INDUCTION OF VARIABILITY THROUGH MUTAGENESIS IN NEELAYAMARI (*Indigofera tinctoria* L.)

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DECLARATION

I hereby declare that the thesis entitled **"Induction of variability through mutagenesis in neelayamari (*Indigofera tinctoria* L.)** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Induction of variability through mutagenesis in neelayamari *Indigofera tinctoria* (L.)** is a record of research work done independently by **Kumanan E** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Dedicated to
My Beloved Parents

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LIST OF ABBREVAIATIONS

%	- Per cent
^{0}C	- Degree Celsius
ANOVA	- Analysis of variance
CD	- Critical difference
CIMAP	- Central institute of medicinal and aromatic plants
cm	- Centimeter
d.f.	- Degrees of freedom
et al.	- And others
Fig.	- Figure
g	- Gram
GA	- Genetic advance
GCV	- Genotypic coefficient of variation
HI	- Harvest index
i.e.	- That is
KAU	- Kerala Agricultural University
kg	- Kilogram
KR	- Kilorads
LAI	- Leaf area index
MSE	- Error mean square
No.	- Number
NS	- Not significant
PCV	- Phenotypic coefficient of variation
SE	- Standard error
SS	- Sum of squares
viz	- Namely

Introduction

1. INTRODUCTION

Neelayamari (*Indigofera tinctoria* L.), commonly known as Indian indigo, is a commercially useful medicinal leguminous plant. It is cultivated in India, China and other countries of the east as a source of indigo, a commercially valuable dye. The shoot of the plant contains 'indigotin', a deep blue dye in the form of a glycoside namely 'indican'. Plant derived dyes like indigotin have been shown to be useful in dyeing clothes as they do not produce "dermatitis", an allergic condition of the skin often caused by synthetic dyes.

The leaves of *Indigofera tinctoria* (L.) are being used in the Ayurvedic system of medicine as an important ingredient in hair tonics like 'Neelibhringadi thailam'. Neelayamari is also reported to be useful in treatment of myelocytic leukemia, inflammatory skin conditions and in the glandular inflammation of the lymph nodes and tonsils. The root of the plant is used for the treatment of hepatitis. An infusion of the root is given as an antidote in case of snake bite and poisoning by arsenic (Nadkarni, 1976).

The present investigation has been aimed at inducing variability in *Indigofera tinctoria* (L.) for leaf and shoot yield. Mutation breeding has been found to be a potent and handy tool to induce new and additional variability in both qualitative and quantitative traits. Ionising and non-ionising radiations play a significant role for permitting favourable permanent changes, there by increasing scope for selection. Reports of comprehensive work on both the fundamental and applied aspects of mutation research in medicinal plants are practically nil.

Therefore an attempt was made in this study to find out the possibility of inducing desirable mutants in this crop by the application of physical mutagen. In the present investigation, attempts were made with the following objectives.

1. Induction of variability by gamma irradiation for higher biomass yield and indigotin content in *Indigofera tinctoria* (L.).

2. To study the effect of mutagen on the various morphological and biochemical parameters of *Indigofera tinctoria* (L.)

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

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

5. Correlation and path analysis to estimate direct and indirect effects of yield contributing characters on yield.

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

Review of Literature

2. REVIEW OF LITERATURE

2.1. ORIGIN AND CLASSIFICATION

Indigofera tinctoria (L.) is a leguminous medicinal plant, belonging to the family Fabaceae/ Leguminosae of the order Rosales. The family Fabaceae is one of the largest families of angiosperms. It comprises of about 600 genera and 12,000 to 18,000 species distributed all over the world. In India, they occupy the third position in the list of ten dominant families (Bairiganjan et. al, 1985). Senn (1938) reported that the basic chromosome number of the genus *Indigofera* is n = 8.

Hooker (1876) recorded 40 species and 3 varieties of *Indigofera* in India. Revisionary study of the genus revealed the occurrence of 60 species and 8 varieties. Of these, 13 species and 2 varieties are found to be endemic.

According to Arora and Chandel (1972) the centre of diversity of *Indigofera* is mainly concentrated in Eastern and Western Himalayas and Eastern and Western Ghats. Out of 44 species distributed in India, 10 species occur throughout plains, ascending Himalayas and hills of India.

According to Benson (1957) the systematic position of Indigofera is as follows.

Division	: Spermatophyta
Class	: Angiospermae
Subclass	: Dicotyledonae
Group	: Calyciflorae
Order	: Rosales
Family	: Fabaceae/ Leguminosae
Sub family	: Papilionoideae
Genera	: *Indigofera*
Species	: *tinctoria*

Of the various species of Indigofera, the chief ones exploited for the dye in India are *I. arrecta*, *I. suffruticosa*, *I. sumatrana* and *I. tinctoria*. Of these, *I. arrecta* and *I. tinctoria* were the most important ones (CSIR, 1959).

The cultivated species of *Indigofera*, their occurrence, habit and floral characteristics are presented in Table 1.

According to Rajwar (1983), in India, *Indigofera tinctoria* was seen in Kerala, Tamilnadu and Himalayan regions. Vijayakumar and Ramayya (1986) have reported 28 species of *Indigofera* in South India. Some species of the genus are rather scarce. Several species of *Indigofera* became enlisted as rare or endangered. *Indigofera tinctoria* is now a threatened species in India (Hajra et al., 1995).

Ramamurthy and Pullaiah (1998) recorded that Indigofera is represented by 25 species in Eastern Ghats. This is the second largest genus after the Crotalaria, which is represented by 55 species in Eastern Ghats.

2.2. GROWTH HABIT

Ramamurthy and Pullaiah (1998) observed that the general features of the genus *Indigofera* are prostrate, suberect or twining herbs, leaves simple, trifoliate and imparipinnate. Flowers axillary raceme, panicles or heads. Keel petals with downward spur on each side. Stamens diadelphous (9+1), pod usually oblong, linear, erect or deflexed, straight, curved, torulose, usually dehiscent.

Indigofera tinctoria Linn. is an erect, suffruticose, pubescent shrub 1.2 to 1.8 m high, with firm woody terete branches, light greenish brown

Table 1. Cultivated species of genus *Indigofera* (CSIR, 1959)

Species	Common name	Area of Cultivation	Leaf characters	Flower characters	Pod characters	Uses
Indigofera arrecta Hochst.	Natal indigo/Java indigo/Bengal indigo	Bihar, U.P.	10-13 cm long, leaflet 7-8 pairs with one odd terminal leaflet	Pinkish red, in axillary racemes	Straight and reflexed	As a cover crop in coffee, tea and rubber plantations. As a green manure crop for rice. Root used as abortifacient (Nyazema, 1987)
I. suffruticosa Mill.	West Indian indigo/Anil indigo	Indonesia, Malaya, Ceylon, Africa	5-8 cm long, leaflets 5-15, oblong, glabrous above and pubescent below	Pale orange in colour, in short racemes of 15-20	Curved. 0.3-0.6 inch long, 2-4 seeded, thickened at sutures	As a cover crop and green manure crop in coffee and tea plantations. The plant is febrifugal, vulnerary, purgative, antispasmodic, diuretic and stomachic
I. sumatrana Gaertn.	-	Andhra Pradesh, Madras	leaflets 9-15, obovate to nearly elliptic	Racemes 3-6 inch long	Obtusely curved, 2-4 cm long	As a green manure crop, preceeding cotton, maize or sugarcane.
I. tinctoria Linn.	Common indigo/ Indian indigo	India, China and other countries of the east	1-3 inch long, 9-13 leaflets	Pink, in nearly sessile spicate raceme, 2-4 inch long	Glabrescent, slightly curved or straight, ¾ - 1 inch long	As a cover crop and green manure crop in coffee plantations and rice fields. Plant extract used in epilepsy and nervous disorders, bronchitis, as an ointment for sores, old ulcers and haemorrhoids.

to somewhat silvery grey in colour bearing alternate pinnate compound leaves 2.5-7.5 cm long, petioles 1.2-2.5 cm long, stipules small and subulate. Leaflets 7-13, opposite, membraneous, bluish green, oblong or oblanceolate, rounded, glabrous above and thinly clothed with appressed hairs beneath. Leaves turn greyish black on drying. Flowers numerous, in nearly sessile lax spicate racemes 5-10 cm long. Calyx teeth triangular, acute. The corolla is vexillary pink, consisting of a rounded emarginate standard petal, brownish and pubescent at the back and two wing petals adherent to the two keel petals which are greenish in colour and furnished with a spur on each side often bending back elastically. The stamens are diadelphous, nine and one, anthers uniform. Ovary is sessile, eight to ten or more ovuled with a short incurved style ending in a capitate stigma. Pods 2-3.2 cm long, linear, straight or slightly curved, thickened at the sutures, glabrous, pale greenish grey when young and dark brown on ripening with 8-12 seeds (CSIR, 1959; Iyer and Kolammal, 1960; Kirtikar and Basu, 1935; Kochhar, 1981; Singh and Khan, 1990; Anitha, 1998).

Jawahar (1996) while listing *Indigofera tinctoria* L. under the medicinal plants of the Kani tribes reported that the plant is usually grown by the tribes and scantily seen in the wild state near the tribal settlements.

2.3. MEAN PERFORMANCE

Kulkarni and Karadge (1991) reported that plant height was found to increase throughout the growth stages in moth bean (*Phaseolus aconitifolium* Jacq.).

A study made by Anitha (1998) showed that a mean of 147 days was needed for 50 per cent flowering in *Indigofera tinctoria*.

While evaluating the genetic stock of *Mucuna pruriens*, Samuel (2000) noticed that the fresh and dry weight of leaves increased considerably during the earlier growth stages, but declined towards later stages.

Nair (2000) and Resmi (2001) observed that in *Clitoria ternatia*, the performance of different accessions differed during various growth stages. In the same work it was also noted that the leaf area and root yield increased during the pre flowering and flowering stage, but decreased during the pod maturation stage. The shoot yield was found to increase through out the growth stages.

Mathew (2002) studied the floral biology and anthesis in *Moringa oleifera* and reported that the anthesis occurred during morning hours.

Neema (2004) observed that in *Indigofera tinctoria*, analysis of variance showed significant differences among the 20 accessions for all the characters studied at all the stages except for root girth at collar region during the pre flowering stage. The mean performance of growth parameter found to increase through out the growth stages.

2.4. VARIABILITY STUDIES

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

in a population can be partitioned into heritable and non heritable components with the aid of genetic parameters such as phenotypic and genotypic variation, heritability, genetic advance and genetic gain which serve as the basis for selection. Burton (1952) introduced a convenient procedure for the calculation of genotypic and phenotypic coefficient of variation.

In *Opium poppy*, Jain et al. (2006) studied the genetic variability in sixty three genotypes of opium poppy for plant height, stem diameter, peduncle length, grain yield per plant, husk yield per plant, latex yield per plant and morphine content. Significant variations among all the genotypes were recorded for all the characters. The estimate of PCV was higher than the GCV. The high GCV were observed for grain yield per plant, husk yield per plant and latex yield per plant. Singh et al. (2003) reported that the highest genotypic and phenotypic coefficient of variability was noticed for capsules per plant followed by opium yield per plant, husk yield per plant and seed yield per plant in *Papaver somniferum.*

Sarada (2004) reported that the high phenotypic and genotypic coefficient of variation was shown by the characters viz. crude indigo content, dry weight of root, harvest index (root yield), dry weight of leaves, net assimilation rate and dry weight of shoot in *Indigofera tinctoria.*

Shukla et al. (2003) reported that in fennel high value of phenotypic and genotypic coefficient of variation were observed for seed yield, stover yield, umbels per plant and umbellets per umbel, which suggested that strong variability existed among the genotypes for these traits.

Shah et al. (2003) reported that the genetic constants for the characters revealed that the magnitude of phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation indicating effect of environment in the expression of character. The high magnitude of GCV and PCV were recorded for 1000 seed weight, oil content, harvest index and seed yield in *Coriandrum sativum.*

Singh and Khanna (1991) reported that the coefficient of variability for the agronomic characters studied in opium poppy was low due to the narrow genetic base of the genotypes. Singh *et al.* (2000) reported that significant variations among genotypes were recorded for the characters such as plant height, number of leaves per plant and number of branches per plant in opium poppy. The estimates of PCV were higher than those of GCV. The yield was positively and significantly associated with number of leaves per plant and number of branches per plant.

Sankaranarayanan et al. (1992) reported that in senna high genotypic coefficient of variation estimates were found for total leaf yield and leaf yield at 90 days.

Misra et al. (1998) reported that the highest phenotypic and genotypic coefficient of variation were recorded for dry root yield followed by plant canopy and the lowest for plant height in *Withania somnifera.*

Twenty two morphometrically diverse genotypes of *Andrographis paniculata* collected from North India were evaluated for genetic variability and the results revealed considerable amount of genetic variability among genotypes. Highest phenotypic and genotypic coefficients of variations were recorded for dry biomass yield, leaf: stem

ratio followed by plant height and lowest for leaf length (Misra et al., 2000).

Fifteen fenugreek genotypes were studied for the presence of 12 quantitative characters for effective selection of the important characters for higher yield (Dash and Kole, 2001). The variability in 29 M-lines of fenugreek (*Trigonella foenum-graecum*) was estimated by Kaushik and Dashora (2001). Significant variability was recorded for plant height and seed yield per plot.

Kalamani and Gomez (2001) while conducting a study on the genetic variability analysis in *Clitoria ternatia* recorded that the seed weight, leaf breadth, number of leaves per plant, leaf length and plant height showed high PCV and GCV estimates. The root length, girth, nodule number and yield of 13 accessions of *Clitoria ternatia* intercropped under shade in a coconut garden, were examined by Nair and Reghunath (2002) during the pre-flowering, flowering, seeding and seed maturation stages of the plant and came to the conclusion that the parameters measured increased from pre-flowering up to the seeding stage and decreased during the seed maturation stage.

The work on the evaluation of *Trigonella foenum-graecum* and *Trigonella corniculata* for leaf yield and its components done by Varalakshmi (2002) revealed that the most promising lines in terms of yield and dry matter content were IIHR-4 that produced the tallest plant and IIHR-9, where the leaf height was highest. Kumar and Choudhary (2003) assessed the magnitude of genetic variability in 12 diverse fenugreek (*Trigonella foenum-graecum*) genotypes. Sufficient genetic variation was found for plant height, plant girth, number of branches per plant, number of leaves per plant and root weight.

A study on the genetic variation and relationship between root yield and biochemical traits of 13 genotypes of safed musli by Bhagat and Jadeja (2003) showed that GCV was lower than PCV for root yield.

A study made by Neema (2004) in *Indigofera tinctoria* revealed that the highest phenotypic and genotypic coefficient of variation was observed for the number of effective root nodule followed by fresh weight of pods. The lowest phenotypic and genotypic coefficient of variation was exhibited by number of branches followed by plant spread.

2.5. HERITABILITY AND GENETIC ADVANCE

Heritability in the broad sense refers to the relative proportion of genotypic variance to phenotypic variance. Coefficient of variation 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.

In asgandh, Misra et al. (1998) reported that the heritability in broad sense (h^2_B) and genetic advance (GA) both are high for dry root yield and plant canopy and these two characters are very amenable to selection.

Misra et al. (2000) assessed the genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance in 22 diverse genotypes of kalmegh (*Andrographis paniculata*). They reported

that the dry biomass yield and plant height had high heritability combined with high genetic advance and these characters were the most suitable for improvement through selection.

Yadav et al. (2004) reported that heritability was highest for capsule weight/plant followed by plant height and capsule size. Genetic advance was highest for capsule/plant followed by opium yield/plant and capsule weight/plant in *Papaver somniferum* and Singh et al. (2003) reported that high heritability coupled with high genetic advance was observed for capsule weight/plant, opium yield/plant, capsules/plant, and seed yield/plant.

Lal et al. (2003) reported that high heritability in broad sense (h^2_{BS}) was observed in oil content followed by leaflet width, branches/plant, leaflets in leaf and leaf length in curry leaf (*Murraya koenigii*).

Krishnamoorthy and Madalageri (2002) studied the range of variability and characters associated with growth, yield and yield attributing traits in 15 genotypes of ajowan (*Trachyspermum ammi*). High heritability coupled with high genetic advance was observed for number of seeds per umbel, essential oil yield per hectare, number of umbels per plant, total dry weight, essential oil content and number of tertiary branches.

Shah et al. (2003) reported that twenty genotypes of coriander showed significant differences among themselves for seed yield and other characters in all the three environments. High heritability estimates coupled with high genetic gain were observed for 1000 seed weight, oil content, plant height and days to flowering which are likely to respond to direct selection.

Shukla et al. (2003) studied the coefficient of variability, heritability and genetic advance for seed yield and its five major contributing traits using 30 genotypes of fennel (*Foeniculum vulgare* Mill.) grown on sodic soils. The high heritability coupled with moderate to high genetic advance was observed for seed yield which suggests that recurrent selections from these traits can be advantageous to achieve higher yield.

Sarada (2004) reported that high heritability and high genetic advance was observed for dry weight of leaves, dry weight of root, net assimilation rate, harvest index (root yield) and crude indigo content. Therefore these traits might be highly amenable to direct selection for their genetic improvement over a short span of time.

Jain et al. (2006) reported that the high genetic advance coupled with high heritability was observed for plant height, grain yield per plant and husk yield per plant in opium poppy (*Papaver somniferum*).

2.6. CORRELATION STUDIES

One of the important objectives in a breeding programme is the incorporation of the genetic potential for high yield in a variety. Since yield is a complex character, it is worthwhile to estimate the influence of the association existing between the variable characters and yield. Correlation studies were conducted to determine the interrelationship among various traits which are useful in making selection. Generally estimates of genetic correlations are of very low precision. A knowledge of the estimates helps to understand how the improvement in one character will cause simultaneous change in other characters. A comparison of phenotypic and genotypic correlations would give an indication of the effect of environment on the genetic performance of individuals of a

population. Galton (1889) conceived the idea of correlation of variables for the first time.

According to Jain et al. (2006) in *Opium poppy*, latex yield per plant was significantly and positively correlated with husk yield per plant and grain yield per plant.

Sarada et al. (2006) reported that in Indian indigo, shoot yield was correlated with leaf dry weight, leaf area index, leaf area, lower glycoside content and indigo content.

In coriander, Shah et al. (2003) reported that the number of seeds per umbel and number of umbels per plant were having positive and significant correlation with seed yield per plant.

Singh et al. (2003) reported that the seed yield had positive and significant correlation with plant height and leaves per plant, capsules per plant, stem diameter, capsule size, and capsule weight per plant and husk yield per plant in *Papaver somniferum*.

Krishnamoorthy and Madalageri (2002) conducted studies to assess the range of variability and character associated with growth, yield and yield attributing traits in fifteen genotypes of ajowan (*Tachyspermum ammi*). The oil yield per hectare was positively correlated with days to flowering, days to harvest, number of umbels per plant and essential oil content. The thymol content in essential oil was positively correlated with days to flowering, days to harvest, essential oil content of seeds and essential oil yield.

Sankaranarayanan et al. (1992) reported that in senna, leaf yield was positively correlated with numerous of branches, length of leaves and

leaves per plant and pod yield was significantly correlated with plant height and number of branches.

In *Catharanthus roseus*, the values of genotypic correlation were higher than the phenotypic and environmental values. Number of primary branches showed highly significant positive genotypic association with total fresh leaf yield, whereas stem width showed highly significant negative genotypic association with number of primary branches. Significant positive genotypic association were observed between plant height, stem width and total dry leaf yield, whereas the association between total fresh leaf yield and total dry leaf yield was non-significant (Dwivedi et al., 1999).

Saini et al. (1999) recorded data on ten quantitative traits in 75 indigenous and exotic genotypes of opium poppy and observed a wide range of phenotypic and genotypic variations of all kind. Shukla et al. (2003) conducted correlation and path coefficient analysis in 22 selections of opium poppy for seed yield. Seed yield showed positive and significant correlation with plant height, leaves per plant and stem diameter.

Mishra et al. (2001) observed large differences among 32 accessions of periwinkle collected from different geographical areas of India, Madagascar, Singapore and Malaysia. Strong correlation was observed between leaf area and leaf yield.

Mulas et al. (2002) observed a positive correlation between leaf width and shoot fresh weight in rosemary (*Rosmarinus officinalis* L.) cultivars.

Lal et al. (2003) observed tremendous variability in quantitative traits such as days to flower, leaf length, main stem diameter, branches per plant among genetic stocks in curry leaf (*Murraya koenigii*).

Neema (2004) reported that the highest phenotypic and genotypic correlation was observed with plant height, plant spread, and fresh weight of pods followed by number of number of branches per plant, number of leaves per plant, fresh weight of leaves, leaf area and fresh weight shoots. Fresh weight of roots and root length showed negative correlation in *Indigofera tinctoria*.

2.7. 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 i.e. the response. This analysis permits the separation of correlation coefficient into components of direct and indirect effects of independent variables on dependent variable (Deway and Lu, 1959).

Dwivedi et al. (1999) reported that the path coefficient analysis revealed that percent total alkaloid (PTA) followed by total herbage yield (THY) had the highest positive and significant effect on total leaf alkaloid (TLA), where as plant height, number of primary branches, leaf area index, total leaf yield(fresh) and percent vincristine showed direct negative association.

In Asganth, Misra et al. (1998) reported that the path coefficient study revealed that the highest direct contribution to root yield was made by root diameter and root length. Direct contribution of other traits to root yield was negative.

Path coefficient analysis specifies causes and measures their relative importance. Seed yield has directly contributed to opium yield to the highest extent and also indicating through plant height followed by capsules per plant. The high direct contribution was made by plant height followed by capsules per plant, both of which also contributed indirectly through seed yield. Husk yield made high direct contribution to opium yield and indirectly through seed yield, capsules per plant and plant height. Thus, from practical point of view seed yield was found to contribute high opium yield directly and indirectly both. However, capsule weight shows greatest negative contribution to opium yield in *Papaver somniferum* (Singh et al., 2003).

Krishnamoorthy and Madalageri (2002) revealed that the path analysis indicated the direct influence of days to harvest, plant height and essential oil content on thymol content and these traits also had indirect effect via days to flowering and plant height. Essential oil yield and 1000 seed weight through days to harvest and total dry matter influenced indirectly on thymol content in *Tachyspermum ammi.*

In opium poppy, Jain et al. (2006) reported that the husk yield which had highest positive correlation with grain yield, also exhibited maximum direct path. It also contributed indirectly through peduncle length and stigmatic rays. The plant height, which had positive genotypic correlation exhibited negative direct path. However negative direct effects are counter balanced through positive direct effect of husk yield per plant, peduncle length, stigmatic rays and stem diameter. In case of opium yield the highest direct path is exhibited by grain yield per plant followed by stigmatic rays and peduncle length. Plant height, husk yield per plant and stem diameter have negative direct effects.

