



## Palynological and Stomatal Characters Influenced by Gamma Rays in Gladiolus Cultivars

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### Abstract

An experiment was conducted at the experimental farm of Department of Floriculture and Landscaping, Punjab Agricultural University Ludhiana (Punjab). Uniform size (4cm) and healthy corms of gladiolus varieties viz. 'Punjab Glance' and 'Sylvia' were irradiated with different doses (0,50,100,125,150 Gy) of gamma rays. Each treatment was replicated thrice. Results revealed that pollen fertility, size, stomata number, size and number of chloroplast decreased with the increasing dose of irradiation. In acetocarmine test, pollen fertility was recorded maximum in non-irradiated pollen in both varieties (91.96, 90.70 %) while minimum viability was observed in both varieties treated with 150 Gy. Maximum pollen size, number of stomata, stomata size and number of chloroplast per guard cell was recorded in 50 Gy and minimum at 150 Gy as compared to control in both varieties. Out of two varieties 'Punjab Glance' exhibits more palynological and stomatal characters.

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### Introduction

Gladiolus (*Gladiolus hybridus* L.) is an important bulbous flower crop and is very popular as cut flower both in domestic and international market. It belongs to family Iridaceae. It is universally acclaimed prestigious cut flower (Ram *et al.*, 2005). It is relatively easy to grow and ideal for bedding and exhibition. In India, gladiolus is commercially grown in West Bengal, Maharashtra, Uttar Pradesh, Punjab, Delhi and Rajasthan (Singh, 2006). Ever increasing demand particularly in cities and towns of India make it an important cut flower which is now available in the Indian markets round the year. It is propagated through corms. Gamma rays are used for improving growth and quality of plants, for their high mutation frequency and can interact with atoms and

molecules, thus producing free radicals in cells that affect morphology, anatomy, biochemistry and physiology of the plants (Chahal and Gosal, 2002). Mutation breeding by gamma rays is one of the most powerful methods for developing new varieties, which is very successful in ornamental crops. Jain (2006) stated that induced mutations are highly effective to enhance natural genetic resources for vegetatively propagated crops. Several improvements in ornamental crops could be achieved by gamma mutation, including characters of flowers, leaves, growth habit and physiological traits (Schum and Prell, 1998). Radiation mediated morphological, structural and functional changes in a plant are governed by the intensity and duration of the gamma rays which generally induce cytological, biochemical, physiological, morphological and

genetically changes in cells and tissues (Chandrashekar *et al.*, 2013).

The literary data on the pollen morphology of Iridaceae family is rather limited. Rodionenko (1961) researched the pollen grain morphology of 73 species of *Iris*, 24 species of *Crocus*, using light microscope. Pollen longevity largely depends on the abundance of sucrose which protects the membrane integrity (Hoekstra *et al.*, 1989). Plant species with short-lived pollen contain low amounts of soluble carbohydrates, while those with long-lived pollen have higher soluble carbohydrate content and include cytoplasmic polysaccharides. The effect of gamma radiation on pigment was studied by Palamine *et al.*, (2005) and Jinxi (2006) on *Anthurium andraeanum* and dracaena and they found that 20 Gray decreased the ratio of chlorophylls a and b found a negative correlation between gamma dose and chlorophyll. Chandrashekar *et al.*, (2013) on *Terminalia arjuna*, observed that chlorophyll content decreased with increasing doses. The aim of this study is to give a complete description of the surface structure of leaves and pollen grains of the gladiolus varieties 'Punjab Glance and Sylvia' through gamma irradiation.

