

रजनीगंधा (*पॉलिएन्थस ट्यूबरओसा* लिन.) की प्रज्वल और फूले
रजनी कृषिजीव जातियों पर गामा किरणन के उत्परिवर्तजन प्रभावों
का अध्ययन

**STUDIES ON MUTAGENIC EFFECT OF GAMMA IRRADIATION
ON TUBEROSE (*POLIANTHES TUBEROSA* LINN.) CULTIVARS
PRAJWAL AND PHULE RAJANI**

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**DIVISION OF FLORICULTURE AND LANDSCAPING
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI – 110012**

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ON TUBEROSE (*POLIANTHES TUBEROSA* LINN.) CULTIVARS
PRAJWAL AND PHULE RAJANI**

By

JYOTHI R.

A Thesis

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CERTIFICATE

This is to certify that the thesis entitled “*Studies on mutagenic effect of gamma irradiation on tuberosse (Polianthes tuberosa Linn.) cultivars Prajwal and Phule Rajani*” submitted to the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **HORTICULTURE**, embodies the results of *bona-fide* research work carried out by Ms. **JYOTHI R. (Roll No. 9429)**, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

She has duly acknowledged all the assistance and help received during the course of investigation.

Dated:

(Krishan Pal Singh)
Chairman
Advisory Committee

Dedicated to
My
Beloved Parents

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LIST OF ABBREVIATIONS

h	:	Hour
g	:	Gram
mg	:	Milligram
CD	:	Critical difference
RBD	:	Randomized Block Design
cm	:	Centimeter
ml	:	Milliliter
FA	:	Ferric oxide
TBA	:	Tertiary Butyl Alcohol
<i>et al.</i>	:	Co-worker
cv./cvs	:	Cultivar/Cultivars
γ ray	:	Gamma ray
^{60}Co	:	Cobalt - 60
Gy	:	Gray
%	:	Per cent
kR	:	kiloRad
r/ R	:	rad/Rad
J/m^2	:	Joule per square meter
LD	:	Lethal dose
CV	:	Covariance
ANOVA	:	Analysis of variance
m	:	Meter
mm	:	Millimeter

1. INTRODUCTION

Floriculture is a fast emerging major venture in the commercial world, growing at the rate of 10-15 per cent. India, despite its diverse eco-geographical regions, varied climatic conditions, rich native flora and access to modern technology, has to make a dent in international market. In India the total area under floricultural crops has risen to approximately 1.67 lakh hectares (Anonymous, 2009).

Ornamental bulbs, one of the most beautiful and variable group of geophytes, have been appreciated from ancient times. Ornamental bulbs belong to numerous botanical taxa and constitute a large family of the monocotyledonous plants. From a horticultural standpoint, they are utilized mainly for commercial flower production, including outdoor and forced cut flowers and potted plants, and for landscaping, including private gardening. Tuberose (*Polianthes tuberosa* Linn.) is one of the most important bulbous perennial flowering plant of tropical and sub-tropical areas belonging to monocots (Rose 1903, Bailely 1939). It occupies second position in area and production after gladiolus in India (Desh Raj, 2006). It is found to be originated at Mexico, Hutchinson (1934) placed it under the family Agavaceae.

The most extensive contributions to new species of *Polianthes* were made by Rose (1903). It is a monotypic genus closely related to *Bravoa*. Some other important species of *Polianthes* other than *Polianthes tuberosa* are *P. palustris* (white), *P. durangensis* (purplish), *P. montana* (white), *P. longiflora* (whitish purple), *P. plaitphylla* (white tinged with red), *P. grandiflora* (deep red), *P. geminiflora* (light orange red), *P. gracillis* (white), *P. blissi*, *P. pringlie* (white), *P. sesiliflora* (white), *P. nelsonill* (white) and *P. graminiflora* (red). These species range in colour from white, orange red and red. All the species are wild with the exception of *P. tuberosa* which has never been found anywhere except under cultivation. These species have been discovered in the cool mountains valleys of western and northwestern Mexico.

Tuberose (*Polianthes tuberosa* Linn.) occupies a prime position owing to its popularity as a cut flower, loose flower and also raw material for extraction of the highly valued natural flower oil. The serene beauty of the flower is because of its tall and straight spikes which bear bright white florets which are loosely arranged on spike that can reach 2-4 feet in height. The flowers are bisexual, funnel shaped with waxy white and fragrant perianth tube. Perianth tube consists of six acute tepals. Stamens are six in number with the anthers dorsifixed in the middle of the tube. Gynoecium has a trilocular ovary with numerous ovules and the fruit is a capsule. Foliage is long, slender and grass-like with little landscape value. Potted plants can be put in decorative container and used for entrance ways and interiors (Sharga, 1977). The loose flowers of Single type are used for making garlands and floral decorations and extraction of concrete, while the cut flowers of Double type are used in vase decoration, hand bunches and bouquets. The natural flower oil of *Polianthes tuberosa* remains today one of the most expensive of the perfumers raw materials (Trueblood, 1973; Guenther, 1952; Singh *et al.*, 2010).

In India, tuberose is being commercially cultivated over 30, 000 ha area and the main growing states are Uttar Pradesh, Uttarakhand, Haryana, West Bengal, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh (Singh *et al.*, 2010). In India, there are about 17 cultivars available, which include Single type, Double type and Variegated ones. The main problem in tuberose breeding is lack of genetic variability. Only two cultivars 'Single' and 'Double' are popular among farmers and are being commercially cultivated. Named cultivars are very few in tuberose and they derive their name from the number of row of tepals each flower possesses *viz.*, Single- cultivar with a single row of tepals; Double-the one which bears more than three rows of tepals; Semi-double-cultivar bears flower with two to three rows of tepals. All the variegated cultivars are merely for novelties and hence not cultivated commercially. All the available cultivars in India bear white colour flowers, even though some of the cultivars bear flower buds which are tinged with pink blush like in cultivars, Prajwal, Shringar, Sikkim Selection, Hyderabad Single, Pearl Double and Suvasini and green tinge in

cultivars Vaibhav, Phule Rajani and Mexican Single, however the fully opened flowers are white in colour in all the cultivars. The genetic variability in tuberose is very limited (Anu *et al.*, 2003) and it has narrow genetic base.

The genetic variability is necessary in any plant breeding programme for crop improvement. Non-availability of genetic variability has become a major constraint in conventional breeding of tuberose. Genetic improvement of tuberose is hampered by meager genetic variability, self incompatibility and seed sterility. These factors impair the conventional improvement programme. Even though there is not much genetic variability in this crop, it is highly heterozygous and hence variability can be created. Seed set is observed only in a few Single type cultivars with limited seed set and poor seed germination. Seed set is not observed in the Double type of cultivars either on selfing or crossing. Seed set is also dependent upon the season. Better seed set observed during August to December months under plains of north Indian condition. Double type of cultivars lack female reproductive organ and hence cannot be used as seed parent. This hampers conventional breeding and reciprocal crosses cannot be made. In India, since not many improved cultivars have been developed and available in this crop, there is a great scope for mutation breeding in this crop. Tuberose has advantage of its vegetative mode of propagation through bulbs and bulblets. Once a superior genotype is identified it can be further multiplied by bulbs and bulblets.

To achieve the rapid evolution needed by the plant breeders for satisfying the frequently changing needs, more rapid generation of new genetic forms is required. Such requirements for large amount of new genetic variability can only be met with the aid of induced mutations as supplements to natural genetic resources. Geneticists know no other way to manufacture new gene forms or alleles other than by mutagenic agents. Induced mutations should become increasingly important sources of genetic variability for plant improvement programme because (1) Sources of natural genetic variability for some crop plants are at various stages of depletion or low and (2) induced mutations represent a new nearly untapped reserve of genetic variability.

The history of mutation is as old as the science of modern genetics. Genetic variability, the raw product for evolution in plant species is replenished by spontaneous mutations. Plant breeding which is a controlled evolution was initially dependent on genetic variability from natural sources. Stadler (1928) discovered that mutations could be induced artificially in plant by physical agent like X-rays. Since then it appeared that the breeders possessed a new tool with which they could create variability at will.

Induction of mutation is an important pathway for the production of new genotypes in vegetatively propagated species (Broertjes, 1972) and to enhance natural genetic resource (Jain, 2006). Induction of mutation in vegetatively propagated crops has attracted considerable attention because the selection of mutations of directly prescribed characteristics like colour, form or size, is generally not difficult. Another reason is that cultivars are heterozygous and show a comparatively high mutation frequency, spontaneous as well as induced. Mutagenic agents have been used to induce useful phenotypic variations in plants for more than 70 years (Foster and Twell, 1996). A large number of mutant lines have been isolated from many plants and these plants are further used for plant research and crop breeding purpose (Evans and Sharp, 1983; Gottschalk, 1964).

Mutation induction with radiation has been the most frequently used method to develop mutant cultivars, accounting for about 90% of obtained cultivars (64% with gamma-rays, 22% with X-rays; Jain, 2005). Physical mutagens like ionizing radiation (X-rays, gamma rays and neutrons) and UV light, and also a series of chemical agents are common examples of mutagens that have high efficiency in generation of mutation in plants, animals as well as bacteria. Initial attempts to induce mutation in plants mostly used X-rays, later more and more gamma rays and also fast and thermal neutrons were used. The advantages of physical mutagens are accurate dosimetry and reasonable reproducibility and high and uniform penetration of multicellular system particularly by gamma rays. Gamma radiation has provided a large high number of useful mutants (Predieri, 2001) and is still showing a higher potential for improving vegetatively propagated plants.

So far, among the 2700 officially released mutant varieties worldwide, from 170 different plant species from 60 countries, 566 mutants are from ornamental plants (<http://www-mvd.iaea.org>). These not only increase biodiversity, but also provide breeding material for conventional plant breeding, thus directly contributing to the conservation and use of plant genetic resources. Close to 90% of these officially released mutant varieties were produced using radiation and contribute billions of dollars of additional income to farmers annually.

Some of the selected traits of the mutant in ornamental plants were flower colour, flower morphology, plant architecture, compact growth, flower type and variegated leaves. Since tuberose is a vegetatively propagated plant there is better scope to induce variability by the use of physical and chemical mutagens. Among mutagens, gamma rays have been commonly used effectively for mutation induction (Jain, 2005). Preliminary study on mutagenic effect of gamma radiation and chemical mutagens on tuberose was done by few of the researchers (Gupta *et al.*, 1984; Adisorn, 1992; Anu *et al.*, 2003). Nevertheless the induced mutants have been released in ornamentals; systematic studies on the radiosensitivity of vegetatively propagated plants are scanty (Abraham and Desai, 1976). Presently, variability is created artificially through tinting using artificial colours /dyes, which are often poisonous. Keeping these in view the present investigation is proposed to be carried out with the following objectives.

Objectives

1. To study the radio sensitivity of gamma irradiation in tuberose cultivar Prajwal and Phule Rajani.
2. To induce novel mutants using gamma irradiation in tuberose and selection of novel types based on important traits and to check the inheritance and stability of characters expressed in vM_1 generation in the next vM_2 generation.

2. BACKGROUND

Mutation breeding is considered as one of the important methods to generate additional variability in crop plants including vegetatively propagated horticultural crops. The main advantage of mutation breeding in vegetatively propagated crops is the ability to alter one or few characters of an otherwise outstanding cultivar, without altering the basic genetic make up of the genotype. Even though there is not much genetic variability in tuberose (*Polianthes tuberosa* Linn.), it is highly heterozygous and variability can be created. Since not many improved varieties have been developed and reported in this crop there is a great scope for mutation breeding in tuberose. The literature pertinent to the induced mutation and mutagenesis effect of gamma, other physical mutagens on tuberose, other bulbous crops and also other related crop species has been reviewed under the following heads for common review further based on research wise reviews were discussed:

2.1 Induced mutation

2.2 Induced mutation by ionizing radiations

2.1 Induced mutation

Mutations are the important source of all variability in organisms. Variability caused by induced mutations are not essentially different from variability caused by spontaneous mutation during evolution. Mutation provides us with raw material for the genetic improvement of economic crops, and occasionally a new cultivar results with only a little further breeding. In tuberose, there is no record of spontaneous mutation but two induced mutants namely; Rajat Rekha from Mexican Single and Swarna Rekha from Pearl Double developed from National Botanical Research Institute, Lucknow (Uttar Pradesh) with variegation in leaves were released (Gupta *et al.*, 1984). In tuberose so far there is no other flower/foliage variation has been observed.

Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either can

not be found in nature or have been lost during evolution (Novak and Brunner, 1992).

The successful use of plant breeding for improving crops requires the existence of genetic variation of useful traits. Unfortunately, the desired variation is often lacking. However, radiation can be used to induce mutations and thereby generate genetic variation from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution (Brunner, 1995).

Since the effect of mutation in ornamental plants is very visible, selection for changed flower colour, shape and size is easy and almost anything, which is of value. Hence mutation techniques have become a major tool for breeding ornamental plants (Maluszynski *et al.*, 1995).

The discovery that X-rays induced mutations in the fruit fly *Drosophila Melanogaster* and in barley initiated a new field- induce mutagenesis, which was later to become the most important tool in locating gene on chromosomes, study gene structure, expression and regulation and for exploring genomes. Soon after this discovery many plant breeders and geneticists started to investigate the use of radiation-induced mutations for changing plant traits (Ahloowalia *et al.*, 2004).

Traditionally, any new variation which is attractive is accepted in ornamental plants. The genetic improvement of ornamental plants for improving or developing new varieties requires genetic variation. However, the desirable genetic variation is most often lacking and that hampers the breeding of ornamental plants. This is due to existing germplasm fail to provide the desirable recombination and it is necessary to restore to other resources of variation. Since spontaneous mutation occurs with extremely low frequency, mutation induction technique provide tool for the rapid creation and increase in variability in crop species. The genetic variability can be induced by mutagenic agents, such as radiation and chemical and from which desired mutants could be selected (Datta, 1997; Jain and Spencer, 2006).

According to the Mutant Variety Database (MVD), mutation techniques were applied for crop improvement in more than 60 countries on all continents. The list of crop and plant species in which the use of induced mutation led to the released of at least one improved variety has recently reached 175 entities. This also included the category of ornamental and decorative plant species. These species have recently become as valuable as traditional crops and very often more important in developing countries for foreign currency income induced mutation have proved to be the most rapid, direct and cheapest approach to develop new and attractive flower or ornamental plant varieties (Maluszynski, 2001).

Ahloowalia and Maluszynski (2001) reported that in vegetatively propagated plants, many of mutants were derived from irradiating rooted stem cuttings, detached leaves and dormant plants. According to the FAO/IAEA database, of the 465 mutants released among the vegetatively propagated plants, most of them are in the floricultural plants and few in fruit trees.

In 2007, search regarding the number of induced mutation varieties in the IAEA database, China was first in the number of described induced mutation varieties at 638; India was second, listing 272 varieties; and Japan was third with 233 varieties. The total number of mutant cultivars, including direct-use mutant cultivars and indirect-use cultivars, exceed this total (Nakagawa, 2009).

2.2 Induced mutation by ionizing radiations

McCormik and Platt (1962) studied the effect of ionizing radiation on a natural plant community. Their results indicated that ionizing radiation has both stimulatory and inhibitory effect upon plant growth and that the ecological and morphological effects are reflected by species interaction at the community level.

2.2.1 Mutation induction by gamma irradiation

Since 1964 the researchers have endeavored to obtain useful novel mutants in flower plants by gamma-ray irradiation. Plenty of annual, perennial and bulbous plants were grown several times under different dosages of chronic gamma-ray irradiation at Ohmiya. Among many mutants obtained in the series of

experiments, especially two mutated strains, semi-double flowering wallflower and white-flowering moss verbena, were obtained (Saito, 1977).

Tindall *et al.* (1988) tried to understand mutagenic specificity of gamma rays and the mutagenic mechanisms by irradiating Lambda Phage and Prophage in the *cl* (repressor) gene. Two-thirds of the mutations in irradiated phage assayed in *recA* host cells (no induction of the SOS response) were G:C to A:T transitions; it was hypothesized that these may arise during DNA replication from adenine mispairing with a cytosine product deaminated by irradiation. For irradiated phage assayed in host cells in which the SOS response had been induced, 85% of the mutations were base substitutions, and in 40 of the 41 base changes, a pre-existing base pair had been replaced by an A:T pair; these might come from damaged bases acting as AP (apurinic or apyrimidinic) sites. The remaining mutations were 1 and 2 base deletions. In irradiated prophage, base change mutations involved the substitution of both A: T and of G: C pairs for the preexisting pairs; the substitution of G: C pairs shows that some base substitution mechanism acts on the cell genome but not on the phage. In the irradiated prophage, frame shifts and a significant number of gross rearrangements were also found.

Two hundred and forty two direct mutant varieties generated by using irradiation, chemical mutagenesis and somaclonal variation. About 61% of these were induced by gamma ray irradiation. This high percentage of gamma-ray irradiated mutants indicates that mutation breeding via gamma ray irradiation is an effective and highly successful approach for the generation of commercial cultivars (Nakagawa, 2009).

Przybyla (1992) reported for the induction of mutations in *Alstroemeria* gamma rays (^{60}Co) and fast neutrons (Cyclotron U-120) were used in the range of 2.5 to 100 Gy. As a result of mutation breeding new cultivars were obtained: Catalina, Ines and Juanita. They had attractive colour of flower and give higher yield.

Anu *et al.* (2003) treated bulbs of four tuberose cultivars *viz.*, Single, Double, Shringar and Suvasini with gamma rays (5.0 to 25.0Gy). Based on

growth parameters and floral characters, four mutants were isolated, *viz.*, dwarf mutants, high tiller mutants, non flowering mutants and compact inflorescence mutants and it has observed that these mutants were retained the particular characters in the vM_2 generation.

Jain (2006) reported that induced mutations are highly effective to enhance natural genetic resources and have successfully assisted in developing improved and new cultivars among both seed and vegetatively propagated crops. Among mutagens, gamma rays have been commonly used effectively for mutation induction. Some of the selected traits of the mutant ornamental plants are flower colour, flower morphology, plant architecture, compact growth, flower type and variegated leaves.

2.2.2 Mutation induction by X - rays

Spencer (1955) reported that effect of X-rays and other ionizing radiation on seed and plant been subjects of continued studies since the last century, little has been recorded about consequences of subjecting asexual productive portions of plant to X-ray irradiation. According to his study bulbs and corms of 16 varieties from 12 genera were irradiated with 5200r of X-ray which resulted in significantly earlier flowering.

Broertjes (1966) found that when rooted cuttings of pot-grown chrysanthemum variety Hortensien Rose were irradiated with X-rays. Fast neutrons, thermal neutrons, resulted in a great number of different flower colours, as well as number of mutations of flower shape and size.

When plants of the chrysanthemum variety New Princess were irradiated with X-and gamma rays at dose rates varying between 500-2000 r X-rays and 1-4 Krad gamma ray, flower colour changes were induced by both types of radiation. These changes were accompanied by change in chromosome number and by chromosome fragmentation (Dowrick and Beyoumi, 1966).

Broertjes and Ballego (1967) studied the irradiation effect of X-ray on garden dahlia cultivars and found that the optimal dose ranges from 2 to 3 krads,

considering the production of root, speed of rooting and the subsequent development of the young plants as well as mutation frequency.

Nakornthap (1971) used distinct clones of the ornamental canna to study somatic mutation induced by irradiating the tuberous rhizomes and young plants with acute gamma rays from cobalt-60 (1000-2700r) induced changes on colour and form were observed on the flower petals.

Broertjes (1976) compared X-ray and fast neutron irradiated three autotetraploid *Achimenes* cultivars with diploid form. They obtained that the mutation frequency after irradiation of the autotetraploid was 20-40 times higher than for the corresponding diploid. They selected several promising mutants even though this phenomenon is hard to explain.

2.2.3 Mutation induction by Fast and Thermal Neutrons and Ion beams

Cormels of the eight varieties of gladiolus were exposed to thermal and X-ray by Jenkins (1961). He reported that even low rates of both types of radiation reduced the sprouting of the cormels and height of the plants. The heavier doses (15,000 to 20,000 R) were lethal and none of the cormels sprouted in this experiment.

Abraham and Desai (1976) studied the effect of fast neutrons on gladiolus at 142,284,426,710,852 and 994 rad taking 100 corms per treatment of the variety Scarlet Double and also used gamma rays from 1 to 10 kR. Fast neutrons dose for survival was found at 852 rad and 5.5% RBE (Radio Biological Effectiveness). LD50 dose for gamma rays in cormels, based on sprouting was reported at 7.2 kR and that for survival at 4.7 kR.

Masao *et al.*, (2008) investigated the biological effects of heavy-ion beam irradiation on cultured tissues of cyclamen by establishing an irradiation-mediated mutation breeding protocol for producing a new variety of cyclamen. Distinct mutants were not obtained in plants regenerated from irradiated cultured materials. Next, they used tuber of cyclamen as a target for irradiation. Irradiation (8-16 Gy) of 8-15 mm diameter tubers produced male sterility, change in petal colour and petal form. They concluded that mutation induction by heavy-ion

beam irradiation to the tuber would be useful for changing flower characteristic of cyclamen.

2.3 Research Area I: Background: Study on sensitivity and optimal level of gamma irradiation dosage for induction of mutation in tuberose cvs. Prajwal and Phule Rajani

Mutation breeding may be the most effective means when applied to ornamental plants. It helps to improve or to modify a few important traits like flower colour, flower morphology and plant architecture, of the already established varieties without disturbing other major characteristics. In recent years, requests of irradiation to plant materials have increased and their plant species were broadened. To prescribe the radiation dosage for each plant species, wide survey of radiation sensitivity become necessary. Radiation levels required, sensitivity to radiation is known to differ according to age of tissue, between varieties and even as a function of single gene differences. In radiosensitivity studies with any plant material it is necessary to establish tolerance levels and optional growth for treatment. In this context some of the previous works done on some of the flower species and other plant species were reviewed below. Because, even though meager mutation study was done in tuberose, a proper study of radiosensitivity was not done, here we have also mentioned a few reviews on tuberose also.

