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Induced mutation breeding in tuberose (*Polianthes tuberosa*) – a review

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Abstract

Tuberose (*Polianthes tuberosa Linn.*) is major bulb crop growing in both tropical and subtropical areas. The popularity of tuberose is due to as it occupies prime position in cut and loose flower, essential oils extraction and landscaping. The main problem in conventional tuberose breeding is lack of genetic variability, self-incompatibility and seed sterility. To achieve the rapid evaluation, induced mutation was opted. It is one of important pathway to find variability in vegetatively propagated species. So far 3,300 officially released mutants available in 170 different species from 60 countries. Mutation induction in tuberose was carried out by physical mutants like X-rays, Gamma Rays and chemical mutagens like EMS and DES. Till now two induced mutants namely, Rajat Rekha (Silver strip) and Swarna Rekha (Golden Strip) with leaf variation have been developed at CSIR-National Botanical Research Institute, Lucknow (Uttar Pradesh). More mutation works was also carried out through induced mutation. In future still there is wide scope opened for induction of different mutants like colour variation, change in petal shape, altered flower arrangement in spike, long and short spike mutants in tuberose.

Key words: Chemical mutagen, Dimethyl sulphate, Ethyl methane sulphonate, Fast neutron, Gamma rays, Induced mutation, Physical mutagen.

Commercially floriculture is a fast-emerging major venture in the world, growing at the rate of 10-15 per cent (Datta, 2019). Among different flower crops, ornamental bulbs are one of the most beautiful and variable group of geophytes, have been appreciated from ancient times. Tuberose (*Polianthes tuberosa Linn.*) is most important bulbous perennial flowering plant, belonging to monocots. It occupies second position in area and production after gladiolus in India. It is found to be originated at Mexico, it was placed under the family Agavaceae.

The most extensive contributions to new species of *Polianthes* were made by Some other important species of *Polianthes* 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.

Tuberose (*Polianthes* tuberose 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 (Singh *et al.* 2021). 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 landscape value (Jyothi and Singh, 2016).

In India, tuberose is being commercially cultivated over 1435 ha area and the main growing states are Uttar Pradesh, Uttarakhand, Haryana, Telangana, West Bengal, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh (NHB, 2018-19). The yield

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maximization and sustainable production was mainly dependent on integrated nutrient management (Bohra and Nautiyal 2019). There are about 20 cultivars available in India, 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. All the variegated cultivars are merely 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 and green tinge, the fully opened flowers are white in colour in all the cultivars. The genetic variability in tuberose is very limited and it has narrow genetic base.

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. Tuberose is highly heterozygous and hence variability can be created (Jyothi and Singh, 2015). In India, since not many improved cultivars have been developed 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 (Jyothi and Singh, 2016).

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 (Datta, 2017).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. 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 and to enhance natural genetic resource. 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. 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. Physical mutagents 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 and is still showing a higher potential for improving vegetatively propagated plants.

Hernandez-Munoz *et al.* (2019) reported that mutagenesis is an important tool in the generation of ornamental plant varieties. By 2017 more than 700 varieties have been registered in the mutant variety database jointly administered by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency. Among the main genera reported are *Chrysanthem, Rosa, Dahlia* and *Alstromeria*, with 283, 67, 35 and 35 registered varieties, respectively. From *Dahlia*, 18 varieties have been registered and a large number of mutant forms have been generated in Asia and Europe, while from *Polianthes* mutant forms have been developed in Iran and India and from *Helianthus* 569 mutant forms have been developed in Bulgaria. These not only increase biodiversity but also provide breeding material for conventional plant breeding, these mutant forms also have ornamental attributes that meet the quality standards demanded in the international market. Preliminary study on mutagenic effect of gamma radiation and chemical mutagens on tuberose was done.

Induced mutation

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 CSIR-National Botanical Research Institute, Lucknow (Uttar Pradesh) with variegation in leaves was released. In tuberose so far there is no other flower/foliage variation has been observed.

