

Production of Embryos on the Stem of *Ranunculus sceleratus* L.

The floral buds of *Ranunculus sceleratus* excised at the primordia stage (showing sepal, petal and stamen primordia) proliferated into an extensive callus on a modified White's medium supplemented with coco-nut milk (10% v/v) and indole-3-acetic acid (1 ppm). Subsequently, the callus differentiated into embryo-like structures (designated embryoids) which developed into seedlings, both

in situ and when excised and transferred to a fresh medium.

A remarkable feature of the 3- to 4-week-old seedlings is that they themselves show supernumerary embryos at the radicular end or along the surface of the stem (Figures 1, 2). The development of embryos is non-synchronous and several developmental stages may be seen on the same seedling. The mature embryos possess a radicle-plumule axis and two (sometimes three) cotyledons characteristic of the embryos which develop from normally fertilized ovules. The embryos germinate in situ and also when excised and transferred individually to a fresh medium.

Portions of the stem of the seedlings, free from embryos, were transferred to White's medium fortified with coco-nut milk (10% v/v). In 2-week-old subcultures, the explants were already studded with new embryos. Microtome sections revealed that the accessory embryos originate from the epidermal cells. Structurally and functionally these embryos are similar to zygotic embryos. To the best of our knowledge this is the first report of the origin of embryos from the epidermal cells of the stem which provides an additional proof of totipotency of the cells¹.

Résumé. Les embryoides se développant à partir du bourgeon de callee de *Ranunculus sceleratus* deviennent des plantules. Ces plantules produisent des embryons sur-numéraires à l'extrémité radiculaire ainsi que tout le long de la surface de la tige. Les études anatomiques révèlent que ces embryons sont d'origine épidermique. C'est une nouvelle preuve de la totipotence des cellules.

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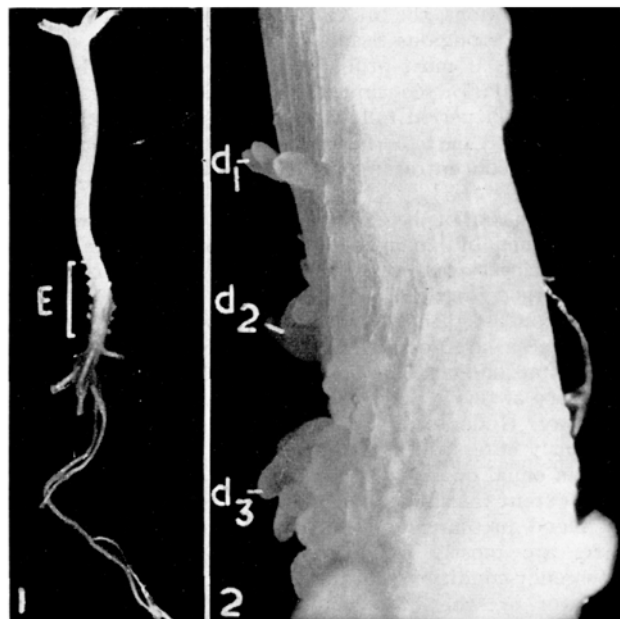


Fig. 1. Seedling with lower portion of the stem studded with accessory embryos. $\times 5$.

Fig. 2. Enlarged view of the portion marked E in Figure 1; note especially the embryos at d_1 , d_2 , d_3 . $\times 65$.

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On the Presence of an External Hemal Pore in *Lymnaea stagnalis* L.

When specimens of the pond snail, *Lymnaea stagnalis* L., are mechanically irritated, they extrude several drops of fluid. This can be demonstrated when a snail has been kept out of water on filter paper for some minutes, by pushing a glass rod against the exposed parts. It can then also be observed quite easily that the fluid escapes from the pneumostome.

The present study was undertaken to determine the nature and the place of exit of this fluid. When studied under the microscope it appeared to contain many amoebocytes, which shows that the main part of the fluid does not consist of urine and of water present in the lung cavity. Therefore it was concluded that the fluid, at least partly, must be blood.

This was confirmed by the fact that when snails were injected in the head or foot with 0.2–0.3 ml of methylene

blue or neutral red solutions the fluid extruded via the pneumostome within 1 sec after the irritation by the injection had the colour of the dye administered.

At first it was thought that the loss of blood had to be explained either by rupture of the pericardium wall, so that blood enters the respiratory cavity through the renopericardial canal and the kidney tube, or by the bursting of the thin-walled blood vessels in the roof of the lung. During careful macroscopic and microscopic examinations, however, no arguments in favour of these two suppositions could be obtained.

The problem was solved in the following way. Full-grown snails (shell length over 30 mm) were injected with 0.2 ml of Indian ink. After the extrusion of black fluid, the animals were narcotized¹ and the lung cavity was

¹ Method described by J. LEVER, J. C. JAGER, and A. WESTERVELD, *Malacologia* 1, 331 (1964).