

## Cytogenetics of Garden Gladiolus I. Pollination Mechanism and Breeding System\*

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The structure and placement of stamens and stigma suggest that *Gladiolus* is essentially outbred. Anthesis occurs between 8.15 to 10.15 hr and anther dehiscence takes place soon after. Stigma becomes receptive nearly 1½ hr after anther dehiscence. Maximum pod development was observed only in hand-pollinated flowers, whereas natural cross-pollinated and bagged flowers showed considerable reduction in pod formation. Flowers are frequently visited by *Apis indica* which goes down the tube for nectar or may also alight directly on the anthers to collect pollen. Self-incompatibility has been found in three taxa, including one elemental species *G. tristis*. There is a graded series from fully self-incompatible to highly self-compatible garden cultivars. Floral biology together with the presence of self-incompatibility, suggest outcrossing. All the garden cultivars despite their hybrid origin depict a uniform breeding system.

**Key Words:** Pollination mechanism, Self-incompatibility, *Gladiolus*

### Introduction

An understanding of the breeding system is very important for tracing the patterns and pathways of evolution and for developing a methodology for genetic improvement. Apart from the presence of self-incompatibility in some taxa of *Gladiolus*, a study of breeding system became essential as different species and garden cultivars show differential response to selfing and crossing.

### Material and Methods

The study involved 40 garden cultivars and 8 species of *Gladiolus*. For pollen germination

studies, the stigma was profusely pollinated; complete pistils were fixed in 1:3 acetic alcohol after 48 hr. After washing, they were transferred to 80 per cent lactic acid at 60°C for 6 hr. After a second washing the material was stained with 1 per cent aqueous solution of fuchsin (acid) for 1 hr. The excess stain was washed out and the styles were mounted in lactic acid. The styles were split lengthwise into three parts before mounting.

### Results

Flowers in *Gladiolus* are borne on a spike and each flower is subtended by two spathe-

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like bracts. The perianth has six petaloid segments in two series and is basally connate. The size of the perianth tube varies among the cultivars from 1.5 cm to 4.5 cm. The three stamens are attached to the outer perianth segments to form an arch in front of dorsal lobe and are placed below the style and stigmatic branches (stylodia). The style is held below the upper central lobe of the perianth; above the stamens, its length varies in different cultivars from 5.0 to 8.0 cm.

A general survey of the cultivars shows that stigma overtops the anthers by 0.5 to 1.0 cm. In bud condition the anthers are adpressed to the stigma and both are at the same level. A shift in their relative position occurs at the time of anthesis as anthers move downwards at their point of attachment to the filament with stigma concurrently overgrowing them by 0.5 to 1.0 cm. (Figure 1).

All the species and cultivars open in the morning excepting the night-blooming *G. tristis*. Flower opening starts at 8.15 hr. and continues up to 10.15 hr. This is immediately followed by anther dehiscence. The stigma become fully receptive after  $1\frac{1}{2}$  hr. of anther dehiscence, which is indicated by the brightly shining papillae on the stylodia, and remains so for the next 24 to 30 hr. after which stigmatic tissue withers and becomes functionless. The mature stigma consists of three stylodia each folded in a V-shape and showing bilateral symmetry with rows of elongate papillae along the outer margins. The time of flower-opening, anther-dehiscence and stigma receptivity, however, depend on the ambient temperature which changes, being advanced on warm days and delayed on cooler days.

To ascertain the type of breeding system the following pollination studies were made:

**Open-pollination:** One hundred and fifty buds on different plants of *G. psittacinus* hybr. Type 1 were tagged and were allowed to open-pollinate and develop capsules under natural conditions. The average distance

between the individual plants was 20.0 cm. Only 32 flowers (24.66%) developed capsules.

**Natural cross-pollination:** To study the extent of natural cross-pollination, 100 buds of *G. psittacinus* hybr. Type 1 were emasculated and exposed to facilitate natural cross-pollination. Only 27 flowers developed into capsules, while the average distance between individual plants was 20 cm.