Mutagenic agents

Yadav *et al.* (2022) stated that the agents used for induction of mutation are known as mutagens. The mutation type comprises of physical mutagents (X-rays, gamma rays, alpha particles and ultraviolet radiations) and chemical mutagents or DNA reactive chemicals (Nitrous acid, dimethyl sulphate (DMS), ethyl nitrosourea (ENU), methyl nitrosourea (MNU), ethyl methane sulfonate (EMS), ethyl ethane sulphonate (EES), base analogs (5-bromourcail and 5-chlorourcail), intercalating agents (acridine orange, ethidium bromide and proflavine) metals (arsenic, cadmium, chromium, nickel and biological agents (virus, transposonand bacteria.

Physical mutagens

Many mutant varieties have been created using X-rays and gamma rays. Gamma radiation in particular has been popular; approximately half of all mutant varieties registered in the FAO/IAEA mutant variety database were created using gamma rays. X-rays only account for about 17% of the registered varieties and chemical mutagenesis has been used for about 10% International Atomic Energy Agency (2021).

Mutation induced by fast neutrons and X-rays

Tuberose cvs Mexican Single and Pearl Double bulbs (small and large size) with 142, 284, 426, 568 and 710 rads fast neutron dose. They reported that Mexican Single cultivar flowered in about 10 weeks and bulb size difference did not delay significantly the flowering (Kaintura and Srivastava 2015 and Kaintura *et al.*, 2016). Studied the effect of X-rays treatment on vegetative and floral chracters of different cultivars of tuberose and isolation of promising mutants was done. The experimental material comprised of four tuberose cultivars, *viz.* Kalyani Single, Kalyani Double, Arka Suvasini and Arka Prajwal with two doses of X-rays (0.6 kr and 1.2 kr) along with untreated sample (control). The results indicated that higher dose was detrimental for vegetative and floral parameters. Six mutants were obtained exhibiting variation in plant height (cv. Arka Prajwal) increase in number of petals per floret (cv. Arka Prajwal) and presence of stamen in Arka Suvasini with bulb treatment with 1.2 kr X-rays.

Mutation induced by gamma irradiation

This type of ray is one of the most important mutagenic agents within ionizing radiation because they have been shown to be highly penetrating and potent in inducing variability in plants (Datta, 2017). Gamma rays are emitted in the disintegration process of the radioisotopes of carbon-14, cobalt-60, caesium-137 and to a lesser extent plutonium-239. Irradiation may be acute (short periods) or cronic (long periods). The efficiency of this radiation is because it has an energy level ranging from 10 keV to several hundred keV, which gives it greater penetration power than alpha and beta rays.

Two gamma rays induced variegated leaf mutants were developed and released under the name Rajat Rekha and Swaran Rekha. Rajat Rekha a single flowered tuberose mutant with white streaks along the middle of the blade, while Swaran Rekha a Double flowered mutant of tuberose with golden yellow streaks along the margins of blade. Tuberose cv. Mexican Single bulbs were irradiatd with 0.5 Kr, 1.5 kr, 2.5 kr and 3.5 kr with gamma rays and planted in beds. The gamma dose of 0.5 kr gave one desirable mutant with bolder flowers compared to the control and plants from bulbs treated with other doses.

The higher doses like 2.5 Kr and 3.5 Kr proved to be more or less lethal for sprouting of bulbs. Irradiated bulbs with acute dose of gamma rays survived upto 2.5 kr. High sensitivity to 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 Mexican Single and Pearl Double cultivars of tuberose 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 variation in leaves were detected in treatment population. It was reported that morphological variants like chlorophyll mutants, non-flowering mutants and compact spike mutants were observed in tuberose cvs Mexican Single, Pearl Double, Arka Srinagar and Arka Suvasini treated with gamma rays (5.0 - 2.5 gy). The Rajat Rekha, mutant of Mexican Single tuberose gave maximum leaf length, leaf width, number of spikes per plant and number of florets per spike as compared to its control. While Swaran Rekha which is mutant of Pearl Double tuberose showed maximum plant height, number of florets per spike, floret length as well as length of spikes.

