

Original Research Article

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Effect of Physical and Chemical Mutagens on Morphological Characters in M_1V_2 Generation of Tuberose (*Polianthes tuberosa* L.)

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ABSTRACT

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An investigation was carried out at the Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2014-2016 on the improvement of tuberose var. Prajwal through mutation breeding. The bulbs were treated with gamma rays, diethyl sulphate (DES) and ethyl methane sulphonate (EMS). The treatments consisted of 0.5, 1.0, 1.5, 2.0 and 2.5 kR of gamma rays, 15, 20, 25 and 30 mM of DES and 30, 45, 60 and 75 mM of EMS and control (untreated). Various morphological and floral characters were observed. In general, the treated population had manifested 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.

Introduction

Floriculture in India is estimated to cover an area of 2.55 lakh ha with a production of 17,54,000 MT of loose flowers. Nearly 77 % of the area under floricultural crops is concentrated in seven states comprising Tamil Nadu, Karnataka, West Bengal, Maharashtra, Haryana, Uttar Pradesh and Delhi. Among different states, Tamil Nadu ranks first in area followed by Karnataka, West Bengal and Maharashtra. In Tamil Nadu 3,43,650 MT of loose flowers are produced in an area of about 55,000 hectares. (Anon., 2015).

Tuberose (*Polianthes tuberosa* L.) is one of the most important flowers used for both cut and loose flower purpose. It is extensively cultivated in many sub-tropical and tropical

parts of the world including India. It is a native of Mexico and belongs to the family Amaryllidaceae. It is a bulbous perennial plant with tuberous roots producing long spikes, bearing waxy white fragrant flowers. It is a crop which flowers profusely throughout the year. Due to the longer keeping quality of flower spikes (Benschop, 1993), they are in great demand for making floral arrangements and bouquets in major cities of India. Three types of tuberose which are used in cultivation are single type with one row of corolla segments, semi- Double type with two to three rows of corolla segments and double type with more than three rows of corolla segments.

The spikes as a whole in double types can be used as cut flowers whereas the florets of single varieties are used for making garlands, veni, gajra, bangles, etc. and also for essential oil extraction. The flower yields a very valuable floral concrete (0.08 – 0.11 per cent) upon solvent extraction (Singh, 1995). The absolute of tuberose (essential oil) extracted from floral concrete is used in the preparation of various high value perfumes and cosmetics.

The main emphasis in flower breeding is to improve the varietal traits *viz.*, colour, flower form, size, number of flowers, shelf life, vase life, year round production and growth habit.

Mutation breeding stands for the genetic improvement of crop plants for various economic characters by using physical and chemical mutagens. Mutation is the sudden heritable change that occurred in an organism. It may be caused by spontaneous or through artificial induction and the resulted mutant shows change in the gene or chromosomes. (De and Bhattacharjee, 2011).

Induced mutagenesis has been most successful in ornamental crops. Both physical and chemical mutagens have been used for improving the desired characters of many ornamental crops including amaryllis, asiatic hybrid lily, bougainvillea, chrysanthemum, dahlia, gladiolus, hibiscus, Lantana, marigold, rose, tuberose, gerbera, narcissus, *etc.* Induced mutations in ornamentals comprise traits, such as altered flower characters (colour, size, morphology, fragrance), leaf characters (form, size, pigmentation), growth habit (compact, climbing, branching) and physiological traits such as changes in photoperiodic response, early flowering, free flowering, keeping quality and tolerance to biotic and abiotic stresses. The main advantage of mutation breeding in vegetatively propagated crops is the ability to change one or a few characters of an

otherwise outstanding variety without altering the unique part of the genotype (Datta, 2014).

In any mutation breeding programme, selection of an effective and efficient mutagen is very essential to produce high frequency desirable mutants. Several factors such as properties of mutagens and pH, duration of treatment, temperature *etc.* play an important role to produce a desirable mutant.

Mutations are induced by physical and chemical mutagen treatment in both seed and vegetative propagated crops. The mechanism of mutation induction is that the mutagen treatment will break the nuclear DNA and during the process of DNA repair mechanism, new mutations may occur randomly and are heritable. It is a simple, efficient, rapid and cheap option for obtaining desired genotypes from recalcitrant species. Induced mutation is one of the most widely used techniques for creating additional variability in desirable character. It can be done by physical and chemical mutagens.

In physical mutagens, atoms are the principle source material. Unstable atoms of same element having different weights giving energy or particles are called radioisotopes and electromagnetic waves associated with nuclear decay are called as radiation and the treatment of an organism or plant with radiation is known as irradiation. It is classified into two groups ionizing and non ionizing radiations. Alpha rays (α), Beta rays (β), X-rays, Gamma ray (γ) and Neutrons belongs to the group of ionizing radiation. Non ionizing radiation includes UV rays only. Ionizing radiations normally causes chromosomal rearrangements and deletions. (Bhat *et al.*, 2007). Gamma rays are electromagnetic radiations having shorter wavelength than X rays with more energy and penetrating power. It is produced by a number of isotopes e.g. ^{14}C , ^{60}Co , ^{137}Cs etc. for

chronic treatments requiring slow irradiation over long periods. (De and Bhattacharjee, 2011).

