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Effect of EMS on Morpho-anatomical Changes in Tuberose (*Polianthes tuberosa* L.)

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ABSTRACT

Tuberose (*Polianthes tuberosa* L.), a perennial ornamental plant of the family Agavaceae (formerly Amaryllidaceae) and a native of Mexico, is commercially cultivated in different parts of India, including West Bengal. This flower is commercially important as a loose and cut flower, and for extraction of essential oils used in the perfumery industry. The experimental materials comprised three indigenous varieties of tuberose, 'Calcutta Double', 'Prajwal' and 'Shringar', which are strictly vegetatively propagated. The bulbs of all three varieties were subjected to mutagenic treatments with 0.5% and 0.25% of ethyl methane sulphonate (EMS) for inducing mutations in qualitative and quantitative characters including anatomical feature of the scape (flower-bearing stalk) and stomatal features on the leaves. Immediate effects of the mutagen were evident with respect to some qualitative characters. Induced variations leading to anatomical changes were evident in all three varieties compared to the untreated control. Variation was also exhibited in the structural organization of stomata.

Keywords: EMS, mutation, morpho-anatomical changes, tuberose

INTRODUCTION

Tuberose (*Polianthes tubeorsa* L.), a perennial plant of the Agavaceae family (formerly known as Amaryllidaceae), is a native of Mexico. It is one a common garden plant, producing tall spikes and bearing white flowers, which emit an exquisite fragrance. Tuberose is commercially cultivated in different parts of India including West Bengal. It is commercially important as a loose flower, cut flower, and for extraction of essential oils. In west Bengal (India) about 4807 ha of land is under tuberose cultivation with the production of 1114.7 million stems per year and productivity 0.23 million stems per ha (Anon 2011). However the species does not have much natural variability either in flower colour or type. Only white flower colour in single, semi-double and double cultivars is commonly available. Much work on mutation breeding has not so far been done in this plant for its improvement. Attempts were made by a few scientists to induce mutations by ionizing radiation with limited success. Similarly limited anatomical studies have been conducted on *P. tuberosa*. 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, commonly associated with seed propagation. The mechanical tissue system in the scape provides stability to the inflorescence by keeping it erect after flowering, which is beneficial in the event of an increase of flower size and spike height. Anderson and Abby (1933) observed some anatomical abnormalities in the vascular bundles of mutant plants of *Aquilegia*, including a deviation in the anatomical structure in comparison to the untreated control. Stem anatomical structures can sometimes help in species identification (Sarkar *et al.* 2011).

MATERIALS AND METHODS

The materials consist of three indigenous varieties of tuberose viz. 'Calcutta Double', 'Prajwal' and 'Shringar'. Uniform size bulbs of each variety were treated for 4 h with 0.5% and 0.25% of a chemical mutagen, EMS (ethyl methane sulphonate; E. Merck, Germany) following a standard chemical mutagen treatment. Twenty bulbs of each treatment along with their untreated control of each variety were grown at the Horticulture Research Station of Bidhan Chandra Krishi Viswavidyalaya, Mondouri, Nadia, West Bengal (India). Data relating to sprouting and survival, vegetative, floral and pollen sterility were recorded from the entire plant population during the M_1 generation. The anatomy of the floral stalk (scape) of different treatments as well as their control were studied from the transverse section (TS) of the scape of *P. tubeorsa* which were passed through different grades of alcohol (Jiangsu Huaxi international Trade Co. Ltd.) starting from 30 to 100% and stained with 50% saffranin (E. Merck Ltd., India) and 95% light green (Loba Chemie Pvt. Ltd.) following a standard anatomical staining procedure. Finally, the sections were kept in clove oil (E. Merck) and xylene (E. Merck) mixture (1:1 v/v) for 1 min for cleaning and mounted on a glass slide in a drop of DPX (E. Merck) medium. The slides were placed on a hot-plate overnight or until the DPX solution fully dried up to make the section permanent for future studies. The anatomical peculiarities were studied and described from this permanent slide and photographs were taken with the help of a ZEISS microscope using a 15X ocular lens and a 40X objective lens. Stomata were extracted from the abaxial and adaxial surface of leaves by peeling and then studied under an Aexioscope 40 (ZEISS) microscope after placing it in 5% glycerin to evaporate water. The length and breadth of guard cells and stomatal were determined under 15X × 40X magnification and a field area of 304 µm × 242 µm.