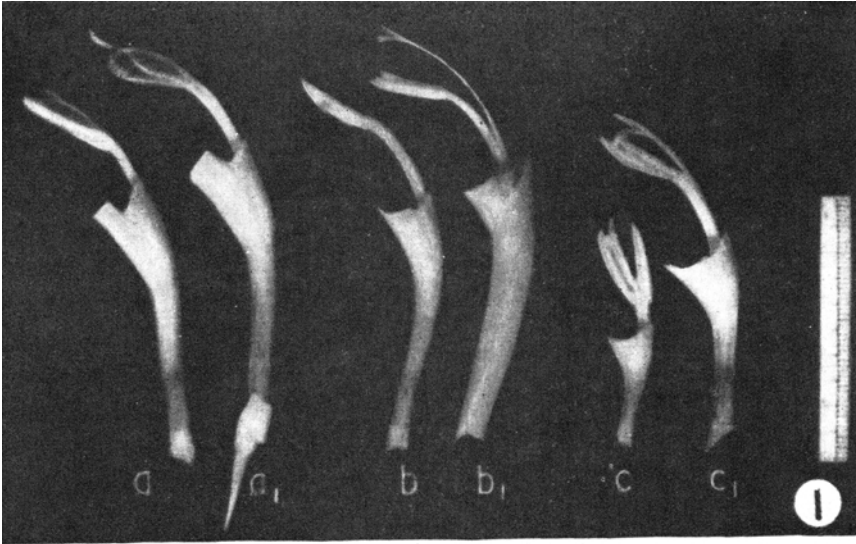
**Bagging:** Fifteen buds each from different cultivars, were bagged one day before anthesis, in order to see the possibility of selfing. None of the flowers developed capsule.

**Self-pollination:** Buds were emasculated and bagged one day before anthesis. Next day hand-pollination was made using pollen from the same or different flower on the same plant. The range of capsule formation on selfing of  $4 \times$  and  $5 \times$  cultivars was 0-86.6 and 33.3-94.1 per cent respectively. Self-incompatibility was observed in *G. tristis* ( $2 \times$ ) and *G.* 'La Paloma' ( $4 \times$ ) (table 1 Figure 2).

**Cross pollination:** Emasculated buds were cross-pollinated under controlled conditions with pollen from different cultivars. The range of capsule formation observed among crosses between  $5 \times \times 5 \times$  (17 combinations),  $5 \times \times 4 \times$  (162 combinations),  $4 \times \times 5 \times$  (15 combinations),  $4 \times \times 4 \times$  (33 combinations) was 20-70, 20-100, 20-80 and 20-80 per cent respectively.

*G.* 'La Paloma' ( $4+$ ) could be crossed with *Acidanthera bicolor* ( $2 \times$ ) when the former was used as female parent; similar was the case with *G.* 'Friendship' ( $4 \times$ )  $\times$  *G. tristis* ( $2 \times$ ) cross as well as *G. psittacinus* ( $5 \times$ )  $\times$  *G. tristis* ( $2 \times$ ) cross (table 1).

The above experiments suggest that gladioli are cross-pollinated. The low frequency of capsule formation under open-pollination and natural cross-pollination may be attributed to the absence of original insect vectors. However, in Lucknow, bees, especially *Apis indica* have been seen to visit



