



Expression of Floral Fasciation in Gamma-ray Induced *Gerbera jamesonii* Mutants

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Abstract

In order to induce variations for floral traits in *Gerbera jamesonii*, seeds obtained from controlled crossing among white gerbera genotypes were irradiated with different doses of gamma rays (1 to 5 kR). Induced floral fasciations were observed in M₁ seed raised plants of gerbera. Ring-fasciation and linear-fasciation were observed in mutant types which lead to deformed and asymmetric flower heads which are mostly male and female sterile. Fasciated flowers are recurrent in the mutant types in comparison to spontaneous floral fasciation in wild type (normal) plants which occurs occasionally and is male and female fertile. An increase in the number and arrangement of vascular bundles in the stalks of fasciated capitula suggests alterations in the shoot apical meristem of the mutated plants. Different phenotypes of fasciated gerbera mutants suggest variable expressivity of the trait and attempts may be made to identify and utilize partially fertile fasciated genotypes in hybridization experiments with wild types for studying the genetic basis of fasciation. Fasciated gerberas can be propagated vegetatively to perpetuate their unusual forms and designated as cultivars of the species.

Key words: *Gerbera seed, linear-fasciation, ring-fasciation, somatic apical meristem, sterility*

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INTRODUCTION

Gerbera (Gerbera jamesonii) is an important commercial cut flower of the trade having extensive demand throughout the world due to its numerous colours, shapes and prolonged vase life. The demand of new types of varieties is always there in the market and mutation breeding in gerbera can effectively result in the development of new variants and possibly varieties for cultivation by flower growers. Mutation breeding has made major contributions in the development of many new colour/shape mutants in ornamental plants (Broertjes and van Harten 1988) and a number of gamma ray induced floret mutants and other morphological mutants have also been induced in other members of Compositae family such as chrysanthemum (Broertjes 1966; Datta et al. 1985, 2005) and sunflower (Jambhulkar 2002). So far, among more than 2300 officially released mutant varieties worldwide, 566 represent ornamental plants (Mohan Jain 2006).

Fasciation (also referred to as fusing or cresting) in plants is characterized by abnormal growth and development which potentially affects all parts (White 1948). Fasciated plant parts become broad ribbon like, often with many parallel ridges extending longitudinally from top to bottom.

A fasciated plant is also referred to as '*fasciata*' or '*cristata*'. Genetically conditioned fasciation is a mutation and widespread in higher plants. Usually, it is a monstrosity having little practical value. This anomaly is often combined with an increased number of flowers accumulated in the top region of the plant. The plants give rise to fasciated stalks resulting in altered floral development. Such mutants can be distinguished into different

types based on their phenotypic expression. Fasciated mutants in *Celosia* and *Salix* are prized and propagated for their ornamental value. The present study is first report of fasciated mutants of gerbera and describes different phenotypes of fasciated mutants induced through gamma irradiation of gerbera seeds. Fasciated gerberas may be considered as unusual forms of ornamental plants.

MATERIAL AND METHODS

Gerbera is a cross pollinated species and seeds were obtained for the study by hand pollinating genotypes of white flower gerbera population being maintained at the Institute of

Himalayan Bioresource Technology (CSIR), Palampur (HP), India. The seeds were subjected to gamma irradiation to induce floral variations. In order to standardize the mutagen treatments and determine the lethal dose (LD 50), seeds were irradiated with five different doses of gamma rays (1 to 5 kR) in Gamma chamber at the Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi, during November, 2007. Irradiated seeds from each dose along with control, were sown in pots under polyhouse condition. Observations were recorded to calculate LD 50 by examination of seedlings to obtain survival percentage. Individual plants obtained from different treatments were shifted to beds in polyhouse and observations were made for further evaluation. Observations were made on floral arrangement of these plants in comparison to wild type plants which formed normal flower heads. Morphological parameters viz., anther dehiscence and seed set after hand pollination were recorded on three capitula for each wild type and fasciated plants and single flower head in case of spontaneous fasciation from the onset of anthesis to maturity of the achenes. Histological analysis of flower stalks was done to determine the arrangement and number of vascular bundles in different types of fasciations by fixing the material for 24 hours in FAA (formalin/glacial acetic acid/ethanol/distilled water, 10: 5: 50: 35 v/v) at room temperature before being transferred to 70% ethanol (Fambrini et al. 2003). Sections of stalks were made from 1 cm below the capitulum of fully open flowers and stained in Delafield's haematoxylin.

RESULTS AND DISCUSSION

Based on survival of the seedlings in each treatment, mortality percentage was calculated and regression analysis was done to determine LD 50 value (2.32 kR). Mortality ranged from (24%) in 2kR treatment to 94% in 5kR with 19% in control. In 5 kR gamma irradiated seeds, only few seedlings survived while the remaining were observed to grow up to the cotyledon stage but failed to grow any further.

