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Identification of variants induced by physical and chemical mutagens in tuberose (*Polianthes tuberosa* L.)

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ABSTRACT

The present investigation was carried out at Horticultural Research Station, Mandouri, Nadia, West Bengal during the year 2014-16 to isolated some putative mutants in tuberose. Different doses of ^{60}Co gamma rays (to bulbs) and varied concentrations of EMS (to seeds) were applied to tuberose cv. Calcutta Double and Arka Nirantara respectively. Mutation having tall and branched spike and other almost one and half times taller (1.6m) than the untreated control (1.01m)could be scored from 10kr gamma rays treatment. The third mutant scored from bulbs treated with 10 kr gamma rays was a unique chlorophyll variegated leaf mutant designated as Pranta Rekha that has green leaves with white margins. One dwarf and other with altered floral characteristics resulted from 1% EMS treatment from the cv.Arka Nirantra. The branched mutant will provide the advantage of harvesting more number of flowers at a time and the leaf variegated mutant would have ornamental values. New mutant genes could be scored therefore from the mutagen treated population in tuberose. Gamma rays were exhibiting more mutagenic efficiency than EMS as far as commercial value is concern.

***Keywords*: Chlorophyll, EMS, gamma rays, mutants, tuberose and variegated**

Tuberose a perennial plant of family Agavaceae is native of Mexico from where it spread to the different parts of the world during the 16th century. It is commercially cultivated in different parts of India including West Bengal. The crop is commercially important as a cut flower, loose flower and extraction of essential oils. In spite of its great importance in floriculture the species does not have much natural variability either in flower color or type although, some varieties show pinkish tinge at bud stage. The bulbs of tuberose are used as the major propagating / planting material of the crop though, developments of plant materials from the seed are rare but not uncommon (Hemanta, 2015 and Rosalind *et al.*, 2018). To develop more variation in biotic and abiotic traits such as disease resistance, flower shape, flower colour, vase life, *etc*. in tuberose, there is an urgent need of well-planned breeding programmes using conventional and non-conventional breeding techniques.

There is limitation of conventional breeding methods involving hybridization in tuberose due to self-incompatibility (Sreethramu *et al.,* 2000). Mutation breeding appears to be a well standardized, efficient and cost-effective technique that can be exploited for the creation of novel ornamental cultivars of commercial importance in tuberose. Induced mutations have been playing an important role in the origin of new cultivars of many ornamental plants (Kayalvizhi *et al.*, 2017b). Ionizing radiations particularly gamma rays have been more successfully used for the development of novel ornamental varieties in contrast to chemical mutagens through induce mutation (Patil and Patil,2009).

Major objective of any mutation breeding programme is to obtain new and better genotypes through the creation of genetic variability in the existing gene pool. 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). As the cultivars selected for the present experiment are strictly vegetatively propagated, any mutant recovered will have the advantage of perpetual propagation without any alteration of character, unlike that of propagation through seeds.

Attempts have been made for genetic improvements in respect of 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 and improved keeping quality in numerous ornamental crops like Amaryllis (Kaicker and Singh, 1979), Begonia (Ito *et al*., 2008), Bougainvillea (Datta and Banerjee, 1994; Swaroop and Jain, 2016), carnation (Richter and Singleton,1995), Chrysanthemum (Singh and Bala, 2015; Telem *et al.*, 2015), Dahlia (Pal, 2015), Gladiolus (Kashyap *et al*., 2018), rose (Bala and Singh, 2015) *etc*.

Much works on mutation breeding has not so far been done in tuberose for its genetic improvement. Attempts were made by a few scientists and perceived different types of morphological abnormalities like changes in shape size, margin apex, fission and fusion of leaves and chlorophyll variegation in leaves while using gamma

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rays (Abraham and Desai, 1976; Younis and Abraham, 1975; Krasaechai, 1976; Krasaechai, 1992; Shukla and Datta, 1993 and Navabi *et al*., 2016), EMS (Singh *et al*., 2015) and X-rays (Kainthura *et al*., 2016) as mutagen. However, structural and numerical changes in stomata and change in vascular bundle anatomy have been observed in three cultivars of tuberose *viz*., Calcutta Double, Prajwal and Shringar treated with different concentration of EMS (Singh *et al*, 2013).

Due to existence of self-incompatibility in tuberose,induced mutagenesis thus seems to be an ideal methodology for the induction of desirable genetic variability and selection of several putative mutants. The treatment of bulbs per seeds with different mutagens, both physical and chemical mutagens may help in developing some plant showing elite characteristics. One of objectives of the present study was therefore contemplated to develop good planting materials as new mutants.