2.8. ECONOMIC IMPORTANCE OF *INDIGOFERA SP.*

The plant commonly known as 'Neeli' or 'Amari' is found both in the cultivated and wild condition in Bengal. It was cultivated on a large scale in many parts of Northern India for extracting the dye 'indigo' from its leaves, before the introduction of synthetic dyes.

The colouring matter of the plant, indigotin ($C_{16}H_{10}O_2N_2$), is present in the form of a glucoside, indican ($C_{14}H_{17}O_6N$), particularly in the lamina and to a small extent in the midrib or rachis. The dye is extracted from the leaves by fermentation, when indican hydrolyses into glucose and indoxyl (C_8H_7ON), the latter on oxidation yields indigotin. Natural indigo, contains in addition to indigotin, varying proportions of a red dye, indirubin, a resinous impurity, indigobrown, indigogluten and other substances.

Indigo finds use in dyeing and printing cotton and rayon and for dyeing wool. It has been employed in the preparation of pigments for paints, lacquers, rubber and printing inks. It is used also to a small extent by artists and in wall paper decoration. It possesses excellent fastness to light and washing, dyeings on wool are faster than those on cotton. Eventhough the importance and use of this natural dye has dwindled in India with the advent of the synthetic products, large quantities of the leaves of this plant are being exported to different parts of the world. The residual parts of the plants, left after the extraction of the dye, are rich in available nitrogen and are used as manure for cereals, oil seeds, sugarcane and tobacco.

Indigofera tinctoria (L.) is of much medicinal importance too. According to Bhavaprakasa, 'neeli' is purgative in action, bitter, hot or destructive. It improves hair growth and premature greying. It cures or is useful in meha (diseases with excessive urine) and giddiness, abdominal enlargement, enlargement of the spleen, vatarakta, kapha, vata, amavata, udavarta, alcoholic intoxication and very severe poison. The leaves have bitter taste. They are used to lessen inflammation, cure chronic bronchitis and asthma (especially of children), piles, leucoderma, bites of insects and reptiles, burns, scalds, ulcers and skin diseases. It is useful in lumbago, enlargement of liver and flatulence. Applied to the naval, it acts as a diuretic and cathartic (Yunani). An infusion of the root is given as an antitode in cases of poisoning by arsenic. Indigo is applied to reduce swelling in the body due to the bites and stings of venomous insects and reptiles (Nadkarni, 1976). It is used as a domestic remedy for the stings of bees and wasps in England and China. In Indo China, the leaves are made into an ointment which is applied to contused, inflamed or itchy parts. It is also used against haemorrhoids. The juice of the leaves is prophylactic and is used against hydrophobia. In Cambodia, the leaves are given in decoction for hennorrhagia. Roots are also used against hepatitis. The roots pounded and macerated in water are drunk for urinary complaints by the Mundas of Chota Nagpur (Kirtikar and Basu, 1935).

The plant is also known for its use in cosmetic preparation from time immemorial. Mention of this plant had been made by Charaka, Susrutha and in Ashtangahridaya in the preparation of medicines for grey hair and constipation. Neeli is one of the main ingredients of Neeliyakadya Thailam (Sreekanta Murthy, 1984) and Neelibhringadi Thaila, which are popular hair oils. Neelibhringadi oil is used not only for inducing abundant growth of hair, but also for maintaining its natural black colour. The extract of *indigofera tinctoria* (L.) (whole plant excluding root) shows hypoglycaemic effect in rats and CNS depressant

effects and potentiation of pentabarbitone induced hypnosis in mice. The LD_{50} of the extract was less than 1000 mg/kg i.p. in mice. The petroleum extract of the whole plant had shown antifungal activity against *Helminthosporium sativum* (Bhatnagar *et al.*, 1961). The alcoholic extract of the aerial parts of *Indigofera tinctoria* (L.) had exhibited marked protection against carbontetrachloride induced liver damage in rats, suggesting stimulation of microsomal enzymes of the liver. The minimum lethal dose was less than 1000 mg/kg i.p. in mice (Anand *et al.*, 1979). Histopathological studies also confirmed the productive effect of the alcoholic extract of *Indigofera tinctoria* (L.) against cc ¼ induced liver damage in rats. This effect was more pronounced in male rats than in female rats (Anand *et al.*, 1981). Various pharmacological studies carried out in the leaf and stem extract of *Indigofera tinctoria* (L.) on the central nervous system had revealed that they had significant depressant action. The leaf extracts of *Indigofera tinctoria* (L.) has significantly protected experimental mice from strychnine induced convulsions, showing a beneficial anti-convalescent action (Rahamathullah et al., 1990).

2.9. MUTAGENESIS

Induced mutation is one of the fast developing links between cytogenetics and morphology. In mutation studies, cytological analysis serves as one of the most dependable indices in assessing the potency of a mutagen on the one hand and mutagen response of a genotype on the other. As a result of the various mutagenic treatments which damage absolutely all the intracellular structures, a multitude of the most diverse responses can be registered in a cell, viz. a delay in division, suppression of DNA synthesis, membrane damage etc. The extent of these responses depends upon the phase of the cell cycle in which the mutagenic treatment was performed. The cytological effects include chromosome breakages,

which can be studied from mitosis as well as meiosis. Chromosomal aberrations and chlorophyll mutations are essential parameters for estimating the index of the mutation frequency and for studying the biological effects of mutation (Kihlman 1975, Grover and Tyagi 1979, Singh, et al., 1980). Chromosomal aberrations play a major role in the induction of sterility which in its turn influences the evolution of mutants. The studies on the effect of ionising radiations and chemical mutagens on chromosome breakage, sterility and chlorophyll mutations have received much attention due to the fact that they are of prime importance in plant breeding. Induction of structural rearrangements is one of the ways of achieving new recombinations, rarely obtained by more conventional methods (Sree Ramulu 1971).

Plant breeders have been successful in developing a large number of high yielding varieties of many crop plants through mutagenesis. Nilsson Ehle (1929) and Gustafsson (1954) had done pioneering works in this field. The mutant varieties developed include the high yielding rice variety, Remei in Japan (Futsuhara et al., 1967). Later on other investigators (Kaul, 1980 and Vairavan and Arumugachamy, 1995) had also evolved high yielding varieties of rice. Other crops included wheat (Goud, 1967; Mehta, 1972; Larik, 1975); sorghum (Sree Ramulu, 1974); *Corchorus olitorius* (Ghosh and sen, 1974); *Arachis hypogea* (Patil, 1971, 1972); *Brassica campestris* (Kumar and Das, 1977); legumes (Gottschalk and Patil, 1971; Gottschalk and Hussein, 1975; Nerkar, 1976; Kesavan and Khan, 1977; Muller and Gottschalk, 1978; Prasad and Das, 1980); tobacco (Rao et al., 1995); sweet potato (Bai and Nayar, 1995).

Most often, quantitative traits are of special concern to the plant breeder, and the possibilities of crop improvement have been exploited upto the hilt by several workers (Gregory, 1955; Lawrence, 1955; Brock, 1965 and 1971; Ehrenberg et al., 1961 and Gaul, 1964), as a valuable tool in plant breeding programmes. Apart from producing high yielding mutants and those with better quantitative characters, disease resistant varieties of many crop plants were also derived by Frey and Browning (1955), Konzak (1956) and Gregory (1956).

Mutagenesis has also contributed much to the evolution of early maturing varieties of crop plants, varieties resistant to frost, heat, drought, lodging etc. Early flowering varieties have been isolated in barley (Hussein et al., 1980); rice and other cereals (Goud, 1972; Khan, 1973); legumes (Zacharias, 1967; Prasad and Das, 1980) etc. Varieties adaptable to different environmental conditions are some of the other mutants of practical application.

Another important aspect in applied mutagenesis is the quantitative and qualitative alteration of seed storage substances, such as proteins and carbohydrates, to some extent also of specific other substances deposited in various plant organs. Special emphasis is directed to seed proteins because a part of diseases due to malnutrition and undernourishment is related to insufficient protein supply. An increase and improvement of these substances would bridge the "protein gap", in the present set up. These considerations especially became valid, under the aspect that 70 per cent of the consumed proteins are derived from plants (IAEA, 1979). Recent investigations had revealed that there are possibilities of enhancing the protein content in crop plants like rice (Tanaka et al., 1970; Harn et al 1973); Triticum (Muntz et al., 1979); legumes (Gottschalk et al., 1975) etc. Significant results had been obtained with regard to essential amino acids. Some of the mutants had exhibited high productivity, but the quality of the seed proteins was low as the amount of essential amino acids had

decreased. On the contrary, some investigators were able to isolate strains with high protein content with an increase in essential amino acids, eventhough the mutants did not reach the yielding capacity of the initial line. It would also be possible to remove or reduce the quantity of undesirable amino acids present in certain crop plants like *Lathyrus sativus* (Nerkar, 1972). Besides these, plants with greater vitamin content, alkaloid content and those with increased quantity of other chemically beneficial constituents could be produced by mutagenesis.

Despite the various advantages of the mutants over the exciting cultivated lines, mutation breeding has got its own limitations too. An evaluation of a comprehensive collection of mutants belonging to different species had shown a negative selection value for most of the mutant genes as a generally valid rule. As far as the breeding values of the mutants are concerned, only a very small proportion were comparable or even superior in their characteristics to the initial lines. Therefore, it is very important that mutagenesis be carried out on a very broad base, the larger the number of mutants, the greater the possibility that a few of them may be beneficial for practical breeding. Pleiotropism, one of the greatest handicaps of mutation breeding, forms the basis behind the negative selection value. The agronomically useful traits of the mutant may be accompanied by one or several negative traits in the majority of cases, thereby reducing the breeding value of the strains involved. It is not only of theoretical, but also of practical interest that the pleiotropic pattern of a mutant gene can be altered to some extent by transferring it into a specific genotypic background (Konzak, 1976 in wheat; Khvostova, 1978; Sidorova, 1981 in peas). If a really important and valuable new character is part of a pleiotropic spectrum, crossing of the mutant with a large number of different strains to find out whether the intensity of the negative characters of the spectrum could be reduced by selecting a specific genotypic composition. The solution to the problem lies in the

fact that hybridization be done with a number of exciting lines or with various mutant lines themselves, inorder to combine their useful characters. Recent advances like recombination DNA technology could also help in achieving this goal.

2.9.1 INDUCED MUTATION IN MEDICINAL PLANTS

Mutation breeding has been found to be a potent and handy tool to induce new and additional variability in both qualitative and quantitative traits. 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 applied aspects of mutation research in medicinal plants are practically nil.

In *Plantago ovata*, Lal and Sharma (2002) reported that irradiation at more than 50 KR doses reduced seed germination. Generally a delay in germination percentage was noticed consequent to irradiation at higher doses as reported by Gupta et al. (1982) in Costus. Anitha (1998) studied that in *Indigofera tinctoria* all the group of mutagen treatments had exhibited significantly reduced values for the percentage of germination. Arya (1999) reported that the sprouting was very much delayed at higher doses and the increase in percentage lethality was more at higher doses of mutagen in *Plumbago rosea.* Treatment with various physical and chemical mutagens had been reported to affect the percentage of germination in *Withania somnifera* (Chandralekha, 1995); in *Cassia angustifolia* (Geetha, 1995).

Lal and Sharma (2002) reported that the survival percentage and pollen fertility declined with increased doses of irradiation as compared to control in *Plantago ovata.* Anitha (1998) reported that the combination treatments of 0.01 percent $NaNo_3$ with different doses of gamma rays, no survival could be observed and higher doses of gamma rays, when tried either alone or in combination with EMS or $NaNo_3$ had also failed to exhibit and survival in *Indigofera tinctoria.* Mareen Abraham (2002) reported that the survival percentage was very less in the doses 40 and 60 GY in *Coleus parviflorus.*

Vasudevan and Jos (1992) showed that in *Dioscorea alata* and *Dioscorea esculenta,* LD_{50} for gamma ray treatment was between 2-3 KR. Lal and Sharma (2002) reported that in *Plantago ovata,* LD_{50} was found to be between 40-50 Krad doses.

The diosgenin content in *Costus speciosus* increased as a result of 2.0 KR gamma ray treatments where as it decreased at 30 KR (Gupta et al. 1982). Arya (1999) reported that in *Plumbago rosea*, 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. In *Coleus parviflorus* the yield per plant was lower in mutants but had uniform and average sized tubers (Vasudevan and Jos, 1988).

Anitha (1998) reported that in *Indigofera tinctoria*, the treated population had exhibited different kinds of abnormalities like variation size and colour of the cotyledons, foliar abnormalities, changes in the height and number of branches, etc. In *Dioscorea alata* and *Dioscorea esculenta,* few clones were identified as dwarf plant types and early maturing types (8 months) as the traditional crop takes 10 months for maturity (Vasudevan and Jos, 1990). Mareen Abraham (2002) reported that in *Coleus parviflorus*, the selected mutants showed photoinsensitivity

to tuberization, an acceptable qualitative change. Arya (1999) reported that dwarf mutants and tall mutants could be observed in the gamma ray treated population in *Plumbago rosea*. Lal and Sharma (2002) reported that in *Plantago ovata*, a promising mutant FEA-5 at 20 Krad dose (early maturity with high seed and husk yield) could be identified for commercial cultivation in North Indian plain conditions.

Materials and Methods

3. MATERIALS AND METHODS

The study titled 'Induction of variability through mutagenesis in neelayamari (*Indigofera tinctoria* L.)' was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during the period 2006-2008.

3.1. Materials

3.1.1. Biological material

The crop selected for the present study was local variety of neelayamari (*Indigofera tinctoria* L.) namely Vellanikkara local. Seeds of the cultivar were obtained from the AINP on Medicinal and Aromatic plants, College of Horticulture, Vellanikkara, Thrissur.

3.1.2. Mutagens

Only one physical mutagens was used for the study. The physical mutagen employed was gamma rays. The gamma irradiation facilities available at the Radio Tracer Laboratory, Kerala Agricultural University, Vellanikkara were utilised. ^{60}Co gamma chamber was employed for irradiation. The source was operating at a dose rate of 5000 rads per minute.

3.2. Methods

3.2.1. Estimation of LD_{50} of gamma rays

The moisture content of the seeds was approximately 12-13 percent. Seeds of uniform size were sorted out. One hundred seeds were irradiated at gamma ray doses of 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 KR. The seeds were sown in pots, germination and survival were recorded at ten days intervals and the LD_{50} was calculated. LD_{50} for gamma rays was found to be 20 KR. Based on this, the doses of gamma ray irradiation were selected as 15.0, 17.5, 20.0, 22.5 and 25.0 KR.

3.2.2. Gamma irradiation

Seeds of uniform size were sorted out. The moisture content of the seeds was approximately 12-13 percent. Five samples of 250 seeds each were irradiated with the selected doses of gamma ray irradiation *viz.,* 15.0, 17.5, 20.0, 22.5 and 25.0 KR.

3.2.3. Study of the M_1 generation

The gamma irradiated seeds were sown in the field on the next day of treatment along with the unirradiated control. The experiment was laid out in Randomised Block Design with six treatments and four replications. The treatments were as follows; T_1 (15.0KR), T_2 (17.5KR), T_3 (20.0KR), T_4 (22.5KR), T_5 (25.0KR) and T_6 (control). The six treatments of *Indigofera tinctoria* are given in Plate 1. The following observations were recorded from the M_1 generation.

A. Germination of seeds

Number of seeds germinating on each day was counted to estimate the percentage of germination.

B. Survival

The number of plants which had survived upto the time of flowering was recorded.

C. Plant height (cm)

Height of the plant from the collar region to the tip was measured using measuring scale at pod maturation stage.

D. Number of inflorescence

The total number of inflorescence produced by the plant was taken.

E. Pollen sterility

The Aceto-carmine technique was utilised to find out the percentage of pollen sterility. Five freshly opened flowers were collected at random, from each treatment. The pollen grains from each flower were dusted onto a clean microslide. A drop of 2 percent Aceto-carmine-glycerine mixture (1:1) was placed over the pollen grains and then mounted with a cover slip. The microslides were

Plate 1. Different treatments of Indigofera tinctoria - M_1 generation

A) T_1 - 15.0 KR

B) T_2 - 17.5 KR

C) T_3 - 20.0 KR

Plate 1. Continued

D) T_4 - 22.5 KR

E) T_5 - 25.0 KR

F) T_6 - Control

observed after 10 minutes. Pollen grains, which were fully stained, were taken as fertile; whereas those which were unstained and shrunken were scored as sterile.

F. Chlorophyll chimeras

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

G. Morphological abnormalities

The population was examined at regular intervals for the presence of other morphological variations due to the direct effect of the mutagen, such as dwarf plants and the plants with alterations in number, size and shape of leaflets in the early formed secondary leaves.

H. Leaf area index (LAI)

Leaf area of observational plants, were calculated by adopting punch method. Leaf area index was worked out using the formula proposed by Watson (1952),

$$LAI = \frac{\text{Leaf area of the plant } (cm^2)}{\text{Ground area occupied } (cm^2)}$$

I. Girth of stem

Measurement was taken round the stem at a height of 3 cm from the soil surface using a tag and the length of the tag was taken as the stem girth.

J. Fresh weight of leaves (g)

The total weight of leaves of the observational plants in each treatment was recorded.

K. Dry weight of leaves (g)

The leaves were oven dried at 70 ± 5 ° C to a constant weight and the dry weight was recorded.

3.2.4. Study of the M_2 generation

The M_2 generation was raised by sowing the seeds collected from the selected plants of the M_1 generation. The seeds of each treatment were collected, bulked and kept separately. Fifty seeds from every treatment were sown in

Randomised Block Design with four replications. The crop was raised as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2002). Seeds were sown at a spacing of 60 × 30 cm. The experimental field and six treatments of *Indigofera tinctoria* are given in Plate 2 and 3. The following observations were recorded from the M2 generation.

I. Morphological characters

A. Growth characters

1. Germination of seeds

Number of seeds germinating on each day was counted to estimate the percentage of germination.

2. Survival

The number of plants which had survived upto the time of flowering was recorded.

3. Plant height (cm)

Height of the plant from the collar region to the tip was measured using measuring tape at pre flowering, flowering and pod maturation stage.

4. Plant spread (cm)

The distance covered by the plant was measured using a measuring tape in the North- South and in East- West directions from the axis and the average was recorded at pre flowering, flowering and pod maturation stage.

5. Number of branches

The total number of branches of the plant was counted at pre flowering, flowering and pod maturation stage.

6. Stem colour

The colour of stem of the observational plants in each treatment was recorded.

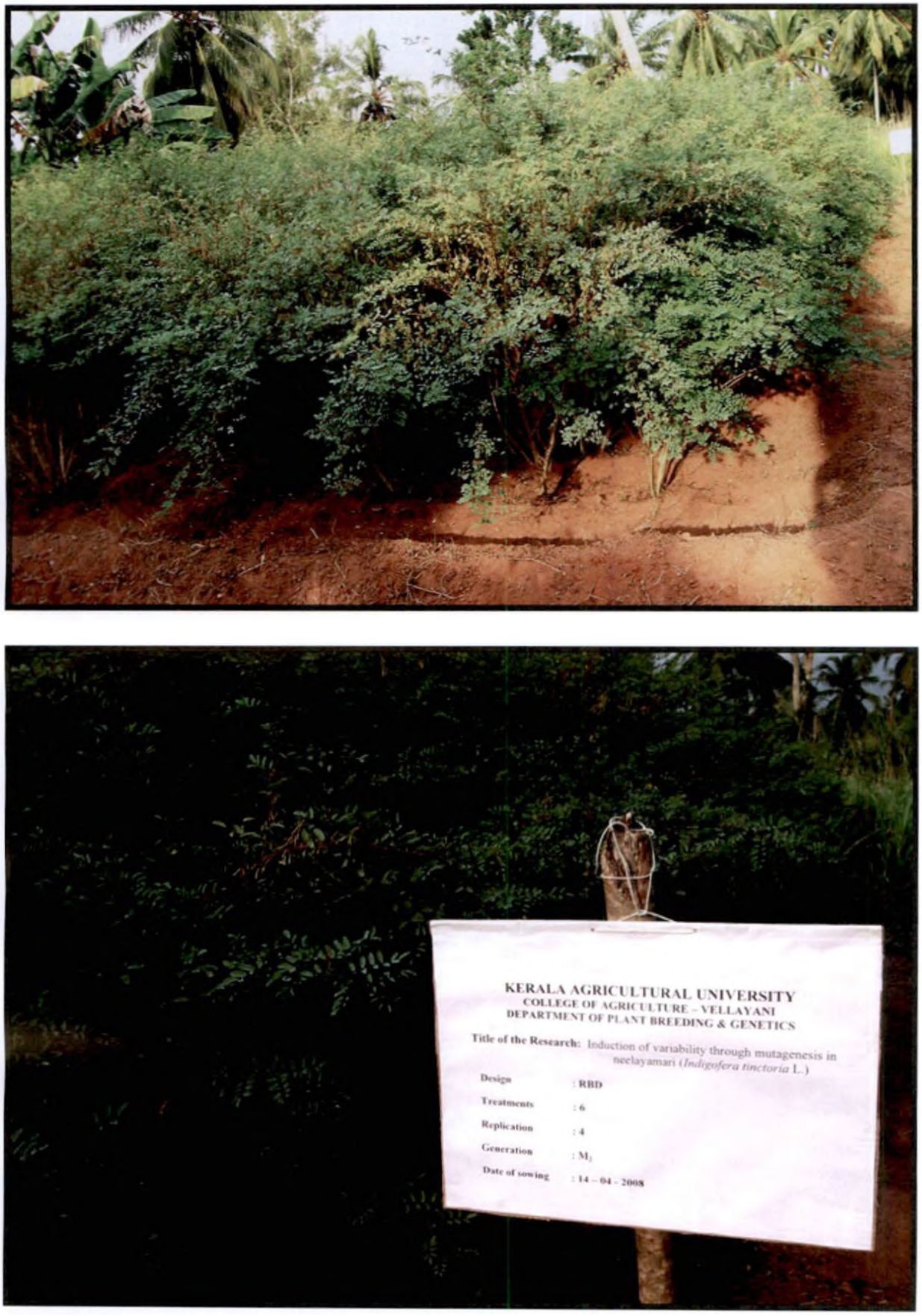
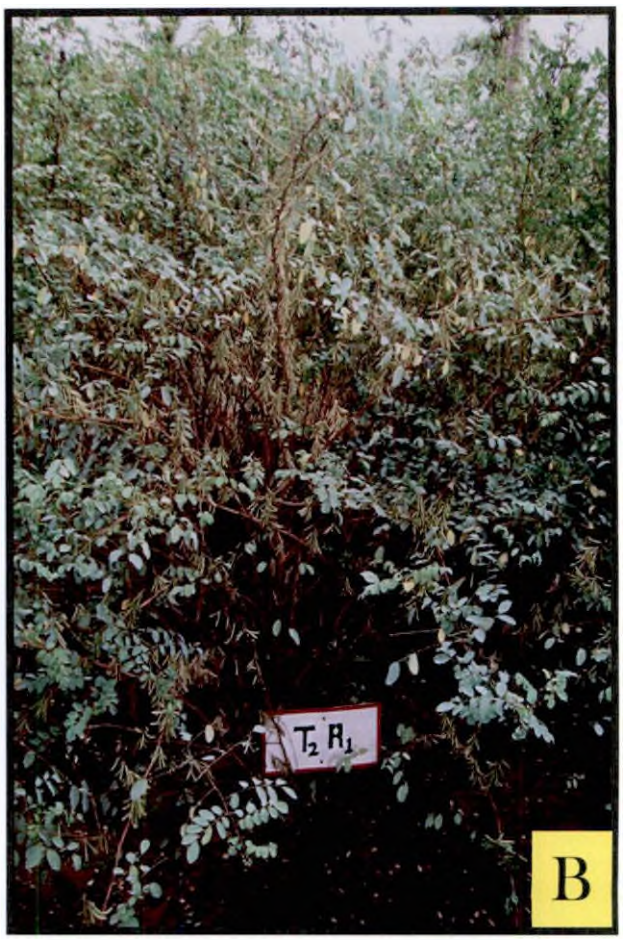


