CHARACTERISATION AND GENETIC IMPROVEMENT IN ROSE (*ROSA* SPP.) THROUGH MUTAGENESIS

by

BRUNDA S.M.

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"CHARACTERISATION AND GENETIC IMPROVEMENT IN ROSE (*ROSA* SPP.) THROUGH MUTAGENESIS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"CHARACTERISATION AND GENETIC IMPROVEMENT IN ROSE (*ROSA* SPP.) THROUGH MUTAGENESIS"** is a record of research work done independently by Ms. Brunda S.M. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

%	-	per cent
μ	-	Mean
ADS	-	Adenine disulphide
AFLP	-	Amplified Fragment Length Polymorphism
ANOVA	-	Analysis of Variance
bp	-	base pairs
BA	-	Benzyl Adenine
cm	-	centimeter
d.f	-	degrees of freedom
DMSO	-	Dimethyl Sulphoxide
DNA	-	Deoxy Ribose Nucleic Acid
EMS	-	Ethyl Methane Sulphonate
et al.	-	and co-workers/co-authors
M_1	-	Mutated generation
MS	-	Murashige and Skoog
Fig.	-	Figure
Gy	-	Gamma ray
g	-	gram
ha	-	hectare
i.e.	-	that is
IAA	-	Indole Acetic Acid
IBA	-	Indole Butyric Acid
kg	-	kilogram
LAF	-	Laminar Air flow
LD_{50}	-	Lethal Dose
m	-	meter
min	-	minutes

PCR	-	polymerase chain reaction
Psi	-	Pounds per square inch
QTL	-	Quantitative Trait Loci
RAPD	-	Random amplified polymorphic marker
RFLP	-	Restricted Fragment Length polymorphism
rpm	-	revolutions per minute
S.E(m)	-	Standard Error Mean
SE	-	Standard Error
spp.	-	Species
v/v	-	Volume/ volume
viz.	-	namely
w/v	-	weight/volume

Introduction

1. INTRODUCTION

Rose 'The Queen of Flowers' is a beautiful ornamental plant of immense horticultural importance, due to its commercial value. Cultivation of roses was initiated nearly 5000 years ago by ancient civilizations in China, Western Asia and Northern Africa (Gudin, 2000). The genus *Rosa* originated in China. Roses are usually propagated by cuttings, although they can also be propagated by budding and grafting. Reliance on season and slow multiplication rate are the main limiting reasons in conventional propagation (Pati *et al.*, 2006). Rose belongs to family *Rosaceae* and genus *Rosa* contains 130 recognized species (Cairns, 2000). The most important commercial products of rose plant are rose oil, rose water, and rose conserve having very high economic value in international markets (Hussain and Khan, 2004). Rose products are very commonly used in cosmetics, perfumery and pharmaceutical industry.

India has maximum area under ornamental crops (88,600 ha) followed by China (59,527 ha), Japan (21,218 ha) and USA (16400 ha). The cut rose accounts for nearly 60 per cent of cut flower trade in global market nearly one lakh hectare of land is estimated to be under production in India. The leading flower is jasmine which is grown in 6270 hectares followed by rose with 5564 hectares and chrysanthemum 3870 hectares. The major rose growing states are Maharashtra, Karnataka, Tamilnadu, Rajasthan, Uttar Pradesh and West Bengal.

Some important rose species *i.e., Rosa borboniana*, *Rosa gruss-an-teplitz* and *Rosa centifolia* are cultivated in Pakistan but main areas of rose cultivation in province of Punjab are Pattoki, Kallar Kahar, Choha Syedan Shah, Chakwal, Sahiwal (Sargodha), Faisalabad and Islamabad. Some *Rosa* species having essential oil are also being grown in Sindh Province (Farooq *et al.*, 2011). Among these species *Rosa borboniana* has flowers of light pink colour, good fragrance and blooms profusely in spring season. Another important rose species, *Rosa grussan-teplitz* has double flowers of red colour blooms in clusters and has a strong fragrance.

Although these species have unique characteristics, their large plant size reduces their ornamental beauty and does not fulfill the rising trends of high density plantations (Hashmi, 2005). So there is a need of reduced stature rose plants that can be used in urban landscape and home conditions with less space. Furthermore, the emphasis in breeding programmes is focused on ornamental characters such as flower colour, flower size and shape, scent and morphology, recurrent blooming, inflorescence structure and plant habit (Debener and Linde, 2009).

In older times, the duration for the selection of a new cultivar could be as long as ten years but nowadays it can be reduced to five years, though selections requiring seven to eight years are still being performed. This is due to the increase in competition, high investment costs and demand in the market (Nybom *et al.,* 2009). Though desirable traits were introduced by classical breeding, there were some limitations to this technique; firstly, because of the restricted gene pool, secondly, due to incompatibility arising because of differences in ploidy level between putative parents in distant crosses and thirdly, due to polygenic nature of characteristics such as uniform growth and simultaneous flowering. Lastly the seed which is finally produced either fails to germinate or exhibits a very low germination rate.

The information related to rose genotypes is fundamental for the choice of parents to hybridise. Databases where hybridisation and seed production data are recorded are a source of information that characterises the fertility of a genotype. In the same way, database of the phenotypic traits, such as flower colour, flower shape and fragrance or disease resistance, is a useful strategy for the initial choice of parents to hybridise (Nybom *et al.,* 2009). Hence characterization and evaluation of the germplasm collections proves to be significant. The genetic diversity available within *Rosa* species is vast and varied. Its utilization in rose improvement depends on the systematic characterization of genetic resources and on the study of possible hybridization mechanisms. Morphological markers are the phenotypic traits of any organism and are the earliest markers used to describe

the observable characters of an organism. Each species of the genus *Rosa* has a wide and overlapping range of morphological variation that are influenced by environmental conditions. India has a very diverse climate, which ranges from subtropical to temperate and allows the cultivation of almost all kinds of plants. Therefore, we cannot negate the possibility of a large diversity in roses grown in India. The first part of the present study is the systematic characterization of rose germplasm with the help of morphological characters.

The occurrence of spontaneous mutations or 'budsports' is at the mercy of nature and is quite often retrogressive. Hence, induction of variation by artificial means has been resorted inorder to increase the frequency of such events. The rapid growth in theoretical understanding and technical advancement in the induction and recovery of mutations have triggered a dynamic phase in the use of induced mutations as a supplement to, or rather a substitute for, the conventional methods of breeding. The mutants are of direct use in the floriculture trade. However, most mutants deviate from the original variety only in minor characteristics and may thus be very difficult to distinguish genetically.

For the induction of mutations, physical and chemical mutagens have been used. Radiation types available for induced mutagenesis are ionizing radiations (gamma-rays, X-rays, protons, neutrons, alpha and beta particles,) and ultraviolet radiations. Gamma rays are mostly used for this purpose and are considered to be more suitable for obtaining mutants with less radiation damage (Yamaguchi *et al.*, 2010). Chemical mutagens also induce heritable variations. It has been used as a breeding tool in the enhancement of ornamental characteristics such as leaf thickness, plant size, and flower size (Shao *et al.*, 2003). *In vitro* mutagenesis is a combined effect of tissue culture and induced mutation which offer an opportunity to increase variation in commercially important cultivars.

One of the major difficulties in mutation breeding in higher plants is the formation of chimeras. Plants with drastic induced variation have been naturally eliminated due to vigorous diplontic selection. These problems can be solved to a

great extent by adopting *in vivo* and *in vitro* propagation methods which help in the production of solid mutants, that can be recognized and propagated more easily. Moreover, some *in vitro* induced changes cannot be noticed, as the gene's structural differences and protein do not alter the biological activity always to such an extent that it can be recorded phenotypically. In this case, variation can be recorded by DNA analysis (Maria Jesus, 2010). The main application of molecular markers are genotype or cultivar identification, phylogenetic studies, construction of chromosome maps, mapping of morphological and physiological characters, and Quantitative trait loci (QTL) analysis of major traits (Debener and Linde, 2009; Nybom *et al.,* 2009). However, morphological observations have several limitations such as extensive evaluation and time needed for assessment. So use of present day molecular markers in addition to the classical methods provides more positive identification of new mutants.

Restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP), though used for screening of genetic diversity, are laborious, usually involves radioactivity, and are not suited for routine application of cultivar identification. Random amplification of polymorphic DNA (RAPD) requires only small amounts of starting DNA, does not require prior DNA sequence information nor involves radioactivity (Williams *et al.,* 1990), while data can be generated faster with less labour than other methods such as RFLP. There are also some examples where an RAPD-based fingerprint technique has been used for mutant discrimination. But there is no information available on genetic diversity studies of mutant cultivars of rose evolved through induced mutagenesis. Therefore, the main objective was to discriminate the mutants from their respective parents and to understand the genetic diversity using RAPD.

Against this brackdrop, the current study was performed with the objective of assessing the variability available in Hybrid Tea and Floribunda groups of roses, analysing the effectiveness of gamma rays and EMS on inducing variability in roses and studying variations of selected mutants at molecular level using RAPD techniques.

Review of Literature

2. REVIEW OF LITERATURE

The literature pertaining to the present investigation has been reviewed here under the following heads:

2.1 Evaluation and characterisation of germplasm

2.2 Induced mutagenesis

2.3 Molecular characterisation studies

2.1 EVALUATION AND CHARACTERISATION OF GERMPLASM

Genetic diversity forms the basis for improvement of crop species. The effective utilization of any species in breeding and its adaptation to different environments depend on the level of genetic diversity it holds. Characterization and evaluation of genetic divergence and relatedness in the breeding material has significant implications for the improvement of crop plants. Besides providing predictive estimates of genetic variation in different species, it facilitates the planning of new breeding approaches for cultivar development. Diverse parental combinations could be used either for hybrid breeding or for the creation of selectable genetic variability in the segregating populations.

2.1.1 The morphological diversity reviews are discussed below:

Mohapatra *et al.*, (2000) evaluated 17 chrysanthemum varieties for suitability as pot plants for north Indian conditions at Ludhiana (1996-98). Significant differences were observed between varieties for all the morphological and floral characters evaluated. Cultivars Pol Rose, Arun Singar, Sharad Singar, Suhag Singar and Bindiya were found suitable for pot culture.

Sirohi and Behera (2000) studied variability among 57 genotypes of chrysanthemum (*Dendranthema*) and reported that the phenotypic coefficients of variation (PCV) were higher than those of genotypic coefficients of variation

(GCV) for all the characters studied. High heritability with high genetic advance was observed for number of branches per plant, disc diameter, number of petals per flower and flower yield.

Deka and Paswan (2001) evaluated six standard cultivars *viz.,* Alfred Simson, Cossa Grandi, Grape Bowl, Litter Pink, Snow Ball and Temptation of chrysanthemum [*D. grandiflora* (*D. morifolium*)] to ascertain their suitability for cultivation as ornamental pot plants under agro-climatic conditions of Jorhat, Assam, India. Significant differences were observed among the cultivars for three morphological characters (plant height, number of leaves per plant and leaf area) and eight floral characters. Analysis of these characters revealed that the cultivars Snow Ball and Temptation were the best cultivars suitable as pot plants for exhibition/decoration purpose.

Pal and George (2002) found significant differences for all characters (plant height, number of leaves, individual leaf area, length of leaf, width of leaf, number of lobes per leaf, days to flower bud emergence, stalk length, stalk diameter, flower diameter, flower weight and shelf life of flowers) among two indigenous (Sonar Bangla and Pride of Jamshedpore) and 10 exotic cultivars of chrysanthemum (*Dendranthema morifolium*). High heritability associated with high genetic advances as the percentage of mean was observed for leaf area and flower weight, indicating the presence of additive gene action. The other traits exhibited high heritability associated with moderate and low genetic advance, indicating the presence of non-additive gene action. Most of the correlations were positive. Flower diameter and weight were significantly correlated with plant height, leaf number and area, and stalk length and diameter.

Talukdar *et al.* (2003) studied genetic variation for growth and flower characteristics in 11 chrysanthemum cultivars. The highest estimates of genotypic coefficient of variation, phenotype coefficient of variation and heritability were recorded for number of leaves and number of flowers per

plant. The number of flowers per plant exhibited the greatest genetic variation and genetic advance. Based on mean plant height, the cultivars were classified as tall (>50 cm), medium (40-50 cm) and short or dwarf (40 cm). The tallest (73.12 cm) cultivar, Purple Decorative was superior for plant spread, number of primary branches, internode length, stem thickness, flower size, fresh and dry weights of flowers, and number of ray florets. Among the medium cultivars, Charming was superior in terms of number of leaves, plant spread, number of primary branches and flower size. Among the dwarf cultivars, Yellow Button recorded the highest number of leaves, plant spread, and number of primary branches, number of flowers and the lowest number of disk florets. However the cultivar Prof. Harris was the most promising in terms of flower size, number of ray florets, internode length and earliness.

Kunigunda (2004) conducted an experiment to evaluate the variability and heritability of morphological traits in seven chrysanthemum cultivars. The estimates of heritability for different traits varied between 0.781 (plant height) and 0.922 (breadth of leaves), suggesting the highly significant genetic control on these traits. The cultivars having high mean values for different traits were Lameet Bright for diameter of flowers, White Criquette and Nivalachs for petal length and La Cagouille and Yellow Criquette for breadth of petals. These cultivars can be used to develop novel varieties with superior qualitative traits.

Ghimiray *et al.* (2005) studied genetic variability in 12 chrysanthemum cultivars for growth and floral characters under two environmental conditions. The estimates of phenotypic coefficient of variation (PCV) were higher than those of genotypic coefficients of variation (GCV) for all the characters. High heritability coupled with high genetic advances were observed for flower yield per plant (g) and the number of branches per plant over environments, flower freshness after full bloom (days) under open field and flower diameter (cm) under protected condition. This

indicated the greater influence of additive gene effects in controlling these traits. The non-additive gene effects were evident in most of the characters, which exhibited moderate to low genetic advance in spite of having high heritability estimates.

Misra *et al.* (2006) evaluated 27 spray type chrysanthemum (*Chrysanthemum morifolium*) cultivars and found wide range of variability for flower yield and yield related characters. The high estimates of genotypic coefficients of variation (GCV), heritability (broad sense) and genetic advance exhibited for number of branches per plant, average flower weight and number of flowers per plant suggest the presence of additive gene action in the expression of these characters.

The genetic diversity among forty landraces of damask rose in Iran was characterized by Aghdaei *et al.* (2007) in Iran for different traits such as, flower weight, flower diameter, peduncle length, number of petals, number of stamens and oil content. The correlation between oil content and other botanical characteristics was not statistically significant whereas it was significant for all the characters.

Tabaei (2007) evaluated morphological traits *viz.,* flower weight, flower diameter, peduncle length, number of petals, number of stamens and oil content among Damask rose landraces from different regions of Iran. Pearson's coefficient showed that the number of petals was positively correlated with flower weight and number of stamens, whereas its correlation with peduncle length was negative. A negative correlation was obtained between oil content and number of stamens which is beneficial for indirect selection of landraces with low number of stamens and therefore, higher oil content.

Koobaz *et al.* (2008) investigated fourteen populations representing different *Rosa* spp. Cluster analysis indicated that inter- and intra-sectional variations exist. Factor analysis and ordination based on principal component analysis revealed that intraspecific variation was present for both quantitative and

qualitative characters. Traits such as presence or absence of hair on pedicel, prickle density and hip shape were useful in the classification of these roses.

Fu *et al.* (2008) studied 33 quantitative characters in 22 *Dianthus chinensis* inbred lines and one accession each of *D. barbatus* and *D. superbus*. Principal component analysis revealed that the first component accounted for 20.7% of total variation and grouped the accessions mainly based on the plant height. The second component accounted for 18.8% variation and grouped the accessions based on the leaf shape parameters. The third component accounted for 12.4% variation and grouped the accessions mainly based on plant shape.

Zeinali *et al.* (2009) studied *Rosa damascena* genotypes and found that they varied significantly for all traits except for fresh weight of petals per flower. Phenotypic and genotypic coefficients of variation for flower yield per plant (48.03%, 36.49%), number of flowers per plant (40.65%, 26.99%) and number of petals per flower (37.56%, 32.31%) were higher than the coefficients for other tested traits. Cluster analysis revealed that Khuzestan and Shiraz were the most related. The most independent ones were the western and eastern Azerbaijan genotypes.

Panwar *et al.* (2010) analysed genetic diversity in thirty two rose genotypes using morphological markers. Genetic divergence ascertained by Mahalanobis D^2 analysis classified the genotypes into seven distinct clusters with the cluster means revealing the best cluster for various growth and flowering traits. This estimate helped in identifying the potential lines from different clusters as parents in hybridization programmes.

Based on morphological characters, Riaz *et al.* (2011) found that five plant genotypes collected from different locations belonged to two different species (*R. webbiana* and *R. brunonii*) and these species belonged to sections *Cinnamonae* and *Synstylae,* respectively. Dendrogram based on morphological markers showed 50% similarity in two clusters. One of the clusters contained

three genotypes of *R. brunonii*, while the other cluster contained two genotypes of *R.webbiana.*

Farooq *et al.* (2011) evaluated eight landraces of Damask rose to determine the diversity on the basis of morphology and oil yield. Pearson's coefficients showed a negative association of flower weight with peduncle length (r = -0.3348) whereas a strong positive correlation was observed for flower weight with the all other morphological characters analysed. Flower diameter showed a positive but weak correlation with peduncle length, number of petals and number of stamens with the values of r = 0.0733, r = 0.5302 and r =0.1241, respectively. Dendrogram was created by cluster analysis based on morphological characters. The study indicated a lack of relationship between genetic variation and collection sites among the landraces.

Henukarai *et al.* (2015) found genetic diversity among twenty three genotypes of rose (*Rosa* species) using morphological characters. Morphological characterization was done using 16 different morphological traits and genetic diversity of rose species was determined using Jaccard's pair wise similarity coefficient. *R.damascena* var. Rani Sahiba and *R. moschata* showed the highest diversity.

Singh *et al.* (2016) reported that high degree of variations observed among the accessions of *R. multiflora* group as compared to variations among the accessions of *R. moschata*, *R. brunonii* and *R. cathayensis* groups suggests high level of out-breeding in *R. multiflora*. Desirable variations were observed for flower colour, petal number, lack of prickles, perpetual flowering, flower diameter and fruit size which contribute significantly to diversity and have potential use in breeding programs.

Baydar *et al.* (2016) evaluated eighty three seed-derived plants for floral characteristics and scent composition. A wide variation in flower

characteristics was identified, with different petal colors from white to red and petal numbers from 5 to 115 in rose.

Gogoi (2016) reported that ten cultivars of rose formed seven clusters based on selected growth and flower characters. A maximum of three cultivars fell under Cluster II of medium plant height and minimum number of flowers per year. Cluster V consisted of two cultivars with taller plant and higher number of flowers per plant per year. Other five clusters comprised of only one cultivar each. The cultivars also exhibited significant variation for the other characters.

2.2 INDUCED MUTAGENESIS

2.2.1 Induced mutagenesis using gamma rays on budwood

According to Fehr (1987), artificial or induced mutation can be a practical and efficient genetic breeding technique to be used with cultivated plants. Artificial mutation induction is carried out using physical and chemical mutagens which can increase the mutation frequency when compared to its spontaneous occurrence. It is used mainly to obtain mutants for qualitative genes and, on a smaller scale, for quantitative genes. However, the breeding process for quantitative genes has been successful in detriment of the genetic variability found in the populations used. High efficiency of mutant production is essential for its extensive use in plant breeding. Thus the use of any mutagenic agent depends not only on its mutagenic effectiveness but also on its efficiency. Mutagenic efficiency is the production of desirable changes free of association with unwanted genetic alterations. There are several ways of using mutation induction in plant breeding. In recent times, developments in *in vitro* mutation induction and advances in cell and molecular biology techniques in plant breeding have appeared very quickly. Mutagenesis proved favourable for mutation induction in tissue cultures. Positive results were obtained when induced mutagenesis and tissue culture techniques were combined.

2.2.1.1 Effect of gamma rays on sprouting:

Rather *et al.* (2002) treated uniform sized bulbs of Dutch iris (*Iris hollandica* cv. Prof. Blaauw) with different doses of gamma rays and observed that sprouting of bulbs was delayed by 60 days in VM_1 generation and by 9 days in VM_2 generation. Bulb survival reduced by 30.52% in V M_1 generation as compared to non – significant reduction in VM_2.

Among the treated population of *Crossandra infundibuliformis* var. Danica, a single individual from the 30 Gy gamma radiation treatment produced a solid mutant, named "Savindi", with altered leaf shape and flower colour. The plants maintained the new phenotypic characters even after five cycles of vegetative propagation indicating the potential to develop as a novel ornamental product (Hewawasam *et al.*, 2004).

Datta *et al.* (2005) carried out *in vitro* mutagenesis in chrysanthemum and found that the frequency of regeneration decreased with increase in dose of gamma rays in all the cultivars except Flirt at 500 Rad treatment where the regeneration frequency remained comparable with that of control. Hundred percent rooting was observed in all the cultivars. All the shoots (both treated and untreated) survived under field conditions and flowered.

Salahbiah and Rusli (2006) observed that dormant axillary bud explants in roses subjected to increasing doses of gamma rays showed a decrease in regeneration capacity, which was completely suppressed at 100 Gy. The lethal dose for 50% of the regenerating explants (LD_{50}) for both cut and miniature roses were observed to be between 20 and 40Gy. A few new flower mutants, with novel colour and attractive shape were selected for further testing in order to produce stable mutants and this had to be micro propagated for a few generations. Thus, using axillary bud explants for the induction of mutation through *in vitro* shoot regeneration, several potential stable mutants of horticultural value could be isolated.

Srivastava *et al.* (2007) studied the response of gamma radiation (Co_{60}) on sprouting and survival of gladiolus corms of different cultivars and found that sprouting of corms was adversely affected by irradiation but was unaffected by cultivar. Maximum sprouting was observed in 20 Gy dose treatment whereas minimum sprouting was in 80 Gy dose.

Shangwen and Xian (2007) irradiated *Gladiolus hybridus* with 0, 25, 50, 75, 100 and 150 Gy Co_{60} gamma rays, to understand the change of main quality indices and biological characters. The heterogeneity in gladiolus increased with the increase of radiation dosage but low dosage radiation could promote germination.

Berenschot *et al.* (2008) carried out mutagenesis in *Petunia x hybrida* Vilm. and observed that seedling survival rates decreased to 55% after treatment with gamma rays as compared to control.

Tiwari *et al.* (2010) carried out a three year mutagenesis programme using physical mutagens (gamma rays) on four cultivars of gladiolus *viz.,* Peter Pear , Advance Red , White Prosperity and Nova Lux which were irradiated with 50, 100 and 150 Gy of gamma rays at N.B.R.I., Lucknow. Reduction in survival rate was observed with increase in exposure of gamma rays.

Tiwari and Kumar (2011) studied gamma ray induced morphological changes in (*Calendula officinalis*) and observed higher percentage of survival at lower doses and poor survival at higher doses in all the generations of *Calendula officinalis*. However, reduced survival upto the second generation (M_2) and no survival after that were exhibited by the plant treated with 5 kR gamma rays.

2.2.1.2 Effect of gamma irradiation on vegetative and floral parameters

Gupta and Singh (1970) reported that gamma ray treatment of budwood of rose cultivars Montezuma and Super Star at 40, 50 and 60 Gy before budding generally led to reductions in bud take, plant survival and plant height. Somatic

mutations were induced in flower colour and shape in Montezuma, 50 Gy being the most effective dose. Super Star was the more radiation-sensitive of the 2 cultivars.

Lata and Gupta (1971) exposed both bud woods (40 Gy) and stem cuttings (20, 40, 60 and 80 Gy) to gamma rays. Effect of gamma rays on oil content of some scented cultivars of hybrid tea roses, survival, and cytological feature of original and mutant cultivars including their breeding behaviour was studied. They concluded that flowers from irradiated plants were generally smaller and contained less oil than those of control.

Kaicker and Swarup (1972) stated that gamma irradiation induced colour mutations in the rose cvs. Christian Dior, Queen Elizabeth and Kiss of Fire. Then dormant buds were treated with 50-100 Gy of gamma rays. 50 Gy was found as the best treatment while higher doses were lethal. Out of various chemical bud dips, N-nitroso-n-methyl urethane induced colour mutants in rose cv. Christian Dior and EMS induced mutation with low petal numbers in cv. Kiss of Fire.

Bud woods of rose cv. Gulzar with gamma rays from a cobalt-60 source or dipped in EMS and the treated buds were T-budded on Edward rootstock. Two mutants were obtained, the better of which, induced by 0.25 EMS treatment, had blue strip and was released under the name Madhosh (Kaicker and Swarup, 1978).

Lata (1980) subjected the budwood from seven rose cultivars exhibiting five different flower colours to gamma irradiation at 0, 30, 40 and 50 Gy. Three mutations, one in growth habit and two in flower colour were successfully isolated and propagated. The results suggested that Floribunda rose Pink Parfait was the most suitable for induction of mutations.

Datta and Gupta (1982) irradiated bud woods of rose *cv.* Junior Miss with 30, 40 or 50 Gy and these buds were budded on *Rosa indica* var. *odorata.* No mutation in flower color was detected in the first year but one plant from the 30 Gy treatment showed a mutation from pink to white in the second year after heavy

pruning. This mutant was isolated and propagated by repeated budding. The flower diameter and petal size were significantly reduced in the mutant but the petal number was increased. No significant differences in the numbers of stomata or their size were noticed.

Datta and Gupta (1984) revealed that the 'Saroda' cultivar of rose was induced by exposing bud woods of cv. Queen Elizabeth to 30 Gy gamma rays that produced almost white flowers. Another new cultivar producing very light pink flowers named Sukumari was induced by exposing bud woods of cv. America's Junior Miss to 30 Gy. These two mutants detected mutants in VM_1 were isolated and multiplied by repeated budding.

Beneteka (1985) irradiated rose single bud cuttings with 0, 20, 30, 40 and 60 Gy gamma rays and subsequently observed four bud-propagated generations, and the optimal doses were 40 and 50 Gy and chimerism decreased with successive generations. However, chimerism remains the main constraint in mutation breeding of vegetatively propagated crops.

Bud woods of nine cultivars were irradiated with gamma rays at 30, 40 or 50 Gy and these buds were budded on *Rosa indica* var. *odorata* was irradiated by Datta (1985). He observed reduction in sprouting and survival which increased as the dose increased with the cv. Orange Sensation which was the most sensitive and cv. Kiss of Fire, being the most resistant one. Reduction in height was also noted with cv. Kiss of Fire being the least and cv. Zambra the most affected. Somatic mutations in flower colour were noted in all cultivars except cv. Happiness.

Gamma irradiations at 30 Gy was applied to the green shoot of several rose cultivars including Crimson Glory, Super Star, Condesa de Sastago, Peace, Pink Peace and South Seas by Huang and Chen (1986). In the M_1V_1 , M_1V_2 and M_1V_3 generations, mutants were selected for leaf and flower characteristics. Four new culitvars named Ji Guang, Xia Guang Wan Doa, Zhen Jie and Nan Hai Lang Hua were established from stable mutant clones.

Smilansky *et al.* (1986) exposed stem cuttings of rose cv. Mercedes to various doses of gamma rays and ethyl methane sulphonate (EMS). This resulted in a range of petal color changes, from light orange cream through pink, salmon, orange and red to dark red. The appearance of mutations such as changes in flower size, shape, number of petals, thorniness, etc. was less frequent.

Datta, (1989) reported that budwood of 32 rose cultivars (*Rosa* spp.) were exposed to 30-40 Gy of gamma rays and eyes were grafted on *Rosa indica* var. *odorata* root stock. Somatic mutations in flower colour/shape were detected as chimera in 21 cultivars. The size of the mutant sector varied from a narrow streak on a petal to a whole flower and from a portion of a branch to an entire branch. Fourteen mutants were detected in M_1V_1, four in M_1V_2 and three in M_1V_3. Maximum number of mutations was detected following 30 Gy treatment. Nine mutants were obtained *viz.,* 'Sharada', 'Sukumari', 'Tangerine Contempo', 'Yellow Contempo', 'Pink Contempo', 'Striped Contempo', 'Twinkle', 'Curio' and 'Light Pink Prize' .

Arnold *et al.* (1998) studied the effect of various gamma ray doses on four cultivars of miniature roses and found that radiation significantly decreased the mean number of spines per 10 cm of stem length at 50 Gy and increased the mean petiole length at 50 and 100 Gy in Portluck cultivar. It reduced mean spine number in Mountie cultivar. Mean petal number in Blue Blood cultivar was significantly reduced at 50 and 200 Gy. Plant height decreased with increase in dose of gamma rays in all the four cultivars of rose *i.e.,* Portluck, Blue Blood, Mountie and Dark Red Mountie. None of the plant growth parameters were affected by the radiation doses studied in Dark Red Mountie cultivar nor were the length and width of the terminal leaflet of any of the cultivars.

Neville *et al.* (1998) reported that absorbed gamma ray doses of 50, 100, and 200 Gy were used to produce mutations in miniature rose. The mean spine

number, petiole length and petal number were reduced significantly in all cultivars. The range in color increased sustainably for all the cultivars.

Datta *et al.* (2001) carried out an experiment with *Dendranthema grandiflora* cv. 'Puja'. The rooted cuttings were treated with different doses of gamma rays. Sectorial somatic mutations were detected in all the doses. Original and mutated ray florets were cultured on MS medium. When transferred to the field, regenerated plants flowered true to explant floret colour and shape. The isolated ray floret colour mutants with tubular florets were maintained vegetatively which proved to be true to type in two successive generations.

To induce flower colour mutants, 3 cm long shoots of chrysanthemum were irradiated using a Co_{60} source at 0, 5, 10, 15 and 20 Gy doses. Different parameters like survival percentage, number of leaves per shoot and growth rate at 7, 14, 21 and 28 days after irradiation was studied. Different colour mutants were found after irradiation (Otahola *et al.*, 2001).

Banerji and Datta (2002a) conducted an experiment to study the effects of gamma irradiation on chrysanthemum [*Chrysanthemum morifolium* (*Dendranthema morifolium*)] cv. 'Lalima' with particular reference to somatic mutations. Rooted cuttings were irradiated with 0, 15, 20, 25 Gy of gamma rays. Results revealed a significant reduction in survival, growth rate, plant height, number of branches and leaves per plant and leaf and flower size, whereas increase in morphological, floral and chromosomal abnormalities were recorded after irradiation with gamma rays.

The rooted cuttings of chrysanthemum cv. 'Surekha' were exposed to 0, 150, 200 and 250 Gy of Co_{60} gamma rays. Reduction in survival, plant height and plant growth, number of branches, leaves, flowers, and size of flower heads was observed after irradiation. Increase in foliage, flower abnormalities, chromosomal aberrations and delay in flowering were

observed after exposure (Banerji and Datta, 2002b).

Cantor *et al.* (2002) irradiated the corms and cormels of three gladiolus cultivars (Her Majesty, Applause and Speranta) for 72 hrs with 137 Cs gamma source on cylindrical exposure geometry. Significant effect in length of roots was obtained in the variants which were irradiated with 1 Gy gamma radiation and 3 Gauss magnetic field.

A new *Chrysanthemum multiflorum* cultivar 'Samco' was developed utilising a spontaneous mutant from cv. 'Veria Dark'. Plant height of 'Samco' was much reduced with small flower heads comprising of white coloured ray florets with yellow disc florets (Pieters, 2002).

Shobha *et al.* (2002) conducted pigmentation studies in rose cv. 'Paradise' and its induced mutants and reported that gamma irradiation (30 to 60 Gy) resulted in reduction in flavanol, anthocyanin and leucoanthocyanin contents.

Dilta *et al.* (2003) treated rooted cuttings of various cultivars of *Dendranthema grandiflora* with gamma rays at 0 and 20 Gy. Changes were observed after gamma ray treatment in different vegetative and floral characters. Plant survival, plant height, number of branches, plant spread and number of flowers decreased after treatment. An increase in the magnitude of plant abnormalities was recorded. Delay in number of days to bud formation and days to harvest were recorded in treated plants as compared to control.

Goo *et al.* (2003) carried out *in vitro* experiments to investigate colour change in flowers of six chrysanthemum cultivars irradiated with gamma rays. Fifty percent lethality was found at the dose of 40 Gy of gamma rays. Flower colour and shape were changed by gamma irradiation of 20-40 Gy. Among various colour changes, white was changed to pink and yellow; yellow to red; and brown to yellow and purple to white, pink and red. The changes were observed both in ray florets and disc florets, but the changes were more in ray

florets.

Hu Chao *et al.* (2003) investigated the effect of Co_{60} gamma ray (10, 15, 25 and 30 Gy) treatments on the physiology and biochemistry of chrysanthemum plant. The chlorophyll content in the irradiated plants was higher than that in control. Photosynthetic ratios of irradiated chrysanthemum were higher than those of the controls.

Nagatomi *et al.* (2003) irradiated young plants of chrysanthemum cv. 'Taihei'. The petals and buds from the irradiated plants were cultured *in vitro* and one flower colour mutant was selected from regenerated plants. The growth of mutant cultivar was similar to that of cv. 'Taihai' which was suitable for production of medium sized cut flowers opening in early and mid-November. Mutant showed general resistance to insect pests and diseases.

Maximum time for all growth milestones was taken by 60 Gy treated protocorms. Among the various treatments, significant difference was not noticed with regard to the number of leaves. Among the treated population of *Crossandra infundibuliformis* var. Danica, a single individual from the 30 Gy gamma radiation treatment produced a solid mutant, named "Savindi", with altered leaf shape and flower colour. The plants maintained the new phenotypic characters even after five cycles of vegetative propagation indicating the potential to develop as a novel ornamental product (Hewawasam *et al.*, 2004).

Datta and Chakrabarty (2005) reported that the mutations were mostly in flower color and shape as a result of both chemical and physical mutagens. More than 30 rose mutant varieties have been released and commercialized mainly for changed flower color, higher oil content and better oil quality.

Kole and Meher (2005) performed an experiment using different doses of gamma rays in two varieties of zinnia (Suttons Giant Double Orange and Yellow). There was significant reduction in flower number in both the varieties at 100 Gy

and 150 Gy while non-significant difference was recorded between control and 50 Gy. Appearance of flowering was significantly delayed in all the three doses (50, 100, 150 Gy) of gamma rays over control in both the varieties. Flower retention in days was highest in control. Largest flower diameter was observed in 50 Gy. Significant reduction in flower diameter was observed at 100 and 150 Gy over control and 50 Gy in Yellow variety.

Boersen *et al.* (2006) studied the effect of various rates of gamma radiation (0, 10.0, 12.5, 15.0, 17.5, 20.0 and 30.0 Gy) on the frequency of mutations in inflorescence colour and type of chimeras in non-rooted cuttings of *Dendranthema grandiflora* cv. 'Cherry Dark'. A linear decrease in plant height and a quadratic tendency in survival percentage were observed as the rate of the mutagen increased. Higher frequency of mutants, including inflorescence colour, were obtained with gamma radiation at 10 and 12.5 Gy

Salahbiah *et al.* (2006) observed that dormant axillary bud explants subjected to increasing doses of gamma rays showed a decrease in regeneration capacity, which was completely suppressed at 100 Gy. The lethal dose for 50% of the regenerating explants (LD_{50}) for both cut and miniature roses were observed between 20-40Gy. A few flower mutants, with novel colour and shape were selected and tested further for producing stable mutants which were micro propagated for a few generations. Thus, using *in vitro* axillary bud explants for the induction of mutation several potential stable mutants of horticultural value were isolated.

Berenschot *et al.* (2008) irradiated the seeds of petunia with different doses of gamma rays and observed significant reduction in height at 40 and 60 Gy. Plant surviral rate and the frequency of morphological effects at the adult vegetative stage were higher for the M_1.

Lee *et al.* (2008) investigated the structural genes and their transcripts for anthocyanin synthesis in *Dendranthema grandiflorum* 'Argus'. Colour

mutations in chrysanthemum were obtained through gamma irradiation. Normal florets were pinkish, but the mutants had white or purple ray florets and purple or yellow -green disc florets. Irradiation modified both flower size and the number of ray florets. Level of anthocyanin in the mutants ranged from 4 times lower to 6 times higher for the disk florets as compared to control.

Misra *et al.* (2009) studied the effect of gamma irradiation on chrysanthemum cultivar 'Pooja' with particular reference to induction of somatic mutations in flower colour and form. Rooted cuttings were irradiated with 0, 10, 15, 20 and 25 Gy of gamma rays. A reduction in number of branches, leaves and flower head per plant was recorded. An increase in different types of morphological abnormalities was observed with an increased gamma ray dose. Most of the plants remained in juvenile stage and were unable to differentiate flower buds.

Yamaguchi *et al.* (2009) compared the effect of ion beam and gamma ray treatment on mutations induced in axillary buds of chrysanthemum. Axillary buds were irradiated with carbon ions at 2 Gy, Helium ions at 10 Gy and gamma rays at 80 Gy. All of these had a similar effect on survival. All the flower colour mutants induced with gamma rays were periclinal chimaeras. Solid mutants were also obtained when irradiated with 5 Gy of Helium ions, which had less effect on survival and mutation than gamma ray treatment.

Patil *et al.* (2010) irradiated the corms of three cultivars of gladiolus with different gamma doses and observed that plant height was the highest at 2 kR treatment in both the generations. Furthermore, 1 kR and 3 kR also produced taller plants than that were produced by control and other higher doses. Among all varieties, cv. Novalux produced the tallest plants while the shortest plants were observed in cv. American Beauty. The number of leaves were significantly affected and maximum number of leaves were produced by the corms treated with 2 kR dose of gamma rays. The leaf number decreased as the dose of gamma rays increased.

Patil *et al.* (2010a) treated the corms of gladiolus with gamma rays and observed that 2 kR treatment produced maximum number of florets per spike and maximum number of florets remained open at a time in both succeeding generations. Among all varieties cv. Novalux produced maximum number of florets and maximum florets remained open at a time whereas the other two cultivars performed poorly in both VM_1 and VM_2 generations. Spike initiation was delayed with increase in doses and maximum number of days for spike initiation was taken by corms which were treated with the dose of 5 kR gamma rays. Spike length was more in treatments with lower doses i.e from 1 kR to 3 kR as compared to control but started to decrease as the gamma radiation doses increased and shortest spikes were noted at 5 kR in both the generations. Among the cultivars cv. NovaLux produced the longest spikes.

Mahure *et al.* (2010) irradiated unrooted cuttings of chrysanthemum cultivar 'Red Gold' with 10, 20 and 30 Gy gamma rays to induce favorable variation. It was found that lower doses of gamma irradiation and induced encouraging novelties while the higher doses often induced high degree of abnormalities and consequent mortality. The colour novelties induced by the mutagens were isolated and purified in M_2 generation.

Tiwari *et al.* (2010) carried out a three year mutagenesis programme using physical mutagens (gamma rays) on four cultivars of gladiolus *viz.* Peter Pear, Advance Red, White Prosperity and Nova Lux which were irradiated with 5, 10 and 15 kR of gamma rays at N.B.R.I., Lucknow. Reduction in number of spikes/plant, number of florets/spike, days to flower and shelf life were observed with increase in exposure of gamma rays. Positive correlation existed between doses of gamma rays and number of spikes/plant. Florets /spike was positively correlated to 5 kR dose and it became negative at higher dose of irradiation.