## Materials and Methods

The present investigation was carried out during 2014-2015 and 2015-2016 at experimental farm PAU Ludhiana, Punjab during winter season. The geographical location of PAU is at 30° 54' North (latitude) and 75 ° 48 East (longitude) at the height of 247 m above the sea level. Uniform size (4cm) and healthy corms of gladiolus varieties *viz.* 'Punjab Glance' and 'Sylvia' were irradiated with different doses (0,50,100,125, 150 Gy) of gamma rays at College Orchard, Department of Fruit Science, Punjab Agricultural University, Ludhiana using gamma rays Low Dose Irradiator 2000 ANSI-N 433.1. These corms were planted in the field with in 24 hrs of treatment with spacing of row to row 30cm and corm to corm 20cm. The experiment was laid out in Randomized Block Design with three replications. Observations on palynological (pollen fertility and size) and stomata characters (number of stomata, stomata size, number of chloroplast per guard cell and chlorophyll content) were recorded.

To determine the pollen fertility (%), pollen grains collected soon after anthesis were stained with drop of acetocarmine and viewed under microscope (Leica Bright Field Research Microscope) at 200X. Uniformly

stained and round pollens were considered as viable whereas unstained and broken as non-viable and the percent pollen fertility was worked out. Size of well-stained pollen grains (in  $\mu\text{m}$ ) was recorded under microscope for observing stomata characteristics.

A drop of an adhesive (Quick fix) was applied to the abaxial (lower) surface of leaf and spread thoroughly over an area of 1-2 cm<sup>2</sup> during mid-day (11:00 -13:00 pm) while they are still attached to plant. When solidified, the replica containing impressions of epidermis and stomata was stripped off the leaf and observed under microscope.

To determine the number of chloroplasts per guard cell, a drop of I-KI was placed on the peel, removed from abaxial surface of leaf and allowed to react for >5 min. The peel was then washed with deionised water. Starch containing regions (chloroplasts) were stained. After washing, the peel was observed under microscope at 400X.

To determine the chlorophyll content (Hiscox and Israelstam, 1979) freshly removed leaves were finely chopped and 50 mg portion was dipped in a test tube containing 10 ml of DMSO (Dimethyl Sulphoxide). The test tubes were then placed in an oven at 60 °C for about two hours to facilitate the extraction of pigments. After requisite period, the extract was allowed to reach the room temperature and absorbance was read at 645 and 665nm on a spectrophotometer (SL- 171). The chlorophyll content was quantified by using the following equation:

$$\text{Total Chlorophyll} = [20.2(A_{645}) + 8.02(665)] \times V/1000 \times W$$

Total Soluble Sugar Content (mg/g) was determined based on the method given by (Dubois *et al.*, 1951). Dried leaves (0.1g) were added with 5 ml of 80 % ethanol to test tubes, placed in water bath, and heated for 1 hour at 80 °C. Then, 1 ml of the sample extract was taken in another set of test tubes and mixed with 1 ml each of 18 % phenol and distilled water, and then allowed to stand at room temperature for an hour. Finally, 5 ml of sulphuric acid was added and the whole mixture was vortexed. The absorbance was read at 490 nm wavelength on the UV spectrophotometer. Ethanol 80 % was used as blank sample. During 1<sup>st</sup> and 2<sup>nd</sup> years pooled data for all the parameters were statistical analyzed using Duncan Multiple Range Test, (Duncan 1955).

## Results and Discussion

### Radiation effect on Palynological characters

To study the effect of gamma rays on palynological characters, observations were recorded on parameters viz. pollen fertility and pollen size are presented in Table 1.

The appraisal of table elucidate that gamma ray treatment have significantly affected the pollen fertility in both varieties in Plate 1. At higher doses of gamma rays more pollen grains were remain unstained which shows reduction in fertility. Maximum pollen fertility (91.33 %) was recorded in untreated corms i.e. control which was at par with 50 Gy (81.56 %) and 100 Gy (70.37 %). The results also indicate that pollen fertility decreased significantly at 150 Gy gamma rays treatment (42.50 %). Out of two varieties, maximum pollen fertility was recorded in variety 'Punjab Glance' (70.61 %) and it was 67.13 % in 'Sylvia'. The data of interaction reveals that among varieties and gamma rays treatments that maximum pollen fertility (92.74 %) in variety 'Punjab Glance' during the second year in control followed by (91.19 %) in first year at control. Pollen Fertility was decreased as the dose of gamma rays was increased to 150 Gy. Minimum fertility of pollen was observed in 'Sylvia' (40.41 %) during first year.

