MUTATION BREEDING IN ROSE AND PIGMENTATION STUDIES IN ROSE AND HIBISCUS

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Affectionately Dedicated to

My Beloved Parents

DIVISION OF HORTICULTURE
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BANGALORE

C E R T I F I C A T E

This is to certify that the thesis entitled " MUTATION BREEDING IN ROSE AND PIGMENTATION STUDIES IN ROSE AND HIBISCUS" submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in HORTICULTURE to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by MISS K .S . SHOBHA, under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

I . INTRODUCTION

Rose the queen of flowers, belongs to the genus Rosa and the family Rosaceae. It comprises of nearly 120 species (Pal , 1972) and is one of the first flower to be domesticated and it has spread with civilization. The earliest domestication of roses appears to have taken place in the far East and Persia, when roses were rather simple in form with a limited colour range. Towards the close of 19th century rose enjoyed a lot of improvement with respect to its size, form, colour and duration of flowering. Rose is grown both for its beauty and utility. It also finds an important place in modern landscaping. Besides, it is one of the most ideal flowering shrub suitable for growing as pot plants and commercially grown for the production of cut flowers and in the perfume trade for the production of rose oil, rose water and gulkand.

The present day rose varieties are complex hybrids involving interspecific hybridization, polyploidy with high male sterility. Cytological abnormality and heterogenous genotypes perpetuated by vegetative propagation. They do not hence afford much success in achieving the derived objectives in breeding like other sexually propagated species. Thus new cultivars are evolved by hybridization , spontaneous mutation and to certain extent by induced mutations. Mutations are sudden heritable changes in the genetic material which may be chromosomal, cytoplasmic or gene mutations. Induction of mutation is one of the means for producing genetic variability in vegetatively propagated crops. Most of the genetic variability available in plant collection is the prior evolution involving some genetic recombination and exposure to forces of natural selections.

Rose breeding is practiced extensively all over the world for the creation of new forms and novelty types. Spontaneous somatic mutations or bud sports have

played an important role in the evolution of many new cultivars in garden roses. Spontaneous mutations occur frequently causing a valuable increase in the genetic variation. Due to polyploidy and complex heterozygosity mutation breeding in general is a supplement rather than a substitute to other methods of breeding. Sometimes such sudden changes are stable, while they are also liable to revert wholly or partly to the original conditions.

Ionizing radiations have also been successfully used for developing new varieties by induced somatic mutations. Radiation induces very few mutations which can be detected as an alteration of electrophoretic viability. It induces mutations that lead to altered enzyme activity ranging from point mutations to intragenic DNA deletions, multilocus deletions or rearrangements. The main promising aspect of induction of mutation is the ability to change one or a few characters of an otherwise good cultivar without altering the remaining and often unique genotypes. The usefulness of cross breeding is often limited due to high degree of heterozygosity that causes a complicated segregation and makes the recovery of a rare recombinant very difficult on the basis for producing genetic variability in vegetatively propagated sterile crop and in obligate apomixis (Broertjes , 1977).

Thus, the successful utilization of induced mutations as a method of plant breeding is dependent on various factors like the choice of parents, characters to be improved, the type of mutagens and its dosages used, experimental procedures chosen and the efficiency of detection of mutations. Thus through radiation it is possible to induce genetic variation for quantitative characters that is heritable of sufficient magnitude and frequency to be of interest in the breeding pr gramme.

Breeding involves pollination of selected parents and sufficient attention to promote seed development. Pollen grains as of late gained lot of importance as one

of the integrated components of plant genetic resources. Greater importance is to be given on higher degree of pollen fertility while selecting a cultivar as male parent. Pollen fertility of different cultivars can be estimated by the stainability tests. while female fertility can be judged by their seed setting ability. The study of pollen fertility is of great importance to plant breeders and geneticists in eliminating the time problem encountered in artificial pollination.

Colour is the major contributing factor to the total ornamental value of a flower. Flower colour is predominantly due to two types of pigments, flavonoids and carotenoids (Michael Dalling , 1992). Flavonoids contribute to a range of colours from yellow, red to blue. Carotenoids impart an orange or yellow tinge and are commonly the only pigment in yellow to orange flowers. The flavonoid molecules which make the major contribution to flower colour are the anthocyanin glycosylated derivatives of cyanidin (red), dephinidin (blue) and pelargonidin (brick red). It is a well known fact that red, yellow and ivory pigments alone or in combinatiions give various shades of colour and bicolour patterns in roses. Thus there is possibility of manipulating the concentration and composition in the most desirable genetic background.

Evaluation of pigments, their sound biosynthetic pathways aid in understanding the flower colour expression and also creates new avenues for evolving new colours. Pigments present in different flower colours would be useful in the choice of parents for hybridization and elaborate biochemical analysis of pigments Hibiscus the rose of sharon or the shoe flower (Hibiscus rosa sinensis Linn), belongs to the family Malvaceae. It is an ornamental shrub grown in the tropical and sub tropical gardens. The present day garden hibiscus comprises of multicoloured shades including orange, pink, mauve, red, scarlet, yellow etc. In hibiscus the identified anthocyanin was hibiscin, gossypicyanin, delphinidin and

cyanidin. The yellow part of the petals contains gossypetrin and gossypetin whereas the purple parts contains cyanidin, pelargonidin and delphinidin (Nair et al ., 1962).

The biosynthesis of these pigments and hence their relative proportions determines the eventual flower colour. As the work with respect to the pigment estimation and composition is very meagre both in rose and Hibiscus species, possibilities on the improvement of flower colour was laid greater emphasis for the present investigation. The range of variability in flower colour which is of potential value could be exploited further in breeding new varieties of rose and hibiscus.

Thus, the knowledge of different group of pigments and the different colours they contribute, biosynthetic pathways, mechanisms they undergo is necessary to understand the varied expressions of flower colour. Variation in colours and forms of evolved varieties involves the use of phytochemistry in unraveling the hidden informations about the pigment constituents and their biochemical mechanisms involved in the expression of colours.

A systematic planned mutation breeding programme would play a key role in solving some of the problems in the current breeding process, such as the need for the commercial exploitation of hybrid vigour, development of a usable form of male sterility, various morphological characters including vegetative and floral characters along with pigment contents for different flower colours. It is only in such a path breaking role that mutation breeding is likely to find the most rewarding application in future. Therefore, with regard to the above considerations, the present investigation was undertaken with the following objectives:

1. To study the effects of different levels of irradiation treatments on induced mutants in different rose cultivars.

2. To study the growth parameters of different cultivars on both induced and spontaneous mutants.

3. To study the morphological and floral changes in induced and spontaneous mutants of roses.

4. To study the pigment composition of both induced and spontaneous mutants of rose along with some hibiscus cultivars.

5. To estimate the pollen fertility and viability status of different rose and hibiscus cultivars.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

2.1 MUTATION BREEDING STUDIES IN ROSE

Mutations are sudden heritable changes in the characteristics of the genetic makeup. Mutations can be induced at relatively higher frequencies by treating with certain mutagens. Spontaneous and induced somatic mutations have played an important role in the evolution of many important cultivars of garden roses. Gamma rays have been successfully used for the induction of somatic flower colour or type mutations in roses. The possibility of mutation breeding in vegetatively propagated crops depend on the genetical characters involved, mutagens used, handling of the material, availability of a selective screening method etc.

Rose is called the queen of flowers and is grown widely. Rose breeding is extensively undertaken in France, USA, Canada, Australia, Germany, Newzealand and several other countries. The chromosome number vary from 2n=14 to 2n=56, but most of the species are diploid and tetraploids and commercially grown rose cultivars are triploids. Generally speaking chromosomes in breeding behave in a normal regular fashion. There are times when new characters suddenly arise in a species and found to act as a single dominant but more frequently as a recessive character. Such alterations are probably due to a single gene and thought to be accounted for some modifications in its molecular structure. Spontaneous mutations occur frequently and have caused a valuable increase in the genetic variation of ploidy level, high sterility, seed dormancy accompanied by poor seed germination.

Varietal choice and genetic characters should be carefully chosen. With a view to study the plant responses to low doses of irradiation extending over a

longer period of time and also at different growth phases of plants, facilities of chronic radiation from Co60 source are being developed in many countries. A high degree of optimism is maintained in favour of chronic and semi-acute exposures especially in vegetatively propagated horticultural crops and results obtained so far in producing somatic mutations are much encouraging. Thus, the present investigation was carried out to study the effects of recurrent gamma irradiation along with spontaneously induced mutations in rose genotypes. Thereby, a brief attempt is made to review the literatures available on various morphological aspects involving both vegetative and floral characters, related genetic factors of both induced and spontaneous mutants in rose.

Zimmerman and Hitchcock (1951) reported a new rose sport with single and pink flowers when compared to their double pink parents. When plants of the sports were propagated from stem cuttings, they continued to show the same characteristics, while plants from rooted cuttings reverted back to the original parentage. It was thus said that rose varieties were probably periclinal chimeras. Mutation breeding came into existence only after Stadler's discovery (1928) that higher rates of mutations could be induced by X-rays in both maize and barley. Although hybridization technique is well known since centuries (Allard, 1960). The ability to produce mutations artificially attracted the attention of several workers and considerable efforts have been diverted towards utilisation of this technique in crop improvement.

There are several ways in which the new roses have evolved. The two major being the occurrance of natural or induced mutations or sports, and the raising of roses from seeds produced as a result of artificial or natural cross pollination. In natural mutation, a shoot with different genetic behaviour is observed on an existing plant, which when vegetatively propagated, produces a new cultivar.

Natural mutants have been observed for a very long time. The double flowered variants found in many species, the dwarf habit in Rosa chinensis and other species are good examples of natural mutations or sports. The famous dwarf polyantha Paul Crampel, which has flowers of a unique geranium-red tint, frequently back mutates to scarlet of pale pink. A major component of breeding programme has been (Allard, 1960) focused on rose with the aim of combining winter hardiness, perpetual flowering and ornamental value to the flower.

Sarkow (1961) reported the production of rose varieties through bud mutations. Analysis of occurrence showed the number of mutations found per variety within the groups, the colours produced and the age at which varieties have been mutated. Special attention was paid to the use of in-vivo or in-vitro adventitious bud techniques by which solid non chimeral mutants were obtained resulting in an easy and early selection. Gupta (1966) reported some interesting results after irradiation of hybrid tea roses with different acute exposures to gamma rays. Single flowers with 5 petals were produced after irradiating rose varieties 'Crimson', 'Mirandy' and 'Super Star'.

The most suitable rates of acute gamma irradiation recommended was 5000-6000 rads for rose budwoods. Gupta and Shukla (1970) subjected rose budwoods of cv 'Montezuma' and 'Super Star' to different gamma ray treatments and recorded the data on plant height, percentage of flowers with morphological abnormalities, colour and shape of mutated flowers. Gamma irradiation induced colour mutations in the cv 'Christion Dior', 'Queen Elizabeth' and 'Kiss of Fire' by treating the dormant buds with 5-10 Kr gamma rays which was found to be the best treatment where higher dosages was found to be lethal. Davis (1971) obtained spontaneous mutants 'Gabriella' and 'Jaguar' from 'Mercedes'. These two mutants produced many variegated flowers and were periclinal chimeras in nature.

Lunstad (1975) evaluated 45 new cultivars of floribunda and polyantha roses. Based on the results the cvs 'Jan Speck', 'Tip Top' and 'Pernille Poulsen' were recommended to be the best varieties. Three common mutants were produced namely 'Desi'(3Kr), 'Permoser'(1.5Kr) and 'Flamingo Queen'(7-8Kr) released in Canada. Improved flower colour attributes such as intense colour of the petal margins, stripes etc. were observed. Le Greice (1976) depicted the new colour break among roses which were first seen in polyantha rose sports from 'Red Orleans'. Swarup et al .(1971,1973,1978) have described the various morphological characters of 21 new rose cultivars evolved at IARI, New Delhi. Cultivars 'Mridula' and 'Chitralekha' showed maximum and minimum plant heights. The colour of the flowers varied from creamy white in 'Navneet', phlox pink in 'Surabhi' and deep ruby red in 'Chitralekha', respectively.

Kaicker and Swarup (1978) reported that the mutants obtained by treating the budwoods of rose cv 'Gulzar' had blue stripes on deep magenta flowers. Morphological characters of some new varieties of floribunda and hybrid teas were studied (Lata, 1980) with 3,4 and 5Kr gamma irradiation. Significant differences were recorded but however, 3Kr exposure was the least harmful to the production of shoots. The decline in shoot length was correlated with increase in exposure. In another experiment she stated that LD50 dosage for white and mauve flowered cultivars was lower than yellow, red or pink flowered ones, the last being more prone to mutations. Only 3 mutations, one in growth habit, 2 in flower colour were successfully identified. Among them 'Pink Parfait' was found to be most suitable for further induction of mutations.

Irulappan and Rao (1981) reported the effects of gamma rays and EMS on 'Edward Rose' assessed in two generations. During VM1 generation individual flower weight and number increased in all mutagenic treatments. In VM2 generation increased mean values for number of flowers and weight of

flowers/plant was observed. Budwoods of rose cv 'Junior Miss' was irradiated with 3,4 and 5Kr gamma rays (Datta and Gupta, 1982). No mutants were detected in the first year but in the second and subsequent years after heavy pruning pink to white flower colour mutants at 3Kr treatments were observed. Flower diameter and stomatal size was reduced with increase in petal number. Kaicker (1982) listed 50 rose cultivars which were either naturally occurring or induced mutants with striped or multi coloured petals along with their mutated characteristics.

Datta and Gupta (1983) irradiated the budwoods of rose cv 'Contempo' with 3,4 and 5Kr of gamma rays. Three somatic flower colour mutants were isolated and multiplied in the pure form. Results indicated that the changes in the flower colour was due to qualitative and quantitative changes in pigments by irradiation. Cuttings of 'Grussan Berlin', 'Super Star' and 'John Strong' were irradiated at 2,4,6 and 8Kr gamma rays (Guo et al., 1983). Growth of the callus, stems, roots, leaves and flowers were inhibited when irradiation dosage was more than 6Kr. Thus the optimum dosage recommended was 4Kr gamma rays. In another experiment 'Saroda' and 'Sukumari' were the two mutants obtained by exposing budwoods of cv 'Queen Elizabeth' to 3Kr gamma rays, which produced almost white coloured flower mutant.

Datta (1985,1989) observed the reduction of sprouting and survival with increase in exposure to gamma rays in rose. Among the cultivars used 'Orange Sensation' was found to be more sensitive. Stimulation in sprouting was observed in some cultivars after treatment with lower doses. He also studied on the differential radiosensitivity, where somatic mutations in flower colour and type were recorded in chimeric forms. Three mutants viz., 'Light Pink Price', 'Curio' and 'Twinkle' were released as new cultivars. Benetka (1985) reported some experiences on the methodology with the isolation of somatic mutations in rose cv 'Sonia'. Optimum irradiation dose was found to be between 4 and 5Kr and with

increased bud generations the number of chimeral mutants decreased. The progeny of these shoots had a lower frequency of chimeras which would seem to be the most suitable propagation material to obtain non-chimeral mutants.

The improvement on miniature roses was reported (Datta, 1985) on sprouting percentage, survival and plant height which decreased with an increase in the dosage. Somatic mutation in flower colour was detected in both the cultivars 'Windy City' and 'Magic Carrousel'. Repeated recurrent gamma irradiation reduced sprouting, survival and plant height but increased the somatic mutations. Mutant colours thus obtained includes light, orange, pink, yellow and orange with a yellow streak. Kaicker and Dhyani (1986) irradiated the budwoods of two rose cultivars 'Folklore' and 'Davis Tystermann' with 2.5, 4 and 5Kr gamma rays. The effective dosage was found to be 2.5Kr for 'Folklore' and produced two mutants viz., orange and orange with tipped pink petals. Green shoots of rose cv 'Crimson Glory', 'Super Star', 'Peace' etc. were irradiated at 3Kr gamma rays (Huang and Chen, 1986). Mutants were selected for leaf and flower characteristics from these varieties and four new cultivars were established from the stable mutant clones.

(Rukmanski, 1988) gave a general classification on mutation based on 4 main criteria :

- **Inducing Factors** : Distinguishes spontaneous and induced mutations along with mutations induced by physical, chemical and biological factors.
- **Change in Genotype** : Distinguishes various types of gene mutations and chromosome mutations.
- **Change in Phenotype** : Distinguishes such types as morphological/ physiological/ biochemical/ somatic/ gametic/ direct/ back/ recessive/ dominant/ micromutations/ macromutations/ fertile/ semifertile/ sterile/ vital/ lethal and semilethal mutations.
- **Usefulness** : Distinguishes deleterious neutral and useful mutations.

Datta (1989) evaluated per cent sprouting, survival and plant height after gamma irradiation in different cultivars of garden roses. Reduction in all the characters was seen in all the cultivars except in 'Neelambari' which was found to be resistant to higher exposures upto 4Kr. Of the eleven cultivars, somatic mutations in flower colour was detected in four cultivars namely, 'Salmon Beauty', 'Raja Surendra Singh of Nalagarh', 'Arjuna' and 'Mrinalini'.

Most colour changes depicted the reappearance of previous colour mutations but in sport cultivars, change towards the original colour occurred. Histological observations could supplement visual observations when assessing mutations for breeding. (Zykov and klimenko, 1989) studied the prospects of shortening the time to breed new garden rose cultivars. He suggested that induction of mutation using gamma irradiation of heterozygotic forms was especially suitable for rose breeding, resulting in a more rapid and intensive display of useful recessive characters. Gamma irradiation combined with distant hybridization produced perpetual flowering, highly ornamental and climbing forms. In preliminary varietal trails, 20 promising mutants of rose were identified. Stem cuttings of hibiscus, chrysanthemum, rose and tuberose determined radiosensitivity on the basis of cytological and morphological indices of somatic mutations (Datta, 1990a). The mutation frequency and the spectrum varied with the cultivar and gamma ray dosages.

Cytogenetical studies on the cv 'Folklore' and its mutants were carried out by Datta (1991a). Control cv 'Folklore' was found to be tetraploid with 2n=28 and meiotic analysis revealed a maximum number of multivalents. Euploidy was most commonly found in both control and its 12 induced mutants with exceptional cells of aneuploid numbers. Variation in chromosomal pairing reflected varying degrees of expression with additional number of micronuclei and chromosomal mosaicism. These factors are responsible through the use of low dosage of gamma rays at 2.5 -

5Kr. Datta (1991b) carried out the experiment to assess the bud uptake of 35 rose cultivars on to Rosa indica var. Odorata rootstocks. Exposing the buds to gamma rays determines the LD50 dosage which is a prerequisite for mutation breeding programme. Different cultivars of different groups were differentially sensitive to gamma rays indicating that radiosensitivity was genotypically dependent.

Comparative study on recurrent gamma ray induced dwarf mutants of cv 'Folklore' revealed that there was marked difference in terms of morphological characters between dwarf mutants and the original variety (Kaicker and Kumar, 1992). Induced somatic mutations in flower colour and shape was noted in 21 cultivars, out of which 19 mutants were isolated . The mutant sector varied from a narrow streak on a petal to an entire flower and from a portion of a branch to an entire branch with the most suitable dosage of 3Kr gamma irradiation.

Meilland (1993) stated that a new and distinct variety of hybrid tea roses abundantly forms an excellent capacity for reblooming attractive double long lasting flowers which are venatian pink in colour. The new variety originated as a spontaneous mutant exhibiting upright growth habit, vigorous vegetation and was well suited for cut flower production under greenhouse conditions. Datta (1990b,1995) subjected cultivars 'Zorina' and 'Mrinalini' to gamma rays and observed reduced sprouting, change in flower colour and increased morphological abnormalities.

Datta (1994) irradiated budwoods of rose cv 'Salmon Beauty' which showed various abnormalities. The mutant tissues, striped in nature was isolated and established in the pure form which reduced sprouting and survivality after irradiation. Surendra Kumar and Kaicker (1994) studied on three different types of striped induced mutants of cv 'Folklore' which revealed that all morphological variations may be a direct consequence of irradiation. Datta (1995) reported

gamma ray induced mutant 'Sukumari' from the original cv 'America's Junior Miss'. Changes in the petal number was recorded in some of the induced mutants. The number significantly decreased among the mutants yellow, striped contempo and windy city, but the petal number increased in 'Twinkle' and its mutant 'Salmon Beauty'. Thus spontaneous as well as artificial mutations play an important role in the evolution of new garden roses. From the above literature it is clear that roses are very much suitable for mutation breeding, since novelty of flower colour and other desirable mutations can be produced in outstanding cultivars without loosing any of their original desirable characters.

2.1.1 INHERANT / GENETIC FACTORS

In self fertilised crops hybridization stands as a conventional methodology by which favourable genes available in different genotypes could be combined to a single genotype through genetic recombinations. Alternatively mutation breeding emerged as a non-conventional breeding method of improving crops not only by tapping variability due to the new gene recombinations which might have lost due to selection and the types never evolved due to variability already existing in the natural populations (Brock, 1958 and Brady, 1960).

Characters like plant height, growth habit, appearance, position and colour of flowers etc., are governed by genes. Only a few genes may be required to determine the length of the stem, while there may be several genes which are collectively responsible for the colour of a specific cultivar (Sparrow, 1961). The duration of flowering in roses is determined by a gene which in its dominant form restricts blooming to once a year. The recessive form of this gene giving a far more extended period of flowering is obviously of great importance (Pal, 1972). The characters in rose was dependent primarily on a single dominant genes like glossy leaves (vs.dull), climbing habit (vs.dwarf), double flowers (vs.single), while the

characters dependent on an interaction of many genes are vigour, fragrance, thorniness, strength of neck, width of leaf, length of cutting stem and shape of bud and open flowers.

The basic characters of any plant are determined by its genotype, which, although identical in all plants of a single clone, can give different responses according to the environment in which the plant is grown. New genotypes are produced by sexual reproduction and by mutation occurring either naturally or by induction. The inheritance of fragrance did not follow a definite pattern as its expression is governed by the ultimate effect of many genes. Therefore, considerable decrease or increase in intensity of fragrance was noticed in some of the hybrid flowers (Swarup et al., 1973). The presence of fragrance in the blooms of hybrid progenies in the crosses between non-fragrant parents suggests the presence of complementary genes for fragrance in the parents.

Like other plants, growth and flowering behaviour of roses is governed by genetic factors. Significant differences in the growth and flowering of different rose species and cultivars have been observed which are attributed to the inherent genetic variation existing amongst these species and cultivars (Jicinska, 1975). The range of variability in roses is very great. Fuji and Matsumura (1967) observed that the productivity was determined mainly by the additive effect of genes. Genes with additive effect had the principal role in flower character inheritance. They estimated the selfed progeny of gerbera for genetic components of variance where he found high heritability for inflorescense, diameter and number of ray florets and relatively low for disc diameter and stem length. The heritability of ligule length and stalk legth showed a high level of heterosis. Ligule length was correlated with certain other flower characters.

A correlation coefficient at genotypic and phenotypic levels among the seven traits studied by Jicinska (1981) showed the same trend and genotypic correlations were on the higher side. The estimates of heritability and genetic advance were quite high for all the characters except the number of leaves per plant. Path analysis of thirty nine different genotypes indicated that the flower diameter, plant height, longevity of flowers and number of branches were important component characters for the number of flowers per plant.

Studies on phenotypic variability, heritability and genetic advance, made by Broertjies (1978) indicated the existence of high heritability for several characters. High heritability was accompanied with high genetic advance for the number of branches, spikes, flower buds and petals and for average flower weight, showing an additive gene effect. High heritability and lower genetic advance showing a non-additive gene effect was obtained for flower diameter and plant height while high heritability with moderate values of genetic advance was obtained for plant height. High degree of heritability for number of branches and thorns, branch angle and plant height was also reported by Micke (1975). The leaf length and new shoot colour, however, had a low degree of heritability.

The genes located on a given chromosome are said to be linked together as these are transmitted in the progeny as a group, provided crossing over does not occur at meiosis. In gladiolus heavy substance of segments and heavy ruffling, heavy ruffling and short flower heads, blue-violet or red flower colour and certain pathogenic organisms, large floret size and loose floret attachment, flower colour and corm pigmentation, production of large cormels and late blooming, etc., are said to be linked characters (Griesbach, 1982). In case of very closely linked genes, large number of seedlings of a cross is grown for getting more number of desired combinations.

According to Bhal and Gupta (1982) inheritance studies indicated that the characters were controlled by recessive genes. In general, mutagenic treatment widened the range and increased the mean values for all the traits. Variability generated was not dependent on dosage, but it varied with the trait. They also reported the variability and genetic advance after selection in quantitative traits. Variability generated was found to be dependent on characters, treatment and also generations.

Jong (1984) found 70% heritability of characters, like number of days to flower and number of flowers per plant, in 79 F1 population from 15 parents. He also studied the inheritance of flower doubleness and floret corolla shape in 70 F1 population from 16 parents. Flower doubleness and corolla shape were not linked in chrysanthemum. Heritability, repeatability and components of variance for 68 morphological characters describing the inflorescence, scape and florets of Gerbera hybrida were tabulated by Drennan et al. (1986). Heritability estimates were generally moderate to high for simple dimention characters and characters which were the mean of several measurements. For most characters the variance amongst inflorescence from the same plant was the largest portion of the total phenotypic variance.

Colour in roses, as in other flowers is governed by the expression of water soluble pigments called anthocyanidin. The three important anthocyanidins are pelargonidin present in orange-red to scarlet flowers, cyanidin present in crimson to bluish-red flowers and delphinidin present in blue and violet flowers. Besides these anthocyanidins, there are a number of other pigments such as flavonols and carotenoids for white, yellow or brown pigments in the flowers. Earlier work of Connors (1914) reveals that in crossing of yellow with white, red being present as a latent characters and white is dominant over yellow or red. In some yellow carnations the presence of red is associated with the presence of perfect sexual

organs. Genetics and chemistry of flower colour of carnation have further been studied by Handerson (1953). Six independent factors are responsible for expression of self-colour in carnation.

Inheritance of different characters of chrysanthemum have been studied by many workers. The somatic genetic analysis of the apical layers of 16 chimeral sports of the Indianapolis cultivars led to the understanding of complex nature of colour inheritance (Stewart and Derman, 1970). It was shown that a cultivar can be genetically of one colour in L1 and of another colour in L2. As the sex cells arise from L2, a cultivar which is white in L1 and pink in L2 would have white colour but breed as pink and vice versa. They also studied the inheritance of characters, cell sap anthocyanin and chromoplast carotene production. Segregation of flower colour was suggested by them to help in the identification of parental genotype.

It may not be possible to get blue roses till these pigments are incorporated by some means. Each pigment was highly heritable from the seed parent. All pigments, particularly cyanin and peonin showed quantitative inheritance. The inheritance of each of these colour pigments is reported to be mainly controlled by additive gene action (Vries et al., 1980). The genetical studies on the inheritance of colour in roses has indicated that the dominant colour in rose is magenta, pink or tyrian-rose to orange-red. The pink colour is dominant over dark red, orange-yellow, yellow, white and scarlet. Deep yellow colour is recessive to light yellow and white is recessive to cream and light yellow.

In certain cases, inhibitor genes are present which inhibit the pigments of lavender-red-purple anthocyanins of gladiolus present in their stamens or inner portions of the petals and if there is simultaneous presence of one or more inhibitor genes, the effect of inhibitor genes to a certain degree may be prevented

consequently. The cultivars of rose having inhibitor genes may be pure white, cream or yellow which express their inhibited colours under certain temperature and light conditions. Such inhibitor genes may or may not inherit in the offsprings, if inherited the colour may be suppressed, otherwise the colours in the flowers are expressed (Zykov et al., 1991a).

2.2 POLLEN STUDIES IN ROSE AND HIBISCUS

Ornamental crops in the recent years are rapidly emerging as potential export products. Among them rose is one of the most important commercial crop grown for its cut flowers. Rose breeding is now carried out on a large scale in France, Germany, Netherlands, UK, USA, Canada and other advanced countries. Success of rose breeding is comparatively less as rose is a composite hybrid involving interspecific hybridization, polyploidy with high female and male sterility, chromosomal abnormalities, heterozygous genotypes etc. In addition to this, poor germination of seeds further limits the success of rose breeding.

On the other hand, hibiscus is also one of the most fascinating ornamental shrub grown in the tropical and sub tropical gardens. In reality it is a highly polymorphic cross compatible group of species with complex hybrids and their derivatives. Almost all the cultivars are sexually sterile which may be due to genic, structural, segregational or environmental causes. The basic information regarding pollen fetility, seedset, pollen storage and viability in most of the cultivars of rose and hibiscus cultivars is lacking. Thus available information in view of these aspects have been briefly reviewed and summarised below.

2.2.1 POLLEN FERTILITY AND VIABILITY

As early as in 1951, Calvino conducted a series of studies in relation to the pollen fertility of rose, where he described various aspects on pollen fertility such as morphological, biochemical characters of pollen grains, percent pollens aborted, number of chromosomes with certain somatic characters, reserve substances in pollen germinability, viability and methods to prolong the viability of pollen grains. Pollen sterility in 96 varieties of garden roses was studied by (Sahare and Shastry, 1963), of which 67 varieties were of hybrid class, in which cv 'Kaiserin August Victoria' had the highest pollen sterility percentage (98%), with 'Ena Harkness' and 'General Mr Arthur' having the lowest pollen sterility (10%). It was also observed that three varieties each of triploids and anueploid hybrid teas had very high pollen sterility. Pollen stainability and meiosis observations were made with the help of acetocarmine stain.

Nair (1965) studied the significance of pollen morphology in relation to the taxonomical evolution of cultivated plants. A preliminary investigation of pollen morphology and key to their identification of some rose species were undertaken by Reitsma (1966). Vilasini et al. (1966) conducted studies on pollen morphology, production and viability in different varieties of Hibiscus rosa-sinensis. The pollen grains of all the varieties were similiar in shape, but considerable variations in size existed both between and within varieties. Germination in sucrose media is enhanced by the addition of 100 ppm boric acid. Cytochemical localization of hydrolytic enzymes in the walls of pollen grains were studied by Knox and Harrison (1969). The site of deposition was the cellulosic intine over the walls.

Preliminary survey on pollen sterility status of six strongly scented hybrid tea roses using acetocarmine stain was carried out by Lata (1971). Shrivelled, unstained and empty pollen grains were considered as sterile, with an average

pollen fertility varying from 19 to 36 percent during winter season. The viability test was conducted by Nakajima (1973) on six essential oil rose cultivars. Pollens from the results indicated to have been stored successfully for upto 70 days in sealed test tubes at 5-7^{0}C.

Pollen viability in garden roses was high when they were irradiated with 5-20Kr gamma rays by Klimenko et al. (1974). The pollens of the cv 'Kordes Sondermeldum' and 'Dortmund' showed outstanding resistance to irradiation so far as the viability was concerned. Lata and Gupta (1975) studied the effect of ionising radiations on roses and determined pollen fertility status with acetocarmine stain in cv 'Montezuma' along with its pink and reddish orange flowered mutants. Unstained and shrivelled grains were treated as sterile. Fertility decreased in pink flowered mutants as compared to control whereas, an increase was recorded in reddish mutants. Kosh et al. (1976) evaluated the effects of temperature and RH on the pollen viability of six rose species. In most of the species the highest pollen viability was attained after 2-3 weeks, with an optimum RH of 50 or 70 percent at 0 and 25^{0}C, respectively.

Pollen viability of hybrid tea roses grown outdoor and in the greenhouses were studied by Surina (1976). Pollens from outdoor plants were more viable than from greenhouse grown plants. Gowda et al. (1977) observed the percentage of pollen fertility or stainability in 8 hybrid tea roses by acetocarmine stain. Pollen fertility varied from 12-56 percent. It was observed that the percentage of pollen fertility in controlled pollination and pollen stainability were almost similiar. Pollen viability was shown to be inherited additively in hybrid tea roses originating from 31 crosses (Visser et al., 1977a). Swim (1979) determined pollen fertility in different cultivars with a total of 184 cross combinations attempting hybridization between hybrid teas and floribunda roses. Pollens for use in hybridization remained viable for atleast 9 months when rose pollens were stored at 1^{0}C

temperature and 1 to 20 percent RH (Dubois and De Vries, 1979). Good pollen fertility percentage in three new cultivars of roses, viz., 'Spotless Gold', 'Spotless Yellow' and 'Spotless Pink' which were selections from F3 population were recorded by Semeniuk (1979).

Lata (1980) while studying the effect of ionising radiations on 7 rose cultivars determined pollen fertility status in all the cultivars on the basis of acetocarmine stainability test. It was observed that irradiation brought about a loss in pollen fertility. Sterility in Hibiscus rosa-sinensis cv 'Scarlet' was attributed due to a combination of pre-fertilization (placental inhibition) and post-fertilization (embryo abortion) as stated by Rajan and Namboodari (1982). Viability of fresh pollens in rose cultivars were evaluated by Pearson and Harney (1984). Though they observed a positive and significant correlation between pollen staining and germination, they concluded that absolute pollen viability was a better indicator than staining.

Pollen grains of Hibiscus species were examined by (Shim et al., 1988). The shape of the pollens were all spherical, echinate and perforate. Single flowered cultivars had larger pollen grains than double flowered cultivars . Zykov and Klimenko (1988) studied pollen viability in mutant forms of rose. Irradiation of heterozygous rose cuttings alters the flower colour through the loss of dominant genes controlling colour and manifestation of recessive alleles. Pollen yield of some ornamental trees and bushes from Rosaceae family was recorded by Szklanowska (1992). In most rose species the pollen productivity was 3-10 kg/ha, but in Rosa rugosa it was upto 20 kg/ha and in Rosa multiflora 150-500 Kg/ha was estimated with 80 - 90 per cent sterile pollen grains.

Brewbaker (1992) showed no visible differences in pollen tube growth between self and cross pollinated Hibiscus species. Snow and Spira (1993)

demonstrated the potential for unpredictable effects of pollen competition on individual selfing rates in Hibiscus moscheutos which in turn may effect progeny vigour. This complex situation contrasts outcross pollens which consistently outcompetes self pollens. Cryopreserved pollen showed to retain its ability for fertilization according to Marchant et al. (1993). Successful long term storage of pollens will facilitate hybridization of rose species and cultivars that do not flower synchronously. Normal anthers with functional pollens that could be used for hybridization was reported by Vaidya (1994). The mutant was recessive for normal female fertility characteristics and was designated as female sterile.

2.2.2 POLLEN GERMINATION

Pollen germination was studied in artificial media of sucrose, agar and lactose (Singh, 1961). Giberrellins when added to the media, stimulated the germination of fresh pollens in certain woody plants. In some cases, highest concentration inhibited germination. Both the rate and percentage of germination along with the length of the pollen tubes increased by 0.005% GA solution and remained viable than on the media without it (Kaurov and Vakula, 1961). Changes in the composition of sugars and enzyme activity during the storage of rose pollens were recorded by Zolovitch et al. (1964). Pollen germination declined sharply after 50 days and ceased after 80 days of storage.

Cochis (1965) suggested that the best artificial medium for invitro germination of hibiscus pollen was found to be 1 per cent agar and 40 percent of sucrose with no additives. GA and 2,4-D were found to inhibit in-vitro pollen germination in 4 varieties of hibiscus. Differences in varietal responses to the treatments were ascribed to differences in pollen viability. The compounds promoted pollen tube elongation, but the effect reduced with increasing concentration of the compounds (Vilasini et al., 1967). Maximum pollen

germination and pollen tube lengths occurred at temperatures 30, 35 or 40^{0}C (Kancalova, 1975) and also observed the branched pollen tubes occasionally in the germinating pollens of individual Rosa species. The optimum pH reported was 6.5-7.0 for pollen germination and optimum temperature was 70-85^{0}F.

Jicinska et al. (1976) evaluated the percentage of morphologically normal pollen grains and compared with the viability and actual in-vitro germinability of the pollens from 18 shrubs of wild Czechoslovak roses. Visser (1977a) reported that the germination capacity and staining ability of pollens were greatly impeded on dehydration during storage with less RH. This effect was said to be corrected by humidifying the pollens beforehand for about one hour. This pre-treatment increases the percentage of pollen germination more than the per cent stained. Visser et al. (1977b) reported that pollen grains germinated well in 15 percent sucrose solution and 40 ppm boric acid. Gupta et al . (1979) carried out artificial germination of pollen grains by using sucrose. Highest percentage of pollen germination and longest pollen tube growth was recorded in 'Christian Dior' and a minimum in 'Super Star'. In another study the fresh pollen germination and pollen tube growth in 13 rose cultivars were examined. Pollens of the cv 'Hansa' exhibited the best germination percentage (70%).

The percentage of pollen fertility was estimated by using acetocarmine stain and versatile stain suggested by Alexander (1980). According to him acetocarmine stain has certain limitations since it stains only non aborted pollen grains, while the aborted pollens were identified by the unstained and empty pollen walls. The versatile stain suggested overcomes all these problems and gives a clear differentiation between non aborted and aborted pollen grains. The non aborted grains take crimson red and aborted ones green stain. Meynet et al. (1994) recorded dihaploid plants obtained by parthenogenesis induced using irradiated pollen and cultured in-vitro on modified MS media.

Voyiatzi (1995) germinated pollen grains of 5 hybrid tea roses in-vitro to determine whether these cultivars were suitable as pollen donors in the breeding programme. Pollen grains of all five cultivars germinated poorly in the medium containing only sucrose. Highest germinability was observed in 'Ferry Porche' and 'Lady X' on 15 per cent sucrose + 50ppm boric acid and on 20 per cent sucrose+100ppm boric acid, respectively. Addition of calcium nitrate reduced germination whereas effect of pH on the medium was cultivar and composition dependent. The addition of boric acid decreased the responsiveness of the pollens of all cultivars to the pH changes of the medium. Results indicates that cvs 'Lady X' and 'Ferry Porche' were the best suitable pollen donors.

2.3 PIGMENT STUDIES IN ROSE AND HIBISCUS

Rose is one of the most important commercial flower crop cultivated throughout the world for their cut flowers and garden decoration purposes. Floral colours are of great assistance in giving both breadth and depth to our understanding of the visual sensations excited by polychromatic radiations. In numerous cases, the efforts of horticulturists to introduce improved varieties of flowers enjoy popular colour resulted in producing flower forms which exhibit more attractive colours.

Large number of hybrids and open pollinated seedlings are being raised every year by nurserymen, various institutions and ameatures for selecting desirable traits from them for commercial release. The creation of new colour patterns in rose species has always been a major aspect of classical flower breeding which has been accompanied by a detailed chemical and biochemical analysis of the components responsible. The major chemical components which influences colour formation in most of the ornamentals are carotenoids, flavonols, chalcones, aurones and especially anthocyanins.

The new colour break among roses was first seen in polyantha rose sports from red 'Orleans'. Normally the base material in red roses were cyanidin, but here the new colour pelargonidin appeared. The same change occurred in seedlings raised on vivid roses such as 'Independence' and many others. The sport has proved itself capable of breeding a whole race of brilliant colours in hybrid teas, floribunda and shrub roses. As pelargonidin is always present with cyanidin breeding results are not predictable. Differences in the perceived colours can arise as a consequence of the same floral pigment being present but in different quantities on rose petals. On the other hand, differences in the colour appears in various cases which is ascribable to the actual differences in the nature of the floral pigments.

In this regard, a brief literature on pigmentation details of rose and hibiscus flower colours including the contributing factors for varied colour expressions are briefly reviewed below

2.3.1 BIOCHEMICAL AND GENETICAL CHARACTERS OF FLOWER COLOURS

Arisumi (1963) has described the flower colours in Rosa species with special reference to biochemical and genetical analysis and their application of the results in practical breeding. Chromatographic studies have shown cyanin to be the commonest of several anthocyanins present in both wild and cultivated roses. Ahuja et al. (1963) identified anthocyanins in the petals of rose cv 'Pink Coronet' and 'Happiness'. The anthocyanin identified was cyanidin glucoside as cyanidin 3,5 - diglucoside. Studies on flower colours in rose with special reference to biochemical and genetic analysis was undertaken by Arisumi (1964). Breeding trails reported in floribunda roses revealed that it contained pelargonin when crossed with yellow acyanic varieties. When yellow roses containing kaempferol were used, all the offsprings contained pelargonin, but almost no pelargonin was found in the progeny whose yellow parent contained quercetin.

Studies on the evaluation of morphological characters after the effect of ionizing radiations have been conducted by many workers. Vega and Martin (1967) isolated an unusually high yielding anthocyanin from the rose cv 'Paula Scarlet' as cyanidin 3, 5 - diglucoside. The expression and relationship between anthocyanin content and the expression of colour tone in 2 groups of red rose varieties were studied by Yasuda (1967a). Pigments are not altered but the mutants showed an increase or decrease of one or several of the pigments found in control Heslot (1968). Colorimetric study of the quantitative effect of anthocyanin and the role of surface reflection was also undertaken. Results indicated that surface reflection plays an essential role in the expression of petal colours. This is presumably because most interesting mutations are hidden as recessive alleles in heterozygous condition. Flowers of 10 rose cultivars were identified by Valadon and Mummery (1968). All contained /3 - carotene and /3 - carotene oxide mutachrome. Lutein was present in small amounts. A new yellow pigment termed rosaxanthin was found in 6 varieties. It was suggested that rose flowers are yellow or orange depending on carotenoids present and also non carotenoid pigments mainly anthocyanin.

Petals of 670 rose cultivars and 8 species were grouped according to their anthocyanin composition (Yokoi, 1974). The pigments identified were, cyanidin 3 - glucoside and 3,5 - diglucoside, pelargonidin 3 - glucoside and 3,5 - diglucoside and peonidin 3,5 - diglucoside. A large number of floribunda cultivars contained 4 pigments. More the pigment in the flower more scarlet or orange was the colour. Peonidin was found in large amounts in many species and hybrids, even in floribundas, hybrid teas and miniature roses. One of the rose shrubs contained all the five pigments. New genetic resource in peonin pigment and a new combination of anthocyanins were found in rose species. Peonins were transfered from the native tetraploid to fertile tetraploid hybrids with floribunda and hybrid tea roses (Marshall, 1975). A separate pair of blue fluorescing spot was associated with

each of these 3 anthocyanins. Yellow flourescent spots, probably flavonoids were present when the anthocyanins were almost absent.

A great deal of information is known about the flavonoid pigments (Harborne, 1976). The flavonoid pigments can be subdivided into two groups like anthocyanidins and the copigments. All the copigments have a light yellow or cream colour, while the anthocyanidins are coloured yellow/ orange through blue. Yellow anthocyanidins which are not very common, usually are combined with carotenoids to give yellow flower colours. There are six major non yellow anthocyanidins viz.,pelargonidin, cyanidin, delphinidin, malvidin, petunidin and peonidin. Specific anthocyanidin molecules differ in the type and number of side groups which are attached to the basic anthocyanidins which are glycosylated and they are called the anthocyanins. These sugars sometimes are acylated with organic acids like caffeic, cinnamic or benzoic acids. Glycosylation and acylation were known to stabilize the colour of the anthocyanidins under cellular conditions.

Orchid flower colour is the result of a mixture of two types of pigments. The production of each pigment type is the result of an independent sequence of biochemical reactions. A block in the flavonoid pathway resulted in the absence of red or blue colour, but will not affect the carotenoid pathway of yellow / orange colours. This is why jewel box flowers are red when the parents are orange (aurantiaca) and purple (Harborne, 1976). Biosynthesis of anthocyanidin, cyanidin 3-monoglucoside in the developing flower buds of cv 'fresham' rose and its progeny were studied by Asen (1976). Glucoside biosynthesis in flower buds of the progeny was different from flower buds of plants grown under dark Vries et al. (1980) and the inheritance of each pigment was found to be mainly controlled by additive gene actions.

Datta and Gupta (1983) reported that mutation may result in both synthesis of new pigments and blocking of the development of one or more existing pigments. This is associated with either increase or decrease in the concentration and composition of pigments ultimately leading to the formation of new colours. Greisbach (1984) reported that the flower colour is usually due to at least two different types of pigments. One is lipid soluble and includes the carotenoids. These pigments are located within the plastids and are responsible for yellow orange colours. The other type of pigment is the water soluble flavonoids located within the vacuoles and are responsible for yellow / orange through blue colours. The flavonoids are usually found in the cells of the upper and lower epidermis while carotenoids are found in the cells scattered throughout the tissues.

Pigment extracts of 5 floribunda cultivars, 5 hybrid teas were subjected to irradiation by gamma rays (Lata, 1987). A gradual loss of pigment (anthocyanin) occurred as radiation dosage increased with all the cultivars except for hybrid teas. Both gamma rays and chemical mutagens showed no correlation in relation to colour intensity. About 10% of the plants from treated seeds produced white flowers which were devoid of anthocyanin pigments. Mol et al. (1989) suggested different methods for genetic manipulation of floral pigments. The first case was by recombinant DNA technology, biosynthesis by antisense and sense genes. Future prospects are by using catalytic RNA, competing enzymes and regulatory genes. Zykov et al. (1990) examined biochemical pathways of flavonoid pigment synthesis in rose and their relative scores in the contents of flavonols, quercetin, kaempferol, anthocyanidins, cyanidins, peonidin and pelargonidin in the mutants. Results indicated that the primary flavonoids is quercetin and the primary anthocyanidins are cyanidin, both being formed from a common precursor.

The petal flavonoid patterns of 4 modern rose cultivars and six of their spontaneous mutants were characterised from HPLC analysis. Native glycosides of

cyanidin, pelargonidin, quercetin and kaempferol were precisely quantified (Biolley et al. 1991). The co-occurrence of kaempferol - quercetin - pelargonidin or quercetin - pelargonidin - cyanidin was observed with a dominant pelargonidin unassociated with a dominant quercetin. Mutants producing cyanidin and kaempferol in almost equal amounts exhibited a modified pattern of mono and disubstituted flavonoids. An addition to one hydroxyl group of the orange pelargonidin it also resulted in the creation of purple cyanidin (Kim and Fajieda, 1991). By varying the ratio of the carotenoids to flavonoids different colours can be produced. Brown colour can thus result from a high concentration of purple flavonoids. An almost equal amount of the two pigments resulted in bronze shades, while red shade produced by high concentration of purple flavonoids and low concentration of yellow carotenoids

Manson and Krinkel (1994) isolated key genes affecting blue and red colouration and controlled the flavonoid pigments, notably anthocyanins. They reported that delphinidin pigment is responsible for the blue colour of the flowers in many ornamentals including rose which cannot synthesise this pigment owing to the deficiency of an enzyme. Thus genes responsible with appropriate enzyme for the conversion could lead to the production of blue roses henceforth.

2.3.2 FACTORS AFFECTING FLOWER COLOUR VARIATIONS

Ahuja (1962) identified and estimated anthocyanidins in the petals of rose cv 'Pink Coronet' and 'Happiness'. Amount of cyanidin was found to increase from the outer petal to the central petals in the buds and partly opened flowers, but decreased in fully opened flowers. Cyanidin contents expressed on the dry weight basis differed significantly suggesting that moisture and cyanidin contents may be related. Brock (1964) have dealt with the chemistry and the distribution of

anthocyanins in higher plants which is confined to those aspects of chemistry of anthocyanins that relate to flower colour variations.

Shisha and Takano (1964) studied the effects of temperature and light on the colour of rose flowers. Chromatographic analysis showed that the pigments consisted mainly cyanin. In the acid media with a pH of 3 or less, the colour of anthocyanins are determined by the degree of hydroxylation in the /3 ring. The greater the substitution bluer the colour. Due to the presence of hydroxyl groups, glycosides of cyanidin are bluer than those of pelargonidins and the glycosides of delphinidin are bluer than those of cyanidins. On the basis of these structural modifications it has generally been assumed that pink, scarlet and orange-red flowers contain pelargonidin glycosides whereas mauve and blue flowers contain delphinidin glycosides.

Asen et al. (1975) identified peonidin 3, 5 - diglucoside from 'Heavenly Blue' flowers. Thus glycosides of 5 of the 6 commonly occuring anthocyanidins have now been reported as the principal pigments in blue flowers. Therefore, with the assumption that delphinidin was necessary for the blue flower colour, and found to be enormous for most anthocyanins with the exception of pelargonidin glycosides having the capacity in producing blue flowers. Dommergues (1976) stated that mutation may be due to a change in the nature of the pigment themselves, although most of them are already present in the plants. Anthocyanin accumulation was most rapid in combination with kinetin, visible light and UV lights (Nakamura et al., 1980). No accumulation occurred under UV light in the absence of kinetin and it appeared that visible light did not directly aid the action of kinetin but increased the activity of enzymes in anthocyanin biosynthesis.

2.3.3 pH

Many factors affect the colour of specific anthocyanins. Some of these factors are genetic while others are environmental. Higher the pH bluer the colour and lower the absorption of anthocyanin or copigment complex. A factor external to the anthocyanins is the acidity or alkalinity of the medium in which it is dissolved. The pH of the flower (Shibato et al. ,1949), irrespective of the colour was acidic in nature and within the range of 2.8 to 6.2 pH. This is because, the colour of true flower petals are largely confined to the epidermal cells. Gunckel and Sparrow (1961) reported that with an increase in the anthocyanins they are converted to very unstable purplish anhydro bases that are almost immediately converted to colourless carbinol bases. Thus there is equilibrium reaction between the three anthocyanin forms and the colour of the solution is pH dependent.

Valadon and Mummery (1968) demonstrated that oxygenated carotenoid level in rose petals increased with age. They also considered this change as a sign of uncontrolled disintegration during senescence. Co-pigmentation of cyanidin 3, 5 - glucoside with quercetin or keampferol glycosides in the petals of 'Better Time' roses was found to depend upon the pH. A slight change in the pH of the vacuole influenced the co-pigmentation of anthocyanidins. The blueing, a well recognized phenomenon in senescing red roses was demonstrated to be due to an increase in the pH caused by breakdown of protein and accumulation of free ammonia. In certain varieties, blackening or browning of the petals was caused by the oxidation of flavones, leucoanthocyanins, phenols and accumulation of tannins.

Stewart (1969) conducted studies on the relation of flower colours to the OD spectra of intact tissues and of anthocyanin extracts. The main genetic factors known to control the type, amount and distribution of anthocyanins in a flower is due to the genes affecting colour by controlling structural modifications of

individual anthocyanins through shifts in pH, metal chelation and co-pigmentation. It was reported that pH greatly affected the colour of aluminium - cyanidin - 3, diglucoside chelates Asen et al. (1969). Below 3 pH, there was little or no chelation and only a slight colour change was observed. As the pH was increased from 3 to 4 , flower colour changed from red to blue violet. This change was so sensitive that was visible with an increase of less than 0.1 pH unit.

De loose (1978) reported that bluish shades are caused by an increase in pH and reddish shade by a decrease in the pH of the medium. Yokoi et al. (1979) reported that in neutral aqueous solutions, anthocyanins acylated with p-coumaric acid were unstable and that those acylated with caffeic acid was stable. The vicinal hydroxyl grouping of caffeic acid apparently is important to the stability of anthocyanins particularly at above 3 pH range.

2.3.4 METAL CHELATES

Bayer et al. (1966) reported that certain metals can chelate with anthocyanins that contain an ortho-dihydroxyl system to form highly coloured, stable metal complexes at a pH range where anthocyanins are virtually colourless. Of the six commonly occurring anthocyanidins only cyanidin, delphinidin, petunidin and their glycosides form metal chelates. No intensely coloured chelates were formed with bivalent ions of metals. It is likely that metals other than iron or aluminium may be involved in the flower colours. Metal chelation occurs with only three of the six commonly occurring anthocyanidins and their glycosides and therefore cannot explain the range of flower colours which contain only glycosides of pelargonidin, peonidin or malvidin derivatives. Asen et al. (1971a) and Saito (1976) and isolated iron present in the blue pigments of corn flowers. Presumably iron was part of the peptide macro molecules associated with the blue pigment and

was not available for chelation with the anthocyanin. Thus metal chelates do not play an important role in the flower colour.

2.3.5 CO-PIGMENTATION

In the past much attention has been paid to the inheritance of anthocyanins which have a minor role in the colour and little attention to the other factors like co-pigmentation, carotenoids and pH which have a major role in the flower colour. In vitro copigmentation, the association of flavonoids and other related compounds with anthocyanins were observed by Robinson and Robinson (1931, 1932) and Lawrence (1932). It would thus appear that co-pigmentation was not involved in the black colouration of some petals but the extract showed an obvious spectral shift with certain flavonoids, tannins and compounds of metals. The shift is thought to be due to the presence of pyrogallol tannin. Yasuda (1968) studied the expression of colour tone in rose petals with the spectral transmission curves of extracts in the red petals of cv ' Happiness' and 'Bome Nuit'. The copigments had a light yellow or cream colour, while anthocyanins were coloured yellow or orange through blue.

Co-pigmentation in roses (Asen et al., 1971a) were the flavonoids, kaempferol and quercetin glycosides. Other factors affecting copigmentation are the concentration of anthocyanin and the molar ratio of copigments in anthocyanins. Asen et al. (1971b) reported that at lower anthocyanin concentrations, a shift of similar magnitude was obtained only by increasing the molar ratio of co-pigment to anthocyanins. The colour of the anthocyanin copigment complex was extremely sensitive to slight changes in pH.

