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The Course of Root Differentiation from Root Primordia in Poplar Stems

MÁRIA LUXOVÁ and A. LUX

Institute of Experimental Biology and Ecology, Slovak Academy of Sciences, Bratislava*

Abstract. Upon rooting of poplar stem cuttings the total inductive stimulation does not take place, but the individual root primordia maintain a relative independence. This becomes evident by various time parameters of their differentiation. Under experimental conditions, in dormant cuttings from one year individuals of the hybrid I 214 the activation of the first root primordia occurs after 24 h, the pre-emergent development of the roots formed was completed after 72 h.

The activated root primordium is divided into two regions. In the distal region with the predominating cell division the root apex with histogens is formed by the action of initials. From peripheral cell layers of the distal region the so-called "Wurzeltasche" develops which covers the root cap. Due to cell elongation in the proximal region the root apex is pushed up towards the stem surface. The beginning differentiation of the connective vascular tissue is a preparative step for the connection of the vascular system of the developing root with the secondary vascular system of the maternal stem. Following the penetration of the root through the peripheral stem tissues this connection is realized with progressing development. In the developed root the protoxylem elements differentiate continuously and acropetally in direct continuity with tracheids of the basal connective region.

The property which positively facilitates the vegetative propagation of plants is the capacity of forming root primordia in stems. According to BARANOVA (1951), their formation results from a special plant adaptability to the periodic change in environmental conditions. Root primordia are formed in those species the historical evolution of which occurred in moist stands and river bays of the temperate zone, regularily flooded at the time of spring thaws. Under favourable conditions, their existence enables a rapid development of adventitious roots. In an opposite case the root primordia remain in the stems in a latent state.

Our preceding paper (LUXOVÁ and LUX 1981) dealt with structural aspects of the initiation and development of root primordia in poplar stems. The inner organization of primordia is not advanced in this genus. The primordia from which roots develop under normal conditions of rhizogenesis (hence the term "root primordia"), may give rise to shoots after a specific hormonal treatment (LUX and LUXOVÁ, unpublished data). The present paper aims

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* *Address:* Dúbravská cesta 26, 885 34 Bratislava, Czechoslovakia.

at elucidating the mode of root formation from the primordia mentioned as well as the course of differentiation of their vascular connection with the secondary vascular tissue of the maternal stem.

MATERIAL AND METHODS

The central stem cuttings of the poplar hybrid I 214, cut from one-year individuals at the period of dormancy (January), were loosely wrapped up in a moist filter paper. They were placed in a vertical position into cylinders with water on the bottom, to keep the filter paper moist by capillary elevation. Rhizogenesis took place in an incubator in the dark at a temperature of 30 °C. Following a 24-, 48-, 72-, 96-, and 120-h exposure of the cuttings to moisture and temperature, the initiating roots along with adjacent tissues of the maternal stem were fixed in Navashin's solution in the vacuum and then transferred through methylsalicylate in paraffin. The series of 7–10 μm thick cross- and longitudinal sections prepared on a microtome were stained with Heidenhain's hematoxyline and mounted in Euparal. Altogether 50 roots were processed in this way.

RESULTS

During rhizogenesis the total inductive stimulation does not occur even within one single cutting. The individual root primordia maintain a relative independence, and considerable differences may exist between the time parameters of their differentiation (Fig. 1A). As early as after a 24-h induction, the beginning of the elongation growth of cells and their vacuolation can be observed in the first activated root primordia, and that in their proximal region, adjacent to the cambium. The cells elongate in the direction perpendicular to the longitudinal axis of the maternal stem. In the central region of the primordium the cells are thin-walled, on the periphery they have irregularly thickened primary cell walls (Fig. 1B). In certain primordia the cells divide. A greater number of proliferating cells occurs in the central subdistal region which is the site of organization of the endogenously developing apical meristem of the root (Fig. 1C).

The repeated periclinal and anticlinal cell division brings about the periclinal zoning in the distal region of the primordium. On the base of the central region cell elongation and vacuolation continues. Cell division is less abundant there, the cells divide mostly cross-wise and form longitudinal cell columns. The enlargement of the differentiating root primordium results in an obliteration of cells in adjacent tissues, especially in the cortex.