Koh *et al.* (2010) irradiated rooted cuttings of two roses, 'Spidella' and 'Cabernet' with different gamma rays doses (30, 50, 70, 90, 110, 130, 150 and 170 Gy) from a cobalt-60 source. They observed that 50% lethal doses (LD_{50})

were 110 Gy for 'Spidella' and 150 Gy for 'Cabernet', respectively. 50% reduction in shoot length was observed at 70-90 Gy dose for 'Spidella', and 110 Gy dose for 'Cabernet'. Solid, chimeric and mosaic petal mutants with various colors were induced from 'Spidella' and 'Cabernet' when 30-170 Gy dose was used. The mutants obtained from 'Spidella' had white, ivory, pinky ivory, light pink and deep pink petal colors. The mutants obtained from 'Cabernet' had pink, deep pink, purple red (magneta), orange red and purple petal colors.

Koh (2011) irradiated forty four rooted cuttings of rose variety 'Kardinal' at 70 Gy gamma-ray dose from a 60Co source to induce mutants. Four different kinds of mutant twigs each with a different color flower were obtained from the irradiated 'Kardinal' with red petals. Characteristics of shoot, leaf, etc. from the four mutants were also different from the ones of 'Kardinal'.The line KA1 was the shortest in shoot, internode and peduncle length, and the lowest in prickle number. The reverse side of leaves was reddish green color in 'Kardinal' as well as in the line KA4, but it was green in color in the lines KA1, KA2, and KA3.

Kolar *et al.* (2011) performed an experiment using gamma rays in delphinium to induce chlorophyll mutations and observed that out of the 11 different types of mutations maximum (10) was induced by 10 kR gamma ray treatment followed by 5 kR. Among the mutants recorded, viridis type was the most predominant followed by xantha and striata. The mutants contained significantly less chlorophyll than normal plants. Among the mutants, xantha and aurea contained the least chlorophyll.

Tiwari and Kumar (2011) studied gamma ray induced morphological changes in pot marigold and found that the percentage of abnormalties increased with increase in exposure to gamma rays. In M_1 generation, drastic reduction in abnormalities were recorded whereas no significant abnormalties were recorded in M_3 generation. Significant delay in flowering occurred in M_1 generation. Maximum delay in flowering was noticed at 5 kR gamma irradiation in M_1 generation. However the irradiation effect was non-significant after second

generation. In M_1 generation maximum total fresh weight of flower / m^2 was recorded at 2.5 kR gamma rays whereas minimum was observed minimum at 5.0 kR gamma irradiation. There was no effect of gamma irradiation on fresh weight of flower/ m^2 in the second generation and later generation.

Singh and Kumar (2013) carried out an experiment to study the effect of gamma irradiation on morphological characters of ten different varieties of gladiolus. It was observed that treatment with 2.0 kR gamma rays in cv. J.V. Gold showed the best performance with respect to number of leaves per plant

Kaicker (2015) studied the cytology of 12 distinct induced colour mutants obtained by both EMS and gamma-rays in cv. 'Folklore'. The parent cv. 'Folklore' was observed to be 4n. Cytological variants seem to be responsible for mutations of colour and habit.

Ketpet *et al.* (2015) irradiated four varieties of roses by applying X-rays at doses 0 and 100 Gy to bud segments. The result showed that X-rays at 10 Gy affected the growth of rose mutants and reduced the flower size, the number of petals and the size of receptacle but increased the length of flower necks and flower stem. It showed that variety Kardinal gave 2 mutants (pink and salmon pink) and First Red gave one mutant (deep pink) with different color, flower size, stem length, size of receptacle and number of petals.

The rose cultivar 'Marcia' was treated with gamma radiation of doses 20, 30, 40, 50, 60, 70, 80 and 90 Gy. Results of ANOVA showed that treatment with various levels of gamma radiation had significant effect on seedling height, number of offsets, number of leaf as well as fresh weight and dry weight of rose seedlings. It was concluded that treatment with various dozes of gamma rays destroyed few seedlings and the effect of various doses was different for different characteristics of seedlings as recorded by Moharrami *et al.* (2015).

Sadhukhan *et al.* (2015) evaluated the stock plants of three varieties viz. BC-8-05, Winter Queen and Bidhan Shova of chrysanthemum. These were then

subjected to different levels of gamma irradiation viz. 0, 10, 15, 20, 25 and 30 Gy to study the rooting potential. Number of roots, as well as root length increased significantly in several cases at 10 Gy in comparison to control. Unrooted cuttings of Winter Queen recorded the highest LD_{50} (20.1 Gy), whereas it was found to be the lowest in Bidhan Shova (10.2Gy)

2.2.2 Induced *in vitro* mutagenesis using EMS

2.2.2.1 Effect of EMS on sprouting:

Roychowdhary and Tah (2011) treated healthy seeds of Dianthus with different concentrations of EMS and observed that germination percentage was lower in EMS treated plants as compared to control. Survival rate of the treated seeds reduced with increased dose of mutagen in M_1 generation. The lowest laboratory germination of 51% with the lowest survival seedling (153 out of 300) was recorded in 0.7% EMS. Survival at flowering stage or at maturity due to different mutagenic doses ranged between 41.7 and 69.6% with EMS treatments whereas it was 91.67% in control.

2.2.2.2 Effect of EMS on vegetative and floral parameters:

Nonomura *et al.* (2001) reported that when apical buds of lateral branches were treated with methyl-N-nitro-N-nitrosoguanidine mutagens, variations in size, shape, color and number of petals were detected in the flowers. The chemical mutagen N-methyl-N-nitro-N-nitrosoguanidine at 100 µg per ml was found to be effective.

Latado *et al.* (2004) used chemical (EMS) mutation in immature floral pedicels to develop new cultivars of chrysanthemum (*Dendranthema grandiflora* Tzvelev). Immature pedicels of chrysanthemum cv. Ingrid were treated with 0.77% (0.075 M) EMS solution for 1 hr and 45 min, followed by rinsing in water for 15 min and then cultivated in MS medium (salts and vitamins) amended with 1 g/l of hydrolysed casein, 1 mg/l BAP, and 2 mg/l IAA. Out of the total (910)

plants obtained from the pedicels treated with EMS, 48 (5.2%) mutants were obtained with change in petal color (pink-salmon, light-pink, bronze, white, yellow, and salmon color). Most of them (89.6% of the total) were phenotypically uniform.

Datta *et al.* (2005) treated the ray florets of *Chrysanthemum morifolium* with different doses of gamma rays, cultured them on MS- medium and observed somatic mutations in flower colour and floret shape in gamma ray treated population. No chimeric nature of mutation was detected in any plant. However, no change in floret colour was observed in cv. Maghi but the shape of the ray floret was changed at 10Gy treatment. Flowering period (days to flower bud initiation, first colour showing and full bloom) was slightly delayed in treated population. Number of flowers per plant decreased significantly in regenerated plants except in cv. Maghi where the trend was reversed but floret size (length and width) remained the same in all cases.

Senapati *et al.* (2008) reported that apical and axillary meristems of *Rosa hybrida* cv. 'Pusa Gaurav' were pretreated with various concentrations of oryzalin to induce variation *in vitro*. The lethal dose (LD_{50}) was 20 µM oryzalin pretreated for 24 hr. Oryzalin-treated microshoots were cultured on Murashige and Skoog (MS) medium supplemented with BAP, IAA, ADS and oryzalin. Elongated shoots were rooted in half-strength MS medium supplemented with IBA and about 65% rooted plants survived in the greenhouse. Minor variations in some morphological characters such as plant height, number of branches, foliage size, thorn density, flower diameter, flower depth and number of petals were observed.

Mekela *et al.* (2010) treated the semi hard wood cuttings of *Jasminum sambac* cv. Gundumalli with different doses of gamma rays and EMS and found that at higher dose of 2.5 kR gamma rays +30 mM EMS, there was reduction in flower length, bud length, stalk length and flower weight.

Kolar *et al.* (2011) studied induced chlorophyll mutations in *Delphinium malabaricum* (Huth) Munz. and observed that the highest frequency of chlorophyll mutations was recorded in 0.25% EMS treatment.

Roychowdhury and Tah (2011) in Dianthus studied chemical mutagenic action on seed germination and related agro- metrical traits in M_1 generation and observed that plant height, days to seed germination, number of leaves per plant and stem diameter decreased with increase in dose of EMS concentration.

Senapati and Rout (2011) reported that cultivar First Red was pretreated with various concentrations of oryzalin to induce variation *in vitro*. The present results indicate that LD_{50} was obtained in 20 µm oryzalin. Both the treated and untreated meristems were cultured in Murashige and Skoog (MS) basal medium supplemented with BAP, IAA, ADS and oryzalin. The elongated shoots were rooted in the half strength MS basal medium supplemented with IBA and about 60% rooted plants survived in the green house.

Bhajantri and Patil (2013) treated the corms of gladiolus with Ethyl methyl sulphonate and observed that number of leaves per plant and plant height recorded high phenotypic and genotypic coefficients of variation at 0.50% EMS treatment in both Ethyl Cavcole and White Prosperity genotypes. Low PCV and GCV were recorded for characters such as leaf width, interfloret length and floret diameter in all the treated populations of Ethyl Cavcole whereas White Prosperity showed moderate variability for inter floret length in all treated populations.

Toyoda *et al.* (2015) reported that apical buds of lateral rose branches (*Rosa hybrida* 'Carl Red') were asexually propagated by cutting and treated with chemical mutagens. Variations in size, shape, colour, and the number of petals were detected most frequently in flowers produced from apical buds treated with 100 µg/ml N-methyl-N-nitro-N-nitrosoguanidine. The variant petals were cultured on Murashige and Skoog (MS) medium supplemented with α-naphthalene acetic

acid (NAA) and 6-benzylaminopurine (BAP) for *in vitro* isolation and the multiplication of morphologically altered rose plants.

2.2.3 Induced mutagenesis using gamma rays and EMS

Wilson (1993) conducted induced mutagenesis in Hybrid Tea with three cultivars adopting *in vivo* and *in vitro* culture. Bud woods were exposed to gamma ray doses of 20,30,40,50 and 60 Gy and budded onto root stocks as *in vivo* treatments. Bud woods after exposure to the same doses of gamma rays were cultured *in vitro* using Murashige and Skoog (MS) basal medium supplemented with growth regulators. The cultivar Folklore proved to be the most responsive. From 30 Gy treated population one flower colour mutant and from 40 Gy treatment one mutant with increased number of petals could be isolated.

Rooted cuttings of Chrysanthemum morifolium cv. 'Maghi', were treated with different doses of gamma rays. As a result, mutations in flower colour and chlorophyll variegation were detected. Attempts were made to standardize a micro technique for plant regeneration from mutated tissue. Explants were cultured on MS medium with different combinations of growth regulators. Plants with variegated leaves and two new flower colours i.e. mauve and white were isolated in pure form (Mandal *et al.* 2000a)

Ray florets of *Chrysanthemum morifolium* Ramat cv. 'Lalima' were irradiated with gamma ray doses of 0.5 Gy and 1 Gy after inoculation on MS medium supplemented with α- naphthalene acetic acid and benzyladenine. After transplanting in field, two mutants were obtained in 0.5 Gy treated plants. Both the mutants were yellow coloured but one had flat spoon shaped ray florets similar to the original cultivar while the other had tubular florets (Misra *et al.* 2003)

Sobhana and Rajeevan (2003) carried out *in vitro* mutagenesis studies by using gamma rays. Dosages upto 60 Gy were employed for irradiating the protocorms of a *Dendrobium* hybrid, Sonia 28 x Emma White. They found that significant difference was not observed in the survival percentage of irradiated and control protocorms.

A protocol for *in vitro* mutagenesis for chrysanthemum was established by Dao *et al.* (2006). LD_{50} was 5 kR for callus irradiation. Various growth, developmental, morphological, colour and abnormal shape mutations were identified in M_1V_4 generation.

Wang and Yu (2006) irradiated micro-shoots of *in vitro* cultured *C. morifolium* cv. 'Qiuzhishan' with 5 to 30 Gy gamma rays. The plant growth and flower characters were investigated after transplanting regenerated plantlets. The results showed that 20 Gy was the lethal dose of gamma rays for *in vitro* culture, while 10 Gy was the optimum dose to induce mutations. In contrast with the bright yellow flower of the control, different shades of red colour were observed in mutant flowers.

Misra and Datta (2007) selected a greenish white, large flowered chrysanthemum cultivar 'Madam E Rogar' for *in vitro* propagation and mutagenesis to induce further genetic variability. A protocol was standardized to develop large scale quality planting material for commercial exploitation. Genetic variability in the form of a solid mutant of yellow colour was obtained from this cultivar, when freshly inoculated ray florets were treated with 10 Gy gamma radiation dose.

To induce variability in rose cv. Folklore irradiation of axillary buds followed by *in vitro* culture was employed. Gamma irradiation at 20, 30 and 40 Gy was done. The *in vitro* induced micro shoots were subcultured on a rooting medium consisting of MS basal medium supplemented with growth regulators and

activated charcoal. Rooting percentage was the lowest in 40 Gy treatment. Gamma irradiation at 30 and 40 Gy significantly delayed root initiation, reduced the number of roots per culture and root length (Wilson and Nayar, 2007).

Kumar *et al.* (2012) carried out *in vitro* mutation induction and selection in chrysanthemum lines for improved resistance to *Septoria obese* Syd and observed that the percentage of surviving calli decreased with increasing gamma ray doses. No calli was recovered at gamma ray dose 30 Gy while 22.2% of the calli survived at 20 Gy dose of gamma irradiation.

Hybrid Tea rose (*Rosa hybrida* L.) cultivar Pusa Mohit was subjected to *in vitro* mutagenesis. Single node cuttings were irradiated with different doses of gamma rays (0, 5, 10, 15, 25, 40, 55, 65, 70, and 80 Gy). The irradiated explants were cultured aseptically on MS basal medium supplemented with growth regulators to induce sprouting and shoot proliferation. 40 Gy treatment was determined to be the LD_{50} dose. Explants treated with higher doses (65, 70 and 80 Gy) showed deleterious effects of ionizing radiations (Madhubala and Singh, 2013).

Two mutant lines of rose, *Rosa centifolia* and *Rosa gruss-an-teplitz,* which were treated with different levels of gamma rays and colchicine were evaluated. Data on various parameters such as plant height, shoot length, fresh leaf weight, dry leaf weight, flower diameter, number of shoots, number of flowers/plant/week, weight of 10 flowers and number of petals was recorded. Statistical analysis of obtained data showed significant variation. When compared to control, gamma radiations showed greater improvement in *Rosa centifolia* but colchicine impact was more pronounced on *Rosa gruss-an-teplitz* as observed by Butt *et al.* (2014).

2.3. MOLECULAR CHARACTERISATION STUDIES

2.3.1 Identification and analysis of genetic variation

Mutants that show morphological characteristics better than parents and show the existence of a genetic difference are expected to be developed into new varieties which are superior. The success of mutation can be observed through changes in morphology, anatomy, and also at the DNA level (Widiastuti *et al.* 2010). Presently, there are various methods available which can be used to detect and monitor tissue culture-derived plants and cultivar identification. The most reliable methods are the molecular marker techniques that identify the variance depending on the plant proteins, which are expressed from defined regions of DNA or DNA polymorphisms (Gaafar and Saker, 2006). Different DNA markers, such as RFLPs, RAPDs and microsatellites, have been used to assess genetic variation induced in plants (Devarumath *et al.*, 2002; Pew and Deng, 2005). RAPD is a powerful technique for identification of genetic variation. It has the distinct advantage of being technically simple and quick to perform, requiring only small amounts of DNA (Gaafar and Saker, 2006).

RAPD markers are considered to be a rapid tool for assessing genetic diversity at molecular level and this technique has further advantages over other systems of genetic documentation because it has a universal set of primers and no preliminary work such as probe isolation, filter preparation, or nucleotide sequencing is necessary in various plant species (Millan *et al.* 1996). Any genetic change induced by *in vitro* conditions of tissue culture is expected to generate stable plants carrying interesting heritable traits. Therefore, their early detection is considered to be very useful in plant tissue culture and transformation studies.

In vitro grown meristems of *Rosa hybrida* cultivars First Red, Cri Cri and Pusa Gaurav were treated with various concentrations of EMS to develop mutants. The maximum rate of shoot multiplication was obtained on MS medium supplemented with growth regulators. LD_{50} was observed to be 1% EMS treatment for 6 hours. Genetic variation was detected among the *in vitro* raised

plants by using RAPD markers. The result showed that 22.9, 19.2 and 27.1 % polymorphism was obtained among the *in vitro* raised plants of First Red, Cri Cri and Pusa Gaurav respectively (Senapati and Rout, 2008).

2.3.2 Analysis of mutants by using RAPD

Ya Hui *et al.* (2003) irradiated five chrysanthemum cultivars with different 60_{Co} gamma dosage and subjected to RAPD analysis to determine genetic relationships among the cultivars. Six polymorphic primers selected from 25 arbitrary primers generated 72 amplified bands which identified different chrysanthemum cultivars and lines. Compared with the control, differences in the cultivars were observed at the molecular level.

Random amplified polymorphic DNA (RAPD) markers were used to study the molecular characterization of 10 new radio mutants of chrysanthemum. The original cultivar 'Richmond' differed in genetic distance from its Lady Group mutants. The analysis of genetic similarity indices revealed low diversity within the radio mutants. The Lady Group cultivars, derived from one original cultivar by radio mutation, could be distinguished from each other by using RAPD markers of only a single primer or sets of two or three primers. Polymerase chain reaction analysis proved the efficiency of the RAPD method for DNA fingerprinting of the original cultivar 'Richmond' and its new radio mutants (Lema-Ruminska *et al.*, 2004).

In the earlier study on chrysanthemum radio mutants (*Dendranthema grandiflora* Tzvelev) and their original cultivars of two cultivar groups, Nero and Wonder, the Ney similarity index revealed a high diversity between the cultivars and in all of the cases was less than 0.829. Eight of twenty primers resulted in polymorphic bands which allowed for distinguishing between the chrysanthemums cultivars examined. A single primer or set of two primers resulted in specific fixed and repeatable pattern of bands in each original cultivar and in each mutant (Lema-Ruminska *et al.*, 2005).

An attempt was made to understand the molecular systematics and genetic difference between 10 original chrysanthemum cultivars and 11 mutants. The similarity among the cultivars and mutants varied from 0.17 to 0.90 using RAPD analyses, a simple but efficient method to distinguish cultivars and to assess parentage. Two distinct groups were found. In one of these, two cultivars were present, showing that they were different from all other cultivars. Mutants with different flower colours could be distinguished at the molecular level using RAPD technique, holding promise to identify unique genes as SCAR markers. A high genetic distance among the different chrysanthemums showed that there exists a possibility of introgressing new and novel genes from the chrysanthemum gene pool (Bhattacharya and Silva, 2006).

Kumar *et al.* (2006) characterized eleven radio mutants from two chrysanthemum cultivars Ajay and Thai Chen Queen by RAPD to understand the extent of diversity and relatedness. Out of 40 random primers screened, 21 gave reproducible polymorphic bands. Their study revealed that RAPD molecular markers can be used to assess polymorphism among the radio mutants and can be a useful tool to supplement the distinctness, uniformity and stability analysis for plant variety protection.

Genetic variation of regenerated plantlets following *in vitro* mutation was detected *via.* RAPD in chrysanthemum. RAPD bands produced from eighteen 10-mer arbitrary primers were used to assess the genetic variation of plantlets regenerated from floret-derived calli treated with 0, 10, 15 and 20 Gy gamma rays. These primers generated a total of 167 reproducible RAPD bands ranging in size from 0.3 to 2.0 kb, of which 61.7% were polymorphic and 38.3% monomorphic. The number of bands per primer ranged from 4 to 14, with an average of 9.3. The further analysis of RAPD results showed that genetic variation of generated plantlets was proportional to the dosage of

gamma rays. The 15 and 20 Gy treatments were not significantly different. This finding is consistent with the common conception that genetic variation of radio mutants is usually proportional to the dosage of mutagen within a certain range (Teng *et al.*, 2008).

Barakat *et al.* (2010) induced mutation in *Chrysanthemum morifolium* cv. Delistar White' through *in vitro* mutagenesis by treating the ray florets with 0.5 and 1.0 Gy of gamma irradiation. RAPD analysis was applied for the detection of genetic polymorphism among chrysanthemum mutants and their parents. Shoot length decreased with gamma ray treatment in comparison to control. 0.5 Gy was found to be the most effective dose in inducing mutation in flower shape, number of florets per flower head and conversion from tubular florets to spoon shaped florets. No change in flower colour was observed.

Shufang *et al.* (2010) carried out ISSR Analysis of M_1 generation of *Gladious hybridus* Hort. treated with EMS and found that with an increase in EMS concentration, the mutagenic rate was increased. However no close relationship between the dose of mutagen and the divergence of plant genomes in treated plants was observed.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled 'Characterisation and genetic improvement in Rose (*Rosa* spp.) through mutagenesis' was carried out at Regional Agricultural Research Station, Ambalavayal during the period 2014-2017. The materials used and the methodologies adopted in this study are described below.

3.1 EVALUATION AND CHARACTERISATION OF GERMPLASM

3.1.1 Experimental Material

In the present study, twenty five varieties each coming under Hybrid Tea and Floribunda groups were evaluated separately with respect to morphological characters. These genotypes were selected, budded, established and maintained at the Rose garden, Regional Agricultural Research Station, Ambalavayal. Completely Randomized Design with 10 single plant replications was employed. The genotypes used for evaluation are listed in Table 1.

3.1.2 Observations Recorded

Observations belonging to the following characters were recorded on the basis of average of ten plants per replication in each treatment.

1. **Number of leaves at first flower**

   The total number of compound leaves per plant was counted at first flower and recorded.

2. **Number of days to first flower**

   The days taken to flowering from the date of budding was recorded from ten plants per treatment and the mean was worked out.

Plate 1. General view of experimental plot

Table 1. List of Rose genotypes used for characterisation

Sl. No	Code	Hybrid Tea genotypes	Sl. No	Code	Floribunda genotypes
1	M 185	Madame George Delbard	1	1783	Versailles
2	A 9	Aishwarya	2	1696	Tickled Pink
3	H 107	Chirst of Colomb	3	1490	Rosarale de Chateau
4	M 242	Pink Panther	4	1857	Rose Mary Gandhi
5	R 78	Roughe Miland	5	1598	Princess de Monaco
6	S 170	Shrewsbury Show	6	1624	Ochi di Fita
7	A 172	Alaine Souchen	7	1627	Carry Free Beauty
8	A 83	Amara	8	S280	Sterntaler
9	B 158	Fryat	9	1324	Orange N Lemon
10	S 262	Perfume Perfect	10	1682	Lisa
11	L 89	Silver Star	11	T77	The McCartney Rose
12	A 129	Lincoln Cathedral	12	C 258	Chesire
13	M 48	A Tago	13	1740	Monnalisa
14	S 280	Demestra	14	1589	Carolanne
15	M 184	Golden Fairy Sport	15	1650	City of Glasgow
16	T 85	Mary Jean	16	1489	Messara
17	P 124	Toplesse	17	1684	Michel Fish
18	M 228	Priority Pride	18	1609	Mini Pink
19	P 194	Majestic	19	1593	Sans Souci
20	C 168	Prince Jardiner	20	S 292	Schloss Elutin
21	L 123	Cel b Lau	21	L 122	Lasting Piece
22	M219	Lois Wilson	22	1790	Plantein on Blumen
23	A 71	Mom's Rose	23	W 60	Winchester Cathedral
24	-	Alabama	24	1581	Golden Fairy
25	-	Josepha	25	-	Prosperity

3. **Prickle density (per five cm)**

The number of well-developed prickles was counted over a length of five cm in the middle portion of the stem and the prickle density expressed as prickles per five cm of shoot length.

4. **Flower size (cm)**

Size of three flowers per plant was measured at the point of maximum breadth at full bloom stage and the mean was recorded.

5. **Flower weight (g)**

Fresh flower weight was taken on the day of flower opening immediately after excision to prevent water loss.

6. **Pedicel length (cm)**

The length of pedicel was taken from the origin of the stalk from the main stem to the neck of the flower.

7. **Number of petals per flower**

The number of petals was counted from ten flowers in each treatment and the mean was calculated.

8. **Size of petals (cm)**

A random petal in the flower was taken for measuring the size of the petals. Size of the petal was found out using the formula length x breadth and expressed in cm.

9. Number of flowers per plant/bunch

The total number of flowers produced in ten sample plants of each treatment was recorded separately for a period of six months and the means were calculated.

10. Fragrance

Fragrance was recorded as high/ moderate/ low.

11. Flower colour

The exact shade of the flower was recorded at full bloom stage.

12. Seed setting ability

Seed setting ability was recorded as seed setting / non seed setting.

13. Vase life/ longevity (days)

Flowers were taken to a room soon after harvest, where they were cut to 50 cm and placed with cut ends dipped in water for 12 hours. The next day, flowers were observed for any withered appearance. The number of days the flower remained fresh in plain water was considered as the vase life.

3.2 INDUCED MUTAGENESIS

The experimental materials were obtained from the Rose garden, RARS, Ambalavayal, Wayanad. Two varieties each selected from Hybrid Tea and Floribunda groups were subjected to gamma ray and Ethyl Methane Sulphonate (EMS) treatments. The investigation envisaged on studying the differential sensitivity of rose varieties by subjecting them to different doses of physical (Gamma rays) and chemical mutagens (Ethyl Methane Sulphonate).

3.2.1 (a) Induced mutagenesis using gamma rays on budwood

Steps followed in gamma ray treatment

Collection of budwood

↓

Preparation of budwood 6-7 cm

↓

Gamma irradiation of budwood

↓

Budding to the root stock

↓

Observing for bud take (%) and survival (%)

↓

Fixing doses based on LD_{50} value

↓

Administering doses to the material

↓

Field evaluation of M_1 generation

3.2.1.1 Collection of materials

The material used for irradiation was budwood. Buds were collected only from healthy shoots. Budwood of eight to ten cm length having three to five dormant buds were used for irradiation. While collecting budwood, three buds immediately below the flower were discarded.

3.2.1.2 Treatment with gamma rays

The Budwood of size 5-8 cm length at appropriate stage were used for treatment. Gamma irradiation was done in the gamma chamber installed at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore where Co_{60} serves as the source of gamma rays. Budwood was irradiated at ten different doses from 20-100 Gy with an interval of 10 Gy.

3.2.1.3 Method of budding

On the same day of irradiation the treated buds and control were separated and patch budding on rooted rootstocks was done. Two months after establishment, they were transferred to earthern pots. Uniform cultural and management practices were adopted during the entire growth period.

3.2.1.4 Fixation of LD_{50} value

LD_{50} is a common parameter to decide the effective doses of mutagens. LD_{50} is a dose which results in 50 per cent mortality of treated buds. LD_{50} statistic value was determined for each variety. Based on this, five doses including control was fixed separately for each variety and was administered to the genotypes.

Table 2. Physical mutagen treatment details

SI. No	Genotype	Mutagen	Dose employed
1	Pink Panther	Gamma rays	Control
2.	Demestra		20 Gy
3.	Monnalisa		30 Gy
4..	Golden Fairy		40 Gy
			50 Gy
			60 Gy
			70 Gy
			80 Gy
			90 Gy
			100 Gy

3.2.1.5 Administering gamma ray doses to the budwood

3.2.1.5.1 Collection of materials

The material for irradiation was budwood. Buds were collected only from healthy shoots. Budwood of eight to ten cm length having three to five dormant buds were used for irradiation. While collecting budwood, three buds immediately below the flower were discarded.

3.2.1.5.2 Treatment with gamma rays

The Budwood of size 5-8 cm length at appropriate stage were used for treatment. Gamma irradiation was done in the gamma chamber installed at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore where Co_{60} serves as the source of gamma rays. Five doses including control selected based on LD_{50} value were employed for the experiment. The Varieties used were Monnalisa, Pink Panther, Demestra and Golden Fairy.

3.2.1.5.3 Method of budding

On the same day of irradiation the treated buds and control were separated and patch budding on rooted rootstocks was done. Two months after establishment, they were transferred to earthern pots. Uniform cultural and management practices were adopted during the entire growth period.

Field evaluation of gamma ray treated M_1 generation was done. Four genotypes at five doses including control. All the thirteen morphological characters studied for characterisation were studied for the field evaluation of gamma ray treated M_1 generation.

3.2.2 Induced *in vitro* mutagenesis using EMS

Chemical mutagen – EMS

The chemical mutagen, Ethyl Methane Sulphonate ($CH_3SO_2OC_2H_5$, Molecular weight 124.16 and density D_4^{25} =1.203 g / ml) stored in room temperature was used for the experiment. Twelve different concentrations of EMS ranging from 0.2-1.2% v/v with 0.1% v/v interval were used to treat tissue culture mediated multiple shoots of Schloss Elutin, Jogan, Josepha and Morning Sun at a duration of 30, 60 and 90 minutes with replications. The type of explants used for induction of callus and establishment of shoot cultures mainly depends upon the juvenility of explants. To develop the methods for propagation through tissue culture, it has been suggested to select highly juvenile explants as starting

material. Surface sterilized, young and juvenile shoot tip explants of the four rose varieties were inoculated on MS medium.

Initially thirteen varieties were tried for EMS treatment. They include Giselle, September Mourn, Sweet Lady, Alliance, Ancher Dom, Pink Panther, Monnalisa, Demestra, Golden Fairy, Schloss Elutin, Jogan, Morning Sun and Josepha. Among them only these Schloss Elutin, Jogan, Josepha and Morning Sun varieties were able to survive up to the last stages and hence they were used for the present research.

3.2.2.1 Standardization of culture media

The study was carried out in the Tissue Culture Laboratory, RARS, Ambalavayal, Kerala Agricultural University during 2015-2017. Budsticks having three to four young nodal segments along with prominent bud was selected. They were cut into the size of 1.5 cm length with one bud. The nodal segments were then subjected to different pretreatments. The explants were washed with running tap water for 15 minutes, followed by five minutes of detergent wash, 20 min of bavistin (5%) dip followed by washing with Tween-20(2-3 ml)+NaHocl solution(3-5 ml) for 30 minutes and then finally washing with distilled water. The pre-treated explants were then surface sterilized in LAF with 0.02% mercuric chloride for four minutes followed by two to three rinsing with autoclaved distilled water. The explants were cut into suitable size before inoculating and again treated with 0.02% mercuric chloride for two minutes. This was followed by two to three washings of explants with autoclaved distilled water.

The surface sterilized explants were cultured on MS medium supplemented with different concentrations of BA (1.0 and 2.0 mg/l), and ADS (25.0 and 50.0 mg/l) to find out the best treatment combination for initial culture establishment. For culture initiation, 20- 25 explants were inoculated per treatment in three replications along with control. The cultures were maintained at 25±1°C under fluorescent white light at a photoperiod of 16/8 h light and dark cycles throughout. The sprouted shoots were then sub-cultured onto MS medium

supplemented with different concentrations of BA (1.0 and 2.0 mg/l), IAA (0.1 and 0.25 mg/l) and ADS (25.0 and 50.0 mg/l) to find out the best treatment combination for shoot proliferation. The multiplied shoots were again sub-cultured on the same proliferation medium. Elongated shoots were then transferred individually in culture vessels containing half-strength MS medium supplemented with IBA (0.25mg/L), activated charcoal (5%) and sucrose (2 and 3%) for rooting. Initiation of rooting was observed after 2-3 weeks. After 6-7 weeks, rooted plantlets were deflasked and multiple shoots were taken for EMS treatment.

3.2.2.2 Steps followed in EMS treatment

Inoculation of the explant

↓

Subculturing of the explant

↓

EMS treatment in LAF

↓

Washing with sterile water

↓

In vitro culture establishment

↓

In vitro shoot multiplication

↓

In vitro rooting

↓

Deflasking of the plants

↓

Hardening of the explants

↓

Transferring to small cups

↓

Establishment of the plants in Humid chamber

↓

Field evaluation of M_1 generation

3.2.2.3 Preparation of EMS solution:

EMS solution was prepared in two phases:

In the first phase, the required volumes of water and 2% (v/v) DMSO were mixed and autoclaved at 120 ^{0}C for 15min at 103.5 kPa (15 psi). The mixture was left to cool to room temperature.

In the second phase, which was carried out in a LAF cabinet, EMS was added to the water–DMSO mixture. When ready to incubate the target materials in the mutagen, a sterile syringe was used to add the required volume of EMS solution to the mixture. The resulting solution was shaken vigorously to give a homogeneous emulsion.

3.2.2.4 EMS treatment to the tissues

In vitro derived multiple shoots were cut and made into suitable size and then treated with the EMS solution on a gyratory shaker for three durations (30, 60 and 90 minutes). The treated shoots were washed thoroughly in double distilled autoclaved water three times to remove all traces of EMS and cultured on the media for shoot proliferation. After 30 days, sub culturing was done again and they were transferred to the multiplication media. Once the plants were well established in the media they were transferred to the rooting media. After rooting,

they were kept for hardening. During the process, the plantlets were dipped in bavistin (0.1%) for 5 min. and planted in potrays into a mixture of compost: soil: sand 1:1:1 (v/v). Acclimatization in a humidity chamber was done. Protrays with established seedlings were successfully transferred to the green house.

Procedure of treatment:

1. Remove the leaves from the plants and cut the stems into explants each with two nodes.

2. Keep these nodal segments in a sterile plastic or glass petri dish containing sterile distilled water and seal with parafilm to avoid contamination.

3. Transfer the explants from water into the homogeneous EMS solution under aseptic conditions in the LAF.

4. Leave the explants immersed in EMS solution for the desired predetermined time. In order to enhance the viability of the explants, the setup is made to stand on a gyratory shaker (80–120 rpm).

5. After treatment, wash the explants in sterile distilled water under aseptic conditions. Washing is done by passing the explants onto a sterile sieve and transferring into a conical flask or beaker containing sterile water and shaking thoroughly. The process of transferring to a fresh sterile sieve and washing through shaking in sterile water is repeated at least 3 times to remove all traces of EMS.

6. Collect the EMS and the wash solutions for appropriate disposal as hazard wastes.

7. Transfer the washed explants into medium and then incubate on a horizontal gyratory shaker at 60 rpm under continuous light at 26°C.

3.2.2.5 Fixation of LD_{50} value

LD_{50} is a common parameter to decide the effective doses of mutagens. LD_{50} is a dose which results in 50 per cent mortality of treated buds survive.

Table 3. EMS treatment details

SI. No	Genotypes	Mutagen	Dose employed (%)	Duration (minutes)
1	Schloss Elutin	EMS	Control	30,60 and 90
2.	Jogan		0.2	
3.	Josepha		0.3	
4..	Morning Sun		0.4	
			0.5	
			0.6	
			0.7	
			0.8	
			0.9	
			1.0	
			1.1	

			1.2	

3.2.2.6 Effect of EMS on Morphological Characters

After fixing the LD_{50}, five doses including control selected based on LD_{50} value were employed for the experiment. The entire procedure of *in vitro* culture establishment and EMS treatment to the tissues was repeated. Field evaluation of EMS treated M_1 generation was studied on different doses of EMS on four genotypes at five doses including control. All the thirteen morphological characters studied for characterisation was studied for the field evaluation of EMS treated M_1 generation also.

3.2.2.7 Observations recorded on Mutants

Observations were made on M_1 flowers to assess induced abnormalities if any, on variation in colour, shape, size or number of petals.

1. Mutants in plant architecture

Each plant was observed critically and the observations were recorded on the basis of any deviation from normal plant growth, spread, architecture and flowering behavior (abnormal flowers and non-formation of flower heads).

2. Mutants in leaf characters

Each plant was critically observed and observations were recorded with respect to changes in leaf colour (variegation), size, shape, margin, apex, fission and fusion of leaves.

3. Mutants in floral characters

The flower heads were critically observed for any fasciation/ asymmetrical or lopsided shape and variations were recorded.

4. Bud take (%)

Number of plants which take the bud in each treatment was counted after 15 days of planting and it was expressed as percentage.

5. Survival at 30 days

Number of surviving plants in each treatment was counted after 30 days of planting and it was expressed as percentage.

6. Meristem regeneration (%)

Number of meristems which regenerate in each treatment was counted after 15 days of planting and it was expressed as percentage.

3.3 MOLECULAR CHARACTERIZATION

The study was conducted to analyse the variations at molecular level of the parent cultivars and selected mutants by using RAPD.

3.3.1 Plant material

The leaf sample was collected from the experimental field maintained at RARS Ambalavayal, *viz.*, Monnalisa, Pink Panther and Demestra. The LD_{50} values of Monnalisa mutants were analysed for variation they are M_1, M_2, M_3 and M_4. (M_1 -40, M_2-50, M_3-60 and M_4-70 Gy).

3.3.2 Molecular markers

Reported ten decamer primers were selected to study the parental polymorphism in the three selected genotypes of rose. The sequence and name of each marker is given in Table 4.

Table 4. List of primers used for RAPD parental polymorphism analysis

Sl. No.	Primer Name	Primer Sequence (5'-3')	Sl. No.	Primer Name	Primer Sequence (5'-3')
1	OPA-1	CAGGCCCTTC	6	OPN-7	CAGCCCAGAG
2	OPA-2	TGCCGAGCTG	7	OPN-15	CAGCGACTGT
3	OPA-4	AATCGGGCTG	8	OPV-17	ACCGGCTTGT
4	OPD-8	GTGTGCCCCA	9	OPV-10	GGACCTGCTG
5	OPN-2	ACCAGGGGCA	10	P-4	GGGTAACGCC

3.3.3 Isolation of genomic DNA

Genomic DNA from these accessions were isolated using the procedure of Himedia kit method. 100 mg of the finely cut leaves were weighed and ground properly in 400 µl Lysis Buffer using a mortar and pestle. DNA isolation protocol was followed.

1. Transfer the above mixture to 2.0 ml collection tube using a clean spatula. Add 4µl of RNase A Solution and vortex vigorously.

2. Incubate the above mixture for 10 minutes at 65°C with intermittent vortexing of the tube every 3-4 minutes.

3. Add 130 µl of precipitation buffer to the above mixture, mix and incubate for 5 minutes on ice. Centrifuge at 14,000 rpm for 5 minutes.

4. Add the lysate onto the HiShredder placed in a 2.0 ml collection tube and centrifuge for 2 minutes at14,000 rpm.

5. Transfer the flow through fraction from step 4 to a new 2.0 ml collection tube without disturbing the cell debris pellet.

6. Add 1.5 volumes of binding buffer to the above mixture obtained from step 5 and mix by pipetting.

7. Add 650 µl of the mixture from step 6, including any precipitate that may have formed, onto the HiElute Miniprep spin column placed in a 2.0 ml collection tube. Centrifuge for 1 minute at 8,000 rpm. Discard the flow through fraction.

8. Repeat step 7 with the remaining sample. Transfer the column in a new 2.0 ml collection tube and discard the flow through fraction and the 2.0 ml collection tube.

9. To the HiElute Miniprep spin column placed in a new 2.0 ml collection tube, add 500 µl of wash solution. Centrifuge for 1 minute at 8,000 rpm.

10. Add another 500 µl of the wash solution to the HiElute Miniprep Spin Column and centrifuge for 2 minutes at 14,000 rpm.

