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Embryo Rescue in *Ornithogalum*

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Abstract. **Techniques are described to determine whether embryos are formed in ovules of incompatible crosses between *Ornithogalum* (L.) plants, and to rescue embryos in cases where the development of embryos is halted following fertilization. By using Herr's clearing liquid, it can be ascertained within 5 hours whether hybrid embryos have been formed. Such embryos can be rescued by culturing them in ovulo on basal medium containing 70 g sucrose/liter and no added growth regulators. The embryos' requirement for sucrose changes as they develop; therefore, cultured ovules are transferred after 14 days to a medium containing 10 g sucrose/liter, where germination occurs.**

Ornithogalum spp. are of commercial importance in the cut flower industry. A breeding program to develop improved cultivars was initiated at the Vegetable and Ornamental Plant Research Inst., Pretoria, Republic of South Africa, but was hampered by incompatibility between species. In several unsuccessful crosses, ovaries of- pollinated flowers enlarged, but turned yellow and died after 1 week. Fertilization possibly took place in such ovaries, but the embryo did not develop or was halted following fertilization.

In this report, we describe two techniques: 1) determining within a few hours whether embryos were formed, and to what stage their development appeared normal; 2) rescuing embryos to obtain seedlings. *Ornithogalum dubium* (Houtt.) was chosen because it is self-compatible, has good seed set, and is useful to our horticultural industry for its glossy, orange flowers.

Mature plants were cultivated at 22C in a commercial glasshouse during their normal growing season (winter). They received natural light, and daylength followed the natural seasonal pattern in the Southern Hemisphere. Flowers were emasculated before anthesis and pollinated once—when stigmas appeared woolly and were receptive.

The breeder can determine whether hybrid embryos have been formed in the ovules of incompatible crosses by the following method. After pollination, young seeds (fertilized ovules) of *O. dubium* were fixed for 4 hr in Camoy's fixative at daily intervals. The fixed seeds were stored in 70% ethanol if necessary. For observation, seeds were removed from the fixative or 70% ethanol and mounted in a drop of Herr's clearing liquid (Herr, 1971). Slides were examined at x 100 (Fig. 1, top left) using phase contrast illumination to localize the embryo. The oil-immersion objective was used for observing details. In seeds with normal embryo development, stages of pro-embryo development 4 to 10 days after pollination (DAP), as described earlier (Van Rensburg and Robbertse, 1988) for self-pollinated *O. dubium*, were observed (Fig. 1, top right; bottom left, center, and right). Preparations were not permanent, as digestion by the clearing liquid continued after the embryos became visible. As seed and endosperm development progressed, the period for clearing increased. In the case of pro-embryos of 4 to 5 DAP, clearing took place in 10 to 15 see, whereas this period was ≈1 min for globular embryos of 10 DAP. No embryo of a more advanced stage than the globular stage at 10 DAP could be observed when this clearing method was used. In unfertilized ovules, unfertilized egg apparati (EA) were not observed and embryo sacs appeared empty. Van Rensburg and Robbertse (1988) described walls of the EA of *O. dubium* before fertilization as thin or absent. This situation probably explains why no EA was observed in unfertilized ovules in our study. We suggest that, in the application of this technique, ovules that may contain embryos are examined from 4 DAP, since this was the first stage at which embryos were observed in *O. dubium.*

An embryo culture technique that can be used to rescue hybrid embryos was developed using *O. dubium* embryos. The basal medium (BM) used consisted of Murashige and Skoog (1962) inorganic salts to which the following compounds were added (mg·liter^{-1}): FeNaEDTA, 25; glutamine, 400; cysteine, 20; glycine, 10; arginine, 10; and myoinositol, 100. The pH was adjusted to 5.8 after 6 g agar/liter was added; then the medium was sterilized at 121C for 15 min. The following were added to the medium: sucrose at O, 1, 10, 30, 50, or 70 g·liter^{-1}; benzylamino purine (BA), naphthaleneacetic acid (NAA), and gibberellic acid (GA_3), each at 0, 0.01, 0.1, or 1 mg·liter^{-1}. Before dissection of ovules, ovaries were surface ster-