2.3.1 Radiosensitivity study in ornamental plant species.

Sparrow and Gunckel (1956) have studied radiation tolerance in 79 species of plants. They reported that a dose of 30r per day damaged the trumpet lily, whereas 6000r per day was required for damage of gladiolus.

According to Vaartaja (1963) there would be large difference exist between plant species in their response to ionizing radiation. When two species of *Hyacinthus* treated under identical condition with the same X-ray dose, many abnormal cells occurred in *H. orientalis*, the species with the considering larger chromosomes than *H. dalmaticus*.

In a comparative study on the effect of gamma irradiation on two species of tomato, it was found that *L. pimpinellifolium* expressed a greater sensitivity to chronic gamma irradiation than that of *L. esculentum*. It was concluded that the relative sensitivity of the two species to chronic gamma irradiation may be completely reversed depending on stages irradiated and criteria observed (Nettancourt and Contant, 1966).

Dry seeds of some of pine species were exposed to chronic ^{60}Co gamma irradiation, germination and survival parameters were studied. They observed that LD_0 varied from approximately 400 to 3700R and LD_{100} varied from 1500 to 11,200R. From this study it was showed that white pine and white spruce found to be most sensitive with an LD_{50} of 575R and Jack pine the most radio-resistant with LD_{50} of 2950R (Clark *et al.*, 1968).

Diploid and tetraploid seedling of marigold, zinnia and petunia were irradiated with increased exposures of X-rays and then scored for survival (LD_{50} and LD_{100}). The observed LD_{50} for each of diploid and tetraploid form was: 2n marigold - 7.3kR, 4n marigold - 6.4kR; 2n zinnia - 2.2kR, 4n zinnia - 7.2kR; 2n petunia - 3.5kr, 4n petunia - 4.0kR. The corresponding LD_{100} was 2n marigold- 12kR, 4n marigold- 14kR; 2n zinnia- 3kR, 4n Zinnia-15kR; and 2n Petunia-9kR, 4n Petunia-10kR (Miller, 1970).

Gupta and Shukla (1971) studied the effect of gamma irradiation on some scented roses. They irradiated bud-wood of twelve scented cultivars of garden rose with 4kR. The percentage of bud take, survival and height of plants decreased after irradiation. They found that level of auxin concentration in plant drops after exposure to ionizing radiation.

Abraham and Desai (1976) found that high sensitivities of bulbs to radiations particularly to gamma rays may be due to high moisture content of the buds in the bulb. In tuberose the number of leaves, plant height, number of plants flowered and survival decreased as the dose increased i.e. 2.5 kR of gamma rays.

Rasamee (1993) found that when bulblets of lily cv. Mont Blanc were irradiated with gamma ray (0-10 Gy), survival percent decreased with increasing dose (46.58) at 3 Gy.

The radio sensitivity of *Bambusa arundinaceae* was assessed by exposing the seeds collected from a deciduous forest to gamma rays with 10 to 150 kR doses at 10kR interval. Gamma rays enhanced seed germination in 10 kR treatment compared to the control, there was a drastically reduced and delayed germination was observed at 20 and 30kR treatments. LD₅₀ for seed germination was 19.3kR, 26.30kR for shoot length and 22.04 kR for root length indicated the high sensitivity of *B. arundinaceae* (Lokesha 1996).

Hussin *et al.* (2000) in their study found that *Brachiaria decumbens* was less sensitive to gamma radiation. At a dose of 800Gy, the percentage reduction in growth was about 40%.

Thapa (2004) studied the effect of acute gamma rays on *Pinus kesiga* and *P. wallichiana*. Seeds of these two pinus species were irradiated with different levels (1.0, 2.5, 5, 10, 15, & 30Gy) of gamma rays, germination of both control and treated seeds were recorded. According to the study *Pinus kesiga* seeds exposed to 30kR got germinated but in *P. wallichiana* 30kR was found lethal for seed germination and it was restricted upto 20kR only. With increased exposure hypocotyle and epicotyle elongation get decreased in both the species.

Majeed *et al.* (2010) found that mean germination time (MGT) of *Lepidum sativum* was significantly affected and delayed at higher doses of gamma rays. The other growth parameters like shoot and root length, number of branches and leaves per plant were showed declining tendency with increased doses of gamma irradiation.

2.3.2 Radiosensitivity study in some agricultural plant species

According to Mc Crory and Grun (1969) 26 per cent of potato plants those received 6 and 9kR survived and appeared to grow normally than 83 per cent in case of plants received 0 and 3kR. Further survival and vigor was increased among most of the plants whose ancestors had received doses of 6 and 9kR of radiation, this increase was attributed the hereditary transmission of radiation-induced genetic damage in the form of recessive lethals and detrimental. It was

concluded that gamma irradiation dosage of 6 and 9kR would be useful in generating mutated population.

Bottino and Sparrow (1971) studied the sensitivity of lima bean (*Phaseolus limensis* Macf.) to ⁶⁰Co gamma radiation with three stages of reproductive development. The decreasing order of sensitivity was measured as seed yield was buds, flowers in anthesis and large pods. They found that about a 15-fold difference between the most sensitive and most resistant stages.

From the results of the comparative study of radio sensitivity by gamma rays (⁶⁰Co) in autopolyploid series in rice, it has been observed that the dose response varies with the total dose and with different ploidy levels. Haploid plants showed no rooting at all in all the doses exposed. Confirming highest sensitivity for irradiation. Diploids were less sensitive than haploids at 20kR. Tetraploids were found to be highly radio-resistant and triploids been the intermediate, in comparison with diploids and tetraploids (Katayama, 1972).

Irradiation of *in vitro* explants and subsequent adventitious regeneration has been tested for 4 commercially important varieties of pear (*Pyrus communis*) with the aim to create mutants with a reduced susceptibility to fire-blight (*Erwinia amylovora*). LD₅₀ dose of both gamma irradiation and ultraviolet radiation were also studied. Histological investigations showed leaves with flattened epidermal cells after ultraviolet irradiation and slack spongy parenchyma after gamma irradiation (Leblay *et al.*, 1992).

Ellyfa *et al.* (2007) found that increased dosage of gamma irradiation was accompanied by decreased in height, root length, shoot and survival of snap bean. It was found that the LD increased with increased gamma irradiation.

During the radiation sensitivity study of *Citrus sinensis* height increment of irradiated and non-irradiated plantlets was studied after three weeks of irradiation. The plantlet height was inhibited with increased does of gamma irradiation. For the height percentage of irradiated plantlets to reach 50%, the gamma doses administered was 27Gy (Kiong Ling *et al.*, 2008).

A strategic approach was made to estimate the optimal treatment conditions (doses of mutagens) through relating the extent of damage in seedling

progeny to the exposure levels of the initiating propagule to mutagens. Seeds of 15 elite sunflower genotypes commonly used as breeding stocks and grown on commercial scale were treated with a range of mutagens *viz.*, gamma rays, fast neutrons and with ethyl-methane-sulfonate at different treatment doses. It was found that LD₅₀ (LD₃₀) values for the sunflower lines ranged from 120 to 325 Gy (5 to 207Gy) for gamma irradiation (Gvozdenovic *et al.*, 2009).

In a study on effect of gamma irradiation on germination, seedling height and survival rate of two sesame (*Sesamum indicum* L.) cultivars were seen. It was observed that cultivar 32-15 was more sensitive to gamma irradiation than cultivar 38-1-7. The effective dose which caused 50% growth reduction was 645Gy for cultivars 32-15 and 740 Gy for cultivar 38-1-7. The lethal dose (LD₅₀) observed was 550 and 740Gy for 32-15 and 38-1-7 cultivars, respectively (Boureima *et al.*, 2009).

2.3.3 Histological study of gamma irradiated tissues

Histology studies on pea (*Pisum sativum*) roots sonicated by a broad beam of ultrasound indicated that most of the damage was occurred in the elongated region. They found microscopic evidence on cell destruction in the meristem. Histological observations on sections of treated roots indicated necrotic nuclei within the region of sonication (Law *et al.*, 1978).

When *Drosophila* larvae were irradiated with 1300-1500 R of gamma rays both apoptotic and necrotic cell death were observed in imaginal wing discs. The ultrastructure of cell death by apoptosis was characterized by fragmentation of dead cells into highly condensed, membrane-bound particles. The ultrastructure of cell death by necrosis was characterized by cell lysis and organelle degeneration. Marked contrast was also seen in the distribution of the two types of cell death: apoptosis was universal in irradiated discs and affected widely distributed single cells, or small groups of cells, whereas necrosis formed lesions by afflicting large numbers of contiguous cells (Abbott, 1983).

During the study of effect of gamma and ultraviolet irradiation on adventitious regeneration of pear leaves, it was observed that after ultraviolet

irradiation as low as 62.5 J/m^2 , the leaves become crumbly and rolled up and their metabolism seemed to be altered. Histology investigations showed leaves with flattened epidermal cells after ultrasound and slack spongy parenchyma after gamma irradiation (Leblay *et al.*, 1992).

In September 1990, samples of wood and bark were collected from *Pinus sylvestris* L. at three locations exposed to different levels of radioactive fallout from the 1986 accident at the Chernobyl nuclear power plant (NPP). Cross-sections of wood from the most exposed location showed a distinct change in histology in the annual ring of 1986, a consequence of the accident on 26 April. The width of annual rings decreased after the accident, and the relative width of latewood in annual rings increased transiently in 1986 and subsequently decreased in 1987. In 1987, an increase in the number of vertical resin ducts was observed, related to contamination at the location and the number of radial rays decreased at the two locations of higher contamination. The present work suggests that detailed studies of dose-effect relationships after exposure to different dose rates and radiation qualities may establish the usefulness of pine trees as in situ, time-recording differential dosimeters of ionizing radiation (Skuterud *et al.*, 1994).

2.4 Research Area II: Background: Effect of acute gamma irradiation on flower and bulb parameters and stability study of mutants in tuberose (*Polinathes tuberosa* Linn.)

Man has been growing ornamental plants since ages for all occasions. Commercial cut flowers have high value due to high economic returns. Induced mutations should become increasingly important sources of genetic variability for plant improvement programme because; Sources of natural genetic variability for some crop plants are at various stages of depletion or low and induced mutations represent a new nearly untapped reserve of genetic variability. Induction of mutation is an important pathway for the production of new genotypes in vegetatively propagated species. Even though there is not much genetic variability in tuberose, it is highly heterozygous and hence variability can be created. In tuberose there is very scanty work has been done on study of mutation

breeding. Here very concentrating on mutation work done in some of the important bulbous flowering plants and other ornamental species including tuberose as main focus.

2.4.1 Mutation studies in tuberose

Two gamma ray induced variegated leaf mutants were developed and released under the name Rajat Rekha and Swarna Rekha. Rajat Rekha a Single flowered tuberose mutant with white streaks along the middle of the blade, while Swarna Rekha a Double flowered mutant of tuberose with golden yellow streaks along the margins of the blade (Gupta *et al.*, 1974).

Abraham and Desai (1976) found that high sensitivities of bulbs to radiations particularly to gamma rays may be due to high moisture content of the buds in the bulb. In tuberose the number of leaves, plant height, number of plants flowered and survival decreased as the dose increased i.e., 2.5 kR of gamma rays.

When bulbs of both Single and Double cultivars of *Polianthes tuberosa* L. were exposed to 500, 1000 and 1500 rad of gamma rays for induction of genetic variability. Different types of morphological abnormalities like shape, size, margin, apex, fission and fusion of leaves and chlorophyll variegation in leaves were detected in treatment population (Shukla and Datta, 1993).

Anu *et al.*(2003) reported that morphological variants like chlorophyll mutants, non flowering mutants and compact spike mutants were observed in tuberose cultivars Mexican Single, Pearl Double, Shringar and Suvasini treated with gamma rays (5.0, to 25.0 Gy).

2.4.2 Mutation studies in ornamental bulbous plants

The fate of somatic mutations induced by γ -rays in gladiolus cv. Oscar was followed through the life of the plant. When dormant corms were treated with 4000 R and 6000 R and mutation frequencies per plant and per flower order, as well as sector area distribution, were calculated. Data on mutated sector sizes suggested that a petal could derive from either 1, 2 or possibly more initial cells. Mutations were found to cover more than one flower and to be induced before

flower differentiation in the spike primordium of the dormant corms (Buaitti *et al.*, 1969).

Przybyl (1992) found that induction of mutation with ionising radiation appeared to be a useful method to obtain new cvs of *Alestromeria*. He suggested that from technical point of view gamma rays are more convenient and doses optimal for induction of useful mutants were rather low, in the range of 3 to 7 Gy. Mutations of frequent occurrence were: change of flower colour and change of design on the flower petals. Less frequent were mutation of flower petals shape, change of flower size, mutation of growth vigour, productivity and season of bloom.

Exposure of storage roots of *Ipomoea batatas* L. to gamma irradiation to 90, 180 and 240 Gy resulted in delayed rooting, formation of rosette like shoots and stems less shoots without apical meristems (Celso and Maria, 1992).

The corms measuring about 4.5 cm in diameter of four gladiolus cvs. *viz.*, Green Finch, Mayur, Rose Memento and Wind Song were subjected to 0.0, 15.0, 30.0, 45.0 and 60.0 Gy doses of ⁶⁰CO gamma rays and planted in randomized block design with three replications, putting 10 corms in each replication. The data were recorded in vM₁ on different parameters, and up to vM₈ all those plants showing abnormality were grown in observational rows to see if the changes occurring in vM₁ persist or some new ones appear (Misra, 1998).

Bulbs of wedgewood iris were irradiated with cobalt-60 gamma irradiation (100-1500 r) at various stages of bud development. It was observed that irradiated bulbs accelerated flowering by upto 21 days. A few of the irradiated flowers were abnormal. Foliage length and bulb yield were progressively reduced by increasing doses of irradiation (Halevy and Shoub 2005).

Due to the absence of sexuality, mutation breeding approach is another way of creating genetic variability in saffron. An attempt was made to create new variants for economic characters through the induction of mutation using gamma irradiation. Existing saffron population was irradiated with gamma rays from a Co 60 source at 0.25, 0.50, 0.75 and 1 kr doses. Delayed sprouting was observed in

higher dose (1 kr). Higher dose was also associated with decreased corm yield and dry pistil weight/plant due to strong reduction in percentage of flowering plants and number of flowers/plants (Nehvi *et al.*, 2007).

2.4.3 Mutation studies on ornamental plants

A research was carried out on the induction by gamma rays of somatic mutations in carnation. It was observed from this study that decreased number of flower per branch and of branches per plant. The number of petals did not show any significant effect of the treatment but revealed an increased in variability roughly proportion to doses (Buiatti and Ragazzini, 1965).

When shoot apical meristems of *Antirrhinum majus* were irradiated for ten days with 50-6000 R/20 h- day with ⁶⁰Co gamma rays and effects investigated in detail. Induced somatic mutations appearing in the petals were scored (Sekiguchi *et al.*, 1971).

Seven mutants of perennial *Portulaca grandiflora* as a result of gamma rays treatment revealed that there was a considerable variation in number, size, shape and distribution of stamens and carpels along with petal characteristics (Lata and Gupta, 1971).

Jain *et al.* (1998) reported that gamma irradiation (10 and 20 Gy) of two gerbera cultivars produced mutants showing flower colour, flower morphology and plant morphology traits with an average of 8.6% mutation frequency.

Arunee and Praparat (2000) found that the variations in phenotypic characters of flower colour, form and size were noticed when stem cuttings of two perennial *Portulaca grandiflora* varieties, Double Orange and Double Pink were gamma irradiated with the doses of 0, 10, 20 and 40 Gy.

Stem cuttings of two perennial *Portulaca grandiflora* varieties, Double Orange and Double Pink were gamma irradiated with the doses of 0, 10, 20 and 40 gray. Variations in phenotypic characters of flower colour, form and size were noticed (Arunee and Praparat, 2000).

Rooted cuttings of *Dendrathera grandiflora* cv. Puja were treated with different dose of gamma rays. Sectorial somatic mutations both in flower colour

and shape were detected in all the doses. One of the mutant has showed tubular floret upon original flat spoon shape (Datta *et al.*, 2001).

Lamseejan *et al.* (2003) used chrysanthemum cv. Taivei for mutation induction with chronic and acute gamma irradiation treatment and they obtained flower mutants of different traits such as flower colour, flower form and size. They observed that the mutation frequencies of these traits were also differed.

Misra *et al.* (2004) developed two chrysanthemum mutants by gamma irradiation (0.5 Gy). They found that both the mutants were of yellow colour, in that one mutant had tubular shaped floret which was completely different from original cultivar.

Srivastava and Mishra (2005) found that at 10 Gy, a mutant of *Hibiscus rosa-sinensis* was detected, which produced flower of significantly small in size compared to the control when the cuttings were irradiated with gamma radiation at 5, 10, 20, 30, 50, 70 and 100 Gy to induce mutation. Floral variants obtained at other doses showed variation in the length and orientation of the style, number and size of anthers and also flower colour.

The *Celosia cristata* was irradiated with ^{60}Co - γ ray and its germination rate and seedling growth were observed. The results showed that there was a positive correlation between irradiation dosage and germination rate, but there was a negative relationship between the length of roots, the height of seedling and the irradiation dosage. And the results also indicated that though it could improve the germination rate, it restricted the seedling growth (Qiang, 2005).

Recently, under the INS/5/031 TC project, Indonesians released three rose mutant varieties- Rosmarun, Yulikara and Rosanda-with specific colour and shape induced by gamma radiation treatment (Jain and Spencer, 2006).

The seeds of *Tagetes patula* were irradiated with ^{60}Co - γ ray of the different dosages and the seed germination rate and seedling growth were observed. The result showed that the relation between the germination and the radiant dosage were negatively correlated; the relation between the length of bud and the radiant dosage were positive correlation and the relation between the length of root and the radiant dosage were tiny negative correlation. Irradiation

could improve the growth of *Tagetes patula* but it could restrict the rate of seed germination (Zhou Zhen-chun 2006).

According to Thohirah *et al.* (2009) gamma irradiation had exerted various effects on growth of *C. alismatifolia*, including extension of days to shoot emergence, plant height, leaves and shoot number as well as modification in plant morphology and flower development.

3. MATERIALS AND METHODS

The present investigation entitled “**Studies on mutagenesis effect of gamma irradiation on tuberose (*Polianthes tuberosa* Linn.) cvs. Prajwal and Phule Rajani**” was conducted at the Research Farm of Division of Floriculture and Landscaping, Indian agricultural Research Institute, New Delhi, during 2008-2010. Histological work was carried out at Cryogenic Laboratory of National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi. The different experimental materials and methods adopted for carrying out the experiments are dealt hereunder.

3.1 Materials

3.1.1 Plant material

The present study of mutagenic effect of gamma irradiation was conducted on two tuberose cultivars *viz.*, Prajwal and Phule Rajani (Plate.1). Both these cultivars are of Single type with high superior quality of loose and cut flower production. Bulbs of these two cultivars were collected from Research Farm of Division of Floriculture and Landscaping, IARI, New Delhi.

Cultivars description

3.1.2 Prajwal:

Prajwal is a cross between Shringar x Mexican Single cultivars. This cultivar bears Single type of flowers on tall, stiff spikes. The flower buds are attractive with slightly pinkish colour while the open flowers are white. The individual florets are heavier in weight and larger in size compared to Mexican Single and also all available Single petalled cultivars. It yields 22% more loose flowers than cv. Shringar. This cultivar is best suited for loose flower and cut flower trade. It was released by Indian Institute Horticultural Research, Bengaluru, (Srinivas, 2004).

3.1.3 Phule Rajani:

Phule Rajani is a cross between Mexican Single x Shringar cultivars. This cultivar was released during 2008 by Mahatama Phule Krishi Vidhyapeeth Rahuri, Regional Research Station – National Agricultural Research Project,

Ganeshkhind, Pune (Maharashtra). This cultivar bears Single type of greenish white flower bud and flower spikes remain straight in field and in vase. The cultivar has appropriate spike length and rachis length about half of the spike length with well distributed 46-48 florets on the spike. The flower buds are greenish white which are very much acceptable to the consumers for both, loose flowers as well as cut flower trade (Katwate *et al.*, 2006).

3.1.4 Different bulb stages

For our investigation three stages daughter bulbs uprooted/harvested from each of two tuberose cultivars were selected for planting:

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

3.2 Field layout

A field layout was prepared during last week of December 2008 with 2x2m blocks with six blocks in row with three replicates of the same according to the randomized block design (Figures 1&2). Prepared blocks made into fine tilth using farm tools. To these blocks approximately one kg of farm yard manure was added and mixed properly, it was left upto planting time and weeding was done in between.

3.3 Treatment with gamma rays

Six gamma irradiation treatments along with control were finalized:

G₀ = 0 Gy (control)

G₁ = 2.5 Gy

G₂ = 5.0 Gy

G₃ = 7.5 Gy

G₄ = 10.0 Gy

G₅ = 15.0 Gy

3.4 Irradiation frequency

In our study we used acute gamma rays from Cobalt -60 source

S₀ = Single dose (One time)

- 3.5 **Design of experiment:** Randomized Block Design
- 3.6 **Total number of treatment combinations:** $2 \times 6 \times 1 \times 3 = 36$ (Table.1)
- 3.7 **Total number of bulbs irradiated:** $36 \times 36 \times 3 = 3888$
- 3.8 **Research Area I: Study on sensitivity and optimal level of gamma irradiation dosage for induction of mutation in tuberose cvs. Prajwal and Phule Rajani**

3.8.1 Preparation of bulb stages

Three bulb stages from each of two tuberose cultivars were selected *viz.*, B₀- Freshly harvested bulbs; 2. B₁- Three weeks after storage; 3. B₂- Six weeks after storage. For B₀ stage the bulbs of both cultivars Prajwal and Phule Rajani were harvested on 9th March 2009. For both B₁ and B₂ stages bulbs of both Prajwal and Phule Rajani cultivars were harvested on 19th February 2009.