11. Centrifuge the tube with HiElute Miniprep Spin Column for an additional two minutes at 14,000 rpm to remove traces.

12. Pipette 100 µl of the Elution Buffer directly onto the column without spilling to the sides of the column. Incubate for 1 minute at room temperature. Centrifuge at 10,000 rpm for 1 minute to elute the DNA.

3.3.4 PCR amplification

PCR amplification reactions were carried out in a 20 µl reaction volume.

2x DyNAzyme II PCR Master Mix : 10 µl

Primer (100 µM)	:	1 µl
Distilled Water	:	7 µl
DNA	:	2 µl

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile

95 °C	-	5.00 min	
94 °C	-	0.45 min	}
35 °C	-	1.00 min	} 35 cycles
72 °C	-	1.30 min	
72 °C	-	10.00 min	
4 °C	-	∞	

3.3.5 Agarose gel electrophoresis of PCR products

The PCR products were checked in 1.5% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 4 µl of 6X loading dye was mixed with 20 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB).

The gels were visualized in a UV transilluminator and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.6 Quantification of DNA

After ensuring the presence of DNA in samples by electrophoresis the quality and quantity of DNA was measured as follows.

5µl of DNA dissolved in 0.1X TAE was added to 3ml of distilled water and read against distilled water used as blank at an absorbance of 260 nm and 280 nm, in an UV spectrophotometer. The concentration of DNA in sample was calculated using the formula;

$$\text{Amount of DNA (ng/ml)} = A260 \times \frac{\text{Volume of dist.water (µl)}}{\text{Amount of DNA (µl)}} \times 0.05 \times 1000$$

Where, A260 = Absorbance at 260nm.

The quality of DNA was judged from ratio of absorbance values at 260 nm and 280 nm. A ratio of 1.8-2.0 indicated best quality of DNA.

3.4 STATISTICAL ANALYSIS

The data were processed with the help of various standard statistical procedures as mentioned below.

3.4.1 Analysis of variance

The biometric observations recorded from the field evaluation were subjected to analysis of variance for the comparison among various accessions and to estimate variance components. The significance of mean sum of squares for each character was tested against the corresponding error degrees of freedom using F test (Fisher and Yates, 1967).

3.4.2 Estimation of genetic parameters

For each character, the phenotypic and genotypic components of variance were estimated by equating the expected value of mean squares (MS) to the respective variance components. The variance components were estimated based on this. It is as follows:

i. Genotypic variance (GV)= MST-MSE/r

ii. Environmental variance (EV) = MSE

iii. Phenotypic variance (PV) = GV + EV

Where, MSE=Mean sum of squares for error, MST= Mean sum of squares for treatments.

3.4.2.2 Phenotypic and genotypic coefficient of variation

The method suggested by Burton and Devane (1953) was followed for computation of the following parameters.

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sigma_g}{\bar{X}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

σ_p = Phenotypic standard deviation

σ_g = Genotypic standard deviation

$\overline{X}$ = Grand mean

GCV and PCV values were categorized as low (0-10%), moderate (10-20%) and high (>20%).

3.4.2.3 Heritability in broad sense

Heritability in broad sense (h^2) was calculated as a ratio of genotypic variance to the phenotypic variance (Hanson *et al.* 1956).

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

The heritability percentage was categorized as low (0-20%), moderate (30-60%) and high (>60%) as given by Robinson *et al.* (1949).

3.4.2.4 Genetic advance

This was computed according to the method suggested by Johnson *et al.* (1955).

Genetic advance (GA) = $i\sigma p\ h^2$

Where,

i = 2.06 when top 5% individuals are selected

σ_p = Phenotypic standard deviation

h^2 = Heritability in broad sense

3.4.2.5 Genetic advance as percentage of mean (GAM)

This was calculated by using the formula given below.

$$GAM = \frac{GA}{\overline{X}} \times 100$$

Where,

GA = Genetic advance

$\overline{X}$ = General mean

Genetic advance as per cent of mean was categorized as low (0-10%), moderate (10-20%) and high (>20%) by Johnson *et al.* (1955).

3.4.5 Genetic divergence analysis

The genetic divergence between genotypes was estimated using Mahalanobis D^2 statistic (1936). The distance D from the sample was computed using the formula.

$$D^2p = d^1 S^{-1} d$$

Where,

D^2p = Square of distance considering 'p' variables

d^1 =Vector observed differences of the mean values of all the characters

S^{-1}= inverse of variance and covariance matrix

3.4.5.1 Clustering of the D^2 values

All the genotypes used were clustered into different groups following Tocher's method (Rao, 1952). The intra and inter distance were also computed. The criterion used in clustering to the same cluster is that it should at least on the average, show a smaller D^2 value than those belonging to different clusters.

The device suggested by Tocher (Rao, 1952) was started with two closely associated populations and a third population was found which had the smallest average of D^2 from the first two. Similarly, the fourth was chosen to have a smallest average D^2 value from the first three and so on. The permissible increase in the D^2 value was shown by a population to the nearest population. If at any stage increase in average D^2 value exceeded the average of already included, because of the addition of a new genotype, then that genotypes was deleted. The genotypes that are included already in that group were considered as the first cluster. This procedure was repeated till D^2 values of the other genotypes were exhausted omitting those that were already included in the former clusters and grouping them into different clusters.

3.4.5.2 Intra cluster distance

The average intra cluster distances were calculated by the formula given by Singh and Chaudhary (1977).

Square of intra cluster distance = $\Sigma Di^2/n$

Where,

ΣDi^2 = sum of distance between all possible combinations.

n= number of all possible combinations

3.4.5.3 Inter cluster distance

The average inter cluster distance was calculated by the formula described by Singh and Chaudhary (1977).

Square of inter cluster distance = $\Sigma Di^2/n_i n_j$

Where,

ΣDi^2 = Sum of distances between all possible combinations ($n_i n_j$) of the entries included in the cluster study.

n_i = Number of entries in cluster i

n_j = Number of entries in cluster j

3.4.5.4 Contribution of individual characters towards genetic divergence

The character contribution towards genetic divergence was computed using method given by Singh and Chaudhary (1977). In all the combinations, each character was ranked on the basis of $di = y_i^j - y_i^k$ values.

Where,

di = mean deviation

y_i^j = mean value of the j^{th} genotype for the i^{th} character and

y_i^k = mean value of the k^{th} genotype for the i^{th} character.

Rank 'I' is given to the highest mean difference and rank p is given to the lowest mean difference

Where,

P is the total number of characters.

Finally, another Table giving information on number of times that each character appeared in the first rank is prepared and per cent contribution of characters towards divergence was calculated.

3.4.5.5 LD_{50} value

The LD_{50} dose of irradiation was calculated by probit analysis method using observations on mortality percentage as described by Sharma (1998).

Results

4. RESULTS

The present investigation entitled "Characterisation and genetic improvement in Rose (*Rosa* spp.) through mutagenesis", was carried out in College of Agriculture Vellayani and RARS Ambalavayal during the period 2014-2017. The purpose of the present study was to analyze the genetic diversity among different rose genotypes using morphological characters and to analyse the effectiveness of gamma rays and EMS on inducing variability in roses. The salient findings as revealed from the investigation are presented here.

4.1 EVALUATION AND CHARACTERIZATION OF GERMPLASM.

Under this experiment, 25 varieties each coming under Hybrid Tea and Floribunda groups were evaluated separately for morphological characters. In order to build up a base population of uniform age, bud wood from the selected varieties were budded on to root stocks. The population was evaluated in a CRD with ten replications.

4.1.1 Analysis of variance

The mean performances for nine quantitative characters in 25 genotypes of both Hybrid Tea and Floribunda groups of roses are presented in Tables 5 and 6. Highly significant differences were observed among the genotypes for all the characters indicating the presence of considerable genetic variability.

4.1.2 Mean performance of Hybrid Tea genotypes

The mean performance of all the Hybrid Tea genotypes for various quantitative characters are given below. The range in mean values are presented in Table 7.

Table 5. Analysis of variance for nine characters in 25 genotypes of Hybrid Tea Roses

Sources of variation	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
MSS Treatment	1790.51**	2883.80**	35.59**	522.02**	12.88**	4.70**	968.98**	89.21**	1.02**
MSS Error	5.1	2.16	1.08	1.93	0.6	0.22	2.79	0.3	0.46
F value	351.08**	31.82**	32.79**	270.30**	21.32**	21.20**	347.05**	296.62**	2.19**

Table 6. Analysis of variance for nine characters in 25 genotypes of Floribunda Roses

Sources of variation	Number of leaves at first flower	Number of days to first flower	Prickle density/ 5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
MSS Treatment	2642.48**	1007.68**	78.17**	265.01**	15.41**	6.93**	682.97**	44.59**	2.06**
MSS Error	4.51	1.95	1.09	1.25	0.21	0.08	3.5	0.3	0.67
F value	585.31**	514.82**	71.32 **	210.36	72.57**	78.11**	194.91**	147.17**	3.06**

*Significant at 5% level

**Significant at 1% level

Table 7. Mean performance for nine characters in 25 genotypes of Hybrid Tea Roses

Sl. No.	Hybrid Tea Genotypes	Number of leaves at first flower	Number of days to first flower	Prickle density /5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
1	Madame George Delbard	25.66	55.83	6.50	8.50	3.17	4.71	18.00	3.27	22.50
2	Aishwarya	42.50	51.66	9.33	9.21	6.87	4.46	25.33	5.09	19.95
3	Chirst of Colomb	28.50	42.50	6.00	6.35	4.97	6.91	28.66	3.37	21.45
4	Pink Panther	28.50	53.83	2.83	6.73	4.63	6.46	27.83	2.71	22.50
5	Roughe Miland	52.66	64.67	2.33	6.92	4.51	5.83	33.00	2.60	22.50
6	Shrewsbury Show	61.66	63.16	6.50	8.32	7.61	6.66	55.00	2.71	30.00
7	Alaine Souchen	24.33	63.16	6.33	7.41	6.08	5.85	45.16	2.96	24.90
8	Amara	59.66	67.50	3.50	10.30	7.17	7.30	17.33	3.65	30.00
9	Fryat	54.16	20.00	6.33	6.90	4.89	5.85	33.66	3.68	30.00
10	Perfume Perfect	61.50	52.33	2.00	7.27	4.99	8.06	32.50	3.85	30.00
11	Silver Star	24.66	42.83	1.83	8.70	5.60	4.91	25.50	4.82	22.50
12	Lincoln Cathedral	57.66	71.00	3.66	6.83	4.80	4.98	23.66	2.66	22.50
13	A Tago	53.33	64.66	3.16	8.08	6.98	6.25	62.33	3.21	24.90
14	Demestra	46.00	24.00	4.00	9.13	1.76	4.11	18.16	1.69	22.45
15	Golden Fairy Sport	31.16	42.00	5.83	8.11	2.29	6.15	13.66	5.37	32.95
16	Mary Jean	68.66	57.00	11.66	8.06	6.13	5.51	23.83	3.81	30.00
17	Toplesse	17.66	30.00	2.33	6.72	5.80	5.80	22.83	4.41	22.50

Contd...

Sl. No.	Hybrid Tea Genotypes	Number of leaves at first flower	Number of days to first flower	Prickle density/ 5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
18	Priority Pride	66.16	38.16	3.33	6.67	5.18	5.71	43.50	3.29	19.95
19	Majestic	26.50	50.33	4.33	6.84	7.09	5.53	14.83	3.64	34.95
20	Prince Jardiner	55.83	51.83	3.16	8.17	3.79	5.68	18.00	4.24	30.00
21	Cel b Lau	53.00	65.66	8.50	6.90	6.35	5.85	31.00	3.26	27.45
22	Lois Wilson	34.33	25.83	4.33	9.91	5.97	6.48	26.00	4.56	15.00
23	Mom's Rose	43.66	26.50	4.00	9.38	5.06	4.93	23.00	4.23	19.95
24	Alabama	65.66	30.00	3.83	9.20	5.83	5.98	14.66	5.95	39.95
25	Josepha	25.33	63.16	6.33	7.29	6.05	6.01	45.16	2.98	22.50
	C.D @5%	2.55	1.66	1.17	1.57	0.87	0.53	1.89	0.62	0.77
	C.D @1%	3.35	2.18	1.54	2.06	1.15	0.70	2.48	0.81	1.01
	Mean	44.91	48.70	4.88	7.92	5.35	5.84	28.91	3.68	1.71
	S.E.	1.00	0.65	0.46	0.62	0.34	0.20	0.74	0.24	0.30

1. Number of leaves at first flower

Range of variation for number of leaves at first flowering in Hybrid Tea was from 17.66 to 68.66. In Hybrid Tea, the genotype Toplesse (17.66) had lowest number of leaves out of the 25 genotypes followed by Alaine Souchen (24.33) and Silver Star (24.66). The highest number of leaves at first flower was recorded in the genotype Mary Jean (68.66) which was followed by Priority Pride (66.16) and Alabama (65.66).

2. Number of days to first flower

Number of days taken by the plants to initiate flowering in the 25 genotypes varied from 20 to 71 days. The early flowering genotypes were *viz.,* Fryat, Demestra and Lois Wilson taking 20.00, 24.00 and 25.83 days respectively to flower. The late flowering genotypes were Lincoln Cathedral, Amara and Cel b Lau taking 71.00, 67.5 and 65.66 days respectively to flower.

3. Prickle density (per five cm)

Prickle density exhibited a wide range of variation from 1.83 to 11.66 in Hybrid Tea. In this group, the genotype, Silver Star (1.83 cm) had the lowest prickle density followed by Perfume Perfect (2 cm) and Roughe Miland (2.3cm). The highest prickle density was found in Mary Jean (11.66 cm) followed by Aishwarya (9.33cm) and Cel b Lau (8.50 cm).

4. Flower size (cm)

Flower size ranged between 6.35 to 10.30 cm. Among the 25 genotypes, bigger flower size was observed in Amara (10.30 cm), Lois Wilson (9.91 cm) and Mom's Rose (9.38 cm). Smaller flowers were recorded in Chirst of Colomb (6.35cm), followed by Priority Pride (6.67cm) and Toplesse (6.72cm).

Table 9. Estimates of range in mean values for nine characters in 25 Hybrid Tea and Floribunda Roses

Sl. No.	Traits	Hybrid Tea Roses	Floribunda Roses
1	Number of leaves at first flower	17.66-68.66 (Toplesse-Mary Jean)	17.16-93.16 (Ochi di Fita-Sans Souchi)
2	Number of days to first flower	20-71 (Fryat-Lincoln Cathedral)	28.50-71.50 (City of Glasgow-Messara)
3	Prickle density/5 cm	1.83-11.66 (Silver star-Mary Jean)	2.00-13.16 (Princess de Monaco-Winchester Cathedral)
4	Flower size (cm)	6.35-10.30 (Chirst of Colomb-Amara)	4.73-8.66 (Chesire-Carolanne)
5	Flower weight(g)	1.76-7.61 (Demestra-Shrewsbury Show)	1.76-6.49 (Rose Mary Gandhi-Monnalisa)
6	Pedicel length(cm)	4.11-8.06 (Demestra -Perfume Perfect)	2.61-6.93 (Michel Fish-Sterntaler)
7	Number of petals per flower	13.66-62.33 (Golden Fairy sport-A Tago)	11.00-54.67 (Messara-Monnalisa)
8	Size of petals (cm)	1.69-5.95(Demestra-Alabama)	3.32-7.70(Rose Mary Gandhi-Lasting Piece)
9	Number of flowers per plant/bunch	15.00-39.95 (Lois Wilson- Alabama)	17.40-42.45 (Rosarale De Chateau-Orange N Lemon)

5. Flower weight (g)

Flower weight ranged from 1.76 to 7.61g. Among the 25 genotypes flower weight was low among Demestra (1.76 g) followed by Golden Fairy Sport (2.29 g) and Madame George Delbard (3.17g). Flower weight was high in Shrewsbury Show (7.61g) , Amara (7.17g) and Majestic (7.09g).

6. Pedicel length (cm)

Considerable variation existed between the genotypes for this trait as is seen by mean values ranging from 4.11 to 8.06 cm in Hybrid Tea. Pedicel length was the highest in the genotype Perfume Perfect (8.06 cm) followed by Amara (7.30 cm) and Chirst of Colomb (6.91cm) whereas the genotypes Demestra, (4.11cm), Aishwarya (4.46 cm) and Madame George Delbard (4.71cm) recorded low pedicel lengths.

7. Number of petals per flower

Significant variation was observed for number of petals per flower as is evidenced by the large differences in their mean values ranging from 13.66 to 62.33. The highest number of petals per flower was recorded in the genotype A Tago (62.33) followed by Shrewsbury Show (55.00) and Josepha (45.16). Lowest number of petals per flower was recorded in Golden Fairy Sport (13.66) followed by Alabama (14.66) and Majestic (14.83).

8. Size of petals (cm)

Size of petals ranged from 1.69 to 5.95 cm. Among the 25 genotypes, higher petal size was seen in Alabama (5.95cm) followed by Golden Fairy Sport (5.37) and Aishwarya (5.09) whereas lower petal size was recorded in Demestra (1.69) followed by Roughe Miland (2.60) and Lincoln Cathedral (2.66).

Table 10. Performance for qualitative traits for Hybrid Tea Roses

Sl. No.		Name of genotype	Fragrance	Flower colour	Seed setting ability	Vase life/ longevity (days)
1	M185	Madame George Delbard	Medium	Red	No	1.4
2	A 9	Aishwarya	Medium	Light rose	No	1.8
3	H 107	Chirst of Colomb	Low	Creamy white	No	1.6
4	M242	Pink Panther	Medium	Pink	No	2.0
5	R 78	Roughe Miland	Medium	Light pink	No	1.8
6	S 170	Shrewsbury Show	Medium	Medium pink	No	2.8
7	A 172	Alaine Souchen	Medium	Red	No	2.2
8	A 83	Amara	Medium	Red	No	1.6
9	B 158	Fryat	Medium	Yellow	Yes	1.4
10	S 262	Perfume Perfect	Low	Very light pink	No	1.2
11	L 89	Silver Star	High	Very light pink	No	1.8
12	A 129	Lincoln Cathedral	Medium	Red	No	1.8
13	M 48	A Tago	Medium	Yellow	No	2.0
14	S 280	Demestra	Medium	Yellow	No	3.2
15	M 184	Golden Fairy Sport	Medium	Yellow	Yes	2.6
16	T 85	Mary Jean	Low	Light orange	No	2.0
17	P 124	Toplesse	High	Cream white	Yes	1.4
18	M 228	Priority Pride	High	Pinkish	No	1.8
19	P 194	Majestic	Medium	Light rose	No	2.6
20	C 168	Prince Jardiner	Medium	Light violet	No	1.6
21	L 123	Cel b Lau	Low	Pink	No	2.6
22	M219	Lois Wilson	Low	Cream	No	1.8
23	A 71	Mom's Rose	Medium	Orange	No	2.0
24	-	Alabama	High	Light pink	No	1.6
25	-	Josepha	Low	Pink	No	2.0

9. Number of flowers per plant

Number of flowers per plant among of all the 25 Hybrid Tea genotypes ranged between 15.00 and 39.95 and genotypes Alabama (39.95), Majestic (34.95) and Golden Fairy Sport (32.95) have recorded higher number of flowers per plant. On the other hand Lois Wilson (15.00) followed by Aishwarya (19.95) and Chirst of Colomb (21.45) have recorded lower number of flowers.

10. Fragrance

Among the 25 Hybrid Tea genotypes studied, fragrance ranged from low through medium to high. For majority of genotypes, fragrance was medium (15 genotypes) whereas for six genotypes it was low. Four genotypes recorded high fragrance. It is presented in Table 10.

11. Flower colour

Flower colour among the Hybrid Tea genotypes ranged from light violet in Prince Jardiner through the various rainbow colours to red in four genotypes. Three genotypes were creamy white or cream in colour whereas four genotypes were yellow. Mary Jean was light orange whereas Mom's Rose was orange in colour. Nine genotypes exhibited different shades of pink, ranging from very light pink, light pink and medium pink to pink. In six genotypes the colour ranged from light rose to red. In total 15 genotypes were different shades of pink or red. It is presented in Table 10 and Plate 2.

12. Seed setting ability

None of the 25 genotypes of Hybrid Tea groups of roses had seed setting ability.

13. Vase life/ longevity (days)

It is evident from the data presented in Table 10 that there is a variation in flower longevity among different accessions of rose. The maximum flower

Table 8. Mean performance for nine characters in 25 genotypes of Floribunda Roses

Sl. No.	Floribunda Genotypes	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
1	Versailles	27.45	54.00	2.66	6.67	1.82	3.60	25.00	3.89	27.45
2	Tickled Pink	27.45	65.66	3.50	6.59	3.74	4.45	35.00	3.95	27.45
3	Rosarale de Chateau	17.4	31.33	3.16	7.07	4.90	4.25	37.33	4.40	17.40
4	Rose Mary Gandhi	19.95	67.16	9.16	5.31	1.41	4.41	24.50	3.32	19.95
5	Princess de Monaco	30	55.00	2.00	6.51	4.42	5.23	33.67	5.26	30.00
6	Ochi di Fita	30	58.66	8.00	5.16	1.60	4.68	30.83	3.73	30.00
7	Carry Free Beauty	22.5	31.66	2.33	5.48	3.07	3.53	45.50	3.87	22.50
8	Sterntaler	24.9	43.00	6.83	6.82	5.86	6.93	33.67	4.49	24.90
9	Orange N Lemon	57.45	41.66	8.50	6.32	5.39	6.40	18.83	4.71	42.45
10	Lisa	34.95	56.33	3.00	6.10	2.14	4.51	22.83	3.95	34.95
11	The Mccartney Rose	54.00	58.83	12.16	6.75	6.21	5.73	16.00	5.21	24.90
12	Chesire	53.50	58.83	12.16	4.73	1.60	4.00	16.67	4.48	27.45
13	Monnalisa	52.66	63.50	3.16	7.14	6.49	4.35	54.67	3.84	32.40
14	Carolanne	45.83	56.33	2.33	8.66	5.48	4.38	14.67	4.48	37.50
15	City of Glasgow	32.16	28.50	2.33	6.89	3.46	4.45	22.83	4.80	22.50
16	Messara	32.16	71.50	3.16	6.32	3.48	4.96	11.00	4.37	34.95
17	Michel Fish	50.16	34.00	5.33	7.10	3.38	2.61	14.50	4.82	40.95

Contd...

Sl. No.	Floribunda Genotypes	Number of leaves at first flower	Number of days to first flower	Prickled ensity/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
18	Mini Pink	22.83	53.50	3.00	6.90	4.42	3.60	25.16	4.49	24.90
19	Sans Souci	93.16	30.80	3.83	8.38	6.21	6.33	33.83	6.25	24.90
20	Schloss Elutin	83.83	63.16	7.16	6.74	5.60	4.61	34.67	4.55	34.95
21	Lasting Piece	17.16	46.00	11.50	8.11	5.58	6.78	16.17	7.70	24.90
22	Plantein on Blumen	27.50	53.83	6.16	5.64	5.07	4.71	23.67	4.45	27.45
23	Winchester Cathedral	57.50	32.50	13.16	6.49	5.47	5.06	24.83	4.23	34.95
24	Golden Fairy	74.83	51.16	2.66	8.21	3.20	3.80	12.17	6.89	30.00
25	Prosperity	56.66	57.50	7.33	6.49	4.68	3.60	25.33	4.45	24.90
	C.D @ 5%	2.42	1.59	1.19	1.28	0.52	0.34	2.13	0.62	0.93
	C.D @ 1%	3.16	2.08	1.55	1.66	0.68	0.44	2.78	0.81	1.22
	Mean	50.69	50.54	5.79	6.66	4.21	4.68	25.77	4.66	2.00
	S.E.	0.94	0.62	0.46	0.50	0.20	0.12	0.83	0.24	0.36

longevity was exhibited in Demestra (3.20 days) followed by Shrewsbury Show (2.8 days) and Cel b Lau (2.6 days). The minimum flower longevity was recorded in Perfume Perfect (1.2 days) followed by 1.4 days each in Madame George Delbard, Fryat and Toplesse.

4.1.3 Mean performance of Floribunda genotypes

The mean performance of all the Floribunda genotypes for various quantitative characters are given below. The range in mean values are presented in Table 8.

1. Number of leaves at first flower

Range in variation for number of leaves at first flower in Floribunda genotypes was from 17.16 to 93.16.In Floribunda, the genotype Lasting Piece (17.16) had the lowest number of leaves among 25 genotypes followed by Rosarale de Chateau (17.40) and Rose Mary Gandhi (19.95). The maximum number of leaves at first flower was recorded in the genotype Sans Souci (93.16) which was followed by Schloss Elutin (83.83) and Golden Fairy (74.83).

2. Number of days to first flower

Number of days taken by the plants to initiate flowering in the 25 genotypes varied from 28.50 to 71.50 days. The early flowering ones among the twenty five genotypes were City of Glasgow (28.50), Sans Souci (30.80), and Rosarale de Chateau (31.33). The late flowering genotypes were Messara, Rose Mary Gandhi and Tickled Pink taking 71.50, 67.16 and 65.66 days respectively to produce flowers.

3. Prickle density (per five cm)

Prickle density exhibited a wide variation of 2.00 to 13.16 in Floribunda groups of roses. The genotype, Princess de Monaco (2.00) had the lowest prickle density among the 25 genotypes followed by Carry Free Beauty and Carolanne

(2.33 each). The highest prickle density was found in Winchester Cathedral (13.16) followed by Chesire and The McCartney Rose (12.16 each).

4. Flower size (cm)

Flower size ranged between 4.73 and 8.66 cm. The highest flower size was recorded in the genotype Carolanne (8.66 cm) followed by Sans Souci (8.38 cm) and Golden Fairy (8.21 cm).The lowest flower size was recorded in Chesire (4.73 cm) followed by Ochi di Fita (5.16 cm) and Rose Mary Gandhi (5.31cm).

5. Flower weight (g)

Flower weight ranged from 1.76 and 6.49 g. Flower weight was high among the genotypes Monnalisa (6.49 g), Sans Souci and The McCartney Rose (6.21g each). Flower weight was low among the genotypes Rose Mary Gandhi (1.41 g), Ochi di Fita and Chesire (1.60 g each).

6. Pedicel length (cm)

Pedicel length ranged between 2.61 and 6.93 cm. Pedicel length was the highest in the genotype Sterntaler (6.93 cm) followed by Lasting Piece (6.78 cm) and Orange N Lemon (6.4 cm) whereas the genotypes Michel Fish (2.61cm), Carry Free Beauty (3.53cm) and Versailles (3.60cm) recorded lower pedicel lengths.

7. Number of petals per flower

Significant variation was observed for number of petals per flower as is evident from the large differences in their mean values ranging from 11.00 to 54.67. The highest number of petals per flower was recorded in Monnalisa (54.67) followed by Carry Free Beauty (45.50) and Rosarale de Chateau (37.33). Lowest number of petals per flower was recorded in Messara (11.00), Golden Fairy (12.17) and Michel Fish (14.50).

Table 11. Performance for qualitative traits for Floribunda Roses

Sl. No.	Codes	Name of the genotype	Fragrance	Flower colour	Seed setting ability	Vase life/ longevity (days)
1	1783	Versailles	Medium	Pinkish orange	No	1.5
2	1696	Tickled Pink	Medium	Pink	No	1.4
3	1490	Rosarale de Chateau	Medium	Light pink	No	1.6
4	1857	Rose Mary Gandhi	Medium	Creamy saffron	No	1.2
5	1598	Princess de Monaco	Medium	Light pink	No	2.0
6	1624	Ochi di Fita	Medium	Orange	No	2.2
7	1627	Carry Free Beauty	Medium	Light rose	No	2.0
8	S280	Sterntaler	Medium	Yellow	No	2.2
9	1324	Orange N Lemon	Low	Dark pink	No	1.8
10	1682	Lisa	High	Dark peach	No	1.8
11	T77	The Mccartney Rose	High	Pink	No	2.0
12	C 258	Chesire	Medium	Red	No	1.6
13	1740	Monnalisa	Low	Red	No	2.4
14	1589	Carolanne	Low	Yellow	No	2.0
15	1650	City of Glasgow	Medium	Cream	No	2.0
16	1489	Messara	Medium	Pink outer petals white in middle	No	1.8
17	1684	Michel Fish	Medium	Light pink	No	2.6
18	1609	Mini Pink	Medium	Light rose	No	2.6
19	1593	Sans Souci	High	Creamy orange	No	1.6
20	S 292	Schloss Elutin	Medium	Creamy orange	No	3.0
21	L 122	Lasting Piece	Medium	Orange	No	2.4
22	1790	Plantein on Blumen	Medium	Red	No	1.8
23	W 60	Winchester Cathedral	High	Cream	No	2.0
24	1581	Golden Fairy	Medium	Yellow	No	2.2
25	-	Prosperity	High	White	No	2.4

Plate 2. Hybrid Tea varieties-details of single flower

Plate 3. Floribunda varieties-details of single flower

8. Size of petals (cm)

Size of petals exhibited a range in value from 3.32 to 7.70 cm. Among the 25 genotypes, the highest petal size was seen in the genotype Lasting Piece (7.70 cm) followed by Golden Fairy (6.89 cm) and Sans Souci (6.25 cm) whereas the lowest petal size was recorded in Rose Mary Gandhi (3.32 cm) followed by Ochi di Fita (3.73 cm) and Monnalisa (3.84 cm).

9. Number of flowers per plant

Number of flowers per plant ranged between 17.40 and 42.45. Further, the number of flowers per plant did not vary much. Genotype Orange N Lemon (42.45), Michel Fish (40.95), and Carolanne (37.50) recorded higher number of flowers per plant. On the other hand lower number of flowers were seen in Rosarale de Chateau (17.40), Rose Mary Gandhi (19.95), and Carry Free Beauty (22.50).

10. Fragrance

Among the 25 Floribunda genotypes studied, fragrance ranged from low through medium to high. For majority of genotypes, fragrance was medium (17 genotypes) whereas for three genotypes it was low. Five genotypes recorded high fragrance. It is presented in Table 11.

11. Flower colour

The wide range of flower colours among the Floribunda genotypes was from white in Prosperity through the various rainbow colours to red in three genotypes. Four genotypes were creamy white or different blends of cream in colour whereas three genotypes were yellow. Two genotypes were with orange colour whereas Schloss Elutin and Sans Souci were creamy orange in colour. Versailles was pinkish orange in colour. Six genotypes exhibited different shades of pink, ranging from very light pink, light pink and medium pink to pink. Lisa genotype was dark peach in colour. In five genotypes, the colour ranged from

Table 12. Estimates of variability, heritability and genetic advance for nine traits in Hybrid Tea Roses

Sl. No	Characters	Genotypic variance $\sigma^2 g$	Phenotypic variance $\sigma^2 p$	Genotypic coefficient of variation GCV (%)	Phenotypic coefficient of variation PCV (%)	Heritability (%)	Genetic advance	GA as percent of mean
1	Number of leaves at first flower	297.56	302.66	38.40	38.73	98.31	35.23	78.45
2	Number of days to first flower	480.27	482.43	11.76	11.78	99.55	45.04	24.17
3	Prickle density/5 cm	5.75	683.62	49.14	53.57	84.12	45.31	92.84
4	Flower size (cm)	86.68	88.61	29.13	29.45	97.82	18.96	59.35
5	Flower weight (g)	2.04	2.65	26.75	30.44	77.21	2.58	48.42
6	Pedicel length (cm)	0.74	0.96	14.79	16.84	77.10	1.56	26.76
7	Number of petals per flower	161.03	163.82	43.89	44.27	98.30	25.91	89.65
8	Size of petals (cm)	14.81	15.12	53.19	53.72	98.01	7.85	108.47
9	Number of flowers per plant/bunch	0.09	0.55	41.76	43.59	96.60	6.25	94.90

light rose to red. In total 13 genotypes were having different shades of pink or red. It is presented in Table 11 and Plate 3.

12. Seed setting ability

Among the 25 genotypes of Floribunda groups of roses none had seed setting ability.

13. Vase life/ longevity (days)

It is evident from the data presented in table 11 that there is a variation in flower longevity among different accessions of rose. The maximum flower longevity was exhibited in Schloss Elutin (3.00 days) followed by Michel Fish and Mini Pink (2.6 days each). The minimum flower longevity was recorded in Rose Mary Gandhi (1.2 days) followed by 1.4 days each in Tickled Pink and 1.5 days in Versailles.

4.1.6 Estimation of genetic parameters

4.1.6.1 Genetic variability in Hybrid Tea genotypes

To know the extent of genetic variability existing in the diverse genotypes, the data on phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance as percent of mean were estimated and presented in Tables 12 and 13.

1. **Number of leaves at first flower:** The phenotypic (38.73%) and genotypic, (38.40%) co-efficients of variation for number of leaves at first flower were moderate accompanied by high heritability of 98.31per cent and high genetic advance of 78.45 as percent of mean.

2. **Number of days to first flower:** For number of days to first flower, PCV (11.78%), and GCV (11.76%) were low coupled with high heritability of 99.55 percent and genetic advance of 24.17 as percent of mean.

3. **Prickle density (per five cm):** High PCV (53.57%) and GCV (49.14) for prickle density were recorded, with a high heritability of 84.12 per cent and a high genetic advance of 92.84 as percent mean.

4. **Flower size (cm):** Moderate PCV (29.45%) and GCV (29.13%) values were observed in the genotypes for flower size with high heritability (97.82%) and genetic advance as per cent of mean of 59.35.

5. **Flower weight (g):** Moderate PCV (30.44%) and GCV (26.75%) values were observed in the genotypes for flower weight with high heritability (77.21%) and genetic advance as per cent of mean (48.42).

6. **Pedicel length (cm):** Moderate PCV (16.84%) and GCV (14.79%) values were observed in the genotypes for pedicel length with high heritability (77.10%) and genetic advance as per cent of mean (26.76).

7. **Number of petals per flower:** High estimates of phenotypic (44.27%) and genotypic co- efficients of variation (43.89%) were recorded with high heritability (98.30%) and genetic advance as per cent mean of 89.65 for the character number of petals per flower.

8. **Size of petals (cm):** Size of petals recorded the highest PCV (53.72%) and GCV (53.19%) values with high heritability (98.01%) and genetic advance as per cent of mean (108.47).

9. **Number of flowers per plant (g):** Genotypes had high PCV and GCV accompanied with high heritability and genetic advance as per cent mean for number of flowers per plant. The character exhibited high PCV (43.59%) and GCV (41.76%) values with high heritability (96.60%) and genetic advance as percent of mean (94.90).

Table 13. Estimates of variability, heritability and genetic advance for nine traits in Floribunda Roses

Sl. No	Characters	Genotypic variance $\sigma^2 g$	Phenotypic variance $\sigma^2 p$	Genotypic coefficient of variation GCV (%)	Phenotypic coefficient of variation PCV (%)	Heritability (%)	Genetic advance	GA as percent of mean
1	Number of leaves at first flower	439.66	444.17	41.36	41.57	98.98	42.97	84.77
2	Number of days to first flower	167.62	169.57	67.94	68.34	98.85	26.51	131.91
3	Prickle density/5 cm	12.84	13.94	61.93	64.52	92.14	7.08	122.47
4	Flower size (cm)	43.95	45.21	29.24	29.66	97.21	13.46	59.40
5	Flower weight (g)	2.53	2.74	37.97	39.53	92.27	3.15	75.13
6	Pedicel length (cm)	1.14	1.22	22.81	23.68	92.78	2.11	45.27
7	Number of petals per flower	113.24	116.74	40.72	41.34	97.00	21.59	82.61
8	Size of petals (cm)	7.38	7.68	47.85	48.82	96.06	5.48	96.61
9	Number of flowers per plant/bunch	6.23	6.90	44.10	47.62	95.63	5.50	95.13

4.1.6.2 Genetic variability in Floribunda genotypes

1. **Number of leaves at first flower:** The phenotypic (41.57%) and genotypic (41.36%) co- efficients of variation for number of leaves at first flower were high accompanied with high heritability of 98.98per cent and high genetic advance of 84.77 as per cent of mean.

2. **Number of days to first flower:** In Floribunda, PCV (68.34%), and GCV (67.94%) for number of days to first flower were high coupled with high heritability of 98.85per cent and genetic advance of 131.91 per cent.

3. **Prickle density (per five cm):** High PCV (64.52%) and GCV (61.93%)) were recorded for prickle density, with a high heritability of 92.14percent and with a high genetic advance as percent mean of 122.47.

4. **Flower size (cm):** Moderate PCV (29.66%) and GCV (29.24%) values were observed for flower size for the genotypes with high heritability (97.21%) and genetic advance as per cent of mean (59.40).

5. **Flower weight (g):** Moderate PCV (39.53%) and GCV (37.97%) values were observed for the genotypes with high heritability (92.27%) and genetic advance as per cent of mean (75.13).

6. **Pedicel length (cm):** Moderate PCV (23.68%) and GCV (22.81%) values were recorded by the genotypes for pedicel length with high heritability (92.78%) and genetic advance as per cent of mean (45.27).

7. **Number of petals per flower:** High estimates of phenotypic (41.34%) and genotypic (40.72%)) co-efficients of variation were recorded with high heritability (97.00%) and genetic advance as per cent mean of 82.61.

Table 15. Clustering of 25 genotypes of Hybrid Tea Roses

Cluster	Name of the genotypes	No of genotypes
I	Madame George Delbard, Chirst of Colomb, Pink Panther, Priority Pride and Prince Jardiner	5
II	Roughe Miland, Shrewsbury Show, A Tago, Demestra and Lois Wilson	5
III	Aishwarya, Lincoln Cathedral and Mary Jean	3
IV	Silver Star, Toplesse and Cel b Lau	3
V	Alaine Souchen and Fryat	2
VI	Perfume Perfect and Majestic	2
VII	Mom's Rose and Alabama	2
VIII	Amara	1
IX	Golden Fairy Sport	1
X	Josepha	1

Table 16. Clustering of 25 genotypes of Floribunda Roses

Clusters	Name of the genotypes	No of genotypes
I	Tickled Pink,Rose Mary Gandhi, Princess de Monaco, Ochi di Fita, The McCartney Rose, Chesire, Messara , Sans Souci, Plantein on Blumen and Prosperity	10
II	Rosarale de Chateau, Carry Free Beauty, City of Glasgow and Michel Fish	4
III	Versailles, Lisa and Schloss Elutin	3
IV	Sterntaler, Orange N Lemon and Winchester Cathedral	3
V	Monnalisa	1
VI	Carolanne	1
VII	Mini Pink	1
VIII	Lasting piece	1
IX	Golden Fairy	1

8. **Size of petals (cm):** Size of petals recorded high PCV (48.82%) and GCV (47.85%) values with high heritability (96.06%) and genetic advance as per cent of mean (96.61).

9. **Number of flowers per plant :** The character number of flowers per plant exhibited high PCV (47.62%) and GCV (44.10%) values with high heritability (95.63%) and genetic advance as per cent of mean (95.13).

10. 4.1.7 Genetic divergence studies

4.1.7.1 Clustering of genotypes by D^2 statistics in Hybrid Tea

Procedure suggested by Tocher's method (Rao1952) was used to group 25 rose genotypes into ten clusters by treating the estimated D^2 values as the square of the generalized distance.