Mutation can also be induced chemically with alkylating agents such as Diethyl sulphate (DES) and Ethyl methane sulphonate (EMS) etc. The alkyl group of chemical mutagens reacts with DNA which may change the nucleotide sequence and cause a point mutation. (Broertjies and Harten, 1988). EMS alkylates are guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions (Bhat *et al.*, 2007).

Tuberose being a cross pollinated crop, there is need for high yielding variety with improved fragrance to overcome farmer's predicament. Mutation breeding is the most effective and commonly employed tool to induce acceptable variations in the existing cultivars *viz.*, high yielding and better quality of genotypes (Bhattacharjee, 2006). The present study was undertaken to induce desirable variations in tuberose using physical and chemical mutagens.

Materials and Methods

A study was conducted at the Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2015-16 on the improvement of tuberose (*Polianthes tuberosa* L.) var. Prajwal through mutation breeding. The experimental site is geographically situated at an altitude of 426.72 metres above mean sea level (MSL) and between 11°02' North Latitude and 76°57' East Longitude. Bulbs of tuberose var. Prajwal (2-3 cm diameter) were subjected to gamma and chemical mutagens treatment.

Prajwal is a hybrid of tuberose (Shringar x Mexican Single) developed by IIHR, Bangalore. It is a single type with greenish

white flower buds with dark pink tinge at the tips. It yields 18 t/ha/ year. It is ideal as loose flower and cut flower.

One physical mutagen (gamma ray) and two chemical mutagens *viz.*, Diethyl Sulphate (DES) and Ethyl Methane Sulphonate (EMS) were used in the study.

For gamma irradiation of bulbs, the Gamma chamber - 1200 available at the Centre for Plant Breeding and Genetics of Tamil Nadu Agricultural University, Coimbatore, installed and maintained by the Board of Radiation and Isotope Technology (BRIT), DAE, Mumbai. Cobalt - ⁶⁰(Co) emitting 5000 rads per minute at the time of irradiation was used. The formula suggested by Kodym and Afza (2003) was used for the calculation of duration of exposure.

Diethyl Sulphate (C₂H₂O₂ SO₂) with a molecular weight of 154.19, boiling point 208° C (lit.), density =1.777 g/ ml at 25°C (lit.) was obtained from M/s Sigma-Aldrich Company, U.S.A. Prior to use, it was removed from refrigerator and placed in a desiccator with calcium chloride until room temperature was reached.

Ethyl Methane Sulphonate (CH₃ SO₂ OC₂ H₅) procured from M/s. Sigma-Aldrich Company, U.S.A was used. It has a Molecular weight-124.16, boiling point of 80/100 mm Hg and Density of D₄²⁵ = 1.203 g/ml). It was stored in dry air at 0° C to maintain its purity. Prior to use, it was removed from refrigerator and placed in a desiccator with calcium chloride until it reached room temperature. The treatment details are furnished below (Table 1).

Cultural practices were followed as per the Crop Production Manual, TNAU, 2012. The recommended fertilizer dose of 200:200:200 kg ha⁻¹ of NPK was applied. Half of the RDF was applied as basal and the remaining half

was applied in two splits at 30 and 45 days after planting respectively. Foliar spray of micronutrients (H_3BO_3 @ 0.1 % + $ZnSO_4$ @ 0.5 % + $FeSO_4$ @ 0.2 %) was given four times at 60, 120 and 180 days after planting (Ganesh, 2010). The observations recorded on growth, yield and quality parameters in M_1V_2 generation. For individual plants, the morphological variations were observed upto 200 days after planting.

Results and Discussion

Various morphological and floral characters were observed for the mutagen treated plants. In general, the treated population had manifested reduced expression than the control (untreated population) in most of the morphological and floral characters. Higher the dose of mutagens, there was reduction in the expressivity of the traits.

The expression of the morphological characters plant height, number of leaves, leaf length, leaf width and leaf thickness increased

in the lower doses and decreased in the higher doses in the M_1V_2 generation. Similarly, days taken for spike emergence and first floret opening were observed earlier at lower doses. The floral and yield characters namely spike length, rachis length, floret length and diameter, number of spikes per plant, number of florets per spike, weight of single floret and flower yield per m^2 were observed maximum at lower doses and were minimum in the higher doses.

A total of 1617 plants were examined until 200 days to isolate the chlorophyll mutants. Chlorophyll mutants noticed in M_1V_2 generation are of four types viz. 'albino' 'chlorina' 'striata' and 'xantha' (Plate 1). The treatment T_3 (1.0 kR gamma ray) recorded maximum number of chlorophyll mutants and it was followed by T_4 (1.5 kR gamma ray) and T_{12} (45 mM of EMS). Similar observations were made by Sambanthamurthi, (1983) and Kumar *et al.*, (2013) in tuberose; Banerji and Datta (1998) and Sisodia and Singh (2014) in gladiolus.

