GENETIC IMPROVEMENT THROUGH INDUCED MUTATION IN DAHLIA
(*Dahlia variabilis* Desf.)

by

MANU R.
(2015-11-109)

THESIS

Submitted in partial fulfilment of the

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DECLARATION

I, hereby declare that this thesis entitled **"GENETIC IMPROVEMENT THROUGH INDUCED MUTATION IN DAHLIA (*Dahlia variabilis* Desf.)"** 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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LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA	Analysis of Variance
&	and
%	Per cent
CD	Critical difference
cm	Centimetre
et al.,	And others or co-workers
Fig.	Figure
g	Gram
ha	Hectare
ha^{-1}	Per hectare
KAU	Kerala Agricultural University
Gy	Gray
krad	Kilo rad
m	metre
Krad	Kilo rad
NS	Non -significant
No.	Number
$Plant^{-1}$	Per Plant
Sl.	Serial
cv	Cultivar
Kr	Kilo rad

LD	Lethal dose
C0-60	Cobalt-60
WM	White mutant
PM	Purple mutant
i.e.,	That is
viz.,	Namely
SE	Standard error

Introduction

1. INTRODUCTION

Flowers are the nature's best creation to express human sentiments. Whether it is love or envy, happiness or sorrow, friendship or courtship, there are flowers for every occasion, thought and emotion.

Floriculture is the branch of horticulture that deals with the cultivation of flowering and ornamental plants for sales or for use as raw materials in cosmetic industry. Demands for floricultural products are steadily increasing both in the domestic as well as export markets. India has made significant improvement in the production of flowers, particularly cut flowers, which have good potential for export. Globally more than 145 countries are involved in floriculture industry and the global floriculture trade. In India, commercial floriculture gained momentum in the 1990's. The development of Indian commercial floriculture has centered around the production of Rose, Marigold, Gerbera, Chrysanthemum, Gladiolous, Anthurium, Carnation, Orchid, Tuberose, Lilium, Alstroemeria etc. (Misra and Ghosh, 2016). Area under flower crops in India is 309.26 ha and production of cut flowers is 593 MT and of loose flowers is 1653 MT. The area under flower crops in Kerala is around 26.56 ha with cut flowers production of 0.03 MT and loose flower production of 0.67 MT in the year 2015.

Dahlias (*Dahlia variabilis* Desf.) are one of the most important perennial herbaceous flowering and tuberous rooted plants, known for their gorgeous attractive spectacular flowers. This plant is being grown in many parts of the world for its beautiful blooms of various shades of colour for the gardens, for cut flowers and as a loose flower. Dahlias are easy to grow both in open field and in pot. All types of dahlias are widely used for exhibition, garden display and home decoration. Dwarf growing types are mostly suitable for flower beds and borders, whereas large flowering types can be grown in pots as well as in field conditions. They are popular

for terrace garden or verandah display and long stemmed flowers of various forms and colours are used in flower arrangements. Cut flowers of pompon and miniature types stay fresh in flower vases for many days and are good to make moderately good garlands and as individual loose flower. This tuberous rooted, half-hardy herbaceous perennial belongs to the family Asteraceae. Dahlia originated in Mexico and was named by Cavanilles in the year 1791, to commemorate the work of a Swedish Botanist Dr. Andreas Dahl, a pupil of Linneaus. The aerial plant part die each winter and are replaced by new growth from ground level in favourable climate spring. In less favourable climates the dahlia is mainly treated as an annual (Hammett, 1980).

Among the twelve species of dahlia, the commonly found eight species *viz*., *Dahlia variabilis, D. imperialis, D. exelsa, D. coronata, D. coccinea, D. merkii, D. zuarezii* and *D. rosea* are generally important. Out of these eight species, *D. variabilis* and *D. rosea* are of horticultural importance, which include showy, fancy types, anemone flowered, cactus and semi cactus types, peony, decorative, ball types, fimbriated, water lily, star types and also the parents of most of the pompon cultivars.

The present day *Dahlia variabilis* varieties are higher order hybrids with several species in their lineage. *Dahlia coccinea, Dahlia imperialis* and *Dahlia pinnata* are the major contributors. Chromosome numbers of cultivated dahlias vary from diploid (2n=16) to tetraploids and octaploids along with different types of aneuploids (Darlington, 1973). Giannasi (1975) has concluded that cultivated dahlias are mostly tetraploids. Sorensen (1969) has commented upon "an innate tendency to mutate in *Dahlia coccinea",* the chief progenitor of *Dahlia variabilis.* Originally, the flower was single and with few bored ray florets and the colour range was white, red, pink and purple. In modern day dahlias, however, the colour change range includes white, cream, yellow, orange, bronze, pink, red, lavender and purple as single colours, or combination of two or more colours with a wide range of shapes and growth habits (Rowell, 1981). Propagation of dahlia is by seeds, tubers, or stem cuttings from a shoot or sprouted tubers. All modern dahlia varieties are propagated

vegetatively to ensure true-to-type progeny. Seed progeny never breed true due to inherent self-sterility (75%), high heterozygosity and polyploidy (Vinayananda, 1985). Small scale vegetative propagation is through tuber separation and large scale propagation is through sprout cuttings.

The flower of dahlia is a capitate inflorescence consisting of a swollen receptacle with a few hundred florets, surrounded by involucral bracts. The florets are of two types, the outermost florets are called ray florets, distinguished by being female or neutral, and corolla is extended on one side to form a coloured ligule and the central florets called disc are hermaphrodite in nature and have a symmetrical corolla divided into five teeth. The inflorescence can be classified as either 'single', in which an outer ring of coloured, showy florets surrounds a central disc of smaller, yellow florets, or 'double', in which coloured florets predominate. Single flowered dahlia has a single row of ray florets surrounding the central disc. In semi-double flowers, more than one row of ray florets occurs and the central disc remains visible (Hammett, 1980). There are many sizes and shapes of dahlia which makes it almost impossible to characterize them. However there are some classifications done by National Dahlia Society and Royal Horticultural Society, England such as anemone flowered, collarette, paeony-flowered, formal decorative, informal decorative, showy, pompon, cactus, semi-cactus and star shaped (Hammett, 1980).

Dahlias are very easy to grow either in the pot or in the field and are extensively used for exhibition, garden display and home decoration. Certain species of dahlias have medicinal and nutritional uses. Tubers of this plant contain significant amount of insulin and fructose and small quantities of medicinally active compounds such as phytin and benzoic acid. Insulin extract from tuber of dahlia is used in diagnosis of renal function. Seeds are a good source of fats and proteins which also contain more than 16 per cent oil and 20.9 to 47.0 per cent protein. The root exudate is nemato toxic.

In floriculture industry, there is a constant demand for novelty in existing crops. Development of new cultivars through conventional or modern techniques has been an objective in commercial floriculture industry. Though there are many species and many cultivars of dahlia with different number, placement, texture, colour and size of florets with different peduncle length, symmetry and vigour, there is still scope for improving these characters through breeding.

From the past 50 years, the use of induced mutation (both physical and chemical mutagens) has played a significant role in development of superior crop varieties. Mutation breeding has paved way to create genetic diversity and induce desirable characters in existing varieties or creating new genotypes altogether. The utilization of induced mutations for the improvement of crop plants has yielded several mutants which have been used directly as new cultivars (Gottschalk, 1983). As in traditional cross breeding, induced mutants are passed through several generations of selfing or clonal propagation, usually through *in vitro* techniques which leads to fixation of mutation event.

Mutation is a single cell event and different mutagens have different mechanisms of action. The possible genetic consequences on DNA, after mutagen treatment with radiation may be rupture of hydrogen bonds between base pairs, breaking phosphate bonds, double stranded breaks, single stranded breaks, breaking of other covalent bonds of the nucleotides and cross linkage between and within single strands.

Mutation breeding has become more successful in ornamental plants due to some additional advantages. Changes in any phenotypic characteristics such as colour, shape or size of flower and chlorophyll variegation in leaves can be easily detected. Effect of mutations in ornamentals is very visible, and selection for changed flower colour, shape, and size is easy. Almost anything novel is of value. Hence mutations are a major tool for breeding of ornamentals (Maluszynki *et al.*, 1995).The combined method of irradiation with floral organ *in vitro* cultures is of great use in

mutation breeding (Nagatomi and Degi, 2009). Furthermore, any ornamental species are heterozygous and are often propagated vegetatively thus allowing the detection, selection and conservation of mutants within M_1V_1 generation (Van Harten, 2002).In ornamentals, the artificially induced commercial mutant cv. 'Faraday', a flower colour mutant in tulip was released in 1949 in the Netherlands by W. E. De Mol from X-ray irradiated bulbs of cv. 'Fantasy', following irradiation in 1936. A second flower colour mutant cultivar in tulip cv. 'Estella Rijnveld' was released by the same scientist in 1954 (Van Harten, 2002).

In Dahlia, freshly harvested tubers are more suitable for irradiation. For many reasons *D. variabilis* must be considered as a promising species for mutation breeding. The high polyploidy and the great number of flower colour genes brings attention to this species (Broertjes, 1967). Flower colour and other distinguishable mutations ranging from dominant to recessive can be observed in the material due to the high degree of heterozygosity and vegetative propagation. Furthermore, genetic composition of a given cultivar is not altered significantly. For this reason, mutation breeding is an important way for developing new varieties. It is of crucial importance to irradiate the buds at the earliest possible stage of development. Irradiation should therefore take place immediately after harvest, when no visible eyes can be detected on these so-called dormant tubers.

Keeping in view the above facts the present study was carried out with the following broad objectives;

1. To induce variability in dahlia for plant architecture using gamma rays.
2. To induce variability in dahlia for floral characters using the same physical mutagen.

Review of Literature

2. REVIEW OF LITERATURE

Dahlia is one of the important flower crops of hill zone, getting importance in these days because of its multi coloured flowers with different shapes. Information on genetic architecture of various quantitative traits, particularly of those that contribute to growth, yield and quality would be most useful in planning breeding programme so as to make effective selections. There are many factors which account for variation in yields, as it is very much influenced by environment and genotypic potential of a crop plant. Therefore knowledge on the pattern of genetic variation will enable the breeder to know the magnitude of genetic variation available for selection and utilisation in further breeding programmes for future improvement.

Mutation is defined as a sudden heritable discontinous change in the characteristics of an organism. The term 'mutation' was introduced by Hugo de Vries in 1900. But mutations were known to occur in animals and plants much before this time. The discovery of mutagenic action of X-rays on Drosophila by Muller in 1927 and in barley by Stadler in 1928 initiated a new field of induced mutagenesis. The first commercial mutant cultivar, 'Chlorine' was obtained in tobacco after X-ray irradiation by Goodspeed in 1929. Mutation breeding involves the use of either spontaneous or artificially induced mutations for improvement of plant varieties. Among these, induced mutations have been used extensively in ornamentals to develop novelties. Mutations are generally recessive but dominant mutations also occur. Most of the mutations have deleterious effects, but a small portion of them are beneficial. Mutations are random, recurrent and have low frequencies in nature *i.e.*, they may occur in any gene and the same mutation may occur again and again.

Mutation breeding is an established method for crop improvement and has played a major role in the development of many new flower colour/shape mutant varieties in ornamentals. Induced mutagenesis has been used for crop improvement,

whereby plant genes are altered by treating seeds or other plant parts with chemical or physical mutagens. Voluminous work has been done worldwide for the improvement of both seed and vegetatively propagated crops through induced mutation.

The component of the present investigation concerning the induction of genetic variability through gamma radiations in dahlia (*Dahlia variabilis* Desf.) has been studied by few workers. It will be useful to review their works in dahlia as well as related ornamental flower crops. Some of the most relevant works have been presented for vegetative, floral and mutation attributes.

2.1 EFFECT OF VARIOUS MUTAGEN ON DAHLIA

Broertjes and Ballego (1967) irradiated tubers of a number of garden dahlia cultivars with various doses of X-rays. The optimal dose range was from 2 to 3 Kr, considering the production of rooted cuttings, the speed of rooting, the subsequent development of young plants as well as mutation frequency. A great number of mutations for flower colour and shape were observed in the irradiated varieties 'Salmon Rays, 'Arthur Godfrey' and 'Eldorado'. Four mutants of 'Salmon Rays' have been awarded, named, registered as new varieties and put on the market.

Five radiation induced mutants of the cultivar 'Arthur Godfrey,' a decorative dahlia with flaming red orange flowers of good form and habit have been commercialized as reported by Broertjes and Ballego (1968).

A spontaneous mutant for flower colour and shape in a white flowering dahlia was studied by Singh (1970). The mutant described arose in the cultivar 'White Pearl' and produced rosy-magenta flowers of a more attractive shape than those of the parent. Multiplication by softwood cuttings did not lead to any changes

in characters and it was suggested that a mutation or an inhibitor gene was responsible for the new type.

Das *et al.* (1980) conducted induced mutagenesis trials in fourteen dahlia cultivars. Gamma rays in doses upto 80 Gy were employed on tubers. Nineteen mutants were recorded. Out of these, eleven mutants have been released. The optimal dose for mutation induction was observed to be 20-30 Gy.

Tubers of fourteen leading varieties of dahlia were irradiated with gamma rays with doses from 1-8 Kr. The results indicated that with the increase in doses from 2 Kr there was decrease in growth of tubers. LD-50 was found to be in the range of 3-4 Kr and the optimum dose for the induction of mutation was observed to be 2-3 Kr. Maximum number of mutants were found at 2 Kr dose. Nineteen mutants were recorded. Out of these 19 mutants, 11 mutants were named for release (Das *et al.,* 1980).

Forkmann and Stotz (1984) studied the selection and characterization of flavonoids 3-hydroxylase mutants of dahlia, streptocarpus, verbena and zinnia. Precursor experiments and chromatographic studies indicate that the hydroxylation of flavanones in the 3-position to dihydro flavonols was blocked in special white-flowering mutants of dahlia, streptocarpus, verbena and zinnia. The activity of the enzyme flavanone 3-hydroxylase was completely absent in flower extracts of the corresponding acyanic mutants.

Tubers of nine cultivars of dahlia, corms of ten cultivars of gladiolus and bulbs of three cultivars of narcissus were treated with ^{60}Co γ radiation (doses were in the range 0-45 Gy) and were evaluated for eight vegetative and floral characters. After 5 Gy treatment four dahlia cultivars showed increased plant height (Misra, 1990).

Hamatani *et al.* (2001) gave N-heavy ion beam treatment (10 Gy) to small shoots (1 cm long) of dahlia (*Dahlia pinnata*) and observed vigorous growth of shoots *in vitro* as well as in the experimental field, and showed the highest frequency of mutations, e.g., floral diameter (3-12 cm), flower colour variation such as darker or paler coloured petals or white tipped petals. Flowering frequency declined with increasing heavy-ion beam dose and the same phenomenon was observed with gamma radiation treatment.

Dahlia cultivar 'Pinki' was irradiated with 0, 2.5, 5, 10 and 15 Gy gamma rays. Reduction in survival, plant height, leaf number and size, length of peduncle, flower size and floret number and size was observed after irradiation and with increase in dosage of gamma rays. Mutation appeared in foliage in the form of chlorophyll variegation and in the flower head in the form of white sectors in ray florets in V_1M_1 generation. The dosage recommended was 5 and 10 Gy gamma rays for induction of somatic mutations in dahlia cultivar 'Pinki' (Dwivedi and Banerji, 2008).

2.2 EFFECT OF VARIOUS MUTAGENS ON ROSES

Lata and Gupta (1971) exposed both bud woods (4 Kr) and stem cuttings of roses (2, 4, 6 and 8 Kr) to gamma rays. Effect of gamma rays on oil content of some scented cultivars of hybrid tea roses, survival, and cytological feature of original mutant cultivars including their breeding behavior was studied they concluded that flowers from irradiated plants were generally smaller and contained less oil than those of control.

Gamma irradiation induced colour mutations in rose cvs. 'Christian Dior', 'Queen Elizabeth' and 'Kiss of Fire' were obtained when dormant buds were treated with 5-10 Kr of gamma rays. 5 Kr was found to be 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 mutations with low petal numbers in cv. 'Kiss of Fire' (Kaicker and Swarup, 1972).

Bud woods of rose cv. 'Gulzar' were treated 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 strips and was released under the name 'Madhosh' (Kaicker and Swarup, 1978).

Bud woods of rose cv. 'Junior Miss' were irradiated with 3, 4 and 5 Kr gamma rays and these buds were budded on *Rosa indica* var. 'Odorata'. No mutation in flower colour was detected in the first year but one plant from the 3 Kr 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, 1982).

Datta and Gupta (1984) revealed that the white flowered 'Saroda' cultivar of rose was induced by exposing bud woods of cv. Queen Elizabeth to 3 Kr gamma rays. Another new cultivar produced very light pink flowers and was named 'Sukumari'. It was induced by exposing bud woods of cv. 'America's Junior Miss' to 3 Kr gamma rays. These two mutants detected in VM_1 were isolated and multiplied by repeated budding.

Bud woods of 9 cultivars were irradiated with gamma rays at 3, 4 and 5 Kr gamma rays and these buds were budded on *Rosa indica* var. 'Odorata' (Datta, 1985). Reduction in sprouting was observed. 'Orange Sensation' was the most sensitive and cv. 'Kiss of Fire', was the most resistant one. Reduction in height was also noted with

cv. 'Kiss of Fire' being the least and cv. 'Zambra' being the most affected. Somatic mutations in flower colour were noted in all cultivars except cv. 'Happiness'.

Beneteka (1985) irradiated rose single bud cuttings with 0, 20, 30, 40 and 60 Gy gamma rays and subsequently observed four bud-propagated generations 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.

Gamma radiations at 3 Kr 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.

Arnold *et al.* (1998) studied the effect of various gamma doses on four cultivars of miniature roses and found that radiation significantly decreased the number of spines per 10 cm stem length at 50 Gy and increased the mean petiole length at 50 and 100 Gy in 'Portluck' cultivar although 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 roses *i.e,* 'Portluck', 'Blue Blood', 'Mountie' and 'Dark Red Mountie'. None of the other plant growth parameters were affected by radiation doses. The length and width of the terminal leaflet was not affected by mutagen treatment in any of the cultivars.