The critical rummage of data indicates that different doses of gamma rays had significant effect on pollen size in gladiolus varieties 'Punjab Glance' and 'Sylvia'. Maximum pollen size (34.34  $\mu\text{m}$ ) was recorded in treated corms at 50 Gy which were at par with control (33.60  $\mu\text{m}$ ) and 100 Gy (26.82  $\mu\text{m}$ ). Pollen size was decreased significantly at 150 Gy (16.13  $\mu\text{m}$ ). Out of two varieties, maximum pollen size was recorded in variety 'Punjab Glance' (28.64  $\mu\text{m}$ ). It was 26.42  $\mu\text{m}$  in 'Sylvia'. The data of interaction reveals that among varieties, gamma rays treatment and years indicate that maximum pollen size (36.48  $\mu\text{m}$ ) was found in variety 'Punjab Glance' during the second year at 50 Gy followed (36.00  $\mu\text{m}$ ) in second year at 0 Gy. Pollen size decreased as the dose of gamma rays were increased to 150 Gy. The minimum pollen size was observed in (15.37  $\mu\text{m}$ ) in 'Sylvia' during first year.

### Radiation effect on Stomatal characters

The appraisal of table elucidate that gamma rays treatment have significantly affected the number of stomata in both varieties are presented in Table 1.

Maximum stomata number (15.11  $\text{mm}^2$ ) was recorded at 50 Gy which was at par with control (14.59  $\text{mm}^2$ ) and 100 Gy (12.26  $\text{mm}^2$ ). Number of stomata was decreased significantly at 150 Gy gamma rays treatment (5.12  $\text{mm}^2$ ).

Out of two varieties, maximum stomata number was recorded in variety 'Punjab Glance' (11.58  $\text{mm}^2$ ). It was 10.60  $\text{mm}^2$  in 'Sylvia'.

The data of interaction reveals that among varieties, gamma rays treatment and years indicate that maximum stomata size (15.89  $\text{mm}^2$ ) in variety 'Punjab Glance' during the second year at 50 Gy followed by (15.61  $\text{mm}^2$ ) in second year at 0 Gy. Number of stomata was decreased as the dose of gamma rays were increased to 150 Gy that is minimum number of stomata was recorded in (4.78  $\text{mm}^2$ ) in 'Sylvia' during first year.

The appraisal of table elucidates that gamma rays treatment have significantly affected the stomata size in both varieties are presented in Fig 1. Maximum stomata size (31.70  $\mu\text{m}$ ) was recorded in treated corms which were at par with control (25.90  $\mu\text{m}$ ) and 100 Gy (21.09  $\mu\text{m}$ ).

Stomata size was decreased significantly at 150 Gy gamma rays treatment (8.88  $\mu\text{m}$ ). Out of two varieties, maximum stomata size was recorded in variety 'Punjab Glance' (21.57  $\mu\text{m}$ ). It was 20.20  $\mu\text{m}$  in 'Sylvia'.

A significant difference was recorded in number of chloroplast per guard cell, after gamma ray treatment in both varieties. Maximum number of chloroplast per guard cell (6.66) was recorded at 50 Gy which was at par with control (5.46) and 100 Gy (4.79). It is evident from the results that chloroplast number per guard cell decreased significantly at 150 Gy gamma rays treatment (2.72). Out of two varieties, maximum number of chloroplast was recorded in variety 'Punjab Glance' (4.86). It was 4.44 in 'Sylvia'.

The observation recorded on chlorophyll content of gladiolus varieties 'Punjab Glance' and 'Sylvia' treated with different doses of gamma rays are presented in Fig. 2 Maximum chlorophyll content (0.067  $\text{mg g}^{-1}$ ) was recorded at 50 Gy which was at par with 0 Gy (0.063  $\text{mg g}^{-1}$ ) and 100 Gy (0.058  $\text{mg g}^{-1}$ ). Chlorophyll content was decreased significantly at 150 Gy gamma rays treatment (0.034  $\text{mg g}^{-1}$ ). Out of two varieties, maximum chlorophyll content was recorded in variety 'Punjab Glance' (0.598  $\text{mg g}^{-1}$ ). It was 0.474  $\text{mg g}^{-1}$  in 'Sylvia'.