Plate 2. General view of experimental field – M_2 generation

Plate 2. Continued



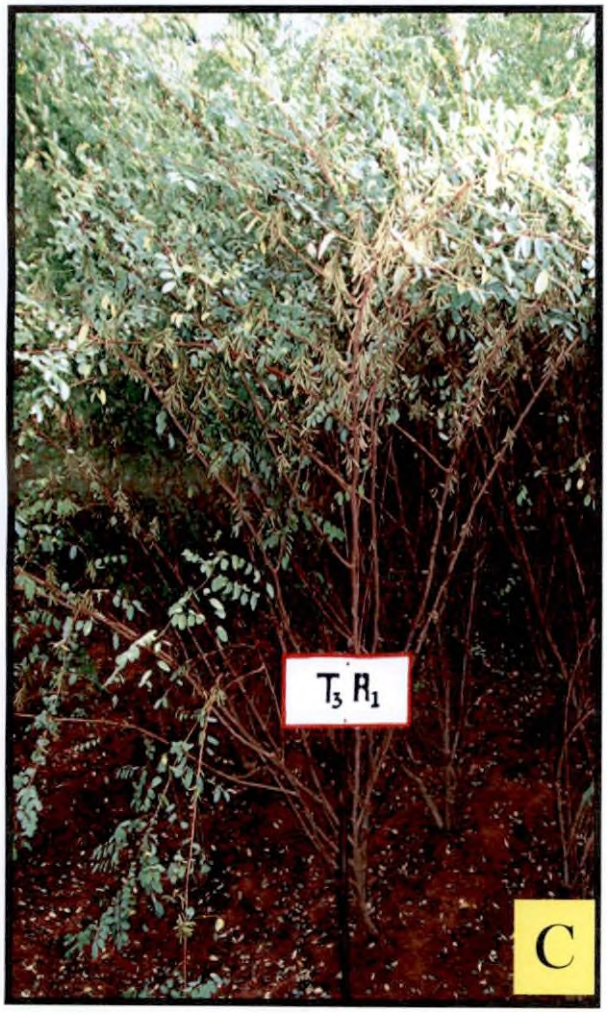
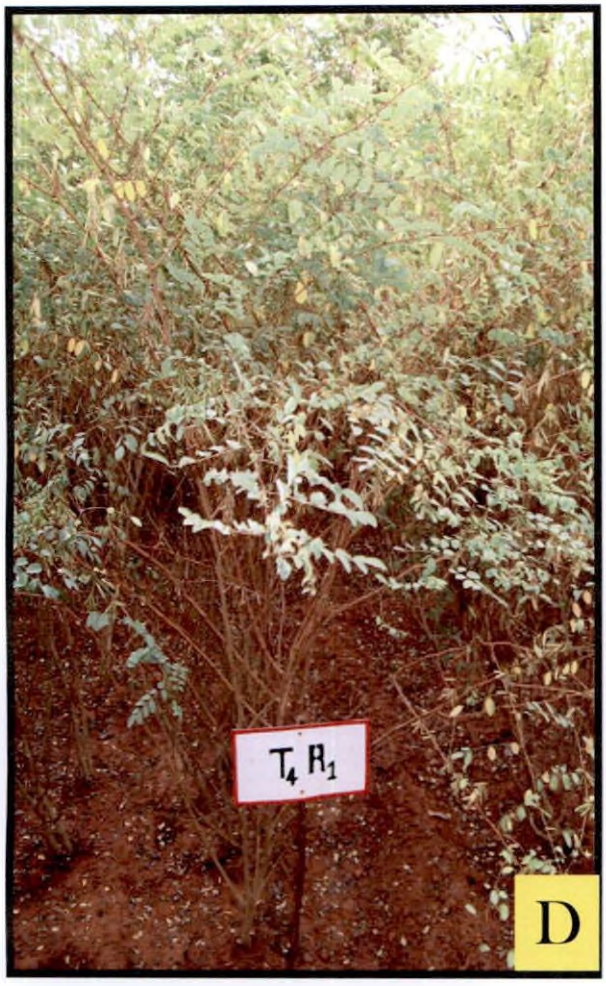


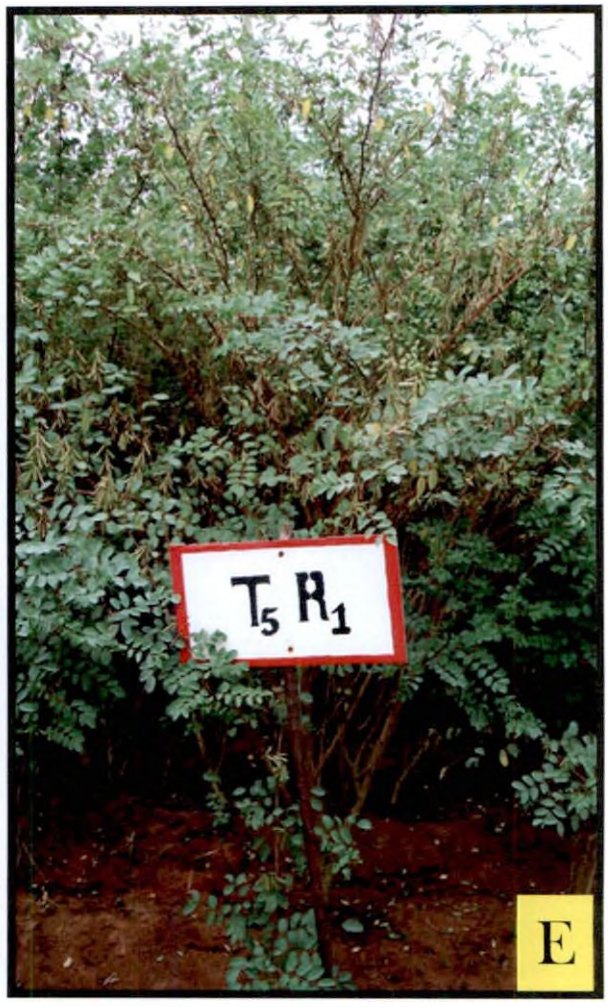
Plate 3. Different treatments of Indigofera tinctoria - M_2 generation

A) T1 - 15.0 KR

B) T2 - 17.5 KR

C) T3 - 20.0 KR





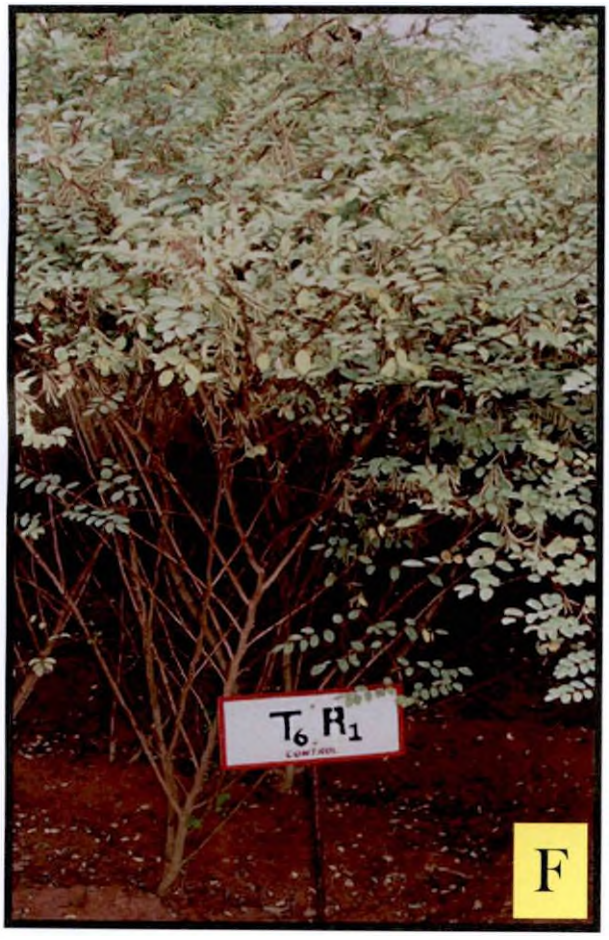


Plate 3. Continued

D) T4 – 22.5 KR

E) T5 – 25.0 KR

F) T6 - Control

7. Girth of stem (cm)

Measurement was taken round the stem at a height of 3 cm from the soil surface using a tag and the length of the tag was taken as the stem girth at pre flowering, flowering and pod maturation stage.

8. Number of leaves per plant

Total number of leaves from the base to the tip of the plant including the branches was counted at 90, 150 and 240 days after sowing. Dropped leaves were counted by their respective nodes.

9. Length of leaves (cm)

The average length of leaves selected at random from the observational plant was recorded.

10. Width of leaves (cm)

The average width of leaves selected at random from the observational plant was recorded.

11. Colour of leaves

The colour of leaves of the observational plants in each treatment was recorded.

12. Leaf area (cm^2)

The leaf area was calculated by adopting punch method. Fifty punches were made. The discs as well as leaves were dried in hot air oven at 70 ° C and their respective dry weights were recorded. From the data of leaf dry weight leaf area per plant was computed and recorded (Watson, 1952).

13. Leaf area index (LAI)

Leaf area of observational plants, were calculated by adopting punch method. Leaf area index was worked out using the formula proposed by Watson (1952),

$$LAI = \frac{\text{Leaf area of the plant } (cm^2)}{\text{Ground area occupied } (cm^2)}$$

14. 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 preflowering, flowering and pod formation stage and recorded as internodal length in cm.

15. Harvest Index (HI)

Harvest index was calculated at final harvest using the formula

$$HI = \frac{\text{Y econ.}}{\text{Y biol.}} \times 100$$

Where,

Y econ. - dry weight of officinal part

Y biol. - total dry weight of plant

B. Yield and Yield attributes

1. Fresh weight of leaves (g)

The total weight of leaves of the observational plants in each treatment was recorded at pre flowering, flowering and pod maturation stage.

2. Dry weight of leaves (g)

The leaves were oven dried at 70 ± 5 ° C to a constant weight and the dry weight was recorded at pre flowering, flowering and pod maturation stage.

3. Fresh weight of shoots (g)

Weight of the stem and leaves of the observational plants from each treatment was recorded at pre flowering, flowering and pod maturation stage.

4. Dry weight of shoots (g)

The shoots were oven dried at 70 ± 5 ° C to a constant weight and the dry weight of shoot was recorded at pre flowering, flowering and pod maturation stage.

5. Fresh weight of pods (g)

The fresh weight of the pods of each treatment during harvesting stage was recorded.

6. Dry weight of pods (g)

The pods with seeds were oven dried at 70 ± 5 ° C to a constant weight and the dry weight of pods was recorded.

II. Reproductive biology

1. Number of days for flowering

Total number of days required for commencing flowering from the date of sowing in fifty per cent of plants in a plot was recorded in each treatment.

2. Time of anthesis

Ten mature flower buds in every treatment in a replication were tagged at 6 am and the time of flower opening was noted and the mean value recorded.

3. Time of anther dehiscence

Flower buds were tagged in group of ten at the time of anthesis. They were observed with a hand lens at two hour interval for anther dehiscence. Appearance of longitudinal splits in the pollen sac indicated the commencement of anther dehiscence. When more than three anthers in a flower liberated pollen grains it was reckoned as having completed anther dehiscence. The observation was repeated for three days with another group of flowers and the average was worked out (Mathew, 2002).

4. Pollen sterility

The Aceto-carmine technique was utilised to find out the percentage of pollen sterility. Five freshly opened flowers were collected at random, from each treatment. The pollen grains from each flower were dusted onto a clean microslide. A drop of 2 percent Aceto-carmine-glycerine mixture (1:1) was placed over the pollen grains and then mounted with a cover slip. The microslides were observed after 10 minutes. Pollen grains, which were fully stained, were taken as fertile; whereas those which were unstained and shrunken were scored as sterile.

5. Percentage of pod set

The total number of pods produced in an inflorescence in each replication in a treatment was noted in the observational plants. The percentage of pod set was calculated using the total number of flowers produced per inflorescence.

6. Number of days for pods harvesting

Number of days taken for harvest from the first day of flowering was recorded in each treatment.

7. Hundred seed weight

Seeds were taken from the pods collected from each plant of each treatment, hundred seed weight determined and for each treatment the mean weight worked out.

8. Seed colour

The colour of seeds of the observational plants in each treatment was recorded.

III. Biochemical analysis

Indigotin content

The indigotin content was estimated using a modified procedure of Dai et al. (1987).

Procedure

1. Leaves of plants showing morphological variation in the M_1 generation were collected and dried. The dried leaves of each sample were powdered and then dried for 2 hours at 150 ° C.
2. 80 mg powder was weighed out into a clean beaker.
3. 50 ml chloroform was added to the powder and covered with a tin foil.
4. Kept in a water bath at 80 °.C for 20 minutes.
5. The contents of the beaker were allowed to cool down to room temperature using cold water without shaking.
6. The supernatant was transferred to a 100 ml volumetric flask.
7. Repeated the same procedure, and collected the supernatant in the same volumetric flask.
8. Made up the volume upto 100 ml using chloroform.
9. The absorbancy of the solution was read at 325 nm, against the solvent (chloroform) as blank, using a UV visible recording spectrophotometer (Shimadzu – Model – UV – 2100S)

10. Comparison of the values of the samples and the control was done.

Statistical analysis

The data collected for the various characters in the M_1 and M_2 generations were tabulated and mean values were subjected to statistical analysis. Computations were carried out using the software GENRES (developed at the Tamilnadu Agricultural University, Coimbatore).

Components of variance

The mean squares between treatments consisted of variances attributable to genotype, environment and phenotype (Singh and Chaudhary, 1985).

For each character the phenotypic and genotypic components of variance were estimated by equating the expected value of mean squares (MS) to the respective variance components (Jain, 1982). Based on this the following variance components were estimated.

i) Genotypic variance, $\sigma^2 g = \frac{MST - MSE}{r}$

ii) Environmental variance, $\sigma^2 e = MSE$

iii) Phenotypic variance, $\sigma^2 p = \sigma^2 g + \sigma^2 e$

Co efficient of variation

It is a unit of measurement used for comparison of variation of different characters measured in different units. Genotypic and phenotypic coefficients of variation were worked out using the estimate of $\sigma^2 g$ and $\sigma^2 p$ and expressed in percentage (Burton, 1952) for each trait.

i) Phenotypic coefficient of variation (PCV)

$$= \frac{\sigma p}{\text{Mean}} \times 100$$

ii) Genotypic coefficient of variation (GCV)

$$= \frac{\sigma g}{\text{Mean}} \times 100$$

Heritability

For each trait heritability (broad sense) was estimated as the ratio of genotypic variance to phenotypic variance and expressed as percentage (Jain, 1982).

$$\text{Heritability } (H^2) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Heritability per cent was categorized as suggested by Johnson *et al.* (1955) *viz.*, low (0 – 30), moderate (30 – 60) and high (above 60).

Genetic Advance (Johnson et al. 1955 and Allard 1960)

Genetic advance which measures the change in mean genotypic level of the population brought about by selection depends upon standardised selection differential, heritability and phenotypic standard deviation (Allard, 1960).

Genetic advance as percentage of mean was estimated as per the method suggested by Lush (1940) and Johnson et al. (1955) for each trait as

$$\text{Genetic advance, GA} = \frac{k\, H^2 \sigma p}{\overline{X}} \times 100$$

Where, k is the standardised selection differential (k = 2.06) at five per cent selection intensity and $\overline{X}$ is the mean of the character over all accessions. Genetic advance was categorized into low (below 10 per cent), moderate (10-20 per cent) and high (above 20 per cent) as suggested by Johnson et al. (1955).

Correlation Analysis

Phenotypic, genotypic and environmental correlation coefficients were worked out for two characters X_i and X_j as

$$\text{Genotypic correlation } (r_{gij}) = \frac{\sigma_{g_{ij}}}{\sigma_{g_i} \times \sigma_{g_j}}$$

$$\text{Phenotypic correlation } (r_{pij}) = \frac{\sigma_{p_{ij}}}{\sigma_{p_i} \times \sigma_{p_j}}$$

$$\text{Environmental correlation } (r_{eij}) = \frac{\sigma_{e_{ij}}}{\sigma_{e_i} \times \sigma_{e_j}}$$

where, σ_{gij}, σ_{pij} and σ_{eij} denote the genotypic, phenotypic and error covariances between two traits X_i and X_j respectively.

Path coefficient Analysis

Path analysis is applied to identify relatively important component characters (which are the independent v variables) as 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

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

Where A is the genotypic inter-correlation matrix with respect to independent variables, B is the column vector of path coefficients and C is the column vector of genotypic correlation coefficient between the dependent and independent variables. Vector B provides estimates of path coefficient 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 from unknown and uncontrollable factor was also estimated using vector B (Dabholkar, 1992).

The direct and indirect effects were calculated and classified into very high (> 1), high (0.30 – 0.99), moderate (0.20 – 0.29), low (0.10 – 0.19) and negligible (0.00 – 0.09) (Lenka and Mishra, 1973).

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

$I = b_1x_1 + b_2x_2 + \ldots + b_nx_n$ Where n characters were involved and the genetic worth H, of a plant is defined as $H = a_1G_1 + a_2G_2 + \ldots + a_nG_n$ Where G_1 $G_2 \ldots G_n$ represents the genotypic value of the characters and a_1, a_2 $\ldots a_n$ 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 P b = G a where P and G are the phenotypic and genotypic variance – covariance matrix respectively, b is the column vector of b coefficients and 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.

Results

4. RESULTS

The effect of gamma irradiation on neelayamari (*Indigofera tinctoria* L.) in the M_1 and M_2 generation was studied and the results are presented below.

4.1. Sensitivity study

The effect of various doses of mutagen in causing mutagenic changes in *Indigofera tinctoria* (L.) was studied. The germination percentage was used to calculate the LD_{50} of the mutagen under the experimental condition.

Different doses of gamma rays were tried under laboratory conditions, based on the percentage of variation of germination of the seeds over the control subjected to irradiation (Table 2).

Table 2. Effect of varying doses of gamma rays on the germination and survival of seedlings on *Indigofera tinctoria* (Laboratory conditions)

Dose of gamma irradiation (KR)	Germination %	Depression of germination over control (%)	Survival %	Depression of survival over control (%)
Control	82.00	0	70.50	0
5	61.40	74.88	62.30	88.37
10	55.20	67.32	57.50	81.56
15	52.00	63.42	52.65	74.68
20	58.50	71.34	47.24	67.00
25	52.75	64.33	41.48	58.84
30	40.25	49.09	38.25	54.25
35	34.28	41.81	35.50	50.36
40	31.60	38.54	33.00	46.81

As the dose increased up to 20 KR, the percentage of variation of germination over the control was 71.34 per cent and thereafter there was a decline of germination percentage. Hence LD_{50} of the gamma rays was fixed as 20 KR. Therefore five doses of mutagen at regular intervals namely, 15.0, 17.5, 20.0, 22.5 and 25.0 KR were applied on the seeds and tried in field trials.

4.2. M_1 Generation

4.2.1. Mean performance

The analysis of variance revealed significant differences among the treatments for all the fourteen characters studied (Table 3).

The mean value of the six treatments for all the characters namely germination percentage, survival percentage, plant height, stem girth, number of branches, number of leaves, leaf area index, fresh weight of leaves, dry weight of leaves, days to first flowering, number of inflorescence, pollen sterility, dry weight of shoot and fresh weight of shoot are presented in Table 4.

The germination percentage of the treatments was ranged from 46.18 (25.0 KR) to 78.42 (control) with an average of 56.67 percentage. The survival percentage of the treatments was ranged from 83.28 (25.0 KR) to 89.33 (Control) with an average of 86.17 percentage.

The plant height of the treatments was maximum in control (229.35 cm) and minimum in 25.0 KR (173.64 cm) with an average of 196.95 cm. The girth of stem of the treatments was raged from 6.20 cm (25.0 KR) to 6.55 cm (control) with an average of 6.34 cm. The number of branches of the treatments was ranged from 18.84 (25.0 KR) to 26.03 (control0 with an average of 22.37. The number of leaves of the treatments was ranged from 683.37 (25.0 KR) to 854.75 (control) with an average of 764.22.