Majumdar *et al.* (2013) exposed tuberose cvs. Arka Prajwal and Arka Vaibhav bulbs to different doses of gamma radiation (2.5, 5.0, 7.5, 10.0 and 12.5 Gy) for induction of genetic variability. Bulb sprouting in cv. Arka Vaibhav was stimulated with lower dose (2.5 Gy) of irradiation. The number of leaf and its length, spike length and number of flowers reduced whereas, days to spike initiation increased with the increment of radiation doses in both the cultivars. The LD_{50} was found in higher dose (12.5 Gy) in both of them. Different types of morphological abnormalities like changes in shape, size, margin, apex fission and fusion of leaves and chlorophyll variegation in leaves were detected in the population. Frequency of morphological abnormalities and chromosomal abnormalities like bridge, fragments, early separation and clumbing increased with higher doses.

Pohare *et al.* (2013) carried out induction of genetic variability in in vitro regenerated *Polianthes tuberosa* in Local cultivar, with five different doses of gamma radiations (10, 20, 30, 40 and 50 Gy). Non-significant differences were observed in survival rates among the untreated (control) and treated plants with different doses of gamma irradiation. But significant differences were recorded in plant morphological characters upon gamma radiation treatments of 30 Gy in terms of plant height, number of leaves, leaf size and colour as compared to the control plant. Kainthura and Srivastava (2015) and Kainthura *et al.* 2016 treated four tuberose cvs. *viz.*, Kalyani Single, Kalyani Double, Arka Suvasini and Arka Prajwal with gamma rays (0.5 kr and 1.5 kr). The results indicated that mutagenic treatments at lower doses has significant simulative effect on some parameters i.e. sprouting percentage, days taken to sprouting whereas most of the parameters showed decrease from desired parameters i.e. survival rate, leaf length, number of spikes per plant, number of florets per spike, flowering duration and vase life. Higher dose was detrimental for vegetative and floral parameters.

Jyothi and Singh (2015) conducted a field trial in tuberose cvs Arka Prajwal and Phule Rajani with a view to evaluate the sensitivity, proper bulb stage and optimal gamma irradiation dose to induce mutant. Tuberose cvs Arka Prajwal and Phule Rajani with three different bulb stages, *viz.*, freshly harvested bulb, three weeks after uprooting and six weeks after uprooting were irradiated by 2.5 Gy, 5.0 Gy, 7.5 Gy, 10.0 Gy and 15.0 Gy of gamma rays and control untreated. Probable LD_{50} dose of gamma irradiation was between 10.0 Gy to 12.0 Gy for freshly harvested bulbs and bulbs after six weeks after uprooting in cv. Arka Prajwal and in all the bulb stags of cv. Phule Rajani. For bulbs after three weeks of uprooting probable LD_{50} dose was 3.25 Gy in cv. Arka Prajwal and 10.25 Gy in cv. Phule Rajani. Non sprouting of Arka Prajwal bulbs at three weeks after uprooting beyond 2.5 Gy was supported by histological results. All three stages of bulbs have showed different response over gamma irradiation. In general, sprouting and all vegetative parameters (plant height, number of tillers per clump, number of leaves per clump, length of leaves and width of leaves) decreased over increased irradiation level. Freshly harvested bulbs responded comparably high to the gamma irradiation doses followed by bulbs after six weeks of uprooting in both cultivars. It was concluded that gamma irradiation dose 7.5 Gy to 11.5 Gy could yield attractive and useful mutants in tuberose.

Furthermore, Jyothi and Singh (2017) with above experiment (Jyothi and Singh, 2015) assessed the effect of flower and bulb parameters in M_1 generation. They reported that with increase in doses of gamma irradiation, gradual reduction in number of spikes, flower number, number of bulb, weight of bulb and bulb diameter was observed. In some cases lower dose was found 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 M_1 population.