3.8.2 Gamma irradiation treatment

For gamma irradiation tuberose bulbs of cvs. Prajwal and Phule Rajani of different stages were packed in LDPE bags (9 bulbs in each packet), total of 4 bags were prepared to get 36 bulbs treated for each block planting. These different stages were subjected to gamma irradiation of different dosage levels (2.5, 5.0, 7.5, 10.0 and 15.0 Gy) at Gamma Chamber, Nuclear Research Laboratory, I.A.R.I, New Delhi. The B₀ and B₁ stages bulbs of both tuberose cultivars were irradiated on 12th March 2009. The B₂ stage bulbs of both tuberose cultivars were irradiated on 2nd April 2009.

3.8.3 Planting

The experiment was laid out in randomized block design with three replication. The treated bulbs as well as untreated bulbs (control) were planted on the same day. The B₀ and B₁ stages bulbs of both tuberose cultivars were planted on 12th March 2009. The B₂ stage bulbs of both tuberose cultivars were planted on 2nd April 2009.

3.8.4 Observations recorded

Sprouting per cent

This was recorded by counting the total number of sprouted bulbs in each treatment after forty days of planting upon total number of bulbs planted.

Survival per cent

The total number of plants survived among the sprouted bulbs in each treatment was recorded. LD₅₀ (Lethal dose-50) was determined on the basis of the plants sprouted in each treatment.

3.8.4.1 Vegetative growth parameters

For taking the observation on vegetative parameters only four gamma irradiation treatments (2.5 Gy, 5.0 Gy, 7.5 Gy and 10.0 Gy) were taken into consideration along with the control. Because gamma irradiation dose 15.0 Gy had showed 100 per cent lethality in all the bulb stages of both the cultivars.

Days to sprouting of bulbs

Sprouting of bulbs was recorded at 20 days interval up to 40 days, as under normal condition sprouting ceases after this period. Keeping this in view, stimulation for early sprouting and delayed sprouting were determined in each treatment.

Plant height (cm)

This parameter was recorded when plants attained their maximum height with the appearance of the last flower on the spike or plants attained their maximum height in the case where plants did not flower. The height was measured from the ground level to the tip of last floret of the spike. Total fifteen plants were measured with respect to treatment and replication in all the bulb stages of two cultivars of tuberose.

Number of tillers per clump

The number of tillers were sprouted out near the base of a plant was recorded. This was recorded after the first flowering was over. This parameter was observed for fifteen plants with respect to treatment and replication of both the tuberose cultivars.

Number of leaves per clump

The total number of leaves per clump at the time of spike initiation was recorded. This parameter was recorded for five plants each in three replication.

Length of leaves (cm)

For this parameter longest leaf of a plant was selected and measured. Total of five plants were selected in a particular treatment. Over all fifteen plants were measured for this character.

Width of leaves (cm)

Width of the longest leaf was measured which has been selected to measure the length. Here also five plants were selected in a particular treatment. Over all fifteen plants were measured.

Stem and leaf variation

Any variations appearing in the treated plants different from the control were recorded. The plants growth was observed regularly for the variation such as differences in size, shape or chlorophyll distribution or any other variations of vegetative origin were recorded.

3.8.5 Preparation of material for histology study

The histology was studied for one of the problem which has occurred during the optimal gamma irradiation level experiment in cv. Prajwal. It was observed that there was zero per cent sprouting when B₁ (three weeks after uprooting) bulbs of cultivar Prajwal were irradiated with gamma ray doses 5.0, 7.5, 10.0 and 15.0 Gy. In order to find out the answer histology experiment was undertaken. Here bulbs of cv. Prajwal were uprooted and stored for three weeks under normal room temperature. After completion of three weeks the bulbs were irradiated with the gamma-rays with the same dosage level i.e. 5.0Gy, 7.5Gy and 10.0Gy. From this irradiated bulbs growing buds were excised which use to be situated in the center of bulbs and the axillary buds which were grown around the basal plate of the bulbs were also excised.

Fixing the tissue:

The excised tissues were transferred to FA (Ferric oxide) solution in the small glass vial for 48 h. After this these tissues were transferred to 70% alcohol

solution. By this method tissues have been fixed, which were further used for the next procedures of histological study.

Dehydration:

These fixed tissues were subjected to two standard dehydration protocol using TBA series (Johansen, 1940). Once the tissues passed with the dehydration procedure blocks of these tissues were made using 1:1 ratios of paraffin wax and bee wax. These blocks are cut into equal shape of either square or rectangle cubes.

Microtomy:

Tissue contained cubes fixed to the microtomy equipment (LEICA RM 2162, Leica) by melting some amount of wax on it. The equipment was adjusted to cut the tissue contained cubes at 0.6 μ thickness. In between microtomy blade was washed by xylene to dissolve extra wax which use to adhere during cutting process.

Spreading:

The tissues which were in the thin layer of wax in the ribbon form were spread on the clear slide using Haupt's adhesive. These slides were then dried on the hot plates at 80°C for 1-2 min and it is left for over night on the polyethylene sheet.

Staining:

To visualize the cells properly the slides which contained the thin tissues were made to follow the standard staining procedure using fast green and tolvidin blue stains (Johansen, 1940). Once the slides were done with staining DPX mounting was did, left for over night in room temperature to dry up and final slides ready to observe under microscope.

3.8.5.1 Microscopic observations

Below mentioned observations were recorded for the histology study with respect to per cent damage to the cells;

1. Shape for the cells
2. Shape of the nucleus
3. Size of the cells

3.9 Research Area II: Effect of acute gamma irradiation on flower and bulb parameters and stability study of mutants in tuberose (*Polinathes tuberosa* Linn.)

Study on effect of gamma irradiation on plant growth characters viz., vegetative, flowering and bulb characters is very important to study the frequency of mutagenic effects. In our study we subjected bulbs of three different stages of tuberose cvs. Prajwal and Phule Rajani to acute gamma irradiation. This study was continued once the radiosensitivity and optimal level of acute gamma irradiation on these two cultivars were observed. Here gamma irradiation doses, 2.5, 5.0, 7.5 and 10.0 Gy were taken into consideration because in our First Research Area we had observed that 15.0 Gy was completely given the lethal effect (100 per cent) with respect to both the cultivars and in all three bulb stages of tuberose.

3.9.1 Observations in vM₁ generation

The following observations were recorded to determine the effects of gamma irradiation on flower and bulb characters of tuberose cultivar Prajwal and Phule Rajani in all the bulb stages. A known number of random plants per treatment per replication were selected for recording these morphological observations. Any abnormality or variations in different treatments were also recorded.

3.9.1.1 Flowering characters

Days to spike appearance

Number of days required for emergence of first spike was recorded from the date of planting of the bulbs and average value of five plants data in each treatment, replication and in each bulb stage was computed.

Days for opening of first pair of florets

Number of days required for blooming of five plants in each treatment and in each bulb stage were recorded from the date of spike appearance to opening of the first pair of florets in a treatment and average value was computed. For this

observation same plants were selected for which the first observation i.e. days to spike appearance was recorded.

Flowering duration (day)

This parameter was recorded by observing the date of opening of the first floret and date of drying of last top floret in a spike under field condition. For this total of three plants were selected from each treatment and replication in all the bulb stages, because we observed that total number of spikes were less with increased gamma irradiation.

Number of florets per spike

The total number of florets per spike was counted on the day when the last floret started opening. For this observation also total of three plants were selected in each treatment in all the bulb stages and average value was computed.

Vase life (day)

Vase life of a cut spike was recorded by counting the number of days they remain fresh under room condition in normal water. For this spikes were selected when the first floret was about to open. The average days were computed. Vase life of spikes from 10.0 Gy gamma irradiated plants were not been selected because they were with negligible length with malformed flowers.

Number of spikes per plot

Total number of spikes was recorded by counting number of spike per clump in each replication of a treatment in all bulb stages.

Spike length (cm)

Length of the spike was recorded by measuring the distance between first flag leaf and the uppermost floret of the spike at the time of its opening.

Fresh weight of spike (g)

Fresh weight of three spikes in each treatment and replication in all the bulb stages was recorded. Individual spike was weighed used a portable 500 g weighing balance.

Average floret diameter (cm)

Diameter was recorded for first five florets of three plants each from a treatment and replication in all the bulb stages. Diameter was recorded as the

distance from the apex of one petal to the opposite. It was measured by using digital vernier caliper.

Average floret length (cm)

Length was recorded for the first five florets of three plants each from a treatment and replication in all the bulb stages. Length was recorded from the base of the floret tube to the apex of the petal.

Average fresh weight of individual floret (g)

Fresh weight of individual floret was recorded for the first five florets of three selected plants each from a treatment and replication in all the three bulb stages. Weight was measured by using electronic balance.

Variation in floral colour, size, shape, number of floral organs and variation in spike stem

Careful observations were made to record any change in flower character of gamma irradiated plants with respect to each treatment. On the basis of this also some useful mutants were selected.

3.9.1.2 Bulb Characters

Number of bulbs per clump

The average number of bulbs at the time of lifting after first flowering in each treatment, in each bulb stage were recorded.

Weight of bulbs per clump (g)

The weight of one bulb was found out as average weight by weighing all the bulbs of a plant divided by their number to derive the average values. For this three plants were selected from each replication in all the bulb stages.

Average diameter of bulb (cm)

The diameter of bulbs were measured with the help of Vernier Callipers and average of five bulbs was computed.

Average length of bulbs (cm)

The length of bulb was measured with the help of Vernier Callipers and average of five bulbs was computed from each replication from all the bulb stages.

3.10 Design of experiment

The experiment was laid out in randomized block design with three replications having 5 gamma irradiation treatments. Three bulb stages *viz.*, B₀ (Freshly harvested bulbs), B₁ (Three weeks after storage), B₂ (six weeks after storage) of two cultivars Prajwal and Phule Rajani were used each at three different dosage level of gamma irradiation treatment. A control of untreated bulbs was also maintained with each replication.

3.11 Statistical analysis

The experiment data were analyzed statistically by analysis of variance (ANOVA) as applicable to randomized block design, (Snedecor and Cochran, 1967) using a computerized package (Windostat).

3.12 Stability study of the mutants in vM₂ generation

For the stability bulbs from the plants which have showed variation in the vM₁ generation were uprooted on first week of January 2010. These bulbs are stored under normal room temperature for about 40 days. The bulbs of particular plant which had showed the variation are planted in 1x1 m plots on 25th of February 2010. The following observations were recorded:

1. Per cent sprouting
2. Per cent survival
3. Variation in the form of presence and absence manner.

The first generation plants which not showed good and acceptable variation were also taken care to observe their performance in the vM₂ generation.

Study on sensitivity and optimal level of gamma irradiation dosage for induction of mutation in tuberose cvs. Prajwal and Phule Rajani

4.1.1 Abstract

Present experiment was conducted to find out the radiosensitivity of tuberose cultivars used, proper bulb stage and optimal gamma irradiation dosage. Cultivars Prajwal and Phule Rajani with three different bulb stages *viz.*, freshly harvested bulb (B₀), three weeks after uprooting (B₁) and six weeks after uprooting (B₂) were irradiated by 2.5Gy, 5.0Gy, 7.5Gy, 10.0Gy and 15.0 Gy of gamma rays. LD₅₀ dose of gamma irradiation was estimated between 10.0 Gy to 12.0 Gy for freshly harvested bulbs and bulbs after six weeks of uprooting in cv. Prajwal and in all the three bulb stages of cv. Phule Rajani. For the three weeks after uprooted bulbs LD₅₀ dose of 3.25 Gy in cv. Prajwal was estimated, this bulb stage was found very much sensitive to gamma irradiation which was also supported by the histological result obtained. The all three stages of bulbs have showed different response over gamma irradiation. In general we have observed decreased sprouting and inferior vegetative parameters with increased irradiation level. Out of three bulb stages tested freshly harvested bulbs (B₀) responded comparably high to the gamma irradiation doses followed by bulbs after six weeks of uprooting (B₂) in both cvs. Prajwal and Phule Rajani of tuberose.

Key words: Bulb stages, gamma radiation, radiosensitivity, spike, tuberose

4.1.2 Introduction

The dose to be applied for obtaining a high mutagenic efficiency generally depends on the specific properties of the radiation type, biological system and the radiation facility. The current study utilizes physical mutagen and gamma radiation, to create variation in tuberose. Gamma radiation treatment has the following advantages over the chemical mutagen like there is no residual effect; uniformity in penetration, less time consuming and a large number of samples can be treated in less time. Apart from the choice of the proper mutagenic agent, the dosage and the treatment conditions are also important. The plant material and the

stage of plant material to be irradiated are the important criteria to enhance maximum mutation efficiency. For example: the stage in the life cycle of the plant and plant organs; the sensitivity of the plant species to the effects of the mutagenic agents; the possible genotypic differences in sensitivity in the mutagenic treatments etc. A precise method for the determination of the radiation dose absorbed has been proposed by Neville *et al.* (1998). However, the common procedure in assessing the most appropriate dosage is based on radiosensitivity, which is estimated through the response of the irradiated material.

Many vegetatively propagated plants including tuberose are with complicated physiology (e.g. dormancy) and complex genetics (e.g. high degree of heterozygosity, self-incompatible). In tuberose spontaneous mutations-‘sports’ have not been recorded till date. Though, a huge scientific literature is available in this regard but the current study is to find the most appropriate bulb stage and dose of gamma radiation required to obtain large number of mutants. With this back ground the present study was taken up with the aim to find out radiosensitivity stage and optimal gamma irradiation doses for the induction of some useful mutants in tuberose cultivars Prajwal and Phule Rajani.

The two cultivars used in this study are the popular commercial cultivars in India. This experiment would yield the LD₅₀ dose for tuberose. The LD₅₀ is a dose at which 50% of the plant material survives is generally accepted as the optimum irradiation dosage for the purpose of creating new varieties because an acceptable number of cuttings survive while a large number of mutations are obtained.

4.1.3 Materials and Methods

The present investigation was carried out during 2008-2010. Two commercial cultivars of tuberose *viz.*, Prajwal and Phule Rajani were used. Three different stages of tuberose bulbs of both the cultivars were selected for irradiation *viz.*, Freshly harvested bulbs (B₀); Three weeks after uprooting (B₁) and Six weeks after uprooting (B₂). These three different bulb stages were irradiated with five levels of gamma radiation along with one control which is not irradiated. Following is the level of gamma radiation used in the study - G₀ = 0

Gy (Control), $G_1 = 2.5$ Gy, $G_2 = 5.0$ Gy, $G_3 = 7.5$ Gy, $G_4 = 10.0$ Gy, $G_5 = 15.0$ Gy. Gamma irradiation chamber facility at Nuclear Research Laboratory of IARI, New Delhi, was used. Considering each bulb stage as treatment and stage of bulbs as block the layout was designed according to randomized block design with 2x2 m plot size. The bulbs were planted in plots at 30x30 cm spacing and 5-7 cm depth in three replications.

The data were recorded on the following characters to find out the sensitivity and optimal level of gamma irradiation- sprouting per cent, survival per cent and vegetative growth parameters like days to sprouting of bulbs, plant height (cm) and number of tillers per clump, number of leaves per clump, length of leaves (cm) and width of leaves (cm). Any variations appearing in the treated plants different from the control were also recorded. The plant growth was monitored regularly and variations of any form, floral or vegetative, were recorded meticulously.

In order to find out the cause for non-sprouting of cv. Prajwal bulbs (B_1 stage) beyond 2.5 Gy of gamma irradiation (Plate.2), histology study was done. For histological study three weeks old bulbs of cv. Prajwal were selected and irradiated with the levels of gamma radiation i.e. 5.0Gy, 7.5Gy and 10.0Gy. The central growing buds of these irradiated bulbs were fixed by keeping into FA solution for 48 hours. The fixed samples are stored in 70 per cent alcohol. These fixed tissues are subjected to standard protocol of dehydration and staining by Johansen (1940). The prepared blocks were cut into equal sized cubes and microtomy performed using microtome machine LEICA RM 2162, Leica. And the slides were prepared using Houpts solution. These slides were made to follow standard staining protocol using fast green. DPX mounting was done for stained slides. These permanent slides were observed under compound microscope (Imager 1A, AX10) to notice the changes in the cells which was induced by gamma irradiation in cv. Prajwal.

The experimental data were subjected to analysis of variance (ANOVA) as applicable to randomized block design (Snedecor and Cochman, 1967) using a computerized package (Windostat).

4.1.4 Results

Radiosensitivity study of tuberose in cvs. Prajwal and Phule Rajani was carried out based on the data recorded on per cent sprouting, per cent survival and all the vegetative parameters. In order to support the non-sprouting of Prajwal above 2.5Gy radiation histological study was carried out.

Per cent sprouting

Per cent sprouting was studied after forty days of bulb planting (Figure 3) (CD for per cent sprouting was calculated based on the raw data not through arc sin transformation because our pre cent values were ranged from 100% to 60%). It was observed that the sprouting per cent was 100% at lower dose of gamma irradiation (2.5 Gy). There after linear reduction in sprouting was observed from 5.0 Gy to 7.5 Gy in freshly harvested bulbs (B₀) of cv. Prajwal. But, at 10.0 Gy there was drastic reduction in the pre cent sprouting (61.11 %). Bulbs after three weeks of uprooting (B₁) were found very much sensitive to gamma irradiation. It was observed that at 2.5 Gy dose of gamma irradiation, sprouting reduced upto 60 per cent and beyond 2.5 Gy (5.0Gy, 7.5 Gy, 10.0 Gy and 15.0 Gy) there was complete mortality. In bulbs after six weeks of uprooting (B₂) gamma irradiation doses 2.5 Gy and 5.0 Gy did not affect the sprouting per cent (100 %). At 7.5 Gy and 10.0 Gy the per cent sprouting was 80.56% and 54.63%, respectively. In case of Phule Rajani (Figure.4) (CD for per cent sprouting was calculated based on the raw data not through arc sin transformation because our pre cent values were ranged from 100% to 60%) the sprouting per cent was not affected by gamma irradiation up to 7.5 Gy but at 10.0 Gy drastic reduction (54.63%) was observed in freshly harvested bulbs (B₀). In three weeks after uprooted bulbs (B₁) decreased sprouting per cent was observed beyond 2.5 Gy. In the six weeks after uprooted bulbs (B₂) 7.5 Gy and 10.0 Gy showed reduced sprouting. In general 15.0 Gy resulted in complete mortality across the bulb stages and cultivars.

Survival per cent

In our study we observed least mortality after sprouting across the bulb stages of both the tuberose cultivars so the survival per cent was calculated based

on the number of bulbs planted upon number of plants survived. By using this we estimated the LD₅₀ dose of gamma radiation for three bulbs stage of cvs. Prajwal and Phule Rajani. The LD₅₀ dose for freshly harvested bulbs (B₀) of cv. Prajwal is 10.25 Gy and for cv. Phule Rajani is 11.25 Gy (Figure 5). In three weeks after uprooted bulbs (B₁) LD₅₀ dose 3.25 Gy for cv. Prajwal and 10.25 Gy for cv. Phule Rajani (Figure 6) was observed. The LD₅₀ dose for six weeks after uprooted bulbs (B₂) of cv. Prajwal is 10.25 Gy and for cv. Phule Rajani is 11.5 Gy (Figure 7) was estimated.

Results of histological study

Histology study was conducted to find out effect of gamma irradiation beyond 2.5 Gy for three weeks after uprooted bulbs (B₁) of cv. Prajwal that had resulted in complete mortality. The result of the histology study is presented in Plates 12 and 13. We have observed that cells irradiated with 5.0 Gy, 7.5 Gy and 10.0 Gy gamma radiation severely affected (Plate. 3). In non irradiated tissue we observed the cells were arranged properly with nucleus in them. But, after irradiation about 90% cells were without nucleus. The cells become bigger in size and the shape deformed. Irradiation with 10.0 Gy of gamma irradiation caused complete damage of outer epidermal layer (Plate.4). Most of the bundle sheath cells were damaged at 5.0 Gy and above doses of gamma radiation. In one of the slide we observed cells with more vaculation were also devoid of nucleus.

In order to study the effect of irradiation on vegetative parameters data were recorded on days to sprouting, plant height and number of tillers per clump (Table.2). These parameters would support our study on gamma radiation sensitivity of tuberose.

Days to sprouting

In general sprouting delayed with increased gamma irradiation doses across the bulb stages and tuberose cultivars. In freshly harvested bulb stage (B₀) gamma irradiation doses 2.5 Gy, 5.0 Gy and 7.5 Gy were on par with control for days to sprouting in cv. Prajwal. In the six weeks after uprooted bulb stage (B₂) 5.0 Gy (17.67) and control (16.67) were on par. In cv. Phule Rajani non

significant difference was observed between control (19.00) and 2.5 Gy (20.67) in three weeks after uprooted bulb stage (B_1) and non significant difference observed between the treatments in B_0 for days to sprouting. The days to sprouting found highest in freshly harvested bulb stage (B_0) for cultivars Prajwal (32.67) and Phule Rajani (31.33) at 10.0 Gy of gamma irradiation. The high CV for days to sprouting in six weeks after uprooted bulb stage (B_2) implies the response for gamma irradiation was comparably high in this stage in both the cultivars for this trait.