Table 3. Analysis of variance for fourteen characters in *Indigofera tinctoria*

Sl. No	Characters	Mean square			
		Replication	Treatments	Errors	F-value
1	Germination percentage	4.87	555.50	0.63	879.99
2	Survival percentage	0.87	25.52	0.80	31.85
3	Plant height	1.33	1573.40	1.24	1265.75
4	Girth of Stem	0.0093	0.09	0.0054	16.52
5	Number of branches	0.09	21.19	0.32	67.11
6	Number of leaves	1.00	16028.00	29.47	543.94
7	Leaf area index	0.003	1.27	0.0051	250.62
8	Fresh weight of leaves	110.33	15303.90	49.40	309.80
9	Dry weight of leaves	18.06	1570.43	25.47	61.66
10	Days to first flowering	4.38	242.94	1.54	157.66
11	Number of inflorescence	13.44	15774.20	55.18	285.88
12	Pollen sterility	1.18	1280.75	2.80	458.25
13	Dry weight of shoots	44.14	9043.95	38.55	234.62
14	Fresh weight of shoots	478.22	116000.00	147.02	789.00

Table 4. Treatment differences with respect to various characters in *Indigofera tinctoria* – M_1 generation

Treatments	Germination %	Survival %	Plant height (cm)	Girth of Stem (cm)	No. of branches	No. of leaves	LAI	Fresh wt. of leaves (g)	Dry wt. of leaves (g)	Days to first flowering	No. of inflores -cence	Pollen sterility	Dry wt. of shoot (g)	Fresh wt. of shoot (g)
15.0 KR	57.13	88.38	207.08	6.285	22.92	804.16	4.964	602.07	138.19	137.5	648.13	39.69	316.25	1734.75
17.5 KR	53.01	87.47	199.32	6.230	22.43	783.32	4.556	566.55	125.56	141.0	616.34	50.55	266.80	1679.37
20.0 KR	51.19	84.35	190.10	6.500	21.90	752.03	4.285	555.56	119.79	142.3	588.48	58.49	247.27	1617.19
22.5 KR	48.09	84.25	182.18	6.250	22.08	707.69	4.388	561.43	127.72	145.8	557.78	64.07	243.93	1539.70
25.0 KR	46.18	83.28	173.64	6.200	18.84	683.37	4.270	451.05	101.89	148.8	526.73	69.79	230.54	1510.53
Control	78.42	89.33	229.35	6.550	26.03	854.75	5.719	633.81	161.33	126.0	700.45	21.11	351.36	1979.52
Mean	55.67	86.17	196.95	6.336	22.37	764.22	4.697	561.72	129.08	140.3	606.32	50.62	276.02	1676.84
SE	0.562	0.633	0.788	0.052	0.397	3.838	0.050	4.970	3.569	0.878	5.253	1.182	4.390	8.574
CD	1.197	1.349	1.680	0.111	0.847	8.181	0.107	10.593	7.607	1.871	11.196	2.520	9.358	18.275

SE - Standard error of mean
CD - Critical difference at 5 per cent level

The leaf area index of the treatments was maximum in control (5.72 cm^2) and minimum in 25.0 KR (4.27 cm^2) with an average of 4.70 cm^2.

The fresh weight of leaves of the treatments was ranged from 451.05 g (25.0 KR) to 633.81 g (control) with an average of 561.72 g. The dry weight of leaves of the treatments was ranged from 101.89 g (25.0 KR) to 161.33 g (control) with an average of 129.08 g.

The days to first flowering of the treatments was maximum in 25.0 KR (148.8) and minimum in control (126.0) with an average of 140.3.

Number of inflorescence of the treatments was maximum in control (700.45) and minimum in 25.0 KR (526.73) with an average of 606.32. The pollen sterility of the treatments was maximum in 25.0 KR (69.79) and minimum in control (21.11) with an average of 50.62.

The dry weight of shoot of the treatments was ranged from 230.54 g (25.0 KR) to 351.36 g (control) with an average of 276.02. The fresh weight of shoot of the treatments was ranged from 1510.53 g (25.0 KR) to 1979.52 g (control) with an average of 1676.84 g.

4.2.2. Genetic Parameters

The phenotypic, genotypic and environmental variances for the various characters are presented in Table 5. Estimates of variance makes up the major part of the phenotypic variance with very little contribution by the environment.

4.2.2.1. Coefficient of Variation

The phenotypic coefficient of variation, genotypic coefficient of variation and environmental coefficient of variation are presented in Table 5. The

Table 5. Estimates of genetic parameters for fourteen characters in 6 treatments of *Indigofera tinctoria* – M_1 generation

Sl. No.	Characters	Variance			Coefficient of variation (%)		Heritability as % (H^2)	Genetic advance as % of mean
		σ_g^2	σ_p^2	σ_e^2	PCV	GCV		
1	Germination %	138.72	139.35	0.63	21.21	21.16	99.55	43.49
2	Survival %	6.18	6.98	0.80	3.07	2.89	88.52	5.59
3	Plant height	393.04	394.28	1.24	10.08	10.07	99.68	20.70
4	Girth of stem	0.021	0.027	0.005	2.57	2.29	79.51	4.21
5	No. of branches	5.22	5.53	0.32	10.52	10.21	94.29	20.43
6	No. of leaves	3999.63	4029.10	29.47	8.31	8.28	99.27	16.98
7	LAI	0.315	0.320	0.005	12.05	11.96	98.42	24.43
8	Fresh wt of leaves	3813.63	3863.03	49.40	11.07	10.99	98.72	22.50
9	Dry wt of leaves	386.24	411.71	25.47	15.72	15.23	93.81	30.38
10	Days to first flowering	60.35	61.89	1.54	5.61	5.54	97.51	11.26
11	No. of inflorescence	3929.76	3984.93	55.18	10.41	10.34	98.62	21.15
12	Pollen sterility	319.49	322.28	2.80	35.47	35.31	99.13	72.43
13	Dry wt of shoots	2251.35	2289.90	38.55	17.34	17.19	98.32	35.11
14	Fresh wt of shoots	28963.24	29110.27	147.02	10.18	10.15	99.49	20.85

environmental coefficients of variation values do not exhibit much variation. The PCV and GCV of the characters are given in the Table 5 and Fig. 1.

4.2.2.1.1. Phenotypic Coefficient of Variation (PCV)

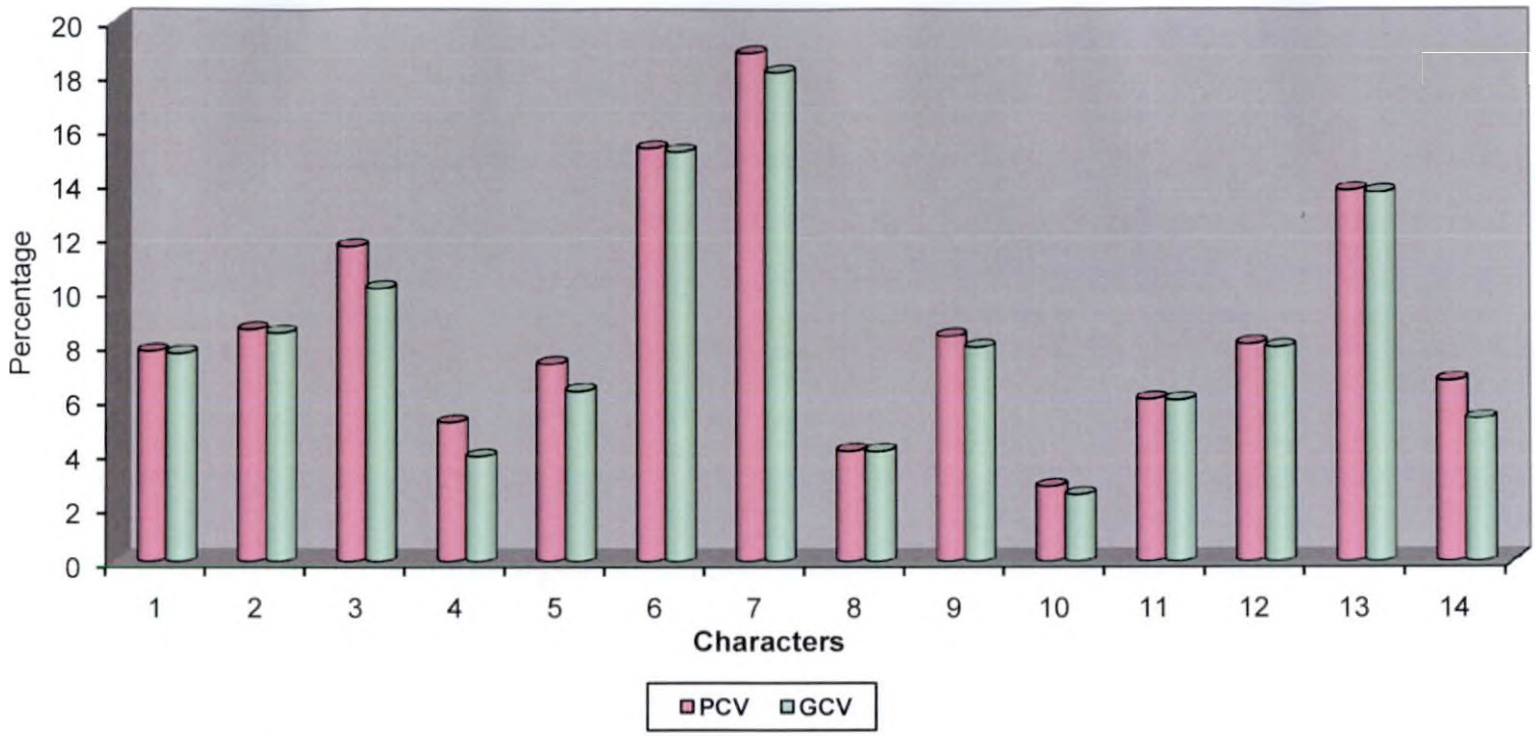
The PCV was maximum for pollen sterility (35.47). The germination percentage (21.21), dry weight of shoot (17.34), dry weight of leaves (15.72), leaf area index (12.05) and fresh weight of leaves (11.07) also had high PCV indicating high degrees of variation. PCV was very less for stem girth (2.57) and survival percentage (3.07).

4.2.2.1.2. Genotypic Coefficient of Variation (GCV)

The highest value of GCV was observed for pollen sterility (35.31); germination percentage (21.16), dry weight of shoot (17.19), dry weight of leaves (15.23), leaf area index (11.96) and fresh weight of leaves (10.99) also recorded high values. Stem girth and survival percentage had less GCV of 2.29 and 2.89 respectively.

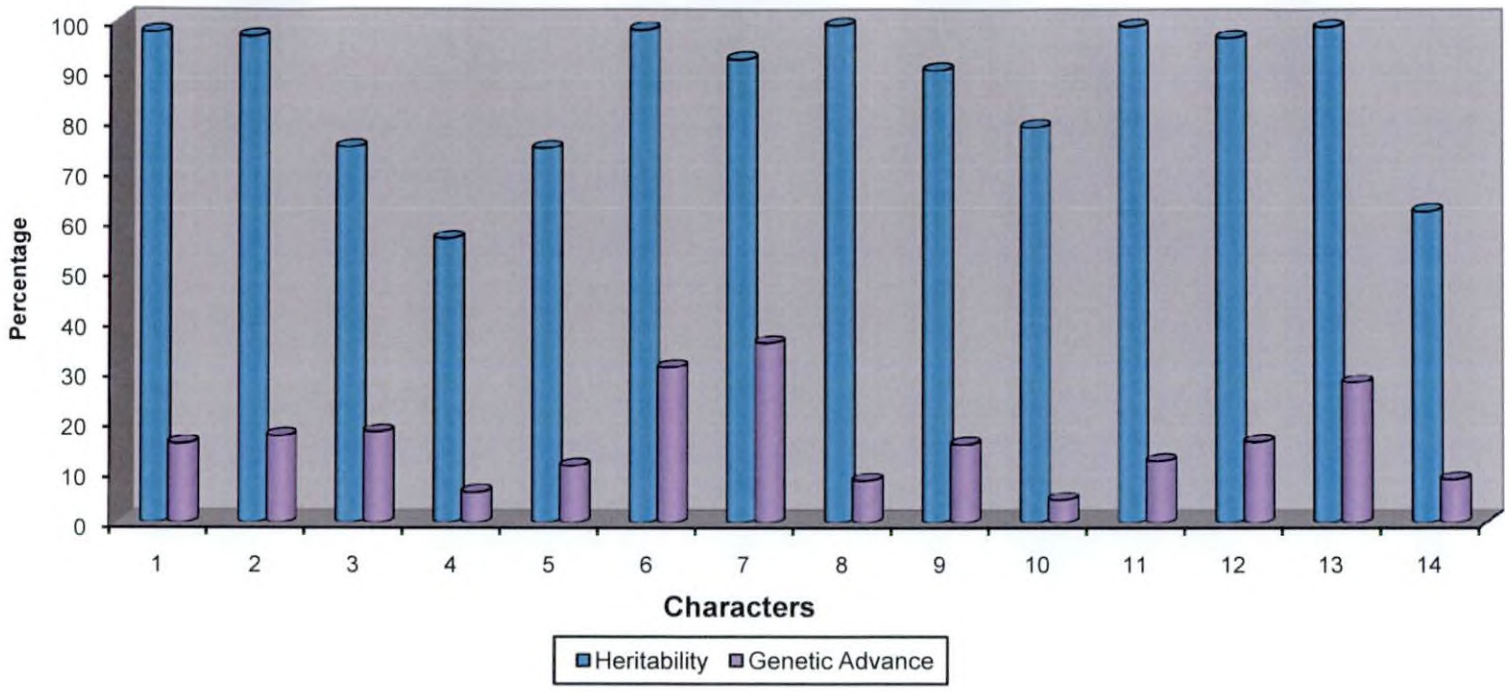
4.2.2.2. Heritability (broad sense)

The heritability estimate recorded for the various characters are given in Table 5 and Fig. 2. According to the classification suggested by Robinson et al. (1949) in this work germination percentage, survival percentage, plant height, stem girth, number of branches, number of leaves, leaf area index, fresh weight of leaves, dry weight of leaves, days to first flowering, number of inflorescence, pollen sterility, dry weight of shoot and fresh weight of shoot had high heritability estimates.



1. Germination
2. Survival
3. Plant height
4. Girth of stem
5. No. of branches
6. No. of leaves
7. Leaf area index
8. Fresh weight of leaves
9. Dry weight of leaves
10. Days to first flowering
11. No. of inflorescence
12. Pollen sterility
13. Dry weight of shoots
14. Fresh weight of shoots

Fig 1. PCV & GCV for the characters with different doses of gamma rays - M1 generation



1. Germination
2. Survival
3. Plant height
4. Girth of stem
5. No. of branches
6. No. of leaves
7. Leaf area index
8. Fresh weight of leaves
9. Dry weight of leaves
10. Days to first flowering
11. No. of inflorescence
12. Pollen sterility
13. Dry weight of shoots
14. Fresh weight of shoots

Fig 2. Heritability and Genetic advance for the characters with different doses of gamma rays - M1 generation

4.2.2.3. Genetic Advance (as percentage of mean)

The genetic advance estimates of the various characters is presented as percentage of mean and given in Table 5 and Fig. 2. The highest estimate of genetic advance was observed for pollen sterility (72.43). According to the classification of Robinson et al. (1949) germination percentage, dry weight of shoot, dry weight of leaves, leaf area index, fresh weight of leaves, fresh weight of shoot, plant height and number of branches had high genetic advance, while number of leaves and days to first flowering had moderate genetic advance. The low genetic advance was observed for stem girth (4.21) and survival percentage (5.59).

High heritability coupled with high genetic advance was observed for plant height, germination percentage, fresh weight of shoot, number of leaves, pollen sterility, fresh weight of leaves, number of inflorescence, leaf area index, days to first flowering, number of branches and dry weight of leaves.

4.2.3. Morphological abnormalities

The gamma ray treated population had exhibited different kinds of abnormalities like variation in the size and colour of the cotyledons, foliar abnormalities, changes in the height and number of branches, etc.

4.2.4. Chlorophyll chimeras

Plants with chlorophyll deficient patches on the leaves were noticed in the 22.5 KR and 25.0 KR doses of gamma rays, while whole chimeric plants were completely absent in the M1 generation in all the treatments used in the present study. The chlorophyll deficient patches in the plants later disappeared.

4.3. M_2 Generation

Morphological and yield attributes of plants that received various doses of mutagen in M_2 generation were compared statistically and the results are presented below.

4.3.1. Mean performance of growth parameters

The analysis of variance showed significant differences among the treatments for all the characters studied at all the stages.

4.3.1.1. Germination of seeds

The data on mean percentage of germination of seeds for various treatments in the M_2 generation are presented in Table 6. The germination percentage was affected by gamma rays. A progressive decrease in the germination percentage was observed with increasing doses of gamma rays. The treatment 25.0 KR exhibited a low germination percentage. The germination percentage ranged from 45.50 (25.0 KR) to 75.25 (control) with an average of 54.97.

4.3.1.2. Survival of plants

The data on mean percentage of survival of plants in the M_2 generation are presented in Table 6. At all three stages i.e. 30^{th}, 60^{th} and 90^{th} days after sowing, survival percentage increased with increases in the doses of mutagen. The treatment 25.0 KR exhibited a low survival percentage. The survival percentage of the plants in various treatments ranged from 59.13 (25.0 KR) to 82.90 (control) with an average of 69.93.

Table 6. Treatment differences with respect to various characters in *Indigofera tinctoria* – M_2 generation

Treatments	Germination %	Survival %	Length of leaves (cm)	Width of leaves (cm)	Leaf area (cm^2)	LAI	Internodal length (cm)	Harvest index	Fresh weight of pods (g)	Dry weight of pods (g)	100 seed weight (g)	Indigotin content (%)
15.0 KR	57.38	71.78	2.533	1.250	7048.78	3.388	7.01	0.377	567.18	184.88	0.389	1.827
17.5 KR	53.50	70.20	2.482	1.250	7016.51	3.285	6.60	0.394	562.30	181.18	0.368	1.812
20.0 KR	48.75	69.10	2.362	1.225	7051.54	3.556	6.87	0.427	551.79	177.25	0.387	1.862
22.5 KR	49.45	66.50	2.485	1.350	7031.84	3.964	6.55	0.400	531.42	168.48	0.354	1.996
25.0 KR	45.50	59.13	2.110	1.275	7009.48	3.270	6.48	0.376	507.37	147.34	0.322	2.048
Control	75.25	82.90	2.563	1.300	7138.55	4.719	7.58	0.250	639.05	222.60	0.401	1.788
Mean	54.97	69.93	2.422	1.275	7049.45	3.697	6.85	0.371	559.85	180.29	0.370	1.889
SE	1.404	1.301	0.083	0.051	3.373	0.50	0.085	0.031	5.703	1.825	0.018	0.055
CD	2.993	2.774	0.178	0.108	7.190	0.107	0.182	0.065	12.155	3.890	0.039	0.177
F- value	117.30	71.02	8.07	1.57	385.88	250.79	46.35	8.15	122.50	365.86	5.06	7.58

SE - Standard error of mean
CD - Critical difference at 5 per cent level

4.3.1.3. Plant height

The mean value of the various treatments for the character plant height in various growth stages are presented in Table 7.

At the pre flowering stage the maximum height was observed for control (104.98 cm) and minimum for 25.0 KR (66.86 cm) with an average of 78.33 am. At flowering stage the maximum height was observed for the treatment 22.5 KR (162.40 cm) and minimum for control (137.40 cm) with an average of 148.42 cm. At pod maturation stage the maximum height was observed for the control (271.20 cm) and minimum for 25.0 KR (218.76 cm) with an average of 236.72 cm.

4.3.1.4. Plant spread

The mean value of the various treatments for the character plant spread in various growth stages are given in Table 8.

Mean plant spread exhibited a range of 56.40 cm (25.0 KR) to 78.67 cm (control) with an average of 63.82 cm in the pre flowering stage. The treatment 20.0 KR showed the maximum plant spread of 145.95 cm and control showed the minimum plant spread of 110.70 cm with an average of 122.69 cm during the flowering stage. In pod maturation stage the variation between the plant spread ranged from 148.71 cm (15.0 KR) to 185.19 cm (22.5 KR) with an average of 160.18 cm.

4.3.1.5. Girth of stem

The mean value of the various treatments for the character, girth of stem in various growth stages are presented in Table 9.

Table 7. Treatment differences for the character plant height (cm) at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering (90 DAS)	Flowering (150 DAS)	Pod maturation (220 DAS)
15.0 KR	75.05	141.60	229.18
17.5 KR	77.40	145.55	226.50
20.0 KR	75.71	156.55	239.40
22.5 KR	69.99	162.40	235.28
25.0 KR	66.86	147.03	218.76
Control	104.98	137.40	271.20
Mean	78.33	148.42	236.72
SE	1.738	2.714	1.923
CD	3.704	5.786	4.098
F- value	123.18	23.87	181.92

SE - Standard error of mean
CD - Critical difference at 5 per cent level

Table 8. Treatment differences for the character plant spread (cm) at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering (90 DAS)	Flowering (150 DAS)	Pod maturation (220 DAS)
15.0 KR	62.72	114.65	148.71
17.5 KR	60.61	121.33	154.40
20.0 KR	67.13	145.95	163.92
22.5 KR	57.40	127.20	185.19
25.0 KR	56.40	116.30	159.86
Control	78.67	110.70	149.02
Mean	63.82	122.69	160.18
SE	1.128	2.499	1.638
CD	2.405	5.326	3.492
F- value	106.53	52.05	138.53

SE - Standard error of mean
CD - Critical difference at 5 per cent level

Table 9. Treatment differences for the character girth of stem (cm) at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering (90 DAS)	Flowering (150 DAS)	Pod maturation (220 DAS)
15.0 KR	2.614	5.87	8.35
17.5 KR	3.124	5.99	8.93
20.0 KR	2.368	6.88	9.33
22.5 KR	2.329	6.10	9.00
25.0 KR	2.159	6.06	8.75
Control	3.397	5.08	8.40
Mean	2.665	5.99	8.79
SE	0.041	0.089	0.211
CD	0.088	0.190	0.449
F- value	284.79	83.19	6.26

SE - Standard error of mean
CD - Critical difference at 5 per cent level

At the pre flowering stage the maximum girth was observed for the control (3.40 cm) and minimum for 25.0 KR (2.16 cm) with an average of 2.67 cm. During the flowering stage the range observed was between 5.08 cm (control) and 6.88 cm (20.0 KR) with an average of 5.99 cm. In the pod maturation stage, the stem girth varied between 8.35 cm (15.0 KR) and 9.33 cm (20.0 KR) with an average of 8.79 cm.