The mutants of VM_1 generation not found stable in VM_2 generation, but some more mutants from the maintained gamma irradiation population of VM_1 generation related to change in flower shape. In general, freshly harvested bulbs responded more to gamma irradiation. From this study, it has found that gamma irradiation level 7.5 Gy and 10.0 Gy were found optimal for mutation in cvs Arka Prajwal and Phule Rajani. Furthermore, based on experiment conducted by Jyothi and Singh (2015), Jyothi *et al.* (2019) reported that gamma radiation affected the sprouting in cv. Arka Prajwal where above 2.5 Gy there was no bulb sprouting observed.

This was supported by the histological study were cells become bigger in size, deformed shape of cell, complete damage of outer epidermal layer and cells with more vacuolation was observed. New mutants were isolated from VM_1 generation *viz.*, tall flowering mutant, dwarf mutant, flower colour mutant, double spike head mutant, eventhough these mutants were novel but they were not found stable in next generation. In VM_1

populations mutants were derived from the primary gamma irradiated population which was maintained after observing the gamma irradiation effect of VM_1 generation *viz.*, flower shape mutant, tall mutant, flower colour mutant, mutant with higher rachis length and variegated leaf mutant. This study revealed that freshly harvested bulbs of cvs Arka Prajwal and Phule Rajani are highly suitable for mutation induction either in vivo and also in vitro condition.

Bulbs of tuberose cv. Arka Prajwal were subjected to treatments at different doses of gamma rays (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 KR). Leaf abnormalities (life texture and chlorophyll variation) were noticed in 1.0 KR and 1.5 KR treated bulbs. Gamma rays at 0.5 KR resulted in economic traits namely, number of spikes per plant (three number) and number of florets per spike (55 Nos.) as reported by Kayalvizhi *et al.* (2016a). Jyothi and Singh in 2016 concluded that freshly harvested bulbs of Prajwal Na Phule Rajani responded more to gamma irradiation. Gamma radiation at 7.5 Gy and 10.0 Gy. Optimal for mutation induction in these two studied cultivars. Based on this experiment Kayalvizhi *et al.* (2017b) also reported that in gamma rays 1.0 and 1.5 KR treated plants produced chlorophyll mutants such as Striata and also broad leaf mutants observed in 1.5 KR gamma ray dosages. Gamma ray treated plants produced branched spikes in tuberose.

An experiment was conducted by Sah *et al.* (2017) to observe the influence of gamma dose i.e. 2 KR and 4 KR alongwith untreated control in different cultivars of tuberose. 4 KR dose of gamma ray bulbs do not sprout due to lethal effect. Maximum sprouting percentage was recorded with control followed by 2 KR dose of gamma irradiation. Early flowering was noticed due to gamma irradiation dose at 2 KR which was significantly earlier than control. Maximum internodal length of spike, duration of flowering and weight of bulbs was recorded in control than treated bulbs.

Ghosh *et al.* (2017) treated tuberose cv. Calcutta Single loose flower with gamma irradiation (0.01, 0.02, 0.05, 0.10, 0.50, 1.0 and 2.5 KGY) and generally regarded as safe (GRAS) flower preservative solution for extending shelf life. The flowers were packed in low density polyethylene bags, heat sealed and stored at 23±2oC, 80% relative humidity (RH) and ±1oC, 40% RH, respectively. Flowers stored at these two temperature regimes were subjected to sensory evaluation and biochemical analysis. From these assessments, the longest shelf life of loose flowers was found to be 8 days at 23±2oC, 80% RH (compared to 4 days for control) and 24 days at 4±1oC, 40% RH (compared to 8 days for control) using combination treatment of low dose gamma irradiation (0.02 kGY) and preservative solutions (4% sucrose and 0.02% $CaCl_2$)

Singh and Sadhukhan (2019) carried out an investigation to isolate some putative mutants in tuberose cv. Calcutta Double. Different doses of 60 co gamma rays (to bulbs) were applied. Mutation having tall and branched spike and other almost one and half times taller (1.6 m) than the untreated control (1.01 m) could be scored from 10 KR gamma ray treatment. The third mutant scored from bulbs treated with 10 KR gamma ray was a unique chlorophyll variegated leaf mutant designated as Pranta Rekha that has green leaves with white margins. The branched mutant will provide the advantage of harvesting more number of flowers at a time than the leaf variegated mutant would have ornamental values. New mutant genes could be scored therefore form the mutagen treated population in tuberose.