**Table.1** Effect of different doses of gamma rays on palynological and stomatal studies of gladiolus varieties ‘Punjab Glance’ and ‘Sylvia’

Doses of gamma rays (Gy)	Pollen fertility (%)					Pollen Size (µm)					Number of Stomata (mm <sup>2</sup> )				
	Punjab Glance		Sylvia		Mean	Punjab Glance		Sylvia		Mean	Punjab Glance		Sylvia		Mean
	2014 - 15	2015 - 16	2014 - 15	2015 - 16		2014 - 15	2015 - 16	2014 - 15	2015 - 16		2014 - 15	2015 - 16	2014 - 15	2015 - 16	
0 (control)	91.19	92.74	90.34	91.07	91.33 <sup>a</sup>	34.41	36.00	31.15	32.85	33.60 <sup>a</sup>	14.98	15.61	13.78	14.00	14.59 <sup>a</sup>
50	82.74	85.15	78.45	79.93	81.56 <sup>b</sup>	35.25	36.48	32.19	33.45	34.34 <sup>a</sup>	15.26	15.89	14.52	14.81	15.11 <sup>a</sup>
100	70.00	72.48	69.08	69.93	70.37 <sup>c</sup>	27.11	27.48	25.52	27.19	26.82 <sup>b</sup>	12.37	12.82	11.82	12.07	12.26 <sup>b</sup>
125	54.52	57.37	51.59	53.00	54.11 <sup>d</sup>	24.63	26.37	21.00	22.34	23.58 <sup>c</sup>	7.93	8.19	6.97	7.11	7.55 <sup>c</sup>
150	42.52	45.34	40.41	41.74	42.50 <sup>e</sup>	15.97	16.89	15.37	16.30	16.13 <sup>d</sup>	5.26	5.41	4.78	5.04	5.12 <sup>d</sup>
Mean	68.19	70.61	65.97	67.13	67.97	27.47	28.64	25.04	26.42	26.89	11.16	11.58	10.37	10.60	10.92
LSD (p=0.05)	Treatments (A) = 1.84; Varieties (B) = 1.16; A x B = 1.64					Treatments (A) = 1.88; Varieties (B) = 1.19; A x B = 1.68					Treatments (A) = 0.68; Varieties (B) = 0.43; A x B = 0.61				

**Fig.1** Effect of gamma irradiation on Stomata Size and Number of chloroplasts in gladiolus varieties ‘Punjab Glance’ and ‘Sylvia’

