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THE OCCURRENCE OF NEWLY INDUCED PHLOEM IN SHOOT-PRODUCING EXPLANTS OF ROOT TISSUE OF CICHORIUM INTYBUS L. VAR. SATIVA BISCH. CULTIVATED *IN VITRO*

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In a lecture given at the International Conference of Plant Tissue Culture at State College, Pa. (U.S.A.) in 1963, KARSTENS (1964) expressed the hope that more study would be given to the formation of phloem *in vitro* and to the structure of this newly-formed tissue. The literature contains repeated mention of the formation of phloem by plant tissue cultured *in vitro*, especially in explants but also in established strains. It is striking, however, to find that phloem is only mentioned; no structural details are given and, apart from a few exceptions and those very sketchy, no illustrations are given.

CAMUS (1949) has demonstrated in material cultured *in vitro* that shoots developing on explants of root tissue of *Cichorium intybus* induce bundles of vascular tissue in the underlying tissue. He reports that these bundles are partially composed of phloem. It should be noted in this connection that:

a. except for topographic figures, no pictures of either the newly-formed phloem or that initially present in the explant are given;

b. only transverse sections could be studied because the path taken by the bundles could not be determined externally and it was therefore impossible to make properly oriented longitudinal sections.

c. most of the bundles observed ran through the phloem already present in the explants or along the already present cambium, which must have made it difficult to determine whether the sieve tubes observed really belonged to the induced bundles.

BUVAT (1948) described the cytology of similar root-tissue explants, but referred to Camus for the vascular tissue induced by the shoots.

Although there need of course be no question whatever about the accuracy of the observations made by and the conclusions drawn by Camus, it seemed of sufficient interest, in respect of the remarks in the first paragraph above, to attempt to obtain supplementary information. This proved feasible by making use of a microtechnical method designed especially for the purpose, to be described in detail elsewhere (GALAVAZI, 1964). By this method, which leaves the cell contents intact, the material is made transparent and the xylem stained, thus

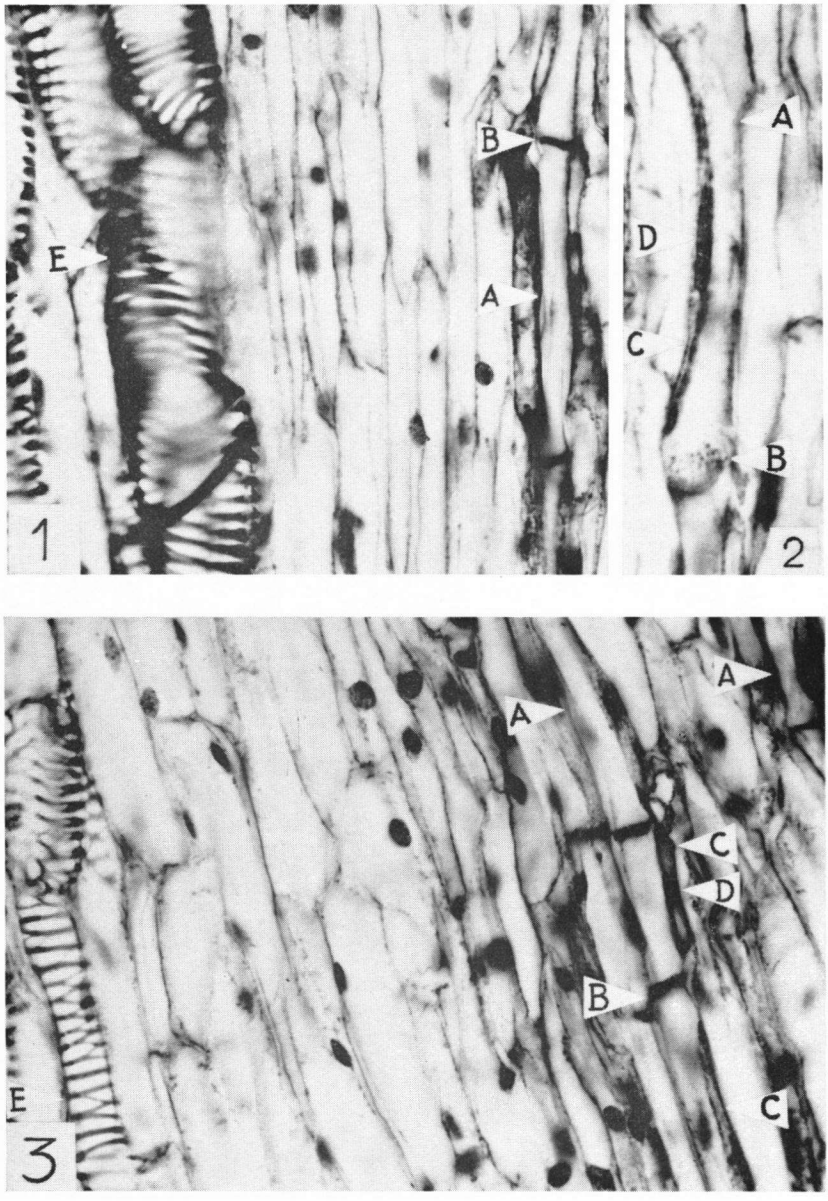

Plate I. 1, 2, 3.

Cichorium intybus L. var. *sativa* Bisch. Longitudinal sections through bundles of vascular tissue induced in parenchymatous proliferations of root tissue explants. 15μ. Safranin-fast green.

A: sieve tube; B: sieve plate; C: companion cell; D: nucleus of companion cell; E: xylem elements. 1 and 3: 350 ×; 2: 530 ×.

enabling determination of the path taken by the induced bundles. This is important because it permits accurate positioning of the material for making properly oriented longitudinal sections. The fact that the contents of the cells are left undisturbed makes it possible in addition to use the routine microtechnical methods to make very satisfactory microscopical preparations from the material.

Some of the *Cichorium* explants used in this work form considerable callus proliferations composed entirely of parenchymatous tissue. When a shoot subsequently develops on one of these proliferations, the bundle of vascular tissue induced by the shoot takes a course over an appreciable distance through homogeneous parenchyma. In such cases it is naturally impossible to confuse newly-formed vascular elements, whether xylem or phloem, with similar elements initially present.

Figures 1, 2, and 3 show photomicrographs of longitudinal sections through bundles of vascular tissue in callus proliferations obtained with our method. It is clear in the first place that this method indeed produces true longitudinal sections. Further, the Figures show that phloem elements are unquestionably present in the induced vascular bundles. Both enucleate, protoplasm-poor sieve tubes with distinct sieve plates and protoplasm-rich companion cells with distinct nuclei are visible.

On the basis of unequivocal illustrative material it may therefore be concluded that in the case described here phloem with a normal structure was indeed formed *in vitro*.

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