Navabi *et al.* (2016) irradiated the mature bulbs of tuberose with gamma irradiation dose from 10 Gy to 400 Gy. The results showed that at the dose treatment of 10 Gy, all plants sprouted with a time delay, and at doses of 50 and 100 Gy, 57% and 29% of plants were sprouted and grew, respectively. Though, at dosages of 200 and 40 Gy, none of the plants survived. In this experiment, changes in plant morphology were observed according to the different treatments, but no changes were observed in flower colour.

Abhang Rao *et al.* (2019) treated tuberose cv. Phule Rajan bulb with five doses of 0.5 Kr, 1.0 Kr, 1.5 Kr, 2.0 Kr and 2.5 Kr alongwith untreated sample (control). The results indicated that the mutagenic treatment at lower doses had significant stimulative effect on some parameters that is, sprouting of bulb percentage, leaf length, chlorophyll content, rachis length, spike length, number of opened florets whereas most of the parameters have showed decreased at desired content, that is number of days for bulb sprouting, survival percentage, plant height, leaf area, stem diameter, number of unopened florets, weight of flower. Higher doses of mutagen were determental for growth parameters and lower doses of mutagen were beneficial for quality parameters. Seven mutants were obtained in VM_1 generation.

Sharavani *et al.* (2019) exposed tuberose cv. Hyderabad Single bulbs to different doses of gamma rays *viz.*, 0, 5, 10, 15, 20, 25 and 30 Gy representing treatments. The results revealed that sprouting percentage was 100% for control and 5 Gy treatments. Maximum plant height, number of leaves, leaf width and number of tillers were recorded for bulbs treated with 5 Gy followed by control plants recorded maximum leaf area and chlorophyll content. All bulb parameter (bulb-weight, diameter, number) were recorded maximum in control. By increasing the dose of gamma rays from 10 Gy there was a significant reduction in vegetative growth parameters. Variegated leaves and albino mutants were recorded for bulbs treated with 20 Gy.

Further more (Sharavani *et al.*, 2019b), to avoid excessive loss of actual experimental materials, radio-sensitivity tests were conducted to determine LD_{50} doses before massive irradiation of similar material. Tuberose cv. Hyderabad Single bulbs were exposed to six doses of gamma radiation (5, 10, 15, 20, 25 and 30 Gy) and 0 as control. Significant reduction of in floral parameters was observed with increased dose of gamma irradiation. Bulbs treated with doses 5, 10, 15, 20 Gy and control plants have shown flowering Control plants recorded maximum values for all floral attributes followed by 5Gy. The probit analysis based on sprouting percentage and mortality values for bulbs exhibited that LD_{50} value of gamma irradiation for tuberose cv. Hyderabad single was 20 Gy.

Tuberose bulbs were subjected to gamma radiation in order to identify variants with altered scent profile. The results indicated that significantly decreased in plants sprouted from bulbs exposed to higher dose of gamma rays. Floral volatile emission rate was increased in the lowest dose (10 Gy) as compared to control plants. The phenomenon of hormesis was observed in the plants since bulbs exposed to lower dose (10 Gy) showed enhanced growth rate, higher volatile content in comparison with control plants. Plants sprouted from bulbs exposed to higher dose of radiation showed lethal effects. In order to study genetic variation among control and plants sprouted from irradiated bulbs, inter simple sequence repeat marker analysis was also carried out and it was observed that out of 74 loci, 67 were polymorphic (90.54%). The genetic similarity coefficient values were also calculated among control and variant lines thus validating morpho-physiological variation (Kutty *et al.*, 2020).