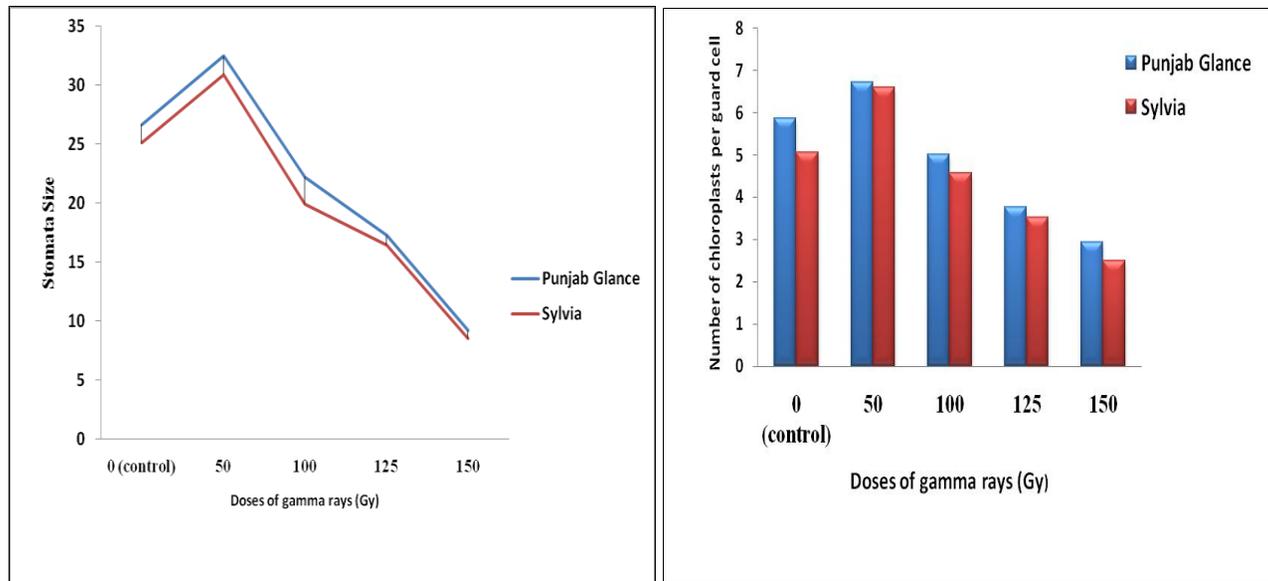


Fig.2 Effect of gamma irradiation on chlorophyll content ( $\text{mg g}^{-1}$ ) and Total Soluble Sugars of gladiolus varieties 'Punjab Glance' and 'Sylvia'

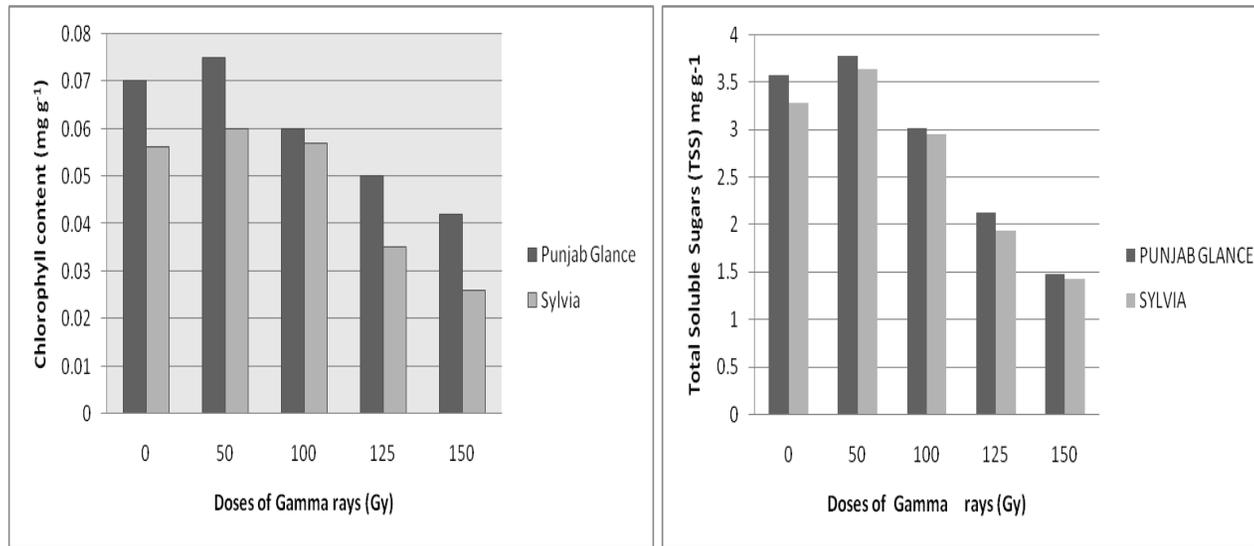
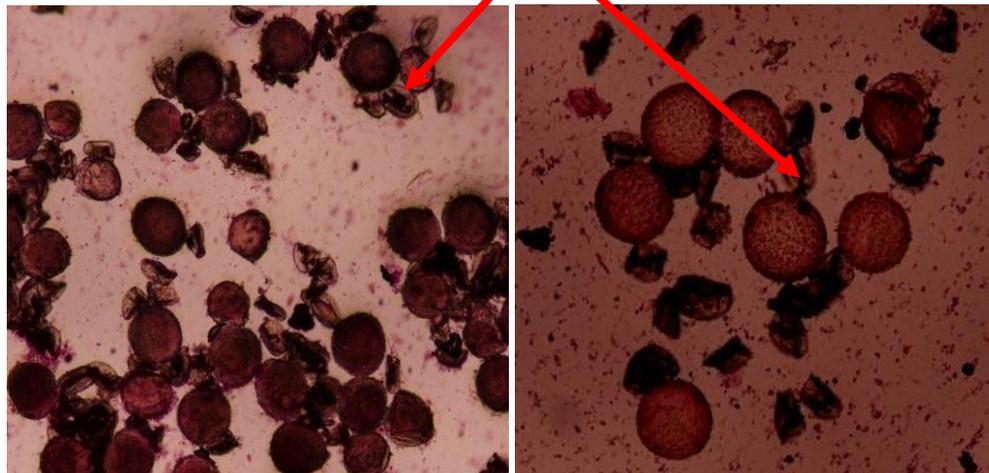


