Genotypic and Environmental Variation in Production of *2n*-gametes of Oriental x Asiatic Lily Hybrids

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Abstract

It is attractive to use *2n* gametes in breeding for three reasons: a) they can overcome the sterility of F_1 hybrids between distantly related species, b) facilitate homoeologous recombination between alien chromosomes and c) generate a large number of different genotypes of *2n* gametes from a single F_1 hybrid. Such genetic variation can be potentially useful for breeding. However, there is one drawback of using *2n* gametes in breeding. The frequency of *2n* gamete-producing genotypes OA-hybrids, is very low. By producing a large number of interspecific hybrids followed by careful screening, we have selected few *2n* gamete producing F_1 hybrids between different groups of *Lilium* species. This screening for *2n* pollen production revealed enormous variation in *2n* pollen production frequencies between the different genotypes and between the same genotypes grown in different environments. The fluctuations in greenhouse temperature appeared to influence *2n* gametes frequency considerably. To study this during several years, four normally complete sterile genotypes were exposed to a heat shock treatment in phytotron. Three out of the four genotypes produced viable *2n* pollen. In total 2% of the treated flowers became fertile due to this temperature-induced stimulation of *2n* pollen formation.

INTRODUCTION

During interspecific hybridization of distantly related *Lilium* species, multiple crossing barriers occur. Several techniques have been developed in the past to overcome these barriers. Pre-fertilization barriers can be overcome by using pollination techniques, like the cut style method, the grafted-style method and in vitro isolated ovule pollination technique (Asano and Myodo, 1977ab; Van Tuyl et al., 1991). Post-fertilization barriers that occur during the development of the hybrid embryo can be circumvented using in vitro rescue methods like embryo, ovary-slice and ovule culture (Asano, 1980; Van Tuyl et al., 1991; Okazaki et al. 1994). Later a second type of post-fertilization barrier occurs; F_1-sterility. The fertility of such diploid F_1 hybrids can be restored using either mitotic (chromosome doubling) or meiotic polyploidization techniques (Van Tuyl et al., 1992; Van Tuyl, 1993; Van Tuyl and Lim, 2003). Subsequently a third type of post-fertilization barrier is encountered (especially when using mitotic polyploidization) in the form of a lack of introgression (Van Tuyl et al., 2002).

The use of unreduced or *2n* gametes is an attractive approach to overcome these last two post-fertilization barriers (Van Tuyl et al, 2002). First of all because *2n* gametes can overcome the sterility of F_1 hybrids between distantly related species; secondly because *2n* gametes facilitate homoeologous recombination between alien chromosomes (Karlov et al., 1999; Lim et al., 2000); and a third advantage is the fact that a large number of different genotypes of *2n* gametes can be generated from a single F_1 hybrid. Such genetic variation can be potentially useful for breeding.

Unfortunately the number of *2n* gamete-producing genotypes among Oriental x Asiatic (OA) hybrids is very low. This paper describes the results of our efforts to detect new *2n* gamete-producing genotypes among an existing collection of OA F_1 hybrids using a careful screening method. Furthermore an attempt to induce the production of *2n* pollen in sterile OA-hybrids using heat shock treatment is described.

MATERIALS AND METHODS

Over one hundred F_1 hybrid OA genotypes were grown in unheated greenhouses (creating an environment with temperature fluctuation due to the daily changes in outside temperature) some were also grown in a heated greenhouse (causing an environment with a more or less stable temperature). Additionally, plants of four normally sterile F_1 hybrid OA genotypes were grown in a phytotron for 6 weeks and exposed daily to the following (extreme) temperature fluctuation regime: 8 hours at 10°C followed by 8 hours at 30°C under artificial lighting and 4 hours at 10 °C followed by 4 hours at 30°C in the dark, creating a day/night ratio of 16 : 8 hours.

Upon flowering all genotypes were screened for *2n* pollen production using a pollen germination test: Pollen was cultured on artificial bacteriological agar medium (100 g sucrose, 5 g bacteriological agar, 20 mg boric acid and 200 mg calcium nitrate per litre) over night at 25°C. After 24 hours the pollen germination (i.e. viable *2n* pollen) percentage was observed using a stereo microscope. The pollen was classified as large (*2n*), small (*n*) and empty. The pollen germination percentage was scored counting only the large germinated pollen grains.

