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Short Communication

Development and evaluation of intervarietal hybrids of Asiatic lily (*Lilium* x *elegans* Thunb.)

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Abstract

Intervarietal crosses in Asiatic hybrid lilies (*Lilium x elegans* Thunb) were evaluated under Indian environmental conditions for their commercial value. Rapid and highest germination of 57.7% was obtained in Shiraj x Sumplon cross combination, whereas the hybrid Pollyanna x Stargater recorded 21% crossability. A total of 68 progenies were tested for their ploidy levels of which 48.5% were diploid, 10.3% triploid and 41.2% were mixoploids. A high magnitude of variation for different floral traits was recorded in the clones. Majority of triploids and most of the mixoploids produced large flowers with heavy textures. Triploids were considered superior to diploids. These observations indicated the possibility of developing indigenous hybrids of Asiatic *Lilium* for commercialization.

Key words: *Lilium* Asiatic**,** intervarietal crosses, ploidy, flow cytometery

The genus *Lilium* (2n= 2x=24) comprises of more than 90 species [1] and is classified into six sections. The Asiatic hybrid lily, one of the most important ornamental plants world wide, is derived from interspecific crosses. Among species of the section Sinomartagon *L. dauricum, L. maculatum, L. concolor, L. leichtlinii, L. davidii* and *L. cernum* are prominent and distributed in East Asia [2]. Cultivars of hybrid lily have wide range of flower colour, shape, size and morphological characteristics. As a cut flower, lily is now ranked as the fourth most important crop in the Netherlands. Lilies of ornamental importance are the results of crosses within three sections and interspecific hybrids within sections have been bred and cultivated widely since early 1800's [3]. Lilies breeders have tried to broaden the genetic base by a number of approaches, such as mitotic polyploidization [4] and sexual polyploidization from unreduced (2n) gametes [5-7]. Although both modes can double chromosome number, their relative efficacy is very different in genetic variability, epistasis, heterozygosity and ability of transferring specific genetic traits [6, 8]. However, intervarietal hybridization has also become a very effective way to increase genetic variability at ploidy levels within the sections. Asiatic cultivars belongs to diploid (2n=2x=24), triploid (2n=2x=36) and tetraploid (2n=2x=48) group [9].

Lilies are used as cut flowers and are very popular in India. Asiatic and Oriental lilies are commercially grown in hilly areas and in mild and tropical climates. To reduce the cost of bulb import and to make interesting lily cultivars available for Indian lily growers, a genetic improvement programme focused on suitability for Indian conditions and new commercial traits was initiated at Indian Agricultural Research Institute, Regional Station, Katrain during 2007 by attempting different intervarietal crosses in Asiatic hybrid lilies, *Lilium* x *elegans* Thunb. Katrain is situated at a latitude of 31^{o}58'N, longitude 77^{o}06'4" E and altitude of 1500 m in district Kullu of Himachal Pradesh. It has a typical temperate climate area with temperature ranging from –3.33 to 33.3^{o}C. It receives 100-125cm rainfall per year. The bulbs of the Asiatic hybrid lily cultivars were obtained from Model Floriculture Farm Chail (Solan), Department of Horticulture, Himachal Pradesh and

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some from the Van der Zen Export B.V. The Netherlands, supplied by Florence Flora, India, during 2000-2002. Bulbs of these cultivars were maintained and multiplied at Sarsai Farm of the IARI, Regional Station, Katrain.

Asiatic lily cultivars *viz*., Pollyanna, Shiraj, Navona, Brunello, Prato, Stargater and Sumplon were used in diallelic cross (partial and incomplete). A few reciprocal crosses were also made. The pollinations were performed manually as normal stigmatic pollination techniques to overcome pre-fertilization barriers [10]. Five flowers for each combination were pollinated with freshly dehisced anthers of the male parents. Flowers of male and female parents were emasculated approximately 24 hours before anthesis and immediately cross pollinated. Pollination was effected by dusting the anthers on the stigma and caps of butter paper were then placed over the pistils to prevent accidental contamination. Capsules resulting from hand pollination were allowed to grow on the plants until they reached the stage of maturity.

