INDUCTION AND EVALUATION OF GENETIC VARIABILITY IN CHETHIKODUVELI

(Plumbago rosea L.*)*

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Induction and evaluation of genetic variability in Chethikoduveli (*Plumbago rosea* L.)"** is a bona fide 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, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Induction and evaluation of genetic variability in Chethikoduveli (*Plumbago rosea* L.)"** is a record of research work done independently by **Smt. Arya. K.** 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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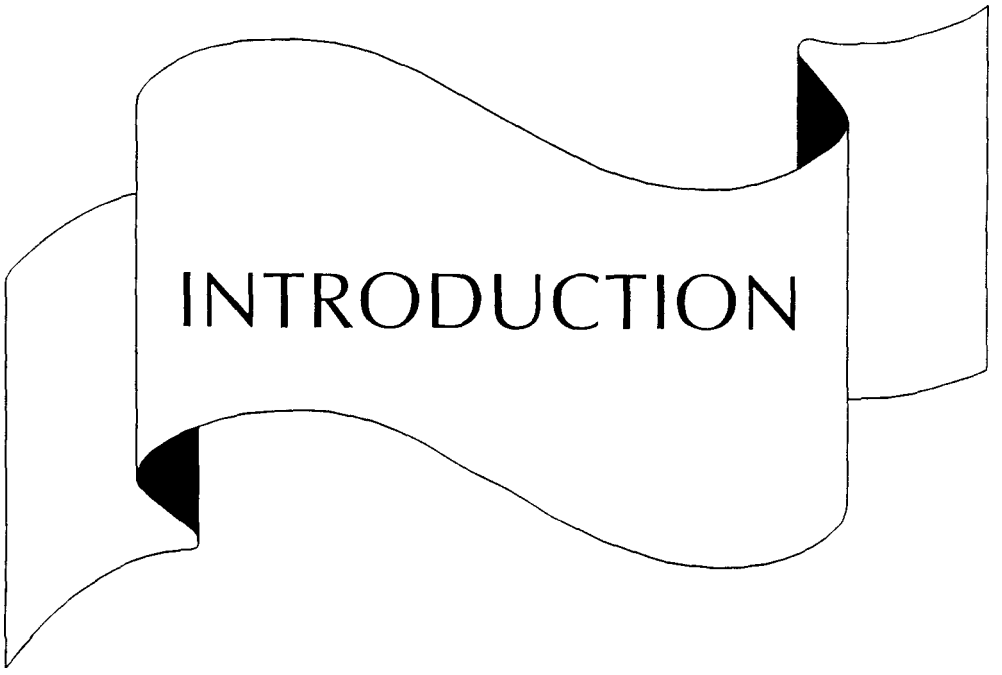

INTRODUCTION

INTRODUCTION

Plants have been used as medicinal agents from the earliest days of man's existence. The ancient Indian System of Medicine is predominantly a plant based materia medica, making use of our native plants. It caters to almost the entire rural population of our country. A perusal of literature showed that Indian medicinal plants attracted the attention of various scholars both from within the country and abroad.

Indian plants have always provided a base for upgradation and synthesis of biologically active drugs. For example, the discovery of reserpine from *Rauvolfia serpentina* and its role as an effective antihypertensive and tranquilizing agent was a major breakthrough in the first half of the twentieth century. Later isolation of vinblastine and vincristine from *Catharanthus roseus* as anticancer drugs and discovery of natural drugs like diosgenin from *Dioscorea deltoidea* and solasodine, from *Solanum aviculare* and *S. laciniatum* used in the synthesis of steroid hormones, denoted the importance of herbal medicines. Similarly other uses of natural drugs for treatment of leucoderma, cardiac diseases, liver cirrhosis, blood pressure etc. attracted world attention for herbal

medicines as effective remedies. Consequently, during the last decade there has been an ever increasing demand from the developed countries for more and more therapeutically active alkaloids viz., quinones, steroids, glycosides and terpenoid derivatives. This has necessitated the cultivation of important medicinal plants on commercial scale and development of new varieties suited for this purpose.

'Chethikoduveli', *Plumbago rosea* L. belonging to the family Plumbaginaceae is a highly valued perennial shrub distributed throughout the plains of India. It finds its main use in chemotherapeutics. The roots of this plant contain an acrid crystalline principle called 'plumbagin'. 2-methyl 4-hydroxy 1-4 napthoquinone which is extensively used in the treatment of early cases of chronic skin disease leucoderma, as an abortifacient, for lowering blood pressure and has also got antifungal, antimicrobial and anticancerous properties. Apart from its medicinal and antimicrobial properties, plumbagin can also be used as preservative for non alcoholic drinks and wine. The commercial cultivation of this crop is gaining importance owing to its varied uses and the ease with which it can be grown in the tropics.

Inspite of the medicinal importance of 'Chethikoduveli', not much work has been done in improving the genetic potential of this crop. Systematic breeding programmes have not been undertaken and no promising varieties have so far been reported. Absence of extensive

natural genetic variability in this crop has been the limiting factor in varietal improvement.

Collection of a large number of genotypes of a crop species and its wild relatives play an important role in evolution and genetic improvement of crop plants. The germplasm collections furnish the richest source of variability and crop improvement depend upon the availability of this variability.

The available literature on 'Chethikoduveli' does not reveal any information on varietal availability in this crop. Hence a preliminary attempt has been done for the first time to assemble representative samples of clones from the major growing centres of 'Chethikoduveli' within Kerala State. The collection of these clones was used for basic genetic analysis of the existing variation in different populations of the crop. Rapid strides through recombination breeding in this crop are restricted for want of seed production. The multiplication of this crop is done entirely through vegetative means and so the variation has remained more or less constant. Available literature does not throw any light on the cause of non production of seeds in Chethikoduveli. Basic studies on floral biology, anthesis, pollination etc. are also lacking in this crop. Hence such studies were also undertaken to explore the possibilities of inducing seed fertility in the crop.

Genetic improvement has always been difficult in 'Chethikoduveli' due to absence of sexual reproduction. In such situations, mutation breeding has been found to be a potent and handy tool to induce new and additional variability in both qualitative and quantitative traits.

The history of mutation is as old as the science of modern genetics. Plant mutation research has not only produced valuable results in terms of varieties, but also it has stimulated progress in related sciences, such as plant genetics and plant physiology. Spontaneous mutations have played a considerable role in the improvement of vegetatively propagated crops. The greater constancy of these plants preserved through clonal propagation permits the detection of even slight phenotypic changes. Mutation is the most important means to produce genetic variability in sterile crops. The main advantage of mutation induction in vegetatively propagated crops is the ability to change one or a few characters of an otherwise outstanding cultivar, without altering the remaining part of the genotype. The high degree of heterozygosity and polyploidy in vegetatively propagated crops which are serious handicaps in conventional breeding are advantageous in mutation breeding, as large variations can often be observed in the irradiated plants. Ionising and non-ionising radiations play a significant role for permitting favourable permanent changes, thereby increasing scope for selection. Therefore an attempt was made in the present study to find out the possibility of inducing desirable mutant in this crop by the application of physical mutagen.

Reports of comprehensive work on both the fundamental and the applied aspects of mutation research in medicinal plants are practically nil.

Keeping in view of the information gap on the breeding aspect on one hand and the practical feasibility of evaluating the germplasm and the micro-mutational approach for yield improvement on the other, the present study in *Plumbago rosea* was undertaken with the following objectives:

1. To study the floral morphology, time of anthesis and pollination mechanisms in 'Chethikoduveli' (*Plumbago rosea*).

2. To assess the viability of pollen through palynological studies.

3. Pollination studies to explore the possibility of seed set in 'Chethikoduveli'.

4. Dormancy tests to find out the germination of seed in *Plumbago zeylanica* ('Vellakoduveli').

5. Cytological studies to identify the meiotic behaviour in 'Chethikoduveli'.

6. To find out the extent of variability present in the population by estimating the parameters like genotypic coefficient of variation, heritability and genetic advance.

7. To find out the association of different characters with yield and also among themselves

8. To select adaptable and high yielding ecotypes based on the selection index prepared using major characters.

9. To identify the optimum dose of physical mutagen for the induction of variability

10. To induce variability by physical mutagen like gamma rays in Chethikoduveli.


REVIEW OF
LITERATURE

REVIEW OF LITERATURE

Origin and Classification

Chethikoduveli, *Plumbago rosea* L. is a dicotyledonous plant, belonging to the family Plumbaginaceae of the order Plumbaginales. It comes under the series Heteromerae (Bentham and Hooker, 1884). Hutchinson (1973) and Engler (1973) reported that *Plumbago* comes under the order Plumbaginales. There are seven related genera *viz.*, *Plumbago, Plumbagella, Ceratostigma, Armeria, Limonium, Goniolimon* and *Acantholimon* reported in the family Plumbaginaceae (Darlington and Wylic, 1961). The genus *Plumbago* includes 19 species other than *Plumbago rosea* L. The systematic position of *Plumbago rosea* is as follows.

Class	:	Dicotyledons
Sub class	:	Gamopetalae
Series	:	Heteromerae
Order	:	Plumbaginales
Family	:	Plumbaginaceae
Genera	:	Plumbago
Species	:	Rosea

The species *Plumbago rosea* is reported to be indigenous to Sikkim and Khasi hills. Its cultivation is always associated with anthropogenic localities in both north and south India indicating its use as a tribal medicine (CSIR, 1969). The crop is highly suited to Kerala conditions and is an important ingredient in the indigenous drugs. Albeit a lot of research has been done on chemical constituents and their properties basic studies on the morphology and crop improvement are meagre. The available literature on *Plumbago rosea* is limited. Hence the literature citations for the present work were broadened to cover vegetatively propagated crops belonging to a wide range of economic use such as sugar, tubers, medicinal, aromatic and ornamental.

Flowering

Krizek and Semeniuk (1972) studied the effect of day and night temperature on flowering of *Limonium* an ornamental plant in the family Plumbaginaceae. They reported that a low temperature of 16-13°C was required for flower initiation.

Long days and cool night temperatures increased the percentage of flowering in *Limonium* cultivars (Semeniuk and Krizek, 1972). Application of gibberellic acid 500 ppm accelerated flowering of *Limonium* (Wilfret and Raulston, 1975). Escher *et al.* (1988) reported that *Plumbago indica* requires a day length of nine hours for flowering.

The successful pollination and fertilization in some members of Plumbaginaceae are reported by many authors. Russell (1982) reported the ultrastructure of synergids lacking megagametophyte and successful fertilization in *Plumbago zeylanica*. The successful pollination and developmental phases of sperms in syngamy and triple fusion are reported in *Plumbago zeylanica* (Russell, 1983).

Baker (1966) reported pappillate type and non pappillate type (cob) stigma in several species of Plumbaginaceae. Dulberger (1975) reported that the stigma and pollen dimorphism reflects the incompatibility mechanisms in the family Plumbaginaceae and the inhibition of pollen growth occurs at the stigmatic surface. Bahadur (1978) reported distyly as common in several members of the family Plumbaginaceae. Mattsson (1983) emphasized the significance of pollen derived substances and the interaction between pollen and stigmatic surface in successful pollination. The elongation of pollen tube in the pistil tissues is promoted and guided by pistil derived nutrients and directional cues (Heslop-Harrison, 1987 and Mascarenhas, 1993).

Heritability and Genetic advance

Heritability in the broad sense refers to the relative proportion of genotypic variance to phenotypic variance. Coefficient of variation (CV) is used to compare the relative variation among different metric traits which are measured in different units.

Lush (1937) and Johnson *et al.* (1955) developed accurate procedures for the calculation of genetic advance under specified intensities of selection which in metric traits largely depends on the heritability, phenotypic variability of the trait under selection and the selection differential expressed as phenotypic standard deviation.

Reddy (1980) estimated heritability in broad sense and genetic advance in respect of cane yield, number of millable canes, single cane weight, length of millable cane and juice quality in ten varieties of sugarcane. He found the number of millable canes recorded maximum heritability and genetic advance indicating that this character is less vulnerable to environmental influence and could be relied upon as one of the important criterion for selection. However, Kadian *et al.* (1997) reported that highest heritability was observed for leaf width and single cane weight when 32 genotypes of sugarcane were assessed for nine yield components. He found that high heritability coupled with high genetic advance were associated with single cane weight and cane yield / clump indicating that yields could be improved by direct selection for single cane weight combined with indirect selection for cane productivity.

Ali *et al.* (1994) assessed the genotypic coefficient of variation, heritability and genetic advance in 16 genotypes of ginger. They reported

that the number of leaves per plant and number of nodes per plant had high heritability combined with high genetic advance and these characters were the most suitable for improvement through selection.

In turmeric, Jalgaonkar and Jamdogni (1989) assessed the yield and eleven yield related characters and reported that except weight of mother rhizome and yield of primary and secondary fingers per plant all other characters showed high heritability and high genetic advance. Nandi (1991) reported that the phenotypic coefficient of variation and heritability coefficient of variation and heritability were found to be maximum for yield per plant and lowest for the weight of single mother rhizome in turmeric. Maximum genetic potentiality in turmeric was observed in rhizome yield, length of secondary and primary fingers, weight of mother rhizome and internodal distance of primary fingers and moderate value of heritability was found for nodes, girth, number of secondary fingers, nodes in primary finger, mother rhizome and plant height (Indiresh *et al.*, 1992).

Bindroo and Bhat (1988) reported that, in *Dioscorea deltoidea* diosgenin content, tuber girth, stem thickness and leaf breadth had high heritability while number of branches per tuber and number of nodes in 50 cm stem length were low in heritability.

In *Colocasia esculenta* (L.) Schott, a high heritability estimate was noticed for number of cormels per plant, weight of cormels per plant

and tuber yield (Kumar *et al.*, 1995). Pandey *et al.* (1996) evaluated 31 genotypes for eight yield contributing characters. They reported high heritability combined with high genetic advance for weight of mother cormel, weight of cormels and yield per plant.

In Kacholam, Kanakamany (1998) reported that heritability was highest for leaf length followed by leaf breadth and tiller number and lowest for rhizome weight. Genetic advance was highest for number of leaves followed by rhizome number and lowest for leaf length.

Correlation studies

Correlation studies to determine the inter-relationship among various traits, are useful in making selection. Information on the association of plant characters with yield and also on the intercorrelations are available in crops like ginger, turmeric, sugarcane, colocasia and dioscorea.

Nybe (1978) reported that in ginger, length of leaf blade, length of petiole, leaf area index and number, length and girth of primary and secondary fingers were positively correlated with yield. According to Mohanty and Sharma (1979) in ginger, rhizome yield was positively and significantly correlated with number of leaves, secondary rhizome fingers, tertiary rhizome fingers, total rhizome fingers and number and weight of adventitious roots. Pandey and Donbhal (1993) reported that rhizome

yield per plant was positively associated with plant height, number of fingers per plant, weight of fingers and weight of primary rhizome. The inter-character association pattern showed that in ginger the oleoresin content is positively correlated with gingerol and shogaol content (Zachariah *et al.*, 1993).

Nambiar (1979) estimated the inter correlation among the morphological characters and yield in turmeric and the results showed that number of tillers, plant height and number of fingers had highly significant positive correlation with yield. Number of fingers per plant, number of tillers per plant, height, rhizome length and dry matter percentage contributed four per cent towards yield of turmeric rhizome. Mukhopadhyay and Roy (1986) observed a high correlation between plant height and yield per plant at both the phenotypic and genotypic levels. The yield of cured turmeric was found to be significantly correlated with yield of secondary fingers (Jalgaonkar *et al.*, 1990).

In sugarcane, Das *et al.* (1996) reported that the cane yield was positively and significantly associated with commercial cane sugar, stalk weight, stalk diameter and number of internodes per cane whereas the number of millable canes was negatively and significantly associated with stalk weight, height of millable cane and sucrose percentage. Das *et al.* (1997) evaluated 27 sugarcane cultivars in another study and reported that sugar yield was positively and significantly associated with cane productivity, individual cane weight, purity of juice, commercial cane

sugar percentage and cane height at maturity, whereas number of millable cane was negatively correlated with individual cane weight and its length. Kundu and Gupta (1997) examined 41 sugarcane clones and found that sucrose, brix and commercial cane sugar percentage were positively correlated with sugar yield and cane productivity had a significant positive correlation with number of millabe canes, stalk height, stalk weight and internodal length. The cane yield had a significant correlation with stalk number in sugarcane (Sukhchain *et al.*, 1997). They also found that stalk length had significant correlation with stalk weight and internodal length and commercial cane sugar was positively and significantly correlated with cane yield and stalk number.

In *Colocasia esculenta* (L.) Schott, Sarkar *et al.* (1996) reported that the plant height, number of tillers, number of cormels and weight of cormels were positively correlated with yield.

Bindroo (1988) reported that in *Dioscorea deltoidea* a negative correlation was noticed between the diosgenin content and moisture content of the tubers. He also reported that white fleshed tubers had a higher concentration of diosgenin, lower moisture content and were thicker in tuber girth than yellow tubers and tubers of intermediate colour.

In *Kaempferia galanga*, a significant positive association of yield with number of leaves, tillers, leaf length, plant spread and rhizome number was noticed (Kanakamany, 1998).

Path coefficient analysis

Path coefficient analysis is standardized partial regression coefficient analysis and as such measures the direct effect of one variable and indirect effect via other variables on the dependent variable ie., the response. This analysis permits the separation of correlation coefficients into components of direct and indirect effects of independent variables on dependent variable (Dewey and Lu, 1959).

In ginger, path coefficient analysis (Ratnambal, 1979) revealed that the phenotypic correlation between yield of rhizome and height of pseudostem was quite high and so also the direct effect of height towards the correlation. The direct effect of number of leaves on yield was found to be low. Eventhough length of leaf had a negative direct effect, it was compensated by a high positive correlation between plant height and final yield. Pandey and Donbhal (1993) reported that weight of finger, width of fingers and leaf width were the strongest forces influencing yield in ginger.

Path coefficient analysis in turmeric (Nambiar, 1979) indicated that plant height (of pseudostem) was a single important morphological character for which selection for yield could be made. The height of the plant and length of secondary fingers were the major contributors towards rhizome yield. Direct effects of number of leaves per tiller and girth of mother rhizome were positive whereas number of nodes per primary finger

and petiole length had high negative direct effect on rhizome yield (Geetha, 1985). The rhizome length and number of fingers per rhizome had highest direct and positive effect on rhizome yield (Lal *et al.*, 1986). Mukhopadhyay and Roy (1986) recommended tillers per clump, leaves per shoot and plant height as selection criteria for improving yield.

In *Kaempferia galanga*, Presannakumari *et al.* (1994) recommended the rhizome yield and oil content as selection criteria for crop improvement. Path coefficient analysis of important yield attributes indicated that the number of rhizomes had the maximum direct effect on yield in Kacholam (Kanakamany, 1998).

In *Colocasia esculenta*, Pillai *et al.* (1995) examined 22 accessions and found that number of cormels per plant had the maximum direct effect on yield followed by mean cormel weight. Pandey *et al.* (1996) assessed 31 genotypes of taro and found that weight of mother corm had the highest direct effect and weight of cormels had higher indirect effect on yield and so could be used as selection criterion for higher yielding genotypes. Number of tillers per plant had highest positive direct effect on cormel yield and plant height whereas number of cormels per plant had indirect effect on cormel yield (Sarkar *et al.*, 1996).

In 24 sugarcane varieties, path analysis revealed that commercial cane sugar had highest direct positive effect on cane yield followed by number of millable canes per hectare, stalk weight and stalk diameter.

Commercial cane sugar percentage, sugar percentage in juice, stalk weight and diameter are indirectly associated with cane yield via commercial cane sugar (Das *et al.*, 1996). Path coefficient analysis in 50 sugarcane genotypes revealed that stalk weight had greatest direct effect on stalk yield followed by number of millable canes per hectare (Das *et al.*, 1997). Sukhchain *et al.* (1997) evaluated 17 diverse clones of sugarcane and found that stalk number and stalk weight had relatively high positive direct effect on cane yield and suggested that selection for improvement of cane yield could be based on stalk number and stalk weight.

Yield of plumbagin

Plumbagin is present to a maximum of about 0.91 per cent in the roots of all the species of *Plumbago* seen in India (CSIR, 1969). The proportion of plumbagin varies with the locality, growth, age, soil conditions and season of the year. In general, it was found that older the plant and drier the soil, the greater is the quantity of active principle found in the roots. It has also been reported that the fresh roots yield a much greater proportion of plumbagin than roots which have been stored for longer periods.

Pharmacological properties of plumbagin

Krishnaswamy and Purushothaman (1980) isolated plumbagin from the roots of *Plumbago zeylanica* and reported that it is a potential anticancer drug and is used in reducing the tumour growth in fibrosarcoma

and also against lymphocytic leukaemia. The antiviral activity of Liv 52, a powdered mixture of 18 plants in which *Plumbago zeylanica* is a component was studied by Singh *et al.* (1983). The antifertility activity of plumbagin isolated from the roots of *Plumbago zeylanica* was reported by Bhargava (1984). Gujar (1990) reported the use of Plumbagin in the treatment of liver disorders. The ethanolic root extract of *Plumbago rosea* showed an increase in life span of mice bearing ascites sarcoma-180 tumor (Solomon *et al.*, 1993). Devi *et al.* (1994) suggested that the ethanolic root extract of *Plumbago rosea* as a good candidate for use with radiation to enhance tumour killing effect. Plumbagin obtained from various species of the genus *Plumbago* possesses potent anti-fertility activity (Dhar and Rao, 1995). The anti inflammatory properties of root extracts of genus *Plumbago* were reported by Rimbau *et al.* (1996).

Other biological activities

Plumbagin, a yellow pigment in the species of *Plumbago* is reported to exhibit various insecticidal, antibacterial and antifungal activities. Gujar and Mehrotra (1988) reported that Plumbagin possessed high contact toxicity to *Dysdercus koenigii*. Joshi *et al.* (1988) reported that retarded growth, delayed metamorphosis using deformation and moulting were inhibited when red cotton bug was treated topically with plumbagin. Plumbagin isolated from the roots of *Plumbago indica* was found to have high larvicidal action against mosquito larvae (Chockalingam *et al.*, 1990). The mortality and survival of the parasitic nematode

Haemonchus controtus and the embryonation of *Ascaris sum* were inhibited when plumbagin extracted from the plants of *Plumbago* spp was applied (Fetterer and Fleming, 1991). Villavicencio and Perez-escandon (1992) reported the antifeedant effect of the extracts of *Plumbago* spp. on three species of orthopteran acridids. Gujar *et al.* (1994) studied the bioactivity of the extracts of plants in the genus *Plumbago* and reported that the inhibition of growth by plumbagin in nymphs of *Dysdercus koenigii* was dose dependent and in adults the mating behaviour and reproduction were affected. Plumbagin significantly affected both chitin and cuticular protein when applied topically to the larvae of noctuid *Helicoverpa armigera* (Krishnayya and Rao, 1995). Saxena *et al.* (1996) reported that topical application of plumbagin dissolved in acetone, killed the adults of *Musca domestica*, a dipteran insect in high concentration and also induced sterility in medium concentrations.

Krishnaswamy and Purushothaman (1980) reported that plumbagin extracted from the two species of *Plumbago* viz., *rosea* and *zeylanica* showed antibacterial activity. It inhibits the growth of both gram positive and gram negative bacteria at 20 mg/ml.

The antifungal activity of plumbagin against *Rhizopus nigricans*, *Pencillium notatum*, *P. canadense*, *Epidermophyton floccosum* and *Microsporum nanum* at 10 mg/ml was reported by Krishnaswamy and Purushothaman (1980).

Bharathi *et al.* (1994) reported that plumbagin was 80-100 per cent effective in eliminating plasmids coding for nodulation and nitrogenase activity found in *Rhizobium meliloti* 4013, *Rhizobium leguminosarum* 2001 and *Bradyrhizobium* sp. IC3342.

Mutagens

Both physical and chemical mutagenic agents are valuable for induction of mutation in plants. Physical mutagens are more convenient to use on bulky plant materials like stem cuttings and tubers. Among the physical mutagens, X-rays and gamma rays are extensively used by the plant breeders.

Physical mutagens

Among the variety of ionising radiations available, the most common ones used are X-rays and gamma rays. The critical doses of gamma rays depended on the genotype of the irradiated plants. Favourable changes brought about by lower doses of gamma irradiation have been successfully exploited in vegetatively propagated crops (Desai and Abraham, 1974 and Raghava *et al.*, 1988).

The use of X-rays and gamma rays is the most practical method of inducing mutation of vegetatively propagated plants with all kinds of starting materials, such as whole plants, stem cuttings, tubers, bulbs, rhizomes and detached leaves. Low dosages of irradiation are preferred

if the goal is to change only one gene in an otherwise undisturbed genetic background (Broertjes and Harten, 1978).

Mutations induced by gamma rays for creating desirable combination of traits have been reported in many ornamental like carnation (D´Amato *et al.*, 1964), crocus (Mitsukini and Arai, 1965), gladiolus (Buiatti and Tesi, 1968), *Polyanthes tuberosa* (Gupta *et al.*, 1974), Chrysanthemums (Broertjes and Harten, 1978) and in plants like cassava (Vasudevan *et al.*, 1967), potato (Upadhya and Purohit, 1973), turmeric (Reghupathy *et al.*, 1976), ginger (Raju *et al.*, 1980; Giridharan, 1984 and Jayachandran and Mohanakumaran, 1992), sweet potato (Kukimura and Kouyama, 1982) and coleus (Vasudevan and Jos, 1988 and 1989). Generally gamma rays in lower dosage causes stimulatory effects (Gupta *et al.*, 1974) and in higher dosage induces mutagenic changes (Ono, 1971).

The main limitations in irradiation of vegetatively propagated plants, where vegetative parts have to be irradiated are chimera formation and diplontic selection both causing complications in selection due to multicellular nature of bud apex. The result is a relatively low mutation frequency and limited mutation spectrum, while selection procedures cannot be applied before the stable periclinal chimera stage has been reached. These difficulties can be largely restricted or avoided by the use of *in vivo* or *in vitro* adventitious bud techniques, by which large number of solid, non chimeral mutants can be produced if explants are irradiated before regeneration of the adventitious shoots (Broertjes and

Harten, 1978). Plant breeders generally prefer ionizing radiation because it is easily applicable, with good penetration and high mutation frequency (Broertjes and Harten, 1978). In case of vegetatively propagated plants, the main advantage is that once a good genotype is obtained it can be propagated and made use of directly (Broertjes and Harten, 1978).

Gaul (1970) gave the following account on the effects of irradiation on vegetatively propagated plant materials. After irradiation of plant and plant parts, a range of effects can be observed depending on the character and stage of material as well as on the radiation treatment given. Only a few plants reveal mutation effects, because the naturally occurring material when subjected to artificial changes responds very little on account of its well established stabilisation in constitution. Mutation effects may differ even within the same plant. The main effects of mutagens include physiological changes (primary injury), point mutations or gene mutations and chromosomal aberrations. Primary injury is restricted to M_1 generation whereas the latter two are transferred to the succeeding generations. In chromosomal mutations all the plant features are affected, while in other types of mutations only few or single characters are affected.

Sparrow (1961) and Gaul (1970) have reported plant injury or lethality as the major consequence of mutagenic treatments on vegetative parts. These effects vary very much according to genotype of plant type

and dose of mutagen employed and modifying factors present. Sparrow (1961) and Evans (1962) have reported that cytological changes are also met with as a result of mutagenic treatments. The type of induced chromosomal mutations their mitotic and meiotic behaviour and genetic consequences have been reported by Sparrow (1961). Most of the radiations induced sterility in M_1 and further generations is haplontic (Muntzing, 1930).

Mutation frequencies

Mutations are generally found to occur at random with differences in mutability between different loci and regions of chromosomes. For potato, Heiken (1958) has summarized mutation frequencies as one in 10^{-6} or 10^{-7} plants showing spontaneous mutation for the leaf character and yellow margin. According to many studies polyploids exhibit lower mutation frequencies than diploids (Broertjes, 1976). In polyploids mutagenic treatments lead to gross chromosomal damage with a dominant expression for certain traits (Broertjes, 1976). Information about frequencies of spontaneous and induced mutations in vegetatively propagated crops is rather scarce. Most authors express mutation frequencies for vegetatively propagated plants as the percentage of plants showing one or more mutations for number of visible characters.

The effect of mutagen treatment on different traits under study in some important vegetatively propagated crops is reviewed here.

Sprouting

A delay in sprouting and reduction in germination percentage are noticed consequent to irradiation at higher doses as reported by Sparrow and Christenson (1950) in potato tubers, Vijayalakshmi and Rao (1960) and Jalaja (1971) in sugarcane, Vasudevan *et al.* (1967) and Thamburaj *et al.* (1985) in cassava, Vasudevan *et al.* (1967) in colocasia, Gupta and Shukla (1971) in rose and Natarajan (1975) in turmeric.

Abraham (1970) reported in cassava that maximum sprouting of buds from irradiated stem cuttings was obtained at doses of less than 1.5 kR whereas no sprouts were produced at all at doses of 5.0 kR and more. The percentage of sprouting decreased as the dose of gamma rays increased in tuberose. At 0.5 kR the sprouting percentage was 96, which reduced to 72 at 2.5 kR (Sambandamurthi, 1983). Sumabai and Nayar (1992) observed in sweet potato that the gamma ray induced population took more number of days for sprout initiation and completion of sprouting.