Three of the variant plants in M_1 generation (from 2 and 3kR irradiation treatment), formed abnormal floral shapes and the flowers were observed male and female sterile. However, the variants were distinct from spontaneous anomalies in floral shapes (fasciations) which occur occasionally in the wild types. While all variants share some phenotypic floral features, they can be distinguished into three types which are being designated as spontaneous fasciation, ring fasciation and linear fasciation. In comparison

to normal flower type (Fig. 1 a, b, c, d and e) spontaneous fasciation (Fig. 1 f, g, h, i and j) possibly results from fusion of two floral primordia at a developmental stage prior to floral bud formation. The bud appears compressed and at this stage a cleavage is formed in the centre of the disc. Flowers have distorted shape and it appears as if two flowers are joined in the centre. There is presence of two conjoined stalks which are visible up to the dorsal side of the capitulum. In case of spontaneous fasciation fertility is not affected and normal seed set is obtained on hand pollination.

Ring fasciations were observed in two plants, one each under mutagenic treatments of 2 and 3 kR doses of gamma rays. Compared to spontaneous fasciation, a ring shaped membranous invagination is formed at the centre of the disc in the floral bud which becomes prominent and assumes triangular or Y shape (tri-radiate types) distorting the floral plane (Fig. 1 k, l, m, n and o). The floral structure appears deformed and lignification of the central membranous ring takes place at a later stage. The disc florets are few and deformed and no anthers were observed protruding from them. Even seed set was not obtained upon hand pollination of the stigma emerging from ray florets.

Linear fasciation was observed in one plant under mutagenic treatment of 2 kR dose of gamma rays. Linear fasciation combined features of both spontaneous and ring fasciations (Fig. 1 p, q, r, s and t). Floral buds form a cluster of multiple inflorescences. The overall floral cluster appears as a large deformed flower and the membranous invagination is present at the centre of each inflorescence which becomes lignified at a later stage. Anthers were not observed in the cluster of capitula and no seed was obtained upon hand pollination. Multiple (three or four) stalks were observed on flowers exhibiting linear fasciation.

Other morphological features of fasciated flowers were also affected such as the fasciated plants formed a high number of ray flowers (Table 1) and the shape of receptacle turned wavy and invaginated. Except for spontaneous fasciation disc florets were mostly deformed and/or missing and were found in deeply invaginated and narrow spaces of the capitulum. The stalks of fasciated flowers had more number of vascular bundles than normal plants (Fig. 1 e, j, o and t). The floral features have been observed to be recurrent in both ring and linear fasciations as compared to spontaneous fasciation which is non-recurrent



Figure 1 Floral development in normal (a, b, c, d & e), spontaneous (f, g, h, i & j), ring (k, l, m, n & o) and linear fasciated (p, q, r, s & t) plants of *Gerbera jamesonii*. Flower buds (a, f, k and p), flower shapes (b, g, l and q), presence of fertile stigma and anthers (c and h), absence of fertile stigma and anthers (m and r), receptacle shapes (d, i, n and s). Transverse sections of flower stalks, stained in Delafield's haematoxylin. Scale bars: e = 1 mm; j = 1 mm; o = 1 mm; t = 1.5 mm

Table 1 Floral characters of fasciated mutant plants in comparison to wild type

Floral characters	Wild	Spontaneous	Fasciation types in M ₁ generation	
			Ring	Linear
Number of ray florets	48	91	97	247
Receptacle shape	Round	Elongated	Wavy	Wavy and invaginated
Disc	Normal	Normal	Deformed	Deformed
Mean number of vascular bundles	24	36	40	64
Range & standard deviation of vascular bundles	22-27 (2.64)	36 (-)	37-44 (3.60)	63-66 (1.73)

Fasciation is a rare trait overall, but has been observed in more than 100 vascular plant species, affecting dicots and monocots in 39 plant families and 86 genera (Tang and Knap 1998). The linear fasciation with a flat, ribbon shaped appearance is common while ring fasciation (the growing point fuses to form a funnel shape) also occurs. Considering the expression of fasciations in M_1 generation, it appears that the trait may be dominant. In *Celosia argentea*, spontaneously arisen fasciated lines have been used in horticulture for many decades referred to as crested cockscomb celosias. *Celosia argentea* var. *crispata* carries its fasciation via seed, being a genetically mutated tetraploid plant. Similar genotypes, obtained in mutagenic treatments, are available in *Amaranthus hypochondriacus* and could become of economic interest, as in the cockscomb (Behera and Patnaik 1979). Among cultivated plants, fasciated genotypes are of some importance for breeding purposes in *Pisum sativum*, which are well known since Mendel's experiments (*Pisum umbellatum*) and have been studied for theoretical and practical reasons (Gottschalk 1971).

