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Use of *in vitro* methods in intersection hybridisation of *Lilium* L.

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Abstract

For the study, 16 lily genotypes were chosen, including five Latvian varieties well adapted to the Latvian climate. The genotypes belong to three different sections and two intersection hybrids. Ovaries were cut in the 5th, 10th, 14th, 20th and 23rd days after pollination and cultured on Murashige and Skoog basal medium supplemented with 9 % sucrose, 0.7 % agar and 1.0 mg l^{-1} naphthaleneacetic acid. In 30 till 40 days, seeds were excised and cultured on embryo culture medium. The pollen fertility of tested genotypes was very different, ranging from 5 % till 83 %. The period of pollen germination depended on the genotype. Most of the genotypes had maximum pollen germination after 16 to 24 h, but for variety 'Pārgalvīgā' only after 40 h. For the major part of genotypes used in the investigation, 10-day-old ovaries were more successful. Hybrid embryos were established on 22 crosses, and bulblets were obtained from seven cross combinations, including four combinations from in-section hybrid crosses and three from intersection crosses.

Key words: intersection hybridisation, *in vitro*, *Lilium*, ovary culture.

Introduction

The conventional practice in lily breeding is in-section hybridisation. Most of widely grown cultivars of Asiatic hybrid lilies originated from crosses between species of the *Sinomartagon* section. The next popular hybrid group is the Oriental hybrid lilies, obtained from crosses within the section *Archelirion*. In the section *Leucolirion Lilium longiflorum* cultivars are an economically important group of lilies. *Aurelian* hybrids, originated from crosses between species *L. regale*, *L. sargentiae* and *L. henryi* of the section *Leucolirion* are suitable for outdoor conditions. Traditionally in lily breeding, cultivars from the *Sinomartagon*, *Archelirion* and *Leucolirion* sections are used for in-section crosses (De Jong 1974; Schenk 1990; Van Creij et al. 1990). However, this method cannot be used to combine traits from cultivars belonging to different sections.

To improve the assortment of lily by traits transmission from *Lilium* species of different sections, intersection breeding was begun in the 1970s. This was achieved in connection with the development of *in vitro* methods, because using crosses of genetically distant forms resulted in problems of fertilisation and growth ability of embryos. Several techniques, such as cutting of style, the grafted-style, *in vitro* isolated ovule pollination technique, embryo, ovary-slice and ovule *in vitro* culture methods, have been developed to overcome the pre-and post-fertilization barriers (Van Tuyl et al. 1991; Roh et al. 1996;

Van Tuyl et al. 1998).

Many lily breeding programmes has been carried out to obtain cultivars with improved flower longevity (Van der Meulen–Muisers, Van Oeveren 1990), highly ornamental flowers, and resistance to virus (Dapkuniene et al. 2000), bulb rot (caused by a fungus *Fusarium oxysporum* f. sp. *lilii*) (Löffler et al. 1990) and *Botrytis* (Doss et al. 1986; Koster, Meer 1993) etc. In the Latvian climatic conditions breeding for resistance against *Botrytis* is especially important. Ecological plasticity and flower longevity together with special flower colour and shapes also are important traits for Latvian lily breeders. The aim of the investigation was to elaborate a tissue culture method for obtaining in a short time new initial material for ornamental lily cultivars with good resistance to *Botrytis* and ecological plasticity.

Materials and methods

For the study 16 lily genotypes were chosen, including five Latvian varieties well adapted to the Latvian climate. The genotypes belong to three different sections and include two inter-section hybrids (Table 1). All of the used varieties were complex in-section hybrids. The bulbs of species *L. henryi*, *L. henryi* var. *citrinum* and Latvian lily varieties were obtained from the collection of the Botanical Garden of the University of Latvia. The hybrids *L. longiflorum* × *L. auratum* was obtained from Lindel Lilies Ltd, Canada, and the cultivars 'Casa Blanca', 'Gran Paradiso', 'Leslie Woodriff', 'Tetra Oriental', 'Aristo', 'Longistar', 'LA hybrids' from Bishoff-Tulleken Lilies, Holland. Some of the used genotypes ('Aristo', 'Eksotika', *L. henryi*, *L. henryi* var. *citrinum*) have a high level of resistance against *Botrytis* infection. Chromosome analysis was carried out for determination of chromosome number in root-tip cells (De Jong 1974).