The exposure of gamma rays reduced the sprouting percentage also. Giridharan and Balakrishnan (1992) noticed decreased sprouting percentage at increased dosages of gamma rays in ginger.

Survival

In colocasia, many plants germinated normally but failed to survive after gamma irradiation (Vasudevan *et al.*, 1968). Gupta *et al.* (1974)

found that exposing tuberose to doses above 2.0 kR gave no survival of plants. In gladiolus, post germination lethality occurred and 50 per cent survival of plants was obtained at 4.7 kR gamma rays (Abraham and Desai, 1976). In *Costus speciosus* a reduction in survival resulted on gamma ray treatment at higher doses of 3.0 kR (Gupta *et al.*, 1982).

Flowering

The number of days for flowering was very much influenced by mutagenic treatments. Many workers demonstrated the effect of gamma rays in modifying the flowering behaviour of rhizomatous and allied crops such as canna (Nakornthap, 1965), iris, (Broertjes, 1968), sugarcane (Singh, 1970 and Das *et al.*, 1975), tuberose (Gupta *et al.*, 1974 and Sambandamurthi, 1983) and gladiolus (Misra, 1976 and Raghava *et al.*, 1988). Induction of non flowering characters has been reported in sugarcane by Walker and Sisodia (1969) and Rao (1974).

In the case of potato tuber treatment with physical and chemical mutagens led to increase in fertility. The flowering time in Kalanchoe was very much influenced by mutation (Broertjes and Harten, 1978). Rao (1974) obtained sugarcane clones with little or no flowering when the cuttings were treated with 3.0 and 5.0 kR gamma rays followed by three cycles of propagation and selection for absence of flowering. Jagathesan (1977) could produce flowerless mutants in sugarcane. In sweet potato flowering mutants were produced by Kukimura and Kouyama

(1982). Flowering behaviour of ginger could not be altered by the levels of gamma irradiation (Giridharan and Balakrishnan, 1992).

Morphological variants

A number of useful morphological variants have been reported in vegetatively propagated crops in general and ornamentals in particular.

In sugarcane, the mutagenic treatments resulted in a number of foliar abnormalities (Prasad, 1959). Vijayalakshmi and Rao (1960) irradiated species of Saccharum and hybrids and obtained several morphological mutants. Hrishi *et al.* (1968) found that the treatment of buds and growing meristems of sugarcane with different chemical mutagens produced morphological variants. Mutants for glabrous leaf sheath in pubescent varieties of sugarcane Co-527 and Co-419 were observed by Jagathesan and Sreenivasan (1970). Escober and Lopez (1970) irradiated sugarcane seed pieces with gamma rays and produced abnormalities of the growing point, malformation of the leaves as well as stunting and reduction in the size of the stalk. Jagathesan (1977) produced a number of variants in sugarcane like dwarfs, those that are flowerless, and those with glabrous leaves, increased girth rate and yield.

In potato, the gamma irradiation of tubers resulted in different aberrant types like light green types with several kinds of leaf deformities (Heiken, 1961). Kishore *et al.* (1963) and Jauhar (1969) also studied

the effect of gamma irradiation in potato tubers and observed morphological differences in foliage shape, size and vigour of plants as well as tuber production. In potato a few mutants of practical importance such as the yellow skinned tuber mutants from an outstanding red skinned seedling (Broertjes and Harten, 1978) and tuber mutants in commercial varieties Kufri Sindhuri and Kufri Red (Jauhar and Swaminathan, 1967) are well known.

Masima and S ato (1959) observed variations in leaves, stems and tubers in the X_1 generation of the X-ray irradiated young shoots of sweet potato. Hernandez *et al.* (1964) observed somatic mutations such as changes in skin colour and flesh colour of roots, due to irradiation of gamma rays in sweet potato. Soriano (1972) could irradiate buds of sweet potato with gamma rays (0.8-3.2 kR) and obtained leaf mutants with ovate cordate and serrate types. Sumabai (1989) found that in sweet potato the vine length and branch number per vine varied in treated population.

In cassava, Vasudevan *et al.* (1967) obtained viable morphological mutants by treating the stem cuttings with gamma rays at a dose range from 4.0 kR and 7.0 kR. Moh (1976) obtained somatic mutants in cassava with 4.0 kR gamma rays.

Gupta (1966) and Gupta and Shukla (1970) noted that, radiation treatments not only affected the flower shape and size in rose, but

significant changes were produced in essential oil content also. Kaicher and Swarup (1972) observed the occurrence of deformed shoots with puckered, thickened and chlorophyll mosaic leaves and leaves with forked and united leaflets in 5.0 kR gamma ray irradiated Christian Dior Rose.

Desai (1973) exposed the cuttings of chrysanthemum to both acute and chronic gamma irradiation and observed acute exposure to be more effective than chronic ones. Gupta *et al.* (1974) induced and multiplied a large number of flower colour mutants in chrysanthemum.

Raju *et al.* (1980) observed formation of weak and elongated underground rhizomes in ginger on treatment with 20 kR gamma rays. In turmeric and mango ginger, the same treatment showed almost normal growth, but the leaves showed abnormalities (Raju *et al.*, 1980).

Sharma and Mukherjee (1973) produced mutants in grape variegata by gamma irradiation of a Pusa seedless cutting. Donini (1976) presented several cherry scion mutants by the induction of X-rays, γ-rays and thermal neutrons.

Rhizomes of young plants of canna irradiated with gamma rays at 1.0, 1.5 and 2.7 kR resulted in stunted plants with variegated leaves (Nakornthap, 1965). In dahlia a large number of mutations for flower colour and form have been reported (Broertjes and Ballego, 1967). Gupta *et al.* (1982) reported that in cactus the number of branches per plant increased at 1.5 kR but decreased at 3.0 kR.

Chlorophyll deficient mutants

In carnation, chlorophyll deficient sectors were observed when rooted cuttings were gamma irradiated (Buiatti *et al.*, 1965). In tapioca, Vasudevan *et al.* (1967) produced chlorophyll deficient plants at 0.3 to 10 kR gamma ray exposure. Vasudevan *et al.* (1968) also reported the occurrence of chlorophyll deficient plants in *Colocasia esculenta* as a result of gamma ray irradiation at 0.5 kR to 10 kR.

In other vegetatively propagated crops namely, canna (Nakornthap, 1965), colocasia (Vasudevan *et al.*, 1968), mentha (Ono, 1971) and tuberose (Gupta *et al.*, 1974; Konzak, 1966 and Sambandamurthi, 1983), leaf variation due to gamma ray irradiation has been reported. Giridharan and Balakrishnan (1992) reported appearance of yellow stripes as a result of gamma irradiation in the ginger cv. Rio-de-janeiro, and Maran. In colocaisa, Vasudevan *et al.* (1987) observed chlorinas and other leaf abnormalities.

Yield and quality attributes

A large number of useful types with high yield are obtained as a result of mutation in vegetatively propagated crops.

Vasudevan *et al.* (1967) could observe mutants in cassava with high starch content and with decreased HCN content, which would enhance the industrial value of cassava. Abraham (1970) and Nayar (1975) obtained

mutants for high yield in cassava. Moh (1976) obtained somatic mutants in cassava with high tuber yield.

In sweet potato, cold tolerant types were obtained when exposed to gamma rays (Miu, 1973). Kukimura and Takemata (1975) reported that mutants with increased as well as reduced sugar content were obtained after treatments of shoots, dormant root tubers and seeds of sweet potato with Co^{60} gamma rays. The higher exposure (2.0 - 2.5 kR) of gamma rays was effective in increasing the tuber yield and tuber number in sweet potato (Sumabai and Nayar,1995).

The diosgenin content in costus increased as a result of 2.0 kR gamma ray treatment whereas it decreased at 30 kR (Gupta *et al.*, 1982).

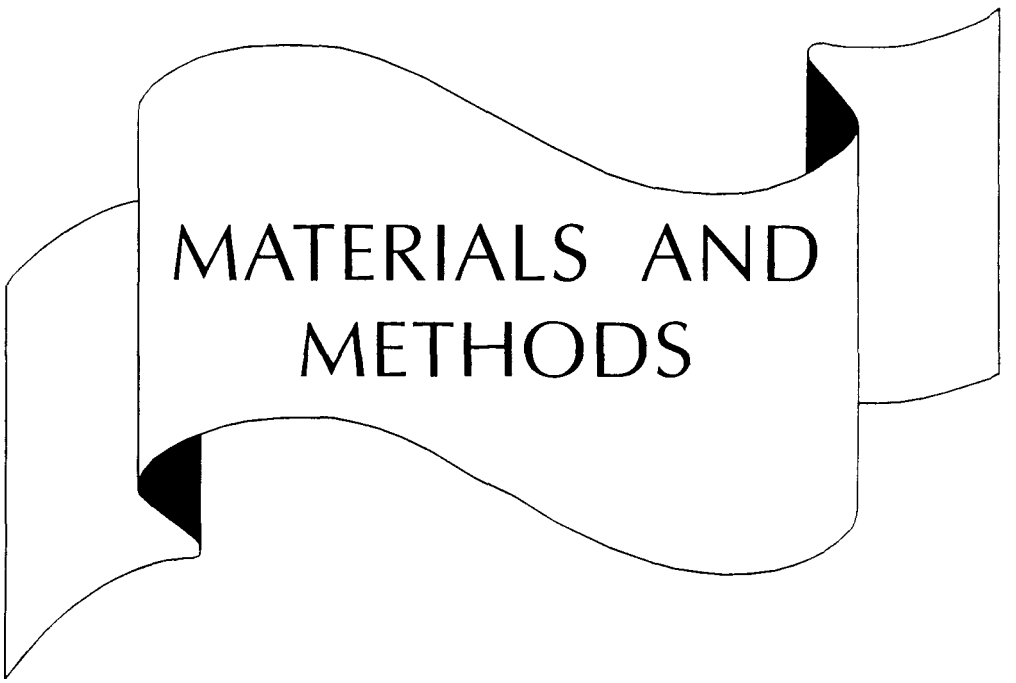
In turmeric, Reghupathy *et al.* (1976) obtained mutants resistant to scales (*Aspidiotus hartio*) through gamma irradiation. Stimulatory and mutagenic effects of ionising radiations can be exploited commercially in turmeric (Shah *et al.*, 1982). Rangaswamy (1986) obtained mutants in *Curcuma longa* by X-irradiation, with orange yellow rhizomes with a high curing percentage and curcumin content.

Dormant single budded suckers of *Mentha arvensis* var piperascens were subjected to various X-ray and gamma ray treatments and mutants with improved oil constituents were obtained (Kak and Kaul, 1979). Pavlovic *et al.* (1983) observed a positive correlation between

irradiation dose and essential oil content in *Mentha piperata*. Nair (1969) isolated lemon grass mutants with high essential oil content by gamma ray treatment.

In ginger Giridharan (1984) found that the quality in terms of spice oil and oleoresin content was not altered by irradiation with gamma rays. In coleus the yield per plant was lower in mutants but had uniform and average sized tubers (Vasudevan and Jos, 1988).

Lata and Gupta (1971) and Irulappan (1979) were able to isolate some mutant clones of roses with qualitative and quantitative changes in their essential oils.


MATERIALS AND
METHODS

MATERIALS AND METHODS

The investigations reported herein on the "Induction and evaluation of genetic variability in 'Chethikoduveli' (*Plumbago rosea* L.)" were carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during the period 1995-1998. Field experiments were conducted in the garden attached to the Department of Plant Breeding and Genetics. The phytochemical analyses were carried out at the Phytochemistry Laboratory of the Tropical Botanic Garden and Research Institute, Pacha, Palode.

Materials

The study was undertaken through the conduct of the following two different field experiments

Experiment - 1 Germplasm collection and evaluation

Experiment - 2 Induction of variability

For the first experiment seven ecotypes of *Plumbago rosea* collected from different parts of Kerala and one related species *Plumbago zeylanica* L.

collected from College of Agriculture, Vellayani were used. The details of these plant materials are presented in Table 1.

Table 1. Details of plant materials used for germplasm collection and evaluation

Sl. No.	Species	District	Source	Type designation
1.	*Plumbago rosea*	Trivandrum	Tropical Botanic Garden and Research Institute, Palode	Et_1
2.	*Plumbago rosea*	Kottayam	Farmer's field at Kozha	Et_2
3.	*Plumbago rosea*	Malappuram	Medicinal garden of Arya Vaidyasala	Et_3
4.	*Plumbago rosea*	Trivandrum	Farmer's field at Neyyattinkara	Et_4
5.	*Plumbago rosea*	Trichur	College of Horticulture Vellanikkara	Et_5
6.	*Plumbago rosea*	Ernakulam	Farmer's field at Muvattupuzha	Et_6
7.	*Plumbago rosea*	Palghat	Medicinal garden of Arya Vaidya Pharmacy	Et_7
8.	*Plumbago zeylanica*	Trivandrum	College of Agriculture Vellayani	Et_8

For the second experiment plant material from Sl. No. 5 (Et_5) was used.

Methods

Germplasm collection and evaluation

Two noded cuttings excised from the semi-hardwood portion were planted with one node above soil surface in polybags (12 x 18 cm with 150 guage thickness) filled with potting mixture (soil, sand and cowdung in equal proportions). In each bag four cuttings were planted. The potted bags were kept under shade and judiciously watered. One node of each cutting was dipped in 500 ppm IBA (Indole butyric acid) for 30 seconds before planting. This was done to facilitate easy rooting. Termite attack observed in the nursery was effectively controlled by drenching BHC 50 WP at 0.2 per cent.

Planting

The land was dug well and the soil was brought to a fine tilth. After the onset of Southwest monsoon, three month old rooted cuttings of the eight ecotypes were raised in a Randomised Block Design (RBD) with three replications. Thirty plants were planted at a spacing of 50 x 15 cm. At the time of planting, FYM @ 10t/ha was applied and incorporated into soil. Fertilizers were applied at the rate of 50:25:50 kg NPK/ha. Two thirds of the total dose was given at two months after planting and the remaining one third five months after planting. Fertilizer application was followed

by earthing up. The plot was hand weeded as and when necessary and the crop was raised as irrigated field crop.

Leaf blight and die back diseases were noticed during summer periods and three sprays of Indofil M.45 0.3 per cent were given to control them. Mite attack was observed and was controlled by Kelthane 0.2 per cent spray. Detailed studies on the floral biology, cytology, compatibility and biometrics were made.

Study of floral biology

In order to find out the causes for the failure of seed set in *Plumbago rosea* and lack of seed germination in *Plumbago zeylanica*, detailed observations on the following aspects were done.

Days taken for first flower opening

Number of days taken to start flowering was recorded by counting the days taken from planting to the first flower opening in a plant.

Time of anthesis

Flower opening was closely observed at intervals of five minutes from 6.00 AM to 10.00 AM in ten plants in each replication in every treatment.

Floral morphology

The morphological features of the inflorescence and the flower were closely observed with the help of hand lens and dissection microscope.

Pollen size and fertility

Measurement on pollen was taken by using micrometer. Freshly collected pollen were stained with aceto-carmine-glycerine mixture (1:1) and the fertility percentage on the basis of stainability was estimated as the percentage of stained pollen over the total number of pollen grains (Shivanna and Johri, 1985) as average of 10 fields counted for each treatment.

Pollen viability

***In vitro* studies**

Freshly collected pollen grains were incubated in the following media to study the viability.

i) Distilled water
ii) 0.5 % sucrose
iii) 1.0 % sucrose
iv) 1.5 % sucrose
v) 2.0 % sucrose
vi) Standard Brew Baker's medium

Boric acid	-	100 mg
Potassium nitrate	-	100 mg
Magnesium sulphate	-	100 mg
Calcium nitrate	-	150 mg
Sucrose	-	5 per cent

Pollen grains were placed in a drop of the culture solution on a slide and incubated in the following manner. A pair of petridishes were lined with moist filter paper. Two match sticks were placed parallel in the bottom half on which two slides containing a drop of the germination medium with the pollen grains were placed and was covered by the top half. This was left undisturbed at room temperature for 24 hours. The percentage of pollen grains germinated was recorded after staining in one per cent cotton blue. The germinated grains were counted from ten different fields per treatment per replication and the percentage of germination was calculated as

$$\text{Percentage of germination} = \frac{\text{No. of pollen grains germinated}}{\text{Total number of pollen grains}} \times 100$$

***In vivo* studies**

To study the pollen germination and tube growth *in vivo*, freshly opened flowers were pollinated with appropriate pollen using a clean sterile brush.

The following method was adopted to study the pollen germination and tube growth *in vivo*. At the desired time after pollination, the pistil was dissected out from the flower and fixed in Carnoy's fluid (1:3 acetic

acid - alcohol). After keeping the pistils for one to two days in the fixative they were transferred to slides into which few drops of lactophenol-cotton blue mixture (1:1) were added and the pistils were warmed upto to 60°C until the stigmatic tissue became soft. A cover glass was placed on the material, gently pressed and observed under microscope.

Compatibility studies

In order to explore the possibility of widening the gene pool in *Plumbago*, different systems of pollination were tried with the eight ecotypes and the seed set in each was assessed as shown below.

Artificial self pollination	-	by using pollen from the same flower
Artificial sibbing	-	by using pollen from separate flower of the same plant
Artificial cross pollination	-	by using pollen from other ecotypes
Bud pollination	-	by pollinating the previous day of flower opening
Mentor pollination	-	by using a mixture of pollen grains, ie.. from irradiated and normal plants
Chemically aided pollination	-	by spraying the stigmatic surfaces with five per cent sucrose and pollinating with pollen of another plant

Pollination on decapitated pistil - by removing the stigma with scalpel and applying pollen grains on the cut surface of the style.

Twenty flowers were randomly selected from each treatment and used for each pollination technique. Per cent seed set was recorded later.

Cytology

To trace the cytological background of the two species, viz., *Plumbago rosea* and *Plumbago zeylanica* flower buds of varying age were fixed in Carnoy's fluid. Meiosis was studied using acetocarmine as stain.

Dormancy studies

To test the seed dormancy, mature seeds of *Plumbago zeylanica* were collected and kept for germination both under laboratory and field conditions. Fruit with peristant calyx, seeds with intact seed coat and seeds after mechanical scarification were subjected for germination studies.

Biometric analysis

The data collected for the various characters were tabulated and mean values were subjected to statistical analysis. Analysis of variance and covariance was done to estimate the phenotypic, genotypic and environmental components of variance. The estimates of coefficients of variation, correlation coefficients heritability coefficient and genetic advance were computed from the formulae given below.

Phenotypic variance

$$V_{(P)} = V_{(G)} + V_{(E)}$$

where $V_{(G)}$ = Genotypic variance

$V_{(E)}$ = Environmental variance estimated as mean square due to error

Genotypic variance

$$V_{(G)} = \frac{\text{Meansquare (Treatment)} - \text{Mean square (Error)}}{\text{Number of replication}}$$

The phenotypic and genotypic coefficients of variation were worked out for each character by making use of the estimates of $V_{(P)}$ and $V_{(G)}$ defined above

$$\text{Phenotypic coefficient of variation (PCV \%)} = \frac{\sqrt{V_{(P)}}}{\text{Mean}} \times 100$$

$$\text{Genotypic coefficient of variation (GCV \%)} = \frac{\sqrt{V_{(G)}}}{\text{Mean}} \times 100$$

where mean indicate the mean of a character taken over all the treatments.

Heritability (in broad sense)

It is defined as the ratio of the genotypic variance to the phenotypic variance and was estimated for each character as

$$\text{Heritability } (H^2) = \frac{V_{(G)}}{V_{(P)}} \quad \text{or} \quad \frac{V_{(G)}}{V_{(P)}} \times 100$$

(in percentage)

Genetic advance

The expected genetic improvement by selection was given by the genetic advance (GA) which was worked out as

$$GA = k \cdot h^2 \cdot \sqrt{V_{(P)}}$$

where 'k' is the standardised selection differential which is equal to 2.06 in the case of five per cent selection in large samples.

Phenotypic, genotypic and environmental correlations

These correlations were computed by completing the analysis of covariance tables between each pair of observations. The phenotypic correlation coefficient between two characters x and y was estimated as $r_p(x,y)$

$$r_p(x,y) = \frac{Cov_p(x,y)}{\sqrt{V_{(P)}x} \times \sqrt{V_{(P)}y}}$$

where $Cov_P(x,y)$ denoted the phenotypic covariance between characters x and y. This was obtained by equating the respective expected values of mean sum of products. $V_{(P)}x$ and $V_{(P)}y$ denoted the estimated phenotypic variance for x and y respectively.

The genotypic correlation coefficient $r_g(x,y)$ and environmental correlation coefficient $r_e(x,y)$ were also computed from the analysis of covariance tables. The above formula was used in this case also with the phenotypic covariances and variances replaced by the genotypic or environmental covariances and variances.

The significance of the phenotypic correlation coefficients was tested with reference to the critical value of r at (n-2) degrees of freedom where n is the number of pairs of observations used (Snedecor and Cocharan, 1980). Tests are not available for the genotypic correlation coefficient.

Path coefficient analysis

Path analysis is applied to identify relatively important component characters (which are the independent variables) of a dependent variable on the basis of their direct and indirect effects and it helps the plant breeder to lay emphasis on component characters during selection. The solution of the matrix equation,

$$\underset{\sim}{A}\ \underline{B} = \underline{C}$$

where, $\underset{\sim}{A}$ is the genotypic inter-correlation matrix with respect to independent variables, $\underline{B}$ is the column vector of path coefficients and $\underline{C}$ is

the column vector of genotypic correlation coefficients between the dependent and independent variables. Vector $\underline{B}$ provides estimates of path coefficients which means the direct effect of the independent variable on the dependent variable, and also the indirect effect of each independent variable on dependent variable through other variables. Residual variation which could arise form unknown and uncontrallable factor was also estimated using Vector $\underline{B}$ (Dabholkar, 1992).

Selection Index

Selection index proposed by Smith (1936) based on discriminant function of the observable characters was used to select the genotypes for crop improvement. The phenotype was expressed as

$I = b_1x_1 + b_2x_2 + ... + b_nx_n$ where n characters were involved and the genetic worth H, of a plant is defined as $H = a_1G_1 + a_2G_2 + ... + a_nG_n$ where G_1 G_2 .... G_n represent the genotypic value of the characters and a_1, a_2 .... a_n denote the weights to be assigned to each character. The 'b' coefficients were determined such that the correlation between H and I is maximum, so that, maximum gain can be expected in the selection of the phenotype. This will lead to the solution of the system of matrix equations given by $\underset{\sim}{P}\ \underline{b} = \underset{\sim}{G}\ \underline{a}$ where, $\underset{\sim}{P}$ and $\underset{\sim}{G}$ are the phenotypic and genotypic variance - covariance matrix respectively, $\underline{b}$ is the column vector of b coefficients and $\underline{a}$ the column vector of assigned weights which are taken as unit in the present case without distinguishing the relative importance of each of the component characters. Selection indices were

calculated for all the ecotypes and those with the highest values could be considered for further breeding programmes.

Experiment 2. Induction of variability

Gamma irradiation was done in the CO^{60} gamma chamber available at the Radio Tracer Laboratory attached to the College of Horticulture, Vellanikkara. The details of the source, dose rate / half life and mode of action of mutagen are given in Table 2.

Table 2. Source, dose rate and mode of action of mutagen

Mutagen	Source	Dose rate/ Half life	Mode of action
Gamma rays	Radio Tracer Laboratory College of Horticulture, Vellanikkara.	5000 rads / min.	Ionization

Two noded semi-hardwood cuttings of *Plumbago rosea* of uniform size collected from College of Horticulture, Vellanikkara were subjected to different doses of the mutagen. Detailed studies on the qualitative and quantitative aspects were made in the M_1V_1 generation.

Sensitivity studies

In order to find out the optimum dose of gamma irradiation a preliminary study using two noded cuttings of 'Chethikoduveli' was done. Twenty samples each were at first subjected to eight different doses of gamma rays, viz., 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0 and 40.0 kR. The irradiated cuttings along with control were planted immediately in polybags filled with potting mixture. Sprouting count was observed for 45 days from the date of planting. Since none of the cuttings sprouted, a second set of irradiation treatment was given to another set of cuttings with ten different doses from 0.5 to 5.0 kR at 0.5 kR intervals. The irradiated cuttings were planted and sprouting counts were taken daily for 45 days from the date of planting. Percentage of sprouting was worked out. The values of sprouting were then transformed into angles and statistically analysed to find out the significance of difference between the treatments. From the data on percentage sprouting, the ED_{50} (the dose which gave fifty per cent and above survival rate) was found out by employing the method of probit analysis. Based on the result thus obtained six doses of gamma rays at regular intervals with ED_{50} as highest dose were fixed as follows 0.25, 0.50, 0.75, 1.0, 1.25 and 1.50 kR.

Field studies

Effect of mutagen on M_1V_1 generation

The field experiment was laid out with the two-noded semi-hardwood cuttings of *Plumbago rosea* collected from Vellanikkara (Et_5).

The cuttings which were exposed to the six doses of gamma rays viz., 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kR along with an untreated control were planted in polybags filled with potting mixture. Two hundred and fifty cuttings were used for each treatment. After three months the sprouted cuttings were planted at a spacing of 50 x 15 cm in raised beds with 25 plants per plot. The experiment was laid out in RBD with seven treatments and four replications. The cultural and manurial practices were done as described earlier in Experiment 1. Irrigation was provided uniformly.

The observations were taken on the survival of plants in the nursery (on 30th, 60th and 90th days) and one month after transplanting (on 120th days after planting [DAP]) and the morphological variants obtained were recorded.

Survival count on 30th, 60th, 90th and 120th day of planting

Survival of plants in the field was determined on the 30th, 60th, 90th and 120th day of planting the cuttings by counting the surviving plants in the field on that particular day and the percentage value computed from the total number of plants planted for each treatment.

Chlorophyll and other morphological variants

Chlorophyll chimeras

The plants were examined at periodic intervals for chimeric expressions of chlorophyll deficient patches or sectors on their leaves.

Other morphological variants

The population was examined at regular intervals for the presence of other morphological variations due to the direct effect of the mutagen.

Other observations

The observations on various other characters were recorded from ten randomly selected plants for each treatment, both in the nursery and in the mainfield, for the two experiments.

Nursery observations

Days taken for sprouting

Number of days taken to start sprouting was calculated from the date of planting to the date of emergence of the sprout from the buds.

Days taken to complete sprouting

Number of days taken to complete sprouting was calculated from the date of planting to the day after which no further sprouting was observed.

Sprouting percentage

Sprouting counts were taken daily from the date of planting to the day after which no further sprouting was observed. Total sprouting

percentage was estimated from the values taken on the day after which no further sprouting was observed.

Number of leaves per plant

The number of leaves per plant was counted for each observational plant from 45 days after planting (DAP) in polybags to 90 DAP at 15 day intervals and the average number of leaves per plant was worked out.

Main field observations

Plant height

The height of plant was measured at monthly intervals from the nursery till harvest. The measurements were taken from the ground level to the tip of the top most leaf and expressed in centimeter (cm).

Internodal length

The distance between the point of attachment of the first fully opened leaf and that of the next lower leaf was measured at monthly intervals from 90 DAP in the nursery till harvest and recorded as internodal length in cm.

Number of suckers per plant

The number of suckers per plant was counted from 90 DAP in the nursery till harvest at monthly intervals.

Days taken for blooming

Number of days taken to start flowering was recorded by counting the days taken from planting to the first flower opening in a plant.

Post harvest observations

Harvesting was done fifteen months after transplanting to the main field. All the ten observational plants from each plot were dug out separately. Then the roots and shoots were washed in flowing water to remove the adhering soil particles and the data on the following parameters were recorded.

Length of root

The length of the longest root was measured separately for each observational plant and mean worked out and expressed in cm.

Number of roots

The number of primary roots in each observational plant was counted separately and mean worked out.

Girth of root

The girth of the thickest root was measured separately for each observational plant and mean worked out and expressed in cm.

Fresh weight of roots

Fresh weight of roots for each observational plant was recorded separately and the mean per plant was worked out and expressed in gram.

Fresh weight of shoot

The fresh weight of shoot for each observational plant was recorded separately and the mean shoot weight per plant was worked out and expressed in gram.

Dry weight of roots

The roots were dried in shade for one week and the dry weight of roots were recorded separately and mean dry weight of root per plant was worked out and expressed in gram.

Dry weight of shoot

The shoot samples after recording the fresh weight were dried in a hot air oven at 80°C for 48 hrs. Then the samples were weighed separately and mean dry weight was computed and expressed in grams.

Biochemical studies

Extraction of plumbagin

A sample of root from each of the selected ten observational plants was collected and pooled to get a composite sample. These bits of roots

were dried and powdered. Samples of the powder was extracted with chloroform using a Toshniwal Soxhlet extractor or apparatus for 8 hours at room temperature until all the pigments were leached out of the samples. The chloroform extract was separated through Whatman No. 1 filter paper and the filtrate was concentrated under vacuum in Buchi Rotavapor - M. The concentrated samples were used for chromatographic analysis.

