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# Note on the Culture of Lychnis Embryos

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The additional literature cited in two recent reviews by White (2, 3) indicates the growing interest in tissue culture since his original review (1) of the subject in 1931. Although embryo culture is not tissue culture in the true sense of the word, the approach to the problems and the technics employed, are so similar that White has summarized the literature on embryo culture, and all other studies approaching a true tissue culture, in these articles.

The present study was begun in an attempt to obtain normal plants from immature *Lychnis* embryos dissected from developing seeds. Although immature embryos of other genera have been cultured, primarily those of the Gramineae and Cruciferae, apparently no effort has been made to culture embryos of any genera of the Caryophyllaceae.

Ten days after pollination, *Lychnis* seeds measure about 1.5 mm. in length. The embryo has begun to curve around the perisperm. Cotyledons, hypocotyl, radicle, and stem tip are differentiated. The diameter of the embryo through the stem tip region averages 110 micra. As the embryo matures, it becomes more curved and increases in diameter until, at the time of capsule dehiscence, it averages 250 micra in diameter.

Immature embryos were dissected from developing seeds under a binocular microscope. Young seeds were anchored by a sterile dissecting needle inserted in the perisperm while another sterile needle was used to chip away a groove around the periphery of the ovule. Half of the perisperm was then lifted away, exposing the embryo. The embryo was immediately transferred to White's nutrient medium made solid by the addition of one-half percent agar.

Embryos were started on agar slants in test tubes. When four or more leaves were evident, the seedlings were usually transferred to the same nutrient in 250 cc. Erlenmeyer flasks. After six to eight leaves had been formed the seedlings were transferred to sand and latr to soil.

Embryos removed from ovules nine days after pollination and cultured on artificial media elongated in two days to about 4 mm. in length. The curved embryos straightened and the cotyledons began to spread out at right angles to the hypocotyl. Three days later the cotyledons were green and the seedlings 9 mm. long. Nine days after culturing there was a healthy top growth of six leaves but a poorly developed root system. Seedlings grown from nine day embryos never developed beyond this point.

Embryos excised from seeds ten days after pollination were successfully grown to maturity. The rate of growth and development appeared the same as for nine day embryos. A healthier root growth, however, apparently made it possible to culture them beyond the

six leaf stage. Seedlings from ten day embryos were potted three weeks after excision, and were in the vernal stage seven weeks later.

Embryos dissected from seeds eleven days after pollination and later, were the same size (4 mm.) as the younger ones after two days growth on agar. The cotyledons were spreading at right angles to the hypocotylroot axis. All older embryos elongated more rapidly than the nine day ones. The largest twelve day embryos were 12-13 mm. long when the nine day embryos were only 9 mm. long.

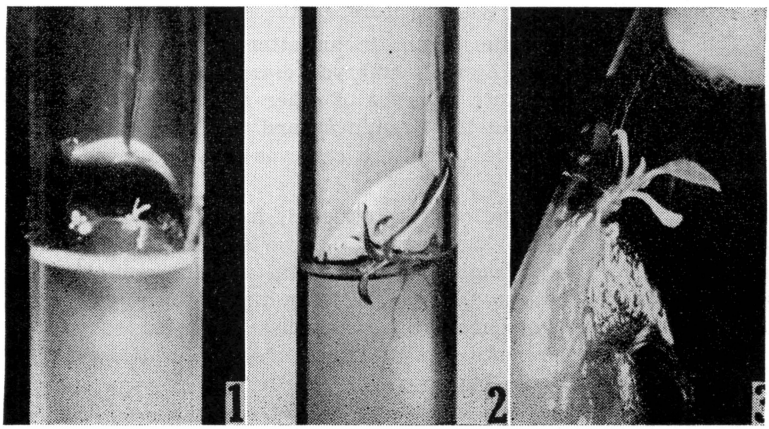


PLATE I

Embryo dissected from seed 22 days after pollination.
Figure 1. Embryo growing on agar 4 days.
Figure 2. 11 days.
Figure 3. 28 days.

Embryos twenty-two days after pollination or about three days prior to capsule dehiscence, when grown on nutrient agar, approximate the development of embryos from mature seeds. After four days on nutrient agar, the cotyledons became green, the hypocotyl was erect, and roots deeply penetrated the agar (Fig. 1). Secondary leaves were evident after eight days of culturing. Three days later (Fig. 2), the seedlings had four leaves in addition to the cotyledonary leaves and a much branched root system. At this time the plants (Fig. 3) were potted and later developed into vigorously growing rosettes. One month later the plants were blooming.

Except for the more rapid growth of the older embryos, no difference was observed between the growth pattern of immature embryos dissected ten to twenty-two days after pollination. Plants obtained from immature embryos resembled young plants growing outdoors. Seedling seldom developed more than eight leaves on artificial media even if transferred to fresh nutrient, but appeared to remain in a static condition.

The rapid development of *Lychnis* embryos following pollination and the ease with which embryos can be removed from seeds and grown on solid nutrient media, suggests that *Lychnis* may offer suitable material for further anatomical study of embryo and seedling development. More extended study of the technic of culturing younger embryos on artificial media should make it possible to grow mature plants from embryos younger than ten days after pollination.

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