Plate.1 Pollen grains under Leica Bright Field Research Microscope

Non-Viable pollen



Punjab Glance (100 Gy)

Sylvia (100)

It is evident from the Fig 2 indicates that treatment of corms with gamma rays have significant effect on sugars in varieties 'Punjab Gance' and 'Sylvia'. Maximum soluble sugars ( $2.40 \text{ mg g}^{-1}$ ) were recorded at 50 Gy which was at par with 0 gy ( $2.10 \text{ mg g}^{-1}$ ) and 100 Gy ( $1.90 \text{ mg g}^{-1}$ ). Sugar content was decreased significantly at 150 Gy gamma rays treatment ( $1.06 \text{ mg g}^{-1}$ ). Out of two varieties maximum sugar content was recorded in variety 'Punjab Gance' ( $1.91 \text{ mg g}^{-1}$ ). It was  $1.64 \text{ mg g}^{-1}$  in 'Sylvia'.

The decrease in pollen viability is mostly caused by excessive nutrient consumption due to high intensity of irradiation. Low dose of gamma rays contains starch and has high amount of sugars which provides the respiratory substrates for pollen and extends pollen viability. The reduction in pollen fertility is due to chromosomal aberrations and the rate of reduction in pollen fertility increased at higher doses. This might be due to high amount of chromosomal damage by gamma irradiation (Kumar *et al.*, 2013). Irregular meiosis is known to result in high pollen sterility following gamma rays treatment in gladiolus (Buiatti and Ragazzini 1965). Datta (1988) also obtained similar palynological investigation of mutants developed after gamma irradiation and colchicines treatment in chrysanthemum. Dhaduk (1992) had similar results in which he found reduction in pollen fertility in cvs. 'Melody', 'Rose Supreme' with 7 kR and 10 kR of gladiolus. Matsuo and Onozawa (1961) reported that gamma radiation generally reduces the reproductive ability of the plant and increases the number of sterile pollen much more than the environmental effects.

Significant decrease in number of stomata has been recorded in both varieties after gamma irradiation. Banerji *et al.*, (1996) irradiated rooted cutting of chrysanthemum cv. 'Navneet' with 1.5, 2.0, 2.5, 4.0, 6.0 and 8.0 kRad of gamma rays and observed reduction in stomata number. Significant difference was recorded by Datta and Banerji (1993) for number of chloroplasts per guard cell in mutants developed after gamma rays treatment.

Irradiation caused some breakdown in chloroplastic structure resulting more degradation of chlorophyll. Hasbullah *et al.*, (2012) reported that chlorophyll was insensitive at low doses. The chlorophyll content of plants decreased after irradiation which might be due to the release of chlorophyll from its protein complex (Saha *et al.*, 2010).

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