A study was conducted on pigmentation in rose cv. 'Paradise' and its induced mutants by Shobha *et al.* (2002) and they reported that gamma irradiation (3 to 6 Kr) resulted in reduction in flavanol, anthocyanin and leucoanthocyanin contents.

The mutations in roses were mostly in flower colour 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 colour, higher oil content and better oil quality as reported by Datta and Chakraborthy (2005).

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. 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 colours were induced from 'Spidella' and 'Cabernet' when 30-170 Gy dose was employed. The mutants obtained from 'Spidella' had white, ivory, pinky ivory, light pink and deep pink petal colours. The mutants obtained from 'Cabernet' had pink, deep pink, purple red (magneta), orange red and purple petal colours.

Hybrid Tea rose (*Rosa hybrida* L.) cv. 'Pusa Mohit' was subjected to *in vitro* mutagenesis. Single node cuttings were irradiated with different doses of gamma rays (0, 5, 10, 15, 20, 25, 40, 55, 65, 70, and 80 Gy). 40 Gy treatment was determined to be the LD_{50} dose. Explants treated with higher doses showed deleterious effects of ionizing radiations (Madhubala and Singh, 2013).

2.3 EFFECT OF VARIOUS MUTAGENS ON CHRYSANTHEMUM

Rooted cutting of chrysanthemum cv. 'Anupam' were irradiated with 1.5, 2.0 and 2.5 Kr of gamma rays (Co_{60}) by Banerji and Datta (1990) and a significant reduction in survival, number of branches, number of leaves and number of flower heads and leaf and flower head size was recorded. The leaf and flower abnormalities increased upon irradiation over the control. They also observed significant delay in flower bud initiation, colour showing and full blooming in treated plants as compared

to control. Somatic flower colour mutations were also detected in VM_1 generation at 1.5 and 2.0 Kr doses of gamma rays.

Datta and Banerji (1995) carried out karyotype analysis of various chrysanthemum cultivars along with their gamma ray induced somatic flower colour and shape mutants. The change in flower colour or shape may be considered due to gene mutation. It was neither through change in chromosome number nor due to change in the karyomorphology. They also irradiated rooted cuttings of four chrysanthemum cvs. 'Bhima', 'Fish Tail', 'Lalima' and 'M-71' with 1.5, 2.0 and 2.5 Kr doses of gamma rays (Co-60) and observed significant reduction in plant survival, plant height, number of branches, leaves and flower head size. The foliage and floral abnormalities and days to first flower increased upon gamma irradiation. Promising somatic flower colour and shape mutations were detected in VM_1 generation which were released as new cultivars, 'Jugno' and 'Kumkum', respectively.

Data on survival, plant height and leaf, flower and floret number and pollen fertility. They observed increased abnormalities in foliage, floral and stomatal characters after irradiating rooted cuttings of *Chrysanthemum morifolium* (*Dendrathema morifolium*) cv. 'Navneet' with gamma rays at 1.5, 2.0 and 2.5 and 2, 4, 6 and 8 Kr was studied by Banerji *et al.* (1996). Percentage survival in cv. Navneet dropped from 83 to 33 percentage following radiation in 2.5 Kr. The frequency of abnormalities increased with increasing dosage. Flower colour mutation ranged from small sectors in ray florets to whole rays florets and capitula to all capitula on whole branches. Wild type colour of 'Navneet' is creamy white and a yellow flowered mutant was isolated, multiplied and released as 'Navneet Yellow'.

Banerji and Datta (2001) exposed the rooted cuttings of *Chrysanthemum morifolium* cv. 'Surekha' to 150, 200 and 250 Gy doses of gamma rays (Co-60) and observed significant reduction in plant height, number of branches, leaves and flower

heads as well as leaf size and flower head size. The foliage and floral abnormalities as well as chromosomal aberrations increased in the treated plants as compared to control. They detected various flower colour mutations in VM_1 generation as 'sectoral chimaeras'.

An experiment was carried out with *Dendranthema grandiflorum* cv. 'Puja'. The rooted cuttings treated with 1.0, 1.5 and 2.0 Kr gamma rays resulted in sectorial somatic mutations in all doses. Original and mutated ray florets cultured on MS medium and transferred to the field resulted in regenerated plants that 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 (Datta *et al.,* 2001).

Dilta *et al.* (2003) treated rooted cuttings of various cultivars of *Dendranthema grandiflora* cvs. 'Ajay', 'Baggi', 'Bright Golden Anne', 'Ellen Van Langen', 'Glance', 'Mountaineer', 'Nanako', 'Shyama' and 'Snow Ball' with gamma rays at 0 and 2 Kr doses. Plant survival, plant height, number of branches, plant spread and number of flowers decreased after gamma irradiation treatment. Delay in number of days to bud formation and days to harvest was recorded in treated plants as compared to untreated. The somatic colour mutations were induced in 'Ellen Van Langen', 'Gulmohar', 'Snowball' and 'Shyama'.

The petals and buds from the irradiated plants of chrysanthemum cv. 'Taihei' were cultured *in vitro* and one flower colour mutant was detected from regenerated plants. The growth of the 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. The mutant showed general resistance to insect pests and diseases as studied by Nagatomi *et al.* (2003).

Datta *et al.* (2005) treated ray florets of *Chrysantemum morifolium* cultivars 'Flirt', 'Puja', 'Maghi' and 'Sunil' with 500 and 1000 Kr doses of gamma rays and cultured on MS medium supplemented with different combinations of growth regulators. A decrease in frequency of direct shoot regeneration was observed in gamma ray treated florets. Five solid flower colour and floret shape mutants with slight changes in ray floret morphology were detected and established.

Irradiated micro-shoots of *in vitro* cultured *Chrysanthemum morifolium* cv. 'Qiuzhishan' with 5-30 Gy doses of gamma rays were studied for plant growth and flower characters by Wang and Yu (2006). 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.

Liu *et al.* (2000) treated buds of orange flower colour ground cover chrysanthemum 'Cheng Yun' with electron beam irradiation at 20, 40, 60 and 80 Gy. The irradiated buds were dissected and their florets were inoculated on MS medium for callus induction. It was found that doses of 40-60 Gy was optimal for inducing mutations. The altered flower colours ranged from yellow to red-purple and the flowering was 25 days earlier or 15-20 days later than that of the control.

A greenish white large flowered chrysanthemum cultivar 'Madam E Rogar' was selected for *in vitro* propagation and mutagenesis to induce 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 1.0 Gy gamma radiation dose (Misra and Datta, 2007).

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

Mahure *et al.* (2010) reported that shoot length was decreased with gamma ray treatment in comparison to the control in *Chrysanthemum morifolium* cv. 'Delister White' through *in vitro* mutagenesis by treating ray florets with 0.5 and 1.0 Gy of gamma irradiation. The irradiation dose 0.5 Gy was found to be very effective in inducing mutations in flower shape, number of florets flower^{-1} head and conversion from tubular florets to spoon shaped florets. No change in flower colour was observed. The colour novelties induced by gamma rays treatments were isolated and purified in cultivar 'Red Gold'.

A study on chrysanthemum variety 'Otome Pink' recorded that at higher doses of gamma rays, the number of flower heads per plant as well as diameter of flowers head was reduced (Kumari *et al.,* 2013). Flower head faciation and wide range of flower colour mutations such as yellow, orange, light pink and purple were recorded at 10 and 15 Gy dose. A quilled petal type mutant was also obtained after gamma irradiation.

2.4 EFFECT OF VARIOUS MUTAGENS ON GLADIOLUS

Misra and Choudhary (1979) irradiated corms of gladiolus cultivars 'Himaprabha' and 'Jo Wagenaar' by gamma irradiation (3 and 4 Kr) and recorded advance sprouting in the former variety and malformed leaves, reduced plant height

and number of florets on the spikes and fascination and increase in floral parts in the latter variety.

The effect of gamma rays in three gladiolus cultivars namely 'Litlle Giant, Monsoer' and 'Wild Rosev' was studied by Raghava *et al.* (1981). They irradiated corms at 1, 2.5, 5, 10 and 15 Kr doses. Sprouting was affected significantly in 10 and 15 Kr treatments. LD_{50} was found to be between 10 and 15 Kr. Doses of 10 Kr and above proved to be detrimental for vegetative and floral traits. Plants treated with 10 Kr remained blind (without flower) whereas the plants at 15 Kr died after sprouting. Flowering was delayed significantly at above 5 Kr treatments. Radiation treatment caused decrease in spike length, number of florets per spike and floret size.

Banerji *et al.* (1981) irradiated dormant corms of *Gladiolus psittacinus* var. 'Hookeri' cultivars 'Orange' and 'Red' with 2.5, 5, 7.5, 10 and 12.5 Kr gamma rays. They noted reduction in sprouting, plant height, sprout number, spike length, number of leaves per corm, number of florets per spike, corm size and number of corms and cormels when treated with gamma radiation. Morphological abnormalities and chromosomal aberrations like early separations, bridges, fragments and laggards increased with increased exposure to gamma rays. Sprouting, emergence of spikes and first floret opening were delayed in higher doses while flowering completely ceased at the highest dose. LD_{50} on survival basis was found to fall in between 10 and 12.5 Kr. From the experiment, one mutant was isolated at 2.5 Kr treatment.

The effect of gamma rays at 0-10 Kr and Ethyl methane sulphonate (EMS), diethyl stilbestrol (DES) and n-methyl-N-nitroso urea (MNU) on corm production of gladiolus was studied by Misra and Bajpal (1983). A dose of 3 Kr was found to be effective in increasing the number of corms in cvs. 'Himprabha, 'Ratna's Butterfly', 'Sylvia' and 'Muielae'.

A studied was conducted on effect of gamma rays on different varieties of gladiolus by Mahesh and Misra (1983). They reported that 2-5 Kr dose was injurious for corm production. However, changes in colour of florets, increase and decrease in number of floral organs and their fasciations and spike bifurcation were observed with treatments 3.5 to 4.0 Kr in cvs. 'Christian Jane' and 'Oscar'.

Banerji *et al.* (1994) irradiated dormant corms of gladiolus cv. White Friendship with 250, 500, 750, 1000 and 1250 Gy gamma rays. They observed reduction in survival, plant height, number of leaves and florets, spike length and corm size and delayed flowering after irradiation. Morphological abnormalities in foliage and chromosomal aberrations during root tip mitosis increased with increase in dose. Flowering ceased after treatment with 1250 Gy. MV_2 and MV_3 generations also followed the pattern of abnormalities exhibited by MV_1 plants. Pink flower colour was detected in a few plants in a sectorial chimeric form in the MV_2 population. In the MV_3, from 750 Gy, one plant produced a spike with light pink florets. This mutant has been isolated in the pure form.

In a study conducted by Srivastava *et al.* (2007) showed that gamma irradiation treatments adversely affected all the morphological characters in gladiolus cvs. 'Sylvia' and 'Eurovision'. The maximum sprouting, plant height (57.7 cm), number of leaves (8.0), leaf length and leaf breadth (5.1 cm and 3.0 respectively), spike length (65.0), rachis length (48.4 cm), number of florets per spike (13.5) and floret diameter (9.0 cm) were recorded at 20 Gy radiation which were lower than control while days to corm sprout (10.7 days) and days to flowering (93.6 days) were significantly reduced over the control (11.8 days and 102.8 days, respectively). As the doses increased, all the characters were adversely affected and 80 Gy dose was lethal. Comparing the two cultivars, 'Eurovision' was more resistant to irradiation and positively responded to mutagenic treatments at lower doses than cv. 'Sylvia'.

Zhang and Wang (2008) radiate the corms of two cultivars of gladiolus with electron beam ('Beauty Queen' and 'Rose Supreme') with 3 MeV energy in order to test the feasibility of electron beam for induction of mutations in gladiolus and to study effects of various doses of electron beam on growth and development of gladiolus. They concluded that electron irradiation could inhibit the growth and development of plants in the shoot stage and initial bloom period.

Doses of 6 Kr and 7 Kr gamma rays proved injurious in gladiolus as the number of florets and length of spike reduced drastically (Patil and Dhaduk, 2009). Colour variations were observed in florets as well as fasciations and spike bifurcation when treated with 4 Kr to 7 Kr gamma rays in all varieties. Treatments of 5 Kr and 6 Kr were found to be good for induction of mutants and four mutants were isolated in VM_2 generation.

Reduction in number of florets, number of spikes, days to flower, shelf life, vase life and size of florets was observed in four gladiolus cultivars due to gamma irradiation. Positive correlation was recorded between doses of gamma rays and number of spikes per plant in M_1 generation. Number of florets per spike was positively correlated with doses upto 5 Kr gamma radiation and it became negative at higher doses of irradiation (Tiwari *et al.,* 2010).

2.5 EFFECT OF VARIOUS MUTAGENS ON TUBEROSE

Bulblets of *Polianthus tuberosa* were irradiated with different doses of gamma rays *i.e,* 5, 10, 15, 20, 25 and 30 Gy by Krasaechai (1992). They revealed that doses of 10 Gy and above reduced the growth rate and percent bulblet survival was zero after 15 and 20 weeks for those irradiated with 25 or 30 Gy, respectively. Leaf chimera was found in the irradiated plants but lower colour mutant was not reported.

Shukla and Datta (1993) reported that sprouting of bulbs in tuberose was stimulated in both cultivars i.e. 'Single' and 'Double' at 500 rad but got reduced at higher doses. Different types of morphological abnormalities such as change in shape, size, margin, apex, fission and fusion and chlorophyll variegation in leaves were detected at higher at higher doses. In cytological study, different types of abnormalities such as bridges, fragments, early separation and clumping were observed during mitosis in both cultivars. Frequency of morphological abnormalities and chromosomal aberrations increased with the increase in doses. It was concluded that cv. 'Single' was more sensitive to mutagenic treatment than cv. 'Double'.

Mutant plants having a wide range of variation in flower colour, flower shape and floret number per flower spike were obtained in plants raised by using florets from sodium azide treated spikes as explants (Shen and Huang, 2001). They treated harvested tuberose spikes with 1 to 1.5 mM of sodium azide by soaking spikes in it for 60, 90 and 120 minutes, young florets were excised as explants and cultured in MS medium. Survival rate was highest with 90 minute treatment.

Krishnan *et al.* (2003) treated bulbs of tuberose cultivars *viz.*, 'Single', 'Double', 'Suvasini' with gamma radiations (5.0 to 25 Gy) and noted that sprouting percentage was decreased and days taken for sprouting was increased using gamma rays. Plants irradiated with 5.0 Gy showed stimulatory effect on length of corolla, length of floret, vase life and bulb characters. Morphological variants such as chlorophyll mutants, non-flowering mutants and compact spike mutants were observed in different cultivars at different levels of mutagen. Based on growth and floral parameters, four mutants were isolated *viz.*, dwarf mutants, high tiller mutants, non-flowering mutants and compact inflorescence mutants. They retained these characters in M_2 generation also. Moreover, high heritability and genetic gain were

noticed for number of flowers per spike, spike length, flower diameter, leaf length and leaf width in VM_2 generation.

Bulbs of *Polianthus tuberosa* were treatted with different doses of colchicine by dipping them in 200, 400, 600, 800 and 1000 ppm of colchicine for 16 hrs. Treatment with colchicine at 400 ppm and 600 ppm resulted in the highest values for plant height, number of leaves, spike diameter, length of spike, number of florets per spike and length of rachis per spike. Weight of spike and 100 floret weights were highest with 600 ppm and 400 ppm colchicine treatments (Boora *et al.*, 2003).

2.6 EFFECT OF VARIOUS MUTAGENS ON OTHER FLOWER CROPS

Laneri *et al.* (1990) obtained several flower colour mutants in gerbera by gamma irradiation and suggested that shoots should undergo 3-4 cycles of micropropagation after mutagenic treatment in order to minimize chimerism.

Gamma irradiation (10 and 20 Gy doses) of two gerbera cultivars produced mutants showing flower colour, flower morphology and plant morphology variations (Jain *et al.*, 1998).

Matsubara (1975) conducted a trial to determine the effect of gamma rays on bulbs of tulip at different stages of flower development. Doses of 0.5, 1.0 and 3.0 Kr were applied. Marked delay in floral organ development occurred during or immediately after flower differentiation. Stunting, petal malformation and colour changes were observed in the different treatments.

Materials and Methods

3. MATERIALS AND METHODS

An investigation to study "Genetic improvement through induced mutation in Dahlia (*Dahlia variabilis* Desf.)" was carried out at College of Agriculture, Vellayani, Kerala Agricultural University, during 2015 to 2017. The materials used, techniques adopted for collection, analysis and interpretation of data in the present study are furnished in this chapter.

3.1 MATERIALS USED

Dahlia cultivars were collected from Regional Agricultural Research Station, Ambalavayal. Details of the materials used are listed below.

3.2. EXPERIMENTAL DETAILS

Design	: Randomized Block Design
Replications	: Three
Number of treatments	: Eighteen (three varieties and six doses for each variety)
Spacing	: 60 cm × 40 cm
Location	: College of Agriculture, Vellayani

3.2.1 Fixing the Dose of Mutagen

Mutagen dose was fixed based on reports regarding the optimum dose of gamma rays that can be used to induce variability in floral and plant architectural characters in dahlia. The relevant reports are enlisted in the Table 3.

Based on these reports, the doses selected for the present investigation were 5, 10, 15, 20 and 25 Gy along with control for each of the three varieties.