RESULTS AND DISCUSSION

In total 12 genotypes were found to produce *2n* pollen in notable frequencies. The results of the pollen germination tests is shown in Table 1. The data from the pollen germination tests show considerable variation in germination percentages. Not only variation between the different genotypes but also variation between clones of the same genotype growing in different environments was observed. There was even considerable variation among different clones of one genotype within one environment and even among the different flowers of one clone.

Four out of twelve genotypes (notably accessions 951502-1, 951584-1, 952400-1 & 962433-1) that had been grown under both environmental conditions were found to produce *2n* pollen. All four of these had already been identified as *2n* pollen producers in previous years. The other eight genotypes that were detected as *2n* pollen producers in the unheated greenhouse had up until then always been grown in heated greenhouses. Pollen germination tests had shown them to be sterile for several years in a row.

All flowers of the plants from the heat shock treatment were tested for pollen germination. The results can be found in Table 2. In total 2% of the flowers responded to the treatment. But among the four genotypes the response (see Table 2.) as well as the pollen germination percentage varied. The pollen germination percentages varied between 1% and 5%. Three out of the four genotypes produced viable 2*n* pollen.

CONCLUSION

These results suggest that the variation in *2n* pollen production is of both environmental and genetic origin. Temperature fluctuation, both natural (the unheated greenhouse) and artificial (the phytotron), seems to be an agent that stimulates the production of *2n* gametes. But not all results corroborate with this assumption: some genotypes showed higher pollen germination percentages in the heated greenhouse. Because of the large variation in *2n* pollen frequencies and the inconsistent nature of this variation, it is advisable to repeat screening and screen under different environments. It is also demonstrated that heat shock treatment can be used successfully to induce *2n* pollen production for breeding purposes.

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Tables

Table 1. Detected *2n* pollen producing OA F_1 hybrids.

Crossing number	Parentage		Pollen germination (%) (range %)	
	Oriental Hybrid	Asiatic Hybrid	heated greenhouse	unheated greenhouse
951462-1	'Romero Star'	'Connecticut King'	—	31.4 (0 – 75)
951447-1	'Bel Paso'	'Gran Sasso'	—	2.0 (n.a.)
951502-1	'Pesaro'	'Connecticut King'	4.9 (0 – 10)	16.6 (0 – 100)
951584-1	'Acapulco'	'Sancerre'	35.1 (0 – 90)	23.3 (0 – 60)
952088-1	'Expression'	'Au Revoir'	—	25.0 (n.a.)
952381-5	'Mero Star'	'Connecticut King'	—	2.6 (0 – 10)
952400-1	'Mero Star'	'Gran Sasso'	7.6 (0 – 75)	0.53 (0 – 20)
952462-1	'San Marco'	'Connecticut King'	—	37.5 (20 – 50)
962119-1	'Acapulco'	'Connecticut King'	—	6.0 (0 – 40)
962160-1	'Bernini'	'Connecticut King'	—	1.9 (0 – 25)
962254-2	'Tenerife'	'Lanzorote'	—	2.1 (0 – 30)
962433-1	'Sissi'	'Mirella'	12.2 (0 – 60)	24.4 (0 – 75)

n.a. = not available; — = genotype was not present

Table 2. Effect of heat shock treatment on the fertility of four sterile OA F_1 hybrids.

Crossing Number	Number of flowers treated	Dead buds (%)	Sterile flowers (%)	Fertile flowers (%)
951301-5	340	8.82 (n=30)	88.82 (n=302)	2.35 (n=8)
951914-1	196	11.73 (n=23)	86.73 (n=170)	1.53 (n=3)
953508-1	91	3.30 (n=3)	96.70 (n=88)	0.00 (n=0)
951462-1	44	2.27 (n=1)	90.91 (n=40)	6.82 (n=3)
Total	671	8.49 (n=57)	89.42 (n=600)	2.09 (n=14)