Plant height (cm)

For plant height non significant difference observed between control (74.82) and 2.5 Gy (70.86) in cv. Prajwal at B_0 stage. In three weeks after uprooted bulb stage (B_1) the plant height was significantly low because the surviving plants did not flower at all. In bulbs of six weeks after uprooted (B_2) all the gamma irradiation doses showed inhibitory effect on plant height when compared to control. In cv. Phule Rajani across the bulb stages 2.5 Gy did not caused any effect on plant height. There was drastic reduction in the plant height across the bulb stages at 10.0 Gy of gamma irradiation in both the cultivars. For plant height the response of gamma irradiation was comparably high in freshly harvested bulb stage (B_0) of both the cultivars.

Number of tillers per clump

The non significant difference observed between control and 2.5 Gy for number of tillers per clump in cv. Prajwal in both freshly harvested (B_0) and six weeks after uprooted bulb stage (B_2). But, the numbers of tillers per clump were least at 2.5 Gy in B_1 bulb stage (three weeks after uprooting). In all the bulb stages there was non significant difference observed between control and 2.5 Gy in cv. Phule Rajani. The number of tillers drastically reduced across the bulb stages and cultivars at 10.0 Gy of gamma irradiation. A high CV was observed for the number of tillers per clump in B_1 stage of cv. Prajwal and B_2 stage of cv. Phule Rajani. This implies the effect of radiation was high in these two bulb stages.

The effect of gamma irradiation on leaf parameters were also studied (Table. 3). In tuberose leaf parameters are very important because they ultimately affect the flowering and bulb production parameters.

Number of leaves per clump

In freshly harvested bulb stage (B_0) of cv. Prajwal gamma irradiation resulted in more reduction of leaf number. But at the same time freshly harvested bulbs (B_0) of cv. Phule Rajani have not showed any critical difference at all the doses of gamma irradiation for number of leaves per clump. The non significant difference between control (33.40) and 2.5 Gy (33.50), 5.0 Gy (28.73) and also between control (30.40) and 2.5 Gy (27.73) was observed in bulbs of six weeks after uprooted (B_2) stage of cvs. Prajwal and Phule Rajani, respectively. Freshly harvested bulbs of cvs. Prajwal and Phule Rajani have showed comparably high response to gamma irradiation.

Length of leaf (cm)

The length of leaf was not affected by 2.5 Gy and 5.0 Gy in freshly harvested (B_0) and six weeks after uprooted (B_2) bulb stages in cv. Prajwal and in cv. Phule Rajani B_0 and B_2 bulb stages were not affected by 2.5 Gy of gamma radiation. Freshly harvested bulb stage (B_0) of cvs. Prajwal and Phule Rajani showed higher response to gamma irradiation.

Width of leaf (cm)

Even though there was increase in the leaf width at 2.5 Gy in freshly harvested bulb stage (B_0) in both Prajwal (2.49 cm) and Phule Rajani (2.15) cultivars but the increase was not significant. Non significant difference observed between control and 5.0 Gy in B_2 bulb stage in both the cultivars. Leaf width was not affected by 2.5 Gy of gamma radiation in B_1 and B_2 bulb stages of cv. Phule Rajani. Leaf width was affected more by gamma radiation in freshly harvested bulb stage (B_0) of cvs. Prajwal and Phule Rajani.

Leaf and plant abnormalities

At higher gamma irradiation dose (10.0 Gy) we have observed leaf abnormalities like crinkled leaf, golden yellow and white margin in one side of the leaf (Plate. 5). Plant abnormalities like reduced plant height, rosette form of

plant and multiple sprouting from single bulbs (Plate. 6) were observed. It was observed that at lower dose of gamma irradiation 2.5 Gy and 5.0 Gy there was no reduction in the chlorophyll content across the bulbs stages in both tuberose cultivars. There was slight reduction of the chlorophyll content was observed at 7.5 and 10.0 Gy of gamma irradiation doses.

4.1.5 Discussion

Radiation level and sensitivity to radiation is known to differ according to age of tissue, between varieties. In irradiation study with any plant material it is necessary to establish the tolerance levels and optimum growth for gamma radiation treatment. In the present study of radiosensitivity of tuberose cultivars, Prajwal and Phule Rajani, we recorded observations on sprouting per cent, survival per cent and the effect on vegetative parameters.

The sprouting per cent in freshly harvested bulbs (B_0) of both the cultivars are not much affected by the lower dose of gamma rays. But at the same time the lower dose of gamma irradiation (2.5 Gy and 5 Gy) had no effect on the sprouting per cent of freshly harvested bulbs of cv. Prajwal than the control and in higher doses 7.5 Gy and 10.0 Gy of gamma irradiation decreased the sprouting per cent in both the cultivars. Our study is in conformity with the findings of Sheehan and Sagawa (1959); Dryagina (1962, 1964); Uzenbaev and Nazarenko (1970); Seilluer (1975); Misra and Choudhury (1978). Their work revealed that lower doses of gamma irradiation enhance the sprouting while higher doses delayed the sprouting.

B_1 bulb stage (three weeks after uprooting) of cv. Prajwal was found highly sensitive to gamma irradiation, there was no sprouting observed beyond 2.5 Gy. But, in cv. Phule Rajani the three weeks after uprooted bulbs stage (B_1) responded to all the doses of gamma irradiation and significant decrease in sprouting was observed above 2.5 Gy. This higher radiosensitivity could be due to their less developed meristems (Katagiri and Lapins, 1974).

In B_2 (six weeks after uprooting) bulb stage 100 per cent sprouting was observed at 2.5 Gy and 5.0 Gy and it found decreased at 7.5 Gy and 10.0 Gy in

both the tuberose cultivars. This could be due to bulbs may have a large cell population, greater apical size during recovery and faster rate of growth increase the tolerance of apices to the lower dose of irradiations (Langenauer *et al.*, 1973). The fact for lower sprouting at higher dose of gamma irradiation could be level of auxin concentration in plants which drops after exposure to ionizing radiation (Skooge 1935, Gorden 1957). Over all we observed that there was complete mortality at 15.0 Gy gamma radiation dose across the bulb stages and cultivars. Abraham and Desai (1976) concluded from their study that high sensitivity of bulbs to radiation particularly to gamma rays may be due to high moisture content of the buds in the bulbs, they also said that this may be the main reason for low RBE values obtained in bulbs plant they used (tuberose, gladiolus, amaryllis). In some of previous radiation studies they stated that tuberose can tolerate gamma irradiation upto 25.0 Gy (Anu *et al.*, 2003). But, in our study the tolerance limit was only upto 12.0 Gy. This might be due to the varied radiosensitivity of the varieties as reported by Nilan (1956).

Survival per cent in our study was not affected much by gamma irradiation across the bulb stages and cultivars. We had observed mortality of fewer sprouted plants. We had calculated the survival per cent based on number of bulbs planted and number of plants survived. It was seen that survival per cent affected at higher dose of gamma irradiation i.e. 7.5 Gy and 10.0 Gy. This is in conformity with the work of Banerji *et al.* (1987). LD₅₀ (Lethal Dose 50) has been ascertained to be beyond 10.0 Gy for freshly harvested bulbs (B₀) and bulbs of six weeks after uprooting (B₂) in both the tuberose cultivars, which is in conformity to the finding of Raghava *et al.* (1988). But in our study B₁ bulb stage (three weeks after uprooting) was found highly sensitive to gamma irradiation in cv. Prajwal, the LD₅₀ observed for this bulb stage was 3.25 Gy. This may be the first finding in which different bulb stage of tuberose cvs. Prajwal and Phule Rajani were used to study the response to gamma irradiation.

Effect of gamma irradiation on vegetative parameters

The effect of gamma irradiation on days to sprouting of bulbs revealed general increase in days to sprouting with increased gamma radiation across the

bulb stages and cultivars of tuberose. But the low doses of gamma irradiation (2.5 Gy and 5.0 Gy) did not affect the days to sprouting. The days to sprouting of bulbs increased at 10.0 Gy in B₀ bulb stage of cvs. Prajwal (32.67) and Phule Rajani (33.33). It revealed that higher doses of gamma radiation suppressed sprouting and the time taken for sprouting was increased. This result was found in conformity to the mutation work in tuberose carried out by Anu *et al.*, (2003) and Sambendamurthy (1983) and Giridharan (1984) in ginger. Similar results were reported in gladiolus by many workers (Moes 1966, Buiatti *et al.*, 1967, Dryagina 1970, Banerji *et al.*, 1981, Fuji and Matsumura 1958).

The plant height was not much affected by lower doses of gamma radiation (2.5 Gy) across the bulb stages and cultivars of tuberose. In general, with the increase in gamma irradiation dose there was decrease in plant height. At 10.0 Gy the plant height was severely affected in all the bulb stages of both the tuberose cultivars. Similar results were reported earlier by Banerji *et al.*, (1994) in gladiolus. This may be caused by the reduced amount of endogenous growth regulators, especially the cytokinin, because of breakdown or lack of synthesis due to irradiation (Omar, 1988). Reduction in plant height due to gamma radiation treatment can be explained on the basis of harmful physiological effects which bring about a reduction in meiotic activity of the cells and retarding cell division, chromosomal aberrations and also damage to auxins (Gray, 1956). Reduction in growth can also be explained as differential killing of meristematic cells due to genetic injury, with damage cells producing less cell progenies (Catchside 1948, Sparrow *et al.*, 1952, Hagberg and Nybom, 1954).

Like other plant parameters which are discussed above the number of tillers reduced with increased gamma irradiation doses in all the bulb stages and across the cultivars of tuberose. But in contrast to our result Anu *et al.*, (2003) found high tiller mutants in tuberose.

With respect to leaf parameters a general decrease in general all three leaf characters (Number of leaves/clump, length of leaf and width of leaf) was observed with increase in gamma irradiation doses in all three bulb stages of cvs. Prajwal and Phule Rajani. The lower dose of gamma irradiation (2.5 Gy) did not

affect the leaf parameters significantly. The freshly harvested bulbs (B₀) of cv. Phule Rajani did not show any significant difference for number of leaves per plant in different gamma rays treatments. With respect to leaf parameters, freshly harvested bulbs (B₀) of both the tuberose cultivars responded comparably high to gamma irradiation. There was significant reduction in the length and width of leaf in 10.0 Gy gamma irradiation dose across the bulb stages and cultivars. Similar results were reported by Banerji *et al.*, (1994) in gladiolus; Anu *et al.*, (2003) in tuberose and by Marek (1954), Misra (1978) and Banarji *et al.*, (1981, 1987) in gladiolus.

Plant and leaf abnormalities

Increased gamma irradiation resulted in some of the leaf abnormalities like leathery leaves, formation of white and golden yellow bands on one side of the leaf margins only in few leaves. Presumably this effect might be due to radiation damage to the cells themselves and cell division. A similar phenomenon was reported by Gunkel and Sparrow (1954). In plant abnormalities like rosetting of all the leaves of the plants and multiple sprouting shoots from single bulb was observed at 10.0 Gy. It has been demonstrated that the degree of chromosomal aberration in somatic or meiotic cells generally increase as the degree of growth inhibition become more severe (Lea, 1947, Catecheside, 1948). These abnormalities are determined to the behaviour of tissues at different phases of development (Hansel, 1966). These are mainly due to the mutagenic effects on cell division (Gupta and Samata, 1967), chromosomal breakage and damage (Sparrow *et al.*, 1952), damage to auxins (Gorden, 1957) or due to change in biochemical products of the plant (Steinberg 1956). Kawai and Sato (1966) reported that the increase in mutation frequency has been shown to be accomplished by the injuries in M₁ plants. From this report it can be concluded that higher doses of gamma irradiation ranging from 7.5 Gy to 11.5 Gy could yield some variants in cv. prajwal and Phule Rajani in relation to leaf, flower and bulb characters. It has been observed that these deformities became normal in vM₂ generation. Similar results are reported by Arunee and Prapararat (2000) in *Portulaca grandiflora*. Chlorophyll content was not significantly affected by 2.5

Gy and 5.0 Gy in all the bulb stages of tuberose cultivars Prajwal and Phule Rajani. The reduction in chlorophyll content was observed at 7.5 Gy and 10.0 Gy of gamma irradiation doses. This chlorophyll deficiency could be attributed to mineral deficiency caused by biochemical changes.

4.1.9 Conclusion

In freshly harvested bulbs of cv. Prajwal gamma irradiation dose 2.5 Gy showed some stimulatory effect with respect to per cent sprouting. Higher dose of gamma irradiation (7.5 Gy and 10.0 Gy) reduced the per cent sprouting across the bulb stages in both the cultivars. Survival per cent was not much affected by the gamma irradiation doses. Three weeks of uprooted bulb stage (B₁) cv. Prajwal was found very much sensitive to gamma irradiation doses beyond 2.5 Gy. In general 7.5 Gy and 10.0 Gy doses of gamma irradiation have showed more effect on vegetative parameters across the bulb stage in both the cultivars Prajwal and Phule Rajani and 2.5 Gy and 5.0 Gy have not made any significant effect and the response of bulbs stages were in the order B₀> B₂>B₁, respectively. The optimal dose for mutation induction in these two tuberose cultivars would be from 7.5 Gy to 11.5 Gy.

Effect of acute gamma irradiation on flower and bulb parameters and stability study of mutants in tuberose (*Polinathes tuberosa* Linn.)

4.2.1 Abstract

Genetic variability is the prerequisite for any crop to be improved. Mutation breeding is a best method identified to enhance the genetic variability in crops within short time. Knowing the limited variability in tuberose, present investigation was undertaken for the improvement in this crop by utilizing the commercial cultivars Prajwal and Phule Rajani. To induce variation, gamma radiation (2.5 – 15.0 Gy) was applied and the effects on flower and bulb parameters were assessed in vM₁ generation. With increase in doses of gamma irradiation treatment, gradual reduction in number of spikes, floret number per spike and number of bulbs was observed. In some case lower dose was found to be simulative, while higher dose had inhibitory effect on morphological variation. Various macro mutations were scored for uniform flowering, reduced number of bulbs and spike number in M₁ population. The mutants of vM₁ generation not found stable in vM₂ generation. But we could able to find the some more mutants from the maintained gamma irradiated population of vM₁ generation related to change in flower shape. In general, freshly harvested tuberose bulbs of cvs. Prajwal and Phule Rajani responded more to gamma irradiation. From this study it has found that gamma irradiation level, 7.5Gy and 10.0Gy were found optimal for mutation induction in these two tuberose cultivars.

Key words: Bulb, gamma radiation, mutation, spike, tuberose

4.2.2 Introduction

The growth of floriculture industry has taken long strides worldwide. The industry must meet the demands of consumer by providing new value added flower which are cost effective and unique in flower or plant characters e.g., flower colour, flower shape, plant architecture, plant morphology and so on.

Tuberose (*Polianthes tuberosa* L.) is one of the most important bulbous flowering plant of tropical and subtropical areas, it is monocot genus, perennial bulbous plant (Rose 1903, Bailely 1939). It is found to be originated at Mexico, Hutchinson (1960) placed it under the family Agavaceae. Among commercial bulbous flowers tuberose occupies second position in area and production after gladiolus in India. Tuberose occupies a prime position owing to its popularity as a cut flower, loose flower, landscape gardening and also for extraction of the highly valued natural flower oil. The serene beauty of the flower is because of its tall and straight spikes which bear bright white florets in pair. There are three types of tuberose; Single with one row of corolla segment, Semi-double bearing flowers with two to three rows of segments and Double having more than three rows of corolla segments. In an seventeen cultivars which are available in India, some of the cultivars bears flower bud with pink tinge e.g., Prajwal, Shringar, Suvasini, Hyderabad Single, Pearl Double and Sikkim Selection and some cultivars bears flower bud with green tinge e.g., Vaibhav, Phule Rajani and Mexican Single.

New commercially interesting cultivars can be bred in two ways. A cross between two existing cultivars resulted in highly variable off spring from which good cultivars can be selected. In case of tuberose seed setting is a difficult process and it observed mainly in Single type of cultivars but not in all the Single type of cultivars and it has observed in a quite erratic manner, seed germination also quite low and seed setting is not observed in Double type. So one has to select always Single type of tuberose cultivar as a female parent for breeding programme. In sexual method of breeding in tuberose seed takes about three seasons to come into blooming and further many years for multiplying the stock.

Genetic variability is necessary in any plant breeding programme for crop improvement. The genetic variability in tuberose is very limited (Anu *et al.*, 2003) and it has narrow genetic base. Genetic improvement of tuberose is hampered by meager variability, self incompatibility and seed sterility. Even though there is not much genetic variability in this plant, it is highly heterozygous and hence variability can be created by any quickest means of breeding approaches like mutation induction by gamma irradiation. In this context mutation assisted

breeding (MAB) can contribute greatly for genetic improvement of tuberose. Mutation breeding may also provide unusual strains of curiosity (Misra and Choudhury, 1979) which is normally difficult through conventional breeding methods, therefore, the present study for inducing variability through mutation breeding which offers a comparatively shorter duration was taken up. With this above background our present study was emphasized on mutagenic effect of gamma irradiation of different parameters of tuberose cultivars Prajwal and Phule Rajani.

4.2.3 Materials and Methods

The present study was done using two tuberose cultivars Prajwal and Phule Rajani. Three bulb stages *viz.* Freshly harvested bulbs (B₀); Three weeks after uprooting (B₁) and Six weeks after uprooting (B₂) were evaluated. This study was continued once the radiosensitivity and optimal level of acute gamma irradiation on these two cultivars were observed. Based on the previous study results we irradiated each bulb stage of both cultivars with four levels of gamma radiation (2.5, 5.0, 7.5 and 10.0 Gy). In the previous study results revealed that 15.0 Gy caused complete mortality across the bulb stage and varieties.

To study the effect of radiation on flower and bulb parameters the data were recorded on following traits- days to flower spike appearance, opening of first pair of floret, flowering duration, number of florets per spike, vase life, total number of spikes, spike length, fresh weight of spike, floret diameter and length, fresh weight of individual floret and bulb parameters like number of bulbs, fresh weight of bulbs, length and diameter of bulbs. Any abnormality or variations were also recorded. The experimental data were subjected to analysis of variance (ANOVA) as applicable to randomized block design, (Snedecor and Cochman, 1967) using a computerized package (Windostat).

4.2.4 Results

The effect of radiation on flowering and bulb parameters is studied in the present experiment. The results are described in following headings.

Effect of gamma irradiation on days to spike appearance and flowering

The effect of gamma irradiation on the days to spike appearance and the days to flowering is presented in Table 4. In general spike appearance in tuberose will occur in 2-4 months from the date of planting of bulbs. The gamma radiation has not affected the days taken to spike appearance in both the cultivars except in the freshly harvested bulbs (B_0) of cv. Prajwal where significant difference between gamma radiation treatments was observed. But, in the three weeks after uprooted bulb stage (B_1) of cv. Prajwal there was no spike initiation occurred at 2.5 Gy. When we observed the gamma radiation effect on the days to flowering, in both freshly harvested (B_0) and six weeks after uprooted (B_2) bulb stages of cv. Prajwal all the gamma irradiation doses except 2.5 Gy (in B_2 bulb stage) decreased the days to flowering. In cv. Phule Rajani across the bulb stages (B_0 , B_1 and B_2) 2.5 Gy and 5.0 Gy doses the days to flowering was on par with control. The days to flowering were least in 10.0 Gy of gamma irradiation across the bulb stages and cultivars because the spikes bear few (1-4) floret buds (Plate 11a). High CV was observed for these two flowering parameters in B_0 bulb stage of both cvs. Prajwal and Phule Rajani.

Effect of gamma irradiation on number of florets per spike, flowering duration and vase life of cut spike

The effect of gamma irradiation doses was a gradual decrease in number of florets/ spike, duration of flowering under field and vase life of cut spike (Table 5) with increase in dosage. 2.5 Gy of gamma radiation had no effect on number of florets in B_0 and B_2 bulb stages of cv. Prajwal. In freshly harvested bulb stage (B_0) of cv. Phule Rajani, 2.5 Gy made a slight increase in the number of florets (41.07) as compared to control (38.00). Though, non significant difference was found between control and 2.5 Gy and 5.0 Gy. In general 10.0 Gy of gamma radiation reduced the number of florets to a greater extent in all the bulb stages of cvs. Prajwal and Phule Rajani.

In cv. Prajwal except 2.5 Gy in all other gamma irradiation doses (5.0 Gy, 7.5 Gy and 10.0 Gy) there was significant decrease in the flowering duration in B_0 and B_2 bulb stages. In cv. Phule Rajani across the bulb stages, 2.5 and 5.0 Gy

showed non significant difference. Flowering duration was least at 10.0 Gy across the bulb stages and cultivars, because very few florets appeared in each spike, as it mentioned earlier also. High CV was observed in B₂ bulb stage of cv. Prajwal and B₀ bulb stage of cv. Phule Rajani.

In general vase life of cut tuberose spike of the irradiated plants from all the bulb stages of both the cultivars got reduced, because spikes from irradiated plants were with less number of florets. In B₀ and B₂ bulb stages of cv. Prajwal 2.5 Gy has not affected the vase life of the cut spikes. In cv. Phule Rajani vase life of cut spikes were not affected in 2.5 Gy and 5.0 Gy dose of gamma irradiation in B₁ bulb stage. In B₀ bulb stage of cv. Phule Rajani at 2.5 Gy an increased vase life of cut spikes (8.83 day) was observed than the control (8.07 day). In B₂ bulb stage we found gradual decrease of vase life with increased gamma irradiation doses.

Effect of gamma irradiation on number of spikes per plot, length (cm) and fresh weight of spike (g)

Cut spikes are the economic part of the tuberose so we have also studied the effect of gamma irradiation on spike parameters like number of spikes per plot, length of spike and fresh weight of spike (Table. 6). There was drastic reduction in the number of spikes per plot with the increased gamma irradiation doses in B₀ and B₂ bulb stages of cv. Prajwal. In B₁ bulb stage of cv. Prajwal all the plants were remained in the vegetative stage.