Asen et al. (1972) reported that co-pigmentation not only caused blueing but also stabilized and enhanced the colour of anthocyanins at a pH range which was

virtually colourless. The greatest copigment effects were obtained with flavonols and c-glycosyl flavones and the smallest effects with amino acids and benzoic acid, respectively. Asen et al. (1970, 1975) established that co-pigmentation was a naturally occuring phenomenon. By manipulating pH, anthocyanin concentration and molar ratio of co-pigment to anthocyanin, the visible absorption spectra of many flowers can be stimulated in-vitro. The phenomenon of co-pigmentation is the explanation for the infinite variations in pink to blue flowers that exists at a pH range where anthocyanins were virtually colourless.

Holton and Tanaka (1994) noted the history of search for blue roses which was apparently unstainable by traditional breeding methods. Conversion of dihydromyricetin (DHM) from petunia into pelargonidin or cyanidin producing rose cultivars should divert anthocyanin biosynthetic pathway. It might be possible to raise the pH of the vacuole using co-suppression techniques, which could also increase blue coloration by affecting co-pigmented anthocyanins.

2.3.6 QUALITATIVE AND QUANTITATIVE ESTIMATION OF PIGMENTS

2.3.6.1 ROSE

Ahuja et al . (1964) identified that the anthocyanidins in the petals of rose cv 'Happiness' as cyanidin through chromatographic technique. Spectroscopic behaviour of roses of different colours was reported by Raman (1965a) by extracting floral pigments. Vega (1967) studied spectrophotometric light absorption of anthocyanin containing tissues of fresh flowers by the use of the opal glass transmission method. The type of the curve obtained was related to the pigment anthocyanin composition which gives a chemical basis on flower colour variation. Yasuda (1967b) studied on the development of colour tone in rose petals of the cultivar 'Happiness' , 'Karl Herbt' and 'Radar' with a comparison of spectro reflectance of red rose petals and spectro- transmittance of anthocyanin solutions.

Transmittance curve solutions containing high levels of cyanins or cyanidin solutions saturated with rutin appeared to be more similar to the reflectance curve in the petals.

Jennen (1972, 1973) described the structure and properties of anthocyanins responsible for flower colour. Using thin layer chromatography the anthocyanins of red rose varieties were separated, purified and identified as cyanin 3-glycoside and cyanidin 3, 5-diglucoside. Structural, spectral and chromatographic properties of flavonoids along with the glycosides of red or yellow roses were separated and identified (1973). Quercetin and kaempferol 3- glycoside contributed only slightly to the colour of yellow roses. Isolation and identification of anthocyanins are responsible for contributing the colour (Lodh and Selvaraj, 1973) in Bangalore blue grapes.

Asen (1976) identified flavonoid chemical markers in roses and their high pressure liquid chromatographic quantitative resolution for cultivar identification. Changes in the rate of fertilizer application and daylength affected only the concentration of flavonoids and the ratio of each compound where the total flavonoids remained fairly constant. Yokoi et al.(1979) stated the relationship between the quantitative measurement of true flower colours of rose cultivars. The chromatographic, colorimetric and spectrometric absorption spectra, lightness and the colour values of flower were estimated. In order to completely understand the flower colour it is necessary to know the pigment composition along with the quantitative colour values.

The numerous quantitative or qualitative differences provided 20 chemical markers with an objective for flower colour identification. Asen (1982) reported that the changes in the environment can influence the biosynthesis of flavonols. Even if a single flavonoid chemical marker is lost, sufficient number of other

flavonoids were present for positive cultivar identification. New flower colours can be produced by breeding through carotenoid or anthocyanin combinations. Plants with true red and even pink flowers have now been produced by breeding with mutants having decreased levels of carotenoids.

More than 1200 progeny from 47 families of roses were analysed for anthocyanin pigments during a breeding programme (Marshall et al., 1983). Results indicated that each pigment was highly heritable from seed or pollen parents and all pigments particularly cyanin and peonin, showed quantitative inheritance. A system is proposed to explain most of the synthetic pathways and control of anthocyanin production. Datta (1983) reported the differences in flower colours between few rose cultivar and its mutants due to quantitative changes in pigments. He suggested that gamma irradiation was capable in modifying the system which controls pigment biosynthesis.

Biolley et al. (1991) observed carotenoid pigments which were isolated from the yellow petals along with the relative amounts each varying with the climatic conditions. Both pigments together with some related compounds were synthesised and characterised by spectral data. Flavonoid pigment variation in the flower development of garden rose varieties and their mutants were derived by Zykov et al. (1991a). The first to appear during ontogeny was flavonol, kaempferol, quercetin and then anthocyanidins in the order of cyanidin, peonidin and pelargonidin. Mutations led to a synchronous shift of anthocyanidin synthesis, leading to changes in the quantitative and qualitative composition of pigments. Kaicker (1992) observed that the amount of cyanidin was maximum in the normal coloured flowers of cv 'Folklore' and decreased considerably in the induced mutants. In induced gamma irradiation there was higher cyanidin content at the bud stage and minimum in the petals of fully opened flowers in all the cultivars

tried. A higher colour mutant was always associated with reduction of cyanidin content (Dohare and Mathew, 1993).

2.3.6.2 HIBISCUS

Hibiscus rosa-sinensis L. is a widespread shrub, 5-8 feet high with brilliant shining thick foliage. It is constantly in bloom with large brilliant scarlet red flowers which have pretty columns of pistil and stamens projecting from the centre with large number of intervarietal hybrids and innumerable colours in common. A direct vision spectroscopy view exhibits a wavelength greater than 600nm to full strength, while shorter wavelengths suffer a practically complete extinction. Immersion of the petals in acetone results in rapid extraction of the colour. Extraction exhibits visible absorption with a yellow green sector followed by a strong general absorption in the blue region. It will be seen that apart from the three absorption bands in yellow, green and blue green characteristic of florachrome B, two other bands are noticeable in the blue and violet regions of the spectrum. It may be inferred that besides florachrome B which is responsible for the red colour, there is also present a yellow pigment which has a strong absorption band in the short region of the spectrum.

Using short absorption paths it allows light of smaller wavelengths to come through a strong absorption band between 580 and 590nm. Longer columns showed a change in colour from rose-red to a deep red colour expected in the circumstances. These inferences receives support from the fact that the colour of acetone extract does not fade away completely after 24 hours, but exhibits a residual yellow colour which makes it possible to separate the red and yellow pigments by chromatographic methods. Thereby, a brief review of literature is made on the floral characters of hibiscus and their pigments involved for different colour spectra formation.

Hayashi (1942) reported the presence of anthocyanin pigment from the red flowers of Hibiscus rosa-sinensis. Subramanian and Swamy (1961) identified the pigments of the flowers of Hibiscus tiliaceus. Gossypetin was seen to be the major component with some quercetin and traces of kaempferol pigments along with the flavonoid gossypetin and gossypitrin glycosides. In Hibiscus surratensis the yellow part of the petals contain gossypetrin and gossypetin and in the purple parts cyanidin, pelargonidin and delphinidins were present (Nair et al., 1964). In the flowers of Hibiscus mutabilis creamy yellow colour of the flowers in the morning proved to be due to quercemitrin and meritin and their pink-red colouration in the evenings were due to the presence of cyanin (Subramanian and Swamy , 1964).

Raman (1965b,1969) found pelargonidin floral pigments through spectrographic methods present in hibiscus and their perception of colour spectrum. The absorption spectra through spectrophotometry of floral pigment extracts of red Hibiscus rosa-sinensis were also estimated with increased anthocyanin contents. Subramanian and Nair (1970) reported the colour change of the flowers of Hibiscus mutabilis. Cyanin and cyanidin 3-rutinoside, 5-glucoside were present when flowers were picked at noon and their concentration had tribled. The colour change of the flowers from ivory white to light rose during the day is ascribed to anthocyanin pigment synthesis. Lowry (1971) identified free cyanidin in the flowers of Hibiscus mutabilis. The pink basal blotch in this species is mainly due to the presence of cyanidin which was estimated to be the first case of free anthocyanidins occurring in hibiscus flowers.

Hanny et al. (1972) identified the quantitative carotenoid constituents in hibiscus species. Those most abundant in the buds and leaves were /3-carotene and lutein. Lutein 5,6 epoxide predominated in the flowers wherein carotene hydrocarbons comprised 19% of the total carotenoids in the flowers. Egolf and Santamour (1975) isolated anthocyanin pigments from the flowers of Hibiscus

syriacus, which consisted mainly of 3 - glucoside of delphinidin, petunidin and malvidin. These pigments were normally associated with mauve, violet and blue flower hues. But one of the cultivars of hibiscus contained cyanidin 3-glucoside associated with crimson magenta hues as the major petal pigment. Thus the development of the cultivars with true red flowers is remotely present in Hibiscus syriacus species.

Floral anthocyanins in some Malaysian Hibiscus species was reported by Lowry (1976). From among the 14 species the most common floral anthocyanin was cyanidin 3 - sambubioside. Contrarily, flavonols and anthocyanins of Hibiscus mutabilis were of the same glycosidic types. The origin of some new varieties of Hibiscus rosa-sinensis belonging to the family Malvaceae was described by Bhat (1979). Ornamental hibiscus is in reality a highly polymorphic cross compatible group of species with complex hybrids and their derivatives. Rigorous screening of large population of hibiscus hybrid seedlings resulted in the final selection of 14 very promising once in varied spectra of colour combinations. Five flavonol glycosides in pink petals of Hibiscus mutabilis were noted by Ishikura(1982) as quercitin 3-sambubioside, isoquercitrin, hyperin, guaijaverin and a compound containing kaempferol, glucose, galactose and xylose. Pouget et al. (1990) identified and measured two main anthocyanins, namely hibiscin and gossypicyanin using thin layer chromatographic method. The anthocyanidins and sugars identified were delphinidin, cyanidin and as glucose, fructose and xylose.

The major anthocyanin in the petals of Hibiscus rosa-sinensis cv 'Brilliant' used as a food colorent was confirmed to be cyanidin 3-sophoroside by means of chromatographic and NMR analysis (Nakamura et al., 1990). Absorption spectra, anthocyanins, pH and copigment contents of petals were analysed to characterize flower colour variation in 47 cyanic cultivars of Hibiscus syriacus (kim and Fajieda, 1991). On the basis of visual evaluation petal colours were associated

with the nature and relative content of anthocyanins. Increase of bathochromic shifts and blueing of the petals were in good agreement with increase of flavone/ anthocyanin ratios at 550 - 640nm, malvidin derivatives, malonated anthocyanins and the cell sap pH. Increase in the these pigment factors enhanced the copigmentation effects considerably.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigation has been undertaken to study the effect of radiation sensitivity on induced and spontaneous mutants, qualitative and quantitative estimation of flower colour pigments along with the pollen studies on rose and hibiscus cultivars maintained in the germplasm collection block at the Indian institute of Horticultural Research, Hessaraghatta, Bangalore. The experiment was conducted during the period from 1994-97.

The institute is located at 26 Km North of Bangalore. It is situated approximately between the North latitude of $13^0 7'40"$ - $13^0 8'$ and East longitude $77^0 29'$ - $77^0 29'30"$, respectively. The altitude is at 863 metres above the mean sea level.

The following rose genotypes and their respective mutants were selected for the present study of investigation (Bhat and Tejaswini , 1996).

3.1 VARIETAL DESCRIPTION

3.1.1 ROSE

Rose is an ornamental shrub with upright climbing habit, alternate leaves, oddly pinnate with stipules adherent to the leaf stalks and alternate. Flowers are solitary or in corymbs. Calyx five lobed, petals and sepals generally are 5 in number, but many more in other cultivars due to stamen transformation. The present day garden roses are complex hybrids involving interspecific hybridization, polyploidy with high female and male sterility. Rose, because of its utility occupies a prominent place amongst the flower crops and is one of the oldest of fragrant flowers to be cultivated.

3.1.1.1 INDUCED MUTANTS

PARADISE(Weeks., 1979) : 'Swarthmore' x Unnamed seedling. One of the most distinctive roses, developing a most unusual colour combination of striking beauty. Paradise is one of the impressive novelty called as 'Sterling Silver' and 'Blue Moon' with beautifully shaped buds opening into elegant, large blooms of opalascent lavender with magenta at the petal edges and delicately brushed with vivid pink at the centre. On ageing the lavender deepens and pink becomes ruby red. Vigorous and healthy, with large glossy green foliage and long lasting quality with fragrance.

RAJA SURENDRA SINGH OF NALAGARH (Pal., 1977) : 'Scarlet Knight' x 'Montezuma'. Large sized, good high centred flowers of classic shape and dazzling orange salmon pink colour. Buds are extremely beautiful, fully double large flowered with imbricated petals having long lasting qualities.

SINDOOR (IARI, 1980) : 'Sea Pearl' x 'Suryodaya'. Long pointed buds open to geranium lake, well formed double and large blooms in single or double clusters, tall and vigorous growing bush with clear and attractive foliage.

3.1.1.2 SPONTANEOUS MUTANTS

AKITO (Tantau., 1971) : Floribunda class of roses, extremely hardy and vigorous. Moderate plant height, abundant flowers with wonderful range of colours, long lasting flowers. Spotless, gracefully white, very double with repeated flowering.

EIFFEL TOWER (Armstrong., 1965) : 'First love' x unnamed seedling. Very long, tapering buds, opening into double, elegant, flowers medium pink to neyron rose, fragrant carried on long stems. Growth is vigorous and upright with semi glossy, light green foliage.

FIRST FEDERAL GOLD (Boemer., 1968) : A hybrid tea seedling. Bright yellow flowers with high centered, double full, moderately fragrant. A delightful rose with large urn shaped buds, elegant huge, full blooms and alluring fragrance.

KRONENBOURG (Colley., 1961) : Floribunda class of roses, bright yellow to creamish white flowers. Spotless, very double with repeated flowering.

TZIGANE (Merrand and shetja., 1967) : A hybrid tea seedling, flowers are medium brown to yellowish in colour. A delightful rose with vigorous growth habit.

WOUBURN GOLD (Cobley & Sydney., 1962) : Floribunda class of roses, abundant flowering, bears long lasting flowers, hardy and vigorous. Flowers are vivid golden tangerine orange, fragrant and large clusters.

3.1.2 HIBISCUS

The shoe flower or rose of sharon (Hibiscus rosa-sinensis Linn) belongs to the family malvaceae. This is a widespread shrub 5-8 feet high with bright shining thick foliage. It is constantly in bloom with large brilliant scarlet flowers which have pretty columns of pistils and stamens projecting from their centres. Flowers are single or double, saucer or cup shaped. Petals are attractive, recurved margins. The shrub with its flowers has an attractive appearance and hence it is very effective as an ornamental hedge. The varieties under study are moderate to vigorous in growth with profuse erect lateral branches. Highly floriferous, woody stem, leaves are simple, alternate, cordate-ovate, acute, serrate, entire margined incurved , undulating and slightly pubescent and shinning in nature.

The following varieties developed at IIHR, Hessaraghatta was used for the pigment estimation studies (Bhat, 1979 and Bhat and Verma , 1980).

AIKTA ('Debby Ann' X 'H.S.203') : Corolla is post office red (45B) with mandarine red (40C) border. Basal portion of the corolla is prominent cardinal red (53D) which spreads upto 4cm and terminates as red purple rays along the veins.

ARUNODAYA ('IIHR-H-2' X 'Rachiah') : Corolla is nasturtium orange (25B) and the basal part is rose bengal (57B) which spreads upto 3cm and terminates with light mauve pink stripes along the veins.

ASHIRWAD ('IIHR-H-2' X 'Rachiah') : Corolla is orange yellow (29A) with cardinal red centre (53A) spreads upto 3cm and terminates as red rays along the veins.

BANAZEER ('H.S.Red' X 'H.S.123') : Corolla is bright yellow. The basal portion is neyron rose (55B) which spreads upto 3.5cm and terminates as whitish purple rays along the veins.

BASANT ('IIHR-H-1' X 'Rachiah') : Corolla is sulphur yellow (6A) without any conspicuous centre.

CHITRALEKHA ('Debby Ann' X 'H.S.203') : Corolla is china rose (58D) with slighly frilled margin. Characteristic variegation with cardinal red (53A) basal portion spreads upto 2cm.

DILRUBA ('Debby Ann' X 'H.S.203') : Corolla is dark golden buff with slighly reddish brown towards the margin. Basal portion of the corolla is dark red with azalea pink (39D) border which spreads upto 3.5cm.

NARTAKI ('H.S.Red' X 'H.S.123') : Corolla is marigold orange (28B). The basal part is cardinal red (53B) which spreads upto 2cm.

NAZNEEN ('H.S.203' X 'Rastrapati') : Corolla is tangerine orange (24B). Basal part of corolla is red with light mauve border which spreads upto 3cm.

PRIYA ('IIHR-H-2' X 'Rachiah') : Corolla is rose bengal (57C) and the basal part of the corolla is cardinal red (53A) which spreads upto 2.5cm.

PHULKARI ('H.S.139' X 'H.S.181') : Corolla is delft rose (46D) with prominent light purple rays. Border of the petals lemon yellow (13B) which spreads nearly 2cm. Basal portion of the corolla is shinning red with spinel red (54B) border.

RATNA ('IIHR-H-1' X 'Rachiah') : Corolla is mandarine red (50A) with orange stripes and white centre.

RED SATURN ('IIHR-H-2' X 'Crombell') : Corolla is turkey red (52A) without any conspicuous centre.

SHANTI ('IIHR-H-1' X 'Rachiah') : Corolla is primrose yellow (4D) and the basal part is tyrian purple (57A) spreading upto 2cms.

TRIBAL QUEEN ('IIHR-H-1' X 'H.S.481') : Corolla is cardinal red (53C) and the basal part is dark purple red spreading upto 2.5cm.

3.2 DETAILS OF THE EXPERIMENTAL PROCEDURES

3.2.1 EXPERIMENT 1 : MUTATION BREEDING IN ROSE

3.2.1.1 INDUCED MUTATION

Genotypes Used : Paradise, Raja Surendra Singh of Nalagarh and Sindoor

Replication : 7

Treatments : 5

T1 = Control

T2 = 3Kr gamma ray

T3 = 4Kr gamma ray

T4 = 5Kr gamma ray

T5 = 6Kr gamma ray

3.2.1.2 SPONTANEOUS MUTATION

Genotypes Used : Akito, Eiffel Tower, First Federal Gold, Kronenbourg, Tzigane, Wouburn Gold.

Replication : 5

Treatments : 12

Design : RCBD

3.2.1.3 METHODOLOGY

Roots stocks of Rosa multiflora was raised initially by ploughing the land and making into fine tilth. Periodically rootstocks were maintained by allowing only a single branch to grow by pinching the side sprouts and branches. This will help in developing a well sturdy and good rootstock for budding purpose. Constant weeding, irrigation, fertilizer application and spray scheduling for the incidence of pests and diseases were also timely undertaken. Contrarily, on the other hand varieties identified for budding in the germplasm was also well maintained. Synchrony between rootstocks and bud development should be maintained.

When once the buds are well developed(plumpy but not burst), simultaneously the strong stems of the rootstocks of pencil thickness should also be ready for budding purpose. Then 35 buds each was collected from each variety, irradiated by exposing the buds to gamma rays from the radiation chamber situated at IIHR, Hessaraghatta, Bangalore. The calibrated timing was 80 rads/minute or 12.5 minutes/Kr (initial capacity) for all the above said three varieties used. The buds were inserted at about 5 -7 cms above the ground level by removing the thorns and leaves about 2cms off the stalk. Budding was carried out by the 'T-shaped' method carefully for better bud union. After budding utmost care should be taken with respect to all the cultural operations and pinching of side shoots which deviates the nutrient availability to the developing young irradiated buds.

Likewise spontaneous mutants selected for the present study was identified in the germplasm. Pruning operations were regularly undertaken, after which during the time of full fledged flowering period regular observations on all the morphological and floral characteristics were recorded. Here also all the cultural operations were followed as per schedule. Along with this a very light pruning was given to induced mutants for putting forth new flush of growth. Gamma ray induced and spontaneous mutants were closely observed for very minute changes in any of its growth habit.

3.2.1.4 OBSERVATIONS RECORDED

The following are the parameters studied during the period of investigation in detail

1. **Growth habit :** Growth habit was recorded after the full establishment of rose plants with respective cultivars and mutants used.

2. **Plant height :** The height of the plant was measured from bottom to the top of the vegetative growth point before pruning.

3. **Number of branches :** The total number of branches before pruning is taken as a measure of count.

4. **Number of leaflets / branch :** Total number of leaflets present per individual branch was recorded.

5. **Thorn characters :** The shape of the thorn whether hooked or straight and the colour whether brown or green were observed visually. The density of thorns on the stem after full maturity was recorded. For noting this observation, cuttings of 10cm length stems from the basal portion were taken in each cultivar and their mutants. Average no of thorns per ten cm area was recorded.

6. **Leaf characters :** The foliage size was noted as large, medium or small whereas, leaf glossyness as normal to glossy in appearance. The colour of the matured leaves whether light green, normal,deep or coppery was recorded in each cultivar and its mutants on the basis of visual observation.

7. **Floriferousness :** With respect to the growth habit and appearance floriferousness was recorded as good, medium or poor.

8. **Fragrance :** The fragrance of the flower was recorded as high, moderate, slight or no fragrance by sensing or smelling the fresh flowers in each cultivars.

9. **Stem diameter :** The range in stem diameter was recorded by measuring the circumference of the sturdy stems in cms.

10. **Internodal length :** It is the length between the two nodes along the stem which is recorded in cms.

11. **Leaf area :** Individual and random sampled leaf area was recorded using leaf area meter in sq.cms.

12. **Flower characters**

- Total number of flowers present per plant.
- Total number of petals present per individual flower.
- Total number of flower clusters present per plant. All the above characters were recorded as a measure of count.
- Flower diameter was measured from one end to the other end of the petals diagonally across the fully opened flowers expressed as cm.
- Flower characters were visually observed as striped, spotted or shaded in nature.
- Flower weight in gms were recorded on individual fresh weight basis.
- Colour of the flower was recorded with the help of Horticultural Colour Charts published by the Royal Horticultural Society, England.

13. **Bud forms :** Individual plants were observed and the shape of the buds were recorded as ovoid, pointed or globular.

14. **Sprouting percentage :** The total number of buds sprouted during the initial stages of development was recorded as sprouting percentage.

15. **Survival percentage :** Overall plants survived from the sprouted buds during the long run was recorded as survival percentage.

3.2.1.5 EXTRACTION AND ESTIMATION OF CHLOROPHYL FROM LEAF EXTRACT

Fresh leaves of 0.1gm was homogenized by placing the leaves in 7ml of dimethyl sulphoxide solution (DMSO). The extract is then placed at 65^0C for 4hrs in hot air oven for the complete extraction of chlorophyll from the leaves. When once the leaf becomes colourless, filter the extract and transfer the contents into buchner funnel. The filterate is pooled and the final volume should be made upto 10ml by further diluting the extract. Always extract thefresh plant tissues and estimate chlorophyll immediately. The OD values are measured at 643 and 663nm for the determination of chlorophyll A and B along with the total chlorophyll contents.

The total chlorophyll content can be calculated on the fresh weight basis employing the following formulae:

$$\text{Total chl (mg/g)} = \frac{20.2\ A643 - 8.02\ A663}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll A (mg/g)} = \frac{12.7\ A663 - 2.69\ A643}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll B (mg/g)} = \frac{22.9\ A643 - 4.68\ A663}{a \times 1000 \times w} \times V$$

Where, a = Length of path light in the cell

V = Volume of the extract in ml

w = Fresh weight of the sample in gms.

3.2.1.6 STATISTICAL ANALYSIS

Data tabulated from among all the induced and spontaneous mutants were analysed in a completely randomized block design method. The mean values of each character was calculated and the level of significance of their difference (original vs mutant) was determined by 't' test along with Duncan's multiple range test to compare the treatment means.

Co-efficient of variation (CV) : $CV = \frac{EMS/2}{GM} \times 100$

Phenotypic and Genotypic variances (PV & GV) : (Singh and Chowdary)

$$GV (\sim g^2) = \frac{MSST - MSSE}{R}$$

$$PV (\sim p^2) = \sim g^2 + \sim e^2$$

Where $\sim e^2$ = Environmental variance

Phenotypic and Genotypic Co-efficient of variance :

$$PCV = \frac{\sim p^2/2}{GM} \times 100$$

$$GCV = \frac{\sim g^2/2}{GM} \times 100$$

Heritability (h^2) : In the broad sense it was estimated as a ratio of genotypic to phenotypic variation expressed in percentage as suggested by Hanson (1963).

$$h^2 = \frac{g^2}{p^2} \times 100$$

where g^2 & p^2 are genotypic and phenotypic variance

Genetic advance (GA) : GA = H.P.K.

H = Heritability $g=g^2/p^2$

P = Phenotypic standard deviation

K = Standard selection differential and it is 2.06 at 0.5% significance

Correlation Co-efficients : It was tested against 'r' values given by Fisher and Yates (1963) at (N-2) df at 5% and 1% significance.

- Phenotypic correlation (rp)

$$rp = \frac{P\ x.y}{(P^2x \,.\, P^2y)/2}$$

- Genotypic correlation (rg)

$$rg = \frac{G\ x.y}{(G^2x \,.\, G^2y)/2}$$

Where P x.y = Phenotypic co-variance between characters x & y

G x.y = Genotypic co-variance between characters x & y

P^2x and P^2y + G^2x and G^2y = Phenotypic and genotypic variance of characters x and y.

3.2.2 EXPERIMENT II : POLLEN STUDIES IN ROSE AND HIBISCUS

3.2.2.1 ROSE

Induced mutants : Paradise (3kr, 4kr, 5kr and 6kr) and Raja Surendra Singh of Nalagarh.

Spontaneous mutants : Akito, Eiffel Tower, First Federal Gold, Kronenbourg, Tzigane, Wouburn Gold.

Replication = 3

Treatments =17

3.2.2.2 HIBISCUS

Varieties used : Aikta, Ashirwad, Basant, Chitralekha, Dilruba, Phulkari and Priya

Treatments = 7

Replication = 3

Design = CRD

3.2.2.3 METHODOLOGY

The parameters studied during the study of investigation are detailed below

3.2.2.3.1 POLLEN COLLECTION

The procedure involved in pollen collection depends on the type of species, inflorescence and peak anthesis period. Since most of the ornamental flowers are hermophrodite, pollen collection needs careful attention. For pollens having poor viability profiles manual collection is the only alternative, which is done on the day of pollen collection on the potential female parent.

In heterozygous species like rose and hibiscus, the flowers were bagged one day prior to pollen collection in order to maintain purity and avoid insect foraging. The following day, pollens are collected from the anthers directly by clipping off the entire flowers for pollen collection. Fully opened flowers were processed in the laboratory and the pollens were collected by gentle tapping over the petri plates. Pollens were then collected in the form of fine dust. Variability in the quality of the collected pollens may arise due to changes in the environmental conditions, genotypic differences, vigour and physiological status of the plant (Shivanna and Mohan, 1993).

3.2.2.3.2 VIABILITY ASSESSMENT

Monitoring pollen viability ie. the ability of matured pollens to deliver functional sperm cells to the embryo sac following compatible pollination is a pre requisite factor. Due to the lack of information regarding viability, germinability and fertility status in rose and Hibiscus species , a brief study to standardize the media was undertaken. The most commonly used technique was as follows

3.2.2.3.3 INVITRO GERMINATION BY HANGING DROP METHOD

Pollen grains were smeared onto a drop of the medium containing sucrose as a carbohydrate source, boric acid, calcium nitrate, magnesium sulphate and potassium nitrate. In some cases gibberillins was also added in minute quantities. For rose pollen germination we used 15% sucrose, 300ppm calcium nitrate, 200ppm magnesium sulphate, 100ppm boric acid and 100ppm potassium nitrate; whereas for hibiscus, 45% sucrose along with 100ppm boric acid was recommended. The solution was prepared and stored under refrigeration. The hanging drop with pollen were placed on a coverslip and covered with the cavity slide. Several such drops with pollens is prepared and incubated in the dark at specific temperature (25^0C) with 100% humid condition. After 3hrs in rose and 6hrs in hibiscus cultivars of incubation the coverslips were removed carefully. By using the versatile nuclear stain fertility status was estimated as follows

3.2.2.3.4 POLLEN FERTILITY ESTIMATION BY USING A VERSATILE STAIN

For the present study pollen fertility was estimated by using a versatile stain suggested by Alexander (1980). The stain is prepared with ethyl alcohol(95%) - 20ml, malachite green - 20mg (2ml of 1% solution in 95% alcohol), distilled water 50ml, glycerol 40ml, acid fuchsin - 100mg (10ml of 1% aqueous solution), phenol 5gms and lactic acid 3ml.

Freshly dehised pollen grains were first subjected to germination by hanging drop method. A drop of Alexander's stain was added over the pollen by warming the slide slightly over a flame 3 to 4 times. Cover slips were carefully placed to avoid air bubbles and leave it for about 5 to 6 min for proper staining. The pollen grains which were shrivelled and stained green were recorded as sterile, whereas the ones which were round and stained crimson red was recorded as fertile. The average pollen fertility has been estimated in terms of percentage. After 24 hours the pollen grains were examined microscopically and the percent viability was determined by counting the number which had germinated from a minimum sample count. Germination was scored when the length of the emerging pollen tube was greater than the diameter of the pollen grains.

3.2.2.4 STATISTICAL ANALYSIS

The percent decline or change in the pollen viability was calculated based on the pretreatment viability values. The statistical significance of the various treatments were determined using Duncan's multiple test.

3.2.3 EXPERIMENT III : QUANTITATIVE AND QUALITATIVE ESTIMATION OF PIGMENTS IN ROSE AND HIBISCUS

3.2.3.1 ROSE

Genotypes used : Paradise and its induced mutants (3kr, 4kr, 5kr & 6kr)

Spontaneous mutants : Akito, Eiffel tower, First Federal Gold, Kronenbourg and Wouburn Gold.

Total number analysed :17 genotypes (SE $\pm$ 3 samples in each samples)

3.2.3.2 HIBISCUS

Varieties used : Aikta, Ashirwad, Banazeer, Basant, Chitralekha, Dilruba, Nartaki, Nazneen, Priya, Phulkari, Ratna, Red Saturn, Shanti, Tribal Queen.

Total number analysed : 14 genotypes (SE $\pm$ 3 samples in each samples)

3.2.3.3 METHODOLOGY FOR THE ESTIMATION OF PIGMENTS

3.2.3.3.1 ROSE

The flowers of respective genotypes in rose cultivars were collected three months after the pruning operation ie., during the peak flowering stage. Keeping in view the uniformity in the flower opening partially opened flowers which are free from pests and desease incidence are collected at random both in spontaneous and induced mutants for the extraction of pigments.

3.2.3.3.2 HIBISCUS

The flowers of all the 14 varieties undertaken for the estimation of pigments were collected in the full bloom stage especially during morning hours when the flowers have just opened. The stage of harvest depends upon the prior bud initiation. April to May is very congenial for flower collection which is the peak flowering stage. The entire flower was used for pigment extraction and estimation.

3.2.3.3.3 EXTRACTION OF ROSE AND HIBISCUS PETALS IN ALCOHOL

Petals are extracted in different solvents for different lengths of time where some of the solvents are capable of penetrating the tissues rapidly and stop enzyme activity. Plant constituents possess different degrees of solubility in different solvents, where water is the best solvent for a number of constituents present in the tissues but does not penetrate quickly to stop enzyme activity. Alcohol is highly effective in penetrating tissues and stopping enzyme activity. Hence in the present study, rose and hibiscus petals for analysis was extracted in alcohol solution.

3.2.3.3.4 ESTIMATION OF ANTHOCYANIN

Five hundred mg of petal extracts were homoginated with acidic methanol (HCl : methanol 1:99 ratio) and kept for 72hrs. Filter the residue and the filter paper

was washed with acidic metanol. The volume of the filtrate was made up to 100ml with acidic methanol. The colour intensity was recorded at 530nm adjusting 100% transmission against acidic methanol.

3.2.3.3.5 ESTIMATION OF LEUCOANTHOCYANIN

One ml of the sample (alcohol extract) is taken in a glass stoppered test tube. Reduce the volume to 0.5ml by placing it on a hot water bath so that the sample does not contain more than 0.5ml of ethanol. Add 0.5ml water followed by 10ml of leucoanthocyanin reagent (dilute 25ml of 36% HCl to 500ml with n-Butanol) and the contents were mixed thoroughly. The tubes were heated on a water bath at $97\pm 1^{0}C$ for one minute without covering the tubes. Then cover the tubes with the stopper and heat for about 40 minutes and cool it under running tap water. Similarly, a blank was also maintained with the extract but without heating. Absorbance of the solution was measured in a 1.0cm cell at 550nm in a colorimeter and the results were expressed as OD at 550nm.

3.2.3.3.6 EXTRACTION OF FLAVONOL

One ml of petal extract containing not more than 0.1ml of ethanol was taken in 25ml conical flask and diluted to 2ml with water. Four ml of vanillin reagent (dissolve 1gm of recrystallised vanillin in 100ml of 70% conc. Sulphuric acid) was added from the burette rapidly within 10 - 15 sec to flask A and 4ml of 70% sulphuric acid to flask B + 2ml of water. A blank was prepared in flask C containing 4ml vanillin reagent and 2ml of water. The content of the flask A and B were shaken well and kept in water bath maintained at $35^{0}C$. The flasks were then kept under room temperature for exactly 15min. The optical density (OD) values of the contents of flask A, B and C were measured at 1.0cm cell at 500nm against 47% sulphuric acid (Flask D) (To prepare 47% sulphuric acid add 4ml of 70% conc. sulphuric acid to 2ml of water). The OD values of B and C was subtracted

from that of A. Flavonol content was calculated by using a standard curve prepared with phloroglucinol and results were expressed as mg per cent.

3.2.3.3.7 EXTRACTION AND ESTIMATION OF CAROTENOIDS

Freshly harvested flower petals weighing 10gms were chopped and tranferred to 50ml of 80% acetone solution for colour extraction. It was homogenized thoroughly and the extract is decanted. Filter the extract through Whatman No. 42 filter paper. Repeat the extraction until the tissues were free of pigments. The filterate was pooled and partitioned with equal quantity of peroxide free ether thrice using a separatory funnel. Water is added to produce two layers during the initial ether extraction. The ether phase contained carotenoids to which add 5ml of 20% alcoholic. Shake well, allow it to stand for 30 minutes, wash repeatedly with water to remove excess of KOH completely. Dry it over anhydrous sodium sulphate and make up the desired volume with the solvent and measure the aborbance at 448nm. Carotenoid is calculated as /3- carotene and expressed on mg per cent basis.

3.2.3.3.8 PAPER CHROMATOGRAPHIC ANALYSIS OF ANTHOCYANIN PIGMENTS

Random samples of each of the variety in both rose and hibiscus were taken for the extraction of pigments. The petals were ground with diethyl ether in a precooled waring blender for 5 minutes after removing the ether fraction. The residual mass was treated with 1% acidic methanol kept overnight and then filtered with a celite bed. This clear anthocyanin extract in acidic methanol was used to determine the absorption maxima in visible range. Then the extract was concentrated under reduced pressure and temperature 40 $\pm$ 1^{0}C, and the concentrated extract was used for paper chromatographic separation.

The concentrated extract was spotted on Whatman No - 3 chromatographic sheet as a long streak of one cm width and fractionation of the pigments was made

using n-Butanol : Acetic acid : water (4:1:5) as an ascending solvent. After developing the chromatogram for about 60 hrs (solvent front 18"), air dry it under diffused light till the paper is free from the solvent vapour. Individual colour bands Rf values were calculated using the formula

$$Rf = \frac{\text{Distance moved by solute (cm)}}{\text{Distance moved by solvent (cm) front}}$$

Rf values obtained were compared with those of authentic standards.

3.2.3.4 BAND COLOUR INTENSITY

The colour intensity of individual bands separated by paper chromatography was measured at 530nm both in rose and hibiscus genotypes.

3.2.3.5 VISUAL FLOWER PETAL COLOUR

The colour of the flower petals were recorded by matching it with the Royal Horticultural Society Colour Chart, published by the Royal Horticultural Society, London, in association with the Flower Council of Holland, Leiden.

3.2.3.6 STATISTICAL ANALYSIS

The data recorded during each replication were statistically analysed and there were three independent samples for each analysis and the values represented were mean $\pm$ SE of three samples each in case of quantitative and qualitative pigment estimation.

RESULTS

IV . RESULTS

It is a well known fact that exposure of plants to mutagenic agents induces various types of morphological, physiological and cytological changes. Mutagen sensitivity has been known to be influenced by variety of factors such as type of mutagen dosage used, treatment conditions used, genotypes and other environmental factors. So in any mutation breeding programme, identification of the crop species as well as adopting suitable methods and duration of treatment is very essential.

The results of the present findings on spontaneous mutations in cultivars viz., Akito, Eiffel Tower, First Federal Gold, Kronenbourg, Tzigane and Wouburn Gold and on induced mutations in cultivars viz., Paradise, Raja Surendra Singh of Nalagarh and Sindoor were as follows

4.1 EXPERIMENT I : MUTATION BREEDING STUDIES IN ROSE

4.1.1 MORPHOLOGICAL STUDIES ON INDUCED MUTATION

Artificial induction of mutation offers a new possibility in crop improvement. The mutants thus obtained can be used either directly as a new variety or it can be used indirectly or directly in hybridization programme. Mutation breeding has shown to be particularly beneficial in changing a single character without altering the background genotype in breaking the tight linkages in diplodization of artificial polyploids and in releasing new varieties by bringing in new records.

In the present study, rose cultivars Paradise, Raja Surendrasingh of Nalagarh and Sindoor were subjected to different doses of gamma rays to study the effect on several growth parameters and to assess the magnitude and nature of

genetic variability induced by gamma irradiation. The results are in accordance with the following observations

4.1.1.1 Growth Habit

The study on all the genotypes of induced mutants revealed that almost all the cultivars had spreading habit which was on par with the parent (control) irrespective of the treatment differences in all the three generations under study (Table 1). It was observed that 6kr treated buds exhibited upright habit whereas all the mutants showed vigorous and bushy growth habit. Paradise and Raja Surendra Singh of Nalagarh were found to be comparatively more vigorous, compact than their respective mutants.

4.1.1.2 Sprouting and Survival Percentage

The sprouting and survival percentage of irradiated plants varied significantly among treated cultivars. It was observed that at 3kr irradiation of the cultivar Paradise, sprouting started during the 3rd week with more number of sprouting percentage, followed by 4 and 5kr in 5th and 7th week showing less sprouting percentage as depicted in the Table 2. In both the cultivars of Raja Surendra Singh of Nalagarh and Sindoor sprouting was initiated from 6th and 3rd week in 3 and 4kr irradiated plants whereas, 5 and 6kr treated plants showed lesser and late sprouting percentage.

In all the three irradiated cultivars highest percentage of sprouting was observed at 3kr (62.86%) in Paradise followed by Raja Surendra Singh of Nalagarh and Sindoor (40.00 &45.71%). From the Table 2 it is clear that higher the dosage lesser is the sprouting percentage which was found to be evident at 5 and 6kr irradiated plants in all the three cultivars. Sindoor was drastically affected at higher dosages of 4 to 6kr where the percentage of sprouting was found to be

Table 1 : Effect of induced mutation on the growth habit of rose cultivars

Sl. No	Treatment	Growth habit	Spine characters		Foliage characters		Glossy-ness	Florife-rousness	Bud form	Fragra-nce
			Shape	Colour	Size	Colour				
1	**Paradise**									
	Control	Upright	Hooked	Brown	Medium	Coppery	Normal	Good	Ovoid	No
	3kr	Upright	Hooked	Brown	Medium	Light green	Normal	Good	Ovoid	No
	4kr	Spreading	Hooked	Brown	Medium	Normal	Normal	Poor	Ovoid	No
	5kr	Spreading	Hooked	Brown	Medium	Light green	Normal	Medium	Ovoid	No
	6kr	Upright	Hooked	Brown	Large	Light green	Normal	Medium	Ovoid	No
2	**Raja Surendra Singh of Nalagarh**									
	Control	Upright	Straight	Brown	Medium	Dark green	Normal	Medium	Pointed	Moderate
	3kr	Upright	Straight	Brown	Medium	Dark green	Normal	Medium	Globular	No
	4kr	Upright	Straight	Brown	Medium	Dark green	Normal	Medium	Globular	No
	5kr	Upright	Straight	Brown	Medium	Dark green	Normal	Medium	Ovoid	Slight
	6kr	Upright	Straight	Brown	Small	Dark green	Normal	Medium	Ovoid	Slight
3	**Sindoor**									
	Control	Spreading	Hooked	Brown	Small	Light green	Normal	Good	Pointed	No
	3kr	Spreading	Hooked	Brown	Small	Light green	Glossy	Good	Ovoid	No
	4kr	Spreading	Straight	Brown	Small	Light green	Normal	Good	Ovoid	No
	5kr	Spreading	Straight	Brown	Small	Normal	Normal	Good	Ovoid	No
	6kr	Upright	Hooked	Brown	Small	Normal	Glossy	Good	Ovoid	No
4	**Mutants**									
	M1	Spreading	Hooked	Brown	Medium	Light green	Normal	Good	Ovoid	Slight
	M2	Spreading	Hooked	Brown	Medium	Normal	Normal	Good	Ovoid	Slight
	M3	Spreading	Hooked	Brown	Medium	Light green	Normal	Good	Ovoid	Slight
	M4	Spreading	Straight	Brown	Medium	dark green	Normal	Good	Ovoid	Slight

M1 = Paradise 3kr variegated flower mutant
M2 = Paradise 3kr thornless mutant
M3 = Paradise 4kr miniature mutant
M4 = Raja Surendra Singh of Nalagarh 6kr variegated flower mutant

Plate 1: Photograph showing the general view of the experimental plot

almost nil. Thus gamma rays affected sprouting percentage bearing certain deviations in the present study. Differential response of genotypes for irradiation was also recorded to be evident. All the three treated varieties showed stimulatory effects at 3kr gamma irradiation (Fig 1).

Sprouting and survival percentage showed direct correlation with each other. Survival after sprouting was always very less (10-30%). On the basis of 50% survival the LD50 dosage for all the three cultivars undertaken for irradiation treatments was determined. LD50 dosage varies accordingly from cultivar to cultivar. Reduction in the survival percentage was recorded with increased exposure to gamma rays except for the cultivar Raja Surendra Singh of Nalagarh, were at higher dosage of 6kr it showed good survival percentage (Fig 2). The cultivars Paradise and Sindoor were found to be most sensitive to increased dosages of gamma irradiation with respect to survival and sprouting percentage (Table 2). Thus the LD50 dosage reccomended for the cultivars Paradise and Sindoor was 3-4kr whereas, for Raja Surendra Singh of Nalagarh it varied from 5-6kr, respectively.

4.1.1.3 Plant Height

In all the three cultivars subjected to gamma rays there was a drastic and significant difference among different treatments within cultivars. Plant height was reduced considerably with increase in gamma irradiation in all the three cultivars irrespective of the generation(Tables 3,4,5). Maximum plant height was observed in the third generation of cultivars Paradise (60.00cm), Raja Surendra Singh of Nalagarh (39.20cm) and Sindoor (49.20cm) at 3kr irradiation, compared to their respective control treatments (79.40, 61.20 and 68.00cms), followed by 4kr in all the three cultivars used for the present investigation and the least was at higher dosage of 5 to 6kr gamma rays (Fig 3).

Table 2 : Effect of induced mutation by gamma rays on the days taken for sprouting and survival of three rose cultivars

Sl. No	Treatments	Days for sprouting(weeks)	Sprouting Percent	Survival Percent
I	Paradise			
	Control	2	85.71	71.43
	3k	3	62.86	57.14
	4kr	5	42.86	28.57
	5kr	7	28.57	17.14
	6kr	8	8.57	2.86
	CD at 5%	-	2.613**	3.122**
	SEM	-	0.379	1.051
II	Raja Surendra Singh of Nalagarh			
	Control	2	82.86	62.86
	3k	6	40.00	28.57
	4kr	7	22.86	20.00
	5kr	6	34.28	28.57
	6kr	5	42.86	31.43
	CD at 5%	-	2.286**	2.816**
	SEM	-	0.799	0.948
III	Sindoor			
	Control	2	62.86	54.28
	3k	3	45.71	37.14
	4kr	4	28.57	22.86
	5kr	6	11.43	5.71
	6kr	8	2.86	0.00
	CD at 5%	-	2.932**	2.168**
	SEM	-	0.718	0.729

** Significant at 1% level

Fig 1: Effect of gamma irradiation on sprouting percentage of rose genotypes

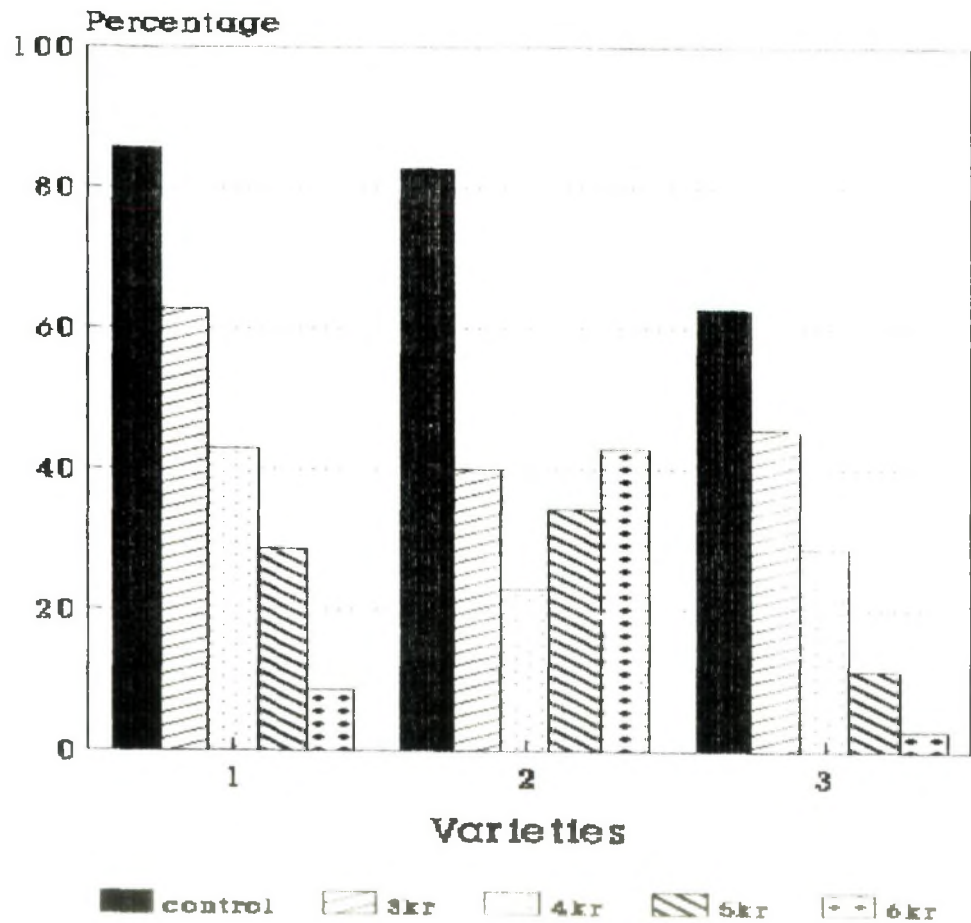
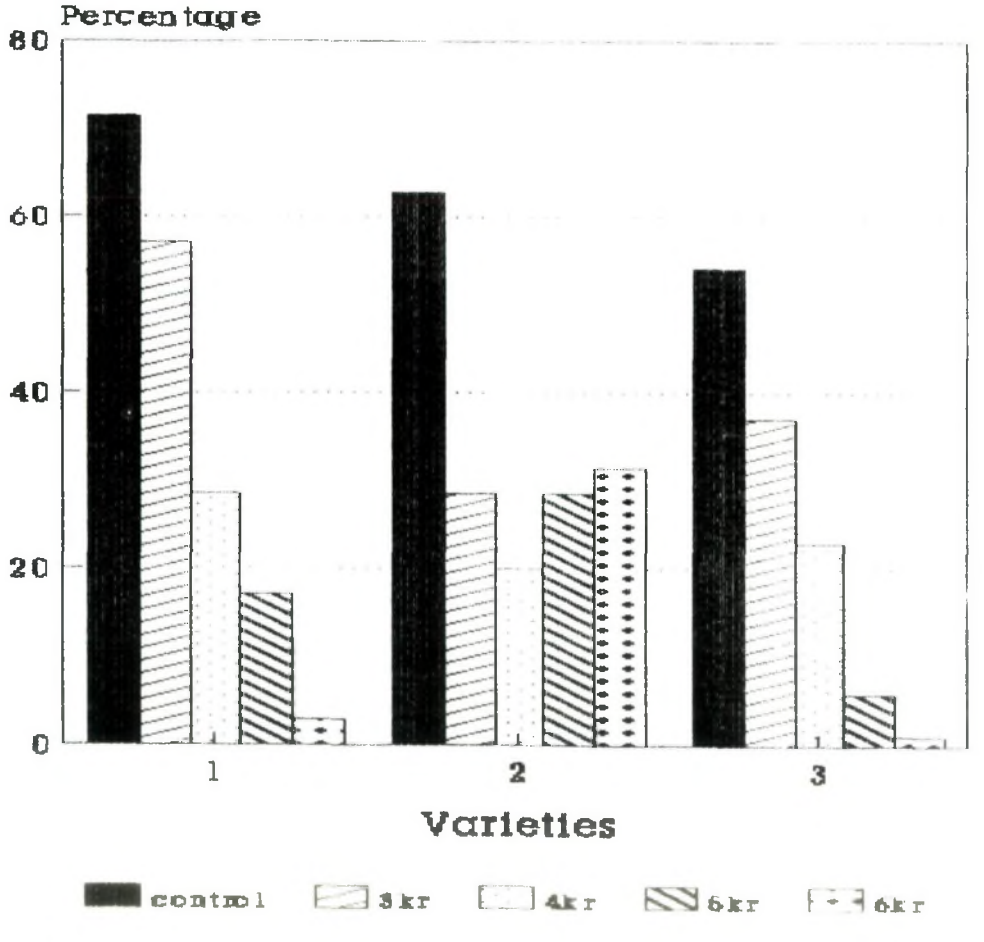


Fig 2: Effect of gamma irradiation on survival percentage of rose genotypes



4.1.1.4 Stem Diameter and Internodal Length

In all the three generations no significant difference was observed with respect to stem diameter by gamma ray irradiation. There was no treatment difference between the cultivars whereas, all the observations recorded within the cultivar were on par with each other. Irrespective of the dosage there was no difference with respect to the season or the varieties used. Similarly internodal length also did not show any treatment variation (Tables 3,4,5) within the varieties and even among the varieties in all the three seasons. Significant variation was not observed with respect to the treatment dosages induced by gamma rays on all the three cultivars in the consecutive generations.

4.1.1.5 Spine Characters

Variation in the colour of the spine among different rose mutants and its respective parents were not found to be considerable. It was observed that in most of the genotypes the colour of the young stem was either light green with reddish brown tinge or light brown with greenish tinge. When matured in all the genotypes brown to green coloured spines were observed under the study. The shape was straight to hooked in nature in all the three cultivars (Table 1).

There was inverse relationship with respect to the number of spines present in 10cm area of the stem on an average. Gamma irradiation at higher levels drastically reduced the number of spines. It was found that (Tables 3,4,5) higher the gamma irradiation lesser was the spine count and vice-versa. High spine count was recorded in Raja Surendra Singh of Nalagarh (16.40) , Sindoor (11.40) and 3kr Paradise (10.40) followed by 4kr treated plants. Least spine count was observed at 5kr treated buds of Paradise, Raja Surendra Singh of Nalagarh and Sindoor (4.40, 10.00 and 9.00), respectively (Fig 4).

Table 3 : Effect of gamma irradiation on the morphological characters of three rose cultivars in VM1 generation

Treatment	Plant height (cm)	Branches /plant	Leaflets /branch	Spine count	Stem Diameter (cm)	Internodal length (cm)
I Paradise						
Control	82.20	8.80	17.20	10.20	3.30	5.56
3kr	57.80	9.00	13.80	10.40	4.08	4.90
4kr	42.80	9.80	14.80	7.00	3.22	5.26
5kr	48.80	12.40	14.40	4.40	3.40	4.16
6kr	43.60	11.20	13.20	7.00	3.20	4.44
CD at 5%	8.420**	2.018*	1.829*	1.180*	0.376	0.439
CV	10.64	14.70	9.29	11.29	7.93	6.74
SEM	3.67	0.95	0.86	0.56	0.18	0.21
II Raja Surendra Singh of Nalagarh						
Control	62.20	7.40	16.40	24.00	4.22	2.78
3kr	38.80	6.00	14.60	18.40	3.18	2.38
4kr	33.20	5.00	10.80	9.40	2.78	2.22
5kr	39.40	6.20	10.20	12.60	2.38	2.96
6kr	33.80	6.00	11.00	13.00	3.26	3.86
CD at 5%	3.606**	1.047*	1.484*	3.038**	0.278	0.362
CV	6.48	12.76	8.78	14.64	6.55	9.51
SEM	1.70	0.49	0.78	1.43	0.13	0.17
III Sindoor						
Control	67.80	8.20	15.80	18.00	3/14	4.80
3kr	53.20	5.00	13.80	8.60	2.52	3.82
4kr	47.00	6.20	12.80	11.00	2.78	4.88
5kr	44.60	5.40	12.20	11.80	3.10	4.34
6kr	39.80	6.00	10.20	9.60	2.68	5.00
CD at 5%	4.047**	0.837*	1.508*	1.642*	0.311	0.224
CV	5.98	10.14	8.68	10.38	8.14	3.65
SEM	1.91	0.39	0.71	0.77	0.15	0.11

*Significant at 5% level

**Significant at 1% level

Table 4 : Effect of gamma irradiation on the morphological characters of three rose cultivars in VM2 generation.