The pattern of distribution of genotypes into various clusters in Hybrid Tea is presented in Table 15. In Hybrid Tea, among the ten clusters formed, Cluster I and II were the biggest, consisting of five genotypes each followed by Clusters III and IV each comprising of three genotypes. Cluster I included varieties Madame George Delbard, Chirst of Colomb, Pink Panther, Priority Pride and Prince Jardiner. Cluster II consisted of Roughe Miland, Shrewsbury Show, A Tago, Demestra and Lois Wilson. Cluster III comprised of Aishwarya, Lincoln Cathedral and Mary Jean, whereas cluster IV consisted of Silver Star, Toplesse and Cel b Lau. Cluster V contained Alaine Souchen and Fryat. Cluster VI included Perfume Perfect and Majestic. Cluster VII contained Mom's Rose and Alabama. Clusters VIII, IX and X contained solitary genotypes *viz.*, Amara, Golden Fairy Sport and Josepha respectively.

The pattern of distribution of genotypes into various clusters in Floribunda is presented in the Table 16. In Floribunda, among the nine clusters formed Cluster I was the biggest cluster consisting of ten genotypes *viz.*, Tickled Pink, Rose Mary Gandhi, Princess de Monaco, Ochi di Fita, The McCartney Rose,

Table 17. Average intra and inter cluster distance values of 25 genotypes of Hybrid Tea Roses

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	278.58	506.47	480.99	750.24	480.99	927.58	969.22	1151.15	1107.36	5895.32
II	0.00	324.20	999.14	554.80	465.24	1127.45	1630.20	792.10	1693.21	7432.01
III		0.00	195.47	815.08	940.19	974.64	481.35	774.90	1041.97	4650.33
IV			0.00	256.32	1108.09	664.87	866.14	500.79	965.98	5095.53
V				0.00	1.07	1452.47	1760.39	1344.90	2013.32	8159.59
VI					0.00	271.20	613.74	1656.31	540.11	3430.09
VII						0.00	71.41	1219.41	349.30	2652.81
VIII							0.00	0.00	1489.01	6520.64
IX									0.00	2975.20
X										0.00

Table 18. Average intra and inter cluster distance values of 25 genotypes of Floribunda Roses

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	338.29	840.06	585.30	523.31	556.07	529.81	1610.47	1645.49	1023.73
II	0.00	364.94	832.76	387.40	910.33	968.47	1041.18	2279.14	1378.19
III		0.00	170.04	539.14	562.77	712.74	834.28	2647.54	881.52
IV			0.00	203.25	701.36	629.48	797.97	1742.18	1004.15
V				0.00	0.00	726.36	706.40	2397.25	1459.17
VI						0.00	1170.80	1211.78	500.98
VII							0.00	2457.28	771.08
VIII								0.00	955.48
IX									0.00

Chesire, Messara, Sans Souci, Plantein on Blumen and Prosperity. Cluster II consisted of Rosarale de Chateau, Carry Free Beauty, City of Glasgow and Michel Fish. Cluster III included Versailles, Lisa and Schloss Elutin. Cluster IV comprised of Sterntaler, Orange N Lemon and Winchester Cathedral. Cluster V, VI, VII, VIII and IX contained solitary genotypes *viz.,* Monnalisa, Carolanne, Mini Pink, Lasting Piece and Golden Fairy respectively.

4.1.7.2 Inter relation of clusters

The intra and inter cluster D^2 and D values among the ten clusters of Hybrid Tea are given in the Table 17. The intra cluster D^2 values indicated that, Cluster II (D^2 = 324.20) had the maximum genetic diversity within the genotypes of that group followed by Cluster I (D^2 = 278.58) and Cluster VI (D^2 = 271.20). The inter cluster D^2 values of the ten clusters revealed that the highest inter cluster generalized distance (D^2 = 8159.59) was between Cluster X and Cluster V while, the lowest (D^2 = 349.30) was between clusters IX and VII.

The intra and inter cluster D^2 and D values among the nine clusters of Floribunda are given in the Table 18. The intra cluster D^2 values indicated that Cluster II (D^2 = 364.94) had the maximum genetic diversity followed by Cluster I (D^2 = 338.29) and Cluster IV (D^2 = 203.25) .The inter cluster D^2 values of the nine clusters revealed that the highest inter cluster generalized distance (D^2 = 2647.54) was between Cluster VIII and Cluster III while, the lowest (D^2 = 387.40) was between Clusters IV and II.

4.1.7.3 Cluster means for different characters studied

The mean values of the nine quantitative morphological characters studied in 25 Hybrid Tea genotypes grouped in ten clusters are presented in Table 19. Cluster IV had the highest number of leaves at first flower (66.67), while the lowest number of leaves at first flower was recorded in Cluster V (24.83). Cluster VIII had the late flowering genotypes (67.50 days) whereas cluster VII had the early flowering genotypes (26.17). The genotypes of (26.17) Cluster V had the

Table 19. Cluster means of various characters of 25 genotypes of Hybrid Tea Roses

Clusters	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
I	25.37	46.50	4.40	7.07	5.14	5.89	22.43	3.53	24.45
II	50.77	63.47	3.70	7.41	5.71	6.04	40.37	2.79	22.45
III	32.78	45.50	5.67	8.68	4.92	5.18	21.50	5.10	25.80
IV	66.67	53.72	5.61	7.84	4.97	6.42	24.78	3.97	30.00
V	24.83	63.17	6.33	7.35	6.07	5.93	45.17	2.97	23.70
VI	60.17	29.08	4.83	6.79	5.04	5.78	38.58	3.49	25.05
VII	39.00	26.17	4.17	9.65	5.52	5.71	24.50	4.40	17.55
VIII	59.67	67.50	3.50	10.30	7.17	7.30	17.33	3.65	30.00
IX	46.00	46.50	4.00	9.13	1.76	4.12	18.17	1.69	42.45
X	65.67	63.47	3.83	9.20	5.83	5.98	14.67	5.95	19.95

Table 20. Cluster means of various characters values of 25 genotypes of Floribunda Roses

Clusters	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight(g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
I	41.72	60.05	6.67	6.08	3.71	4.54	23.27	3.12	27.30
II	45.71	31.38	3.29	6.67	3.70	3.71	30.04	3.18	28.20
III	79.44	57.83	4.28	6.51	3.19	4.24	27.50	2.93	32.55
IV	48.44	39.06	9.50	6.55	5.58	6.13	25.78	3.17	39.15
V	52.67	63.50	3.17	7.14	6.50	4.35	54.67	2.72	32.55
VI	45.83	56.33	2.33	8.66	5.49	4.38	14.67	3.17	37.50
VII	22.83	53.50	3.00	6.91	4.43	3.60	25.17	3.18	25.00
VIII	17.17	46.00	11.50	8.11	5.58	6.78	16.17	5.44	25.05
IX	74.83	51.17	2.67	8.21	3.20	3.80	12.17	4.88	30.00

highest prickle density (6.33), while genotypes of Cluster VIII had the lowest prickle density (3.50) when compared to other clusters. Lowest flower size was noticed in cluster VI (6.79 cm) while the genotypes having higher flower size belonged to cluster VIII (10.30 cm). Cluster IX had the genotypes with low flower weight with a mean of 1.76 g whereas highest flower weight with a mean of 7.17 g was observed in (3.50) Cluster VIII. The genotypes in Cluster IX had low pedicel length (4.12cm) while genotypes of cluster VIII had high pedicel length with a mean of 7.30 cm. Cluster X had the lowest number of petals per flower with a mean of 14.67 cm while Cluster V had the highest number of petals per flower (45.17). Cluster IX recorded the lowest size of petals with a mean of 1.69 cm, whereas Cluster X recorded the highest size of petals with a mean of 5.95 cm. Highest number of flowers per plant was observed in Cluster IX with a mean of 42.45, whereas lowest number of flowers per plant was recorded in Cluster VII (17.55).

The mean values of nine characters studied in 25 Floribunda genotypes grouped in nine clusters are presented in Table 20. Cluster III had the highest number of leaves at first flower (79.44), while the lowest number of leaves at first flower was recorded in Cluster VIII (17.17).Cluster V had the late flowering genotypes (63.50 days) whereas Cluster II had the early flowering genotypes (31.38 days). The genotypes of Cluster VIII had the highest prickle density (mean of 11.50/5 cm), while genotypes of Cluster VI had the lowest prickle density (mean of 2.33), when compared to other clusters. Lowest flower size was noticed in Cluster I (6.08 cm) whereas highest flower size was observed in Cluster VI (8.66 cm). Cluster III had the genotypes with low flower weight with a mean of 3.19 g and high flower weight (mean of 6.50 g) was noticed in Cluster V. The genotypes in Cluster VII had low pedicel length of 3.60cm while genotypes in Cluster VIII had high pedicel length with a mean of 6.78cm. Cluster IX had the lowest number of petals per flower (12.17) while cluster V had the highest number of petals per flower (54.67). Cluster V recorded the lowest size of petals (2.72 cm) whereas cluster VIII recorded the highest size of petals (5.44 cm).

Table 14. Contribution of nine characters towards divergence in 25 genotypes of Hybrid Tea and Floribunda Roses

Sl. No.	Characters	Hybrid Tea Roses (%contribution)	Floribunda Roses (%contribution)
1	Number of leaves at first flower	22	34
2	Number of days to first flower	39	32
3	Prickle density/5 cm	0	0
4	Flower size (cm)	12	8
5	Flower weight(g)	0	2
6	Pedicel length(cm)	0	2
7	Number of petals per flower	14	11
8	Size of petals (cm)	12	10
9	Number of flowers per plant/bunch	1	1

Highest number of flowers per plant was observed in Cluster IV (39.15), whereas lowest number of flowers per plant was recorded in the Cluster VII (25.00).

4.1.7.4 Contribution of different characters towards divergence

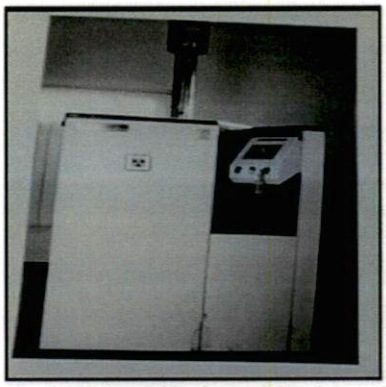
The percent contribution of each character towards divergence in case of Hybrid Tea is presented in Table 14. It was observed that number of days to first flower (39%) was the largest contributor towards divergence followed by number of leaves at first flower (22%), number of petals per flower (14%), flower size and size of petals, (12% each). The remaining characters did not contribute significantly to the total divergence.

The per cent contribution of each character towards divergence in case of Floribunda is presented in Table 14. It is evident that number of leaves at first flower (34%) was the largest contributor towards divergence followed by number of days to first flower (32%), number of petals per flower (11%) and size of petals (10%).The remaining characters *viz.*, pedicel length, prickle density and flower weight did not contribute significantly to the total divergence.

4.2 INDUCED MUTAGENESIS

Induced mutagenesis was carried out as a part of the investigation entitled "Characterisation and genetic improvement in Rose (*Rosa* spp.) through mutagenesis". In this study, two genotypes each selected from Hybrid Tea and Floribunda groups were subjected to gamma rays and Ethyl Methane Sulphonate (EMS) treatments. The genotypes selected were Pink Panther and Demestra under Hybrid Tea and Golden Fairy and Monnalisa under Floribunda. The objective of the study was to create variation in roses using gamma radiations and EMS. The results obtained during the experimentation were statistically analysed, tabulated and reported as under:

Collection of budwood



Gamma irradiation of budwood

Preparation for budding

Budding onto the rootstock

Sprouting of bud

Transfer to the Field establishment in pots

Plant in vegetative stage

Treated plant with flower

Plate 4. Steps in gamma treatment

Plate 5. Development of M_1 generation

Plate 6. M_1 generation Field view

4.2.1 Induced mutagenesis using gamma rays on budwood

4.2.1.1 Fixing of LD_{50} value

The trial for fixing LD_{50} dose for each genotype was carried out separately. For fixing LD_{50} value, trials were conducted employing ten doses (including control) from 20 to 100 Gy gamma rays. Effect of different doses of gamma radiations studied on survival percentage and bud take percentage in the four genotypes are presented in Table.

1. Bud take percentage

Data on bud take percentage as influenced by gamma irradiation is presented in Table 21. In Pink Panther, the maximum bud take (93.33%) was recorded in control while minimum (6.67%) was recorded in100 Gy gamma ray treatment. All the treatments were significantly different from each other. Monnalisa had significantly different data with respect to bud take percentage having cent per cent bud take in control and minimum (13.33%) with 100 Gy, which was significantly different from all other gamma ray treatments as well as other genotypes.

In Demestra, 93.33% bud take was recorded in control while minimum was recorded in (0.00%) 100 Gy, gamma ray treatment. All the treatments were significantly different from each other. In Golden Fairy, the maximum per cent bud take (93.33%) was recorded in control, which was significantly different from all other treatments, while the minimum (6.67%) was with 100 Gy gamma ray treatment.

2. Survival percentage at 30 days

The data pertaining to survival of plants at 30 days after gamma radiations is presented in Table 21. It is evident from the data that survival percentage decreased with increase in dose of gamma rays.

Table 21. Effect of gamma irradiation on Bud take (%) and Survival (%) at 30 days in Rose genotypes

Sl. no	Bud take (%)					Survival (%) at 30 days			
	Dose of gamma rays (Gy)	Pink Panther	Monnalisa	Demestra	Golden Fairy	Pink Panther	Monnalisa	Demestra	Golden Fairy
1	20	86.67	93.33	86.67	86.67	80.00	86.67	86.67	86.67
2	30	80.00	86.67	80.00	66.67	66.67	86.67	73.33	60.00
3	40	73.33	80.00	66.67	60.00	60.00	73.33	60.00	53.33
4	50	60.00	73.33	46.67	33.33	46.67	66.67	40.00	26.67
5	60	33.33	66.67	33.33	33.33	13.33	46.67	26.67	0.00
6	70	26.67	60.00	20.00	26.67	6.67	20.00	0.00	0.00
7	80	20.00	33.33	13.33	20.00	0.00	13.33	0.00	0.00
8	90	13.33	20.00	6.67	13.33	0.00	6.67	0.00	0.00
9	100	6.67	13.33	0.00	6.67	0.00	0.00	0.00	0.00
10	Control	93.33	100.00	93.33	93.33	86.67	93.33	93.33	93.33

Table 22. Fixation of gamma ray doses based on LD_{50} value for bud take (%) and survival (%) in Rose genotypes

	Doses fixed (Gy)				
Name of genotype	LD_{50} stat.value	(1)	(2)	(3)	(4)
Pink Panther	39	30	40	50	60
Monnalisa	51	40	50	60	70
Demestra	43	30	40	50	60
Golden Fairy	32	20	30	40	50

In Pink Panther the maximum per cent plant survival (86.67%) was recorded in control while no plants survived in 80, 90 and 100 Gy gamma rays. All the treatments were significantly different from each other. Monnalisa had significantly different data with respect to plant survival having 93.33% plant survival in control and no survival under 100 Gy, which was significantly different from all other gamma ray doses. In Demestra, 93.33% plant survival was recorded in control while 70,80,90 and 100 Gy gamma rays recorded no survival. All the treatments were significantly different from each other. In Golden Fairy, the maximum per cent plant survival (93.33%) was recorded in control, which was significantly different from all other treatments, while the minimum was with 60,70,80,90 and 100 Gy being no survival at all. No survival at 30 days was observed at higher doses in the different varieties. In Golden Fairy no survival was there for 5 doses from 60 to 100 Gy, and in Demestra no survival was not there for 4 doses from 70 to 100 Gy. In Pink Panther, the highest doses did not survive at 30 days and in Monnalisa, the highest dose tried did not survive at 30 days.

It was observed that the various varieties showed significant difference in survival percentage. Analysis indicated the extrapolated LD_{50} value based on bud take (%) and survival (%)as 39 Gy in Pink Panther as 51 Gy in Monnalisa as 43 Gy in Demestra and 32 Gy in Golden Fairy (Table 22).

4.2.1.2 Effect of Gamma Radiation on Morphological Characters

4.2.1.2 a. Direct effect of gamma ray treatment on M_1

Based on LD_{50} value five doses including control were selected separately for each of the four varieties and treatments were given. Initial field evaluation of gamma ray treated M_1 generation was done to study the direct effect of mutagen on M_1 with respect to these characters .The data pertaining to number of days to bud take, number of days to first leaf and size of first flower has been presented in Tables 23, 24, 25 and 26.

Table 23. Field evaluation of gamma ray treated M_1 generation of Demestra

Sl.no	Dose of gamma rays (Gy)	No of days to Bud take	No of days to first leaf	Size of first flower
1	30	22.67	28.33	7.43
2	40	27.33	31.00	7.12
3	50	32.33	34.00	6.85
4	60	34.33	34.67	5.31
5	Control (0)	19.00	27.00	8.16
	SE (m)	0.91	1.43	1.45
	C.D (5%)	2.86	4.51	4.21
	C.V	5.79	8.20	7.28

Table 24. Field evaluation of gamma ray treated M_1 generation of Pink Panther

Sl.no	Dose of gamma rays (Gy)	No of days to Bud take	No of days to first leaf	Size of first flower
1	30	24.00	30.67	9.01
2	40	29.67	32.33	8.65
3	50	31.33	33.38	8.42
4	60	32.61	34.00	7.91
5	Control (0)	22.33	30.00	9.51
	SE (m)	2.10	1.61	1.34
	C.D (5%)	6.63	5.08	4.71
	C.V	13.29	10.02	11.25

Table 25. Field evaluation of gamma ray treated M_1 generation of Golden Fairy

Sl.no	Dose of gamma rays (Gy)	No of days to Bud take	No of days to first leaf	Size of first flower
1	20	29.00	27.67	7.64
2	30	30.67	30.33	7.24
3	40	34.00	31.67	6.21
4	50	37.00	32.00	5.43
5	Control (0)	24.67	20.33	8.10
	SE (m)	0.71	1.27	0.14
	C.D (5%)	2.25	4.01	0.38
	C.V	3.99	8.36	6.14

Table 26. Field evaluation of gamma ray treated M_1 generation of Monnalisa

Sl.no	Dose of gamma rays (Gy)	No of days to Bud take	No of days to first leaf	Size of first flower
1	40	25.00	28.00	6.78
2	50	26.33	30.33	5.21
3	60	29.84	31.67	5.14
4	70	31.33	34.00	4.35
5	Control (0)	23.00	22.12	7.01
	SE (m)	0.95	0.95	0.68
	C.D (5%)	3.01	3.01	1.85
	C.V	6.17	5.74	10.34

1. Number of days to bud take

Data presented in Tables 23 to 26 depicts number of days to bud take as influenced by gamma irradiation in the different genotypes. In the genotype Pink Panther, control took 22.33 days to bud break, whereas the dose 60 Gy took 32.61 days to bud take, followed by 50, 40 and 30 Gy taking 31.33, 29.67 and 24.00 days respectively to bud break. In Monnalisa, control took 23.00 days for bud break, but the treatments 40, 50 and 60 Gy were on par taking 25.00, 26.33 and 29.84 days respectively for bud break. The dose 70 Gy took maximum days to bud take (31.33). In the genotype Demestra, control took 19.00 days for bud take whereas 30Gy took 22.67 days. But maximum days to bud take was recorded by 60 Gy followed by 50 and 40 Gy, being 34.33, 32.33 and 27.33 days respectively. In Golden Fairy, the minimum number of days to bud take was 24.67 (Control) whereas the maximum days taken was recorded in 50 Gy (37.00 days). It was followed by 40,30 and 20 Gy being 34.00, 30.67 and 29.00 days respectively.

2. Number of days to first leaf

Data presented in Tables 23 to 26 indicated the number of days to first leaf as influenced by gamma irradiation. In the genotype Pink Panther, control took 30.00 days to first leaf, whereas the dose 60 Gy took 34.00 days followed by 50, 40 and 30 Gy requiring 33.38, 32.33 and 30.67 days respectively to produce the first leaf. In Monnalisa, control took 22.12 days for first leaf, but the treatments 40, 50 and 60 Gy which were on par took 28.00, 30.33 and 31.67 days respectively to first leaf. The dose 70 Gy took maximum days to first leaf (34.00 days). In the genotype Demestra control took (27.00) days for bud take whereas 30Gy took 28.33 days. Highest number of days to first leaf was recorded by 60 Gy followed by 50 and 40 Gy, being 34.67, 34.00 and 31.00 days respectively. In Golden Fairy, the minimum number of days to first leaf was 20.33 whereas the maximum days taken was recorded in 50 Gy (32.00 days) followed by 40, 30 and 20 Gy being 31.67, 30.33 and 27.67 days respectively.

3. Size of first flower

The data pertaining to size of first flower are presented in Tables 23 to 26. It is evident from the data that differences for this character were recorded in all the genotypes used. The size of the first flower was comparatively larger than the succeeding flowers.

The data revealed that irrespective of genotypes, un-irradiated plants had significantly larger first flowers. In Pink Panther, no significant difference in flower size between the treated and untreated plants was observed. Control showed a flower size of (9.51 cm) whereas the 30, 40, 50 and 60 Gy recorded sizes of 9.01, 8.65 and 8.42 and 7.91 cm respectively. In Monnalisa, the control flower recorded a size of 7.01 cm whereas the irradiation treatment 40 Gy was on par with control recording 6.78 cm. The treatments such as 50, 60 and 70 Gy recorded flower sizes of 5.21, 5.14 and 4.35 cm respectively.

In Demestra flower size was 8.16 cm in 0 Gy. The treatments 30, 40 and 50 Gy were on par with each other with flowers of sizes 7.43, 7.12 and 6.85 cm respectively whereas 60 Gy showed significant difference (5.31 cm) when compared with control. Exposure to gamma rays reduced first flower size significantly. In Golden Fairy, the untreated flower showed a size of 8.10 cm whereas the lowest flower size was recorded in 50 Gy (5.43cm) followed by 40, 30 and 20 Gy with flower sizes of 6.21, 7.24 and 7.64 cm respectively.

4.2.1.2 b. Field evaluation of gamma ray treated M_1 generation.

The observations on nine morphological characters of the four varieties of gamma ray treated M_1 generation is presented in Tables 27, 28, 29 and 30 and the same has been discussed below.

1. Number of leaves at first flower

The data pertaining to this attribute has been presented and an appraisal of the data elucidates that gamma ray treatment had a significant effect on number of

Table 27. Mean performance for morphological characters of mutants developed through gamma radiation in Demestra

Dose of gamma rays (Gy)	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight(g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
30	96.40	110.20	4.00	9.42	4.40	4.08	28.20	2.15	12.60
40	94.40	110.80	3.00	9.19	4.36	3.98	25.20	1.59	9.00
50	93.20	111.00	4.60	8.97	4.25	3.84	23.60	1.53	7.80
60	91.80	113.20	2.80	7.72	4.14	3.82	23.20	1.43	7.20
Control (0)	109.40	116.40	5.80	10.48	5.50	5.28	35.40	3.15	15.60
SE(m)	2.66	1.27	0.54	1.45	0.80	0.25	1.13	0.14	0.49
C.D (5%)	7.84	3.74	1.58	4.29	2.35	0.73	3.32	0.40	1.45
C.V	5.88	2.42	28.51	7.37	3.77	12.74	8.91	13.67	30.22

Table 28. Mean performance for morphological characters of mutants developed after gamma radiation in Pink Panther

Dose of gamma rays (Gy)	Number of leaves at first flower	Number of days to first flower	Prickle density/ 5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
30	46.60	129.20	6.20	8.05	6.53	5.70	29.40	5.83	6.60
40	44.40	131.00	4.40	7.69	5.89	5.00	28.20	5.50	6.10
50	42.40	131.80	4.60	7.60	5.30	4.54	28.00	5.28	6.00
60	40.40	133.00	5.00	7.31	4.65	4.52	27.00	5.14	5.40
Control	66.60	122.20	7.00	8.33	7.28	6.36	33.40	6.01	10.80
SE(m)	4.35	0.99	0.59	0.12	0.34	0.27	1.37	0.91	0.35
C.D (5%)	12.85	2.92	1.75	1.08	1.01	0.81	4.07	2.70	1.05
C.V	19.46	1.64	23.41	10.11	12.44	12.34	10.15	23.69	33.10

Table 29. Mean performance for morphological characters of mutants developed after gamma radiation in Golden Fairy

Dose of gamma rays (Gy)	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
20	95.00	162.40	6.00	7.25	6.80	3.32	62.00	4.71	6.80
30	81.20	164.40	6.60	7.05	6.72	2.82	59.60	4.06	6.60
40	72.20	165.40	5.40	7.04	6.63	2.64	57.60	4.00	5.40
50	70.00	167.60	4.40	6.95	6.32	2.46	56.80	3.94	4.20
Control	103.60	159.60	7.40	7.96	7.06	3.58	65.80	4.98	10.20
SE(m)	6.30	0.85	0.64	0.17	0.15	0.11	2.05	0.42	0.38
C.D (5%)	18.58	2.50	1.88	1.44	0.43	0.32	6.05	1.25	1.13
C.V	16.01	11.11	23.01	9.49	4.71	7.89	7.29	20.41	37.54

Table 30. Mean performance for morphological characters of mutants developed after gamma radiation in Monnalisa

Dose of gamma rays (Gy)	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
40	102.00	163.00	7.80	6.07	3.73	4.30	38.80	2.90	9.00
50	100.40	166.60	6.40	5.30	3.14	4.00	36.40	2.74	6.80
60	99.40	169.00	6.20	5.21	2.85	3.98	35.20	2.47	5.40
70	98.60	169.20	5.40	4.63	2.76	3.94	33.00	1.45	4.20
Control	115.40	159.40	10.20	6.13	4.26	5.04	52.40	3.53	14.40
SE(m)	2.91	0.64	0.78	1.10	0.18	0.21	1.01	0.07	0.40
C.D (5%)	8.58	1.89	2.30	3.10	0.53	0.61	2.98	0.22	1.19
C.V	6.06	8.83	20.92	6.40	11.61	10.39	5.54	6.04	35.05

leaves at first flower over the control in all the genotypes tried.

In Demestra, among the different gamma ray doses, maximum number of leaves at first flower of 109.40 was recorded in control which was found to be significantly different from all other treatments, while the minimum number of leaves (91.80) was observed with 60Gy followed by 50 Gy (93.20), 40 Gy (94.40) and 30 Gy (96.40). Data of Pink Panther revealed that among the different gamma ray doses, maximum number of leaves at first flower (66.60) was recorded with control which was found to be significantly different from all other treatments, while the 30, 40 and 50 Gy recorded 46.60, 44.40 and 42.40 leaves. Minimum number of leaves at first flower (40.40) was recorded with 60 Gy. In Golden Fairy, among the different gamma ray treatments, maximum number of leaves at first flower (103.60) was recorded in control which was found to be significantly different from all other treatments. The treatment 20 Gy recorded 95.00 leaves at first flower. It was followed by 30 Gy with 81.20 leaves. Lower number of leaves of 70.00 and 72.20 were recorded with 50 and 40 Gy treatments respectively. In Monnalisa, among the different gamma ray doses, 115.40 leaves at first flower was recorded in control. In the treatments 40,50 and 60 Gy it was 102.00, 100.40 and 99.40 respectively but the lowest number of leaves at first flower of 98.60days was recorded with 70 Gy treatment.

2. Number of days to first flower

The data pertaining to this attribute has been presented and an appraisal of the data elucidates that gamma ray treatment delayed flowering significantly in all the rose genotypes.

In Demestra, among the different gamma ray treatments, minimum days taken to first flower was 116.40 days recorded with 0 Gy which was found to be significantly different from all other treatments. The maximum (113.20 days) time was taken by 60 Gy followed by 50, 40 and 30 Gy 111.00, 110.80 and 110.20 days respectively.Data of Pink Panther revealed that among the different gamma rays treatments earliest flowering (122.20 days) was recorded with control which

was found to be significantly different from all other treatments. The maximum number of days to first flower (133.00 days) was recorded with 60 Gy, whereas 50, 40 and 30 Gy took 131.80, 131.00 and 129.20 days respectively to flower.

In Golden Fairy, among the different gamma ray doses, the minimum days taken to full bloom of 159.60 days was recorded under control which was found to be different from all other treatments. The maximum of 167.60 days was recorded with 50 Gy treatment whereas the doses 20, 30 and 40 Gy recorded 162.40, 164.40 and 165.40 days respectively to flower. In Monnalisa, earliest flowering under gamma ray treatment of 159.40 days was recorded in control. The longest period to first bloom (169.20 days) was recorded in 70 Gy treatment.

3. Prickle density (per 5 cm)

The perusal of the data for prickle density indicates that there was a general reduction in prickle density over control among all the varieties of roses with increase in gamma ray doses.

In Demestra, it was evident from the data that the maximum prickle density (5.80 /5cm) was recorded with control which was significantly different from all the other doses of gamma rays. 30, 40 and 50 Gy recorded 4.00/5cm, 3.00/5cm and 4.60/5cm. The minimum prickle density (2.80/5cm) was with 60 Gy. Data of Pink Panther revealed that among the different gamma ray doses the maximum prickle density of 7.00/5cm was recorded in control which was found to be significantly different from all other treatments. 30 Gy recorded 6.20/5cm, 40 Gy recorded 4.40/5cm and 50 Gy recorded 4.60/5cm but the lowest prickle density of 5.00/5cm was recorded with 60 Gy gamma radiation.

In Golden Fairy, among the different gamma ray doses a prickle density of 7.40/5cm was recorded in control which was found to be different from all other treatments. The 20, 30 and 40 Gy treatments recorded prickle densities of 6.00/5cm, 6.60/5cm and 5.40/5cm respectively. The lowest prickle density of 4.40/5cm was recorded with 50 Gy treatment. The prickle density in Monnalisa,

among the different gamma ray doses recorded a maximum value of 10.20/5cm in control followed by 7.80/5cm, 6.40/5cm, 6.20/5cm and 5.40/5cm in 40, 50, 60 and 70 Gy respectively. A significant difference was found to exist between the control and all other treatments.

4. Flower size (cm)

The data pertaining to flower size in the field evaluation of M_1 after gamma ray treatment is presented in Tables 27 to 30. It is evident from the data that significant differences for this character were recorded in all the genotypes studied.

The data revealed that irrespective of genotypes, un-irradiated plants had significantly larger flowers. In Demestra control flowers had a size of 10.48 cm. The treatments 30, 40 and 50 Gy were on par with each other *i.e.,* 9.42, 9.19 and 8.97 cm respectively whereas 60 Gy showed significant difference (7.72 cm) when compared with control. In Pink Panther, there was no significant difference between the treated and untreated flowers. The control showed a flower size of 8.33 cm whereas the 30, 40, 50 and 60 Gy recorded sizes of 8.05, 7.69, 7.60 and 7.31 cm respectively. In Golden Fairy, the untreated flower showed a size of (7.96 cm) whereas the lowest flower size was recorded in 50 Gy (6.95 cm) followed by 40, 30 and 20 Gy with flower sizes of 7.04, 7.05 and 7.25 cm respectively. In Monnalisa, the control flower recorded a size of 6.13 cm whereas the irradiation treatment 40 Gy was on par with control, recording 6.07 cm. The treatments 50, 60 and 70 Gy recorded 5.30, 5.21 and 4.63 cm respectively as flower size.

5. Flower weight (g)

The data pertaining to flower weight is presented in Tables 27 to 30. It is evident from the data that significant differences for this character were recorded in all the genotypes used.

Un-irradiated plants of Demestra had significantly heavier flowers of 5.50 g under control, whereas all the treatments 30, 40, 50 Gy and 60 Gy were on par with each other with weights of 4.40, 4.36, 4.25 and 4.14 g respectively. Pink Panther showed a significant difference in flower weight between the treated and untreated ones. The control showed a flower weight of 7.28 g whereas the 30, 40, 50 and 60 Gy recorded weights of 6.53, 5.89, 5.30 and 4.65 g respectively.

In Golden Fairy, the untreated flower showed a weight of 7.06 g whereas the other treatments were on par with each other. In Monnalisa, the control flower recorded the maximum weight of 4.26 g whereas the irradiation treatment with 70 Gy recorded a minimum weight of 2.76 g. The treatments 60, 50 and 40 Gy recorded 2.85g, 3.14g and 3.73g respectively. This shows that exposure to gamma rays reduced the flower weight significantly in almost all the genotypes.

6. Pedicel length (cm)

A critical rummage of the data on pedicel length indicated that treatment with gamma rays reduced the length of the pedicel and length decreased proportionately with increase in dose.

In Demestra, the highest pedicel length of 5.28 cm was observed in control and the lowest pedicel length was observed in 60 Gy (3.82 cm), which was on par with 50 Gy and 40 Gy being 3.84 and 3.98 cm respectively. Pedicel length was 4.08 cm in 30 Gy. In Pink Panther, the highest pedicel length of 6.36 cm was observed in control. Among the treatments 30 Gy recorded the highest pedicel length of 5.70 cm followed by 40, 50 and 60 Gy which recorded lengths of 5.00, 4.54 and 4.52 cm respectively.

In Golden Fairy, the maximum pedicel length (3.58 cm) was observed in control, and the minimum pedicel length was observed in 50 Gy (2.46 cm) which was on par with the treatments 40 and 30 Gy which recorded 2.64 and 2.82 cm respectively. 20 Gy recorded a pedicel length of 3.32 cm. In the variety Monnalisa, the highest pedicel length of 5.04 cm was observed in control, whereas all the other

treatments were statistically on par with each other.

7. Number of petals per flower

The data presented in Tables 27 to 30 reveals that there was significant effect of gamma radiation and genotypes on the number of petals per flower.

The radiation of plants with gamma rays, irrespective of genotypes caused significant reduction in number of petals per flower. In case of Demestra, the highest number of petals per flower of 35.40 was recorded in control. In the treatments, maximum number of petals per flower of 28.20 was recorded in 30 Gy followed by 40,50 and 60 Gy being 25.20, 23.60 and 23.20 respectively. In Pink Panther, control exhibited the highest number of petals of 33.40 whereas the rest of the treatments were on par with each other. 30 Gy recorded 29.40 followed by 40, 50 and 60 Gy being 28.20, 28.00 and 27.00 respectively.

In Golden Fairy, the maximum number of petals (65.80) was observed in control, and the minimum number of petals was observed in 50 Gy (56.80) which was on par with the treatments 40 Gy (57.60) and 30 Gy (59.60). 20 Gy recorded 62.00 petals per flower.

In Monnalisa, the maximum number of petals (52.40) was recorded in control and the minimum number of petals were recorded in 70 Gy (33.00), whereas the other treatments 40, 50 and 60 Gy showed 38.80, 36.40 and 35.20 petals per flower respectively.

8. Size of petals (cm)

The data related to petal size has been compiled in Tables 27 to 30. The data revealed that petal size decreased with increase in dose of gamma rays.

In case of Demestra, maximum size of petals (3.15 cm) was recorded in control. In the treatments maximum size of petals (2.15 cm) was recorded in 30 Gy followed by 40, 50 and 60 Gy being 1.59, 1.53 and 1.43 cm respectively. In Pink

Panther, control exhibited highest size of petals of 6.01 cm whereas the rest of the treatments were on par with each other. 30 Gy recorded 5.83 cm followed by 40, 50 and 60 Gy being 5.50 cm, 5.28 cm and 5.14 cm respectively.

In Golden Fairy, the highest size of petals (4.98 cm) was observed in control, and the minimum size of petals was observed in 50 Gy (3.94cm) followed by 40 Gy , 30Gy and 20 Gy recording 4.00,4.06 and 4.71cm respectively. In Monnalisa, the highest size of petals of 3.53 cm was recorded in control and the lowest size of petals was recorded in 70 Gy (1.45cm), whereas in the other treatments *viz.,* 40, 50 and 60 Gy it was 2.90, 2.74 and 2.47cm respectively.

9. Number of flowers per plant /bunch

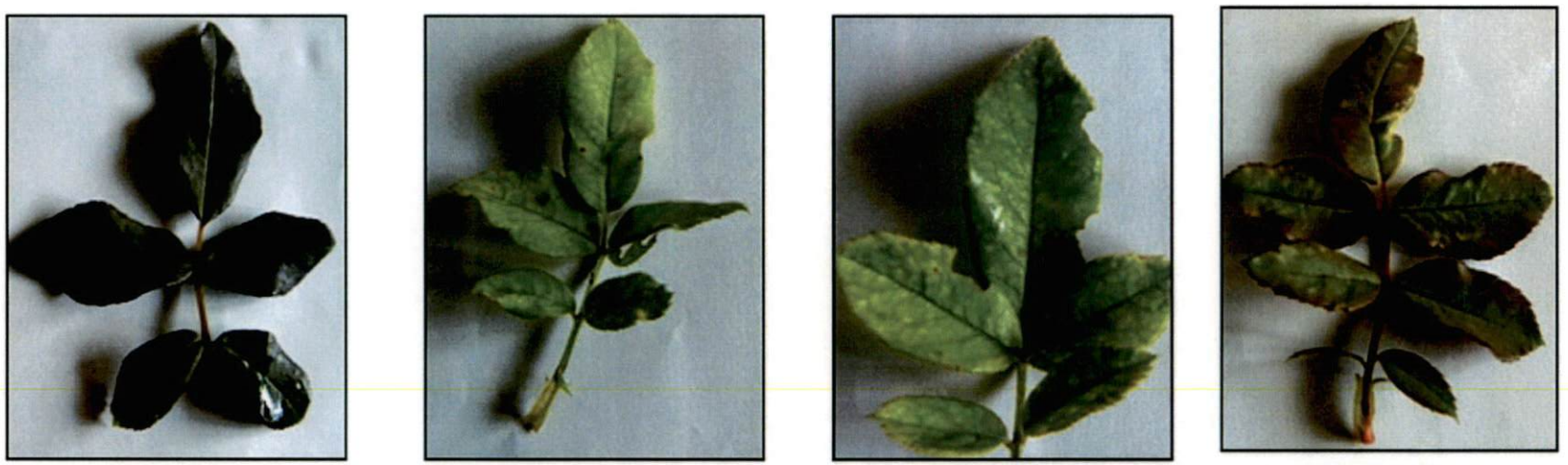
The data pertaining to the effect of gamma rays on the number of flowers per plant is presented in Tables 27 to 30. It is evident from the data that significant differences for this character were recorded in all the genotypes used.

Un-irradiated plants of Demestra had the highest number of flowers of 15.60. The treatments 30, 40, 50 Gy and 60 Gy produced 12.60, 9.00, 7.80 and 7.20 flowers respectively. In Pink Panther, there was a significant difference in flower number between the treated and untreated ones. Control showed the highest flower number of 10.80 whereas 30, 40, 50 and 60 Gy recorded the numbers of 6.60, 6.10, 6.00 and 5.40 respectively. In Golden Fairy, the control produced the highest number of flowers (10.20) whereas the other treatments recorded 6.80, 6.60, 5.40 and 4.20 flowers in 20, 30, 40 and 50 Gy respectively.

In Monnalisa, the control produced the highest number of flowers (14.40) Irradiation with a dose of 40 Gy recorded 9.00 flowers, whereas the treatments 50, 60 and 70 Gy recorded 6.80, 5.40 and 4.20 flowers respectively.