Thin Layer Chromatography (TLC)

The crude plumbagin extracts from the roots were analysed by TLC. TLC plates were prepared using silica gel G-60 (Hi-media Laboratories, Bombay) as the adsorbent. A slurry of silica gel was prepared by mixing silica gel with distilled water in 1:2 (w/v) ratio and spread on 5mm thick glass plate (20 x 20 cm) with the help of a TLC applicator which was adjusted to a thickness of 1.0 mm. The plates were dried at room temperature and activated at 100°C for 30 minutes in a thermoregulated air oven before use.

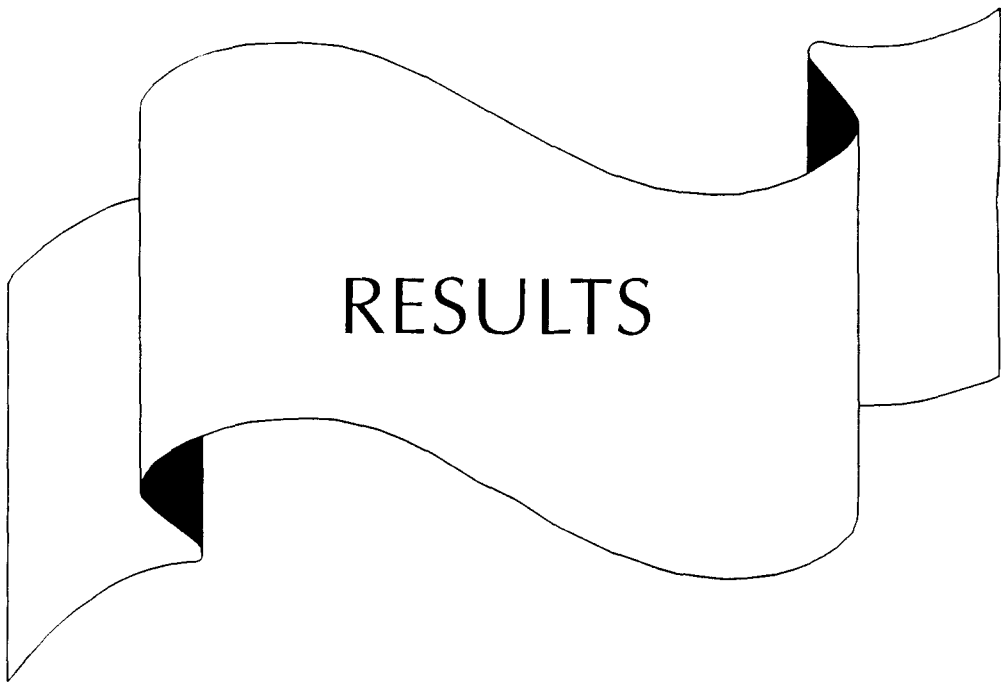
Benzene was used as the solvent to run TLC. Rectangular glass chromatography chambers (30 x 20 x 25 cm) were lined with filter paper to provide a saturated atmosphere. The chromatographic run was carried out at room temperature.

Standard plumbagin (Sigma - Aldrich Co. Ltd., USA) and chloroform extract were applied on TLC plates, 2cm from the bottom of the plates using separate microsyringes. When the spots were thoroughly dried, the

plates were placed in the chromatographic chambers and sealed with the lid. The solvent was allowed to run until its front reached the top of the plate. The chromatograms were then removed, the solvent front was marked and allowed to dry. The co-chromatographed authentic sample of plumbagin was used to detect the presence of plumbagin in the sample on the TLC plate.

Purification and Quantification of Plumbagin

The bright yellow fluorescent band corresponding to the R.F. value 0.67 of standard plumbagin was scraped using thin spatula and eluted with chloroform. After the removal of the sedimenting silica gel by centrifugation, the final volume of the supernatent was adjusted to 10-20 ml depending on the intensity of yellow colour. The amount of plumbagin in the chloroform solution was determined by measuring absorbance in the u.v. and visible ranges (254 n m and 415 mm respectively) using a Shimadzu 2100 Spectrometer. The concentration of plumbagin was calculated from a standard graph. The standard curve was drawn using standard plumbagin of various concentrations which were run in the TLC and eluted as above. In each separate experiment, a known quantity was also run along with the sample to check the reproductivity of the extraction. The recovery was always more than 99 per cent. Analar grade chloroform was used as the blank. On drying at room temperature, plumbagin present in chloroform solution eluted from TLC plates got crystallized.


RESULTS

RESULTS

Experiment 1 Germplasm collection and evaluation

Two noded semi-hardwood cuttings of seven ecotypes of *Plumbago rosea* and one ecotype of *Plumbago zeylanica* were collected from different locations of Kerala and evaluated for their variability. The results are presented below :

Floral biology

The failure of seed set in *Plumbago rosea* and seed germination in *Plumbago zeylanica* were examined by studying the following aspects and results are presented in Tables 3 to 5.

Days taken to first flowering

The mean number of days for flowering of different ecotypes is given in Table 3. Significant variation in the mean number of days was observed among ecotypes.

Number of days for flowering ranged from 110.7 days (Et_8) to 231 days (Et_7) among the ecotypes. Et_8 took the minimum number of

Plate 1. Field showing experiment plot

days for flowering which was significantly low compared to other ecotypes. All the other ecotypes took more than 195 days for flowering. The ecotypes Et_5 and Et_4 were on par for first flowering with mean number of days 217.7 and 216.3 respectively. The ecotypes Et_3 and Et_6 were also on par with mean days for flowering as 226 and 225.3 days respectively. The flowering in all ecotypes of *P. rosea* were seasonal and it occurred only during December - January months whereas flowering in *P. zeylanica* occurred after every new flush.

Time of anthesis

Results pertaining to the time of flower opening are presented in Table 3. The anthesis of all ecotypes of *Plumbago rosea* was found significantly earlier when compared to *Plumbago zeylanica*.

The anthesis in plants of *Plumbago zeylanica* was around 8.53 A.M. while that of *Plumbago rosea* was earlier between 6.45 (Et_1) and 7.12 A.M. (Et_3). Though the earliest anthesis among *rosea* was shown by Et_1 and the latest by Et_7, they were all on par in this character.

Floral morphology

In *Plumbago rosea* the inflorescence is a terminal or axillary raceme whereas in *Plumbago zeylanica* it is an elongated spike. The

Table 3. Floral characteristics of different ecotypes of *Plumbago*

Ecotypes	Days for flowering	Time of anthesis (F/N hours)	Pollen stainability (%)	Pollen size (μm)	Style length (mm)
Et_1	195.3	6.45	73.11 (58.74)	5.3	16.3
Et_2	211.3	6.57	74.25 (59.48)	5.4	16.3
Et_3	226.0	7.12	68.74 (55.98)	5.2	16.8
Et_4	216.3	6.47	73.74 (59.15)	5.3	16.3
Et_5	217.7	6.52	74.40 (59.58)	5.3	16.4
Et_6	225.3	7.00	71.51 (57.71)	5.1	16.6
Et_7	231.0	7.13	69.51 (56.46)	5.3	16.8
Et_8	110.7	8.53	88.38 (70.04)	6.9	20.8
CD(0.05)	4.13	0.889	2.696	0.382	0.428

Numbers in parenthesis denote transformed percentages in angles

Plate 2. Inflorescence of *Plumbago rosea*

Plate 3. Inflorescence of *Plumbago zeylanica*

flowers of the former are scarlet red in colour whereas in latter they are white (Plates 2 and 3). In both the species the flower is hypogynous, actinomorphic and bisexual. The calyx in both the species is five-ribbed, gamosepalous, tubular with unbranched glandular hairs which carry stalked glands at the tip. The calyx is persistent and it completely covers the fruit inside in *P. zeylanica*. The glands in calyx secrete a gummy substance. Corolla in both the species is five in number gamopetalous with basal part tubular and twisted. Stamens are five with swollen basal part of filaments carrying oblong dorsifixed, anthers at tip. In *P. zeylanica* the stigma is papillate and five-lobed whereas in *P. rosea* it is cob type and five-lobed. The style length varies among the two species (Plates 4 and 5). In all ecotypes of *P. rosea*, the style length ranged from 16.3 mm to 16.8 millimeter (mm) whereas in *P. zeylanica* it was round about 20.8 mm (Table 3). The maximum style length among the ecotypes of *Plumbago rosea* was shown by Et_3 and Et_7 (16.8 mm) which was followed by and on par with the ecotypes Et_6, Et_5 (16.4 mm), Et_1, Et_2 and Et_4 (16.3 mm). The gynoecium consists of a superior, pentacarpellary, syncarpous ovary with a single pendulous ovule with ascending funicle in both the species. In *P. zeylanica* seeds were formed but all ecotypes of *P. rosea* were found sterile with no seed formation. The seeds of *P. zeylanica* were found completely covered with the sticky persistent calyx.

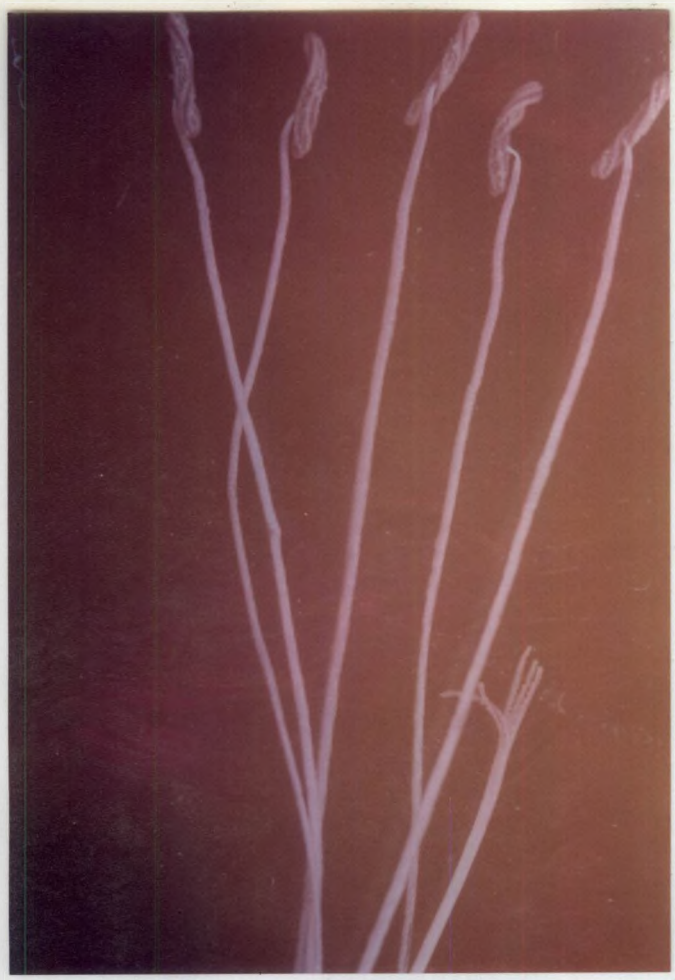


Plate 4. Position of stamen and stigma in *Plumbago rosea*

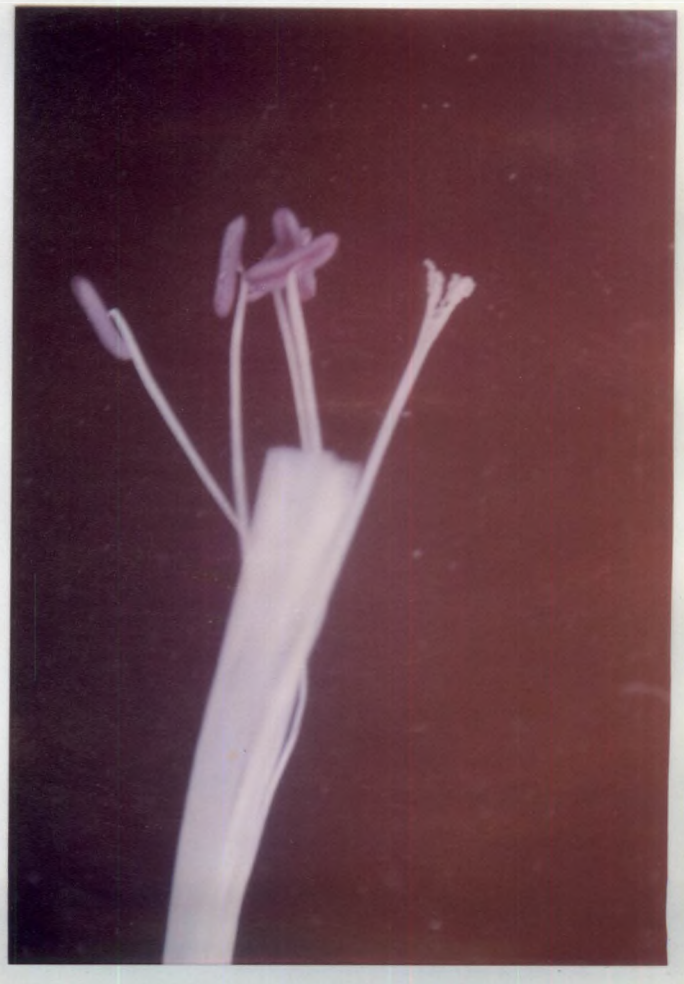


Plate 5. Position of stamen and stigma in *Plumbago zeylanica*

Pollen size

Mean size of pollen grains among the different ecotypes is furnished in Table 3. Statistical analysis of the data showed significant variation among the two species of *Plumbago*. Size of the pollen grains ranged from 5.1 mm in Et_6 to 6.9 mm in Et_8. The ecotypes of *P. rosea* collected from different parts of Kerala showed no significant difference in the size of pollen grains. The pollen grains of both the species are tricolpate with a large colpus extending from pole to pole (Plates 6 and 7).

Pollen fertility

The fertility of pollen grains based on their stainability in acetocarmine was assessed and results are furnished in Table 3. Significant variation was observed among the ecotypes in stainability of pollen grains. As a group, *P. zeylanica* with 88.38 per cent was found to show significantly higher degree of stainability over all the ecotypes of *P. rosea*. The least percentage of stainability was noticed in Et_3 (68.74) which was on par with Et_7 (69.51) and Et_6 (71.51). The remaining ecotypes of *P. rosea* were significantly superior to Et_3 in stainability percentage and these were all on par. Et_5 gave the highest stainability percentage among *P. rosea* ecotypes.

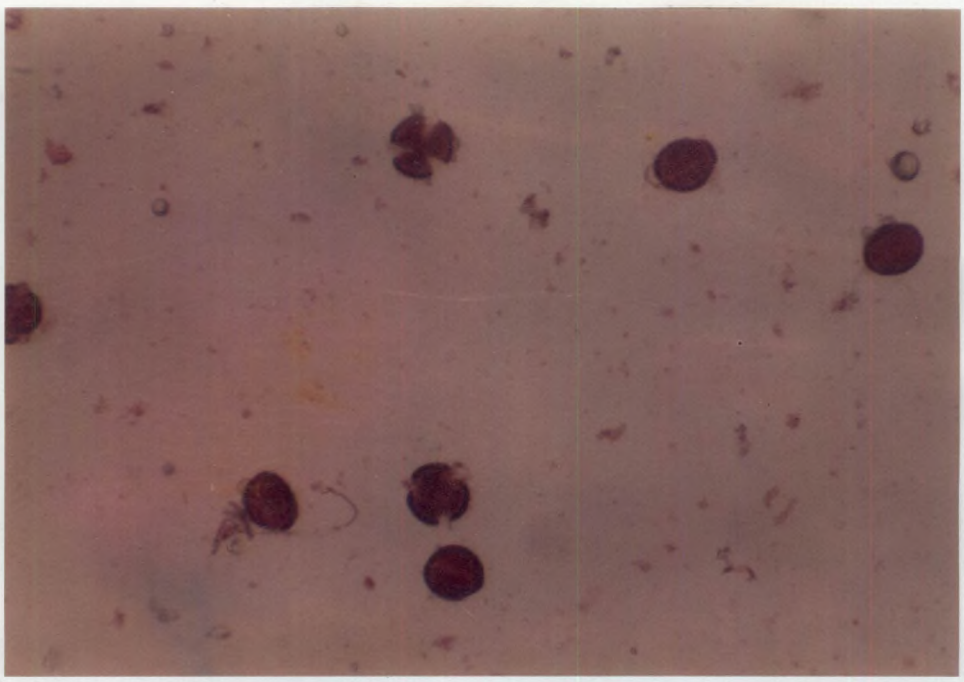
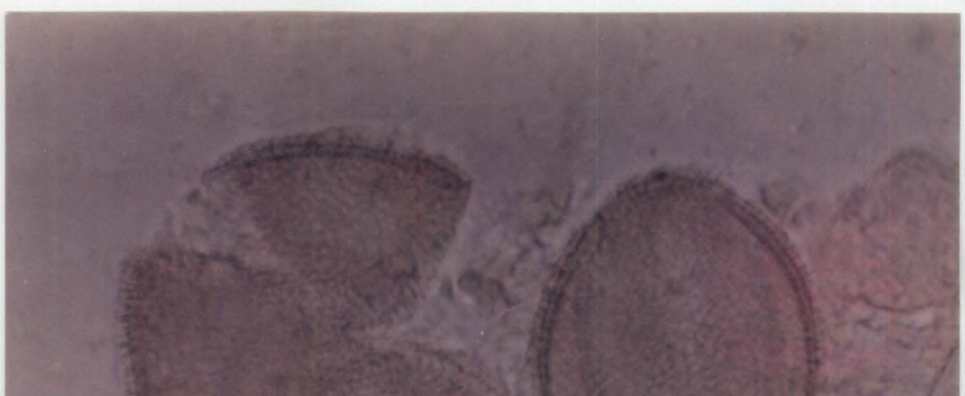


Plate 6. Pollen grains of *Plumbago rosea*



Pollen viability

***In vitro* studies**

The viability of pollen grains was estimated in different germination media and the results were as follows (Table 4).

The pollen grains belonging to all the ecotypes of *Plumbago rosea* did not germinate in any of the media tried whereas the pollen grains of *P. zeylanica* germinated in all the different germination media tested. The maximum percentage of germination was observed in Brew Baker's medium (72.4 per cent) and minimum germination in distilled water (42.0 per cent). The germination media containing 0.5, 1.0, 1.5 and 2.0 per cent sucrose showed 50.1, 52.4, 52.6 and 54.0 per cent germination of pollen grains respectively.

***In vivo* studies**

The pollen germination was estimated by *in vivo* methods using freshly opened flowers and results are furnished in Table 4.

In *in vivo* studies also the pollen grains from all the ecotypes of *P. rosea* failed to germinate while that of *P. zeylanica* germinated in freshly opened flowers (90.0 per cent). The pollen grains of *P. rosea* did not adhere to the stigma whereas abundant adhesion of pollen grains on papillate stigma of *P. zeylanica* was observed (Plates 8 and 9).

Table 4. Pollen germination in different media

Ecotypes	Pollen germination (%)						
	In vitro						*In vivo*
	DW	0.5 % sucrose	1.0 % sucrose	1.5 % sucrose	2.0 % sucrose	Brew Baker's medium	Freshly opened flowers
Et_1	0	0	0	0	0	0	0
Et_2	0	0	0	0	0	0	0
Et_3	0	0	0	0	0	0	0
Et_4	0	0	0	0	0	0	0
Et_5	0	0	0	0	0	0	0
Et_6	0	0	0	0	0	0	0
Et_7	0	0	0	0	0	0	0
Et_8	42.0	50.1	52.4	52.6	54.0	72.4	90.0

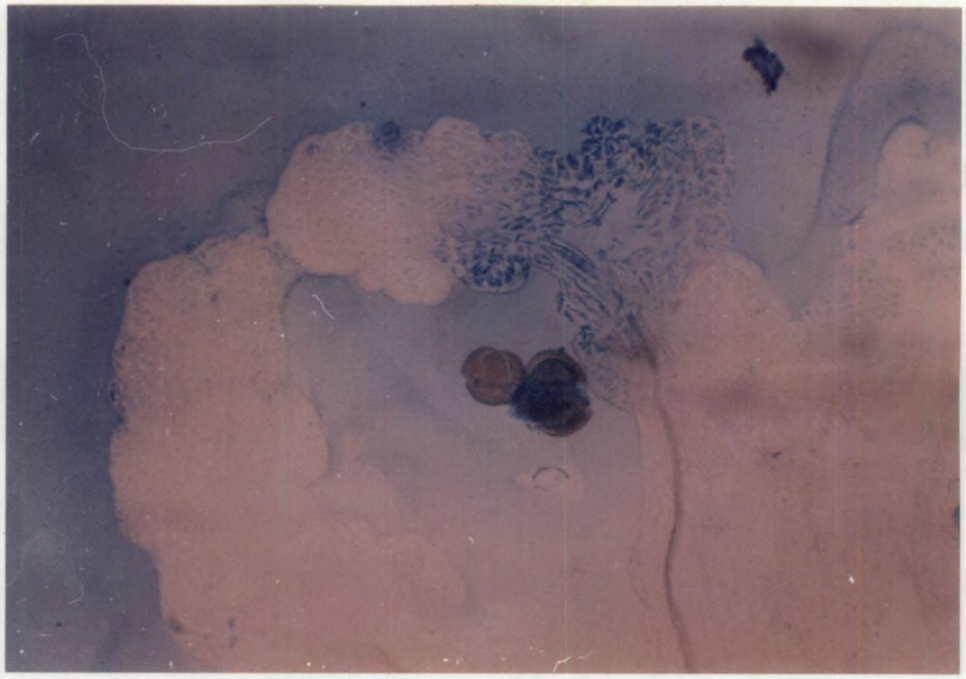


Plate 8. Non-adhesion of pollen grains on stigmatic surface in *Plumbago rosea*

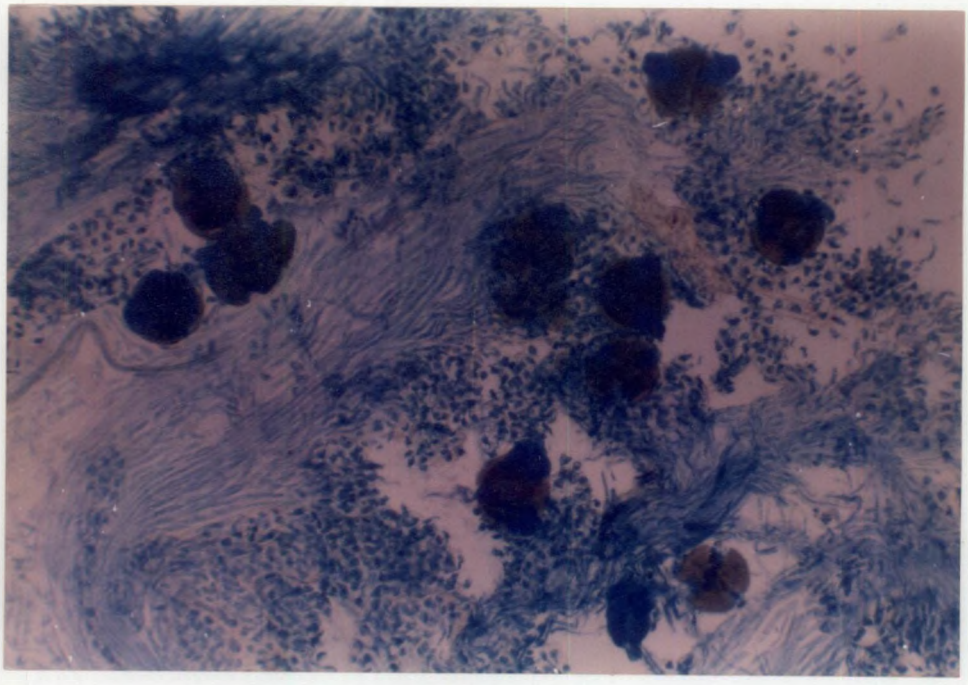


Plate 9. Adhesion of pollen grain on stigmatic surface of *Plumbago zeylanica*

Compatibility studies

In order to find out the causes for failure of seed set in *P. rosea*, different pollination techniques like artificial self pollination, artificial sibbing, artificial cross pollination, bud pollination, mentor pollination, chemically aided pollination and pollination on decapitated pistil were employed to overcome the barriers in seed set. All the techniques failed to give any positive result on seed set in *P. rosea*. In *P. zeylanica* seed set was observed under natural conditions whereas when it was cross pollinated with pollen from *P. rosea* no seeds were obtained (Table 5).

Cytology

The flower buds of *P. rosea* and *P. zeylanica* were stained with acetocarmine and the meiotic cells were observed. Normal pairing was observed in *P. rosea* and *P. zeylanica*. In *P. rosea* six bivalents and in *P. zeylanica* twelve bivalents were found in the metaphase stage.

Dormancy studies

In order to find the failure of seed germination in *P. zeylanica* different methods to overcome the dormancy of mature seeds were employed and the results are presented in Table 6.

Significant variation among treatments were observed. When the seeds were kept for germination with intact calyx, the seeds failed to

Plate 10. Seed germination after scarification in *P. zeylanica*

Table 5. Percentage of seed set under different pollination mechanisms

Ecotypes	Artificial SP	Artificial Sibbing	Artificial CP	Bud Pollination	Mentor Pollination	Chemically aided	On decapitated pistil
Et_1	0	0	0	0	0	0	0
Et_2	0	0	0	0	0	0	0
Et_3	0	0	0	0	0	0	0
Et_4	0	0	0	0	0	0	0
Et_5	0	0	0	0	0	0	0
Et_6	0	0	0	0	0	0	0
Et_7	0	0	0	0	0	0	0
Et_8	62.5	60.5	0	NA	NA	NA	NA

Table 6. Seed germination in *Plumbago zeylanica*

Treatments	Germination (%)	Days taken for 50 % germination
Fruits with persistant calyx	0 (0)	—
Seeds with intact seed coat (T1)	5.57 (13.63)	20
Seeds with tip cut (T2)	98.86 (83.85)	3.17
Seeds with base cut (T3)	85.36 (67.47)	3.67
CD (0.05)	(9.177)	$T_1V_sT_2$ & T_3 = 1.105 $T_2V_sT_3$ = 0.988

Numbers in parenthesis denote transformed percentages in angles

germinate whereas when the calyx was removed the seeds germinated (Plate 10). Among the three treatments in which the seeds germinated, the maximum percentage of seed germination was observed when the tip of the seeds was cut (98.86 per cent) followed by the seeds with base cut (85.36 per cent) and the minimum percentage germination with intact seed coat (5.57 per cent).

Time taken for 50% of the seeds with intact seed coat to germinate was 20 days while seeds with the tip and base cut germinated more quickly with the mean number of days being 3.17 to 3.67 respectively (Table 6).

Biometric analysis

Observations were taken for different quantitative characters for each ecotype both in the nursery and in the field and the results are presented below.

Nursery observations

Observations on various quantitative characters in the nursery such as days taken to sprouting, sprouting percentage, number of leaves, internodal length and plant height are presented in Tables 7, 8 and 9.

Table 7. Aspects of sprouting of cutting of different ecotypes of *Plumbago*

Ecotypes	Number of days to start sprouting (Mean period in days)	Number of days to complete sprouting (Mean period in days)	Duration of sprouting (Mean period in days)	Sprouting percentage
Et_1	20.00	23.80	3.80	95.62 (77.88)
Et_2	14.03	16.20	2.17	82.23 (65.04)
Et_3	13.33	15.00	1.67	68.88 (56.07)
Et_4	10.03	11.93	1.90	95.62 (77.88)
Et_5	10.67	12.60	1.93	67.74 (55.37)
Et_6	11.40	13.47	2.07	67.74 (55.37)
Et_7	11.27	13.60	2.33	66.66 (54.71)
Et_8	9.90	11.80	1.90	85.53 (67.62)
CD(0.05)	0.626	0.414	0.764	2.645

Figures in parenthesis denote the transformed percentages in angles

Sprouting

The various aspects of sprouting of cuttings taken from different ecotypes of *Plumbago* spp. are presented in Table 7.

Number of days to start sprouting

The mean number of days to start sprouting showed significant variation among the different ecotypes.

The number of days to start sprouting ranged from 9.9 to 20 days. The minimum number of days for sprouting (9.9 days) was recorded by Et_8 which was on par with Et_4 whereas the maximum number (20.0 days) was observed in Et_1. Except Et_4, all the ecotypes of *P. rosea* showed significantly different expressions in this character when compared to *P. zeylanica*. The ecotype Et_1 showed a significantly higher duration for sprouting when compared to all other ecotypes. Et_6 and Et_7 were on par in this character while all other ecotypes showed independent expressions.

Number of days to complete sprouting

In this character also Et_8 gave the least value which was significantly lower to those of all the ecotypes of *P. rosea* except Et_4. Among the ecotypes Et_1 showed the maximum days for completion of sprouting (23.8 days) which was significantly higher to all other ecotypes

and species. Et_6 and Et_7 were found to be on par for this character which were also significantly higher than Et_4, Et_5 and Et_8.

Duration of sprouting

The duration of sprouting (interval between first and last sprouting within a treatment) is presented in Table 7.

The results revealed significant variation among the ecotypes. The mean period of duration was found to be highest in the cuttings from Et_1 (3.8 days). The minimum period of duration of 1.67 days was recorded for Et_3 which was on par with all the other ecotypes.

Sprouting percentage

The comparison of different ecotypes for the percentage of sprouting is given in Table 7.