Treatment	Plant height (cm)	Branches /plant	Leaflets /branch	Spine count	Stem Diameter (cm)	Inter. length (cm)
I Paradise						
Control	79.60	9.00	16.80	10.00	3.26	5.50
3kr	57.20	8.80	14.60	11.20	3.86	4.92
4kr	41.00	10.00	14.80	7.20	3.26	5.26
5kr	49.00	12.00	13.80	4.40	3.90	4.26
6kr	41.80	10.40	13.20	6.80	3.24	4.34
CD at 5%	7.274**	1.623*	1.432*	1.207*	0.347	0.485
CV	9.40	12.06	7.29	11.36	7.40	7.45
SEM	3.43	0.77	0.68	0.57	0.16	0.23
II Raja Surendra Singh of Nalagarh						
Control	60.40	7.20	16.00	24.40	4.20	2.98
3kr	39.20	5.80	14.40	19.20	3.10	2.48
4kr	33.20	5.00	10.80	9.80	2.84	2.38
5kr	37.60	6.20	9.80	12.80	2.44	3.06
6kr	36.00	6.00	11.00	12.20	3.30	4.18
CD at 5%	3.753**	1.134*	2.022**	2.847**	0.388	0.330
CV	6.78	14.00	12.16	13.54	9.11	8.17
SEM	1.77	0.53	0.95	1.34	0.18	0.16
III Sindoor						
Control	67.40	8.20	15.80	18.20	3.30	4.76
3kr	51.40	5.20	13.20	8.60	2.44	3.84
4kr	46.40	6.20	12.80	10.80	2.54	4.80
5kr	44.60	5.60	12.40	11.20	3.16	4.36
6kr	39.40	6.00	9.80	9.20	2.76	5.00
CD at 5%	4.470**	1.073*	1.422*	1.628*	0.358	0.409
CV	6.69	12.82	8.29	10.47	9.40	6.70
SEM	2.11	0.51	0.67	0.77	0.17	0.19

*Significant at 5% level

**Significant at 1% level

Table 5 : Effect of gamma irradiation on the morphological characters of three rose cultivars in VM3 generation.

Treatment	Plant height (cm)	Branches /plant	Leaflets/ branch	Thorn count	Stem Diameter (cm)	Inter. length (cm)
I Paradise						
Control	79.40	9.60	17.80	10.20	3.40	4.86
3kr	60.00	9.00	14.20	10.40	3.98	5.14
4kr	47.80	10.80	15.00	7.20	3.26	5.10
5kr	40.80	10.20	14.40	4.40	3.92	5.10
6kr	40.40	10.60	13.20	6.80	3.50	4.18
CD at 5%	7.692**	1.192*	2.081**	1,236*	0.535	0.5152
CV	9.95	8.85	10.40	11.82	11.05	8.22
SEM	3.63	0.56	0.98	0.58	0.25	0.24
II Raja Surendra Singh of Nalagarh						
Control	61.20	7.20	16.20	22.80	4.18	2.52
3kr	39.20	6.20	14.80	16.40	3.08	2.40
4kr	32.00	5.60	11.20	13.20	2.76	2.10
5kr	39.20	5.80	10.20	10.00	2.58	2.69
6kr	37.00	6.00	11.60	11.40	3.16	3.92
CD at 5%	4.642**	1.134*	2.445*	2.239**	0.440	0.343
CV	8.30	13.73	14.25	11.32	10.42	9.22
SEM	2.19	0.53	1.15	1.06	0.21	0.19
III Sindoor						
Control	68.00	8.00	16.00	17.60	2.96	4.56
3kr	49.20	5.00	13.60	10.80	2.62	3.85
4kr	46.20	6.20	12.40	11.40	2.58	4.24
5kr	45.00	5.00	12.80	9.00	3.26	4.32
6kr	37.40	6.00	10.20	9.20	2.66	4.66
CD at 5%	5.345**	0.962*	1.601*	1.748*	0.459	0.540
CV	8.11	11.88	9.18	11.24	12.17	9.31
SEM	2.52	0.45	0.75	0.82	0.22	0.25

* Significant at 5% level

** Significant at 1% level

Fig 3: Effect of induced mutation on plant height of rose genotypes

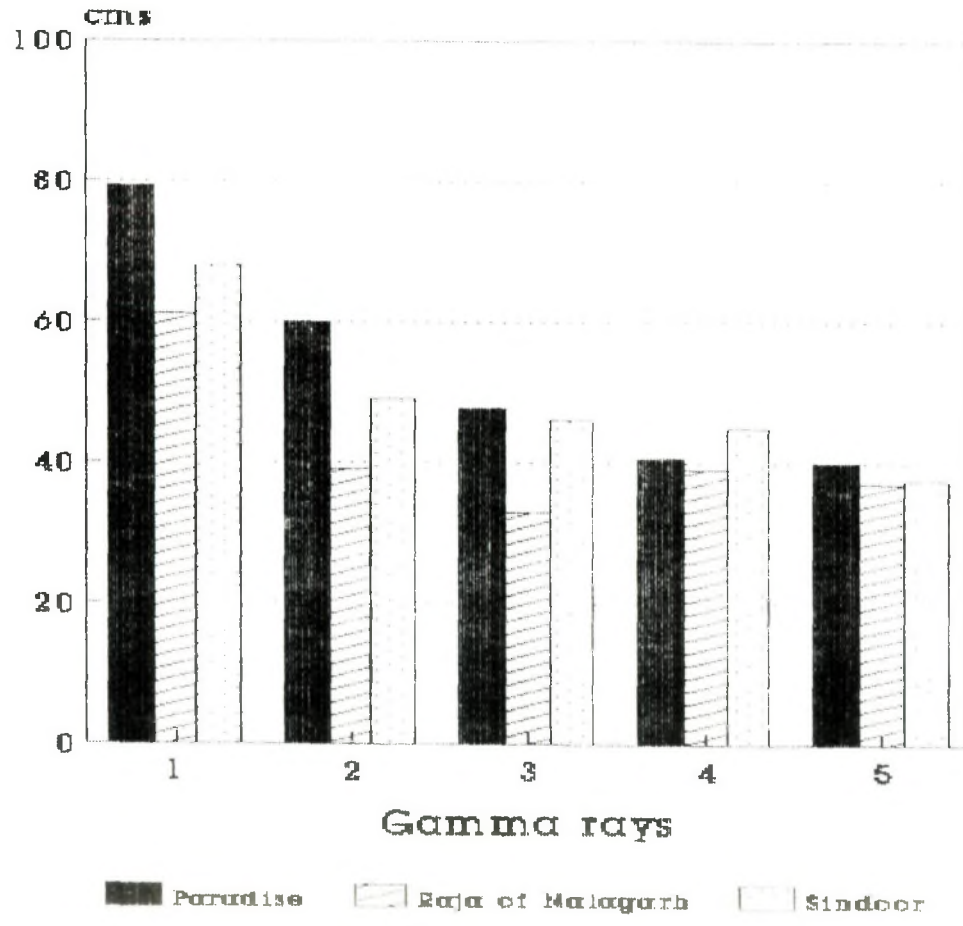


Fig 4: Effect of gamma irradiation on spine count in rose genotypes

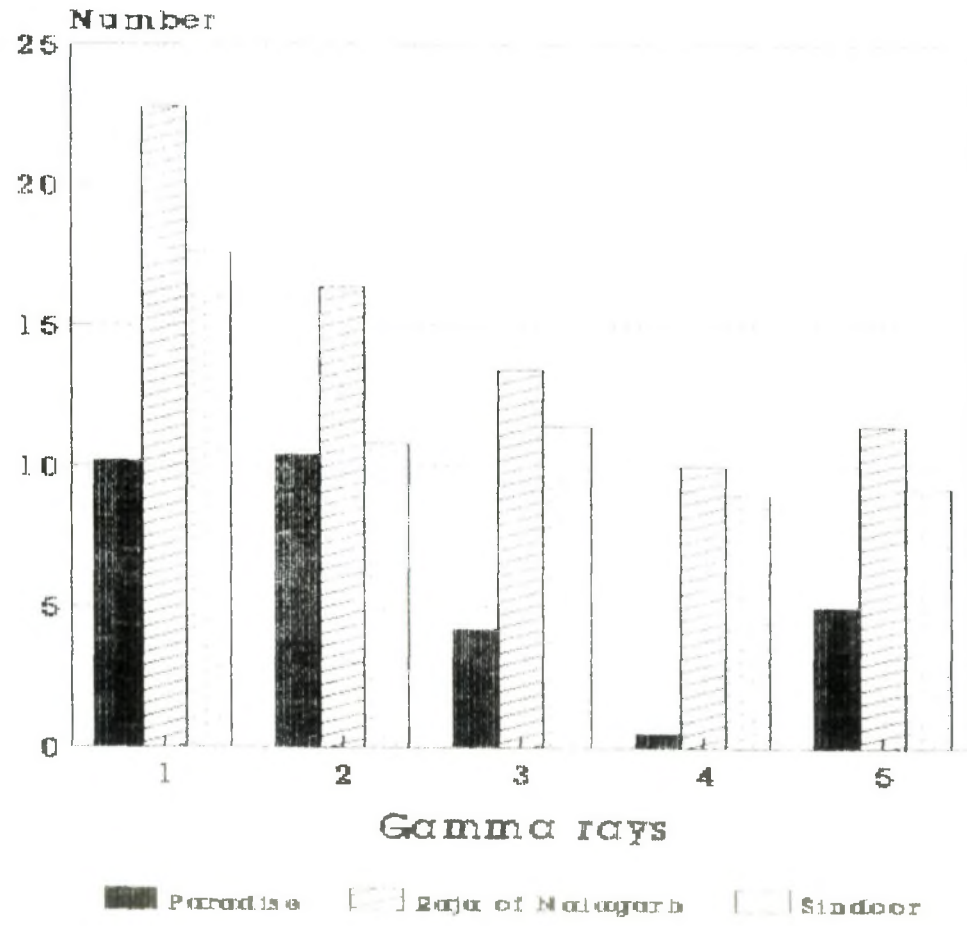


Plate 2: 3krad gamma ray induced mutant of Paradise and its parent cultivar

Plate 3: 4krad gamma ray induced mutant of Paradise and its parent cultivar

Plate 4: 5krad gamma ray induced mutant of Paradise and its parent cultivar

4.1.1.6 Number of Branches

With respect to the number of branches/plant recorded in all the three cultivars there was not much of variation to be noted among the different treatment dosages and found to be on par with each other. However, in cultivar Paradise (10.80) and Sindoor (6.20) maximum number of branches was recorded in the plants exposed to 4kr gamma rays followed by higher dosage compared to control treatments (9.60 and 800), whereas in Raja Surendra Singh of Nalagarh 3kr recorded (6.20) more number of branches per plant. Minimum number of branches were observed at 3kr (Tables 3,4,5) in Paradise, Raja Surendra Singh of Nalagarh and Sindoor (9.60, 6.20 and 5.00), respectively.

4.1.1.7 Number of Leaflets/Branch

Similar to the number of branches plant there was not much of treatment differences observed among the cultivars. But reduction in the number of leaflets was recorded at higher levels of gamma ray treatment dosages in all the three consecutive generation. Thus the maximum number of leaflets was observed at 3kr gamma rays in Paradise (14.20), Raja Surendra Singh of Nalagarh (15.00) and Sindoor (13.60) followed by 4kr treated dosages. Lowest number of leaflets was observed at 6kr treated buds in Paradise (13.20) and Raja Surendra Singh of Nalagarh (10.20) and Sindoor irrespective of the generations under study (Tables 3,4,5).

4.1.1.8 Foliage Characters

In the present study it was observed that there was clear cut variation in the foliage characters. The colour of the young foliage was ranging from dark brownish red to reddish brown (Table 1). Colour of the matured leaves was light to dark greenish. The variation was observed to be drastic in varieties having green

foliage which is bronze tinted as observed in cultivar Paradise giving a coppery shade to the leaves. The leaves were small to medium sized except in 6kr treated mutant of Paradise recording large sized leaves. Leaf floriferousness was medium, good or poor whereas, leaves were normal to glossy in nature.

Individual and random leaf area depicted significant differences among the treatments and even among the different cultivars irradiated during the period of experimentation. They also showed inverse relationship with respect to the treatment dosages. Higher the dosage lesser was the leaf area (Tables 6,7,8) as observed in all the three consecutive generations. However, maximum leaf area was observed in 3kr irradiated plants of Paradise (15.75) at random (290.99), whereas in Raja Surendra Singh of Nalagarh (10.69 and 180.64) and Sindoor (7.69 and 170.30) it was found to be on par with the control treatments. The minimum leaf area in Paradise, Raja Surendra Singh of Nalagarh and Sindoor was observed to be higher at higher dosage of 6kr treated plants both for individual and at random leaf area (11.68 and 218.37, 8.01 and 147.35, 6.74 and 148.85) in all the three consecutive generations recorded.

The leaf samples estimated for the total chlorophyll content varied considerably and was greatly influenced by the irradiation dosage. There was indirect correlation of the total chlorophyll content in irradiated treatments. Maximum chlorophyll content was recorded in 4Kr treated Paradise buds (0.7111) and the minimum (0.2905) at higher dosage of 6kr in comparison with the parent cultivars (Table 12). The miniature Paradise mutant also produced considerably moderate amount of chlorophyll content. Thus as the treatment dosages increase there is a proportionate decrease in the total chlorophyll content among the induced mutants.

Table 6 : Effect of gamma irradiation on the floral characters of three rose cultivars in VM1 generation

Treatment	Petals/ flower	Flower clusters	Flowers/ plant	Individ. LA (sq.cm)	Radom LA (sq.cm)	Flower diameter (cm)	Flower weight (gm)
I Paradise							
Control	34.00	7.40	41.60	12.73	250.57	22.00	11.28
3kr	24.80	7.20	38.40	7.44	175.16	19.20	10.08
4kr	36.60	8.00	57.00	9.24	202.05	20.80	12.36
5kr	38.80	9.20	63.60	10.62	248.31	21.70	10.54
6kr	52.20	6.20	16.00	7.31	231.24	21.40	11.38
CD at 5%	3.232**	1.017*	3.460**	1.407*	14.363**	0.986*	0.743*
CV	6.47	9.98	5.96	11.80	4.84	3.50	4.98
SEM	1.52	0.48	1.63	0.66	6.77	0.47	0.35
II Raja Surendra Singh of Nalagarh							
Control	54.80	7.00	27.80	8.26	140.45	17.30	10.64
3kr	41.20	6.40	18.40	7.18	136.12	16.40	10.38
4kr	45.20	6.00	18.20	6.72	123.12	15.20	8.12
5kr	38.20	5.40	21.80	7.16	112.49	15.40	8.70
6kr	49.40	7.00	19.20	4.40	127.71	15.90	10.14
CD at 5%	3.90**	0.889*	3.588**	1.252*	10.703**	0.920*	0.631*
CV	6.37	10.43	12.67	13.84	6.24	4.28	4.91
SEM	1.84	0.42	1.69	0.59	5.05	0.43	0.30
III Sindoor							
Control	32.00	5.20	33.00	5.56	131.16	17.70	5.98
3kr	28.60	4.80	29.00	4.44	120.39	16.10	5.84
4kr	25.60	5.20	22.20	5.42	133.07	12.94	5.52
5kr	20.20	4.80	14.80	4.98	115.82	13.20	5.58
6kr	22.80	3.60	11.20	5.26	119.09	11.60	4.54
CD at 5%	3.455**	1.169*	3.396**	0.499	10.058**	0.865*	0.664*
CV	9.97	18.47	11.49	7.27	6.05	4.51	9.02
SEM	1.63	0.55	1.60	0.24	4.74	0.41	0.31

*Significant at 5% level

Table 7 : Effect of gamma irradiation on the floral characters of three rose cultivars in VM2 generation.

Treatment	Petals/ flower	Flower clusters	Flowers /plant	Individ. LA (sq.cm)	Radom LA (sq.cm)	Flower dia. cm)	Flower weight (gm)
I Paradise							
Control	31.80	7.00	43.00	21.50	366.04	22.90	11.12
3kr	25.20	7.40	39.40	10.86	218.01	19.80	9.92
4kr	35.20	7.60	59.80	14.27	268.49	21.50	12.42
5kr	38.40	8.80	61.40	15.50	288.50	22.00	9.98
6kr	52.20	5.80	16.80	11.94	285.42	21.00	11.98
CD at 5%	4.559**	1.296*	5.611**	1.514*	12.249**	0.877*	0.875*
CV	9.30	13.21	9.49	7.62	3.20	3.05	5.89
SEM	2.15	0.61	2.65	0.71	5.78	0.41	0.41
II Raja Surendra Singh of Nalagarh							
Control	57.40	7.20	27.40	11.01	187.66	17.30	10.38
3kr	39.60	6.40	20.00	11.12	182.07	16.10	10.88
4kr	44.20	6.20	16.80	10.11	163.52	15.40	7.94
5kr	37.20	5.60	21.80	9.62	149.65	15.70	8.72
6kr	50.00	6.80	19.20	7.56	155.44	15.70	9.76
CD at 5%	5.276**	1.134*	3.837**	0.826*	6.918**	1.247*	0.713*
CV	8.61	13.13	13.60	6.23	3.08	5.80	5.58
SEM	2.49	0.53	1.81	0.39	3.26	0.59	0.34
III Sindoor							
Control	31.20	5.00	31.00	8.79	186.71	17.90	5.94
3kr	27.80	4.80	29.40	6.84	162.38	15.50	5.63
4kr	23.40	5.00	22.60	8.03	170.45	12.70	5.60
5kr	19.20	5.00	15.80	6.99	152.96	13.40	5.60
6kr	25.40	3.40	10.40	6.99	149.47	11.00	4.24
CD at 5%	3.857**	1.008*	3.077**	0.485	7.589**	1.137*	0.630
CV	11.33	16.20	10.51	4.81	3.44	6.01	8.70
SEM	1.82	0.48	1.45	0.23	3.58	0.54	0.30

* Significant at 5% level

** Significant at 1% level

Table 8 : Effect of gamma irradiation on the floral characters of three rose cultivars in VM3 generation.

Treatment	Petals/ flower	Flower clusters	Flowers/ plant	Individ. LA (sq.cm)	Radom LA (sq.cm)	Flower dia.(cm)	Flower weight (gm)
I Paradise							
Control	36.20	7.20	40.20	22.62	363.16	22.30	11.42
3kr	30.00	9.60	58.60	15.75	280.37	21.50	11.06
4kr	37.20	7.20	59.20	14.14	263.44	21.00	11,90
5kr	40.00	9.00	40.40	10.95	218.42	19.80	10.74
6kr	49.80	6.00	15.40	11.68	163.64	21.40	10.24
CD at 5%	3.706**	1.060*	4.475**	1.168*	11.39**	1.161*	0.886
CV	7.38	10.98	7.81	5.80	2.98	4.09	5.97
SEM	1.75	0.50	2.11	0.55	5.34	0.55	0.42
II Raja Surendra Singh of Nalagarh							
Control	57.00	6.80	25.80	10.84	184.69	17.20	10.82
3kr	39.00	6.60	19.60	10.69	180.64	16.50	10.92
4kr	47.80	6.00	16.20	9.85	164.69	15.50	7.70
5kr	38.00	5.60	18.80	8.89	160.42	15.70	8.88
6kr	51.00	4.80	21.20	8.01	147.35	15.50	9.80
CD at 5%	6.055**	1.114*	3.204**	0.703	8.282**	1.243*	0.909*
CV	9.69	13.06	11.76	5.43	3.69	5.77	7.04
SEM	2.86	0.53	1.51	0.33	3.91	0.59	0.43
III Sindoor							
Control	32.80	5.00	31.20	8.92	186.34	17.90	5.80
3kr	28.80	5.80	29.20	7.66	170.30	15.70	5.84
4kr	28.00	5.20	21.40	7.29	167.41	12.80	5.32
5kr	22.30	4.40	17.00	7.27	166.71	13.20	5.74
6kr	21.20	3.20	10.60	6.74	146.85	11.50	4.26
CD at 5%	4.127**	1.192*	3.253**	0.621	11.910**	0.804*	0.568
CV	11.49	18.67	11.09	6.12	5.30	4.22	7.86
SEM	1.95	0.56	1.53	0.29	5.62	0.38	0.27

* Significant at 5% level

** Significant at 1% level

Fig 5: Effect of gamma irradiation on the number of flowers in rose genotypes

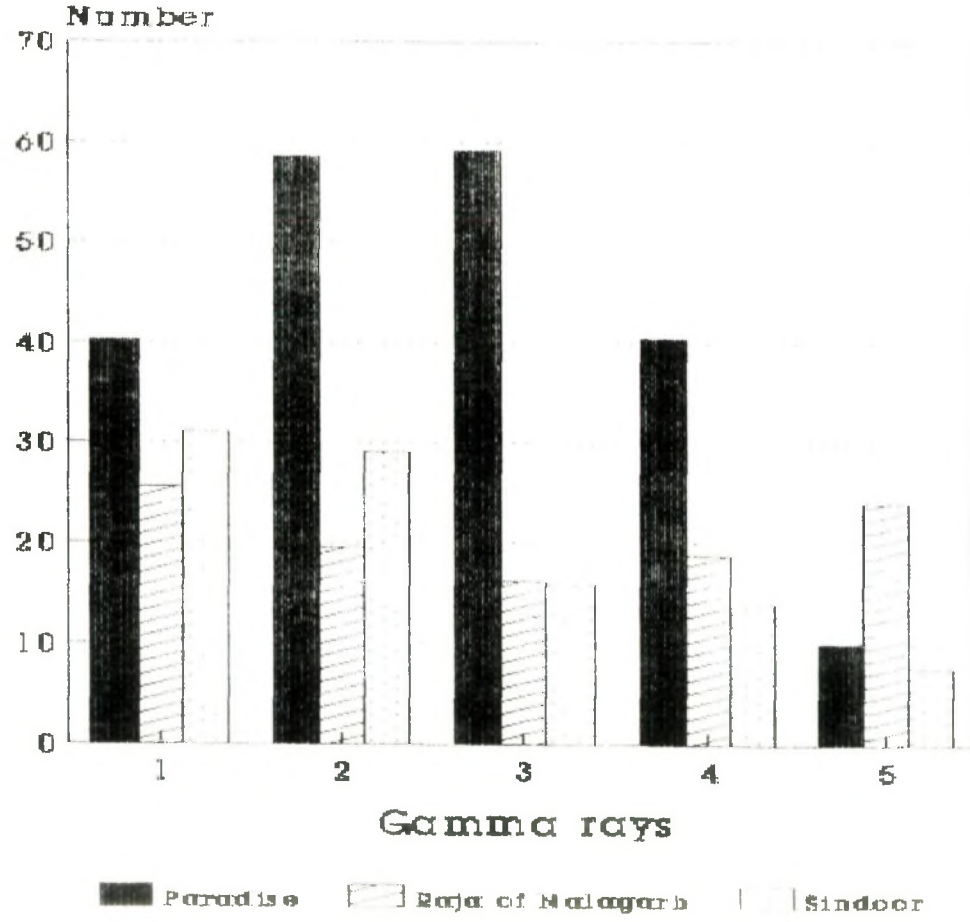
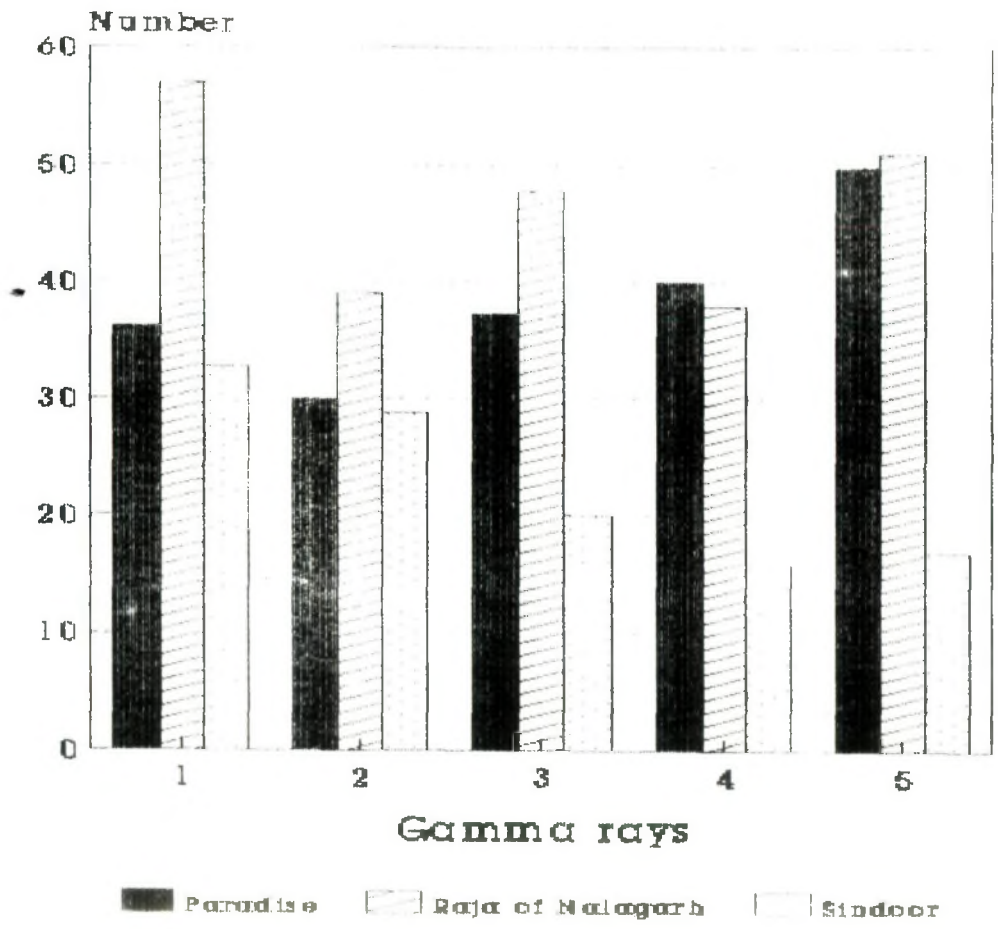


Fig 6: Effect of gamma irradiation on petal number of rose genotypes



4.1.1.9 Flower Diameter

There was not much of variation with respect to the flower diameter and it recorded a decrease in the flower diameter with increase in gamma irradiation. The maximum flower diameter in cultivars Paradise (21.50cm), Raja Surendra Singh of Nalagarh (16.50cm) and Sindoor (15.70cm) was recorded at 3kr gamma ray treated plants followed by 4kr gamma irradiation. Minimum flower diameter was observed at 5kr and 6kr treatments (Tables 6,7,8) in all the three cultivars and in almost all the three seasons.

4.1.1.10 Flower Weight

Flower weight was found to be non significant with respect to the effect of gamma irradiation. Fresh weight of the flowers was found to be maximum at 3 to 4kr treated plants of Paradise (11.90gm) and Sindoor (5.84gm). The least was found to be recorded at 6kr in Paradise (10.24gm) and Sindoor (4.26gm). In Raja Surendra Singh of Nalagarh higher the dosage better was the fresh weight and the maximum flower weight was observed at 3kr (10.92gm) followed by 6kr (9.80gm), respectively in all the three generations under observations (Tables 6,7,8).

4.1.1.11 Number of Flowers/plant

There was drastic difference among treatments within varieties used with respect to the number of flowers/plant. Maximum number was observed at 3kr treated plants in both Paradise (59.20) and Sindoor (29.20) but in Raja Surendra Singh of Nalagarh (21.20) it gave maximum number of flowers at higher dosages of 6kr followed by 4kr in Paradise (58.60) and Sindoor (21.40), whereas in Raja Surendra Singh of Nalagarh at 3kr (19.60) treated plants (Tables 6,7,8). Thus both

flower clusters/plant and the total number of flowers produced/plant had similar correlation results with respect to the mutagen treatment (Fig 5).

4.1.1.12 Number of Petals/Flower

Significant difference was found on the number of petals produced per flower within the treatments. Maximum number of petals was observed at higher dosage of 6kr in Paradise (49.80) and Raja Surendra Singh of Nalagarh (51.00) cultivars, whereas Sindoor exhibited increased petal number at 3kr dosage (28.80) compared to the control treatments (Tables 6,7,8). In Raja Surendra Singh of Nalagarh and Paradise higher the dosage more was the number of petals observed and was vice-versa in the cultivar Sindoor. In all the three generations the total number of petal variation with respect to gamma irradiation was significantly high between the treatments and also among the cultivars used (Fig 6).

4.1.1.13 Number of Flower Clusters

Number of flower clusters and flowers/plant were interrelated and found to be highly significant. In both the cases higher the dosage lesser was the number of flower clusters and flowers produced. The maximum number of flower cluster was observed at 3kr and 4kr treatments among all the three cultivars studied viz., Paradise (9.60), Raja Surendra Singh of Nalagarh (6.60) and Sindoor (5.80) followed by 4 to 5kr treated plants compared to the control treatments (Tables 13,14,15). Minimum flower clusters observed was in Paradise (6.00), Raja Surendra Singh of Nalagarh (3.20) at 6kr treated plants.

4.1.2 SOMATIC MUTATIONS

Many variegated, incurved flowers, colour change in the flowers, spinelessness, small size flowers were detected in irradiated plants. These changes

persisted in subsequent vegetative generations and were considered to be cases of somatic mutations. Highest percentage of flowers with somatic mutations was observed in the buds which were exposed to 3kr gamma rays. The percentage decreased with an increase in the exposure of gamma rays. The percentage of the flowers with mutations was the highest at the young flower bud stage after exposures to 3 to 6kr gamma irradiation (Tables 9,10,11).

Second generation was scored for the detection of viable mutations throughout the crop growth period in all the cultivars used. Among various doses of gamma rays used in the present study 3 to 4kr in Paradise, 6kr in Raja Surendra Singh of Nalagarh induced increased frequency of viable mutations in both second and third generation on plant basis. The lowest frequency of viable mutations on the plant basis was observed in 5-6kr treatments, except in case of Raja Surendra Singh of Nalagarh with the spectrum of viable mutations, growth habit, flower size, colour etc. The following are the desirable mutants obtained during the period of investigation which found to be very desirable and prominent. A brief morphological description of each of the desirable mutant types are given below.

4.1.2.1 Paradise 3kr Variegated Flower Mutant

The new mutant flower colour was lavender mauve with white streaks or variegations on the ventral and dorsal surface of the petals. The mutated plants were observed carefully from the VM2 generation onwards and in the further generations it was found to be very prominent with a plant height of 59cms, upright, compact and bushy habit. With respect to morphological characters it was found that the change was significant compared to control treatments (Table 9,10,11). Number of branches recorded was 10 and leaflets 13.60, with moderate spine count (11.20) and stem diameter. Internodal length was onpar with the control treatments. Leaf area was drastically reduced with individual (7.38) and random leaf area (176.86), respectively.

From among the floral characteristics total number of petals were reduced to 29 with very low flower production (6.40) which was onpar with number of flowers produced/plant under control treatments (40.00). Flower weight (9.92) and flower diameter (19.00) was not much affected when compared to its parents. This particular mutants observed had white variegation all over the petals which was found to be unique when compared to its original parent (Fig 7).

4.1.2.2 Paradise 5kr Thornless Mutant

Another mutant was observed in the cultivar Paradise by irradiating the plants with 5kr gamma irradiation. Though the spine count was moderate during first season but at VM2 generation it exhibited completely spineless characters which was very unique compared to the parent cultivar. The plant height was increased along with the flower weight, whereas contrarily number of branches per plant, leaflets, stem diameter, flowers produced and flower weight was on par with the control treatments whereas number of petals and number of flower clusters were found to be drastically reduced. Spine count was the main criteria for selection. It was dramatically reduced from 15.42 (control) to 3.20 when irradiated at 5kr gamma rays. In VM1 and VM2 generations the flowers obtained were slightly mauve coloured, whereas in VM3 generation the colour of the flower along with the spineless character was changed completely to white (Fig 7). Though other morphological traits were found to be the same with the original genotype the two major contrasting changes were with respect to the change of white flower colour and spinelessness observed during the period of study (Tables 9,10,11).

4.1.2.3 Paradise 4kr Miniature Mutant

A dwarf mutant was observed by irradiating the plants at 4kr gamma rays in the cultivar Paradise and subsequent reduction in various characters was obtained. A decrease in plant height (40.40) was observed compared to the control (60cm).

Table 9 : Effect of gamma irradiation on the morphological and floral characters of four desirable mutants observed in VM2 generation of three rose cultivars

Mutant	Flower cluster	Flower weight (gm)	Flowers /plant	Individ. LA (sq.cm)	Rand. LA (sq.cm)	Flower Dia. (cm)	Plant height (cm)	Branch /plant	Leaflets /branch	Spine count	Stem Dia. (cm)	Inter. Len. (cm)	Petal/ flower
M1	6.40	9.92	40.00	7.38	176.86	19.00	59.20	10.00	13.60	11.20	3.94	5.14	29.00
M2	5.80	10.32	41.60	8.24	175.84	17.00	60.20	10.40	13.40	3.20	3.88	5.28	28.00
M3	9.60	4.06	70.40	5.22	119.02	7.70	40.40	10.20	15.20	7.40	1.82	4.96	20.00
M4	6.20	10.12	18.80	4.36	124.62	16.00	34.00	6.40	11.00	13.40	2.82	4.22	43.60
CD(5%)	1.349**	0.569	4.987**	1.202*	21.24**	1.124*	2.789*	1.271*	1.516*	1.214*	0.395	0.368	4.08**
CV	13.98	4.79	8.47	13.84	10.33	5.28	4.18	9.97	8.26	10.00	9.19	5.45	9.59
SEM	0.62	0.26	2.29	0.00	9.74	0.52	1.28	0.58	0.70	0.56	0.18	0.17	1.84

M1 = **Paradise 3kr variegated flower mutant**

M2 = **Paradise 5kr thornless mutant**

M3 = **Paradise 4kr miniature mutant**

M4 = **Raja Surendra Singh of Nalagarh 6kr variegated flower mutant**

Table 10 : Effect of gamma irradiation on the morphological and floral characters of four desirable mutants observed in VM3 generation of three rose cultivars

Mutant	Flower cluster	Flower weight (gm)	Flowers /plant	Individ LA (sq.cm)	Rand. LA (sq.cm)	Flower Dia. (cm)	Plant height (cm)	Branch /plant	Leaflets /branch	Spine count	Stem Dia. (cm)	Inter. Len. (cm)	Petal/ flower
M1	6.20	9.80	40.00	10.59	224.70	19.70	57.80	10.00	13.40	10.80	3.84	5.00	29.40
M2	6.30	10.72	40.60	10.68	223.83	19.00	59.60	10.40	13.80	1.40	3.58	5.18	28.80
M3	9.60	3.64	65.80	7.68	146.89	7.40	39.60	9.80	15.20	7.20	1.80	4.68	19.00
M4	6.40	10.14	18.40	7.71	158.48	15.50	34.60	6.20	10.60	13.00	2.86	4.00	45.20
CD (5%)	1.174*	0.797	6.606**	0.940	9.78**	1.364*	5.19**	1.407	2.304*	1.489*	0.42	0.403	4.165**
CV	11.99	6.74	11.63	7.44	3.77	6.39	7.86	11.22	12.61	13.33	10.14	6.19	9.87
SEM	0.54	0.37	3.03	0.43	4.49	0.63	2.36	0.65	1.06	0.68	0.19	0.18	1.91

M1 = **Paradise 3kr variegated flower mutant**

M2 = **Paradise 5kr thornless mutant**

M3 = **Paradise 4kr miniature mutant**

M4 = **Raja Surendra Singh of Nalagarh 6kr variegated flower mutant**

Table 11 : Effect of gamma irradiation on the morphological and floral characters of four desirable mutants observed in VM4 generation of three rose cultivars

Mutant	Flower cluster	Flower weight (gm)	Flowers /plant	Individ. LA (sq.cm)	Rand. LA (sq.cm)	Flower Dia. (cm)	Plant height (cm)	Branch /plant	Leaflets /branch	Spine count	Stem Dia. (cm)	Inter. Len. (cm)	Petal/ flower
M1	6.20	9.86	39.40	10.90	216.01	19.30	58.00	10.20	13.60	11.00	3.64	4.80	30.60
M2	6.60	10.74	40.60	11.12	221.74	19.40	60.20	11.20	13.80	0.20	3.88	5.12	29.20
M3	8.60	3.76	64.40	7.97	148.69	7.30	29.20	8.80	16.80	7.20	1.74	4.42	19.00
M4	6.40	10.06	18.80	8.14	148.07	15.00	30.40	6.00	9.80	12.80	2.78	4.60	40.00
CD(5%)	0.950*	0.700	5.169**	0.698	13.52**	1.047*	6.363**	1.239*	1.547*	1.526*	0.646	0.546	3.504**
CV	9.92	5.90	9.19	5.31	5.34	4.98	10.38	9.93	8.31	13.75	15.56	8.37	8.53
SEM	0.44	0.32	2.37	0.32	6.21	0.48	2.92	0.57	0.71	0.70	0.30	0.25	1.61

M1 = **Paradise 3kr variegated flower mutant**

M2 = **Paradise 5kr thornless mutant**

M3 = **Paradise 4kr miniature mutant**

M4 = **Raja Surendra Singh of Nalagarh 6kr variegated flower mutant**

Fig 7: Effect of gamma irradiation on morphological characters on M1 & M2 mutants observed in rose genotypes

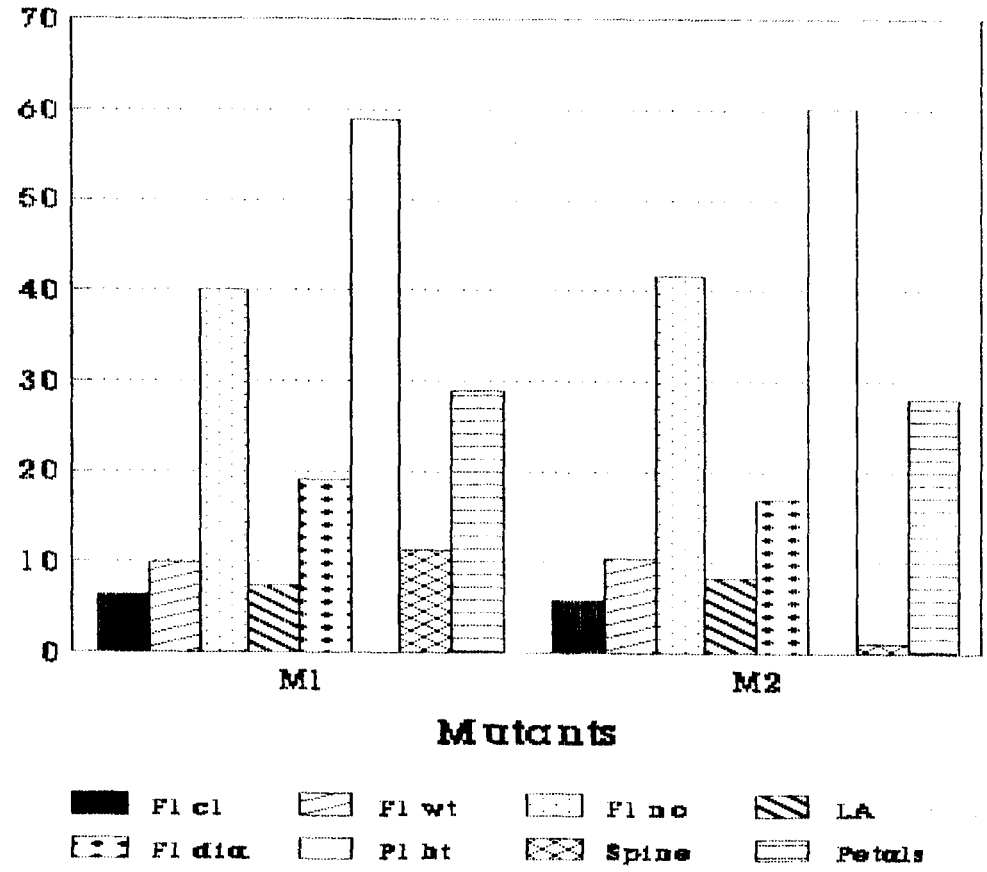


Fig 8: Effect of gamma irradiation on morphological characters of M3 & M4 mutants observed in rose genotypes

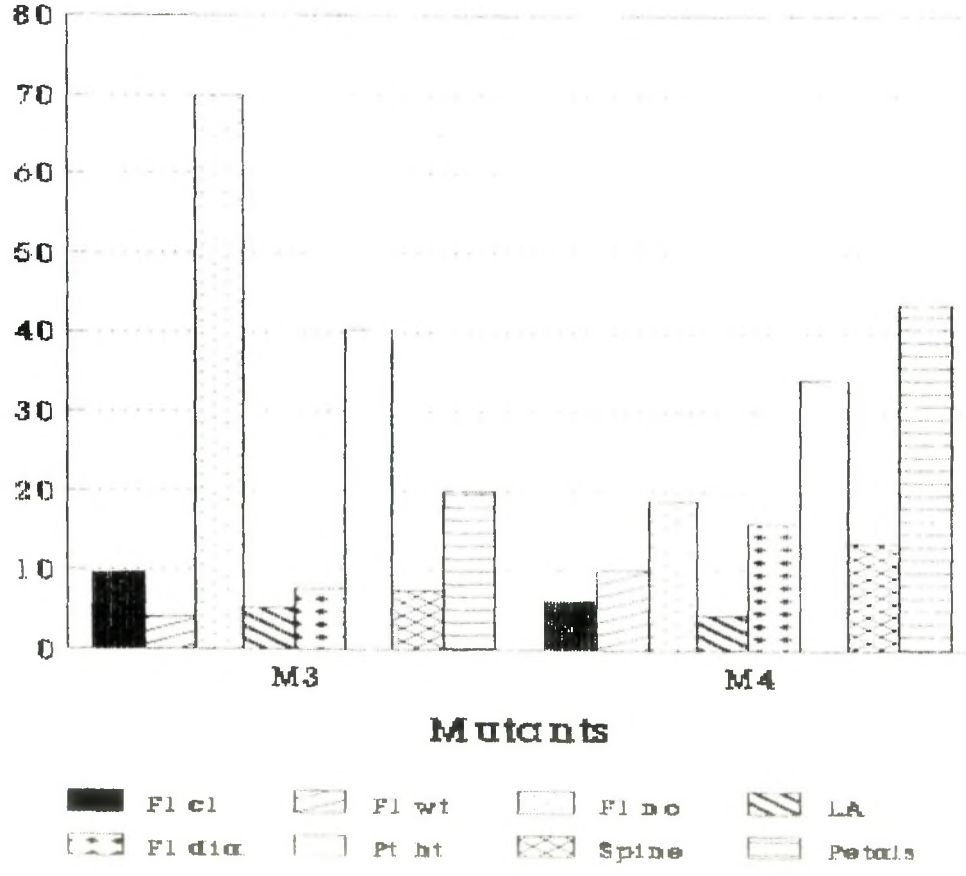


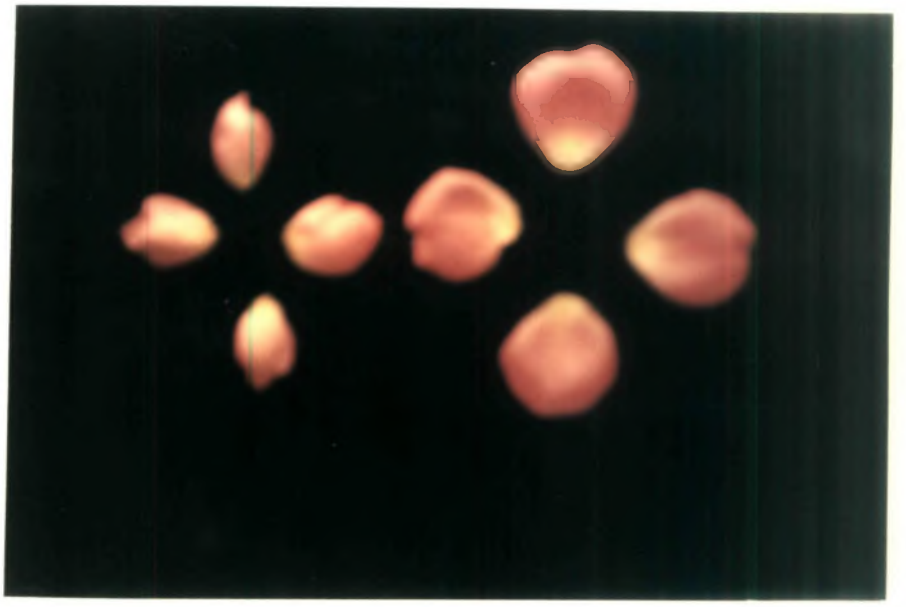
Plate 5: 6krad gamma ray induced mutant of Paradise and its parent cultivar

Plate 6: Miniature flowered dwarf mutant at 4krad gamma rays of cultivar Paradise

Plate 7: Ventral view of flower petal variegation at 3krad gamma ray induced mutant of Paradise

Plate 8: Reduced petal size in the dwarf mutant of 4krad gamma ray of cultivar paradise

Plate 9: Ventral view of flower petal variegation at 6krad induced mutant of Raja Surendra Singh of Nalagarh



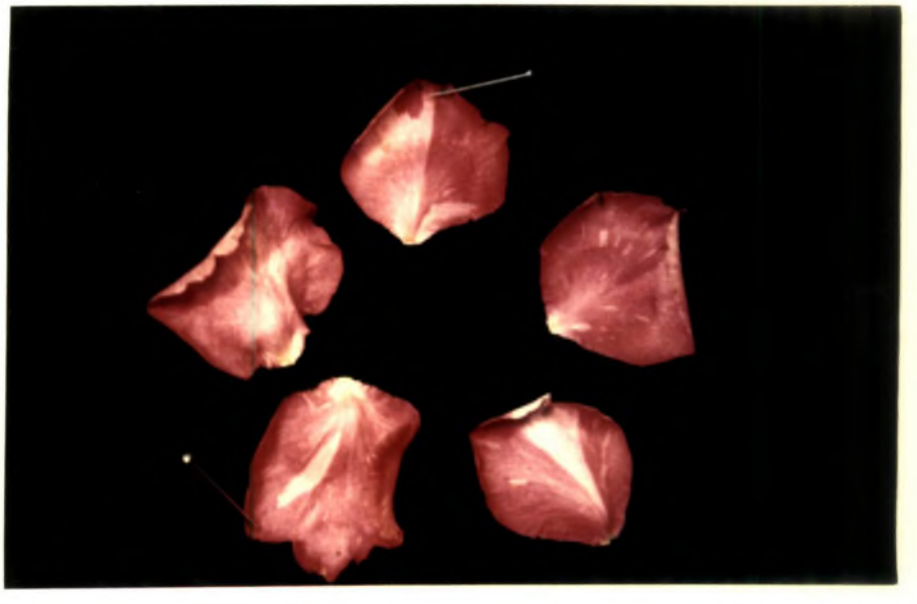


Plate 10: Thornless mutant at 5krad gamma ray of cultivar Paradise along with other treatments from left - control, 3krad, 4krad, 5krad (Thornless) and 6krad induced plants showing variation in spine count

Plate 11: Photograph showing the variation in the size and shape of leaf in gamma ray induced mutant of Paradise cultivar from left - control, 3krad, 4krad, 5krad and 6krad treatments


1
2
3
4
5


1
2
3
4
5

Plate 12 Plate depicting the flower colour of cultivar Sindoor

Plate 13 Plate depicting the flower colour of cultivar Raja Surendra Singh o Nalagarh

Table 12 : Estimated chlorophyll content in different spontaneous and induced mutants of rose genotypes

Sl. No	Genotypes	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total Chlorophyll (mg/g)
1	Paradise	0.3440	0.0675	0.5776
2	3kr	0.1318	0.2433	0.6163
3	4kr	0.0732	0.1380	0.7111
4	5kr	0.2769	0.4158	0.3265
5	6kr	0.2824	0. 0526	0.2905
6	Raja Surendra Singh of Nalagarh	0.3986	0.1127	0.4568
7	3kr	0.3893	0.1327	0.6143
8	4kr	0.2779	0.0915	0.5665
9	5kr	0.2263	0.1599	0.3369
10	6kr	0.3035	0. 0865	0.8418
11	Sindoor	0.5453	0.1832	0.5112
12	3kr	0.3386	0.1031	0.5216
13	4kr	0.7919	0.2292	0.3692
14	5kr	0.2483	0.0729	0.3858
15	6kr	0.2278	0.0629	0.3441
16	Paradise 4kr miniature mutant	0.3865	0. 1132	0.4487
17	Akito (P)	0.0627	0.1806	0.9002
18	Akito (M)	0.5860	0.1082	0.7662
19	Eiffel Tower (P)	0.4185	0.0457	0.5311
20	Eiffel Tower (M)	0.1405	0.2031	0.1861
21	First Federal Gold (P)	0.5009	0.3476	0.7062
22	First Federal Gold (M)	0.3593	0.4672	0.8432
23	Kronenbourg (P)	0.4095	0.1158	0.4939
24	Kronenbourg (M)	0.3765	0.1551	0. 6555
25	Wouburn Gold (P)	0.3873	0.1638	0.4951
26	Wouburn Gold (M)	0.6019	0.2458	0.6547
27	Tzigane (P)	0.4260	0.1963	0.9620
28	Tzigane (M)	0.3770	0.2442	1.0401

(P) = Parent

(M) = Mutant

Moderate to less number of branches and spine count was found to be increased. Number of leaflets upto 15.20, drastic reduction in stem diameter (1.82) and flower diameter (7.70) was very unique, with moderate reduction in the number of petals (20.00), flower weight (4.06), internodal length (4.96) and leaf area (5.22 and 119.02), respectively. Contrarily number of flower clusters/plant and number of flowers/plant was found to be enormously increased. Overall, the mutant obtained was of dwarf growth habit, compact and with very small sized flowers, leaf area and stem diameter. The total number of flowers increased by two folds and the size of the flower was very small compared to the parent treatments (Fig 8).

4.1.2.4 Raja Surendra Singh of Nalagarh 6kr Variegated Flower Mutant

Variegated flower mutant was observed in cultivar Raja Surendra Singh of Nalagarh at 6kr gamma rays. In VM2 generation slightly variegated flowers were obtained with prominent white streaks on ventral side of the flower. Variegation was very prominent in the further generations as observed and found desirable for further perpetuation. Morphologically, plants were upright, compact with a plant height of 34cm, less number of branches (6.40cm) and stem diameter (2.82cm). Number of leaflets, spines and internodal length was found to be moderate and onpar with the parent. Flower clusters and number of flowers per plant was moderate and interrelated.

Petal number was increased (43.60) along with the increase in diameter (16.00). However, leaf area was considerably reduced due to higher irradiation effect. Though the original colour of the genotype was predominantly darker in shade, higher dosages persisted a prominent mutant with white streaks which was lightly orange shaded (Fig 8). Therefore, petal Variegation was observed in Paradise at 3kr with lower dosage Raja Surendra Singh of Nalagarh exhibited flower colour variation at higher doses of 6kr which was inversely related (Tables 9,10,11).

4.1.3 MORPHOLOGICAL STUDIES ON SPONTANEOUS MUTATION

Spontaneous mutations or sports have contributed considerably to the variation among vegetatively propagated plants. Efforts to copy this variation by means of artificially induced mutations have met with varying success in different plants. The possibilities of mutation breeding are dependent on the genetical constitution of the crops involved and the degree of heterozygosity.

The results of the present findings on spontaneous mutations in cultivars viz., Akito, Eiffel Tower, First Federal Gold, Kronenbourg, Tzigane and Wouburn Gold were as follows

4.1.3.1 Growth Habit

The study on all the genotypes of spontaneous mutants revealed that the growth habit of Eiffel Tower and Tzigane were found to be erect, upright, whereas all other genotypes were of spreading types. All the mutants showed vigorous and bushy growth whereas Wouburn Gold and Akito were comparatively more vigorous, compact compared to their respective mutants (Table 12A).