Table 1. Details of genotypes used in the study

Sl.No.	Name of cultivar	Colour	R.H.S. Colour chart reading
1.	Amb-white	White	White group N155 A
2.	Amb-purple	Purple	Red purple group N 74 C
3.	AMmb-red	Dark red	Red group 46 A

Table 2. Details of gamma ray treatment doses and treatment time (sec)

Sl. No.	Dose (Gy)	Duration of exposure (sec)
1	0	No exposure
2	5	13
3	10	26
4	15	39
5	20	52
6	25	65

Table 3. Reports regarding to fixing of dose

Dose of gamma rays	Reports in Dahlia
20 – 30 Gy	Broertjes and Ballego (1967)
20 – 30 Gy	Das *et al.*, (1980)
2.5 – 15 Gy	Dwivedi and Banerji (2008)
Upto 22.5 Gy	Das *et al.*, (1977)

3.3 PREPARATION OF EXPERIMENTAL SITE

Soil was thoroughly dug, all the weeds, stubbles, stones, were completely removed, experimental plot was incorporated with well decomposed farm yard manure. The experimental area was laid out as per plan adopting randomized block design, the layout made with the help of rope and whole field was divided into three parts for replicating the experiment thrice. Each plot was divided into plots for allotment of various treatments. Plots of 1×3.6 m^2 were laid out to accommodate all treatments replicated thrice.

3.4 IRRADIATION AND PLANTING

Tubers of dahlia were irradiated with gamma ray of doses ranging from 5-25 Gy at the interval of 5 Gy. 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. Tubers were planted in the field after irradiation.

3.5 CULTURAL OPERATIONS

3.5.1 Irrigation

Experimental plots were irrigated for the complete cropped area as and when needed depending upon the soil moisture level.

3.5.2 Inter Cultural Operations

Experimental plot was kept free from weeds by regular hand weeding as and when weeds emerged. Staking was given to all the plants with sticks to avoid lodging of crop. Pinching was done to enhance more laterals at 30 days after transplanting.

3.5.3 Harvesting and Cleaning of Tubers

When flowering was over and the leaves were almost dried up and the colour of the stem turned yellow, the plants were cut leaving only 15 cm stem from the ground. The tubers were taken out with a forked hoe and allowed to dry for 5-6 days in a shady area. Then the tubers were cleaned to remove adhering soil and kept in a cool and dry place.

3.6 BIOMETRICAL OBSERVATIONS

The data was collected on various parameters during vegetative and flowering stages from five plants randomly tagged in each plot for each replication and average values were used for analysis (Plate 1A & 1B).

3.6.1 M_1 Damage

Observations include sprouting (%), survival at 15 days after planting and plant height at 30 days after planting.

3.6.1.1 Sprouting (%)

Number of tubers sprouted for each treatment in M_1V_1 were counted in open field condition and expressed in percentage.

3.6.1.2 Survival at 15 Days (%)

Number of tubers that survived in each treatment in M_1V_1 were counted in open field condition at 15 days after planting and expressed in per cent.

Plate 1. (A). Planting and vegetative stage of Dahlia

Plate 1. (B) Vegetative and Flowering stage of dahlia

3.6.2 Vegetative Characters

3.6.2.1 Plant Height at 30 Days Interval (cm)

The height of the plant was measured from the ground to the growing tip of the main stem with the help of scale for all randomly tagged five plants and average was worked out and expressed in centimeter. The plant height was taken at an interval of 30 days *viz*., at 30, 60, 90 and 120 days after planting.

3.6.2.2 Plant Spread (cm)

The plant spread was measured as the distance between outer most side shoot in east to west direction at maturity.

3.6.2.3 Number of Branches Plant^{-1}

Branches arising from the main stem upto the top portion of central stem in each plant was counted from the five tagged plants and average was worked out. Number of branches were taken at an interval of 30 days *viz*., at 60, 90, 120 days after planting.

3.6.2.4 Number of Leaves Plant^{-1}

The total number of leaves^{-1} plant were counted at 30, 60, 90 and 120 days after planting.

3.6.2.5 Leaf Length (cm)

The length of the leaf was measured from the highest shoulder point to the tip of the leaf during its peak stage of growth randomly from five tagged plants, average was worked out and expressed in centimeter.

3.6.2.6 Leaf Width (cm)

The width of the leaf was measured at the maximum width point of the leaf during its peak stage of growth from five randomly tagged plants, average was worked out and expressed in centimeter.

3.6.2.7 Internodal Length (cm)

The distance between two successive nodes was measured randomly from five tagged plants and average was worked out and the gap between two internodes was expressed in centimeter.

3.6.2.8 Thickness of Internode (cm)

The thickness of internode was taken at a point midway between two nodes in the central region of the stem with the help of vernier calipers from five tagged plants and average was worked out.

3.6.2.9 Thickness at Node (cm)

The thickness of the node was taken at the node in the central region of the stem with the help of vernier calipers from five tagged plants and average was worked out.

3.6.3 Floral Characters

3.6.3.1 Number of Days Taken to Flowering

This was recorded by counting the number of days from the date of planting to the stage at which the first flower bud bloomed in each genotype. This was recorded from tagged plants and average was worked out.

3.6.3.2 Number of Flowers Plant^{-1}

Number of flowers produced in each of the tagged plants were recorded and the average number of flowers produced per plant was worked out.

3.6.3.3 Size of Flower (cm)

Size of five flowers on each plant was recorded as average of distance in east to west and north to south directions.

3.6.3.4 Number of Florets (Disc and Ray Florets)

The number of florets flower^{-1} head was counted in each plant. Number of florets of five fully opened flowers from each cultivar was counted and average was worked out to get average number of florets.

3.6.3.5 Length of Floret (cm)

A random petal in the flower of each tagged plant was taken for measuring the length of the petals and the average was worked out.

3.6.3.6 Breadth of Florets (cm)

A random petal in the flower of each tagged plant was taken for measuring the breadth of the petals and the average was worked out.

3.6.3.7 Longevity of the Intact Flower in the Plant (Days)

The number of days the flower remained fresh in the plant were counted and longevity of intact flowers were recorded.

3.6.3.8 Longevity of the Cut Flower (Days)

Flowers were taken to a room soon after harvest, where the stem was cut to 30 cm and placed with cut ends dipped in water for 12 hours. During the next few days, the flowers were observed for any withered appearance. The number of days the flower remained fresh in plain water was considered as the vase life.

3.6.3.9 Flower Colour

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

3.7 SCREENING OF MUTANTS FOR ORNAMENTAL TRAITS

Screening of mutants was carried out in M_1 V_1 and M_1 V_2 generations, as reported by Broertjes and Van Harten in 1988.

3.8 STATISTICAL ANALYSIS

The statistical analysis of the quantitative data obtained during the course of investigation was done by following statistical model.

Analysis of Variance

The data were statistically analyzed by using factorial Randomized Block Design with two factors in accordance with the procedure outlined by Gomez and Gomez (1984). The significance of difference among treatment means were tested by F-test. Wherever, F-test was found significant, critical difference (C.D.) at 5 per cent level of significance was calculated. The results are presented in the form of graphs and tables with photographs at appropriate places for proper interpretation.

Results

4. RESULTS

The present investigation entitled "Genetic improvement through induced mutation in Dahlia (*Dahlia variabilis* Desf.)" was carried out at College of Agriculture, Vellayani, Kerala Agricultural University, during 2015 to 2017. The purpose of the present study was to induce variability in dahlia for plant architecture and floral characters through gamma irradiation. The salient findings as revealed from the investigation are presented here.

4.1 EFFECT OF GAMMA RADIATIONS ON M_1 DAMAGES AND VEGETATIVE CHARACTERS

Effect of gamma rays on percent of sprouting, survival at 15 days and plant height at 30 days was studied as a manifestation of M_1 damage. M_1 generation was evaluated with respect to 21 quantitative morphological characters including 8 vegetative characters, 9 floral characters and 4 tuber characters. The results are presented below.

The doses above 15 Gy *i.e.* 20 and 25 Gy were lethal and showed no sprouting.

The results of statistical analysis for different vegetative characters are presented in Table 4. The table reveals that the treatment related to plant height (cm), branches at maturity, leaves at maturity, plant spread (cm) and internodal length were significantly different from one another. Interaction effect between treatment and variety was also found to be significant for all these characters except for intermodal length at 5% level of significance.

Table 4. ANOVA table for vegetative characters in dahlia

	D F	Plant height	Branches at maturity	Leaves at maturity	Plant spread	Internodal length	Thickness of internode	Thickness at node
		MSS	MSS	MSS	MSS	MSS	MSS	MSS
Replication	2	13.25	0.048	2.78	6.46	0.02	0.0035	0.0055
Treatment	3	475.32**	5.34**	267.26**	159.492**	6.423**	0.035	0.035
Variety	2	93.02**	3.30	17.512**	311.208**	5.21**	0.102	0.144
T x V	6	5.21**	0.666	3.453**	18.823**	0.544	0.009	0.05
Error	22	3.99	0.114	0.386	2.442	0.035	0.002	0.001

4.1.1 Sprouting and Survival (%)

The data related to sprouting and survival of plants after gamma radiations are shown in Table 5. Significant differences were observed in sprouting (%) of plants in the three varieties (Amb-white, Amb-purple and Amb-red) with respect to different doses of gamma irradiation.

Sprouting percentage decreased as the dose of gamma rays increased. Maximum sprouting was observed in 5 Gy (88.86 %) whereas, 15 Gy showed minimum sprouting (16.6 %). No sprouting was observed in 20 Gy and 25 Gy (0 %), recording cent per cent mortality.

Survival percentage was dependant on the dose rate used for treatment. Hundred percent survival was recorded in plants which were untreated. Plants treated with 5 Gy showed maximum survival (88.86 %) whereas plants treated with 15 Gy dose of gamma rays showed a minimum survival percentage of 16.6.

Table 5. Effect of gamma radiations on sprouting and survival percentage in dahlia varieties

Varieties	Sprouting and survival (%)			
	Control	5 Gy	10 Gy	15 Gy
Amb-white	100	77.73	49.97	16.60
Amb-purple	100	94.43	72.17	16.60
Amb-red	100	94.43	61.06	16.60
Mean	100	88.86	61.06	16.60
CD at 5%	14.82			
Sem±	4.90			

Note: 20 Gy and 25 Gy: No sprouting

Table 6. Effect of gamma radiations on plant height at 30 days after planting

Varieties	Plant height at 30 days (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	22.21	18.45	16.69	13.37	17.68
Amb-purple	17.85	13.35	13.03	9.50	13.43
Amb-red	21.71	14.75	13.99	13.67	16.03
Mean	20.59	15.52	14.57	12.18	-
	CD at 5%		SE m±		
Treatments (T)	1.20		0.40		
Variety (V)	1.03		0.35		
T*V	NS		0.70		

Note: 20 Gy and 25 Gy: No sprouting

4.1.3 Plant height (cm) (at 30 Days Interval)

The results related to the effect of gamma rays on plant height recorded at 30 days after planting are presented in Table 6.

A gradual decrease in plant height was recorded with gradual increase in dose of gamma rays. Untreated plants showed a height of 20.59 cm which is greater than treated plants. Among the treatments, plants treated with a dose of 5 Gy showed the maximum height of 15.72 cm, whereas plants treated with a dose of 15 Gy showed a significant decline in height (12.18 cm) at 30 days after planting.

Plant height at 60 days after planting revealed that the maximum height was recorded in control (64.03 cm) and minimum height was shown by plants which were treated with the highest dose of 15 Gy (Table 7).

At 90 days after planting, plants treated with the doses of 5, 10 and 15 Gy showed heights of 75.4, 67.12 and 60.61 cm respectively (Table 8). Maximum height was noted in plants which were untreated (83.45 cm).

At 120 days after planting, control plants showed a height of 99.21 cm (Table 9). Among the treatments, plants treated with a dose of 5 Gy showed a height of 91.75 cm, whereas plants treated with a dose of 15 Gy showed a significant decline in height (76.83 cm).

4.1.4 Plant Spread (cm)

The data recorded in Table 10 shows that maximum plant spread was found in untreated plants. Among the treatments, plants treated with a dose of 5 Gy showed a spread of 33.25 cm which was the highest as compared to other treatments with

Table 7. Effect of gamma radiations on plant height at 60 days after planting

Variety	Plant height at 60 days (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	67.08	60.56	54.31	52.46	58.60
Amb-purple	61.43	57.54	49.12	47.63	55.93
Amb-red	63.60	57.86	48.15	45.00	53.65
Mean	64.03	58.58	48.98	48.36	-
	CD at 5%		SE m±		
Treatments (T)	1.96		0.66		
Variety (V)	1.70		0.57		
T*V	NS		1.15		

Note: 20 Gy and 25 Gy: No sprouting

Table 8. Effect of gamma radiations on plant height at 90 days after planting

Variety	Plant height at 90 days (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	84.73	80.32	77.52	64.93	76.88
Amb-purple	81.58	71.11	62.41	59.00	68.52
Amb-red	84.04	74.78	61.45	57.90	69.54
Mean	83.45	75.40	67.12	60.61	-
	CD at 5%		SE m±		
Treatments (T)	2.44		0.82		
Variety (V)	2.11		0.71		
T*V	4.23		1.43		

Note: 20 Gy and 25 Gy: No sprouting

Table 9. Effect of gamma radiations on plant height at 120 days after planting

Variety	Plant height at 120 days (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	104.78	97.98	89.21	77.27	92.31
Amb-purple	90.75	83.312	78.97	74.63	81.91
Amb-red	102.11	93.97	82.70	78.6	89.34
Mean	99.21	91.75	83.62	76.83	-
	CD AT 5%		SE m±		
Treatments (T)	2.14		0.72		
Variety (V)	1.85		0.62		
T*V	3.71		1.25		

Note: 20 Gy and 25 Gy: No sprouting

Table 10. Effect of gamma radiations on plant spread (cm) in dahlia

Variety	Plant spread (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	34.39	34.91	30.48	25.10	32.22
Amb-purple	39.43	37.68	33.94	31.12	36.79
Amb-red	29.14	27.18	25.59	24.58	26.62
Mean	34.32	33.25	30.00	26.93	-
	CD AT 5%		SE m±		
Treatments (T)	1.53		0.52		
Variety (V)	1.33		0.45		
T*V	2.63		0.90		

Note: 20 Gy and 25 Gy: No sprouting

higher doses. The least plant spread (22.93 cm) was found in plants treated with the highest dose of gamma rays 15 Gy.

4.1.5 Number of Branches Plant^{-1} (at 30 Days Interval)

Data recorded in Table 11 revealed that more number of branches were found in untreated plants as compared to treated plants at 30 days after planting. Maximum number of branches were found in plants which were treated with lower doses and minimum number of branches were found in plants treated with higher doses.

Number of branches were found to be more in control plants (3.99) as compared to plants treated with 5, 10 and 15 Gy dose of gamma rays, as they showed lower number of branches i.e., 3.11 ,2.46 and 2.00 branches respectively at 60 days after planting (Table 12).

Plants treated with doses of 5, 10 and 15 Gy showed 6.12, 5.03 and 3.89 branches respectively at 90 days after planting (Table 13). Maximum number of branches was noted in plants which were untreated (8.15).

Data recorded in Table 14 reveal that more number of branches were found in untreated plants as compared to treated plants at 120 days after planting. Maximum number of branches was found in plants which were treated with the lower dose *i.e.* 5 Gy (12.03) and minimum number of branches was found in plants treated with the highest dose *i.e.* 15 Gy (9.89).

Table 11. Effect of gamma radiations on number of branches at 30 days after planting

Variety	Branches at 30 days				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	2.17	1.65	1.11	1.00	1.48
Amb-purple	2.38	2.01	1.07	1.00	1.61
Amb-red	2.39	1.59	1.07	1.00	1.51
Mean	2.31	1.75	1.08	1.00	-
	CD at 5%		SE m±		
Treatments (T)	0.12		0.04		
Variety (V)	0.12		0.04		
T*V	0.21		0.07		

Note: 20 Gy and 25 Gy: No sprouting

Table 12. Effect of gamma radiations on number of branches at 60 days after planting

Variety	Branches at 60 days				Mean
	control	5 Gy	10 Gy	15 Gy	
Amb-white	3.38	2.65	2.36	2.00	2.59
Amb-purple	5.16	3.94	2.56	2.00	3.41
Amb-red	3.44	2.76	2.46	2.00	2.66
Mean	3.99	3.11	2.46	2.00	-
	CD AT 5%		SE m±		
Treatments (T)	0.34		0.11		
Variety (V)	0.34		0.11		
T*V	0.59		0.19		

Note: 20 Gy and 25 Gy: No sprouting

Table 13. Effect of gamma radiations on number of branches at 90 days after planting

Variety	Branches at 90 days				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	7.50	5.58	4.86	3.67	5.40
Amb-purple	10.06	7.28	5.17	4.00	6.62
Amb-red	6.89	5.52	5.07	4.00	5.37
Mean	8.15	6.12	5.03	3.89	-
	CD at 5%		SE m±		
Treatments (T)	0.56		0.19		
Variety (V)	0.48		0.16		
T*V	0.97		0.32		

Note: 20 Gy and 25 Gy: No sprouting

Table 14. Effect of gamma radiations on number of branches at 120 days after planting

Variety	Branches at 120 days				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	11.72	11.77	10.11	9.00	10.65
Amb-purple	12.78	12.36	11.80	10.67	11.90
Amb-red	11.61	11.36	11.13	10.00	11.02
Mean	12.03	11.83	11.01	9.89	-
	CD AT 5%		SE m±		
Treatments (T)	0.78		0.26		
Variety (V)	0.67		0.23		
T*V	NS		0.46		

Note: 20 Gy and 25 Gy: No sprouting

4.1.6 Number of Leaves Plant^{-1} (at 30 Days Interval)

Data presented in Table 15 revealed that number of leaves were significantly different between treatments at 30 days after planting. Maximum number of leaves were found in control (9.46) and minimum number was found in 15 Gy (2.21).

Number of leaves were found to be more in untreated plants (20.51) as compared to plants treated with 5, 10 and 15 Gy dose of gamma rays, as they showed lower number of leaves *i.e.*, 15.70, 13.30 and 8.88 respectively at 60 days after planting (Table 16).