In cv. Phule Rajani 2.5 Gy showed stimulatory effect on spikes initiation on B₀ bulb stage. The result was an increased number of spikes per plot. The number of spikes per plot was 27.33 (Plate. 7) which three times to the control (9.33). But, the number of spikes per plot decreased with increased gamma irradiation doses. In B₁ and B₂ bulb stages the number of spikes decreased with increased gamma radiation doses. B₀ bulb stage in both the cultivars of tuberose has showed higher response to gamma irradiation for number of spikes per plot.

In general spike length got decreased with increased gamma irradiation doses. Spike length was not affected by 2.5 Gy of gamma irradiation at B₀ bulb stage of cv. Prajwal. But, in case on B₂ bulb stage of cv. Prajwal there was a gradual decrease in spike length noticed with increased doses of gamma

irradiation. Across the bulb stages of cv. Phule Rajani, 2.5 Gy had no effect on spike length and showed non-significant difference with control. Spike length got reduced very much at 10.0 Gy across the bulb stages and cultivars. The response to gamma radiation was comparably high in B₀ bulb stage in both the tuberose cultivars.

The gradual decrease in the fresh weight of cut spike has observed with increased gamma irradiation doses in B₀ and B₂ bulb stages of cv. Prajwal. In B₀ bulb stage of cv. Phule Rajani, 2.5 Gy resulted in giving more fresh weight of cut spike (94.00 g) as compared to control (76.76 g). In B₁ and B₂ bulb stages of cv. Phule Rajani 2.5 and 5.0 Gy has showed non significant difference for fresh weight of cut spike. Beyond this radiation level i.e. 7.5 and 10.0 Gy, the fresh weight of cut spike got reduced. B₀ bulb stage of both the tuberose cultivars was showed comparably higher response to gamma irradiation.

Effect of gamma irradiation on individual floret parameters

In the present study effect of gamma irradiation on individual floret parameters was also studied (Table. 7). For floret length and diameter, 2.5 Gy has not showed the significant difference in B₀ and B₂ bulb stages of cv. Prajwal. B₀, B₁ and B₂ bulb stages not responded significantly to 2.5 Gy and 5.0 Gy in cv. Phule Rajani for floret length and diameter. Fresh weight of individual floret not affected much by gamma irradiation (2.5 Gy, 5.0 Gy and 7.5 Gy) across the bulb stages and cultivars.

Effect of gamma irradiation on bulb parameters

The effects of gamma irradiation on bulb parameters were studied in both cvs. Prajwal and Phule Rajani (Tables 8 & 9). No significant difference observed for number of bulbs at 2.5 Gy in cv. Prajwal and 2.5 and 5.0 Gy in cv. Phule Rajani. Plants of 7.5 Gy and 10.0 Gy were yielded in less number of bulbs across the bulb stages and cultivars (Plate. 8). All the gamma irradiation doses did not show any significant difference for fresh weight of bulb in freshly harvested (B₀) and six weeks after uprooted (B₂) bulbs stages of cv. Prajwal and freshly harvested (B₀) bulbs of cv. Phule Rajani. Fresh weight of bulbs at 10.0 Gy was found less in all three bulb stages of both the cultivars. Three weeks after

uprooted (B_1) and six weeks after uprooted (B_2) bulbs of cv. Phule Rajani showed significant difference for fresh weight of bulbs between the gamma irradiation doses. There was non significant difference for diameter of the bulbs between control and gamma irradiation doses in freshly harvested (B_0) and six weeks after uprooted bulbs (B_2) of cv. Prajwal and cv. Phule Rajani. In general, the length of bulbs was also not affected, across the bulb stages and cultivars, by 2.5 Gy, 5.0 Gy and 7.5 Gy gamma irradiation doses. At 10.0 Gy significant reduction in length of bulb was observed.

Flower and spike variations in vM_1 generation

In the present study variations in flower were recorded like reduced petal number and increased petal number than normal (Plate. 9) and change in the flower tube shape in both the cultivars (Plate. 10). Deformed spikes and change in the floret arrangement was also observed in some irradiated plants (Plate. 11).

Variants during vM_1 generation and their stability

1. Tall flowering variant

This variants was identified in 7.5 Gy gamma irradiated freshly harvested bulb stage (B_0) of cv. Prajwal. The spike was of 146 cm height (Plate 12a) which is 34 cm more than the normal Prajwal spike (112 cm). The spike also contained more number of florets (76) which is also more than the normal Prajwal spike (42). The spike remained straight and no lodging was observed up to opening of last floret.

2. Flower colour variant

Flower colour variants i.e. slightly pinkish was derived from cv. Prajwal after 7.5 Gy gamma irradiation in B_0 bulb stage (Plate. 13). The colour was more intense than the normal flower bud of cv. Prajwal which also shows pinkish ting at bud stage. But this mutant retained the colour even after opening of the flower. But the opened florets were not in good form and spike was also not straight.

3. Dwarf variant

This dwarf variant was derived from cv. Phule Rajani in three weeks after uprooted (B_1) bulb stage at 7.5 Gy gamma irradiation. The spike length was

reduced up to 34 cm. The number of florets per spike were found as same as that of normal Phule Rajani spike. But the length of the floret got reduced (Plate. 12b).

4. Double spike head variant

This variant was derived from cv. Prajwal in six weeks after uprooted bulb stage (B_2) at 7.5 Gy of gamma irradiation dose (Plate. 14). It was observed that two spike heads were fused from the neck and there was no demarcation on the stock of the spike. It was yielded more number of florets per spike (54) then the control (42).

Bulbs of these variants were uprooted during mid of January 2010 and stored for 30 days and planted in 1x1 m sized plots at the end of February 2010. There was no reduction in the per cent germination and survival of the bulbs observed. But all the four mutants were not found stable.

Mutants in vM_2 generation from the primary gamma irradiated population

The below mentioned mutants were derived from the primary gamma irradiated population which was maintained after observing the gamma irradiation effect of vM_1 generation.

1. Flower shape mutant:

Two flower mutants change in flower shape was derived from cv. Prajwal. In one of the mutant the shape of the petal has been changed from elongated to round shape (Plate. 15). All the florets of the spike have showed the same petal shape. This was observed in B_2 bulb stage i.e. six weeks after uprooting of bulbs at 10.0 Gy of gamma irradiation dose. In the second flower shape mutant increased petal length was observed (Plate. 16). And the florets were very big than the normal floret of cv. Prajwal. This was observed in B_1 bulb stage i.e. three weeks after uprooting at 2.5 Gy of gamma irradiation dose.

2. Tall mutant:

This mutant was derived from the cv. Phule Rajani from freshly harvested bulb stage (B_0) at 7.5 Gy gamma irradiation dose. The spikes were grown up to 94 cm (Plate. 17) whereas normal spike is around 70-74 cm in general.

3. Flower colour mutant:

This mutant was same as that one which we observed in vM₁ generation. This was derived from the same plot at 7.5 Gy gamma irradiation dose in cv. Prajwal from freshly harvested bulb stage (B₀) (Plate. 18).

4. Mutant with higher rachis length:

This mutant was derived from cv. Prajwal from freshly harvested bulb stage (B₀) at 7.5 Gy gamma irradiation dose. In this mutant the individual florets become small and are arranged at equal space intervals (Plate. 19).

5. Variegated leaf mutant:

This mutant was derived from cv. Phule Rajani from freshly harvested bulb stage (B₀) at 10.0 Gy gamma irradiation dose (Plate. 20). In this mutant white colour band has formed at the middle of the leaf. All the leaves which has emerged from the bulb were showed the same pattern. This mutant was separated from the mother bulb and transferred to pot and maintained.

When we observed the response of different bulb stages of both the tuberose cultivars for mutation induction by gamma irradiation (Figure 8), the more number of mutants were isolated from the freshly harvested bulbs (B₀) of both the tuberose cultivars which was flowered by six weeks after uprooted bulbs (B₂) and we have not observed any mutation in three weeks after uprooted bulbs (B₁).

4.2.5 Discussion

Gamma irradiation did not affect doses on days to spike appearance and days to flowering on two cultivars of tuberose *viz.*, Prajwal and Phule Rajani, it was observed that in general days to spike appearance was increased with increase in gamma irradiation in all the bulb stages (B₀, B₁ and B₂) of tuberose cultivars used even though there was no critical difference found. Radiations may indirectly delay sprouting and slows down the growth rate during early period of growth or it may be due to the deleterious effects of radiation on growth hormones like auxins and gibberellins induction. Similar results were earlier described in tuberose by Anu *et al.* (2003). They reported that cv. Suvasini took

maximum number of days (200.92) for first spike emergence. But in our study we were not found these many days delay in spike appearance, the maximum days found was around 133.43 in freshly harvested bulb stage of cultivar Prajwal. In cultivar Phule Rajani at 10.0 Gy we had observed early spike appearance but these spikes head were found abnormal. In three weeks after uprooted bulb stage (B₁) none of the surviving plants produced the spike at 2.5 Gy. This bulb stage was found highly sensitive to gamma irradiation as no survival was observed beyond 2.5 Gy. Similar results were reported by Banerji *et al.* (1981) in gladiolus and in their study the survived plants did not produce any spike after exposure of 12.5 kR of gamma rays.

In case of days to flowering it was observed that it got decreased with increased gamma irradiation dose. This effect could be due to less number of florets per spike in irradiated plants than number of florets in spikes of control plants. It was also observed that lower doses of gamma irradiation (2.5 and 5.0 Gy) had no effect on days to flowering in cv. Phule Rajani.

The effect of gamma irradiation on average number of florets per spike was found to be proportional to irradiation dose. Increase in the gamma irradiation dose beyond 2.5 Gy resulted in drastic reduction in the number of florets per spike in cv. Prajwal. The reduction in number of florets per spike was observed at 2.5 Gy in B₀ and B₂ bulb stages of cv. Prajwal and at 2.5 Gy and 5.0 Gy in all the bulb stages of cv. Phule Rajani, although the reduction was non significant. Number of florets per spike was severely affected after 5.0 Gy of gamma irradiation. Exception was observed at 2.5 Gy in B₀ bulb stage of cv. Phule Rajani where little stimulatory effect of gamma irradiation was observed. Similar results were reported by Anu *et al.* (2003) in tuberose cv. Suvasini with increased number of florets (34.42) than the control plant and also in gladiolus by Haskins and Moore (1935); Johnson (1948); Spancer (1955). Reduction in number of florets per spike following radiation was reported earlier by Seilleur (1975); Gupta and Banerji (1977) in gladiolus. The decreased number of florets as a consequence of somatic competition (Nybom, 1970) takes place in the course of intra somatic selection.

Flowering duration in field and vase life of cut spikes were found in correlation with each other. It was observed that flower duration in field and vase life of cut spikes were not affected by the low dose of gamma irradiation (2.5 Gy) in B₀ and B₂ bulb stages of cv. Prajwal. At the same time in all the bulb stages of cv. Phule Rajani flowering duration and vase life was not significantly affected by 2.5 Gy and 5.0 Gy of gamma irradiation. Both these flower parameters were affected significantly at 7.5 and 10.0 Gy. Lower flowering duration was observed in 10.0 Gy across the bulb stages and cultivars of tuberose. The reduced longevity of spike following the treatment of gamma rays was also reported by Misra and Bajpai (1983) in gladiolus. The stimulatory effect of gamma irradiation was observed for both the parameters at 2.5 Gy in B₀ bulb stage of cv. Prajwal but the deference was not found significant.

In general the number of spikes per plot decreased with increase in gamma irradiation. In B₀ and B₁ bulb stages of cv. Prajwal the drastic reduction in the spike number was observed in all the gamma irradiation doses. In cv. Phule Rajani the stimulatory effect of lower dose of gamma irradiation (2.5 Gy) on number of spikes per plot in B₀ bulb stage was observed. But similar effect was not observed in B₁ and B₂ bulb stages. Over all at 10.0 Gy we had observed only one or two spikes per plot across the bulb stages of tuberose cultivars and these spike were abnormal in shape. Similar results were reported by Misra and Mahesh (1993) in gladiolus. In their study the number of spikes per plot were reduced above 4.0 kR and remaining plants remained in the vegetative phase only.

Spike length and fresh weight were not affected by low dose of gamma irradiation (2.5 Gy) in all the bulb stages of cv Prajwal and Phule Rajani. Above 2.5 Gy a decreased spike length and fresh weight was observed. Spike length was reduced sharply at 10.0 Gy across the bulb stages in both the tuberose cultivars. Similar results were reported by Anu *et al.* (2003) in tuberose; Misra and Mahesh (1993); Banerji *et al.* (1994) in gladiolus.

With respect to individual flower parameters, flower diameter was not affected by gamma radiation upto 5.0 Gy in B₀ and B₂ bulb stages of cv. Prajwal and up to 7.5 Gy in B₁ and B₂ bulb stages of cv. Phule Rajani. Flower diameter

was increased up to some extent in B₀ bulb stage of cv. Phule Rajani at 2.5 Gy but increase was not significant.

Similarly, length of floret was also not affected by lower dose of gamma irradiation (2.5 Gy) in both the cultivars in all the bulb stages. The decreased length of florets was observed above the 5.0 Gy of gamma irradiation.

We have not observed any significant reduction of fresh weight of individual floret at lower dose of gamma irradiation i.e. 2.5 Gy and 5.0 Gy across the bulb stages of tuberose cultivars. Increased dose of gamma radiation (7.5 Gy and 10.0 Gy) showed a negative effect on the fresh weight of floret.

Enhancement of flower size in Edward rose with 2.4 kR and below was reported by Irulappan and Rao (1981) and Valsamma (1989). Desai and Adraham (1978) found that low doses of radiation produced bigger flowers in rose.

Decrease in flower size can be attributed to poor growth of flowers on irradiated plants due to radiation damage (Gaul 1970). Gupta and Shukla (1971); Lata (1973); Datta and Gupta (1982) reported reduction in flower size following irradiation in rose. Decrease in the diameter and length at higher dose of gamma irradiation (7.5 Gy and 10.0 Gy) could be the main reason for reduction in the fresh weight of individual flower at these doses, because all these parameters correlate with each other. Reduction in the diameter and length of florets at high dose of gamma irradiation (7.5 Gy and 10.0 Gy) could be the main reason for reduction in the fresh weight of individual florets. This signifies that all these parameters correlate with each other.

When we observed the effect of gamma irradiation on bulb parameters i.e., number of bulbs per clump, fresh weight of bulbs and diameter and length of the bulbs in each treatment, the number of bulbs per clump was not affected by the lower dose of irradiation up to 5.0 Gy. Plants with 7.5 Gy and 10 Gy yielded less number of bulbs per clump across the bulb stages of both the tuberose cultivars. All the gamma irradiation doses did not show any significant effect on fresh weight of bulbs in most of the bulb stages of tuberose cultivars. In general length and diameter of the bulbs was also not affected by irradiation doses up to 7.5 Gy. The reduction in production of bulbs could be because of the ill-effect of gamma

irradiation, as it hampered the root system, the roots become thicker and stouter. The cessation of growth of the axillary buds in the bulbs could be due to the inactivation of enzymes and auxins responsible for such growth. Similar result was observed by Grabowska and Mynett, (1970).

In our study we have observed some of the morphological changes in the florets. Generally cv. Prajwal bears florets of six petals, during our investigation we come across florets from two petals to eight petals. It was observed that the frequency of this kind of petals was more in lower florets of the spike and upper florets had normal petal number. Similar study observed with respect to mutation frequency in gladiolus (Buiatti *et al.*, 1969), when they calculated the mutation frequency per flower it decreased from the bottom to the top of the spike and this was not found to be constant throughout the plants. Abnormal growth of the spikes at 10.0 Gy in both the cultivars of tuberose was observed. These types of abnormalities were observed by Marek (1959) in gladiolus. The assumption one can make that the damage would be caused at the tip of the flower primordial differentiation. Complete blindness of plants shows severe damage in the synthesis of growth substance like auxins and other growth regulators (Skoog, 1935). There recovery to normal form in the next generation vM₂ indicates that the physiological effect on plant height following radiation was temporary phenomenon. Because we observed normal flowering phase in these blind plants

In vM₁ generation total four variants were observed *viz.*, longer spike length variant, double spikehead variant and flower colour variant of cv. Prajwal and shorter spike length variant of cv. Phule Rajani. When stability of these mutated characters was observed in the next generation we have not come across with the expression of similar character in none of these mutants, all the mutants were revert back into original form. The main reason for this could be diplontic selection, which is competition between the mutated cell and the non mutated neighbor cells. Mutated cell often can not express itself because it loses the competition within the cell layer (Broerties, 1966). From the above presentation it can be conclude that in order to over come these difficulties growing complete plant from single cell either *in vitro* or *in vivo* using adventitious bulbs could be

done. From this study we can conclude that freshly harvested bulb stage (B_0) is very much useful for mutation induction under *in vitro* condition because it contain less number of proliferation cell. We observed some of the useful mutants like change in floret shape, increased rachis length, tall mutants and variegated leaf in the vM_2 generation from the primary irradiated population. This mutant could be of putatively stable because these are isolated after the second cycle of the bulb propagation.

4.2.6 Conclusion

From this study it was found that freshly harvested bulbs of both the tuberose cultivars Prajwal and Phule Rajani are highly suitable for mutation induction either *in vivo* and also *in vitro* condition. The gamma irradiation dose 7.5 Gy and 10.0 Gy were found effective for the mutation induction in tuberose cultivars Prajwal and Phule Rajani.

5. DISCUSSION

Induction of somatic mutations has an important role as a means of crop improvement (Micke and Donini, 1973) in ornamental plants. The highly heterozygous nature, narrow genetic constituent, self incompatibility and less seed setting and seed sterility of tuberose make it more amenable to mutation breeding. Hence, induced mutations assume special significance in the improvement of tuberose. Besides, in an ornamental crop like tuberose, any change that gives novel flower colour and form becomes more acceptable to the growers. Mutations of interest, once recovered can be maintained by means of asexual propagation (bulbs) in this crop. Radiation levels required, sensitivity to radiation is known to differ according to age of tissue, between varieties and even as a function of single gene differences. In radiosensitivity studies with any plant material it is necessary to establish tolerance levels and optimal growth for treatment. In tuberose eventhough meager mutation study was done, a proper study of radiosensitivity has not been done. With this background the objectives of the present studies were conducted to study the radiosensitivity of two commercially cultivating tuberose cultivars Prajwal and Phule Rajani using three different bulb stages (B_0 B_1 and B_2) and to see the effect of the acute gamma irradiation on flowering and bulb parameters. The salient results are discussed here under.

During mutation study to prescribe the radiation dosage for each plant species, wide survey of radiation sensitivity become necessary. In our study one of the immediate effects of gamma irradiation was inhibition of sprouting of bulbs in all the bulb stages of Prajwal and Phule Rajani at higher doses (7.5 Gy and 10.0 Gy). At the same time the lower dose of gamma irradiation (2.5 Gy and 5.0 Gy) increased the sprouting per cent of freshly harvested bulbs of cv. Prajwal. The fact for lower sprouting at higher dose of gamma irradiation could be level of auxin concentration in plants which drops after exposure to ionizing radiation (Skooge, 1935; Gorden, 1957). In general lower doses of gamma irradiation enhanced the sprouting while higher doses delayed the sprouting. B_1 bulb stage (three weeks after uprooting) of cv. Prajwal was highly sensitive to gamma irradiation, as

sprouting was absent beyond 2.5 Gy. Similar situation was not observed in cv. Phule Rajani with same bulb stage (B₁ – three weeks after uprooting). This shows the difference in the cultivar response to gamma irradiation. This higher radiosensitivity could be due to their less developed meristems (Katagiri and Lapins, 1974). Over all we observed complete mortality at 15.0 Gy gamma irradiation dose across the bulb stages and cultivars.

The estimated LD₅₀ (Lethal dose 50) for B₀ and B₂ bulb stages of cultivar Prajwal was 10.25 Gy, for B₁ bulb stage the LD₅₀ dose was 3.25 Gy. This shows that B₁ bulb stage of cv Prajwal was more sensitive to gamma irradiation. For cultivar Phule Rajani the estimated LD₅₀ dose was 11.25 Gy, 10.25 Gy and 11.5 Gy for B₀, B₁ and B₂ bulb stages, respectively. This shows the cultivars difference to gamma radiation.

We studied the response of plant parameters like days to sprouting of bulbs, plant height and number of tillers per clump in three bulb stages (B₀, B₁ and B₂) of tuberose cultivars Prajwal and Phule Rajani. These three vegetative parameters were in general not affected by gamma irradiation dose up to 5.0Gy across the bulb stages of both the cultivars. The sprouting was delayed when the freshly harvested (B₀) bulbs were irradiated at 10.0 Gy, the plant height and number of tillers per clump was also adversely affected in all the bulb stages of both the cultivars at 10.0 Gy. It revealed that higher doses of gamma irradiation suppressed sprouting and the time taken for sprouting was increased. This result is in correlation with mutation study conducted by Anu *et al.* (2003) in tuberose. The plant height reduction could be due to reduced amount of endogenous growth regulators especially cytokinin as a result of breakdown or lack of synthesis due to irradiation. Significant reduction in the leaf parameters like number of leaves per clump, length of leaf and width of leaf was observed at 7.5 Gy and 10.0 Gy. Similar result was reported by Banerji *et al.* (1994) in gladiolus. The chlorophyll deficiency which observed at 7.5 Gy and 10.0 Gy which could be due to mineral deficiency and biochemical changes.