4.1.3.2 Plant Height

There was significant difference among the parent and the mutants with regard to the plant height in all the three vegetative generations under observation. It was seen that in almost all the genotypes taken for the present study, the mutants showed reduced plant height in comparison to their respective parents (Tables 13,14,15). Differences in the plant height was observed between some of the original genotypes irrespective of the varieties. In most cases there was not much of variation in the plant height except in Eiffel Tower and Wouburn Gold

Table 12A : Effect of spontaneous mutation on the growth habit of rose genotypes

Sl. No	Treatment	Growth habit	Spine characters		Foliage characters		Glossyness	Florifero-usness	Bud form	Fragrance
			Shape	Colour	Size	Colour				
1	AP	Upright	Straight	Brown	Medium	Dark Green	Normal	Good	Ovoid	Slight
2	AM	Spreading	Straight	Brown	Medium	Dark green	Glossy	Good	Globular	No
3	ETP	Upright	Straight	Brown	Medium	Light green	Normal	Poor	Ovoid	High
4	ETM	Upright	Straight	Brown	Medium	Light green	Normal	Poor	Pointed	High
5	FPGP	Spreading	Straight	Brown	Small	Dark Green	Normal	Medium	Ovoid	No
6	FPGM	Spreading	Straight	Brown	Medium	Light green	Normal	Medium	Globular	Slight
7	KP	Spreading	Hooked	Brown	Medium	Light green	Glossy	Medium	Ovoid	Moderate
8	KM	Spreading	Hooked	Brown	Large	Light green	Glossy	Medium	Ovoid	No
9	TP	Upright	Hooked	Green	Medium	Light green	Normal	Poor	Ovoid	No
10	TM	Upright	Straight	Brown	Medium	Green	Normal	Poor	Ovoid	No
11	WGP	Spreading	Hooked	Brown	Medium	Light green	Normal	Medium	Ovoid	Moderate
12	WGM	Spreading	Hooked	Brown	Medium	Dark green	Glossy	Good	Globular	Moderate

ETP = Eiffel Tower Parent
ETM = Eiffel Tower Mutant
AP = Akito Parent
AM = Akito Mutant
WGP = Wouburn Gold Parent
WGM = Wouburn Gold Mutant
KP = Kronenbourg Parent
KM = Kronenbourg Mutant
FPGP = First Federal Gold Parent
FPGM = First Federal Gold Parent
TP = Tzigane Parent
TM = Tzigane Mutant

genotypes. It was observed that only mutant of First Federal Gold exhibited increased plant height than its parent (Fig 9).

From among the different mutants studied Eiffel Tower (1 ⅛6.39) had the maximum height followed by Wouburn Gold (87.20). Most of the genotypes exhibited moderate height ranging from 33 to 150cm, whereas the genotypes of Eiffel Tower and its mutant was tall and Tzigane recording a very poor growth. It was also noted that the height ranged from 33 to 138cm at the first peak flowering stage, 47 to 146cm at second peak flowering stage and 47 to 141cm in the third peak flowering stage, indicating that the plant height also varied from season to season.

4.1.3.3 Stem Diameter

There was not much of difference observed among the genotypes and their mutants with respect to the stem diameter (Tables 13,14,15). Almost all the cultivars showed similar diameter with a maximum diameter recorded in Wouburn Gold (4.78cm) followed by Kronenbourg mutant (3.58cm) and the least to be found in Eiffel Tower (2.82cm).

4.1.3.4 Internodal Length

Internodal length was also recorded to be non significant with the maximum length observed in Wouburn Gold parent (5.86cm) followed by Eiffel Tower (5.60cm) and the minimum length was observed in First Federal Gold (2.66cm). All the mutants showed proportional increase in the internodal length over the parents (Tables 13,14,15).

Table 13 : Effect of spontaneous mutation on morphological characters of different rose genotypes in the VM1 generation.

Genotypes	Plant height (cm)	Branches/ plant	Leaflets/ branch	Spine count	Stem Diameter (cm)	Intern. Length (cm)
Akito (P)	49.00	7.40	11.60	10.20	2.74	4.86
Akito (M)	50.80	10.00	14.80	24.80	3.04	5.20
Eiffel Tower (P)	138.00	6.60	17.80	10.20	2.20	5.60
Eiffel Tower (M)	111.40	8.00	20.80	11.20	2.54	4.94
First Federal Gold (P)	44.00	6.60	17.20	21.00	3.34	3.08
First Federal Gold (M)	49.20	6.40	14.80	24.00	2.84	3.18
Kronenbourg (P)	50.60	5.40	13.00	33.20	4.68	3.90
Kronenbourg (M)	33.80	6.40	11.60	44.40	3.32	5.86
Tzigane (P)	32.00	4.23	17.60	19.87	3.40	3.00
Tzigane (M)	39.40	4.40	15.00	21.80	2.70	3.86
Wouburn Gold (P)	66.00	8.00	18.80	17.80	2.76	5.48
Wouburn Gold (M)	51.00	8.20	19.60	32.00	4.22	4.20
CD at 5%	7.195**	1.662*	2.103**	2.673**	0.360	0.504
CV	9.281	18.012	10.798	9.399	9.038	8.742
SEM	2.596	0.599	0.759	0.964	0.130	0.181

(P) = Parent

(M) = Mutant

* **Significant at 5% level**

** **Significant at 1% level**

Table 14 : Effect of spontaneous mutation on morphological characters of different rose genotypes in the VM2 generation.

Genotypes	Plant height (cm)	Branches /plant	Leaflets/ branch	Spine count	Stem Diameter (cm)	Intern. Length (cm)
Akito (P)	69.00	6.80	11.20	10.00	3.16	4.84
Akito (M)	68.80	9.80	13.40	23.40	2.76	5.44
Eiffel Tower (P)	146.39	6.60	17.40	10.00	2.82	5.42
Eiffel Tower (M)	121.80	7.80	21.00	11.00	2.96	4.84
First Federal Gold (P)	58.60	6.40	14.80	20.80	2.54	2.64
First Federal Gold (M)	66.40	6.40	14.40	22.40	2.64	3.26
Kronenbourg (P)	67.40	5.80	12.20	29.00	3.16	3.84
Kronenbourg (M)	47.00	6.40	10.80	42.60	3.46	5.74
Tzigane (P)	39.80	5.66	13.90	18.97	2.77	3.45
Tzigane (M)	49.80	5.80	14.80	20.00	2.92	4.24
Wouburn Gold (P)	87.20	7.40	18.20	16.40	4.02	5.68
Wouburn Gold (M)	74.80	8.40	19.80	29.00	4.56	4.68
CD at 5%	7.992**	1.462*	2.156*	2.628**	0.403	0.467
CV	8.139	15.967	11.663	9.849	9.949	8.093
SEM	2.883	0.527	0.778	0.948	0.145	0.168

(P) = Parent

(M) = Mutant

* Significant at 5% level

** Significant at 1% level

Table 15 : Effect of spontaneous mutation on morphological characters of different rose genotypes in the VM3 generation.

Genotypes	Plant height (cm)	Branches /plant	Leaflets/ branch	Spine count	Stem Dia.(cm)	Intern. Length (cm)
Akito (P)	71.40	6.80	10.80	10.80	3.02	4.96
Akito (M)	69.60	9.80	15.00	25.00	2.76	5.04
Eiffel Tower (P)	141.80	7.20	17.60	9.40	2.46	5.56
Eiffel Tower (M)	120.40	8.20	21.00	11.20	2.96	5.02
First Federal Gold (P)	57.00	6.80	16.60	20.40	2.62	2.66
First Federal Gold (M)	65.00	6.40	14.80	22.00	2.86	3.46
Kronenbourg (P)	69.80	5.80	13.00	32.60	3.26	3.98
Kronenbourg (M)	47.40	6.20	10.80	45.00	3.58	5.64
Tzigane (P)	45.00	6.20	17.56	19.87	2.54	2.98
Tzigane (M)	51.80	6.60	15.60	21.60	2.92	3.58
Wouburn Gold (P)	86.60	7.20	19.00	18.60	4.14	5.58
Wouburn Gold (M)	72.00	8.00	18.80	30.80	4.78	4.26
CD at 5%	7.739**	1.689*	1.701**	2.89*	0.375	0.388
CV	7.919	18.307	8.765	10.282	9.118	6.733
SEM	2.792	0.609	0.614	1.041	0.135	0.140

(P) = Parent

(M) = Mutant

* Significant at 5% level

** Significant at 1% level

Fig 9 : Spontaneous mutation on plant height of rose genotypes

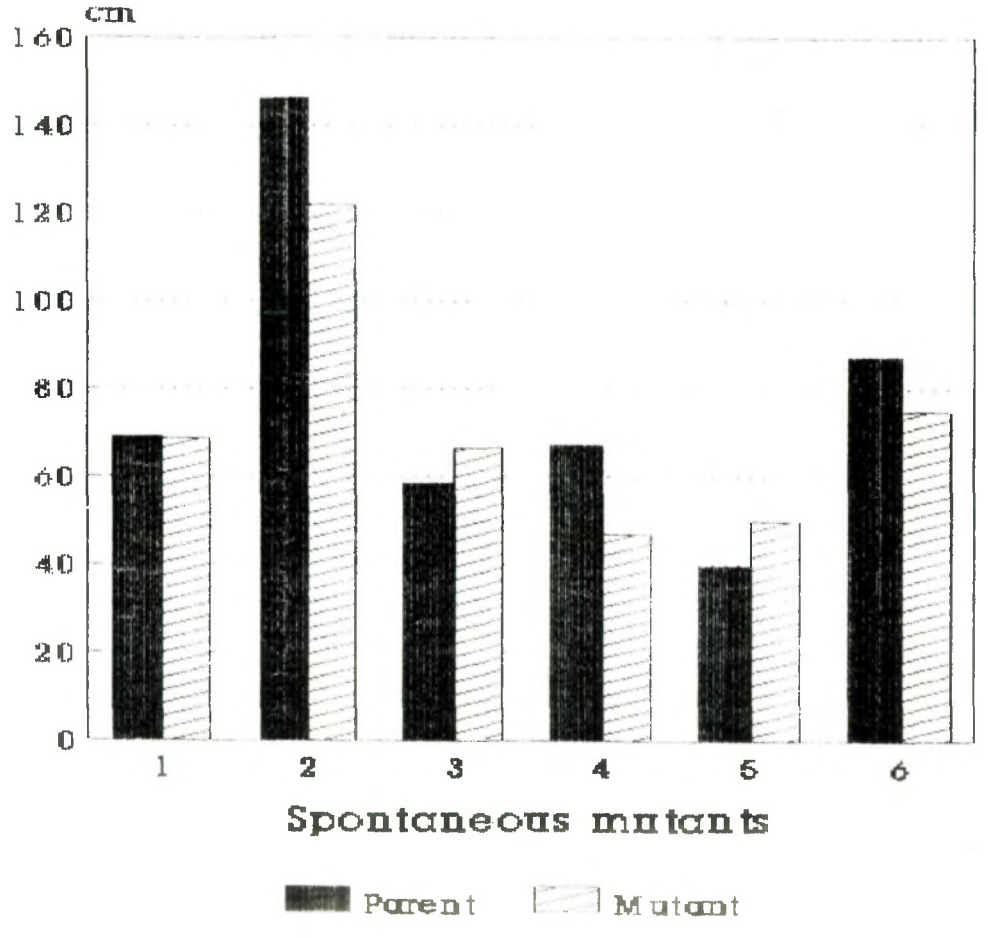


Fig 10: Spontaneous mutation on the spine count in rose Genotypes

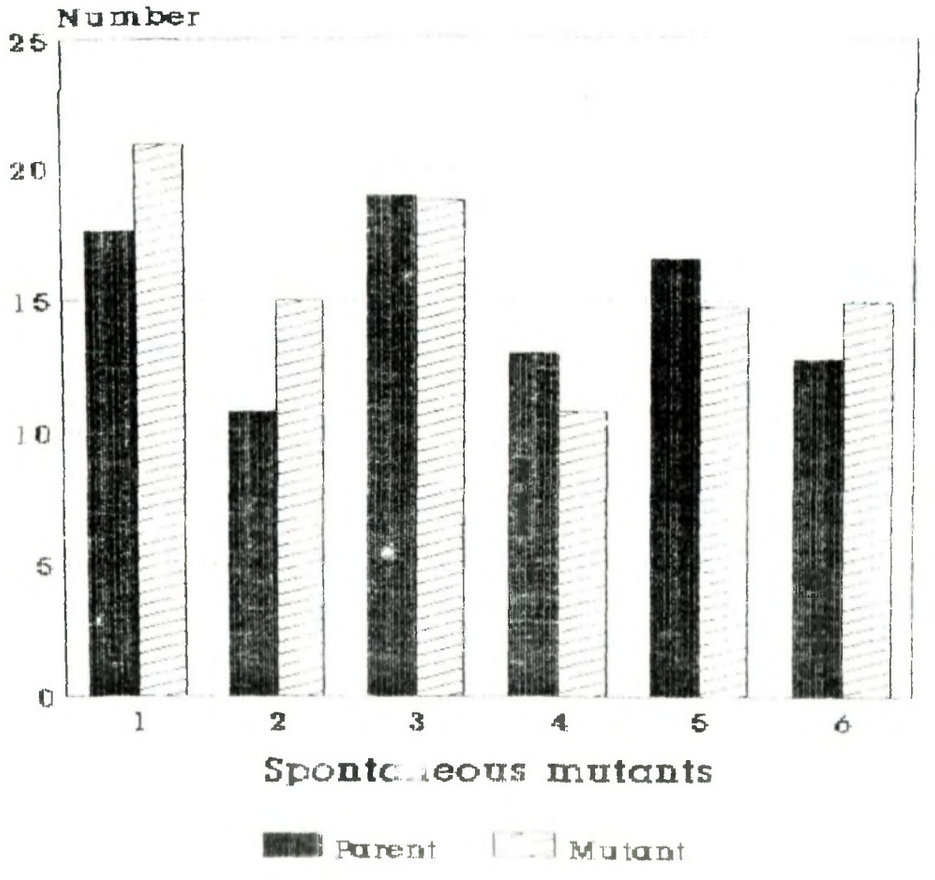
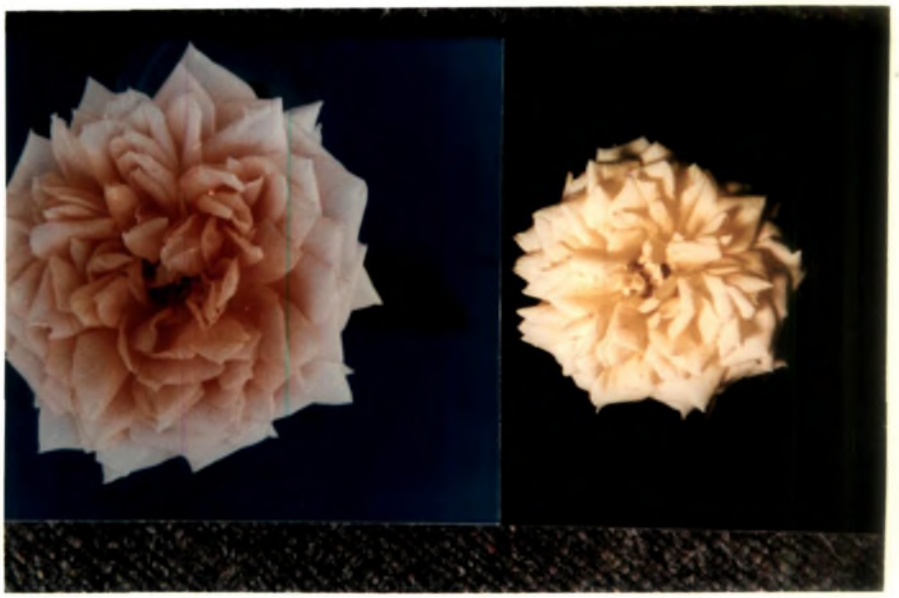


Plate 14: Spontaneous mutant of Akito and its parent genotype

Plate 15 : Spontaneous mutant of Eiffel Tower and its parent genotype

Plate 16: Spontaneous mutant of First Federal Gold and its parent genotype



4.1.3.5 Spine Characters

Variation in the colour of the spine among different rose mutants and its respective parents were not found to be considerable. It was observed that in most of the genotypes the colour of the young stem was either light green with reddish brown tinge or light brown with greenish tinge. When matured in all the genotypes brown to green coloured spines were observed under the study. The shape was straight to hooked in nature in all the genotypes (Table 12).

Most of the genotypes were moderate to highly spiny and the number of spines ranged from 10 to 44 per 10cm cuttings at the base of the stem (Tables 13,14,15). The natural mutants showed increase in the spine number, but there was not much of difference between the parent and the mutants, except in Akito mutant (25.00) and parent (10.80) along with Wouburn Gold mutant (32.00) and its parents (17.80) respectively. Kronenbourg mutant had maximum number of spines (45.00) followed by Wouburn Gold (32.60) when compared to other genotypes (Fig 10).

4.1.3.6 Number of Branches and Leaflets

There was not much variation in the number of branches and leaflets produced/plant. Maximum number of branches was observed in Akito mutant (9.80) followed by mutant of Wouburn Gold mutant (8.00) and the least to be in Tzigane (4.40) and even in kronenbourg (5.40). Maximum leaflets were recorded in Eiffel Tower mutant (21.00) followed by Wouburn Gold Mutant (19.80) and the least in Kronenbourg and Akito mutants in all the three seasons under study (Tables 13,14,15). There was significant difference among the parent and mutants, but among all the genotypes the variation was not considerably significant.

4.1.3.7 Foliage Characters

In the present study it was observed that there was clear cut variation in the foliage characters. The colour of the young foliage was ranging from dark brownish red to reddish brown (Table 12A). Colour of the matured leaves revealed that 3 out of 9 genotypes had dark green , 4 with green and 2 with light green. The variation was observed to be drastic in genotypes having green foliage which is bronze tinted giving a coppery shade to the leaves. The leaves were small to medium sized except in Kronenbourg recording large sized leaves. Leaf floriferousness was medium, good or poor whereas, leaves were normal to glossy in nature.

Individual and random leaf area was found to be highly significant between individual genotypes though not among their respective mutants. Maximum leaf area was recorded in Kronenbourg (18.37) individually and concurrently (335.39) at random followed by Eiffel Tower mutant (17.68 & 328.44) and Wouburn Gold mutant (16.98 & 320.87). With individual increase in the leaf area there was a significant increase at random with respect to the total leaf area among the different genotypes. In comparison to other genotypes minimum area was recorded in the genotypes First Federal Gold (10.69 & 279.41) followed by Akito (11.83 & 251.56), respectively (Tables 16,17,18).

It was observed that in spontaneous mutants the maximum chlorophyll content was recorded in Tzigane mutant (1.0401) followed by Akito (0.9002). In the cultivars Wouburn Gold, First Federal Gold mutant and Tzigane mutants, significant increase in the chlorophyll content among the mutants was noted whereas, it was found to be lesser in Eiffel Tower, Akito and Kronenbourg. From among all the genotypes under study there was significant difference among the estimated chlorophyll A and Chlorophyll B contents (Table 12), which was in correspondence with the total chlorophyll content.

4.1.3.8 Flower Fragrance

Variation in the fragrance of rose mutants was moderate among all the genotypes, except in Eiffel Tower and Paradise genotypes. It was observed that these genotypes were most outstanding with high fragrant flowers and kronenbourg and wouburn Gold mutants had moderate fragrance. Some of the genotypes had slight fragrance like in the mutants of Akito and First Federal Gold(Table 12A). Lack of fragrance was noted in the mutants of Akito, Kronenbourg and Tzigane genotypes.

4.1.3.9 Flower Diameter

The flower diameter between the cultivars and their respective mutants did not vary to a great extent (Tables 16,17,18) in all the three vegetative generations. However, the genotypes Kronenbourg mutant (25.50cm) recorded maximum flower diameter followed by First Federal Gold mutant (22.80cm) and Eiffel Tower mutant (18.20cm) compared to other genotypes. The minimum flower diameter was recorded in Akito (15.50cm) and Eiffel Tower (15.78cm). The other genotypes exhibited more or less equal to moderate flower diameter. Significant difference was observed between the genotypes but among the parent and mutants there was not much difference observed (Fig 11).

4.1.3.10 Flower Clusters

Number of flower clusters produced per plant was found to have significant difference between the genotypes. All the mutants showed increase in the number of flower clusters when compared to the parents (Tables 16,17,18). Maximum flower clusters was recorded in Akito mutant (8.80) followed by Wouburn Gold mutant (18.80), whereas the least number of flower clusters was found in the mutant Tzigane (1.40) when compared to their mutants.

Table 16 : Effect of spontaneous mutation on the floral characters of different rose genotypes in the VM1 generation.

Genotypes	Petals/ flower	Flower cluster	Flower weight (gm)	Flowers /plant	Individ. LA (sq.cm)	Rand. LA (sq.c)	Flower dia. (cm)
Akito (P)	36.00	8.80	4.88	52.80	11.83	251.56	16.10
Akito (M)	45.40	12.80	5.66	82.00	12.19	280.35	15.50
Eiffel Tower (P)	33.40	4.20	8.56	35.20	13.98	276.47	15.78
Eiffel Tower (M)	42.00	5.00	8.46	31.40	17.68	328.44	18.20
First Federal Gold(P)	88.40	2.60	10.24	9.60	10.69	279.41	21.70
FirstFederal Gold (M)	105.40	2.60	9.92	10.80	11.19	319.92	22.80
Kronenbourg (P)	48.60	3.00	12.84	25.60	18.37	335.39	20.40
Kronenbourg (M)	57.00	4.00	16.20	29.20	15.02	293.84	25.50
Tzigane (P)	46.79	1.22	8.56	3.98	10.88	190.78	10.88
Tzigane (M)	52.40	1.40	7.88	4.20	12.54	202.27	13.80
Wouburn Gold (P)	43.80	7.80	5.04	56.60	13.60	300.69	14.50
Wouburn Gold (M)	38.60	8.80	6.30	73.60	16.93	320.87	17.30
CD at 5%	3.460**	1.013*	0.753*	3.013**	1.259*	11.78**	1.091*
CV	5.469	14.412	6.833	6.528	7.299	3.395	4.788
SEM	1.248	0.365	0.272	1.087	0.454	4.249	0.394

(P) = Parent

(M) = Mutant

* Significant at 5% level

** Significant at 1% level

Table 17 : Effect of spontaneous mutation on the floral characters of different rose genotypes in the VM2 generation

Genotypes	Petals/ flower	Flower cluster	Flower weight (gm)	Flowers /plant	Individ .LA (sq.cm)	Rand. LA (sq.c)	Flower diam. (cm)
Akito (P)	34.60	8.80	4.82	51.20	12.23	296.57	15.90
Akito (M)	45.00	11.60	5.26	75.20	11.57	252.44	15.60
Eiffel Tower (P)	32.00	4.80	8.18	35.00	13.80	267.77	21.70
Eiffel Tower (M)	40.40	5.20	8.58	27.80	17.06	314.96	21.70
First Federal Gold (P)	81.80	2.20	11.10	9.40	10.12	280.13	16.00
First Federal Gold (M)	101.80	2.60	9.86	10.40	11.15	317.47	18.40
Kronenbourg (P)	50.00	2.80	12.46	27.00	18.09	283.76	20.90
Kronenbourg (M)	54.40	4.40	16.74	28.40	15.19	322.98	25.40
Tzigane (P)	45.89	1.24	9.60	3.57	10.67	157.90	11.00
Tzigane (M)	52.40	1.60	7.54	4.80	11.92	196.83	13.10
Wouburn Gold (P)	42.00	8.00	5.34	54.20	13.19	294.76	14.40
Wouburn Gold (M)	36.80	8.60	6.22	73.20	16.51	298.34	17.60
CD at 5%	3.319**	1.048*	0.966*	4.463*	1.140*	15.98**	0.940*
CV	5.375	15.021	8.613	10.120	6.707	4.699	4.120
SEM	1.197	0.378	0.349	1.610	0.411	5.766	0.339

(P) = Parent

(M) = Mutant

* Significant at 5% level

** Significant at 1% level

Table 18 : Effect of spontaneous mutation on floral characters of different rose genotypes in the VM3 generation.

Genotypes	Petals/ flower	Flower cluster	Flower weight (gm)	Flowers /plant	Individ .LA (sq.cm)	Rand. LA (sq.c)	Flower dia. (cm)
Akito (P)	35.20	12.40	5.76	50.20	12.04	278.59	15.60
Akito (M)	44.60	8.00	5.24	81.00	12.23	240.79	15.40
Eiffel Tower (P)	33.20	6.00	8.56	33.00	14.39	282.26	21.10
Eiffel Tower (M)	40.00	8.40	4.84	30.40	17.21	319.69	21.10
First Federal Gold (P)	86.20	2.40	7.54	9.80	9.99	277.68	16.30
First Federal Gold (M)	107.60	2.40	9.90	10.80	11.08	317.38	17.80
Kronenbourg (P)	50.40	4.00	16.08	25.00	17.97	285.76	20.30
Kronenbourg (M)	56.60	4.00	9.98	29.20	14.19	325.06	24.60
Tzigane (P)	49.80	1.22	6.08	3.54	9.94	65.00	10.45
Tzigane (M)	52.00	1.40	7.98	4.00	11.98	93.69	12.60
Wouburn Gold (P)	41.60	8.80	6.38	55.60	13.50	296.25	14.30
Wouburn Gold (M)	37.80	3.00	12.78	73.20	16.76	307.23	17.20
CD at 5%	3.174**	1.176*	0.699*	3.673**	1.074*	10.34**	1.146*
CV	5.049	16.690	6.383	8.119	6.312	3.042	5.154
SEM	1.145	0.424	0.252	1.325	0.387	3.733	0.414

(P) = Parent

(M) = Mutant

* Significant at 5% level

** Significant at 1% level

Fig 11: Spontaneous mutation on floral characters in rose genotypes

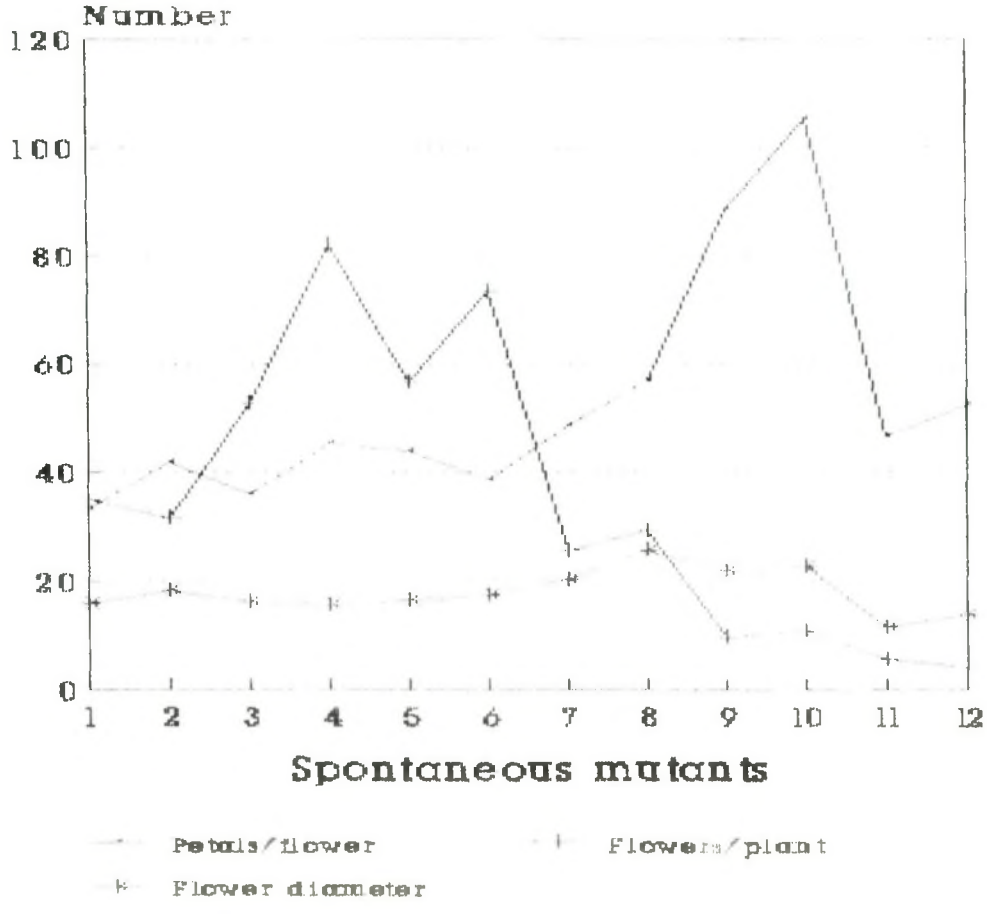


Plate 17: Spontaneous mutant of Kronenbourg and its parent genotype

Plate 18: Spontaneous mutant of Tzigane and its parent genotype

Plate 19: Spontaneous mutant of Wouburn Gold and its parent genotype

4.1.3.11 Number of Petals / Flower

Changes in the petal number was recorded between the original genotype and the mutants (Tables 16,17,18). In almost all the genotypes the petal numbers significantly varied among the genotypes and the mutants in all the three seasons. Highest number of petal was recorded (105.40) in First Federal Gold mutant compared to its parent (88.40) followed by Kronenbourg (57.00), respectively. The least petal count was observed in Eiffel Tower (33.40) followed by Akito (36.00) (Fig 11).

4.1.3.12 Flower Weight

Fresh weight of individual flowers were not found to be significant, and was not much influenced by the mutational events, but there was a difference in the variation observed among the spontaneous mutants. The highest flower weight was recorded in Kronenbourg (16.20) followed by First Federal Gold (10.24). Tremendous reduction in the flower weight was recorded in Akito (4.88) and Wouburn Gold (5.04), respectively (Tables 16,17,18).

4.1.3.13 Number of Flowers/Plant

Significant difference was observed in the number of flowers/plant contrary to the cultivars and between their mutants. In almost all the cases the number of flowers increased significantly in the mutants compared to their respective parents (Tables 16,17,18). Considerable increase in the flower number was seen in the mutants, and a maximum (82.00) was recorded in Akito mutant compared to its parent (52.80) followed by Wouburn Gold mutant (73.60) and its parent (56.60). A high reduction in the number of flowers was recorded in Tzigane mutant (4.20) followed by First Federal Gold (9.60) and its mutants (10.80) , respectively (Fig 11).

4.1.4 ANALYSIS OF VARIANCE

Any of the variations for different quantitative characters of the mutant lines are selected in general based on some desirable characters like heritability estimates and other parameters. Therefore, the range , variability, heritability and genetic advance for different quantitative characters are presented in the Tables 19 - 36.

4.1.4.1 Coefficient of variance

The values of genotypic and phenotypic coefficient of variability, heritability, genetic advance and genotypic, phenotypic variances was observed in both induced and spontaneous mutants. However, average or the least PCV (19.35) in flower diameter and GCV (14.67) in the number of branches/plant showed lesser variability with respect to induced mutations and all other characters exhibited moderate PCV range (20.56 to 49.42) and GCV (18.64 to 47.70) range, respectively. It was in the number of leaflets which showed the least variability with the PCV (17.34) and GCV (15.24) in all the subsequent three seasons (Tables 19,20,21).

All the characters among spontaneous mutants showed little deviation of GCV from PCV indicating the reliability of PCV for selecting the genotypes. The highest PCV and GCV range (60.60 to 62.09) was observed for number of flowers/plant in all the three seasons along with the number of flower clusters (51.58 to 56.85), in spontaneous mutants. In all the remaining characters of spontaneous mutants a moderate PVC range (20.44 to 47.05) and GCV range (18.10 to 45.92) was observed (Tables 22,23,24). In induced mutant the highest range of PCV and GCV (52.19 to 54.75) was observed for number of flowers/plant in all the three successive generations (Fig 12).

Table 19 : Effect of induced mutation by gamma rays on the phenotypic and genotypic variance, heritability and genetic advance of different morphological characters in rose genotypes under VM1 generation

Treatments	Phenotypic variance	Genotypic variance	Phenotypic C . O . V	Genotypic C . O . V	Heritability (Broad sense)	Genetic advance (5%)
Plant height	194.93	178.57	27.95	26.76	91.60	26.34
Number of branches	6.05	5.08	31.24	28.63	84.03	4.25
Number of leaflets	5.39	4.05	17.35	15.03	75.11	3.59
Thorn count	26.37	24.39	46.33	44.56	92.51	9.78
Stem diameter	0.44	0.38	21.14	19.53	85.38	1.78
Internodal length	0.97	0.88	22.93	21.93	91.47	1.85
Random leaf area	2241.29	2119.85	30.36	29.52	94.58	92.24
Individual leaf area	6.06	5.20	35.91	33.26	85.82	4.35
Flowers/plant	302.17	292.95	54.75	53.91	96.95	34.71
Petals/flower	119.29	112.33	31.16	30.24	94.16	21.18
Flower clusters/plant	2.74	2.12	25.91	22.80	77.38	2.64
Flower weight	6.98	6.72	30.34	29.77	96.27	5.24
Flower diameter	14.43	13.85	22.67	22.21	95.99	7.51

C.O.V = Coefficient of variance

Table 20 : Effect of induced mutation by gamma rays on the phenotypic and genotypic variance, heritability and genetic advance of different morphological characters in rose genotypes under VM2 generation

Treatments	Phenotypic variance	Genotypic variance	Phenotypic C . O . V	Genotypic C . O . V	Heritability (Broad sense)	Genetic advance (5%)
Plant height	176.60	160.91	26.98	25.75	91.11	24.94
Number of branches	5.52	4.55	30.15	27.38	82.52	3.99
Number of leaflets	5.69	4.09	17.98	15.24	71.85	3.53
Thorn count	29.39	27.45	49.42	47.77	93.41	10.43
Stem diameter	0.42	0.34	20.56	18.64	81.20	1.09
Internodal length	1.06	0.97	24.35	23.44	92.65	1.96
Random leaf area	3754.76	3704.87	30.32	30.11	98.67	124.55
Individual leaf area	13.48	12.97	35.27	34.60	96.21	7.40
Flowers/plant	287.57	274.96	53.73	52.54	95.61	33.40
Petals/flower	133.00	121.89	33.20	31.78	91.65	21.77
Flower clusters/plant	2.635	1.89	25.65	21.77	72.02	2.4-
Flower weight	7.58	7.26	31.82	31.13	95.71	5.43
Flower diameter	15.98	15.41	23.67	23.24	96.44	7.94

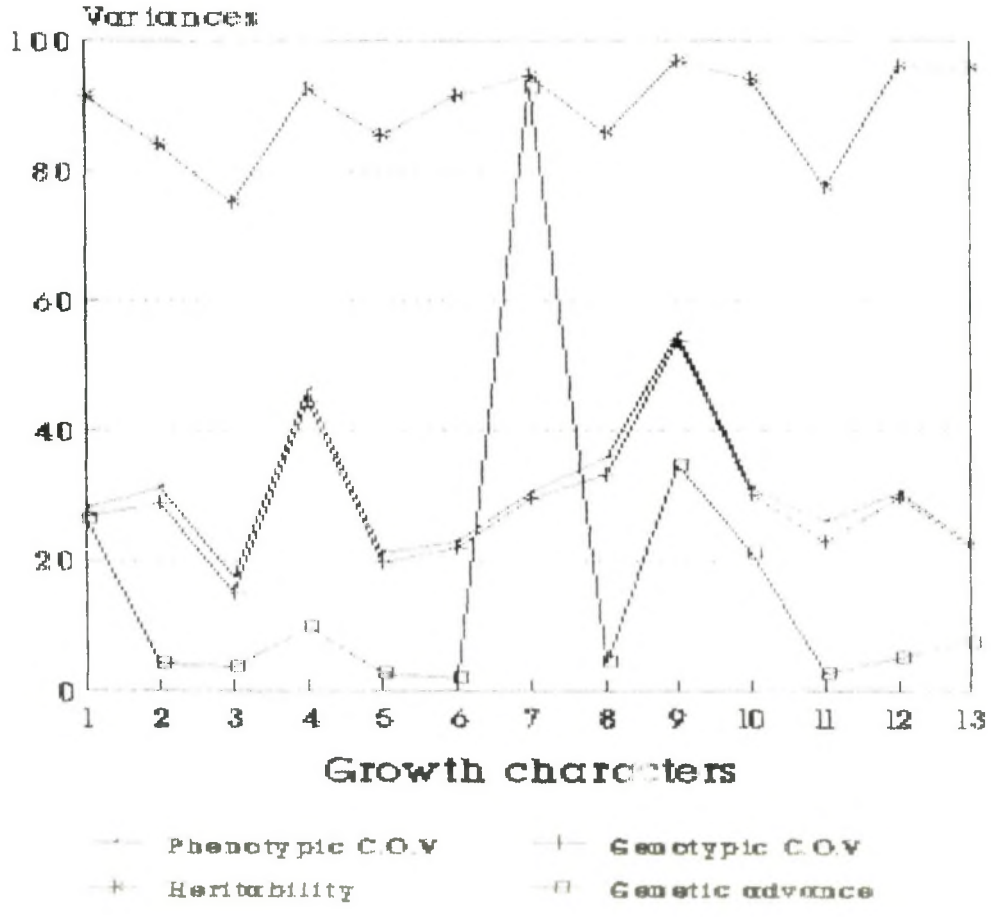
C.O.V = Coefficient of variance

Table 21 : Effect of induced mutation by gamma rays on the phenotypic and genotypic variance, heritability and genetic advance of different morphological characters in rose genotypes under VM3 generation

Treatments	Phenotypic variance	Genotypic variance	Phenotypic C . O . V	Genotypic C . O . V	Heritability (Broad sense)	Genetic advance (5%)
Plant height	208.74	189.19	29.81	28.38	90.63	26.97
Number of branches	5.36	4.58	29.86	27.59	85.35	4.07
Number of leaflets	7.09	4.77	19.64	16.12	67.35	3.69
Thorn count	24.54	22.52	46.36	44.41	91.75	9.36
Stem diameter	0.49	0.35	22.31	18.73	70.47	1.02
Internodal length	0.83	0.70	22.13	20.27	83.88	1.58
Random leaf area	3619.44	3544.70	29.86	29.55	97.93	121.37
Individual leaf area	14.58	14.16	36.39	35.86	97.11	7.64
Flowers/plant	270.01	260.89	53.91	52.19	96.62	32.70
Petals/flower	120.41	109.50	30.24	29.51	90.93	20.55
Flower clusters/plant	2.23	1.57	22.80	19.95	70.47	2.16
Flower weight	7.56	7.20	29.77	30.93	95.18	5.39
Flower diameter	15.97	15.18	22.21	23.30	95.11	7.82

C.O.V = Coefficient of variance

Fig 12: Effect of induced mutation on phenotypic and genotypic variances



4.1.4.2 Variance

The highest recorded phenotypic and genotypic variations from among different characters in induced mutations at random leaf area was (3619.44 & 3544.70) followed by the characters viz., plant height (203.74 & 189.19), number of flowers/plant (270.01 & 260.89) and the number of petals/flower (120.41 to 109.50) in all the three seasons (Tables 19,20,21). Similarly, in spontaneous mutants (Tables 22,23,24) large phenotypic and genotypic variations were observed in random leaf area (3318.00 & 3227.69) followed by plant height at a range of (844.49 & 810.80), spine count (111.90 & 107.25), number of petals/flower (482.75 & 474.91) and number of flowers/plant (526.95 & 521.04), respectively. The remaining characters showed low phenotypic and genotypic variability indicating that they are influenced by environmental factors rather than genetic factors (Fig 12).

4.1.4.3 Heritability

The broad sense heritability estimates were high for almost all the characters in spontaneous and induced mutants with the exception of number of branches/plant I58.56%) in spontaneous mutants in all three seasons under observation. The maximum heritability estimates was observed with respect to the flower characters and leaf area and the minimum heritability was recorded in the number of leaflets and stem diameter (Table 19-24).

4.1.4.4 Genetic Advance

Among induced mutants (Tables 19,20,21) genetic advance as per cent mean was found to be the lowest in the characters of stem diameter (1.02) and the highest for leaf area at random (124.55) followed by plant height, number of flowers/plant, spine count and number of petals/flower. Similarly, the genetic

Table 22 : Effect of spontaneous mutation on the phenotypic and genotypic variance, heritability and genetic advance of different morphological characters in rose genotypes under VM1 generation

Treatments	Phenotypic variance	Genotypic variance	Phenotypic C . O . V	Genotypic C . O . V	Heritability (Broad sense)	Genetic advance (5%)
Plant height	844.49	810.80	46.46	45.52	96.01	57.47
Number of branches	4.33	2.54	27.98	21.41	58.56	2.51
Number of leaflets	11.10	8.22	21.20	18.25	74.07	5.08
Thorn count	111.90	107.25	46.10	45.13	95.84	20.88
Stem diameter	0.57	0.48	23.54	21.73	85.26	1.32
Internodal length	1.44	1.27	25.84	24.73	88.55	2.19
Random leaf area	482.70	474.91	43.05	42.70	98.38	44.52
Individual leaf area	10.39	9.72	56.85	54.99	93.57	6.21
Flowers/plant	11.20	10.83	37.65	37.03	96.70	6.66
Petals/flower	526.95	521.04	61.66	61.31	98.87	46.75
Flower clusters/plant	14.24	13.21	27.11	26.31	92.75	7.21
Flower weight	3318.00	3227.69	20.57	20.29	97.27	115.43
Flower diameter	12.66	11.88	19.35	18.75	93.88	6.88

C.O.V = Coefficient of variance

Table 23 : Effect of spontaneous mutation on the phenotypic and genotypic variance, heritability and genetic advance of different morphological characters in rose genotypes under VM2 generation

Treatments	Phenotypic variance	Genotypic variance	Phenotypic C . O . V	Genotypic C . O . V	Heritability (Broad sense)	Genetic advance (5%)
Plant height	689.67	650.70	33.31	32.35	94.34	51.04
Number of branches	3.04	1.19	23.46	14.67	39.11	1.40
Number of leaflets	11.01	9.12	21.19	19.29	82.89	5.66
Thorn count	113.53	108.11	47.05	45.29	95.22	20.90
Stem diameter	0.53	0.44	22.00	20.03	82.83	1.24
Internodal length	1.49	1.39	26.27	25.39	93.42	2.35
Random leaf area	496.84	490.28	43.95	43.66	98.68	45.31
Individual leaf area	10.31	9.41	56.49	53.97	91.27	6.03
Flowers/plant	11.06	10.74	37.65	37.11	97.27	6.65
Petals/flower	513.74	504.96	62.09	61.56	98.29	45.89
Flower clusters/plant	13.80	13.05	27.08	26.33	94.56	7.23
Flower weight	3263.54	3193.86	20.81	20.59	97.86	115.16
Flower diameter	12.44	11.58	19.65	18.97	93.12	6.76

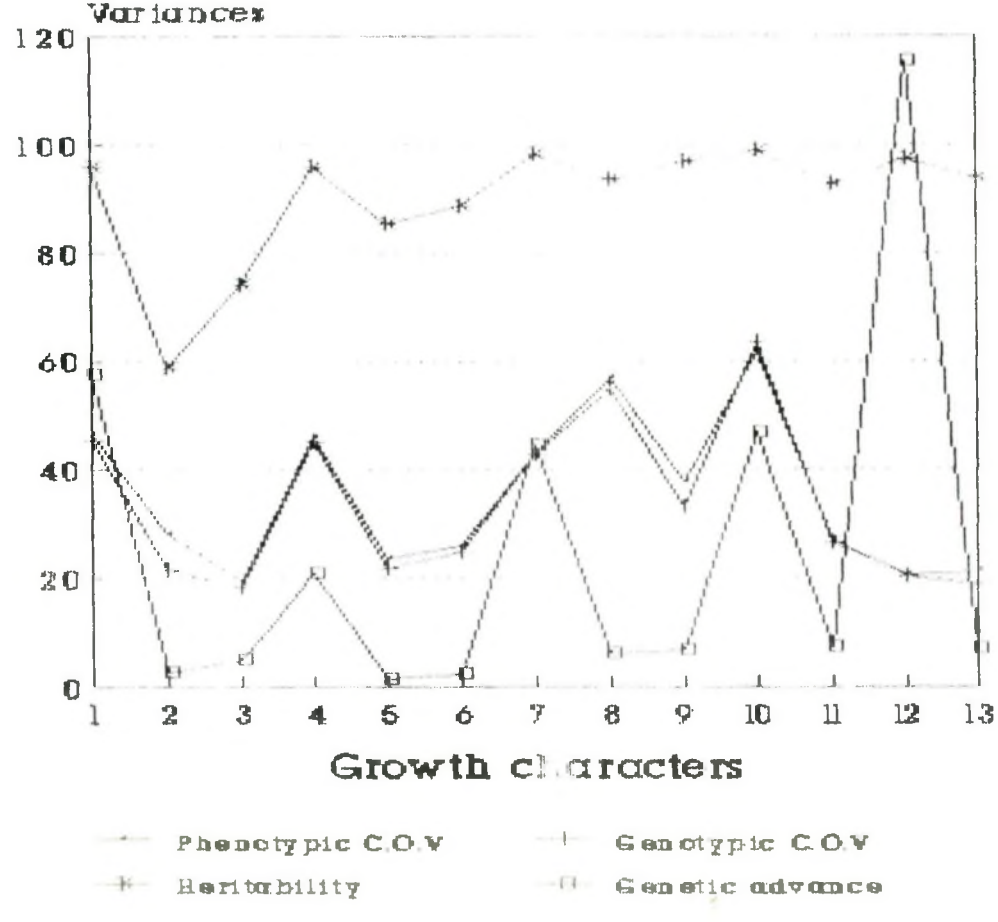
C.O.V = Coefficient of variance

Table 24 : Effect of spontaneous mutation on the phenotypic and genotypic variance, heritability and genetic advance of different morphological characters in rose genotypes under VM3 generation

Treatments	Phenotypic variance	Genotypic variance	Phenotypic C . O . V	Genotypic C . O . V	Heritability (Broad sense)	Genetic advance (5%)
Plant height	758.71	717.14	34.77	33.80	94.52	53.63
Number of branches	3.14	1.75	24.01	17.93	55.79	2.03
Number of leaflets	12.62	9.59	23.82	20.77	76.02	5.56
Thorn count	93.86	89.36	45.00	43.91	95.21	19.00
Stem diameter	0.45	0.35	20.65	18.10	76.79	1.06
Internodal length	1.53	1.38	26.57	25.31	90.72	2.31
Random leaf area	429.15	421.99	41.58	41.23	98.32	41.96
Individual leaf area	9.14	8.43	53.74	51.59	92.18	5.74
Flowers/plant	11.393	10.78	37.29	36.28	94.66	6.58
Petals/flower	477.72	464.76	61.44	60.60	97.28	43.80
Flower clusters/plant	14.08	13.23	27.35	26.52	93.98	7.26
Flower weight	3145.82	2979.59	20.44	19.89	94.71	109.43
Flower diameter	13.06	12.48	19.63	19.19	95.59	7.11

C.O.V = Coefficient of variance

Fig 13: Effect of spontaneous mutation on phenotypic and genotypic variances



advance in spontaneous mutants as per cent mean was lowest for stem diameter (1.32) and highest for leaf area random followed by the plant height, number of flowers/plant, number of petals and spines (Tables 22,23,24). Moderate genetic advance was recorded in almost all the characters under study. A low genetic advance in all the seasons was recorded at a range (1.06 - 7.26). All the other characters exhibited a lower genetic advance at a range (1.02 to 10.43) in all the three generations for both induced and spontaneous mutations.

4.1.5 PATH COEFFICIENT ANALYSIS

The path coefficient analysis at phenotypic level was worked out to determine the direct and indirect contribution of physiological and morphological characteristics to the flower yield.

4.1.5.1 Path Effects on Induced and Spontaneous Mutations

The nature and extent of direct and indirect contributions of different characteristics, viz., plant height, leaflets, internodal length, stem diameter, spine count, number of petals, flower clusters, flowers, flower weight, flower diameter, leaf area and branches/plant were presented for all the three seasons under observation in the Tables 25 to 30.

Number of flowers/plant had the highest direct effect on the flower yield both in spontaneous and induced mutants (1.1272 & 0.7785) followed by the number of flower clusters (0.8268 & 0.7469). In spontaneous mutants the number of leaflets (0.4771), petals (0.8756), flowers/cluster (0.5713) and flower diameter (0.8756) showed positively direct effects on flower yield and was negatively correlated with plant height (-0.0821), flower weight (-0.4784) and leaf area (-0.6737), respectively. Similarly, in induced mutants the flower weight, number of flower clusters, flower diameter exhibited positively direct effect, whereas the direct

Table 25 : Path coefficient analysis for different characters on the flower yield in induced mutants of rose cultivars in VM1 generation

	X1	X2	X3	X4	X5	X6	X7	X8
X1	-0.2045	0.1909	-0.1826	0.1150	0.9436	0.2366	-0.1872	0.1043
X2	-0.1240	0.3150	-0.1416	0.1026	0.2279	0.1836	-0.1537	0.6252
X3	-0.1146	0.1370	-0.3257	0.1702	-0.4221	0.1899	-0.4971	0.1488
X4	-0.1064	0.1463	-0.2509	0.2210	-0.4699	0.2007	-0.4877	0.1304
X5	0.8469	-0.3150	-0.6032	0.4557	-0.2279	0.6458	-0.4894	0.7874
X6	0.1141	0.1364	-0.1459	0.1046	-0.3471	0.4241	-0.2800	0.3770
X7	-0.4909	0.6209	-0.2075	0.1381	-0.1429	0.1522	-0. 7803	0.1522
X8	-0.1130	0.1042	-0.2567	0.1526	-0.9502	0.1522	-0.7600	0.1642

X1 = Plant height
X2 = Flower diameter
X3 = Number of leaflets/branch
X4 = Number of flower clusters/plant
X5 = Flower diameter
X6 = Number of petals/ flower
X7 = Leaf area
X8 = Number of flowers/plant

Table 26 : Path coefficient analysis for different characters on the flower yield in induced mutants of rose cultivars in VM2 generation

	X1	X2	X3	X4	X5	X6	X7	X8
X1	-0.2372	0.2228	-0.3195	0.1802	0.7294	0.6135	-0.6449	0.2405
X2	-0.1432	0.3691	-0.2390	0.1626	0.1103	0.1379	-0.4917	0.1471
X3	-0.1556	0.1811	-0.4872	0.3147	-0.1711	0.1195	-0.1648	0.3506
X4	-0.1238	0.1739	-0.4442	0.3452	-0.2417	0.1491	-0.1654	0.3186
X5	-0.1276	-0.3004	-0.6148	0.6154	-0.1355	0.6181	-0.1580	0.1441
X6	-0.3796	0.1327	-0.1519	0.1343	-0.2185	0.3834	-0.8303	0.7296
X7	-0.5763	0.6835	-0.3024	0.2151	-0.8068	0.1199	-0.2655	0.3410
X8	-0.1333	0.1269	-0.3991	0.2570	-0.4567	0.6537	-0.2115	0.4279

X1 = Plant height
X2 = Flower diameter
X3 = Number of leaflets/branch
X4 = Number of flower clusters/plant
X5 = Flower diameter
X6 = Number of petals/ flower
X7 = Leaf area
X8 = Number of flowers/plant

Table 27 : Path coefficient analysis for different characters on the flower yield in induced mutants of rose cultivars in VM3 generation

	X1	X2	X3	X4	X5	X6	X7	X8
X1	-0.2889	0.1509	-0.2561	0.1952	-0.3769	-0.2123	-0.2737	0.6955
X2	-0.1391	0.3133	-0.1806	0.1697	-0.1376	0.2448	-0.3209	0.7785
X3	-0.1853	0.1418	-0.2989	0.3180	-0.1757	0.1445	-0.1626	0.3450
X4	-0.1619	0.1528	-0.2646	0.3749	-0.2634	0.1745	-0.1667	0.3204
X5	-0.5629	-0.2230	-0.3626	0.4742	-0.1932	0.4050	-0.1529	0.7136
X6	-0.1616	0.1196	-0.1519	0.1601	-0.3593	0.3792	-0.1035	0.7469
X7	-0.9249	0.2687	-0.2379	0.2126	-0.1083	0.1439	-0.2727	0.3483
X8	-0.1817	0.7855	-0.3148	0.2469	-0.7070	0.9121	-0.2180	0.3105

X1 = Plant height
X2 = Flower diameter
X3 = Number of leaflets/branch
X4 = Number of flower clusters/plant
X5 = Flower diameter
X6 = Number of petals/ flower
X7 = Leaf area
X8 = Number of flowers/plant

Table 28 : Path coefficient analysis for different characters on the flower yield in VM1 generation of spontaneous rose mutants

	X1	X2	X3	X4	X5	X6	X7	X8
X1	-0.0826	-0.0437	0.0325	0.0014	0.0143	-0.0171	-0.0082	0.0875
X2	0.2523	0.4771	-0.0220	0.0526	-0.1127	0.1685	0.1641	0.5251
X3	-0.3441	-0.0404	0.8756	-0.3919	03017	-0.3046	0.3046	1.1275
X4	-0.0273	0.1734	-0.7029	0.5713	-0.1976	0.1213	0.1213	0.8268
X5	0.0827	0.1130	-0.1648	0.2429	-0.4784	-0.1749	-0.1749	0.5440
X6	0.0380	0.0650	-0.0640	0.0142	0.0673	0.1839	0.1839	0.2086
X7	-0.0665	-0.2318	-0.0572	-0.0797	0.2289	-0.4976	-0.4976	0.3873
X8	0.2118	-0.0335	-0.0924	-0.1323	0.4575	0.3756	0.3756	0.6362

X1 = Plant height
X2 = Flower diameter
X3 = Number of leaflets/branch
X4 = Number of flower clusters/plant
X5 = Flower diameter
X6 = Number of petals/ flower
X7 = Leaf area
X8 = Number of flowers/plant

Table 29 : Path coefficient analysis for different characters on the flower yield in VM2 generation of spontaneous rose mutants.

