Acta Universitatis Latviensis, 2008, Vol. 745, Biology, pp. 37–41

The embryogenesis and development of newly obtained interspecific lily hybrids *in vitro*

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Abstract

Interspecific hybridization is the most important source for variation in ornamental breeding. Tissue culture methods are used to overcome fertilization barriers in these hybrids. The aim of the present study was to establish a microcultivation system for explanted zygotic embryos of lilies from interspecific crossings of Asiatic, Oriental, Trumpet hybrids and *Lilium longiflorum* in different combinations and their backcross derivatives based on the embryo rescue method. Five combinations of lily crosses in 2002, and 11 of 16 combinations in 2004 gave positive results, although with different percent of germination and embryo survival.

Key words: embryogenesis, lily hybrids, *in vitro*.

Introduction

The breeding strategies of ornamental plants e.a. lilies include a wide range of *in vitro* methods. Three most important groups of modern commercial lilies are the Asiatic hybrids (A), originating from interspecific crosses within the *Sinomartagon* section, the Oriental hybrids (O), obtained from crosses within the *Archelirion* section, and the *Longiflorum* group (L; Van Creij et al. 1993). Pedigree analysis shows that the majority of lily cultivars from the group LA (*Lilium longiflorum* Thunb. × Asiatic hybrid) were derived after back crossing of alloploid hybrids producing non-reduced 2*n* gametes on diploid Asiatic cultivars (Proscevičus 2004). In the genus *Lilium*, interspecific hybridization has been conducted to produce novel hybrids that can combine resistance of Orientals against *Botrytis*, virus resistance of Asiatics, and *Fusarium* resistance of Trumpets, with attractive flowers and good cultivation qualities (Chi 2002).

The Asiatic lilies are most resistant in open areas in wet and cool maritime climates but the flowers have average ornamental value and have no fragrance. Most ornamental lilies with enjoyable delicate fragrance are from the Oriental group. However, they are completely unadapted for the above-mentioned climate. Therefore, it is necessary to perform interspecific crossings between different groups of lilies, which do not produce fertile seeds in natural conditions or do not produce seeds at all. Because of pre- and post-fertilization barriers, successful interspecific crosses have not been reported for *Lilium* using an Asiatic hybrid as a mother plant in crossings with Oriental or *Lilium longiflorum* as a pollen source (Chi 2000). To overcome pre-fertilization barriers, the cut-style method,

the grafted style method and the placenta pollination method have been tested (Van Tuyl et al. 1991; Janson et al. 1993; Willemse et al. 1995). Embryo rescue, ovary-slicing, and ovule culture have been used to overcome post-fertilization barriers (Van Tuyl et al. 1991; Okazaki et al. 1992; Chi 2002; Ikeda et al. 2003).

The purpose of the present study was to establish a microcultivation system for explanted zygotic embryos of lilies from interspecific crossings of Asiatic, Oriental, Trumpet hybrids (T) and *Lilium longiflorum* in different combinations and their backcross derivatives based on the embryo rescue method.

Materials and methods

The *in situ* hybridization method was used for this study. Five combinations used in 2002 were: (I) *Lilium longiflorum* × Asiatic hybrid, *3n*) × Asiatic hybrid, 4*n* (LAA); (II to V) Oriental hybrids × Trumpet hybrids (OT). In 2004, four crossing combinations were used: OT × OT – (three crossings); OT × T (Trumpet) – (nine crossings); TA (Trumpet hybrids × Asiatic hybrids) × A – (two crossings); and TA × T- (two crossings). Seed buds were isolated from capsules which were sterilized by briefly dipping them in 96 % ethanol and passing them through the flame. Seed coats were removed aseptically, and embryos with endosperm were placed on filter paper bridges in test tubes (19 mm diameter) with liquid initial medium for 4 days (Fig. 1A). Then, embryos were removed from browned endosperm (Fig. 1B, C) and placed on a new medium for further development (Fig.1 D). Both media were made after an original protocol designed by the Tissue Culture Department of the National Botanical Garden (Jakobsone, unpublished data).

All cultures were maintained in a growth chamber in continuous darkness at 23 to 25 °C until the formation of a first leaf. From this stage all the plant material was cultivated as bulblets on agar-solidified modified Murashige and Skoog (1962) medium supplemented with 0.08 mg L^{-1} α-naphthalene acetic acid. Plantlets were placed in an illuminated chamber with white fluorescent lamps at 23 to 25 °C. Further transplantations were carried out depending on the stage of development. Regenerants with well developed bulbs were transplanted *ex vitro* on soil for further regrowth and selection.

Results and discussion

Interspecific hybridization is the most important source for variation in ornamental breeding. It is indispensable to combine diverse gene pools allowing to add a new characteristics into the current cultivars. Interspecific hybrids have an enormous potential to extend not only their qualitative but also quantitative traits such as the type of flower, plant phenotypes, other single dominant traits from parent species related to environmental adaptation etc. While natural hybrids can exist between species with overlapping flowering times, pre- and post-fertilization barriers hinder the frequency of these hybrids (Van Tuyl et al. 2003). Establishment of a microcultivation system for the rescue of zygotic embryos is necessary to overcome post-fertilization barriers. In our experiments, 60 to 80 days after pollination was too long, and did not allow to prevent embryo abortion occurring at very early developmental stages. Van Tuyl et al. (1991) noted that embryos at 8th day after pollination were successfully rescued by ovary slice culture in an interspecific cross of *L. longiflorum*.