The percentage of sprouting of the cuttings in different ecotypes differed significantly. The percentage sprouting ranged from 66.66 in Et_7 which was on par with Et_6, Et_5 and Et_3 to 95.62 in Et_1 and Et_4. In the expression of this character Et_2 and Et_8 have shown independent stand whereas Et_1 and Et_4 have remained as the highest sprouting group against Et_3, Et_5, Et_6 and Et_7 as the lowest sprouting group.

Number of leaves per plant

The mean number of leaves per plant produced by the different ecotypes in the nursery is furnished in Table 8.

The mean number of leaves per plant at 45 DAP ranged from 1.3 (Et_6) to 1.8 (Et_8) among the different ecotypes. The minimum number of leaves at 45 DAP was recorded by the ecotype Et_6 (1.3) which was on par with Et_1 and Et_3 (1.4) and Et_4 (1.5). The maximum number of leaves per plant (1.8) at 45 DAP was observed in Et_8. There was no significant difference among these values except between Et_8 and the group Et_1, Et_3, Et_4 and Et_6. Et_2, Et_5 and Et_7 were on par among themselves as well as with Et_8. At 60 DAP, the ecotype Et_6 produced less number of leaves than all other ecotypes. Maximum number of leaves per plant was produced by Et_8 followed by Et_2. At 75 DAP, maximum leaves were seen in Et_8 which was on par with Et_2 and minimum number of leaves by Et_6, Et_7, Et_1 and Et_5. At 90 DAP, the mean number of leaves per plant ranged from 4.3 to 5.7 among the ecotypes. The minimum number of leaves per plant (4.3) was recorded by Et_7 which was on par with Et_3 and the maximum by Et_8 (*P. zeylanica*). At 90 DAP, Et_8 showed significant superiority over all other ecotypes in this character followed by Et_2. Et_4 and Et_6 were on par but superior to Et_7, Et_3 and Et_1. In the overall performance of this character also Et_8 was significantly superior to all other ecotypes followed by Et_2. The performance of Et_4, Et_6 and Et_7 were on par when the general mean of leaf production in nursery was taken into consideration.

Table 8. Mean values of the number of leaves in the nursery

Ecotypes	45 DAP	60 DAP	75 DAP	90 DAP	Mean
Et_1	1.4	3.0	3.7	4.5	3.17
Et_2	1.7	3.8	4.5	5.0	3.75
Et_3	1.4	3.2	4.1	4.4	3.28
Et_4	1.5	3.2	4.3	4.7	3.44
Et_5	1.6	3.4	3.8	4.6	3.34
Et_6	1.3	2.8	3.6	4.7	3.11
Et_7	1.6	3.3	3.6	4.3	3.18
Et_8	1.8	4.2	4.6	5.7	4.08
CD(0.05)	0.217	0.316	0.241	0.191	0.109

Internodal length

Table 9 gives the internodal length of different ecotypes in the nursery. Among the different ecotypes this character ranged from 1.81 to 2.27 cm. The maximum internodal length was observed in Et_8 which was on par with, Et_5 (2.25 cm). Et_6, Et_1 and Et_4 were found to be on par for this character.

Plant height

Data pertaining to the mean values of plant height in the nursery for the different ecotypes are furnished in Table 9.

Maximum plant height was recorded by Et_8 (10.27 cm) which was significantly superior to all other ecotypes followed by Et_2 (9.93 cm) and minimum plant height was for Et_6 (7.04 cm) and Et_3 (7.11 cm). The plant height of other ecotypes ranged from 7.04 (Et_6) to 9.93 cm (Et_2) with each having an independent stand in this character.

Number of suckers per plant

The comparison on the number of suckers produced by different ecotypes in the nursery can be viewed from Table 9. This number ranged between 1.00 and 1.50. The maximum number of suckers was noticed in Et_8 (1.50) which was on par with Et_2 (1.33) and Et_7 (1.40). The minimum number of suckers (1.00) was noticed in Et_1, Et_6 and Et_3 (1.03).

Table 9. Mean value on different nursery characters at 90 DAP

Ecotypes	Internodal length (cm)	Plant height (cm)	Numbers of suckers
Et_1	1.850	7.910	1.000
Et_2	2.190	9.930	1.330
Et_3	2.070	7.110	1.030
Et_4	1.860	8.190	1.270
Et_5	2.250	9.190	1.300
Et_6	1.810	7.040	1.000
Et_7	2.140	9.320	1.400
Et_8	2.270	10.270	1.500
CD(0.05)	0.066	0.222	0.195

Field observations

Internodal length

The mean values of internodal length of different ecotypes during the different growth period are given in Table 10.

The results showed significant variation for this character among the different ecotypes. The mean value of internodal length over the growth period ranged from 3.91 to 6.02 cm. The maximum mean value for the character was recorded by Et_8 (6.02 cm) which was significantly superior to all other ecotypes. Except Et_1 and Et_4 which were on par for this character, all other ecotypes have shown independent expressions.

Plant height

The data on plant height for the eight ecotypes over a period of one and a half years are presented in Table 11.

Plant height was significantly influenced by the different growing seasons. Maximum plant growth was seen during rainy season whereas it was almost standstill during summer months. The maximum upsurge of growth was shown during the period from July to December where by almost three fourth of the total growth was achieved by majority of the ecotypes. This maximum mean growth of 253.43 cm at the 18th month of growth was achieved by Et_8 which was highly and significantly superior to all other ecotypes.

Table 10. Mean value of internodal length at different growth period

Ecotypes	Internodal length (cm)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Overall Mean
Et_1	2.32	2.41	2.41	2.41	2.43	2.73	3.45	5.17	6.04	6.93	8.11	8.92	4.494
Et_2	2.57	2.65	2.65	2.65	2.68	2.93	3.88	5.72	7.27	7.73	8.75	9.71	4.93
Et_3	2.19	2.26	2.26	2.26	2.34	2.61	3.01	4.46	5.15	6.13	7.46	8.60	4.06
Et_4	2.29	2.37	2.37	2.37	2.43	2.82	3.69	5.13	5.91	6.41	7.76	9.05	4.38
Et_5	2.64	2.73	2.73	2.73	2.76	3.0	3.87	5.73	6.90	7.32	8.43	9.11	4.83
Et_6	2.11	2.24	2.24	2.24	2.34	2.67	3.45	4.46	5.38	5.64	6.66	7.45	3.91
Et_7	2.67	2.81	2.81	2.81	2.85	3.00	3.85	5.15	5.95	6.37	7.88	9.10	4.60
Et_8	2.85	2.96	3.04	3.64	3.35	3.75	5.07	7.58	8.62	9.64	10.63	11.75	6.02

CD(0.05) Treatments = 0.060 Treatments x months = 0.154

Table 11. Mean values of plant height at different growth periods

Ecotypes	Plant height (cm)																	
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Overall mean
Et_1	4.80	6.10	6.75	7.91	10.53	11.96	12.36	12.43	12.49	12.59	14.82	19.09	26.19	37.19	47.05	54.77	67.53	21.45
Et_2	4.64	5.88	8.03	9.93	15.31	17.55	20.15	20.45	20.62	20.79	23.14	30.01	35.12	47.57	65.91	77.26	88.75	30.07
Et_3	3.66	4.76	5.06	7.11	10.05	11.87	11.93	12.11	12.27	12.39	14.43	18.17	25.37	36.38	39.28	43.10	49.20	18.66
Et_4	4.83	6.09	6.90	8.19	11.19	12.72	14.16	14.51	14.71	14.80	17.0	21.48	28.91	40.40	53.66	59.22	71.83	23.57
Et_5	3.78	4.90	6.41	9.19	14.02	16.33	18.22	18.65	18.73	18.84	20.97	25.17	32.47	44.56	59.02	67.0	83.50	27.16
Et_6	3.06	4.19	5.01	7.04	9.50	11.59	11.83	11.94	12.12	12.27	13.46	17.59	23.90	34.42	36.09	38.90	45.48	17.55
Et_7	3.11	4.15	6.36	9.32	11.73	14.02	16.66	16.30	16.35	16.45	18.71	22.63	26.73	43.15	55.95	62.95	77.00	24.76
Et_8	5.23	7.54	8.31	10.27	16.13	20.47	24.22	27.22	30.29	30.38	34.19	39.38	48.82	68.06	114.70	186.67	253.43	54.43

CD(0.05) Treatment = 0.117 Treatment x months = 0.754

Among the *P. rosea* ecotypes maximum height was obtained for Et_2 (88.75 cm) followed by Et_5 (83.5 cm). The minimum growth as determined by height was found in Et_6 which was significantly inferior to all other ecotypes. Each ecotype was found significantly unique in this character.

When the overall mean of plant height was considered the maximum mean value (54.43 cm) was recorded by Et_8 which was followed by the ecotypes Et_2 (30.07 cm) and Et_5 (27.16 cm). The minimum plant height was recorded by the ecotype Et_6 (17.55 cm) followed by Et_3 (18.66 cm). Each ecotype was found significantly unique for this character.

Number of suckers per plant

The influence of different growing seasons on total number of suckers produced per plant was observed and presented in Table 12.

With regard to sucker production, the different growing seasons had significant impact among the ecotypes. During the initial growth periods the sucker production was considerably low but during the peak rainy season (June to October) the number of suckers produced per plant increased. The maximum sucker production was attained by the month of October (tail end of north east monsoon). At this stage maximum sucker production (8.4) was shown by Et_8 and Et_2 followed by Et_4 (7.4), Et_5 (6.9) and Et_7 (6.7). For this character also each ecotype showed significant uniqueness except Et_8 and Et_2 which were on par.

Table 12. Mean value on number of suckers at different growth periods

Ecotypes	Number of suckers												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Overall mean
Et_1	1.5	1.7	1.7	1.7	1.7	1.9	2.5	3.2	4.7	5.4	5.4	5.4	3.07
Et_2	1.7	1.8	1.8	1.8	1.8	2.1	2.6	3.6	7.3	8.4	8.4	8.4	4.14
Et_3	1.5	1.7	1.7	1.7	1.7	2.0	2.5	3.4	4.7	5.6	5.6	5.6	3.14
Et_4	1.6	1.8	1.8	1.8	1.8	2.0	2.7	3.6	6.5	7.4	7.4	7.4	3.82
Et_5	1.7	1.8	1.8	1.8	1.8	2.0	2.6	3.6	5.3	6.9	6.9	6.9	3.59
Et_6	1.5	1.6	1.6	1.6	1.6	2.0	2.5	3.3	4.7	5.7	5.7	5.7	3.13
Et_7	1.7	1.8	1.8	1.8	1.8	2.0	3.0	3.7	5.4	6.7	6.7	6.7	3.59
Et_8	1.8	1.8	1.8	1.8	1.8	2.2	3.2	4.1	7.6	8.4	8.4	8.4	4.28

CD(0.05) Treatment = 0.095 Treatment x months = 0.185

The overall mean number of suckers per plant ranged from 3.07 to 4.28 with the maximum number in Et_8 (4.28) followed by Et_2 (4.14). The ecotypes Et_4 and Et_7 as one group (3.82 and 3.59) and ecotypes Et_3, Et_6 and Et_1 as another group (3.14, 3.13 and 3.07) were on par within each group whereas they differed significantly between groups.

Fresh weight of shoot

Data pertaining to the fresh weight of shoot among the different ecotypes are presented in Table 13.

There was significant variation in the fresh weight of shoot among the ecotypes. The mean value of fresh weight of shoot ranged from 2.67 kg to 127.77 g. The maximum value of fresh weight of shoot was recorded by Et_8 (2.67 kg) followed by Et_2 (422.50 g) which was on par with the ecotypes Et_5 and Et_4 (384 g and 379.33 g). The minimum fresh weight for the shoot was on par with Et_1, Et_3 and Et_7 (148.63g, 149.03g and 201.83g).

Dry weight of shoot

The data relating to the dry weight of shoot are presented in Table 13.

Statistical analysis of the data on mean value of shoot on dry weight basis showed significant variation among the ecotypes.

Table 13. Vegetative characters at harvest

Ecotypes	Internodal length (cm)	Plant height (cm)	Number of suckers	Fresh weight of shoot (g)	Dry weight of shoot (g)
Et_1	8.92	67.53	5.40	148.63	72.67
Et_2	9.71	88.75	8.40	422.50	203.17
Et_3	8.60	49.20	5.60	149.03	70.33
Et_4	9.05	71.83	7.40	379.33	184.33
Et_5	9.11	83.50	6.93	384.00	186.50
Et_6	7.45	45.48	5.67	127.77	61.33
Et_7	9.10	77.00	6.73	201.83	99.83
Et_8	11.75	253.43	8.40	2666.67	1400.00
CD(0.05)	0.340	0.848	0.320	178.697	61.867

Et_8 recorded the highest dry weight of shoot (1.4 kg) which was significantly superior to all other ecotypes. The lowest dry weight was recorded by Et_6 (61.33 g) which was on par with Et_3 (70.33 g). Et_1 (72.67 g) and Et_7 (99.83 g). The ecotypes Et_2 (203.17 g), Et_5 (186.50 g) and Et_4 (184.33 g) were on par for this character.

Length of root

The data relating to the root length of plant is presented in Table 14.

The root length varied significantly among the ecotypes. The value for this character ranged from 40.60 cm to 86.37 cm. The maximum root length was recorded by Et_8 (86.37 cm) which was significantly superior to all other ecotypes. The minimum root length (40.60 cm) was recorded by Et_3 which was on par with Et_7 (40.83 cm). All other ecotypes showed significantly unique values for this character.

Number of roots per plant

From the data presented in Table 14 it could be seen that number of roots per plant differed significantly among the ecotypes. The values of this character ranged from 10.73 to 22.47.

The maximum expression was shown by Et_2 (22.47) which was significantly superior to all other values. The minimum root number was shown by Et_7 (10.73) which was also significantly lower to all other

Table 14. Root characters at harvest

Ecotypes	Length of root (cm)	Number of root / plant	Girth of root (cm)	Fresh weight (g)	Dry weight (g)	Plumbagin content (%)
Et_1	61.5	20.40	3.50	248.27	124.67	1.40
Et_2	73.47	22.47	2.67	252.33	130.16	1.20
Et_3	40.60	14.80	2.83	194.20	101.50	0.98
Et_4	49.0	20.13	2.50	248.13	124.67	1.20
Et_5	46.63	13.53	2.33	163.50	93.67	1.00
Et_6	55.37	18.27	2.50	235.47	115.17	1.00
Et_7	40.83	10.73	2.67	141.07	78.50	0.97
Et_8	86.37	16.07	3.17	321.43	138.83	0.61
CD (0.05)	1.582	0.627	0.536	2.151	3.985	0.317

values. Except Et_1 and Et_4 which were showing on par values (20.40 and 20.13 respectively) all other ecotypes were significantly unique in expression for this character.

Girth of root

The mean girth of root for different ecotypes tested is presented in Table 14.

There was significant difference among the ecotypes for this character. The values ranged from 2.33 cm to 3.50 cm. Et_1 showed maximum value for this character (3.50 cm) which was on par with that of Et_8 (3.17 cm). All other ecotypes were on par for this character with values ranging from 2.33 cm (Et_5) to 2.83 cm (Et_3).

Fresh weight of roots

Data pertaining to the fresh weight of root are given in Table 14. There was significant variation for this character among the ecotypes, ranging from 141.07 g to 321.43 g. The maximum value was recorded by Et_8 (321.43 g) which was significantly superior to those of all other ecotypes. Among the *P. rosea* ecotypes Et_2 was the best (252.33 g) followed by Et_1 (248.27 g) and Et_4 (248.13 g) which were on par but significantly inferior to Et_2. The minimum value was recorded by Et_7 (141.07 g). Each of the remaining ecotypes were significantly different among themselves for this character.

Dry weight of roots

The dry weights of roots of different ecotypes are presented in Table 14. The mean value ranged from 78.50 g to 138.83 g. The maximum dry weight of root (138.83 g) was recorded by Et_8 and minimum (78.50 g) by Et_7. Et_8 was significantly superior to all other ecotypes. Except Et_1 and Et_4, which were on par, all other ecotypes showed significant individuality for the expression of this character.

Plumbagin content

Data pertaining to the purified plumbagin content among the different ecotypes are furnished in Table 14.

There was significant difference among the ecotypes for this character. The mean value ranged from 0.61 per cent (Et_8) to 1.4 per cent (Et_1). The ecotype Et_8 (*P. zeylanica*) was significantly inferior to all other ecotypes in plumbagin content. Among the *P. rosea* types Et_1 with 1.4 per cent of plumbagin in its roots was found significantly superior to all other ecotypes except Et_2 and Et_4 which were on par.

Genetic parameters

Genetic parameters were estimated for different characters. Table 15 indicates the phenotypic and genotypic variances and coefficients of variation for ten different characters.

Table 15. Phenotypic and genotypic variance and coefficient of variation

Sl. No.	Character	Variance		Coefficient of variation	
		Phenotypic	Genotypic	Phenotypic	Genotypic
1.	Days taken for sprouting	11.25	11.13	26.67	26.52
2.	Duration of sprouting	0.574	0.384	34.11	27.89
3.	Plant height (cm)	4481.55	4481.32	72.69	72.69
4.	Internodal length (cm)	1.50	1.46	13.30	13.13
5.	Number of suckers per plant	1.48	1.44	17.83	17.63
6.	Length of root (cm)	275.69	274.88	29.47	29.45
7.	Girth of root (cm)	0.21	0.12	16.65	12.45
8.	Number of roots per plant	15.78	15.65	23.30	23.20
9.	Dry weight of roots (root yield) (g)	422.59	417.42	18.13	18.02
10.	Plumbagin content (%)	0.090	0.057	27.90	22.23

Phenotypic and genotypic variance

The maximum value for phenotypic variance was given by plant height (4481.55) which was followed by root yield (422.59) and root length (275.69). Plumbagin content showed the minimum phenotypic variance (0.090).

Plant height recorded maximum genotypic variance (4481.32) followed by root yield (417.42) and length of root (274.88). In genotypic variance also the plumbagin content expressed the least value (0.057).

Phenotypic and genotypic coefficient of variation

Maximum phenotypic coefficient of variation was recorded for plant height (72.69) followed by sprouting duration (34.11), length of root (29.47), plumbagin content in root (27.90), days to start sprouting (26.67) and number of roots per plant (23.30).

Maximum genotypic coefficient of variation was also reported for plant height (72.69) followed by length of root (29.45), duration of sprouting (27.89), days to start sprouting (26.52), number of roots per plant (23.20) and plumbagin content in roots (22.23).

Heritability and genetic advance

Heritability and genetic advance were computed for ten different characters which are presented in Table 16.

Table 16. Heritability and genetic advance

Sl. No.	Character	Heritability (%)	Genetic advance (%)
1.	Days taken for sprouting	98.87	6.83
2.	Duration of sprouting	66.85	1.04
3.	Internodal length	97.50	2.46
4.	Plant height	99.90	137.90
5.	Number of suckers	97.74	2.45
6.	Length of root	99.70	34.10
7.	Girth of root	55.94	0.53
8.	Number of rootsper plant	99.10	8.11
9.	Dry weight of roots (root yield)	98.78	41.83
10.	Plumbagin content in roots	63.50	0.39

High heritability estimates were observed for almost all the characters studied. The highest heritability values were recorded for plant height (99.9 %), length of root (99.7 %), number of roots per plant (99.10 %), days to start sprouting (98.87 %), root yield (98.78 %), number of suckers per plant (97.74 %) and internodal length (97.50 %). Medium values were obtained for duration of sprouting (66.85 %) plumbagin content in roots (63.50 %) and root girth (55.94 %). All the characters are heritable in nature. Environment influence is least for all characters except for duration of sprouting, plumbagin content and root girth. For these three characters, 33 per cent, 36 per cent and 45 per cent of the variability is accounted by environment.

Genetic advance as percentage was maximum for plant height (137.90 %) and minimum for plumbagin content in roots (0.39 %). Root yield (41.83 %) and root length (34.10 %) recorded moderately high genetic advance while plumbagin content in roots and root girth recorded lower values. Considering the two parameters heritability and genetic advance together, plant height, root length and root yield had comparatively higher values for both.

Correlation

Phenotypic and genotypic correlations of dry root yield with other characters and their *inter se* association were worked out. The data on correlations have been split up under the following categories.

Table 17. Phenotypic and genotypic correlation coefficients of dry root yield with other characters

Sl. No.	Characters	Coefficient of correlation	
		Genotypic	Phenotypic
1.	Days taken for sprouting	0.1756	0.1728
2.	Days to complete sprouting	0.1720	0.1714
3.	Plant height	0.4875	0.4844*
4.	Number of leaves	0.7760	0.7492**
5.	Internodal length	0.4527	0.4406*
6.	Number of suckers per plant	0.4125	0.3983*
7.	Root length	0.8444	0.8399**
8.	Root girth	0.5000	0.3653*
9.	Number of roots per plant	0.8078	0.7998**
10.	Fresh weight of shoot	0.5279	0.5197**
11.	Fresh weight of root	0.9710	0.9651**

* Significant at 0.05 level of probability

** Significant at 0.01 level of probability

Correlation between dry root yield and other components

The estimatesof correlation coefficient at the phenotypic and genotypic levels are given in the Table 17.

All the genotypic correlations between yield and other characters were positive. Fresh weight of root had the highest positive correlation with dry root yield (0.9710) followed by root length (0.8444) and number of roots per plant (0.8078). Days taken for sprouting and days taken to complete sprouting had no significant correlation with dry root yield. Root girth, plant height, internodal length and number of suckers per plant were significant only at 5 per cent level of probability.

At the phenotypic level also fresh weight of root had highest significant positive correlation with yield (0.9651). Root length and number of roots per plant had high positive correlation with yield (0.8399 and 0.7998).

Correlation between pair of characters, other than those with yield

Table 18 gives the data on correlation amongst the characters in all possible combinations.

At the genotypic and phenotypic level, days taken to complete sprouting had high positive and significant correlation with days taken to sprouting (0.9989 and 0.9912).

Table 18. Correlation between pair of characters, other than those with yield

Characters													
Days taken to sprouting	P	1.0000											
	G	1.0000											
Days to complete sprouting	P	0.9912	1.0000										
	G	0.9989	1.0000										
Plant height	P	-0.3237	-0.3042	1.0000									
	G	-0.3256	-0.3046	1.0000									
Internodal length	P	-0.1977	-0.1924	0.9227	1.0000								
	G	-0.2112	-0.1952	0.9346	1.0000								
Number of suckers	P	-0.4745	-0.4723	0.6657	0.7578	1.0000							
	G	-0.4864	-0.4786	0.6734	0.7778	1.0000							
Root length	P	0.0922	0.1009	0.7546	0.6952	0.5889	1.0000						
	G	0.0906	0.1010	0.7560	0.7048	0.5961	1.0000						
Root girth	P	0.5985	0.5853	0.3104	0.3242	-0.1104	0.4216	1.0000					
	G	0.7573	0.7878	0.4147	0.4383	-0.2024	0.5534	1.0000					
Number of roots	P	0.4227	0.4118	-0.0839	-0.0301	0.1639	0.5090	0.1998	1.0000				
	G	0.4265	0.4143	-0.0842	-0.0269	0.1659	0.5111	0.2396	1.0000				
Fresh weight of roots	P	0.0651	0.0685	0.6348	0.5393	0.4132	0.8811	0.4140	0.6465	1.0000			
	G	0.0652	0.0683	0.6349	0.5442	0.4187	0.8825	0.5565	0.6496	1.0000			
Fresh weight of shoot	P	-0.3629	-0.3527	0.9852	0.8792	0.6266	0.7475	0.3090	-0.0584	0.6772	1.0000		
	G	-0.3728	-0.3551	0.9923	0.8952	0.6377	0.7500	0.3734	-0.0634	0.6817	1.0000		
Dry weight of shoot	P	-0.3631	-0.3486	0.9874	0.8806	0.6228	0.7459	0.2953	-0.0678	0.6782	0.9932	1.0000	
	G	-0.3677	-0.3519	0.9906	0.8902	0.6271	0.7484	0.3929	-0.0654	0.6800	0.9954	1.0000	
Plumbagin content	P	0.4942	0.4789	-0.5700	-0.3722	0.2315	0.2396	0.0133	0.5236	-0.0834	-0.5983	-0.5933	1.0000
	G	0.5850	0.6036	-0.7172	-0.5450	0.2965	0.2876	0.0154	0.6553	-0.1077	-0.7388	-0.7171	1.0000

The height of the plant had negative correlation with days taken to start and complete sprouting at both genotypic and phenotypic level.

The internodal length had positive correlation with plant height at genotypic as well as phenotypic level (0.9346 and 0.9227) whereas it showed negative correlation with the sprouting both for starting and completion.

The number of suckers produced per plant showed a positive correlation with plant height and internodal length (0.6734 and 0.7778) at genotypic level whereas a negative correlation was found with days taken to sprouting and days taken to complete sprouting.

At genotypic level the length of the root showed maximum positive correlation with plant height (0.7560) followed by internodal length (0.7048) and number of suckers per plant (0.5961).

Root girth was positively correlated with days taken to start and complete sprouting and root length (0.7573, 0.7878 and 0.5534) whereas it showed a negative correlation with number of suckers produced per plant.

Number of roots per plant showed positive and significant (at five per cent) correlation with root length, days taken to sprouting and to complete sprouting at genotypic level (0.5111, 0.4265 and 0.4143).

The fresh weight of root was significantly and positively associated with root length (0.8825) followed by number of roots (0.6496), plant height (0.6349), girth of the root (0.5565) and internodal length (0.5442). But this character showed no significant correlation with days taken to sprouting and days taken for complete sprouting.

The fresh weight of shoot showed a positive and significant correlation with plant height, internodal length, number of suckers produced per plant, length of root and fresh yield of roots at genotypic level. The maximum positive significant correlation was found to be associated with plant height (0.9923) followed by internodal length (0.8952) and root length (0.7500).

At genotypic level the dry weight of shoot also showed positive correlation with fresh yield of shoot, plant height, internodal length, root length, fresh yield of roots and number of suckers produced per plant. The maximum positive correlation was found with fresh weight of shoot (0.9954) followed by plant height (0.9906) and internodal length (0.8902).

The plumbagin content in roots showed both positive and negative correlations with other characters. At genotypic level, the plumbagin content in roots had a positive correlation with number of roots per plant (0.6553) followed by days taken to complete sprouting (0.6036) and start sprouting (0.5850). This character has shown a negative

correlation with fresh weight of shoot (0.7388), plant height (0.7172), dry weight of shoot (0.7171) and internodal length (0.5450).

Path coefficient analysis

Based on the correlation of yield and its components, path coefficient analysis was carried out to elicit further information through direct and indirect effects of important yield attributes. The results are presented in Tables 19 to 21.

Table 19 reveals the direct and indirect effects of characters on plumbagin content of roots (Y). The correlation between plumbagin content and plant height (X_1) was negative (-0.7172) and the direct effect was also negative (-0.3419). Though the indirect effect via internodal length (X_2) and dry yield of roots (X_8) was positive (2.9152), the negative indirect effect via number of suckers (X_3), root length (X_4), root girth (X_5), number of roots (X_6) and fresh yield of roots (X_7) (-3.2905) was dominant over the positive indirect effect. This along with the negative direct effect was responsible for this correlation. The negative indirect effect via fresh yield of roots was dominant.

The direct effect of X_2 on Y was positive and high (1.0791) while its correlation with Y was 0.5450. This negative indirect effect especially via X_7 and X_3 was mainly responsible for the reduction in the magnitude of correlation, though positive indirect effect via X_8 was high. X_1, X_3, X_4, X_5, X_6 and X_7 also exhibited negative indirect effect.

Table 19. Path coefficient analysis showing direct and indirect effect of characters on plumbagin content in roots

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	r
X_1	-0.3419	1.0085	-0.7151	-0.1340	-0.1945	-0.0135	-2.2334	1.9067	-0.7172
X_2	-0.3195	1.0791	-0.8260	-0.1249	-0.2056	-0.0043	-1.9143	1.7706	0.5450
X_3	-0.2302	0.8393	-1.0620	-0.1057	0.0949	0.0266	-1.4728	1.6133	0.2965
X_4	-0.2584	0.7605	-0.6330	-0.1773	-0.2596	0.0820	-3.1043	3.3026	0.2876
X_5	-0.1418	0.4730	0.2149	-0.0981	-0.4691	0.0384	-1.9576	1.9556	0.0154
X_6	0.0288	-0.0290	-0.1762	-0.0906	-0.1124	0.1604	-2.2851	3.1594	0.6553
X_7	-0.2170	0.5873	-0.4447	-0.1564	-0.2610	0.1042	-3.5177	3.7977	0.1077
X_8	-0.1667	0.4885	-0.4381	-0.1497	-0.2345	0.1296	-3.4156	3.9111	0.1246

Residue = 11.4 %

X_1 = Plant height
X_2 = Internodal length
X_3 = Number of suckers
X_4 = Root length
X_5 = Root girth
X_6 = Number of roots
X_7 = Fresh root yield
X_8 = Dry root yield

The magnitude of correlation between X_3 and Y was less (0.2965) and positive while the direct effect of X_3 was negative and high (-1.0620). Though the indirect effect of X_2 and X_8 was relatively high and positive, the negative indirect effect via X_7 along with the direct effect was mainly responsible for this correlation. X_4 had also directly contributed less in negative direction and its correlation with Y was also small (0.2876). The relatively higher negative indirect effect via X_7 and X_3 and positive indirect effect via X_2 and X_8 were the main contributors of this correlation.