Maximum plant and leaf abnormalities were observed at 10.0 Gy gamma irradiation dose in freshly harvested bulbs of tuberose cultivars used like rosette

of all the leaves of the plants, multiple sprouted shoots from single bulb leathery leaves and formation of white and golden yellow bands on one side of the leaf margins only in few leaves. These kind of abnormalities were mainly due to the mutagenic effects on cell division (Gupta and Samata, 1967), chromosomal breakage and damage (Sparrow *et al.*, 1952), damage to auxins (Gordon, 1957) or due to change in biochemical products of the plant (Steinberg, 1956). From this first experiment it is concluded that higher doses of gamma irradiation ranging from 10.0 Gy to 11.5 Gy can yield some variants in cvs. Prajwal and Phule Rajani.

The possibilities of creating new and even unusual forms of tuberose are immense and the breeder will always have future goals to work for creating new forms and novel colours. During mutation study it is important to observe all the characters of a plant to find out the most useful variation. In all three bulb stages (B₀, B₁ and B₂) of tuberose cultivars, days to spike appearance got delayed with increase in gamma irradiation doses, fairly at 7.5 Gy and 10.0 Gy doses. But there was no critical difference observed. Radiation may indirectly delay the sprouting rate during early period of growth. Opposite to delayed spike appearance the days to flowering was earlier in all the bulb stages of both the tuberose cultivars. This effect could be due to less number of florets per spike in irradiated plants. At the same time lower dose of gamma irradiation (2.5 Gy and 5.0 Gy) did not affect both of these flowering parameters.

We have observed correlation between number of florets per spike, flower duration in the field and vase life of cut spikes in relation to gamma irradiation doses. The reduced number of florets per spike, lower flower duration and vase life was observed beyond 2.5 Gy of gamma irradiation in all the bulb stages of cv. Prajwal and Phule Rajani. The decreased number of florets could be as a consequence of somatic competition (Nybom, 1970). The reduced longevity of spikes in the field and their vase life is due to less number of florets per spike in the gamma irradiated plants (5.0 Gy, 7.5 Gy and 10.0 Gy). Similar result was observed in gladiolus by Misra and Bajpai (1983). The stimulatory effect in number of florets per spike, only in freshly harvested bulb stage (B₀) of cv.

Prajwal was observed. This signifies that this particular bulb stage responded more to gamma irradiation for this trait.

Number of spikes per plot in general determine the yield, reduced with increase in gamma irradiation doses across the bulb stages of both the tuberose cultivars. At 10.0 Gy most of the plants remained in the vegetative phase in both the cultivars. Stimulatory effect on number of spikes per plot was observed in B₀ bulb stage of cv. Phule Rajani at 2.5 Gy. This shows the different response of the cultivars to gamma irradiation in relation to their developmental stages. Spike length and fresh weight of spike are the most important parameters as Single type tuberose cultivars some time used as cut flower. In the present study these two flower parameters was not found affected by gamma irradiation doses 2.5 Gy and 5.0 Gy in all the bulb stages (B₀, B₁ and B₂) of both the tuberose cultivars. At the same time 7.5 Gy and 10.0 Gy of gamma irradiation doses sharp reduction of both length and fresh weight of spike was observed. A similar kind of result was reported by Anu *et al.* (2003) in tuberose cv. Suvasini.

Individual flower parameters like fresh weight of floret, length and diameter of individual floret of a spike are most important in case of Single type of tuberose cultivars because these are mostly used as loose flowers. In Prajwal and Phule Rajani which are Single type of tuberose cultivars the length and fresh weight of individual florets were not affected by gamma irradiation up to 5.0 Gy in all three bulb stages (B₀, B₁ and B₂). Flower diameter got affected beyond 5.0 Gy in cv. Prajwal and at 10.0 Gy in cv. Phule Rajani. Decrease in flower size can be attributed to irradiation damage in the flower primordia (Gaul, 1970). The non significant increase in the flower diameter was observed in freshly harvested bulb stage (B₀) of cv. Phule Rajani at 2.5 Gy. Similar report on increase in flower size of Edword rose was observed by Irulappan and Rao (1981).

All the bulb parameters *viz.*, number of bulbs per clump, length of bulbs, diameter of bulbs and fresh weight of bulbs per clumps were affected maximum at 10.0 Gy in all three bulb stages (B₀, B₁ and B₂) of cv. Prajwal and Phule Rajani. The reduction in production of bulbs could be caused by ill-effect of gamma irradiation, as it hampered the root system, the roots became thicker and stouter as

we observed in present study. The cessation of growth of the axillary buds in the bulb by the inactivation of enzymes and auxins could also be responsible for reduction in the growth of the bulbs (Grabowska and Mynett, 1970).

In the present study cv. Prajwal showed more of morphological variations than cv. Phule Rajani. The four variants identified in vM₁ generation viz, tall spike variant, double spike head variant, short spike variant and coloured variant were did not express in the next generation (vM₂). The main reason could be diplontic selection, which is competition between mutated cells and the non mutated neighbor cells (Broerties, 1966). We have observed five putatively stable mutants viz., change in floret shape, increased rachis length, tall spike mutant and variegated leaf mutant in vM₂ generation from the primarily irradiated population. From the above presentation the non stability of the mutants in tuberose can be over come by growing complete plants from single irradiated cell under *in vitro* or by irradiated adventitious bulbs under *in vivo* condition.

6. SUMMARY

The present investigation carried out to study the mutagenic effect of gamma irradiation in tuberose cultivars Prajwal and phule Rajani. The experiment was conducted in the Division of Floriculture and Landscaping, Indian Agricultural Research Institute, New Delhi, during 2008-2010. The main objectives of the study were to find out the radiosensitivity and optimal dose of gamma irradiation for these two commercial cultivars of tuberose with three different bulb stages (B₀- freshly harvested bulb, B₁- three weeks after uprooting and B₂- six weeks after uprooting) and also to find out the mutagenic effect of gamma irradiation on flowering and bulb parameters, stability study of mutants obtained from vM₁ generation. An attempt also was made to find out the cause for non sprouting of the three weeks after uprooted bulbs of cv. Prajwal beyond 2.5 Gy by histological procedure.

The experiment was conducted in a randomized block design, six RBD layout was prepared for each of three bulb stages of both Prajwal and Phule Rajani cultivars. For all these bulb stages of tuberose cultivars effect of acute dose of gamma irradiation (0.00 Gy, 2.5 Gy, 5.0 Gy, 7.5 Gy, 10.0 Gy and 15.0 Gy) was given. Radio-biological effect on per cent sprouting, per cent survival, vegetative parameters, flower parameters and bulb parameters in vM₁ generation was investigated. The mutants obtained in vM₁ generation were planted in the vM₂ generation to notice the stability of characters which were expressed in vM₁ generation. The primary population of the irradiated plants was also maintained to isolate some of the mutants which were seen after second cycle of the propagation of the irradiated bulbs.

Research Area I: Study on sensitivity and optimal level of gamma irradiation dosage for induction of mutation in tuberose cvs. Prajwal and Phule Rajani

The use of gamma irradiation has been shown to affect the per cent sprouting and per cent survival. From the present experiment we found out that,

the higher gamma irradiation doses (7.5 Gy and 10.0 Gy) reduced the sprouting per cent across the bulb stages in both cv. Prajwal and cv. Phule Rajani. The stimulatory effect of gamma radiation (2.5 Gy) observed only in B₀ bulb stage (freshly harvested bulb stage) of cv. Prajwal. From the survival per cent we estimated the LD₅₀ values for all three bulb stages of both the cultivars. It can be concluded that, it is possible to get the useful mutant in tuberose between gamma radiation 7.5.0 Gy to 11.5 Gy treatment. In general 15.0 Gy gamma radiation is the maximum limit for cvs Prajwal and Phule Rajani. From histological study prophase cell stage of only cv. Prajwal i.e. three weeks after uprooting (B₁) is very much sensitive to gamma irradiation. This shows the difference in the cultivar response to gamma irradiation levels.

In vegetative parameters, days to sprouting increased with increase in gamma irradiation, exception observed was freshly harvested bulbs of cv. Prajwal where up to 7.5 Gy days to sprouting was not affected. Drastic reduction in plant height and number of tillers per clump was observed at 10.0 Gy. Eventhough stimulatory effect of (2.5 Gy) gamma irradiation was observed for number of leaves per clump and width of leaves in freshly harvested bulbs of cv. Phule Rajani but the response was non significant. Variation in vegetative parameters was comparably high in freshly harvested bulb (B₀) in both the tuberose cultivars.

From present study we can expect more of variation in flower and bulb parameters from freshly harvested bulb stage and six weeks after uprooted bulb stages of tuberose cultivars Prajwal and Phule Rajani at gamma radiation level between 10.0 Gy to 11.5 Gy.

Reseach Area II: Effect of acute gamma irradiation on flower and bulb parameters and stability study of mutants in tuberose (*Polinathes tuberosa* Linn.)

When the flower and bulb parameters studied in relation with gamma irradiation in tuberose cultivars Prajwal and Phule Rajani using three different bulb stages we observed that, days to spike appearance following gamma irradiation doses showed significant difference only in freshly harvested bulbs

(B₀) of cv. Prajwal. We observed significant reduction in days to opening of first pair of florets at 7.5 Gy and 10.0 Gy because less number of florets per spike across the bulb stage of both the cultivars. Number of florets per spike decreased beyond 2.5 Gy in cv. Prajwal and beyond 5.0 Gy in cv. Phule Rajani across the bulb stages. Flower duration in the field and vase life of cut spike affected severely by 7.5 Gy and 10.0 Gy doses of gamma irradiation in all the bulb stages of tuberose cultivars Prajwal and Phule Rajani. Drastic reduction in number of spikes per plot observed starting from 2.5 Gy in freshly harvested (B₀) and six weeks after uprooted (B₂) bulb stages of cv. Prajwal but in cv. Phule Rajani the gradual reduction was observed. At 10.0 Gy most of the plants remain in the vegetative Phase in both the cultivars across the bulb stages. Up to 7.5 Gy no effect of gamma irradiation on individual floret parameter was observed. In bulb parameters, number of bulbs per plant was affected severely at 10.0 Gy in all the bulb stages of both the cultivars. In general fresh weight of bulbs, length and diameter of bulbs were not affected much by gamma irradiation doses up to 7.5 Gy.

The four variants were isolated during the first generation *viz.*, tall spike variant, double spike head variant, short spike variant and coloured variant were found unstable in vM₂ generation as they revert back to original cultivar form. During second generation we identified some of the attractive mutants which were assumed to be putatively stable because they were from second cycle of propagation of the primarily irradiated bulbs *viz.*, round and elongated petal shape mutants, coloured mutant, high rachis length mutant from the cultivar Prajwal. Variegated leaf mutants and tall spike mutant from cultivar Phule Rajani. In general freshly harvested bulbs comparably showed higher response to gamma irradiation in most of the flower and bulb parameters in both the tuberose cultivars. Most of the mutants isolated in the present study were general from 7.5 Gy and 10.0 Gy doses of gamma irradiation. In future we can able to get solid stable mutants with these attractive characters from *in vitro* mutagenesis method using meristamatic tissues from freshly harvested bulbs of tuberose.

Studies on mutagenic effect of gamma irradiation on tuberose (*Polianthes tuberosa* L.) cultivars Prajwal and Phule Rajani

Abstract

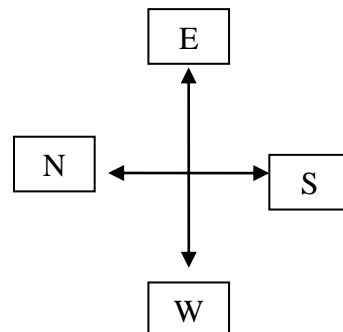
Genetic variability is the prerequisite for any crop to be improved. Mutation breeding is a best method identified to enhance the genetic variability in crop within short period. During mutation breeding it is very important to find out the radiosensitivity of the particular cultivar along with the biological material used to irradiate, in order to find out the optimal radiation dose for the induction of high mutation frequency. Keeping in mind the limited variability present in tuberose the present investigation was undertaken for the improvement in this plant by utilizing two commercial cultivars *viz.*, Prajwal and Phule Rajani. Three different bulb stages *viz.*, freshly harvested bulb (B₀), three weeks after uprooting (B₁) and six weeks after uprooting (B₂) of both the cultivars were irradiated by 0.00Gy (control), 2.5Gy, 5.0Gy, 7.5Gy, 10.0Gy and 15.0 Gy of Cobalt-60 gamma rays. LD₅₀ was between 10.0 to 11.75 Gy for freshly harvested bulbs and six weeks of uprooted bulbs of cultivar Prajwal and for all three bulb stages of cultivar Phule Rajani. The bulbs of three weeks after uprooting found very much sensitive to gamma irradiation, this was supported by histological study, which showed the adverse irradiation effect on cells and nucleus of the irradiated tissues. In some case lower dose was found to be simulative, while higher dose had inhibitory effect on morphological parameters. Various macro mutations were scored for increased and reduced spikes number per plot, reduced leaf number and bulbs per clump in vM₁ population. The variants observed in the vM₁ generation were reverted back in the next generation. But we isolated some of the useful putatively stable mutants from primarily irradiated population after second cycle of bulb propagation *viz.*, round and elongated petal shape mutant, higher rachis length mutant, longer spike length mutant and variegated leaf mutant. Out of three bulb stages tested freshly harvested bulbs (B₀) and gamma irradiation doses 7.5 Gy and 10.0 Gy were found suitable for mutation induction in tuberose cultivars Prajwal and Phule Rajani.

रजनीगंधा (*पॉलिएन्थस ट्यूबरओसा* लिन.) की प्रज्वल और फूले रजनी कृषिजीव जातियों पर
गामा किरणन के उत्परिवर्तजन प्रभावों का अध्ययन

सारांश

किसी भी फसल के सुधार के लिए आनुवंशिक विभिन्नता एक पूर्व-निर्धारित तथ्य है। फसल में अल्पावधि में आनुवंशिक विभिन्नता में सुधार लाने हेतु उत्परिवर्तन प्रजनन एक सर्वश्रेष्ठ चिन्हित उपाय है। उत्परिवर्तन प्रजनन के दौरान विकिरण के लिए उपयोग की गई जैविक सामग्री के साथ विशिष्ट किस्म की रेडियो संवेदनशीलता का पता लगाना अत्यंत महत्वपूर्ण है ताकि उच्च उत्परिवर्तन आवर्ती के प्रेरण हेतु अनुकूलनतम विकिरण मात्रा का पता लगाया जा सके। रजनीगंधा में मौजूद सीमित विभिन्नता को ध्यान में रखते हुए दो व्यावसायिक कृषि जीवजातियों यथा प्रज्वल और फूले रजनी का उपयोग करते हुए इस पौधे में सुधार के लिए वर्तमान अध्ययन किया गया। दोनों किस्मों के तीन भिन्न शल्ककंद स्थितियों जैसे कि ताजा कटाई किए गए शल्ककंद (Bo), उखाड़े जाने के तीन सप्ताह पश्चात् (B₁) तथा उखाड़े जाने के छः सप्ताह पश्चात् (B₂) वाले शल्ककंद को कोबाल्ट-60 गामा किरणों से 0.00Gy (नियंत्रित), 2.5Gy, 5.0Gy, 7.5Gy, 10.0Gy तथा 15.0Gy द्वारा विकिरण किया गया। प्रज्वल कृषि जीवजातियों के ताजा उखाड़े गए शल्ककंदों एवं उखाड़े जाने के छः सप्ताह पश्चात् वाले शल्ककंदों तथा फूले रजनी कृषि जीवजातियों के सभी तीन स्थितियों वाले शल्ककंदों के लिए घातक मात्रा 50 (LD₅₀) 10.0 से 11.75 Gy के बीच था। उखाड़े जाने के तीन सप्ताह पश्चात् वाली स्थिति के शल्ककंद (B₁) गामा विकिरण के प्रति अत्यधिक संवेदी पाए गए जिसका प्रभाव पूर्व अध्ययन द्वारा किया गया जिससे विकिरण ऊतकों की कोषिकाओं एवं केन्द्रक पर प्रतिकूल विकिरण प्रभाव का पता चला। कुछ मामलों में कम मात्रा भी उत्प्रेरित पाई गई जबकि अधिक मात्रा का आकृतिक प्रांचलों पर निरोधक प्रभाव था। VM₁ जनसंख्या में प्रति क्यारी में कणियों की बढ़ी हुई तथा घटी हुई संख्या, घटी हुई पत्तियों की संख्या तथा प्रति झुरमुट शल्ककंद संख्या के लिए विभिन्न महा-उत्परिवर्तन स्कोर किए गए। VM₁ पीढ़ी में महसूस किए गए उत्परिवर्तन अगली पीढ़ी में विपरीत थे। लेकिन हमने शल्ककंद प्रवर्धन के दूसरे चक्र के पश्चात् मुख्यतः विकिरण जनसंख्या से कुछ उपयोगी स्थिर उत्परिवर्ती पृथक किए जैसे कि गोलाकार एवं दीर्घकृत पंखुड़ी आकार उत्परिवर्ती, कहीं अधिक पिच्छाक की लम्बाई उत्परिवर्ती, कहीं बड़े अधिकतर कणियों की लंबाई वाले उत्परिवर्ती तथा रंग-बिरंगी पत्तियों वाले उत्परिवर्ती। जांच की गई तीन शल्ककंद स्थितियों में से ताजा कटाई किए गए शल्ककंद (Bo) व गामा विकिरण मात्रा 7.5Gy एवं 10.0Gy रजनीगंधा की प्रज्वल व फूले रजनी कृषि जीवजातियों में उत्परिवर्तन प्रेरण के लिए उपयुक्त पाए गए।

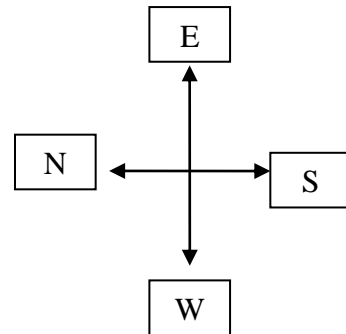
B0G2P	B0G4P	B0G1P
B0G4P	B0G5P	B0G0P
B0G1P	B0G0P	B0G5P
B0G0P	B0G1P	B0G4P
B0G5P	B0G2P	B0G3P
B0G3P	B0G3P	B0G2P



B1G5P	B1G1P	B1G3P	B1G0P	B1G2P	B1G4P
B1G4P	B1G5P	B1G0P	B1G3P	B1G2P	B1G1P
B1G4P	B1G0P	B1G2P	B1G1P	B1G5P	B1G3P
B2G5P	B2G3P	B2G1P	B2G2P	B2G4P	B2G0P
B2G3P	B2G2P	B2G5P	B2G0P	B2G1P	B2G4P
B2G4P	B2G1P	B2G0P	B2G5P	B2G2P	B2G3P

Figure1. Field Layout of tuberose cultivar Prajwal

B0G4PR	B0G5PR	B0G0PR
B0G1PR	B0G1PR	B0G1PR
B0G3PR	B0G3PR	B0G3PR
B0G5PR	B0G0PR	B0G5PR
B0G2PR	B0G4PR	B0G2PR
B0G0PR	B0G2PR	B0G4PR



B1G4PR	B1G1PR	B1G0PR	B1G2P	B1G5PR	B1G3PR
B1G3PR	B1G2PR	B1G1PR	B1G5PR	B1G0PR	B1G4PR
B1G3PR	B1G0PR	B1G5PR	B1G2PR	B1G4PR	B1G1PR
B2G0PR	B2G5PR	B2G4PR	B2G2PR	B2G3PR	B2G1PR
B2G2PR	B2G1PR	B2G5PR	B2G0PR	B2G3PR	B2G4PR
B2G3PR	B2G4PR	B2G0PR	B2G2PR	B2G5PR	B2G1PR

Figure 2. Field Layout of tuberose cultivar Phule Rajani

Table 1. Treatment combinations used under mutagenic study of tuberose cvs. Prajwal and Phule Rajani

Freshly harvest bulbs	3 weeks after uprooting	6 weeks after uprooting
B0G0P	B1G0P	B2G0P
B0G1P	B1G1P	B2G1P
B0G2P	B1G2P	B2G2P
B0G3P	B1G3P	B2G3P
B0G4P	B1G4P	B2G4P
B0G5P	B1G5P	B2G5P
B0G0PR	B1G0PR	B2G0PR
B0G1PR	B1G1PR	B2G1PR
B0G2PR	B1G2PR	B2G2PR
B0G3PR	B1G3PR	B2G3PR
B0G4PR	B1G4PR	B2G4PR
B0G5PR	B1G5PR	B2G5PR

9 - APPENDICES

Meteorological data (2008-2010) recorded at IARI, New Delhi

Longitude: 77°12'E; Latitude: 28°40'N

Height above mean sea level: 228.16m

Month	Temperature(°C)		Relative Humidity (%)		Rainfall (Total mm)	sunshine hours hrs/day	Evaporation (mm)
	Max.(°C)	Min.(°C)	Morning	Evening			
2008							
October	32.9	14.1	78	41	0	7.9	6.4
November	28.2	10.6	91	62	0	3.7	3.5
December	21.7	6.1	92	62	0	4	2.6
2009							
January	20.5	5.1	89	38	0	5.2	2.7
February	23.1	10.3	93	65	55.8	5	3.3
March	26.9	13.4	84	59	54.4	7.7	4.3
April	37.3	20.2	67	35	0	8.7	9.8
May	38.2	23.6	66	44	40.4	7.9	10.1
June	37.6	27.2	70	55	83.8	6.6	11.6
July	34.9	26.5	81	68	83.6	5.5	8.5
August	33.6	25.9	86	74	216.8	5.8	7.2
September	33.4	24.1	85	71	72.8	5.4	7.5
October	32.9	14.1	78	41	0	7.9	6.4
November	28.2	10.6	91	62	0	3.7	3.5
December	21.7	6.1	92	62	0	4	2.6
2010							
January	19.5	5.4	89	68	1.8	4.9	2.6
February	22.1	7.2	78	37	0	5.2	3.5
March	32.1	14.3	76	27	0	6.9	6.1

