

Mitotic and Meiotic Polyploidization in Lily Hybrids for Transferring *Botrytis* Resistance

C.M. Beers, R. Barba-Gonzalez, A.A. van Silfhout, M.S. Ramanna and J.M. van Tuyl
Plant Research International, Wageningen University and Research Centre
P.O. Box 16, 6700 AA Wageningen
The Netherlands

Keywords: leaf tip test, breeding, OA-hybrid, intergenomic recombination, transgression

Abstract

In an effort to transfer *Botrytis* resistance from Oriental lilies to Asiatic hybrids (*Lilium*, $2n=2x=24$) we made a large number of F_1 hybrids between these two distantly related species. Because these species belong to two different taxonomic sections, the F_1 hybrids were totally sterile and could not be directly used in breeding. Therefore, two approaches were used for utilizing the F_1 hybrids. First, the somatic chromosome number of the F_1 s was doubled by treating with oryzalin that resulted in allotetraploids (mitotic doubling). These allotetraploids were used for crossing with the parents. Second, $2n$ gametes were used directly for crossing with the parents for producing sexual polyploids (meiotic doubling). The two types of BC_1 progenies were monitored for resistance against *Botrytis elliptica* through a "leaf tip test". Disease severity was evaluated on a nominal scale, ranging from 1 resistant (no lesions) to 6 (a high degree of necrosis with mycelium or even spores). In both populations the resistance varied from very susceptible to highly resistant. However, the occurrence of transgression of resistance, meaning that the degree of resistance in some seedlings exceeded that of the parent, was higher in meiotically doubled polyploids as compared to those derived from mitotic doubling. This was explained from the fact that the typical allotetraploids produce uniformly a single genotype of $2x$ gametes containing both parental genomes because of autosyndetic pairing so that there is no scope for genetic variation. On the contrary, in the case of meiotic polyploids intergenomic recombination occurs between the alien chromosomes that could lead to considerable amount of genetic variation. This phenomenon might be an explanation for the observed transgression of *Botrytis* resistance in the meiotic polyploid progenies.

INTRODUCTION

Lily (*Lilium* L.) is cultivated world wide as a cut flower, pot plant and garden plant. The lily is an economically important flower crop. Last year 11.8 billion cut flowers were offered to the Dutch auctions and 3.5% of the cut flowers were lilies. The acreage of bulb production in The Netherlands is more than 4000 ha. In order to grow bulbs during the summer, 10-15 fungicidal sprays are needed to protect the plants against infection by *Botrytis elliptica*. To lower the use of chemical fungicides in the culture of lilies, breeding for resistance to *Botrytis* should have a high priority. In the lily assortment, resistance to *Botrytis* is especially present in the Oriental lilies, while resistance to *Fusarium oxysporum* and Lily Mottle Virus occurs only in the Asiatic hybrid group. To combine the resistances, OA-hybrids (Oriental x Asiatic) are developed. A large number of F_1 -hybrids have been produced, but most are in generally sterile. To overcome this sterility two approaches were used. First, the somatic chromosome number of the F_1 s was doubled by treating with oryzalin to produce allotetraploids (mitotic doubling). These allotetraploids were used for crossing with Asiatic and Oriental hybrids. Second, $2n$ gametes were used directly for crossing with the parents for producing sexual polyploids (meiotic doubling). It is typical for allotetraploids to uniformly produce a single genotype of $2x$ gametes containing both parental genomes because of autosyndetic pairing so that there is limited genetic variation. On the contrary, in the case of meiotic polyploids intergenomic recombination occurs between the alien chromosomes that could lead to a considerable

amount of genetic variation (Lim et al., 2001, 2003; Lokker et al., 2005; Barba-Gonzalez et al., 2005). The two types of BC₁ progenies were monitored for resistance against *Botrytis elliptica* through a “leaf tip test”. In this paper the segregation of *Botrytis* resistance in the different BC₁-populations is described.

MATERIALS AND METHODS

Plant Material

The plant material used is presented in Tables 1 and 2.

***Botrytis* Leaf Tip Test**

The fungus *Botrytis elliptica* was grown on lily leaf-agar medium for over 2-3 weeks under continuous black light (Philips 8 W/08) at 18°C. A spore suspension with a concentration of 2x10⁵ spores/ml was used for inoculation.

Leaves were taken above the middle of the plants that had not yet flowered or that were flowering. Due to the smaller size plants, leaves from seedlings were taken from the whole plant. Leaf tips had a size of 5 to 6 cm when the leaves were cut. In the Well's plates the leaf tips had a size of 3 to 4 cm because 1 or 2 cm of the leaf tip was removed because of the possibility of stress.

Six leaf tips per genotype in two replications were tested in Well's plates. The Well's plates were filled with tap water. The Well's plates were placed in boxes where an air humidity of >90% was reached by spraying the leaves after inoculation and paper inside the boxes with water. The boxes were transferred to a climate room at 20°C. A 2 µl drop of spore suspension was placed on every abaxial side of each leaf tip. The leaf tips were screened for symptoms 3-4 days after inoculation. The DSS-values (disease severity score) which were used, ranged from 1 = no lesion to 6 = highest degree of infection (Fig. 1).

RESULTS AND DISCUSSION

***Botrytis* Response of the Mitotic Populations**

A large variation from very susceptible to resistant was found. Eight out of 101 genotypes of the progeny showed transgression. The other genotypes of the progeny showed a response between the values of the parents.