Before pollination the pollen viability was checked. The pollen was collected from all genotypes used in the hybridisation. The pollen was put on Petri dishes in a culture medium (20 g l^{-1} sucrose, 75 mg l^{-1} gibberellic acid, 7 g l^{-1} agar) and cultivated 16 to 40 h at 25 °C in the dark. Germinated pollen was counted after 16, 24, 32 and 40 h. The viability of pollen was expressed as a percentage. In hybridisation only genotypes with a pollen viability 5 % and higher were used. In total 22 different crosses were made (Table 2). Hybridisation was conducted from mid July till early August both in a greenhouse and outdoor. For better pollination efficiency, stigmata were sprayed with proline solution in water (200 mg l^{-1}) two days after castration. Pollination was done on the third day after

Table 1. Lily genotypes used for hybridisation. *, lily varieties of Latvian origin; **, intersection hybrids

Section	Genotype
Sinomartagon	'Aristo', 'Banga', 'Gran Paradiso', 'Orfejs'*
Archelirion	'Casa Blanca', 'Liesma'*, 'Tetra Oriental', 'Leslie Woodriff'
Leucolirion	'Eksotika'*, 'Līksna'*, 'Pārgalvīgā', *L. henryi*, *L. henryi* var. *citrinum*
Sinomartagon × *Leucolirion*	'Longistar'**, 'LA hybrid'**
Leucolirion × *Archelirion*	*L. longiflorum* × *L. auratum*

castration. Repeatedly three days after pollination, stigmata were sprayed with a 75 mg l^{-1} gibberellic acid solution and 15 mg l^{-1} boric acid in water. The temperature ranged from 20 °C to 25 °C during pollination. For each cross, one to three ovaries were cut on stage when ovaries looked soft and were intensively green, which was found to be optimal in preliminary experiments (Table 2).

Sterilisation methods described earlier were used (Ornicāne, Rashal 1997). For *in vitro* cultivation the method described by Van Creij et al. (1990) with some modification was used. Ovaries were transversely cut in six to eight 2-to 3-mm-thick slices. Six to eight slices were placed on a Petri dish with Murashige and Skoog (1962; MS) basal medium, supplemented with 9 % sucrose, 0.7 % agar and 1.0 mg l^{-1} naphthaleneacetic acid. The medium was adjusted to pH 5.8 by NaOH and sterilised two times by autoclaving for 20 min. Ovary slices were incubated at 25 °C in the dark. After 30 to 40 days culture, establishing seeds were excised from the ovary slices and cultured on germinating medium: MS medium with 5 % sucrose, 0.7 % agar, 1.0 mg l^{-1} naphthaleneacetic acid (pH 5.5 before autoclaving). Seeds were incubated at 17 °C in the dark about a month, till embryos formed. Embryos were transferred on Petri dishes with growth stimulation

Table 2. Hybrid combinations used and the time after pollination, when ovaries were cut. First member of a combination represents a mother plant

Cross combination	Time after pollination (days)				
	5	10	14	20	23
'Aristo' × 'LA hybrid'					×
'LA hybrid' × 'Aristo'					×
'Banga' × 'Aristo'	×				×
'Orfejs' × 'Aristo'				×	
'Longistar' × 'Aristo'				×	
'Pārgalvīgā' × 'Casa Blanca'	×		×		
'Pārgalvīgā' × *L. henryi* var. *citrinum*	×		×		
'Tetra Oriental' × 'Līksna'	×				
'Liesma' × 'Tetra Oriental'	×				
'Tetra Oriental' × 'Gran Paradiso'		×			
L. henryi var. *citrinum* × 'Pārgalvīgā'		×			
'Pārgalvīgā' × (*L. longiflorum* × *L. auratum*)		×			
(*L. longiflorum* × *L. auratum*) × 'Pārgalvīgā'		×			
'Eksotika' × 'Pārgalvīgā'		×			
'Eksotika' × *L. henryi* var. *citrinum*		×			
'Casa Blanca' × 'LA hybrid'		×			
'Casa Blanca' × *L. henryi* var. *citrinum*		×			
'Casa Blanca' × 'Tetra Oriental'		×			
'Tetra Oriental' × 'Pārgalvīgā'		×			
'Tetra Oriental' × 'Eksotika'		×			
'Leslie Woodriff' × 'Eksotika'		×			
'LA hybrid' × 'Pārgalvīgā'		×			

medium: MS medium with 2 % sucrose, 0.7 % agar, 1.0 mg l^{-1} naphthaleneacetic acid (pH 5.0 before autoclaving). Embryos were incubated at 25 °C in the light (16 h day, light 3000 lx). After developing a leaf and root system, bulblets were transferred to pots with soil mix (one part sand / one part peat; v/v) and placed in a growth chamber (16 h day, light 3000 lx, 18 °C to 22 °C). In three to four weeks bulblets were planted in a greenhouse.

Results

The number of chromosomes was determined for genotypes with absent preliminary information (Table 3). It was found that most of those genotypes had 24 chromosomes (2n), three of them were mixoploids with fractions of cells with 24 and 36 chromosomes (3n).

The pollen fertility of genotypes included in the experiment was very different and ranged from 5 % ('Pārgalvīgā', 'Leslie Woodriff') to 83 % (*L. henryi* var. *citrinum*). The period of pollen germination depended on the genotype. Most of the genotypes had the maximum pollen germination after 16 to 24 h, but the variety 'Pārgalvīgā' only after 40 h.