Preparation of TBA (Tertiary Butyl Alcohol) in per cent for Dehydration – TBA series - 2

%	D/W	95% Alcohol	TBA	100% Alcohol
50	50	40	10	-
70	30	50	20	-
85	-	45	55	-
100	-	-	75	25

Preparation of TBA (Tertiary Butyl Alcohol) in per cent for Dehydration – TBA series - 1

%	TBA (ml)	95% Alcohol	Water
10	10	40	50
20	20	50	30
35	35	50	15
55	55	45	-
75	75	25	-
Pure	100	-	-

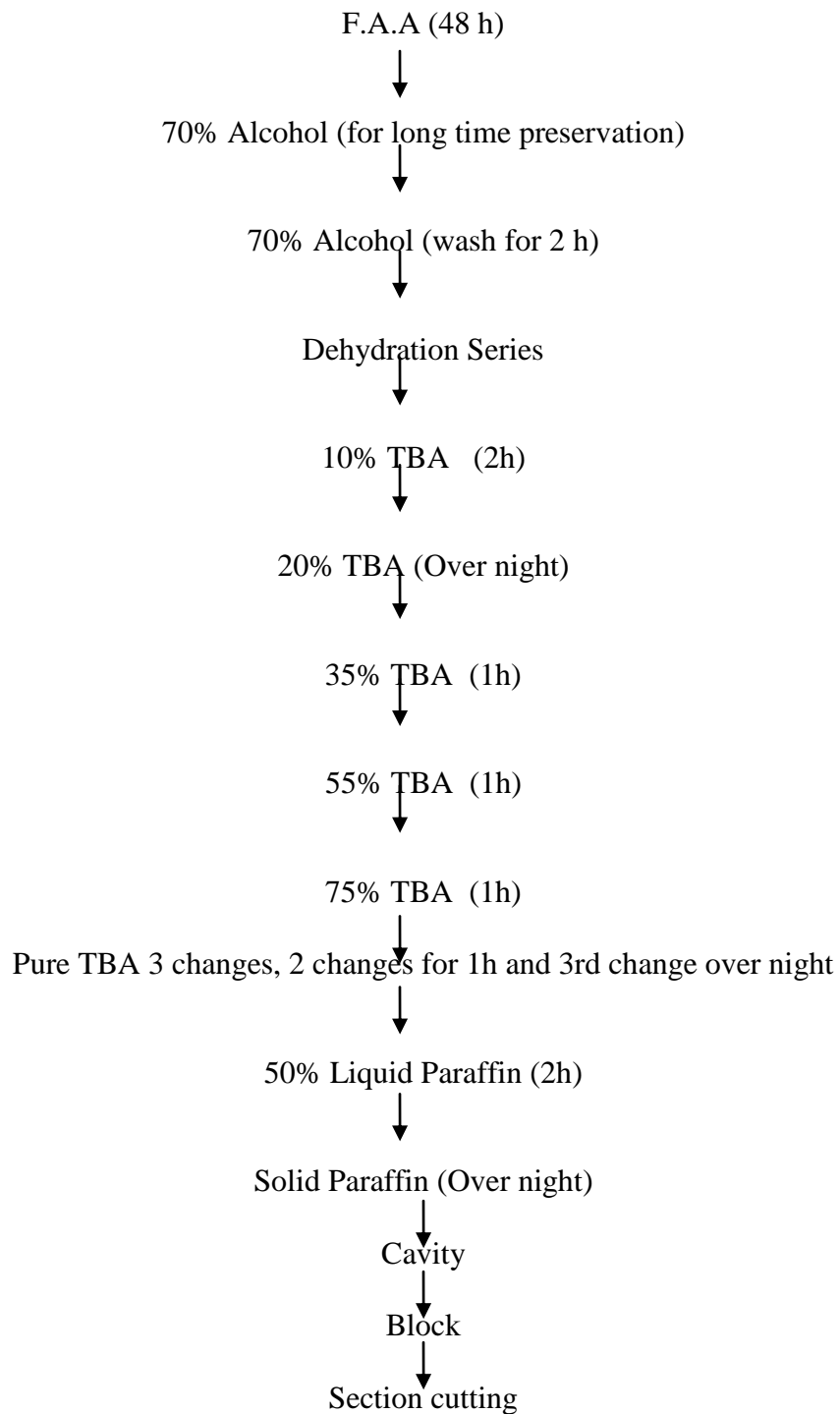
Preparation of Alcohol for staining procedure

%	Rectified Spirit	D/W
30	31.2	68.8
50	52.1	47.9
60	62.5	37.5
70	72.9	27.1
80	83.5	16.7
90	93.5	6.5

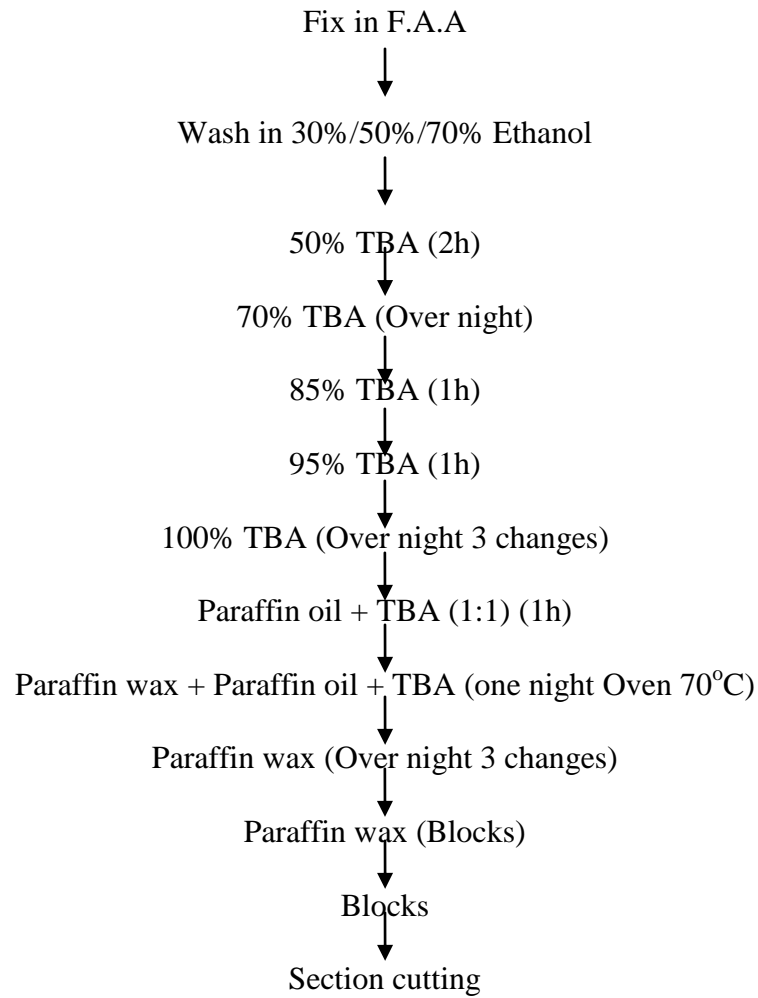
Ingredients for preparation of Haupt's fluid (Adhesive)

Ingredients	Quantity
Distilled water	100 ml
Gelatin	1 g
Phenol	2 g
Glycerin	15 ml

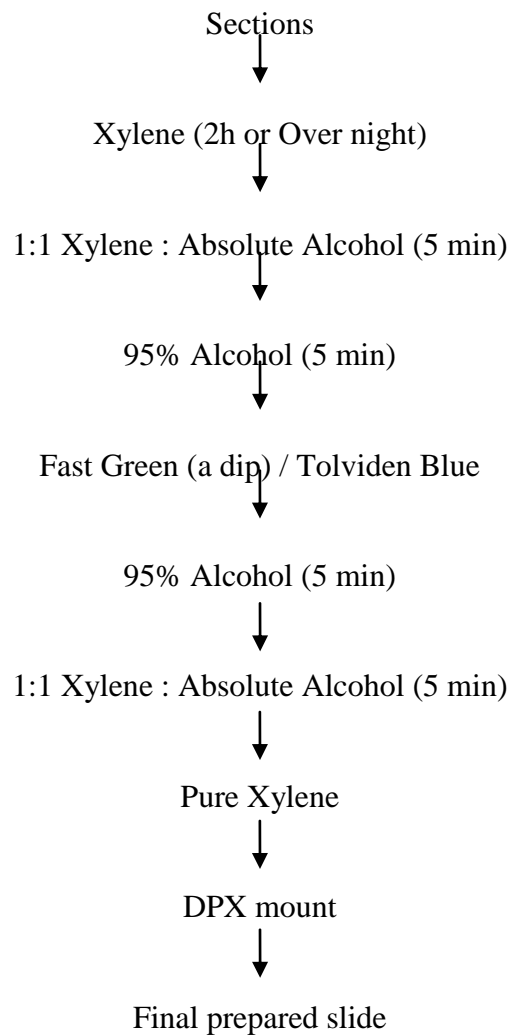
Dehydration – TBA Series 1



Dehydration – TBA Series 2



STAINING TECHNIQUE



Raw data:

Number of bulbs sprouted and survival for percent sprouting and per cent survival

Treatment	Means					
	B ₀ P	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
0	30	36	36	35.333	36	36
2.5	36	22.667	36	36	36	36
5	33	0	36	36	27.333	36
7.5	35	0	29	35.667	21	30.333
10	22	0	19.667	19.667	9.333	24
15	0	0	0	0	0	0

B₀- Freshly harvested bulbs, B₁- Three weeks after uprooting, B₂- Six weeks after uprooting.

(T₁) G₀ = 0 Gy (control), (T₂) G₁ = 2.5 Gy, (T₃) G₂ = 5.0 Gy, (T₄) G₃ = 7.5 Gy (T₅) G₄ = 10.0 Gy

P- Prajwal, PR- Phule Rajani

Days to sprouting of bulbs in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	28	20	15	29	19	17
T1R2	29	21	18	28	19	15
T1R3	29	20	17	27	19	15
T2R1	25	25	20	23	20	23
T2R2	27	26	19	26	20	17
T2R3	28	26	20	30	22	16
T3R1	29	0	20	26	25	22
T3R2	26	0	17	27	22	19
T3R3	27	0	16	27	22	20
T4R1	30	0	23	30	27	22
T4R2	28	0	20	30	30	22
T4R3	30	0	20	30	28	22
T5R1	35	0	24	33	29	25
T5R2	31	0	23	31	29	22
T5R3	32	0	23	30	29	22

Plant height (cm) of cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	75.83	79.8	88.65	72.12	68.36	77.58
T1R2	73.56	81.53	92.13	68.33	72.34	68.49
T1R3	75.06	83.6	93.27	72.92	68.92	70.72
T2R1	71.83	0	79.93	61.86	68.76	73.78
T2R2	70.76	0	80.46	71.67	60.35	75.72
T2R3	70	0	79.22	65.92	60.85	71.58
T3R1	53.77	0	72.2	57.32	59.68	61.95
T3R2	66.06	0	74.56	57.61	60	62.22
T3R3	55.8	0	77.73	64.93	58.22	63.22
T4R1	55.86	0	57.03	59.63	56.22	43.05
T4R2	51.9	0	66.5	50.38	52.42	58.88
T4R3	37.66	0	53.91	48.45	51.55	58.38
T5R1	19.8	0	13.4	16.1	0	11.82
T5R2	7.5	0	6.1	0	20.16	0
T5R3	11.6	0	5.81	18.78	7.38	6.11

Number of tillers per clump in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	8.8	8.2	9.8	10.4	10.2	12.2
T1R2	8.2	8.4	9.3	10	10	12.1
T1R3	8.6	8.5	8.5	10.1	10	12.3
T2R1	7.6	0	8.5	10	9.7	9
T2R2	8.2	0	8.3	10.6	9.2	10.2
T2R3	8	1	7.8	10.6	8.6	11.5
T3R1	6.6	0	6.7	7.4	7.8	6.6
T3R2	7.6	0	8.1	9.8	9.2	5.8
T3R3	7.2	0	5.3	8.7	8.5	7.6
T4R1	5.3	0	7	5.8	3.3	11.6
T4R2	4.7	0	6.2	9	4.8	10.3
T4R3	4.8	0	6.1	7.2	3.1	9.8
T5R1	2.6	0	2.6	3.6	0	5.2
T5R2	3.4	0	3.8	2	1	2.1
T5R3	3.2	0	4.2	2.8	1	2.7

Number of leaves per clump of cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	28.2	28	35.6	14.6	23.8	28.4
T1R2	23	30	28.2	16	26.2	30.2
T1R3	27.8	27.2	36.4	17.6	25.4	32.6
T2R1	15	17.8	34.2	25	18.2	28.2
T2R2	16.2	22.3	36.1	22.8	20.8	26.2
T2R3	15.2	25.8	30.2	17.4	23	28.8
T3R1	14.2	0	29.6	17	17.6	28.4
T3R2	19.4	0	30.2	18.6	17.4	23.6
T3R3	15	0	26.4	16.4	19.8	22.8
T4R1	15.8	0	27.4	17.6	17.2	19.8
T4R2	11.4	0	23.4	15.2	16.2	20
T4R3	12.2	0	25.8	16.6	15.4	20.2
T5R1	11.2	0	15.6	16.6	14	17.1
T5R2	10.4	0	12.8	18.6	13.2	15.8
T5R3	12.4	0	16.8	18	17.1	19.2

Length of leaves (cm) of cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	46.66	52.2	57.08	52.14	53.06	53.08
T1R2	51.62	56.54	60.52	54.5	55.1	57.83
T1R3	54.54	55.68	59.28	56.1	55.18	59.89
T2R1	51.06	18.1	56.74	49.76	43.78	53.96
T2R2	51.96	17.64	57.83	49.36	50.35	52.36
T2R3	51.78	20.2	58.03	48.98	50.36	55.1
T3R1	44.76	0	55.13	36.06	43.01	53.12
T3R2	53.32	0	54.26	39.64	47.13	49.98
T3R3	47.58	0	55.78	40.98	50.23	50.62
T4R1	46.32	0	49.58	42.68	38.68	49.36
T4R2	40.36	0	50.72	38.38	33.48	43.68
T4R3	46.26	0	52.18	39.5	46.15	45.18
T5R1	24	0	3.86	18.88	15	30.16
T5R2	19.8	0	42.08	20.74	20.18	27.56
T5R3	25.5	0	32.63	30.14	22.63	31.58

Width of leaves of cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	2.44	2.86	2.888	2	2.4	2.42
T1R2	2.2	2.94	2.98	2.14	2.32	2.38
T1R3	2.54	2.82	3.1	2.06	2.38	2.38
T2R1	2.4	1.88	2.56	2.38	2.22	2.38
T2R2	2.5	1.4	2.72	2.16	2.22	2.3
T2R3	2.58	1.2	2.66	1.92	2.22	2.28
T3R1	2.26	0	2.46	2.02	1.92	2.26
T3R2	2.34	0	2.54	1.98	2.2	2.22
T3R3	2.28	0	2.5	1.88	2.18	2.2
T4R1	2.12	0	2.2	1.82	1.82	2.1
T4R2	2.34	0	2.4	1.88	1.76	1.9
T4R3	2.26	0	2.46	1.64	1.56	1.82
T5R1	1.6	0	1.96	1.6	1.42	1.5
T5R2	1.62	0	2.12	1.54	1.5	1.6
T5R3	1.92	0	2	1.54	1.41	1.48

Days to spike appearance in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	110.33	118.44	101	108.33	126.66	99
T1R2	121.67	124.67	106.6	113.66	131.33	101.33
T1R3	123.33	126.33	103	120	128.66	105
T2R1	125	0	107	113.66	127.66	106.33
T2R2	134.66	0	106	122.33	128.66	108
T2R3	127.33	0	102	125.66	142	109
T3R1	128.33	0	101	130.66	133.33	106.33
T3R2	132.66	0	100	126.66	128	109.33
T3R3	139.33	0	105	141.66	137.33	105
T4R1	121.33	0	98	135.66	132	107.66
T4R2	98	0	106	130.3	126.33	103
T4R3	131	0	103.67	134.33	136.33	101
T5R1	128.33	0	110.33	132.33	0	105.33
T5R2	129.33	0	111.33	0	131	0
T5R3	131	0	110.67	134	131.33	106

Days to flowering in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	13.3	16.2	14.66	18.8	13.2	15.4
T1R2	15.6	12.4	16	13.6	15.4	18.8
T1R3	12.6	13	16.66	15.6	14.6	18.4
T2R1	11.6	0	12.2	16.6	13.4	16.8
T2R2	10.2	0	15.33	18.6	12.8	16.4
T2R3	8	0	14.33	15	14.8	16
T3R1	6	0	10.9	15.8	13.2	16.2
T3R2	6	0	7	15	12.6	16.4
T3R3	8	0	7.6	16	13.8	14.4
T4R1	3.6	0	8.6	11.4	8.6	13.4
T4R2	4	0	8	9.6	10.6	10.3
T4R3	4.3	0	12.2	9.8	8.8	9.6
T5R1	2	0	3.2	1	0	3.6
T5R2	1	0	1	0	2	0
T5R3	2	0	1	2	3.4	2

Number of florets/spike in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	33.3	36.2	34.66	44.8	33.2	39.4
T1R2	35.3	32.4	38	33.6	39.4	43.8
T1R3	32.6	33	39.66	35.6	38.6	44.4
T2R1	31.6	0	32.2	41.6	33.4	40.8
T2R2	30.3	0	35.33	44.6	32.8	39.6
T2R3	28.6	0	34.33	37	37.4	39.8
T3R1	26	0	29.8	35.8	36.2	37.4
T3R2	24	0	27	37	33.6	38.2
T3R3	28.6	0	27.6	41	35	38.6
T4R1	12.6	0	21.6	31.4	28.6	35.4
T4R2	18	0	20.2	29.6	30.6	33.3
T4R3	18.3	0	32.2	29.8	28.6	29.6
T5R1	2.2	0	8.2	2	0	8.6
T5R2	0.8	0	1	0	5	0
T5R3	2.4	0	1	3	5.4	3.2

Flowering duration in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	16.65	18.1	17.33	19.4	13.6	16.7
T1R2	17.65	16.2	19	13.8	16.7	18.9
T1R3	16.3	16.5	19.83	15.3	16.3	19.2
T2R1	15.8	0	16.1	17.8	13.7	17.4
T2R2	15.15	0	17.66	19.3	13.4	16.8
T2R3	14.3	0	17.16	16	15.7	16.8
T3R1	13	0	14.9	14.9	15.1	15.7
T3R2	12	0	13.5	15.5	13.8	16.1
T3R3	14.3	0	13.8	17.5	14.5	16.4
T4R1	6.3	0	10.8	13.2	11.3	14.7
T4R2	9	0	10.1	12.8	12.3	13.6
T4R3	9.15	0	16.1	12.9	11.3	11.8
T5R1	1.1	0	4.1	1	0	2.3
T5R2	1	0	1	0	2.5	0
T5R3	1.2	0	1	1	2.4	2.1

Vase life of cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	8.8	9.5	8.5	9.7	6.8	8
T1R2	8.8	8.1	9.5	6.9	8.3	9
T1R3	8.1	8.25	9.9	7.6	8.1	9.5
T2R1	7.9	0	8	8.9	6.8	8.5
T2R2	7.5	0	8.5	9.6	6.7	8
T2R3	7.1	0	8.5	8	7.8	8
T3R1	6.5	0	7	7	7.5	7.5
T3R2	6	0	6.6	7.7	6.5	8
T3R3	7.1	0	6.5	8.5	7	8.3
T4R1	3.1	0	5.4	6.6	5.5	7
T4R2	3	0	5	6.3	6	6.5
T4R3	5.4	0	8	6.3	5.5	5.9
T5R1	0	0	0	0	0	0
T5R2	0	0	0	0	0	0
T5R3	0	0	0	0	0	0

Number of spikes/plot in cvs. Prajwal and phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	13	10	29	7	34	34
T1R2	26	13	27	5	30	36
T1R3	28	10	33	16	25	35
T2R1	8	0	15	25	22	26
T2R2	5	0	12	35	28	35
T2R3	5	0	10	22	27	32
T3R1	8	0	16	21	6	23
T3R2	14	0	7	24	21	24
T3R3	5	0	5	22	6	27
T4R1	9	0	5	16	12	20
T4R2	5	0	8	17	8	13
T4R3	5	0	8	17	12	6
T5R1	2	0	3	1	0	3
T5R2	1	0	1	0	15	0
T5R3	2	0	1	5	5	2

Spike length (cm) in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	72.82	76.6	85.45	69	65.26	74.36
T1R2	70.36	78.43	89.13	65.23	69.23	65.27
T1R3	71.86	80.5	90.27	69.8	65.7	67.5
T2R1	68.83	0	76.93	58.76	65.43	70.56
T2R2	67.36	0	77.36	68.67	57.13	72.5
T2R3	67	0	76.1	62.8	57.73	68.36
T3R1	50.67	0	69	54	56.46	58.73
T3R2	62.96	0	71.36	54.5	56.67	59
T3R3	52.7	0	74.73	61.93	55.1	60
T4R1	52.86	0	53.83	56.43	53	39.83
T4R2	48.6	0	63.5	47.16	49.3	55.66
T4R3	34.46	0	50.7	45.35	48.33	55.16
T5R1	16.7	0	10.2	13	0	8.6
T5R2	4.5	0	3	0	17.16	0
T5R3	8.6	0	2.6	15.67	4.16	2.78