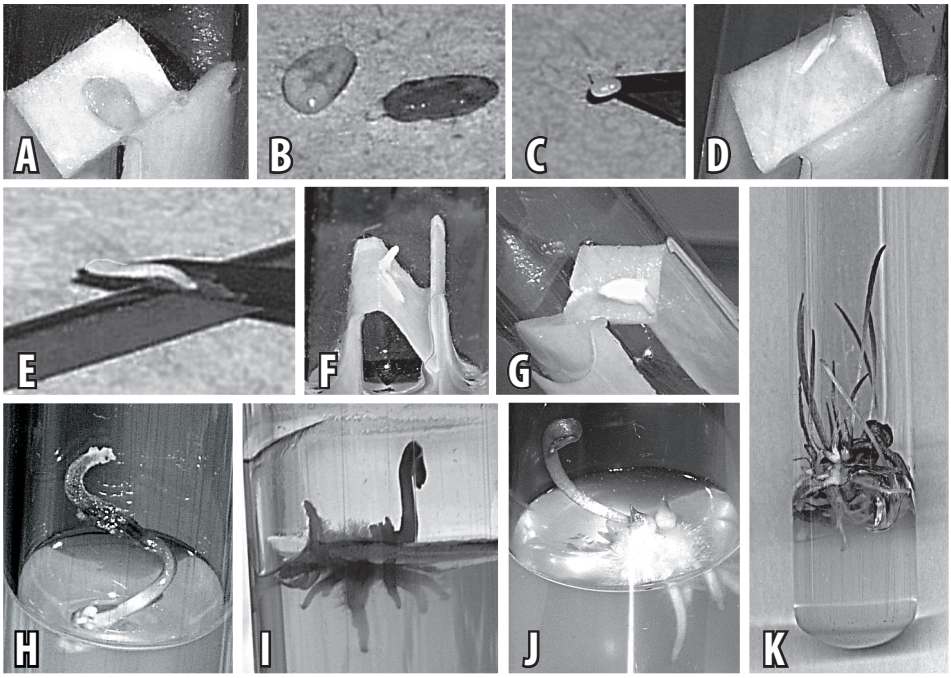

Fig. 1. Embryo- and morphogenesis of interspecific lily hybrids *in vitro*. A, hybrid seed-bud without seed-coat transplanted *in vitro*. B, 4 days after transplantation. C, zygotic embryo, after a globular stage. D, elongated embryo. E, transplantation of embryo, torpedo stage. F, polarization of embryo. G, formation of cotyledon. H, development of the shoot. I, rooting phase. J, formation of bulblets. K, normally developed microplant of lily *in vitro*.

Five combinations of lily crosses in 2002, and 11 out of 16 combinations in 2004 gave positive results although with different percent germination and embryo survival. In general the method used in the present study was more successful than the cutting of seed vessels into slices, as described in the literature (Chi 2002).

In our experiments, a torpedo stage (Fig. 1E) started in a short time after isolation of embryos, approximately in a week. The embryogenesis stage *in vitro* ended with formation of a bipolar structure – embryo –prepared to form a completely new plant body (Preťová, Olbert 2006). As shown in Fig. 1F, the polarization started immediately after the torpedo stage.

The polarization stage ended with formation of cotyledon (Fig. 1G) further developing into first shoot (Fig. 1H) which appeared after about 2.5 to 3 months. After that explants could be cultivated in the light. Rooting (Fig. 1I) and bulb formation (Fig. 1J) took place only in light conditions. The proliferation and normal *in vitro* cultivation of lilies (Fig. 1K) was achieved after five-six months. Transplanting to soil was performed in August, 10 months after the initiation of sterile culture.

The OA lily hybrids (Oriental hybrids × Asiatic hybrids) are of practical interest in horticulture as well as of basic scientific importance. Distantly related species have been used in crop improvement where the traditional approach was to produce an alloploid from

F_1 hybrid through somatic chromosome doubling. Such allopolyploids are appropriately called "permanent hybrids" because the parental characteristics almost never segregate in their progenies (Barba-Gonzalez et al. 2005).

The condition of embryos cultured *in vitro* and subsequent quality of bulblets was very variable within one crossing, and clones from one seed showed different developmental quality as well. The first flowering of hybrids obtained *in vitro* was noticed in 2006 and will be a source of further selection outdoors. The method used suggested as a very important wide-range possibility in the breeding process. We conclude that this approach can be successful in selecting the most quality clones in *in vitro* cultivation. We must assess whether these differences are observed in the field, including the proliferation rate.

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Jauniegūtu starpsugu liliju hibrīdu embrioģenēze un attīstība *in vitro*

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Kopsavilkums

Starpsugu hibridizācija ir vissvarīgākais izmaiņu avots dekoratīvo augu selekcijā. Audu kultūru metodes izmanto, lai pārvarētu apaugļošanās barjeras šiem hibrīdiem. Pētījuma mērķis bija izveidot mikrokultivēšanas sistēmu zigotisko embriju eksplantiem no starpsugu krustojumiem starp Āzijas, orientālo un trompešliliju grupu lilijām un atgriezenisko krustojumu derivātiem pamatojoties uz embriju izglābšanas metodi. Piecas liliju krustojumu kombinācijas 2002. gadā un 11 no 16 kombinācijām 2004. gadā deva pozitīvus rezultātus, lai gan ar atšķirīgu dīgšanas un embriju izdzīvošanas procentu.