Short Communication Plant Tissue Culture Letters, **12**(3), 317-319(1995)

Comparison of Seedling Production among Several Embryo-rescue Techniques in *Lilium formosanum* Wallace

Yoshiji NIIMI, Masaru NAKANO and Masashi GOTO*

Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Niigata 950-21, Japan
* Present address: *Sakata Seed Corp.*, 2-7 Nakachodai, Tsuzuki-ku, Yokohama 224, Japan

(Received May 27, 1995)
(Accepted August 5, 1995)

In the genus *Lilium*, interspecific hybridization has been conducted for producing novel cultivars, and various embryo-rescue techniques, for example, embryo culture[4,5], ovule culture[4,5], ovary culture[5,6], and ovary-slice culture[1-3] have successfully been applied to overcome post-fertilization barriers of cross incompatibility. Although, among these techniques, embryo culture has been employed most frequently, it requires highly skilled manipulation to deal with a small embryo, and hardly be applied to very young embryos. In the present study, a comparison of several embryo-rescue techniques was made in order to establish a simple and efficient method for obtaining seedlings from young embryos in *Lilium formosanum* Wallace.

Ovaries of *L. formosanum* havested 10, 20, 30 and 40 days after self-pollination were surface-disinfected with 70% ethanol for 1 min. and then with a commercial bleach solution containing 1.8% chlorine for 10 min. followed by 3 rinses with sterilized distilled water. Ovary-slice disks were prepared by cutting ovaries transversely into slices 5 mm thick. Ovules were excised from ovaries with or without placenta, and embryos were picked up under a dissecting microscope. Four different explants, embryo, ovule, ovule with placenta, and ovary-slice disk, were placed on a

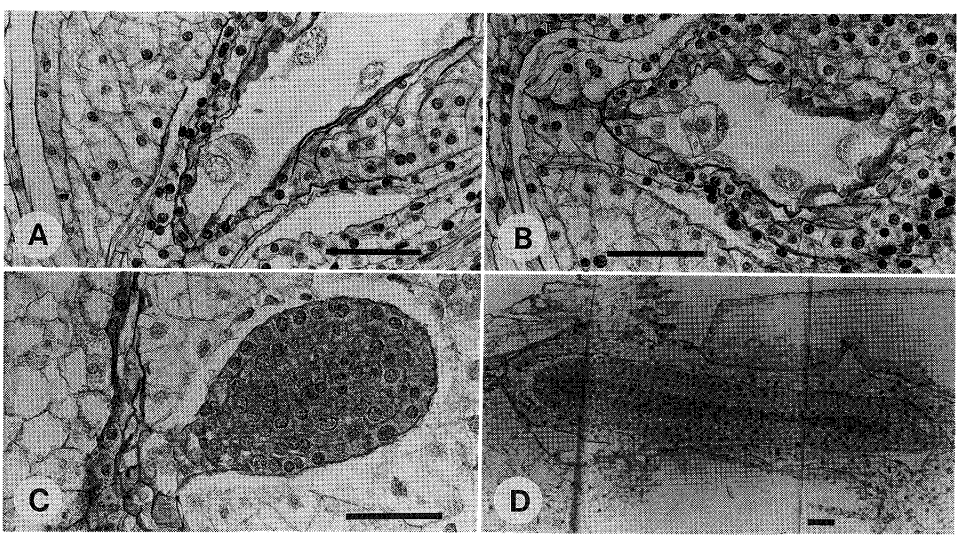

Fig. 1 Longitudinal sections of ovules 10-(A), 20-(B), 30-(C), and 40-(D) days after self-pollination. Bar=0.1 mm.

medium containing major salts of B5[7], minor salts of Murashige and Skoog[8] at half strength, amino acids of Prakash and Giles[9], 0.1 mg/*l* α-naphthaleneacetic acid, 5% sucrose and 0.7% agar, and adjusted to pH 5.7. Cultures were maintained at 25°C in the dark. Examination of serial longitudinal sections of ovules showed that ovules sampled 10 and 20 days after pollination contained two-celled (below 0.1 mm in length) and four-celled (*ca.* 0.1 mm in length) pro-embryos, respectively (**Fig. 1-A, B**). Ovules 30 and 40 days after pollination had embryos *ca.* 0.3 mm and *ca.* 1.6 mm in length, respectively (**Fig. 1-C, D**).