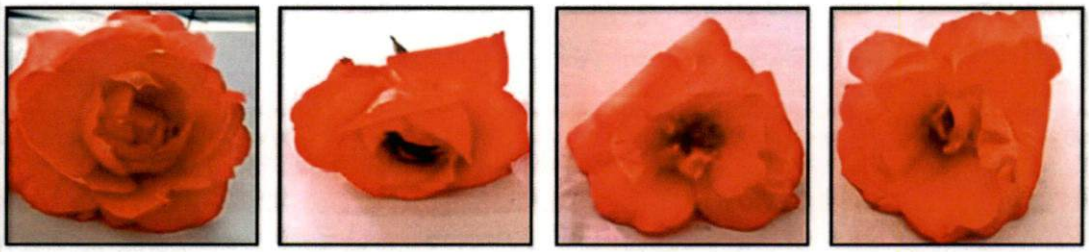
Control D_1-30 Gy D_2-40 Gy D_3-50 Gy

Plate 7. Mutants in floral characters Demestra using gamma rays



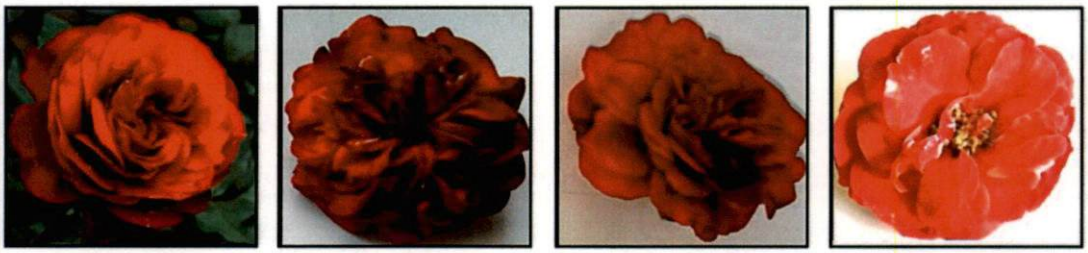
Control D_1-30 Gy D_2-40 Gy D_3-50 Gy

Plate 8. Mutants in leaf charactersDemestra using gamma rays



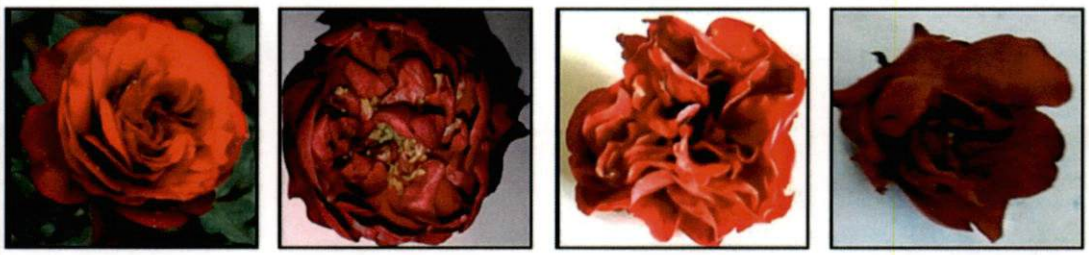
Control P_1-20 Gy P_2-30 Gy P_3-40 Gy

Plate 9. Mutants in floral characters-Pink Panther using gamma rays



Control Mn_1-30 Gy Mn_2-40 Gy Mn_3-50 Gy

Plate 10. Mutants in floral charactersMonnalisa using gamma rays



Control Mn_4-70 Gy Mn_5-80 Gy Mn_6-80 Gy

Plate 11. Abnormalities in floral charactersMonnalisa using gamma rays

Control Mn_1-40 Gy Mn_2-50 Gy Mn_3-60 Gy

Plate 12. Mutants in leaf charactersMonnalisa using gamma rays

10. Fragrance

In the genotypes Demestra, Pink Panther and Golden Fairy control and radiated ones record a medium fragrance, in Monnalisa low fragrance was recorded. It has been presented in Table 46.

11. Flower and leaf (colour, form, size and shape)

The unirradiated flower of Demestra (Table 44 and Plate 7) was light yellow with dark golden center with a medium sized flower of normal shape. Light yellow buds open out to large flowers which has beautiful form and exquisite colour. Mutant D_1 was induced by 30 Gy gamma ray dose. All flowers were pure homozygote (solid mutant) producing medium sized, compact flower with golden yellow colour. Flower was star shaped and the petals had rims folded inside. D_2 (40 Gy) Mutant was creamy pale orange in colour with a cup shaped flower where the inner petals were folded inside and giving a cup like appearance to the flower. Mutant D_3 (50 Gy) was golden yellow in colour. The petals were arranged in a haphazard distribution with a very small center and the flower arrangement was flat and plate like.

In Demestra, (Plate 8) the control leaf was dark green with a glossy and thick texture. Shiny, leathery appearance was seen. In D_1, the leaves were light green in colour with a spread of yellow. In D_2, green and yellow colour was seen in leaf. In D_3, the leaves were narrow and looking deformed the entire colour of the leaf was dark green and the margin of the leaf was copper coloured.

In Pink Panther, (Table 44 and Plate 9) the flower was large, well shaped and deep pink in colour. In Mutant (P_1) 20 Gy the colour is deep pink with a very shapely flowers and of semi-open type. Petals are intact and medium size. In P_2 (30Gy) the flower recorded triangular appearance. Petal shape has changed and size is of petal has reduced. The wavy margin is not prominent in the petals. Some petals are deformed. The arrangement of the petals of the flower is in haphazard manner. In P_3 (40 Gy), the centre is slightly lopsided. The haphazard distribution

of the petals is giving the flower an abnormal look with unequal distribution of petals throughout the flower and also decreased number of petals. In all the doses there is no significant change in flower color or the intensity of pinkness is the same in both control and treated ones.

In Golden Fairy, the control flower was large, well formed and bright yellow. The blooms opened fast and colour tends to fade. Dense arrangement of petals gave a showy in nature to the flowers pink dots were present on the last whorl of petals. In (G_1) 20 Gy, the center was diffused with random arrangement of uniform sized petals thickly around the centre. In G_2 (30 Gy), the yellow flower with compact arrangement of the inner petals which was yellowish in color, whereas the outer petals started fading away as the days progressed.

In Monnalisa, (Table 44 and Plate 10) the control flower is magnificent deep velvety vibrant red and is very showy, open type with appealing blooms which are produced on a medium vigorous hardy bush. In M_{n1} (30 Gy) the flower is deeper velvetty red and white streaks in centre but the inner petals were arranged in a most compact manner. In 40 Gy, (M_{n2}) the flower is deep velvetty red and lopsided in form, the flower is misshaped in appearance when compared with the control. In 50 Gy, (M_{n3}) the flower is deep pink in colour with white streaks in outer petals. Petals had serrated margin. The intensity of redness had decreased and it was showing a blend of dark pink which means that redness had started fading away and as a result the white color had started creeping in. Abnormalities were also recorded in Monnalisa (Plate 11) with respect to floral characters. The abnormalities were noticed in different doses such as 70 and 80 Gy. In 70 Gy (M_{n4}) the center has spread or enlarged throughout the flower and the petals at the outer whorl are showing a cup like arrangement. In 80 Gy (M_{n5}), the petals were crinkled and randomly arranged. In 80 Gy (M_{n6}), the flower failed to open.

In Monnalisa (Plate 12) control, the leaves of the plant are dark green, broad and the margin of the leaves serrated normally. In M_{n2}, the margin of the

Table 44. Variations in floral characters in M_1 generation-gamma ray treatment

Variety and mutants derived	Variations in colour	Variations in form	Variations in size		Variations in shape	
			Flower	Petals	Flower	Petals
Demestra-Control	Light yellow with dark golden center	Normal	Medium	Medium	Normal shaped	Normal
D_1-30 Gy	Golden yellow	Star shaped	Medium	Medium	Star shaped	Rim folded inside
D_2-40 Gy	Creamy pale orange	Cup shaped	Medium	Medium	Cup shaped	Rim folded inside
D_3-50 Gy	Golden yellow	Normal	Medium	Medium	Plate shaped	Normal
Pink Panther-Control	Deep pink	Well formed	Large	Large	Circular	Normal
P_1-20 Gy	Deep pink	Very shapely flowers	Normal	Intact and Medium	Semi-open type	Normal
P_2-30 Gy	Deep pink	Triangular appearance	Small	Small	Circular	Normal
P_3-40 Gy	Deep pink	Slightly lopsided	Normal	Intact, medium	Circular	Normal
Golden Fairy-Control	Bright yellow	Well formed	Large	Large	Circular	Medium
G_1-20 Gy	Yellow	Misformed	Large	Small and needle shaped	Circular	Medium
G_2 -30 Gy	Yellow	Well formed	Medium	Medium	Circular	Medium
Monnalisa-Control	Deep velvety Red	Normal, open	Medium	Medium	Misshaped	Petals intact
Mn_1-30 Gy	Deeper velvetty red with white streaks in center	Normal	Medium	Petal number reduced	Normal	Margins serrated
Mn_2-40 Gy	Deeper, velvetty red	Lopsided	Medium	Medium	Misshaped	Margins serrated
Mn_3-50 Gy	Deep pink with white streaks in outer petals	Normal	Medium	Medium	Normal	Margins serrated

Table 46. Performance for qualitative traits in M_1 generation-gamma ray treatment

Variety and mutants derived	Fragrance	Seed setting ability	Vase life/ longevity (days)
Demestra-Control	Medium	No	3.2
D_1-30 Gy	Medium	No	3.0
D_2-40 Gy	Medium	No	2.6
D_3-50 Gy	Medium	No	2.2
Pink Panther- Control	Medium	No	2.0
P_1-20 Gy	Medium	No	1.8
P_2-30 Gy	Medium	No	1.4
P_3-40 Gy	Medium	No	1.0
Golden Fairy- Control	Medium	No	2.2
G_1-20 Gy	Medium	No	2.0
G_2 -30 Gy	Medium	No	1.8
Monnalisa-Control	Low	No	2.4
Mn_1-30 Gy	Low	No	2.2
Mn_2-40 Gy	Low	No	2.0
Mn_3-50 Gy	Low	No	1.8

leaves were not as in control, the leaves were pale green with a copper red color found only in the serrated margin of leaves. In M_{n3}, the leaves were small, crinkled and with light green color in the middle of the leaf and a copper color was at the margin. The shape of the leaves is also altered or deformities in the shape was seen with dot like variegation on the surface of the leaf. In M_{n4}, the leaf arrangement itself is different compared to the rest, the leaves are jet copper in colour.

12. Seed setting ability

Most of the cultivars failed to set seed in the control flowers. Seed setting in the gamma ray treatment was nil in all the four genotypes of roses at all the doses tried.

13. Vase life/longevity (days)

The vase life recorded in Demestra control (Table 46) was 3.2 days. Mutant D_1 recorded a vase life of 3.0 days, mutant D_2 recorded 2.6 days and Mutant D_3 recorded 2.2 days. In Pink Panther control, the vase life recorded was 2.0 days. In Mutant P_1 it was 1.8 days, in P_2 it was 1.4 days and in P_3 vase life was 1.0 day. In Golden Fairy, the control flower recorded 2.2 days as vase life. In G_1 the flower had a vase life of 2.0 days and in G_2 it was 1.8 days. In Monnalisa, the control flower recorded a vase life of 2.4 days, M_{n1} recorded 2.2 days, M_{n2} recorded 2.0 days and M_{n3} faded in 1.8 days.

4.2.2 Induced *in vitro* mutagenesis using EMS

The present investigations were carried out to standardize efficient protocol for *in vitro* multiplication of rose genotypes Schloss Elutin, Jogan, Josepha and Morning Sun using EMS under *in vitro* conditions.

4.2.2.1 Standardization of culture medium

a. **Standardization of growth regulators for culture establishment**

In order to standardize the culture establishment medium, MS medium supplemented with different combinations of BA (1.0 and 2.0 mg /l) and ADS (25 and 50 mg/l) were used. Pre-treated and surface sterilized nodal explants were cultured on these media and data was recorded on the following parameters:

1. Percent explant survival

The perusal of data presented in Table 31 indicated that among the media tried for culture establishment, the maximum explant survival (63.80%) was registered with the media comprising of MS+BA (2.0 mg/l)+ADS (25 mg/l) followed by MS+BA (2.0 mg/l)+ ADS (50 mg/l) (60.20%) . MS+BA (1.0 mg/l) + ADS (50 mg/l) gave 52.60% survival, MS+ BA (1.0 mg/l) + ADS (25mg/l) gave 45.80% and MS+ BA (2.0 mg/l) gave 39.60% survival. The medium devoid of hormones (control) gave the minimum explant survival (36.20 %).

2. Percent bud sprouting

Bud sprouting was found to be significantly influenced by the combination of growth regulators (Table 31). The highest bud sprouting was recorded with MS+BA (2.0 mg/l) +ADS (25 mg/l) (75.60%) followed by MS+BA (2.0 mg/l) + ADS (50 mg/l) (62.40%). MS+BA (1.0 mg/l) + ADS (50 mg/l) gave 60.80% sprouting, MS+ BA (1.0 mg/l) +25 ADS (25mg/l) gave 53.60% sprouting and MS+ BA (2.0 mg/l) gave 50.40%. All these treatments were statistically different from other combinations. Minimum sprouting was recorded in control (36.80%).

3. Days to bud sprouting

The days taken for bud sprouting was found to be significantly influenced by the different growth regulator combinations. The perusal of data clearly suggests that the treatments can be ranked as follows with respect to earliness in

Table 31. Effect of growth regulators on *in vitro* culture establishment in Rose genotypes

Sl.no	Treatment (mg/L)	Explant survival (%)	Bud sprouting (%)	Days to bud sprouting
1	MS + Control	36.20	36.80	11.00
2	MS+2BA	39.60	50.40	10.20
3	MS+1BA+25 ADS	45.80	53.60	10.00
4	MS+2BA+25 ADS	63.80	75.60	7.40
5	MS+1BA+50 ADS	52.60	60.80	9.80
6	MS+2BA+50 ADS	60.20	62.40	8.40
	SE(m)	2.81	4.07	0.80
	C.D (5%)	8.19	11.89	2.32
	C.V	12.63	16.09	18.80

Table 32. Effect of growth regulators on *in vitro* shoot proliferation in Rose genotypes

Sl.no	Treatments (mg/L)	No. of shoots proliferated/explant	
		First sub-culture	Second sub-culture
1	MS devoid of hormones (Control)	1.40	2.00
2	MS+2BA	1.80	2.20
3	MS+2BA+0.1IAA+25 ADS	2.00	2.40
4	MS+2BA+0.25IAA+25 ADS	3.20	3.40
5	MS+2BA+0.1IAA+50 ADS	2.10	2.80
6	MS+2BA+0.25IAA+50 ADS	2.20	3.00
	SE(m)	0.37	0.35
	C.D (5%)	1.08	1.01
	C.V	38.75	26.71

sprouting. MS+BA (2.0 mg/l)+ADS (25 mg/l) (7.40 days) followed by MS+BA (2.0 mg/l)+ ADS (50 mg/l) (8.40 days), MS+BA(1.0 mg/l)+ ADS (50 mg/l) (9.80 days), MS+ BA (1.0 mg/l)+25 ADS (10.00 days) and MS+ BA (2.0 mg/l) (10.20 days). The duration required for bud sprouting was the highest in control (11.00 days). Since the treatment MS+BA (2.0 mg/l)+ADS (25 mg/l) gave significantly superior explant survival and bud sprouting along with superior earliness in sprouting, it was selected as the most suitable medium for culture establishment for further studies.

b. Standardization of growth regulators for shoot proliferation

In order to standardize the shoot proliferation medium, the shoots were transferred to multiplication media comprising of basal MS medium supplemented with different concentrations of various growth regulators such as BA (2.0 mg/l), IAA (0.1 and 0.25 mg/l)) and ADS (25 and 50 mg/l) (Table 32). The proliferated shoots were sub-cultured upto two sub-cultures and the prolificacy was adjudged in each medium. Significant response in respect of shoot proliferation and growth of cultures was observed and the following observations were recorded.

Number of shoots sprouted per explant in first and second sub-cultures

The number of shoots proliferated per explant was shown to be affected by the growth regulator combinations in the first subculture and is presented in (Table 32). From the data it is clear that the treatment of MS+BA (2.0 mg/l), +IAA (0.25 mg/l)+ADS (25 mg/l) gave the maximum number of shoots sprouted per explant (3.20) followed by the treatments MS+ BA (2.0 mg/l), +IAA (0.25 mg/l)+ADS (50 mg/l) (2.20), MS+BA(2.0 mg/l)+IAA (0.1 mg/l)+ADS (50 mg/l) (2.10), MS+BA (2.0 mg/l) +IAA (0.1 mg/l)+ADS (25 mg/l) (2.00 days) and MS+BA(2.0 mg/l) (1.80 days) as recorded after first subculture. Minimum number (1.40) of shoots sprouted in hormone free medium.

Table 33 . Effect of growth regulators on *in vitro* rooting in Rose genotypes

Sl.no	Treatment (mg/L)	Days to root initiation	Rooting (%)	Root Length (cm)	No. of roots per shoot
1	1/2M.S+0.2IBA+2% Sucrose	25.00	55.00	4.33	3.67
2	1/2 M.S+1IBA+3% Sucrose+50 ml coconut water	22.33	62.33	4.67	4.00
3	1/2 M.S+0.2 IBA+2% Sucrose+2BA	16.33	71.67	6.00	6.00
4	1/2 M.S+0.2 IBA+2 % Sucrose+0.2NAA +2BA	20.00	66.33	5.67	5.00
5	MS devoid of hormones(Control)	31.00	40.33	3.00	2.00
	SE(m)	0.71	2.17	0.26	0.15
	C.D (5%)	2.25	6.82	0.81	0.47
	C.V	5.40	6.34	9.45	6.25

The number of shoots proliferated per explant was shown to be affected by the growth regulator combinations in the second subculture and is presented in (Table 32). From the data it is clear that the treatment of MS+BA (2.0 mg/l) +IAA (0.25 mg/l)+ADS (25 mg/l) gave the maximum number of shoots sprouted per explant (3.40 days) followed by the treatments MS+ BA (2.0 mg/l), +IAA (0.25 mg/l)+ADS (50 mg/l) (3.00 days), MS+BA(2.0 mg/l)+IAA (0.1 mg/l)+ADS (50 mg/l) (2.80 days), MS+BA (2.0 mg/l), +IAA (0.1 mg/l)+ADS (25 mg/l) (2.40 days) and MS+BA(2.0 mg/l) (2.20 days) as recorded after second subculture. The minimum (2.00 days) number of shoots sprouted in hormone free medium.

The treatment combination MS+BA (2.0 mg/l)+IAA (0.25 mg/l)+ADS (25 mg/l) was significantly superior to other treatments in terms of number of shoots proliferated during first and second sub cultures and hence employed for further experiments.

c. Standardization of auxins for root induction

In order to standardize the root inducing medium, the effect of different concentrations of sucrose (2% and 3%), coconut water (50 ml/l), BA(2.0 mg/l), IBA (1.00 mg/l) and 0.25 mg/l), NAA (0.2 mg/l) and activated charcoal (5%) in combination supplemented to half MS was studied and the data was recorded with the following observations:

1. Days to root initiation

It is evident from the data given in Table.33 that shoots transferred on to half MS medium supplemented with different doses of auxins (NAA and IBA) individually or in combinations showed earlier root initiation as compared to full strength MS medium supplemented with different doses of auxins individually or in combinations. For successful rooting, four different concentrations of auxins were tested and were found to be statistically different. Out of the different treatments tried, the earliest root initiation (16.33 days) was registered with the treatment ½ M.S+IBA (0.2 mg/l) +sucrose (2%) + BA (2.0 mg/l) followed by ½

M.S+IBA(0.2 mg/l)+ Sucrose (2%) + NAA (0.2 mg/l)+ BA (2.0 mg/l) (20.00 days), ½ M.S+ IBA(1.0 mg/l)+Sucrose(3%) + coconut water(50 ml) (22.33 days) and ½ M.S+ IBA(0.2 mg/l)+2% Sucrose (25.00 days).The maximum number of days (31.00 days) taken for root initiation was recorded with control *i.e.* MS medium devoid of auxins.

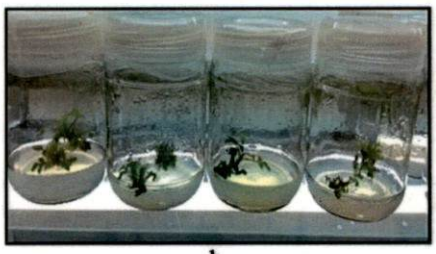
2. Per cent rooting

Data presented in (Table 33) revealed that rooting percentage was significantly influenced by the different treatment combinations. The highest percentage of rooting (71.67%) was observed when the micro-shoots were transferred to half-strength MS medium supplemented with IBA(0.2 mg/l) +sucrose(2%)+BA(2.0 mg/l) which was significantly superior over other treatments. The other effective treatments were half-strength MS medium supplemented with ½ M.S+IBA(0.2 mg/l)+sucrose(2%)+NAA(0.2 mg/l)+ BA (2.0 mg/l) (66.33%), ½ M.S+ IBA(1.0 mg/l)+sucrose(3%) +coconut water(50 ml) (62.33 %), ½ M.S+IBA(0.2 mg/l)+2% sucrose (55.00 %). Hormone–free full strength MS medium (control) gave only 40.33% rooting.

3. Root length (cm)

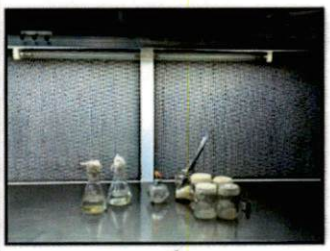
Root length was observed to be significantly influenced by the different treatments (Table 33). As is evident from the data, the highest root length (6.00 cm) was reported with half-strength MS medium supplemented with IBA (0.2 mg/l) +sucrose (2%) + BA (2.0 mg/l) which was significantly superior over other treatments. The other effective treatments were half-strength MS medium supplemented with IBA(0.2 mg/l)+ sucrose (2%) + NAA (0.2 mg/l)+ BA (2.0 mg/l) (5.67 cm), IBA(1.0 mg/l)+sucrose(3%) + coconut water (50 ml) (4.67 cm) and IBA(0.2 mg/l)+2% sucrose (4.33 cm). All the treatments resulted in significant increase over the control. The lowest root length was recorded in full strength MS medium devoid of auxin (3.00 cm).

a.



b.

c.



d.

e.

f.

g.

h.

i.

j.

k.

l.

m.

a. Inoculation of explant
b. Subculturing
c. Preparation of *invitro* shoot for EMS treatment in LAF
d. Introduction of prepared shoot into EMS treatment in LAF
e. EMS treatment on gyratory shaker
f. Inoculation of EMS treated shoots
g. Subculturing
h. Invitro rooting
i. Deflasking and Bavistin treatment
j. Exvitro establishment in Humidity chamber
k. Transferring to the Field Establishment in pots
l. Plant in vegetative stage
m. Treated plant with flower

Plate 13. Steps in EMS treatment

4. Number of roots per shoot

The effect of different basal media in combination with auxin treatments was observed to be significant in respect to number of roots per shoot (Table 33). The maximum number of roots per shoot (6.00) was recorded on half-strength MS medium supplemented with IBA (0.2 mg/l) +sucrose (2%) + BA (2.0 mg/l) followed by half-strength MS medium supplemented with IBA(0.2 mg/l)+ sucrose (2%)+ NAA (0.2 mg/l)+ BA (2.0 mg/l) (5.00), IBA(1.0 mg/l)+sucrose(3%)+ coconut water(50 ml) (4.00) and IBA(0.2 mg/l)+2% sucrose (3.67). The minimum number of roots (2.00) was recorded in half strength MS medium devoid of growth regulators.

The treatment combination half M.S+ IBA (0.2 mg/l) +sucrose (2%) + BA (2.0 mg/l) was found to be significantly superior to control and all the other treatments and hence was chosen for further experiments.

4.2.2.2 In vitro mutagenesis

For induction of mutations under *in vitro* conditions, all the four genotypes *i.e.*, Schloss Elutin, Jogan, Josepha and Morning Sun were subjected to EMS treatment. The details of experiments are as follows:

EMS treatment of *in vitro* established cultures

In this experiment, the nodal explants of rose genotypes Schloss Elutin, Jogan, Josepha and Morning Sun were proliferated under aseptic conditions in the selected medium. The proliferated shoots were made into small segments and were subjected to treatment with different doses of EMS. After mutagenic treatment, the shoots were cultured on the selected *in vitro* root induction medium which was followed by transfer of rooted plantlets to pots, their hardening and field transfer. The following observations were recorded for the genotypes, Schloss Elutin, Jogan, Josepha and Morning Sun under *in vitro* conditions.

Table 34. Effect of EMS treatment on Meristem regeneration (%) and Survival at 30 days (%) in Schloss Elutin genotype

		Meristem regeneration (%)			Survival at 30 days (%)		
Sl.no	Dose of EMS (%)	30 minutes treatment	60 minutes treatment	90 minutes treatment	30 minutes treatment	60 minutes treatment	90 minutes treatment
1	0.2	93.33	80.00	73.33	86.67	66.67	60.00
2	0.3	86.67	73.33	66.67	80.00	60.00	53.33
3	0.4	80.00	66.67	60.00	73.33	60.00	53.33
4	0.5	66.67	60.00	53.33	60.00	46.67	46.67
5	0.6	60.00	60.00	46.67	53.33	46.67	33.33
6	0.7	60.00	53.33	46.67	53.33	40.00	33.33
7	0.8	53.33	46.67	33.33	46.67	33.33	20.00
8	0.9	46.67	26.67	26.67	33.33	20.00	13.33
9	1	26.67	20.00	13.33	13.33	6.67	6.67
10	1.1	20.00	13.33	6.67	6.67	0.00	0.00
11	1.2	13.33	6.67	0.00	0.00	0.00	0.00
12	Control	93.33	93.33	93.33	93.33	93.33	93.33

Table 35. Effect of EMS treatment on Meristem regeneration (%) and Survival at 30 days (%) in in Jogan genotype

		Meristem regeneration (%)			Survival at 30 days (%)		
Sl.no	Dose of EMS (%)	30 minutes treatment	60 minutes treatment	90 minutes treatment	30 minutes treatment	60 minutes treatment	90 minutes treatment
1	0.2	86.67	80.00	73.33	93.33	86.67	60.00
2	0.3	86.67	80.00	66.67	86.67	73.33	53.33
3	0.4	80.00	73.33	60.00	80.00	66.67	53.33
4	0.5	80.00	73.33	60.00	73.33	66.67	46.67
5	0.6	73.33	66.67	53.33	73.33	60.00	46.67
6	0.7	73.33	66.67	46.67	66.67	60.00	40.00
7	0.8	66.67	60.00	46.67	66.67	53.33	40.00
8	0.9	66.67	60.00	40.00	60.00	53.33	33.33
9	1	60.00	53.33	40.00	53.33	46.67	33.33
10	1.1	60.00	46.67	33.33	53.33	40.00	26.67
11	1.2	53.33	40.00	26.67	40.00	33.33	20.00
12	Control	93.33	93.33	93.33	80.00	80.00	80.00

Table 36. Effect of EMS treatment on Meristem regeneration (%) and Survival at 30 days (%) in Josepha genotype

		Meristem regeneration (%)			Survival at 30 days (%)		
Sl.no	Dose of EMS (%)	30 minutes treatment	60 minutes treatment	90 minutes treatment	30 minutes treatment	60 minutes treatment	90 minutes treatment
1	0.2	86.67	80.00	73.33	86.67	80.00	60.00
2	0.3	80.00	80.00	66.67	80.00	66.67	53.33
3	0.4	80.00	73.33	60.00	73.33	66.67	53.33
4	0.5	73.33	66.67	60.00	66.67	60.00	46.67
5	0.6	73.33	66.67	53.33	66.67	60.00	46.67
6	0.7	66.67	60.00	53.33	66.67	53.33	40.00
7	0.8	66.67	60.00	46.67	60.00	46.67	40.00
8	0.9	60.00	53.33	46.67	53.33	46.67	40.00
9	1	53.33	46.67	40.00	46.67	40.00	33.33
10	1.1	46.67	40.00	33.33	33.33	33.33	26.67
11	1.2	40.00	33.33	26.67	26.67	20.00	13.33
12	Control	93.33	93.33	93.33	93.33	93.33	93.33

Table 37. Effect of EMS treatment on Meristem regeneration (%) and Survival at 30 days (%) in Morning Sun genotype

		Meristem regeneration (%)			Survival at 30 days (%)		
Sl.no	Dose of EMS (%)	30 minutes treatment	60 minutes treatment	90 minutes treatment	30 minutes treatment	60 minutes treatment	90 minutes treatment
1	0.2	86.67	73.33	66.67	80.00	60.00	53.33
2	0.3	80.00	66.67	60.00	73.33	53.33	53.33
3	0.4	80.00	66.67	53.33	73.33	46.67	46.67
4	0.5	73.33	60.00	46.67	66.67	46.67	40.00
5	0.6	66.67	53.33	46.67	66.67	40.00	33.33
6	0.7	66.67	53.33	40.00	60.00	40.00	33.33
7	0.8	60.00	46.67	33.33	60.00	33.33	26.67
8	0.9	60.00	33.33	26.67	46.67	26.67	13.33
9	1	53.33	26.67	13.33	40.00	20.00	6.67
10	1.1	46.67	13.33	6.67	33.33	0.00	0.00
11	1.2	33.33	6.67	0.00	20.00	0.00	0.00
12	Control	93.33	93.33	93.33	86.67	86.67	86.67

Table 38. Fixation of doses of EMS based on LD_{50} value for meristem regeneration (%) and survival (%) in Rose genotypes for 30 minutes

	Doses fixed (%)				
Name of genotype	LD_{50} stat.value	(1)	(2)	(3)	(4)
Schloss Elutin	0.58	0.5	0.6	0.7	0.8
Jogan	0.85	0.8	0.9	1.0	1.1
Josepha	0.77	0.7	0.8	0.9	1.0
Morning Sun	0.85	0.8	0.9	1.0	1.1

4.2.2.3 Fixing of LD_{50} value

For fixing LD_{50} value, the proliferated shoots were cut into small segments and exposed to EMS at (0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9,1.0,1.1,1.2 % and control). The analysis of LD_{50} dose for individual genotypes was carried out separately and is presented in Tables 34, 35, 36 and 37. The data indicated that explant survival decreased linearly with increase in EMS doses. The highest explant survival was noted in control in all the genotypes which was 93.33% in Schloss Elutin, and 80.00% in Jogan and 93.33% in Josepha and 86.67% in Morning Sun.

1. Meristem regeneration (%)

With increasing dose of EMS concentrations, there was a marked reduction in meristem regeneration. In Schloss Elutin the non-irradiated explants (control) gave highest meristem regeneration (93.33%) in all the three durations *viz.,* 30, 60 and 90 minutes. Among the 11 treatments, highest meristem regeneration was recorded in 0.2% in 30^1 (93.33%). It was 80.00% in 60^1 and 73.33% in 90^1. The minimum was recorded in 1.2% with a meristem regeneration rate of 13.33% at 30^1, (6.67%) in 60^1 and no regeneration at 90^1. 1.1% EMS recorded second lowest (%) value for meristem regeneration *i.e.,* 20.00% at 30^1, 13.33% in 60^1 and 6.67% at 90^1 . In Jogan, the non-irradiated explants (control) gave highest meristem regeneration (93.33%) in all the three durations (30, 60 and 90 minutes. Among the 11 treatments, 0.2% and 0.3% in 30^1 (86.67%) recorded the highest meristem regeneration. The minimum regeneration was recorded in 1.2% with a meristem regeneration rate of 53.33% at 30^1, 40.00% in 60^1 and 26.67 % at 90^1 .

In Josepha, the non-irradiated explants (control) gave highest meristem regeneration (93.33%) in all the three durations (30, 60 and 90 minutes. Among the 11 treatments, 0.2% in 30^1 recorded the highest meristem regeneration (86.67%). The minimum meristem regeneration was recorded in 1.2% with a rate of 40.00% at 30^1, 33.33% in 60^1, 26.67 % at 90^1. In Morning Sun, the non-irradiated explants (control) gave highest meristem regeneration (93.33%) in all

the three durations (30, 60 and 90 minutes. Among the 11 treatments, 0.2% in 30^1 (86.67%) recorded the highest meristem regeneration. The minimum was recorded in 1.2% with a meristem regeneration rate of 33.33% at 30^1, 6.67% in 60^1 and no regeneration at 90^1.

2. Survival at 30 days

It is clear from the data that there was reduction in explant survival with increasing EMS doses. In Schloss Elutin the non-irradiated explants (control) gave the highest survival of (93.33%) in all the three durations (30, 60 and 90 minutes). Among the 11 treatments, 0.2% in 30^1 (86.67%) recorded highest survival. At 1.2% none of the plants survived in all the three durations of 30, 60 and 90 minutes. In Jogan, the non-irradiated explants (control) gave highest survival (80.00%) in all the three durations (30, 60 and 90 minutes. Among the 11 treatments, 0.2% in 30^1 (93.33%) recorded highest survival of 86.67% in 60^1. The minimum was recorded in 1.2% with a survival rate of 40.00% at 30^1, 33.33% in 60^1, 20.00% at 90^1.

In Josepha, the non-irradiated explants (control) gave highest survival (93.33%) in all the three duration (30, 60 and 90 minutes). Among the 11 treatments, 0.2% in 30^1 (86.67%) recorded the highest survival. The minimum was recorded in 1.2% with a survival rate of 26.67% at 30^1, 20.00% in 60^1, 13.33% at 90^1. In Morning Sun, the non-irradiated explants (control) gave highest survival (86.67%) in all the three durations (30, 60 and 90 minutes). Among the 11 treatments, 0.2% in 30^1 recorded the highest survival (80.00%). The minimum was recorded in 1.2% with a survival rate of 20.00 % at 30^1 and no survival at 60^1 and 90^1.

Based on LD_{50} value, five doses including control were selected for each genotype. For Schloss Elutin the doses were 0.5, 0.6, 0.7% and 0.8%. For Josepha the doses were 0.7, 0.8, 0.9 and 1.0%. In Jogan, 0.8, 0.9, 1.0 and 1.1% were selected. In Morning Sun the doses were 0.8, 0.9, 1.0 and 1.1%. Duration of 30 minutes was considered for the rest of the experiments. Since higher durations

Table 39. Field evaluation of EMS treated M_1 generation for the size of first flower

Dose of EMS (%)	Schloss Elutin	Dose of EMS (%))	Josepha	Dose of EMS (%)	Jogan	Morning Sun
0.5	6.54	0.7	6.53	0.8	7.47	7.48
0.6	6.12	0.8	6.12	0.9	6.80	6.13
0.7	5.28	0.9	5.87	1.0	5.80	5.50
0.8	4.24	1.0	5.41	1.1	5.63	4.51
Control(0)	6.84		7.41		8.40	8.12
SE (m)	1.95		1.68		0.34	0.95
C.D (5%)	5.01		4.85		4.01	2.01
C.V	8.17		8.43		10.17	6.74

resulted in mortality. The analysis indicated the extrapolated LD_{50} value based on survival (%) for the various rose genotypes. For Schloss Elutin it was 0.58%, for Jogan it was 0.85%, for Josepha it was 0.77% and for Morning Sun it was 0.85% (Table 38).

4.2.2.5 Effect of EMS on Morphological Characters

(a). Direct effect of EMS treatment on M_1

Field evaluation of EMS treated M_1 generation was done on four genotypes at five doses including control.

1. Size of first flower

The data pertaining to the field evaluation of EMS treatment on the size of first flower is presented in Table 39. It is evident from the data that differences for this character were recorded in all the genotypes studied.

In general, un-treated plants had significantly larger first flowers. In, Schloss Elutin first flower was 6.84cm in control. In the treatments 0.5, 0.6 and 0.7%, first flowers were on par with each other being 6.54, 6.12 and 5.28 cm respectively, whereas 0.8% showed a significant reduction to 4.24 cm when compared with control. In Josepha, there was no significant difference between the treated and untreated first flowers. The control showed a flower size of 7.41cm whereas the 0.7, 0.8, 0.9 and 1.0% doses of EMS recorded the sizes of 6.53, 6.12, 5.87 and 5.41 cm respectively.

In Jogan, the untreated flowers showed a size of 8.40cm whereas the lowest flower size of 5.63cm was recorded in 1.1% followed by 0.8, 0.9 and 1.0 % with flower sizes of 7.47, 6.80 and 5.80 cm respectively. In Morning Sun, the control flower recorded a size of 8.12 cm. The EMS treatment of 0.8 % was on par with control, recording 7.48cm. The treatments 0.9, 1.0 and 1.1 % recorded 6.13, 5.50 and 4.51 cm respectively as the size of the first flower.

Table 40. Mean performance for morphological characters of mutants developed after EMS treatment in Schloss Elutin

Dose of EMS (%)	Number of leaves at first flower	Number of days to first flower	Prickle density/ 5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
0.5	36.60	60.20	8.20	7.93	6.12	7.70	29.80	4.83	7.20
0.6	34.40	62.00	7.40	7.36	5.98	7.50	28.40	4.30	6.60
0.7	32.40	64.80	6.60	7.15	5.15	6.54	28.00	3.28	6.00
0.8	30.40	66.00	6.35	6.81	4.84	6.12	27.40	3.14	4.80
Control	38.60	40.20	9.15	8.12	7.28	8.36	30.40	5.01	7.80
SE(m)	3.35	0.99	0.59	0.12	0.28	0.27	1.37	0.74	0.25
C.D (5%)	1.85	2.92	1.63	1.08	1.01	0.81	4.07	2.48	1.15
C.V	16.46	11.64	10.41	10.11	11.44	12.34	10.15	20.45	23.10

Table 41. Mean performance for morphological characters of mutants developed after EMS treatment in Josepha

Dose of EMS (%)	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
0.7	35.00	58.40	6.60	7.71	4.80	4.32	38.00	4.71	6.10
0.8	32.20	54.40	6.00	7.65	4.08	3.92	36.60	3.06	6.00
0.9	31.40	45.40	5.40	6.87	3.63	3.64	31.60	3.00	5.40
1.0	30.00	44.60	4.40	6.54	3.32	2.46	25.40	2.64	4.80
Control	41.60	61.60	7.20	8.41	5.41	4.58	40.80	4.98	6.30
SE(m)	5.30	1.85	0.64	0.17	0.10	0.11	1.05	0.42	0.83
C.D (5%)	16.58	3.50	1.48	1.44	0.33	0.32	4.05	1.25	1.31
C.V	14.01	21.11	13.01	9.49	4.14	7.89	5.29	20.41	34.54

Table 42. Mean performance for morphological characters of mutants developed after EMS treatment in Jogan

Dose of EMS (%)	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
0.8	32.33	62.33	6.50	6.47	5.07	10.10	22.67	4.20	6.69
0.9	30.67	62.67	5.47	5.80	4.43	9.33	21.33	3.23	6.30
1.0	27.67	66.33	5.03	5.60	3.90	8.83	20.00	2.60	4.11
1.1	24.00	70.00	4.20	4.63	3.23	7.60	19.00	2.23	3.81
Control	36.33	57.33	7.26	7.60	5.17	10.66	25.67	5.40	8.22
SE(m)	0.83	1.07	0.16	0.15	0.20	0.28	0.86	0.14	0.16
C.D (5%)	2.62	3.39	0.51	0.49	0.63	0.88	2.70	0.44	0.51
C.V	4.76	2.92	4.89	4.44	7.94	5.30	6.82	6.78	4.42

Table 43. Mean performance for morphological characters of mutants developed after EMS treatment in Morning Sun

Dose of EMS (%)	Number of leaves at first flower	Number of days to first flower	Prickle density/5 cm	Flower size (cm)	Flower weight (g)	Pedicel length (cm)	Number of petals per flower	Size of petals (cm)	Number of flowers per plant/ bunch
0.8	32.33	44.00	6.80	5.40	3.23	10.11	18.00	4.23	6.00
0.9	31.00	46.00	6.22	4.83	3.10	9.33	16.00	3.32	5.49
1.0	29.00	47.67	6.07	4.50	2.70	8.11	13.00	3.11	4.29
1.1	28.00	54.00	5.77	4.37	2.27	7.62	12.33	2.89	3.21
Control	38.00	36.33	7.60	5.97	3.80	11.32	21.00	5.31	6.99
SE(m)	0.65	1.33	0.13	0.14	0.12	0.34	0.79	0.13	0.16
C.D (5%)	2.05	4.20	0.41	0.45	0.38	1.09	2.49	0.40	0.51
C.V	3.55	5.06	3.44	4.89	6.89	6.41	8.50	5.81	16.18

(b) Field evaluation of EMS treated M_1 generation

The observations on the morphological characters of the four varieties of EMS treated M_1 generation is presented below. (Tables 40, 41, 42 and 43)

1. Number of leaves at first flower

The data pertaining to this attribute has been presented and an appraisal of the data elucidates that EMS treatment had a significant effect on number of leaves at first flower in all the genotypes of rose.