MATERIALS AND METHODS

The present investigation was carried out at Horticultural Research Station, Mandouri, Nadia, West Bengal during the year 2014-15 and 2015-16. The experimental site was geographicallylocated at 23.5^0N latitude and 89^0E longitude with an average altitude of 9.75 m above sea level.This zone falls under the subtropical humid climate where summer and winter both are short and mild/moderate. So, this zone is not subjected to condition of extreme winter.The experimental materials comprised of an indigenous double petal variety *i.e*. Calcutta double and a single petal variety *i.e*. Arka Nirantara released by Indian Institute of Horticultural Research (IIHR), Bangalore. In the present study physical mutagen (gamma rays) and chemical mutagen (Ethyl Methane Sulphonate) were used to induce mutation.

Sixty number of healthy and uniform sized bulbs (1.5-2.0 cm in diameter)of variety Calcutta double were irradiated with different doses (10Gy, 20Gy and 30Gy) of γ-rays at UGC- DAE consortium for scientific research, Kolkata centre (south campus of Jadavpur University, salt lake, Kolkata). The source of gamma rays was ^{60}Co and the dose rate was 7.12 Gy minute^{-1}.The formula suggested by Kodym and Afza (2003) was used for the calculation of duration of exposure.

Ethyl Methane Sulphonate ($CH_3SO_2OC_2H_5$) procured from M/s. Sigma-Aldrich Company, U.S.A was used. It has a Molecular weight of 124.16, boiling point of 80/100 mm Hg and density of D_4^{25} = 1.203 g ml^{-1}). All solutions of the chemical mutagens were prepared in freshly prepared phosphate buffer having pH-7. Presoaked (9h in distilled water) 150 seeds for each treatment of variety Arka Nirantara were treated with different concentration (0.25%, 0.5% and 1%) of EMS for 4h at ±25°C.Treated seeds washed thoroughly with running tap water for 1 hour to remove the residues of the chemical.

The irradiated bulbs (20 bulbs replication^{-1}) and EMS treated seeds (50 seeds replication^{-1}) were sown in experimental field on the same dayalong with their respective controls in randomized block design (RBD) with three replications to develop the M_1generation (2014-15).Cultural practices were followed as per the Crop Production Manual, BCKV. Survival percentage anddata on different yield contributing characters like plant height, number of shoots clumps^{-1}, spike length, rachis length, number of florets spike^{-1}, spike yield plot^{-1}, spike clump^{-1}, weight of cut spike and weight of individual floretwererecorded at different morphogenetic stages during entire growth period.Individual plants were observed for the morphological variations throughout the growth period after planting. Morphologically variant plants selected and isolated during first year were grown separately in plant to row methodalong with their respective control in subsequent years (2015-16) to check their fidelity. Five plants data only for morphologically variant characters and yield were recorded.

RESULTS AND DISCUSSION

A total five mutants with novel morphological characteristics could be isolated from the present investigation. Out of which three were isolated from Calcutta double variety (gamma rays irradiated) while remaining two from the variety ArkaNirantara (EMS treated). A brief narrative of these novel mutants have been presented under following heads.

Bulbs treated with gamma rays

Leaf variegated mutant

So far two leaf mutants in tuberose like Swarna Rekha and Rajat Rekha has been reported in double and single flowered type respectively (Gupta *et al.*,1974). One mutant of double flowered tuberose (Swarna Rekha) has golden yellow streaks on the edge of the leaves (Fig.1.a) while the single flowered tuberose RajatRekha has white streaks longitudinally in center of the leaves (mid axis) (Fig.1.b). In the present study abnormality in the form of leaf variegation in one plant could be isolated in M_1 generation and the characters are observed to be true to the mutant types in M_2 generation that is true breeding. In contrast to Swarna Rekha, the leaf mutant isolated from 20 Gy gamma rays in the present study is having white colour in the leaf margin (Fig. 1.c, d). This new leaf mutant is named as Pranta (margin) Rekha (streak). So far there is no report of such mutant in tuberose.Though, Kayalvizhi *et al*. (2017a) observed

Table 1: Different vegetative and reproductive characters of branched mutants and untreated control

Characters	Control	Mutant (Branched)
Spike height (cm)	101 ± 0.20	160 ± 0.44
No. of flowers in main spike	52 ± 0.26	58 ± 0.53
Rachis height (cm)	33 ± 0.19	110.5 ± 0.38
No. of branch	01 ± 0	03 ± 0.05
First branch		
Spike height (cm)	-	37.5 ± 0.27
Rachis height (cm)	-	22 ± 0.21
No. of flowers	-	20 ± 0.30
Second branch	-	
Spike height (cm)	-	22.2 ± 0.18
Rachis height (cm)	-	10.5 ± 0.22
No. of flowers	-	13 ± 0.26
Third branch	-	
Spike height (cm)	-	13.5 ± 0.32
Rachis height (cm)	-	03.5± 0.13
No. of flowers	-	03 ± 0.09

Note: ± Standard Error

four different types of unstable chlorophyll mutants viz. 'albino' 'chlorina' 'striata' and 'xantha' from gamma ray and EMS treated bulbs of tuberose cv. Prajwal in M_1 generation.