Figures 1-2 Relative position of stigma and stamens in (a, a<sub>1</sub>) *G. psittacinus* hybr. Type 1, (b, b<sub>1</sub>) *G. psittacinus* hybr. Type 3, (c, c<sub>1</sub>) 'Friendship' (a, b, c) Before anthesis (a<sub>1</sub>, b<sub>1</sub>, c<sub>1</sub>) After anthesis; 2 Pollen tubes of *G. tristis*, 48 hr after selfing (note swollen apices and inhibition of growth in stylar tissue) ×1500

the flowers attracted by the presence of profuse pollen as well as nectar. The three processes, viz. flower opening, anther dehiscence, and nectar formation occur almost simultaneously. The lower perianth segments act as landing sites; these also have nectar guides in the form of the streaks or blotches leading the bees to the base of the perianth where nectar is stored. The bees being small cannot bridge the gap between the landing site or the site of nectar collection and anthers and stigma. Consequently, nectar collection does not result in pollination. However, bees have also been seen to alight directly on the anthers and scuffle over them to collect pollen and while doing so they may cause self- or cross-pollination. The foraging of pollen may be so heavy that the entire anther sac is emptied within a short time after dehiscence. A breeder should be cautious in collecting the anthers in time. Besides bees, small Hymenoptera are also seen at the base of perianth tube. They only act as nectar robbers and do not make contact with anthers or stigma.

An important fact that has emerged from

the present study is the presence of self-incompatibility in *G. tristis* ( $2n = 30$ ), cvs. 'La Paloma' and 'Debonair' ( $2n = 60$ ). The cultivars form a graded series with respect to seed set under selfing with a totally self-sterile 'La Paloma' and 'Debonair' to a highly, self-fertile 'Solid Gold'. The pollen germination studies reveal that whereas in the self-incompatible taxa the pollen grains germinate on the stigma and penetrate stigmatic tissues, the tips of their tubes become swollen in the stylar tissue and stop growing further (figure 2). A few pollen tubes failed to penetrate the stigma but grew erratically on the stigmatic surface. In *G. psittacinus* hybr. Type 1 which is self-fertile, many pollen tubes were seen to stop growing at various levels in the style even 60 hr after self-pollination.

Cross-pollination of *Acidanthera bicolor* with various cultivars also gave interesting results. Its pollen was able to germinate on the stigma of all the cultivars, but did not penetrate the stigmatic tissue except that of 'La Paloma' and 'Pacifica' where it effected fertilization and the production of intergeneric hybrids (table 1).

Table 1 Results of reciprocal  $4 \times \times 2 \times$ ,  $5 \times \times 2 \times$  and  $5 \times \times 4 \times$  crosses

Ploidy ♀	♂	2×			4×		5× G. Psittacinus		
		<i>G. tristis</i>	<i>A. bicolor</i>	'La Paloma'	'Pacifica'	'Friendship'	Type 1	Type 2	Type 3
2×	<i>G. tristis</i>	SI	—	0	0	0	0	0	0
	<i>A. bicolor</i>	—	SC	0	0	0	0	0	0
4×	'La Paloma'	—	77.08*	SI	—	—	—	—	—
	'Pacifica'	—	25.0	—	SC	—	—	—	—
	'Friendship'	28.33	0	—	—	SC	—	—	18.14
5×	Type 1	60.44	0	15.94	11.26	6.0	SC	6.89	4.46
	Type 2	60.0	0	33.3	10.2	3.02	8.33	SC	12.06
	Type 3	50.0	0	4.54	4.0	6.12	1.58	8.5	SC

\*Percentage of seed germination; SI = Self-incompatible; SC = Self-compatible; 0 = Crosses unsuccessful; — = Crosses not attempted