Control plants showed the highest number of leaves (33.13) than treated plants (Table 17) at 0 days after planting. Among the treatments, plants treated with a dose of 5 Gy showed the maximum number of 27.27 leaves, whereas plants treated with a dose of 15 Gy (19.63) showed a significant drop in number of leaves.

A gradual decrease in number of leaves was recorded with gradual increase in the dose of gamma rays. Untreated plants showed the highest number of leaves of 47.79. Among the treatments, plants treated with a dose of 5 Gy showed the maximum number of leaves (47.61), whereas plants treated with the dose of 15 Gy (42.89) showed a significant drop in number of leaves at 120 days after planting (Table 18).

4.1.7 Leaf Length and Leaf Width (cm)

The data related to leaf length is compiled in Table 19. The data recorded revealed that the effect of gamma rays on leaf length was highly significant between the untreated plants and the treated plants. Untreated plants showed a leaf length of 7.34 cm. Treated plants showed a gradual decrease in length as the dose of gamma

Table 15. Effect of gamma radiations on number of leaves at 30 days after planting

Variety	Leaves at 30 days				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	9.11	4.65	2.56	3.00	4.82
Amb-purple	10.11	5.00	2.62	3.33	5.26
Amb-red	9.17	5.16	2.27	2.67	4.81
Mean	9.46	4.93	2.48	2.21	-
	CD at 5%		SE m±		
Treatments (T)	0.32		0.12		
Variety (V)	0.28		0.09		
T*V	NS		0.19		

Note: 20 Gy and 25 Gy: No sprouting

Table 16. Effect of gamma radiations on number of leaves at 60 days after planting

Variety	Leaves at 60 days				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	20.22	16.12	8.92	8.33	13.40
Amb-purple	22.94	17.16	10.80	9.33	15.06
Amb-red	18.39	13.84	9.60	9.00	12.71
Mean	20.51	15.70	13.30	8.88	-
	CD at 5%		SE m±		
Treatments (T)	0.61		0.20		
Variety (V)	0.52		0.17		
T*V	1.05		0.35		

Note: 20 Gy and 25 Gy: No sprouting

Table 17. Effect of gamma radiations on number of leaves at 90 days after planting

Variety	Leaves at 90 days				Mean
	control	5 Gy	10 Gy	15 Gy	
Amb-white	34.89	29.93	24.14	22.33	27.82
Amb-purple	30.28	25.49	20.03	18.00	23.45
Amb-red	34.22	26.40	19.47	18.67	24.69
Mean	33.13	27.27	21.21	19.63	-
	CD at 5%		SE m±		
Treatments (T)	0.84		0.28		
Variety (V)	0.73		0.24		
T*V	1.46		0.49		

Note: 20 Gy and 25 Gy: No sprouting

Table 18. Effect of gamma radiations on number of leaves at 120 days after planting

Variety	Leaves at 120 days				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	46.61	46.34	43.03	40.67	44.65
Amb-purple	47.11	47.02	44.38	43.00	45.73
Amb-red	49.66	49.48	47.13	45.00	48.30
Mean	47.79	47.61	44.84	42.89	-
	CD at 5%		SE m±		
Treatments (T)	1.03		0.35		
Variety (V)	0.90		0.30		
T*V	NS		0.61		

Note: 20 Gy and 25 Gy: No sprouting

rays increased. The plants treated with 5 Gy showed a leaf length of 6.59 cm which was significantly higher as compared to plants treated with a dose of 15 Gy (5.45 cm).

A gradual decrease in leaf width was recorded with gradual increase in the dose of gamma rays (Table 20). Leaf width ranged from 2.3 cm in 15 Gy to 3.18 cm in control. 5 Gy showed an intermediate width of 2.81 cm.

4.1.8 Internodal Length (cm)

Observations on intermodal length were recorded at maturity and data are presented in Table 21. The effect of gamma rays on intermodal length was found to be significant between treated and untreated plants. Untreated plants recorded the highest internodal length of 6.92 cm as compared to treated plants. 15 Gy treatment recorded the least length (4.92 cm). As the dose of gamma rays increased, there was significant decline in the length of internode.

4.1.9 Thickness of Internode and Node (cm)

The data related to thickness of internode is compiled in Table 22. Untreated plants showed a thickness of 1.28 cm as compared to treated plants. The plants treated with 5 Gy showed a thickness of 1.23 cm which was significantly higher as compared to plants treated with a dose of 15 Gy (1.14 cm).

The data recorded in Table 23 showed that the maximum thickness at node was found in untreated plants. Among the treatments, plants treated with a dose of 5 Gy showed a thickness at node of 1.37 cm which was the highest as compared to treatments with higher doses. The least thickness was found in plants treated with a higher dose of gamma rays of 15 Gy (1.22 cm).

Table 19. Effect of gamma radiations on leaf length (cm)

Variety	Leaf length (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	7.62	6.84	5.84	5.17	6.37
Amb-purple	7.75	6.61	5.95	5.53	6.46
Amb-red	6.66	6.32	5.94	5.67	6.15
Mean	7.34	6.59	5.91	5.45	-
	CD at 5%		SE m±		
Treatments (T)	0.14		0.04		
Variety (V)	0.12		0.04		
T*V	0.25		0.08		

Note: 20 Gy and 25 Gy: No sprouting

Table 20. Effect of gamma radiations on leaf width (cm)

Variety	Leaf width (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	4.00	3.17	2.91	2.57	3.16
Amb-purple	3.83	3.30	2.66	2.60	3.10
Amb-red	2.37	1.96	1.83	1.73	1.97
Mean	3.18	2.81	2.46	2.30	-
	CD AT 5%		SE m±		
Treatments (T)	0.11		0.04		
Variety (V)	0.10		0.03		
T*V	0.20		0.06		

Note: 20 Gy and 25 Gy: No sprouting

Table 21. Effect of gamma radiations on internodal length (cm)

Variety	Internodal length (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	7.75	6.79	6.27	4.83	6.41
Amb-purple	7.15	6.30	5.53	5.20	6.04
Amb-red	5.88	5.02	4.88	4.73	5.13
Mean	6.92	6.03	5.40	4.92	-
	CD at 5%		SE m±		
Treatments (T)	0.18		0.06		
Variety (V)	0.15		0.05		
T*V	0.31		0.10		

Note: 20 Gy and 25 Gy: No sprouting

Table 22. Effect of gamma radiations on thickness of internode (cm)

Variety	Thickness of internode (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	1.41	1.35	1.33	1.15	1.31
Amb-purple	1.28	1.22	1.20	1.15	1.21
Amb-red	1.17	1.12	1.09	1.12	1.12
Mean	1.28	1.23	1.20	1.14	-
	CD at 5%		SE m±		
Treatments (T)	0.04		0.01		
Variety (V)	0.04		0.01		
T*V	0.08		0.02		

Note: 20 Gy and 25 Gy: No sprouting

4.2 EFFECT OF GAMMA RADIATIONS ON FLORAL CHARACTERS

The observations on floral characters of the three varieties of gamma rays treated M_1 generation is presented below.

The results of statistical analysis for different floral characters are presented in Table 24. The table reveals that the treatments related to days to flowering, number of flowers, size of flower, number of florets, fresh weight of tubers and number of tubers were significantly different from one another. Interaction effect between treatment and variety was also found to be significant for all these characters except for number of tubers, length of tuber and diameter of tuber at 5% level of significance.

Table 24. ANOVA table for floral characters in dahlia

	D F	Days to flowering	Number of flowers	Size of flower (cm)	Number of florets	Fresh weight of tuber (g)	Number of tubers	Length of tuber (cm)	Diameter of tubers (cm)
		MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
Replication	2	1.293	9.166	0.016	0.543	0.056	0.701	0.004	0.003
Treatment	3	61.360**	129.505**	7.303**	550.214**	821.947**	12.736**	2.107	0.113
Variety	2	182.928**	2.537	53.072**	35,611.71**	87.017**	1.563	1.410	0.023
T x V	6	3.467**	9.004**	0.939	24.551**	48.708**	0.989	0.405	0.018
Error	22	1.302	5.064	0.057	8.797	10.223	0.263	0.286	0.007

4.2.1 Number of Days to Flowering

The data pertaining to this attribute has been presented and an appraisal of the data elucidated that gamma ray treatment had a significant effect on number of days to first flowering in all the varieties of dahlia (Table 25).

Among the different treatments, minimum days taken to first flowering was 63.89 days and was recorded in 5 Gy treatment which was found to be significantly different from all other treatments. In the other treatments, it was 67.88 days in 10 Gy followed by 69.55 days in 15 Gy. Number of days to flowering was found lower in plants treated with 5 Gy as compared to untreated plants (64.94 days).

Table 23. Effect of gamma radiations on thickness at node (cm)

Variety	Thickness at node (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	1.48	1.44	1.39	1.33	1.41
Amb-purple	1.37	1.31	1.28	1.22	1.29
Amb-red	1.28	1.20	1.15	1.13	1.19
Mean	1.37	1.31	1.27	1.22	-
	CD at 5%		SE m±		
Treatments (T)	0.03		0.01		
Variety (V)	0.02		0.01		
T*V	NS		0.02		

Note: 20 Gy and 25 Gy: No sprouting

Table 25. Effect of gamma radiations on number days to flowering

Variety	Number days to flowering				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	64.89	63.52	66.83	68.33	65.89
Amb-purple	61.78	61.19	63.88	65.33	63.05
Amb-red	68.16	66.96	72.93	75.00	70.06
Mean	64.94	63.89	67.88	69.55	-
	CD at 5%		SE m±		
Treatments (T)	1.12		0.38		
Variety (V)	0.97		0.32		
T*V	1.94		0.65		

Note: 20 Gy and 25 Gy: No sprouting

4.2.2 Number of Flowers Plant^{-1}

Number of flowers plant^{-1} ranged from 18.66 in 15 Gy to 27.18 in control (Table 26). A dose dependent drop in flower number was noted with 5 Gy recording 25.03 and 10 Gy recording 21.34 flowers.

4.2.3 Size of Flower (cm)

The data pertaining to flower size in M_1V_1 are presented in Table 27. Control plants produced significantly larger flowers (10.02 cm) whereas the highest gamma ray dose, 15 Gy produced the smallest flowers (8.02 cm). Higher doses of gamma rays were found to reduce flower size drastically (Plates 2 & 3).

4.2.4 Number of Florets (Both Disc and Ray Florets)

The data presented in Table 28 revealed that there were significant effects of gamma radiations on number of florets (both disc and ray florets) in M_1V_1.

A gradual decrease in number of florets was recorded with gradual increase in the dose of gamma rays. Untreated plants showed the highest number of florets of 118.46. Among the treatments, plants treated with 5 Gy showed the maximum number of florets (114.61), whereas plants treated with 15 Gy (85.52) showed a significant depletion in number of florets.

4.2.5 Length and Breadth of Floret (cm)

The data presented in Table 29 revealed that length of ray florets differed significantly with treatment. Maximum length of florets was found in plants which were untreated (4.20 cm) and minimum length was found in 15 Gy (3.30 cm).

Breadth of florets (Table 30) was found to be more in untreated plants (1.48 cm) as compared to plants treated with 5, 10 and 15 Gy dose of gamma rays, as they

Table 26. Effect of gamma radiations on number of flowers plant^{-1}

Variety	Number of flowers plant^{-1}				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	29.11	25.37	19.31	16.67	22.61
Amb-purple	25.44	24.54	22.12	20.00	23.03
Amb-red	27.00	25.19	22.6	19.33	23.53
Mean	27.18	25.03	21.34	18.66	-
	CD at 5%		SE m±		
Treatments (T)	2.21		0.75		
Variety (V)	NS		0.65		
T*V	NS		1.29		

Note: 20 Gy and 25 Gy: No sprouting

Table 27. Effect of gamma radiations on size of flower (cm)

Variety	Size of flower (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	12.42	12.07	9.94	9.62	11.01
Amb-purple	10.33	9.22	8.88	8.51	9.23
Amb-red	7.33	7.15	6.86	5.94	6.82
Mean	10.02	9.48	8.56	8.02	-
	CD at 5%		SE m±		
Treatments (T)	0.23		0.08		
Variety (V)	0.24		0.06		
T*V	0.40		0.13		

Note: 20 Gy and 25 Gy: No sprouting

Plate 2. Effect of gamma radiations on size of flower (cm) of Amb-white

Plate 3. Effect of gamma radiations on size of flower (cm) of Amb-purple

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Table 28. Effect of gamma radiations on number of florets

Variety	Number of florets				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	174.50	167.30	157.06	151.67	162.63
Amb-purple	122.67	116.58	111.37	104.90	113.88
Amb-red	58.22	59.97	49.93	47.37	53.87
Mean	118.46	114.61	106.12	85.52	-
	CD at 5%		SE m±		
Treatments (T)	2.91		0.98		
Variety (V)	2.52		0.85		
T*V	5.05		1.71		

Note: 20 Gy and 25 Gy: No sprouting

Table 29. Effect of gamma radiations on length of floret (cm)

Variety	Length of floret (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	4.66	4.5	4.31	4.03	4.38
Amb-purple	4.15	3.99	3.55	3.17	3.72
Amb-red	3.79	3.55	3.36	2.70	3.35
Mean	4.20	4.01	3.74	3.30	-
	CD at 5%		SE m±		
Treatments (T)	0.08		0.03		
Variety (V)	0.07		0.02		
T*V	0.15		0.05		

Note: 20 Gy and 25 Gy: No sprouting

Table 30. Effect of gamma radiations on breadth of floret (cm)

Variety	Breadth of floret (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	1.41	1.34	1.23	1.17	1.29
Amb-purple	1.66	1.51	1.39	1.35	1.47
Amb-red	1.39	1.32	1.17	1.07	1.24
Mean	1.48	1.39	1.26	1.19	-
	CD at 5%		SE m±		
Treatments (T)	0.05		0.01		
Variety (V)	0.04		0.01		
T*V	NS		0.03		

Note: 20 Gy and 25 Gy: No sprouting

Table 31. Effect of gamma radiations on longevity of intact flower (days)

Variety	Longevity of intact flower (days)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	4.81	4.23	3.76	3.57	4.09
Amb-purple	4.16	4.08	3.78	3.57	3.89
Amb-red	4.84	4.13	3.61	3.40	4.00
Mean	4.60	4.14	3.71	3.51	-
	CD at 5%		SE m±		
Treatments (T)	0.10		0.03		
Variety (V)	0.08		0.03		
T*V	0.17		0.06		

Note: 20 Gy and 25 Gy : No sprouting

showed minimized values in breadth which varied from 1.39 and 1.26 to 1.19 cm respectively.

4.2.6 Longevity of Intact and Cut Flowers (Days)

The data pertaining to the effect of gamma ray treatment on longevity of intact flower are presented in Table 31. It was evident from the data that significant differences for this character were recorded in all varieties studied. Maximum longevity of intact flower was found in plants which were untreated (4.6 days) and minimum was found in 15 Gy (3.51 days).

A gradual decrease in longevity of cut flower was recorded with gradual increase in the dose of gamma rays. Untreated plants showed a higher longevity of about 6.36 days. Among the treatments, plants treated with a dose of 5 Gy showed the maximum longevity (6.03 days), whereas plants treated with a dose of 15 Gy (4.67) showed a significant drop in longevity (Table 32). In general, higher doses of gamma radiations reduced longevity of cut flower.

4.2.7 Flower Colour

Data pertaining to flower colour in M_1V_1 generation are tabulated in Table 33. In Amb - white and Amb - purple there were mutants for flower colour and form at 5 Gy and 10 Gy. In M_1V_1 generation, no flower colour mutation was recorded in Amb - red at 5, 10 and 15 doses of gamma rays used.

Table 33: Colour mutants isolated from M_1V_1 generation

Variety	Mutant	Dose of gamma rays
Amb - white	WM1	5 Gy
	WM2	10 Gy
Amb - purple	PM2	5 Gy

Table 32. Effect of gamma radiations on longevity of cut flower (days)

Variety	Longevity of cut flower (days)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	6.11	6.35	5.06	4.03	5.39
Amb-purple	5.18	5.50	4.84	4.47	5.00
Amb-red	7.80	6.26	5.80	5.52	6.34
Mean	6.36	6.03	5.23	4.67	-
	CD at 5%		SE m±		
Treatments (T)	0. 14		0.04		
Variety (V)	0.12		0.04		
T*V	0.24		0.08		

Note: 20 Gy and 25 Gy: No sprouting

Table 34. Effect of gamma radiations on fresh weight of tubers (g) in M_1V_1 generation

Variety	Weight of tubers (g)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	44.94	47.56	35.35	21.21	37.27
Amb-purple	53.66	46.25	38.29	26.78	41.25
Amb-red	40.54	39.82	35.89	28.19	36.11
Mean	46.38	44.54	36.51	25.39	-
	CD at 5%		SE m±		
Treatments (T)	3.14		1.06		
Variety (V)	2.72		0.92		
T*V	5.44		1.84		

Note: 20 Gy and 25 Gy: No sprouting

4.3 EFFECT OF GAMMA RADIATIONS ON TUBER CHARACTERS IN M_1V_1 GENERATION

4.3.1 Fresh Weight of Tubers (g)

The data related to fresh weight of tubers are compiled in Table 34. The data revealed that gamma ray treatment had a significant effect on fresh weight of tubers (g). The plants treated with 5 Gy showed a tuber weight of 44.54g which was significantly higher, as compared to tubers produced by plants treated with a dose of 15 Gy (25.39 g).

4.3.2 Number of Tubers

The data pertaining to the field evaluation of gamma ray treatment on the number of tubers are presented in Table 35. The untreated plants produced the highest number of tubers (7.35) whereas, other treatments recorded 7.07, 5.31 and 4.40 in 5, 10 and 15 Gy doses of gamma rays respectively. The minimum number of tubers was recorded in the treatment with the highest dose of gamma rays *i.e.* at 15 Gy (4.40).

4.3.3 Length and Diameter of Tuber (cm)

The data represented in Table 36 revealed that length of tubers varied significantly with treatment. Maximum length of tubers was found in plants which were treated with a dose of 5 Gy (5.76 cm) and minimum length was found at 15 Gy (4.76 cm).