4.3.1.6. Number of branches

The mean value of the various treatments for the character number of branches in various growth stages are given in Table 10.

At pre flowering stage the number of branches was the highest for control (8.87) and the lowest for 25.0 KR (6.92) with an average of 7.74. At flowering stage the number of branches was the highest for treatment 22.5 KR (22.47) and the lowest for control (14.30) with an average of 18.82. At pod maturation stage the number of branches was the highest for control (28.31) and the lowest for 15.0 KR (21.38) with an average of 24.14.

4.3.1.7. Number of leaves

The mean value of the various treatments for the character number of leaves in various growth stages are presented in Table 11.

The maximum number of leaves in the pre flowering stage was observed for the control (361.73) and minimum for 25.0 KR (302.34) with an average of 327.38. During flowering stage the maximum number of leaves was recorded for the treatment 22.5 KR (823.14) and minimum for control (644.25) with an average of 753.64. In the pod maturation stage the maximum number of leaves was observed for control (1195.59) and minimum for 25.0 KR (980.75) with an average of 1080.26.

Table 10. Treatment differences for the character number of branches at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering (90 DAS)	Flowering (150 DAS)	Pod maturation (220 DAS)
15.0 KR	8.160	17.54	21.38
17.5 KR	7.858	18.93	23.16
20.0 KR	7.447	20.61	25.39
22.5 KR	7.167	22.47	24.60
25.0 KR	6.918	19.07	22.00
Control	8.870	14.30	28.31
Mean	7.737	18.82	24.14
SE	0.009	0.721	0.997
CD	0.020	1.537	2.126
F- value	11969.00	29.77	12.99

SE - Standard error of mean
CD - Critical difference at 5 per cent level

Table 11. Treatment differences for the character number of leaves at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering (90 DAS)	Flowering (150 DAS)	Pod maturation (220 DAS)
15.0 KR	323.77	728.59	1042.91
17.5 KR	332.07	749.73	1079.18
20.0 KR	327.00	795.13	1107.10
22.5 KR	317.35	823.14	1076.02
25.0 KR	302.34	780.98	980.75
Control	361.73	644.25	1195.59
Mean	327.38	753.64	1080.26
SE	2.213	2.803	28.061
CD	4.717	5.974	59.811
F- value	158.69	1013.69	12.87

SE - Standard error of mean
CD - Critical difference at 5 per cent level

4.3.1.8. Length of leaves

The mean values of the treatments are presented in Table 6. The length of leaves was found to vary between 2.11 cm (25.0 KR) to 2.56 cm (control) with an average of 2.42 cm.

4.3.1.9. Width of leaves

The mean values of the treatments are given in Table 6. The mean leaf width exhibited a range of 1.23 cm (20.KR) to 1.35 cm (22.5 KR) with an average of 1.28 cm.

4.3.1.10. Internodal length

The mean values of the treatments are presented in Table 6. The maximum internodal length was observed for control (7.58 cm) and minimum for 25.0 KR (6.48 cm) with an average of 6.85 cm.

4.3.1.11. Leaf area

The mean value of the treatments for the character leaf area is given in Table 6. Among the six treatments including one control studied the maximum leaf area was found for control (7138.55 cm^2) and minimum for 25.0 KR (7009.48 cm^2) with an average of 7049.45 cm^2.

4.3.1.12. Leaf area index

The mean value of the treatments is given in Table 6. The maximum leaf area index was observed for control (4.72 cm^2) and minimum for 25.0 KR (3.27 cm^2) with an average of 3.70 cm^2.

4.3.1.13. Colour of leaves

There is no difference in colour of leaves between the treatments.

4.3.1.14. Stem colour

There is no difference in colour of stem between the treatments.

4.3.1.15. Harvest index

The mean value of the treatments for the character of harvest index for leaf yield is presented in Table 6. The maximum harvest index was observed for the treatment 20.0 KR (0.427) and minimum for control (0.250) with an average of 0.371.

4.3.2. Mean performance of yield characters

4.3.2.1 Fresh weight of leaves

The mean value of the treatments for the character fresh weight of leaves in various growth stages are presented in Table 12.

The fresh weight of leaves ranged from 66.98 g (25.0 KR) to 109.24 g (control) with an average of 77.18 g in the pre flowering stage. At flowering stage the mean range was from 410.25 g (control) to 548.78 g (22.5 KR) with an average of 479.08 g. At pod maturation stage the mean range was from 749.84 g (15.0 KR) to 841.58 g (control) with an average of 782.91 g. The fresh weight of leaves at pod maturation stage are shown in Fig. 3.

4.3.2.2. Dry weight of leaves

The mean value of the treatments for the character fresh weight of leaves in different growth stages are given in Table 13.

The dry weight of leaves ranged from 20.18 g (17.5 KR) to 43.29 g (control) with an average of 25.44g in the pre flowering stage. In the flowering stage the values ranged from 129.47 g (control) to 204.31 g (22.5 KR) with an average of 176.48 g. In pod maturation stage the values ranged from 161.15 g

Table 12. Treatment differences for the character fresh weight (g) of leaves at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering	Flowering	Pod maturation
15.0 KR	74.82	451.48	749.84
17.5 KR	75.79	469.94	776.78
20.0 KR	66.19	498.09	759.59
22.5 KR	70.07	548.78	785.23
25.0 KR	66.98	495.96	784.48
Control	109.24	410.25	841.58
Mean	77.18	479.08	782.91
SE	2.173	6.763	1.986
CD	4.631	14.415	4.233
F- value	111.06	96.95	519.69

SE - Standard error of mean
CD - Critical difference at 5 per cent level

Table 13. Treatment differences for the character dry weight (g) of leaves at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering	Flowering	Pod maturation
15.0 KR	21.41	163.29	166.47
17.5 KR	20.18	174.46	166.18
20.0 KR	26.29	193.06	161.15
22.5 KR	20.83	204.31	171.68
25.0 KR	20.61	194.26	167.48
Control	43.29	129.47	199.47
Mean	25.44	176.48	172.07
SE	1.704	3.863	3.156
CD	3.632	8.234	6.728
F- value	56.16	100.61	38.43

SE - Standard error of mean
CD - Critical difference at 5 per cent level

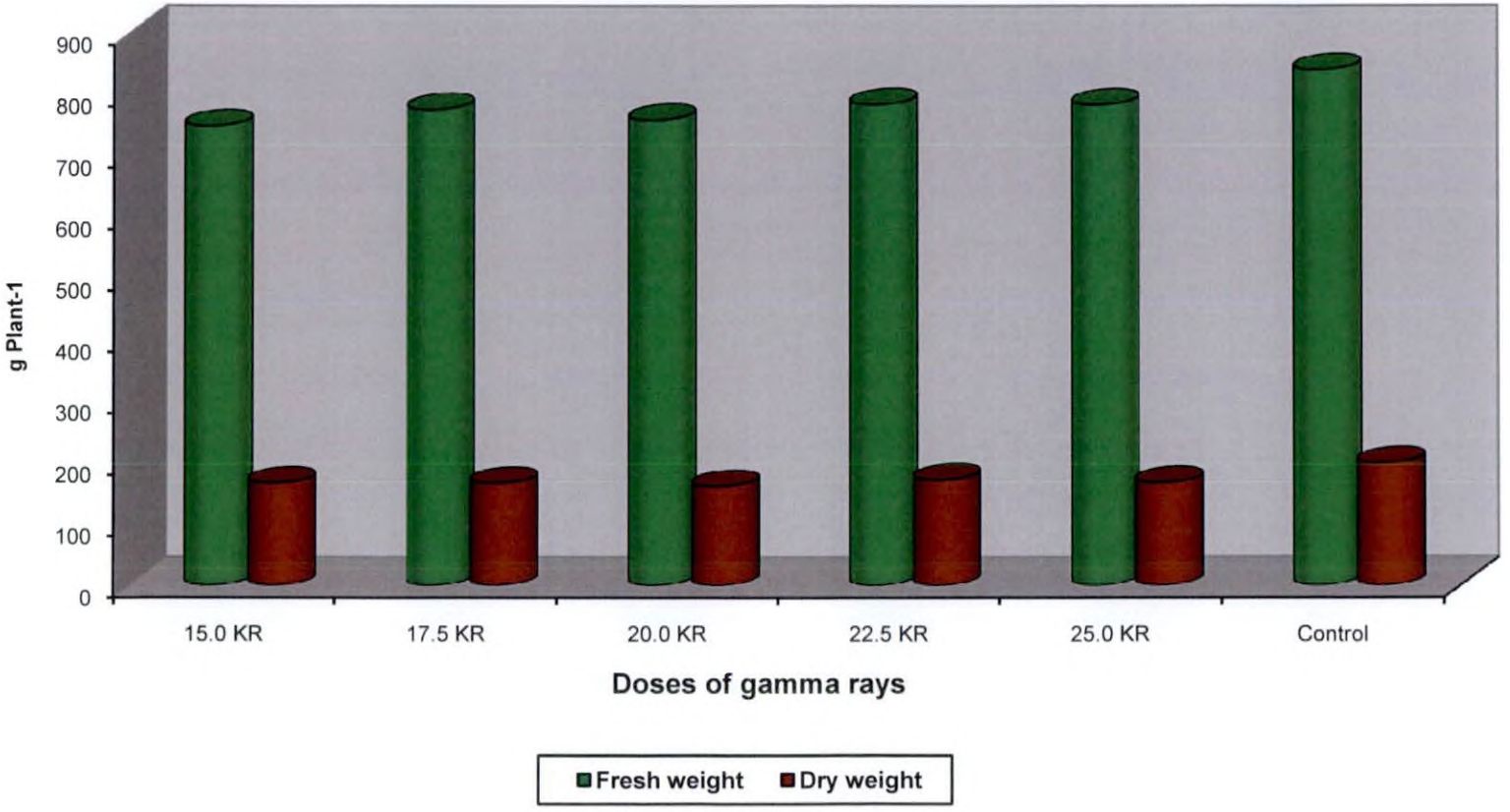


Fig 3. Leaf yield of different doses of gamma rays

(20.0 KR) to 199.47 g (control) with an average of 172.07 g. The dry weight of leaves at pod maturation stage is shown in Fig 3.

4.3.2.3. Fresh weight of shoots

The mean value of the treatments for the character fresh weight of shoot at various growth stages is presented in Table 14. The fresh weight of shoot was greatest for control (401.86 g) and lowest for 25.0 KR (276.39 g) with an average of 316.09 g in the pre flowering stage. The fresh weight of shoot was maximum for 22.5 KR (988.95 g) and minimum for control (890.94) with an average of 955.48 g in the flowering stage. At pod maturation stage the fresh weight of shoot was greatest for control (2508.71 g) and lowest for 17.5 KR (2319.37 g) with an average of 2411.17 g. The fresh weight of shoot at pod maturation stage is shown in Fig 4.

4.3.2.4. Dry weight of shoots

The mean value of the treatments for the character dry weight of shoots in different growth stages is given in Table 15. Dry weight of shoot was found to vary between 63.80 g (25.0 KR) to 105.61 g (control) with an average of 78.95 g in the pre flowering stage. In the flowering stage the range was between 310.30 g (control) to 397.66 g (20.0 KR) with an average of 352.77 g and in the pod maturation stage the range was between 718.06 g (control) to 643.61 g (20.0 KR) with an average of 644.26 g. The dry weight of shoots at pod maturation stage is shown in Fig 4.

4.3.2.5. Fresh and dry weight of pods

The mean value of the treatments for the characters fresh and dry weight of pods is given in Table 6 and Fig 5.

Table 14. Treatment differences for the character fresh weight (g) of shoots at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering	Flowering	Pod maturation
15.0 KR	314.86	932.29	2419.75
17.5 KR	301.79	962.04	2319.37
20.0 KR	306.05	988.83	2399.45
22.5 KR	295.63	988.95	2428.71
25.0 KR	276.39	969.95	2391.03
Control	401.86	890.94	2508.71
Mean	316.09	955.48	2411.17
SE	1.997	2.933	21.685
CD	4.256	6.251	46.220
F- value	969.23	333.42	16.05

SE - Standard error of mean
CD - Critical difference at 5 per cent level

Table 15. Treatment differences for the character dry weight (g) of shoots at different growth stages in *Indigofera tinctoria*

Treatments (Dose of gamma rays)	Stages of plant growth		
	Pre flowering	Flowering	Pod maturation
15.0 KR	74.46	338.24	605.81
17.5 KR	76.99	353.21	637.12
20.0 KR	81.49	397.66	643.61
22.5 KR	71.37	362.30	638.68
25.0 KR	63.80	354.91	622.27
Control	105.61	310.30	718.06
Mean	78.95	352.77	644.26
SE	1.769	3.641	2.692
CD	3.771	7.761	5.737
F- value	131.40	124.47	413.59

SE - Standard error of mean
CD - Critical difference at 5 per cent level

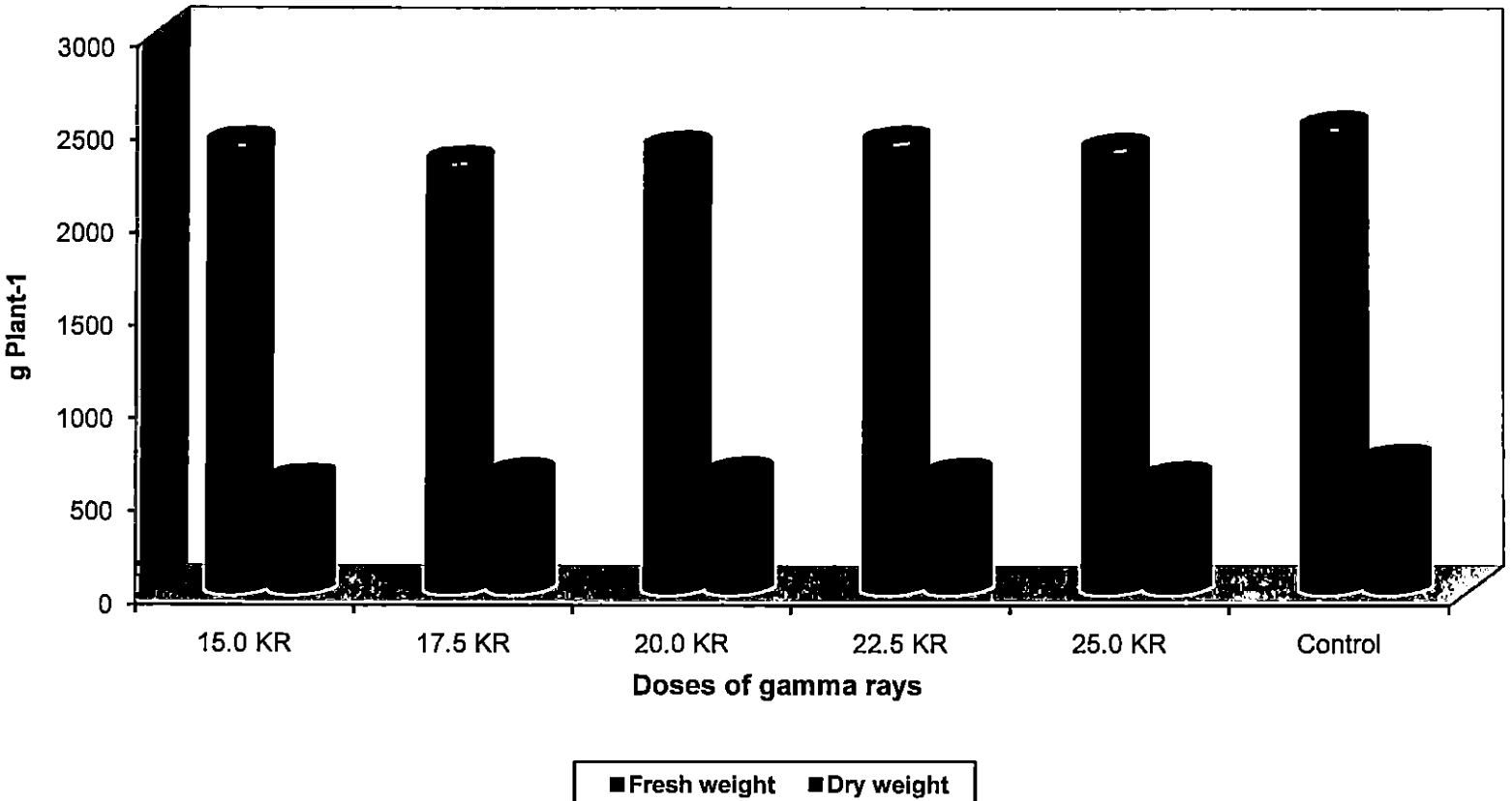


Fig 4. Shoot yield of different doses of gamma rays

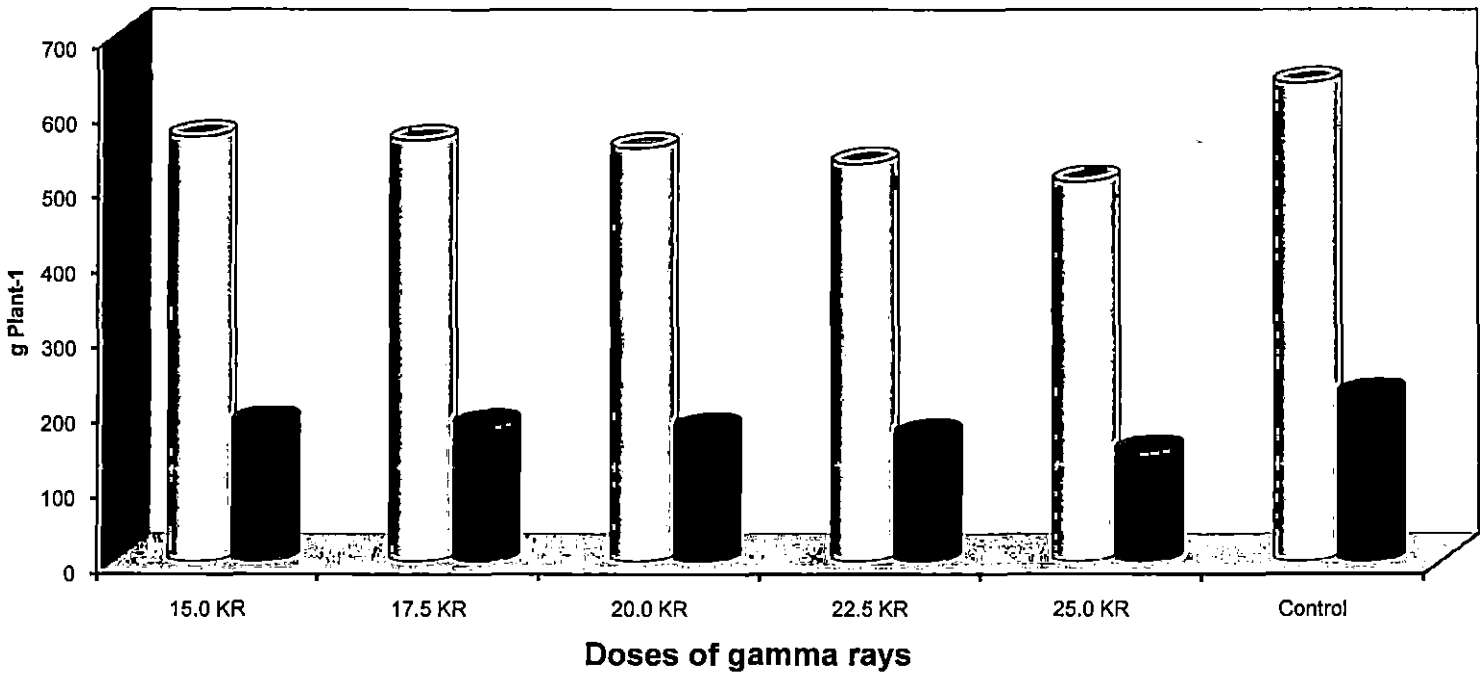


Fig 5. Pod yield of different doses of gamma rays

The fresh weight of the pods was highest for control (639.05 g) and lowest for the treatment 25.0 KR (507.37 g) with an average of 559.85 g. The maximum dry weight was recorded for control (222.60 g) and the treatment 25.0 KR recorded the minimum dry pod weight of 147.34 g with an average of 180.29 g.

4.3.3. Reproductive parameters

4.3.3.1 Floral biology

Flowers numerous, in nearly sessile lax spicate racemes 5 – 10 cm long. Calyx teeth triangular, acute. The corolla is vexillary pink, consisting of a rounded emarginate standard petal, brownish and pubescent at the back and two wing petals adherent to the two keel petals which are greenish in colour and furnished with a spur on each side often bending back elastically. The stamens are di-adelphous, nine unite and one free, anthers uniform. Ovary is sessile, eight to ten or more ovuled with a short incurved style ending in a captite stigma. The stages and development of inflorescence are given in plate 4.

4.3.3.2. Floral character

Floral characters such as number of days for flowering, time of anthesis, time of anther dehiscence, pollen sterility, percentage of pod set, number of days for pods harvesting is shown in Table 16.

4.3.3.3. Number of days for flowering

Number of days for flowering ranged from129.75 to 155.0 days. The control flowered early while the treatment 25.0 KR flowered last, with an average of 146.25 days.

4.3.3.4. Time of anthesis

Time of anthesis was between 8.52 a.m (control) to 10.31 a.m (22.5 KR).

Table 16. Floral characteristics of *Indigofera tinctoria* in different treatments

Treatments	Days to first flowering	Time of anthesis (a.m.)	Time of anther dehiscence (a.m.)	Pollen sterility	Pod set (%)	Days for pod harvesting
15.0 KR	145.00	9.34	11.11	39.30	47.61	94.47
17.5 KR	147.00	9.59	11.25	47.14	42.94	91.44
20.0 KR	150.25	9.51	10.51	53.84	40.38	94.30
22.5 KR	150.50	10.31	10.57	56.43	35.26	94.48
25.0 KR	155.00	10.15	11.08	59.28	34.63	95.39
Control	129.75	8.52	11.20	17.71	56.26	94.41
Mean	146.25	9.49	11.35	45.62	42.85	94.08
SE	1.065	0.063	0.031	1.480	1.459	0.648
CD	2.269	0.135	0.065	3.155	3.110	1.380
F- value	135.88	156.55	226.86	217.71	62.71	8.77

SE - Standard error of mean
CD - Critical difference at 5 per cent level

Plate 4. Stages of development of *Indigofera tinctoria*

A) A branch with Inflorescence

B) A branch with Inflorescence & Pods

4.3.3.5. Time of anther dehiscence

Time of anther dehiscence was between 10.51 a.m (20.0 KR) and 11.25 a.m (17.5 KR).