	X1	X2	X3	X4	X5	X6	X7	X8
X1	0.5662	0.3101	-0.2606	0.0717	-0.1478	0.1023	0.0313	0.1651
X2	-0.0980	-0.1789	0.0158	-0.0222	0.0673	-0.0366	-0.0233	0.0164
X3	0.1429	0.0274	-0.3103	0.1509	-0.0997	0.1144	-0.0126	0.0459
X4	0.0880	0.0861	-0.3377	0.6947	-0.3857	0.0590	0.0649	0.1187
X5	0.0555	0.0800	-0.0683	0.1180	-0.2126	-0.0633	-0.0489	0.1520
X6	0.1339	0.0383	-0.0690	0.0159	0.0558	0.1873	0.1291	0.1167
X7	0.0253	0.0569	0.0186	0.0427	0.1052	0.3153	0.4573	0.2488
X8	-0.1540	0.0484	-0.0781	-0.0903	0.4776	0.3290	0.3873	0.5280

X1 = Plant height
X2 = Flower diameter
X3 = Number of leaflets/branch
X4 = Number of flower clusters/plant
X5 = Flower diameter
X6 = Number of petals/ flower
X7 = Leaf area
X8 = Number of flowers/plant

Table 30 : Path coefficient analysis for different characters on the flower yield in VM3 generation of spontaneous rose mutants.

	X1	X2	X3	X4	X5	X6	X7	X8
X1	-0.2089	-0.1090	0.0983	-0.0810	0.0482	-0.0531	-0.0249	0.0580
X2	0.3194	0.6123	-0.0483	0.0091	-0.0494	0.2017	0.1159	0.0413
X3	0.3889	0.0652	-0.8265	0.4244	-0.1248	0.3237	-0.0371	0.0864
X4	0.3479	0.0134	-0.4610	0.8979	-0.3943	0.1163	0.0896	0.0154
X5	-0.2132	-0.0746	0.1396	-0.4060	0.9245	0.4549	0.2113	0.3379
X6	-0.2428	-0.3149	0.3745	-0.1238	-0.4704	-0.9561	-0.6717	0.1805
X7	0.1248	0.1983	0.0471	0.1046	0.2395	0.7361	1.0479	0.4802
X8	-0.1283	0.0312	-0.0483	-0.0079	0.4689	0.3806	0.3000	0.6622

X1 = Plant height
X2 = Flower diameter
X3 = Number of leaflets/branch
X4 = Number of flower clusters/plant
X5 = Flower diameter
X6 = Number of petals/ flower
X7 = Leaf area
X8 = Number of flowers/plant

effects on flower yield was negatively correlated for plant height, number of petals, number of leaflets and leaf area in all the three seasons observed consequently.

The indirect contributions of all the characters in spontaneous mutants at flowering was maximum through number of flowers/plant (0.0875, 0.5251, 0.8268, 0.5440, 0.2086, 0.6362). The number of flowers produced had a maximum of petals/flower (0.8756) followed by the flower weight (0.3017). Similarly, in induced mutations also the indirect contribution of all characters at flowering period was maximum through the total number of flowers produced per plant (0.6955, 0.3450, 0.3202, 0.7136, 0.7469, 0.3483 and 0.3105) undertaken during the period of study.

4.1.6 CORRELATION AND PATH ANALYSIS

The phenotypic and genotypic correlation coefficients were determined to know the extent and nature of relationship between morphological and physiological attributes, yield based and flowering pattern separately both in induced and spontaneous mutants. The correlation coefficient values are presented in Tables 31 to 36.

4.1.6.1 Induced Mutation

The phenotypic and genotypic correlation co-efficients between morphological and physiological attributes in induced mutations exhibited highly significant positive correlation among all the 13 characters recorded for the present study of investigation (Tables 31,32,33).

The characters like flower diameter, flower weight, number of flower clusters, flower number, internodal length, leaflets, branches and plant height was positively correlated with respect to the yield attributes. Flower diameter at high

Table 31 : Phenotypic and genotypic correlation coefficient on different morphological characters in VM1 of generation induced mutants in rose genotypes

Phenotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.8061**	0.1996	0.4169*	0.1760	0.6906**	0.7883**	0.1401	0.6441**	-0.1203	0.3310*	0.4750**	0.5525*
X2			0.3589*	0.6272**	0.1297	0.6251**	0.6371**	-0.0562	0.5955**	-0.0037	0.1971	0.3787*	0.2400
X3				0.1523	0.7189**	0.4734*	0.4480*	0.1105	0.1542	-0.1903	0.4331*	0.5456**	0.0558
X4					-0.2624	0.2062	0.1852	-0.5150**	0.2349	0.3414*	-0.0100	-0.0092	-0.0414
X5						0.4592*	0.4451*	0.4313*	0.1041	-0.3914*	0.5102**	0.6754**	0.2161
X6							0.7704**	0.1314	0.4396*	-0.1751	0.4645*	0.5321**	0.5206**
X7								0.3800*	0.4908*	-0.4050*	0.4350*	0.6965**	0.5606**
X8									0.0879	-0.4433*	0.2258	0.4747*	0.4091*
X9										0.1211	0.2787	0.3926*	0.4411*
X10											0.1609	-0.4179*	0.0346
X11												0.3652*	0.6062**
X12													0.4345*
X13													

Genotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.8368**	0.2361	0.4319*	0.1831	0.7691**	0.8236**	0.1355	0.7074**	-0.1289	0.3939*	0.5331**	0.5805**
X2			0.4214*	0.6531**	0.1305	0.6896**	0.6657**	-0.0511	0.6547**	-0.0012	0.2406	0.4316*	0.2451
X3				0.1834	0.8304**	0.5353**	0.5061**	0.1351	0.1804	-0.1999	0.5237**	0.6760**	0.0420
X4					-0.2726	0.2390	0.1956	-0.5713**	0.2827	0.3692*	-0.0162	0.0013	-0.0436
X5						0.5141**	0.4654*	0.4535**	0.1121	-0.4258*	0.6068**	0.7462**	0.2235
X6							0.8539**	0.1497	0.5207**	-0.1871	0.6285**	0.6080**	0.5842**
X7								0.4197*	0.5326**	-0.4310*	0.5078**	0.7993**	0.5986**
X8									0.1269	-0.4980*	0.2633	0.5358**	0.4348*
X9										0.1498	0.3732*	0.4222*	0.4919*
X10											0.1935	-0.4771*	0.0438
X11												0.5266*	0.7439**
X12													0.4681*
X13													

X1 = Plant height
X2 = Number of branches/plant
X3 = Number of leaflets/branch
X4 = Thorn count
X5 = Stem diameter
X6 = Internodal length
X7 = Number of petals/ flower
X8 = Number of flower clusters/plant
X9 = Flower weight
X10 = Number of flowers/ plant
X11 = Individual leaf area
X12 = Random leaf area
X13 = Flower diameter

Table 32 : Phenotypic and genotypic correlation coefficient on different morphological characters in VM2 generation of induced mutants in rose genotypes

Phenotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.7969**	0.1795	0.3369*	0.2653	0.7445**	0.8193**	0.2034	0.6650**	-0.1242	0.3439*	0.5080**	0.5620*
X2			0.3127*	0.5951**	0.1257	0.6232**	0.6209**	-0.0506	0.5589**	0.0054	0.1852	0.3761*	0.2429
X3				0.1612	0.6587**	0.3891*	0.3119*	0.0037	0.1760	-0.1372	0.3598*	0.4591*	0.0016
X4					-0.2708	0.1783	0.1262	-0.5164**	0.3331*	0.3619*	-0.0814	-0.0508	-0.0538
X5						0.4462*	0.4556*	0.4062*	0.1112	-0.3796*	0.5432**	0.6971**	0.2234
X6							0.9117**	0.2248	0.3922*	-0.1435	0.4713*	0.5087**	0.5221**
X7								0.4362*	0.4463*	-0.3082*	0.4906*	0.6477**	0.6559**
X8									0.0570	0.4801*	0.2604	0.4815*	0.4545*
X9										0.1647	0.3157*	0.3413*	0.4147*
X10											0.1409	-0.4348*	0.0360
X11												0.3759*	0.6039**
X12													0.4293*
X13													

Genotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.8201**	0.2119	0.3624*	0.2668	0.7640**	0.8411**	0.2215	0.7383**	-0.1274	0.4150*	0.5547**	0.6022**
X2			0.3753*	0.6303**	0.1417	0.6413**	0.6411**	-0.0608	0.6442**	0.0086	0.2400	0.4128*	0.2531
X3				0.1626	0.8141**	0.4588*	0.3650*	-0.0012	0.1867	-0.1372	0.5143**	0.6276**	0.0104
X4					-0.2875	0.1898	0.1405	-0.5666**	0.3920*	0.3868*	-0.0934	-0.0475	-0.0422
X5						0.4737*	0.4677*	0.4365*	0.1056	-0.3986*	0.6269**	0.7844**	0.2335
X6							0.9318**	0.2371	0.4327*	-0.1502	0.5715**	0.5721**	0.5704*
X7								0.4525*	0.4872*	-0.3138*	0.5823**	0.7175**	0.6835**
X8									0.0818	-0.5178**	0.3279*	0.5772**	0.4803*
X9										0.2044	0.3742*	0.4317*	0.5049**
X10											0.1819	-0.4855*	0.0426
X11												0.5784**	0.7489**
X12													0.4990*
X13													

X1 = Plant height
X2 = Number of branches/plant
X3 = Number of leaflets/branch
X4 = Thorn count
X5 = Stem diameter
X6 = Internodal length
X7 = Number of petals/ flower
X8 = Number of flower clusters/plant
X9 = Flower weight
X10 = Number of flowers/ plant
X11 = Individual leaf area
X12 = Random leaf area
X13 = Flower diameter

Table 33 : Phenotypic and genotypic correlation coefficient on different morphological characters in VM3 generation of induced mutants in rose genotypes

Phenotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.7996**	0.2405	0.3658*	0.2209	0.7097**	0.7892**	0.1930	0.6665**	-0.1290	0.2507	0.5934**	0.6296**
X2			0.3796*	0.5607**	0.1211	0.6112**	0.5965**	-0.0100	0.5847**	-0.0215	0.1177	0.4565*	0.3204*
X3				0.1859	0.6427**	0.4603*	0.3810*	0.0292	0.1501	-0.2375	0.3816*	0.4437*	-0.6056
X4					-0.2608	0.1363	0.0909	-0.4791*	0.3391*	0.3553*	-0.0712	-0.0134	0.0195
X5						0.4521*	0.4352*	0.4041*	0.0968	-0.3877*	0.5456**	0.6381**	0.1398
X6							0.9142**	0.1968	0.4156*	-0.1742	0.4878*	0.5802**	0.5611**
X7								0.3356*	0.4842*	-0.3298*	0.4528*	0.7002**	0.6420**
X8									0.1493	-0.4414*	0.1468	0.4758*	0.3156*
X9										0.0386	0.2058	0.4149*	0.5177**
X10											0.1301	-0.4451*	0.0928
X11												0.3862*	0.4819*
X12													0.4696*
X13													

Genotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.8253**	0.2949	0.4067*	0.2322	0.7446**	0.8193**	0.2203	0.8132**	-0.1361	0.3209*	0.6457**	0.6814**
X2			0.4695*	0.6211**	0.1317	0.6406**	0.6187**	-0.0032	0.7481**	-0.0182	0.1809	0.5236**	0.3513*
X3				0.2150	0.7688**	0.5440**	0.4577*	0.0305	0.2688	-0.2363	0.5645**	0.5790**	-0.0050
X4					-0.2901	0.1411	0.1000	-0.5772**	0.4083*	0.3753*	-0.1308	-0.0087	0.0038
X5						0.4594*	0.4430*	0.4567*	0.1094	-0.4180*	0.6683**	0.6971**	0.1359
X6							0.9360**	0.2083	0.4970*	-0.1885	0.6007**	0.6386**	0.5854**
X7								0.3800*	0.5740**	-0.3404*	0.5833**	0.7577**	0.6769**
X8									0.1508	-0.5246**	0.1987	0.5658**	0.3419*
X9										0.0199	0.2734	0.5427**	0.6480**
X10											0.0876	-0.5035**	0.0967
X11												0.5636**	0.6220**
X12													0.5180**
X13													

X1 = Plant height
X2 = Number of branches/plant
X3 = Number of leaflets/branch
X4 = Thorn count
X5 = Stem diameter
X6 = Internodal length
X7 = Number of petals/ flower
X8 = Number of flower clusters/plant
X9 = Flower weight
X10 = Number of flowers/ plant
X11 = Individual leaf area
X12 = Random leaf area
X13 = Flower diameter

level of significance was positively correlated with plant height, internodal length, leaf area and flower clusters, whereas flowers/plant and flower weight was correlated with 5% level. Flower weight on the other hand was positively correlated with plant height, leaflets, stem diameter, leaf area at 1% level, whereas at 5% level of significance number of branches, leaf area, flowers, petals, flower clusters and flower diameter was found to be correlated.

Positive correlation and high level of significance in the number of flower clusters was observed with stem and flower diameter, whereas at 5% level it was correlated with characters like plant height, internodal length, leaf area and flower weight. At 5% level of significance number of petals /flower was positively correlated with thorn count and negatively with stem diameter, individual leaf area along with flower weight, respectively. Only positive correlation was observed in the number of flowers with internodal length, leaf area, flower weight and flower diameter at 5% level and number of branches at 1% level of significance (Tables 31,32,33).

Individual leaf area was negatively correlated and highly significant with thorn count. At 5% level positive correlation was observed with stem diameter, leaf area, flower diameter, flower weight and negatively with flower clusters. At random the leaf area was positively correlated at 1% level with plant height, number of branches, internodal length, flower weight, flower diameter and number of petals /flower. At 5% level correlation was with leaflets, stem diameter, leaf area, number of flowers and clusters/plant accordingly.

Internodal length was positively correlated with leaflets, stem diameter, flowers and flower clusters at 5% level, whereas with plant height, number of branches, leaf area, flower weight and flower diameter at 1% level of significancy. A positive correlation and high level of significance was associated with the characters like leaflets, flower clusters, petals, flower weight whereas at 5% level

internodal length, random leaf area was correlated. The correlation on thorn count was not significant in association with other characters. However, a negative correlation was observed with flowers /plant at 1% significant level and positive correlation with petals and plant height at 5% level of significancy whereas, number of leaflets, branches and plant height were all positively correlated. With respect to the number of leaflets, stem diameter and flower weight at 1% level, the number of branches, internodal length, leaf area and flower clusters were correlated at 5% level (Tables 31,32,33) of significance.

Similarly number of branches/plant was correlated with plant height, thorn count, internodal length, leaf area and flowers/plant at 1% significant levels whereas, leaflets and flower weights was correlated at 5% level (Tables 31,32,33). Lastly, plant height was highly significant and positively correlated with number of branches, internodal length, leaf area, flowers, flower diameter and flower weight correlation at 5% level. All the above observations recorded were in accordance with both phenotypic and genotypic correlation coefficient in the three consecutive generations of the present investigation.

4.1.6.2 Spontaneous Mutants

Positive correlation and high level of significance was observed in the plant height and the number of leaflets with respect to the number of branches/plant along with stem diameter and individual leaf area at 5% level of significance. Stem diameter was found to be positively correlated with plant height at 1% level of significance. At 5% level stem diameter was positively correlated with number of branches/plant, leaflets, petals and flowers/plant and negatively correlated with flower diameter, leaf area, internodal length and spine count, whereas, internodal length was highly significant and positively correlated with spine count and leaf area at random and negatively correlated with stem diameter and number of petals/flower at 5% level of significance (Tables 34,35,36).

Table 34 : Phenotypic and genotypic correlation coefficient on different morphological characters in VM1 generation of spontaneous mutants in rose genotypes

Phenotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1.0000	0.5617**	0.5648**	-0.1206	0.6962**	-0.1921	-0.1468	0.3083*	0.0236	0.1275	-0.0272	-0.0927	0.3141*
X2			0.7067**	0.1853	0.3345*	0.1045	0.0858	0.0921	-0.0870	-0.1131	0.3173*	-0. 0529	0.0958
X3				0.1685	0.3481*	0.0882	-0.3342*	0.0972	0.0808	-0.1659	0.3247*	-0.1361	-0.1909
X4					-0.4827*	0.8269**	-0.5366**	0.3572*	0.0766	-0.0470	0.1610	0.5421**	-0.0584
X5						-0.4760*	0.3334*	-0.1884	0.1756	0.4159*	-0.2148	-0.3712*	0.1692
X6							-0.4370*	0.2844	-0.0246	-0.1389	0.0932	0.5600**	-0.0262
X7								-0.6726**	-0.0732	0.1959	-0.0386	-0.3494*	-0.3879*
X8									-0.0453	-0.0227	-0.1456	0.3928*	-0.3164*
X9										0.6334**	-0.2375	0.1560	0.4010*
X10											-0.4110*	-0.0091	0.5664**
X11												0.1254	0.4750*
X12													0.1400
X13													1.0000

Genotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1.0000	0.5784**	0.5974**	-0.1252	-0.7251**	-0.2122	-01464.	0.3542*	-0.0296	0.1405	-0.0604	-0.0904	0.3380*
X2			0.7471**	0.1883	0.3411*	0.1218	0.0847	0.1048	0.0884	-0.1057	0.3526*	-0.0666	0.0094
X3				0.1764	0.3703*	0.0742	-0.3514*	0.1247	0.1066	-0.1691	0.3623*	-0.1624	0.2109
X4					-0.4933*	0.9640**	-0.5443**	0.3779*	0.0903	-0.0478	0.1835	0.6953**	0.0571
X5						-0.5160**	0.3473*	-0.1928	0.2022	0.4380*	-0.2429	-0.5168**	-0.1738
X6							-0.4500*	0.2994	-0.0243	-0.1500	0.1157	0.7447**	-0.0150
X7								-0.7287**	-0.0856	0.2048	-0.0484	-0.4423*	-0.3943*
X8									-0.0597	-0.0133	-0.1711	0.5982**	0.3410*
X9										0.7039**	-0.3143*	0.1674	-0.4567*
X10											-0.4659*	-0.0247	-0.5888**
X11												0.0427	0.5458**
X12													0.1543
X13													1.0000

X1 = Plant height
X2 = Number of branches/plant
X3 = Number of leaflets/branch
X4 = Thorn count
X5 = Stem diameter
X6 = Internodal length
X7 = Number of petals/ flower
X8 = Number of flower clusters/plant
X9 = Flower weight
X10 = Number of flowers/ plant
X11 = Individual leaf area
X12 = Random leaf area
X13 = Flower diameter

Table 35 : Phenotypic and genotypic correlation coefficient on different morphological characters in VM2 generation of spontaneous mutants in rose genotypes

Phenotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1.0000	0.5260**	0.5998**	-0.1013	0.6804**	-0.1583	-0.1458	0.2277	-0.0741	0.1624	-0.0790	-0.1111	0.2795
X2			0.6801**	0.1448	0.2224	0.0792	0.0376	0.0992	-0.1270	-0.2093	0.0915	-0.2131	0.0473
X3				0.1719	0.2869	0.0853	-0.3553*	0.1556	0.1107	-0.1634	0.1997	-0.1399	0.1763
X4					-0.5100**	0.9078**	-0.5430**	0.4156*	0.4520*	-0.0320	0.1664	0.4531*	0.1387
X5						-0.5195**	0.3121*	-0.1793	-0.0809	0.5135**	-0.3357*	-0.2748	-0.2485
X6							-0.4678*	0.3501*	0.3410*	-0.1466	0.1132	0.4584*	0.1142
X7								-0.7179**	-0.2754	0.2582	-0.0724	-0.3552*	-0.4450**
X8									0.0892	-0.0493	-0.0910	0.4395*	0.3122*
X9										0.3173*	0.2249	0.1856	0.0015
X10											-0.3087*	0.0274	-0.5608**
X11												-0.0065	0.4979*
X12													0.1850
X13													1.0000

Genotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1.0000	0.5488**	0.6292**	-0.1093	0.7243**	-0.1743	-0.1485	0.2507	-0.0910	0.1801	-0.0955	-0.1258	0.2947
X2			0.6972**	0.1392	0.2319	0.0973	0.0415	0.0923	-0.1622	-0.2144	0.1343	-0.2803	0.0574
X3				0.1824	0.3008*	0.0849	-0.3721*	0.1629	0.1117	-0.1641	0.2063	-0.1962	0.1819
X4					-0.5360**	0.9513**	-0.5534**	0.4449*	0.5117**	-0.0239	0.1906	0.6030**	0.1469
X5						-0.5648**	0.3238*	-0.1790	-0.1292	0.5435**	-0.3889*	-0.4986*	-0.2643
X6							-0.4910*	0.3868*	0.4285*	-0.1411	0.1273	0.6330**	0.1299
X7								-0.7562**	-0.3270*	0.2725	-0.0930	-0.4787*	-0.4643*
X8									0.1261	-0.0521	-0.1191	0.5927**	0.3296*
X9										0.3815*	0.3053*	0.2536	-0.0632
X10											-0.3729*	0.0478	-0.5899**
X11												0.0377	0.5632**
X12													0.2356
X13													1.0000

X1 = Plant height
X2 = Number of branches
X3 = Number of leaflets/branch
X4 = Thorn count
X5 = Stem diameter
X6 = Internodal length
X7 = Number of petals/ flower
X8 = Number of flower clusters/plant
X9 = Flower weight
X10 = Number of flowers/ plant
X11 = Individual leaf area
X12 = Random leaf area
X13 = Flower diameter

Table 36 : Phenotypic and genotypic correlation coefficient on different morphological characters in VM3 generation of spontaneous mutants in rose genotypes

Phenotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.6262**	0.5767**	-0.0886	0.3427*	-0.0221	-0.0981	0.3240*	-0.0905	0.1195	-0.0463	-0.1104	0.2550
X2			0.6824**	0.0998	0.2273	0.0918	0.0480	0.1673	-0.0593	-0.2153	0.1840	-0.1646	0.1171
X3				0.2649	0.4773*	0.1245	-0.3792*	0.1837	0.1401	-0.2101	0.3008*	-0.0417	0.2522
X4					-0.1145	0.5204**	-0.5325**	0.3745*	0.4042*	0.0020	0.1295	0.3817*	0.1295
X5						-0.4186*	0.1484	-0.3229*	0.3540*	0.3559*	-0.0760	-0.3484*	-0.2262
X6							-0.4948*	0.3941*	0.4140*	-0.4707*	0.0137	0.1038	0.3515*
X7								-0.6961*	-0.2003	0.1750	-0.0672	-0.3224*	-0.4554*
X8									0.0377	-0.0154	-0.1481	0.3629*	0.3957*
X9										0.4014*	0.1392	-0.0065	-0.1712
X10											-0.4332*	-0.0407	-0.5585**
X11												0.1039	0.4690*
X12													0.2500
X13													

Genotypic correlation coefficient

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1		0.6552**	0.6153**	-0.0985	0.3715*	-0.0158	-0.1063	0.3378*	-0.0957	0.1156	-0.0735	-0.1872	0.2837
X2			0.7077**	0.1056	0.2289	0.1019	0.0441	0.1772	-0.0580	-0.2235	0.1909	-0.2991	0.1196
X3				0.2204	0.4959*	0.1308	-0.3949*	0.2066	0.1344	-0.2270	0.3375*	-0.1322	0.2544
X4					-0.1153	0.5469**	-0.5428**	0.3949*	0.4409*	0.0013	0.1458	0.6299**	0.1417
X5						-0.4451*	0.1521	-0.3341*	0.3742*	0.3642*	-0.0821	-0.5581**	-0.2317
X6							-0.5184**	0.4284*	0.0033	-0.4954*	0.0153	0.3015*	0.3972*
X7								-0.7218**	-0.2184	0.1809	-0.0822	-0.5530**	-0.4744*
X8									0.0373	-0.0139	-0.1586	0.6050**	0.4397*
X9										0.4350*	0.1864	0.0848	-0.1791
X10											-0.4926*	-0.0882	-0.5851**
X11												0.2203	0.5367**
X12													0.3428*
X13													

X1 = Plant height
X2 = Number of branches/plant
X3 = Number of leaflets/branch
X4 = Thorn count
X5 = Stem diameter
X6 = Internodal length
X7 = Number of petals/ flower
X8 = Number of flower clusters/plant
X9 = Flower weight
X10 = Number of flowers/ plant
X11 = Individual leaf area
X12 = Random leaf area
X13 = Flower diameter

High level of significance and positive correlation in spine count was observed with internodal length and leaf area at random and negatively correlated with number of petals/flower. At 5% level of significance positive correlation was observed with internodal length and negative correlation with stem diameter. Leaf area at random was positively correlated with number of flower clusters/plant and negatively with stem diameter, number of petals/flower, whereas at 1% level it was positively correlated with spine count and internodal length. Individual leaf area was positively correlated at 5% level with number of branches, leaflets and flower diameter, whereas number of flowers/plant was negatively correlated (Tables 34,35,36).

With respect to the flower characters flower diameter was found to be positively and significantly correlated with number of leaflets, internodal length and flowers at 5% level and negatively correlated with stem diameter and number of petals. At 1% level of significance it was negatively correlated with the spine count. Number of flowers/plant was positively correlated with flower weight at 1% level, whereas flower diameter was negatively correlated with individual leaf area and stem diameter at 5% level. Contrarily, flower weight was positively correlated with number of flowers at 1% and negatively with flower diameter at 5% level.

Number of petals /flower was negatively correlated with high significancy in spine count and number of flower clusters / plant, whereas at 5% level it was correlated with number of leaflets, internodal length, leaf area at random and flower diameter. Only stem diameter was found to be positively correlated whereas the number of flower clusters/plant with plant height, spine count, flower diameter and leaf area at random recorded positive correlation at 5% level and negatively correlated with high significancy in the number of petals/flower. Thus, correlation effects which was observed in all the three seasons among all the genotypes under study of spontaneous mutants with respect to the phenotypic and genotypic effects were found to be onpar with each other (Tables 34,35,36).

4.2 EXPERIMENT II : POLLEN STUDIES

4.2.1 Pollen Studies on Rose and Hibiscus

The pollen fertility status differs from cultivar to cultivar both in rose and hibiscus. There is a dormant period for rose growth and flower production in Indian plains as the winter not being severe whereas, in hibiscus all the cultivars are sexually sterile. This complex is indeed unique in supporting different chromosome lines because of vegetative propagation.

Hibiscus is a self compatible, but spatial separation of anthers from stigmas prevents the automatic self pollination. The large flat stigma, long style, large pollen grains and the stylar branches/pistil makes it attractive for pollen growth studies. However, detailed information based on indepth studies are lacking on many of these aspects. Thus in the present study, investigation was conducted with the objective of knowing the pollen germination, pollen fertility, sterility and viability status of rose and hibiscus genotypes by using nuclear stains invitro. The results of the findings are described below as follows

4.2.2 Media Standardization

The pollen fertility status of rose and hibiscus was estimated by using nuclear stain suggested by Alexander (1980). Aborted pollen grains did not take any colour from the stain and they were empty, small in size and deformed, whereas non aborted grains were large, round and stained blue in colour. Germinating pollens are stained and assessed when the size of the pollen tube growth is larger than the diameter of the pollens with acute estimates. The non aborted pollen grains were round with definite outline in contrast to the deformed sterile pollen grains. Percentage of aborted and non aborted pollen grains in all the rose mutants are presented in the Table 37. The data from the table revealed the status among natural and induced mutants.

Table 37 : Effect of spontaneous and induced mutations on the pollen fertility, viability and germinability of different rose genotypes

Sl. No	Genotypes	Aborted pollens	Non aborted pollens	Pollen germination (%)	Pollen size	Pollen viability(%)	Pollen fertility(%)
1	Akito (P)	15.07	34.02	72.43	Long	94.43	70.03
2	Akito (M)	33.03	43.60	52.00	Medium	66.33	47.14
3	Eiffel Tower (P)	72.35	27.20	20.00	Small	36.28	78.68
4	Eiffel Tower (M)	80.68	14.03	0.00	Small	0.00	85.19
5	First Federal Gold(P)	65.73	37.14	22.32	Small	36.93	20.47
6	FirstFederal Gold(M)	42.63	51.90	59.71	Medium	77.35	52.76
7	Kronenbourg (P)	29.40	63.02	44.46	Medium	17.72	39.77
8	Kronenbourg (M)	33.03	65.95	40.92	Medium	54.37	32.44
9	Raja of Nalagarh	87.96	63.49	0.00	Medium	0.00	0.00
10	Sindoor	77.65	76.09	10.40	Small	26.04	8.62
11	Tzigane (P)	32.98	49.07	34.00	Medium	36.98	28.77
12	Tzigane (M)	40.49	57.40	36.42	Medium	48.98	26.32
13	Wouburn Gold (P)	29.40	70.58	66.33	Medium	78.68	67.44
14	Wouburn Gold (M)	23.00	66.72	69.79	Long	85.19	62.39
15	Paradise	16.42	56.94	72.96	Long	89.42	70.42
16	3kr Paradise	36.45	62.69	57.03	Medium	70.03	53.47
17	4kr Paradise	57.03	42.20	54.16	Medium	67.85	50.12
	CD at 5%	7.73**	6.42**	6.79**	-	7.04**	7.04**
	CV	8.42	7.14	7.60	-	8.79	8.06
	SE	2.06	1.92	2.93	-	2.44	2.59

(P) = Parent

(M) = Mutant

Fig 14: Pollen viability, fertility & germination of spontaneous rose mutants

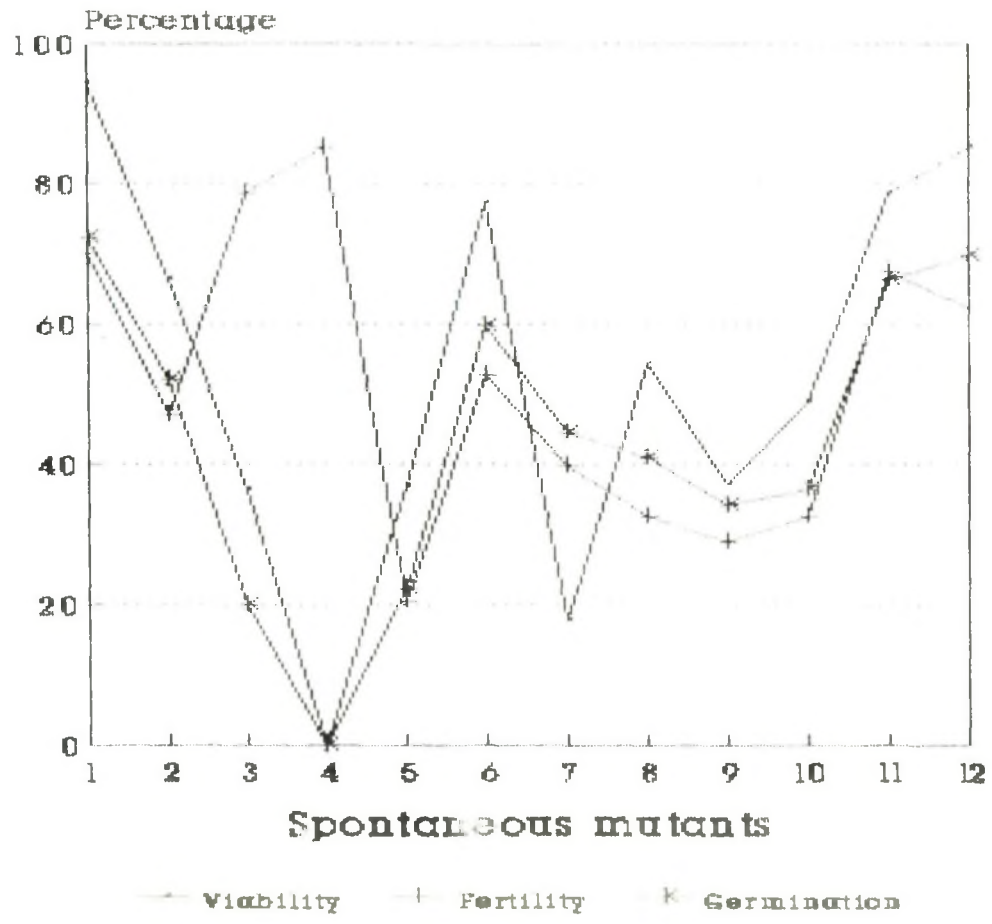
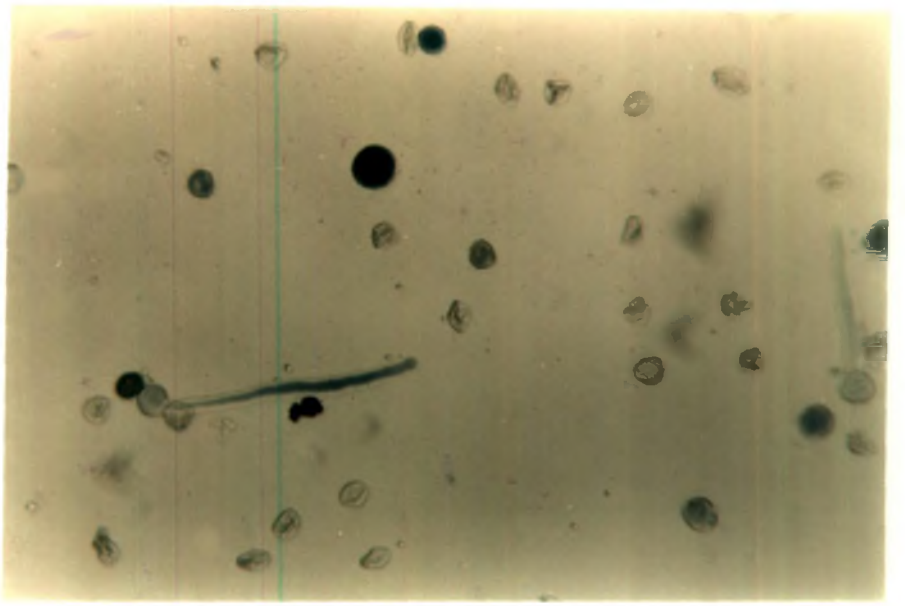
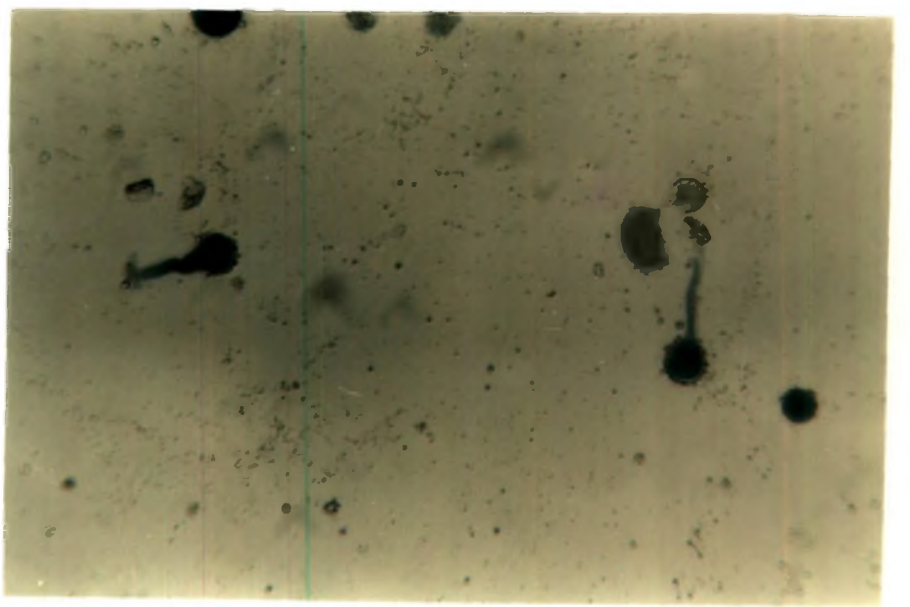


Plate 20: Photograph showing the effect of gamma irradiation on pollen germination and pollen tube growth in the cultivar Paradise

Plate 21: Photograph showing the Variation in pollen germination and pollen tube growth at 3krad gamma ray induced mutant of cultivar Paradis

Plate 22: Photograph showing the Variation in pollen germination and pollen tube growth at 4krad gamma ray induced mutant of cultivar Paradis





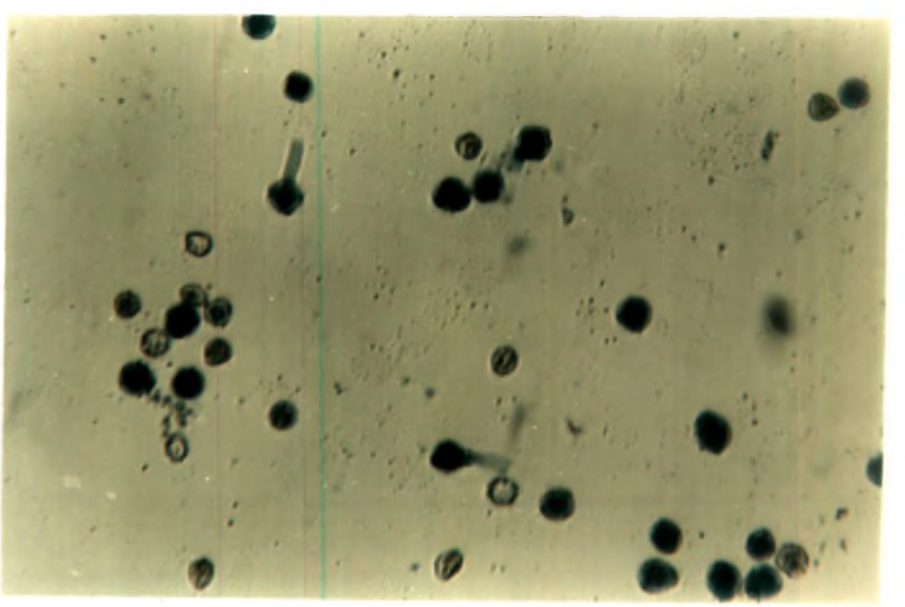
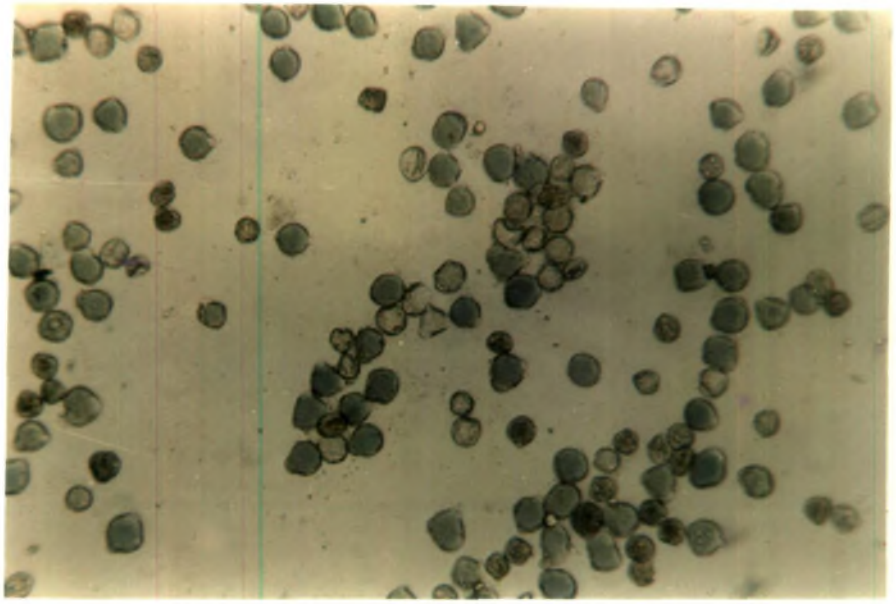
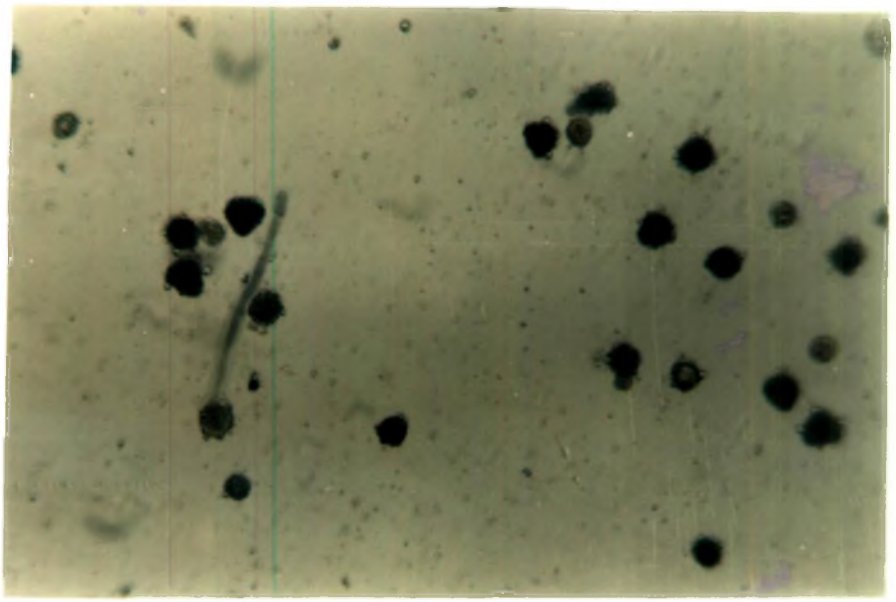


Plate 23: Photograph showing the Variation in pollen germination and pollen tube growth at 5krad gamma ray induced mutant of cultivar Paradise

Plate 24: Photograph showing the Variation in pollen germination and pollen tube growth of Akito genotype

Plate 25: Photograph showing the Variation in pollen germination and pollen tube growth in the spontaneous mutant of Akito genotype





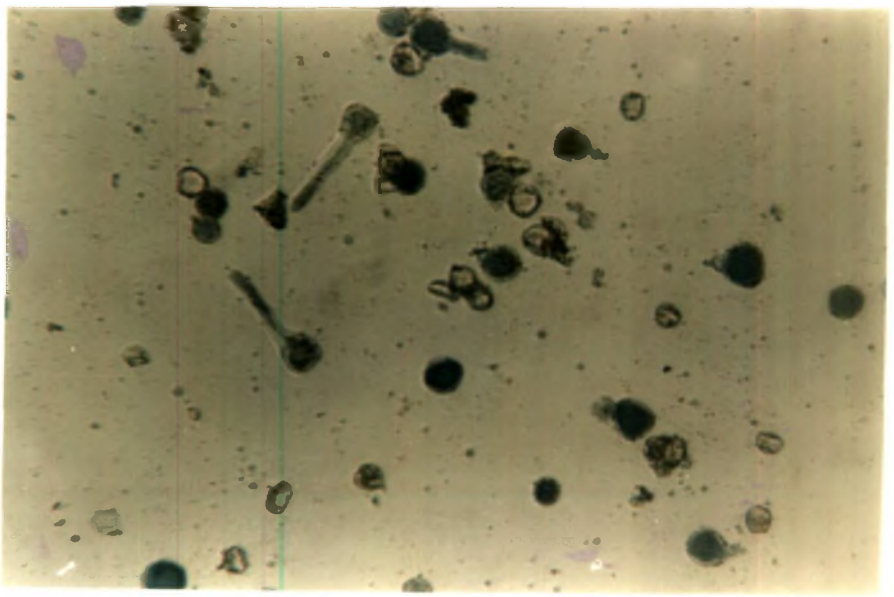
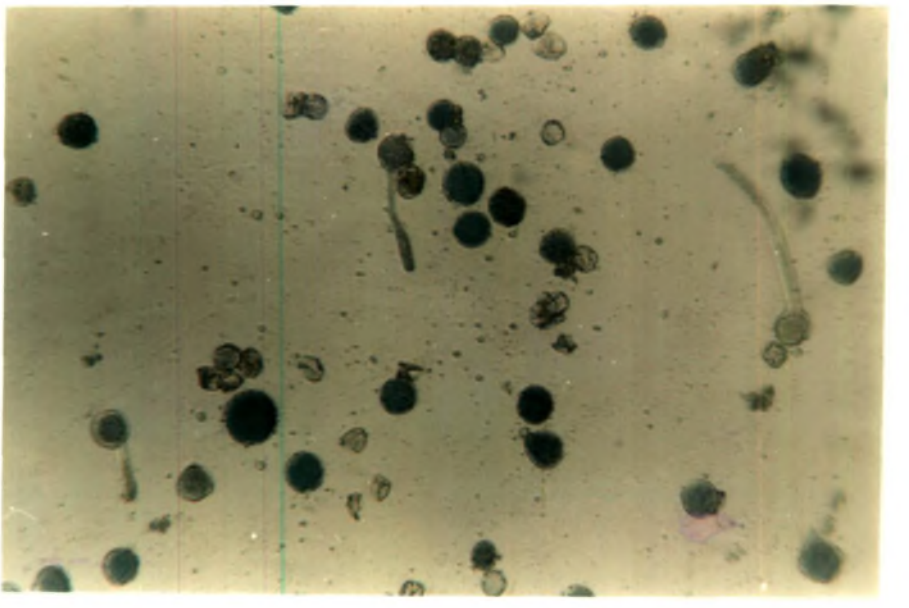


Plate 26: Photograph showing the Variation in pollen germination and pollen tube growth in Wouburn Gold genotype

Plate 27: Photograph showing the Variation in pollen germination and pollen tube growth in the spontaneous mutant of Wouburn Gold genotype



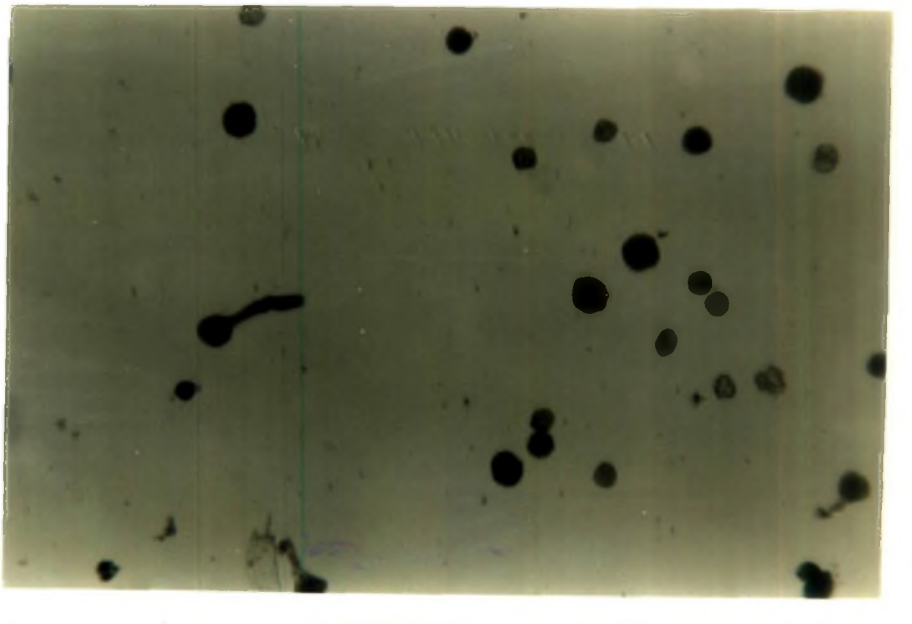
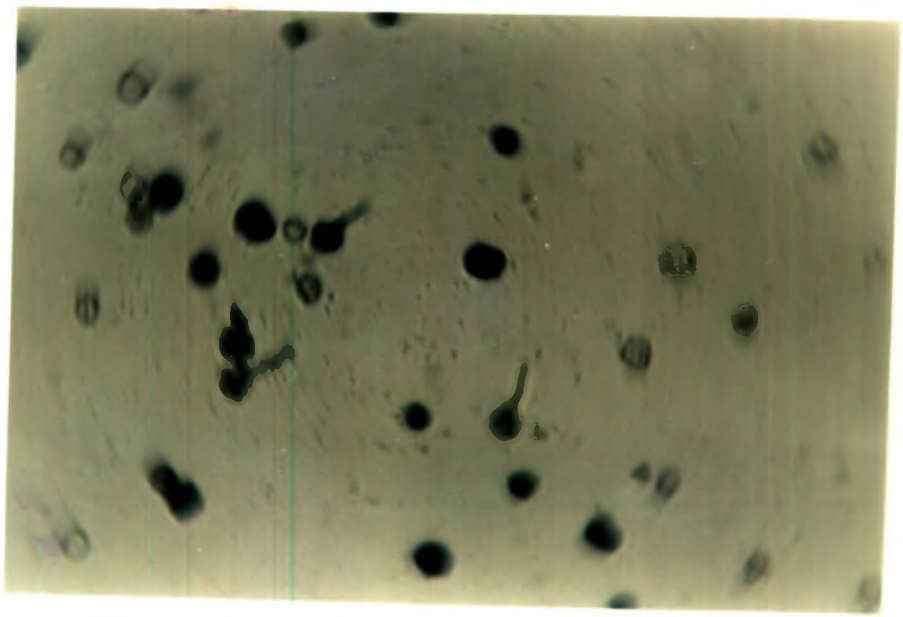
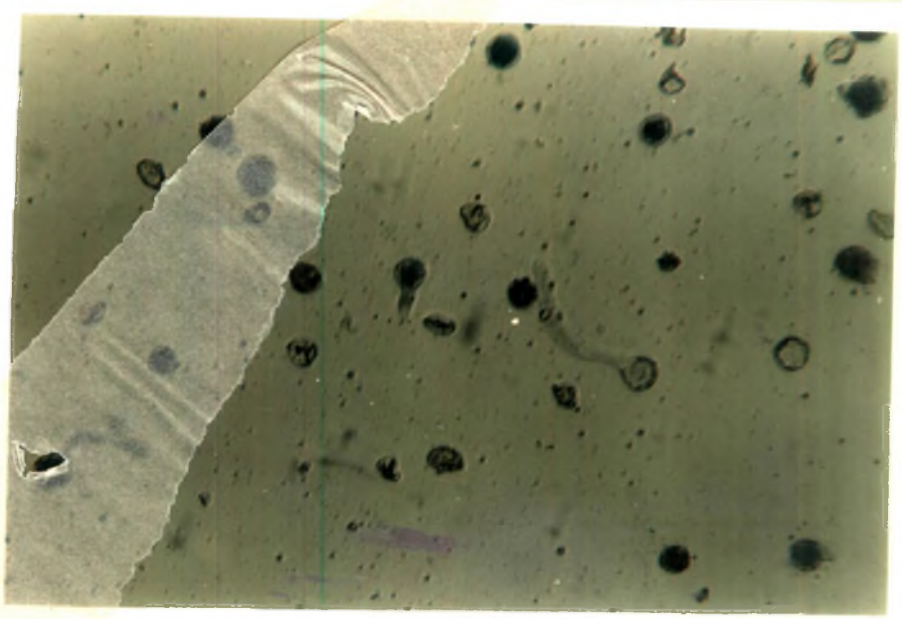


Plate 28 : Photograph showing the Variation in pollen germination and pollen tube growth in First Federal Gold genotype

Plate 29 : Photograph showing the Variation in pollen germination and pollen tube growth in the spontaneous mutant of First Federal Gold genotype





Among rose mutants minimum fertility status was observed in Sindoor (8.62%) followed by Eiffel Tower mutant (19.20%), whereas a maximum of 70 per cent pollen fertility was observed both in Paradise and Akito mutant. It was observed that there was significant difference among the parent and mutants in pollen germination aspect. Maximum pollen germination was observed in Paradise (72.96%) and Akito mutant (72.43%) followed by Wouburn parent and mutant (69%). Least pollen germination was recorded in most of the mutants. From the Table 37 it is clear that in all the rose genotypes, the mutants which were produced naturally had higher and better fertility and viability status when in comparison to their parents. Contrarily with induced mutants fertility and viability status was found to be more in parents rather than in the mutants induced by gamma irradiation treatments (Fig 14).

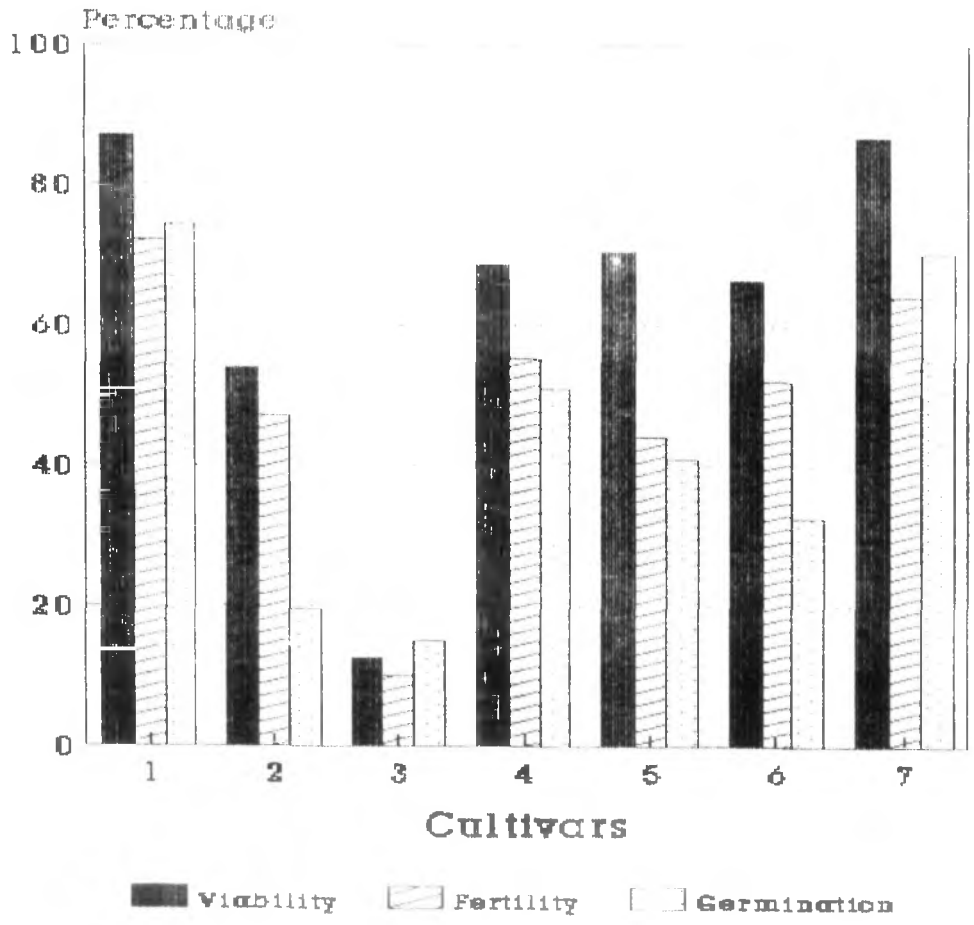
The size of the pollens germinated were small to medium long among hybrid teas and floribunda mutants used in the present study. Based on the degree of pollen stainability rose genotypes were grouped into highly fertile (70.42%). moderately fertile (53.47%) and fertile (39.77%) genotypes, when germinated under the media containing 150gm sucrose supplemented with 300mg calcium nitrate, 200mg magnesium sulphate, 100mg potassium nitrate and 100mg boric acid incubated at 25 $\pm$ 2^{0}C for 4hrs in dark enclosed in a moist chamber providing 100 per cent RH. Hibiscus pollen grains appeared as powdery mass to the naked eye and their colour varied from white, creamy white or yellowish. The outer surface of pollens were surrounded by a yellow substance and individual pollen grains were pantaporate, spheriodal and spinose in shape. In general, pollen production per flower depended on the number of anthers/flower and it was observed that it ranged from 4 to 148 in number. Pollen fertility ranged between 4.6 to 97.4 per cent (Table 38).

