

LT-0019

IN VITRO POLLINATION AND OVARY CULTURE AS A BREEDING TOOL IN WIDE
HYBRIDIZATION OF *LILIUM* AND *NERINE*

Jaap M. van Tuyl, Marjan G.M. van Creijl and Maria P. van Diën
Centre for Plant Breeding and Reproduction Research (CPRO-DLO),
P.O. Box 16
6700 AA Wageningen, The Netherlands

Abstract

Using lily (*Lilium* L.) as a model crop, an in vitro pollination, fertilization and embryo rescue system has been developed. By combining pollination techniques to overcome pre-fertilization barriers with in vitro methods to overcome post-fertilization barriers, wide interspecific lily crosses could be made more efficiently. In vitro cut-style pollination and in vitro grafted style techniques were successfully applied in various interspecific crosses. To overcome post-fertilization barriers, methods for ovary culture, ovary-slice culture and ovule culture were generated. Five *Lilium* species were used *L. longiflorum*, *L. henryi*, *L. concolor*, *L. dauricum*, *L. candidum*. The application of these methods resulted in many new hybrids between these species. To overcome intergeneric crossing barriers between the genera *Nerine*, *Amaryllis* and *Lycoris* in vitro pollination and ovary culture techniques have been developed analogous to lily. Results are promising.

1. Introduction

The bulb crops lily (*Lilium* L.) and *Nerine* Herb., which are grown for both bulb and cut flower production, represent an important economic value in The Netherlands. Although the cut flowers are appreciated world-wide, the assortment can still be improved greatly by introducing traits from allied species. In lily are topics of current breeding programs: introduction of resistances against viral diseases, bulb rot (caused by *Fusarium oxysporum*) and *Botrytis*, good forcing ability, tolerances for suboptimal growing conditions together with special flower colours and shapes. In *Nerine* the assortment is dominated by *Nerine bowdenii*, which is only available in the colour pink. New colours might be obtained from related species and genera, e.g. white and orange from *N. sarniensis* and yellow from *Lycoris aurea*. Another species, *Amaryllis belladonna* might be used as parent for a larger flower size (Van Tuyl et al., 1988).

Interspecific crossings are mostly limited by both pre- and post-fertilization barriers. In lily, many studies have dealt with methods for overcoming the pre-fertilization barriers (Myodo, 1963; Asano and Myodo, 1977). In a comparison of several pollination methods, it was concluded that in lily most pre-fertilization barriers can be bypassed by using the cut-style technique (Van Tuyl et al., 1988). This method comprises the deposition of pollen on the stylar surface after cutting the style with stigma, and allows pollen to circumvent stylar barriers which normally inhibit pollen tube growth (Myodo, 1963; Asano and Myodo, 1977). Seedset however is low, probably caused by the

sterilized in 70% ethanol (1 min) and bleached with 2% chlorine (15 min) and subsequently rinsed three times in sterile water. After rinsing, petals and anthers were dissected with a scalpel, and complete pistils were placed vertically in a long test tube (20 cm) partly filled with medium. Aseptic pollen was obtained by dehiscence of sterilized flowers under sanitary conditions. The viability of aseptic pollen was determined by in vitro germination tests. For lily all three types of pollination, N, GSH and GSM, were applied in vitro.

Success of in vitro pollination was scored for lily 30-45 days after pollination (DAP) by slicing the ovaries and careful dissection of embryos from the ovules, or 60-90 DAP by counting the number of mature seeds with embryos and endosperm; for *Nerine* the seeds were measured, counted and sown in the soil or placed on ovule culture medium (Table 2).

3.2. Ovary-ovule culture and embryo rescue technique

Ovary-ovule culture was applied with compatible crosses within *Lilium* and *Nerine* using the species and cultivars mentioned in Table 1. The method was also used in incongruent crosses within *Lilium* (crosses of *L. longiflorum* with 'Whillito', *L. dauricum*, *L. concolor* and *L. henryi*; 'Connecticut King' with 'Star Gazet' reciprocally) and *Nerine* (crosses with *Amaryllis* and *Lycoris*). Ovary culture was started 5-8 DAP (lily) and 2-5 DAP (*Nerine*). Ovaries were sterilized as described for buds above. Subsequently, for lily ovaries were transversely sectioned in about six to eight 3-4 mm thick sections, slices were polarly placed on the medium. The development of the different media used in this study are described elsewhere (Van Tuyt et al., 1991) and summarized in Table 2.