4.3.3.6. Pollen sterility

The pollen fertility was affected by gamma rays. A decrease in pollen fertility with increase in the doses of mutagen was observed. The treatment 25.0 KR exhibited high pollen sterility (59.28) and control exhibited low pollen sterility (17.71).

4.3.3.7. Percentage of pod set

The highest percentage pod set was observed for control (56.26) and lowest for 25.0 KR (34.63).

4.3.3.8. Number of days for pods harvesting

The number of days for pod harvesting ranged from 94.30 (17.5 KR) to 95.39 (25.0 KR).

4.3.3.9. Hundred seed weight

The mean value of the treatments is given in Table 6. The hundred seed weight was maximum for control (0.401 g) and minimum for 25.0 KR (0.322 g) with an average of 0.370 g.

4.3.3.10. Seed colour

There is no difference in colour of seed between the treatments.

4.3.4. Indigotin content

The mean value of the treatments for indigotin content is given in Table 6. The highest indigotin content was recorded for the treatment 25.0 KR (2.048) followed by 22.5 KR (1.996), 20.0 KR (1.862) and 15.0 KR (1.827). The lowest indigotin content for control (1.788).

4.3.4. Genetic parameters

The phenotypic, genotypic and environmental variances of plant characters such as plant height, plant spread, girth of stem, number of branches, number of leaves, leaf area index, fresh weight of leaves, dry weight of leaves, fresh weight of shoot, dry weight of shoot, fresh weight of pods, dry weight of pods, harvest index and indigotin content are presented in the Table 17. Estimates of variance showed that for all the characters, genetic variance makes up the major part of the phenotypic variance with very little contribution by the environment.

4.3.4.1. Co efficient of variation

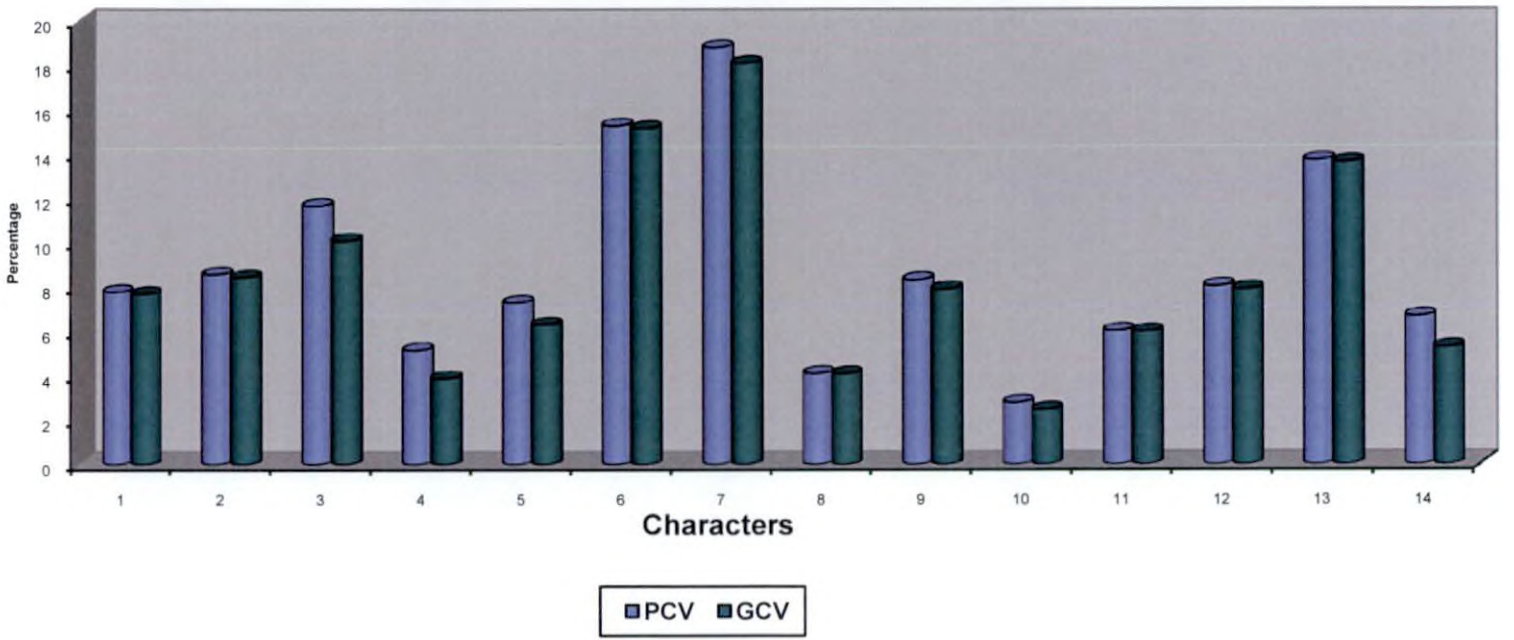
The phenotypic coefficient of variation, genotypic coefficient of variation and environmental coefficient of variation were worked out. The environmental coefficients of variation values do not exhibit much variation. The PCV and GCV of the characters are given in the Table 17 and Fig 6.

4.3.4.1.1. Phenotypic coefficient of variation (PCV)

The PCV was maximum for harvest index (18.84). The leaf area index (15.31), dry weight of pods (13.75), number of branches (11.68) also had high PCV indicating a high degree of variation. PCV was very less for fresh weight of shoot (2.78) and fresh weight of leaves (4.10).

Table 17. Estimates of genetic parameters for various characters of *Indigofera tinctoria* – M_2 generation

Sl. No.	Characters	Variance			Coefficient of variation (%)		Heritability as % (H^2)	Genetic advance as % of mean
		σ_g^2	σ_p^2	σ_e^2	PCV	GCV		
1	Plant height	334.45	341.84	7.39	7.81	7.73	97.84	15.74
2	Plant spread	184.52	189.89	5.37	8.60	8.48	97.17	17.22
3	No. of branches	5.96	7.95	1.99	11.68	10.12	74.99	18.05
4	Girth of stem	0.117	0.206	0.089	5.16	3.89	56.80	6.04
5	No. of leaves	4673.99	6248.83	1574.84	7.32	6.33	74.80	11.28
6	Leaf area index	0.32	0.32	0.005	15.31	15.19	98.42	31.04
7	Harvest index	0.01	0.01	0.00	18.84	18.13	92.57	35.93
8	Fresh wt of leaves	1022.98	1030.87	7.89	4.10	4.09	99.23	8.38
9	Dry wt. of leaves	186.46	206.39	19.93	8.35	7.94	90.35	15.54
10	Fresh wt of shoots	3538.49	4478.93	940.44	2.78	2.47	79.00	4.52
11	Dry wt. of shoots	1494.48	1508.97	14.49	6.03	6.00	99.04	12.30
12	Fresh wt. of pods	1975.76	2040.81	65.04	8.07	7.94	96.81	16.09
13	Dry wt. of pods	607.60	614.26	6.66	13.75	13.67	98.92	28.01
14	Indigotin content	0.01	0.02	0.01	6.71	5.29	62.19	8.60



1. Plant height
2. Plant spread
3. No. of branches
4. Girth of stem
5. No. of leaves
6. Leaf area index
7. Harvest index
8. Fresh weight of leaves
9. Dry weight of leaves
10. Fresh weight of shoots
11. Dry weight of shoots
12. Fresh weight of pods
13. Dry weight of pods
14. Indigotin content

Fig 6. PCV and GCV for the characters with different doses of gamma rays - M2 generation

4.3.4.1.2. Genotypic coefficient of variation (GCV)

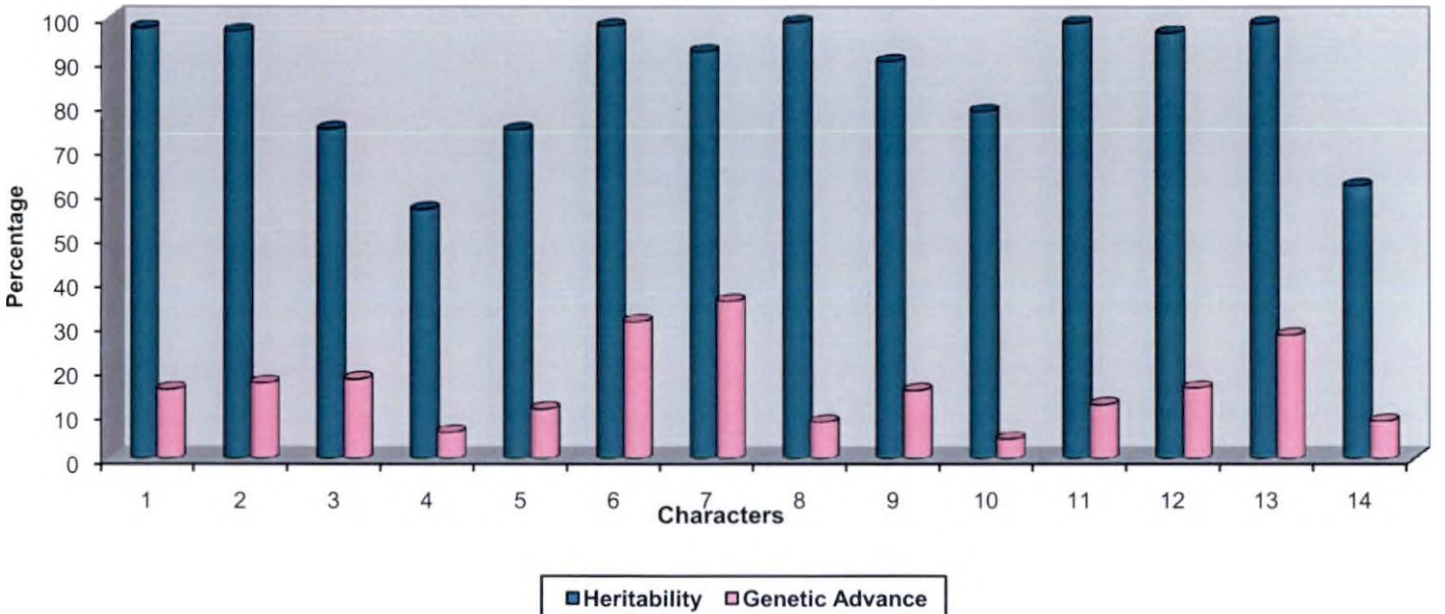
The highest genotypic coefficient of variation was observed for the harvest index (18.13) followed by leaf area index (15.19), dry weight of pods (13.67) and number of branches (10.12). The lowest genotypic coefficient of variation was exhibited by fresh weight of shoot (2.41) and fresh weight of leaves (4.09).

4.3.4.2 Heritability (broad sense)

The heritability estimate recorded for the various characters are given in Table 17 and Fig 7. Very high heritability estimate was observed for fresh weight of leaves (99.23). According to the classification suggested by Robinson et al (1949) in this work dry weight of shoots, dry weight of pods, leaf area index, plant height, plant spread, fresh weight of pods, harvest index, dry weight of leaves, fresh weight of shoots, number of branches, number of leaves and indigotin content also had high heritability estimates. Girth of stem had moderate heritability estimates.

4.3.4.3. Genetic advance

The genetic advance estimates of the various characters as percentage of mean is given in Table 17 and Fig 7. The highest estimate of genetic advance was observed for harvest index (35.93) followed by leaf area index (31.04) and dry weight of pods (28.01). Number of branches, plant spread, fresh weight of pods, plant height, dry weight of leaves, dry weight of shoot and number of leaves had moderate genetic advance. The low genetic advance was observed for fresh weight of shoots (4.52) followed by girth of stem (6.04), fresh weight of leaves (8.38) and indigotin content (8.60).



1. Plant height
2. Plant spread
3. No. of branches
4. Girth of stem
5. No. of leaves
6. Leaf area index
7. Harvest index
8. Fresh weight of leaves
9. Dry weight of leaves
10. Fresh weight of shoots
11. Dry weight of shoots
12. Fresh weight of pods
13. Dry weight of pods
14. Indigotin content

Fig 7. Heritability and Genetic advance for the characters with different doses of gamma rays - M2 generation

High heritability coupled with high genetic advance was observed for leaf area index, dry weight of shoots, dry weight of pods, plant spread, plant height, fresh weight of pods, harvest index, fresh weight of leaves, dry weight of leaves, number of branches and number of leaves.

4.3.5. Correlation studies

The phenotypic, genotypic and environmental correlation coefficients were estimated for 14 characters viz., plant height, plant spread, number of branches, girth of stem, number of leaves, leaf area index, harvest index, fresh weight of leaves, dry weight of leaves, fresh weight of shoots, dry weight of shoots, fresh weight of pods, dry weight of pods and indigotin content.

The results of the correlation analysis are presented under the following subtitles.

a) Correlation between yield and other characters

b) Correlation among the yield components

4.3.5.1. Correlation between yield and other characters

The phenotypic, genotypic and environmental correlation coefficients of yield with other characters are presented in Table 18.

The phenotypic correlation was found to be highly significant and positive for plant height (0.918), leaf area index (0.893), fresh weight of leaves (0.900), number of branches (0.849), number of leaves (0.785) and dry weight of leaves (0.857) where as harvest index (-0.768) showed significant negative correlation. All the characters except plant spread, girth of stem and indigotin content recorded positive correlation with fresh weight of shoots.

The genotypic correlation was found to be highly significant and positive for dry weight of shoots (0.905) followed by leaf area index (0.896), plant height

Table 18. Phenotypic, genotypic and environmental correlation coefficients between yield and other characters in *Indigofera tinctoria*

Sl. No	Characters	Correlation coefficients		
		Phenotypic	Genotypic	Environmental
1	Plant height	0.918**	0.835*	-0.017
2	Plant spread	-0.173	-0.065	0.076
3	No. of branches	0.849*	0.697	0.068
4	Stem girth	-0.171	-0.480	-0.623
5	No. of leaves	0.785*	0.612	0.133
6	Leaf area index	0.893**	0.896**	0.046
7	Harvest index	-0.768*	-0.831*	0.015
8	Fresh wt of leaves	0.900**	0.660	0.080
9	Dry wt. of leaves	0.857*	0.835*	-0.063
10	Dry wt. of shoots	0.696	0.905**	0.031
11	Fresh wt. of pods	0.754	0.810*	-0.061
12	Dry wt. of pods	0.742	0.782*	0.087
13	Indigotin content	-0.287	-0.352	-0.125

(0.835), dry weight of leaves (0.835), fresh weight of pods (0.810) and dry weight of pods (0.782) where as harvest index (-0.831) showed significant negative correlation. The genotypic correlation of yield with all the characters except plant spread, girth of stem and indigotin content were found to be positive.

The environmental correlation coefficients were found to be negligible between yield and its component characters.

4.3.5.2. Correlation among the yield components

The phenotypic, genotypic and environmental correlations among the various yield components were studied and the coefficients are given in Tables 19, 20 and 21.

Plant height

At phenotypic level significant positive phenotypic correlation was observed with leaf area index (0.936), fresh weight of shoots (0.918), dry weight of pods (0.880), number of leaves (0.840), fresh weight of pods (0.865), number of branches (0.831) and dry weight of leaves (0.824) while harvest index (-0.773) recorded significant negative correlation. The characters fresh weight of leaves and dry weight of shoots were found to be positive correlation.

Plant height showed significant genotypic correlation with number of leaves (0.981), number of branches (0.965), leaf area index (0.946), dry weight of shoots (0.937), dry weight of pods (0.894), fresh weight of pods (0.889), dry weight of leaves (0.883), fresh weight of shoots (0.835) and fresh weight of leaves (0.767) while harvest index (-0.808) recorded significant negative correlation. Environmental correlation coefficients were found to be negligible among the yield components.

Table 19. Estimates of phenotypic correlation coefficients among the yield components of *Indigofera tinctoria*

Character	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
X_1	1.000	-0.208	0.831*	-0.236	0.840*	0.936**	-0.773*	0.754	0.824*	0.918**	0.733	0.865*	0.880**	-0.447
X_2		1.000	0.008	0.501	-0.177	0.020	0.415	-0.122	-0.269	-0.173	-0.051	-0.554	-0.486	0.546
X_3			1.000	-0.011	0.825*	0.795*	-0.472	0.689	0.647	0.849*	0.552	0.608	0.643	-0.348
X_4				1.000	-0.197	-0.272	0.570	-0.274	-0.375	-0.171	-0.509	-0.396	-0.357	0.255
X_5					1.000	0.753	-0.498	0.551	0.577	0.785*	0.501	0.779*	0.795*	-0.602
X_6						1.000	-0.801*	0.835*	0.865*	0.893**	0.793*	0.730	0.749	-0.235
X_7							1.000	-0.832*	-0.898**	-0.768*	-0.709	-0.770*	-0.733	0.264
X_8								1.000	0.909**	0.900**	0.587	0.611	0.585	-0.105
X_9									1.000	0.857*	0.696	0.754	0.742	-0.287
X_{10}										1.000	0.609	0.781 *	0.774 *	-0.350
X_{11}											1.000	0.552	0.553	-0.181
X_{12}												1.000	0.988**	-0.684
X_{13}													1.000	-0.702
X_{14}														1.000

* Significant at 5 per cent level
** Significant at 1 per cent level

X1 - Plant height
X2 - Plant spread
X3 - Number of branches
X4 - Stem girth
X5 - Number of leaves
X6 - Leaf area index
X7 - Harvest index
X8 - Fresh weight of leaves
X9 - Dry weight of leaves
X10 - Fresh weight of shoot
X11 - Dry weight of shoot
X12 - Fresh weight of pods
X13 - Dry weight of pods
X14 - Indigotin content

Table 20. Estimates of genotypic correlation coefficients among the yield components of *Indigofera tinctoria*

Character	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
X_1	1.000	-0.207	0.965**	-0.307	0.981**	0.946**	-0.808*	0.767*	0.883 **	0.835*	0.937**	0.889**	0.894**	-0.586
X_2		1.000	0.058	0.687	-0.168	0.021	0.459	-0.124	-0.273	-0.065	-0.183	-0.563	-0.493	0.713
X_3			1.000	0.102	0.957**	0.932**	-0.662	0.794*	0.790*	0.697	0.984**	0.737	0.744	-0.377
X_4				1.000	-0.028	-0.324	0.813*	-0.396	-0.668	-0.480	-0.196	-0.559	-0.512	0.311
X_5					1.000	0.870*	-0.658	0.667	0.777*	0.612	0.922**	0.922**	0.951**	-0.732
X_6						1.000	-0.825*	0.846*	0.931**	0.896**	0.905**	0.751	0.764*	-0.319
X_7							1.000	-0.872*	-0.995**	-0.831*	-0.805*	-0.804*	-0.765*	0.402
X_8								1.000	0.947**	0.660	0.908**	0.623	0.587	-0.147
X_9									1.000	0.835*	0.905**	0.810*	0.782*	-0.352
X_{10}										1.000	0.684	0.618	0.624	-0.110
X_{11}											1.000	0.799 *	0.784*	-0.447
X_{12}												1.000	0.996**	-0.897**
X_{13}													1.000	-0.900**
X_{14}														1.000

* Significant at 5 per cent level
** Significant at 1 per cent level

X1 - Plant height
X2 - Plant spread
X3 - Number of branches
X4 - Stem girth
X5 - Number of leaves
X6 - Leaf area index
X7 - Harvest index
X8 - Fresh weight of leaves
X9 - Dry weight of leaves
X10 - Fresh weight of shoot
X11 - Dry weight of shoot
X12 - Fresh weight of pods
X13 - Dry weight of pods
X14 - Indigotin content

Table 21. Estimates of environmental correlation coefficients among the yield components of *Indigofera tinctoria*

Character	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
X_1	1.000	-0.245	0.057	-0.073	0.016	0.417	-0.101	-0.118	-0.140	-0.017	-0.293	0.001	0.046	0.112
X_2		1.000	-0.503	-0.086	-0.398	-0.033	-0.434	-0.033	-0.245	0.076	0.412	-0.269	-0.191	-0.083
X_3			1.000	-0.235	0.432	-0.099	0.583	0.090	-0.019	0.068	0.025	-0.224	0.044	-0.295
X_4				1.000	-0.541	-0.365	-0.109	0.401	0.508	-0.623	-0.374	0.158	0.392	0.174
X_5					1.000	0.107	0.365	-0.543	-0.396	0.133	-0.181	-0.056	-0.428	-0.332
X_6						1.000	-0.381	-0.057	-0.319	0.046	-0.014	-0.108	-0.332	0.193
X_7							1.000	0.139	0.141	0.015	0.090	-0.174	-0.020	-0.245
X_8								1.000	0.461	0.080	-0.021	-0.014	0.342	0.185
X_9									1.000	-0.063	0.031	-0.061	0.087	-0.125
X_{10}										1.000	0.076	0.133	0.046	-0.368
X_{11}											1.000	-0.110	-0.111	0.010
X_{12}												1.000	0.709	0.109
X_{13}													1.000	0.057
X_{14}														1.000

* Significant at 5 per cent level
** Significant at 1 per cent level

X1 - Plant height
X2 - Plant spread
X3 - Number of branches
X4 - Stem girth
X5 - Number of leaves
X6 - Leaf area index
X7 - Harvest index
X8 - Fresh weight of leaves
X9 - Dry weight of leaves
X10 - Fresh weight of shoot
X11 - Dry weight of shoot
X12 - Fresh weight of pods
X13 - Dry weight of pods
X14 - Indigotin content

Plant spread

Plant spread showed positive phenotypic correlation with indigotin content (0.546), girth of stem (0.501), harvest index (0.415), leaf area index (0.415) and number of branches (0.008). Genotypic correlation was positive for indigotin content (0.713), girth of stem (0.687), harvest index (0.459), leaf area index (0.021) and number of branches (0.058).

Number of branches

Number of branches showed significant positive phenotypic correlation with fresh weight of shoot (0.849), plant height (0.831), number of leaves (0.825) and leaf area index (0.795). Fresh weight of leaves (0.689), dry weight of leaves (0.647), dry weight of shoots (0.552), fresh weight of pods (0.608) and dry weight of pods (0.643) were positively correlated with number of branches. At genotypic level dry weight of shoot (0.984), plant height (0.965), number of leaves (0.957), leaf area index (0.932), fresh weight of leaves (0.794) and dry weight of leaves (0.790) showed significant positive correlation. Dry weight of pods (0.744), fresh weight of pods (0.737), fresh weight of shoots (0.697) and girth of stem (0.102) was positively correlated with number of branches.