There was no germination under distilled water. It was found that sucrose, agar and boric acid content in the growth media had profound influence on

Table 38 : Pollen viability, fertility and germinability on different Hibiscus cultivars

Sl. No	Cultivars	Aborted pollens	Non aborted pollens	Pollen viability (%)	Pollen germination (%)	Pollen fertility (%)
1	Ashirwad	15.74	69.09	87.04	74.36	72.03
2	Arunodaya	63.30	31.40	53.90	19.60	47.10
3	Basant	71.17	22.20	12.42	15.00	10.00
4	Chitralekha	36.98	58.74	68.66	50.98	55.22
5	Dilruba	43.06	55.72	70.44	41.00	44.03
6	Phulkari	58.59	36.69	66.32	32.24	52.14
7	Priya	20.32	66.31	86.82	70.98	64.16
	CD at 5%	7.49**	6.14**	6.77**	5.44**	7.14**
	CV	8.41	7.62	7.46	6.02	8.72
	SE	2.03	2.14	1.97	0.43	1.99

Fig 15: Pollen viability, fertility & germination in hibiscus cultivars



germination and tube elongation in hibiscus pollen grains. A media containing sucrose 20 per cent, agar 1 per cent and boric acid 100ppm was found to be the best for pollen germination as well as for better pollen tube growth. The beneficial effects of agar might be attributed to the supply of moisture, carbohydrates and other nutrients. Boron helps in oxygen uptake in addition to the synthesis of pectic substances required for the formation of the walls of germinating tubes. The different varieties used exhibited wide variation in pollen size, pollen outgrowth, fertility, germination and pollen tube growth. Standardization of media for pollen germination and tube growth indicates that a medium containing 20 per cent sucrose, 1 per cent agar and 100ppm boric acid was the best for rose pollen germination (Fig 15).

The analysis of data showed that the intervarietal variation in the germination was highly significant. Pollen grains of Ashirwad (74.36%) gave the highest per cent germination followed by Priya (70.98%). Pollen tube growth was found to vary among different cultivars (Table 38). Significant difference in viability of pollen grains between cultivars was noted. Maximum viability was recorded in Ashirwad (87.04%) followed by Priya (86.82%) with the least (12.42) to be recorded in Basant. The per cent of aborted pollens in all the cultivars was significantly minimum with higher percentage of non aborted pollens. Pollen germination was found to be enormously profuse in Ashirwad (74.36%) and the least in the cultivar Basant (15%). Thus a wide range of germinability of pollens in different varieties was met with. Pollen fertility and viability was found to be onpar with each other with significant difference among different cultivars undertaken.

It was seen from the Table 38 that the variety Phulkari which gave 66.32 per cent viability as per nuclear test gave only 32.24 per cent germinability test in the nutrient media. Also Arunodaya recorded 53.90 per cent viability test, but gave 19.60 per cent germination in the nutrient media. These results indicate that

certain varieties which gave increased viability in nuclear tests gives either poor or no germination at all. But none of the cultivars showed increase in pollen viability in the germination test method. These results are in agreement with the views of determining pollen viability status.

Among the various concentrations used or tried it was found that from 0-20 per cent pollen grains failed to germinate, whereas from 20 per cent onwards pollen germination was observed but germination per cent was very low and pollen tube failed to elongate. Boric acid was found to increase the percent germination and tube elongation. Pollens were of polysiphonous natured in majority of the hibiscus cultivars. The data on the statistical analysis indicated that varietal variation in pollen viability was highly significant both in rose and hibiscus.

4.3 EXPERIMENT III : PIGMENTATION STUDIES

Phytochemical analysis contributed in addition to identifying and quantifying different pigment classes and had also been used in unravelling the genetics of colour inheritance in the genotypes of garden plants. The present study investigated the qualitative and quantitative distribution of anthocyanins, leucoanthocyanins, flavonols and carotenoids in 17 rose genotypes including 4 induced mutants and 5 spontaneous mutants and 15 Hibiscus cultivars.

4.3.1 Rose Genotypes and their Mutants

4.3.1.1 Anthocyanin

The anthocyanins in induced mutants of cultivar Paradise reduced considerably with increased irradiation dosage. Paradise cultivar with red purple petals contained the maximum anthocyanin content (1.9236), 3kr mutant with purple and variegated white petals had 1.4462, 4kr mutant with pink purple petals had 0.4266, 5kr mutant with purple violet petals had 0.1973 and 6kr mutant with

white petals had 0.0821 anthocyanin contents, respectively. The reduction in the anthocyanin content in the irradiated mutants resulted in the lighter petal colours.

The anthocyanin content of rose flowers of eight genotypes varied considerably depending on the petal colour (Table 39). Cultivar Raja Surendra Singh of Nalagarh with orange salmon pink petal colour recorded maximum anthocyanin content (3.1413) followed by Wouburn Gold with golden yellow petal colour (3.0442), Sindoor with geranium lake (2.2467), Akito with white (2.0342), Eiffel Tower with neyron rose (1.9732), Paradise with red purple (1.9236) and First Federal Gold with yellow (1.8172) petal colours, respectively. Mutant of Kronenbourg with yellow petals recorded minimum anthocyanins (0.2556) among the eight genotypes studied (Fig 16).

Studies on anthocyanin content in 5 genotypes and their spontaneous mutants had indicated that except in the genotypes Eiffel Tower and Kronenbourg where the anthocyanin content decreased in the mutant all other genotypes viz., Akito, Wouburn Gold, Kronenbourg and First Federal Gold had increased amount of anthocyanin content in their respective spontaneous mutants. This increase in the anthocyanin content among spontaneous mutants had contributed azalea pink petal colour in Akito, tangerine orange petal colour in Wouburn Gold and red brown petal colour in First Federal Gold.

In the present study, it was observed that induced mutants had reduced anthocyanin content and spontaneous mutants in general had higher anthocyanin content as compared to their parents (Table 39).

4.3.1.2 Leucoanthocyanin

With regard to induced mutants of cultivar Paradise the results indicated that the amount of leucoanthocyanin content decreased with increase in irradiation

Table 39 : Petal colour, Anthocyanin, leucoanthocyanin, Flavonol and Carotenoid contents in Rose genotypes and their mutants (Values mean ± SE of 3 analysis)

Genotypes	Petal Colour*	Anthocyanin**(OD at 530 nm)	Leucoanthocyanin (OD at 550nm)	Flavonol (mg% as phloroglucinol)	Carotenoid (mg/100g as /3-Carotene)
Paradise	Red Purple(82D)	1.9236 ± 0.4269	0.2193 ±0.1266	22.36 ± 0.4506	0.0302 ± 0.0174
3kr	Purple with white variegation(73C)	1.4462 ± 0.3923	0.1613 ±0.0931	24.16 ± 0.4851	0.0417 ± 0.0241
4kr	Pink Purple(62D)	0.4266 ± 0.3642	0.1549 ±0.0893	17.32 ± 0.3032	0.0393 ± 0.0227
5kr	Purple Violet(62A)	0.1973 ± 0.0832	0.0044 ±0.0025	25.83 ± 0.3944	0.0462 ± 0.0267
6kr	White(65D)	0.0821 ± 0.0032	0.0034 ± 0.0019	16.03 ± 0.3362	0.0473 ± 0.0273
Akito (P)	White(37A)	2.0342 ± 0.6634	0.0866 ±0.0501	37.40 ± 0.9047	0.0675 ± 0.0389
Akito (M)	Azalea Pink(28B)	3.1623 ± 0.8781	0.0103 ±0.0059	17.09 ± 0.4096	0.1623 ± 0.0937
Eiffel Tower (P)	Neyron Rose(45A)	1.9732 ± 0.4142	0.2560 ±0.1478	28.42 ± 0.5120	0.2695 ± 0.1556
Eiffel Tower (M)	Orient Pink(70A)	1.6720 ± 0.3921	0.0703 ±0.0406	14.64 ± 0.2391	0.3432 ± 0.1981
First Federal Gold (P)	Yellow(65A)	1.8172 ± 0.3973	0.2406 ±0.1389	36.40 ± 0.8103	0.0752 ± 0.0434
First Federal Gold (M)	Red Brown(70B)	1.9266 ± 0.4463	0.1678 ±0.0968	23.52 ± 0.4187	0.0187 ± 0.0108
Kronenbourg (P)	Crimson Yellow (64A)	0.5816 ± 0.2436	0.0850 ±0.0491	22.76 ± 0.4977	0.3051 ± 0.1761
Kronenbourg (M)	Yellow(29B)	0.2556 ± 0.1430	0.1876 ±0.1083	35.70 ± 0.7361	0.4965 ± 0.2867
Wouburn Gold (P)	Golden Yellow(23A)	3.0442 ± 0.8572	0.2767 ±0.1597	19.44 ± 0.3178	0.3432 ± 0.1981
Wouburn Gold(M)	Tangerine Orange(35B)	4.1233 ± 0.0972	0.1226 ±0.0708	21.20 ± 0.3942	0.4915 ± 0.2838
Sindoor	Geranium Lake(40A)	2.2467 ± 0.5241	0.2393 ±0.1381	32.42 ± 0.7047	0.0973 ± 0.0562
Raja Surendra Singh of Nalagarh	Orange Salmon Pink(41B)	3.1413 ± 0.7440	0.1413 ±0.0816	39.33 ± 0.9236	0.1256 ± 0.0725

(P) = Parent

(M) = Mutant

* Based on R. H. S colour chart

** 0.5g petal in 100ml 1% acidic methanol

Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 16: Anthocyanin and flavonol pigment contents in rose genotypes

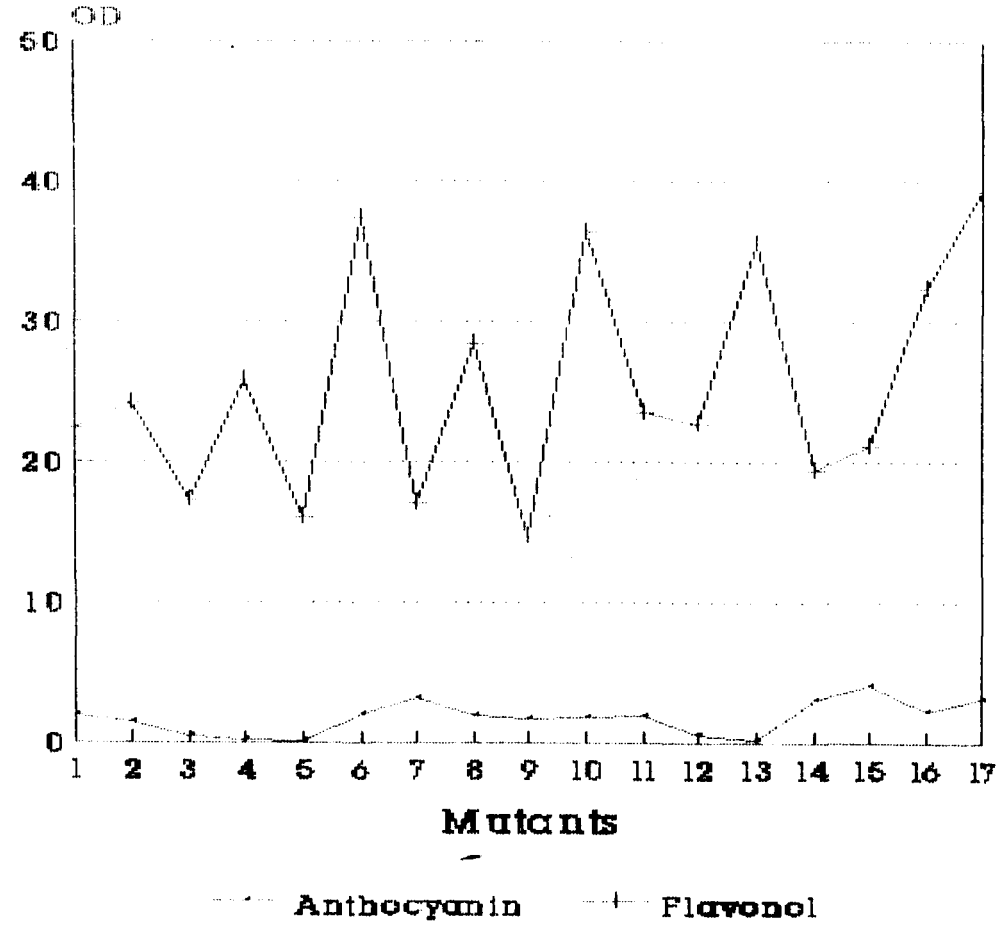
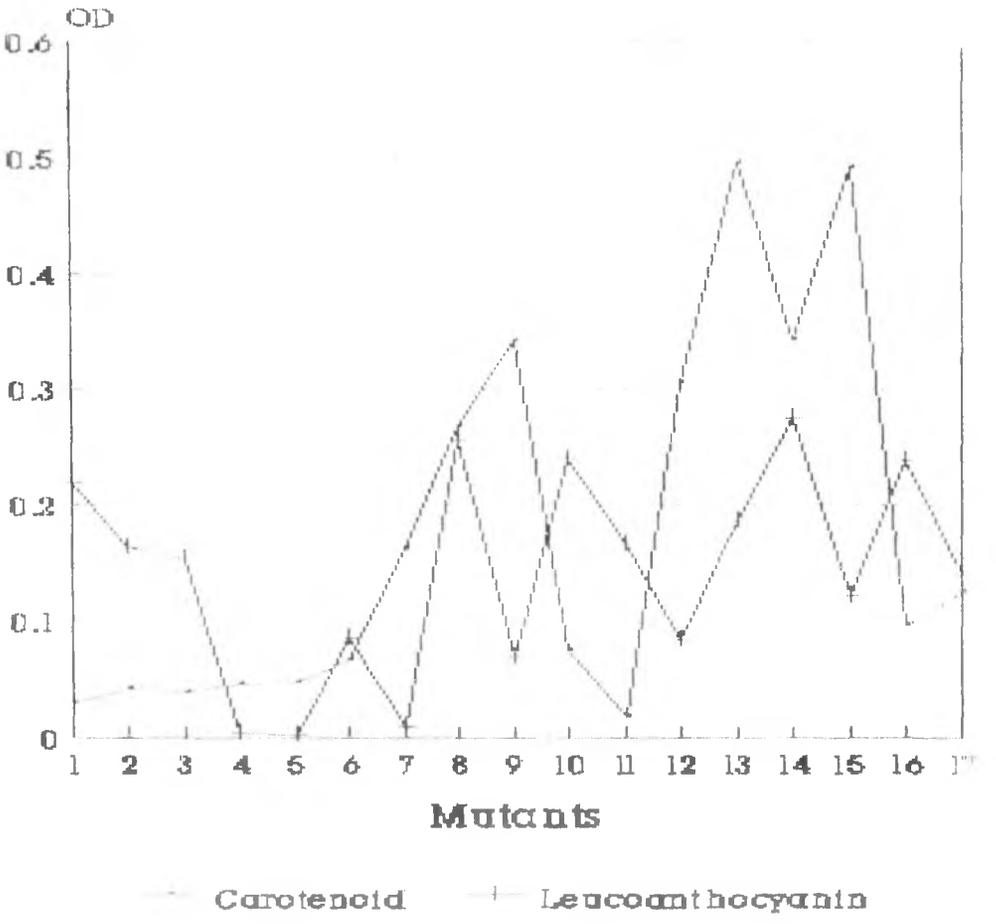


Fig 17: Carotenoid and leucoanthocyanin pigment contents in rose genotypes



dosages. Higher the irradiation dosage lower was the leucoanthocyanin content in all the induced mutant under study. Maximum amount of leucoanthocyanin was recorded at 3kr gamma ray treated plants (0.1613) having purple white petal colour and a considerable reduction was observed at 6kr treated buds (0.0034) resulting in the white petal colour as compared to its parent genotypes (0.2193) with red purple petal colours, respectively (Table 39).

Variation in the leucoanthocyanin content depending on the petal colour was observed among the eight rose genotypes studied. Maximum leucoanthocyanin content was recorded in Wouburn Gold (0.2767) and Eiffel Tower (0.2560) with golden yellow and neyron rose petal colour. Minimum amount was observed in Akito mutant (0.0103) with azalea pink petal colour. The data indicated that dark petal coloured genotypes had high leucoanthocyanin content when compared to light petal coloured genotypes (Fig 17).

The amount of leucoanthocyanins decreased considerably in all the spontaneous mutants compared with their respective parents. Among the spontaneous mutants First Federal Gold with red brown petal colour had maximum (0.1678) content and Akito mutant with azalea pink petal colour had minimum (0.0103) leucoanthocyanin content. The data indicated that the amount of leucoanthocyanins decreased considerably in both induced and spontaneous mutants of rose genotypes.

4.3.1.3 Flavonols

The flavonol content with respect to induced mutants higher or lower gamma radiation dosages did not show any regular pattern of increase or decrease in the flavonol content. Out of the different irradiation treatments maximum flavonol content (28.83) was recorded at 5kr resulting in the purple violet coloured

flowers and minimum (17.32) flavonol content at 6kr resulting in the white shaded flower colours(Table 39).

The flavonol content of rose petals varied among 8 different genotypes under the study. Among the 8 genotypes of rose Raja Surendra Singh of Nalagarh with orange salmon pink petals had the highest (39.33) flavonol content followed by Akito mutant (37.40) with azalea pink petal colour and First Federal Gold (36.40) with yellow white flower colour. The other genotypes with varying petal colours studied have recorded comparatively lesser amount of flavonols (Fig 16).

The data indicated that there was considerable decrease in the flavonol content among the spontaneous mutants, except Wouburn Gold mutant which had higher flavonol content as compared to its parent genotypes. Flavonol content among the five mutants varied from 14.64 to 23.52 mg% where First Federal Gold with red brown petal colour had maximum and Eiffel Tower mutant with orient pink petal colour recorded minimum flavonol content, respectively. The data indicated that in rose the flavonol content in general decreased among the spontaneous mutants as compared to their respective parents.

4.3.1.4 Carotenoids

Data indicated that increase in the radiation dosage in general, increased total carotenoid content as compared with the parent cultivar Paradise. Maximum amount of carotenoid content was recorded at 6kr with white petal colour (0.0473) and the minimum at 4kr (0.0393) with pink purple petal colour, respectively.

Total carotenoid content varied considerably among the 8 rose genotypes studied. Mutant of Kronenbourg with yellow petal colour had 0.4965 mg% and Wouburn Gold with golden yellow petals had 0.3432 mg% of total carotenoids indicating that more the yellow colour in the petals higher will be the total

carotenoid content. Cultivar First Federal Gold recorded lower (0.0187) carotenoid content with red brown petal colour indicating that darker shades resulted in lesser carotenoid content (Fig 17).

The total carotenoid content among spontaneous mutants was more as compared to their respective parents in the cultivars Eiffel Tower, Akito and Wouburn Gold and Kronenbourg and it was found to be less in First Federal Gold. It was observed that the mutant of First Federal Gold recorded the minimum carotenoid content (0.0187) with red brown petal colour and mutant of Wouburn Gold with tangerine orange colour had maximum of total carotenoids (0.4915) among the spontaneous mutants. The results indicated the total carotenoids was more in yellow coloured genotypes and their mutants.

4.3.1.5 Paper Chromatographic Separation of Anthocyanin Pigments in Rose Genotypes

Paper chromatographic studies was undertaken to understand the qualitative and quantitative differences within the anthocyanins in rose genotypes and their induced and spontaneous mutants. The data are presented in Tables 40 to 47. Authentic standards of anthocyanins, namely, cyanin chloride, pelargonin chloride as well as anthocyanidins, pelargonidin chloride were also co-chromatographed for the identification of separated anthocyanin bands from rose genotypes.

4.3.1.5.1 Induced Mutants

The cultivar Paradise with its four induced mutants showed considerable variation in the number of anthocyanin pigments. The parent cultivar Paradise had three bands with red purple band colour which might have given rise to the red purple petal colour. Contrarily in all the induced mutants the above three bands were absent. Induced mutation using gamma rays resulted in the reduction of the

Table 40 : Paper chromatographic separation of anthocyanin pigments in the petals of Paradise cultivar and their induced mutants
(Rf values mean ± SE of 3 analysis)

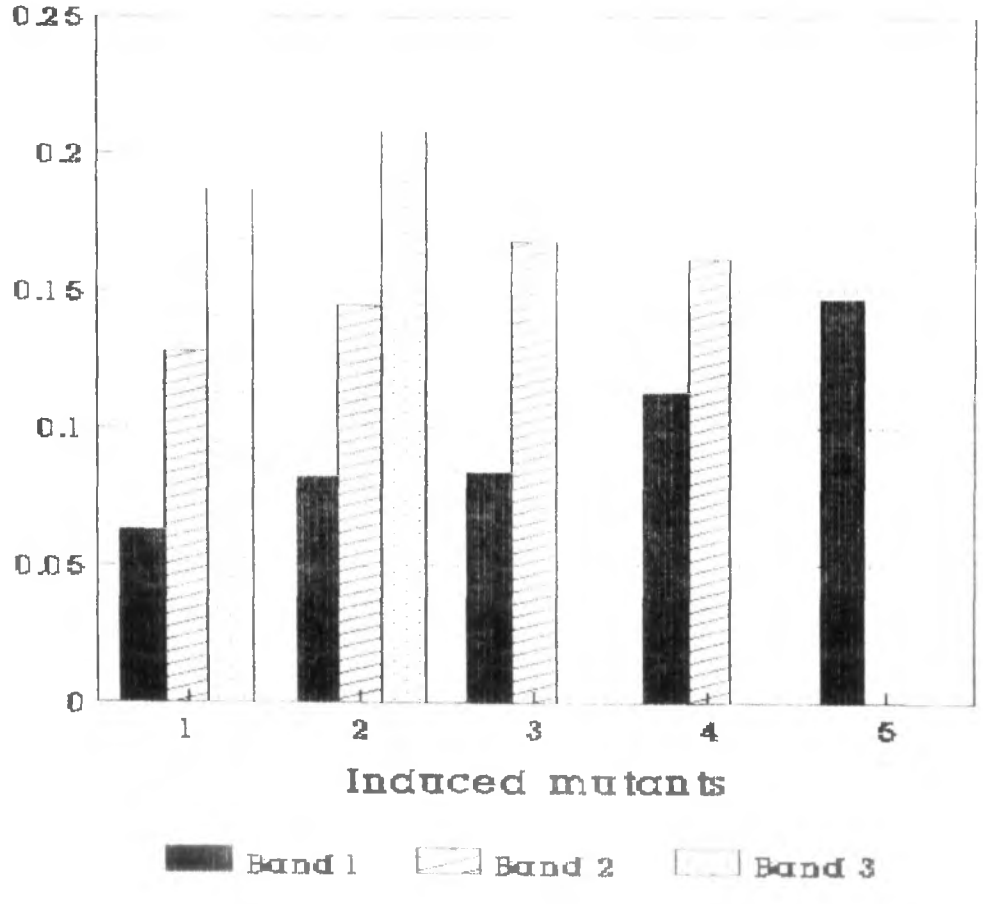
No of band	Paradise	3kr Paradise	4kr Paradise	5kr Paradise	6kr Paradise	Band Colour*
1	0.063 ±0.036(70A)	-	-	-	-	Red purple
2	-	0.082 ± 0.474(71B)	0.084 ±0.044(68C)	-	-	Red purple
3	-	-	-	0.113 ±0.012(68D)	-	Red purple
4	0.128 ±0.074(70D)	-	-	-	-	Red purple
5	-	0.145 ±0.084(70C)	-	-	0.147 ±0.079(65D)	Red purple
6	-	-	0.168 ±0.097(63C)	0.162 ±0.114(63D)	-	Red purple
7	0.187 ±0.108(82D)	-	-	-	-	Red purple
8	-	0.208 ±0.121(80C)	-	-	-	Red purple
Petal Colour*	Red Purple(82D)	Purple White(73C)	Pink Purple(62D)	Purple Violet(62A)	White(65D)	

*Based on the R.H.S colour chart
- Absent
Values within paranthesis indicate band numbers as per R.H.S colour chart

Authentic Standards

Cyanin Chloride	0.165 ± 0.2120
Pelargonin Chloride	0.305 ± 0.1060
Pelargonidin Chloride	0.910 ± 0.2820

Fig 18: Paper chromatographic separation of anthocyanin in the induced mutant of cultivar Paradise



number of anthocyanin bands, where the higher dosage of 5kr gamma rays resulted in the minimum number of bands (Fig 18).

With respect to 3kr variegated induced mutant of Paradise, it recorded 3 new anthocyanin bands having red purple band colour which might have lead to the petal variegation. The second band of 3kr Paradise was also present in the 4kr mutant along with a new anthocyanin band having red purple band colour which indicates lighter petal colour shades, whereas 6kr induced mutant of Paradise had only one new anthocyanin band which was not present in other induced mutants, resulting in the complete petal colour change over to white (Table 40).

Quantitative estimation of flower petal colour (anthocyanin bands) in Paradise cultivar indicated that when compared to the parent cultivars the induced mutants had lesser concentration of anthocyanins which inturn reflects lesser hue in imparting colour to the petals. The parent cultivar and 3kr induced mutant had darker petal colour shades of red purple having higher concentration of three anthocyanin bands. The results indicated that the irradiation dosages altered qualitatively and quantitatively the anthocyanin pigments in the petals of parent cultivar Paradise resulting in the lighter petal colours in induced mutants (Table 47).

4.3.1.5.2 Spontaneous Mutants

Akito : The parent genotype Akito which was completely white in colour had two anthocyanin bands of red colour which was also present in the mutant (Fig 19). Apart from the two anthocyanin bands present in both the mutants and parent, the mutant had two new anthocyanin bands with orange red colour imparting to the azalea pink mutant petal colour (Table 41). Quantitative estimation indicated that the parent had less anthocyanin concentration of lighter petal colour shade as

Table 41 : Paper chromatographic separation of anthocyanin pigments in the petals of Akito and its spontaneous mutant (Rf values mean ± SE of 3 analysis)

No of bands	Akito (Parent)	Akito (Mutant)	Band Colour*
1	0.086 ± 0.049 (37C)	0.073 ± 0.042 (48C)	Red
2	-	0.106 ± 0.061 (28D)	Orange Red
3	0.164 ± 0.094 (37D)	0.165 ± 0.095 (51D)	Red
4	-	0.212 ± 0.122 (28C)	Orange
Petal Colour*	**White (37A)**	**Azalea pink (28B)**	

Table 42 : Paper chromatographic separation of anthocyanin pigments in Eiffel Tower and its spontaneous mutant (Rf values mean ± SE of 3 analysis)

No of bands	Eiffel Tower(Parent)	Eiffel Tower(Mutant)	Band Colour
1	0.086 ± 0.049 (88D)	-	Violet
2	0.108 ± 0.062 (70D)	0.102 ± 0.058 (73D)	Red purple
3	-	0.123 ± 0.071 (70B)	Red purple
4	0.419 ± 0.068 (43D)	-	Red purple
5	-	0.191 ± 0.114 (70D)	Red
Petal Colour*	**Neyron rose (45A)**	**Orient Pink (70A)**	

***Based on the R.H.S colour chart**
- Absent
Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 19: Paper chromatographic separation of anthocyanin in the spontaneous mutant of Akito and Eiffel Tower

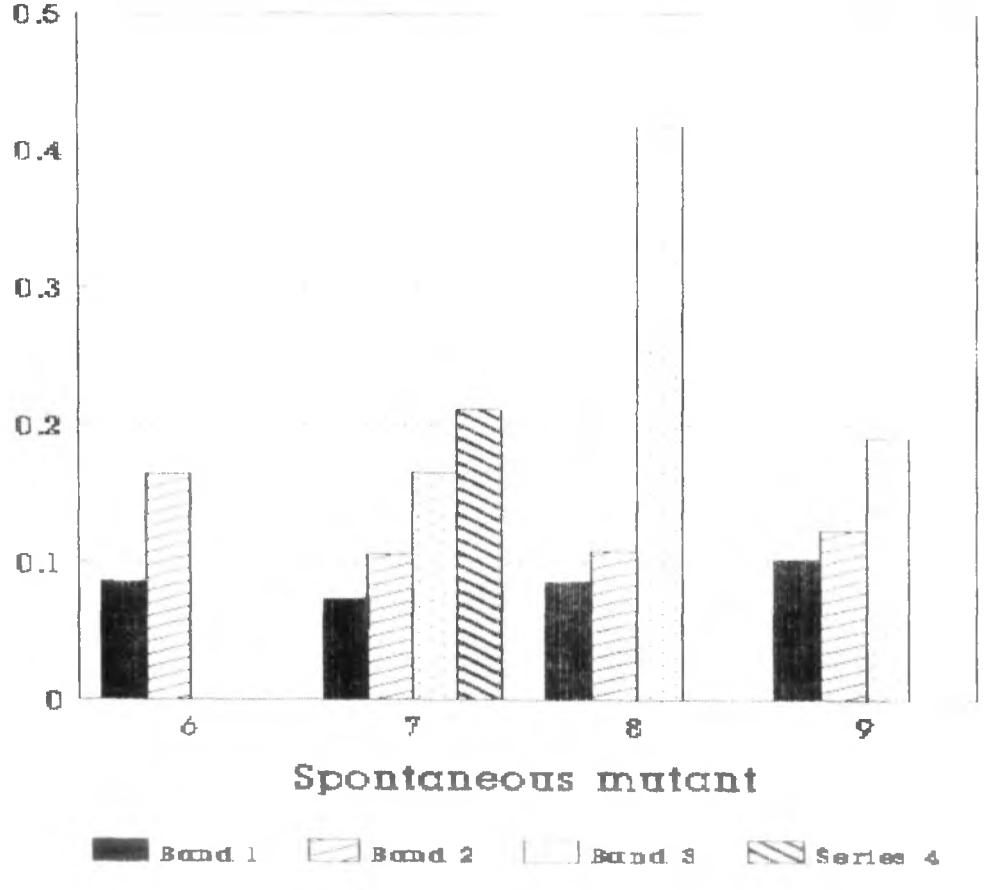


Table 43 : Paper chromatographic separation of anthocyanin pigments in the petals of First Federal Gold and its spontaneous mutant (Rf values mean ± SE of 3 analysis)

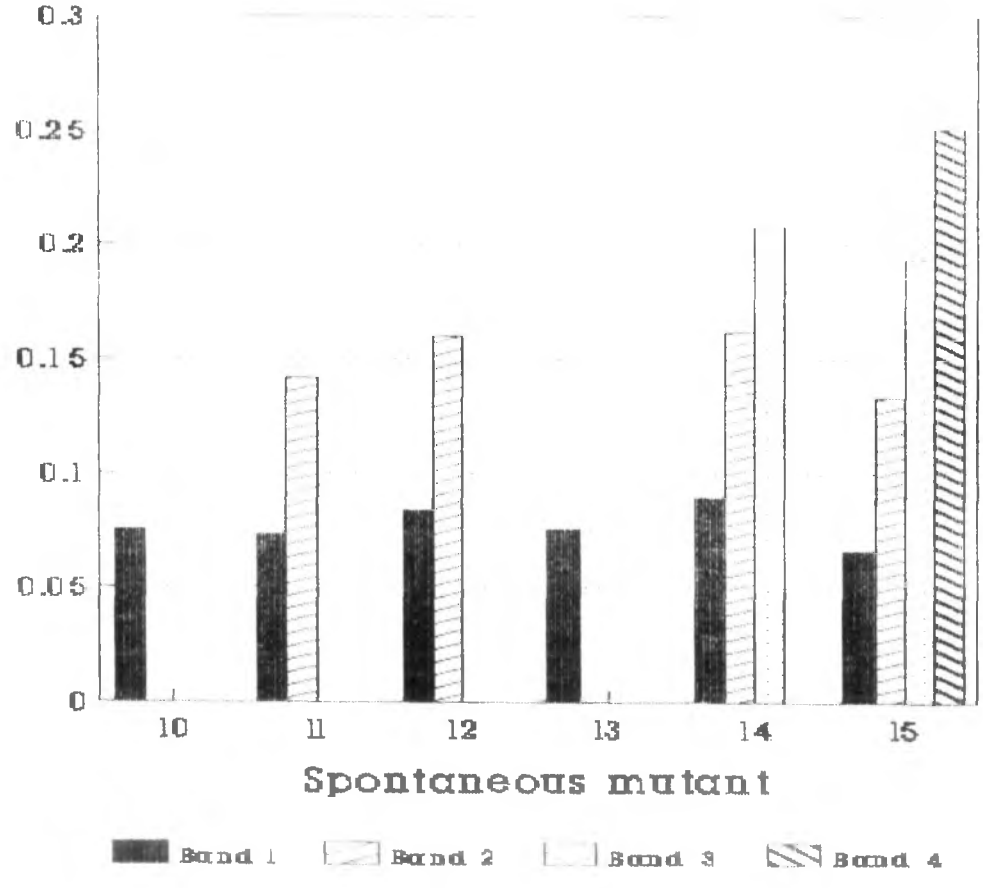
No of bands	First Federal Gold(Parent)	First Federal Gold (Mutant)	Band Colour*
1	0.075 ± 0. 043 (65D)	0.073 ± 0.042 (72C)	Red purple
2	-	0.142 ± 0.081 (70D)	Red purple
Petal Colour*	Yellow (65A)	Red Brown (70B)	

Table 44 : Paper chromatographic separation of anthocyanin pigments in the petals of Kronenbourg and its spontaneous mutant(Rf values mean ± SE of 3 analysis)

No of bands	Kronenbourg (Parent)	Kronenbourg (Mutant)	Band Colour*
1	-	0.075 ± 0.044 (29B)	Yellow orange
2	0.084 ± 0.048 (65A)	-	Red purple
3	0.160 ± 0.092 (64C)	-	Red purple
Petal Colour*	Crimson yellow (64A)	Yellow (29B)	

*Based on the R.H.S colour chart
- Absent
Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 20:Paper chromatographic separation of anthocyanin in First Federal Gold, Kronenbourg and Woubarn Gold mutants



compared to its spontaneous mutant having increased concentration giving rise to pink mutant petal colour (Table 47).

Eiffel Tower : The parent genotype Eiffel Tower had three anthocyanin bands with violet to red purple band colour, whereas the mutant also had 3 anthocyanin bands of which only one band was common in both the parent and the mutant (Fig 19). The mutant had two new anthocyanin bands (Table 42).Quantitative estimation indicated that Eiffel Tower parent had higher anthocyanin concentration compared to its corresponding spontaneous mutant which might have lead to the darker flower colour shade in the parent and light pink shade in the mutant (Table 47).

First Federal Gold : First Federal Gold had only one anthocyanin band with red purple band colour whereas its spontaneous mutant had one more new anthocyanin band with red purple petal colour (Table 43). The pigment concentration was found to be very less in the parent genotype indicating a lighter flower colour shade as compared to the higher concentration in the mutants resulting in red brown petal colour (Table 47) (Fig 20).

Kronenbourg : The mutant of Kronenbourg was yellow with only one anthocyanin band, whereas in the parent genotype with crimson yellow petal colour, this particular band was absent and had two new anthocyanin bands with red purple colour (Table 44). Quantitative estimation of flower petal colour in Kronenbourg and its spontaneous mutant showed that the mutant which had yellow petal (Fig 20) colour with one band had low concentration of pigments, whereas the parent which had two new bands had higher concentration of red purple bands resulting in crimson yellow petal colour, respectively (Table 47).

Table 45 : Paper chromatographic separation of anthocyanin pigments in the petals of Wouburn Gold and its spontaneous mutant(Rf values mean ± SE of 3 analysis)

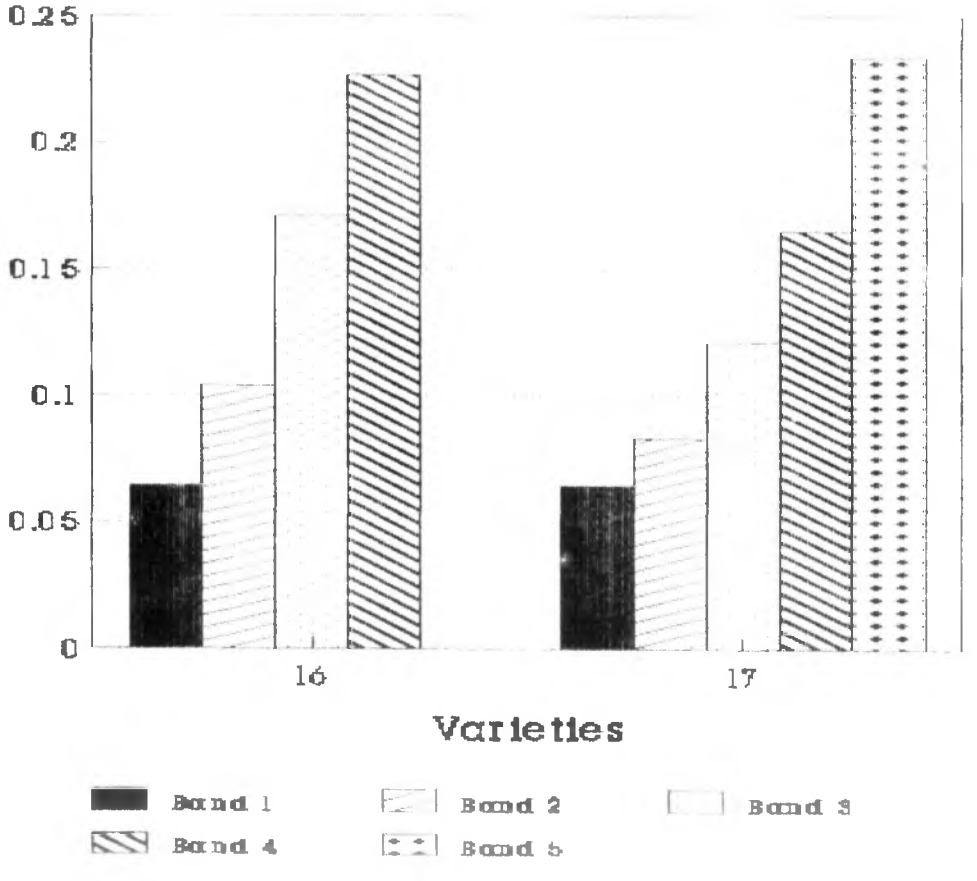
No of bands	Wouburn Gold(Parent)	Wouburn Gold(Mutant)	Band Colour*
1	-	0.066 ± 0.038 (70C)	Red purple
2	0.089 ± 0.051 (23D)	-	Orange red
3	-	0.133 ± 0. 076 (33C)	Orange red
4	0.162 ± 0.093 (29B)	-	Orange
5	-	0.194 ± 0.112 (68B)	Red purple
6	0.208 ± 0.120 (22D)	-	Yellow orange
7	-	0.251 ± 0.145 (35B)	Orange red
Petal Colour*	Golden yellow (22A)	Tangerine orange (35B)	

Table 46 : Paper chromatographic separation of anthocyanin pigments in the petals of Sindoor & Raja Surendra Singh of Nalagarh (Rf values mean ± SE of 3 analysis)

No of bands	Sindoor	Raja Surendra Singh of Nalagarh	Band colour*
1	0.064 ± 0.037 (70C)	0.064 ± 0.036 (70C)	Red Purple
2	-	0.083 ± 0.048 (70B)	Red Purple
3	0.104 ± 0.060 (43C)	-	Red Purple
4	-	0.121 ± 0.069 (41B)	Red Purple
6	0.171 ± 0.099 (41C)	0.165 ± 0.957 (29C)	Red Violet
7	0.227 ± 0.131 (33A)	0.234 ± 0.135 (32C)	Orange Red
Petal Colour*	Geranium lake (40A)	Orange salmon pink (41B)	

*Based on the R.H.S colour chart
- Absent
Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 21: Paper chromatographic separation of anthocyanin pigment in Sindoor and Raja Surendra Singh of Nalagarh



Wouburn Gold : Both the parent genotype and its spontaneous mutant of Wouburn Gold had different anthocyanin band patterns. Wouburn Gold with golden yellow petal colour had three anthocyanin bands having orange red to yellow orange colour giving rise to yellow petal colour (Fig 20). Spontaneous mutant of Wouburn Gold exhibited four new anthocyanin bands which was not present in the parent cultivar imparting more of orange red band colour. The new bands present in the mutant has resulted in tangerine orange petal colour (Table 45).

Quantitative estimation of the petal colour indicated that all the three bands present in the parent genotype had lesser concentration whereas its corresponding spontaneous mutant exhibited 4 new bands having higher concentration which resulted in the tangerine orange colour of the mutant (Table 47).

Sindoor and Raja Surendra Singh of Nalagarh

The cultivars Sindoor and Raja Surendra Singh of Nalagarh both had red orange as the flower petal colour. Cultivar Sindoor had four anthocyanin bands with the first three bands having red purple colour whereas the fourth band had orange red colour (Fig 21). Cultivar Raja Surendra Singh of Nalagarh also had I , III and IV anthocyanin bands of Sindoor in addition to the two new bands having red purple band colour imparting to geranium lake in Sindoor and orange salmon pink petal colours in Raja Surendra Singh of Nalagarh, respectively (Table 46).

4.3.1.6 Chemical Identification of Anthocyanin Pigments in Rose

Paper chromatography of anthocyanin pigments in different rose cultivars used for the present investigation has indicated varietal differences in the number of

Table 47 : Colour intensity of anthocyanin bands separated by paper chromatography in the petals of Rose genotypes and their mutants (OD values at 530nm)

Genotype	OD values at 530nm									
Paradise	0.398	-	-	0.444	-	-	0.495	-	-	-
3kr	-	0.244	-	-	0.282	-	-	0.334	-	-
4kr	-	0.143	0.155	-	-	0.169	-	-	-	-
5kr	-	-	0.066	-	-	0.114	-	-	-	-
6kr	-	-	-	-	0.022	-	-	-	-	-
Akito(P)	-	0.086	-	-	-	0.093	-	-	-	-
Akito(M)	0.143	-	0.180	-	-	0.268	-	0.396	-	-
Eiffel Tower(P)	-	0.276	0.301	-	0.362	-	-	-	-	-
Eiffel Tower(M)	-	-	0.161	0.223	-	-	0.240	-	-	-
First Federal Gold(P)	0.119	-	-	-	-	-	-	-	-	-
First Federal Gold(M)	0.150	-	-	-	0.229	-	-	-	-	-
Kronenbourg(P)	-	0.157	-	-	-	0.204	-	-	-	-
Kronenbourg(M)	0.102	-	-	-	-	-	-	-	-	-
Wouburn Gold(P)	-	0.192	-	-	-	0.249	-	0.286	-	-
Wouburn Gold(M)	0.152	-	-	0.234	-	-	0.318	-	-	0.344
RON**	0.359	0.412	-	0.452	-	0.467	-	-	0.509	-
Sindoor	0.377	-	0.429	-	-	0.444	-	-	0.512	-
Rf values	0.06-0.07	0.08	0.09-0.11	0.12-0.13	0.14	0.16-0.17	0.19	0.21	0.22-0.23	0.25

*Based on the R.H.S colour chart
P = Parent
M = Spontaneous mutant
RON = Raja Surendra Singh of Nalagarh

pigments having Rf values ranging from 0.06-0.10, 0.11-0.13, 0.14-0.18, 0.19-0.22, 0.23-0.25 and above 0.25 was recorded in the 17 genotypes of rose under the present study. The number of bands present was one in First Federal Gold and mutant of Kronenbourg ; 2 in Akito, Kronenbourg and the mutants of 5kr Paradise and First Federal Gold ; 3 in Paradise, Wouburn Gold, mutant of 4kr and 5kr Paradise, Eiffel Tower parent and mutant ; 4 bands in Sindoor and mutant of Akito and Wouburn Gold ; 5 bands was observed in the cultivar Raja Surendra Singh of Nalagarh, respectively.

Authentic standards of anthocyanins namely cyanin chloride and pelargonidin chloride had Rf values 0.165 $\pm$ 0.2120 and 0.305 $\pm$ 0.1060, respectively. Pigment bands in rose genotypes showing Rf values 0.16 to 0.17 matched with that of cyanin standards. All the genotypes except genotypes Kronenbourg and First Federal Gold had cyanin pigments.

4.3.2 Hibiscus Cultivars

4.3.2.1 Anthocyanin

Considerable variation was observed in the petal colour among the fourteen hibiscus cultivars studied. Based on the petal colour the cultivars are grouped as red, orange/orange yellow and yellow/yellow orange.

With respect to the cultivars Red Saturn, Chitralekha, Priya, Aikta and Tribal Queen grouped under red, showed variation in the anthocyanin content. Out of the five cultivars Red Saturn recorded (3.9342) maximum anthocyanin with turkey red colour followed by Priya with rose bengal (3.0989), Tribal Queen (2.8631) with cardinal red and Aikta (2.3440) with post office red petal colour indicating that darker the petal colour shade higher was the anthocyanin content. Minimum amount of anthocyanin was recorded in Chitralekha (0.7863) with china rose petal colour (Table 48).

The culivars Ashirwad, Arunodaya, Dilruba, Nartaki and Phulkari belonging to orange/orange yellow group also differed considerably in the anthocyanin content. Ashirwad with orange yellow petal colour recorded the highest anthocyanin content (3.2322) followed by Nartaki (2.7632) with marigold orange petal colour and Dilruba with golden buff petal colour recorded minimum (0.6411) anthocyanin content (Fig 22).

Cultivars Basant, Banazeer, Nazneen, Ratna and Shanti grouped under yellow/yellow orange group showed in general, less anthocyanin content compared to the red group. Cultivar Nazneen (3.0741) with tangerine orange petal colour had maximum anthocyanin (3.0741) content and Shanti with primrose yellow petal colour had minimum anthocyanin content (0.0652).

4.3.2.2 Leucoanthocyanin

From among the red group Priya (0.3756) recorded maximum amount of leucoanthocaynin content with rose bengal petal colour followed by Aikta (0.2268) with post office red colour, whereas the other cultivars recorded moderate to low leucoanthocyanin irrespective of the flower colour shades (Fig 23).

With respect to the orange/orange yellow group Ashirwad, Arunodaya, Dilruba, Nartaki and Phulkari showed considerably increased amount of leucoanthocyanin content when compared to the red group of cultivars. Nartaki recorded the maximum (0.3890) leucoanthocyanin followed by Phulkari (0.3262) having marigold orange and delft rose as flower colours, indicating higher leucoanthocyanin content in darker flower colour shades (Table 48).

Out of the 5 cultivars grouped under yellow/ yellow orange, Nazneen (0.2904) followed by Ratna (0.2190) recorded higher leucoanthocyanin content

Table 48 : Petal colour, Anthocyanin, leucoanthocyanin, Flavonol and Carotenoid contents in Hibiscus cultivars (Values mean ± SE of 3 analysis)

Cultivars	Petal Colour*	Anthocyanin** (OD at 530nm)	Leucoanthocyanin (OD at 550nm)	Flavonol (mg% as phlorogluci-nol)	Carotenoids (mg/100g as /3-Carotene)
		Red Group			
Alkta	Post office red (45B) with cardinal red (40C) base	2.3440 ± 0.5604	0.2268 ± 0.1309	34.57 ± 0.8241	0.0030 ± 0.1749
Chitralekha	China rose(58D)	0.7863 ± 0.3219	0.0763 ± 0.0440	33.86 ± 0.7921	0.3106 ± 0.1794
Priya	Rose bengal(57C) with cardinal red(53A) base	3.0989 ± 0.9431	0.3756 ± 0.2168	42.09 ± 0.9436	0.2610 ± 0.1507
Red Saturn	Turkey Red (52A)	3.9342 ± 0.4260	0.1620 ± 0.0935	20.94 ± 0.3783	0.0632 ± 0.0346
Tribal Queen	Cardinal Red(53C) with dark purple red base	2.8631 ± 0.2932	0.0761 ± 0.0439	29.57 ± 0.5977	0.0903 ± 0.0521
		Orange/ Orange Yellow Group			
Ashirwad	Orange yellow(29A) with cardinal red(53A) centre	3.2322 ± 0.8032	0.2642 ± 0.1525	11.76 ± 0.2076	0.2343 ± 0.1354
Arunodaya	Nasturtium orange (25B) with rose bengal base (59B)	2.0461 ± 0.2938	0.2143 ± 0.0659	19.37 ± 0.3391	0.4862 ± 0.2807
Dilruba	Golden Buff(49C) with azalea pink(39D) base	2.0120 ± 0.2643	0.3203 ± 0.1849	32.83 ± 0.7893	0.4293 ± 0.2478
Nartaki	Marigold orange (28B) with cardinal red (53B) base	2.7632 ± 0.3240	0.1890 ± 0.1091	29.20 ± 0.5019	0.3493 ± 0.2016
Phulkari	Delft rose(46D) with spinel red(54B) base	2.0431 ± 0.6941	0.3262 ± 0.1883	19.14 ± 0.3178	0.5023 ± 0.2905
		Yellow / Yellow Orange Group			
Banazeer	Bright yellow with Neyron rose(55B) base	0.1772 ± 0.9450	0.0396 ± 0.0229	14.29 ± 0.2442	0.3420 ± 0.0808
Basant	Sulphur Yellow (6A)	0.4672 ± 0.6485	0.1548 ± 0.0893	6.15 ± 0.1204	0.4646 ± 0.1528
Nazneen	Tangerine Orange(24B) with red & mauve base	3.0741 ± 0.7944	0.2904 ± 0.1678	30.19 ± 0.6108	0.2924 ± 0.0533
Ratna	Mandarin Red(50A) with orange red and white centre	1.8243 ± 0.4382	0.2190 ± 0.1264	18.66 ± 0.3120	0.0510 ± 0.2604
Shanti	Primrose Yellow(4D) with Tyrian purple(57A) base	0.0652 ± 0.3047	0.1217 ± 0.0703	17.22 ± 0.2981	0.1372 ± 0.0791

* Based on R. H. S colour chart

** 0.5g petal in 100ml 1% acidic methanol

Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 22: Anthocyanin and flavonol pigment content in hibiscus cultivars

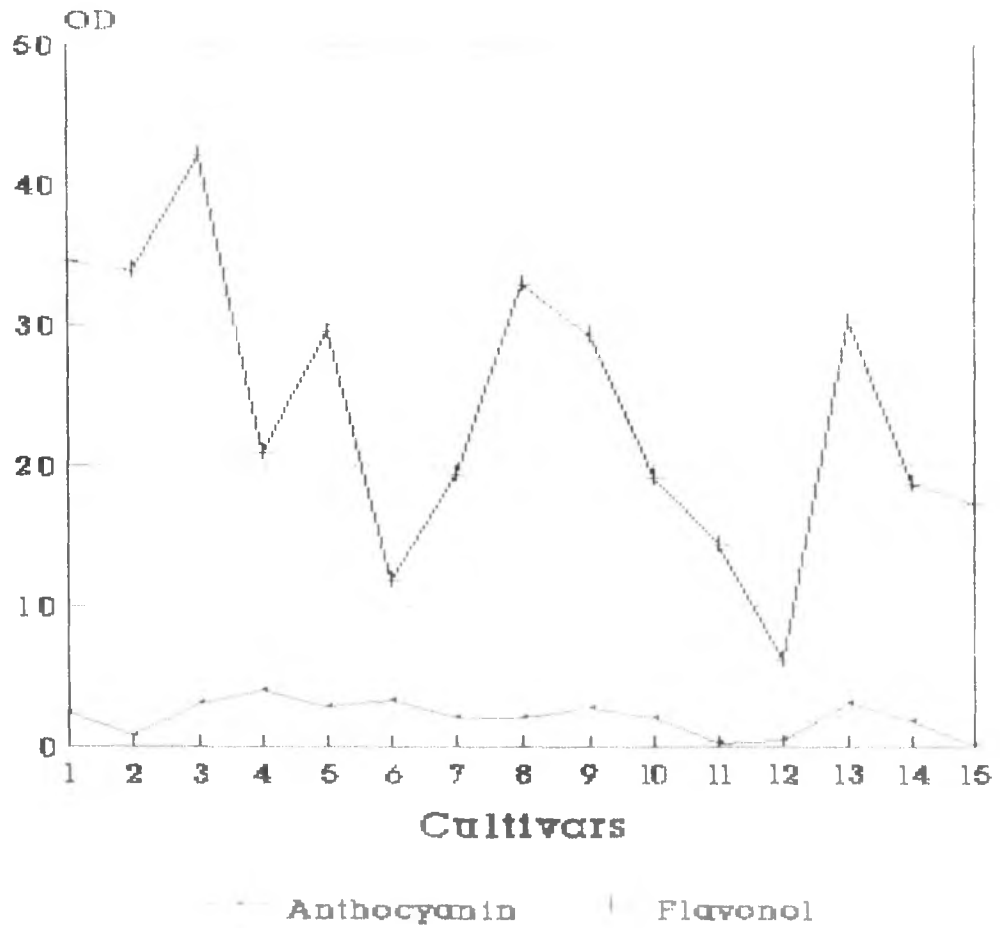
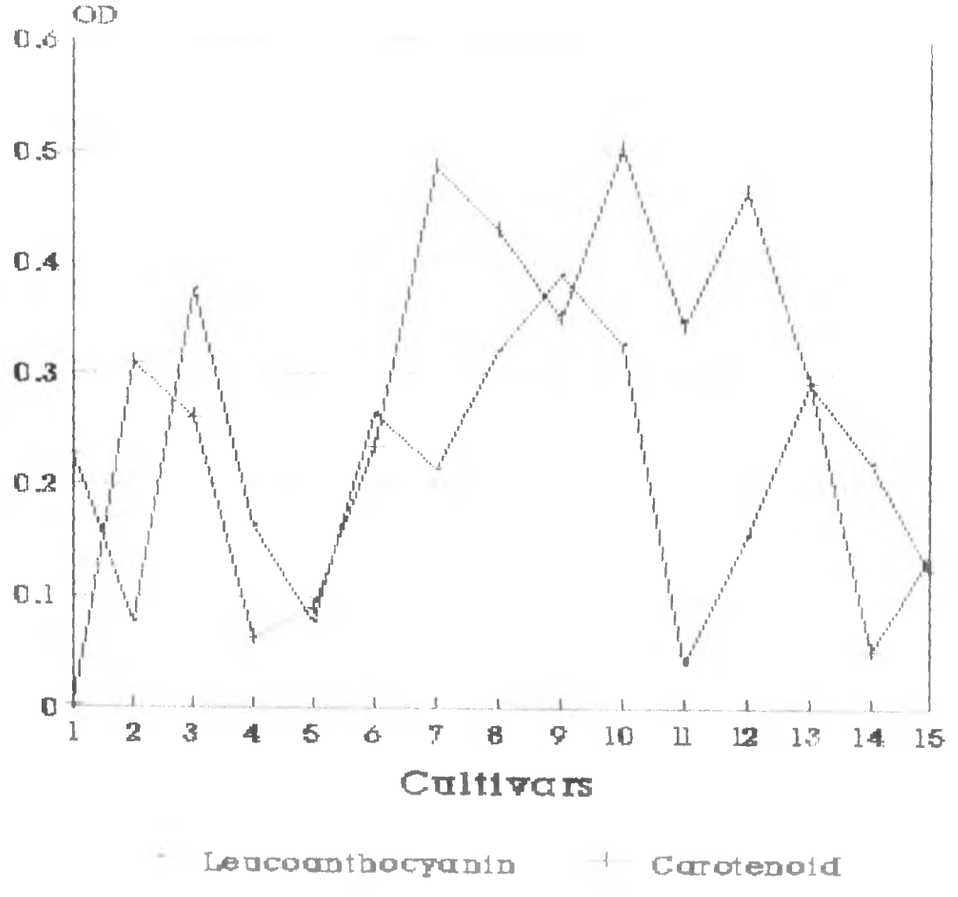


Fig 23: Leucoanthocyanin and carotenoid pigment contents in hibiscus cultivars



with tangerine orange and mandarin red petal colours and Banazeer with light shaded yellow colour exhibited lesser (0.0396) leucoanthocyanin content.

