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Intraspecific Hybridization in Glory Lily

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ABSTRACT

Hybridization between genetically distinct populations of a single species can serve as an important stimulus for the evolution of invasiveness. Such intraspecific hybridization was examined in *Gloriosa superba*, a medicinal and an emerging cut flower species. The direct and reciprocal cross of the parents in the intraspecific crosses made among germplasm of *G. superba* resulted in more than 90% of pod set. The parent GS 09, either as male or female parent, gave maximum pod set, fresh pod weight, number of seeds per pod, fresh seed weight per pod. The F_1 hybrids recorded the maximum mean value for all the characters over the parental mean except number of leaves per plant. The means recorded were 18.28% germination, 51.41 days for germination, 11.01 cm seedling height, 6.06 cm root length, 314.03 for vigour index (VI), 1.74 cm microtuber length, 2.12 cm microtuber girth, and 1.04 g microtuber weight.

Keywords: Glory lily, intraspecific hybridization, crossing, F_1 seeds, seedlings

INTRODUCTION

Hybridization is a strong evolutionary force that can potentially reshape the genetic composition of populations and create novel genotypes that facilitate adaptation to new environments (Stebbins 1950; Anderson and Stebbins 1954; Arnold 1997). The importance of hybridization in evolutionary processes such as speciation has long been acknowledged (Darlington 1940; Stebbins 1969), but its application to the field of invasion biology has only more recently been discussed (Cox 2004; Schierenbeck and Arnouche 2006), as has the larger role of evolution itself (Lavergne and Molofsky 2007; Novak 2007). Hybridization between genetically distinct taxa has been proposed as a mechanism for the evolution of invasiveness in introduced and native species (Ellstrand and Schierenbeck 2000). Alternatively, such hybridization events can also produce outbreeding depression by disrupting co-adapted gene complexes and local adaptation in established species (Arnold 1997). Intraspecific hybridization has been carried out artificially for centuries to improve agriculturally or horticulturally important plant species (Khanduri and Sharma 2002; Johnston *et al*. 2003), but it has rarely been examined in natural populations, with few exceptions (Johansen-Morris and Latta 2006).

Gloriosa superba L., a climber belonging to the Colchicaceae family, is a major high value medicinal crop cultivated in Tamil Nadu. *Gloriosa* derives its name from the Latin word 'gloriosus', which means handsome and *superba* from the word 'superb' means splendid or majestic. The plant is known as glory lily, creeping lily or flame lily in English, Kazhappaikizhangu, Kanvalikizhangu, Karthigaikizhangu or Sengandhal malar in Tamil (Sundar *et al*. 2006). The genus *Gloriosa* comprises of 10 to 15 known species *viz*., *G. superba*, *G. lutea*, *G. plantii*, *G. latifolia*, *G. magnifica*, *G. rothschildiana*, *G. abyssinica*, *G. longifolia* and *G. simplex*. The important species found in India are *G. superba* and *G. rothschildiana* (Tarar and Vishwakarma 1995). Seeds and tubers contain valuable alkaloids *viz*., colchicine and colchicoside as the major constituents, which are used to treat gout and rheumatism (Prabirand Raghunath 1993).

The genetic variability also is low owing to the continued vegetative propagation through tubers which has reduced the vigour, tolerance to biotic and abiotic stress causing low yields. The growing demand for the seeds of *G. superba* in the international market and the wider popularity it has gained among the farmers necessitates attempts to induce new variability with high yield, high colchicine content, dwarf stature and leaf blight resistant of the plant as well. Traditional or conventional breeding has not been attempted so far as there is only one ecotype under cultivation and genetic wealth is limited. Introduction of new variability is the only option for the breeders at present to create new variability for selection of high yielding cultivars (Rajadurai 2001). Although a lot of information is available on the growth and development of *Gloriosa*, a limited attempt has been made to breed new cultivars in *G. superba*.

