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

A.C. Lokker, Rodrigo Barba-Gonzalez, Ki-Byung Lim, M.S. Ramanna and Jaap M. van Tuyl
Plant Research International, Wageningen University and Research Centre, P.O. Box 16, NL-6700 AA Wageningen, The Netherlands
Tel: +31 317 477329
Fax: +31 317 418094
E-mail: jaap.vantuyl@wur.nl

Keywords: Lilium, interspecific hybrids, 2n gametes, pollen germination test

Abstract
It is attractive to use 2n gametes in breeding for three reasons: a) they can overcome the sterility of F1 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 F1 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 F1 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; F1-sterility. The fertility of such diploid F1 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 F1 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 F1 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 F1 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 F1 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 F1 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 2n 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.

ACKNOWLEDGEMENTS

The authors would like to thank the 11 Dutch lily breeding companies, who have supported the lily research at Plant Research International for many years.
Literature Cited
Asano, Y. 1980. Studies on crosses between distantly related species of lilies. IV. The
culture of immature hybrid embryos 0.3 - 0.4 mm long. J. Japan. Soc. Hort. Sci.
49:114-118.
Asano, Y. and Myodo, H. 1977a. Studies on crosses between distantly related species of
Asano, Y. and Myodo, H. 1977b. Studies on crosses between distantly related species of
lilies. II. The culture of immature hybrid embryos. J. Japan. Soc. Hort. Sci. 46:
267-273.
recombination in 2n-gamete producing interspecific hybrids of Lilium (Liliaceae)
Lim, Ki-Byung, Chung, J.D., Van Kronenburg, B.C.E., Ramanna, M.S., De Jong, J.H.
and van Tuyl, J.M. 2000.Introgression of Lilium rubellum Baker chromosomes into L.
longiflorum Thunb.: a genome painting study of the F₁ hybrid, BC₁ and BC₂
‘Oriental’ hybrid and L. ‘Asiatic’ hybrid produced by embryo culture with revised
media. Breeding Science 44: 59-64.
Van Tuyl, J.M. 1993. Survey of research on mitotic and meiotic polyploidization at
and Bino, R.J. 1991. Application of in vitro pollination, ovary culture, ovule culture
and embryo rescue for overcoming incongruity barriers in interspecific Lilium crosses.
Plant Science 74: 115-126.
325: 625-630.
Van Tuyl J.M. and Lim K.B. 2003. Interspecific hybridization and polyploidization as
### Tables

#### Table 1. Detected 2n pollen producing OA F<sub>1</sub> hybrids.

<table>
<thead>
<tr>
<th>Crossing number</th>
<th>Parentage</th>
<th>Pollen germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>heated greenhouse</td>
</tr>
<tr>
<td></td>
<td>Oriental Hybrid</td>
<td>Asiatic Hybrid</td>
</tr>
<tr>
<td>951462-1</td>
<td>‘Romero Star’</td>
<td>‘Connecticut King’</td>
</tr>
<tr>
<td>951447-1</td>
<td>‘Bel Paso’</td>
<td>‘Gran Sasso’</td>
</tr>
<tr>
<td>951502-1</td>
<td>‘Pesaro’</td>
<td>‘Connecticut King’</td>
</tr>
<tr>
<td>951584-1</td>
<td>‘Acapulco’</td>
<td>‘Sancerre’</td>
</tr>
<tr>
<td>952088-1</td>
<td>‘Expression’</td>
<td>‘Au Revoir’</td>
</tr>
<tr>
<td>952381-5</td>
<td>‘Mero Star’</td>
<td>‘Connecticut King’</td>
</tr>
<tr>
<td>952400-1</td>
<td>‘Mero Star’</td>
<td>‘Gran Sasso’</td>
</tr>
<tr>
<td>952462-1</td>
<td>‘San Marco’</td>
<td>‘Connecticut King’</td>
</tr>
<tr>
<td>962119-1</td>
<td>‘Acapulco’</td>
<td>‘Connecticut King’</td>
</tr>
<tr>
<td>962160-1</td>
<td>‘Bernini’</td>
<td>‘Connecticut King’</td>
</tr>
<tr>
<td>962254-2</td>
<td>‘Tenerife’</td>
<td>‘Lanzorote’</td>
</tr>
<tr>
<td>962433-1</td>
<td>‘Sissi’</td>
<td>‘Mirella’</td>
</tr>
</tbody>
</table>

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

#### Table 2. Effect of heat shock treatment on the fertility of four sterile OA F<sub>1</sub> hybrids.

<table>
<thead>
<tr>
<th>Crossing Number</th>
<th>Number of flowers treated</th>
<th>Dead buds (%)</th>
<th>Sterile flowers (%)</th>
<th>Fertile flowers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>951301-5</td>
<td>340</td>
<td>8.82 (n=30)</td>
<td>88.82 (n=302)</td>
<td>2.35 (n=8)</td>
</tr>
<tr>
<td>951914-1</td>
<td>196</td>
<td>11.73 (n=23)</td>
<td>86.73 (n=170)</td>
<td>1.53 (n=3)</td>
</tr>
<tr>
<td>953908-1</td>
<td>91</td>
<td>3.30 (n=3)</td>
<td>96.70 (n=88)</td>
<td>0.00 (n=0)</td>
</tr>
<tr>
<td>951462-1</td>
<td>44</td>
<td>2.27 (n=1)</td>
<td>90.91 (n=40)</td>
<td>6.82 (n=3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>671</strong></td>
<td><strong>8.49 (n=57)</strong></td>
<td><strong>89.42 (n=600)</strong></td>
<td><strong>2.09 (n=14)</strong></td>
</tr>
</tbody>
</table>