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# Application of in vitro pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in interspecific *Lilium* crosses

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The use of a complete and integrated in vitro pollination, fertilization and embryo rescue system in lily was examined. By combining pollination techniques to overcome pre-fertilization barriers with in vitro methods to overcome post-fertilization barriers, interspecific lily crosses could be made more efficiently. In vitro cut-style pollination and in vitro grafted style technique were developed and applied on various interspecific crosses using *Lilium longiflorum*, *L. dauricum*, *L. henryi*, and both Asiatic and Oriental hybrids as the parents. In addition, methods for ovary culture, ovary-slice culture and ovule culture were generated. Ovule swelling score in ovary culture was used to evaluate media effects on ovule development. Using the integrated in vitro pollination and fertilization protocol it was not only possible to raise the total number of hybrid plantlets in a single interspecific cross, but also the number of successful interspecific combinations.

*Key words:* *Lilium* species; Asiatic hybrids; Oriental hybrids; interspecific hybridization; ovary culture; embryo rescue; in vitro grafted style pollination.

## Introduction

The genus *Lilium* comprises about 85 species, classified into seven sections [1]. The majority of cultivated cultivars, i.e. the Asiatic hybrids, originate from interspecific crosses between species of the *Sinomartagon* section and a smaller group of cultivated varieties, the Oriental hybrids, from crosses in the *Archelirion* section. Most cultivars are grown for bulb and cut flower production and the total harvest represents an important economic value in the Netherlands. Whereas lily flowers are appreciated world-wide, the assortment could still be considerably improved by exploiting traits from *Lilium* species of other sections. In particular, introduction of resistances against viral diseases, bulb rot (caused by *Fusarium oxysporum*) and *Botrytis*, and tolerances for suboptimal culture conditions (e.g. low light and low temperature), together with special flower colors and forms are topics of current lily breeding programs [2].

Possibilities for cross combinations in *Lilium* are limited by incompatibility and incongruity. Sexual barriers preventing interspecific hybridization have been separated into pre- and post-fertilization barriers [3]. In lily, many studies have dealt with methods for overcoming the pre-fertilization barriers [4—6]. In a comparison of several pollination methods, it was concluded that pre-fertilization bar-

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*Abbreviations:* BA, 6-benzylaminopurine; CPO, Centre for Plant Breeding Research; CSM, cut style method; cvs, cultivars; DAP, days after pollination; GSM, grafted style method; IAA, indole-3-acetic acid; MS, Murashige and Skoog medium; N, normal pollination; NAA, naphthalene acetic acid; ovar, ovary; ovul, ovule; 2,4-D, 2,4-dichlorophenoxy acetic acid.

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