## Discussion

It is evident from the study of breeding system that the species and cultivars of *Gladiolus* are out-crossers (see also Frankel & Galun 1977), and the latter despite their hybrid origin represent a uniform population with respect to their breeding system. The positional barrier between stigma and anthers, protandry, and self-incompatibility in *G. tristis* ( $2n=30$ ) and cvs., 'La Paloma' and 'Debonair' ( $2n=60$ ) strongly indicate an outbreeding system involving self-incompatibility alleles. This is also supported by Goldblatt (1971) who mentions that all the diploid South African species of *Gladiolus* studied by him are self-sterile. Lewis and Obermeyer (1972) in their comprehensive study of South African species of *Gladiolus* have remarked that in species such as *G. stellatus*, *G. brevitubus*, *G. gueinzii* and *G. floribundus* the perianth is actinomorphic and the stamens are placed symmetrically around central style. In all the other species the stamens are placed dorsally against the upper dorsal lobe with stigmatic branches overtopping them. This arrangement has been observed in all the garden cultivars studied by us. Additionally the anthers move slightly downwards, at the point of attachment to the filament, at the time of dehiscence, thus facilitating outcrossing. Apart from this, some South African species, e.g. *G. calcaratus* and *G. appendiculatus* have a specialized mechanism for insect pollination (Lewis and Obermeyer 1972). In these species the anthers are caudate, with appendages which hang down the throat of perianth tube, the insect while entering the tube pushes these appendages; consequently locules come down and deposit the pollen on the back of insect. Ingram (1967) made a curious observation in *G. grandis* growing in George Division of Cape Province. The flower colour which is brown during the day changes to a glaucous blue at dusk (and brown colour

is regained next morning) along with the development of fragrance to attract nocturnal insects such as moths. Similar may be the case with other night-blooming and scented species such as *G. tristis* and *G. liliaceous*, etc. All these mechanisms superimposed by self-incompatibility strongly facilitate outcrossing in species and garden cultivars of *Gladiolus*. This brings forth another point that the breeding system facilitating outcrossing has not changed under 150 years of domestication in the European gardens; present-day garden cultivars being highly heterozygous show deleterious effects in terms of vigour and fertility when inbred (Klein 1972, Durst 1975 present study). Thus the genetic system of garden cultivars shows a general adaptivity for heterozygosity and that homozygosity caused by inbreeding creates genotypically unfavourable conditions.

Self-incompatibility was first noticed in *Gladiolus* by Rawson (Darwin 1876) who found that crosses between plants of the same variety (clone) of *Gladiolus* did not set seed. According to Clarke et al. (1977) cuticle on the papillae form the sole physical barrier to pollen tube penetration in case of self-compatible pollination. However, the present study has shown that actual inhibition to pollen tube growth occurs inside the style. This means while pollen recognition takes place at stigma surface the inhibition in case of incompatible pollen occurs in the style. The binucleate pollen (Heslop-Harrison & Shivanna 1977) and pollen tube inhibition inside the style (present study) suggest a gametophytic system of self-incompatibility (GSI). Self-incompatibility has broken down in polyploid taxa, as all the polyploid species *G. italicus*, *G. psittacinus*, *G. communis* and *G. segetum* and garden cultivars (except 'La Paloma' and 'Debonair') studied presently are self-compatible. This was also pointed out by Lewis and Obermeyer (1972). The breakdown of self-incompatibility in polyploid taxa is

understandable in view of dominance/interaction of S-alleles in diploid pollen, and it is the general feature of the species having GSI (see Nettancourt 1977, pp. 113–114). The differential response to selfing shown by the tetraploid garden cultivars is due to their complex ancestry, because of which they possess a polyallelic series of S-genes which act in competition. The fully self-incompatible garden cultivars may be S-homozygous (see Nettancourt, 1977). This is in accordance with the competition interaction hypothesis of Lewis (1947). Besides this, the genetic background of the pollen-producing tetraploid also determines the self-compatibility behaviour, as has been observed in alsike clover (Brewbaker 1954), *Nicotiana glauca*

(Pandey 1968) and *Lycopersicon peruvianum* (Nettancourt et al. 1974), where different tetraploid lines with identical S-genotypes varied greatly in self-compatibility. The differential response to cross-compatibility of various garden cultivars to *Acidanthera bicolor* (Wright, 1971; present study) may also be a direct outcome of highly heterozygous nature of the former, in which certain cultivars possess such genotypes as are cross-compatible to *A. bicolor* resulting in intergeneric hybrids. However, in the light of the processes involved in pollination of *Gladiolus* as outlined by Knox et al. (1976) detailed biochemical studies are needed to substantiate the foregoing conclusions.

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