Diameter of tubers was found to be more in untreated plants (0.91 cm) as compared to tubers produced by plants treated with 5, 10 and 15 Gy as shown in the Table 37.

Table 35. Effect of gamma radiations on number of tubers (g) in M_1V_1 generation

Variety	Number of tubers (g)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	6.78	7.35	5.19	4.67	6.00
Amb-purple	8.61	7.29	5.62	5.00	6.63
Amb-red	6.67	6.58	5.13	3.33	5.43
Mean	7.35	7.07	5.31	4.40	-
	CD at 5%		SE m±		
Treatments (T)	0.50		0.17		
Variety (V)	0.43		0.14		
T*V	0.87		0.29		

Note: 20 Gy and 25 Gy: No sprouting

Table 36. Effect of gamma radiations on length of tuber (cm) in M_1V_1 generation

Variety	Length of tuber (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	6.19	6.05	4.99	4.73	5.49
Amb-purple	3.55	5.06	4.75	4.68	4.51
Amb-red	5.57	6.18	5.15	4.87	5.44
Mean	5.10	5.76	4.96	4.76	-
	CD at 5%		SE m±		
Treatments (T)	0.52		0.17		
Variety (V)	0.45		0.15		
T*V	NS		0.30		

Note: 20 Gy and 25 Gy: No sprouting

Table 37. Effect of gamma radiations on diameter of tuber (cm) in M_1V_1 generation

Variety	Diameter of tuber (cm)				Mean
	Control	5 Gy	10 Gy	15 Gy	
Amb-white	1.01	0.96	0.80	0.63	0.85
Amb-purple	0.84	0.76	0.77	0.71	0.77
Amb-red	0.89	0.99	0.81	0.67	0.84
Mean	0.91	0.90	0.73	0.67	-
	CD at 5%		SE m±		
Treatments (T)	0.08		0.02		
Variety (V)	NS		0.02		
T*V	NS		0.05		

Note: 20 Gy and 25 Gy : No sprouting

4.4 SCREENING OF MUTANTS IN M_1V_2 GENERATION AND THEIR CHARACTERIZATION

In M_1V_1 generation, a total of three mutants were identified for ornamental traits such as flower colour, petal size and petal shape. These mutants were isolated and carried forwarded to M_1V_2 generation using stem cuttings for studies regarding their vegetative and floral characters and stability for variation observed.

4.4.1 Effect of Gamma Radiations on flower colour and form in Dahlia Varieties

It is evident from Table 38 that some colour and form mutants were noticed in M_1V_1 generation.

Table 38. Effect of gamma radiations on colour and form mutants in dahlia varieties

Varieties	Gamma rays dose	Flower colour mutant	Flower form mutant
Amb-white	5 Gy	1	-
	10 Gy	-	1
Amb-purple	5 Gy	1	-

4.4.2 Mutants of Amb – White

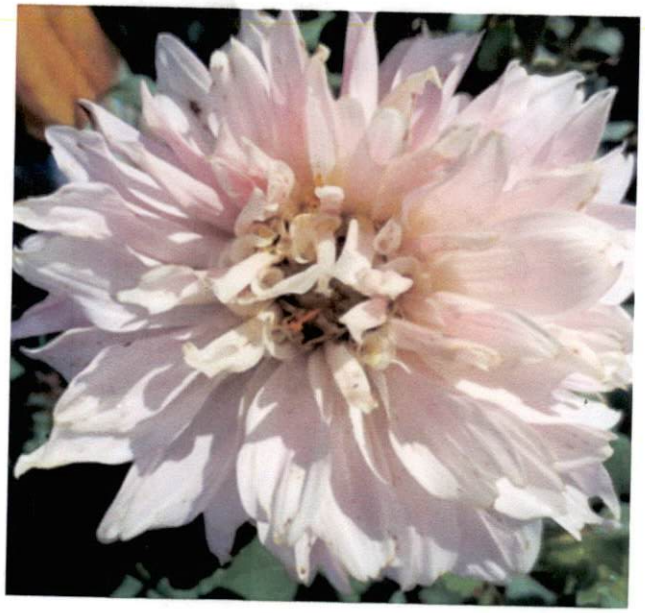
From Amb – white, two mutants were screened, tagged and checked for the stability of characters in M_1V_2 generation. The mutants were developed at 5 Gy and 10 Gy doses of gamma rays (Table 39 and Plate 4 & 5).

Control

WM1 at 5 Gy

WM2 at 10 Gy

Plate 4. Mutants in M_1V_1 generation in Amb-white



WM1 at 5 Gy

WM2 at 10 Gy

Plate 5. Mutants in M_1V_2 generation in Amb-white

4.4.2.1 Mutant WM1

This mutant was generated in Amb – white variety at 5 Gy dose of gamma radiations. It is evident from the data presented in Table 39 that this mutant differed in flower colour and form characters from the control plant. Colour variation (Plate 4) included a change from light brown centre to light yellow centre in WM1. Form variation was manifested as slight spiking of petals.

The mutant differed from control with respect to vegetative characters. Plant height of the mutant was 67.51 cm which was lesser than that of the untreated plants (69.06 cm) at maturity. Number of branches and leaves (3.7, 21.18) at maturity increased in the mutant as compared to control plants (3.54, 20.55). Leaf characters such as leaf length and width of leaf was superior in control plants (7.22, 4.04 cm) as compared to mutant plant which showed reduced size of leaf (6.9, 3.84 cm). Internodal length was higher in control plants (7.85 cm), while the mutant WM_1 showed reduced internodal length (7.56 cm). Thickness of internode and thickness at node were maximum in control plants (1.43, 1.50 cm) whereas, mutant showed reduced thickness at internode as well as at node (1.40, 1.45 cm).

Floral characters such as number of days to flowering was found lower in WM1 mutant (63.40 days), while control plants flowered in 64.97 days. Number of flowers and size of flower were found to be lower in the mutant WM1. The control plants showed the highest number of florets in flowers (175.3), which was much lesser in WM1 mutant (167.4). Length and breadth of florets was reduced in the mutant WM1 (4.52, 1.34 cm respectively, which was higher in control plants (4.67, 1.42 cm). Mutant WM1 showed low longevity for intact flower as compared to control plants, whereas in cut flower, the mutant showed higher longevity (7 days) as compared to control flower (6.20 days). Colour variation included a change from light brown centre to light yellow centre in WM1. Form variation was manifested as slight spiking of petals.

Table 39. Mean performance of screened mutants from Amb – white variety in M_1V_2 generation

Characters	Original	Mutants			
		WM1	Scoring for WM1	WM2	Scoring for WM2
Radiation dose	0.0 Gy	5 Gy		10 Gy	
Plant height at maturity (60 days)	69.06	67.51	-	65.10	-
Number of branches at maturity (60 days)	3.54	3.70	+	2.76	-
Number of leaves at maturity (60 days)	20.55	21.18	+	16.09	-
Plant spread	34.74	36.50	+	31.25	-
Leaf length	7.22	6.90	-	6.02	-
Leaf width	4.04	3.84	-	3.01	-
Internodal length	7.85	7.56	-	7.43	-
Thickness of internode	1.43	1.40	-	1.37	-
Thickness at node	1.50	1.45	-	1.42	-
Number of days to flowering	64.97	63.40	+	67.04	-
Number of flowers plant^{-1}	29.61	26.46	-	25.40	-
Size of flower	12.50	12.14	-	9.97	-
Number of florets (ray and disc)	175.30	167.40	-	158.24	-
Length of florets	4.67	4.52	-	4.33	-
Breadth of florets	1.42	1.34	-	1.24	-
Longevity of intact flower	5.12	4.66	-	4.08	-
Longevity of cut flower	6.20	7.00	+	5.22	-
Flower colour	White with light brown shade in centre	White with yellow shade in centre	+	White with yellow centre	-
Fresh weight of tuber	45.29	49.05	+	36.36	-
Number of tubers	7.16	7.43	+	5.35	-
Length of tubers	6.63	6.24	-	5.13	-
Diameter of tuber	1.13	1.07	-	0.87	-
Radiation advantage	-	-	8	-	0

Tuber characters such as weight of tubers and number of tubers (49.05 g, 7.43) formed was much higher in WM1 as compared to control plants (45.29 g, 7.16). Other tuber characters such as length and breadth of tubers were reduced in WM1 mutant.

4.4.2.2 Mutant WM2

The mutant WM2 was selected from Amb – white variety at 10 Gy dose of gamma rays. This mutant showed variation in floral form characters as compared to the original control plant. The central petals did not open completely, presenting a globular or cabbage head appearance. The mutant was isolated from M_1V_1 generation and vegetatively propagated to M_1V_2 generation for checking its stability in floral characters.

Plant height of the mutant was 65.10 cm which was lower than that of the control plant (69.06 cm) at maturity. Branches and leaves (2.76, 16.09) at maturity have respectively showed decreased numbers in WM2 than in control (3.54, 20.55). Leaf characters such as leaf length and width were superior in control plants (7.22, 4.04 cm) as compared to mutant plants which showed lower values for size of leaf (6.02, 3.01 cm). Intermodal length was highest in untreated plants with a length of 7.85 cm, while mutant WM2 showed a decline in length (7.43 cm). Thickness of internode and thickness at node were maximum in case of control plants (1.43, 1.50 cm) whereas mutant showed reduced thickness at internode as well as at node (1.37, 1.42 cm).

WM2 mutant showed delayed flowering (67.04 days), while control plants flowered earlier at 64.97 days. Number of flowers and size of flower was found higher in case of control plants. Control plants showed a higher number of florets in flowers (175.3), which was much lesser in WM2 mutant (158.24). Length and breadth of floret was reduced in mutant WM2 (4.33, 1.24 cm) which was higher in

control plants (4.67, 1.42 cm). Mutant WM2 showed lowest longevity of intact flower as compared to control plants. Cut flowers showed minimum longevity (5.22 days) than control (6.20 days).

Tuber characters such as weight of tubers and number of tubers (36.36 g, 5.35) found in mutant WM2 had lower values as compared to control plants (45.29 g, 7.16). Other tuber characters such as length and breadth of tubers showed a significant decline in WM2 mutant as compared to control.

4.4.3 Mutant of Amb – purple

4.4.3.1 Mutant PM1

The mutant PM1 was selected from Amb – purple variety at 5 Gy dose of gamma rays. The mutant was isolated from M_1V_1 generation and vegetatively propagated to M_1V_2 generation for checking stability in floral characters. It is evident from the data presented in Table 38 that this mutant differed in floral characters from the original control plants in that the tips of the purple ray florets had a white patch (Table 40 & Plate 6).

Plant height of the mutant was 61.62 cm which was lower than control plants (64.92 cm) at maturity. Number of branches showed the highest value in PM1 mutant (5.61) and number of leaves (19.27) was lesser at maturity as compared to control plants (5.42, 23.01). Leaf characters such as leaf length and width were superior in control plants (7.88, 3.99 cm) as compared to mutant plants which showed reduced leaf size (6.78, 3.36 cm). Internodal length, thickness of internode and thickness at node was maximum in control plants whereas the mutant showed reduced values for all the above characters.

PM1 mutant showed early flowering (61.36 days) than control which flowered in 61.80 days. Number of flowers and size of flower was found highest in

Table 40. Mean performance of screened mutants from Amb – purple variety in M_1V_2 generation

Characters	Original	Mutant	
		PM1	Scoring for PM1
Radiation dose	0 Gy	5 Gy	
Plant height at maturity (60 days)	64.92	61.62	-
Number of branches at maturity (60 days)	5.42	5.61	+
Number of leaves at maturity (60 days)	23.01	19.27	-
Plant spread	39.54	42.92	+
Leaf length	7.88	6.78	-
Leaf width	3.99	3.36	-
Internodal length	7.21	6.46	-
Thickness of internode	1.31	1.25	-
Thickness at node	1.39	1.32	-
Number of days to flowering	61.80	61.36	+
Number of flowers plant^{-1}	25.63	24.33	-
Size of flower	10.35	9.23	-
Number of florets (ray and disc)	123.66	116.66	-
Length of florets	4.17	4.01	-
Breadth of florets	1.66	1.52	-
Longevity of intact flower	4.22	4.00	-
Longevity of cut flower	5.38	6.02	-
Flower colour	Purple petals	Purple base petals with white tip	+
Fresh weight of tuber	54.21	47.11	-
Number of tubers	8.90	7.43	-
Length of tubers	6.04	5.48	-
Diameter of tuber	1.01	0.86	-
Radiation advantage	-	-	4

Control

PM1 at 5 Gy

Plate 6. Mutant in M_1 V_1 generation in Amb-purple

control plants. The control plants showed highest number of florets in flowers (123.66), which was much lower in PM1 mutant (116.66). Length and breadth of florets was reduced in the mutant PM1 (4.01, 1.52 cm). Mutant PM1 showed the lowest longevity for intact flower as compared to control plants, whereas for cut flower mutant showed higher longevity. In PM1 the purple ray florets had a white patch at the tip whereas control plants had a single solid coloured petals of a purple hue.

Tuber characters such as weight of tubers, number of tubers and length and breadth of tubers showed a significant decline in PM1 mutant as compared to control plants.

4.5 RANKING OF MUTANTS FOR UNDERSTANDING RADIATION ADVANTAGE

In general all the mutants showed a decline in value for vegetative as well as floral characters.

The mutant WM1 showed some advantages over its parent Amb-white for characters such as number of branches, number of leaves, plant spread, number of days to flowering, longevity of cut flower, flower colour, fresh weight of tuber and number of tubers after irradiation with 5 Gy dose of gamma rays (Table 39). The flower colour variation observed i.e. from white with light brown colour in the centre to white with yellow shade in the centre was novel and attractive. The WM2 mutant from Amb-white showed no advantageous characters over its control for morphological traits. The form variation from normal flat central disc to globular disc was a noteworthy and prominent novelty.

The mutant PM1 had some beneficial characters over its control parent Amb-purple. The characters such as number of branches, plant spread, number of days to flowering and flower colour were superior in PM1 mutant (Table 40). The flower

colour variation observed, i.e., from solid single colour purple petals with a white patch at tip was a novel, distinct and attractive feature.

4.6 CORRELATION STUDIES

4.6.1 Correlation Studies on Vegetative Characters

Table 41. Correlation studies on vegetative characters

	Plant height at maturity	Branches at maturity	Leaves at maturity	Plant spread (g)	Internodal length (cm)	Thickness of internode (cm)	Thickness at node (cm)
Plant height at maturity	1	0.543**	0.934**	0.756**	0.947**	0.946**	0.893**
Branches at maturity		1	0.914**	0.922**	0.656**	0.777**	0.343*
Leaves at maturity			1	0.845**	0.786**	0.908**	0.905**
Plant spread (g)				1	0.414*	0.913**	0.856**
Internodal length (cm)					1	0.236	0.227
Thickness of internode (cm)						1	0.544**
Thickness at node (cm)							1

Correlation studies for vegetative characters were carried out and is presented Table 41. Plant height was significantly correlated in positive direction with number of branches (0.543), number of leaves (0.934), plant spread (0.756), intermodal length (0.947), thickness of internode (0.946) and thickness at node (0.893) at 1 % level of significance.

Branches at maturity was significantly correlated with number of leaves (0.914), plant spread (0.922), internodal length (0.656), thickness of internode (0.777) at 1% level of significance.

Leaves at maturity was significantly correlated with plant spread (0.845), internodal length (0.908) and thickness of internode (0.905) at 1% level of significance. Plant spread was positively correlated with thickness of internode (0.913) and thickness at node (0.856). Thickness of internode was significantly correlated with thickness at node (0.544) at 1% level of significance (Table 41).

4.6.2 Correlation Studies on Floral Characters

Table 42. Correlation studies on floral characters

	Days to flowering	Flowers plant^{-1}	Size of flower (cm)	Number of florets	Weight of tuber (g)	Number of tubers	Length of tuber (cm)	Diameter of tuber (cm)
Days to flowering	1	0.583**	0.167	0.276	-0.437	0.652**	0.225	0.428
Flowers plant^{-1}		1	0.173	0.408*	-0.636	0.325*	0.376*	0.365*
Size of flower (cm)			1	0.934**	0.094	0.386	0.452**	0.377*
Number of florets				1	0.023	0.442**	0.113	0.374*
Weight of tuber (g)					1	0.875**	0.815**	0.796**
Number of tubers						1	0.637**	0.353*
Length of tuber (cm)							1	0.716**
Diameter of tuber (cm)								1

Days to flowering was positively correlated to number of flowers plant^{-1} (0.583) and number of tubers (0.652) at 1% level of significance. Number of flowers

plant^{-1} was significantly correlated with number of florets (0.408) at 1% level of significance. Size of flower was highly correlated with number of florets (0.934) and length of tuber (0.452) at 1% level of significance (Table 42).

Number of florets was positively correlated to number of tubers (0.442) at 1% level of significance. Fresh weight of tubers was highly significant and positively correlated to number of tubers (0.875), length of tuber (0.815) and diameter of tuber (0.796) at 1 % level of significance. Number of tubers was positively correlated to length of tuber (0.637). Length of tuber was positively correlated to diameter of tuber (0.716) at 1% level of significance.

Discussion

5. DISCUSSION

Dahlia is one of the most popular bulbous flowers grown in many parts of the world for its beautiful ornamental blooms of varying shades of colours for the beautification of gardens and for use as cut flowers. Varieties come in a wide array of sizes/ forms from as low as 1 foot to as tall as 6-8 feet with flowers ranging in size from half-inch to giant sizes. The blooms are curvaceous, spiky with single or double petals. Colours range from white to red, orange to yellow and pink to dark purple.

The present investigations entitled "Genetic improvement through induced mutation in dahlia (*Dahlia variabilis* Desf.)" was carried out to induce variability in three varieties of dahlia namely, Amb-white, Amb-purple and Amb-red using gamma rays under field conditions.. The salient findings of the present investigation are discussed here under the following heads:

5.1 EFFECT OF GAMMA RADIATIONS ON M_1 DAMAGES AND VEGETATIVE CHARACTERS

Evaluation of M_1V_1 generation for M_1 damages and vegetative characters of plants obtained through gamma radiation are discussed below.