Regar *et al.* (2021) studied the response of gamma radiation (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 Gy) alongwith control (untreated) on bulb and bulblet parameters of tuberose cv. Arka Prajwal. The results revealed that lowest decline trends were observed on bulb per clump (2.73, 14.69), bulb fresh weight (68.67 g, 73.86 g), clump weight (275.06g, 574.90g) and propagation coefficient 507.91%, 673.68%) parameters at 2.5 Gy gamma irradiation in V_1M_1 and V_1M_2 generation respectively as compared to control.

Regar *et al.* (2021) Studied the effect of gamma irradiation on bulb and bulblet parameters of tuberose cv. Pearl double. They found that there was increased bulbs per clump which was directly correlated with the fresh weight of the bulb. Same trend was observed in number of bulblets obtained and fresh weight of it. Over all there was increased propagation ecoefficiency at 2.5 Gy. Gamma irradiation.

Regar *et al,* (2022) investigated the response of gamma irradiation on vegetative parameters of tuberose Cv. Pearl double. It has been observed that declining trend in 50% sprouting and sprouting percentage and also in plant survival. In the other way there was an increased in plant height from 35.53 cm to 44.30 cm was observed along with increased number of leaves, leaf length and leaf width at 2.5 Gy radiation. The growth parameters of Cv. Pearl double showed decline trend at 20 Gy. Gamma radiation.

In tuberose, genetic improvement techniques developed in Iran generated mutant forms for flower length and weight from irradiating mature tuberose bulbs with 10 Gy of 60 co gamma rays (Navabi *et al.*, 2016). In India two mutants namely Rajat Rekha and Swarn Rekha with leaf variegation were developed and released (Datta, 2019).

Mutation induction by chemical mutagens

Ethyl methane sulphonate ($CH_3SO_2OC_2H_5$, molecular weight 124.16) among chemical mutagents is the most potent mutagent. It acts as a selective mutagen and induces lethal mutations in lower frequency as compared to gamma rays. It produces random mutations in genetic material by nucleotide substitution, particularly by guanine alkylation (Kayalvizhi *et al.*, 2017). In tuberose, gamma rays were exhibiting more mutagenic efficiency than EMS (chemical mutagen) as far as commercial values are concern (Singh and Sadhukhan, 2019).

Bulbs of three cultivars i.e. Calcutta Double, Arka Prajwal and Arka Shringar of tuberose were subjected to mutagenic treatments with 0.5% and 0.25% of ethyl methane sulphate (EMS) for inducing mutations in quantitative and qualitative characters. Immediate effects of the mutagen were evident with respect to some quantitative characters induced mutations leading to anatomical changes were evident in all three cultivars to untreated control variation was also exhibited in the structural organization of stomata (Singh and Sadhukhan 2013).

Singh *et al.* (2015) treated bulbs of three cultivars i.e. Calcutta Double, Arka Prajwal and Arka Shringar of tuberose with 0.25%, 0.50% and 1% (V/V) ethyl methane sulphate (EMS) concentrations. The results revealed that there was significant reduction observed for sprouting of bulbs, leaf area, spike length and diameter, flower size, number of florets per spike and flower fresh weight in all three cultivars treated with higher dose (1%) of EMS. However number of leaves was increased significantly in most of the EMS treatment and maximum number of leaves was found in 0.5% treatment as compared to their respective control. Minimum sprouting was noticed with higher dose (1%) treatment. Pollen sterility also increases with increasing dose of EMS in both Arka Prajwal and Arka Shringar. Among the cultivars Calcutta Double appeared to be more sensitive to EMS as compared to Arka Prajwal and Arka Shringar.