Branched mutant

This mutant is designated as branched mutant derived from 10 Gy gamma rays treatment from Calcutta double variety. In contrast to normal tuberose varieties having unbranched rachis, the present mutant is having four branches (Fig. 3.a and c). Out of which the main branch is having rachis length of 110.5 cm as compare to 22 cm, 10.5 cm and 3.5 cm respectively of second, third and fourth branch. In case of number of flower per branch the main, second, third and fourth are having 58, 20, 13, and 3 respectively. Hence the cumulative total number of flowers per stick was found to be 94 which were almost double as compare to untreated control (52) (Table 1). Hitherto there is no report of such mutant in tuberose. However, bunched spike in some plants of gladiolus cv. Pusa Kiran with 4.5 kr gamma rays treatment has been reported by Sisodia and Singh (2015).

Tall mutant

This mutant was also isolated from 10 Gy gamma rays treatment of cv. Calcutta Double. The spike length of this mutant is 1.60m as compare to the untreated control having 1.01m (Fig. - 3b and c).A tall and stable mutant isolated by Swaroop and Jain (2016) while studying the effect of ^{60}Co gamma rays in bougainvillea cv. Lady Marry Baring.

Two types of mutants such as dwarf mutants and other mutant involving floral characteristic could be recovered from EMS treated seeds of another variety Arka Nirantara.

Dwarf mutant

This dwarf mutant having the spike length of 43 cm as compare to untreated control (94 cm) has been isolated from 1% EMS treatment. The spike habit is having neck bending and the number of flower appears to be unreduced as compare to untreated control (Fig. 4.a, b). Similar result like stunted plant with only two whorls of petals in cv. Suvasini with 1.5 Kr gamma-ray treatments and plant had extremereduction in height treated with EMS (0.2 %) in cv. Prajwal were observed by Kainthura and Srivastava (2015).

Flower mutant

This mutant is also isolated from 1 % EMS treated seed of the variety Arkanirantara. Both the mutant as well as of control are of equal height but there is visible change in floral characteristics like number of tepals (reduced in case of mutant) and flower appearance. The tepals of mutant are narrower in comparison to untreated control which is broader in nature. Moreover, the mutant showed more synchronization in flowering as compare to untreated control when observed at same age (Fig.- 2.a and b). Kainthura and Srivastava (2015) ware also isolated various flower mutants from gamma rays and EMS treated population of cv. Suvasini and Prajwal.

Fig.1: Leaf variegated mutant. **(a)** Swarna Rekha **(b)** Rajat Rekha **(c and d)** Pranta Rekha, the new mutant. **Fig. 2:** Flower mutant **(a)** EMS treated flower mutant of Arka Nirantra **(b)** Untreated control of Arka Nirantra **Fig. 3:(a)** Branchedmutant of Calcutta Double **(b)** Tall mutant of Calcutta Double **(c)** Untreated control of Calcutta Double **Fig. 4: (a)** EMS treated dwarf mutant of Arka Nirantra **(b)** Control of Arka Nirantra

Seed treated mutants (By EMS)

Similar results were observed by Navabi *et al.* (2016) in gamma rays irradiated population of tuberose. However, in tuberose numerous floret (tepal) variations in respect of number were observed in various treatment of gamma rays (Kayalvizhi *et al*., 2017a) and EMS (Singh *et al*., 2013).

As a result of development of two mutants in particular that is branched mutant and chlorophyll mutant in the variety Calcutta double helped in identification and scoring of new mutant genes. Branched mutant had the advantage of harvesting of almost double the number of flower per spike. Similarly the chlorophyll mutant designated here as Pranto Rekha is new chlorophyll variegated leaf mutant having ornamental value. Both the mutagens were found to be effective as well as efficient in tuberose.It has been found that Gamma rays exhibiting more mutagenic efficiency than EMS. Moreover, three types of mutations (Swaminathan, 1966) such as macro-mutation (here branched and flower mutant), micro- mutation affecting quantitative characters (here tall and dwarf mutants) and chlorophyll mutation (here Pranto Rekha) could be scored from the mutagenic population of tuberose.

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