The correlation between X_5 and Y was negligible. The positive indirect effect via X_8, X_2 and X_3 and negative indirect effect via X_7 were mainly responsible for this negligible correlation. The correlation between X_6 and Y was positive and high (0.6553) while its direct effect was low (0.1604). The positive indirect effect via X_8 (3.1594) and negative indirect effect via X_7 (-2.2851) were the main contributors of this correlation. The direct effect of X_7 was high and negative (-3.5177) while its correlation with Y was negligible (-0.1077). The high positive indirect effect via X_8 (3.7977) is a major contributor for the low magnitude of correlation. The direct effect of X_8 was high (3.9111) while its correlation with Y was very low (0.1246). The high negative indirect effect via X_7 was mainly responsible for this reduced correlation.

To conclude, X_7 is a negative contributing factor and X_8 a positive contributing factor directly and indirectly to the plumbagin content. About

88.6 per cent of the variation in plumbagin content is explained by these eight factors.

Table 20 gives the direct and indirect effects of six characters on fresh weight of roots (Y).

The correlation between fresh weight of roots and other causative factors were positive but the direct effect of internodal length (X_2), number of suckers (X_3), root length (X_4) and root girth (X_5) were negative. The direct effect (1.4830) of plant height (X_1) was positive and high while the indirect effect of X_1 via all the remaining factors were negative. The correlation between X_2 and Y was positive (0.5441) while its direct effect was negative (-0.3263). The indirect effect via X_1 was the main contributor of this positive correlation. The direct effect of X_3 was negative (-0.3634) and its correlation with Y was positive (0.4187). Here also the indirect effect via X_1 was mainly responsible to get a positive correlation. The same trend was observed in the case of X_4 and X_5 also. Both the direct effect of number of roots (X_6) and correlation with Y was positive. While all the indirect effects of X_6 except X_2 was negative. 86.4 per cent of the variation in Y may be determined with these six characters. The direct and indirect effects of eight characters on the dry weight of roots is presented in Table 21. Dry weight of roots (Y) is positively correlated with all the characters while direct effects of internodal length (X_2), root length (X_4), number of roots (X_6) and fresh weight of shoot (X_8) are negative.

Table 20. Path coefficient analysis showing the direct and indirect effects on fresh root yield

	X_1	X_2	X_3	X_4	X_5	X_6	r
X_1	1.4830	-0.3049	-0.2447	-0.1796	-0.0372	-0.0816	0.6359
X_2	1.3860	-0.3263	-0.2827	-0.1675	-0.0393	-0.0261	0.5441
X_3	0.9986	-0.2535	-0.3634	-0.1416	0.0182	0.1607	0.4187
X_4	1.1211	-0.2299	-0.2166	-0.2376	-0.0496	0.4952	0.8826
X_5	0.6150	-0.1430	0.0736	-0.1315	-0.0897	0.2322	0.5566
X_6	-0.1249	0.0088	-0.0603	-0.1214	-0.0215	0.9689	0.6496

Residue = 13.6 %

X_1 = Plant height

X_2 = Internodal length

X_3 = Number of suckers

X_4 = Root length

X_5 = Root girth

X_6 = Number of roots

Table 21. Path coefficient analysis showing the direct effect and indirect effects on dry root yield

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	r
X_1	4.5824	-1.2230	0.5548	-0.7058	0.1332	0.0176	1.3319	-4.2036	0.4875
X_2	4.2827	-1.3086	0.6409	-0.6580	0.1408	0.0056	1.1416	-3.7923	0.4527
X_3	3.0858	-1.0178	0.8239	-0.5565	-0.0650	-0.0347	0.8784	-2.7014	0.4127
X_4	3.4643	-0.9223	0.4911	-0.9336	0.1778	-0.1070	1.8513	-3.1772	0.8444
X_5	1.9003	-0.5736	-0.1668	-0.5167	0.3212	-0.0502	1.1674	-1.5818	0.4994
X_6	-0.3848	0.0352	0.1367	-0.4772	0.0770	-0.2094	1.3627	0.2686	0.8078
X_7	2.9094	-0.7121	0.3450	-0.8239	0.1788	-0.1360	2.0978	-2.8878	0.9712
X_8	4.5471	-1.1715	0.5254	-0.7002	0.1199	0.0133	1.4301	-4.2362	0.5279

Residue = 12.2 %

X_1 = Plant height
X_2 = Internodal length
X_3 = Number of suckers
X_4 = Root length
X_5 = Root girth
X_6 = Number of roots
X_7 = Fresh root yield
X_8 = Fresh shoot yield

The direct effect of plant height (X_1) was high (4.5824) and its correlation with Y was 0.4875. The positive indirect effect through fresh weight of root (X_7) was high (1.3319) followed (0.5548) by number of suckers (X_3) while through X_2 (-1.2230) followed by X_4 (-0.7058) were having high negative indirect effect. These four factors along with X_1 were mainly contributing for this correlation. The direct effect of X_2 was negative and high (-1.3086) and indirect effect through X_8 (-3.7923) followed by X_4 (-0.6580) was also negative and high. The indirect effect through X_1 (4.2827), X_7 (1.1416) and X_3 (0.6409) were positive and high. The direct and indirect contribution of X_2 via X_1, X_3, X_4, X_7 and X_8 was responsible for this correlation. The direct effect of X_3 was positive and high (0.8239) and its correlation with Y is also positive (0.4127). The positive indirect effect through X_1 (3.0858) and X_7 (0.8784) and negative indirect effect via X_2 (-1.0178), X_4 (-0.5565) and X_8 (-2.7014) were the main contributors of this correlation. The direct effect of X_4 was high and negative (-0.9336) but its correlation with Y was positive and high (0.8444). The positive indirect effects via X_4 (3.4643), X_7 (1.8513) and X_3 (0.4911) lead to this positive correlation. X_8 and X_2 have negatively influenced with X_4 on Y. Both the correlation and direct effect of root girth (X_5) on Y were positive. X_1 and X_7 recorded high positive indirect effect (1.9003 and 1.1674) while X_8 and X_4 a high negative indirect effect (-1.5818 and -0.5167). These five factors were mainly responsible for this correlation. High positive correlation was observed for X_6 with Y (0.8078) and its direct effect was negative and low (-0.2094). This

high positive correlation may be attributed to the indirect effects especially via X_7 and X_8 (1.3627 and 0.2686). The negative indirect effect via X_1 (-0.3858) and X_4 (-0.4772) were also high. The correlation is the net effect of X_7, X_8, X_1 and X_4 along with X_6. Both the correlation of X_7 with Y (0.9712) and its direct effect was high (2.0978). X_1 (2.9094), X_3 (0.3450) were the main individual factors which influenced X_7 positively while X_2, X_4 and X_8 influenced in the opposite direction. X_8 was positively correlated with Y though its direct effect was negative. The positive indirect influence via X_1, X_7 and X_3 and negative indirect effect via X_2 and X_4 were the major contributors of this correlation.

12.2 per cent of the residue reveals that 87.8 per cent of the variation in dry weight of roots is contributed by eight factors.

Selection index

Selection index is used for scoring the ecotypes based on the index value based on yield and yield contributing characters. Plant height, internodal length, number of suckers per plant, root length, girth of root, number of roots per plant, fresh weight of root and fresh weight of shoot were adjudged as the major characters contributing to yield and adaptability. So they were selected for the formulation of selection indices along with yield. The selection index prepared based on yield and other characters are presented in Table 22.

Table 22. Selection index for eight ecotypes of *Plumbago*

Sl. No.	Ecotypes		Selection index	Rank No.
1.	Et_1	TBGRI (Palode)	4211	5
2.	Et_2	Farmers field at Kozha (Kottayam)	5611	2
3.	Et_3	Medicinal garden of Arya Vaidyasala (Malappuram)	3740	7
4.	Et_4	Farmers field at Neyyattinkara (Trivandrum)	5281	3
5.	Et_5	College of Horticulture Vellanikkara (Trichur)	4577	4
6.	Et_6	Farmers field at Muvattupuzha (Ernakulam)	4086	6
7.	Et_7	Medicinal garden of Arya Vaidya pharmacy (Palghat)	3701	8
8.	Et_8	(*Plumbago zeylanica*)	16357	1

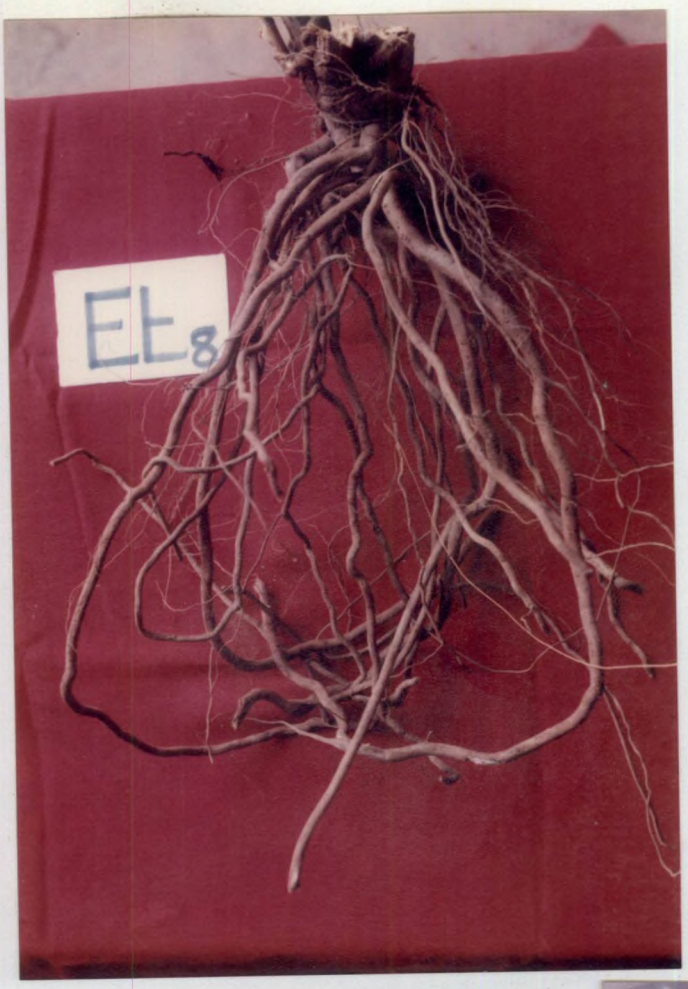


Plate 11. Roots of *Plumbago zeylanica* (Et_8)

Plate 12. High root yielding ecotype of *P. rosea*

Plate 13. High root yielding ecotype of *P. rosea*

Plate 14. High root yielding ecotype of *P. rosea*

The highest index score was recorded by Et_8 (*P. zeylanica*) followed by the ecotype Et_2, Et_4, Et_5, Et_1, Et_6, Et_3 and Et_7 in that order which ranged from 3701 to 5611. The lowest index score were recorded by the ecotype Et_7. The index score of Et_8 was about three times higher than the highest score (Et_2) among the *P. rosea* ecotypes (Plates 11 to 14).

Experiment 2 Induction of variability

Of the several ecotypes of *P. rosea* tested, Et_5 was selected for the experiment because of its easy availability. Two-noded semi-hardwood cuttings collected from College of Horticulture, Vellanikkara (Et_5) were treated with different gamma ray doses. The variability induced by the mutagen was evaluated for one mutational vegetative generation namely M_1V_1 as the duration of the crop was long (18 months) to continue studies. The results are presented below :

Sensitive studies

The efficiency of the mutagen was studied on the basis of the sprouting of cuttings under nursery conditions.

Gamma rays

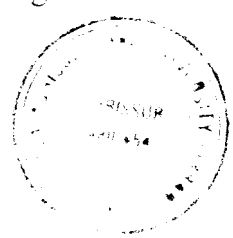
Different doses of gamma rays were tried under nursery conditions on sprouting of cuttings and the results are presented in Table 23.

The survival percentages of cuttings varied from zero to 100. Hundred percentage survival was recorded by the dose 0.5 kR and as the

Table 23. Effect of gamma rays on the sprouting of cuttings (nursery conditions)

Treatments (kR)	Sprouting percentage over control	Percentage variation from control
Control	65.0 (53.73)	0.00
0.50	100.0 (90.0)	53.85
1.00	90.0 (71.56)	38.46
1.50	60.0 (50.77)	-7.69
2.00	5.0 (12.92)	-92.31
2.50	0	—
3.00	0	—
3.50	0	—
4.00	0	—
4.50	0	—
5.00	0	—

Numbers of parenthesis are transformed percentages in angles



dose increased, the survival percentage decreased and none of the cuttings sprouted above a dose of 2.0 kR. Probit analysis of the data provided the relationship between gamma rays and response in terms of probit. the result obtained was as follows

$$Y = -1.556\ X + 7.6212$$

where y = probit which is the transformed value of sprouting percentage

x = logarithm of dosage

Based on the above relationship the ED_{50} which is the dose to provide at least 50 per cent sprouting was calculated to be around 1.685. Therefore six doses of the mutagen at regular intervals with 1.5 as the highest dose viz., 1.5, 1.25, 1.0, 0.75, 0.50 and 0.25 kR were tried in the main field experiments.

Field studies

The mutagen treated populations were compared with the untreated control for 18 characters and the results are presented below.

Effect of mutagen on M_1V_1 generation

Nursery observations

The observations on the following characters in the nursery are presented in Tables 24 to 27.

Sprouting

The effect of different doses of mutagen on sprouting of cuttings under field conditions is furnished in Table 24.

Sprouting percentage

The comparison of the effects of different doses of gamma rays has indicated that the sprouting percentage decreased with increase in doses. The mean values ranged from 58.01 (1.5 kR) to 90.09 (0.25 kR) in the case of gamma irradiation. The sprouting percentage significantly decreased with increasing doses of mutagens in all the treatments. The highest dose of 1.5 kR gamma rays gave a lower sprouting percentage (58.01) than the control (65.01). All other treatments recorded a higher sprouting percentage than the control with the highest percentage (90.09) in the lowest dose (0.25 kR).

Days taken to start sprouting

The number of days taken to start sprouting in gamma rays treated cuttings as compared to control is given in Table 24. Statistical analysis of the data showed significant variation among the treatments.

Sprouting started earlier in the lower doses of 0.25 and 0.50 kR gamma rays where as it was significantly delayed at higher doses (1.25 and 1.50 kR). The intermediate dose of gamma ray (1.0 kR) was on par with control. The mean values ranged from 8.93 (0.25 kR) to 13.88 (1.5 kR).

Table 24. Effect of gamma rays on the sprouting of cuttings (Field experiment)

Treatments kR	Sprouting (%)	Percentage variation over control	No. of days to start sprouting (Mean period in days)	Percentage variation over control	Duration (Mean days)	Percentage variation over control
Control	65.01 (53.71)	0	10.95	0	5.10	0
0.25	90.09 (71.62)	38.58	8.93	-18.45	5.93	16.27
0.50	84.10 (66.48)	29.36	9.03	-17.3	5.83	14.31
0.75	82.04 (64.90)	26.20	9.90	-9.59	5.48	7.45
1.00	77.02 (61.33)	18.47	10.68	-2.47	5.25	2.94
1.25	74.03 (59.34)	13.87	12.00	9.59	4.60	-9.80
1.50	58.01 (49.59)	-10.77	13.88	26.76	4.48	-12.16
CD(0.05)	2.718		0.354		0.288	

Numbers in parenthesis denote transformed percentages in angles

Duration of sprouting

The duration of sprouting (interval between first and last sprouting within a treatment) was found to be reduced with an increase in dose of mutagen (Table 24). Statistical analysis of the results showed significant variations among the treatments. The mean duration of sprouting of 5.25 days noticed at 1.00 kR was on par with control (5.10 days). The mean values ranged from 4.48 (1.5 kR) to 5.93 days (0.25 kR).

Lethality

The effect of gamma rays on lethality at 30, 60, 90 and 120 DAP is presented in Table 25.

Statistical analysis of the data showed significant variation in different doses of the mutagen. The mean values of lethality were same at 30, 60 and 90 DAP. The mean values ranged from 13.3 per cent in control to 20.5 per cent in 1.5 kR. The percentage of lethality increased with increase in dose of mutagen.

The mean values of lethality at 120 DAP, ranged from 1.78 per cent (control) to 27.6 per cent) (1.5 kR)

Number of leaves per plant

The influence of gamma rays on number of leaves per plant in the nursery is presented in Table 26.

Table 25. Effect of gamma rays on the lethality of cuttings

Treatment (kR)	Lethality (Percentage)							
	30 DAP	Percentage variation over control	60 DAP	Percentage variation over control	90 DAP	Percentage variation over control	120 DAP	Percentage variation over control
0	13.33 (21.41)	0	13.33 (21.41)	0	13.33 (21.41)	0	17.79 (24.94)	0
0.25	14.30 (22.21)	7.28	14.30 (22.21)	7.28	14.30 (22.21)	7.28	19.07 (25.88)	7.12
0.50	14.64 (22.49)	9.83	14.63 (22.49)	9.83	14.64 (22.49)	9.82	19.52 (26.21)	9.72
0.75	15.59 (23.25)	16.95	15.59 (23.25)	16.95	15.59 (23.25)	16.95	20.79 (27.11)	16.86
1.00	16.22 (23.74)	21.69	16.22 (23.74)	21.69	16.22 (23.74)	21.69	21.63 (27.71)	21.59
1.25	18.45 (25.44)	38.41	18.45 (25.44)	38.41	18.45 (25.44)	38.41	24.63 (29.74)	38.45
1.50	20.55 (26.96)	54.16	20.55 (26.96)	54.16	20.55 (26.96)	54.16	27.61 (31.69)	55.12
CD (0.05)	0.584		0.584		0.584		0.742	

Figures in parenthesis denote transformed percentages in angles

Table 26. Effect of gamma rays on the mean number of leaves in the nursery

Treatments (kR)	45 DAP	Percentage variation over control	60 DAP	Percentage variation over control	75 DAP	Percentage variation over control	90 DAP	Percentage variation over control	Mean
0	1.30	0	2.5	0	3.8	0	6.5	0	3.53
0.25	1.50	15.38	2.7	8.00	4.0	5.26	6.8	4.62	3.75
0.50	1.78	36.92	2.9	16.00	4.1	7.89	7.1	9.23	3.97
0.75	1.80	38.46	3.0	20.0	4.3	13.16	7.2	10.77	4.08
1.00	1.20	-7.70	2.4	-4.00	3.7	-2.63	6.4	-1.54	3.43
1.25	1.10	-15.38	2.3	-8.00	3.6	-5.26	6.2	-4.62	3.30
1.50	1.00	-23.08	2.1	-16.00	3.3	-13.16	6.1	-6.15	3.13
CD (0.05)	0.112		0.235		0.155		0.227		

At lower doses of gamma rays (0.25 kR to 0.75 kR) leaf production increased over control and at higher doses it decreased. A gradual reduction in mean number of leaves was observed from 1.0 kR gamma rays at all stages of measurement viz., 45, 60, 75 and 90 days after planting. At 45th day after planting the number of leaves ranged from 1.0 (1.5 kR) to 1.8 (0.75 kR). At 60 DAP maximum number of leaves (3.0) was recorded at 0.75 kR treatment and the minimum (2.1) by the highest dose of 1.5 kR. Same was the trend at 75 and 90 DAP with maximum number of leaves recorded by 0.75 kR dose and the minimum by 1.5 kR. The percentage variation over control gave negative values in all higher doses viz., 1.0, 1.25 and 1.5 kR at all stages of observation.

Plant height

The effect of gamma rays on the height of the plant is presented in Table 27.

Significant difference in plant height was noticed among treatments. At 45 DAP, the mean plant height ranged from 2.16 to 4.75 cm. The maximum plant height was recorded at 1.00 kR of gamma rays which was significantly superior to all other treatments and control plants. The plant height in other treatments ranged from 2.16 to 4.12 cm and in control plants mean height was 3.38 cm. At 65 DAP and 90 DAP also, the intermediate dose of gamma rays (1.0 kR) was significantly superior to all other treatments and control with regard to this character. The

Table 27. Effect of gamma rays on the plant height in the nursery

Treatment kR	Plant height (cm)						
	45 DAP	Percentage variation over control	65 DAP	Percentage variation over control	90 DAP	Percentage variation over control	Mean
0	3.38	0	4.38	0	8.86	0	5.54
0.25	2.46	-27.22	3.30	-24.66	6.18	-30.25	3.98
0.50	3.19	-5.62	3.66	-16.44	7.04	-20.54	4.63
0.75	3.78	11.83	5.03	14.84	9.09	2.60	5.97
1.00	4.75	40.53	6.04	37.90	11.14	25.73	7.31
1.25	4.12	21.89	5.13	17.12	8.45	-4.63	5.9
1.50	2.16	-36.10	3.41	-22.15	6.39	-27.88	3.99
CD(0.05)	0.408		0.257		0.978		

percentage variation over control gave negative values for the lower doses of gamma rays (0.25 and 0.50 kR) and for the highest dose (1.5 kR).

Field observations

Flowering

The effect of gamma rays on the number of days to flowering of the plant is presented in Table 28.

Table 28. Effect of gamma ray on number of days to flowering

Treatments kR	Mean number of days	Percentage variation over control
0	219.3	0
0.25	216.5	-1.28
0.50	214.8	-2.05
0.75	211.5	-3.56
1.00	208.8	-4.79
1.25	206.8	-5.70
1.50	203.8	-7.07
CD(0.05)	1.372	

Significant difference in number of days to flowering was noticed due to treatments. There was a gradual decrease in the number of days taken to flowering as the dosage of gamma rays increased. The minimum number of days to flowering was noticed at the highest dose (1.50 kR) and the maximum number of days to flowering in the untreated plants. The maximum number of days to flowering was 219.3 days (control) whereas the minimum number of days was 203.8 days (1.50 kR).

Plant height

The effect of treatments on plant height for the different doses of gamma rays is presented in Table 29.

Plant height was seen significantly influenced by the different doses of gamma rays. Maximum plant growth was seen during rainy season whereas it was almost stand-still during summer months. The maximum plant height was recorded at the lower dose, 0.25 kR (94.23 cm) at the time of harvest and the minimum plant height was exhibited by the control plants (61.86 cm). When the overall mean plant height was considered, the maximum plant height was recorded by the treatment, 1.0 kR (46.20 cm) followed by 1.25 kR) (40.04 cm) and 0.75 kR (38.35 cm). The minimum plant height was exhibited by the control plants (26.55 cm). There was a gradual increase in plant height on the dosage of gamma rays increased upto a certain dose after which the plant height decreased.

Table 29. Effect of gamma rays on the plant height at different growth periods

Plant height (cm)	Treatments (kR)							CD
	0	0.25	0.50	0.75	1.00	1.25	1.50	(0.05)
January	11.88	7.43	10.16	23.14	32.26	22.94	10.03	1.515
% variation over control	0	-37.46	-14.48	94.78	171.55	93.10	-15.57	
February	14.08	8.35	11.65	29.99	39.99	28.93	11.65	1.281
% variation over control	0	-40.70	-17.26	113.00	184.02	105.42	-17.26	
March	17.47	10.13	14.10	34.77	41.31	30.55	15.95	1.331
% variation over control	0	-42.02	-19.29	84.72	136.46	74.87	-8.70	
April	17.47	10.13	14.10	34.77	41.31	30.55	15.95	1.331
% variation over control	0	-42.02	-19.29	84.72	136.46	74.87	-8.70	
May	17.47	10.13	14.10	34.77	41.31	30.55	15.95	1.331
% variation over control	0	-42.02	-19.29	84.72	136.46	74.87	-8.70	
June	18.63	11.57	15.38	35.30	42.22	31.63	17.66	0.647
% variation over control	0	-37.90	-17.45	89.48	126.62	69.78	-5.21	
July	20.39	17.57	18.45	35.96	44.18	37.04	20.90	0.357
% variation over control	0	-13.83	-9.52	77.85	116.68	81.66	2.50	
August	22.56	28.39	24.15	38.41	46.29	42.64	26.15	0.399
% variation over control	0	-25.84	7.05	70.26	105.19	89.01	15.91	
September	30.25	48.04	36.61	40.17	47.32	44.84	38.44	0.516
% variation over control	0	58.81	21.03	32.79	56.43	48.23	27.07	
October	39.27	55.14	50.13	44.73	49.17	49.51	52.42	0.270
% variation over control	0	40.41	27.68	13.90	25.21	26.08	33.49	
November	47.25	72.07	65.18	48.45	58.73	59.68	69.23	0.360
% variation over control	0	52.53	37.95	2.54	24.30	26.31	46.52	
December	61.86	94.23	79.93	62.26	70.30	71.59	80.27	0.350
% variation over control	0	52.33	29.21	0.65	13.64	15.73	29.76	
Mean (overall)	26.55	31.10	29.50	38.35	46.20	40.04	31.22	

The percentage variation gave positive values for all the doses of gamma rays.

Internodal length

The influence of gamma rays on internodal length during different growth period is depicted in Table 30.

The statistical analysis of the data on gamma ray treated population showed significant variation among treatments. During the initial growth period the maximum internodal length was noted at 1.0 kR and the minimum in control population. In the summer season there was no increase in the internodal length. At harvest, the maximum internodal length (10.2 cm) was recorded at the lowest dose (0.25 kR) which was followed by 0.5 kR and 1.5 kR (9.58 cm). The control plants exhibited 8.6 cm whereas the minimum (8.18 cm) was at 0.75 kR.

The mean internodal length when worked out for the entire growth period was highest at 1.0 kR (5.16 cm) which was followed by 0.75 and 1.25 kR gamma rays with mean internodal length of 4.99 and 4.95 cm respectively. Control plants recorded mean internodal length of 4.72 cm which was the lowest among the treatments. The percentage variation gave positive values for the lower doses (0.25 and 0.5 kR) of gamma rays while it gave negative values when the dose increased.

Table 30. Effect of gamma rays on the internodal length at different growth periods

Internodal length (cm)	Treatments (kR)							CD
	0	0.25	0.50	0.75	1.00	1.25	1.50	(0.05)
January	2.28	1.55	1.46	2.44	2.65	2.29	1.53	0.130
% variation over control	0	-58.33	-35.97	7.02	16.23	0.44	-32.90	
February	2.38	2.60	2.46	3.44	3.78	3.46	2.65	0.193
% variation over control	0	9.24	3.36	44.54	58.82	45.38	11.34	
March	2.58	2.76	2.66	3.70	3.81	3.61	2.71	0.138
% variation over control	0	6.98	3.10	43.41	47.67	39.92	5.04	
April	2.58	2.76	2.66	3.70	3.81	3.61	2.71	0.138
% variation over control	0	6.98	3.10	43.41	47.67	39.92	5.04	
May	2.58	2.76	2.66	3.70	3.81	3.61	2.71	0.138
% variation over control	0	6.98	3.010	43.41	47.67	39.92	5.04	
June	3.66	3.19	3.35	4.11	4.33	3.98	3.25	0.290
% variation over control	0	-12.84	-8.47	12.30	18.31	8.74	-11.20	
July	4.16	4.13	4.11	4.39	4.76	4.13	4.44	0.259
% variation over control	0	-0.72	-1.20	5.53	14.42	-0.72	6.73	
August	5.31	5.25	4.89	5.06	5.68	4.90	5.03	0.277
% variation over control	0	-1.13	-7.91	-4.71	6.97	-7.72	-5.27	
September	6.65	7.38	6.74	6.30	6.30	6.19	6.90	0.307
% variation over control	0	10.98	1.35	-5.26	-5.26	-6.92	3.76	
October	7.44	8.24	8.03	7.26	7.25	7.31	8.01	0.309
% variation over control	0	10.75	7.93	-2.55	-2.55	-1.75	7.66	
November	8.43	9.45	8.81	7.65	7.40	7.71	8.76	0.393
% variation over control	0	12.10	4.51	-9.25	-12.22	-8.54	3.92	
December	8.60	10.20	9.58	8.18	8.36	8.64	9.58	0.410
% variation over control	0	18.61	11.40	-4.88	-2.79	0.47	11.40	
Mean (overall)	4.72	5.02	4.78	4.99	5.16	4.95	4.86	

Number of suckers per plant

The effect of gamma rays on number of suckers produced per plant is presented in Table 31.

Chethikoduveli showed an increase in number of suckers per plant with increase in dosage of gamma rays upto 1.0 kR (Table 31) and then decreased with further increase in dosage. However a reduction in number of suckers was observed in control plants, when compared to treated plants (except lowest dose). The maximum number of suckers was produced at 0.75 kR gamma irradiation (9.88) followed by 1.0 and 0.50 kR gamma rays with 9.25 and 8.25 suckers per plant respectively. The statistical analysis of the data over different growth period also showed significant variation except during summer months (Table 32).

The percentage variation gave negative value for the lowest dose (Table 31). The percentage variation gave high values as the dosage of gamma rays increased upto 0.75 kR. All the treatments except the lowest dose (0.25 kR) produced more number of suckers per plant than the control.

Number of roots per plant

The influence of gamma rays on the number of roots per plant is presented in Table 31.