With the continuing development the division of promeristematic cells gives origin to the ground meristem and procambial cylinder, and outwards the initials produce the cells of the root cap (Fig. 1A). This is protected by an adjacent peripheral cell layer of the root primordium which forms a so-called "Wurzeltasche" around it. While in the distal region of the root primordium the root apex is being formed, in its proximal region the connection between the primary vascular tissue of the developing root and the secondary vascular tissue of the maternal stem begins differentiating. The differentiation begins with the formation of short connective reticulately or scalariformly thickened tracheids on the periphery of the root primordium base (Fig. 2B, C). In some cases they are distinguishable even prior to the formation of procambium, in others their development is retarded. In the parenchymatous tissue of the primordium base vascular connectors thus originate which elongate,. due to the differentiation of further vascular elements, acropetally with respect to the forming root apex. The pre-emergent root development is terminated after a 72-h induction.

The penetration of the root through the peripheral tissues of the parent stem is associated with further elongation of the basal region (Fig. 3). The continuing differentiation of connective vascular tissues tends to join up as perfectly as possible the elements of the maternal stems and the elements of the developing root oriented perpendicularly to them (Fig. 4A). Above the subprimordial cambial region the initials of which produce a number of callus-like derivatives during rhizogenesis, the connective disc is differentiated. This has a broad ellipsoid base and its longer axis is oriented parallel to the longitudinal axis of the stem (Fig. 4B). The connective tracheids are arranged irregularly between the parenchymatous cells over the entire area of the base. The development of xylem connectors continues acropetally.

From the parenchymatous cells of the base on the periphery of the disc the cambium is differentiated, which joins up with that of the maternal stem. From the procambium formed in the root, the protoxylem elements begin differentiating acropetally in direct continuity with the connective tracheids. In the root approximatelly 5 mm long the vascular connection with the maternal stem is thus realized. The epidermis is lacking in the connective region which traverses the peripheral stem tissue. By the activity of stem phellogen which is locally destroyed by the penetrating root, a ring of parenchymatous cells with large intercellular spaces is formed around the root base.

DISCUSSION

The formation and development of root primordia in poplar stems is associated chronologically with the period of cambial activity (LUXOVÁ and LUX 1981). The development of roots from these primordia is not only time independent of growth processes of the maternal stem, but is indirectly correlated with them (LUX 1981). In the course of the year the rooting capacity changes – the highest being at the period of dormancy, and along with it the time parameters of root differentiation also change. In addition to the seasonal effect, the time course of differentiation depends on the time and position character of the plant material, *i.e.* on the age of the maternal individual, on that of the stem, and on the position of root primordium on the stem (LUX and LUXOVÁ 1967, LUX 1978).

Upon induction of rhizogenesis the root primordium in the poplar stem is divided into two regions: the distal or subdistal region with a predominating cell division is the site where the root apex is formed; in the proximal portion the volume growth of cells prevails, the majority of which elongate perpendicularly to the surface of the maternal organ. Due to the elongation of the base the root apex is displaced to the stem surface, eventually penetrating outwardly. At the same time the proximal region mediates a connection of the vascular tissue of the developing root with the secondary vascular tissue of the maternal stem. Only diploid cells may participate in the organization of meristems. From this point of view the results of KUBICA (1981) are of interest, dealing with the cytophotometric determination of DNA content of cell nuclei in the poplar root primordia. The dormant root primordium represents a mixoploid cell complex, but in its subdistal region diploid nuclei occur. The finding is obviously associated with the highest mitotic activity as observed in this region during the root primordium formation (LUXOVÁ and LUX 1981), as well as with its function in root organization.

The organization of root apex in the poplar root primordium proceeds rapidly: under the experimental conditions mentioned it is completed approximately within three days from the beginning of induction. As found by CIAMPOROVÁ (in preparation) in a parallel analysed material on an ultrastructural level, a substantial part of lipid supplies of the root primordium cells is utilized in this process.