The M_1V_1 of the three varieties was evaluated with respect to several vegetative characters after exposure to different doses of gamma rays. The characters studied were plant height, number of branches, plant spread, number of leaves, leaf length, leaf width, internodal length, thickness of internode and thickness at node. In general, it was found that the different vegetative 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 (1993) in *Dendrobium* also reported a decrease in vegetative characters with increase in dose of mutagen.

5.1.1 Sprouting (%)

As evident from the data presented in Fig. 1, there was significant reduction in sprouting percentage with increasing gamma ray doses. All the treatments differed significantly with each other with respect to sprouting percentage. Tubers when treated with gamma rays results in ionization and excitation of water molecules which can damage or modify components of plant cells and affect certain physiological and biochemical processes that are vital for sprouting of tubers. According to Kaicker (1992) reduction in survival at higher doses is due to this toxic effect of radiations in rose. Reduction in survival rate at higher doses may also be attributed to genetic loss due to chromosomal aberrations and gene mutations in marigold as reported by Tiwari and Kumar (2011). Significant reduction in sprouting was noticed by Banerji and Datta (1992) in chrysanthemum variety 'Jaya' with 1.5, 2.0 and 2.5 kR gamma rays which is in accordance with the results of the present investigation. Reduction in survival after exposure to gamma rays has been explained as due to disturbances in auxin synthesis (Gordon, 1957) and chromosomal aberration, in chrysanthemum (Gunckle and Sparrow, 1961).

5.1.2 Survival at 15 Days (%)

Significant reduction in survival percentage was observed with increasing gamma ray doses in the present study (Fig. 1). Dilta *et al.* (2003) reported that with an increase in dose of gamma rays the percent survival decreased in chrysanthemum which is in agreement with the current results.

5.1.3 Plant Height (cm) (at 30 Days Interval)

A gradual decrease in plant height was recorded with gradual increase in dose of gamma rays. Maximum height was noted in control plants. Among the treatments higher doses showed a decrease in plant height as compared to lower doses (Fig. 2 & Fig. 3). This general trend was observed with data collected at 30, 60, 90 and 120

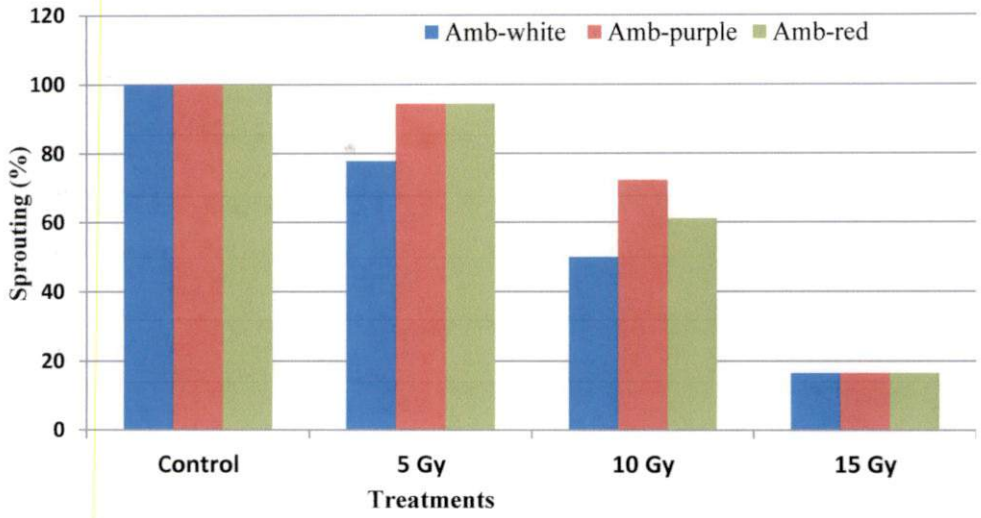


Fig.1. Effect of gamma radiations on sprouting and survival percentage in dahlia varieties

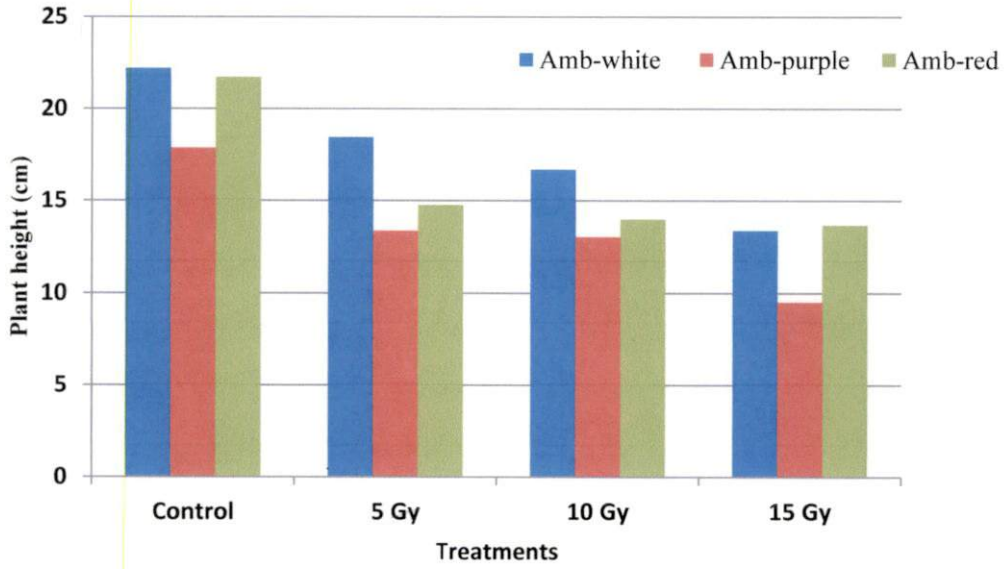


Fig.2. Effect of gamma radiations on plant height at 30 days after planting

days after planting. Significant reduction in plant height was noticed by Banerji and Datta (2001) when they treated the rooted cuttings of chrysanthemum cv. 'Surekha' with 150, 200 and 250 Gy doses of gamma rays. They found that higher doses showed maximum reduction in plant height. The results obtained by Dilta *et al.,* (2003) and Boersen. *et al* (2006) in chrysanthemum confirms the results of the present study. 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.

5.1.4 Plant Spread (cm)

The range of plant spread among the treatments showed that there was a significant reduction in plant spread at higher doses of gamma rays as compared to lower doses and control plants showed good plant spread. This decrease was directly proportional to the dose employed (Fig. 4). 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 vegetative characters with increase in dose of mutagen. Dilta *et al.,* (2003) also observed the decrease in plant spread when rooted cuttings of various cultivars of chrysanthemum were treated with gamma rays.

5.1.5 Number of Branches Plant^{-1} (at 30 Days Interval)

A gradual decrease in number of branches was recorded with gradual increase in dose of gamma rays. Maximum number of branches was noted in plants which were untreated with gamma rays than plants which were treated. Among the treatments, plants which were treated with lower doses showed more number of

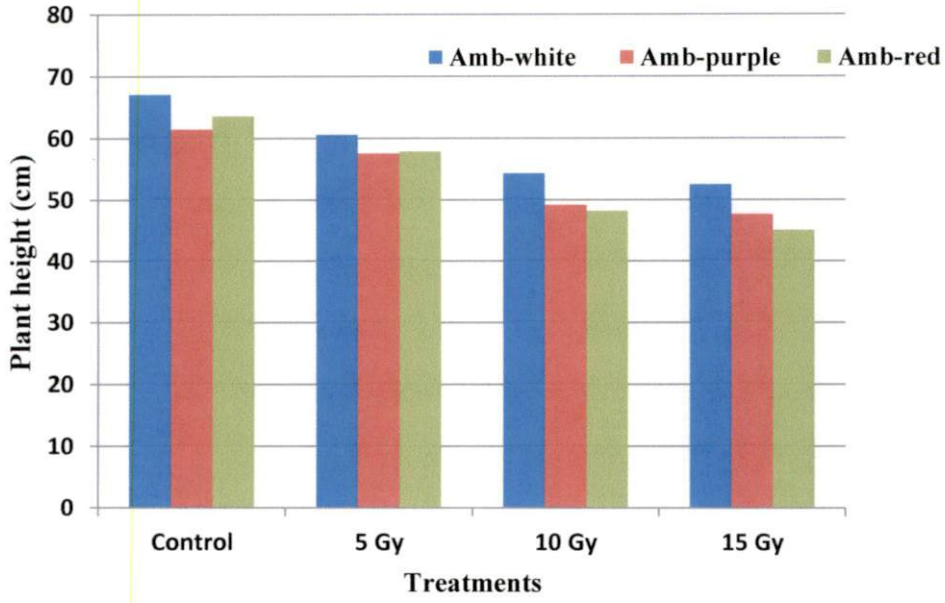


Fig .3. Effect of gamma radiations on plant height at 60 days after planting

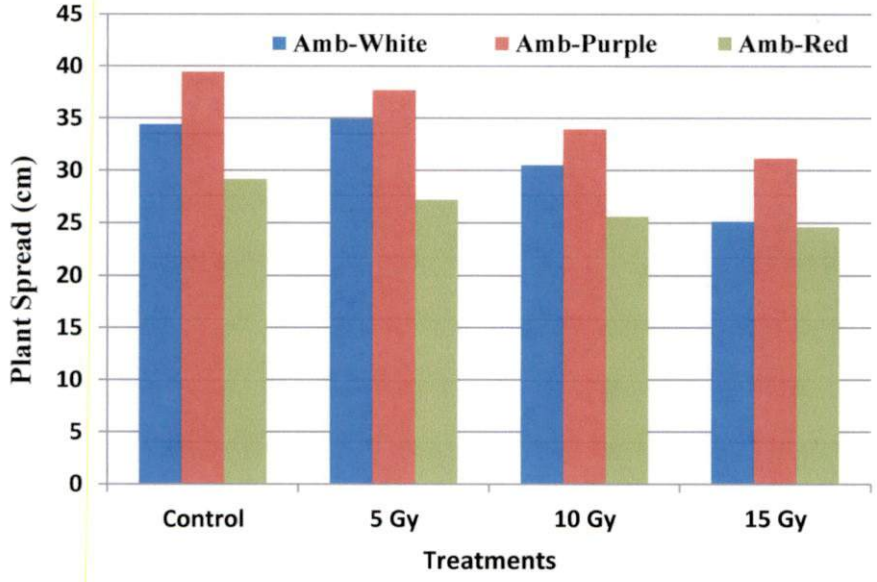


Fig .4. Effect of gamma radiations on plant spread (cm) in dahlia

branches than plants which were treated with higher doses. This general trend was evident at 30, 60, 90 and 120 days after planting (Fig. 5 & Fig. 6). The reduction in number of branches may be due to inhibitory effect of higher mutagenic doses of gamma rays. Datta and Gupta (1980) found significant reduction in number of branches when they treated the rooted cuttings of 'D-5', 'Lalkila' and 'Lilith' cultivars of chrysanthemum with 1.5 and 2.0 kR gamma rays. Similarly, a significant decreased in number of branches was recorded when rooted cutting of chrysanthemum cv. 'Anupam' were exposed to 1.5, 2.0 and 2.5 kR of gamma rays by Banerji and Datta (1990). All these results are in agreement with the results of the present study.

5.1.6 Number of Leaves Plant^{-1} (at 30 Days Interval)

There was a general reduction in number of leaves at higher doses which was evident at all stages viz. at 30, 60, 90 and 120 days after planting (Fig. 7). The decrease was directly proportional to the dose employed. This decrease is mainly due to the decrease in number of branches plant^{-1} as reported by Misra *et al.* (2009) in chrysanthemum cv. 'Pooja'. Reduction in vegetative growth due to gamma irradiation may be due interference of ionized molecules in normal mitotic cell division and also may be due to several chromosome aberrations and consequent reduction in nutrient uptake capacity of plant (Ehrenberg, 1995). Similar results were also reported by (Dwivedi and Banerji, 2008) in Dahlia.

5.1.7 Leaf Length and Leaf Width (cm)

Reduction in vegetative growth due to gamma ray treatment was evident for leaf length and width also (Fig. 8 & Fig. 9). Banerji *et al.,* (1994) had also reported that reduction in leaf number, leaf width and leaf length was found in gladiolous after treating corms with higher dose of gamma rays. Similar results were reported by (Dwivedi and Banerji, 2008) in Dahlia. Datta and Banerji (1993) also observed

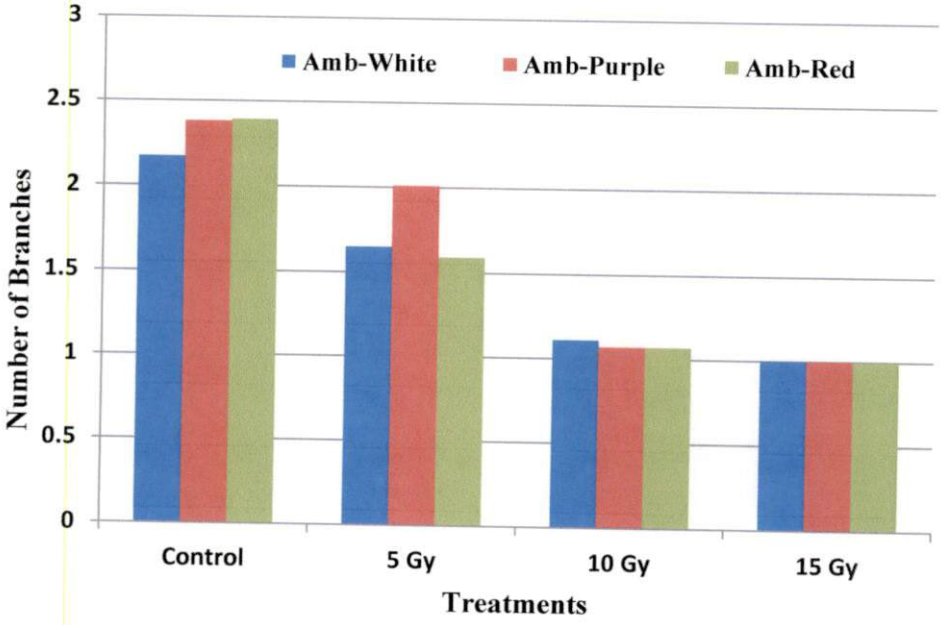


Fig.5. Effect of gamma radiations on number of branches at 30 days after planting

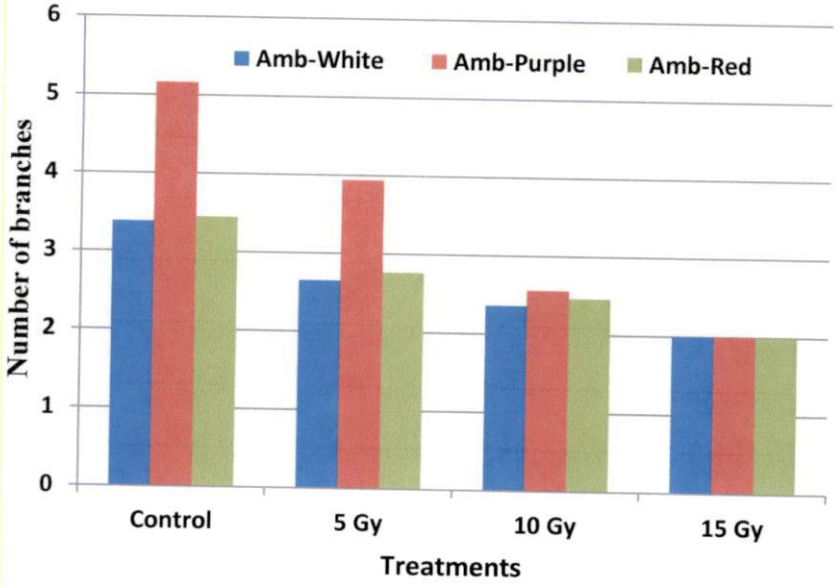


Fig .6. Effect of gamma radiations on number of branches at 60 days after planting

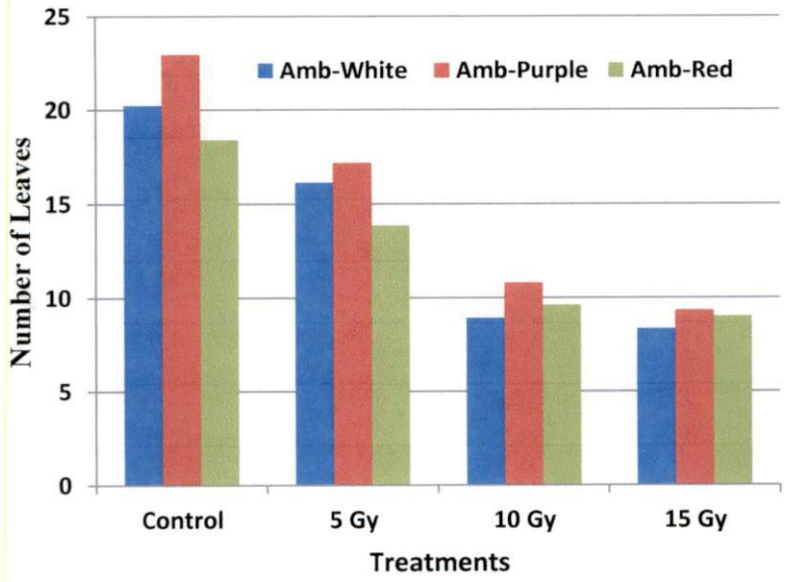


Fig .7. Effect of gamma radiations on number of leaves at 60 days after planting

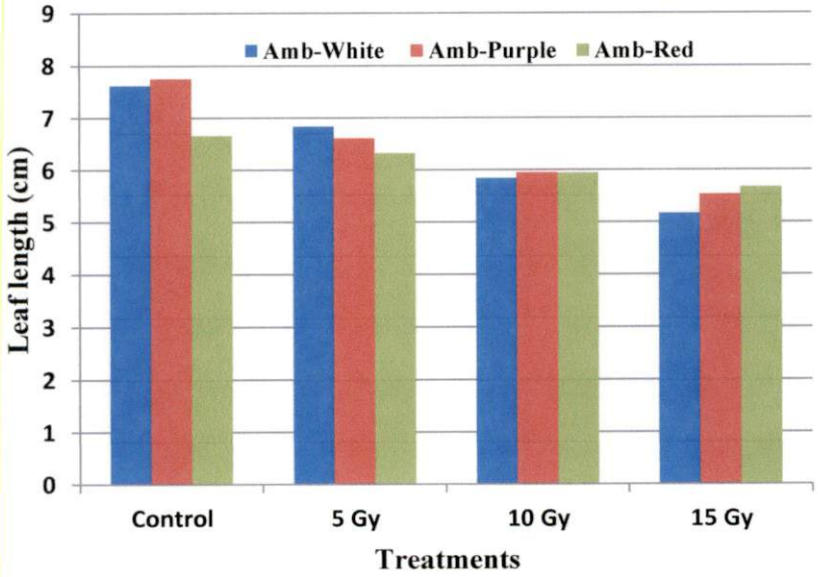


Fig .8. Effect of gamma radiations on leaf length (cm)