Fasciation is also known in tomato fruits (beefsteak tomatoes) and ferns (with names such as 'monstrosa' and 'crispata' and are highly collectable plants). The fasciated willows, *Salix udensis* 'Sekka' and Japanese Fantail willow (*Salix sachalinensis*), are propagated from cuttings and used as ornamental plants. Geneve (1990) described bifurcated fasciations (linear fasciations which split to produce a 'Y' shaped double ribbon), multiradiate (stellate) shaped fasciations (the stem splits into three or more short branches) and ring fasciations (the growing point fuses to form a funnel shape).

Gerbera inflorescences are heterogamous with zygomorphic ray flowers located in the outermost whorl of the capitulum and actinomorphic disc flowers arrayed in arcs radiating from the centre of the capitulum as in sunflower (Berti et al. 2005). However, in the case of fasciated capitulum multiple inflorescences are inserted in a single receptacle (ring fasciations). This observation and other morphological features such as high number of ray flowers, wavy receptacle and increase in number of vascular bundles of the stalks have great resemblance to the floral features of *stem fasciated* recessive mutant in sunflower which possibly results due to enlarged shoot apical meristem constituted by a variable number of juxtaposed meristems (Fambrini et al. 2006). High endogenous levels of auxin are considered to be the cause of enlarged shoot apical meristem and altered phyllotaxis pattern of the fasciated plant. The tri-radiate types of fasciation are common in Compositae (White 1948) and Jambhulkar (2002) considers juxtaposition of meristems as a potential explanation for the phenotypes of another fasciated mutant of sunflower. In case of ring fasciation single stalk is formed suggesting that the shoot apical meristem domes are

not clearly separated, whereas in linear fasciation of gerbera (conjoined multiple stalks with different receptacles joined at the base) shoot apical meristem domes are probably enlarged but separated. Linear fasciation of gerbera exhibits certain features similar to the *Arabidopsis thaliana* mutants *mgo1*, *mgo2* and *mgo3* (Laufs et al. 1998; Guyomarc'h et al. 2004) that display stem bifurcation due to altered shoot apical meristem and root apical meristem. Also, in *Pisum sativum*, *Glycine max* and *Cicer arietinum*, the fasciated genotypes are characterized by a broad stem at the tip (Compton 1911; LaMotte et al. 1988; Knights 1993).

A drastic effect of ring and linear fasciation was observed on the shape and floret number of the capitulum implying high degree of expressivity. This is in accordance to the phenotype of other mutants characterized by enlarged shoot apical meristem such as *clv1* and *clv3* gene mutations in *Arabidopsis* where increase in organ number in all four floral whorls as well as total number of whorls was observed (Leyser and Furner 1992; Clark et al. 1993, 1995).

Regarding sterility of male and female parts in fasciated plants, a negative pleiotropic effect of fasciation is suggested (Fambrini et al. 2006). Male and/or female sterility has previously been reported among fasciated plants (Gottschalk and Hussein 1975). Similarly, sunflower fasciated mutants were also observed to have reduced pollen viability (Fambrini et al. 2006; Shattuck 1985). Compared to ring and linear fasciations, spontaneous fasciation is not a recurrent feature and was observed to occur sporadically in wild types, which otherwise formed normal flowers. Such phenotype may be a result of fasciation with incomplete penetrance and a very low degree of expressivity. The different phenotypes of fasciations in gerbera confirm the observation that the phenotype of fasciation mutants is often complicated by incomplete penetrance and expressivity as in soybean (Albertsen et al. 1983). Variable expressivity of fasciations in gerbera suggests multi-genic/allelic control of the trait. Earlier studies on fasciated mutants of *Pisum* indicate that the mutants are heterogeneous in their morphology as well as their genetic makeup showing strong, linear and weak fasciations (Gottschalk and Wolff 1983).

CONCLUSION

Based on the increase in number of vascular bundles, a possible role of altered shoot apical meristem is involved in expression of ring and linear fasciations of gerbera. Overall, fasciation describes a range of anatomical changes in vascular plants including broadened stems, multiple floral parts and arrangements leading to new types. The observed phenotypes of fasciation in gerbera suggest variable expressivity of the trait and attempts can be made to identify partially fertile fasciations which have potential use for

studying the genetic basis of fasciation. Fasciated gerbera plants can be propagated vegetatively to perpetuate their unusual forms and designated as cultivars of the species.

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