An investigation was carried out by Kayalvizhi *et al.* (2016b) on the induction of mutation in tuberose cv. Arka Prajwal with the objective of examining the effect of chemical mutagens *viz.*, diethyl sulphate (DES) @ 15, 20, 25 and 30 mM and ethyl methane sulphonate (EMS) @ 30, 45, 60 and 75 mM on bulb sprouting, survival percentage and growth parameters. The results revealed that lower doses (15 mM of DES and 30 mM of EMS) were found to favour bulb sprouting and growth parameters of plants. In general, variations in floral characters were observed invariably in all the treatments except control treatment. The LC_{50} values fixed for the chemical mutagens were 25 mM for DES and 60 mM for EMS. It was observed that lower doses of mutagents had recorded higher values for studied morphological parameters (plant height, number of leaves, leaf length and leaf width) and floral parameters (number of florets per spike) spike length, number of spikes per plant, floret length, floret diameter and single floret weight) than untreated control in M_1V_1 generation.

Kaintura *et al.* (2016) conducted an experiment on four cultivars of tuberose *viz.*, Kalyani Single, Kalyani Double, Arka Suvasini and Arka Prajwal to study the mutagen effectiveness of ethyl methane sulphate (EMS) by treating the healthy and uniform bulbs with different doses 0.1% and 0.2% alongwith untreated bulbs as control and evaluated for various vegetative and floral parameters. The findings of the experiment showed that the treatment of EMS at lower dose (0.1%) had significant simulative effect on vegetative parameters *viz.*, sprouting percentage, days to sprouting, while the parameters pertaining to survival rate, leaf length, number of spikes per plant, number of florets per spike, flowering duration and vase life were observed with decreasing trend. High doses had determental effect on studied vegetative and floral parameters.

Kayalvizhi *et al.* (2017b) carried out an experiment on the improvement of tuberose cv. Arka Prajwal through chemical mutagens. The bulbs were treated with diethyl sulphate (DES) ($C_2H_2O_2SO_2$) and ethyl methane sulphate (EMS). The treatment consisted on 15, 20, 25 and 30 mM of DES and 30, 45, 60 and 75 mM of EMS and control (untreated). The results revealed that in general, the treated population had mainifested reduced expression than the control (untreated population) for most of the morphological and floral characters. Higher the dose of mutagens, lower was the expressivity of the traits. Expression of the morphological characters namely, plant height, number of leaves, leaf length, leaf width and leaf thickness increased in the lower doses and decreased in the higher doses in M_1V_2 generation. As chimerism and genetic variability play a key role in the variation observed in mutation treated population, there is a need to identify solid mutants in the future generations. Kayalvizhi *et al.* (2017a) reported that tuberose cv. Arka Prajwal bulbs were treated with chemical mutagen DES at different doses. Nine tepal florets were observed in 30 mM of DES and eleven tepal floret was observed in 15 mM of DES.

Yadav *et al.* (2018) carried out a study in tuberose cv. Arka Prajwal with five treatments (concentrations) of ethyl methane sulphonate (0, 0.25, 0.50, 0.75 and 1.0%) and with three bulb dipping durations (5 min., 4 h and 8 h) resulting in 15 treatments. The results showed that with treatments of bulbs with EMS the number of days taken to initiation of sprouting, complete sprouting, days taken to spike emergence decreased at lower concentration of EMS (0.25%) but at higher doses they delayed the initiation parameters. The lower concentration of EMS (0.25%) also increased plant height, number of leaves, decreased length of leaves while higher doses increased length of leaves and reduced number of leaves and plant height. The dipping duration of 8h was reported effective to increase plant height, number of leaves and reduce length of leaves.

The interaction effect was found highest in treatment combination of 0.25% EMS with 8 h dipping duration for vegetative growth parameters. The interaction effect was non-significant for days to complete sprouting, spike emergence and plant height. With same treatments and treatment combinations (Yadav *et al.*, 2018) in tuberose cv. Arka Prajwal.

Yadav *et al.* (2022) reported that treatment of bulbs with EMS at 0.25% for 8h dipping duration was found best for increase in spike length, rachis length, spike weight, number of opened florets, number of unopened florets and number of spike per clump. Besides that earliness with respect to number of days taken for opening of first florets. Treatments of bulbs with 0.50% EMS for 4h dipping duration in EMS solution shows an increase in number of bulbs per clump, diameter of bulb and weight of bulb. Lower doses of EMS coupled with shorter dipping duration seems promising for extending the vase life of cut flower.