Twelve percent of the ovaries were infected on the growth medium regardless whether the source plants were grown in a greenhouse or in outdoor conditions. Seventeen percent of the planted ovaries either did not start to grow and were damaged after about one week, or formed only calli and did not form seeds. Twenty one percent of the ovaries started to grow and formed seeds, but seeds were without embryos. Fifty percent of the ovary cultures formed seeds with hybrid embryos. Hybrid embryos were established on 14 hybrid combinations (Table 4). Plantlets were obtained only from seven hybrid combinations, including four combinations from in-sections crosses and three from intersection crosses. Hybrid embryos from the other seven crossing combinations were underdeveloped and dead after placing on embryo growth stimulation medium. It was extremely important to determine the stage of ovaries. When ovaries were too young, after planting on medium they failed start to grow or calli were formed. When ovaries were too old, they formed seeds without embryos. For the major part of genotypes used in the investigation, the most successful were 10-day-old ovaries. Our experience showed that cutting time depends both on genotype and growth conditions.

Table 3. Estimated chromosome numbers of different lily genotypes

Genotype	Number of chromosomes (2n)
'Banga'	24
'Līksna'	24
'Liesma'	24
'Orfejs'	24
'Eksotika'	24 + 36
'Pārgalvīgā'	24 + 36
L. longiflorum × *L. auratum*	24 + 36
L. henryi var. *citrinum*	24

Table 4. Embryo formation and number of obtained bulblets of different lily cross combinations. ×, embryos formed; -, no emryo formation

Cross combination	Embryo formation	Number of obtained bulblets
'Aristo' × 'LA hybrid'	×	0
'LA hybrid' × 'Aristo'	-	0
'Banga' × 'Aristo'	-	0
'Orfejs' × 'Aristo'	×	3
'Longistar' × 'Aristo'	-	0
'Pārgalvīgā' × 'Casa Blanca'	×	0
'Pārgalvīgā' × *L. henryi* var. *citrinum*	×	2
'Tetra Oriental' × 'Līksna'	×	1
'Liesma' × 'Tetra Oriental'	-	0
'Tetra Oriental' × 'Gran Paradiso'	-	0
L. henryi var. *citrinum* × 'Pārgalvīgā'	×	0
'Pārgalvīgā' × (*L. longiflorum* × *L. auratum*)	-	0
(*L. longiflorum* × *L. auratum*) × 'Pārgalvīgā'	×	0
'Eksotika' × 'Pārgalvīgā'	×	2
'Eksotika' × *L. henryi* var. *citrinum*	×	6
'Casa Blanca' × 'LA hybrid'	×	12
'Casa Blanca' × *L. henryi* var. *citrinum*	-	0
'Casa Blanca' × 'Tetra Oriental'	-	0
'Tetra Oriental' × 'Pārgalvīgā'	×	0
'Tetra Oriental' × 'Eksotika'	×	0
'Leslie Woodriff' × 'Eksotika'	×	8
'LA hybrid' × 'Pārgalvīgā'	×	0

Discussion

The success of intersection crosses depends on many different factors, such as genotype, hybrid combination, pollen viability, time of ovary cutting, ovary sterilisation method, *in vitro* cultivating conditions, plantlet growth conditions etc.

Knowledge of the chromosome number is critical for choosing genotypes for hybridisation (De Jong 1974). Accessions with 36 chromosomes (3n) usually have unbalanced meiosis and do not produce viable gametes therefore; can not be used for hybridisation (Van Tuyl et al. 1991). Using genotypes that contain both 2n and 3n cells was successful and embryos were obtained in such crosses. The variety 'Pārgalvīgā', used in various combinations, was never successful in outdoor crossings. Probably, this is related to a very long period of pollen germination. In our experiment we obtained embryos from crosses with this genotype. After pollination, stigmata were sprayed with gibberellic and boric acid, which prolonged the time of effective pollination and also stimulated the pollen germination.

In our investigation we mainly used genotypes with a chromosome number 2n or 4n.

One 3n variety ('Longistar') was used as a mother plant only in one cross with 'Aristo', but this cross was not successful: hybrid seeds were without embryos. Probably, sterility in this cross was associated with ploidity of the mother plant, because the cross between 'Aristo' and 'LA hybrid', which is from the same group that 'Longistar', resulted in formation of germinating seeds.

Even in the same hybrid combination the direction of crosses is important, shown by comparison of reciprocal crosses. For example, in the hybrid combination 'Aristo' × 'LA hybrid' and (*L. longiflorum* × *L. auratum*) × 'Pārgalvīgā', embryos were formed, but did not in the reciprocal combinations.

In our investigation, we did not observe a big difference in embryos and plantlets obtaining between in-section and intersection crosses, probable because the genotypes used in in-group crosses were from different species and in nature did not interbreed. The study results showed that it was possible to obtain intersection hybrids from Latvian breeding material, which could be used for creating varieties of lilies with wide ecological plasticity and good disease resistance.

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