Table 31. Effect of gamma rays on the number of suckers and number of roots

Treatment kR	Number of suckers			Number of roots		
	Range	Mean	Percentage variation over control	Range	Mean	Percentage variation over control
0	2-7	5.78	0	9-18	14.38	0
0.25	2-7	5.50	-4.84	7-16	15.20	5.70
0.50	4-10	8.25	42.73	9-22	16.65	15.79
0.75	7-12	9.88	70.93	7-21	18.65	29.69
1.00	7-11	9.25	60.04	16-25	20.60	43.25
1.25	5-9	7.70	33.22	9-19	14.45	0.49
1.50	5-9	7.63	32.01	5-16	11.30	-21.42
CD(0.05)		0.458			0.364	

Table 32. Mean number of suckers produced during different growth period

Treatment kg	Number of suckers												
	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Overall mean
0	1.20	1.30	1.43	1.43	1.43	1.65	2.13	2.75	3.88	4.40	5.78	5.78	2.76
0.25	1.00	1.35	1.48	1.48	1.48	1.90	2.38	2.88	3.38	4.50	5.50	5.50	2.73
0.50	1.35	1.88	2.23	2.23	2.23	2.25	3.05	3.60	4.70	7.25	8.25	8.25	3.96
0.75	1.70	2.15	2.68	2.68	2.68	3.28	4.45	4.98	7.00	8.83	9.88	9.88	5.02
1.00	1.53	2.05	2.48	2.48	2.48	3.03	4.25	4.75	6.5	8.25	9.25	9.25	4.69
1.25	1.28	1.55	1.88	1.88	1.88	2.25	2.88	3.38	4.28	6.70	7.70	7.70	3.61
1.50	1.10	1.33	1.58	1.58	1.58	1.93	2.70	3.23	3.68	6.33	7.63	7.63	3.35

CD(0.05) Treatments = 1.161 Treatment x months = 0.271

Positive shifts in the number of roots per plant was observed due to gamma rays at lower doses. A gradual reduction in the number of roots per plant was observed at higher doses of gamma rays. Maximum number of roots (20.6) was reported by the cuttings treated with 1.0 kR gamma rays. The control gave 14.38 roots per plant. Number of roots reached a minimum of 11.3 at 1.5 kR gamma rays.

In gamma rays treated population, as the dose increased upto 1.0 kR, positive values for percentage variation for number of roots also increased (Table 31). At 1.5 kR the number of roots per plant was significantly lower than the control (11.3).

Fresh weight of shoot

The influence of gamma rays on the fresh weight of shoot is given in Table 33.

The mean weight of shoot in gamma ray treated population ranged from 245.38 g (1.0 kR) to 461.38 g (0.25 kR) and that in control plants is 234.8 g. As the dosage of gamma rays increased there was a gradual decline in the fresh weight of shoot. The maximum weight of the shoot was noticeable at 0.25 kR which was followed by 1.5 and 0.5 kR with a mean weight of shoot of 353.38 and 325.38 g respectively.

Table 33. Effect of gamma rays on the fresh weight and dry weight of shoot

Treatment kR	Fresh weight (gm)			Dry weight (gm)		
	Range	Mean	Percentage variation over control	Range	Mean	Percentage variation over control
0	150-300	234.88	0	70-160	112.88	0
0.25	200-600	461.38	96.43	150-300	237.25	110.18
0.50	200-400	325.38	38.53	80-200	169.63	50.28
0.75	150-400	320.13	36.30	70-200	161.25	42.85
1.00	150-320	245.38	4.47	60-180	122.50	8.52
1.25	180-350	265.75	13.14	70-160	130.13	15.28
1.50	220-400	353.38	50.45	80-200	177.00	56.80
CD(0.05)		3.800			2.911	

The fresh weight of shoot showed positive values for the percentage variation over control when treated with gamma rays. The percentage variation ranged from 4.47 (1.0 kR) to 96.43 per cent (0.25 kR).

Dry weight of shoot

The effect of gamma rays on dry weight of shoot is presented in Table 33.

The mutagenic treatments caused considerable difference in the dry weight of shoot which showed the same trend as in fresh weight of shoot. With increase in dosage of gamma rays the dry weight of shoot decreased upto 1.0 kR after which it again increased. The dry weight of shoot ranged from 112.88 (control) to 237.25 g (0.25 kR). The minimum dry weight of shoot was noticed at 1.0 kR (122.50 g).

The dry weight of shoot also showed positive values for the percentage variation which ranged from 8.52 to 110.18 per cent. In the case of gamma rays the maximum percentage variation (110.18) was recorded at 0.25 kR gamma rays.

Length of root per plant

The influence of gamma rays on the length of root per plant is presented in Table 34.

Table 34. Effect of gamma rays on length and girth of roots

Treatment kR	Root length (cm)			Root girth (cm)		
	Range	Mean	Percentage variation over control	Range	Mean	Percentage variation over control
0	35-66	41.88	0	1.5-2.5	2.25	0
0.25	28-45	39.53	-5.61	1.0-2.5	1.75	-22.22
0.50	55-70	63.85	52.46	1.5-2.5	2.50	11.11
0.75	60-92	76.43	82.50	3.0-4.5	4.30	91.11
1.00	70-105	83.68	99.81	3.0-4.5	3.75	66.66
1.25	60-85	72.68	73.54	2.0-3.5	3.25	44.44
1.50	25-45	37.03	-11.58	1.5-2.5	2.13	-5.33
CD(0.05)		0.736			0.377	

Statistical analysis of the data showed significant variation among treatments. Length of root in case of gamma ray treated population ranged from 37.03 (1.5 kR) to 83.68 cm (1.0 kR). This was followed by 0.75 kR and 1.25 kR with mean root length 76.43 and 72.68 respectively. The control plants recorded 41.88 cm length at the time of harvest.

The percentage variation gave negative values for 0.25 and 1.50 kR whereas for all other treatments the percentage variation was positive. The highest percentage variation was shown by 1.0 kR and lowest by 1.5 kR.

Girth of root

The effect of gamma rays on the girth of root per plant is presented in Table 34.

The girth of roots recorded on the day of harvest showed significant variation among treatments. A gradual increase in the girth of roots was noticeable upto 0.75 kR whereas it declined gradually at higher dosages of gamma rays. The mean values ranged between 1.75 (0.25 kR) and 4.30 cm (0.75 kR) of gamma rays. The control plants recorded a mean girth of 2.25 cm.

The percentage of variation for girth of roots also showed the same trend as followed for root length (Table 34). Negative values for percentage variation was shown by the lowest (0.25 kR) and highest dose (1.5 kR).

Fresh weight of roots

The influence of gamma rays on root weight is given in Table 35. Treatments showed significant difference for this character. Fresh weight of shoots showed an increase with increase in dosage of gamma rays upto 1.0 kR (Table 35) where as it decreased with further increase in dosage. However a reduction in the fresh weight of roots was observed in the lowest dose (0.25 kR) as well as in the highest (1.5 kR) when compared with control. Fresh weight of roots in case of gamma ray treated population ranged between 114.63 g (0.25 kR) an 374.75 g (1.0 kR). The maximum fresh weight of roots in 1.0 kR (374.75 g) was followed by 0.75 and 0.5 kR with mean weight of roots of 266.0 and 187.5 g respectively.

Gamma ray treated population had both negative and positive values for the percentage variation (Table 33). Negative values were shown by 0.25 kR and 1.5 kR while positive values were shown by all other dosages of gamma rays.

Dry weight of roots

The effect of gamma rays on dry weight of roots is presented in Table 35.

Statistical analysis of the data showed significant variation among the treatments. The dry weight of roots ranged from 65.25 (1.5 kR) to

Table 35. Effect of gamma rays on the fresh weight and dry weight of root

Treatment kR	Fresh weight (gm)			Dry weight (gm)		
	Range	Mean	Percentage variation over control	Range	Mean	Percentage variation over control
0	100-220	157.13	0	40-140	83.00	0
0.25	60-180	114.63	-27.05	25-100	71.25	-14.16
0.50	140-250	187.50	19.33	50-150	107.50	29.52
0.75	180-350	266.00	69.29	100-200	152.75	84.04
1.00	200-500	374.75	138.50	140-300	200.25	141.27
1.25	110-250	176.30	12.20	30-130	91.75	10.54
1.50	75-150	116.25	-26.02	25-100	65.25	-21.38
CD(0.05)		3.007			3.360	

200.25 g (1.0 kR). The control plants recorded mean dry weight of roots of 83.0 g. The lowest dose (0.25 kR) and the highest dose (1.50 kR) recorded a lesser mean dry weight of roots (71.25 g and 65.25 g respectively) than control plants.

Positive values of percentage variation were shown by all the dosages of gamma rays except 0.25 and 1.5 kR. The highest percentage variation was given in 1.0 kR (141.27 %) and lowest by 1.5 kR (-21.38 %).

Plumbagin content

The influence of gamma rays on plumbagin content of roots is presented in Table 36.

Statistical analysis of the data showed significant variation among treatments. There was a gradual increase in the content of plumbagin in roots with increase in dosage of gamma rays. The highest concentration of plumbagin was recorded with 1.0 kR (1.49 per cent) which was followed by 0.75 kR and 1.25 kR with a mean plumbagin content of 1.42 and 1.32 per cent respectively. The lower doses of gamma rays (0.25 and 0.50 kR) were on par with each other (1.08 % and 1.10 %). The control plants recorded a mean content of plumbagin of 1.03 per cent. The lowest concentration of plumbagin in roots was recorded by 1.5 kR dosage of gamma rays (0.91 %).

Table 36. Effect of gamma rays on plumbagin content

Treatments kR	Plumbagin (%)	Percentage variation over control
0	1.03	0
0.25	1.08	4.85
0.50	1.10	6.80
0.75	1.42	37.86
1.00	1.49	44.66
1.25	1.32	28.16
1.50	0.91	-11.65
CD(0.05)	0.035	

The percentage variation for the mean plumbagin content gave positive values for all the treatments except the highest dosage of gamma rays which gave a negative percentage variation over control plants.

Optimisation of mutagenic dose

The optimum dosage of gamma rays for different characters of "Chethikoduveli" viz., sprouting of cuttings, number of leaves per plant and plant height in the nursery, important vegetative characters in field and important yield attributes for creating variability were worked out

separately using the quadratic model of regression equation. The values obtained are presented in Tables 37 to 38.

Sprouting of cuttings

The optimum dosage of gamma rays on the sprouting of cuttings is presented in Table 37.

The effective dose of gamma rays for the sprouting of cuttings was around 0.5 kR (Table 37). The optimum dose for number of days to start and complete sprouting was 0.51 kR. For maximum sprouting percentage the optimum dose for duration of sprouting (interval between first and last sprouting within a treatment) was found to be 0.49 kR. 94 and 93 per cent variation in days to start sprouting and days to complete sprouting were attributed respectively to the gamma-irradiation. giving a dose of 0.51 kR for maximum sprouting. 76 per cent of the variation was explained in sprouting percentage with a dose of 0.64 kR for maximum percentage sprouting and 69 per cent of the variation was attributed in duration of sprouting at a dose of 0.49 kR of gamma rays.

Yield contributing characters

The optimum dosages of gamma rays for important attributes related to yield are presented in Table 38.

Table 37. Optimum dose of gamma rays for the sprouting of cuttings

Characters	Quadratic model of response on γ - irradiation	R^2	$F_{1,25}$	Optimum dose kR
Days to start sprouting	$Y = 10.5018 - 4.8964\ \gamma + 4.8429\ \gamma^2$	0.94	186.46	0.51
Days to complete sprouting	$Y = 15.8417 - 3.5858\ \gamma + 3.5048\ \gamma^2$	0.93	168.84	0.51
Sprouting percentage	$Y = 70.4762 + 47.4286\ \gamma - 37.333\ \gamma^2$	0.76	38.64	0.64
Duration	$Y = 5.3399 + 1.3107\ \gamma - 1.3381\ \gamma^2$	0.69	28.28	0.40

Table 38. Optimum dose of gamma rays for root characters

Characters	Quadratic model of response on γ-irradiation	R^2	$F_{1,25}$	Optimum dose kR
Length of roots	$Y = 46.4126 + 52.4421\ \gamma - 32.555\ \gamma^2$	0.16	2.43	0.81
Girth of roots	$Y = 1.9018 + 3.5179\ \gamma - 2.0714\ \gamma^2$	0.29	5.12*	0.85
Number of roots	$Y = 13.3857 + 12.2179\ \gamma - 8.6714\ \gamma^2$	0.39	7.90**	0.71
Fresh weight of roots	$Y = 117.2381 + 349.3929\ \gamma - 221.9524\ \gamma^2$	0.32	5.92*	0.79
Dry weight of roots	$Y = 64.7797 + 201.8214\ \gamma - 130.333\ \gamma^2$	0.39	7.89**	0.77
Plumbagin content	$Y = 0.9149 + 1.0650\ \gamma - 0.6419\ \gamma^2$	0.53	13.97**	0.83

* Significant at 5% level

** Significant at 1% level

Plate 15. Field view of treated (right) and untreated (left) plants

Plate 16. A normal plant of *P. rosea*

For all the yield characters the optimum dose of gamma rays ranged between 0.70 kR and 0.85 kR. The optimum dose of gamma rays for root length was 0.81 kR whereas it was 0.85 and 0.71 kR for root girth and root number respectively. The fresh weight and dry weight for roots had an optimum dose of gamma rays 0.79 and 0.77kR respectively. For maximum plumbagin content in roots the dosage was 0.83 kR. All the fitted regressions were found to be significant. However, the percentage variation explained by the fitted relationships ranged from 16 per cent (length of root) to 53 per cent (plumbagin content).

Appearance of macromutants

Plants of dwarf type, medium dwarf type, tall type, extra tall type, with long slender roots, short round roots and very high yield were observed in M_1V_1 population. Among the gamma ray treated population 1.50 per cent were with dwarf plant type whereas 1.67 per cent were of medium dwarf growth habit (Table 39). Plants which were taller than the normal plant height were also observed in the mutagen treated population. Of the total population, 2 per cent was taller than normal plants and 1.83 per cent was extra tall. These plants were taller than the plants of the related species, *P. zeylanica*. The percentage of long slender roots in gamma ray treated population was 1.50 whereas

Plate 17. Morphological variant No. 1

Plate 18. Morphological variant No. 2

Table 39. Morphological variants in M_1V_1 generation

Treatments (kR)	Morphological variants (%)						
	Dwarf	Medium dwarf	Tall	Extra tall	Long slender root	Short roots	High root yielders (Fresh weight)
0.25	1.00	1.00	0	0	0	3.00	0
0.50	2.00	2.00	0	1.00	0	1.00	0
0.75	4.00	1.00	4.00	3.00	3.00	0	4.00
1.00	0	1.00	5.00	5.00	4.00	0	20.00
1.25	0	0	3.00	2.00	1.00	0	1.00
1.50	0	0	0	0	0	0	0
Mean	1.16	0.83	2.00	1.83	1.50	0.83	4.17

Plate 19. Morphological variant No. 3

Plate 20. Morphological variant No. 4

that of short round roots was 0.83 per cent. The percentage of plants with extra high yield of roots in gamma ray treated population was 4.17 (Plates 17 to 23).

Chlorophyll mutants

The variegation noted on leaves due to chlorlophyll deficiency was considered as chlorlophyll mutants. Such a mutant was observed to occur in M_1V_1 generation in the gamma ray treated plants. Gamma rays at lowest concentration of 0.25 kR produced one albino shoot in one of the treated plants (Plate 24).

Selection of high yielding mutants

Morphological variants for high yield of roots were identified and selected from the treated population. Those plants which gave 50 per cent higher fresh root yield over the highest mean root yield were selected (Table 40).

From the gamma ray treated population, the intermediate doses namely 0.75 and 1.0 kR gave high root yielding. The maximum percentage of high yielders was observed in 1.0 kR (20%) followed by 0.75 kR (4 %). These plants were selected and their fresh weight of roots, dry weight of roots and plumbagin content were recorded separately (Table 40). Among the 0.75 kR treated plants, the maximum

Plate 21 Morphological variant No. 5

Plate 22. Morphological variant No. 6

Table 40. Selected yield mutants

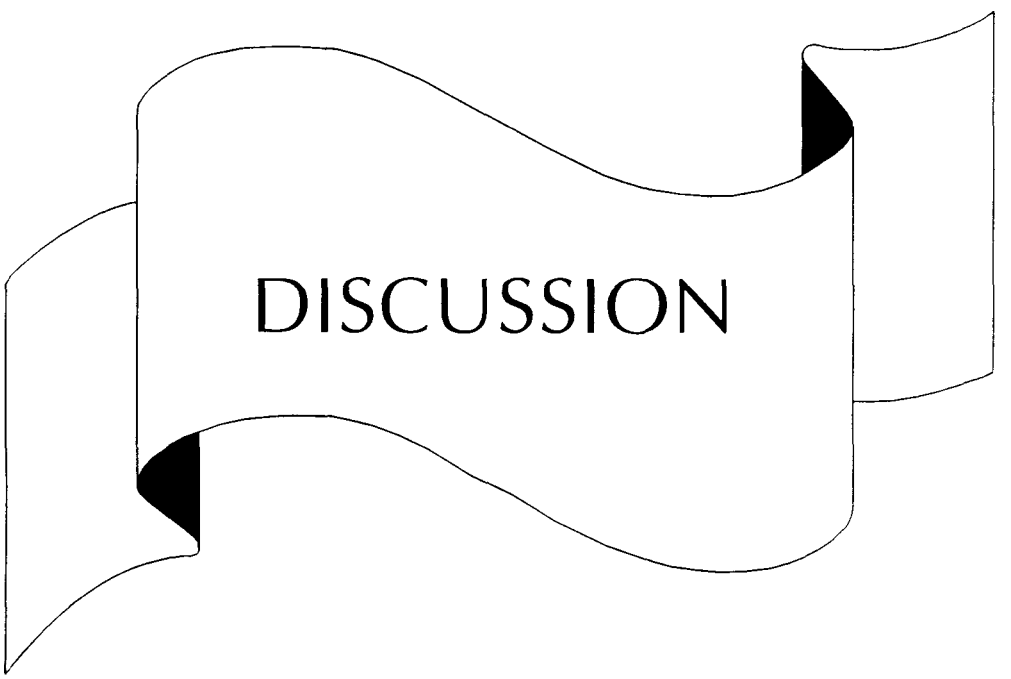
Sl. No.	Mutants	Fresh weight of root (g)	Dry weight root (g)	Plumbagin content (%)
0.75 kR				
1.	PB-3-1	404.00	209.50	1.44
2.	PB-3-2	400.50	198.00	1.43
3.	PB-3-3	406.50	219.50	1.46
4.	PB-3-4	402.00	201.00	1.44
	Mean*	266.00	152.75	1.42
1.0 kR				
1.	PB-4-1	563.00	298.50	1.49
2.	PB-4-2	565.00	305.00	1.50
3.	PB-4-3	568.00	286.00	1.49
4.	PB-4-4	561.50	292.00	1.50
5.	PB-4-5	569.50	290.50	1.50
6.	PB-4-6	562.00	275.25	1.49
7.	PB-4-7	563.00	287.00	1.49
8.	PB-4-8	564.00	298.50	1.51
9.	PB-4-9	562.00	295.00	1.50
10.	PB-4-10	565.50	288.50	1.50
11.	PB-4-11	569.00	295.00	1.51
12.	PB-4-12	564.50	287.75	1.49
13.	PB-4-13	566.00	294.25	1.50
14.	PB-4-14	569.00	295.75	1.51
15.	PB-4-15	563.50	286.00	1.49
	Mean*	374.75	200.25	1.49

* Highest general mean

Plate 23. High yielding variant

Plate 24. Chlorophyll deficient mutant

fresh weight, dry weight and plumbagin content in roots were observed by PB-3-3 (406.50 and 219.50 g and 1.46 per cent respectively). Among the selected high yielders in 1.0 kR treated plants, the maximum fresh weight of roots was observed by PB-4-5 (569-50 g) followed by PB-4-11 and PB-4-14 (569.00 g).


DISCUSSION

DISCUSSION

Medicinal plants constitute an important component of the biodiversity of India. The forest ecosystem of the Western Ghats of Kerala forms one of the major diversity 'hot spot' of medicinal plants in India. *Plumbago rosea* L. (Syn. *Plumbago indica* L.) commonly known as Fire plant or rose leadwort (vernacular : Chethikoduveli) is one of the three most important commercially exploited medicinal plants of Kerala (Nambiar *et al.*, 1985). The roots of this plant possess immense medicinal properties and are used extensively in Ayurvedic medicines. The demand for this drug plant in Kerala, India and even abroad is ever increasing. There is an urgent need to increase the productivity of this medicinal plant to cope with the increased demand. In Kerala, the annual demand for *Plumbago* is 11,000 t. But only ten per cent of this is met by cultivation. The plumbagin content of the existing types of *Plumbago* spp. is only upto a maximum of 0.9 per cent on dry weight basis. The present study envisaged the development of high plumbagin yielding clones of this important medicinal plant species.

Improvement of crop plants largely depends on the extent of genetic variability available within the species. *Plumbago* is propagated only through vegetative means either by offsets or by semi-hardwood

cuttings. In such crops evaluation of variability existing among different ecotypes of the species become a primary work in the crop improvement. Hence, different ecotypes within Kerala state were collected and evaluated. The results were presented in the preceding chapter.

The flowering in *Plumbago rosea* is seasonal and it has never been known to produce viable seeds (CSIR, 1969). No reports are available on the floral biology of this crop. Hence studies on these aspects were also undertaken and results presented.

Various methods adopted to induce seed fertility through selfing and inter se crossing among the different ecotypes have failed to produce any seeds. Hence the induction of mutations through gamma irradiation of stem cuttings was attempted to induce genetic variability in this crop. A total of 1750 semi-hardwood cuttings taken from ecotype Et_5 were subjected to gamma radiation at different doses and M_1V_1 generation was raised in a 7 x 4 RBD field experiment. The results obtained from this trial were also presented in the preceding chapter. Now the results obtained from the above studies are discussed in the following sections of this chapter.

Experiment I. Germplasm collection and evaluation

Floral biology

Information on the floral biology of a plant material is essential to understand the mechanism of pollination and seed set. Several

parameters have been used for finding the sequence and time of flowering in different environmental conditions.

The mean number of days to flowering was significantly lower in *Plumbago zeylanica* when compared to all ecotypes of *P. rosea* examined for this study. Also the flowering occurred after every new branching in *P. zeylanica* whereas it was seasonal in *P. rosea* in which flowering takes place only during December to January. These results show that flowering in *P. rosea* requires short days and cool night temperatures. Similar results were also observed by Escher *et al.* (1988) in *P. indica* and Krizek and Semeniuk (1972) in *Limonium*.

The time of anthesis also differed significantly in the two species of *Plumbago*. The flower opening took place between 6.45 AM and 7.15 AM in all the ecotypes of *P. rosea* while it was well after 8.45 AM in *P. zeylanica*.

The observations on size, stainability and viability of pollen and style length showed marked difference among the ecotypes of *P. rosea* and *P. zeylanica*. The pollen size was bigger for *P. zeylanica* whereas it was smaller in all ecotypes of *P. rosea*. The stainability of the pollen grain in acetocarmine glycerine mixture was greater for *P. zeylanica* than in *P. rosea* ecotypes. The viability of pollen grains also differed significantly between the two species. Both the *in vitro* and *in vivo* studies revealed that there was no pollen germination in all the ecotypes

of *P. rosea* whereas in *P. zeylanica* the pollen grains germinated in all the germination media tried. The findings in the ecotype *P. zeylanica* were similar to the findings of Russell (1982) and Russell and Cass (1983) in *P. zeylanica*. The failure of pollen germination in all the ecotypes of *P. rosea* might be due to lack of pollen-derived substances. Namboodiri *et al.* have pointed out the phenomenon of passive rejection where deficiency of stigmatic or pollen wall constituents cause failure of pollen germination and seed-set (Namboodiri and Tara, 1972; Tara and Namboodiri, 1975 and Namboodiri and Sreedevi, 1976). Other explanations such as inhibition of pollen growth at stigmatic surface (Dulberger, 1975) and lack of pistil derived nutrients (Heslop-Harrison, 1987) were reported for failure of pollen tube growth in many members of Plumbaginaceae.

Compatibility studies

In the present investigation, to overcome the barriers in fruit set and seed set in *Plumbago rosea*, different pollination techniques were employed. Seed set could not be obtained through hand pollination, bud pollination, pollination with mixed pollen, chemically aided pollination and removal of stigma and artificial self pollination, in any of the ecotypes of *P. rosea*. Similar results were obtained by Kanakamany (1998) in *Kaempferia galanga*. In *P. zeylanica* seeds were obtained under natural conditions whereas when it was cross pollinated with pollen of *P. rosea* ecotypes no seeds were obtained.

Cytology

The results of cytological studies revealed that the 'n' number in *P. rosea* was six and that of *P. zeylanica* was 12. These results are in accordance with the earlier reports on these two species (CSIR, 1969).

Dormancy studies

The results of different methods to break the dormancy of seeds of *Plumbago zeylanica* revealed that seeds with persistent calyx did not germinate whereas when the calyx was removed and a small cut was made on the seed coat, the seeds germinated. The observation on days taken for germination revealed that it took 20 days for 50 per cent of seeds with intact seed coat to germinate while the mean number of days for 50 per cent of scarified seeds to germinate was only between three and four. This may probably because scarification broke the intact seed coat thereby allowing the embryo to germinate and help radicle to emerge out more quickly.

Biometric analysis

The mean number of days to start sprouting, to complete sprouting and the duration of sprouting were the lowest for Et_8 (*P. zeylanica*) while they were maximum for Et_1. But in sprouting percentage, Et_1 and Et_4 were found to be the best while Et_8 along with Et_2 remained the poorest. The mean number of leaves produced by the plant in the nursery was

maximum for Et_8 and minimum for Et_7. Significant variation in mean number of leaves was observed between all ecotypes. The increased number of leaves for *P. zeylanica* may be attributed to early sprouting of the cuttings in that species. The other observations recorded in the nursery such as internodal length, plant height and number of suckers have shown significantly higher values in Et_8 when compared to different ecotypes of *P. rosea*. The overall mean of these characters has also shown similar trend. These results indicate the superiority of *P. zeylanica* in early growth habits when compared to different ecotypes of *P. rosea*. Variability in number of leaves has been reported in varieties of ginger by several workers (Nybe, 1978; Mohanty *et al.*, 1981). Similar result was obtained in turmeric also by several workers (Philip, 1978; George, 1981; Mukhopadhyay *et al.*, 1986; Menon *et al.*, 1992).

The mean value of plant height, internodal length and number of suckers produced per plant in the field also showed the superiority of *P. zeylanica* over different ecotypes of *P. rosea*. Significant variability was noticed among the ecotypes for these characters. Similar results were obtained by Nybe (1978) and Mohanty *et al* (1981) in ginger and Philip (1978), George (1981) and Mukhopadhyay *et al.*, (1986) in turmeric.

The mean value on the fresh weight and dry weight of shoot was maximum for ecotype Et_8 followed by Et_2. The superiority of Et_8 in these characters may be due to greater plant height and more number of suckers produced by that species. In general, the superiority of the

species *P. zeylanica* (Et_8) over other ecotypes of *P. rosea* in growth habits during the entire period of their growth resulted in maximum mean value for the above characters.

The mean value on root length was also maximum for Et_8 (*P. zeylanica*) which was significantly superior to all other ecotypes of *P. rosea*. Significant variability for this character was noticed among the different ecotypes of *P. rosea*. Much variability in length of mother rhizome and secondary rhizome in ginger was reported by Nybe (1978). Similar results were obtained in turmeric by George (1981).

Wide range of variability was noticed in the number of roots per plant among the different ecotypes. The maximum number of roots per plant was recorded by Et_2 (22.47) and minimum by Et_7 (10.73). Mohanty *et al.*, (1981) reported variability in the number of rhizome fingers in ginger. Similar results were obtained in turmeric (George, 1981 and Indiresh *et al.*, 1990) also. But Nybe (1978) observed no significant variability in the number of primary fingers in ginger, but secondary rhizomes showed significant variability in that study.

In the present study there was significant difference in the girth of roots among the different ecotypes. Et_1 showed maximum value (3.50 cm) for this character while Et_5 recorded the minimum girth of roots. Similar results were obtained by Nybe (1978) in ginger and George (1981) in turmeric.

The fresh yield and dry yield of roots also showed significant variation among different ecotypes of *P. rosea* as well as *P. zeylanica*. The maximum mean value for fresh and dry yield of roots was recorded by Et_8 whereas minimum value was recorded by Et_7 (141.07 and 78.50 g). Similar variability in rhizome yield was reported by Mohanty *et al.*, (1981) in ginger and Mukhopadhyay *et al.*, (1986) in turmeric.

The plumbagin content in the roots showed significant variation among the two species. All the ecotypes of *P. rosea* recorded a higher percent of plumbagin in their roots whereas *P. zeylanica* was significantly inferior to all other ecotypes in this character.

Genetic parameters

In any crop improvement programme the collection and evaluation of available germplasm and a critical assessment of genetic variability are the basic pre-requirement for devising suitable breeding methods to improve the material. In the present study, the phenotypic and genotypic coefficients of variation were high for plant height and intermediate values for root length, number of roots per plant, plumbagin content, duration of sprouting and days taken to sprouting. Similar results were obtained by Nybe (1978) and Mohanty *et al.*, (1981) in ginger and Philip (1978), George (1981) and Mukhopadhyay *et al.*, (1986) in turmeric.