4.3.2.3 Flavonols

Variation in the flavonol contents among different groups of hibiscus cultivars was observed in the present study. Cultivars belonging to red group namely Priya with rose bengal (42.09) followed by Aikta with post office red (34.57) and Chitralekha with china rose (33.86) flower colours recorded the higher flavonol contents, whereas the other cultivars had moderate amount of flavonols (Fig 22).

The petal flavonol content estimated among the orange/orange yellow group of cultivars also showed moderate amount of flavonol content with the maximum observed in cultivar Dilruba (32.83) having golden buff colour. Darker petal colour shades resulted in higher flavonol content (Table 48).

The yellow/yellow orange group had less flavonol content compared to red and orange groups. Cultivar Nazneen (30.19) with tangerine orange and Ratna (18.66) with mandarin red petal colour recorded higher flavonol content and the cultivar Shanti with primrose yellow flower colour recorded less (6.15) flavonol content among the yellow group.

4.3.2.4 Carotenoids

The total carotenoid content varied considerably among the three groups of hibiscus cultivars and also in the cultivars within a particular colour group. Cultivar Chitralekha (0.3106) with china rose and Priya (0.2610) with rose bengal petal colour exhibited higher carotenoid content when compared to other cultivars. Tribal Queen with cardinal red petal colour (0.0903) recorded minimum

Table 49 : Paper chromatographic separation of anthocyanin pigments in the red grouped petal of Hibiscus cultivars (Rf values mean ± SE of 3 analysis)

RED GROUP

No of bands	Aikta	Chitralekha	Priya	Red Saturn	Tribal Queen
1	0.064 ± 0.037 (Red 43D)	-	-	-	-
2	-	-	0.092 ± 0.053 (Red purple 70B)	-	-
3	-	-	-	0.124 ± 0.071 (Red purple 64B)	0.118 ± 0.068 (Purple 70B)
4	0.135 ± 0.078 (Red 41D)	0.136 ± 0.078 (Purple 58B)	0.139 ± 0.080 (Purple red 81C)	-	0.149 ± 0.086 (Purple 72D)
5	-	-	0.166 ± 0.096 (Red 57B)	0.158 ± 0.091 (Red purple 62B)	-
6	-	0.187 ± 0.108 (Violet 84C)	0.198 ± 0.114 (Greyed orange 170C)	0.191 ± 0.110 (Red purple 65D)	0.185 ± 0.105 (Purple 78C)
7	0.212 ± 0.122 (Red 42D)	-	0.219 ± 0.126 (Greyed orange 170D)	0.217 ± 0.125 (Red 52A)	-
8	-	-	0.233 ± 0.134 (Purple violet 82D)	0.238 ± 0.137 (Red purple 71B)	0.227 ± 0.131 (Red purple 64B)
9	0.253 ± 0.46 (Red purple 21B)	-	-	-	0.251 ± 0.145 (Red purple 53B)
10	0.277 ± 0.160 (Red purple 72D)	0.278 ± 0.161 (Red purple 70B)	-	0.267 ± 0.154 (Red violet 80D)	0.279 ± 0.161 (Red purple 70C)
11	0.326 ± 0.188 (Red purple 71C)	-	0.309 ± 0.178 (Purple violet 80C)	0.326 ± 0.188 (Red violet 71C)	0.322 ± 0.186 (Red purple 41B)
12	0.417 ± 0.240 (Purple violet 80D)	-	-	0.415 ± 0.238 (Violet 85D)	-
13	-	-	-	-	0.460 ± 0.265 (Red 41C)
Petal Colour*	**Post office red (45B) with cardinal red (53C) base**	**China Rose(58D)**	**Rose bengal(57C) with cardinal red(53A) base**	**Turkey red(52A)**	**Cardinal Red(53C) with dark purple red base**

*Based on the R.H.S colour chart
Absent

Fig 24: Paper chromatographic separation of anthocyanin pigments in red grouped hibiscus cultivars

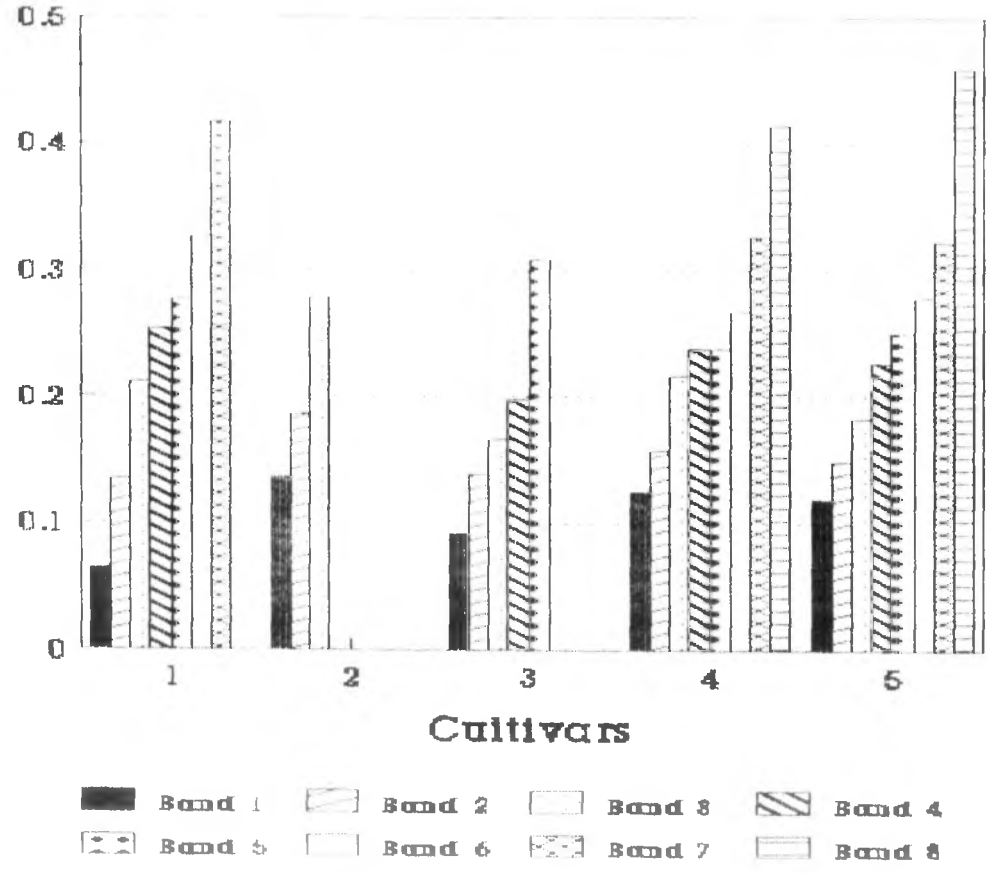


Plate 30 : Photograph showing the flower colour of hibiscus cultivars from left - Phulkari, Basant and Chitralekha

Plate 31 : Photograph showing the flower colour of hibiscus cultivars from left - Ashirwad, Banazeer and Nazneen

Plate 32 : Photograph showing the flower colour of hibiscus cultivars from left - Red Saturn, Tribal Queen and Nartaki

carotenoid content. The red petal colour shaded cultivars had lesser carotenoids as compared to yellow petal coloured cultivars (Fig 23).

In orange/orange yellow group of cultivars Ashirwad, Arunodaya, Dilruba, Nartaki and Phulkari recorded considerably higher amount of total carotenoid contents having orange yellow, nasturtium orange, golden buff, marigold orange and delft rose petal colours, respectively. Maximum carotenoid content was observed in Phulkari (0.5023) followed by Arunodaya (0.4862) having darker petal colour shades (Table 48).

Yellow/yellow orange group of hibiscus cultivars contained more amount of carotenoids. Cultivar Basant (0.4646) with sulphur yellow followed by Banazeer (0.3420) with yellow flower colours had higher carotenoid content and Shanti with primrose white petal colour contained less (0.0791) carotenoid content in this group.

4.3.2.5 Paper Chromatographic Separation of Anthocyanin Pigments in Hibiscus cultivars

4.3.2.5.1 Red Group

Hibiscus cultivars grouped under the red petal colour recorded more number of anthocyanin bands imparting red colour shades. Out of the five cultivars under red group Red Saturn and Tribal Queen had 8 bands each with darker red petal colour shades. Red Saturn showed red purple band colour imparting to turkey red petal colour whereas in Tribal Queen, purple and red purple band colours lead to cardinal red petal colour. Cultivar Aikta had 7 anthocyanin bands ranging from red to red purple band colours responsible for dutch vermillon petal colours (Table 49).

Table 50 : **Paper chromatographic separation of anthocyanin pigments in the 0range/orange yellow group of petals in Hibiscus cultivars**

ORANGE / ORANGE YELLOW GROUP

No of Bands	Ashirwad	Arunodaya	Dilruba	Nartaki	Phulkari
1	-	0.117 ± 0.062 (Orange red 57B)	0.125 ± 0.072 (Orange 26B)	0.111 ± 0.064 (Orange 25D)	0.115 ± 0.066 (Purple 54A)
2	0.136 ± 0.078 (Orange 52B)	-	-	0.147 ± 0.085 (Orange 17B)	-
3	-	-	-	-	0.164 ± 0. 095 (Orange 46D)
4	-	0.188 ± 0.108 (Orange 52C)	0.187 ± 0.108 (Orange 24C)	0.184 ± 0.133 (Orange yellow 48A)	-
5	0.207 ± 0.119 (Yellow orange 16B)	-	0.210 ± 0.121 (Orange red 33C)	-	-
6	-	0.234 ± 0.135 (Orange red 32A)	-	-	0.231 ± 0.133 (Orange red 33C)
7	-	-	0.248 ± 0.143 (Greyed orange 49B)	-	-
8	-	0.268 ± 0.155 (Orange 39B)	0.270 ± 0.156 (Orange 39C)	-	-
9	-	0.306 ± 0.176 (Orange 25C)	0.327 ± 0. 188 (Orange 29C)	-	0.306 ± 0. 176 (Orange 26B)
10	-	-	-	-	0.357 ± 0.202 (Orange 29C)
11	0.382 ± 0.221 (Yellow orange 29B)	-	-	-	-
12	-	0.410 ± 0.237 (Orange red 29C)	-	-	
Petal colour*	**Orange Yellow(29A) with Cardinal Red(53A) centre**	**Nasturtium orange (25B) with rose bengal (57B) base**	**Golden Buff(49C) with azalea pink(39D) base**	**Marigold orange (28B) with cardinal red (53B) base**	**Delft rose(46D) with spinel red(54B) base**

***Based on the R.H.S colour chart**
- Absent
Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 25: Paper chromatographic separation of anthocyanin pigments in orange/orange yellow grouped hibiscus cultivars

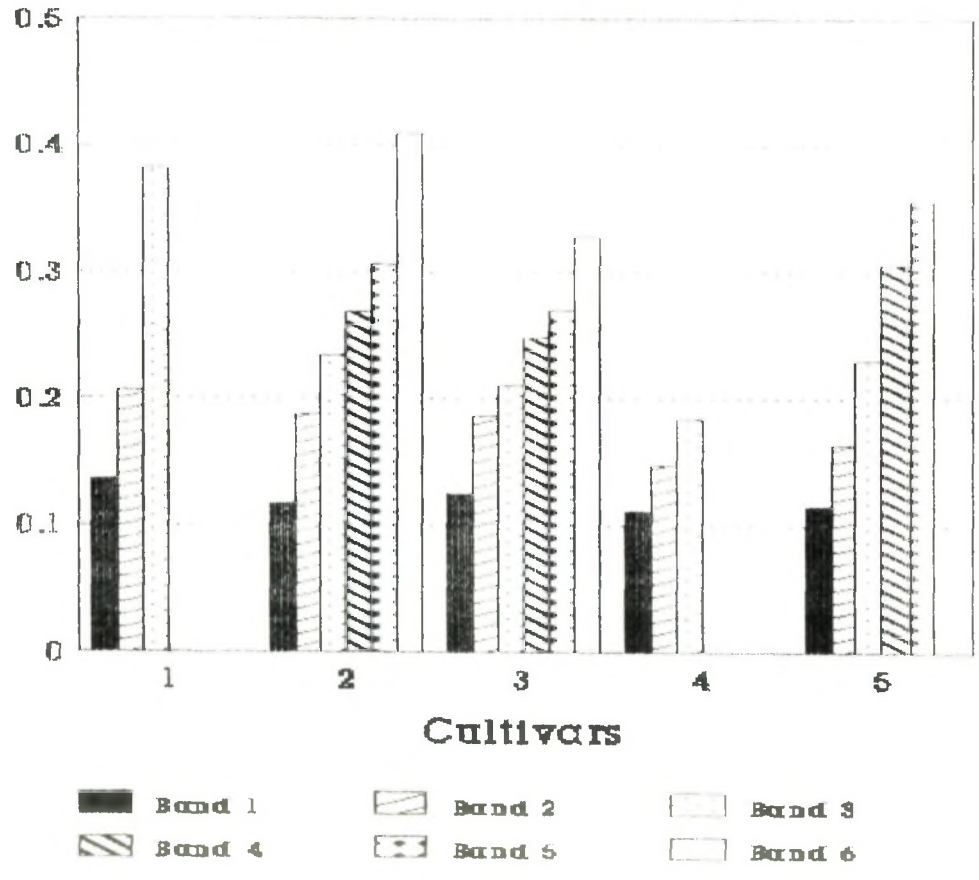


Table 51 : **Paper chromatographic separation of anthocyanin pigments in the yellow/yellow orange grouped petals of Hibiscus cultivars**

YELLOW / YELLOW ORANGE GROUP

No of bands	Banazeer	Basant	Nazneen	Ratna	Shanti
1	0.047 ± 0.027 (Yellow 6C)	-	-	-	-
2	-	-	-	0.109 ± 0. 062 (Orange 26B)	-
3	0.126 ± 0.073 (Yellow 8C)	0.127 ± 0.073 (Yellow 5C)	-	-	-
4	-	-	0.146 ± 0.084 (Orange 26B)	-	-
5	-	0.159 ± 0.092 (Yellow 7D)	-	0.157 ± 0.090 (Yellow Orange 50B)	0.154 ± 0.089 (Yellow 158A)
6	-	0.182 ± 0.105 (Yellow 10C)	-	-	0.180 ± 0.104 (Yellow 149C)
7	-	-	0.235 ± 0.136 (Yellow Orange 11B)	-	
Petal colour*	**Bright yellow with neyron rose(55B) base**	**Sulphur Yellow(6A)**	**Tangerine Orange(24B) with red & mauve base**	**Mandarin Red(50A) with orange red and white centre**	**Primrose yellow(4D) with tyrian purple(57A) base**

***Based on the R.H.S colour chart**

- Absent

Values within paranthesis indicate band numbers as per R.H.S colour chart

Fig 26: Paper chromatographic separation of anthocyanin pigments in yellow/yellow orange grouped hibiscus cultivars

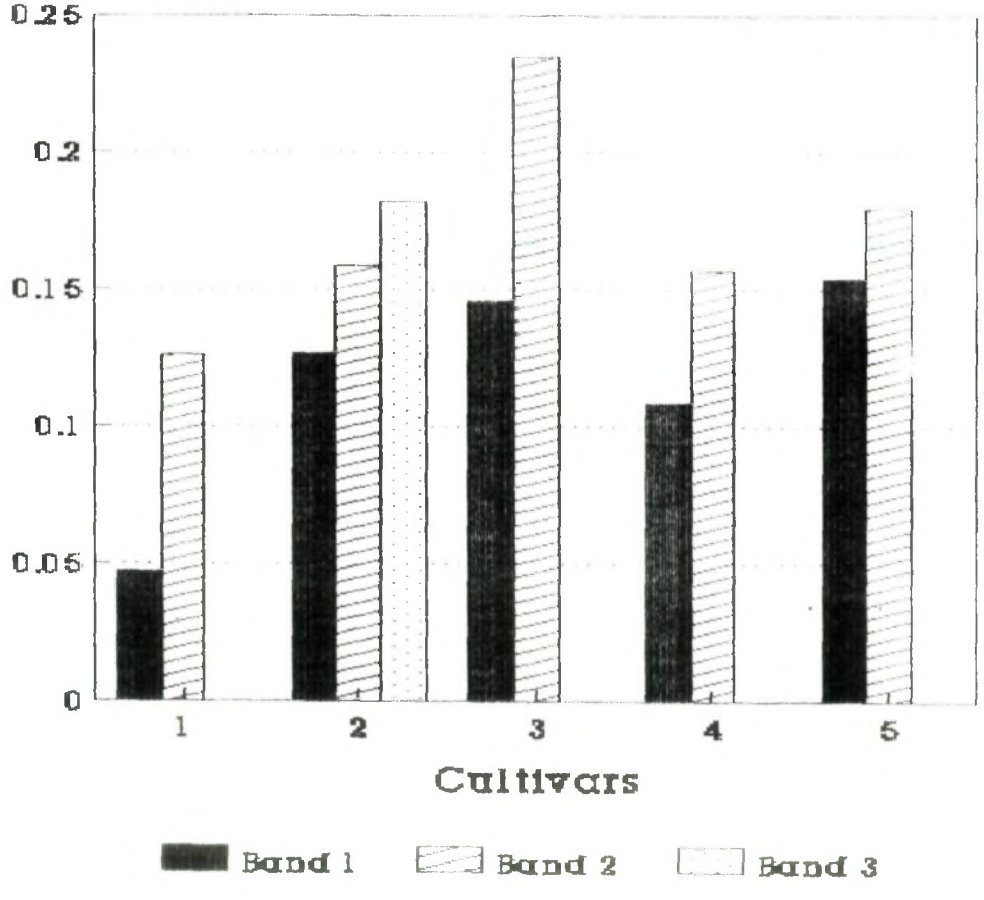


Plate 33 : Photograph showing the flower colour of hibiscus cultivars from left - Aikta, Dilruba and Arunodaya

Plate 34 : Photograph showing the flower colour of hibiscus cultivars from left - Priya, Shanti and Ratna

Cultivar Chitralekha had 3 anthocyanin bands with purple to violet band colour giving rise to china rose petal colour shades. Lesser the colour intensity of petals lesser was the number of anthocyanin bands (Fig 24). Chitralekha with china rose petal colour had lesser concentration of anthocyanins whereas cultivar Red Saturn, Priya, Aikta and Tribal Queen which had darker shades of red as petal colour recorded more anthocyanin concentration (Table 52).

4.3.2.5.2 Orange/Orange Yellow Group

Cultivar Dilruba and Arunodaya had 6 anthocyanin bands with orange colour giving rise to golden buff and nasturtium orange petal colour. But in Phulkari out of the 5 anthocyanin bands though the first band was purple in colour, the other bands were all imparting orange colour which may be the cause for delft rose petal colour(Fig 25). Cultivar Ashirwad and Nartaki had three anthocyanin bands all having orange yellow colours imparting to orange yellow and marigold petal colour (Table 50).

Quantitative estimation of anthocyanin concentration in the orange/orange yellow group of cultivars indicated that it was moderate in Dilruba and Ashirwad which has imparted to the lighter hue of petal colours. Cultivar Phulkari had higher concentration which might have lead to the darker orange petal colour shade in this group of hibiscus cultivars (Table 52).

4.3.2.5.3 Yellow/Yellow Orange Group

The cultivars grouped under yellow/yellow orange petal colours exhibited lesser number of anthocyanin bands as compared to red and orange group of cultivars. Ratna and Nazneen had 2 bands of anthocyanin where two bands were yellow orange in colour and the other two bands gave orange colours (Fig 26). Cultivar Banazeer and Shanti had 2 bands, Basant had 3 anthocyanin bands all of

Table 52 : Colour intensity of anthocyanin bands separated by paper chromatography in the petals of Hibiscus cultivars (OD values at 530nm)

Genotype	OD values at 530nm														
Red Group															
Aikta	0.206	-	-	0.246	-	-	0.290	-	0.332	0.412	0.462	-	-	0.479	-
Chitralekha	-	-	-	0.141	-	0.158	-	-	-	0.207	-	-	-	-	-
Priya	-	0.181	-	0.212	0.252	0.270	0.283	0.294	-	-	0.315	-	-	-	-
Red Saturn	-	-	0.242	-	0.253	0.296	0.302	0.338	-	0.416	0.478	-	-	0.486	-
Tribal Queen	-	-	0.215	0.240	-	0.263	-	0.315	0.336	0.421	0.474	-	-	-	0.542
Orange/Orange Yellow Group															
Ashirwad	-	-	-	0.191	-	-	0.216	-	-	-	-	-	0.252	-	-
Arunodaya	-	-	0.115	0.125	-	0.186	-	-	-	-	-	-	-	-	-
Dilruba	-	-	0.119	-	-	0.147	0.163	-	0.220	0.242	0.280	-	-	-	-
Nartaki	-	-	0.115	0.125	-	0.186	-	-	-	-	-	-	-	-	-
Phulkari	-	-	0.194	-	0.256	-	-	0.276	-	-	0.315	0.352	-	-	-
Yellow/Yellow Orange Group															
Banazeer	0.201	-	0.242	-	-	-	-	-	-	-	-	-	-	-	-
Basant	-	0.206	-	-	0.242	0.260	-	-	-	-	-	-	-	-	-
Nazneen	-	-	-	0.161	-	-	0.215	-	-	-	-	-	-	-	-
Ratna	-	0.125	-	-	0.155	-	-	-	-	-	-	-	-	-	-
Shanti	-	-	-	-	0.061	0.193	-	-	-	-	-	-	-	-	-
Rf values	0.04-0.06	0.09-0.10	0.11-0.12	0.13-0.14	0.15-0.16	0.18-0.19	0.20-0.21	0.22-0.23	0.24-0.25	0.26-0.27	0.30-0.32	0.35-0.36	0.38	0.41	0.46

* Based on R. H. S colour chart

- Absent

Plate 35 . Paper chromatographic separation of flower pigments in Rose cultivars and their mutants

1 . Cv. Paradise
2 . 3kr Induced mutant of Paradise
3 . 4kr Induced mutant of Paradise
4 . 5kr Induced mutant of Paradise
5 . 6kr Induced mutant of Paradise
6 . Eiffel Tower
7 . Spontaneous mutant of Eiffel Tower
8 . Akito
9 . Spontaneous mutant of Akito
10. Wouburn Gold
11. Spontaneous mutant of Wouburn Gold
12. Kronenbourg
13. Spontaneous mutant of Kronenbourg
14. First Federal Gold
15. Spontaneous mutant of First Federal Gold
16. Cv. Sindoor
17. Cv. Raja Surendra Singh of Nalagarh

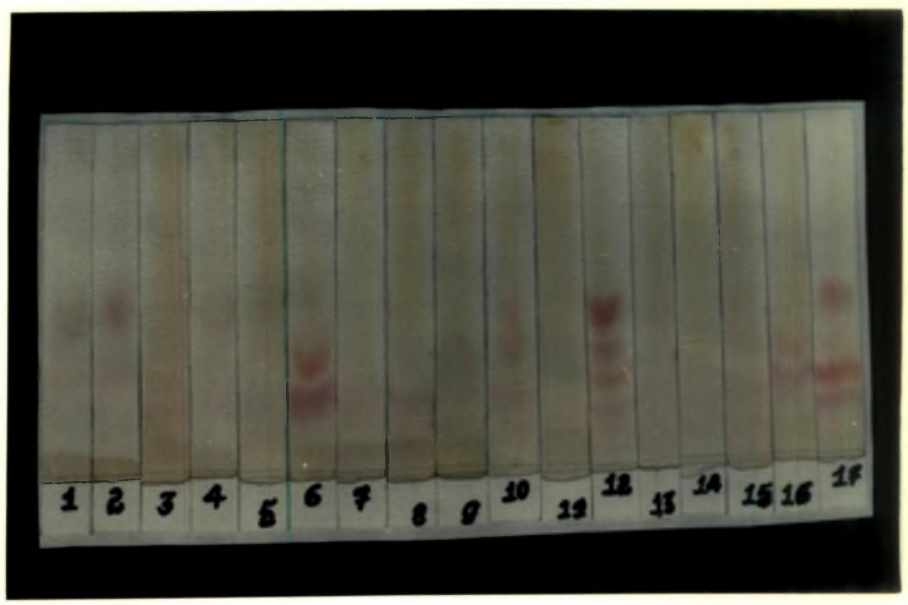

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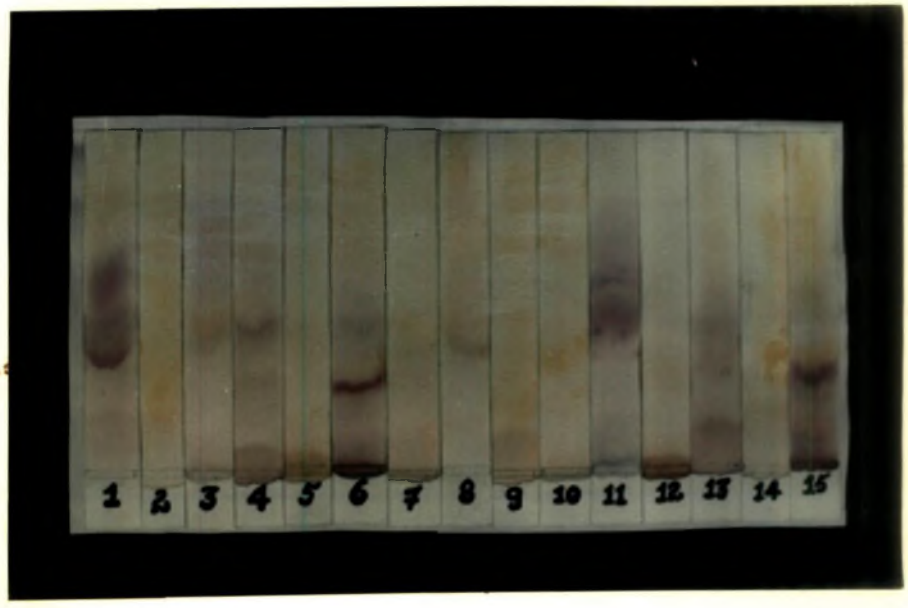
Plate . 36. Paper chromatographic separation of flower pigments in Hibiscus cultivars

1 . Arunodaya
2 . Red Saturn
3 . Dilruba
4 . Chitralekha
5 . Ratna
6 . Nazneen
7 . Ashirwad
8 . Phulkari
9 . Priya
10 . Aikta
11 . Nazneen
12 . Tribal Queen
13 . Shanti
14 . Basant
15 . Nartaki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which had yellow band colour resulting in the appearance of yellow petal colour in Banazeer, primrose yellow in Shanti and sulphur yellow in Basant, respectively (Table 51).

Quantitative determination of anthocyanin contents in the yellow group indicated that the concentration was comparatively moderate in the cultivars Ratna and Nazneen, whereas Basant and Banazeer had higher concentration which might have lead to lighter yellow petal colour shades. Cultivar Shanti recorded lesser concentration with lighter yellow petal colour (Table 52).

4.3.2.6 Chemical identification of anthocyanin pigments in Hibiscus cultivars

Paper chromatography of anthocyanin pigments in different hibiscus cultivars used for the present investigation has indicated varietal differences in the number of anthocyanin pigments present in each cultivars. Two to 8 bands of anthocyanin pigments having Rf values ranging from 0.04-0.09, 0.10-15.0, 16.0-0.21, 0.22-0.27, 0.30-0.33, 0.34-0.37, 0.38-0.42 and 0.43-0.46 was recorded in the 15 cultivars of hibiscus studied.

The number of bands present was two in cultivars Nazneen, Shanti, Banazeer and Ratna ; three in Chitralekha, Ashirwad, Basant, Nartaki ; five bands in Phulkari ; six bands in Dilruba and Arunodaya ; seven bands in Aikta, Priya and eight bands in Red Saturn and Tribal Queen, respectively. Authentic standards of anthocyanins namely cyanin chloride and pelargonidin chloride had Rf values 0.165 ± 0.2120 and 0.305 ± 0.1060. Pigment bands in hibiscus showing Rf values 0.15 to 0.16 and 0.30 to 0.32 matched with that of cyanin and pelargonidin standards.

DISCUSSION

V. DISCUSSION

Mutations provide the material for recombination and selection for plant breeding. All plant breeding programmes now include the production of artificial mutations of crop plants with the help of mutagenic agents. The sensitivity of an organism to a mutagen is not only dependent on the mutagen employed but also on a number of other factors such as genotypic constitution (Abraham and Desai, 1976), chromatin content, physiological state (Strietberg, 1977) and conditions before, during and after treatments. Use of induced mutagenesis appears to be more relevant to rose in view of the constraints in hybridization.

Mutations induced by radiation treatments are being increasingly applied especially with asexually propagated ornamentals. Moreover, the selection of ornamentals is very easy when such visible characters like flower colour, form, size, leaf forms, growth habits etc. are concerned. The mutation spectrum as well as the frequency greatly depends on the genetic constitution of the treated material. Mutations are limited to autonomous layers, hence the final result will nearly always be a periclinal chimera (Sparrow et al ., 1968). Varieties differ in their sensitivity to radiation. which establishes the point that genotypic control occurs within the species in the matter of sensitivity to radiation. The dividing cells are more radiosensitive than the non dividing ones (Das, 1971). Gottschalk (1972) stated that true pleiotropic gene action and the action of simultaneously mutated neighbouring genes are two different phenomenon which normally cannot be distinguished from each other. The detection of the optimal combination of a given mutant gene and distinct genetic background is a matter of chance.

The degree of deviation from existing plant types would vary with the extent of alterations attempted. Mutation breeding has always been valued for inducing one or two changes in a superior genotype. Changing the spectrum of mutations in a predictable manner and thereby achieving directed mutagenesis is an important

goal of current mutation research. The kind of mutation one may expect is completely dependent on the genotype (Broertjies, 1963). First artificial mutation was induced by Sparrow (1964) in woody plants and confirmed that every new plantlet is derived from a single cell of the petiole.

Further, induction of desirable mutation occurred in combination with undesirable changes with several closely linked or neighbouring genes (Nybom and Koch, 1965) This is due to the selection of individuals carrying main mutations, but free from detrimental effects of the other mutations which affects less intensely other agronomic traits. The usefulness of any mutagen depends on mutagenic effectiveness, relation of mutation frequency to doses, the production of desirable changes free from association with undesirable changes (Evans, 1965). Increased efficiency may be expressed either as higher ratios of desirable mutations to chromosomal aberrations or lethality when desirable mutations are sought or as higher ratios of chromosomal aberrations to other parameter, which can be altered by various modifying factors. Hence, appropriate method of induction of mutation are of high relevance to meet the requirement efficiently. Efficiency is defined therefore as the frequency of desirable mutations in relation to non desirable biological alterations.

Artificial induction of mutations offer a new possibility in crop improvement by offering the benefits of changing a single character without altering the background genotype. Such alterations are probably due to a single gene and thought to be accounted for some modifications of molecular structure (Muller, 1927). Mutagenesis reflects not only the structure of DNA, but also the plasm in which it resides. According to Gupta (1966) mutations may be considered as a particular type of biochemical lesions which is a transmissible alteration of the genetic information after cell division.

Other effects include inhibition of DNA synthesis during interphase, inhibition of oxidative phosphorylation, enzymatic destruction of protein structures, delayed mitosis and meiosis and reduction in survival and reproductive capacity. Interphase death is due to a disturbance of mitotic activity and occurs usually at high treatment dosages of gamma irradiation. Gamma rays is known to affect both vegetative and reproductive output in the immediate generations. Numerous histological and cytological changes are said to be induced by ionizing radiations (Swim, 1979). After irradiation one may expect enormous variability for almost any character because of the large heterozygosity and the high ploidy level (Suda and Matsubara, 1977). One must imply the first thing in considering the improvement of directly visible characters. The ploidy and the heterozygosity resulting in a complex pattern of inheritance of most characters, so that the determination of the best starting material is generally a matter of experience.

Variability is the product of spontaneous mutation and hybridization, followed by recombination and natural selection. Since the discovery of Muller (1927) and Stadler (1928) ionizing radiations could induce hereditary changes thereby increasing the rate of mutation many times, than that occurring in nature. In rose spontaneous mutations occur frequently and have caused a valuable increase in genetic variation. Conventional breeding is hampered due to variation in ploidy level, high sterility, seed dormancy accompained with poor seed germination suggested by Donini (1975). Spontaneous mutations arise as a result of errors during DNA replication, repair and recombination. Significant proportion of spontaneous mutations originate from insertion of mobile genetic elements into the genes (Zimmerman and Hitchock, 1951).

The stimulatory action may be due to more physiological activity and the ability of the variety to repair cellular damages after the treatment (Jicinska, 1981). Increased flower yield is due to changes in polygenic architecture of the

genotypes by increasing metabolic activity. Induced mutations contribute by increasing genetic variability. Presently, the investigation was taken up in spontaneous mutants viz.. Akito, Eiffel Tower, First Federal Gold, Kronenbourg, Tzigane and Wouburn Gold and induced mutations on cultivars viz., Paradise, Raja Surendra Singh of Nalagarh and Sindoor along with their respective parents to study the detailed morphological and floral characteristics. The studies were also undertaken on the pigments of these genotypes and their mutants in rose and new cultivars of hibiscus developed at the Indian Institute of Horticultural Research, Bangalore. The detailed morphological aspect and pigment status are discussed along with the available literature as follows.

5.1 EXPERIMENT I : MUTATION BREEDING STUDIES

5.1.1 MORPHOLOGICAL STUDIES ON INDUCED AND SPONTANEOUS MUTATION

5.1.1.1 Growth Habit

The study on growth habit of different rose cultivars revealed either upright or spreading habit. It was observed that in all the three genotypes of induced mutants it exhibited spreading habit which was onpar with the parent irrespective of the treatment differences. It was observed that 6kr treated plants exhibited upright habit whereas all the other mutants showed vigorous and bushy growth, whereas Paradise and Raja Surendra Singh of Nalagarh were found to be comparatively more vigorous, compact than their respective mutants. In the present investigation abnormality in plant growth habit displayed a perfect linearity in the dose. The frequency increased with decrease in the dosage. The reason that could be attributed to is the extra chromosomal breakages (Pal, 1962).

It was observed that natural mutants of Kronenbourg and Wouburn Gold are more vigorous and bushy compared to their parent, whereas mutant of Tzigane

and Eiffel Tower are comparatively less vigorous in growth. In the present study almost all the genotypes was found to have moderate growth habit. Similarly, Hessayon (1981) reported vigorous growth habit under England conditions. Pal (1972) mentioned that the conditions under which roses are cultivated in temperate countries differ very much from those prevailing in other parts of India. The variation in the growth habit perhaps seems to be due to environmental conditions, varietal characters and age of the plants.

Morphologically in induced mutants the growth habit of all the genotypes selected were vigorous, bushy and spreading in nature with an exception of few erect and upright growth habit. Pal (1972) mentioned that the conditions under which roses are cultivated have a direct effect on growth. The variation in the growth habit perhaps seems to be due to environmental conditions, varietal characters and age of the plant. From the results obtained it is clear that the mutants produced significantly more number of branches and leaflets which is a direct effect of desirable trait. With respect to the stem diameter, internodal length, leaflets and branches produced, they were not significantly different among different cultivars or among the mutants.

5.1.1.2 Sprouting and Survival Percentage

In the present study the buds irradiated by gamma rays reduced sprouting with increase in the dosage irrespective of the cultivars used. Among the three varieties used Sindoor was found to be more sensitive to higher exposures. Lower dosage had stimulatory effects from the results obtained. Survival percentage of the irradiated buds in VM1 generation is generally considered as one of the important criteria to estimate the dose levels for a particular mutagen and to a particular crop species. Radiation induced reduction in the activities of hydrolysis, resulting in lack of an energy source which may be the main cause for decreased

bud survival (Ruprecht. 1972). The other reasons may be chromosomal and extrachromosomal damage of the cells. The change was in the form of chimeras in somatic tissues.

In the present study survival percentage of the buds decreased with increase in gamma rays. These observations were in accordance with Zykov and Klimenko (1989), where survival percentage decreased greatly at increased doses and its response was linear between treatments and considerably dependent on sprouting percentage. According to Kaicker (1992a) reduction in sprouting may be due to the toxic effect of higher concentration of gamma rays and hastened metabolic activity at lower dosages. Criteria such as the relationship between lethality or sterility and mutational frequency, chromosomal aberrations in relation to mutations, the size of the mutated section and drastic changes as compared to micromutational events, have been used to assess the superiority of one mutagen over the other (Brock , 1972).

Reduction in sprouting in VM1 generation has been attributed to changes in the level of auxins and ascorbic acid content, physiological and biochemical disturbances, changes in enzymatic activity, chromosomal breakage inhibition with mitotic and DNA synthesis (Nakajima, 1973). The effect of gamma rays was more pronounced at increased doses in all the varieties. Lower doses had stimulatory effects from the results obtained in the present investigation.

In the present study the survival percentage of the buds decreased with increase in dosage of gamma rays. Similar observations have been made by (Zykov and Klimenko, 1988). Survival percentage decreased greatly at increased doses and its response was linear between treatments. Therefore, the buds survived and linearity deviated more at 6kr gamma radiation treatment and similarly displayed in all the other genotypes used. Number of buds killed increased

curvilinearily with increased radiations. Lethality was less at lower doses and increased at higher dosages. Until 4kr dosage lethality was linear, but above 4kr deviation in linearity was noticed. Survival of buds in VM1 generation is considered as one of the important indices to estimate effective dose levels ie the LD 50 level for a particular mutagen induced crops. All the three genotypes responded more or less similar to the effect of gamma rays.

Differential response of genotypes for irradiation was evident where all the three varieties Paradise, Raja Surendra Singh of Nalagarh and Sindoor showed stimulatory effect at lesser dosages of 3kr treatment. Reduction in sprouting may be due to the toxic effect of higher concentration of gamma rays. The variations may be due to the greater tolerance of genotype and it is a fact that different varieties differ in their sensitivity to mutagenic treatments where lower the dosage metabolic activity will be hastened (Kukimara, 1975). Mutation frequency increases with increasing dosages but survival and capacity to regenerate decreased with decrease in dosage (Paparozzi, 1993). One must therefore choose some point between a low dose (100% survival & low mutation frequency) and a higher dosage (low survival & higher mutation frequency). At high doses, too many mutational events may be induced with increased risk of a favourable mutation being accompanied by one or more unfavourable genetic changes (Donini, 1975).

5.1.1.3 Plant Height

Differences in plant height were observed between some of the original genotype and their mutants. In most of the cases considerable variation was observed in plant height among all the gamma ray treated cultivars. With respect to induced mutation increase in radiation dose decreased the plant height irrespective of the variety. It was recorded that at 3kr gamma ray treatment a

maximum plant height was recorded in Paradise (60 cm), Raja Surendra Singh of Nalagarh (39 cm) and Sindoor (53 cm), respectively. There was greater reduction in the number and length of branches as the exposure to gamma rays increased . This may be due to the drop in the level of auxin after exposure to ionizing radiations and chromosomal aberrations induced by radiation (Nakajima, 1965).

It was observed that in spontaneous mutants variation was noted among the mutants and their respective parents except in the cultivar First Federal Gold. Among the spontaneous mutants Eiffel Tower had the maximum height (138 cm) followed by Wouburn Gold (66 cm). But not much difference was observed in all other genotypes in all the three consecutive generations under investigation. This variation in plant height almost supports the observations made by Antonynk (1991) where the plant height of hybrid tea bushes ranged from about 60 to 150 cm. It was observed that the mutants of Eiffel Tower and its parents are comparatively tall. Lata (1973) worked on 53 hybrid tea roses and observed that the plant height ranged from 42 to 98 cm at the first peak flowering, and from 51 to 146 cm at the second peak indicating that plant height also varied from season to season which was in accordance with the present observation recorded.

5.1.1.4 Stem and Spines

Significant difference with regard to the spine count was recorded in induced mutants, where lesser the dosage more was the spine count and viseversa irrespective of the cultivars. It was observed that treated buds exhibited the highest spine count and found to be on par with the control treatments. However, Datta (1990a) reported that there was no difference in spine number between the original cultivar and its gamma ray induced mutants except in Sukumari were the number was significantly high.

Variation in the colour of the stem in different rose genotypes were not found to be considerable. It was recorded that in most of the genotypes the colour of the young stem was either light green with reddish brown tinge or light reddish brown with green tinge. However in First Federal Gold and Tzigane the stems were dark brown colour. Kupuswamy and Sampath (1956 and 1957) also reported slight variation in the colour of tender shoots and matured stem among the 77 rose cultivars.

Most of the genotypes of spontaneous mutation was found to be moderately spiny and number of spines ranged between 10 and 44 per ten centimeter cuttings from the base of the stem. However it was observed that there was not much difference in spine count between parent and spontaneous mutants. Moderate to high thorns were recorded with a range of 10 to 45 a maximum found in Kronenbourg mutant. This variation in the density and size of the spines seems to be due to varietal characters.

But significant difference was observed in the mutant of Akito (24.80) compared to the parent (10.20). The cultivar Wouburn Gold was found to be very spiny with as many as 32 spines compared to all other genotypes with medium sized spines. The spine size of Kronenbourg exhibited large sized spines with First Federal Gold having very small spines. Kuppuswamy and sampath (1956 and 1957) also noticed variation in the size of the spines. This variation in the density of the spines seems to be due to varietal characters. However internodal length and stem diameter both in spontaneous and induced mutants showed no significant difference among the treatments and was found to be on par with each other.

5.1.1.5 Foliage Characters

Hessayon (1981) reported that a typical rose leaf has a smooth surface and made up of three or five or seven leaflets. In the present study, it was observed that there was clear cut variation in foliage ranging from dark brownish red to reddish brown. The colour of the matured leaves in the present investigation revealed that 8 of the 12 genotypes had light green, 3 with dark green and with one coppery to light green leaves. Ueda (1994) reported that a typical rose leaf has a smooth surface and made up of 3 to 7 leaflets. In the present study the colour range was between light to dark green whereas size was medium to small except in Kronenbourg variety which produced huge sized leaves, with normal glossiness and the floriferousness.

There was not much variation in the leaf shape of differnt rose cultivars under the present investigation. The shape was elliptical in 5 cultivars and narrowly elliptical in rest of the genotypes. The number of leaflets per branch varied considerably among mutants and parents along with an increase in the leaf area in the mutants when compared to their parents, whereas in induced mutations with an increase in dosage there was a drastic reduction in the number of leaflets which directly influenced the individual and random leaf area to a considerable extent. In contrast to De loose's (1978) procedures irradiations are generally given during late summer. The doses are chosen to kill the main shoot meristem but to allow the axillary once to remain alive and develop into new shoots. They believe that there is a correlation between radiation damage and frequency of induced mutations. Variations in the leaf area may be due to change in hereditary material, biochemical changes and physiological changes brought about by the mutagen and also due to the random nature of gamma rays to induce mutants in rose genotypes(Nakajima, 1965).

Although 4 of the 12 genotypes had thin and smooth textured leaves, in four genotypes the leaves were leathery in texture and 4 genotypes which had distinctly glossy and shinny leaves. Hessayon (1981) stated that a typical rose leaf had a smooth surface, but the shinyness of the surface varied greatly. Some were highly polished as if they had been recently treated with oil, others were distinctly dull. Many varieties had leaves between these two extremes. Further he mentioned that the surface of the foliage was not always smooth. The rugosa group of shrubs had leaves which were deeply ribbed giving them a characteristically wrinkled effect.

5.1.1.6 Flower Characters

In the present study all the genotypes, with regard to flower characters, was invariably observed and showed remarkable variation. Datta and Gupta (1983) reported that flower colour changes in Contempo were due to quantitative and qualitative changes in pigments induced by irradiation. However, in the present investigation it showed that there was very wide range of variability in the flower characters both in spontaneous and induced mutants. The flower colour also slightly varied from season to season as reported by Pal (1982) in the cultivar 'Michalle Meilland' in which the soft pink colour of the flower changed to apricot-orange in another season.

There was not much of difference in flower diameter of spontaneous mutants. However mutant of Kronenbourg and First Federal Gold showed maximum flower diameter. The other genotypes recorded more or less equal flower diameter. Brock (1971) reported that the differences in petal number, size were observed between some of the original cultivars and their respective mutants. In most of the cases the petal size was reduced over the control and the reduction was significant. Significant increase in petal size was recorded in 'Pink Coronet' and its mutant of 'Salmon Beauty'.

With respect to induced mutations 3kr treatment in all the three cultivars subjected to irradiation was found to be the best in retaining the flower diameter. It was observed that with increase in dosage decrease in the flower diameter was recorded in all the three consecutive generations under study. Fresh weight of the flowers was found to be onpar between the parent and the mutants and with increase in dosage a decreased flower weight was recorded considerably. Thus the flower yield was directly correlated with the number of flower clusters. Increased irradiation dosage reduced drastically the total flower yield and in spontaneous mutants the number of flowers increased compared to their respective parents. Changes in the petal number was recorded between the original genotype and their mutants. Under study, except mutant of First Federal Gold (105) where the number of petal was increased to a greater extent when compared to its parent (88.40) including numerous small ones.

Number of petals in induced mutants was inversely proportional and drastically reduced the petal number in all the three induced mutants. This observation is in accordance with Datta (1994) where he supported that changes in the petal number was recorded in some of the induced mutants. Floral characteristics exhibited significant difference among parent and mutants in spontaneous and induced mutants. Flower diameter, clusters, petal number, number of flowers were all found to be on par with each other. However, Datta (1990b) reported that the differences in petal number were observed between some of the original cultivars and their mutants. Significant increase in the petal number was observed among the mutants compared to their respective parents. Several of the afore mentioned, hampering factors have little or no negative effects when mutation breeding is applied.

Variation in the fragrance of rose flowers was not considerable among all the genotypes under study, except Eiffel Tower which was found to be most

outstanding with high fragrant flowers among all the genotypes under study. Four out of 8 genotypes recorded moderate fragrance and others were found to be slightly fragrant, but however some genotypes lacked fragrance. (Gupta and Shukla, 1971) reported that the flowers were slightly fragrant in 'Doris Tystermann' and 'Lolitha'. In the present investigation it was observed that the flower fragrance seems mainly due to the stage of growth of the plants and weather conditions. Some roses are most fragrant in early flowering and others when fully opened. Similarly the warmth and high air humidity is known to enhance fragrance (Lata and Gupta, 1973) along with few genomic attributes.

5.1.2 Effect of Gamma Irradiation on Somatic Mutations

Many variegated, incurved flowers, small sized flowers were detected in irradiated plants. These changes persisted in subsequent vegetative generations and was considered to be cases of somatic mutations. No mutations was observed in VM1 generation other than few morphological but unstable mutants. According to Sparrow (1961) some of these morphological abnormalities or changes after irradiation might be due to mostly secondary effects of non-genetic physiological disturbances.

Second generation was scored for viable mutations throughout the crop period in all the three cultivars used. Among various doses of gamma rays used in the present study, 3kr to 4kr in Paradise, 6kr in Raja Surendra Singh of Nalagarh induced increased the frequency of viable mutations in both second and subsequent generations on the plant basis. The lowest frequency of viable mutations was observed at 5-6kr treatments, except in case of Raja Surendra Singh of Nalagarh with the spectrum of mutations on the growth habit, flower size, colour etc. The exact nature of induction of somatic mutations cannot be explained with certainty. They may include both gene and chromosomal mutations. Majority of mutational

events are of recessive types either being a true mutation or being the loss of genes. If the character to be improved is dependent on the presence of one or more dominant genes, the irradiation of cultivars recessive for these genes may be less hopeful.

Mutant of the flower petal variegation both in Paradise (3kr) and Raja Surendra Singh of Nalagarh (6kr) was observed. According to Kaicker and Swarup (1975) variegation might have been produced by nuclear or plastid mutations. They were of the view that spontaneous/induced plastid mutations produced a variety of phenotypes, such as cream, white, yellow and various shades of pale similar to those produced by nuclear mutations. This chimeric pattern of variegated petal depends on the occurrence of mutations at different growing points. The colour mutants and sports are generally periclinal chimeras and is genetically different from the original genotype. Consequently, one cannot correlate chromosomal aberrations or difference in the number with a flower colour change, unless one makes completely sure that the tissues compared are genetically identical (Brady,. 1982). This also holds good when irregularities in meiosis are correlated with flower colour changes. On the other hand, a positive correlation between the chromosome number and flower size is well determined in the present study of investigation.

In the present study reduced gamma irradiation in Paradise and Sindoor exhibited better mutation frequency and was vice-versa with respect to Raja Surendra Singh of Nalagarh. Higher doses generated a less wider range for all the characters studied. Range was maximum in M2 generations as observed. Accordingly, larger the mutation step of a given trait, lower will be the average vitality of the mutant, higher will be the probability of the mutants with good improved vitality and appearance (Khanna and Shukla, 1991). When cuttings or budwoods were irradiated with higher doses of ionizing radiations, there is a

greater intrasomatic selection and the mutated cells get eliminated due to competition from the surrounding normal cells.

Useful mutations, once obtained in vegetatively propagated crops can easily be maintained by clonal propagation (Usenbaer and Imankulova, 1974). Clonal formation and competition within a plant between mutated and non-mutated tissues, normally indicated diplontic or intrasomatic selections and are considered as the main bottle necks in mutation breeding of vegetatively propagated plants. The result of these constraints is a relatively low mutation frequency and probably a narrow mutation spectrum, whereas proper artificial induction of mutation by means of physical or chemical mutagens may increase the variation rate by thousand folds. Kaicker and Dhyani (1986). Datta (1991a) reported that chromosomal aberrations and aneuploidy plays an important role and there could be correlation between the dosage and the mutation spectrum in the sense that, an increased dosage would be preferred for instance to induce mutations based on loss or gain of chromosome whereas, low doses would be choosen to induce certain flower colour mutations.