The ovaries or sections were incubated for lily at 25°C and for *Nerine* at 17 or 25°C in the dark. For lily ovule culture was started from 42 DAP after slicing the ovaries and dissection of the ovules to germinate them on the ovule culture medium. Different methods were used for *Nerine*. Three to seven DAP ovaries were placed in vitro, 21-40 DAP ovules were dissected and placed on ovule culture medium. Emerged plantlets were transferred to the embryo rescue medium (Table 2).

4. Results and discussion

4.1. Lily

4.1.1. Ovary-ovule culture

In various incongruent combinations e.g. *L. longiflorum* x 'Whillito', *L. longiflorum* x *L. concolor*, *L. longiflorum* x *L. henryi*, *L. longiflorum* x *L. dauricum* and *L. dauricum* x *L. henryi* this method appeared to be much more successful than the embryo rescue method applied 40 DAP. In average ten times more embryos could be rescued this method as compared with the embryo rescue method applied 40 DAP. Alternative pollination methods for interspecific crosses were

4.3. Conclusions

- In vitro pollination and/or ovary culture techniques are successful in interspecific crosses of *Lilium* and in intergeneric crosses of *Nerine* by delivering considerably more hybrids than in vivo procedures.
- The combination of in vitro pollination and the grafted style technique (GSM) opens new perspectives for overcoming crossing barriers.
- Using in vitro rescue techniques in intergeneric crosses with *Nerine* gives opportunity to overcome F1-sterility by applying in vitro chromosome doubling.

References

- Asano, Y. and Myodo, H., 1977. Studies on crosses between distantly related species of *Lilium*. I. For the intrastylar pollination technique. *J. Japan. Soc. Hort. Sci.* 46(1):59-65.
- 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., 1977. Studies on crosses between distantly related species of lilies. II. The culture of immature hybrid embryos. *J. Japan. Soc. Hort. Sci.* 46:267-273.
- Guha, S., and Johri, B.N., 1966. In vitro development of ovary and ovule of *Allium cepa* L. *Phytomorphology*: 353-364.
- Kanoh, K., Hayashi, M., and Serizawa, Y., 1988. Production of interspecific hybrids between *Lilium longiflorum* and *L. elegance* by ovary slice culture. *Japan. J. Breed.* 38:278-282.
- Myodo, H., 1963. Experimental studies on the sterility of some *Lilium* species. *Journ. Fac. Agr. Univ. Sapporo* 52:70-122.
- Van Roggen, P.M., Wilms, H.J., Kelfzer, C.J. and Van Tuyl, J.M., 1986. Pollen tube growth in an interspecific cross between two *Lilium* species. In: Williams E.G., Knox R.B., Irvine D. (ed.). *Pollination: Proc. Int. Symposium, Melbourne: 240-241.*
- Van Tuyl, J.M., Straathof, Th.P., Bino, R.J. and Kwakkenbos, A.A.M., 1988. Effect of three pollination methods on embryo development and seedset in intra- and interspecific crosses between seven *Lilium* species. *Sex. Plant Reprod.* 1: 119-123.
- Van Tuyl, J.M., Van Diën, M.P., Kroon, G.H. and Kwakkenbos, A.A.M., 1988. Verdieping van het *Nerine* sortiment door soort- en geslachtskruidingen binnen de Amaryllidaceae. *Prophyta* 42(6):157.
- Van Tuyl, J.M., Van Diën, M.P., Van Creijl, M.G.H., Van Kleinwee, T.C.M., Franken, J. 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.
- Van Tuyl, J.M., Meijer, H., and Van Diën, M.P. 1992. The use of oryzalin as an alternative for colchicine in in-vitro chromosome doubling of *Lilium* and *Nerine*. *Proc VI International Bulb Symp.*
- zenkeler, M., 1990. In vitro fertilization and wide hybridization in higher plants. *Critical Reviews in Plant Sciences* 9(3):267-279.