Girth of stem

At phenotypic level girth of stem was positively correlated with harvest index (0.570) and indigotin content (0.255) and all other characters was negatively correlated with girth of stem. Significant positive genotypic correlation was observed with harvest index (0.813).

Number of leaves

Phenotypic correlation was significant and positive for plant height (0.840), number of branches (0.825), fresh weight of shoots (0.785), fresh weight of pods (0.779), dry weight of pods (0.795). Leaf area index (0.753), dry weight leaves (0.577), fresh weight of leaves (0.551) and dry weight of shoot (0.501) was positively correlated with number of leaves.

Genotypic correlation was significant and positive for plant height (0.981), number of branches (0.957), dry weight of pods (0.951), dry weight of shoots (0.922), fresh weight of pods (0.922), dry weight of leaves (0.777) and leaf area index (0.870). Fresh weight of leaves (0.667) and fresh weight of shoots (0.612) was positively correlated with number of leaves.

Leaf area index

At phenotypic level significant positive phenotypic correlation was observed with plant height (0.936), fresh weight of shoots (0.893), dry weight of leaves (0.865), fresh weight of leaves (0.835), number of branches (0.795), dry weight of shoots (0.793) while harvest index (-0.801) recorded significant negative correlation.

Leaf area index showed significant positive genotypic correlation with plant height (0.946), number of branches (0.932), dry weight of leaves (0.931), dry weight of shoots (0.905), fresh weight of shoots (0.896), number of leaves (0.870), fresh weight of leaves (0.846) and dry weight of pods (0.764) while harvest index (-0.825) recorded significant negative correlation.

Harvest index

Harvest index showed significant negative phenotypic correlation with dry weight of leaves (-0.898), fresh weight of leaves (-0.832), plant height (-0.773), leaf area index (-0.801), fresh weight of pods (-0.770) and fresh weight of shoots (-0.768). Significant negative genotypic correlation was observed for dry weight of leaves (-0.995), fresh weight of leaves (-0.872), fresh weight of shoots (-0.831), plant height (-0.808), dry weight of shoots (-0.805), fresh weight of pods (-0.804) and dry weight of pods (-0.765). Significant positive genotypic correlation was observed for girth of stem (0.813).

Fresh weight of leaves

Phenotypic correlation was significant and positive for dry weight of leaves (0.909), fresh weight of shoots (0.900) and leaf area index (0.835). Fresh weight of pods (0.611), dry weight of shoots (0.587) and dry weight of pods (0.585), number of leaves (0.551), plant height (0.754) and number of branches (0.689) was positively correlated with fresh weight of leaves. Genotypic correlation was significant and positive for dry weight of leaves (0.947), dry weight of shoots (0.908), leaf area index (0.846), number of branches (0.794) and plant height (0.767) while harvest index recorded significant negative correlation (-0.872).

Dry weight of leaves

Phenotypic correlation was positive and significant for fresh weight of leaves (0.909), leaf area index (0.865), fresh weight of shoots (0.857) and plant height (0.824) while harvest index (-0.898) recorded significant negative correlation. Fresh weight of leaves (0.947), leaf area index (0.931), dry weight of shoot (0.835), fresh weight of pods (0.810), Number of branches (0.790), dry weight of pods (0.782) and number of leaves (0.777) showed positive and significant genotypic correlation while harvest index (-0.995) showed significant negative correlation.

Fresh weight of shoots

Phenotypic correlation was significant and positive for plant height (0.918), leaf area index (0.893), fresh weight of leaves (0.900), dry weight of leaves (0.857), number of branches (0.849), number of leaves (0.785), fresh weight of pods (0.781) and dry weight of pods (0.774) while harvest index (-0.768) showed significant negative correlation. Genotypic correlation was positive and significant for leaf area index (0.896), plant height (0.835) and dry weight of leaves (0.835) while harvest index (-0.831) showed significant negative correlation.

Dry weight of shoots

Significant positive phenotypic correlation was observed for leaf area index (0.793). Plant height (0.733), number of branches (0.552), number of leaves (0.501), fresh weight of leaves (0.587), dry weight of leaves (0.696), fresh weight of shoots (0.609), fresh weight of pods (0.552) and dry weight of pods (0.553) was positively correlated with dry weight of shoots. Significant positive genotypic correlation was observed for number of branches (0.984), plant height (0.937), number of leaves (0.922), leaf area index (0.905), fresh weight of leaves (0.908), dry weight of leaves (0.905), fresh weight of pods (0.799) and dry weight of pods (0.784) while harvest index (-0.805) recorded significant negative correlation.

Fresh weight of pods

Dry weight of pods (0.996), number of leaves (0.922), plant height (0.889), dry weight of leaves (0.810) and dry weight of shoots (0.799) showed significant and positive genotypic correlation while indigotin content (-0.897) and harvest index (-0.804) recorded significant negative correlation. Dry weight pods (0.988), plant height (0.865), number of leaves (0.779) and fresh weight of pods

(0.781) showed significant and positive phenotypic correlation while harvest index (-0.770) recorded significant negative correlation.

Dry weight of pods

At phenotypic level significant positive correlation was observed with fresh weight of pods (0.988), plant height (0.880), number of leaves (0.795) and fresh weight of shoots (0.774). Genotypic correlation was significant and positive with fresh weight of pods (0.996), plant height (0.894), number of leaves (0.951), leaf area index (0.764), dry weight of leaves (0.782) and dry weight of shoot (0.784) while indigotin content (-0.900) and harvest index (-0.765) recorded significant negative correlation.

Indigotin content

At phenotypic level indigotin content showed negative correlation with all the characters except plant spread (0.546) and girth of stem (0.255). At phenotypic level dry weight of pods (-0.900) and fresh weight of pods (-0.897) showed significant negative correlation.

4.3.6. Path Analysis

In path coefficients analysis, the genotypic coefficients among yield and its component characters were partitioned into different components to find the direct and indirect contribution of each character to fresh weight of shoot.

Fresh weight of shoots was taken as the dependent character and path analysis was done. The characters showing high significant association with yield were selected for the analysis viz., plant height, plant spread, number of branches, number of leaves, harvest index, fresh weight of leaves, dry weight of leaves and

indigotin content. The analysis revealed the direct and indirect effects of various characters on yield as presented in Table 22 and Fig 8.

The highest direct effect was observed for number of branches (1.3842) followed by plant height (0.8044), indigotin content (0.7023), dry weight of leaves (0.0173), fresh weight of leaves (-1.9248), harvest index (-1.7713), number of leaves (-0.8922) and plant spread (-0.0507). Number of branches, plant height, indigotin content and dry weight of leaves had positive direct effect while fresh weight of leaves, harvest index, number of leaves and plant spread had negative direct effect.

Fresh weight of leaves and number of leaves had positive correlation estimates and negative direct effects while harvest index and plant spread had negative correlation estimates and negative direct effects. A positive correlation as well as positive direct effect was noted for plant height, number of branches, dry weight of leaves, while indigotin content had negative correlation estimates and positive direct effects.

Plant height had high direct effect on yield (0.8044) as well as the highest positive genotypic correlation (0.835) with yield. It showed a negative indirect effect (-1.4755) via fresh weight of leaves and also showed a negative indirect effect (-0.8750) via number of leaves.

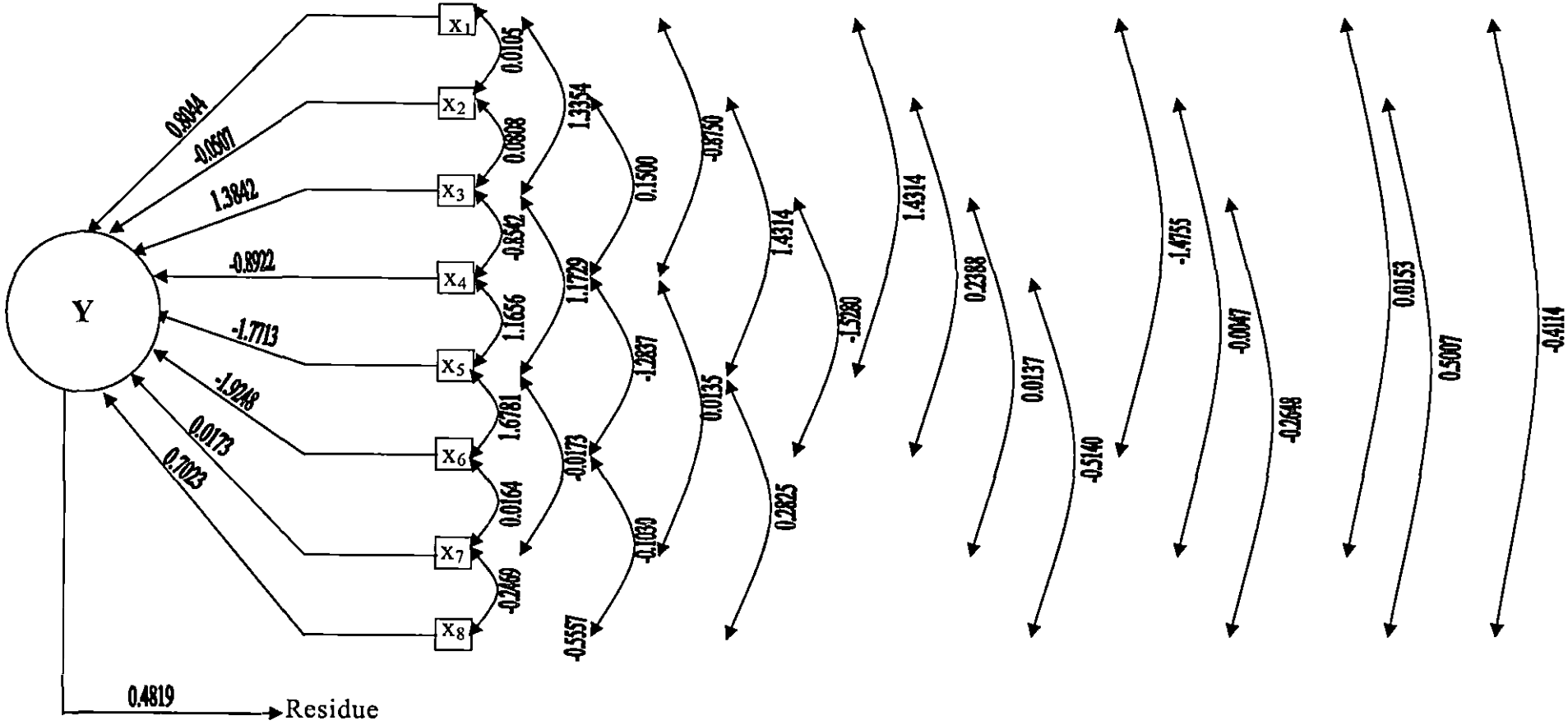
The direct effect of plant spread was negative (-0.0507) and also recorded negative genotypic correlation with yield (-0.065).

Number of branches had high positive direct effect (1.3842) and also positive genotypic correlation (0.697) with yield. It showed a negative indirect effect (-1.5280) via fresh weight of leaves.

Table 22. Direct and indirect effects of components characters on yield in *Indigofera tinctoria*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	Genotypic correlation with yield
Plant height (X1)	**0.8044**	0.0105	1.3354	-0.8750	1.4314	-1.4755	0.0153	-0.4114	0.835
Plant spread (X2)	-0.1669	**-0.0507**	0.0808	0.1500	-0.8128	0.2388	-0.0047	0.5007	-0.065
Number of branches (X3)	0.7760	-0.0030	**1.3842**	-0.8542	1.1729	-1.5280	0.0137	-0.2648	0.697
No. of leaves (X4)	0.7889	0.0085	1.3253	**-0.8922**	1.1656	-1.2837	0.0135	-0.5140	0.612
Harvest index (X5)	-0.6500	-0.0233	-0.9166	0.5871	**-1.7713**	1.6781	-0.0173	0.2825	-0.831
Fresh wt. of leaves (X6)	0.6167	0.0063	1.0988	-0.5950	1.5444	**-1.9248**	0.0164	-0.1030	0.660
Dry wt. of leaves (X7)	0.7106	0.0139	1.0930	-0.6935	1.7631	-1.8228	**0.0173**	-0.2469	0.835
Indigotin content (X8)	-0.4712	-0.0362	-0.5219	0.6529	-0.7126	0.2823	-0.0061	**0.7023**	-0.352

Residual effect = 0.4818732
Direct effects – Diagonal elements
Indirect effects – Off diagonal effects



Direct effects given in straight lines and correlations in curved lines

Y – Yield
X_1 – Plant height
X_2 – Plant spread
X_3 – Number of branches
X_4 – Number of leaves
X_5 – Harvest index
X_6 –Fresh weight of leaves
X_7 – Dry weight of leaves
X_8 – Indigotin content

Fig 8. Path diagram showing direct and indirect effects of components on yield

The direct effect of number of leaves was negative (-0.8922) but it recorded positive genotypic correlation with yield (0.612) due to high positive indirect effect through number of branches (1.3253).

The direct effect of harvest index (-1.7713) as well as negative genotypic correlation (-0.831) was significant with yield.

Fresh weight of leaves had negative direct effect (-1.9248) but it recorded positive genotypic correlation with yield (0.660) due to its positive indirect effect through number of branches (1.0988) and harvest index (1.5444).

The direct effect of dry weight of leaves was low and positive (0.0173) but it recorded high positive genotypic correlation with yield (0.835) due to its positive indirect effect through harvest index (1.7631) and number of branches (1.0930).

Indigotin content had high positive direct effect (0.7023) on yield but the genotypic correlation was negative (-0.352) with yield.

The residual value was 0.4819 indicating that about 52 percent of the variation in yield was contributed by the characters selected for analysis.

4.3.7. Selection Index

A discriminant function analysis was carried out for isolating superior treatments of *Indigofera tinctoria*. The characters selected for the analysis are plant height (X_1), plant spread (X_2), number of branches (X_3), number of leaves (X_4), harvest index (X_5), fresh weight of leaves (X_6), dry weight of leaves (X_7), indigotin content (X_8) and fresh weight of shoots (X_9). The selection index worked out was as follows.

$$I = 9.678989\,X_1 + 1.3659\,X_2 - 1.14816\,X_3 - 0.29348\,X_4 + 300.292\,X_5 + 0.815045\,X_6 + 0.798867\,X_7 + 10.62299\,X_8 + 0.084501\,X_9$$

The scores obtained for the treatments based on selection index are presented in Table 23.

Based on selection index T_6-control ranked first (3593.42), followed by T_4-22.5 KR (3308.932) and T_3-20.0 KR (3287.981). The minimum scores were obtained for T_5-25.0 KR (3134.769).

Table 23. Selection index scores and ranks of *Indigofera tinctoria* treatments

Sl. No	Treatments	Selection index scores	Ranks
1	15.0 KR – T_1	3171.307	4
2	17.5 KR – T_2	3163.798	5
3	20.0 KR – T_3	3287.981	3
4	22.5 KR – T_4	3308.932	2
5	25.0 KR – T_5	3134.769	6
6	0.0 KR - control	3593.42	1

Discussion

5. DISCUSSION

Indigofera tinctoria (L.), is a valuable medicinal plant, which is also utilized as a natural source of the blue dye, indigo. At present the leaf biomass obtained from the genotypes currently cultivated is not applicable. Hence an attempt has to be made to evolve variants with higher leaf yield and indigotin content. Mutation breeding has been attempted in this study with a local variety. Reports of comprehensive work on both the fundamental and the applied aspects of mutation research in medicinal plants are practically nil.

Mutation breeding has been found to be a potent and handy tool to induce new and additional variability in both qualitative and quantitative traits. Ionising and non ionising radiations play a significant role for permitting favourable permanent changes, thereby increasing scope for selection. Therefore the present investigation was carried out with the objective of inducing variability in *Indigofera tinctoria* for leaf and shoot yield.

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. The results are discussed below.

5.1. Sensitivity analysis

Information on the sensitivity of the plant material to the mutagen is essential to arrive at the optimum dose of the mutagen. The unit of the absorbed dose of radiation energy is the KR (kilo rad). The routine procedure in assessing the most appropriate dosage is based on radiosensitivity, which is estimated through the physiological response of the irradiated material (Neville et al, 1998). It involves the determination of dose that causes a 50 percent reduction of growth of the treated material (LD_{50}) when compared with the control (Gaul, 1977). The choice of the dose to be applied for the highest probability of useful mutant rescue

is then left to the breeders experience with the specific plant material, its genetics and its physiology.

Many parameters have been used for determining the sensitivity of the crop plants to different mutagens. Establishment of LD_{50} for gamma rays and EMS is considered as one of the parameter to assessing the sensitivity to the mutagens. This was reported by Sambandamurthi (1983) in tuberose and Jayachandran and Mohanakumaran (1992) in ginger.

In the present study the LD_{50} obtained was 20 KR for gamma rays and the effect of mutagen on indigofera was assessed in terms of germination and survival of the mutant. As established in the studies of early workers increase in the doses of mutagen in the range of 5 to 40 KR, manifested in a decline trend in the germination and survival of treated material. Contrary to 50 per cent survival of plants Heinze and Schmidt (1995) advice to operate at a dose giving (LD_{50} = 10 %) or a dose resulting in 20 per cent survival of the treated material. In due consideration of the above set up information, the effect of the mutagen in the present investigation was evaluated on the basis of the degree of germination of seeds and survival of mutant under laboratory condition.

5.2. Effect of mutagen

By mutagenesis using different physical and chemical agents, several investigators have reported induced changes in the morphology and biochemistry of a variety of plants during the twentieth century. In the present investigation, irradiation with different doses of gamma rays, were found to affect the various characters of *Indigofera tinctoria* (L.) differently.

5.3. Morphological parameters

The M_1 and M_2 generations were evaluated and the results are discussed. The different doses of gamma ray mutagen tried were found to interfere with the percentage of germination and survival in a significant manner. Abnormalities of the seedlings could be observed on treatment with higher doses of gamma rays. This includes size and colour of the cotyledons, foliar abnormalities, etc. The results are in accordance with the findings of Geetha (1995) in *Cassia angustifolia*, Anitha (1998) in *Indigofera tinctoria* and Chandralekha (1995) in *Withania somnifera.*

5.3.1. Germinations of seeds

Treatment with various physical and chemical mutagens had been reported to affect the percentage of germination in a variety of plants by many investigators (Sharma and Sharma, 1992; Viswanathan et al, 1993; Rema et al, 1994; Chandralekha, 1995; Geetha, 1995 and Anitha, 1998).

In the present investigation the germination percentage was found to decrease with increase in the doses of gamma rays in both M_1 and M_2 generation. These results are in accordance with the findings of Anitha (1998) in *Indigofera tinctoria*, Chandralekha (1995) in *Withania somnifera.*

The reduction in the percentage of germination might be due to structural changes in the genetic constitution of the progenies of the treated population which like the synthesis of enzymes, hormones and growth regulators.

Enhancement of the percentage of germination by different doses of gamma rays had been reported by Ehrenberg (1955a, b), Sparrow and Gunckel (1956), Fujii and Matsumura (1958), Matsumura and Fujii (1959) and Basu (1962).

5.3.2. Survival

The survival percentage was found to decrease with increase in the doses of gamma rays in both M_1 and M_2 generation. Similar results have been obtained following seed irradiation by Ojomo and Chheda (1971) and Louis and Kadambavanasundram (1973) in cowpea; Constantin et al (1976) in soybean; Krishnaswami et al (1977) in green gram, Nadarajan et al (1985) in red gram, Anitha (1998) in *Indigofera tinctoria*, Chandralekha (1995) in *Withania somnifera*. The reduction in survival is an index of post germination mortality in the treated plants as a result of cytological and physiological disturbances due to radiation effect. Structural changes in the chromosome are brought by cytological abnormalities caused by irradiation. This interferes with the normal growth and development of organs which leads to a decrease in survival percentages with increasing doses of radiation.

5.3.3. Plant height

A reduction in growth of shoot was observed in the present investigation. The plant height recorded at various growth stages indicated that there was reduction in plant height with increasing doses at all the stages in M_1 generation. These observation are in accordance with the findings of Shivraj and Rao (1963), Patil (1966), Sinha and Roy (1969), Sivasubramanium (1979) and Rathnaswamy (1980) in groundnut. Constantin et al (1976) in soybean, Appa Rao and Jana (1976) in black gram, Khanna and Maherchandani (1980) in gram and Nadarajan et al (1985) in red gram.

In M_2 generation the height of the plants was found to be increased by some of the treatments, where as it was reduced by some others. Early investigators had reported that plant height in some leguminous plants, was increased or decreased and sometimes did not change as a result of irradiation

(Gregory, 1955 and Micke, 1961). Increase in the dose of gamma rays upto 20.0 KR had enhanced the height of the plants (Jha and Sinha, 1977) in *Pennisetum*.

In the present study, variation in plant height induced by the different mutagenic treatments might have resulted from chromosomal aberrations, causing changes in the genetic architecture of the plants, thereby exhibiting variations like biochemical disturbances in the synthesis of auxins and changes in the physiology like mechanism of assimilation in *Indigofera tinctoria* (L.).

5.3.4. Number of branches

In M_1 generation the number of branches per plant was found to be enhanced by certain dose, while it had been reduced by certain others, when compared to that of the control in the present investigation.

The branching pattern of plants, after mutagenic treatments had been studied by Patil (1966) in groundnut, Suhas et al (1977) in *Cassia angustifolia*, Singh et al (1984) in green gram, Patil and Bhalla (1985) in soybean.

In M_2 generation the number of branches per plant was found to be enhanced by certain treatments, while it had been reduced by certain others when compared to that of control in the present investigation. Increased number of branches was reported by Patil (1966) in groundnut after irradiation with X-rays. Suhas et al. (1977) had reported that irradiation with 15 to 25 KR of gamma rays had induced variation in the number of branches in groundnut and Narasimhachary and Bhalla (1988) in pigeon pea. Venkatachalam and Jayabalan (1997) had reported increased and decreased number of branches in *zinia* depending on the doses of the mutagen.

In the present study also, variation in the number of branches per plant after mutagenesis might be attributed to induced changes at the chromosomal, genetic and physiological levels.