***Botrytis* Response of the Meiotic Populations**

Although the number of seedlings was much lower in the meiotic populations, a large variation from very susceptible to resistant was found. Six out of 20 seedlings obtained via meiotic polyploidization showed transgression (Fig. 2). The transgression was estimated because two of the mothers were not available for testing. For cv. Gran Sasso, the tetraploid was tested and the results for cv. Lanzarote were from a previous experiment. The fact that other genotypes that were used in this experiment and in previous experiments had similar results, indicates that the previous results for cv. Lanzarote would have been similar in this test. Also, diploid and tetraploid genotypes were tested in this experiment and the results indicate there was little difference between these genotypes. This indicates that the tetraploid cv. Gran Sasso can be used to see if there is transgression. The transgression for these progenies is estimated and 6 of 20 genotypes showed transgression (Fig. 2).

Although different parents were used to produce the mitotic and meiotic populations, a preliminary conclusion is that due to intergenomic recombination, more variation was found in the meiotic populations than in the mitotic populations. Additional work is needed to determine the actual frequency of intergenomic recombination.

Literature Cited

Barba-Gonzalez, R., Lim, K.B., Ramanna, M.S. and van Tuyl, J.M. 2005. Use of 2n

- gametes for inducing intergenomic recombination in lily hybrids. *Acta Hort.* This volume.
- Lim, K.B., Ramanna, M.S., De Jong, J.H., Jacobsen, E. and van Tuyl, J.M. 2001. Indeterminate meiotic restitution (IMR): a novel type of meiotic nuclear restitution mechanism detected in interspecific lily hybrids by GISH. *Theor. Appl. Genet.* 103:219-230.
- Lim, K.B., Ramanna, M.S., Jacobsen, E. and van Tuyl, J.M. 2003. Evaluation of BC₂ progenies derived from 3 x 2 and 3 x 4 crosses of *Lilium* hybrids: a GISH analysis. *Theor. Appl. Genet.* 106:568-574.
- Lokker, A.C., Barba-Gonzalez, R., Lim, K.B., Ramanna, M.S. and van Tuyl, J.M. 2005. Genotypic and environmental variation in production of 2*n*-gametes of Oriental Asiatic lily hybrids. *Acta Hort.* This volume.

Tables

Table 1. Plant material used for the mitotic populations.

Group	Cross/name	Female	Male
OAA	012062	991102	“Gironde”
OAA	012063	991105	“Gironde”
OAA	012092	991103	“Gironde”
OAA	012105	991102	“Gironde”
OAOA	991102	951301-5	
OAOA	991105	951301-5	
OAOA	991103	951301-5	
OA	951301-5	“Mero Star”	“Conn. King”
AA	“Gironde”		
AA	“Conn. King”		
OO	“Sorbonne”		
OO	“Siberia”		

Table 2. Plant material used for the meiotic populations.

Group	Cross/name	Female	Male
AOA	002526	“Lanzarote”	952400-1
AOA	002531	“Gironde”	952400-1
AOA	002433	“Gran Sasso”	952400-1
OA	952400-1	“Mero Star”	940043 “Gran Sasso”
AAAA	“Gran Sasso”(tetra)		
AA	“Gironde”		
OO	“Siberia”		

Figures



Fig. 1. Disease severity scores used in the leaf tip test.

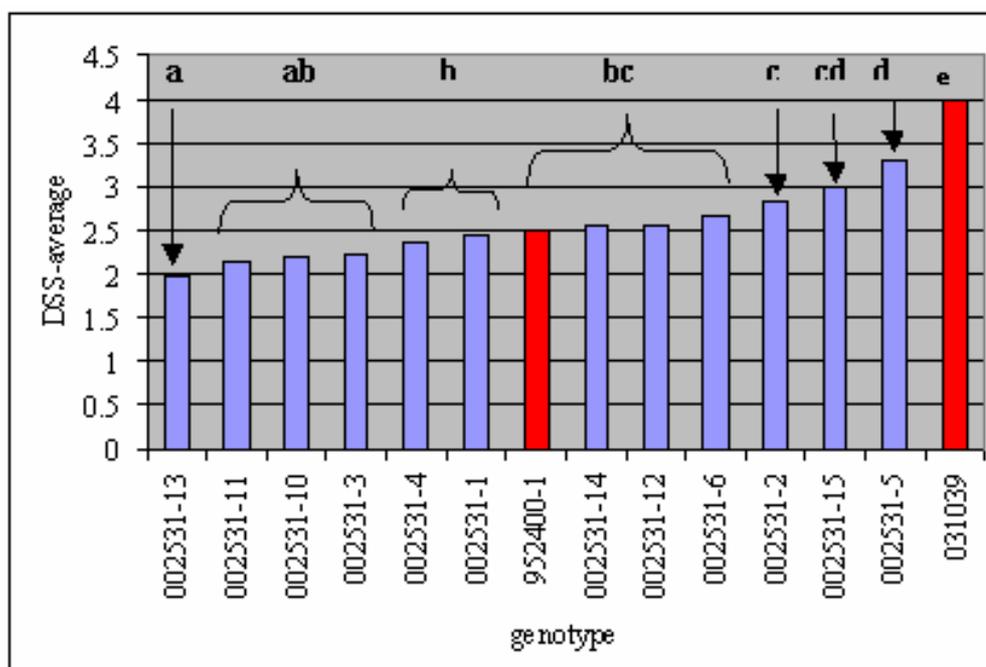


Fig. 2. DSS-score for a meiotic derived population with parents (red) and progeny (blue).