Heritability

High magnitude of heritability combined with genetic advance in respect of yield and yield attributing traits offer scope for identifying good ecotypes of *P. rosea* which will be amenable to selection based on phenotypic characters.

In the present study estimates of heritability (broad sense) ranged from 55.94 per cent (girth of root) to 99.90 percent (plant height). In general, high estimates of heritability were observed for all the characters. The genetic advance expressed as percentage ranged from 0.39 (plumbagin content in roots) to 137.90 (plant height).

High estimates of heritability (broad sense) coupled with high genetic advance were noticed for plant height, root yield and length of root which indicated considerable scope for genetic improvement with respect to these traits. Similar results were reported by Reddy (1980) and Kadian *et al.*, (1997) in sugarcane, Jalgaonkar and Jamdogni (1989), Nandi (1991) and Indiresh *et al.*, (1992) in turmeric.

Correlations and Path analysis

The association analysis based on the correlation coefficients of various components with yield may not give a true picture of the relative merits or demerits of each of the components to final yield. Since an individual component may have direct influence in the improvement of

yield or may have influenced through other components or both, an assessment of the merit of each character by analysing direct and indirect effect of each character towards yield is a valuable information.

This type of correlation and path analysis was effectively used to assess the influence of yield components in vegetatively propagated crops like turmeric (Natarajan, 1975); rose (Irulappan, 1979); cassava (Thamburaj *et al.*, 1985) and tube rose (Sambandamurthi, 1983).

In the present study the correlation coefficients between dry root yield and other characters showed significant positive correlation for almost all characters. The fresh weight of root followed by root length and number of roots per plant had the highest phenotypic and genotypic correlation with dry root yield. Similar results were obtained by Nybe (1978); Mohanty and Sharma (1979); Pandey and Donbhal (1993) and Zachariah *et al.*, (1993) in ginger, Mukhopadhyay and Roy (1986) in turmeric, Das *et al.*, (1996); Sukhchain *et al.*, (1997) in sugarcane, Sarkar *et al.*, (1996) in *Colocasia esculenta* L. and Kanakamany (1998) in Kacholam.

When the correlation between pairs of characters other than dry root yield was worked out, length of root and fresh yield of root showed positive and significant correlation with almost all characters studied. This is in agreement with the findings of Nambiar (1979) in turmeric.

The root length was significantly and positively correlated with plant height, internodal length and number of suckers produced per plant. The girth of root was positively and significantly correlated with root length whereas it was negatively correlated with number of suckers per plant. The plumbagin content in roots was negatively correlated with fresh weight of root whereas it was positively and significantly correlated with number of roots.

Path co-efficient analysis

Path coefficient analysis in the present study indicated that dry yield of roots was a single important morphological character for which selection for highest content of plumbagin, the medicinally important active principle could be made. The direct effects of dry root yield was positive whereas the fresh root yield had a high negative direct effect on plumbagin content. The plant height and number of roots had the highest positive direct effect on fresh yield of roots. Plant height and fresh yield of root had the highest direct effect on dry yield of roots. These results are in agreement with the findings of Presannakumari *et al.*, (1994) and Kanakamany (1998) in *Kaempferia galanga*; Pillai *et al.*, (1995) in *Colocasia esculenta*; Das *et al* (1997) and Sukhchain *et al.*, (1997) in sugarcane.

Selection Index

The highest selection index score was observed in Et_8 followed by Et_2. *Plumbago zeylanica* was found significantly superior in all

characters except plumbagin content of roots when compared to all ecotypes of *P. rosea*. The Et_2 ecotype of *P. rosea* was from Kottayam which ranked first among the *P. rosea* ecotypes with 1.2 per cent plumbagin in its roots. CSIR (1969) has reported only 0.91 per cent of plumbagin content in the samples of *P. rosea* roots. Hence the present study indicates the possibility of selecting clones of *P. rosea* with higher plumbagin content.

Experiment II

Induction of variability

The crop improvement programme of vegetatively propagated crops face many limitations when they are unable to produce any seeds through sexual means. In the conventional sense, creation of genetic variability in such obligatory asexual crops is a difficult proposition which can be overcome only through mutational approaches (excluding of course, the recent developments in biotechnology). The advantages of breeding through induced mutagenesis is the feasibility to change one or a few characters of an otherwise superior genotype without affecting its desirable characters. In this connection, this method can be considered as supplementary to the conventional breeding methods.

Since no literature is available on the effects of induced mutation in *Plumbago* spp., the author is constrained to limit the discussion on

this chapter with literature citations on genera other than *Plumbago*. 'Chethikoduveli' was reported to produce no seeds which was confirmed in this study also. Existing variability among the different ecotypes of *P. rosea* tested in the present study was found to be quite significant. Also there was significant difference between the two species viz. *P. rosea* and *P. zeylanica* tested, for both vegetative as well as floral characters. But unfortunately, *P. rosea* was found both self and cross incompatible in producing fertile seeds. A mutational approach to induce variability was attempted in one of the ecotypes of *P. rosea* namely Et_5 collected from the genetic stock maintained at the College of Horticulture, Vellanikkara, Thrissur.

During the last two to three decades, several new varieties have been developed by induced mutations in vegetatively propagated crops. Several mutants with desirable attributes like high yield, high content of sugar, protein and oil, disease and insect resistance, stress tolerance etc. have been developed in many vegetatively propagated plants like cassava, sweet potato, sugarcane, costus, turmeric, rose, gladioli etc. Vasudevan *et al.*, (1967), Abraham (1970), Nayar (1975) and Moh (1976) obtained desirable mutants in cassava; Kukimura and Takemata (1975) and Sumabai and Nayar (1995) in sweet potato; Gupta *et al.*, (1982) in costus; Reghupathy *et al.*, (1976), Shah *et al.*, (1982) and Rangaswamy (1986) in turmeric; Chan (1966), Gupta and Shukla (1971) and Lata and Gupta, (1971) in rose and Buiatti *et al.*, (1965) in gladioli.

The present investigations were undertaken to create genetic variability in the ecotype Et_5 in *Plumbago rosea* using gamma rays. As a result variability could be induced in characters such as days taken for sprouting, sprouting percentage, duration of sprouting, lethality, yield and yield attributes. The results obtained are discussed in the following sections.

Sensitivity studies

Information on the sensitivity of plant material to the mutagen is essential to find out the optimum dose of mutagen. Many parameters have been used for determining the sensitivity of the crop plants to different mutagens. Sambandamurthi (1983) in tuberose and Jayachandran and Mohanakumaran (1992) in ginger considered sprouting and survival as the parameters useful for assessing the sensitivity to gamma rays. Considering these findings, in the present study the efficiency of the mutagen was studied on the basis of sprouting of the cuttings of *Plumbago rosea* under nursery conditions.

Mutagen treatment of cuttings resulted in decreased sprouting with increase in dosage. Similar results were also observed by Roer (1967) in potato and Jalaja (1971) in sugarcane. The probit analysis on the survival of cuttings revealed that the ED_{50} for *P. rosea* was around 1.685 kR gamma rays. Sambandamurthi (1983) observed 2.5 kR gamma rays as the optimum dose for sprouting in tuberose. The LD_{50} for the percentage of sprouting of cassava sets was between 1.5 and 2.0 kR gamma rays

(Thamburaj *et al.*, 1985). According to Giridharan (1984) the LD_{50} for ginger cultivar Rio-de-janeiro was found to be between 1.0 and 1.5 kR while Jayachandran and Mohanakumaran (1992) observed the LD_{50} to be between 0.5 and 1.0 kR in ginger. These results indicate that the gamma ray sensitivity vary very much according to the species tried.

Effect of mutagen on M_1V_1 generation

The induction of mutations by a mutagen is invariably associated with the production of undesirable changes in the biological material. These were reported to be the direct result of either chromosome aberrations or the indirect toxicity of the mutagen used. These changes can be quantified as M_1 damages such as lethality, injury and sterility. For a particular mutagenic treatment there is a specific correlation between M_1 damages and M_2 mutation frequency. Therefore a quantitative determination of M_1 damage should be a routine procedure in mutation breeding experiments. Damage to plants in the M_1 generation resulting from the biological effects induced by mutagens could be measured by several criteria such as reduction in sprouting and survival, reduction in plant growth, reduction in fertility, increase in the frequency of chromosome aberrations and increase in the frequency of chlorophyll deficient chimeras (Gaul, 1970).

In the present study as revealed by results presented in Table 24, there was a progressive reduction in the mean values for percentage

sprouting with increase in the radiation dose. This result is in agreement with the findings of Roer (1967) in potato, Mukherjee and Khoshoo (1970) in canna, Jalaja (1971) in sugarcane, Gupta *et al.*, (1982) in costus and Giridharan (1984) in ginger. The irradiation treatments stimulated the sprouting except in the highest dose when compared to control. Jasina and Kirsanova (1966) reported that in potato the lower doses stimulated sprouting. Viswanathan *et al.*, (1992) reported that the irradiation treatments at lower dosages viz., 0.5, 0.75 and 1.0 kR produced stimulatory effect on the germination and period taken for germination in *Kaempferia galanga* L.

The lower sprouting rate due to mutagenic treatment with increasing exposures might be attributed to an inactivation of auxin levels in the plant as reported by Skoog (1935). Failure of assimilatory mechanism, production of diffusible growth retarding substances (Mackey, 1951), inhibition of auxin synthesis (Gordon, 1954) and changes in specific activity of the enzymes (Haskins and Chapman, 1956) can also reduce sprouting at higher doses.

In the present study sprouting was very much delayed at higher doses (Table 24). This result is in agreement with the findings by Vijayalakshmi and Rao (1960) and Jalaja (1971) in sugarcane, Vasudevan *et al.*, (1967) in colocasia and Gupta and Shukla (1971) in rose. The delay in sprouting at higher doses might be due to the influence of gamma rays on plant hormones and plant growth regulators in higher plants. In

the present study duration of sprouting was significantly higher in the lower doses of mutagen as compared to control and was significantly lower in the higher doses of gamma rays (Table 24). Similar results were obtained in sweet potato which took more number of days to complete sprouting when treated with gamma rays (Sumabai, 1989). Contrary to these, Kacholam took less number of days to complete sprouting in lower doses of gamma rays (Kanakamany, 1998).

In the present investigation the percentage lethality was more in mutagen treated population compared to control. The increase in percentage lethality was more at higher doses of mutagen. As reported by Skoog (1935) the lower survival rate at higher doses of mutagen might be due to the inactivation of auxin level in the plant with increasing exposures. Sparrow (1961) observed chromosomal aberrations which affected adversely the cell division causing reduction in the survival rate. According to Sato and Gaul (1967) the reduction in survival might be due to physiological disturbances. Mikaelson (1968) noticed that the inhibition of DNA synthesis was responsible for reduction in survival at higher doses of mutagen.

In the present study, leaf production in the nursery was found to be affected by mutagen treatment. An increase in leaf production was observed at lower doses, while higher doses resulted in a reduction in leaf production. Similar trend was observed by Irulappan (1979)in rose, and Thamburaj *et al.*, (1985) in cassava where the lower doses of gamma

rays increased the number of leaves whereas higher doses decreased the leaf production. Gupta *et al.* (1974 and 1982) in tuberose and costus; Sambandamurthi (1983) in tuberose and Giridharan (1984) in ginger observed reduction in leaf production as a result of radiation treatment.

The reduction in number of leaves as a result of high doses of mutagen treatments might be due to a reduction in general growth rate. Other explanations such as auxin destruction (Skoog, 1935), inhibition of auxin synthesis (Gordon, 1954), failure of assimilatory mechanism, production of diffusible growth retarding substance (Mackay, 1951), changes in the specific enzymes (Haskins and Chapman, 1956; Cherry and Leasman, 1967; Endo, 1967), delay in the onset of first mitosis (Natarajan, 1958) and inhibition of DNA synthesis (Mikaelson, 1968) may also have contributed to the reduced growth at various stages following mutagenic treatment.

The observations on the mean height of the plant and internodal length in the present study have revealed that there was an increase in plant height and internodal length in the treated population, as compared to control . At the intermediate doses of gamma rays the height of the plants was higher which made these plants photosynthetically more efficient. The greater plant height at the intermediate doses of gamma rays was responsible for higher root yield as evidenced from the study (Table 29).

The mutagenic treatments have caused the shift in the number of suckers per plant from that of control in both positive and negative directions. At the lowest dose of gamma rays there was a decrease in number of suckers per plant and at intermediate doses the number of suckers per plant was increased. At higher doses the number of suckers was found significantly reduced. Contrary to this, the studies of Natarajan (1975) in turmeric and Sambandamurthi (1983) in tuberose indicated an inversely proportional relationship between sucker production and radiation dose.

In the present study, positive shifts in the fresh weight and dry weight of shoot were observed due to gamma rays at all concentrations, as compared to control. Similar trend was noticed in sugarcane by Das *et al.*, (1996) and in *Colocasia esculenta* by Kundu and Gupta (1997) and Sarkar *et al.*, (1996).

In the present investigation, the observations on the length and girth of roots showed that, at lowest and highest dose of gamma rays, the rate of growth was reduced by the mutagen, as compared to control. Positive shifts in the number, length and girth of roots were observed due to gamma rays at the intermediate doses. A similar trend was noticed with regard to mean fresh weight and dry weight of roots also.

In ginger, Giridharan (1984) reported reduction in yield at higher doses of gamma rays, while reduction in yield at all doses of gamma rays

was observed by Jayachandran and Mohanakumaran (1992). In *Costus speciosus* irradiation of rhizome with gamma rays resulted in decreased yield (Gupta *et al.*, 1982). These varying results by different workers may be due to arbitrary fixation of treatment doses with out any prior sensitivity studies.

Abraham (1970) and Nayar (1975) obtained mutants for high yield in cassava and Kukimura and Kouyama (1982) in sweet potato, through gamma irradiation which lend support to the present findings. Similar increase in yield was reported by Natarajan (1975) in turmeric who obtained high yielding mutants using gamma rays.

In the present study, significant variation in the content of plumbagin in roots was noticed. The mean plumbagin content of roots was significantly higher in all the doses of gamma ray treatments except at the highest dose as compared to untreated population. The highest dose of mutagen resulted in negative shift in plumbagin content. CSIR (1969) reported 0.91 as the maximum percentage of plumbagin content found in various samples of *P. indica* roots. Mutants with alterations in starch content were observed by Vasudevan *et al.*, (1967) in cassava. Kaul and Kak (1973) isolated mutant clones of *Mentha* spp. having increased menthol content. In cassava, mutants with lower HCN was obtained due to gamma irradiation by Moh (1976). Kukimura and Kouyama (1982) obtained mutants with differences in starch content in sweet potato.

The present studies revealed that the highest values for root yield and yield attributing characters were obtained for the treatment with 1.0 kR gamma rays. The treatment with 1.50 kR gamma rays resulted in the lowest yield. At the lowest and highest doses of gamma rays, there were negative shifts in all the yield attributing characters whereas with the intermediary doses of gamma rays positive shifts were observed.

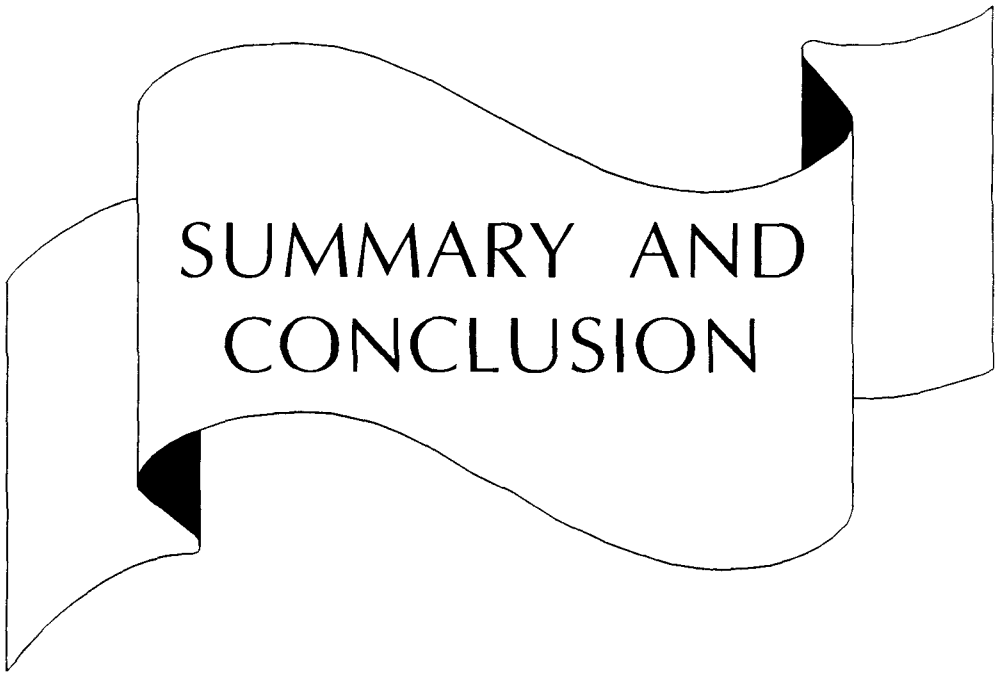
Identification of macromutants

The total screening of the individual plants of the M_1V_1 population resulted in the identification of several mutant variants showing significant positive deviation from the mean values of vegetative and root characters.

Dwarf mutants and tall mutants could be observed in the gamma ray treated population. Such morphological mutants were observed by Jagathesan (1977) in sugarcane and Vasudevan *et al.*, (1967) in cassava. A chlorophyll deficient mutant was also observed in the M_1V_1 population raised in the present study. Similar result was obtained by Vasudevan *et al.*, (1967) in tapioca, Buiatti *et al.*, (1965) in carnation, Gupta *et al.*, (1974) in tuberose and Giridharan and Balakrishnan (1992) in ginger.

Based on the mean values of samples taken, the highest mean fresh weight of roots was observed in 1.00 kR followed by 0.75 kR. Then, every individual plants of those plots was screened for fresh root

weight and all those plants which gave 50 per cent or more fresh root yield over this mean were selected for carrying over to M_1V_2 generation. A total of 24 such high yielders were identified and their dry weight and plumbagin yield were also found out for further evaluation. Raising M_1V_2 progeny lines from each of these plants and evaluation of the lines in comparison with the control plants are suggested as future line of work.


SUMMARY AND
CONCLUSION

SUMMARY

'Chethikoduveli' (*Plumbago rosea* L.) is one of the most important commercially exploited medicinal plant of Kerala. Evaluation of genetic variability existing among different ecotypes grown in Kerala was carried out with seven ecotypes of *P. rosea* and one related species, *Plumbago zeylanica*. Various studies including floral biology, cytology, compatibility and estimation of genetic parameters like heritability, genetic advance, correlation studies, path coefficient analysis and selection index were conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 1995-1998. Induction of genetic variability in *P. rosea* to isolate desirable mutants of economic importance was attempted through induced mutagenesis with gamma rays. The gamma irradiation was done in the Co^{60} gamma chamber, available in the Radiotracer Laboratory attached to the College of Horticulture, Vellanikkara. The performance of the treated plants were evaluated for one generation and the mean performance was assessed based on yield and yield contributing characters. The salient results are presented below :

1. The anthesis in all the ecotypes of *P. rosea* was earlier when compared to the ecotype of *P. zeylanica*.

2. Pollen grains of all ecotypes of *P. rosea* did not germinate under *in vitro* and *in vivo* studies whereas the pollen grains of *P. zeylanica* germinated.

3. Abundant adhesion of pollen grains on the papillate stigma was noticed in *P. zeylanica* whereas pollen grains of all ecotypes of *P. rosea* did not adhere to its stigmatic surface.

4. The scarified seeds of *P. zeylanica* germinated more quickly than seeds with intact seed coat.

5. All the pollination techniques failed to give any positive result on seed set in *P. rosea*.

6. Normal pairing of chromosomes were observed in *P. rosea* and *P. zeylanica*.

7. The analysis on characters like internodal length, plant height, number of suckers produced per plant, fresh weight of shoot, root length and fresh weight of root showed that these were highest for the ecotype of *P. zeylanica*.

8. Plumbagin content was significantly low in *P. zeylanica* when compared to all other ecotypes of *P. rosea*.

9. High estimates of heritability (broad sense) was observed for all the characters studied, the highest being exhibited by plant height (99.90 %).

10. Maximum genetic gain (137.90 %) was observed for plant height while plumbagin content in roots expressed the minimum (0.39 %).

11. Correlation coefficient between dry root yield and its components indicated significant positive association of yield with plant height, number of leaves, internodal length, root length, number of roots, fresh weight of shoot and fresh weight of roots.

12. Path coefficient analysis of important yield attributes indicated that the dry root yield had the maximum direct effect on plumbagin content of roots whereas plant height showed the maximum direct effect on fresh root yield and dry root yield.

13. The selection index score was highest for *P. zeylanica* followed by the ecotype from Kottayam.

14. The exposure of cuttings above 1.685 kR gamma rays reduced the sprouting to 50 per cent and ED_{50} was calculated as 1.685 kR gamma rays.

15. The sprouting percentage of cuttings was significantly decreased with increasing dose of mutagen.

16. Progressive delay in sprouting was noticed as the level of dose increased.

17. Duration of sprouting was found to be reduced with increase in dose of mutagen.

18. The percentage lethality was higher in mutagen treated population than the control.

19. The mean number of days to flowering was reduced as the doses of mutagen increased.

20. Positive shifts in the mean number of suckers, number of roots, root length, root girth, fresh weight of shoot and fresh weight of roots were observed in the immediate doses of mutagen.

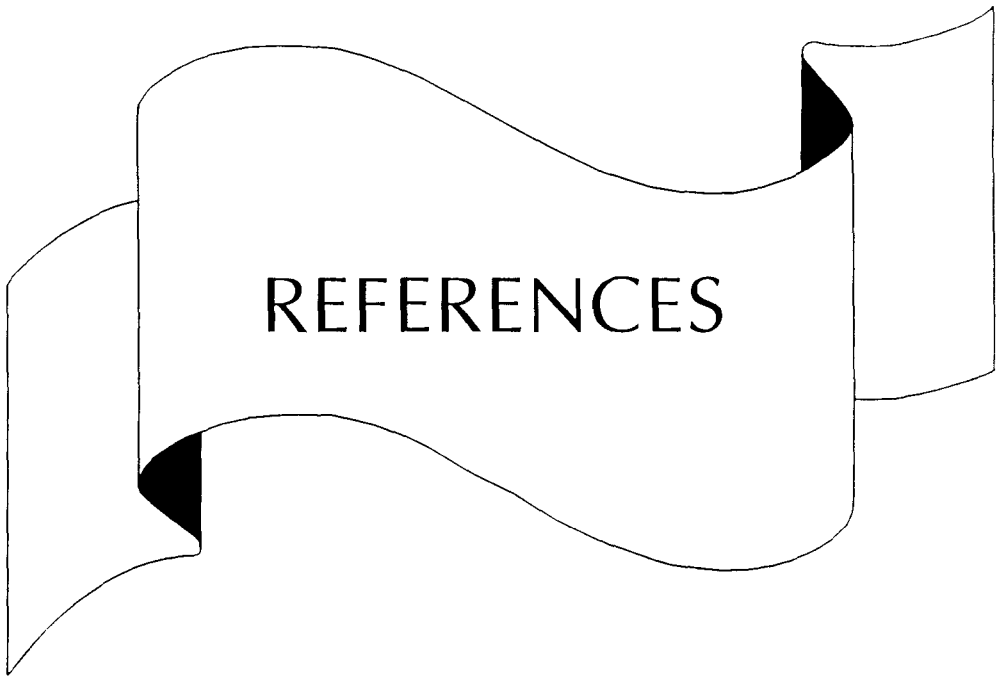
21. A reduction in the mean number of roots, root length, root girth, fresh root yield and plumbagin content was observed at highest dose of mutagen.

22. The optimum dose for maximum yield and yield contributing characters ranged between 0.70 kR and 0.85 kR of mutagen.

23. Morphological mutants were observed in all doses except the highest dose of mutagen.

24. High yielding mutants were observed in plants treated with 0.75 and 1.0 kR of mutagen.

25. Twentyfour high yielding mutants were selected for further evaluation.


REFERENCES

REFERENCES

Abraham, A. 1970. Breeding work on tapioca (cassava) and few other tropical tuber crops. *Tropical Root and Tuber Crops Tomorrow.* **1** : Univ. Hawaii, p. 76-78

Abraham, V. and Desai, B.M. 1976. Biological effectiveness of fast neutrons and gamma rays in some bulbous ornamentals. *Indian J. Genet. Plant Breed.,* **36**(2) : 230-237

Ali, S.A., Mishra, A.K., Mourya, K.N., Kumar, R.K.S. and Tiwari, R.C. 1994. Genetic variability in ginger (*Zingiber officinale* Rose). *Internat. J. Trop. Agric.,* **12** : 282-283

Bahadur, B. 1978. How many tristylous taxa ? *Incompatibility Newslett.* p : 94-104

Baker, H.G. 1966. The evolution, functioning and breakdown of heteromorphic incompatibility system. I. The plumbaginaceae. *Evolution.,* **20** : 349-368

Bentham, G. and Hooker, J.D. 1884. *Genera Plantarum* Vol. III. Reeve and Co., Loudini. p. 627-628

Bharathi, A., Sarada, R.L., Madhari, B. and Polassa, H. 1994. Effect of plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) on plasmid curing of fast growing *Rhizobium* spp. *Indian J. Microbiol.,* **34**(2) : 131-135

Bhargava, S.K. 1984. Effects of plumbagin on reproductive function of male dog. *Indian J. Exptl. Biol.*, **22**(3) : 153-156

Bindroo, B.B. 1988. Association of tuber flesh colour with diosgenin content and other tuber characteristics in *Dioscorea deltoidea* wall. *Research and Development Reporter.* **5**(1-2) : 39-43

Bindroo, B.B. and Bhat, B.K. 1988. Natural variation, heritability and genotypc x year interaction in *Dioscorea deltoidea. Herba Hungarica.*, **27**(1) : 19-26

Broertjes, C. 1968. Dose-rate effects in Saintpaulia. *Mutations in Plant Breeding*, **2** : IAEA, Vienna, p. 63-71

Broertjes, C. 1976. Mutation breeding of auto-tetraploid Achimenes cultivars. *Euphytica*, **25** : 297-304

Broertjes, C. and Ballego, J.M. 1967. Mutation breeding of *Dahlia variabilis. Euphytica*, **16** : 171-176

Broertjes, C. and Van Harten, A.M. 1978. *Application of mutation breeding methods in the improvement of vegetatively propagated crops.* Elsevier Scientific Publishing Co. New York. p. 16

Buiatti, M., Ragazzini, R. and Tognoni, F. 1965. Effects of gamma irradiation on *Gladiolus. Radiat. Bot.*, **5** : 97-98

*Buiatti, M. and Tesi, R. 1968. Gladiolus improvement through radiation induced somatic mutation. Laboratorio di Mutagenesi e differenziamento del C.N.R., Pisa, p. 1-6

Chan, A.P. 1966. Chrysanthemum and rose mutations induced by X-rays. *Proc. Amer. Soc. Hort. Sci.,* **88** : 613-620

Cherry, J.H. and Leasman, K.J. 1967. Comparison of nucleic acids in maize shoots and epicotyl. *Amer. J. Bot.,* **54** : 181-188

Chockalingam, S., Thenmozhi, S., Nalinasundari, M.S. and Sundari, M.S.N. 1990. Larvicidal activity of different products againts mosquito larvae. *J. Envtl. Biol.* 11(2) : 101-104

CSIR. 1969. *The Wealth of India - Raw Materials III.* Publications and Information Directorate, CSIR, New Delhi, p. 162-163

D'Amato, F., Moschini, E. and Pacini, L. 1964. Somatic mutations in carnations induced by gamma rays. *Caryologis,* **17**(1) : 93-101

Dabholkar, A.R. 1992. *Elements of Biometrical Genetics.* Concept publishing company, New Delhi

Darlington, C.D. and Wylic, A.P. 1961. *Chromosome Atlas of Flowering Plants.* George Allen and Unwin Ltd. p. 281

Das, P.K., Dube, S., Ghosh, P. and Dhua, S.P. 1975. Mutation breeding in Dahlia. *Indian J. Ornamental Hort.,* **62**(2) : 3-8

Das, P.K., Jena, B.C. and Nayak, N. 1996. Genetic variability in biometrical traits of early maturing genotypes of sugarcane. *Indian Sugar.,* **46**(9) : 723-726

Das, P.K., Jena, B.C. and Nayak, N. 1997. Character association and path analysis of sugar yield in sugarcane. *Indian Sugar,* **46**(10) : 805-808

Desai, B.M. 1973. Report on mutation experiments on ornamentals at Bhabha Atomic Research Centre, Trombay *2nd workshop on Floriculture,* Agri. Hort. Society, Calcutta (1) : 29-31

Desai, B.M. and Abraham, V. 1974. Radiation induced mutants in canna. *Proceedings of Symposium on the use of radiations and radioisotopes in studies of plant productivity*, Pant Nagar p. 214-217

Devi, P.U., Solomon, F.E. and Sharada, A.C. 1994. *In vivo* tumor inhibitory and radiosensitizing effects of an Indian medicinal plant, *Plumbago rosea* on experimental mouse tumors. *Indian J. Exptl. Biol.,* **32** : 523-528

Dewey, D.R. and Lu, K.H. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J.,* **51** : 515-518

Dhar, S.K. and Rao, P.G. 1995. Hormonal profile of plumbagin. *Fitoterapia.,* **66**(5) : 442-446

*Donini, B. 1976. Breeding methods and applied mutagenesis in fruit plants. *Proc. Workshop Eur. Comm. Israel,* Assoc. Euratom - ITAL, Wagenmgen, p. 445-478

Dulberger, R. 1975. Intermorph structural differences between stigmatic papillae and pollen grains in relation to incompatibility in plumbaginaceae. *Proceedings of Royal Society of London.* **188**(1092) : 257-274

Endo, T. 1967. Comparison of the effects of gamma rays and maleic hydrazide on enzyme systems of maize seeds. *Radiat. Bot.,* 7 : 35-40

Engler, A. 1973. *A dictionary of the flowering plants and ferns.* J.C. Willis, Cambridge University Press, United Kingdom, p. 922

*Escher, F., Rehrmann, P. and Rothert, D. 1988. *Plumbago indica.* Widening the choice of pot plants. *Gartnerborse-und-Gartenwelt.* **88**(18) : 777 (in German)

Escober, J.T. and Lopez, M.E. 1970. A preliminary study on the gamma irradiation of sugarcane seed pieces. *Int. Sugar J.* 72-240

Evans, H.J. 1962. Chromosome aberrations induced by ionizing radiations. *Int. Rev. Cytol.,* **13** : 221-222

Fetterer, R.H. and Fleming, M.W. 1991. Effects of plumbagin on development of the parasitic nematodes *Haemonchus contortus* and *Ascaris summ. Comp. Biochem. Physiol. Comp. Pharm. Toxic.* **100**(3) : 539-542

Gaul, H. 1970. Mutation effects observable in the first generation. *Manual Mutat. Breed.,* IAEA, Vienna, p. 85-95

Geetha, U. 1985. A comparison of path coefficient analysis and principal component analysis in turmeric. M.Sc. (Ag. Stat.) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala.