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Research Note

Table 1 Estimation of stomatal area in control and EMS-treated tuberose bulbs.

Notation	Treatment details	Stomatal area (μm^2)
T_1	Control Calcutta Double (V_1)	1809.96*
T_4	V_1 + 0.5% EMS	1499.19*
T_5	V_1 + 0.25% EMS	1155.65*
T_2	Control Prajwal (V_2)	1727.31*
T_6	V_2 + 0.5% EMS	2141.64*
T_7	V_2 + 0.25% EMS	1719.61
T_3	Control Shringar (V_3)	1589.87*
T_8	V_3 + 0.5% EMS	1439.45
T_9	V_3 + 0.25% EMS	1342.61*
Grand mean		1616.71
SEm (±)		75.77
LSD (0.05)		231.41

Note: * significant

RESULTS AND DISCUSSION

Effect of EMS on morphological characters

Some changes in the number of tepals of individual flowers were observed (5-8) unlike normal tepal number (6), in 'Shringar' and in 'Prajwal'. Variation was observed in 'Prajwal' flowers with 0.5% and 0.25% EMS (**Figs. 1A, 1B, 1D**) and 'Shringar' with 0.25% EMS (**Fig. 1C**).

Effect of EMS on stomatal characters

There were significant differences between stomatal area of leaves of untreated control and 0.25% and 0.5% EMS-treated leaves in 'Calcutta Double' (**Figs. 2.1 A-C**). Similar differences with respect to stomatal area (**Table 1**) were also observed in 'Prajwal' (0.5% and 0.25%, **Figs. 2.2 B, 3C**, respectively) compared to the control (**Fig. 2.2 A**). Similar changes were also visualized in 'Shringer' (**Figs. 2.3 A-C**). Stomatal dimension is an effective method for the determination of ploidy in plants.

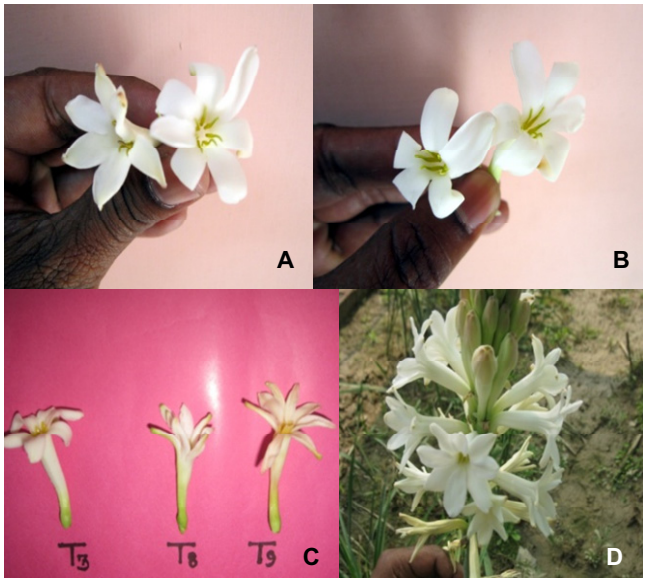

Fig. 1 Morphological changes in *Polianthes* due to treatment of EMS. (**A-B**) Flowers having seven and five tepals in EMS treated progeny of 'Prajwal' (M_1 generation) along with their respective control (T_3); (**C**) individual flower from 0.25% EMS (T_9)-treated 'Shringar' showing 8 tepals with 0.5% EMS (T_8) treatment and untreated control (T_3); (**D**) inflorescence of EMS-treated 'Prajwal' having more than six tepals in the flowers.