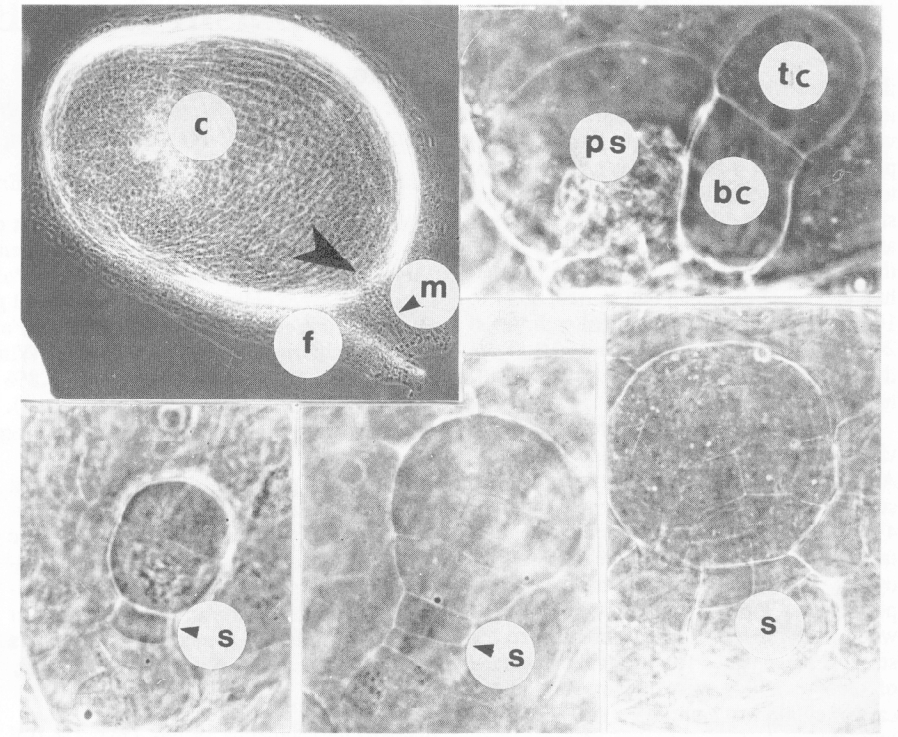

Fig. 1. Observations taken from *Ornithogalum dubium* (Houtt.) embryos in fertilized ovules of young seeds by using a clearing-squash technique. (top left) An ovule (× 100), arrow indicates the position where embryo occurs; (c) chalaza; (f) funicle; (m) micropyle. **(top right)** Two-celled pro-embryo at 4 days after pollination (DAP) (× 1000); (be) basal cell, (tc) terminal cell; (ps) persistent synergid. **(bottom left)** Pro-embryo at 4 DAP (× 1000); (s) suspensor. **(bottom center)** Pro-embryo at 6 DAP (× 1000). **(bottom right)** Globular embryo at 10 DAP (× 1000).

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Table 1. In vitro survival percentage of *Ornithogalum dubium* (Houtt.) embryos at various developmental stages.

Age of embryo (days after pollination)	Stage of development	Survival (%)
4	Pro-embryo, two to 16 cells (in ovulo)	20
6–8	Pro-embryo, 32 to 64 cells (in ovulo)	60
10–12	Globular (in ovulo)	80
14–16	Oval (in ovulo)	80
24	Mature cylindrical (isolated)	100

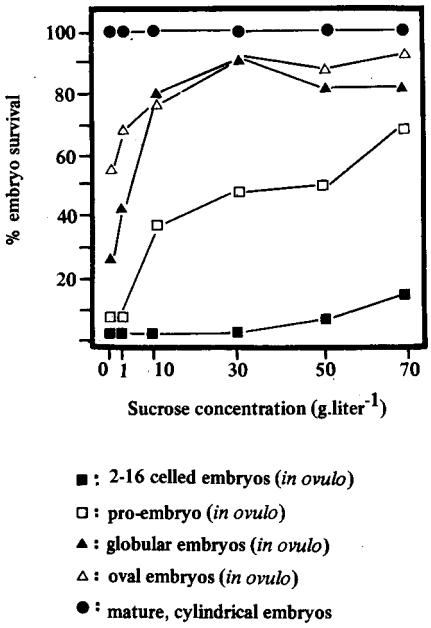

Fig. 2. The in vitro survival percentage of *Ornithogalum dubium* (Houtt.) embryos of various developmental stages cultured on basal medium to which sucrose was added at different concentrations.

ilized-by soaking them in 1% NaOCl for 20 min and rinsing in sterile water. Embryos of various developmental stages were cultured: pro-embryos at 4 DAP that consisted of two to 16 cells; pro-embryos at 6 to 8 DAP consisting of 32 to 64 cells; globular embryos at 10 to 12 DAP; oval embryos, in which the radicle had differentiated and the scutellum and shoot meristem had started, at 14 to 16 DAP; and mature, cylindrical embryos at 24 DAP. Mature embryos were isolated from the ovule and younger embryos were cultured in ovulo.