In Schloss Elutin, among the different EMS treatments, maximum number of leaves at first flower was 38.60, recorded in control, which was found to be significantly different from all other treatments, while the minimum number of leaves (30.40) was with 0.8% followed by 0.7 % (32.40), 0.6 % (34.40) and 0.5 % (36.60).

Data in Josepha reveal that among the different EMS treatments, maximum number of leaves at first flower (41.60) was recorded in control which was found to be significantly different from all other treatments. The doses 0.7, 0.8 and 0.9 % recorded 35.00, 32.20 and 31.40 leaves whereas the minimum number of leaves at first flower 30.00 days was recorded with 1.0 %.

In Jogan, among the different EMS treatments maximum number of leaves at first flower (36.33) was recorded in control which was found to be significantly different from all other treatments. The treatment 0.8% recorded 32.33 leaves, followed by 0.9% (30.67), whereas lower (27.67 and 24.00) values were recorded with 1.0% and 1.1% treatment. In Morning Sun, among the different EMS treatments, 38.00 leaves at first flower was recorded in control. In the treatments 0.8, 0.9 and 1.0% 32.33, 31.00 and 29.00 leaves were recorded. The minimum number of leaves at first flower (28.00) was recorded with 1.1% treatment.

2. Number of days to first flower

The data pertaining to this attribute shows that EMS treatment significantly delayed flowering in all the genotypes of rose studied.

In Schloss Elutin, among the different EMS treatments, minimum days taken to first flower was 40.20 days and was recorded in control which was found to be significantly different from all other treatments. In the other genotypes it was 60.20 days in 0.5 % followed by 0.6, 0.7 and 0.8% being 62.00, 64.80 and 66.00 days respectively. Data in Josepha reveal that among the different EMS treatments, earliest flowering (61.60 days) was recorded with control which was found to be significantly different from all other treatments. The maximum number of days to first flower (58.40 days) was recorded with 0.7 %, whereas 0.8, 0.9 and 1.0 % took 54.40, 45.40 and 44.60 days respectively to first flower.

In Jogan, among the different EMS treatments, minimum days taken to first bloom (57.33days) was recorded with control which was found to be different from all other treatments. The maximum (70.00days) was recorded with 1.1 % treatment whereas the doses 0.8, 0.9 and 1.0 % recorded 62.33, 62.67 and 66.33 days respectively. Earliest flowering in Morning Sun, (36.33days) was recorded in control. The minimum period to first bloom among the different EMS treatments was recorded in 0.8% (44.00 days), followed by 46.00 days in 0.9%, 47.67 days in 1.0 % and in 1.1% it took 54.00 days to flower.

3. Prickle density (per five cm)

The perusal of the data on prickle density indicates that there was reduction in prickle density in all the genotypes of rose over control with increase in EMS concentrations.

In Schloss Elutin, it is evident from the data that the maximum prickle density (9.15/5cm) was recorded in control and was significantly different from all the other doses of EMS. In 0.5, 0.6 and 0.7 % prickle densities of 8.20/5cm, 7.40/5cm

and 6.60/5cm were observed. The lowest prickle density of 6.35/5cm was with 0.8%. Data in Josepha reveal that among the different EMS treatments, the highest prickle density of 7.20/5cm was recorded in control. 0.7% recorded 6.60/5cm, 0.8% recorded 6.00/5cm and 0.9% recorded 5.40/5cm but the lowest prickle density (4.40/5cm) was recorded with 1.0%.

In Jogan, among the different EMS treatments, maximum prickle density of 7.26/5cm was recorded in control. 0.8, 0.9 and 1.0 % doses recorded prickle densities of 6.50/5cm, 5.47/5cm, 5.03/5cm respectively. The lowest prickle density (4.20/5cm) was recorded with 1.1%. The prickle density in Morning Sun, was the highest in control (7.60/5cm) followed by 6.80/5cm, 6.22/5cm, 6.07/5cm and 5.77/5cm in the 0.8 , 0.9, 1.0 and 1.1% doses respectively. There was a significant difference between control and all other treatments.

4. Flower size (cm)

The data pertaining to the field evaluation of EMS treatment on the flower size are presented in Tables 40 to 43. It is evident from the data that differences for this character were recorded in all the genotypes studied.

The data revealed that irrespective of genotypes, un-irradiated plants had significantly larger flower size in Schloss Elutin (8.12 cm). The treatments 0.5%, 0.6% and 0.7% were on par with each showing flower sizes of 7.93, 7.36 and 7.15 cm respectively whereas 0.8 % showed significant difference (6.81 cm) when compared with control. In Josepha, there was a difference between the treated and untreated ones for flower size. The control showed a flower size of 8.41 cm whereas the 0.7, 0.8, 0.9 and 1.0 % recorded the sizes of 7.71, 7.65, 6.87 and 6.54 cm respectively.

In Jogan, the untreated flower showed a size of 7.60 cm whereas the second highest flower size was recorded in 0.8% (6.47cm) followed by 0.9, 1.0 and 1.1 % with flower sizes of 5.80, 5.60 and 4.63 cm respectively. In Morning Sun, the control flower recorded a size of 5.97 cm whereas the EMS treatment at

0.8 % recorded 5.40 cm. The treatments 0.9, 1.0 and 1.1% recorded 4.83, 4.50 and 4.37 cm flower sizes respectively.

5. Flower weight (g)

The data pertaining to the field evaluation of EMS treatment on the flower weight are presented in Tables 40 to 43.

Un-irradiated plants of Schloss Elutin had significantly heavier flowers of weight 7.28 g in control, whereas all the treatments 0.5,0.6,0.7 and 0.8 % were on par with each other with weights of 6.12, 5.98, 5.15 and 4.84 g respectively. In Josepha, there was a significant difference between the treated and untreated ones for flower weight. The control showed a flower weight of 5.41 g whereas the 0.7, 0.8, 0.9 and 1.0 % recorded the weights of 4.80, 4.08, 3.63 and 3.32 g respectively.

In Jogan, the untreated flower showed a weight of 5.17g whereas the other treatments were on par with each other. Flower weight recorded in 0.8 was 5.07g followed by 0.9, 1.0 and 1.1 % with flower weights of 4.43, 3.90 and 3.23g respectively. In Morning Sun, the control flower recorded the highest weight of 3.80g whereas EMS treatment at 1.1 %recorded the lowest weight of 2.27g. The treatments 0.8, 0.9 and 1.0 % recorded 3.23, 3.10 and 2.70 g respectively as flower weights.

6. Pedicel length (cm)

A critical rummage of the data on pedicel length indicates that treatment of the roses with different doses of EMS reduced the length of the pedicel with increase in dose.

In Schloss Elutin the highest pedicel length (8.36 cm) was observed in control. The lowest pedicel length was observed in 0.8 % (6.12 cm), which was on par with 0.5 and 0.6 with lengths of 7.70 and 7.50 cm. Data in Josepha reveal that the highest pedicel length (4.58 cm) was observed in control. Among the treatments

0.7 % recorded the highest pedicel length of 4.32 cm followed by 0.8, 0.9 and 1.0 % which recorded the lengths of 3.92, 3.64 and 2.46 cm respectively.

In Jogan, the highest pedicel length (10.66 cm) was observed in control and the lowest pedicel length was observed in 1.1 % (7.60 cm) which was on par with the treatments 1.0 % and 0.9 % which recorded 8.83and 9.33 cm respectively. The EMS dose of 0.8% recorded 10.10 cm pedicel length. In Morning Sun, the highest pedicel length (11.32 cm) was observed in control. All the other treatments were statistically on par with each other. The lowest pedicel length was observed in 1.1% (7.62cm) which was on par with 0.8, 0.9 and 1.0 % treatments which recorded pedicel lengths of 10.11, 9.33and 8.11cm respectively.

7. Number of petals per flower

The data presented in Tables 40 to 43 revealed that there were significant effects of EMS treatment doses and genotypes on number of petals per flower.

The treatment of plants with EMS, irrespective of genotypes caused significant reduction in number of petals per flower. In case of Schloss Elutin, maximum number of petals per flower (30.40) was recorded in control. In the treatments maximum number of petals per flower (29.80) was recorded in 0.5 % followed by 0.6, 0.7 and 0.8 % being 28.40, 28.00 and 27.40 respectively. In Josepha, the control exhibited the highest number of petals (40.80) whereas the rest of the treatments were on par with each other. The treatment dose 0.7 % recorded 38.00 petals followed by 0.8, 0.9 and 1.0 % recording 36.60, 31.60 and 25.40 cm respectively.

In Jogan, the maximum number of petals (25.67) was observed in control, and the minimum number of petals was observed in 1.1 % (19.00) which was on par with the treatments 1.0 % (20.00) and 0.9 % (21.33). The dose 0.8 %recorded 22.67 petals per flower. In Morning Sun, the highest number of petals (21.00) were recorded in control and the lowest number of petals were recorded in 1.1% (12.33) . In the other treatments *viz*., 0.8, 0.9 and 1.0 % 18.00, 16.00 and 13.00 petals respectively were recorded.

8. Size of petals (cm)

The data related to petal size have been compiled in Tables 40 to 43. The data reveals that the effect of EMS on petal area reduction was significant in all the genotypes. In Schloss Elutin, maximum size of petals 5.01 cm was recorded in control. In the different EMS doses maximum size of petals of 4.83 was recorded in 0.5 % followed by 0.6, 0.7 and 0.8 % 4.30, 3.28 and 3.14 cm respectively.

In Josepha, the control exhibited the highest size of petals with (4.98 cm) whereas the rest of the treatments were on par with each other. In Jogan, the maximum size of petals (5.40) was observed in control, and the minimum size of petals was observed in 0.8% (4.20) followed by 0.9, 1.0 and 1.1 % recording 3.23, 2.60 and 2.23 respectively as the size of petals. In Morning Sun, the maximum size of petals (5.31) was recorded in control and minimum size of petals was recorded in 0.8% (4.23).The remaining treatments *viz.*, 0.9, 1.0 and 1.1 % recorded sizes of 3.32, 3.11 and 2.89 respectively.

9. Number of flowers per plant /bunch

The data pertaining to the field evaluation of EMS treatment on the number of flowers per plant are presented in Tables 40 to 43. It is evident from the data that significant differences for this character were recorded in all the genotypes studied.

In Schloss Elutin, control had the highest number of flowers, being 7.80. The different treatments *viz.*, 0.5, 0.6, 0.7 and 0.8 % produced 7.20, 6.60, 6.00 and 4.80 flowers respectively. In Josepha, there was a significant difference between the treated and untreated plants for this character. The control showed the highest number of flowers of 6.30 whereas the treatments 0.7, 0.8, 0.9 and 1.0 % recorded numbers of 6.10, 6.00, 5.40 and 4.80 respectively.

In Jogan, the untreated plants produced the maximum number of flowers (8.22) whereas the other treatments recorded 6.69, 6.30, 4.11 and 3.81 in 0.8, 0.9,

1.0 and1.1 % respectively. In Morning Sun, the control produced the highest number of flowers of 6.99 whereas the chemical mutagen treatment of 0.8 %recorded 6.00 flowers. The treatments *viz.*, 0.9, 1.0 and 1.1 % recorded 5.49, 4.29 and 3.21 respectively for number of flowers per plant.

10. Fragrance

In the genotypes Schloss Elutin and Jogan control and radiated genotypes recorded a medium fragrance. In Josepha and Morning Sun, low fragrance was recorded. It has been presented in Table 47.

11. Flower colour, form, size and shape

The control flower of Schloss Elutin (Table 45 and Plate 14) flower was dark cream in colour with a thick arrangement of petals. Flower was large and showy in appearance. The petals were medium in size. The mutant flower of Schloss Elutin (S_1- 0.5%) was large Hybrid Tea type flower of good shape and borne singly. It was creamy white with pink shading at the tips of outer petals. Buds open out into large flowers of remarkable beauty. The petals were large and the flower was circular in shape.

The control flower of Josepha (Table 45 and Plate 15) was pink with white center. In J_1 (0.8%) EMS the colour of the flower was with creamy white center and deep pink outer petals. The number of petals had decreased drastically and it was normal in shape. The well formed flower of J_2 (0.9%) had creamy white center and deep pink outer petals where the size of petals had increased. In J_3 (1.0%) the colour of the flower was creamy white with pointed tips.

The control flower of Jogan (Table 45) is creamy white with light yellow center. Its high centered blooms open out profusely and keep their shape for several days. In J_{g1}(0.7%) the colour is creamy white, with a triangular appearance. In Jogan J_{g2} (0.8%) the colour is creamy white with light yellow center and is well formed.

Table 45. Variations in floral characters in M_1 generation-EMS treatment

Variety and mutants derived	Variations in colour	Variations in form	Variations in size		Variations in shape	
			Flower	Petals	Flower	Petals
Schloss Elutin	Dark creamy	Well formed	Large	Medium	Circular	Normal
S_1- 0.5%	Creamy white with pink shading at the tips of outer petals	Well formed	Large	Large	Circular	Normal
Josepha-Control	Pink with white in the center	Well formed	Medium	Large	Circular	Normal
J_1- 0.8%	Creamy white center, deep pink outer petals	Well formed	Medium	Number has reduced drastically	Circular	Normal
J_2- 0.9%	Creamy white center, deep pink outer petals	Well formed	Medium	Size of petals increased	Circular	Normal
J_3- 1.0%	Creamy white center and deep pink outer petals	Well formed	Medium	Petals have pointed tips	Circular	Normal
Jogan- control	Creamy white with light yellow center	Well formed	Medium	Large	Circular	Normal
Jo_1- 0.7%	Creamy white	Triangular appearance	Medium	Large	Circular	Normal
Jo_2- 0.8%	Creamy white, light center yellow	Well formed	Medium	Large	Circular	Normal
Morning Sun-control	Red colour with prominent white streaks	Well formed	Medium	Large	Circular	Normal
Ms_1-0.8%	Red colour with light shades of white streaks	Well formed	Medium	Large	Circular	Normal

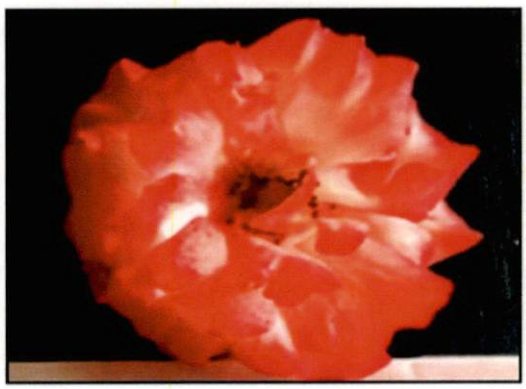
Control

S_1-0.5%

Plate 14. Mutants in floral characters- Schloss Elutin after EMS treatment

Control

J_1- 0.8%



J_2- 0.9%

J_3- 1.0%

Plate 15. Mutants in floral characters- Josepha after EMS treatment

Table 47. Performance for qualitative traits in M_1 generation-EMS treatment

Variety and mutants derived	Fragrance	Seed setting ability	Vase life/ longevity (days)
Schloss Elutin-Control	Medium	No	3.0
S_1- 0.5%	Medium	No	2.8
Josepha-Control	Low	No	2.0
J_1- 0.8%	Low	No	1.6
J_2- 0.9%	Low	No	1.4
J_3- 1.0%	Low	No	1.2
Jogan- control	Medium	No	2.6
Jo_1- 0.7%	Medium	No	2.0
Jo_2- 0.8%	Medium	No	1.8
Morning Sun-control	Low	No	2.4
Mo_1-0.8%	Low	No	2.0

Morning Sun control flower was large, high centered and dark red. Petals were strinkingly edged with a dark red colour with white patches which tended to spread towards the edge of the flower. The flower was cup shaped and plant was a hardy cultivar. The M_{s1}-(0.8%) was red with white streaks and well formed.

12. Seed setting ability

Seed setting in the EMS treatment (Table 47) was nil in all the genotypes of roses at all the doses tried.

13. Vase life/longevity (days)

The vase life recorded in Schloss Elutin (Table 47) was 3.0 days. Mutant S_1 recorded a vase life of 2.8 days. In Josepha control, the vase life recorded was 2.0 days. In Mutant J_1 it was 1.6 days, in J_2 it was 1.4 days and in J_3 vase life was 1.2 days. In Jogan, the control flower recorded 2.6 days as vase life. In J_{g1} the flower had a vase life of 2.0 days and in J_{g2} it was 1.8 days. In Morning Sun, the control flower recorded a vase life of 2.4 days, M_{n1} recorded 2.0 days.

4.3 MOLECULAR CHARACTERIZATION

In the present research work, RAPD analysis was used for molecular characterization. Ten decamer primers were selected based on previous reports. These markers were used for analyzing parental polymorphism in the selected three varieties (Monnalisa, Pink Panther and Demestra). Based on parental polymorphism, two polymorphic primers were selected. Mutants in Monnalisa treated with 40, 50, 60 and 70 Gy of gamma rays were selected based on morphological variations. These mutants namely M_1, M_2, M_3 and M_4 respectively were subjected to molecular analysis.

4.3.1. Isolation of Genomic DNA

Genomic DNA was extracted from leaves of rose plants based on the Hi media kit method. When fresh and tender leaves were used, the purity and yield of

Table 48. Quantity and quality of genomic DNA isolated from parental genotypes of Rose

Sl. No.	Samples	O.D of DNA at A260	O.D of DNA at A280	Ratio of A260/ A280	Quantity of DNA (ng/µl)
1	Demestra-Control	0.009	0.005	1.80	450
2	Monnalisa- Control	0.007	0.004	1.75	350
3	M-40 Gy	0.007	0.004	1.75	350
4	M-50 Gy	0.009	0.005	1.80	450
5	M-60 Gy	0.012	0.006	2.00	600
6	M-70 Gy	0.01	0.006	1.66	500
7	Pink Panther- Control	0.008	0.004	2.00	400

DNA were good. The DNA yield of three parent cultivars of roses ranged from 350 to 450 ng/µl and its purity ranged from 1.75 to 2.00 µg/µl. In Monnalisa mutants the yield of DNA was 350-600 ng/µl and its purity ranged from 1.60 to 2.00 µg/µl. (Table 48). M_1 was the mutant selected from 40 Gy treatment, M_2- from 50 Gy treatment, M_3-from 60 Gy treatment and M_4- from 70 Gy treatment

The ratio of absorbance at 260 nm and 280 nm was 1.80, 1.75 and 2.00 in Demestra, Monnalisa and Pink Panther respectively. Among the mutants M_1 recorded 350 ng/µl, whereas in M_2, recorded 350 ng/µl, M_3 recorded 600 ng/µl and M_4 recorded 500 ng/µl. This showed that Himedia kit protocol yielded high amount of genomic DNA. The quality of genomic DNA of all the genotypes and mutants of Monnalisa was also checked on agarose gel for its base pair size and RNA contamination. This showed that the genomic DNA of the parental genotypes and mutants of Monnalisa was free from any mechanical or enzymatic degradation and was intact and of good quality.

4.3.2. Polymerase Chain Reaction

DNA amplification was carried out. To identify the promising primers for RAPD analysis, 10 decamer primers of series A, D, N, V and P4 were screened. Among them, four primers *viz.,* OPA-4, OPD-8, OPV-10 and P4 showed high polymorphism. In these, two decamer primers were identified for RAPD analysis. Primers that produced highest number of intense, polymorphic and reproducible bands were selected. They were OPA-4 and OPD-8 which were used for further screening of mutants.

The Primer P4 in Demestra produced two polymorphic products at around 300bp and other at around 600bp. In Pink Panther it produced two amplicons. In Monnalisa the primer produced two amplicons one monomorphic band at 200bp and the other at around 800bp. The primer OPN-2 produced one amplicon in all the three genotypes and the band was monomorphic in nature. The primer OPN-7 produced five amplicons in Pink Panther and 6 amplicons in Monnalisa. In the

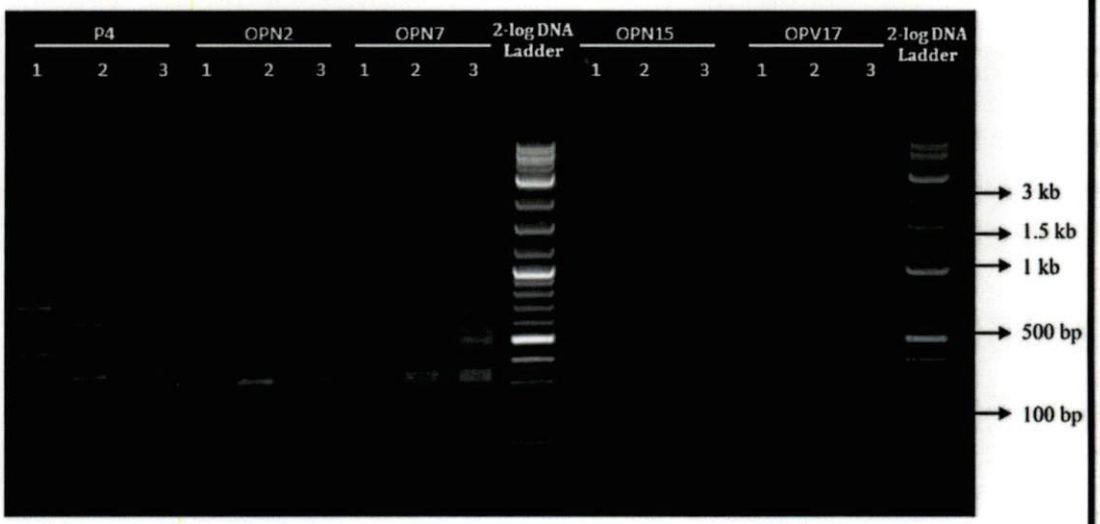


Plate 16. RAPD banding pattern of parent cultivars of rose using decamer primers

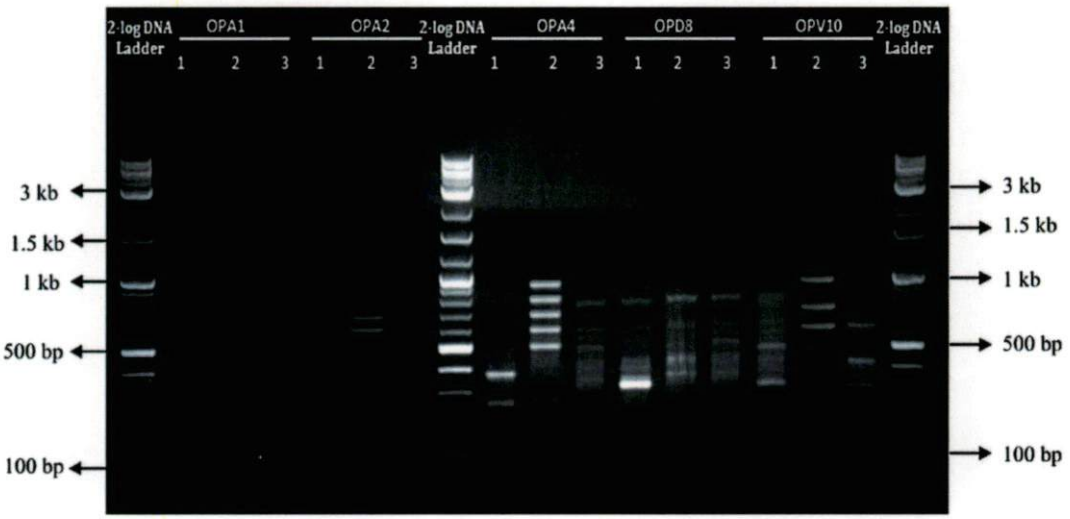


Plate 17. RAPD banding pattern of parent cultivars of rose using decamer primers

1. Demestra 2. Pink Panther 3. Monnalisa

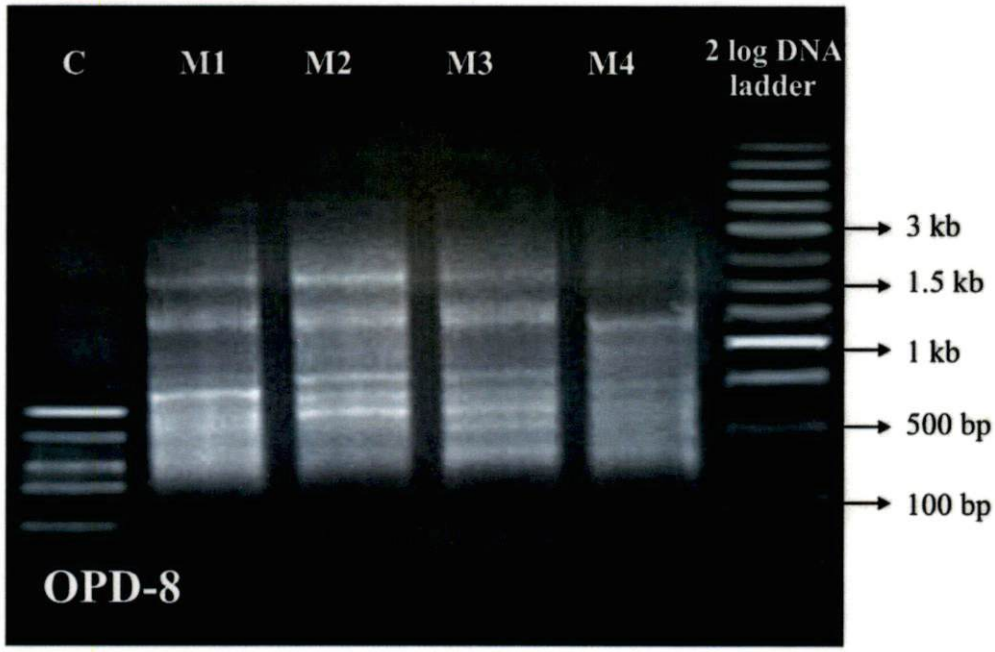


Plate 18. RAPD profile with primer OPD-8 showing polymorphism in control and its variants in Rose

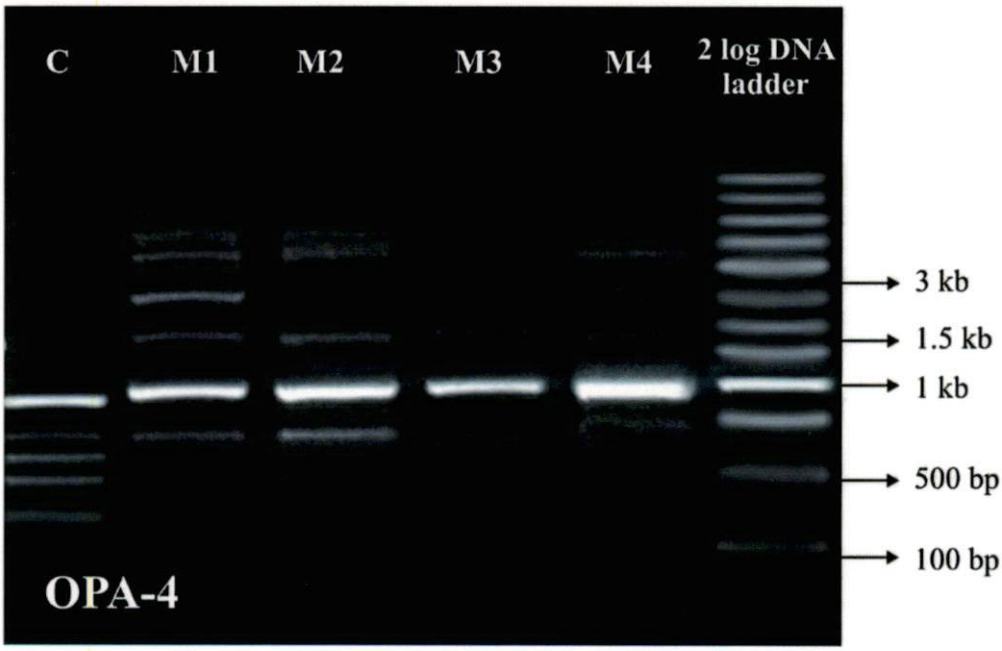


Plate 19. RAPD profile with primer OPA-4 showing polymorphism in control and its variants in Rose

C- Control M1-40 Gy M2-50 Gy M3-60Gy M4-70Gy

Table 49. Analysis of polymorphism of control and mutated plants of Monnalisa using four decamer primers

	Control			M-40 Gy			M-50 Gy		
	Total number of bands	Number of polymorphic bands	%Poly morphism	Total number of bands	Number of polymorphic bands	%Poly morphism	Total number of bands	Number of polymorphic bands	%Poly morphism
OPA-4	12	12	100	12	12	100	12	12	100
OPD-8	10	8	80	10	8	80	10	8	80
Total	22	20		22	20		22	20	
	M-60 Gy			M-70 Gy					
	Total number of bands	Number of polymorphic bands	%Poly morphism	Total number of bands	Number of polymorphic bands	%Poly morphism			
OPA-4	12	12	100	12	12	100			
OPD-8	10	8	80	10	8	80			
Total	22	20		22	20				

primer OPN-15 three monomorphic bands were present in all the three genotypes. In OPN-17 no amplicons were recorded in all the three genotypes. OPA-1 produced one polymorphic band in Demestra at around 500bp whereas in Pink Panther and Monnalisa, the band was monomorphic. In OPA-2 two amplicons were recorded around 600-700bp in Pink Panther. In Demestra OPA-4 produced two polymoprphic amplicons. In Pink Panther OPA-4 produced the maximum number of seven amplicons ranging from 500-900bp. In Monnalisa OPA-4 produced 4 amplicons. In the cultivar Demestra, OPD-8 produced five amplicons and a highly unique polymorphic band was observed in 300-400bp. Pink Panther and Monnalisa produced five amplicons each ranging from 400-800bp. In OPV-10, Demestra produced two amplicons, whereas Pink Panther and Monnalisa produced three amplicons each. Banding pattern is shown in Plates 16 and 17.

In order to check amplification of rose mutants, two primers were used to amplify the DNA of putative mutants of Monnalisa genotype induced by gamma irradiation. The two primers which were used to amplify Monnalisa and its mutants are OPA-4 and OPD-8.The data have been presented in the Table 49.

PCR amplification with primer OPA-4 clearly reveal that four bands were present at 1kb in M_1, M_2, M_3 and M_4 but it was absent in parent cultivar Monnalisa. Similarly most of the bands which are present in mutants are absent in parent cultivar. The total number of amplicons present in both control and mutants is 60, the percentage of polymorphism is 100%. Size of amplified bands ranged from 200bp to more than 3 kb. Banding pattern is shown in Plate.19.The polymorphic band which was present in 1.5 kb was not present in control of Monnalisa but present in its mutants. This can be used to differentiate between the parent and its mutants when RAPD marker OPD-8 was used. The total number of amplicons present was 50 and the percentage of polymorphism is 80%. Size of amplified bands ranged from 300bp -1.5kb. Banding pattern is shown in Plate.18.

In the present study, RAPD analysis using ten primers to detect the parental polymorphism clearly demonstrates the existence of genetic variation

within the three rose genotypes. It also confirms that the mutants of Monnalisa were different from the parental variety in their banding pattern. Hence mutation has occurred as a result of treatment with gamma rays in Monnalisa.

Discussion

5. DISCUSSION

Roses (*Rosa hybrida*) are considered to be one of the most economically important ornamental crops. They have been under cultivation for thousands of years, yet the genetic basis for rose breeding was laid down only recently. Modern cultivars are derived from interspecific crosses followed by selection. Hence wide variation is exhibited by the present day rose cultivars for their growth, size, shape and colour of flowers. Hence there is the need to assess the variation present in this genus before embarking on artificial induction of variability.

Keeping in view the need for development of precise techniques for germplasm characterization in rose, the present investigation was carried out to study the genetic diversity among different Hybrid Tea and Floribunda genotypes of rose on the basis of morphological traits.

5.1 EVALUATION AND CHARACTERISATION OF GERMPLASM

5.1.1. Mean performance of genotypes

The analysis of variance among 25 genotypes under each of the groups of Hybrid Tea and Floribunda roses indicated significant differences among the genotypes for all the nine characters studied. All the genotypes displayed considerable amount of differences in their mean performance with respect to all the characters. This had been exemplified by highly significant mean sum of squares which indicates that the germplasm collections under study were genetically diverse for most of the traits.

Analysis of variances revealed highly significant differences among cultivars of both Hybrid Tea and Floribunda groups of roses for all the characters studied, which revealed that considerable improvement can be made in this crop. The characters studied were number of leaves at first flower, number of days to first flower, prickle density, flower size, flower weight, pedicel length, number of petals per flower, size of petals and number of flowers per plant/bunch. Similar

results showing significant differences for most of the above said characters have been obtained by Tabaei *et al.* (2007) in *Rosa damascena.*

Mean performance of the genotypes in Hybrid Tea group revealed that no single genotype was superior for all the traits. Different genotypes were identified to be superior for each trait. The genotypes Mary Jean, Priority Pride and Alabama were superior in respect of number of leaves at first flower. The desirable genotypes for early flowering were Alabama, Fryat, Demestra and Lois Wilson. Flower size higher for Amara, Lois Wilson and Mom's Rose. The genotypes Shrewsbury Show, Amara and Majestic were superior with respect to flower weight and Perfume Perfect, Amara and Chirst of Colomb for pedicel length. The desirable genotypes in respect of number of petals per flower were A Tago, Shrewsbury Show and Josepha. The genotypes which were having more number of flowers per plant were Alabama, Majestic and Golden Fairy Sport.

Mean performance of the genotypes in Floribunda group of roses revealed that the genotypes Sans Souci, Schloss Elutin and Golden Fairy had maximum number of leaves at first flower. The desirable genotypes for earlier flowering were City of Glasgow, Sans Souci and Rosarale de Chateau. Data regarding days taken to flowering determine whether a genotype is early or late flowering which is an important parameter for selection of suitable variety/genotype for production. The present investigation exhibited significant variation in days taken to flowering. These variations were attributed to genetic characters of the rose. The results obtained by Anuradha and Gowda (2002) in gerbera, Mishra *et al.* (2001) in dahlia and Hegde and Gopinath (2003) in gaillardia also confirmed the presence of significant genetic variation among genotypes studied.

Flower size was higher for Carolanne, Sans Souci and Golden Fairy. The genotypes Monnalisa, Sans Souci and The Mccartney Rose were superior with respect to flower weight and Sterntaler, Lasting Piece and Orange N Lemon for pedicel length. The desirable genotypes in respect of number of petals per flower were Monnalisa, Carry Free Beauty and Rosarale de Chateau. The genotypes

which were having more number of flowers per plant were recorded in Orange N Lemon, Michel Fish and Carolanne. Variation due to additive effect was found controlling the traits flower diameter and number of flowers per plant. This could be improved in the desired direction *via* selection as reported by Verma (2007), in rose. Since different genotypes were identified to be performing differently for qualitative and quantitative traits, diverse genotypes with superior characters could be involved in a hybridization programme for the assembling of desirable traits in a single genotype.

5.1.2 Variability, heritability and genetic advance

The estimates of genotypic coefficient of variation will enable one to have a clear idea about the magnitude of genetic variability present in various characters. In the current investigations the relative magnitude of GCV and PCV, when compared, indicated that PCV values were greater than GCV in respect of all the attributes under observation. The findings of Gantait and Pal (2014), Namita *et al.* (2008), Zeinali *et al.* (2009), Singh and Mishra (2008) and Anuja and Jahnavi (2012) in various crops is similar to these results. In Hybrid Tea genotypes, high estimates of PCV and GCV were observed for size of petals, followed by prickle density, number of petals per flower and number of flowers per plant. In Floribunda genotypes, high estimates of PCV and GCV were observed for number of days to first flower, prickle density and size of petals, followed by number of flowers per plant, number of leaves at first flower and number of petals per flower. The high estimates of PCV and GCV for various characters indicate that selection for these characters would be effective.

Among all the genotypes of rose, phenotypic and genotypic coefficients were higher for thorn density and number of flowers per plant. Narrow differences were observed between genotypic and phenotypic coefficients of variation, showed that variability existed among different genotypes of rose was mainly due to genetic make-up and environmental influences were less in the expression of these traits. Similar results have also been advocated by Verma (2007) and Palai

et al. (2003) in rose for thorn density, plant height and number of flowers per plant.

In the present study, heritability in broad sense was high for all the characters suggesting that selection based on phenotype would be more effective. In both Hybrid Tea and Floribunda groups of roses, high heritability estimates were recorded for prickle density, number of petals per flower, number of days to first flower and flower size indicating that these traits could be improved by simple selection. Similar findings were reported in Hybrid Tea roses by Palai *et al.* (2003), for prickle density, plant height and petal number. Gantait and Pal (2014), Namita *et al.* (2008) and Zeinali *et al.* (2009) also reported high heritability estimates for flower diameter, days taken to flowering and number of flowers per plant in chrysanthemum.

In the present study of Floribunda roses, number of leaves at first flower, number of days to first flower and number of petals per flower exhibited high genetic gain. High coefficient of variation both at the genotypic and phenotypic levels coupled with high heritability and genetic advance was observed for number of days to first flower, prickle density, size of petals and number of flowers per plant. These results indicated that heritability, was due to additive gene effects and selection may be effective. A character exhibiting high heritability may not necessarily give high genetic advance. High value of genetic advance for these characters was indicative of additive gene action. Hence it will be helpful in deciding a breeding procedure for genetic improvement. Similarly high estimates of PCV and GCV coupled with high heritability and genetic gain was reported by Singh and Mishra (2008) and Anuja and Jahnavi (2012) for pedicel length, flower diameter and days to flowering. The characters number of flowers per plant, number of petals per flower and flower weight also recorded high estimates of PCV and GCV coupled with high heritability and genetic gain as reported by Gantait and Pal (2014), Namita *et al.* (2008), Zeinali *et al.* (2009), Singh and Mishra (2008) and Anuja and Jahnavi (2012).

In the present study of Hybrid Tea roses, prickle density, number of petals per flower and number of leaves at first flower exhibited high genetic gain. Palai *et al.* (2003) reported high genetic gain in Hybrid Tea roses for prickle density and petal number. High coefficient of variation both at the genotypic and phenotypic levels coupled with high heritability and genetic advance was observed for the characters *viz.* size of petals, prickle density, number of petals per flower and number of flowers per plant. Hence selection for desired traits may be practiced. Palai *et al.* (2003) also reported similar results in Hybrid Tea roses for petal number.