Hence there is an urgent need to explore the possibilities for developing variability in *Gloriosa* species with high seed yield and improved colchicine content through intraspecific hybridization.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2010-2012. The germplasm of *G. superba*, consisting of 18 ecotypes, were evaluated for genetic diversity in two seasons (2008-2010). Five entries which recorded high yield and quality among the 18 germplasm were selected as parents. Six hybrids were produced by crossing the selected five parents.

Selected parents for intraspecific hybridization

Germplasm	Notation
Nallampalayam cultivated	GS 01
Mulanur cultivated	GS 09
Pudhukottai cultivated	GS 13
Kunjapannai wild	GS 19
Krishnagiri cultivated	GS 20

Wild: Sourced from Kallar natural habitat, Tamil Nadu

Cultivated: Sourced from farmer's field of respective location.

Received: 31 August, 2012. Accepted: 4 October, 2012.

Original Research Paper

Fig. 1 Intraspecific hybridization in *Gloriosa superba*. (**A**) Flowers at pre-anthesis stage; (**B**) Emasculation; (**C**) Dusting of pollen; (**D**) Bagging; (**E**) Matured pods; (**F**) Harvested seeds; (**G**) Evaluation of F_1 hybrid seeds of *G. superba*.

Table 1 Intraspecific hybridization in *Gloriosa superba*.

Crosses	No. of flowers pollinated	Pod set percentage (%)	Days for pod maturity (days)	Fresh pod weight (g)	No. of seeds/pod	Fresh seed weight /pod (g)
GS 01 x GS 09	21	100.00	72.67	11.79	46.40	7.25
GS 09 x GS 01	33	90.90	76.50	16.53	56.80	6.60
GS 09 x GS 13	71	94.36	61.50	13.05	62.20	9.68
GS 13 x GS 09	74	91.89	73.60	10.36	47.00	6.74
GS 19 x GS 20	27	94.11	78.00	7.37	33.80	4.62
GS 20 x GS 19	21	90.47	75.00	7.78	31.60	4.10
Total	247					

GS 01	Nallampalayam cultivated	GS 19	Kunjapannai wild
GS 09	Mulanoor cultivated	GS 20	Krishnagiri cultivated
GS 13	Pudhukottai cultivated		

Crossing technique

A separate crossing block was maintained for the production of F_1 seeds. In the female parent, flowers at the pre-anthesis stage were selected for emasculation. Emasculation was carried out between 7.00 and 9.00 am and bagged with butter paper cover. Similarly, in the male parents, a few selected flower buds at the pre-anthesis stage were bagged without emasculation to avoid contamination by foreign pollen for collection of pollen grains. Pollen from bagged flowers of pollen parents were collected between 8.00 and 9.00 am in the next day morning and dusted on the stigma of emasculated flowers of the respective female parents. The flowers were bagged with butter paper covers and then labeled (**Fig. 1A-D**). The covers were removed after ensuring proper pod set (**Figs. 1E, 1F**). Observations were recorded on pod set percentage (%), days from pollination to pod maturity, fresh pod weight (g), number of seeds per pod, and fresh seed weight per pod (g).

Raising F_1 seeds

Dried seeds of parents and F_1 collected from intraspecific hybridization in *G. superba* were raised to evaluate the F_1 progenies. 500 seeds from each cross were sown after hot water treatment for 1 h (Anandhi 2009). Days to emergence, germination percentage, seedling height (cm), root length (cm), VI, length and girth of microtuber (cm) and fresh weight of microtuber (g) were assessed (**Fig. 1G**).