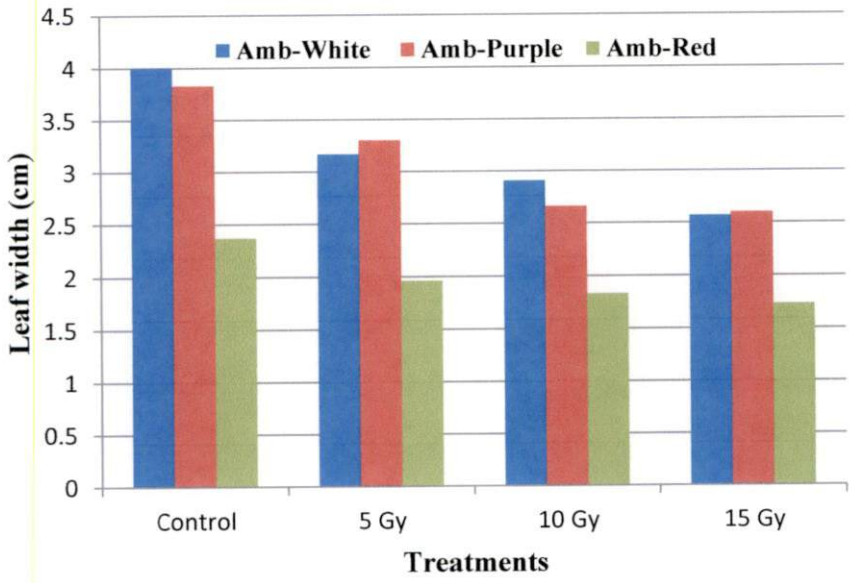


Fig .9. Effect of gamma radiations on leaf width (cm)

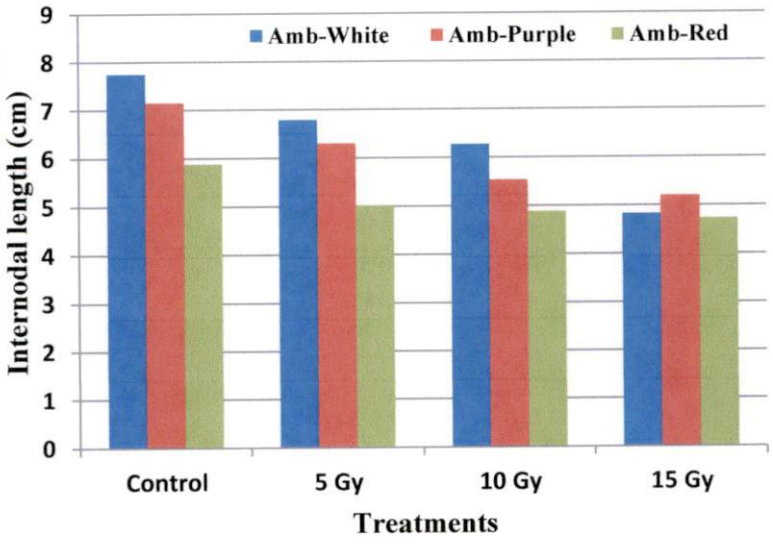


Fig .10. Effect of gamma radiations on intermodal length (cm)

reduction in leaf size after exposing rooted cuttings of chrysanthemum cv. 'Kalyani Mauve' with 150, 200 and 250 Gy doses of gamma rays. All the reports are in agreement with the results of the current study.

5.1.8 Internodal Length (cm) and Thickness of Internode and Node (cm)

The effect of gamma rays and their interaction on intermodal length, thickness of internode and node was found significant among treated and untreated plants. As the dose of gamma rays increased there was a significant reduction for all these metric traits (Fig. 10, Fig. 11 & Fig. 12). This reduction may be due to decrease in plant height and size with increase in gamma ray doses as reported by Dilta *et al.* (2003) in chrysanthemum.

5.2 EFFECT OF GAMMA RADIATIONS ON FLORAL CHARACTERS

5.2.1 Number of Days to Flowering

Early flowering was noticed in lower dose which might be due to increased activities of auxins and gibberellins and other flowering hormones and also may be due to reduced levels of inhibitors. Srivastava *et al.* (2007) observed earlier flower opening in lower doses of gamma rays as compared to control in gladiolous cvs. 'Sylvia' and 'Eurovision' and delayed flowering at higher doses. The delay in flower bud initation may be due to reduction in the rate of various physiological processes with gamma irradiation. Neto and Latado (1996) reported a significant delay in flower bud formation in chrysanthemum variety 'Repin' with 20 Gy dose of gamma rays as compared to control.

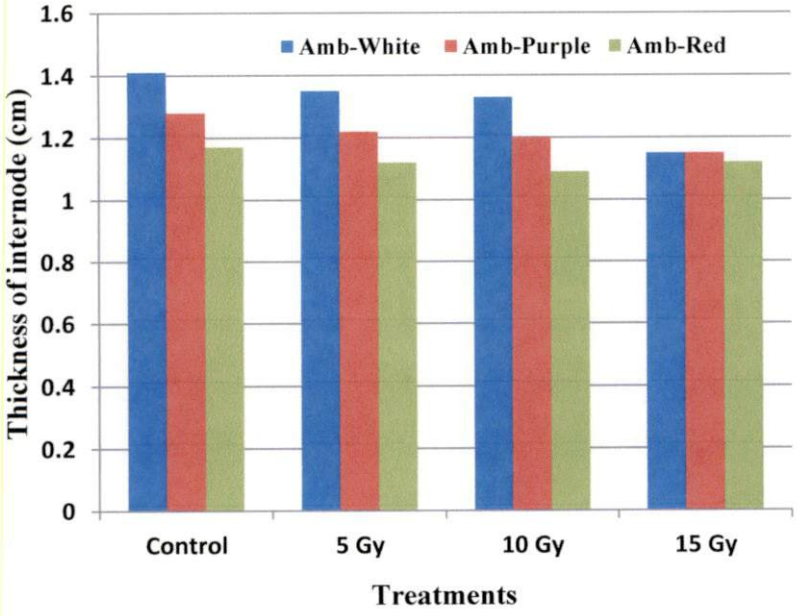


Fig .11. Effect of gamma radiations on thickness of internode (cm)

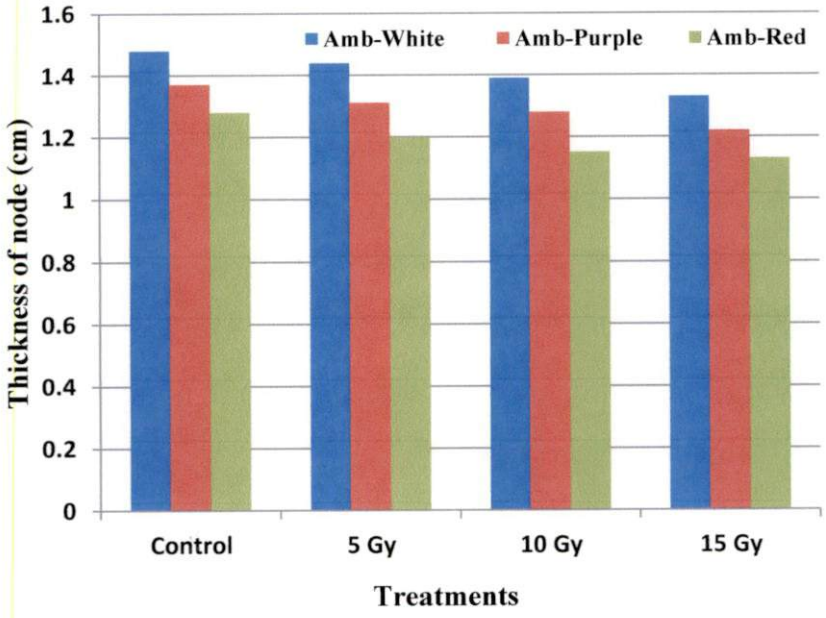


Fig .12. Effect of gamma radiations on thickness at node (cm)

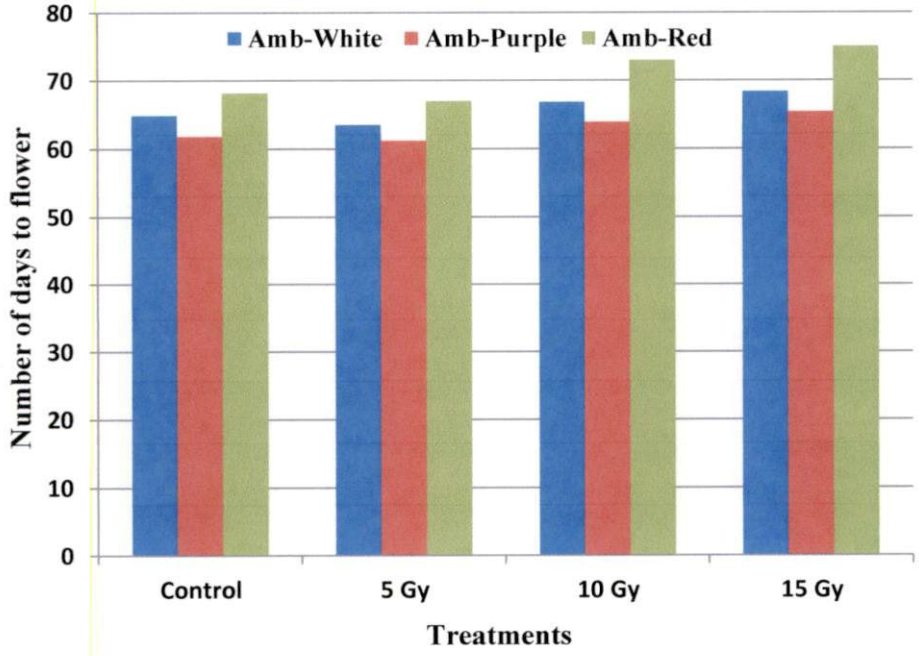


Fig.13. Effect of gamma radiations on number days to flowering

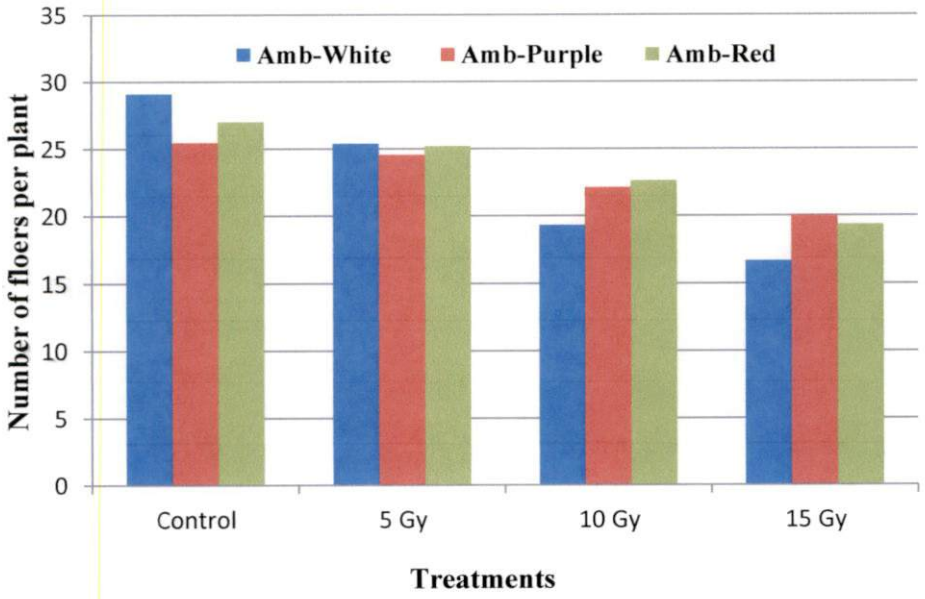


Fig .14. Effect of gamma radiations on number of flowers per plant

5.2.2 Number of Flowers Plant^{-1}

The minimum number of flowers was recorded in the treatment with highest dose of gamma rays. This clearly demonstrates that exposure of plants to higher doses of gamma rays had an adverse effect on floral characters. The decrease in number of flowers^{-1} plant may be due to decrease in number of branches plant^{-1} (Fig. 14). Significant reduction in number of flowers in chrysanthemum when exposed to 1.0, 1.5, 2.0, 2.5 and 3.0 Kr doses of gamma rays were recorded by Dilta *et al.* (2003) which agrees with the current results. The findings of Datta and Gupta (1981) who recorded significant delay in blooming of chrysanthemum variety 'Nimrod' with 15 and 20 Gy gamma ray irradiation are also in line with the results of the present study.

5.2.3 Size of Flower (cm)

In general, higher doses of gamma radiations reduced flower size (Fig. 15). This might be due to reduction in the vegetative growth of the treated plants. Dwivedi and Banerji (2008) reported reduction in flower size in Dahlia cv. 'Pinki' after treatment of rooted cuttings with gamma radiations. At higher doses, the effect of gamma radiations was more pronounced, which resulted in smaller flower size. Gupta and Jugran (1978) also recorded smaller sized flowers in chrysanthemum cvs.cultivar 'Gairik', 'Otome', 'Zakura' and 'Raja' on treating with 1.0, 1.5, 2.0 and 2.5 kR doses of gamma rays. They also concluded that reduction in flower size could be due to physiological, morphological and cytological disturbance by gamma radiation.

5.2.4 Number of Florets (Both Disc and Ray Florets)

Number of florets flower^{-1} was reduced significantly in higher doses of gamma rays (Fig. 16). Sisodia and Singh (2014), observed the maximum number of floret flower^{-1} in the untreated plants and also noted that there was a significant reduction in floret number at higher doses of gamma rays in gladiolus which might be

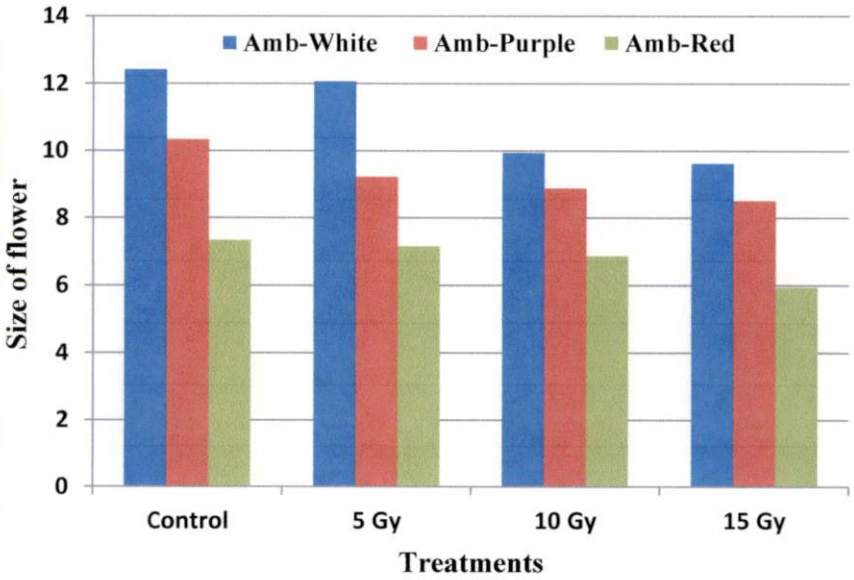


Fig .15. Effect of gamma radiations on size of flower

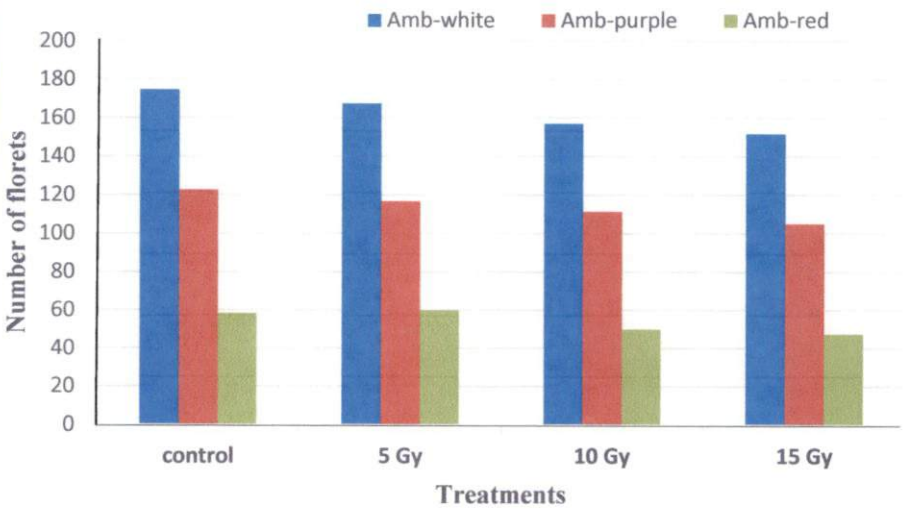


Fig .16. Effect of gamma radiations on number of florets

due to reduction in the vegetative growth of the treated plants and also physiological disturbances which was in line with the present conclusions.