Ability of *O. dubium* embryos to grow in vitro increased with embryo age (Table 1). A small percentage of pro-embryos of 4 DAP survived culture. It should be noted that, at 4 DAP, it was difficult to judge whether certain ovules were bigger than others (bigger ovules were assumed to be fertilized). It is possible that a percentage of ovules cultured were not fertilized and that this affected the survival percentage. The survival percentage of older embryos were higher and, in the case of cylindrical embryos, a 100% survival was obtained. Concentration of sucrose in the nutrient medium had a significant effect on survival and subsequent growth of cultured embryos. Pro-embryos required a relatively high sucrose concentration (70 g·liter^{-1}) to survive (Fig. 2). This requirement decreased as embryos developed; oval embryos survived in the absence of sucrose. Although globular embryos were able to survive at sucrose concentrations of 10 or 30 g·liter^{-1}, they germinated precociously so that malformed seedlings developed. The addition of BA, NAA, or GA_3 at the concentrations given above inhibited embryo growth.

To obtain viable seedlings from pro-embryos, pro-embryos 4 to 10 DAP were cultured initially for 14 days on the BM containing 70 g sucrose/liter. On this medium, embryogenesis continued, although development of the peri-embryonal tissue occurred sporadically. After this period, ovules were placed on BM containing 10 g sucrose/liter. Germination occurred on this medium and resultant seedlings could be transplanted to sterilized soil after 2 months.

In ovulo culture of pro-embryos was used for embryo rescue. The breeding cycle could, however, be shortened by 1 year if mature cylindrical embryos were germinated on the BM containing 10 g sucrose/liter. In this case, embryos were dissected from seeds before seeds were completely dehydrated.

By using Herr's clearing technique, *Ornithogalum* breeders can determine, within 5 hr, whether hybrid embryos have been formed in the ovules of an incompatible cross. Such embryos can be rescued by culturing them in ovulo on a BM containing 70 g sucrose/liter. After an initial culture period of 14 days, ovules are transferred to a BM containing 10 g sucrose/liter for germination.

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Fire Blight Susceptibility in Pyrus Germplasm from Eastern Europe

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Abstract. **During 1976-1980, three plant exploration trips were made throughout eastern Europe in search of native *Pyrus* germplasm. A total of 384 accessions (231 from Yugoslavia, 86 from Romania, 43 from Poland, and 12 each from Hungary and Czechoslovakia) were collected as budwood and propagated at the National Plant Germplasm Quarantine Center in Glenn Dale, Md. Following 8 years of exposure to the fire blight bacterium *[Erwinia amylovora* (Burr.) Winsl. et al.], 17.49" of the accessions remained uninfected, 11.2% rated resistant, 6.8% moderately resistant, and 64.6% blighted severely (26% to 100% of tree blighted). Some of the superior accessions have been released for use in the pear breeding program.**

New pear germplasm has recently been introduced from eastern Europe for preservation and to provide a more-diverse genetic base for breeding improved cultivars. Although the germplasm was thought to be primarily a possible source of resistance to other diseases and insects, the exposure for 8 years to fire blight, caused by *Erwinia amylovora,* allowed us to assess the degree of susceptibility in this gene pool to a bacterial disease of major importance in North America and much of western Europe.

Germplasm collecting. During 1976-1980, three plant exploration trips were made by the senior author to five countries of Eastern Europe: Yugoslavia, Romania, Hungary, Czechoslovakia, and Poland. Pear *(Pyrus communis* L.) germplasm from these countries was nearly absent from North American and most western European repositories and working collections. The region is closer to the Near Eastern center of diversity where domesticated pear is thought to have originated (Vavilov, 1951); in addition, wild

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