George, H. 1981. Variability in the open pollinated progenies of turmeric. M.Sc. (Ag.) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala

Giridharan, M.P. 1984. Effect of Gamma Irradiation in Ginger (*Zingiber officinale* Rosc.) M.Sc. (Ag.) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala

Giridharan, M.P. and Balakrishnan, S. 1992. Gamma ray induced variability in vegetative and floral characters of Ginger. *Indian Cocoa Arecanut and Spices J.*, **15**(3) : 122

Gordon, S.A. 1954. Occurrence, formation and inactivation of auxins. *Ann. Rev. Pl. Physiol.*, **5** : 341-378

Gujar, G.T. 1990. Plumbagin, a naturally occurring naphthoquinone. Its pharmacological and pesticidal activity. *Fitoterapia,* **61**(5) : 387-394

Gujar, G.T. and Mehrotra, K.H. 1988. Toxicity and morphogenetic effects of plumbagin on *Dysdercus koenigii* F. (Het., Pyrrhocoridae). *J. Appl. Ent.*, **105**(5) : 466-470

Gujar, G.T., Rao, R.V.S. and Sankaram, A.V.B. 1994. Bioactivity of naturally occurring plumbaginoids against insects. *Phytophaga,* **6**(2) : 117-119

Gupta, M.N. 1966. Induction of somatic mutations in some ornamental plants. *Proc. All India Symposium in Horticulture.* The Royal Agri-Horticultural Society of India, Calcutta, 107-114

Gupta, M.N. and Shukla, R. 1970. Mutation breeding of garden roses : studies on induction of somatic mutations after gamma irradiation of budwood. *Progve. Hort.*, **2**(3) : 69-83

Gupta, M.N. and Shukla, R. 1971. Mutation breeding of garden roses. *J. Pl. Breed.*, **21**(3) : 129-136

Gupta, M.N., Sumiran, R. and Shukla, R. 1974. Mutation breeding of tuberose (*Polianthes tuberosa* L.) Symp. *Use of Radiations and Radio-isotopes in studies of plant productivity*, Pant Nagar, p. 169-179

Gupta, M.N., Laxmi, V., Dixit, V.S. and Srivastava, S.N. 1982. Gamma ray induced variability in *Costus speciosus*. *Progve. Hort.*, **14**(4) : 193-197

*Haskins, F.A. and H.W. Chapman. 1956. Effects of irradiation, maleic hybrazide, temperature and age on enzyme activity in seedlings of corn (*Zeamays* L.), *Physiologia Pl.*, **9** : 355-362

*Heiken, A. 1958. Aberrant types in potato. *Acta Agr. Scand.*, **8** : 319-358

Heiken, A. 1961. Induction of somatic changes in *Solanum tuberosum* by acute gamma irradiation. *Hereditas*, **47** : 606-614

Hernandez, T.P., Hernandez, T. and Miller, J.C. 1964. Frequency of somatic mutations in several sweet potato varieties. *Proc. Amer. Soc. Hort. Sci.,* **85** : 430-433

Heslop - Harrison, J. 1987. Pollen germination and pollen tube growth. *Int. Rev. Cytol.,* **107** : 1-78

Hrishi, N., Krishnamurthy, T.N. and Marimuthammal, S. 1968. i. Studies in mutagenesis in sugarcane. ii. Induction of visible and micromutations in sugarcane by chemical mutagens. *Proc. Indian Acad. Sci. Sect., B.* **68** : 181-189

Hutchinson, J. 1973. *The families of flowering plants.* Oxford University Press, United Kingdom, p. 560-561

Indiresh, K.M., Uthaiah, B.C., Herle, P.S. and Rao, K.B. 1990. Morphological rhizome and yield characters of different turmeric varieties in coastal Karnataka. *Mysore J. Agric. Sci.,* **24** : 484-490.

Indiresh, K.M., Uthaiah, B.C., Reddy, J.M. and Rao, B. 1992. Genetic variability and heritability studies in turmeric *Curcuma longa* L. *Indian Cocoa Arecanut and Spices J.,* **16**(2) : 52-54

Irulappan, I. 1979. Studies on induced mutations in Edward Rose (*Rose baourboniana Desp.*), Ph.D. Thesis submitted to Tamil Nadu Agricultural University, Coimbatore, India.

Jagathesan, D. 1977. Induction and isolation of mutants in sugarcane. *Mutat. Breed. Newsl.,* **9**(1) : 5-6

Jalaja, N.C. 1971. Studies on the radiosensitivity of *Saccharum* species. Thesis submitted in partial fulfilment of the requirements of Ph.D degree of the University of Madras

Jalgaonkar, R. and Jamdogni, B.M. 1989. Evaluation of turmeric genotypes for yield and yield determining characters. *Ann. Plant Physiol.*, **3**(2) : 222-228

Jalgaonkar, R., Jamdogni, B.M. and Salvi, M.J. 1990. Genetic variability and correlation studies in turmeric. *Indian Cocoa Arecanut and Spices J.*, **14**(1) : 20-22

Jasina, I.M. and Kirsanova, E.V. 1966. Production of hereditary changes in potato by means of gamma rays. *Genetics,* **1** : 53-58

Jauhar, P.P. 1969. Morphological and physiological effects of radiations and radioisotopes on potato, *Solanum tuberosum* L. *Indian J agric. Sci.,* **39** : 88-100

Jauhar, P.P. and Swaminathan, M.S. 1967. Mutational rectification of specific defects in some potato variety. *Curr. Sci.*, **36** : 340-342

Jayachandran, B.K. and Mohanakumaran, K. 1992. Effect of gamma ray irradiation on ginger. *South Indian Hort.*, **40**(5) : 283-288

Johnson, H.W., Robinson, H.F. and Constock, R.E. 1955. Genotypic and phenotypic correlations in soybean and their implications in selection. *Agron. J.*, **47** : 477-482

Joshi, N.K., Lathika, R.M., Banerji, A. and Chadha, M.S. 1988. Effects of plumbagin on growth and development of red cotton bug, *Dysdercus cingulatus* Fab. *Proc. Indian Nat. Sci. Acad. Biol. Sci.*, **54**(1) : 43-46

Kadian, S.P., Chanderkishore and Sabharwal, P.S. 1997. Genetic variability and heritability in sugarcane. *Indian Sugar.*, **46**(12) : 973-975

Kaicher, U.S. and Swarup, V. 1972. Induced mutation in roses. *Indian J. Genet.*, **32** : 257-265

Kak, S.N. and Kaul, B.L. 1979. Induced mutations in the improvement of *Mentha species*. In : *The Role of Induced Mutations in Crop Improvement*. Proc. Symp. Dept. of Atomic Energy, Govt. of India, p. 349-354

Kanakamany, M.T. 1998. Induction of genetic variability in kacholam (*Kaempferia galanga* L.) Ph.D. Thesis submitted to Kerala Agricultural University, Vellanikkara, Thrissur, Kerala.

Kaul, B.L. and Kak, S.N. 1973. Improvement of *Mentha arvensis* L. through induced mutations. In : *Advancing Frontiers in Cytogenetics*. Hindustan Publishing Corporation (India), Delhi. p. 189-195

Kishore, H., Pushkarnath and Singh, G. 1963. The effects of gamma irradiation on potato tubers. *Indian Potato J.*, **5** : 86-92

Konzak, C.F. 1966. Qualitative mutations and plant breeding. *Int. Rice Commn. Newsl.*, **15**(1) : 20-21

Krishnaswamy, M. and Purushothaman, K.K. 1980. Plumbagin : a study of its anticancer, antibacterial and antifungal properties. *Indian J. Exptl. Biol.,* **18**(8) : 876-877

Krishnayya, P.V. and Rao, P.J. 1995. Effect of plumbagin on chitin, cuticular proteins, median neurosecretory cells and corpora allata of *Helicoverpa armigera* Hubner larvae. *Proc. Indian Nat. Sci. Acad. Biol. Sci.,* **61**(2) : 127-135

Krizek, D.T. and Semeniuk, P. 1972. Influencing of day / night temperature under controlled environments on the growth and flowering of Limonium 'Midnight Blue'. *J. Amer. Soc. Hort. Sci.,* **97**(5) : 597-599

Kukimura, H. and Kouyama, Y. 1982. Studies on mutation breeding in sweet potato (*Ipomoea babatas* (L) Lam). In : *Induced Mutations in Vegetatively Propagated Plants,* Coimbatore, India (2) : 11-15

Kukimura, N. and Takemata, T. 1975. Induced quantitative variation of gamma rays and ethylene imine in tuber bearing plants. *Gamma Field Symp.,* **14** : 25-38

Kumar, P.R. and Das, K. 1977. Induced quantitative variation in self incompatible forms in Brassica. *Indian J. Genet.,* **37** : 5-11

Kumar, R., Singh, V.N. and Jain, B.P. 1995. Genetic variability studies in colocasia (*Colocasia esculenta* (L) Schott) *J. Root Crops,* **21**(2) : 116-118

Kundu, S. and Gupta, M.C. 1997. Variability and character correlation studies in *Saccharum officinarum* clones. *Indian Sugar,* **46**(11) : 889-893

Lal, S.D., Shah, A. and Phogat, K.P.S. 1986. Path analysis of productivity in turmeric. *Progve. Hort.,* **18**(1-2) : 101-103

Lata, P. and Gupta, M.N. 1971. Effects of gamma rays on stem cuttings of essential oil bearing Rosa spp. *Flavour Ind.,* **2**(7) : 421-425

Lush, J.L. 1937. *Animal Breeding Plans.* Iowa State College Press. Ames. Iowa. p. 473

Mackay, J. 1951. Neutron and X-ray experiments in barley. *Hereditas,* **37** : 421-464

Mascarenhas, J.P. 1993. Molecular mechanisms of pollen tube growth and differentiation. *Plant Cells,* **5** : 1303-1314

Masima, I. and Sato, H. 1959. X-ray induced mutation in sweet potato. *Japan J. Breed.,* **8** : 233-237

Mattsson, O. 1983. The significance of exine oils in the initial interactions between pollen and stigma in Armeria. In : Malcahy, D.L. and Ottaviano, E. (Eds.) *Pollen Biology And Implications For Plant Breeding.* Elsevier - Biomedical, Oxford pp. 257-264

Menon, R., Valsala, P.A. and Nair, G.S. 1992. Evaluation of open pollinated progenies of turmeric. *South Indian Hort.,* **40** : 90-92

Mikaelsen, K. 1968. Comparison between chromosome aberrations induced by ionizing radiations and alkylating agents at different stages of mitosis in barley seed. *Mutat. Plant Breed.*, IAEA, Vienna, p. 287-290

Misra, R.L. 1976. A colour mutant in Gladiolus L. variety Dicardy. *Sci. Cult.*, **42**(11) : 569-571

*Mitsukini, Y. and Arai, K. 1965. The effect of radiation on crocus. Nippon Univ. *J. Radiat. Med. Biol.*, **1** : 22-30 (in Japanese)

Miu, T.S. 1973. An inquiry into indication of cold tolerance among radiation induced lines of sweet potato. *Genetics*, **74** : 2-11

Moh, C.C. 1976. Screening for acyanogenic mutations in cassava (*Manihor esculenta* Crantz), *Mutat. Breed. Newsl.*, **8** : 10-11

Mohanty, D.C. and Sharma, Y.N. 1979. Genetic variability and correlation for yield and other variables in ginger germplasm. *Indian J. agric. Sci.*, **49** : 250-253

Mohanty, D.C., Das, R.C. and Sharma, Y.N. 1981. Variability of agronomic characters of ginger *Zingiber officinale*. *Orissa J. Hort.*, **9** : 15-17

Mukherjee, I. and Khoshoo, T.N. 1970. Genetic evolutionary studies on cultivated cannas : Parallelism between natural and induced somatic mutations. *Radiat. Bot.*, **10**(4) : 351-364

Mukhopadhyay, S. and Roy, K. 1986. Correlation and path analysis in turmeric. *Indian Agricst.*, **30** : 113-115

Mukhopadhyay, S., Roy, K. and Som, M.G. 1986. Variability in turmeric (*Curcuma longa*) *Exptl. Gen.*, **2** : 16-12

Muntzing, A. 1930. Outlines to a genetic monograph of the genus *Galeopsis* with special reference to the nature and inheritance of partial sterility, *Hereditas.*, **13** : 185-341

Nair, N.V. 1969. Considerations on overcoming intrasomatic selection during mutation breeding of vegetatively propagated plants. *Theor. Appl. Genet.*, **39** : 99-103

Nakornthap, A. 1965. Radiation induced somatic mutations in the ornamental, *Canna. Radiat. Bot.*, **5** (Suppl.) : 707-712

Nambiar, M.C. 1979. Morphological and cytological investigations in the genus *Curcuma* Linn. Ph.D. Thesis, University of Bombay.

Nambiar, V.P.K., Sasidharan, N., Renuca, C. and Balagopalan, M. 1985. Studies on the Medicinal Plants of Kerala Forests. KFRI, Thrissur, Kerala.

Namboodiri, A.N. and Tara, C.P. 1972. Defective stigmatic exudate as a factor in the sterility of *Impatiens*. *Curr. Sci.*, **41** : 340-341

Namboodiri, A.N. and Sreedevi, P. 1976. Pollen and pollinial wall preparations for cytochemical and infra red spectroscopic studies. *IV. Int. Paly. Conf.* Lucknow. **2** : 569-573

Nandi, A. 1991. Genetic variability in turmeric (*Curcuma longa*) Indian J. Agric. Sci. 61(12) : 941-942

Natarajan, A.T. 1958. A cytogenetical study of the effect of mutagen on plants with special reference to the induction of mutations. Ph.D. Thesis submitted to Delhi Univ., India

Natarajan, S.T. 1975. Studies on the yield components and gamma ray induced variability in turmeric (*Curcuma longa* L.) M.Sc. (Ag.). Thesis submitted to Tamil Nadu Agricultural University. Coimbatore, India

Nayar, G.G. 1975. Improving tapioca by mutation breeding. *J. Root Crops*, **1**(2) : 55-58

Nybe, E.V. 1978. Morphological studies and quality evaluation of ginger types (*Zingiber officinale* Rosc.), M.Sc. (Ag.) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala.

*Ono, S. 1971. Studies on the radiation breeding in the genus *Mentha*. Effective irradiation techniques to induce mutation in mint. *Sci. Rep. Fac. Agric.*, Okayama Univ., **37** : 9-15

Pandey, G. and Donbhal, V.K. 1993. Genetic variability, characters association and path analysis for yield components in ginger (*Zingiber officinale*) *J. Spices Arom. Crops.*, **2**(1-2) : 16-20

Pandey, G., Donbhal, V.K. and Sapra, R.L. 1996. Genetic variability correlation and path analysis of taro (*Colocasia esculenta* L.). J. *Hill Res.*, **9**(2) : 299-302

*Pavlovic, N.K., Stojanovic, N. and Milutinovic, M. 1983. Effect of different irradiation doses of mint (*Mentha piperata* L.) quality. *Arhiv za poliopriveredne* Nanke, **44**(154) : 249-254

Philip, J. 1978. Morphologiacl studies and quality evaluation of turmeric types. M.Sc. (Ag.) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala

Pillai, P.K.T., Lakshmi, K.R. and Sheela, M.N. 1995. Correlation and path analysis in taro. *J. Root Crops.,* **21**(2) : 86-89

Prasad, P.R.J. 1959. Studies on the effects of ionizing radiations in plants with special reference to sugarcane. M.Sc. Thesis, Madras University

Presannakumari, K.T., Viswanathan, T.V., Chettattu, G.J. and Augustin, A. 1994. Evaluation of geographical races of *Kaempferia galanga* for yield. *Indian Perfumer,* **38**(2) : 56-59

Raghava, S.P.S., Negi, S.S., Sharma, T.V.R.S. and Balakrishnan, K.A. 1988. Gamma Ray Induced Mutants in Gladiolus. *J. Nuclear Agric. Biol.*, **17** : 5-10

Raju, E.C., Patel, J.D. and Shah, J.J. 1980. Effect of gamma irradiation in morphology of leaf and shoot apex of ginger, turmeric and mango-ginger. *Proc. Indian Acad. Sci. (Plant Sci.),* **89**(3) : 173-178

Rangaswamy, S. 1986. Applied mutation research in field crops at Tamil Nadu Agricultural University. *Mut. Breed. Newsl.,* **28** : 13-14

Rao, P.S. 1974. Mutation breeding for non-flowering in sugarcane. Mutat. Breed. Newsl. **3** : 9

Rao, G.M. and Siddiq, E.A. 1977. Induced variation for yield and its components in rice. *Indian J. Genet. Plant Breed.*, **37**(1) : 12-21

Ratnambal, M.J. 1979. Cytogenetical studies in ginger (*Zingiber officinale* Rosc.), Ph.D Thesis, University of Bombay.

Reddy, C.R. 1980. Studies on heritability and genetic advance in sugarcane. *Maharashtra Sug.*, **5**(6) : 15-18

Reghupathy, A., Santharam, G., Balasubramanian, M. and Arumugam, R. 1976. Occurrence of the scale *Aspidiotus hartii* C. (*Diaspididae Homoptera*) on different types of turmeric, *Curcuma longa* Linn. *J. Pln. Crops,* **4**(2) : 80

Rimbau, V., Riscco, E., Canigueral, S. and Igleslas, J. 1996. Antiinflammatory activity of some extracts from plants used in the traditional medicine of North African Countries. *Phytotherapy Res.*, **10**(5) : 421-423

Roer, L. 1967. Mutation in potatoes induced by gamma irradiation. *Euphytica,* **16** : 283-292

Russell, S.D. 1982. Fertilization in *Plumbago zeylanica* entry and discharge of the pollen tube in the embryo sac. *Can. J. Bot.*, **60** : 2219-2230

Russell, S.D. 1983. Gametic fusion in *Plumbago zeylanica*: ultrastructural evidence for gamete recognition and the preferential transmission of sperm plastids into the zygote. *Meeting of Botanical Society of American and Canadian Botany Association Abstract.* p. 31

Russell, S.D. and Cass, D.D. 1983. Unequal distribution of plastids and mitochondria during sperm cell formation in *Plumbago zeylanica* In : Mulcahy, D.L. and Ottaviano, E. (Eds.) *Pollen biology and implications for plant breeding.* Elsevier Biomedical Oxford, p. 135-140

Sambandamurthi, S. 1983. Studies on induced mutations in tuberose (*Polianthes tuberosa* L.) Ph.D. Thesis submitted to Tamil Nadu Agricultural University, Coimbatore, India

Sarkar, S.K., Kumar, R. and Jain, B.B. 1996. Correlation and path coefficient analysis on yield and yield components of *Colocasis esculenta* (L.) Schott. *In : Tropical Tuber crops. Problems Prospects and Future Strategies.* Science Publishing INV. p. 169-171

Sato, M. and Gaul, H. 1967. Effect of ethyl methane sulphonate on the fertility of barley. *Radiat. Bot.,* 7 : 7-15

Saxena, B.P., Thappa, R.K., Tikku, K., Sharma, A. and Suri, O.P. 1996. Effect of plumbagin on ganadotrophic cycle of the housefly, *Musca domestica* L. *Indian J. Exptl. Biol.,* **34**(8) : 739-744

Semeniuk, P. and Krizek, D.J. 1972. Long days and cool night temperature increase flowering of greenhouse grown *Limonium* cultivars. *Hort. Sci.,* 7(3) : 293

Shah, H.A., Seemanthini, R., Arumugam, R., Muthuswami, S. and Khader, JBMMA. 1982. Co-I Turmeric. A high yielding mutant turmeric. *S. Indian Hort.*, **30**(4) : 276-277

Sharma, R.L. and Mukherjee, S.K. 1973. Some anatomical features of a radiation induced grape variegata. *Vitis,* **12**(3) : 189-191

Shivanna, K.R. and Johri, B.M. 1985. *The Angiosperm pollen : structure and function.* Wiley Eastern Ltd., New Delhi, p. 365

Singh, G. 1970. Chronic gamma irradiation induced resistance to red rot disease in sugarcane var. Co-997. *Sugarcane Pathol. News,* **5** : 24

Singh, V.K., George, C.X., Gupta, K.P. and Gupta, B.M. 1983. Antiviral activity of plant extract Liv 52 in mice experimentally infected with semliki forest encephalitis virus. *Sci. Cult.,* **49**(11) : 354-356

Skoog, F. 1935. The effect of X-irradiation on auxin and plant growth. *J. Cellular Comp. Physiol.,* **7** : 227-270

Smith, H.F. 1936. A discriminant function for plant selection. *Ann. Eugen.,* 7 : 240-250

Snedecor, C.W. and Cochran, W.G. 1980. *Statistical Methods* 7th Ed., the Iowa State Univ. Press

Solomon, F.E., Sharada, A.C. and Devi, P.U. 1993. Toxic effects of crude root extract of *Plumbago rosea* (Rakta Chitraka) on mice and rats. *J. Ethno Pharm.,* **38** : 79-84

Soriano, J.D. 1972. Effect of gamma radiation on buds of sweet potato (*Ipomoea batatas* L.). *Nat. Appl. Sci. Bull.*, **24**(1-2) : 41-53

Sparrow, A.H. and Christensen, E. 1950. Effects of X-ray, neutron and chronic gamma irradiation on growth and yield of potato. *Am. J. Bot.*, **37** : 667

Sparrow, A.M. 1961. Types of ionizing radiation and their cytogentic effects. *Mutat. Plant Breed., NAS-NPC,* **891** : 55-119

Sukhchain, Sandhu, D. and Saini, G.S. 1997. Interelationship among cane yield and commercial cane sugar and their component traits in autumn plant crop of sugarcane. *Euphytica,* **95**(1) : 109-114

Suma Bai, D.I. 1989. Genic manipulations in sweet potato adopting induced muation. Ph.D. Thesis submitted to Kerala Agricultural University, Vellanikkara, Thrissur, Kerala.

Suma Bai, D.I. and Nayar, N.K. 1992. Gamma ray induced growth rate variation in sweet potato genotypes. *Agricultural Science Digest* (Karnal), **12**(3) : 144-146

Suma Bai, D.I. and Nayar, N.K. 1995. Gamma ray induced variation for yield in sweet potato. *Journal of Tropical Agriculture,* **33** : 70-71

Tara, C.P. and Namboodiri, A.N. 1975. Aberrant microsporogenesis and sterility of *Impatiens sultani*. *Am. J. Bot.*, **61** : 585-591

Thamburaj, S., Muthukrishnan, C.R. and Irulappan, I. 1985. Studies on sensitivity of cassava buds to gamma rays and EMS. *S. Indian. Hort.*, **33**(1) : 13-17

Upadhya, M.D. and Purohit, A.N. 1973. Mutation induction and screening procedure for physiological efficiency in potato. *Induced Mutations in Vegetatively Propagated Plants. Proc. Meeting.* FAO/IAEA, p. 61-66

Vasudevan, K.N., Nair, S.G., Jos, J.S., Magoon, M.L. 1967. Radiation induced mutations in cassava. *Indian J. Hort.*, **24**(1-2) : 95-98

Vasudevan, K.N., Nair, S.G., Jos, J.S. and Magoon, M.L. 1968. Radiation induced mutations in *Colocasia esculenta* (L.). *Indian J. Hort.*, **25** : 66-69

Vasudevan, K., Jos, J.S. and Unnikrishnan, M. 1987. Gamma ray induced mutations in Taro (*Colocasia esculenta*) *Bangladesh J. Nuclear Agric.*, **5** : 41-46

Vasudevan, K. and Jos, J.S. 1988. Gamma ray induced mutants in Coleus. *Mutat. Breed. Newsl,*. **32** : 5

Vasudevan, K. and Jos, J.S. 1989. Gamma ray induced day length tolerant mutants in coleus. *Mutat. Breed. Newsl.*, **34** : 5-6

Vijayalakshmi, V. and Rao, J.T. 1960. Effects of gamma rays on germination and growth in some species and hybrids of saccharum. *Curr. Sci.*, **29** : 397-398

Villavicencio, M.A. and Perez-escandon, B.E. 1992. Activity of plumbagin (from *Plumbago pulchella* Boiss : Plumbaginaceae) as an antifeedant in three species of Orthoptera. *Folia - Entomologica - Mexicana.*, **86** : 191-198 (in spanish)

Viswanathan, T.V., Sunil, K.P., Mahato, K.C. and Manuel, J. 1992. Effect of gamma irradiation on the M_1 generation of Kaempferia (*Kaempferia galanga* L.), *South Indian Hort.*, **40** : 146-150

Walker, D.I.T. and Sisodia, N.S. 1969. Induction of non-flowering mutant in sugarcane (*Sacharum* sp.). *Crop Sci.*, **9** : 551-552

Wilfret, G.J. and Raulston, J.C. 1975. Acceleration of flowering of statice (*Limonium sinuatum* Mill) by gibberellic acid (GA_3). *Hort. Sci.*, **10**(1) : 37-38

Zachariah, T.J., Sasikumar, B. and Ravindran, P.N. 1993. Variability in gingerol and shogaol content of ginger accessions. *Indian Perfumer.*, **37**(1) : 87-90

* Originals not seen.

INDUCTION AND EVALUATION OF GENETIC VARIABILITY IN CHETHIKODUVELI

(Plumbago rosea L.*)*

BY

ARYA K.

ABSTRACT OF THE THESIS

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ABSTRACT

The present study "Induction and evaluation of genetic variability in Chethikoduveli (*Plumbago rosea* L.) was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 1995-1998. The study was undertaken through the conduct of two experiments. (i) Germplasm collection and evaluation (ii) induction of variability. Two noded semi-hardwood cuttings of seven ecotypes of *Plumbago rosea* and one related species, *Plumbago zeylanica* were used for the first experiment. The ecotype of *P. rosea* from College of Horticulture, Vellanikkara was used for the second experiment. The cuttings were treated with six doses of gamma rays (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 kR) and M_1V_1 generation was evaluated.

Pollen germination was not noticed in *P. rosea* whereas the pollen grains of *P. zeylanica* germinated. The germination of seeds was more quickly in scarified seeds of *P. zeylanica* than seeds with intact seed coat. All the pollination techniques failed to give any positive result on seed set in *P. rosea*. Normal pairing of chromosomes were observed in meiotic cells of both the species.

All characters studied had significantly high genotypic correlation with yield. High estimates of heritability (broad sense) was observed for all characters studied. High estimates of heritability coupled with high genetic advance was observed for plant height and root length which indicate that direct selection for improvement of these traits will be effective. Path coefficient analysis showed that dry root yield had maximum direct effect on plumbagin content of roots and plant height had maximum direct effect on fresh and dry root yield. The index score for selection was highest for *P. zeylanica* followed by the ecotype of *P. rosea* from Kottayam.

ED_{50} of gamma rays for the stem cuttings of *P. rosea* was 1.685 kR.

The sprouting percentage of cuttings was significantly decreased with increased doses of mutagen. Progressive delay in sprouting was noticed as the level of dose increased. The percentage lethality was higher in mutagen treated population than in the control. The optimum dose for inducing maximum yield and yield attributing characters ranged between 0.70 kR and 0.85 kR of gamma rays. High yielding mutants were observed in plants treated with 1.0 kR of gamma rays.

The highest values for yield and yield attributing characters were obtained for 0.75 and 1.00 kR gamma rays.

Gamma rays at 1.0 kR was most effective in inducing variability for root yield.