

Abnormal organ formation from the hypocotyl of cultured young embryos in *Pharbitis nil* (Preliminary report)

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About two weeks old embryos of *Pharbitis nil* Chois, strain Tendan or Violet, were cultured on the modified White's medium containing a growth-promoting factor obtained from young *Lupinus* seed¹⁾, supplemented with 20g sucrose and 9g agar per liter. For the aseptic isolation of young embryos, young fruits were rinsed with 80 per cent ethanol for three minutes and treated with 10% calcium hypochlorite solution for fifteen minutes. Young seeds were cut open, and spade-shaped green embryos of 0.8 mm were isolated with sterilized needles in a sterilized Petri dish under a dissecting microscope. Isolated embryos were planted 1 mm below the surface of the medium in the test tubes and were maintained at 26°C under continuous illumination of fluorescent lamps. The light intensity at plant level was ca. 3200 lux.

By the end of the two weeks culture, the embryos enlarged ten times in size and remained green and spade-shaped. To avoid the retardation of root initiation which was often observed in the culture of *Datura* embryos on the medium containing *Lupinus* factor¹⁾, they were transferred

to the fresh medium containing 1g casein hydrolysate per liter. The embryos were partially submerged in the medium with the cotyledons kept above the surface. The embryos continued to grow until they attained 3 cm in length in about one month. The hypocotyl elongated and thickened, and the cotyledons expanded. Root and plumule however did not develop. Cotyledons were green while in contact with the culture medium, but in the air it lost chlorophyll and became pale. After about one month many green outgrowths appeared out of the hypocotyl near the radicle. They appeared similar to an ascidial leaf, and grew often downwards in the agar medium. Later some of them developed blade-like expansion with veins (Fig. 1). Microscopic observations of this "leaf blade" revealed that it had no stomata but possessed a number of oil sacs which were found in the cotyledons of this plant. As the development of stomata of the cotyledon itself was strongly suppressed under the culture condition of the present experiment, this adventitious organ seems to be of cotyledonary or foliar nature. This organ formation was observed in all successfully cultured embryos.

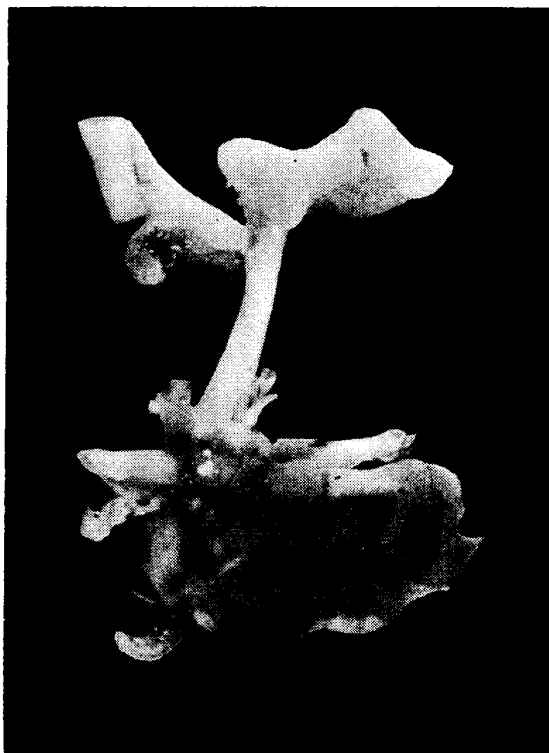


Figure 1. The adventitious organs differentiated from the hypocotyl of cultured embryos of *Pharbitis nil*, ca. 3×

References

1. Matsubara, S. 1962 : Studies on a growth-promoting substance, "embryo factor", necessary for the culture of young embryo of *Datura* in vitro. Bot. Mag. Tokyo 75 : 10-18.

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