RESULTS

Intraspecific hybridization

GS 01 × GS 09 recorded the maximum pod set percentage (100%), followed by GS 09 × GS 13 which registered 94.36%. The cross GS 20 x GS 19 exhibited the minimum percentage (90.47%). The mean number of days for pod maturity ranged from 61.50 to 78.00 days in the intraspecific cross combinations. The days to maturity was minimum (61.50 days) in GS 09 × GS 13, while the duration was longer (78.00 days) in the cross combination GS 19 × GS 20. The fresh weight of the pod ranged from 7.37 g to 16.53 g. The intraspecific cross GS 09 x GS 01 registered the highest fresh pod weight (16.53 g) followed by GS 09 x GS 13 (13.05 g). The cross GS 19 × GS 20 registered the lowest pod weight (7.37 g). The cross combination GS 09 × GS 13 recorded the maximum number of seeds (62.20), fresh seed weight per pod (9.68 g).While, the number of seeds (31.60) and minimum weight (4.10 g) was recorded in GS 20 × GS 19. These results are presented in **Table 1**.

Evaluation of F_1 seeds

The mean data of the seedling characters of F_1 hybrids and parents are presented in **Table 2**.

Seed germination percentage

Among the parents, the seed germination percentage ranged from 11.00% in GS 01 to 18.00 in GS 19. Among the hybrids, seeds of the cross combination GS 01 × GS 09 regis-

Table 2 Mean performance of F_1 seeds of intraspecific hybridization in *Gloriosa superba*.

Crosses	Germination percentage (%)	Days to germination (days)	Seedling height (cm)	No. of leaves /plant	Root length (cm)	VI	Length of microtuber (cm)	Girth of microtuber (cm)	Weight of microtuber (g)	Colchicine content (% dry weight)
Parents										
GS 01	11.00	52.95	10.51	4.05	5.48	175.89	1.87	1.98	0.92	0.168
GS 09	17.50	57.55	10.68	3.80	5.80	288.40	1.75	2.01	0.83	0.170
GS 13	13.75	60.80	10.76	4.25	5.78	227.42	1.93	2.19	0.96	0.184
GS 19	18.00	49.99	9.80	5.05	5.34	272.52	1.38	1.75	0.73	0.162
GS 20	13.47	47.99	10.37	3.82	5.29	210.94	1.56	1.81	0.65	0.155
Mean	14.74	53.85	10.42	4.19	5.53	235.03	1.69	1.94	0.81	0.167
Hybrids										
GS 01 x GS 09	32.75	48.35	11.12	4.00	6.15	565.92	2.05	2.10	0.86	0.214
GS 09 x GS 01	16.25	56.75	10.65	4.40	5.63	264.71	2.84	2.34	1.76	0.161
GS 09 x GS 13	15.25	49.80	10.84	4.10	5.43	248.27	1.14	1.45	0.78	0.139
GS 13 x GS 09	13.75	55.00	10.62	4.15	5.78	225.63	1.73	3.29	1.47	0.242
GS 19 x GS 20	12.20	50.15	11.05	4.15	6.45	214.43	1.31	1.85	0.68	0.162
GS 20 x GS 19	19.50	48.45	11.78	4.00	6.94	365.23	1.39	1.71	0.71	0.173
Mean	18.28	51.41	11.01	4.13	6.06	314.03	1.74	2.12	1.04	0.181

GS 01	Nallampalayam cultivated	GS 19	Kunjapannai wild
GS 09	Mulanoor cultivated	GS 20	Krishnagiri cultivated
GS 13	Pudhukottai cultivated		

tered the highest germination percentage (32.75%); while GS 19 × GS 20 recorded the lowest percentage (12.20%).

Days taken for seed germination

Among the parents, GS 20 recorded earlier seed germination (47.99 days) while GS 13 recorded delayed germination (60.80 days). Among the hybrids, germination was earlier in GS 01 × GS 09 which took 48.35 days for germination while seeds of cross combination GS 09 × GS 01 took 56.75 days to germinate.

Seedling height at 120 days

The seedling height among the parents ranged from 9.80 cm in GS 19 to 10.76 cm in GS 13. Among the hybrids, the highest value for seedling height was recorded in GS 20 × GS 19 (11.78 cm) followed by GS 01 × GS 09 (11.12 cm). The lowest value was recorded in GS 13 × GS 09 (10.62 cm). The mean value of seedling height of hybrid seeds was 11.01 cm.