5.3.5. Number of leaves

The number of leaves was found to be reduced in the plants with different doses of gamma rays. Number of leaves per plant is reduced with increases in the doses of gamma rays, when compared to that of control in M_1 generation. There are several reports regarding the induction of morphological mutations affecting different plants after treatment with physical mutagens (Oka et al, 1958; Ehrenberg et al, 1959; Kawai et al, 1961; Patil, 1966; Choudhary, 1974; Khanolkar, 1977 and Chaudhary, 1978).

In M_2 generation the number of leaves per plant was found to be enhanced by certain treatments (20.0 and 22.5 KR) in the present investigation. An increase in the number of leaves had been reported by Choudhary (1974) in *Trigonella foenum graecum* and Jahagirdar (1975) in *Foenicum vulgare*. Singh et al. (1996) had reported an increase in the number of leaves in *Vigna mungo* on irradiation with gamma rays.

In the present investigation, alterations in the leaf shape and number of leaves might be due to chromosomal aberrations which might have induced changes in the physiology of the plants affecting normal growth.

5.3.6. Leaf area

Some of the mutagenic treatments had increased the leaf area at the time of flowering in M_2 generation. However, a dose dependent increase or decrease in the mean leaf area could not be observed in the present study. According to Schwartz (1954), the leaf area decreased with the increase in the dose of radiation

upto 100 KR on dry maize seeds. Stimulatory effect of irradiation was also reported by Shivraj et al (1963) in groundnut, the frequency of such plants being increased with the increase in the dose of the mutagen. Increased leaf area was also reported by Singh (1996) in *Vigna mungo*.

5.3.7. Leaf area index

A reduction in leaf area index was observed in the present investigation. The leaf area index decreased with the increase in the dose of gamma rays. According to Schwartz (1954), the leaf area index decreased with the increase in the dose of radiation upto 100 KR on dry maize seeds. Stimulatory effect of irradiation was also reported by Shivraj et al (1962) in groundnut, the frequency of such plants being increased with the increase in the dose of the mutagen.

In the present investigation, decrease in leaf area index might be due to induced chromosomal aberrations resulting in genetic variation, or due to mutation induced plasticity of the phenotypes, which might interfere with the metabolic processes like synthesis of auxins.

5.3.8. Length and width of leaves

The length and width of leaves was found to be reduced by certain treatments, while it had been increased by certain others in M_2 generation. Singh (1971) had reported a reduction in the leaf size, after treatment with gamma rays in *Tabernaemontana* and *Portulaca*. Increased size of leaves was observed by Narasimhachary and Bhalla (1988) in mutants of *Cajanus cajan*. There was no variation in leaf, stem and seed colour compared to control after mutagenic treatments.

5.3.9. Pollen sterility

Pollen sterility was observed to decrease with increasing doses of gamma rays in both M_1 and M_2 generation. Decreased fertility with increasing doses of mutagen was reported by Ojomo and Chheda (1971), Louis and Kadambavanasundram (1973), Narsinghani and Kumar (1976) in cowpea and Nadarajan et al (1985) in red gram. Mutagen induced pollen sterility had been reported by Choudhary (1974) in *Trigonella foenum graecum* and Nerkar (1976) in *Lathyrus sativus*.

In the present investigation, increase in pollen sterility might be due to meiotic irregularities or changes in the genetic material governing the pollen fertility or due to additive gene action.

5.3.10. Chlorophyll chimeras

In the present investigation, chlorophyll deficient patches were observed on the leaves of plants treated with 22.5 and 25.0 KR doses of gamma rays in M_1 generation. Similar results have been obtained by Ojomo and Chheda (1971) in cowpea.

5.3.11. Morphological abnormalities

Various types of seedling abnormalities were observed as a result of the different doses of irradiation in *Indigofera tinctoria* (L.). These included the formation of twisted and shrunken cotyledons, stunted growth of the seedlings and yellowing of the cotyledons. In some of the treatments with higher doses seedlings with abnormal or reduced leaflets were also found to occur as a result of gamma irradiation in M_1 generation.

The formation of tricotyledonary leaves had been reported by Ahmed et al (1982) in *Solanum melongena*, and Singh et al (1984) in green gram, after irradiation with gamma rays. Variation in cotyledonary leaves was also reported by Ansari and Siddique (1996) in *Ammi majus* on treatment with ionising radiations. Complete and partial yellowing of the cotyledons were also met with in the present present investigation. Occurrence of chlorophyll mutants had been reported especially in legumes by many investigators Zacharias (1967) and Sjodin (1962).

In the present investigation, development of seedling abnormalities might be due to chromosomal damage, resulting in a changed genetic constitution or physiological disturbances in the interior of the cells, like inhibition or imbalance of the action of growth regulators.

5.3.12. Days taken to flower

The various mutagenic treatments had induced a general delay in flowering when compared to that of the control. Irradiation with gamma rays had induced a delay in flowering in both M_1 and M_2 generation. Similar observations had been reported by Anitha (1998) in *Indigofera tinctoria*.

In the present investigation, delay in flowering might be due to mitotic arrest in the flower primordia or due to mutations taking place in the genes having pleiotropic effects, inducing physiological changes like lower rate of metabolic activities in the plant by the mutagenic agents.

5.3.13. Girth of stem

Mutation affected the girth of stem differentially for varying doses of gamma rays at different growth stages in both M_1 and M_2 generation. It can be

concluded that mutation has changed the plant girth positively and negatively in *Indigofera tinctoria.*

5.3.14. Harvest index

Harvest index of Indigofera was influenced by gamma irradiation. The harvest index could be increased with 20.0 KR. It can be attributed to the interaction of inherent genetic characters of the genotypes to specific doses.

5.3.15. Indigotin content

A comparative study of the indigotin content of the different mutants of *Indigofera tinctoria* in M_2 generation evolved and the control plants have shown that there is considerable variation among the mutants in this aspect. Higher indigotin content was obtained with increased doses of mutagen. This is conformity with the results obtained by Bilquez et al. (1965) in the oil percentage in groundnut. Choudhary (1974) had reported an increase in vitamin C content in *Trigonella foenum graecum* after irradiation with gamma rays. Khalatkar and Bhargava (1987) had observed an increase in th sennoside content in *Cassia angustifolia.* Ilangovan et al. (1991) had also noticed significant differences of sennoside in *Cassia angustifolia.*

5.4. Genetic parameters

5.4.1. Coefficient of variation

Variability is also expressed as the coefficient of variation. Coefficient of variation, phenotypic (PCV) and genotypic (GCV) are better indices for comparison of characters with different units of measurements. The GCV provides a valid basis for comparing and assessing the range of genetic diversity for quantitative characters and PCV measures the extent of total variation.

In the present study, a high PCV was recorded for harvest index, leaf area index, number of branches and dry weight of pods while a low PCV was shown by fresh weight of shoots and fresh weight of leaves.

PCV ranged from 2.78 to 18.84 and GCV ranged from 2.47 to 18.13. The estimates of PCV were higher than that of GCV. This is in conformity with the observations of Singh et al. (2000). In the present study leaf area index, harvest index and dry weight of pods had very high estimates of PCV. Similar results were reported by Sarada et al. (2006) in *Indigofera tinctoria*. GCV is a better tool to understand useful variability, as it is free from the environmental component affecting variability.

In the present study leaf area index, harvest index, dry weight of leaves and dry weight of pods had high estimates of GCV. Similar results were reported by the Sarada et al. (2006) in *Indigofera tinctoria.*

High PCV as well as high GCV were observed for leaf area index, harvest index, number of branches and dry weight of pods. This suggests the scope for improvement of these characters through selection.

5.4.2. Heritability and Genetic advance

While evaluating more than one character their interrelations also have to be worked out and in this context. The parameters like heritability and genetic advance are unavoidable. The phenotypic variance which is due to genotypic variance is expressed by heritability. The magnitude of improvement by selection is detected by genetic advance. High heritability together with genetic advance is an important requirement for selection programme.

High heritability estimates recorded for all the characters except girth of stem. Among yield traits heritability is maximum for fresh weight of leaves

followed by dry weight of shoots, dry weight of pods, leaf area index, plant height, plant spread, fresh weight of pods, harvest index and dry weight of leaves.

High heritability for dry weight of shoots and dry weight of leaves in the present study is in accordance with the findings of Farooqi et al. (1999) in *glory lilly* and Sarada et al. (2006) in *Indigofera tinctoria.*

High genetic advance was noted for harvest index, leaf area index, dry weight of pods, number of branches, plant spread, fresh weight of pods, plant height, dry weight of leaves, dry weight of shoots and number of leaves.

High genetic advance for dry weight of leaves recorded in the present investigation is in accordance with findings of Sarada et al. (2006) in *Indigofera tinctoria.*

High heritability and high genetic advance of characters indicates additive gene action suggesting the possibility of genetic improvement of these characters through selection. The characters leaf area index, dry weight of shoot, dry weight of pods, plant spread, plant height, fresh weight of pods, harvest index, fresh weight of leaves, dry weight of leaves, number of branches and number of leaves.

In the present investigation dry weight of leaves, dry weight of shoots and harvest index recorded high heritability coupled with high genetic advance. Similar results were reported by Sarada et al. (2006) in *Indigofera tinctoria* and Lal et al. (1999) in *Plantago sp.* High heritability coupled with high genetic advance for plant height is in accordance with the findings of Shah et al. (2003) in coriander and Jain et al. (2006) in *Papaver somniferum.*

5.4.3. Correlation studies

Correlation provides information on the nature and extent of association between characters in a population. The component character always shows

interrelationship. When the breeder applies selection on a trait the population under selection is not only improved for that trait but also improve in respect of other characters associated with it. This facilitates simultaneous improvement of two or more characters. Therefore analysis of yield in terms of genotypic and phenotypic correlation coefficient of component characters leads to the understanding of characters that can form the basis of selection. The genotypic correlation between the characters helps to differentiate the vital association useful in breeding from non-vital ones (Falconer, 1981).

In the present study fresh weight of shoot exhibited high positive genotypic correlation with plant height, leaf area index, dry weight of leaves, dry weight of shoots, fresh weight of pods and dry weight of pods. This is in conformity with the studies of Sarada et al. (2006) and Neema (2004) in *Indigofera tinctoria* and Dwivedi et al. (1999) in *Catharanthus roseus*.

In the present study plant height, number of branches, number of leaves, leaf area index, weight of leaves, weight of shoots and weight of pods showed positive phenotypic and genotypic correlations with yield. Similar reports were published by Misra et al. (2000), Singh et al. (2000) and Sangaranarayanan et al. (1992). Among these plant height, leaf area index, weight of leaves, weight of shoots and weight of pods showed high genotypic correlation. The characters plant spread, girth of stem, harvest index and indigotin content exhibited negative correlation with the yield.

Plant height, number of branches, number of leaves, leaf area index, leaf weight and pod weight showed high phenotypic and genotypic correlation with shoot weight. These results showed that selection based on these characters could improve shoot yield, where as plant spread, harvest index and indigotin content recorded negative correlation with shoot yield.

Number of branches is positively correlated with all the characters except harvest index and indigotin content. So selection based on number of branches is effective.

5.4.4. Path analysis

The path analysis reveals whether the association of the component characters with yield is due to their direct effect on yield or is a consequence of their indirect effect via some other traits. Thus path coefficient analysis helps in partitioning the genotypic correlation coefficient into direct and indirect effects of the component characters on the yield. On the basis of which improvement programme can be devised effectively. If the correlation between yield and any of its components is due to the direct effect, it reflects a true relationship between them and selection can be practiced for such character in order to improve yield. But if correlation is mainly due to indirect effect of the character through another component trait, the breeder has to select the latter trait through which th indirect effect is excerted.

In the present investigation, the highest positive and direct effect on yield was exhibited by number of branches followed by plant height, indigotin content and dry weight of leaves, while fresh weight of leaves, harvest index, number of leaves and plant spread had negative direct effects.

High direct effect on number of branches per plant is in accordance with earlier findings of Krishnamoorthy and Madalageri (2002) in *Trachyspermum ammi.*

The positive direct effect of plant height on yield in the study was supported by Krishnamoorthy and Madalageri (2002) in *Trachyspermum ammi*, Dwivedi et al. (1999) in *Catharanthus roseus* and Misra et al. (1998) in *Withania somnifera.*

Plant spread had negative direct effect on yield. Similar results were obtained by Krishnamoorthy and Madalagiri (2002) in *Trachyspermum ammi*.

The residual effect indicated that 48.19 % variation in fresh weight of shoots and the character studied contributed 51.81 % variation. From the present study it is evident that selection of treatments based on number of branches and plant height can be effective for improving yield of the crop.

5.4.5. Selection index

Selection of treatments based on a suitable index is highly efficient in any breeding programme. Selection index scores based on plant height, plant spread, number of branches, number of leaves, harvest index, fresh weight of leaves, dry weight of leaves, indigotin content and fresh weight of shoots were used to identify superior treatments of *Indigofera tinctoria*. An estimation of discriminant function based on reliable and effective characters is a valuable tool for the practical plant breeder. Superior treatments can be selected from different treatments using a selection index employing the discriminant function.

The maximum selection index value was obtained for control followed by 22.5 KR and 20.0 KR. The minimum selection index value obtained for 25.0 KR based on selection index the top ranking treatments were identified to be genetically superior from other treatments, in the production of mutants with higher leaf biomass and indigotin content.

5.5. Identification of Macro mutants

The screening of the individual plants of the M_2 generation population resulted in the identification of some mutants showing significant positive deviation from the mean values for yield and indigotin content.

From the M_2 generation, five mutants were selected based on the morphological variations from the doses 20.0 KR, 22.5 KR and 25.0 KR given in Table 24 and Plate 5.

Highly branched mutants could be observed in the gamma ray treated population. Such morphological mutants were observed by Fursov and Konoplia (1967) in cotton. Nayar and George (1969) had reported an increase in the degree of branching, resulting in an increase in the number of fruits per plant, in a mutant of *Brassica juncea* by X-rays.

A total of five such high yielding mutants were identified and their yield characters and indigotin were also found out for further evaluation (Table 24). Raising M_3 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.

Table 24. Promising mutants with morphological variation in different treatments of *Indigofera tinctoria*

Mutants	Plant height (cm)	Plant spread (cm)	Girth of stem (cm)	Number of branches	Number of leaves	Indigotin content (%)
M-1 (20.0 KR)	298.2	196.0	10.8	47	1700	2.308
M-2 (20.0 KR)	275.5	177.7	10.3	43	1567	2.367
M-3 (22.5 KR)	280.0	185.3	10.6	39	1509	2.317
M-4 (22.5 KR)	291.4	181.5	9.9	36	1500	2.129
M-5 (25.0 KR)	283.6	190.2	10.9	46	1655	2.530
Control	271.20	149.02	8.40	28.31	1195.59	1.788

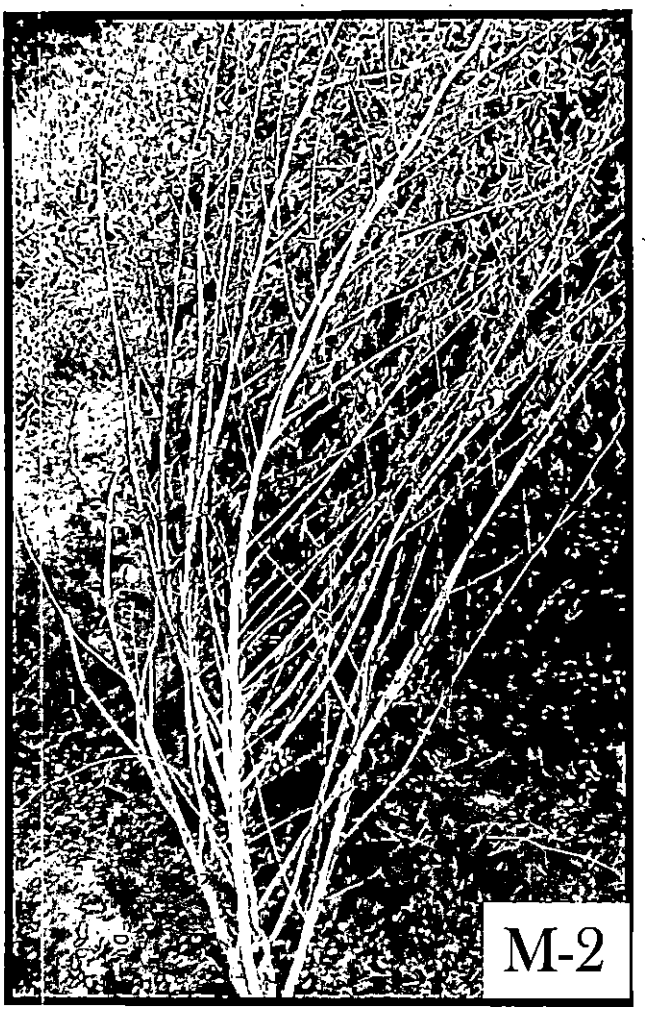


Plate 5. Promising mutants

M-1 = Mutant 1

M-2 = Mutant 2

M-3 = Mutant 3

Plate 5. Continued

M-4 = Mutant 4

M-5 = Mutant 5

Summary

6. SUMMARY

A study was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during the period 2006-2008 to induce variability for higher biomass yield and indigotin content in *Indigofera tinctoria* (L.). The salient findings are summarized below:

1. The exposure of seeds above 20.0 KR gamma rays reduced the germination to 50 % and LD_{50} was calculated as 20.0 KR gamma rays.

2. Progressive decrease in germination and survival was noticed as the level of dose increased in both M_1 and M_2 generation.

3. The mean number of days to first flowering was increased in both M_1 and M_2 generation.

4. The mean performance for all the characters was reduced as the doses of mutagen increased in M_1 generation.

5. In M_2 generation the positive shift in mean plant height, plant spread, girth of stem, number of branches, number of leaves, weight of leaves, weight of shoots, weight of pods and indigotin content were observed in the intermediate dose of mutagen.

6. Indigotin content of leaves was significantly increases with increased doses of gamma rays treatments when compared to control.

7. The optimum dose for maximum yield and yield contributing characters ranged between 17.5 KR and 22.5 KR of gamma rays mutagen.

8. A high magnitude of phenotypic coefficient of variation and genotypic coefficient of variation were noticed for the characters viz., harvest index, leaf area index, dry weight of pods and number of branches suggesting scope for genetic improvement of these traits through selection.

9. High estimates of heritability was observed for all the characters studied. The highest being exhibited by fresh weight of leaves (99.23 %).

10. Maximum genetic advance was observed for the character harvest index (35.93) while fresh weight of shoots expressed the minimum (4.52).

11. Leaf area index, dry weight of shoots, dry weight of pods, plant spread, plant height, fresh weight of leaves, dry weight of leaves, number of branches and number of leaves showed high heritability and high genetic advance which suggest the selection can be resorted for improving these traits.

12. Correlation coefficient between shoot yield and its components indicating significant positive association of yield with plant height, leaf area index, fresh weight of leaves, number of leaves, dry weight of leaves, dry weight of shoots, fresh weight of pods and dry weight of pods, where as harvest index showed significant negative correlation.

13. Path coefficient analysis of important yield attributes indicated that the number of branches, plant height and indigotin content had the maximum direct effect on fresh weight of shoot and minimum for dry weight of leaves.

14. Indigotin content has negative correlation with yield where as it has direct effect on yield through other correlated characters. This indicates that selection for improvement for indigotin content should be carried out with other characters also. It also indicates that there should be an optimum level of biomass yield for higher indigotin content. Frequent harvest will influence the indigotin content

favourably. This has to be determined by assessing the regression coefficient for other characters with indigotin content.

15. The selection index score was highest for control and for the treatment 22.5 KR followed by 20.0 KR of gamma rays.

16. Morphological mutants were observed in 20.0 KR, 22.5 KR and 25.0 KR of gamma ray mutagen.

17. High yielding mutants were observed in plants treated with 20.0 KR of gamma ray mutagen.

18. Five high yielding mutants were selected for further evaluation.

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INDUCTION OF VARIABILITY THROUGH MUTAGENESIS IN NEELAYAMARI (*Indigofera tinctoria* L.)

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ABSTRACT

The study entitled "Induction of variability through mutagenesis in neelayamari (*Indigofera tinctoria* L.)" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during the period 2006-2008, with the objective of inducing variability for higher biomass yield and indigotin content in leaves.

Indigofera tinctoria is a valuable medicinal plant, which is also utilized as a natural source of the blue dye, 'indigo'. At present the leaf biomass obtained from the genotypes currently cultivated is not appreciable. Hence an attempt has to be made to evolve variants with higher leaf yield and indigotin content. Mutation breeding has been attempted in this study with a local variety. The LD_{50} was calculated as 20.0 KR gamma rays. The mean performance for all the characters was reduced as the doses of mutagen increased in M_1 generation. In M_2 generation the positive shift in mean plant height, plant spread, girth of stem, number of branches, number of leaves, weight of leaves, weight of shoots, weight of pods and indigotin content were observed in the intermediate dose of mutagen. Indigotin content of leaves increased significantly with increased doses of gamma rays treatments when compared to control. The optimum dose for maximum yield and yield contributing characters ranged between 17.5 KR and 22.5 KR of gamma rays mutagen.

A high magnitude of phenotypic coefficient of variation and genotypic coefficient of variation were noticed for the characters viz., harvest index, leaf area index, dry weight of pods and number of branches. High estimates of heritability were observed for all the characters studied, the highest being exhibited by fresh weight of leaves. Maximum genetic advance was observed for the character harvest index while fresh weight of shoots expressed the minimum. Correlation coefficient between shoot yield and its components indicated

significant positive association of yield with plant height, leaf area index, fresh weight of leaves, number of leaves, dry weight of leaves, dry weight of shoots, fresh weight of pods and dry weight of pods, where as harvest index showed significant negative correlation. Path coefficient analysis of important yield attributes indicated that the number of branches, plant height and indigotin content had the maximum direct effect on fresh weight of shoot and minimum for dry weight of leaves.

Indigotin content has negative correlation with yield where as it has direct effect on yield through other correlated characters. This indicates that selection for improvement for indigotin content should be carried out with other characters also. It also indicates that there should be an optimum level of biomass yield for higher indigotin content. Frequent harvest will influence the indigotin content favourably. This has to be determined by assessing the regression coefficient for other characters with indigotin content. The selection index score was highest for control followed by the treatment 22.5 KR and 20.0 KR of gamma rays. Selected mutants showed morphological variation over the control. High yielding mutants were observed in plants treated with 20.0 KR of gamma ray. A total of five high yielding mutants were identified and their yield characters and indigotin were also found out for further evaluation. Raising M_3 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.