Seedling production by four different embryo-rescue techniques at various times after self-pollination is summarized in **Table 1**. Embryos 10 and 20 days after pollination were too small (*ca.* or below 0.1 mm in length) to be excised, and therefore, it was impossible to apply embryo culture for these young embryos. It has already been reported that the smallest size for successful embryo culture in *L. longiflorum* was a length of 0.3 mm[3]. In all culture techniques, percentage of seeding production increased as days after pollination at the time of culture increased. Over 50% of embryos 30 and 40 days after pollination could be rescued by employing all four techniques. Seedlings could be obtained from embryos 10 days after pollination by using three embryo-rescue techniques, ovule, ovule with placenta and ovary-slice cultures (**Fig. 2**), although their frequencies were relatively low (below 5%). Although Hayashi *et al.*[10] has already rescued embryos 20 days after pollination by employing an ovary-slice culture technique in *L. formosanum*, to our knowledge, this is the first report on successful production of seedlings from very young embryos 10 days after pollination in the genus *Lilium*. Among these three techniques, ovule with placenta culture seems to be most practical for rescuing young embryos, since, as in the case of embryo culture, ovule culture requires more complicated manipulation especially when young materials are used,

Table 1. Difference in percentage of seedling production among different embryo-rescue techniques in *L. formosanum**1.

Days after pollination	Embryo culture	Ovule culture	Ovule with placenta culture	Ovary-slice culture
10	—*2	4.2	4.9	2.6
20	—	50.0	52.9	28.6
30	53.3	84.6	79.0	82.1
40	100.0	98.1	84.3	89.7

*1 Percentage of seedling production: (No. of germinating embryos)/(No. of cultured embryos) ×100(%). Data were recorded 3 months after pollination.

*2 Embryo culture could not be employed since embryos were too small to be excised.

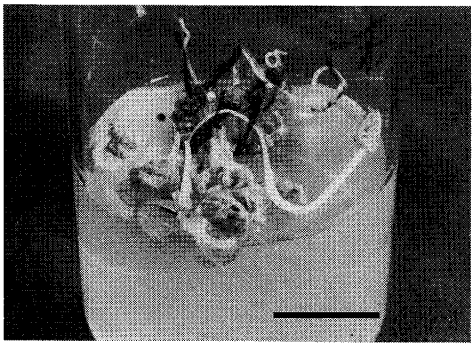

Fig. 2 Seedling production from young embryos 10 days after self-pollination by employing an ovule with placenta culture technique.
Bar=2 cm.

and since bacterial or fungal contamination was frequently observed in ovary-slice culture (data not shown). In addition, ovule with placenta culture can handle a relatively large number of embryos at once.

In the present study, a simple method for obtaining seedlings from very young embryos was established in *L. formosanum* by employing an ovule with placenta culture technique. This method may be applicable for producing novel cultivars from crosses between distantly related species in which death of hybrid embryos occurs at a very early stage.

References

1) North, C., B. Wills, 1969. Euphytica, **18**: 430-434.
2) Asano, Y., H. Myodo, 1977. J. Jpn. Soc. Hort. Sci., **46**: 267-273.
3) Asano, Y., 1980. J. Jpn. Soc. Hort. Sci., **49**: 114-118.
4) Straathof, T. P., J. M. Van Tuyl, C. J. Keijzer, H. J. Wilms, A. A. M. Kwakkenbos, M. P. Van Dien, 1987. Plant Cell Incompat. Newsl., **19**: 69-74.
5) Van Tuyl, J. M., M. P. Van Dien, M. G. M. Van Creij, T. C. M. Van Kleinwee, J. Franken, R. J. Bino, 1991. Plant Sci., **74**: 115-126.
6) Kanoh, K., M. Hayashi, Y. Serizawa, T. Konishi, 1988. Jpn. J. Breed., **38**: 278-282.
7) Gamborg, O. L., R. A. Miller, K. Ojima, 1968. Exp. Cell Res., **50**: 151-158.
8) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
9) Prakash, J., K. L. Giles, 1986. In"Genetic Manipulation in Plant Breeding", p. 335-337, Walter de Gruyter & Co., Berlin, New York.
10) Hayashi, M., K. Kanoh, Y. Serizawa, E. Yoon, 1986. Jpn. J. Breed., **36**: 304-308.

《和文要約》

タカサゴユリ(*Lilium formosanum* Wallace)における簡便で効率的な
実生獲得のための胚救出方法の検討

新美芳二，中野　優，後藤正史*

新潟大学農学部
* 現在：(株)サカタのタネ

タカサゴユリ(*Lilium formosanum*)において，幼胚からの実生獲得方法を確立するために，4通りの胚救出方法(胚培養，胚珠培養，胎座付き胚珠培養，子房輪切り培養)を比較したところ，胚珠培養，胎座付き胚珠培養および子房輪切り培養を行った場合には，自家受粉後10日目の幼胚からも実生を獲得することができた．特に，胎座付き胚珠培養は比較的簡便に行え，また外植片の汚染も少ないといった点から，最も実用的であると考えられた．