Effects of EMS on scape anatomical characters

The internal anatomical structure of the scape of three tuberose varieties (untreated control) exhibited a certain amount of dissimilarity with respect to distribution pattern, number and arrangement of vascular bundles and mechanical tissues. Unlike other monocotyledonous plants, the scape exhibited special features in the mechanical tissues

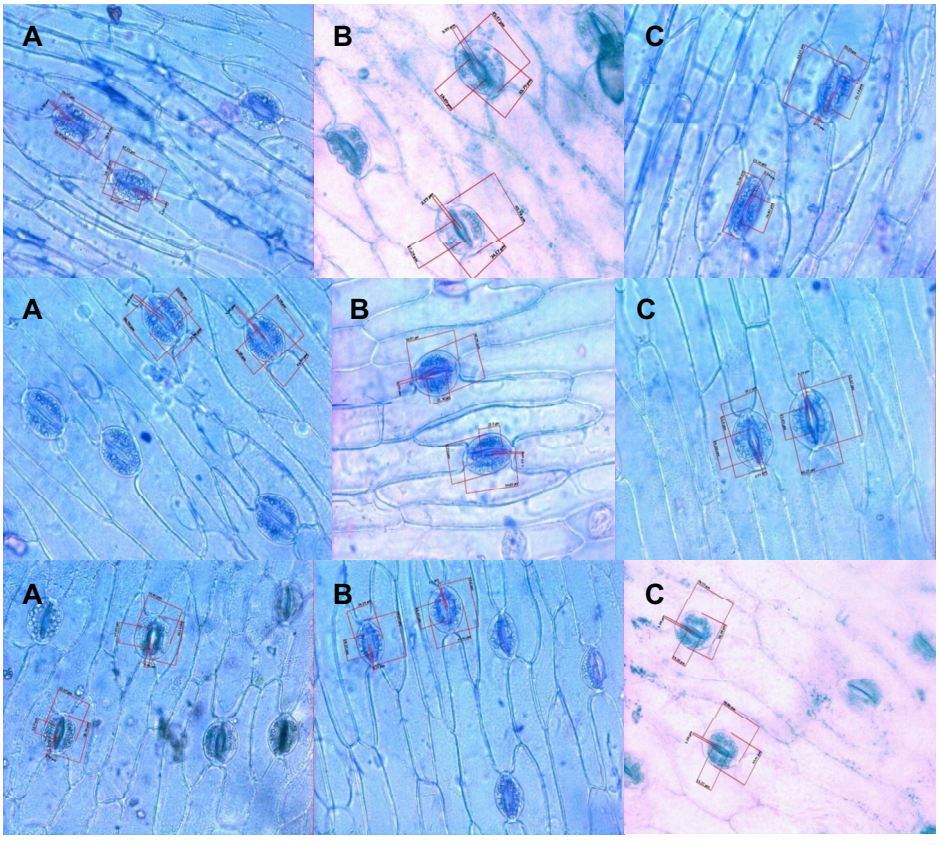

Fig. 2 Leaf anatomy of 2.1 'Calcutta Double' (top row), 2.2 'Prajwal' (center row) and 2.3 'Shringar' (bottom row) showing stomata and their size measurement. (**A**) Untreated control; (**B**) 0.5% EMS treatment; (**C**) 0.25% EMS treatment.