Mean lethal dose or mean reductive dose or median lethal dose-50 (LD_{50})

This dose is the one that reduces survival and growth to 50% in relation to the control treatment and is where most mutations are obtained. Some researchers recommend an interval of 20% higher and lower, while others state that the optimal dose should also lead to the survival of 40 to 60% of the treated material with respect to the untreated material.

According to Jyothi and Singh (2015) the total number of tuberose plants survived among the sprouted bulbs in each treatment was recorded. LD_{50} (Lethal dose-50) was determined on the basis of the plants survived in each treatment.

The survival percent of bulb was calculated using formula given below:

$$\text{Percent survival} = \frac{\text{Total number of bulbs survived}}{\text{Total number of bulbs planted}}$$

Datta (2017) has given LD_{50} value of gamma rays to tuberose (bulb) from 250 Krad to 8 Krad. Jyothi and Singh (2015) calculated the LD_{50} dose for freshly harvested bulbs of tuberose cvs. Arka Prajwal and Phule Rajani. The LD_{50} dose for freshly harvested bulbs of cv. Arka Prajwal was 10.25 Gy and for cv. Phule Rajani it was 11.25 Gy. In three weeks after uprooted bulbs LD_{50} dose was 3.25 Gy for cv. Arka Prajwal and 10.25 Gy for cv. Phule Rajani. The LD_{50} dose for six weeks after uprooted bulbs of cv. Arka Prajwal was 10.25 Gy and for cv. Phule Rajani it was 11.25 Gy. Kayalvizhi *et al.* (2016 b) fixed LD_{50} values for chemical mutagens 25 mM for DES and 60 mM for EMS in tuberose cv. Arka Prajwal. Also Kayalvizhi *et al.* (2016a) fixed LD_{50} value in tuberose cv. Arka Prajwal for gamma rays 2.13 kR (physical mutagen). By increasing the dose/concentration of physical and chemical mutagens beyond LD_{50}, decreased in sprouting percentage of bulbs, survival percentage, plant height and number of leaves per plant decreased. According to Sharavani *et al.* (2019b) the probit analysis based on sprouting percentage and mortality of treated bulbs exhibited that LD_{50} value of gamma irradiation for tuberose cv. Hyderabad single was 20 Gy.

Cytological studies in tuberose for realization of mutation

In one of the study it was reported that 1500 gamma radiations was found to be effective on chromosomal aberrations of root tip mitosis in tuberose cvs. Mexican Single and Pearl Double.

Conclusion

The uses of physical and chemical mutagens in tuberose (*Polianthes tuberosa* Linn.) have shown to be very effective under many qualitative and quantitative parameters. Therefore, mutation breeding has been regarded as a complementary or an alternative method to conventional breeding using physical and chemical mutagens. This has proved to an effective tool for enhancing genetic variability, thus creating grater chances for selection. Several factors should be considered when selecting mutagen treatment conditions. Although chemical mutagens are relatively inexpensive and require little sophisticated equipment, they are regarded as having inferior ability to penetrate deeply into plant tissues or thick seeds.

However, physical mutagens provide consistent treatments but require access to radiation sources, such as X-ray machines, gamma sources, particle accelerators, or nuclear reactors. Other advantages of physical mutagens include easy post-treatment handling of plant tissues or seeds, the ability to treat pollen grains and other fragile materials and the lack of toxic and carcinogenic waste. Another reasons why EMS or other chemicals may be tried in some cases is the fact that they mostly cause single base substitutions, possibly resulting in a series of phenotypically distrinct change of a function mutants for a particular trait. In contrast, physical mutagens usually cause deletions resulting in loss of function mutants. Previously reported experiences with tuberose are valuable when starting mutation breeding for a new species or cultivar. Initial doses can be based on those experiences, thereafter, the treatment conditions may be fine-tuned because different mutagens have different mutagenic efficiencies.

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