Number of leaves per plant

Among the parents, the mean number of leaves ranged from 3.80 to 5.05 in GS 09 and GS 19 respectively. Among the hybrids, the maximum number of leaves (4.40) was recorded in GS 09 × GS 01 while the minimum number (4.00) was recorded in GS 01 × GS 09 and GS 20 × GS 19.

Root length

The mean root length among the parents ranged from 5.29 cm in GS 20 to 5.80 cm in GS 09. The highest value for root length (6.94 cm) among the hybrids was observed in GS 20 × GS 19, while the lowest root length (5.43 cm) was observed in GS 09 × GS 13.

Vigour index

The VI ranged from 175.89 in GS 01 to 288.40 in GS 09 among the parents. In the seedlings of hybrid seeds, GS 01 × GS 09 recorded the highest VI (565.92) while GS 19 × GS 20 recorded the lowest VI (214.43).

Length and girth of the microtuber

The length of the microtuber among the parents ranged from 1.38 cm to 1.93 cm in GS 19 and GS 13. Among the hybrids, GS 09 × GS 01 registered the maximum length of 2.84 cm. The girth of the microtuber among the parents ranged from 1.75 cm to 2.19 cm in GS 19 and GS 13. Among the hybrids, GS 13 × GS 09 registered the maximum girth of 3.29 cm. The minimum length and girth of the microtuber (1.14 cm and 1.45 cm) was registered in GS 09 × GS 13.

Fresh weight of microtuber

The mean fresh weight of the microtuber ranged from 0.65 g in GS 20 to 0.96 g in GS 13 and the overall mean of parent was 0.81 g. Among the hybrids, the weight of the microtuber was maximum (1.76 g) in GS 09 × GS 01 while it was minimum (0.68 g) in GS 19 × GS 20.

DISCUSSION

Intraspecific hybridization is defined as successful matings between individuals from well differentiated populations originally isolated from one another and consequently with different gene frequencies (Stebbins 1950). As such, this process does not pertain to crosses between individuals from the same gene pool that possess different alleles (Arnold 1997). Intraspecific hybridization results in increased genetic variance, altered epistatic interactions, masking or unloading of deleterious alleles, and/or transfer of favorable genes (Lee 2002). If resulting F_1 hybrid individuals or later generation hybrids are fertile, recombination may lead to novel genetic rearrangements which can allow hybrids to expand their ecological tolerance and invade new niche environments (Stebbins 1959; Arnold 1997).

In the present study, total intraspecific cross made among germplasm of *G. superba* were 247, and the number of pods harvested were 221. Almost, all the direct and reciprocal crosses of the parents resulted in more than 90 % of pod set. This may be due to the genetic similarities of the parents (2n=22), that enable the hybridization in both the directions. Similar reports were given by Gupta and Raina (2001), who reported 90 % of pod set under controlled cross pollination in *G. superba*. The reports of Narain (1976) and Sudhendra and Rudre Gowda (1997) in *G. superba* and Omolaja (2009) in *Dioscorea floribunda* also support the present findings.

Evaluation of F_1 seeds

Gloriosa is commercially propagated through tubers. The plants raised from seeds take nearly three to four years or

growing season to flower. In *Gloriosa*, the seed germination is erratic and takes place in three weeks to three months (Farooqi and Sreeramu 2004). The F_1 seeds after germination produce the microtuber during the first growing season (Anandhi 2009).

In the present study, the growths of F_1 seedlings were evaluated for one growing season (6 months). The result showed that F_1 seedlings recorded the maximum mean value for all the characters over the parental mean except number of leaves per plant. The mean values recorded for seed germination was 18.28 %, days taken for germination was 51.41, seedling height was 11.01 cm, root length was 6.06 cm, VI was 314.03, microtuber length was 1.74 cm, microtuber girth was 2.12 cm, micro tuber weight was 1.04 g. Similar observation on seedling characters in glory lily was reported by Anandhi (2009). The F_1 progenies have to be forwarded to the next growing seasons to evaluate yield and quality.

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