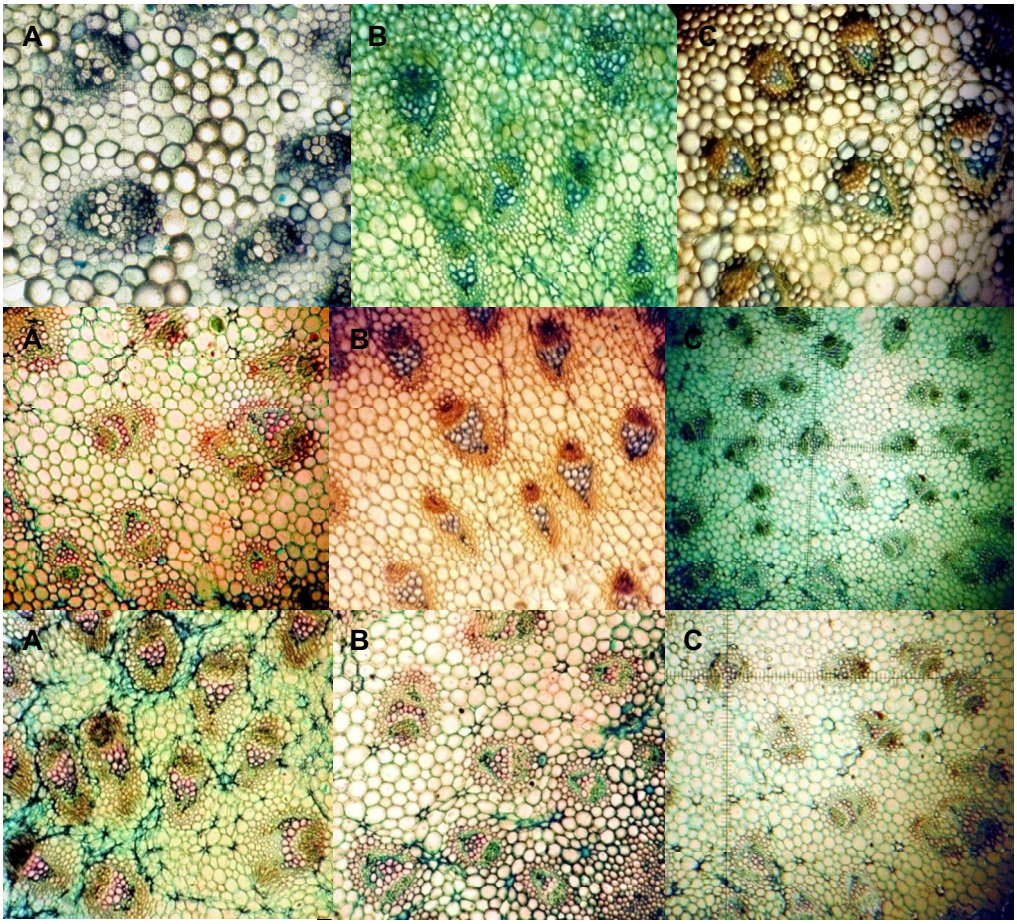

Fig. 3 Anatomical changes in 3.1 'Prajwal' (top row), 3.2 'Shringar' (middle row) and 3.3 'Calcutta Double' (bottom row) after EMS treatement. (**A**) T.S. of scape (untreated control); (**B**) and (**C**) T.S. of 0.5% and 0.25% EMS-treated scape, respectively.

(sclerenchyma) which did not form a sheath surrounding the whole bundle but remained in two patches on the outer and inner side of the bundle. The patches on the outer side on the periphery of the scape were large, forming a cap-like structure while patches on the inner side were proportionally much smaller (**Figs. 3.1 A, 3.2 A, 3.3 A**). The existence of increased protoxylem in the scape anatomy of 0.5% EMS (**Fig. 3.1 B**)-treated material relative to the untreated control suggests better absorption (nutrient) potential from the soil. The localization of sclerenchyma cells surrounding the vascular bundles in the 0.25% EMS-treated sample instead of in two patches as found in the control in 'Prajwal' (**Figs. 3.1 A, C**) is a notable anatomical deviation. In 'Shringar' treated with 0.5% EMS, the xylem was Y-shaped (**Fig. 3.2 B**) and sclerenchyma cells were not prominent, possibly due to damage of mechanical tissues, exhibiting yet another notable feature. However, when treated with 0.25% EMS, sufficient sclerenchyma cells formed, an indication of strong mechanical support to the scape (**Fig. 3.2 C**). In 'Calcutta Double' treated with 0.5% and 0.25% EMS, the vascular bundle was larger than the control, suggesting better functionality of the mechanical tissue (**Figs. 3.3 B, C**). Morpho-anatomical changes were observed in the leaves of *Gladiolus* and *P. tuberosa* infected with foliar nematode (Roy *et al.* 2010, 2011). Stem anatomy, particularly the vascular bundles and their arrangement of different vascular tissues, is helpful for identifying plant species (Sarkar *et al.* 2011). The changes in the scape (stem) anatomy in response to treatment with a mutagen like EMS, including intervarietal and intertreatment differences, indicate that, similar to qualitative and quantitative characters, anatomical features are also amenable to alteration by mutagenic treatment (Anderson and Abby 1933). Since a large number of flowers are borne on the floral axis (scape), strengthening the mechanical tissue system through induced mutagenesis may be a unique tool for improving tuberose.

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