Fresh weight of spike (g) in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	124.38	150.67	150.36	74	95.33	108.56
T1R2	110.08	134.23	178	75.67	87.56	88.33
T1R3	90.78	146.28	156.78	80.6	74.32	85.16
T2R1	89.56	0	100.1	124	86.67	82.13
T2R2	90.2	0	99.59	83	82.52	85.26
T2R3	98.23	0	100	75	79.16	78.63
T3R1	90.1	0	80.48	75.65	70.12	82.33
T3R2	85.85	0	92.67	80.76	70	80.56
T3R3	83.45	0	94.58	73.12	72.86	80.78
T4R1	67.48	0	71.26	63	65.67	50.08
T4R2	57.34	0	59.35	57.54	59.26	59.36
T4R3	78.23	0	50.3	66.65	55.02	60.12
T5R1	8.6	0	18.67	23	0	13.12
T5R2	5.3	0	10	0	35.12	0
T5R3	8.3	0	7.3	32.14	8.06	5.2

Average florets length (cm) in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	5.74	5.11	5.67	6.66	6.67	7.05
T1R2	5.52	5.23	5.74	6.88	6.71	7.27
T1R3	5.5	5.39	5.56	6.67	6.46	7.12
T2R1	5.42	0	5.63	6.94	5.96	6.67
T2R2	4.45	0	5.55	6.52	5.89	6.35
T2R3	5.32	0	5.48	6.87	5.88	6.56
T3R1	5.25	0	5.53	6.99	5.84	6.23
T3R2	5.45	0	5.48	6.62	5.47	6
T3R3	5.3	0	5.5	6.56	4.51	6.12
T4R1	5.28	0	5.31	6.52	6.43	6.1
T4R2	5.28	0	5.38	6.23	6.4	5.83
T4R3	5.32	0	5.42	5.28	5.82	5.8
T5R1	0	0	0	0	0	3.36
T5R2	0	0	0	0	3.56	0
T5R3	0	0	0	0	3.17	0

Average florets diameter (cm) in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	4.46	4.55	4.54	3.52	3.65	3.56
T1R2	4.45	4.5	4.55	3.65	3.67	3.71
T1R3	4.59	4.49	4.54	3.65	3.54	3.66
T2R1	4.5	0	4.4	3.79	3.47	3.59
T2R2	4.43	0	4.49	3.65	3.36	3.39
T2R3	4.51	0	4.47	3.71	3.39	3.57
T3R1	4.36	0	4.5	3.72	3.39	3.49
T3R2	4.35	0	4.48	3.62	3.32	3.38
T3R3	4.33	0	4.48	3.61	2.45	3.45
T4R1	4.26	0	4.36	3.59	3.51	3.45
T4R2	4.38	0	4.4	3.56	3.51	3.39
T4R3	4.28	0	4.32	3.28	3.39	3.37
T5R1	0	0	0	0	0	2.45
T5R2	0	0	0	0	2.15	0
T5R3	0	0	0	0	2.05	0

Average fresh weight of individual floret (g) in cvs. Prajwal an Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	2.3	2.53	2.63	0.74	0.75	0.8
T1R2	3.01	2.6	2.52	0.74	0.74	0.76
T1R3	2.6	2.31	2.38	0.8	0.78	0.76
T2R1	2.63	0	2.46	0.83	0.78	0.76
T2R2	2.52	0	2.51	0.77	0.76	0.73
T2R3	2.46	0	2.49	0.68	0.71	0.75
T3R1	2.41	0	2.42	0.88	0.8	0.81
T3R2	2.42	0	2.35	0.84	0.73	0.77
T3R3	2.35	0	2.41	0.82	0.74	0.73
T4R1	2.48	0	2.33	0.88	0.78	0.8
T4R2	2.5	0	2.15	0.77	0.63	0.71
T4R3	2.15	0	2.3	0.8	0.72	0.71
T5R1	0	0	0	0	0	46
T5R2	0	0	0	0	0.46	0
T5R3	0	0	0	0	0.35	0

Number of bulbs per clump in cvs. Prajwal and phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	13.67	9.67	16	17.33	13	19.67
T1R2	13.33	11.67	15.67	17	17.67	19
T1R3	14.67	13.33	16	15.33	17.67	19
T2R1	12	1.33	15	16.67	14.67	16.33
T2R2	15.67	2	16.33	14.33	14.33	18
T2R3	14	2	14.67	13.33	13.33	18
T3R1	8.33	0	12	13	15	15.33
T3R2	13	0	15	12.67	12.67	15.67
T3R3	11	0	13.67	12.67	15	14
T4R1	6.33	0	15.33	11.67	7.67	14.33
T4R2	8.33	0	15.33	11	4.67	15.33
T4R3	6.33	0	15	12.67	5.67	14
T5R1	4.67	0	4	4.33	1.67	11
T5R2	7	0	4	5	2	5.67
T5R3	4.33	0	4.67	7.33	2	7.33

Fresh weight of bulbs per clump cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	400	315.39	538.36	404	326.14	420.88
T1R2	217	412.09	510.12	300	372.19	376.36
T1R3	584.5	580.14	399.56	369	369.11	398.36
T2R1	320.46	180.58	463.08	270	362.09	378.56
T2R2	363.68	213.8	305.36	359	359.42	348.78
T2R3	358.21	215.54	414.71	347	348.78	392.88
T3R1	245.5	0	273.5	295	356.89	333.33
T3R2	310	0	419.28	287	350.09	332.78
T3R3	186	0	407.86	327	312.14	328.14
T4R1	157	0	415.31	344	262.33	336.56
T4R2	318	0	471.64	416	198.78	386.56
T4R3	267	0	385.72	269.5	200.29	412.11
T5R1	162	0	254.02	95	76.12	328.11
T5R2	176	0	260.13	199	105.35	168.33
T5R3	130	0	310.02	265	98.11	198.89

Average diameter of bulbs in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	27.5	27.33	26.5	28.33	27.56	26.87
T1R2	25.3	30.12	31.03	25.15	23.23	26.95
T1R3	31.85	30.06	33.49	21.09	22.58	24.89
T2R1	24.62	31.08	29.67	24.28	25.8	25.12
T2R2	26.35	32.16	25.73	24.19	23.13	24.44
T2R3	27.78	30.12	29.51	24.09	23.36	26.95
T3R1	22.92	0	25.07	24.31	23.36	23.14
T3R2	25	0	26.74	23.83	23.08	25.08
T3R3	22.18	0	26.45	23.21	23	24.26
T4R1	26.5	0	25.52	23.08	23.58	26.68
T4R2	29.85	0	28.34	26.92	24.96	29.94
T4R3	22.86	0	30.51	26.01	23.18	26.32
T5R1	21.48	0	31.04	20.33	18.13	27.17
T5R2	26.99	0	31.99	24.88	17.88	30.14
T5R3	26.41	0	25.69	24.16	17.32	22.36

Average length of bulbs in cvs. Prajwal and Phule Rajani						
Treatments	BoP	B ₁ P	B ₂ P	B ₀ PR	B ₁ PR	B ₂ PR
T1R1	5.8	5.53	5.03	3.48	3.35	3.6
T1R2	5.98	6.12	6.48	3.28	3.13	3.91
T1R3	5.65	5.62	5.59	3.11	3.27	3.91
T2R1	5.73	3.78	6.17	3.42	3.2	3.13
T2R2	5.88	4.96	3.99	3.24	3.08	3.66
T2R3	6.42	4.89	5.86	3.08	3.14	3.62
T3R1	5.62	0	4.63	3.16	3.16	3.16
T3R2	5.5	0	5.8	3.02	3.08	3.4
T3R3	5.46	0	4.77	3.01	3.1	3.57
T4R1	5.23	0	4.79	3.24	3.21	3.35
T4R2	5.44	0	5.57	3.56	3	3.18
T4R3	5.22	0	5.89	3.12	3	3.44
T5R1	4.76	0	3.97	2.32	2.14	2.86
T5R2	4.25	0	4.29	2.86	2.06	3.1
T5R3	4.3	0	4.41	2.57	2.04	2

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Table 2: Effect of gamma irradiation on vegetative growth parameters in tuberose cvs. Prajwal and Phule Rajani

Treatment	Days to sprouting of bulbs						Plant height (cm)						Number of tillers per clump					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	28.67	20.33	16.67	28.00	19.00	15.67	74.82	81.64	91.35	71.12	69.87	72.26	8.53	8.37	9.20	10.17	10.07	12.20
2.5 Gy	26.67	25.67	19.67	26.33	20.67	18.67	70.86	18.65	79.87	66.48	63.32	73.69	7.93	0.33	8.20	10.40	9.17	10.23
5.0 Gy	27.33	0.00	17.67	26.67	23.00	20.33	58.54	0.00	74.83	59.95	59.30	62.46	7.13	0.00	6.70	8.63	8.50	6.67
7.5 Gy	29.33	0.00	21.00	30.00	28.33	22.00	48.47	0.00	59.15	52.82	53.40	53.44	4.93	0.00	6.43	7.33	3.73	10.57
10.0 Gy	32.67	0.00	23.33	31.33	29.00	23.00	12.97	0.00	8.44	11.63	9.18	5.98	3.07	0.00	3.53	2.80	0.67	3.33
Mean	28.93	9.20	19.67	28.47	24.00	19.93	53.13	20.05	62.73	52.40	51.01	53.57	6.32	1.74	6.81	7.87	6.43	8.60
C.V.	5.07	8.99	7.31	6.92	5.38	7.07	11.04	5.21	6.65	11.98	10.76	11.12	9.43	14.61	12.04	12.61	9.08	12.99
S.E.	0.85	0.20	0.83	1.14	0.75	0.81	3.39	0.49	2.41	3.62	3.17	3.44	0.23	0.15	0.47	0.57	0.34	0.64
C.D. 5%	2.76	0.64	2.71	NS	2.43	2.65	11.04	1.60	7.86	11.82	10.34	11.22	0.75	0.48	1.54	1.87	1.10	2.10

N.S. = Non significant

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 3: Effect of gamma irradiation on leaf parameters in tuberose cvs. Prajwal and Phule Rajani

Treatment	Number of leaves/ clump						Length of leaves (cm)						Width of leaves (cm)					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	26.33	28.40	33.40	16.07	25.13	30.40	50.94	54.81	58.96	54.25	54.45	56.93	2.39	2.87	2.99	2.07	2.37	2.39
2.5 Gy	15.47	21.97	33.50	21.73	20.67	27.73	51.60	18.65	57.53	49.37	48.16	53.81	2.49	1.49	2.65	2.15	2.22	2.32
5.0 Gy	16.20	0.00	28.73	17.33	18.27	24.93	48.55	0.00	55.06	38.89	46.79	51.24	2.29	0.00	2.50	1.96	2.10	2.23
7.5 Gy	13.13	0.00	25.53	16.47	16.27	20.00	44.31	0.00	50.83	40.19	39.44	46.07	2.24	0.00	2.35	1.78	1.71	1.94
10.0 Gy	11.33	0.00	15.07	17.73	14.77	17.37	23.10	0.00	26.19	23.25	19.27	29.77	1.71	0.00	2.03	1.56	1.44	1.53
Mean	16.49	10.07	27.25	17.87	19.02	24.09	43.70	14.69	49.71	41.19	41.62	47.56	2.23	0.87	2.50	1.90	1.97	2.08
C.V.	14.33	19.23	10.86	12.41	7.86	8.06	7.84	7.99	7.32	7.41	7.22	5.06	5.19	8.09	2.42	5.55	5.29	3.10
S.E.	1.36	1.12	1.71	1.28	0.86	1.12	1.98	0.68	0.97	1.76	1.73	1.39	0.07	0.09	0.04	0.06	0.06	0.04
C.D. 5%	4.45	3.65	5.57	NS	2.82	3.66	6.45	2.21	16.21	5.75	5.65	4.53	0.22	0.30	0.11	0.20	0.20	0.12

N.S. = Non significant

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 4. Effect of gamma irradiation on days to spike appearance and days to flowering in tuberose cvs. Prajwal and Phule Rajani

Treatment	Days to spike appearance						Days to flowering					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	118.44	123.15	103.53	114.00	128.88	101.78	13.83	13.87	15.77	16.00	14.40	17.53
2.5 Gy	116.78	0.00	105.00	120.55	132.77	107.78	9.93	0.00	13.95	16.73	13.67	16.40
5 Gy	129.00	0.00	102.00	132.99	132.89	106.89	6.67	0.00	8.50	15.60	13.20	15.67
7.5 Gy	129.55	0.00	102.56	133.43	131.55	103.89	3.97	0.00	9.60	10.27	9.33	11.10
10 Gy	133.44	0.00	110.78	88.78	87.44	70.44	1.67	0.00	1.73	1.00	1.80	1.87
Mean	125.44	24.63	104.77	117.95	122.71	98.15	7.21	2.77	9.91	11.92	10.48	12.51
C.V.	6.94	7.55	6.67	8.71	7.28	8.05	6.78	2.94	6.73	9.76	9.17	9.16
S.E.	3.03	1.07	1.61	3.55	3.33	3.90	0.78	0.53	1.07	0.91	0.59	0.95
C.D. 5%	5.26	3.50	NS	NS	NS	NS	2.55	1.72	3.50	2.96	1.93	3.10

N.S. = Non significant

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 5. Effect of gamma irradiation on number of florets per spike, flowering duration and vase life in tuberose cvs. Prajwal and Phule Rajani

Treatment	Number of florets per spike						Flowering duration (day)						Vase life (day)					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	33.73	33.87	37.44	38.00	37.07	42.53	16.87	16.93	18.72	16.17	15.53	18.27	8.57	8.62	9.30	8.07	7.73	8.83
2.5 Gy	30.17	0.00	33.95	41.07	34.53	40.07	15.08	0.00	16.97	17.70	14.27	17.00	7.50	0.00	8.33	8.83	7.10	8.17
5.0 Gy	26.20	0.00	28.13	37.93	34.93	38.07	13.10	0.00	14.07	15.97	14.47	16.07	6.53	0.00	6.70	7.73	7.00	7.93
7.5 Gy	16.30	0.00	24.67	30.27	29.27	32.77	8.15	0.00	12.33	12.97	11.63	13.37	3.83	0.00	6.13	6.40	5.67	6.47
10.0 Gy	1.80	0.00	3.40	1.67	3.47	3.93	1.10	0.00	2.03	0.67	1.63	1.47	0.00	0.00	0.00	0.00	0.00	0.00
Mean	21.64	6.77	25.52	29.79	27.85	31.47	10.86	3.39	12.83	12.69	11.51	13.23	5.29	1.72	6.09	6.21	5.50	6.28
C.V.	10.19	13.49	15.50	12.36	7.98	9.13	9.92	13.49	10.82	13.69	9.57	9.08	13.96	19.95	12.75	14.31	9.98	8.38
S.E.	1.27	0.53	2.28	2.13	1.28	1.66	0.62	0.26	1.10	1.00	0.64	0.69	0.43	0.20	0.45	0.51	0.32	0.30
C.D. 5%	4.15	1.72	7.45	6.93	4.19	5.41	2.03	0.86	2.88	3.27	2.07	2.26	1.39	0.65	1.46	1.67	1.03	0.99

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 6. Effect of gamma irradiation on spike parameters in tuberose cvs. Prajwal and Phule Rajani

Treatment	Number of spikes per plot						Spike length (cm)						Fresh weight of spikes (g)					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	22.33	11.00	29.67	9.33	29.67	35.00	71.68	78.51	88.28	68.01	66.73	69.04	108.41	143.73	161.71	76.76	85.74	94.02
2.5 Gy	6.00	0.00	12.33	27.33	25.67	31.00	67.73	0.00	76.80	63.41	60.10	70.47	92.66	0.00	99.90	94.00	82.78	82.01
5.0 Gy	9.00	0.00	9.33	22.33	11.00	24.67	55.44	0.00	71.70	56.81	56.08	59.24	86.47	0.00	89.24	76.51	70.99	81.22
7.5 Gy	6.33	0.00	7.00	16.67	10.67	13.00	45.31	0.00	56.01	49.65	50.21	50.22	67.68	0.00	60.30	62.40	59.98	56.52
10.0 Gy	1.67	0.00	1.67	2.00	6.67	1.67	9.93	0.00	5.27	9.56	7.11	3.79	7.40	0.00	11.99	18.38	14.39	6.11
Mean	9.07	2.20	12.00	15.53	16.73	21.07	50.02	15.70	59.61	49.49	48.04	50.55	72.53	28.75	84.63	65.61	62.78	63.97
C.V.	52.30	35.21	27.81	29.05	22.58	20.66	11.74	5.56	7.11	11.70	10.20	11.16	13.45	13.24	11.30	21.78	15.79	11.25
S.E.	2.74	0.45	1.93	2.61	2.15	2.51	3.39	0.50	2.45	3.34	2.83	3.26	3.63	2.20	3.52	3.25	3.72	3.16
C.D. 5%	8.93	1.46	6.28	8.50	10.26	8.20	11.06	1.64	7.98	10.90	9.23	10.62	18.36	7.17	18.01	26.90	18.67	13.55

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 7. Effect of gamma irradiation on average individual floret parameters in tuberose cvs. Prajwal and Phule Rajani

Treatment	Average floret length (cm)						Average floret diameter (cm)						Average fresh weight of individual floret (g)					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	5.59	5.24	5.66	6.74	6.61	7.15	4.50	4.51	4.54	3.61	3.62	3.64	2.64	2.48	2.51	0.76	0.76	0.77
2.5 Gy	5.33	0.00	5.55	6.78	5.91	6.53	4.48	0.00	4.45	3.72	3.41	3.52	2.54	0.00	2.49	0.76	0.75	0.75
5.0 Gy	5.29	0.00	5.50	6.72	5.27	6.12	4.35	0.00	4.49	3.65	3.05	3.44	2.39	0.00	2.39	0.85	0.76	0.77
7.5 Gy	5.06	0.00	5.37	6.01	6.22	5.91	4.31	0.00	4.36	3.48	3.47	3.40	2.38	0.00	2.26	0.82	0.71	0.74
10.0 Gy	0.00	0.00	0.00	0.00	2.24	1.12	0.00	0.00	0.00	0.00	1.40	0.82	0.00	0.00	0.00	0.00	0.27	0.33
Mean	4.26	1.05	4.42	5.25	5.25	5.36	3.53	0.90	3.57	2.89	2.99	2.96	1.99	0.50	1.93	0.64	0.65	0.67
C.V.	6.00	5.99	1.40	5.96	8.82	5.70	1.52	1.59	0.78	3.31	2.88	2.19	6.16	3.64	3.80	6.94	6.48	6.02
S.E.	0.15	0.04	0.04	0.18	0.57	0.49	0.03	0.01	0.02	0.06	0.36	0.36	0.11	0.04	0.04	0.03	0.07	0.08
C.D. 5%	0.48	0.12	0.12	0.59	1.86	1.59	0.10	0.03	0.05	0.18	1.18	1.18	0.34	0.19	0.14	0.08	0.24	NS

N.S. = Non significant

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 8. Effect of gamma irradiation on number of bulbs and fresh weight of bulbs per clump in tuberose cvs. Prajwal and Phule Rajani

Treatment	Number of bulbs/clump						Fresh weight of bulbs/clump					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	13.89	11.56	15.89	16.55	16.11	19.22	400.50	435.87	482.68	357.67	355.81	398.53
2.5 Gy	13.89	1.78	15.33	14.78	14.11	17.44	347.45	203.31	394.38	325.33	356.76	373.41
5.0 Gy	10.78	0.00	13.56	12.78	14.22	15.00	247.17	0.00	366.88	303.00	339.71	331.42
7.5 Gy	7.00	0.00	15.22	11.78	6.00	14.55	247.33	0.00	424.22	343.17	220.47	378.41
10.0 Gy	5.33	0.00	4.22	5.55	1.89	8.00	156.00	0.00	274.72	186.33	93.19	231.78
Mean	10.18	2.67	12.84	12.29	10.47	14.84	279.69	127.84	388.58	303.10	273.19	342.71
C.V.	11.15	9.96	6.09	10.66	6.30	9.99	36.88	45.98	18.74	21.22	9.38	12.99
S.E.	0.66	0.46	0.45	0.76	0.99	0.86	59.56	33.93	42.04	37.13	14.80	25.71
C.D. 5%	2.14	1.50	1.47	2.47	3.21	2.79	NS	110.66	NS	NS	48.27	83.85

N.S.= Non significant

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting

Table 9. Effect of gamma irradiation on average bulb diameter and length in tuberose cvs. Prajwal and Phule Rajani

Treatment	Average diameter of bulb (cm)						Average length of bulb (cm)					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂	B ₀	B ₁	B ₂
Control	28.22	29.17	30.34	24.86	24.46	26.24	5.81	5.76	5.70	3.29	3.25	3.81
2.5 Gy	26.25	31.12	28.30	24.19	24.10	25.50	6.01	4.54	5.34	3.25	3.14	3.47
5.0 Gy	23.37	0.00	26.09	23.78	23.15	24.16	5.53	0.00	5.07	3.06	3.11	3.38
7.5 Gy	26.40	0.00	28.12	25.34	23.91	27.65	5.30	0.00	5.42	3.31	3.07	3.32
10.0 Gy	24.96	0.00	29.57	23.12	17.78	21.18	4.44	0.00	4.22	2.58	2.08	2.65
Mean	25.84	12.06	28.49	24.26	22.68	24.94	5.42	3.34	5.15	3.10	2.93	3.33
C.V.	10.80	6.98	10.05	9.51	5.57	8.99	4.71	9.18	15.34	5.94	1.57	10.04
S.E.	1.61	0.49	1.65	1.33	0.73	1.45	0.15	0.18	0.46	0.11	0.03	0.19
C.D. 5%	NS	1.58	NS	NS	2.38	NS	0.48	0.58	NS	0.35	0.09	0.63

N.S. = Non significant

B₀- Freshly harvested bulbs

B₁- Three weeks after uprooting

B₂- Six weeks after uprooting