Table.1 Treatment details

Treatment	Dose of physical and chemical mutagens
T ₁	Untreated control
T ₂	0.5 kR gamma rays
T ₃	1.0 kR gamma rays
T ₄	1.50 kR gamma rays
T ₅	2.00 kR gamma rays
T ₆	2.50 kR gamma rays
T ₇	15 mM DES
T ₈	20 mM DES
T ₉	25 mM DES
T ₁₀	30 mM DES
T ₁₁	30 mM EMS
T ₁₂	45 mM EMS
T ₁₃	60 mM EMS
T ₁₄	75 mM EMS

Table.2 Morphological abnormalities

Treatment	Variation / morphological abnormalities recorded in M_1V_2 generation
T ₃	Absence of pink tinge on tip of the floret
T ₄	Leaf abnormalities such as merging of two leaves
T ₅	Leaf vein, single floret per node, fusion of two florets, uneven size of florets and broader stamen
T ₆	Two rows of florets in single type variety
T ₇	Sickle shaped leaf and tepal elongation
T ₈	Crinkled, lobed leaf, leaf vein, tepal serration, sharp and bent floret tip and pink colour of whole bud
T ₉	Leaf vein, bent floret tip
T ₁₀	Leaf vein, spike abnormalities
T ₁₁	Uneven size of florets

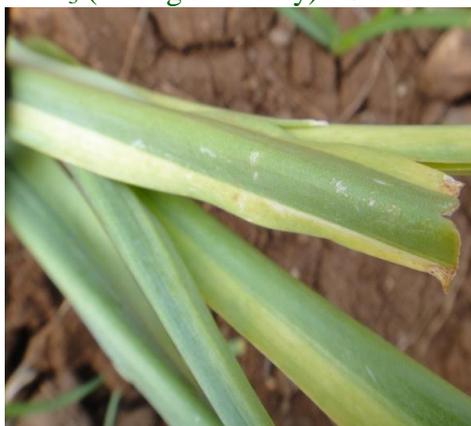
Plate.1 Chlorophyll mutants in M_1V_2 generation



T₃ (1 kR gamma ray) - Striata



T₃ (1 kR gamma ray) - Albino and Xantha

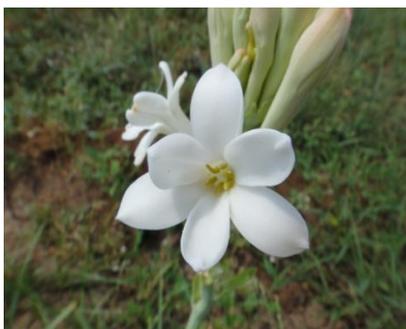


T₄ (1.5 kR gamma ray) - Striata



T₄ (1.5 kR gamma ray) – Broad leaf formation

Plate.2 Floret tepal variations in M_1V_2 generation



Control with six tepal



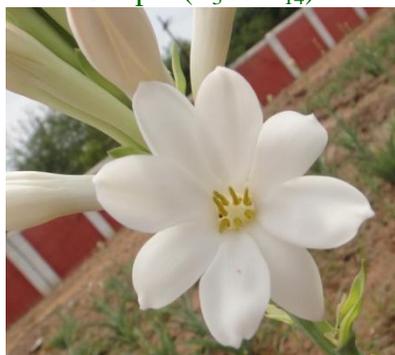
4 tepal (T_3 to T_{14})



5 tepal (T_3 to T_{14})



7 tepal (T_2 to T_{13})



8 tepal (T_2 to T_{14})



T_7 - (15 mM DES) – 11 tepal floret

Broad leaf mutants were observed in 2.5 kR gamma ray with a leaf width of 2.53 cm and T_7 (15 mM DES) 2.87 cm. Branched leaf mutants were observed in 1.0 kR and 2.0 kR. These variations may be useful for landscape purpose twing to their enhanced aesthetic values. These findings are similar to those of Singh *et al.*, (2013) in tuberose.

A non flowering spike was recorded in 25 mM DES. Similar result was reported by Sambanthamurthi (1983).

Floral mutants

Four tepal floral mutants were observed in all the treatments except T_1 (control) and T_2 . Five tepal florets were also observed in all the treatments except T_1 (control), T_2 , T_5 and T_6 . Similarly, all the treatments except T_2 and T_3 produced seven tepal florets but in control plants only six tepal florets were produced. Likewise, eight tepal florets were observed in all the treatments except T_5 . Nine tepal florets were observed in T_{10} (30 mM of DES).

Eleven tepal floret was observed in T₇ (15 mM DES) (Plate 2). These findings are in accordance with those of Datta (1977), Van Harten (1998) and Datta (2000) in ornamentals and Anu *et al.*, (2003) and Kainthura and Srivastava (2015) in tuberose (Table 2).

These findings are in line with the morphological abnormalities in the foliage and florets observed by earlier workers in irradiated material of gladiolus Banerji and Datta (1998), Kumar *et al.*, (2013); Banerji *et al.*, (2000) and Singh *et al.*, 2013 gladiolus.

In conclusion, 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.

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