5.2.5 Length and Breadth of Floret (cm)

Dwivedi and Banerji (2008) reported a reduction in petal area in Dahlia cv. 'Pinki' after treatment with gamma rays. At higher doses, effect of gamma radiations was more pronounced, which resulted in smaller petal size (Fig. 17 & Fig. 18). The reduction in petal size in terms of length and width of petals 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, 1957). The current 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 in Bougainvillea cv. 'Los Banos Beauty'. Such an effect is known to arise due to chromosomal aberrations in addition to genetic mutations.

5.2.6 Longevity of Intact and Cut Flowers (Days)

A gradual decrease in longevity of cut flower was recorded with gradual increase in the dose of gamma rays (Fig. 20). Untreated plants showed a higher longevity than treated plants. The longevity of dahlia was least and there was disharmony of ray florets opening at higher doses. The delay in flowering resulted in a reduced longevity period both in intact and in cut flower condition. The reports of Banerji *et al.* (1994) in gladiolus and Negi *et al.* (1983) in china aster are in line with the findings of the present investigation.

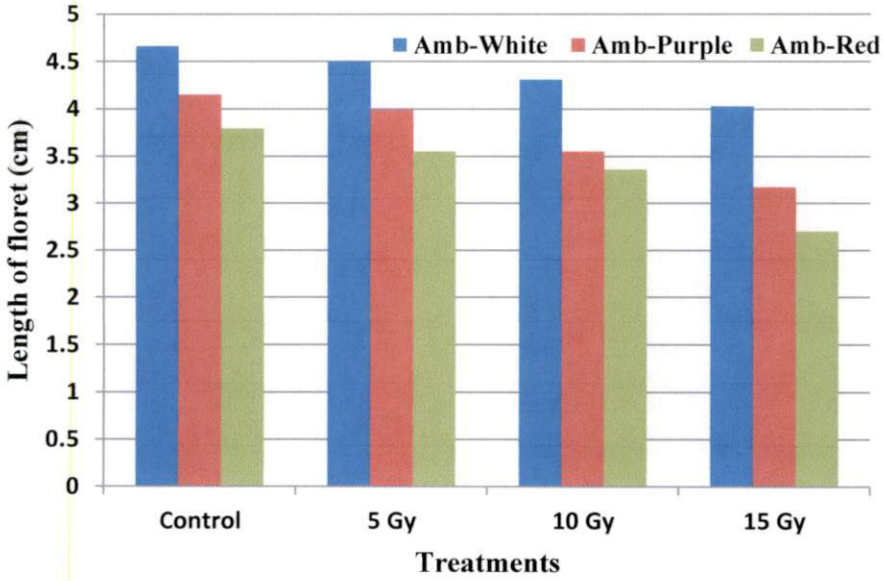


Fig .17. Effect of gamma radiations on length of floret (cm)

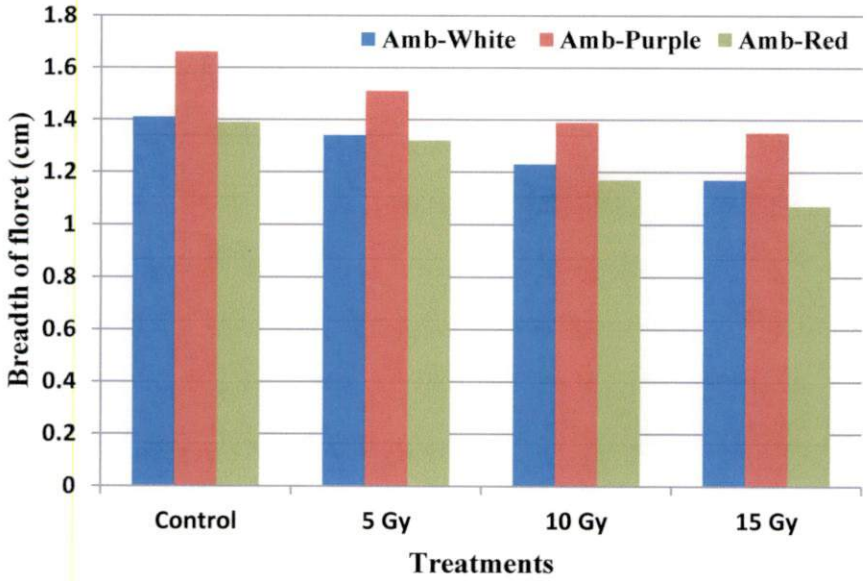


Fig .18. Effect of gamma radiations on breadth of floret (cm)

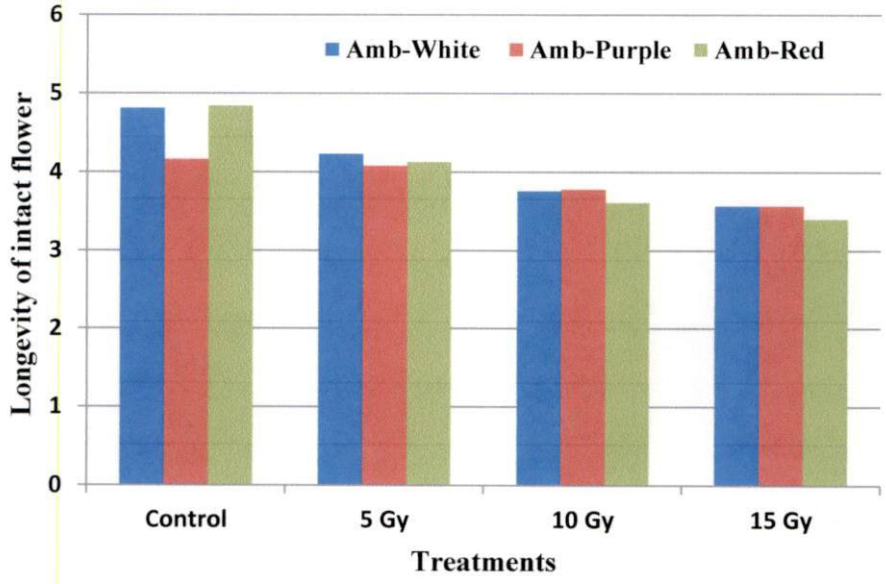


Fig .19. Effect of gamma radiations on longevity of intact flower (days)

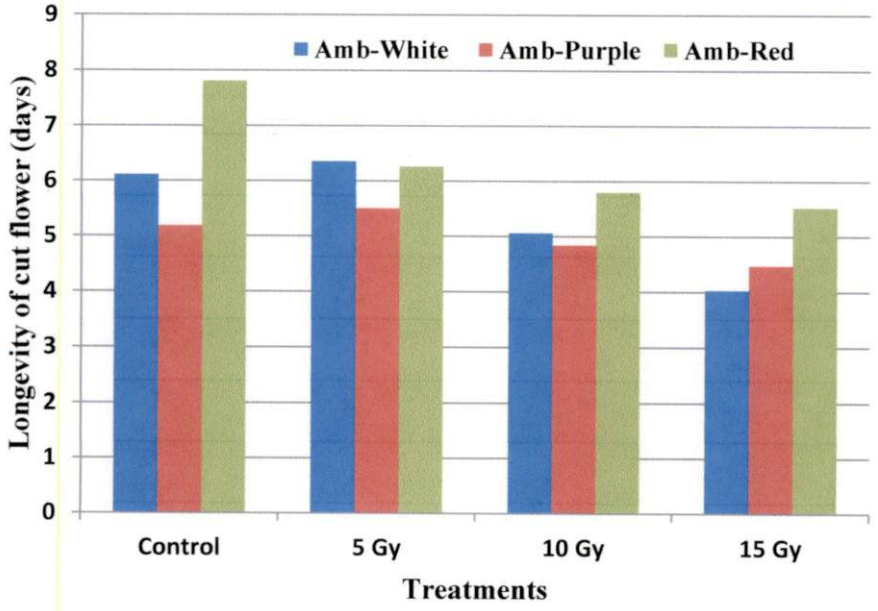


Fig .20. Effect of gamma radiations on longevity of cut flower (days)

5.2.7 Flower Colour and Form

In Amb - white and Amb - purple mutants could be identified in M_1V_1 generation for flower colour at 5 Gy and 10 Gy dose of radiation. Change in the flower colour might be due to the mutations at cellular level. WM1 mutant in Amb-white had a colour change in the central disc. It was brownish in the variety whereas it was light yellow in the mutant, with ray florets being white coloured similar to the original. In WM2 mutant in Amb-white, the central disc was enclosed by a whorl of ray florets presenting an unopened spherical appearance of ray florets in the centre. Towards the pheriphery, the ray florets were broad and arranged in a haphagard manner. These two mutants however showed a tendency to revert back to the original in M_1V_2 generation (Plate 7). PM1 in Amb-purple at 5 Gy had white sectors on the tip of purple ray florets in M_1V_1 generation which showed a stablility in M_1V_2 generation. Dwivedi and Banerji (2008) obtained mutants similar to PM1 with white sectors in ray florets when dahlia cv. 'Pinki' was irradiated with 5-10 Gy gamma rays. Datta *et al.,* (2001) could identify ray floret colour mutants which appeared to be true to type in two successive generations when *Dendranthema grandiflora* cv. 'Puja' was irradiated with 1.0-2.0 krad gamma rays.

5.3 CORRELATION STUDIES ON VEGETATIVE AND FLORAL CHARACTERS

The association between various vegetative and floral parameters was studied in dahlia. Correlations revealed that plant height and branches were significantly correlated in positive direction with number of leaves, plant spread, internodal length, thickness of internode and thickness at node. Days to flowering was positively correlated to number of flowers per plant and number of tubers. Number of flowers per plant was significantly correlated with number of floret. Size of flower was highly correlated with number of florets and length of tuber. Since these associations are in desirable direction and selection of these traits may ultimately improve the yield.

These results are in agreement with the results obtained by Suman *et al.* (1980) in dahlia. In addition, Rao (1982) and Asish *et al.* (2004) reported significant and positive association of plant height with intermodal length in China aster and anthuriums respectively.

Summary

6. SUMMARY

An investigation to study "Genetic improvement through induced mutation in Dahlia (*Dahlia variabilis* Desf.)" was carried out at College of Agriculture, Vellayani, Kerala Agricultural University, during 2015 to 2017. The study was carried out to find out the variability induced by gamma radiations on morphological and plant characters in three varieties namely, Amb-white, Amb-purple and Amb-red under field conditions.

The first part of the programme included induced mutagenesis using gamma rays as potent mutagen. Three varieties were treated with six doses of gamma rays ranging from 5 to 25 Gy along with control. The data recorded on M_1 damages, vegetative characters, floral characters and tuber characters were statistically analysed and significance of results was verified. In the experiment three prominent variants isolated in M_1V_1 generation were advanced to M_1V_2 generation for stability assessment.

The M_1V_1 of the three varieties was evaluated with respect to several vegetative characters after exposure to different doses of gamma rays. The characters studied were plant height, number of branches, plant spread, number of leaves, leaf length, leaf width, internodal length, thickness of internode and thickness at node. In general, it was found that the different vegetative traits showed reduced expression as compared to control on mutagen treatment. This decrease was directly proportional to the dose employed.

The per cent sprouting and survival at 15 days (%) decreased with increase in dose of gamma rays which was exhibited by all the three varieties in the same fashion. The reduction at higher doses might be attributed to genetic loss due to chromosomal aberrations and gene mutation which might be lethal for sprouting and survival.

A gradual decrease in plant height was recorded with increase in dose of gamma rays which was evident in data recorded at 30, 60, 90 and 120 days after planting. Reduction in vegetative growth after exposure to radiations might be due to interference in normal mitosis and frequent occurrence of mitotic aberrations, inhibition of rate of assimilation and also due to changes in auxin level or due to inactivation of auxin.

The range in plant spread among the treatments showed that there was a significant reduction in plant spread at higher doses of gamma rays as compared to lower doses. This decrease was inversly proportional to the dose employed. A gradual decrease in number of branches was recorded with gradual increase in dose of gamma rays. The reduction in number of branches may be due to inhibitory effect of higher mutagenic doses of gamma rays. Leaf length and leaf width were found higher in control plants as compared to treated plants. As dose of gamma radiation increased the length and width of leaf decreased. Internodal length, thickness of internode and thickness at node were found superior in control plants than the plants treated with gamma rays in all the three varieties studied.

Days to flowering however, was earlier at 5 Gy (63.89 days) as compared to control (64.94 days). As dose of radiation increased the days to flowering also increased. Number of flowers plant^{-1} and size of flower (cm) were inversely proportional to mutagen dose. The number of flowers got significantly reduced with increasing rate of gamma irradiation. The decrease in number of flowers per plant may be due to decrease in number of branches plant^{-1}.

Significant reduction in floret number at higher doses of gamma rays was noticed, which might be due to reduction in the vegetative growth of the treated plants and also physiological disturbances. Petal number was also reduced at doses which were high.

The longevity of flower was reduced and there was disharmony of ray floret opening at higher doses. The delay in flowering resulted in a reduced longevity period both in intact and cut flower condition.

In M_1V_1 generation Amb - white and Amb - purple produced mutants for flower colour at 5 Gy and 10 Gy dose of radiation. Flower colour mutant obtained from Amb-white variety at 5 Gy was WM1 which showed a change in colour from wood colour in control flower to yellow colour in the central disc region . In WM2 mutant in Amb-white, the central disc was enclosed by a whorl of ray florets presenting an unopened spherical appearance of ray florets in the centre. Towards the pheriphery, the ray florets were broad and arranged in a haphazard manner. The mutant from Amb-purple PM1 showed white tips with purple base, whereas control flower was single solid purple colour.

Length of tuber was found to be the highest in plants treated with 5 Gy dose as compared to untreated plants. As doses were increased the tuber length showed a diminishing trend. Fresh weight, length and breadth of tubers were inversely proportional to mutagen dose.

Three mutants showing prominent flower character mutations were isolated from M_1V_1 generation. They include WM1 showing flower colour and form variation isolated from Amb-white, WM2 showing form variation isolated from the same parent and PM1 showing colour variation isolated from Amb-purple. They were carried forward to M_1V_2 generation for stability screening. Both WM1 and WM2 showed a tendency to revert back to the original flower form and colour, denoting that they were not stable. PM1, the colour mutant isolated from Amb-purple, showed stable performance in M_1V_2 generation. Although prominent plant architecture mutations were not observed, significant size reduction at higher doses was prevalent in all the three varieties in M_1V_1 and M_1V_2 generations.

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Genetic improvement through induced mutation in dahlia (*Dahlia variabilis* Desf.)

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ABSTRACT

The present investigation entitled "Genetic improvement through induced mutation in dahlia (*Dahlia variabilis* Desf.)" was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2015-2017, with the objective of inducing variability in dahlia for plant architecture and floral characters through gamma irradiation.

The experimental material comprised of three varieties of dahlia (Amb-white, Amb-puple and Amb-red) and the study was carried out in two experiments. In the first experiment, the material was irradiated with six doses of gamma rays (0, 5, 10, 15, 20 and 25 Gy) and field evaluation of M_1V_1 generation was done. The data recorded on M_1 damages, vegetative characters, floral characters and tuber characters and were statistically analysed and significance of results was verified. In the second experiment prominent mutants isolated in M_1V_1 generation were advanced to M_1V_2 generation for stability assessment.

Significant differences were observed in sprouting (%) of tubers in the three varieties when exposed to different doses of gamma irradiation. Sprouting percentage decreased as the dose of gamma rays increased. Maximum sprouting was observed in 5 Gy (88.86 %). 15 Gy showed a sprouting of 16.6 % whereas no sprouting was recorded in 20 and 25 Gy. According to Kalcker (1992) reduction in sprouting at higher doses is due to toxic effect of radiations.

A gradual decrease in plant height was recorded with increase in dose of gamma rays which was evident in data recorded at 30, 60, 90 and 120 days after planting. Control plants were taller than treated plants in both M_1V_1 and M_1V_2 generations. Maximum number of leaves was obtained in control plants (47.79),

while minimum was recorded in the higher dose *i.e,* 15 Gy (42.89) at 120 days. Leaf length and leaf width were found higher in control plants as compared to treated plants. As dose of gamma radiation increased the length and width of leaf decreased. Internodal length (cm), thickness of internode (cm) and thickness at node were found superior in control plants than the plants treated with gamma rays in all the three varieties studied.

Days to flowering, however was earlier at 5 Gy (63.89 days) as compared to control (64.94 days). As dose of radiation increased the days to flowering also increased. Number of flowers plant^{-1} and size of flower (cm) were inversely proportional to mutagen dose. Length of tuber was found to be the highest in plants treated with 5 Gy dose (5.76 cm) as compared to untreated plants (5.10 cm). Fresh weight, length and breadth of tubers were inversely proportional to mutagen dose.

Three mutants showing prominent flower character mutations were isolated from M_1V_1 generation. They include WM1 showing flower color and form variation isolated from Amb-white, WM2 showing mainly form variation isolated from the same parent and PM1 showing mainly colour variation isolated from Amb-purple. They were carried forward to M_1V_2 generation for stability screening. Both WM1 and WM2 showed a tendency to revert back to the original flower form and color, denoting that they were not stable. PM1, the color mutant isolated from Amb-purple, showed stable performance in M_1V_2 generation. Although prominent plant architecture mutations were not observed, significant size reduction at higher doses was prevalent in all the three varieties in M_1V_1 and M_1V_2 generations.

From the study it may be concluded that gamma rays of doses below 15 Gy can be effectively used to induce variability in dahlia for plant size and floral characters. The flower colour mutant PM1 showing performance stability in M_1V_2 may be forwarded further for stability screening.

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