5.1.3 CORRELATION STUDIES

To ensure a resonable change in the recovery of desired kind of mutants,the surviving M1 population is of critical importance. The ideal size of M1 on the other hand, will depend upon the nature of inheritance of the genes for the characters under consideration as well as the expected mutation rate (Valadon and Mummery, 1968). The inheritance of various other characters have also been worked out according to (Ueda, 1994). The characters in rose depends primarily on a single dominant gene with glossy leaves (vs dull), climbing habit (vs dwarf), double flowers (vs single) etc. While the characters dependent on interaction of many genes are vigorous, thornless, fragrant, stength of the neck, width of the leaf,

length of the stem, shape of the bud, flowers etc. Induction of mutations artificially must be the method of choice, providing inheritance to follow similar pattern to that of major genes.

Referring to mutations which brings about small phenotypic changes can be detected to study the population distribution. The phenotypic expression of a mutant allele is like all genetic characteristic, strongly influenced by environment. The requirements for proper expression of a mutant may be distinctly different from the requirements of the parental allele and therefore the mutant phenotype may not develop in a plant grown under the environmental conditions in which the mutagenized variety is normally cultivated (Sarkow, 1961). Correlations of two characters may be either due to similar genetic causes, linkage or environmental influence. If genetic correlations are high, the attempts will provide an hand in hand response for both the characters. Correlation studies will be worth while for dependent characters having high economic value with low heritability compared to its component characters.

Therefore, an attempt has been made to study the characters assosiated in rose induced and spontaneous mutants at phenotypic and genotypic levels. The most useful dosage of irradiation is that which produces the maximum genetic effect (Guo et al., 1983). The doses that cause gross chromosomal changes are less useful in inducing polygenic mutations. Lata(1975) demonstrated that selection of morphologically similar control plants from irradiated population could result in isolation of high yielding genotypes. She also postulated that plants may be carrying large number of small and individually inconsequential heritable changes which could provide sufficient genetic variability for practicing effective selections.

Spontaneous mutations occur frequently (Datta, 1994) and have caused a valuable increase in genetic variation, especially in hybrid tea groups. Semenuik (1971) studied the history of bud mutations in number of cultivars and was able to draw some interesting conclusions concerning the time of mutation in relation to the age of the variety, degree of mutations in relation to the genotype as well as the for flower colour to mutate in relation to the genotype. Red and pink flowers were generally found to be dominant over white, while the degree of filling of the flower becomes less with mutations.

Phenotypic effects cannot be detected in the population because the polygenic traits are extremely sensitive to environmental factors (Heslot, 1968). Smaller additive variations have played significant role in the evolutionary process by bringing about slight phenotypic changes (Irrullappan and Rao, 1981). In general, the genotypic variance induced by a mutagen is known to show a linear relation with dose. Such linearity is more seen when low to medium doses are administered. Linearity seems to deminish with higher doses and it is likely that variances decrease at high dose levels. Such a behaviour is attributed to an association between lethal and mutational events of higher doses.

Complex physiological characters have several component characters. The component characters are positively associated with the ultimate characters, while amomg themselves the association may be positive or negative (Brady, 1982). An understanding of characters under association is useful in achieving desired improvement through selections and also in determining the criteria of selection for developing productive genotypes. In the present study phenotypic and genotypic correlations were studied between 13 characters. The error variances are relatively larger or higher than the genotypic variances. Very high genotypic but moderate phenotypic correlations revealed that they are highly inherent. The phenotypic

expression of the correlation is reduced by the influence of environment (Jicinska, 1975).

It is evident that genotypic correlation coefficients were higher than the suggested negligible effect of environment on the true expression of the characters. Besides this could also be due to relative stability of the genotypes (Khanna and Shukla, 1991). Characters when strongly negatively associated at genotypic levels indicate that there was a strong linkage between the genes controlling these characters and are less influenced by the changes in environmental conditions during the expression. Studies on the phenotypic variability, heritability and genetic advance made by Kukimura et al. (1975) indicated the existence of high heritability for several characters. High heritability was accompanied with high genetic advance for number of branches, leaflets, flowers, petals and for average flower weight showing additive gene effects. High heritability and low genetic advance showed non additive gene action for flower diameter, plant spread, branches while increased heritability with moderate values of genetic advance was obtained for plant height. High degree of heritability with moderate values of genetic advance was obtained for plant height. High degree of heritability for number of branches, spines, plant height was reported by (Lata ,1974). Stem diameter and internodal length however had a low degree of heritability.

The narrow difference between the phenotypic and genotypic coefficient of variance indicates the reliability of the phenotypic selection for that character (Kaicker, 1982). The broad sense heritability was high with low genetic advance. Increased heritability and increased genetic advance infers that the characters are governed by additive gene action and selection will show an improvement for these characters, whereas moderate (Jicinska, 1981) genetic advance is governed by both additive and non additive gene actions and a low genetic advance is expressed by the influence of non additive gene action. Sparrow (1961) inferred that

randomness in the action of radiations, make them capable of inducing variation in most genetically controlled properties. Selection of a genotype which is under cultivation as a starting material is an advantage owing to its superiority for adoption. At lower dosages the chromosomal breakages were less leading to lesser combinations. Contrarily, increase in variance at lower doses may be due to stimulatory effects. At higher doses radiation might have caused more chromosomal abberations leading to higher recombination which increased the variance in the subsequent generation (Streitberg, 1977).

Large phenotypic variance was observed for plant height, number of flowers, petals, spines and leaf area both in induced and spontaneous mutants. The remaining characters showed moderate to low phenotypic variation. Since it includes both genetic and environmental effects, phenotypic variation is not reliable. If the former is more, selection is more effective else its ineffective. It is essential to split the total variance into genetic and non genetic components to ascertain the reliability of characters through the estimates of genetic parameters such as coefficient of variance, heritability and genetic advance (Manson, 1959).

Genotypic variance was high for the characters similar to phenotypic variance indicating that these characters are contributed by genetic factors and are less influenced by environment. The selection of these characters may be effective and probably governed by additive genes. The characters viz., branches, stem diameter, leaflets, internodal length, flower diameter, flower cluster and flower weight recorded decreased genotypic variance but increased phenotypic variance indicating that they are influenced by environmental factors rather than genetic factors (Nakajima, 1965). Skewed distribution of mutant plants hypothesised that the mean of the population deviates in a direction which is opposite to the previous selection history of the parental material. Thus there is less likelihood of early mutant in an early variety.

The proportion of genotypic variance to total variance gives the heritability in a broad sense. Heritability values is of much use to the breeders, since magnitude of heritability indicates the accuracy with which a genotype can be recognized by its phenotypic expression (Muller, 1927). Though heritability estimates indicates the reliability of the characters in selection programme, their scope is restricted as they are prone to change in environment, material etc. However, if heritability values are used in conjunction with genetic advance, they can be important in selection programme (Sahare and Shastry, 1963). High heritability with increased genetic advance for the characters such as plant height, thorn count and number of petals indicates that these characters are controlled by additive gene effects and may be useful for selection both under induced and spontaneous mutants. High heritability coupled with high genetic advance indicates the possibilty of further perpetuation.

Strongly and positive correlation indicates strong linkages between the genes controlling the characters. Weak association indicates that genes controlling these characters are not linked and the characters are considered to be independent (Sarkow, 1961). Low phenotypic correlation values indicates less environmental effects on the expression of the characters and independence. The nature and degree of association of any two characters may be a result of direct and indirect effect of independent variables on the dependent variable through other values. Path analysis helps in partitioning the total effects into direct and indirect effects (Sarro and Zoronoza, 1991). In view of this the correlation among the characters were subjected for analysing their path effects over different variables. Increase in variance coupled with other positive or negative shift in mean with respect of various polygenic traits has been reported as a general phenomenon in the populations of several crop plants after mutagenic treatments.

The correlation values denote only the extent of association existing between the pair of characters. These independent variable controlled by several component characters are themselves mutually associated. This mutational association impairs the true association existing between different components which will disturb the whole network of cause and effects of the sytem dependent variables. Thus the structure of each component can be probed through two parts of action viz., the direct effect on dependent variable and the indirect effect which are not revealed (Swarup et al., 1971). Hence the variabilities can be made use in further selection and isolation of useful morphological mutants for breeding programme in rose improvement as a future line of work.

Like other plants, growth and flowering behaviour of roses is governed by genetic factors. Significant difference in growth is observed which is attributed to the inherent genetic variations existing amongst species and cultivars (Allard, 1960). Naturally occurring mutations are composed of a large number of mutations of many types including mutations due to unequal cross over by meiotic and mitotic recombination between respective elements (Brock, 1972).

Therefore, leading and relatively new commercial varieties from which only a restricted number of sports have developed are generally selected for artificial mutation induction. It is thus important to get the full range of flower colour sports of such fast growing and easily forcible cultivars which also are generally early flowering, easily formed flower buds having other good characteristics. From the above investigation and discussions on the reviews it is clear that roses are the most suitable plants for mutation breeding, since many flower colours and other mutants can be produced without altering any other characters of the original genotypes.

5.2 EXPERIMENT II : POLLEN STUDIES

The present study was undertaken to evaluate the effectiveness of various in vitro assays for estimating pollen viability and fertility status in rose and hibiscus cultivars. Viability is the capability for living or continuing to develop and fertility is a measure of an individual's ability to produce viable offsprings, while *sterility* is a measure of the proportion of abnormal gametes (Pearson and Harney, 1984). Thereby, pollen is a direct product of genetic recombination, from which haploid gene sequences could be isolated and cloned through available recombinant DNA methodology. It is well documented that pollen is the site of intense genetic activity and selection at genic level, gene expression through transcription and translation. Pollens collected from unopened flowers exhibited higher viability profiles in vitro compared to that of pollens collected from fully opened flowers.

Modern garden rose cultivars are tetraploids and complex interspecific hybrids which have arisen from about 10 rose species. New germplasm is needed to increase the hardiness , however as garden roses suffer injury or dealth each winter. One of the initial mechanisms found in this complex appears to be intravarietal and interspecific hybridisation in hibiscus. This has happened in total disregard to sexual sterility. The implications of the results obtained from the present investigations are discussed as follows.

Preserving pollen viability has gained considerable importance for plant breeders, seed companies and more recently in gene banks to manipulate haploid gene pools for genetic resource conservation. Pollen has been identified as one of the components of plant genetic resources due to its easy access, storability, economy of space and convenience for using under different locations when transported (Jackson and Blundell, 1963). Preserved pollens can be effectively used

for intercrossing asynchronous flowering genotypes without any embargo on time and space. Retention of pollen viability varies significantly from species to species. Environmental factors particularly temperature and humidity, greatly affects viability. Infact, deficiency of respiratory substrates, irreversible loss of membrane permeability, inactivation of enzymes, growth hormones, loss of capacity to synthesize RNA and proteins, free radical formation and chromosomal aberrations, have been suggested to operate in pollens that contribute ultimately towards the loss of pollen viability (Han , 1994a).

The loss of pollen viability and low RH might be due to injury caused from loss of water by the pollen grains and loss of viability under high humidity may be the result of increased physiological activity of the pollen grains. Irreversible impairment, of pollen ultra structure including loss of membrane permeability and inactivation of enzymes and growth hormones have been reported as contributing factors for not preserving the viability for long (Rubtsova, 1978). Thus it is concluded that rose pollen grains cannot be viable for very long periods of time. However, if such prolonged period of storage is desired, it might be advisable to reccomend temperature around freezing.

The percentage of fertility estimated by using the versatile stain suggested by Alexander (1980) differs widely with cultivars. Lata (1971) obtained average pollen fertility range of 19 to 36 percent, stating that in the mutants of rose the fertility percentage was very low. The fertility percentage when estimated by versatile stain varied to a considerable extent. In some rose cultivars it was difficult to differentiate between aborted and non aborted pollen grains by using acetocarmine stain. Alexander (1980) opined that the acetocarmine stain has certain limitations since it stains only non aborted pollen grains, while the aborted pollen is identified by the unstained and empty pollen walls. The dry acetocarmine is not effective for the pollen of most of the plants belonging to families, which

have thick and impervious nature of their pollen walls. Nomerov (1972) opined that the procedure involved in vital staining is time consuming, complicated and not always reliable.

Gowda et al. (1977) observed that the pollen fertility percentage was as high as 56.76 in 'White Christmas' and as low as 12.20 in 'Super Star'. Svejda and Poapst (1972) mentioned that roses are complex hybrids involving interspecific hybridisation, polyploidy with high female and male sterility. Sahare and Shastry (1963) opined that high pollen sterility exists in most of the garden roses and observed that the pollen sterility percentage in hybrid tea roses varied from 10 to 98 per cent. The primary objective of the viability assay must therefore be to determine the ability of pollen to germinate, which could precede a fertility test to check gametic transfer, fertilization and seed formation. Loss of viability is due to either crossability factors associated with specific seed parent or inherent ability of cryostored pollens to perform normal fertilization and seed set on specific cultivars indicating the loss of viability (Banerjee, 1969).

The viability of isolated pollen grains was assessed by their ability to germinate invitro. The viability of rose pollen grains has been shown to be highly variable. Pearson and Harney (1984) compared the viability of fresh pollens, as estimated by percentage of germination collected from six Rosa species and obtained significant difference between genotypes with the germination rate ranging from 1.7 - 69.2 per cent. Generally, germinating pollen is able to draw metabolic substrate from the reserve substances which it contains. Nevertheless it has been shown that growing tubes reabsorb style material and use it for building up the tube wall (Alexander, 1987).

In the present investigation the pollen grains of the 7 different cultivars of hibiscus are found to be spherical, 3-4 colporate, polyphorate and provided with

spines. This observation is in agreement with the general description given by Eyster (1941) for the pollens belonging to the family malvaceae. Though there is slight variation in colour, the pollen grains of different varieties have more or less similar shape. Even within the same variety the size of the pollen grains tends to vary. Variation is observed between normal and sterile grains. The pollen grains of hibiscus cultivars showed great variation in germination on nutrient media. Germination trials artificially revealed that a medium containing 45% sucrose, 100ppm boric acid and 1gm agar is more suitable for many of the hibiscus cultivar (Ganeshan and Rajashekaran, 1985).

Among the different concentrations of sucrose, good pollen germination was obtained at 35 to 45 % sucrose solution. In lower concentration the pollen grains tend to burst and in higher concentration they fail to germinate. But pollens of all varieties do not successfully germinate in this concentration, due to a difference between the varieties in their specific requirements. Boric acid enhances pollen germination and only the pollen grains of those varieties which germinate in the nutrient media are found to germinate on the stigma (Allison and Timothy, 1991). It was found that the varieties of shoe flower behave differently with regard to pollen germination on the stigma and pollen tube growth in the style after self pollination.

The successful invivo germination of pollens under perfect favourable conditions is the product of modified metabolic pathways by which pollen metabolises glucose which is required for pollen tube growth (Stougard, 1983). Thus the ability of the pollen to fix carbon dioxide via phosphoenol pyruvate carboxylase subsequently gets transmitted by the entry of carbon dioxide into organic acids to synthesize proteins from amino acids. It becomes evident that the metabolism makes available protein for tube growth which may not be present in the pollens stored for long and also there is loss in pollen germination capacity.

Favourable flow and timely availability of all metabolites (Rubtsova, 1979) coupled with balanced availability and consumption of sucrose brings about intact germinability due to normal respiratory activity. Therefore, the intactness with which germinability at -196^{0}C is conserved, indicates the ability of cryogenic temperature to have normal enzymatic mechanism in pollen and pollen tube growth through the process of intact glucose metabolism and anaerobic respiration. Pollen germination and pollen tube growth rate are sufficiently variable and its competition is a major genetic consequence for plant regulations. Pollen tube growth rates was a good indicator of a pollen donors ability to sow the seed under competitive conditions (Rajashekaran and Ganeshan, 1994).

Pollen sterility is due to interaction of cytoplasmic nuclear genes (Han, 1994). Failure of the formation of functional gametes is one of the aspect related to pollen sterility, whereas the other aspect deals with the process by which the fertility is lost because of the influence of several extrinsic factors leading to the disturbance in intrinsic mechanisms. Fertility, therefore is an index that indicates the extent to which pollen remains fertile when stored under the influence of different temperatures. Such kind of studies are quite essential to understand the process behind induction of sterility in rose and hibiscus pollen.

According to Ganeshan and Rajashekaran (1985) the best germination media for rose was 15% sucrose + 40ppm boric acid by hanging drop method maintained at an ambient temperature of 4 - 6hrs with 0 - 90% RH . Thereby restricting availability of sucrose to a metabolite deficient trinucleate grain resulting in the inhibition of germination, while under similar circumstances binucleate grains germinate, but tube growth would cease when available metabolites in the grain has been utilized (Jicinska et al., 1976). The factors which govern the behaviour of self sterile plants are strictly inherited and are

transmitted in accordance with definite Mendelian mechanisms with numerous crosses.

Sterility may have accentuated further by the accumulation of deletarious mutations over the years. All the cultivars are sexually sterile which may be due to genic, structural, segregational or environmental causes. More than one cause may be involved. Due to lack of sexual reproduction, there is no chance of elimination of sterility factors. Sterility is disadvantageous for any genetic improvement in hibiscus (Allison and Timothy, 1993). High sterility is mainly caused due to high frequency of chromosome dearrangements during meiosis which includes, failure of the bivalent forms, multivalent association and chromatid bridges forming very few viable gametes.

It is ascertained that some of the biochemical deviations, associated with mitochondria are involved in the induction of pollen sterility. It is a known fact that all the pollen produced in the plant are not fully fertile. Soon after dehiscence, there will be certain amount of sterile pollens available in the total pollen pool collected from the anthers. It has been reported by Brewbaker (1992) that the loss in pollen fertlity is accompanied by accumulation of glycine and reduction in the levels of amino acids. Thereby, a combination of freeze drying followed by cryogenic storage of pollen may hold promise for prolonged duration of storage with high viability status and for maintaining fertility profiles in the near future of rose and hibiscus cultivars.

5.3 EXPERIMENT III : PIGMENT STUDIES

The creation of new colour patterns in ornamentals has always been a major aspect of classical flower breeding accompanied by a detailed chemical and biochemical analysis of the components. The chemical components which

influences colour formation in ornamentals are carotenoids, flavonols, chalcones, aurones and specially anthocyanins (Ahuja, 1963). Evaluation of these different group of pigments, their biosynthetic pathways and mechanisms, co-pigmentation effects, pH changes influencing the colour of the flower is neccessary. This will not only aid in understanding the fact behind the expression of the colour but also creates new avenues for evolving new coloured cultivars.

Considerable variations in rose has been brought through spontaneous mutations giving rise to varied colours and forms. The eight rose genotypes studied including the induced mutants for colour change in the present investigation are Akito, Eiffel Tower, First Federal Gold, Kronenbourg, Paradise, Raja Surendra Singh of Nalagarh, Sindoor and Wouburn Gold.

Variation in hibiscus cultivars on the other hand has been mainly brought about through intra and interspecific hybridization. The 15 cultivars namely, Aikta, Ashirwad, Arunodaya, Basant, Banazeer, Chitralekha, Dilruba, Nartaki, Nazneen, Phulkari, Priya, Ratna, Red Saturn, Shanti and Tribal Queen were investigated to study the pigment status which are discussed in the light of the available literature.

5.3.1 Rose Genotypes and their Mutants

5.3.1.1 Anthocyanin

Mutation has lead to a synchronous shift in the start of the anthocyanidin synthesis to an early or later stage, leading to a turn or change in the quantitative and qualitative composition of the pigments (Mol et al ., 1993). The present study showed the estimated anthocyanin content of the genotypes and the mutants to vary considerably. Mutation in the pigment biosynthetic pathway varies the pigment composition and ultimately forms new colour (Asen, 1976). Jennen (1973)

demonstrated that by statistical analysis, as well as by experimental mutation induction the pink flower is the best starting colour for the induction of other flower colours followed by white, bronze, red purple, yellow, salmon, golden orange, yellow bronze, yellow with red and brown, respectively. Nakamura et al . (1980) reported that the accumulation of anthocyanin was rapid with the combination of kinetin and visible light. Visible light did not directly aid the action of kinetin but increased the activity of enzymes in the anthocyanin biosynthesis.

According to Stewart (1969) the main genetic factors known to control type, amount and distribution of anthocyanins in the flower is due to the genes affecting colour by controlling structural modification of individual anthocyanins through shifts in the pH, metal chelation and co-pigmentation. Among the five spontaneous mutants of rose studied, mutant of Wouburn Gold exhibited highest anthocyanin content having tangerine orange petal colour as compared to Kronenbourg with least amount of anthocyanin having crimson yellow flower colour shades. This indicates that darker the flower colour higher will be the anthocyanin concentration which is in agreement with the report of Datta (1983). He reported that somatic flower colour mutation may be associated with either increase or decrease of one or more existing pigments. Lighter the shades lesser was the quantity of pigments present because of irradiation.

With respect to induced mutations the data indicated that with increase in irradiation dosage anthocyanin content reduced considerably and the maximum reduction was obseved in the buds treated with 6kr gamma rays resulting in lighter shades as compared to the parent cultivar Paradise. According to Ahuja et al. (1964) the difference in colour was due to the difference in concentration of the pigments. Lighter flower colour mutants was associated with decreased cyanidin content. Manson (1959) studied the relationship between anthocyanin content and flower colour tone in red petal colours of rose genotypes. He reported that the petal

colour varied between deep or dark purplish red to very dusky red corresponding to anthocyanin content by gamma irradiation. Results indicated that surface reflection plays an essential part in the expression of petal colour.

5.3.1.2 Leucoanthocyanin

Varietal variation was not observed much among the different genotypes studied in rose with respect to leucoanthocyanin content. Results indicated that the amount of leucoanthocyanin content was found to be moderate in all the genotypes under the present study of investigation. With respect to induced mutation, the amount of leucoanthocyanin decreased with increase in irradiation dosages. According to Harborne (1963) on the basis of structural modifications he stated that pink, scarlet, orange red flowers contain pelargonidin glycosides, crimson and magenta flowers contain cyanidin glycosides and mauve to blue colours contain delphinidin glycoside derivatives. The data indicated that both in induced and spontaneous mutants the darker flower shades had higher leucoanthocyanin content when compared to lighter flower shades.

5.3.1.3 Flavonols

Variation in the composition of flavonols and anthocyanidins in the different genotypes was studied by Zykov et al .(1991a). He reported that the first to appear during ontogeny were the flavonols, kaempferol and quercetin. Anthocyanidins on the other hand is in the order of cyanidin, peonidin and pelargonidins. In the present investigation the flavonol content varied considerably among different genotypes of rose studied. With respect to induced mutants gamma irradition did not affect the total flavonol content in the cultivar Paradise. Moderate amount of flavonol content was observed irrespective of the flower colour.

According to Asen et al . (1972) some of the genetic factors modify the pigment and produce copigments or change the cell conditions so as to produce wide range of one or more type of pigments. Irradiation in the present study might have resulted in the change of different factors which suppressed or eliminated the original pigmentation pattern. Differences in the flavonol content was observed in the spontaneous mutants and their respective parents. There was considerable increase in the flavonol content among spontaneous mutants observed in the present stuy of investigation. Mol et al . (1993) studied genetic resources in peonin and a new combination of anthocyanins in Rosa species. They reported that yellow flourescent spots, probably flavonoids were present when the anthocyanins were absent or faint.

Raman (1965b) reported that the changes in environment can influence the biosynthesis of flavonoids. Vries et al . (1980) reported that red shades may be a result of high concentration of purple flavonoids and low concentration of yellow orange carotenoids. They also reported that pink colour is dominant over dark red, orange yellow, yellow, white and scarlet.

5.3.1.4 Carotenoids

The genotypes with more of yellow pigment content imparting to yellow flower colour had more of carotenoid content. According to Forsyth and Simmonds (1954) bright orange roses are derived from a mixture of pigment cyanidin mixed with carotenoids. Thus mixture of pelargonin and carotenoid produces orange shades whereas, bronze colour is due to admixtures of flavonoids in higher concentration with carotenoids. The data recorded on induced mutants indicate that increase in irradiation dose increased the carotenoid content having lighter flower colour shades. These results were in accordance with the study of Vries et al . (1980) where they stated that red shade may be a result of high

concentration of puple flavonoids and low concentration of yellow orange carotenoids or it may be due to high concentration of red flavonoids and the absence of both carotenoids and chlorophyll pigments.

With respect to spontaneous mutants the total carotenoid content was more when compared to their respective parents except in the mutants of Kronenbourg and First Federal Gold as the genotypes had more of yellow petal colour. Meyer et al . (1991) has reported that by varying the ratio of carotenoids to flavonoid pigments different petal colour shades can be produced. According to Manson and Krinkel (1994) the key genes affecting blue and red colouration was isolated and controlled by carotenoid and flavonoid pigments, notably anthocyanins.

5.3.1.5 Paper Chromatographic Separation of Anthocyanin Pigments in Rose Genotypes

Paper chromatographic studies were undertaken to understand the quantitative and qualitative differences within the anthocyanin pigments. According to Arisumi (1963) chomatographic studies on the flower colour of rose with special reference to biochemical and genetical analysis have shown that cyanin to be the commonest of several anthocyanins present in both wild and cultivated roses.

5.3.1.5.1 Induced Mutation

Analysis of pigments indicated that somatic flower colour changes are due to both quantitative and qualitaitve changes in the pigments as a result of mutation frequency induced by gamma rays in the biosynthetic pathways (Datta, 1985). In the present study cultivar Paradise with its four induced mutants showed considerable variation in the number of anthocyanin pigments.

The parent cultivar having three purple bands were absent in all the induced mutants. With respect to induced mutant of Paradise (3kr), it recorded three new anthocyanin bands which might have resulted in the petal colour variegation whereas, the mutant of Paradise (6kr) had only one new anthocyanin band which was not present in other induced mutants. This inturn has completely changed the flower colour from mauve to white. When compared to the parent cultivar the induced mutants had lesser concentration of anthocyanins which reflects lesser hue in imparting more colour to the petals. Thus the data observed indicated that the radiation dosages altered quantitatively and qualitatively the anthocyanin pigments in the petals of parent cultivar resulting in the lighter petal shades.

Studies on the relation of flower colour to the OD spectra of intact tissues and of anthocyanin extract was conducted by Stewart (1969). According to him the main genetic factors known to control type, amount and distribution of anthocyanin in the flower is due to genes affecting colours by controlling structural modifications of individual anthocyanins through the shift in pH, metal chelation and co-pigmentation effects. Therefore, no new pigments occured in few of the cultivars and mutants which might have been due to the stabilization of anhydro bases (Arisumi et al ., 1972).

5.3.1.5.2 Spontaneous mutation

Marshall et al . (1983) analysed and stated that all pigments particularly cyanin and peonin showed quantitative inheritance. The inheritance of each of these colour pigments is reported to be mainly controlled by additive gene action. The genetical studies on the inheritance of flower colour in roses has indicated that the dominant colour in rose is magenta, pink or tyrian-rose to orange red. The pink colour is dominant over dark red, orange yellow, yellow, white and scarlet.

The following are the results obtained on spontaneous mutation studies in the present investigation

Akito : The parent genotype which was completely white had two bands of red colours whereas its mutant had 2 more orange red coloured bands which might have lead to the pink mutant colour with higher anthocyanin concentration.

Eiffel Tower : The parent genotype Eiffel Tower had higher concentration of 3 anthocyanin bands whereas, it spontaneous mutant had three bands of which two were new having lesser anthocyanin concentration which might have lead to light pink shades.

First Federal Gold : Parent genotype with one band gave yellow petal colour whereas its mutant had 2 new anthocyanin bands than the parent having higher concentration of anthocyanin resulting in the red brown petal colour.

Kronenbourg : Mutant of Kronenbourg was yellow with only one anthocyanin band whereas its parent had 2 new bands with high concentration leading to the flower change of crimson yellow.

Wouburn Gold : The parent genotype had three bands whereas, its spontaneous mutant had completely different anthocyanin bands imparting more of orange red colour which might have resulted in red orange mutant colour having higher anthocyanin concentration.

According to Brock (1962) the new colour pigments might result from mutation co-occurring in the biochemical pathway to pigment formation. It was observed that flavonols, quercetin, kaempferol, anthocyanidins, cyanidin, peonidin and pelargonidin were present in the varieties and mutants. Thus the results

suggested that primary flavonols is quercetin and primary anthocyanidin is cyanidin, both being formed by the same precursor. Changes in some character of the mutants in addition to change in the flower colour suggests that the changes were perhaps induced at several independent loci (Heslot, 1968).

Mutation in the pigments of the petals may take place as a result of either increase or decrease of both in the concentration of one or more existing pigments. The difference may be due to blockage of one or more pigment synthesis giving rise to a new pigment (Meyer et al ., 1991). Thus mutation breeding in the pigment biosynthetic pathway alters the parent composition and ultimately results in the formation of new flower colours.

Cultivar Sindoor and Raja Surendra Singh of Nalagarh : Both the cultivars had red orange as the flower colour with red purple and orange red band colours having higher anthocyanin concentration.

5.3.1.6 Chemical identification of anthocyanin pigments in Rose

Paper chromatography of anthocyanin pigments in 17 rose genotypes has indicated varietal differences in the number of anthocyanin pigments present. One to 5 bands of anthocyanin pigments having Rf values were observed. This observation supports the work done by Harborne (1967) where he stated that there are six common anthocyanins distinguished based on the differences in the Rf values and colour.

The comparison of Rf values with the authentic anthocyanins has indicated the presence of anthocyanin, cyanin in rose genotypes. From the data it was reported that the anthocyanin band showing Rf values 0.14 to 0.18 matched with that of cyanin chloride and thus tentatively the pigment was identified as cyanin.

According to Yokoi (1974) petals of 670 rose cultivars and 8 species were grouped on the basis of anthocyanin composition. He identified five anthocyanin viz., cyanidin 3-glucoside & 3,5-diglucoside, pelargonidin 3-glucoside & 3,5-diglucoside and peonidin 3,5-diglucoside, respectively. Shisa and Takano (1964) studied the formation of red pigments in hybrid tea variety 'Crimson Glory'. Chromatographic analysis showed that the pigments consisted mainly of cyanin.

Ahuja et al . (1962) identified the anthocyanin from the petals of rose 'Pink Coronet' and 'Happiness' as cyanidin 3,5-diglucoside, whereas Vega and Martin (1967) identified and isolated cyanidin 3,5-diglucoside anthocyanin from the red rose 'Paula Scarlet'. According to Jennen (1972) using TLC method the anthocyanins of red rose varieties were separated, purified and identified as cyanidin 3-glucoside and cyanidin 3,5-diglucoside.

5.3.2 Hibiscus Cultivars

Flower pigments are of possible interest in hibiscus cultivars as it exhibits a colour pattern of remarkably frequent occurrence and association with a relatively intense colour pattern which may be of biological significance. The main pigments of hibiscus are the various anthocyanins (cyanidin, peonidin and malvidin) with various flavonols as copigments (azaleatin, quercetin and kaempferol) according to Nakamura et al . (1990).

5.3.2.1 Anthocyanin

Naturally occurring anthocyanins include glycosides of only six commonly occurring anthocyanidins viz., pelargonidin, cyanidin, delphinidin, malvidin, petunidin and peonidin that cannot alone be responsible for the infinite colour variation found among hibiscus flowers (Jorgensen and Mol, 1991). Considerable

variation was observed in the anthocyanin content among the 15 hibiscus cultivars. With respect to the cultivars Aikta, Chitralekha, Priya, Red Saturn and Tribal Queen under red group exhibiting darker petal shades had higher anthocyanin content when compared to Chitralekha with light petal colour of china rose shades exhibiting minimum anthocyanin content. According to Han (1994b) anthocyanins were the major pigments present in red flower colours of Hibiscus rosa-sinensis species. Subramanian and Nair (1970) stated that the colour change of the flowers from ivory white to light rose during the day is ascribed to anthocyanin synthesis in the flowers of Hibiscus mutabilis.

The cultivars Ashirwad, Arunodaya, Dilruba, Nartaki and Phulkari grouped under orange/orange yellow differed considerably with respect to anthocyanin content, whereas under yellow/yellow orange group of cultivars Basant, Banazeer, Nazneen, Ratna and Shanthi with yellow as the major flower colour in general showed less anthocyanin content compared to red petal coloured groups. According to Egolf and Santanour (1975) the anthocyanins isolated from the flowers of hibiscus cultivars were 3-glucosides of delphinidin, petunidin and malvidin, which were normally associated with mauve, violet or blue flower hues but one cultivar contained cyanidin 3-glucoside which was associated with crimson magenta hues. Nair et al. (1962) reported that purple coloured portion of the petals had anthocyanins namely cyanidin, pelargonidin and delphinidin.

5.3.2.2 Leucoanthocyanin

Among the red group of flowers Priya recorded maximum amount of leucoanthocyanin whereas, the other cultivars recorded moderate to low leucoanthocyanin irrespective of the petal colours. With respect to orange/orange yellow group of cultivars Ashirwad, Arunodaya, Diltuba, Nartaki and Phulkari showed considerably increased amount of leucoanthocyanin when compared to red

group of cultivars. Out of the six cultivars grouped under yellow/yellow orange, cultivar Ratna recorded high leucoanthocyanin when compared to yellow flowered cultivars.

5.3.2.3 Flavonols

Petal colours in hibiscus cultivars were associated with the nature and relative content of anthocyanins, along with good agreement with increase in flavonol / anthocyanin ratios, malvidin derivatives, malonated anthocyanins and cell sap pH. Increase in the pigment factors enhanced the co-pigmentation effects (Kim and Fujieda , 1991). Variation in the flavonol contents among different groups of hibiscus cultivars were observed in the present study. Cultivars belonging to red group namely, Aikta, Chitralekha, Priya, Red Saturn and Tribal Queen recorded high flavonol content. The results were in accordance with Ishikura (1982), were he identified five flavonol glycoside in the pink petals of Hibiscus mutabilis as quercetin 3-sambubioside, isoquercetin, hyperin, guaijaverin and a compound containing kaempferol, glucose, galactose and xylose.

Darker the flower colour shades resulted in higher flavonol content as estimated among orange/orange yellow group of cultivars which recorded moderate flavonol content, whereas the yellow/yellow orange group of cultivars with lighter petal shades had less flavonol content compared to red and orange groups. Nair (1961) isolated and reported flavonoid gossypetrin and gossypetin in the yellow portion of Hibiscus tiliaceae species.

5.3.2.4 Carotenoids

Hanny et al . (1972) identified and quantified carotenoid constituents present in Hibiscus syriacus species. They reported that lutein 5,6-epoxide

predominated in the flowers and carotene hydrocarbons comprised of 19% of the total carotenoids in the flowers. In the present study the red petal colour shaded cultivars had lesser carotenoids as compared to yellow petal coloured cultivars. In the orange/orange yellow group of cultivars Ashirwad, Arunodaya, Dilruba, Nartaki and Phulkari recorded considerably higher amounts of total carotenoid contents. It was reported by Biron and Halevy (1974) that pink coloured flowers had both cyanidin and pelargonidin and in scarlet colour, pelargonidin is more than cyanidin, whereas in bright orange coloured flowers pelargonidin and cyanidin with kaempferol are present with a high carotenoid content.

In the present study the yellow/yellow orange grouped cultivars namely Basant with sulphur yellow petal colour, Banazeer with yellow and Nazneen with tangerine orange had high carotene content as compared to red grouped cultivars.

5.3.2.5 Paper chromatographic separation of anthocyanin pigments in Hibiscus cultivars

According to Khanna and Shukla (1991) even though anthocyanins are the colour contributing pigments in hibiscus, these pigments consist of a number of components having different chromatographic and electrophoretic mobilities.

5.3.2.5.1 Red Group : Hibiscus cultivars grouped under the red flower colours recorded more number of anthocyanin bands imparting red colour shades with 6 to 8 colour bands. Out of the five cultivars under red group Chitralekha with china rose petal colour had lesser concentration of anthocyanins whereas cultivar Red Saturn, Priya, Aikta and Tribal Queen which had darker shades of red as petal colour recorded more anthocyanin concentration. Nakamura et al. (1990) reported that the anthocyanin pigments present in red flowers of hibiscus cultivars are cyanidin 3-sambubioside and delphinidin 3-sambubioside.

5.3.2.5.2 Orange/orange yellow Group : Anthocyanin concentration in the orange/orange yellow group of cultivars indicated that it was moderate in Dilruba and Ashirwad which imparted lighter hue of petal colour. Cultivar Phulkari, Arunodaya and Nartaki had higher anthocyanin which might have been due to darker flower shade and high concentration. According to Lowry (1971) pink basal blotch in Hibiscus mutabilis is mainly due to the presence of cyanidin. This was observed to be the first case of free anthocyanidin occurring in hibiscus flowers.

5.3.2.5.3 Yellow/yellow orange Group : The cultivars grouped under yellow/yellow orange petal colours exhibited less number of anthocyanin bands as compared to red and orange group of cultivars. Anthocyanin concentration was comparatively moderate in the cultivars Ratna and Nazneen whereas, Basant, Shanti and Banazeer had high concentration which might have lead to lighter yellow petal colour.

5.3.2.6 Chemical identification of anthocyanin pigments in Hibiscus cultivars

Paper chromatography of anthocyanin pigments in 15 different hibiscus cultivars studied has resulted in the varietal differences in the number of anthocyanin pigments present. Two to eight bands of anthocyanin pigments having Rf value were observed. This supported the observations made by Yasuyuki et al. (1990). They reported that the aromatic signals of hibiscus anthocyanins showed good correspondence with those of authentic cyanidin 3-glucoside distinguished based on the differences in Rf values and colour.

The comparison of Rf values with the authentic standards of anthocyanin has indicated the presence of anthocyanins, cyanin and pelargonidin in hibiscus

cultivars. According to Zykov et al. (1991a) the anamalous occurrence of free cyanidin is probably related to the extremely active metabolic situation. Cyanidin 3-sophoroside was previously reported from Hibiscus rosa-sinensis . that it was only anthocyanins which are found in the various cultivated forms (Yasuda , 1967b), whereas cyanidin 3-glucoside was on the other hand found as the dominant anthocyanin only in few species. The petal colours of rose and hibiscus belonged to the anthocyanin group of pigments showed changes in qualitative and quantitative pattern among the cultivars.

The genotypes with white colours in rose and yellow in hibiscus cultivars contained comparatively less anthocyanin content than orange colours in rose and red in hibiscus genotypes. All these results are in conformity with the reports of Khanna and Shukla (1991) who reported that more the concentration dominant is the expression of the colour shades. Genotypes of darker shades have higher anthocyanin content than the genotypes with lighter shades.

The anthocyanin genes have a general effect on its biosynthesis. One of the genes present in the homozygous condition thereby suppresses the anthocyanin synthesis resulting in a white or a very weakly coloured flower. Dominant alleles are required for the synthesis of flavonoids. The genes must be dominant to obtain the ortho dihydroxy substitute (Robinson and Robinson , 1934) pattern leading to cyanidin. If the genes are homozygous recessive, anthocyanin synthesis is suppressed and kaempferol gets accumulated. Floral pigments and perception of colour through spectroscophic studies revealed that the pigments present in hibiscus cultivars was pelargonidin.

The present study revealed that difference in the perceived colours can arise as a consequence of the same floral pigment being present but in different quantities both in the petals of rose and hibiscus. On the other hand, it also emerged that the

differences in the colour appeared in variuos cases which are ascribable to actual differences in the nature of the floral pigments (Sarro and Zoronoza , 1990). According to Nair et al . (1962) he reported that in the yellow parts of the petals gossypetrin and gossypetin were found whereas, in the purple parts cyanidin, pelargonidin and delphinidin pigments were observed.

This broad general information on various pigment pattern in different flower colours would be useful in the choice of the parents for hybridisation in the absence of elaborate phytochemical analysis of pigments. The present study revealed that mutation breeding in the pigment biosynthetic pathway alters the pigment composition and ultimately leads to the new petal colours. Thus phytochemical analysis has contributed to higher plant genetics, not only as a successful tool for unravelling the minute details of vanishingly small amounts of pigments. It has also been a means of identifying and quantifying different classes of pigments like anthocyanins occurring in different coloured genotypes of garden plants.

SUMMARY

VI. SUMMARY

Induction of mutation in the recent years has become an established tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. Hundreds of improved varieties have been released to farmers for many different crop species, demonstrating the economic value of the technology. Besides the primary objective of obtaining the useful mutants of direct value. Mutation breeding is useful in changing single simply inherited characters in highly developed genetic system, in breaking tight linkages thus helping to obtain rare recombinants and enlarged variability to select for qualitative and quantitative characters. Mutation breeding is of great service with the help of which one can change the genotype besides providing raw material for evolution, recombination and selections, which complicates the inheritance of traits and may impose upon number of progenies necessary to recover desirable recombinants.

By induction of mutation and other types of genetic manipulations breeders are endeavor to alter various regulatory systems and develop new cultivars. Any attempt at plant improvement requires genetic variability. Mutations however, whether spontaneous or induced may affect any genes of a nuclear genome and in addition also the ones located in cytoplasmic organelles. Mutagenesis is an unique force in creating variations as it may alter even those genes that are common to all the varieties of a species and therefore do not show any segregation after a cross within that species (Walter, 1977).

The range of variation which is of potential value in the development of new varieties possessing characters, are not commonly found and most of it remains unexploited. Bud sports have been an important source of variation in Horticultural crops because of the ease with which they can be isolated. Thus improvement of rose begins with the genetic manipulation that could be basically through gene recombination or mutations. Induced mutagenesis has been

attempted in the present investigation with the objective of creating useful genetic variability, isolating mutants or segregants which are determinate, photoinsensitive, high yielding and to assess the breeding behaviour as selected mutants.

Six genotypes were selected as spontaneous mutants and three genotype was selected for induced mutation studies based on the preliminary trails from among the available genotypes. The present study reveals that the colour changes in the mutants were all associated with changes in the biochemical characters. Analysis of morphology and pigment contents revealed that thorn count, number of petals, growth habit, leaf area, number of flower, diameter of the flower, number of petals and flower colour was found very sensitive to gamma irradiation, whereas other morphological characters were found to be more or less stable.

Since asexually propagated plants are reproduced by vegetative means genetic segregants and recombinants normally do not occur in successive clonal generations (Allard, 1960). This leads to gradual accumulation of naturally occurring mutants leading to increased heterozygosity and progressive accumulation of deleterious genes. Thus in the present study it gave an idea regarding the differences in the morphological and biochemical characters associated among different genotypes which are useful for varietal identification and further breeding programme. The results of the expressions are summarized below as follows.

With a view to study the plant responses to low dosage of irradiation extending over a longer period of time and also at different growth phases of the plants, it is being facilitated by chronic radiations from cobalt 60 source. A high degree of optimism is maintained in favour of the chronic and semi acute exposures. With the increase in dosage there was a corresponding decrease in linear growth. Low and medium dosages were more effective and efficient than higher dosage which was concomitant with the previous findings. Viable mutants

provide valuable information about the mode of action of different mutagenic agents as well as mutation pattern at specific locus (Datta , 1990b).

With respect to the various morphological characters, sprouting and survival percentage was greatly affected by gamma irradiation barring certain deviations. Differential response for the genotypes was found to be evident with no correlation. Only on the basis of 50% reduction in survival percentage the LD50 dosage for all the three rose cultivars was determined. A range between 3 and 4kr gamma irradiation was found to be very desirable without much of deleterious effects. Floral characters in spontaneous mutants exhibited drastic difference but sterility percentage was high between parent and mutants irrespective of the cultivars used.

Among the different spontaneous mutants used Eiffel Tower produced high fragrance whereas rest of the mutants produced attractive flower colour which was very distinct from their respective parents, with increased petal count, number of flowers , flower colour, flower diameter etc. Based on these characters one can select for perpetuating different cultivars through various breeding methods. Induction of mutation reduced all the characters related under study at higher dosage which may be due to physiological and biochemical disturbances. Gunckel and Sparrow (1961) opined that at higher dosage recovery is lesser due to its deviation from linearity as a result of rigorous diplontic selection. Therefore, magnitude and the extent of genetic variability was higher in irradiated treatments compared to their parents.

Many variegated, miniatured, single flowered, thornless stems were detected on irradiated plants along with normal flowers. These changes persisted in subsequent vegetative generations and were considered to be cases of somatic mutations. In 3kr gamma irradiated plants a petal variegation was observed in cultivar Paradise and a similar type of flower petal variegation was also observed in Raja Surendra Singh of Nalagarh when treated with higher dosage of 6kr

gamma rays which was found to be unique to their respective parent. Thornlessness was another desirable mutant observed at 4kr gamma irradiation exhibiting white flower colour when compared to its original mauve coloured parent. Thirdly, miniature mutant or dwarf mutant with a drastic reduction in plant growth and flower size was observed along with higher amount of flower yield. All these four mutants obtained may be of great value in future breeding programme.

No mutants were obtained in the cultivar Sindoor whereas in case of Raja Surendra Singh of Nalagarh higher the irradiation treatments greater was the frequency of mutants with desirable traits and better was the performance. At 6kr treated plants a variegated flower with white streaks on orange coloured parent flower was found to be very prominent. Some of the mutant types cannot be ruled out because in the mutants so observed in VM2 generation, only a fraction of the originally induced mutational lesions can be eliminated during ontogeny (Micke, 1975).

Fuji and Matsumura (1967) attributed the reasons for radiation to be an intergenic effect. This difference in the spectrum is referred to as a conspicuous difference in group mutability induced by ionizing radiation, induced chromosomal aberrations which would influence selections, recovery and phenotypic expression of induced mutations. Both environmental and genetic factors revealed to have influenced to a greater extent on the evolution of both natural and induced mutants. Variation for different quantitative characters for the mutant line is selected in general, based on some desirable characters from the results obtained by the range, mean, variances, heritability and genetic advance estimates. All the spontaneous mutants showed meagre deviation of genotypic and phenotypic coefficient of variances, indicating the reliability of phenotypic coefficient of variance for selecting the genotypes. Floral characters exhibited an increased variance with respect to selection. Plant height, leaf area and flower characters expressed high phenotypic and genotypic variations. Thus, the traits which have undergone

stabilizing selections are expected to respond to the mutagenic treatments by way of increase in genetic variance without any significant shift in the mean.

Heritability estimates was almost high for all the characters in correlation with high per cent genetic advance. Traits which exhibit higher heritability estimates are less sensitive to environment for expression. Drennan et al. (1986) cautioned that use of heritability was due to the fluctuations in environment, material, season etc. Thus traits with high heritability coupled with higher genetic advance was preferred. In both spontaneous and induced mutants flower characters attributed to the maximum positive direct effects irrespective to the recurrent gamma ray irradiation, whereas all the remaining characters contributed to the overall phenotypic and genotypic characters with a negative path and indirect effects for all the three consecutive generations. Yield being a quantitative character is a resultant of various characters working together during the growth of the crop period. It is therefore desirable to study the association of different characters by ascertaining the degree of correlation existing under a set of environmental conditions. The method of path coefficient was helpful in partitioning the association into direct and indirect effects, so that the relative influence of each component character on the end product could be assessed.

The correlation values denotes only the extent of association existing between pairs of characters, controlled by several componential characters which by themselves mutually are associated. In natural mutants both positive and negative correlations were hand in hand contributing to the overall variation in different characters under observation. Whereas in induced mutations almost all the characters were found to be positively correlated irrespective of irradiation dosages.

The flower colour of a number of garden varieties of plant species are controlled by many genetic factors. Both spontaneous and induced mutants of rose

and hibiscus cultivars were selected to study and estimate the effects of recurrent irradiation and biochemical affects on the presence of different pigments. Considerable variation in the anthocyanin content was found among induced and spontaneous mutants. With increase in radiation dosage the anthocyanin content was drastically reduced. Among the spontaneous mutants of rose Wouburn Gold and Akito and the cultivars Ashirwad and Priya exhibited highest anthocyanins with moderate anthocyanin content observed among other genotypes. Darker the flower colour shades higher was the anthocyanin content observed both in rose and hibiscus genotypes.

Not much of variation was observed both in rose and hibiscus genotypes with respect to leucoanthocyanin content. The amount of leucoanthocyanin content decreased in the mutants compared to their respective parents among rose genotypes, whereas it was found to be moderate among the other hibiscus cultivars estimated under the present investigation. In both induced and spontaneous mutants of rose the total flavonol content varied considerably to some extent. The total flavonol content was increased in the mutants than that of their parents, with a maximum flavonol content recorded in the genotypes of First Federal Gold and Akito, whereas in hibiscus flavonol content varied drastically among different cultivars with the least recommended in Basant and highest in the cultivar Priya.

Total carotenoids increased in the mutants of both spontaneous and induced genotypes and a maximum was recorded in Wouburn Gold, mutant of Kronenbourg along with 6kr induced mutant of Paradise genotypes. Among the different hibiscus groups, yellow grouped cultivars had more of carotenoids as compared to red or orange groups. From the data recorded it is clear that increase in radiation dosage increased the carotenoid content and decreased the total anthocyanins. Higher the yellow flower colour shade more was the carotenoids observed in rose and hibiscus genotypes.

Qualitative and quantitative estimation was undertaken in the present investigation through paper chromatographic method. All the spontaneous mutants obtained had one or two new anthocyanin bands when compared to their respective parents. With increase in the anthocyanin concentration it indicated a drastic flower colour change among the spontaneous mutants especially in Wouburn Gold with golden yellow to tangerine orange coloured mutants and Akito from white to azalea pink flower colour shades.

Among the 15 hibiscus cultivars grouped under red, orange and yellow , the cultivars belonging to red and orange group exhibited more anthocyanin content with increased concentration having darker flower colour shades when compared to the light shaded yellow grouped cultivars. Thus from the present investigation it was clear that darker the flower colour shades higher will be the amount of anthocyanin content as observed in rose and hibiscus genotypes.

From the results obtained and investigated it was noted that in both rose and hibiscus species the major pigment estimated was anthocyanins. Paper chromatographic study indicated varietal differences in the number of anthocyanin pigments present in each rose and hibiscus genotypes. One to five anthocyanin bands in rose and two to eight bands in hibiscus cultivars was observed. By comparing the anthocyanin bands with that of authentic standards, the pigment present in rose was tentatively identified as cyanin, whereas cyanin and pelargonin was identified in hibiscus cultivars, respectively.

Lastly the study on rose and hibiscus pollens were conducted with the objective of knowing the pollen fertility, sterility, germinability and viability status by using nuclear stains invitro and it was observed that aborted pollen grains did not take any stain, whereas the germinating once were stained and assessed when the size of the pollen tube growth was larger and non aborted pollen grains were round with definite outline. Among the rose mutants minimum fertility status was

observed in Sindoor and Eiffel Tower whereas a maximum of 70 per cent fertility was recorded both in Paradise and Akito mutant. Pollen germination was the least in most of the mutants with a maximum recorded in Paradise and Akito genotypes. Spontaneous mutants of rose had higher and better fertility and viability status when compared to their parents. Contrarily in induced mutants fertility and viability status was found to be more in parents rather than in the mutants induced by gamma irradiation treatments. The best recommendation was the media containing 20 per cent sucrose and boric acid supplemented with calcium nitrate, magnesium sulphate and potassium nitrate incubated at 25^0C for 4hrs in dark with 100 per cent RH.

In hibiscus cultivars it was found that agar and boric acid content in the germinating media had profound influence on pollen tube growth. Pollens of Ashirwad and Priya gave high per cent germination with the per cent aborted pollens being significantly minimum in all the other cultivars. Pollen fertility and viability were found to be on par with each other with significant difference among different varieties or cultivars undertaken. Standardization of media for pollen germination and pollen tube growth indicates that a medium containing 20 per cent sucrose, 1 per cent agar and 100ppm boric acid was found to be the best recommendation in hibiscus cultivars.

With this background information and the investigation under study one can recommend mutation breeding when the natural variability does not provide the genes for the desired traits. When tight linkages are undesirable to the genes, when a simply inherited defects in an intense otherwise superior cultivars need to be rectified, for desired variations, inducing changes will still retain the desired attributes. Lastly when it is desired to introduce blocks at specific stages of a biochemical pathway to make alterations in the chemical composition of the produce having economic value with distinct mutation breeding advantage.

In the overall context however, it will be relevant to consider that in what direction should the mutational approach adopt for making continued contribution to crop improvement activity, especially in Rose species. Future mutation breeding is expected to make a contribution primarily as an important adjunct to the conventional breeding approach. Normally, new cultivars arise by spontaneous mutations by crossing or selfing. The use of radiation opens a new approach, but some caution should be exercised in the assessment of the technique. There is a great gap between inducing a mutation and recovering a useful or economically important cultivar. A well formulated breeding programme, with well known inbreed lines as parents, may be the best approach for the development of new cultivars. However, the use of radiation can be a valuable compliment to conventional breeding methods with changes in the flower colour, pigments or any other outstanding characters.

Thus, from the theoretical stand point of view the main objective is to uncover the action mechanism of mutagens and on the other hand, from a practical point of view to establish efficient methods of inducing mutations in plant breeding widens the spectrum of mutations which would be very useful for improving many floricultural crops through mutation breeding.

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