5.1.3 Genetic divergence studies

Divergence analysis is performed to identify diverse genotypes. Mahalanobis's (1936) generalized distance estimated by D^2 statistic has been used as an efficient tool in the quantitative estimation of genetic diversity for a rational choice of potential parents for breeding programme. It was observed that the 25 genotypes of both Hybrid Tea and Floribunda were distributed at random among the clusters formed based on their genetic distance. The inter cluster distance was not consistent with the geographic distribution of varieties. It can be said that soil does not have any effect on distribution of roses in the areas, however other climatic factors may play crucial role in its distribution pattern, as reported by Yan *et al.* (2005).

Based on D^2 values the 25 genotypes of both Hybrid Tea and Floribunda were grouped into ten and nine clusters respectively. In Hybrid Tea cluster I and II had five genotypes each, whereas clusters III and IV had three genotypes each. Similarly in Floribunda, cluster I was the largest containing ten genotypes while cluster II had four genotypes. Wide range of diversity was observed among rose genotypes. Genotypes falling in the highly divergent groups will help in broadening the existing genetic base. Similar conclusions were drawn by Kavitha and Anbuarni (2009) in African marigold and Rakesh *et al.* (2011) in Snapdragon. The distribution of genotypes into different clusters showed no uniformity with

respect to their origin, thus ruling out the association between geographical distribution and genetic divergence. Similar results were reported by earlier workers Swaroop and Janakiram (2010) and Bhajantri and Patil (2013) in gladiolus.

In Hybrid Tea, the intra cluster distances indicated that cluster II had the maximum intra cluster distance followed by cluster I. The maximum inter cluster distance was found between clusters X and V and it was least between cluster IX and VII, whereas in Floribunda, the highest intra cluster distance was recorded in cluster II followed by cluster I. The maximum inter cluster distance was found between clusters VIII and the least between clusters IV and II. Thus it can be concluded that considerable diversity existed among the 25 genotypes of the present study. This could be the result of selection in different directions by nature and human forces. The increasing parental distance implies a great number of contrasting alleles at the desired loci, and crossing of distantly related parents allows recombination of these loci in the next generation thereby increasing the opportunities for effective selection. Thus, crossing of genotypes from distant clusters may produce higher amount of heterotic effect. Crossing of genotypes within the cluster is not expected to yield desirable recombinants. However, theoretically a general notion exists that larger the divergence between genotypes, higher will be the heterosis. Therefore, it would be desirable to attempt crosses between genotypes belonging to distant clusters to obtain genetically divergent genotypes as reported by Lauric and Ries (2001). Thus, genetic divergence can also be used as an indirect parameter in selecting parents to produce heterotic high yielding progenies. The clustering pattern obtained in the present study also revealed that there was no relation between geographic origin and genotypic diversity. Gupta and Singh (1970) and Deeksha *et al.* (2014) was working on different crops had also indicated that geographic diversity cannot always be used as an index of genetic diversity. It is quite possible that many of the genotypes obtained from different geographical sources might have a common origin. Cluster analysis studies were done in gladiolus by Sheikh and Mushtaq (2006).

Similar conclusions were drawn by Kavitha and Anburani (2009) in African marigold and Rakesh *et al*. (2011) in snapdragon.

Contribution of each character towards genetic divergence has been estimated from the number of times that each character scored the first rank. In Hybrid Tea, it was observed that number of days to first flower, number of leaves at first flower, number of petals per flower, size of petals and flower size contributed maximum towards genetic divergence. In Floribunda, it was observed that number of leaves at first flower, number of days to first flower, number of petals per flower and size of petals contributed maximum towards genetic divergence indicating the major role of these characters in building up diversity and differentiating of inter cluster levels. Such characters can be given more emphasis for the purpose of fixing priority of parents for hybridization programmes. The results of the study imply that, in order to select genetically diverse genotypes for hybridization, the material must be screened for important traits such as number of days to first flower, flower size and number of flowers per plant. These results were in good agreement with the findings of Deshraj and Mishra (2000) in gladiolus, Nimbalkar *et al.* (2006) in dahlia and Manjunath *et al.* (2009) in anthurium.

The cluster means reveal the best cluster for various characters. Depending upon the aim of breeding, potential lines can be selected from different clusters as parents in a hybridization programme. In Hybrid Tea roses, if a breeding programme is aimed at more number of flowers per plant, cluster IX which showed highest mean number of flowers per plant could be selected. If a breeding programme is aimed at highest flower weight, cluster V with highest mean flower weight along with highest values for characters like number of petals per flower and prickle density can be selected. Cluster VIII consisted of genotypes exhibiting higher values for number of days to first flower, flower size, flower weight and pedicel length, while characters such as number of leaves at first flower, prickle density, size of petals and number of flowers per plant had moderate expression.

Thus Cluster VIII could be selected for improving most of the desirable traits in the selection programme.

In Floribunda roses, if a breeding programme is aimed at more number of flowers per plant, cluster IV with highest mean number of flowers per plant can be selected. If a breeding programme is aimed at highest flower weight, cluster V showed highest mean for flower weight along with highest values for characters such as number of petals per flower can be selected. Cluster VIII consisted of genotypes exhibiting highest values for prickle density, pedicel length and size of petals, while characters such as flower size and number of petals per plant had moderate expression. Since all kinds of gene actions and interactions are possible in the expression of quantitative traits it is advisable to make crosses between genotypes selected from the clusters with high mean performance to get desirable transgressive segregants (Dwivedi and Mishra 1996). Crosses that involve parents from more divergent clusters are expected to manifest high heterosis and variability in the germplasm (Singh *et al* 1987). However, the chance of getting segregants with high yield level is quite limited when one of the parents has a very low yield level. Thus selection of parents should also consider the special advantage of each cluster and each genotype within a cluster depending on specific objective of hybridization.

Hence it is worthy to note that in calculating cluster means, the superiority of a particular genotype in respect to a given character is diluted by other genotype that are related and grouped in the same cluster but which are inferior or intermediary for that character in question. So, apart from selecting genotypes from the clusters which have high inter cluster distance for hybridization, one can also think of selecting genotypes based on extent of genetic divergence in respect to a particular character of interest. This means to improve the character of interest highly divergent genotypes for traits may be selected.

Based on overall performance of rose varieties, it can be concluded that morphological data gave a better estimate of genetic differences in rose varieties.

There existed significant diversity between different varieties of rose characterized in the present study, which will form an important basis for selection of variability to be used in future rose improvement. Therefore, diversity of morphological-based markers for genetic diversity of *Rosa* species and interaction of environment are expected to be quite high and this can be efficiently used for future breeding programmes as reported by Debener *et al.* (1996), Joublan *et al.* (1996), Mohapatra and Rout (2006) and Yan *et al.* (2005).

5.2 INDUCED MUTAGENESIS

Induced mutagenesis increase the possibilities of creation of new starting material with high ornamentation (Cantor *et al.* 2002). Induced mutation by gamma rays and chemical mutagens in ornamental plants have been used for genetic changes including more number of flowers, disease resistance, early maturity, etc. Gamma rays are known to influence plant growth and development by inducing cytological, genetic, biochemical, physiological and morphogenetic changes in cells and tissues (Abdullah *et al.* 2009). Roses are preferred due to their wide range of adaptability and various coloured flowers of different shapes and sizes and good shelf life. The possibilities for creating new and unusual forms in roses are immense due to its heterozygous genetic constitution which makes it a promising test material for induced physical and chemical mutagenesis.

Among the available crop improvement techniques, it is very clear that induced mutation breeding is well established one for the development of new ornamentals, whereas a molecular technique is in budding stage. In roses, if only one or a few characters can be improved *viz.,* novel flower colour and flower size. without changing the entire genotype, the mutant can be maintained by means of asexual propagation. In the light of the above facts, mutation breeding offers unique opportunity for improvement in roses through induction of genetic variability. The objective of the present investigation was to study the variation caused by chemical and physical mutagens in vegetative and floral characters. The results obtained during the course of study are discussed here under:

5.2.1 Induced mutagenesis using gamma rays on budwood

Various morphological characters were found to be significantly influenced due to application of gamma rays in rose budwood at different levels. Bud take percentage and survival percentage varied greatly due to gamma doses and it was observed that as doses of gamma rays increased sprouting and bud take percentage decreased. Based on these two parameters, LD_{50} value was fixed for each variety.

According to Sparrow *et al.* (1968), radio sensitivity in plant species depends on nuclear volume, number of chromosomes and ploidy level. Hence, in any mutation breeding programme, starting material is exposed to a range of doses to determine the optimum doses *viz.*, LD_{50} at which survival of treated material is reduced to 50 per cent which varied with the variety. Survival was completely suppressed at 100 Gy. In general, LD_{50} for all the four genotypes was beyond 20 Gy gamma rays dose. These results are in conformity with the results of Salabhiah *et al.* (2006) who recorded LD_{50} for auxillary bud explants in roses.

During fixing LD_{50} value, higher doses of gamma irradiation (90 and 100 Gy) failed to produce any sprout in all the varieties. The variety Golden Fairy showed survival (26.67%) at 50 Gy beyond which there was no survival. In Demestra, none of the plants survived beyond 60 Gy. In Pink Panther, the maximum survival was 80.00% in 20 Gy whereas 70 Gy recorded survival of 6.67%. Beyond this dose, none of the plants survived. Monnalisa was resistant upto 90 Gy with 6.67% survival but the maximum survival (86.67%) recorded was in 20 and 30 Gy. Beyond 90 Gy Monnalisa plants could not survive. Similar observations on varying mortality of treated material and variations in radiation sensitivity among genotypes of a crop were reported by Dwivedi and Banerji (2008) when dahlia varieties were subjected to different doses of gamma rays.

The sensitivity of genotypes with respect to bud take % was also recorded. The bud take at 20 Gy, the lowest dose tried was 86.67% in Demestra, Golden Fairy and Pink Panther whereas in Monnalisa it was 93.33%. Monnalisa which

showed bud take even in 100 Gy with a survival of (13.33%) proved to be the hardiest. Gamma irradiation increased the number of days to bud take as compared to control in all the four varieties tried. Further, an increase in dose of mutagen led to an increase in number of days to bud take. Similar results were reported by Gupta *et al.* (1970) when two varieties of roses were treated with four doses of gamma rays. Tiwari *et al.* (2010) and Tiwari and Kumar (2011) have reported similar results with ornamentals.

In the present study, the budwood irradiated with gamma rays showed an increased mortality per cent with increase in dosage irrespective of genotypes used. Among the four genotypes, the sequence of sensitivity was Golden Fairy>Pink Panther> Demestra >Monnalisa. This agrees with the findings of Broertjes and Van Harten (1988) who reported on varietal differences for radiation sensitivity. The dose of mutagen to apply depends on the radio sensitivity of the species, which in turn is dependent on plant part and stage of development. Genotypes differ in their sensitivity to radiation and this fact establishes that the genotypic control occurs within the species and it is the matter of sensitivity to radiation (Pal, 1962). In the present study using Hybrid Tea and Floribunda roses, LD_{50} value ranged from 32-51Gy. Broertjes and Van Harten (1988) have reported that LD_{50} for different vegetatively propagated crops such as gladiolus, chrysanthemum and others varied from 50-150 Gy. Further, Salabhiah *et al.* (2006) had obtained similar results that LD_{50} for both cut and miniature roses were found to vary between 20 and 40 Gy.

5.2.1.1 Effect of Gamma Radiation on Morphological Characters in M_1

Evaluation of M_1 generation for the morphological characters of mutants derived through gamma radiation are discussed below.

The M_1 of the four varieties was evaluated with respect to several morphological characters after exposure to different doses of gamma rays. The characters studied were number of leaves at first flower, number of days to first flower, prickle density, flower size, flower weight, pedicel length, number of

petals per flower, size of petals and number of flowers per plant. In general, it was found that the different morphological traits showed reduced expression as compared to control on mutagen treatment. This decrease was directly proportional to the dose employed. Results similar to the present findings were reported by Patil and Dhaduk (2009) in gladiolus. Sobhana and Rajeevan (2003) in Dendrobium also reported a decrease in morphological characters with increase in dose of mutagen.

Decrease in number of leaves might be due to reduction in the number of vertical cell layers which resulted in shorter internode, reduction in number of internode or any combination of these processes. Morphological observation of this experiment also collaborated with the findings of Patil *et al.* (2010), Patil *et al.* (2010a) and Singh and Kumar (2013) that higher doses of gamma irradiation was not beneficial to improve plant growth. Reduction in vegetative growth after radiations might be due to interference in normal mitosis and frequent occurrence of mitotic aberrations, inhibition of rate of assimilation and consequent change in the nutrient level in the plant (Ehrenberg, 1995) and inactivation of vital enzymes, especially those associated with respiration (Casarett, 1968). Reduction in vegetative growth due to changes in auxin level or due to inactivation of auxin was hypothesized by Datta and Datta (1953), in rose.

Number of days taken to first flower showed a dose dependent increase in the different varieties as compared to control. This increase might be due to the delay in vegetative growth, which was more prevalent in higher doses exposures. These results were in congruence with the observations made by Singh and Kumar (2013), who reported that the delay in flowering was lesser in lower doses as compared to higher doses. Similar results were also reported by Kole and Meher (2005), while studying the effect of gamma rays in zinnia. The delay in bud initiation ultimately resulting in late blooming, observed in the present study may be due to reduction in the rates of various physiological processes and inhibition of growth. The plant remains in juvenile stage and is unable to differentiate flower heads due to gamma irradiation. As a result of irradiation

many biosynthetic pathways are altered which are directly and indirectly associated with the flowering physiology as reported by Mahure *et al.* (2010). Current results agree with the findings of Datta and Gupta (1981) and Dilta *et al.* (2003), who recorded significant delay in blooming of chrysanthemum variety 'Lilith' with 15 and 20 Gy gamma ray irradiation.

The beneficial effects of lower doses of gamma rays on flowering was reported by Misra *et al.* (2006), Patil *et al.* (2010), Srivastava *et al.* (2007) and Kumar *et al.* (2012) in various vegetatively propagated ornamentals. However, higher doses from 30 to 70 Gy were found to be reducing the flowering parameters, which is in accordance with the results of the present study. Since our objective was induction of beneficial mutations rather than growth stimulation, such lower doses were not included in the present investigation. Doses closer to LD_{50} value were employed which gave a general retarding effect. Radiation reduced prickle density and this reduction was directly proportional to the dose employed. This can be reported as a beneficial effect of mutagen treatment. The reduction in prickle density observed was influenced by the sensitivity of the variety also.

Flower size and flower weight are important characters in any crop of floricultural importance. Physical mutagen treatment resulted in a slight reduction in flower size and weight in the different varieties but this decrease was not statistically significant in majority of the cases. Hence this slight reduction may be due to M_1 effects rather than genetic changes. The size of the first flower was observed to be larger in all situations and genotypes. Mutagen treatment induces breaks the nuclear DNA strands and during DNA repair mechanism, mistake leading to new mutations are induced randomly. Changes can happen in DNA is cytoplasmic organelles, also and result in mutations (Jain and Maluszynski 2004). Wongpiyasatid *et al.* (2007) reported decrease in flower diameter of African violet with increase in gamma irradiation. Similarly Lamseejan *et al.* (2000) found variation in flower size of chrysanthemum on treatment with radiation. As a result of irradiation variation in flower size and flower shape were induced in each

genotype (Yamaguchi *et al*. 2003). For the trait flower weight, highest flower weight after control was recorded in Golden Fairy at 20 Gy, Pink Panther at 30 Gy, Demestra at 30 Gy and Monnalisa at 40 Gy each being the lowest dose employed in that variety.

Pedicel length is one of the important commercial characters of the rose. In general, there was a decrease in pedicel length with increase in dose of mutagen. This decrease was significant in certain situations and negligible in others. Gamma rays interact with atoms and molecules to produce free radical in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation level (Wi *et al.* 2007). In general, there was a drastic reduction in number of petals at higher doses of gamma rays as compared to control which was dependent on the mutagen sensitivity of the variety also. This reduction was observed to be statistically significant in most of the cases. Decrease in number of petals with higher doses is mainly due to disturbance in plant physiological processes and reduction in vegetative growth of plant. Kole and Meher (2005) also recorded increase in ray florets number at lower doses of gamma radiations in zinnia, whereas number of florets reduced drastically at higher doses of gamma rays. These results are parallel with the findings of Misra and Choudhary (1979), in gladiolus and Singh *et al.* (2009) in marigold.

The reduction in petal size in terms of length and width in plants treated with higher doses of gamma rays might be due to inactivation or decrease in auxin content or disturbances in auxin synthesis (Gordon, 1956). These results are in line with the findings of Banerji and Datta (1986) who recorded different types of floral abnormalities, mainly reduction in flower size and petal number. Such an effect is known to arise due to chromosomal aberrations in addition to genetic mutations. This trend is quite common in mutagenized populations. Similar results of decreased petal size were also reported earlier by Kaicker and Swarup (1972), Datta and Gupta (1982b) and Dwivedi and Banerji (2008) in rose. The most

economic character, number of the flowers per plant is very important with respect to breeding point of view. The genotype Demestra at 30 Gy recorded highest number of flowers per plant, followed by Monnalisa at 40 Gy. Overall, there was drastic reduction in number of flowers per plant at higher doses of gamma rays as compared to control. The findings of Sisodia and Singh (2014) who recorded maximum number of flowers in control plants and reduction in flower number at higher doses of gamma rays in gladiolus agree with the present results. The findings of Misra and Choudhary (1979) and Dhara and Bhattacharya (1972) in gladiolus are also in line with the current findings Kole and Meher (2005), found increase in flower number at lower doses of gamma irradiation, whereas, number of flowers reduced drastically at higher doses of gamma rays in zinnia. In the present study, the rate of decrease in flower production was found to be related to the mutagen sensitivity of the variety also. Arnold *et al.* (1998) have reported an extreme case of a similar situation. They carried out induced mutation study on four varieties of roses. The finding was that number of petals increased with application of gamma doses in two varieties *i.e.,* Potluck and Dark Red Mountie, whereas, it decreased in other two varieties Blood Red and Mountie. The doses of gamma rays used were 50, 100 and 200 Gy. They further stated that dose response relationship often showed erratic results because gamma ray photons may miss the targets necessary to generate mutation and radio sensitivity depends on the variety.

5.2.2 Induced in vitro mutagenesis using EMS

5.2.2.1 In vitro culture establishment

The present investigations were carried out to standardize efficient protocol for in vitro multiplication of roses like, Schloss Elutin, Jogan, Josepha and Morning Sun using EMS under *in vitro* conditions.

Plant tissue culture is an efficient method to produce vast number of true to type, healthy and disease free plants in a very short time (Aftab *et al.* 2008). Most important step before culture initiation is sterilization of the explants (Allan,

1991) because freedom of explants from microbes is the main prerequisite for determining the culture establishment capacity. On one hand, surface sterilization aims at killing all microbes that can easily grow under lab conditions whereas, on the other hand it should ensure the explant's survival and regeneration capacity which are known to be influenced by the disinfectant level and sterilization time (Yildiz and Er, 2002). A large number of chemicals including NaOCl, ethanol, hydrogen peroxide, bromine water, mercuric chloride, silver nitrate and various antibiotics are used for surface disinfestation (Bloomfield and Arthur, 1991). However, $HgCl_2$ is commonly used for surface sterilization of many plants (Skirvin *et al.* 1990).

In the present research, the nodal explants of Schloss Elutin, Jogan, Josepha and Morning Sun were disinfected with 0.1% $HgCl_2$ for five minutes. Amir, (2011) also exposed explants to 0.1% $HgCl_2$ solution for sterilization. $HgCl_2$ is commonly used with 70% ethanol for surface sterilization of many plants (Skirvin *et al.* 1990), which was followed in the present study. *In vitro* response of plant tissue depends on the genotype, the physiological status of donor plant, the type of explants, the culture medium and their interaction. Different explants such as seeds, apical buds or nodal explants, stem cuttings, corms, ray florets etc. have been tired by several workers Nonomura *et al.* (2001), Toyada *et al.* (2015), Roychowdhury and Tah (2011), Bhajantri and Patil, (2013) and Datta *et al.* (2005) for *in vitro* mutagenesis.

5.2.2.2 Standardization of culture medium

In order to standardize the culture establishment medium, MS medium supplemented with different combinations of BA (1.0 and 2.0 mg /l), ADS (25 and 50 mg/l) were used. It was shown that BA and ADS in optimum dose led to maximum explant survival, whereas the control showed minimum explant survival. Our reports are supported by the findings of Kim *et al.* 2003 that *in vitro* shoot proliferation is largely based on media formulations having cytokinins as best plant growth regenerator. Its presence in the growth medium assisted the

multiplication of shoots in hybrid roses throughout the year (Rout *et al.* 1999). Purine-type cytokinins effectively release the apical dominance in *in vitro* propagated ornamentals (Bollmark *et al.*, 1995; Kapchina *et al.,* 2000). Purine cytokinin N6- benzyladenine (BA) also stimulated axillary bud break and increased the number of open buds of *Rosa hybrida* L. grown under *in vitro* conditions (Kapchina *et al.*, 2000). All the above results support the current findings. Inclusion of BA and ADS in the correct proportion in the culture medium led to early and high percent bud sprouting.

It has been reported that inclusion of BA in the culture medium is important for bud break and shoot multiplication of *R. hybrida* genotypes (Hasegawa, 1980; Wulster and Sacalis, 1980) which is in line with the current findings. There are genes causing increased number of bud initials and shoot multiplication. Moreover, the possible gene involvement in adjusting hormone levels has also been reported (Tantikanjana *et al.*, 2001). Finally it was concluded that the best treatment for explant survival, percent bud sprouting and days to bud sprouting was MS+BA (2.0 mg/l)+ADS (25 mg/l) and this treatment can be successfully used for culture establishment.

In order to standardize the shoot elongation medium basal MS medium supplemented with different concentrations of various growth regulators *viz.*, BA (2.0 mg/l), IAA (0.1 mg/l) and 0.25 mg/l)) and ADS (25 and 50 mg/l) were tried. The treatment MS+BA (2.0 mg/l) +IAA (0.25 mg/l)+ADS (25 mg/l) gave the maximum number of proliferated shoots per explant. Shoot proliferation rate varied in diverse species and was specific to growth medium (Senapati and Rout, 2008). Noteworthy difference in genotypes with respect to shoot prolification has been ascribed to the genotypic influence as depicted in rose by many researchers (Bressan *et al.*, 1982; Valles and Boxus, 1985). Purine type cytokinin BA more positively affects the growth, development and the appearance of micro plants. Elongation decreases with increase in number of shoots at high level of BA (Carelli and Echeverrigary, 2002). This can be caused either by better uptake and more efficient utilization of the purine cytokinin or by the lower level of

endogenous cytokinins (Veneta *et al.,* 2005). Moreover, plants with increased cytokinin content have more branches (Schmulling, 2004), number of shoots, shoot length and number of leaves by initiating cell division and cell elongation (Peres *et al*., 2001).All the above results corroborate the current findings.

The present results indicate that optimal concentrations of IBA, BA, NAA, sucrose and coconut water gives the best root initiation. In the present study, the medium combination Half MS +IBA (0.2mg/l) +Sucrose (2%)+ BA (2.0mg/l) was found to be significantly superior over other treatments for percent rooting, earliness in root initiation, root length and number of roots per shoot. IBA has been reported to increase the cambial growth at the base of microcuttings that result in differentiation of root primordia (Haq *et al*., 2009). IBA is the most frequently used auxin to cause root initiation. Endogenous auxin along with some root inducing factors might occur naturally within the microcuttings which when supplemented may help for rapid root primordia initiation (Haq *et al*., 2009). Furthermore, root inducing factors are believed to be essential for rooting, which combine with auxin to form a complex that directs RNA to activate enzymes that cause root initiation (Hartmann *et al.,* 2007).

In the current study increase in IBA above optimum level showed an inhibiting effect on rooting. IBA appears to be a better auxin but its higher concentrations can reduce the level of root regeneration percentage as it appears to have lethal effect (Iqbal *et al*., 2003). Moreover, IBA is stable and may remain present for a long time and can inhibit the outgrowth of root primordia resulting in massive ethylene accumulation in the tissue culture container (Deklerk, 2002). Hence the exogenous application of IBA is considered to be better suited for inducing rooting of various species (Pati *et al*., 2004).

Auxin is formed in shoots and moved to effect the development of other tissues in the lower part of stem cuttings. It boosts cell elongation and has other growth-regulating effects also (Camellia *et al*., 2009). IBA has also greater ability to promote rooting and induces less callus formation (Panjaitan *et al*., 2007). The

most favourable concentration of IBA which varies with species induces cell enlargement by extruding protons actively into the region of cell wall resulting in a pH decrease and motivation of cell wall loosening enzyme that promotes the breakage of key bonds of cell wall and increases cell wall extensibility thus causing an increase in cell size and elongation (Taiz and Zeiger, 2006). Han *et al.* (2009) reported that auxins induce sprouting of shoot buds which initiate growth substances in the roots for growth and elongation. Iqbal *et al.* (2003) explained that application of IBA brought changes in protein synthesis and RNA production which increase number of roots by stimulating cell division processes (Husen and Pal, 2007).

IBA due to its weak toxicity stimulates roots more efficiently and showed greater stability for root induction (Han *et al.*, 2009). Early stages of lateral root formation are regulated by polar auxin transport. Therefore, lateral root development is initiated by auxin which is known for its capability to support adventitious root development due to cell division stimulation (George *et al.*, 2008). Higher concentrations of IBA induces higher level of degradative metabolites in tissues which might lead to the blockage of root formation process (Baker and Wetzstein, 2004). Asghar *et al.* (2011) also reported reduction in number of roots of orchid at higher concentrations of IBA.

5.2.2.3 In vitro mutagenesis

For induction of mutations under *in vitro* conditions, the genotypes Schloss Elutin, Jogan, Josepha and Morning Sun were subjected to EMS treatment. The results discussed are as follows:

In all the four genotypes, Schloss Elutin, Jogan, Josepha and Morning Sun, while discussing meristem regeneration it was noticed that, the low dose levels of mutagens are responsible for stimulating sprouting substances such as enzymes which were set free by mutagen treatment and play an important role in plant metabolic activities resulting in stimulated plant growth. Higher doses may have harmful effect on auxins and other growth substances, chromosome structure and

cell division, which suppress growth or create lethal effect on the cells of the plant and consequently lead to poor meristem regeneration and low survival of the plant. (Rather *et al.,* 2002, Srivastava *et al.,* 2007 and Kumar *et al.,* 2012. These reports reconfirm our findings that lower doses of EMS enhanced sprouting while higher doses delayed it.

Our results are in conformity with the works of Berenschot *et al.* (2008) who reported that high EMS doses decreased survival rate considerably. Roychowdhury and Tah (2011) also reported that higher doses of EMS cause detrimental effect on survival at flowering stage or at maturity. Similar results were also reported by Tiwari *et al.* (2010) and Tiwari and Kumar (2011). Khan and Tyagi (2009) attributed this decrease to the effects of mutagens on the meristematic tissues. The decrease in sprouting at higher doses of the mutagens may also be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both. Disturbances in the formation of enzymes involved in the sprouting process may be one of the physiological effects caused by mutagenic treatments, particularly chemical mutagens such as EMS leading to decrease in sprouting. EMS mutagenesis gives rise to a high mutation frequency without preference for specific genomic regions, and generates many alleles that allow the isolation of novel phenotypes. Point mutations can be identified by the TILLING (Targeting induced local lesions in genome) method (McCallum *et al.,* 2000 a and b).

In the present study, moderate doses of EMS showed stimulatory effect with respect to some vegetative characters and growth parameters. It may be due to the increased activity of enzymes involved in biosynthesis of hormones such as gibberllins, cytokinins *etc.* (Singh *et al.,* 2000) which enhances cell growth ultimately resulting in an increase in the number of spikes, florets, advanced flowering *etc.* Stimulatory effects of lower concentrations of chemical mutagens have been reported by various workers (Vagera *et al.,* 1976 and Zargar *et al.*, 1994). On the other hand, increase in the dose of chemical mutagens caused

damaging effects on biological activities of plants which may be due to inactivation of cells because of mitotic disturbances/ chromosomal aberrations leading to poor growth of the plants (Zargar *et al.,* 1994).

In the present study the opening of basal florets from the date of planting was delayed with the increase in doses of EMS except 0.2% of EMS treatment. In 0.2% EMS dose, early flowering was observed .With increased doses of EMS, there was a non-significant decrease in duration of flowering. Delay in spike initiation following EMS treatment as observed in the present study has been reported earlier by Roychowdhury and Tah (2011), in Dianthus. They reported that days taken to spike initiation increases with the increase in doses of EMS. Similar result was also observed by Bhajantri and Patil (2013). Delay in flowering might be due to disturbances in biochemical pathway, which assists in synthesis of flower inducing substances.

Roychowdhury and Tah (2011) reported decrease in plant height and other vegetative characters with increase in EMS doses in Dianthus. Alteration in growth parameters due to varying doses was explained by many workers. It may be attributed to one or more of the following reasons (i) the increase in destruction of growth inhibitors (ii) the increase in growth promoters (iii) the sudden increase in metabolic status of corms at certain levels of dose or (iv)it may be due to the induced chromosomal aberrations. These findings are in close agreement with the earlier reports of Wang and Yu (2006), Solanki and Sharma (1999), Solanki and Sharma (2002), Kumar and Selvaraj (2003) and Solanki and Phogat (2005). In general, there was decrease in pedicel length with increased dose of mutagen as compared to control.

In the present study, the decrease in number of spikes per plant and number of florets per spike may be due to deleterious effects of EMS at higher concentration. These are in conformity with results of Bhajantri and Patil (2013) in gladiolus and Roychowdhury and Tah (2011) in Dianthus. Patil and Dhaduk (2009) also reported increase in number of spikes, which might be due to slight

increase in photosynthetic activity that was encouraged by mutagen, which was also supported by the findings of Misra *et al* (2006). Brock (1964) postulated the mutagenic treatments induce differential changes in the polygenic system.

Significant differences were observed for petal size after EMS treatment. Among the four genotypes, Schloss Elutin at 0.5% reported the highest size of petals followed by Josepha at 0.7%, Morning Sun at 0.8 % and Jogan at 0.8%. It is assumed that with increase in dose of EMS, certain growth substances are stimulated which increase diameter of basal florets. Similar stimulatory effect on number of florets per spike was observed by Bhajantri and Patil (2013). The most economic character that is number of the flowers per plant which is very important from the breeding point of view. The genotype Schloss Elutin at 0.5% recorded highest number of flowers per plant, followed by Jogan at 0.8%, whereas the rest two genotypes are almost on par with each other.

Most of the vegetative and floral characters barring a few were decreased in magnitude with increased doses of mutagens. The decrease in quantitative characters has been attributed to physiological disturbances or chromosomal damage of the cells of the plants caused by the mutagens. EMS usually cause point mutations but the loss of a chromosome segment or deletion can also occur. The variations obtained during the experimentation due to EMS may show stability in subsequent generations.

Low and medium fragrance was recorded in both the control and radiated genotypes in all the four cultivars. In EMS treatment also similar trend was observed. No seed setting ability was recorded any of the mutants. After flower colour, floral fragrance is the second most appealing trait to consumers, particularly in cut-flowers. Floral scent is due to volatiles that are primarily derivatives of terpenoids, phenylpropanoids/benzenoids or fatty acids. In some flowers, including rose (much admired for the fragrance of its flowers in garden varieties) natural fragrance has been lost in modern cut flower varieties. This is because fragrance is correlated to poor vase life and breeders have largely

eliminated fragrance during selection for longer vase life. The re-introduction of fragrance into some of the more widely grown cut flowers would be a very useful commercial achievement. Rose is grown for its cut flower, hence longevity, freshness and appealing appearance has paramount importance. The results on post harvest studies indicated that some parameters of the varieties that are under genetic control are responsible for longevity of flower and vase life. In several studies, it has been proved that wide differences in post harvest behaviour and lasting quality among different cultivars of flowers existed. Flowers recorded highest amount of respiration at senescence and hence vase life was minimum in these flowers.

A visual observation on different characters *viz.,* change in flower colour and change in flower form is classified. According to available literature induction of somatic colour mutations in gamma irradiated plant material may be ascribed to the chromosomal aberrations, break down of phosphate metabolites, accumulation of free amino acids and change in the gene sequence. However, the radio-sensitivity of the variety is dependent upon the genotypes (Bowen *et al.*, 1962 and Datta, 2001).The change in flower form was also recorded by Lamseejan *et al.* (2000) in chrysanthemum with chronic and acute gamma irradiation treatment. Misra *et al.* (2003) developed one chrysanthemum mutant with tubular florets by gamma irradiation while the original florets were flat spoon shaped. Anthocyanins are responsible for the blue, red and violet colours on copigments of flavonoids and metals (Jurd and Asen, 1966; Takeda *et al.*, 1994). The other important group of colour compounds is carotenoids. These are the pigments which, occurring in plastids, are responsible for yellow, orange and even red colours (Lema-Ruminska *et al.,* 2005*).* A colour change in mutants involved both qualitative and quantitative pigment modifications. All this suggests that mutation in the mutants obtained resulted in changes in genes of flavonoids biosynthesis involving an inactivation of any of the enzymes on the biosynthesis pathway. Obtaining new inflorescence colours with the use of mutation seems to be related to a genetic material destruction. It can be considered that genes of biosynthesis of concerned

pigments are blocked in original cultivars. Blocking genes occurring in a dominant form effectively block biosynthesis pathways of pigments. Induction of flower colour mutation after gamma irradiation is in conformity with the results obtained earlier for other ornamentals (Datta and Banerji, 1990; Datta, 1997).

Morphological abnormalities in flowers were developed after gamma irradiation. Mutation sectors varied from a small sector of the floret to the entire flower and portion of a branch. The percentage of mutation and spectrum of mutation varied with the different doses. Mandal *et al.* (2000a) detected sectorial flower colour mutations in gamma irradiated plants in chrysanthemum. In gamma irradiated population somatic mutations in floret shape and flower colour were noticed (Datta *et al.*, 2005). Like our results the flower colour of *Dendranthema grandiflora* showed a great tendency towards becoming brighter than towards darkening in irradiated plants (Zalewska and Jerzy, 1997). Chimerism remains the main hurdle in mutation breeding of vegetatively grown crops (Beneteka, 1985). Laneri *et al.* (1990) obtained several flower mutants in gerbera by gamma irradiation in the form of chimeras. Miyazaki *et al.* (2002) isolated variegated flower mutant plants in *Petunia hybrida* by irradiation of Surfinia. As a result of irradiation flower shape varied in each cultivar (Yamaguchi *et al.*, 2003). Wide range of flower colour mutants have been reported not only largely including the outstanding characteristics of the original cultivars, but sometimes even with an appreciable improvement in quality and yield.

Flower colour modification is an obvious application of the technology as flower colour is one of the most important traits for flower breeders and in many species the whole spectrum of flower colours is not available. The genetic manipulation methods used to create potential new flower colours include creation of new flavonoid biosynthesis pathways by introducing genes encoding enzymes that are absent, redirection of pathways by over-expression of genes and/or suppression of pathways by down-regulation of genes. Abnormalities were also recorded in Monnalisa, It is imperative to mention that higher dose of gamma irradiation *i.e.,* 5.5 kR causes abnormalities in several varieties of gladiolus and

found lethal in case of cultivars. At higher dose of gamma rays some plants even failed to produce bud and flowers and remained in vegetative stage and in some species there was no flowering even at lower doses whereas none of the plants with this abnormality were observed in control. Flower heads became fascinated in different form. There was no dose specific fasciation. These abnormalities might be due to chromosomal aberrations and disturbances in the production and/or distribution of growth substances caused by gamma rays (Gunckel, 1957). The formation of fasciated heads after irradiation are in close conformity with the findings of Banerji and Datta (1992) and Dwivedi *et al.* (2000). These abnormalities are genotype dependent and depends on the mechanism involved in the repair of radiation induced damage within the organism (Datta, 1985).

In M_1 generation, most of the mutations were in the form of chimeras and the size of mutated sector varied from cultivar to cultivar from narrow streak on petal to entire petal, a single ray floret on flower to more than one ray floret to whole flower. The results are in parallel line with the findings of Broertjes and Ballego (1967) and Das *et al.* (1978) who reported a great number of mutations for flower colour and shape in the irradiated cultivars. It is clear that radio-sensitivity in different ornamentals is a genotype dependent mechanism. Flower colour is related with different pigment formation pathways and because every variety had a different colour change was different. Like mutation frequency, the spectrum of mutation also varied with cultivars and dose of gamma rays.

Exposure to ionizing radiations had caused little disturbances in pigment synthesis might be the reason for different shades of floret colours in rose cultivars but stable and solid mutants might be due to changes at DNA level. The reversion of some mutants in the original colour shows that changes were merely due to physiological disturbances caused as a result of radiations. These findings are in close conformity with the findings of Datta (2009), who reported that mutations are also detected in M_1 generation. Several cycles of propagation are needed to obtain homo-histones or to dissolve chimeras and to obtain solid mutants in vegetatively propagated plants after mutagen treatment (Ahloowalia

and Maluszynski, 2001). It is well apparent that flower colour is controlled by many genetic and physiological factors. These factors modify the pigment and produce co-pigment or change the cell condition in such a way as to produce a wide range of one type of pigment. Three major pigments (betalain, carotenoid and anthocyanin) are responsible for visual reflection of flower colour. Among them anthocyanin is associated with the majority of orange, red purple and blue hues (Kim *et al*., 2007). Yellow colour of gladiolus florets is due to carotenoids and or chlorophyll (Takamura and Miyajima 1996). The results are also in agreement with the irradiated corms of four cultivars of gladiolus and explained that flower colour was associated with corresponding change in anthocyanin content. Reduction in anthocyanin pigments in the petals was explained due to certain disturbances in the synthesis of these pigments.

The different doses of gamma rays resulted in variations in leaves such as leaf colour, shape, margins, or leaves with less chlorophyll content. Gamma irradiation of budwoods induced different types of abnormalities in leaf especially during the early period of growth. Mutants in leaves were obtained in Monnalisa and Demestra. Variants in the irradiated plants may be due to chromosomal aberrations, disturbance in the production and/or distribution of growth substances, break down of phosphate metabolism and accumulation of free amino acids (Gunckel and Sparrow, 1961). Datta (1988) also reported that the basic cause of abnormal plant growth is associated with non-heritable physiological disturbances of growth substances, changes in enzyme activity, variation in ascorbic acid concentration, breakage of phosphate metabolism, accumulation of free amino acids etc. Development of chlorophyll variegation in leaves or dark green leaves may be due to irregular distribution of chlorophyll in the leaves due to mutagenic treatment (Datta and Basu, 1977), Gunckel and Sparrow, 1961, Banerji and Datta (2001) and Zargar *et al.* (1994) observed similar results.

5.3. MOLECULAR CHARACTERISATION

In the present study, the mutants were phenotypically different from their parents with respect to the morphological characters studied and flower colour. To clarify to what extent this phenotypical variation was related to the genetic level, the present RAPD analysis was carried out. Noticeable differences between parents and their mutants were observed at genetic level.