Seeds of 45 populations (Table1) were sown in plastic pots filled with 1:1:1 (sand: cocapeat: perlite) potting mixture and placed in a greenhouse without temperature control. The observations were taken on number of healthy seeds, percent germination and crossability. The crossability was used to indicate the rate of success of a particular cross and calculated by dividing the number of the seedlings with the number of healthy seeds sown x 100 at seedling stage. After six months of seed germination, bulblets were harvested and placed at 4^{o}C for 8 weeks inside the refrigerator. After 8 weeks all bulblets were disinfected and planted separately in pots filled with a mixture of soil: sand: FYM (well rotten farm yard manure) for further bulb formation.

Table 1. The result of intervarietal crosses using Asiatic hybrids as male and female

Cross combinations	Days to germination (days)	%tage germination (%)	Crossability (%)
Navona x Brunello	14.66	9.33	2.5
Pollyanna x Shiraj	13.33	57.66*	16.0
Shiraj x Pollyanna	13.33	13.66	4.5
Brunello x Navona	13.33	12.00	2.5
Shiraj x Sumplon	12.33*	18.66	4.5
Pollyanna x Stargater	15.00	42.66	21.0
Shiraj x Stargater	14.66	8.33	7.5
Shiraj x Dreamland	27.00	24.33	12.5
Pollyanna x Prato	14.33	5.33	3.0
$CD_{(P = 5\%)}$	1.92	12.40	

*Crossability was calculated with the formula: the number of the seedlings divided by the number of healthy seeds sown x 100)

Ploidy levels of bulblets were determined by flow cytometric analysis [11]. This study was carried out in the Nunhems India Pvt. Ltd. Lab. situated at Kullu-Valley, Himachal Pradesh. Scales of bulblets were used for the measurement of relative DNA contents by a flow cytometer PA (Partec, GmBH, Munster, Germany) for comparison with the DNA content of scales of parental lines. To isolate nuclei, samples were chopped with a razor blade in 0.5 ml of solution A (high resolution DNA kit, type A; Partec GmBH). Nuclei were then stained for 5 min. in dark with 2 ml of solution B (high resolution DNA kit, type B; Partec GmBH) containing 4, 6-diamidino-2-phenylindole (DAPI) prior to flow cytometric analysis. However, due to lack of cytological facilities at the station, the genetic backgrounds of the parental varieties and chromosome number of hybrids has not been analyzed in the present study.

Bulbs of 68 progenies, which represent the product of approximately 45 populations, were planted in pots with growing medium consisting of FYM: sand: soil at the rate of 1, 1 and 1 (by volume) in October, 2010. To mimic the cultivation conditions of most of the flower producers, the study was undertaken without greenhouse temperature control. Once the shoots were 5-7 cm tall, a liquid fertilizer of 20:20:20 (NPK) was applied at the rate of 200 ppm [12] after fifteen days intervals. The observations on average number of days to flowering, average number of flowers/plant, average plant height, flower bud length and average flower diameter were taken at the time of anthesis. The most interesting progenies were selected considering commercial and agronomic traits of relevance to the proposed objectives.

A high degree of self-incompatibility was found in all those tested cultivars. Similar results were reported with some diploid cultivars of *L. longiflorum* [13, 14]. The most rapid (12.33days) and highest seed germination (57.66%) was observed in Shiraj x Sumplon cross (Table 1). Maximum crossability was noticed (21.0%) in Pollyanna x Stargater. Because of the long duration (two or more years), the seedlings were replanted in pots to obtain flowering size bulbs and then grown in polyhouse for evaluation. In the present study,

it could not be concluded that these plants were true hybrids without some certain means of identification, especially since some lilies often produce apomictic seed when pollinated with a wide range of other species [15]. It was not possible to distinguish between hybrids or any apomictic seedlings with any degree of confidence by comparison of morphological characters until flowering. However, the verification of hybrids in an early stage was done using flow cytometry. By crossing Asiatic hybrids both as female and male parents, a total of 68 progenies were obtained of which 33 (48.5%) were diploid, seven (10.3%) triploid and 28 (41.2%) were mixoploids (Table 2) (Profiles of DNA content not shown). This indicated the diverse origin of these Asiatic hybrids. Occurrence of mixoploids might be due to irregularity in meiotic chromosomal distribution as was similarly observed in tetraploid *L. longiflorum* [16].

Table 2. The ploidy levels of some intervarietal crosses evaluated by DNA measurement with flow Cytometry

Crosses	No. of plants analyzed	Ploidy levels		
		Di-ploid	Tri-ploid	Others (poly-ploids)
Pollyanna x Shiraj	32	21	-	11
Pollyanna x Prato	6	1	5	-
Shiraj x Sumplon	14	11	-	3
Navona x Brunello	5	-	2	3
Pollyanna x Stargater	10	-	-	10
Pollyanna x Shiraj	1	-	-	1
Total	68	33	7	28

Fortunately all the tested cultivars were cross fertile and several populations of seedlings obtained by crossing were grown and observed. All were highly variable indicating considerable heterozygosity of the parents. Sixty eight clonal populations were evaluated for their flower quality. The 29 clones were arranged in order of their ploidy levels (Table 3). In general, the triploid clones produced more flowers as compared to diploid and mixoploids. The average number of blooms per plant ranged from 1 to 6. Considerable variation was recorded for height (from 19.0 cm in clone 1 to 69.2 cm in clone 27) and time of blooming (from 153.0 days to 244 days in clone 20 and clone 18, respectively). The clones also varied considerably in flower size, bud length and spread of foliage and stem colour. Typical plants of each three ploidy groups are shown in Fig. 1. Plants a, b and c are representative of clones 2, 18 and 19 and plants d, e and f of clones 20, 23 and 29. Clones

Table 3. Average performance of 19 diploid, 5 triploid and 5 mixoploid clones of intervarietal *Lilium* crosses

Clone nos.	Average no. of flowers	Average plant height (cm)	Average no. of days to bloom (days)	Average bud length (cm)	Average flower diameter (cm)
Diploid					
1	2.0	19.0	159.0	9.0	16.8
2	5.0	50.0	168.0	9.0	15.2
3	2.0	27.0	183.0	7.5	14.1
4	1.0	32.0	181.0	8.2	14.2
5	3.0	42.0	188.0	9.5	15.2
6	1.0	26.0	169.0	7.2	11.7
7	5.0	50.0	154.0	9.0	14.2
8	1.0	35.0	214.0	8.4	15.6
9	1.0	48.0	244.0	6.7	11.7
10	1.0	35.0	227.0	7.0	11.1
11	1.0	52.5	160.0	8.6	15.7
12	2.0	27.0	162.0	8.5	16.3
13	3.0	42.0	202.0	8.6	16.1
14	2.0	45.5	208.0	7.6	14.7
15	4.0	31.0	219.0	6.5	11.5
16	2.0	37.5	227.0	7.3	14.0
17	3.0	28.0	229.0	7.0	12.5
18	3.0	32.0	244.0	7.5	14.7
19	1.0	54.0	259.0	9.8	15.7
Triploid					
20	4.0	60.0	153.0	10.6	13.7
21	4.0	54.0	159.0	9.0	15.5
22	3.0	23.0	175.0	8.0	10.2
23	3.0	33.5	215.0	9.4	17.0
24	6.0	48.0	175.0	8.0	14.7
Mixoploid					
25	2.0	35.0	178.0	8.2	17.0
26	1.0	29.0	187.0	8.4	15.3
27	1.0	69.2	228.0	8.4	15.0
28	2.0	57.0	166.0	5.5	10.2
29	2.0	38.4	215.0	7.2	13.7

Fig. 1. Typical plants of three ploidy groups

19 and 2 were tall enough but suitable for cut stem while clone 23 was satisfactory for both as cut and pot plants. Clones 18 and 29 were attractive plants and useful for pot culture. Majority of mixoploids produced large flowers with heavy textures and short stocks, which are desirable characteristics.

Asiatic hybrid lily has highly heterozygous genome structure [16, 17] and the range of variation occurring in the clones is greater than the parent populations and may be attributed to new combinations of genes from each parent as well as to gene rearrangements. Majority of triploids and most of the mixoploids produced large flowers with heavy textures, which are desirable features. Triploids were considered superior to diploids. It is evident from this research that intervarietal hybridization within a section is a desirable and possible approach to increase genetic variability [18] which can be used to develop indigenous varieties with high heterosis.

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