RAPD bands produced from three genotypes (Demestra, Pink Panther and Monnalisa) and the mutants of Monnalisa *i.e.,* M_1, M_2, M_3 and M_4 were used to assess genetic variation. A total of 12, 24 and 20 bands were generated in the three genotypes that is Demestra, Pink Panther and Monnalisa respectively. On an average, the size of bands ranged from 300- 800 bp in Demestra, in Monnalisa it is 400-900bp and in Pink Panther it is 300-800bp. Parental polymorphism results showed that primer OPN-2 produced a band at 200bp. Mohapatra and Rout (2005) have reported similar results in roses that OPN-2 amplified at 100-270bp. In OPD-8 Demestra showed amplification at 300-800bp, in Pink Panther at 400-800bp and in Monnalisa at 600-800bp whereas Mohapatra and Rout (2005) reported that amplification was around 200-260bp in rose genotypes. This investigation is an understanding of the level and partitioning of genetic variation within the cultivars which would provide an important input into determining appropriate management strategies. This study will help in future breeding programs in roses.

RAPD is a useful technique for the rapid and easy assessment of genetic variation of mutants and may become a potential tool for the quick selection of mutants with great genetic variation during early growth stages (Teng *et al.*, 2008). In primer OPA-4, the total number of amplicons are 60, the percentage of polymorphism is 100%. Size of amplified bands ranged from 300bp to more than 3 kb. Shufang *et al.* 2010 reported that each sample of tetraploid locus produced a total of 63 bands with an average frequency of 3 bands per primer and the amplified products ranged in size from 564 bp to 3,530 bp.

In primer OPD-8, the total number of amplicons which was present was 50, the percentage of polymorphism was 80%. Size of amplified bands ranged from 300bp -1.5kb. Similarly Mohapatra and Rout, (2005) assessed the genetic relationship among 34 cultivars of rose with the aid of 4 RAPD series (A, B, D and N), chosen primers created 162 bands which fell in the range of 100 bp to 3400 bp in size. It was shown that single nucleotide substitutions in ten decamer primers indeed can be reflected on the amplicons profiles. The main changes observed in the RAPD profiles have resulted both in an appearance or disappearance of different bands with variation in their intensity as well. These effects might be connected with structural rearrangements in DNA caused by different types of DNA damages.

Thus, RAPD method is applicable for the detection of changes in the DNA structure after gamma treatments. The variation in band intensity and disappearance of some bands may correlate with level of photoproducts in DNA template after gamma ray treatment, which can reduce the number of binding sites for *Taq* polymerase. Appearance of new bands can be explained as the result of different DNA structural changes (breaks, transpositions, deletions etc). The present RAPD analysis can be used not only for estimating genetic diversity present in different floricultural crops but also for correct identification of mutant/new varieties for their legal protection under plant variety rights.

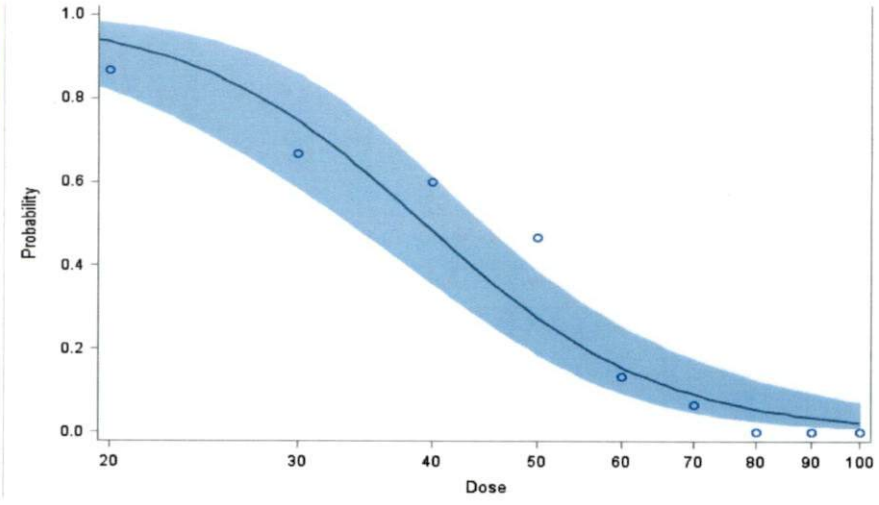


Figure 1. LD $_{50}$ of gamma rays in Pink Panther

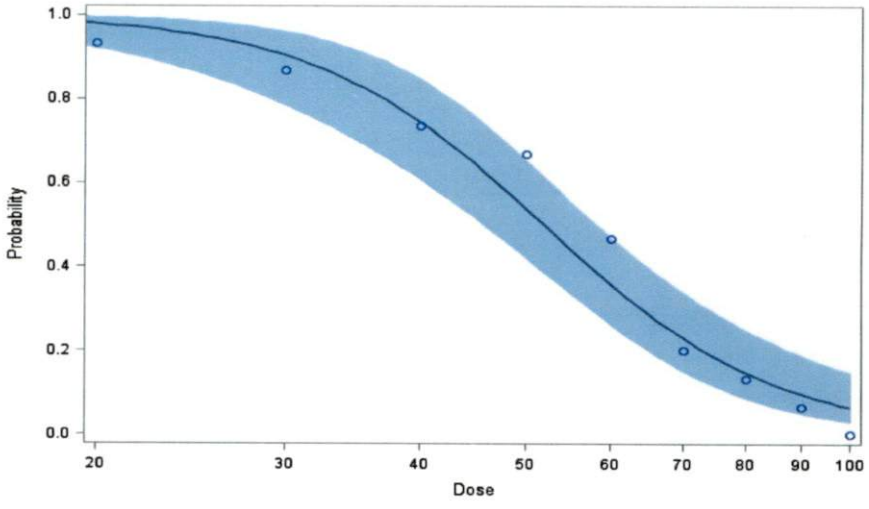


Figure 2. LD $_{50}$ of gamma rays in Monnalisa

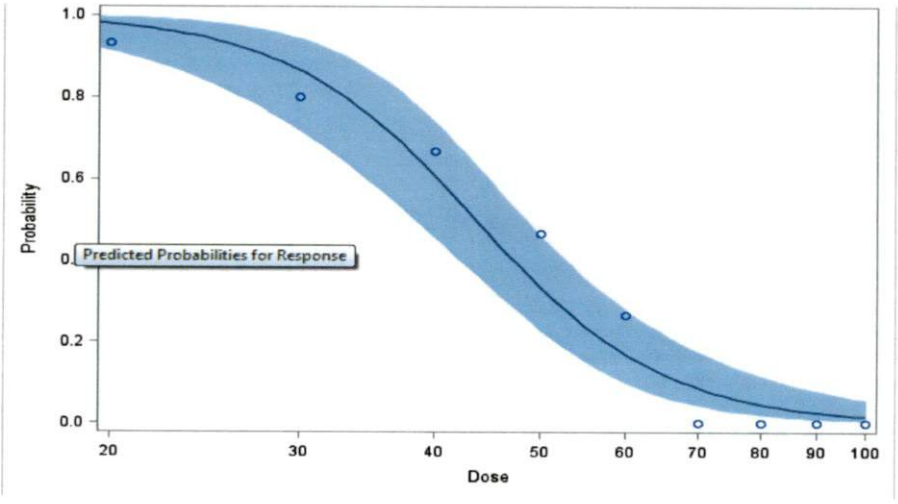


Figure 3. LD $_{50}$ of gamma rays in Demestra

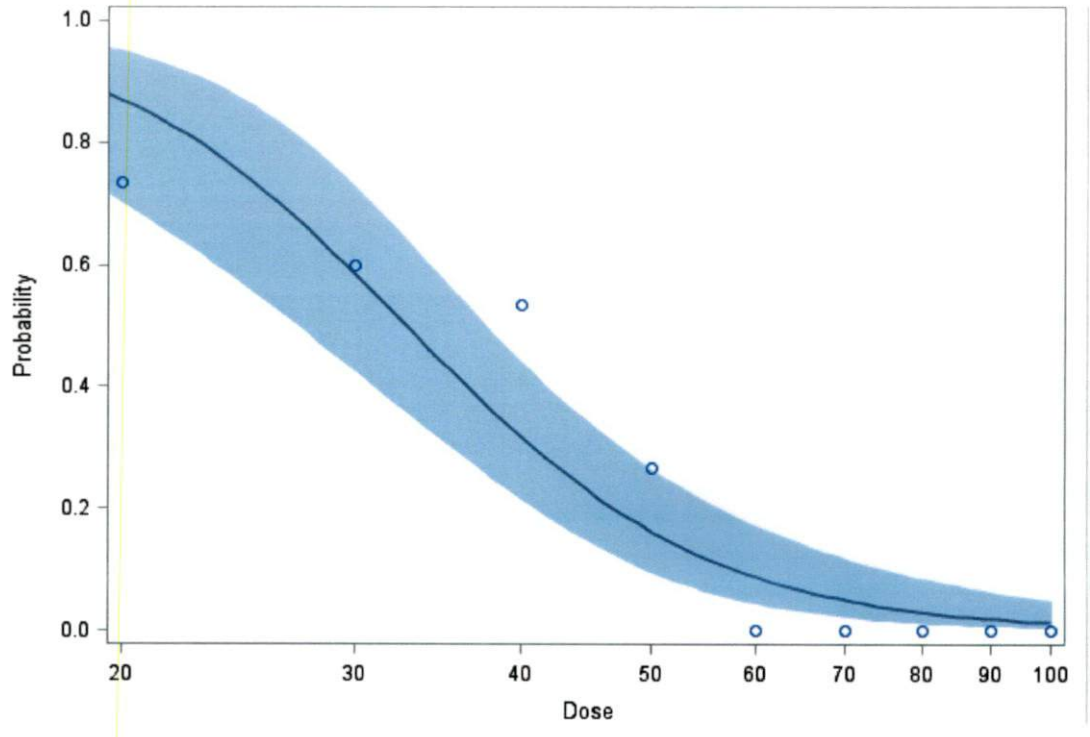


Figure 4. LD_{50} of gamma rays in Golden Fairy

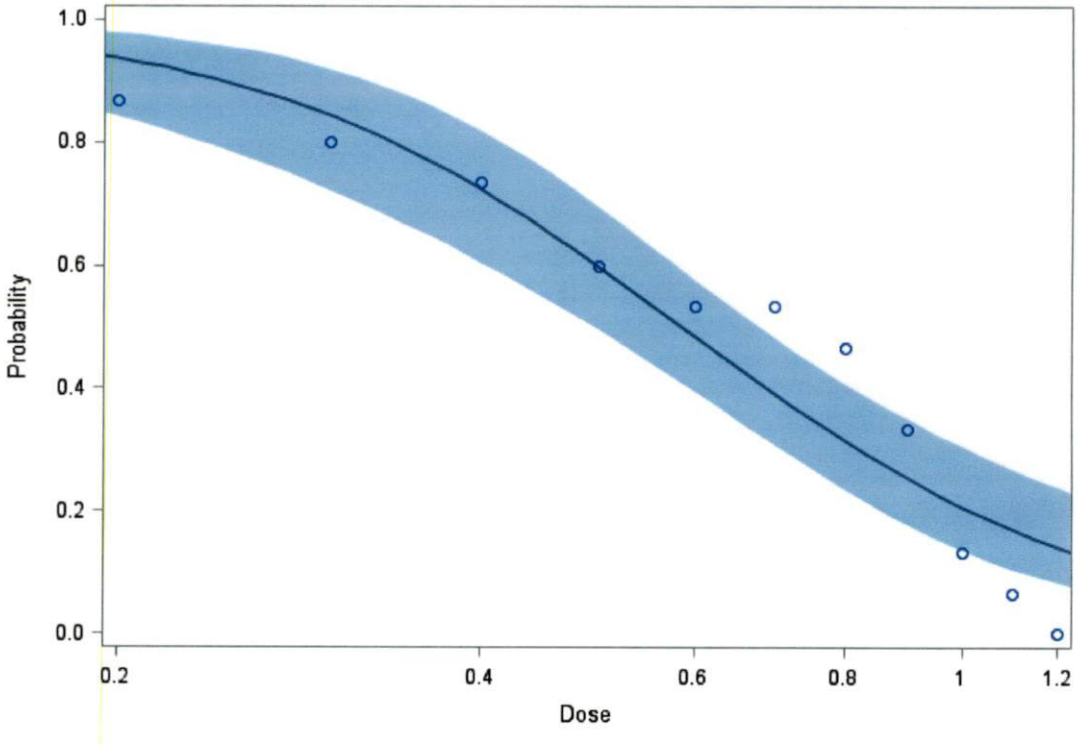


Figure 5. LD_{50} of EMS treatment in Schloss Elutin

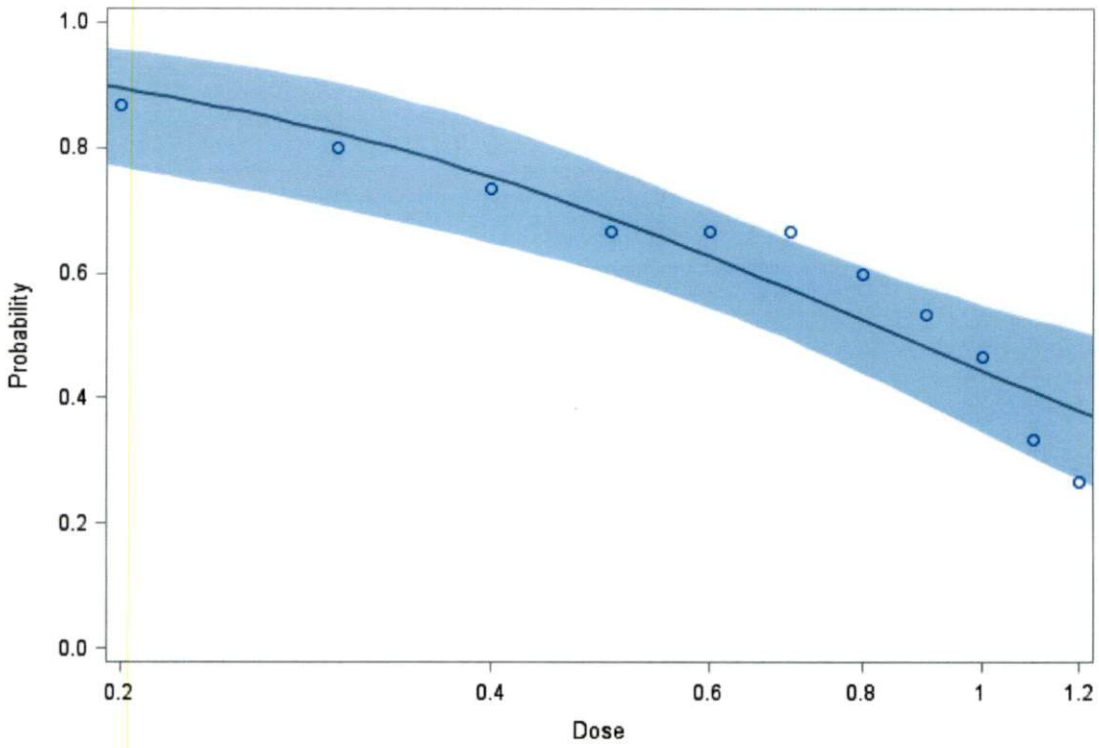


Figure 6. LD_{50} of EMS treatment in Jogan

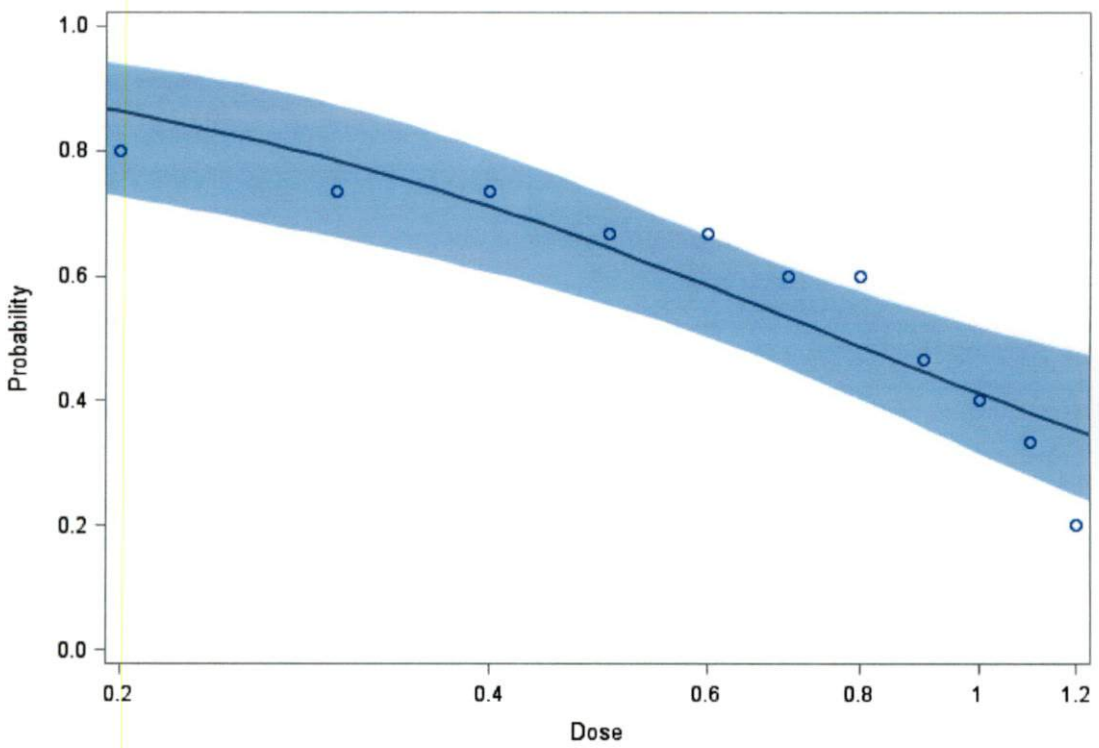


Figure 7. LD_{50} of EMS treatment in Josepha

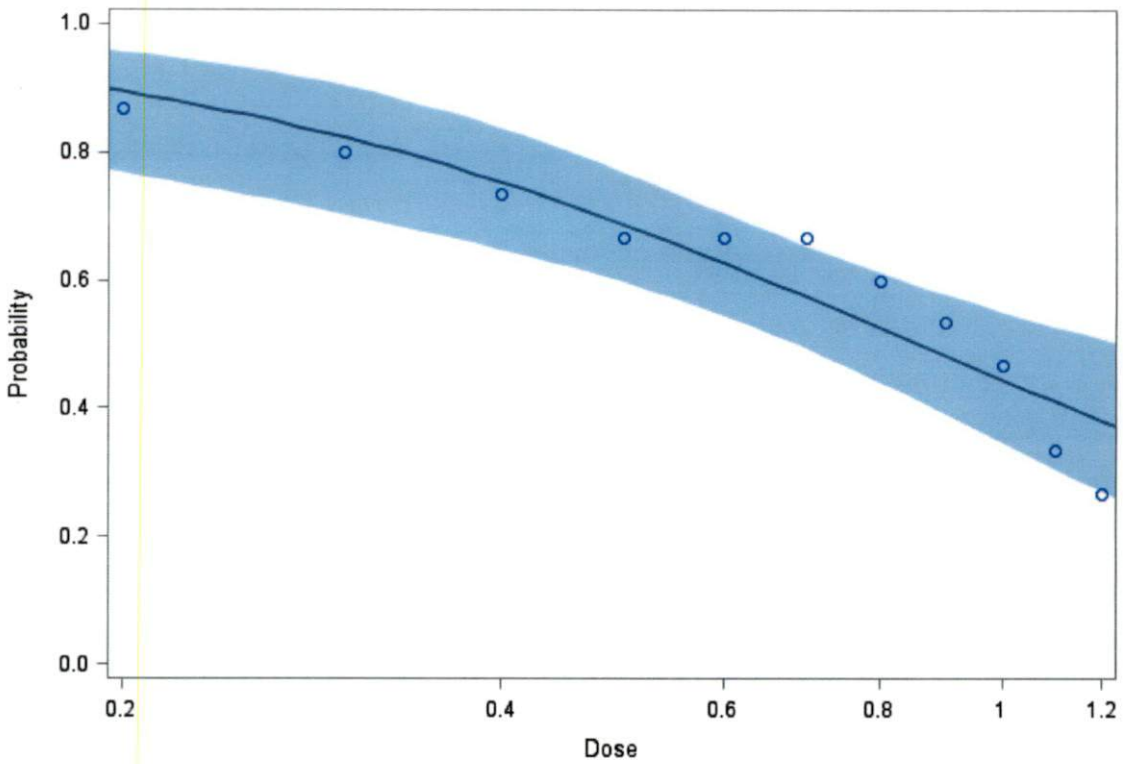


Figure 8. LD_{50} of EMS treatment in Morning Sun

Summary

6. SUMMARY

The present investigation entitled "Characterisation and genetic improvement in Rose (*Rosa* spp.) through mutagenesis" was carried out in College of Agriculture Vellayani and RARS, Ambalavayal during the period 2014-2017.The major objective of the study was to assess the natural variability available in Hybrid Tea and Floribunda groups of roses and to analyze the effectiveness of gamma rays and Ethyl methane sulphonate (EMS) on inducing variability in them for improved plant architecture and floral characters.

The first part of the programme consisted of evaluation and characterization of germplasm of Hybrid Tea and Floribunda groups of roses. For assessing genetic variability and diversity, twenty five genotypes under each group were studied with respect to thirteen morphological characters. The analysis of variance revealed highly significant differences in genotypes for all the characters studied in both the groups indicating the presence of genetic variability for most of the traits. In Hybrid Tea accessions, Highest values of PCV and GCV were obtained for number of petals per flower, size of petals and number of flowers per plant and prickle density. In Floribunda accessions, high estimates were obtained for number of days to first flower and prickle density, moderate estimates for number of leaves at first flower and flower weight. This indicated the existence of comparatively high variability for these traits, which could be exploited for improvement of the traits through selection. In Hybrid Tea, prickle density, number of days to first flower, number of leaves at first flower and number of petals per flower whereas, in Floribunda flower size, number of days to first flower, number of leaves at first flower and number of petals per flower exhibited higher heritability coupled with high genetic advance as per cent of mean. This indicated the scope of selection in the population, since there is a wide range of variation and additive gene action.

The twenty five rose genotypes each under Hybrid Tea and Floribunda were grouped into ten and nine clusters respectively on the basis of Mahalanobis

D^2 statistic. In Hybrid Tea, cluster I and II contained five genotypes each, while clusters III and IV had three genotypes each. Two genotypes were present in clusters V, VI and VII, whereas clusters VIII, IX and X had one genotype each. On the other hand, 25 genotypes of Floribunda were grouped into nine clusters. Cluster I contained ten genotypes and four genotypes belonged to cluster II, while clusters III and IV had three genotypes each. Cluster V, VI, VII, VIII and IX had one genotype each. It would be desirable to attempt hybridization between genotypes belonging to distant clusters to obtain highly heterotic crosses. Inter cluster distances were worked out in Hybrid Tea considering nine characters and the inter cluster distance varied from 349.30 (between clusters IX and VII) to 8159.59 (between clusters X and V). In Floribunda, the inter cluster distance varied from 387.40 (between clusters IV and II) to 2647.54 (between clusters VIII and III). These results suggest the presence of wide diversity between these clusters. Therefore, genotypes from these clusters can be selected for hybridization programme to get desirable heterotic recombinants.

In Hybrid Tea, the intra cluster distances indicated that cluster II had the maximum intra cluster distance followed by cluster I. The maximum inter cluster distance was found to be between clusters X and V. In Floribunda genotypes, cluster II had the maximum intra cluster distance followed by cluster I. The maximum inter cluster distance was found to be between clusters VIII and III, which indicates that considerable diversity existed among 25 genotypes. These results suggested the presence of wide diversity between these clusters. In both the groups of roses, it was observed that per cent contribution towards genetic divergence of number of days to first flower and number of leaves at first flower was maximum, followed by number of petals per flower and size of flower indicating the major role of these characters in determining the genetic diversity of genotypes.

In Hybrid Tea, the cluster means indicated that the genotypes in cluster X were earliest to flower whereas cluster VIII consisted of cultivars exhibiting highest values for number of days to first flower, flower size, flower weight and

pedicel length. Characters such as leaves at first flower, prickle density, size of petals and number of flowers per plant had moderate expression. In Floribunda, the cluster means indicated that the genotypes in cluster II were earliest to flower. Cluster VIII consisted of cultivars exhibiting highest values for prickle density, pedicel length and size of petals. Characters such as flower size and number of petals per plant had moderate expression. It was recorded that no single cluster was superior for all the characters observed.

The second part of the programme included induced mutagenesis using physical and chemical mutagens. The physical mutagen used was gamma rays. For fixing LD_{50} value, budwoods of four varieties *viz.,* Pink Panther and Demestra (both under Hybrid Tea) and Golden Fairy and Monnalisa (both under Floribunda) were treated with nine doses of gamma rays ranging from 20 to100 Gy along with control. The per cent plant survival and bud take (%) decreased with increase in dose of gamma rays. All varieties of roses exhibited this trend. LD_{50} values were fixed statistically considering percent survival and bud take (%) separately for each genotype. The LD_{50} statistic value for gamma rays was 39, 51, 43 and 32 Gy for Pink Panther, Monnalisa, Demestra and Golden Fairy respectively. The doses were fixed specifically based on LD_{50} values and were administered to the four different genotypes.

The M_1 plants were field planted and was evaluated for thirteen morphological characters. Exposure to gamma rays decreased flower size and weight in all the four genotypes and the reduction was directly proportional to the increase in dose. Pedicel length is an important character for all cut flowers, especially Hybrid Tea roses. A dose dependent reduction was observed for this character, which was not significant in several situations. Number of flowers produced per plant is always of economic significance. Exposure to gamma rays significantly reduced the number of flowers produced as compared to control. But the variation between doses was not significant in all the four genotypes. Mutagen sensitivity of the variety mainly determined the reduction in flower number. The reduction in number of petals per flower due to exposure to various doses of

gamma rays was drastic. The dose dependent reduction noticed was significant in two genotypes and not significant in two genotypes.

From the present analysis of original species and their gamma ray induced mutants it was found that in addition to change in flower colour or shape, significant changes in some morphological characters had also occurred in the mutants. Gamma ray induced new flower colour or shape mutants of the present experiment can be very useful in future practical breeding programme and the new mutants can also be directly used for floriculture industry after confirming their acceptance.

Chemical mutagen treatment was done using EMS on *in vitro* cultures of many genotypes *viz.,* Ancher Dom, Pink Panther, Monnalisa, Demestra, Golden Fairy, Sweet lady, Alliance, Schloss Elutin, Jogan, Josepha and Morning Sun. But finally four genotypes were carried upto the last stage namely, Schloss Elutin, Jogan, Josepha and Morning Sun.

The best *in vitro* culture medium for culture establishment comprised of MS+BA (2.0 mg/l)+ADS (25 mg/l).It was found to be the best medium with better explant survival, bud sprouting and minimum days to bud sprouting. This was proved to be the best medium for culture establishment. MS medium supplemented with BA (2.0 mg/l), +0.25 IAA (0.25 mg/l)+ADS (25 mg/l) produced the maximum number of quality shoots both in first and second subcultures. Hence this treatment combination was found to be the best for shoot proliferation in first as well as the second subcultures. Roots produced on half M.S+IBA (0.2 mg/l) +Sucrose (2%)+ BA (2.0 mg/l) media were sturdier and more suitable for rooting than the roots on MS medium devoid of auxins. Half MS medium containing 2% sucrose gave better results over MS without auxins with maximum number of quality roots. Supplementation of IBA and BA to the half MS medium recorded the highest rooting percentage, more number of roots with maximum length and earlier rooting as compared to other treatments and control. Meristem regeneration

and survival percentage decreased as the dose of EMS increased. Hence it was considered as the best rooting medium.

In vitro cultures of genotypes *viz.,* Schloss Elutin, Jogan, Josepha and Morning Sun were treated with twelve doses of EMS ranging from 0.1 to 1.2% along with control for three durations (30,60 and 90 minutes) for fixing LD_{50} value. Maximum survival was recorded in 30 minutes treatment and hence it was selected. The doses were fixed specifically based on LD_{50} values and were administered to the four different genotypes. The LD_{50} value for EMS treatment was 0.58, 0.85, 0.77 and 0.85 for Schloss Elutin, Jogan, Josepha and Morning Sun respectively. Five doses including control were administered to the different genotypes and field planting of M_1 was done. EMS treated *in vitro* derived M_1 was evaluated for thirteen morphological characters.

EMS treatment reduced flower size and weight in all the four genotypes studied. The reduction was proportional to the increase in dose, though not significant in most of the cases. Pedicel length also followed the same trend. The characters number of petals per flower and petal size decreased due to EMS treatment and the reduction was dose dependent. The reduction in number of petals per flower was not as drastic as in gamma ray treatment. Reduction in petal size was significant in all the four varieties studied. Number of flowers produced per plant decreased significantly with EMS treatment. The decrease was directly proportional to the increase in dose.

Induced mutagenesis, both physical and chemical, resulted in variations in form and colour of flowers and leaves in M_1.Variations in form of flowers was recorded in Pink Panther, Demestra and Monnalisa. Out of these, the variation in form isolated in Pink Panther mutant at 20 Gy is a novelty. The mutant had very shapely semi-open flowers in contrast to the fully open flowers of the parent. Demestra at 30 Gy showed form and colour variation. In one of the variants, the flower was found to be star shaped with golden yellow colour in contrast to the

light yellow with dark golden center in the parent. In Monnalisa, the control flower was red in colour, whereas the mutant at 50 Gy had red colour with white streaks at the center. In 70 and 80 Gy doses abnormalities in flower form were recorded in the variety. Among the mutants derived, leaf colour variants were isolated from Demestra at 30,40 and 50 Gy and Monnalisa at 40, 50 and 60 Gy. Under EMS treatment, flower colour variation was observed in Schloss Elutin. Control flower was dark Creamy in colour whereas the mutant derived at 0.5% was creamy white in colour with pink shading at the tips of outer petals. In Josepha, control flower was pink with white colour in the center whereas the mutants at 0.8% and 0.9% were with creamy -white center and deep pink outer petals. No seed setting was observed in M_1.

In the present experiment, RAPD analysis was used for molecular characterisation of three parent cultivars *viz.,* Pink Panther, Demestra and Monnalisa and four morphological mutants in Monnalisa *viz.,* M_1, M_2, M_3 and M_4. Genomic DNA was extracted from leaves of rose plants. The DNA yield of three genotypes ranged from 350-600 ng/µl and its purity ranged from 1.75-2.00 µg/µl. In Monnalisa mutants M_1, M_2, M_3 and M_4 the DNA yielded was 350-500 ng/µl and its purity ranged from 1.60-2.00 µg/µl. RAPD analysis of the selected parental rose genotypes and mutants was done. Ten decamer primers were selected based on previous reports. These markers were used for parental polymorphism in the selected three genotypes. Among them, four primers *viz.,* OPA-4, OPD-8, OPV-10 and P-4 showed high polymorphism. In OPA-4, Demestra produced two polymorphic amplicons. Pink Panther produced the maximum number of seven amplicons ranging from 500-900bp and Monnalisa produced four amplicons. OPD-8 in Demestra produced five amplicons and a highly unique polymorphic band was observed in 300-400bp. Pink Panther and Monnalisa produced five amplicons each ranging from 400-800bp. In these, two decamer primers were identified for RAPD analysis. Primers that produced highest number of intense, polymorphic and reproducible bands were selected. They were OPA4 and OPD8 which were used for further screening of mutants.

Mutants derived from the cultivar Monnalisa that is M_1, M_2, M_3 and M_4 were selected based on morphological characteristics. These mutants were subjected to molecular analysis. The two primers which were used to amplify Monnalisa and its mutants are OPA-4 and OPD-8. In the primer OPA-4, the total number of amplicons present in both control and mutants were 40 and the percentage of polymorphism is 100%. Size of amplified bands ranged from 900bp to more than 3 kb. In the primer OPD-8, the total number of amplicons present was 50, the percentage of polymorphism was 80%. Size of amplified bands ranged from 300bp -1.5kb.

From the research work, it can be pointed out that a high degree of variability existed in Hybrid Tea and Floribunda groups of roses. The diversity was analysed and classified by Mahalanobis D^2 statistics. It will serve as a reference material in future studies aimed at the utilization of valuable germplasm in rose improvement. The gamma ray and EMS treatments induced variations in form and colour of flowers and colour of leaves. Among all the M_1 mutants obtained, six colour variants *viz.*, in Schloss Elutin at 0.5% in Josepha at 0.8%, 0.9% and 1.0% in Demestra at 30 Gy and in Monnalisa at 50 Gy and two form variants one in Pink Panther at 20 Gy and one in Demestra at 30 Gy were strikingly different from control. Out of these, three flower colour variants *viz.,* in Demestra at 30 Gy, in Schloss Elutin at 0.5% and in Josepha at 0.8% and one flower form variant in Pink Panther at 20 Gy were found to be promising. The M_1 mutants isolated could be carried forwarded by *in vitro* means by taking explant from the plant part showing variation to see if solid mutants can be developed. These may be released directly as varieties or utilised in further breeding programme. On the basis of the present study, it can be concluded that induced mutagenesis technique is a better option for improvement of rose and other vegetatively propagated crops as it offers the opportunities for crop improvement within a shorter span of time as compared to conventional methods. This study adds to the ongoing efforts to increase diversification in flower colour and size of

rose flowers. The outcome of the study will be helpful for future studies regarding induction of mutations in rose. In RAPD analysis parental polymorphism was detected and clearly demonstrated the existence of genetic variation within the three rose genotypes. It also showed noticeable differences and polymorphism present between parents and their mutants in the cultivar Monnalisa. Thus the phenotypic variation found in the mutants of Monnalisa was confirmed at genetic level.

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CHARACTERISATION AND GENETIC IMPROVEMENT IN ROSE (*ROSA* SPP.) THROUGH MUTAGENESIS

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ABSTRACT

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ABSTRACT

The present investigation entitled "Characterisation and genetic improvement in Rose(*Rosa* spp.) through mutagenesis" was carried out in College of Agriculture Vellayani and RARS, Ambalavayal during the period 2014-2017.The major objective of the study was to assess the natural variability available in Hybrid Tea and Floribunda groups of roses and to analyze the effectiveness of gamma rays and Ethyl methane sulphonate (EMS) on inducing variability in them for improved plant architecture and floral characters.

The first part of the programme consisted of evaluation and characterization of germplasm of Hybrid Tea and Floribunda groups of roses. For assessing genetic variability and diversity, twenty five varieties under each group were studied with respect to thirteen morphological characters. ANOVA revealed highly significant differences among the genotypes for all the traits analysed. In Hybrid Tea accessions, moderate values of PCV and GCV were obtained for number of leaves at first flower, number of petals per flower, size of petals and number of flowers per plant. In Floribunda accessions, high estimates of PCV and GCV were obtained for number of days to first flower and prickle density. High heritability coupled with high genetic advance as percent of mean was observed for number of days to first flower, number of leaves at first flower and number of petals per flower in accessions belonging to Hybrid Tea group whereas in Floribunda group, flower size, number of days to first flower, number of leaves at first flower and number of petals per flower exhibited high heritability and genetic advance.

The twenty five rose genotypes each under Hybrid Tea and Floribunda were grouped into ten and nine clusters respectively on the basis of Mahalanobis D^2 statistic. The higher number of clusters obtained under each group is an indication of the genetic diversity within the group. In both the groups of roses, it was observed that the characters number of days to first flower and number of leaves

at first flower contributed maximum towards genetic divergence. Cluster means of economically important characters were determined.

The second part of the programme included induced mutagenesis using physical and chemical mutagens. The physical mutagen used was gamma rays. For fixing LD_{50} value, budwoods of four varieties *viz.,* Pink Panther and Demestra (both under Hybrid Tea) and Golden Fairy and Monnalisa, (both under Floribunda) were treated with nine doses of gamma rays ranging from 20 to100 Gy along with control. The doses were fixed specifically based on LD_{50} values and were administered to the four different varieties. M_1 was field planted and was evaluated for thirteen morphological characters. Statistical analysis of quantitative characters showed that for an increase in gamma ray doses, a proportionate significant decrease for various plant growth parameters was evident. Mutants in plant architecture, leaf characters and floral characters were also recorded.

Chemical mutagen treatment was done using EMS on *in vitro* cultures of four varieties *viz.*, Schloss Elutin, Jogan, Josepha and Morning Sun. Regeneration protocols for nodal explants were first standardized. The best *in vitro* culture medium for culture establishment comprised of MS+BA(2.0 mg/l)+ADS(25 mg/l).The best medium for shoot proliferation was BA(2.0 mg/l)+IAA(0.25 mg/l)+ADS(25mg/l) and the best medium for root induction was identified as ½ M.S+IBA(0.2 mg/l)+sucrose(2%)+BA(2.0mg/l). *In vitro* cultures of genotypes *viz.*, Schloss Elutin, Jogan, Josepha and Morning Sun were treated with twelve doses of EMS ranging from 0.1to1.2% along with control for three durations (30,60 and 90 minutes) for fixing LD_{50} value. Maximum survival was recorded in 30 minutes treatment and hence it was selected. The doses were fixed specifically for the four varieties based on LD_{50} values. Five doses including control were administered to the different varieties and field planting of M_1 was done. EMS treated *in vitro* derived M_1 was evaluated for thirteen morphological characters. Most of the vegetative and floral parameters showed a decrease in mean values with increase in doses of mutagen.

Induced mutagenesis, both physical and chemical, resulted in variations in form and colour of flowers and leaves in M_1.Variations in form of flowers was recorded in Pink Panther, Demestra and Monnalisa. Out of these, the variation in form isolated in Pink Panther at 20 Gy is a novelty. The mutant had very shapely semi-open flowers in contrast to the fully open flowers of the parent. Demestra at 30 Gy showed form and colour variation. In one of the variants, the flower was found to be star shaped with pale orange colour in contrast to the pale yellow with dark golden yellow centre in the parent. Among the mutants derived, two leaf variants were also isolated from Demestra and Monnalisa through gamma rays. In EMS treatment, Schloss Elutin and Josepha yielded desirable colour mutants. No seed setting was observed in M_1.

RAPD analysis of the selected parental rose varieties and mutants was done. Based on the presence or absence of polymorphic bands, variation was detected at the molecular level. Ten RAPD decamer primers were used to check parental polymorphism. Among them, two primers *viz.,* OPA-4 and OPD-8 that were highly polymorphic. Polymorphism between the parent cultivar Monnalisa and its mutants could be demonstrated using two polymorphic primers *viz.,* OPD-8 and OPA-4.

In conclusion, the extent of variability in Hybrid Tea and Floribunda groups of roses was found to be high as is evident from clustering based on Mahalanobis D^2 statistic. Both gamma rays and EMS could induce variations in form and colour of flowers and colour of leaves. Among the mutants derived three flower colour variants *viz.,* in Demestra at 30 Gy, Schloss Elutin at 0.5% and Josepha at 0.8% and one flower form variant in Pink Panther at 20 Gy were found to be promising and were carried forwarded to the M_1V_1 generation by budding. The M_1 mutants isolated can be carried forwarded by *in vitro* means by taking explant from the plant part showing variation to see if solid mutants can be developed. These may be released directly as varieties or utilised in further breeding programme.