In the proximal region of the root primordium which develops as a connective region, the differentiation of connective tracheids need not occur synchronously with the development of root apex. A similar observation is also referred to by CARLSON (1933). According to her the differentiation of connective xylem in the rose may begin very early, or may be delayed till the tissue differentiation in the root primordium proper begins. We observed that in certain cases the induction of elongation of the connective region did not take place, though the connective xylem was differentiated and the root apex was developed normally. The development of such roots ceases at the pre-emergent stage.

From the histogenetic point of view the connective region is thus not a product of the root apical meristem. It is not separated by the epidermis from the peripheral tissues of the maternal stem through which it traverses. Its function is similar to the basal region of the adventitious sphaeroblastic shoots the development of which was described by BALDINI and MOSSE (1956) in the apple. The sphaeroblasts are formed, similarly as root primordia, in the stems as the result of the local meristematic activity. Unlike them they have the character of isolated meristematic centres in bark tissues. Upon induction of shoot development the apical meristem is formed in the sphaeroblast, while from the central adjacent region of peripheral tissues the basal region develops. It is formed by a callus-like tissue with radially elongated elements between the sphaeroblast base and the xylem of the maternal stem. Also here in the basal region the vascular cambium is formed, similarly as already observed upon development of stem roots. BYRNE *et al.* (1977a) were the first to describe the origin of the vascular cambium in secondary roots of the species *Glycine max.*

In the material analysed the differentiation of the connective xylem and further of the primary root xylem proceeds continuously and acropetally. However, the results obtained do not answer the question as to whether the vascular differentiation in the developing root is influenced by mature tissue of the maternal stem (induction hypothesis) or by the apical meristem of the root (self-determination hypothesis). Nevertheless, we incline to the view of BYRNE *et al.* (1977b) that the induction affects primarily the differentiation of the basal region, whereas with continuing growth of the root the control of determination is gradually shifted acropetally to the root apex.

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Figures at the end of the issue.

BOOK REVIEW

BIKHELE, Z. N., MOLDAU, KH. A., ROSS, YU. K.: MATEMATICHESKOE MODELIROVANIE TRANSPIRATSII I FOTOSINTEZA RASTENIÏ PRI NEDOSTATKE POCHVENNOÏ VLAGI. [MATHEMATICAL MODELLING OF TRANSPIRATION AND PHOTOSYNTHESIS IN PLANTS AFFECTED BY SOIL MOISTURE STRESS.] — Gidrometeoizdat, Leningrad 1980. 223 pp., 2 Rbl., in Russian with English Summary.

Mathematical modelling, relying on the use of high-speed computers, has been developed to provide a comprehensive quantitative description of the behaviour of dynamic systems. In this monograph the simulation techniques are applicated to the analysis of the effects of meteorological factors on the processes influencing plant production and efficiency of water utilization. In the first chapter the authors critically evaluated basic concepts and formulae concerning the energy and mass exchange between the plant and environment and review models of the production process. The second chapter describes the mathematical model of transpiration and photosynthesis. It is very important to study these processes also under conditions of water stress because in the existing production models the influence of water deficit is often neglected. Special attention is paid to stomatal regulation. The model includes about 30 parameters, their values being derived from authors' own experiments or from data available in the literature. The model plant has evenly illuminated non-growing leaves and non-growing roots evenly distributed in the soil. Calculations obtained with the use of this model allow the authors to predict the dependence of leaf water potential, stomatal resistance, transpiration rate, photosynthetic rate, transpiration coefficient and productivity on soil moisture, air humidity, temperature, wind speed and photon fluence rate (Chapter 3), or the daily and seasonal courses of leaf water potential, stomatal resistance, transpiration rate, photosynthetic rate and heat balance components under various real meteorological conditions (Chapter 4). In the fifth chapter the authors analyse the choice of parameters and results of calculations and propose possibilities of the further improvements of such models.

The book is addressed to all research workers in agrometeorology, ecology, plant physiology, agriculture, forestry, *etc.* who are rather interested in a theoretically based than completely empirical approach to the research.

JANA POSPÍŠILOVÁ (*Praha*)

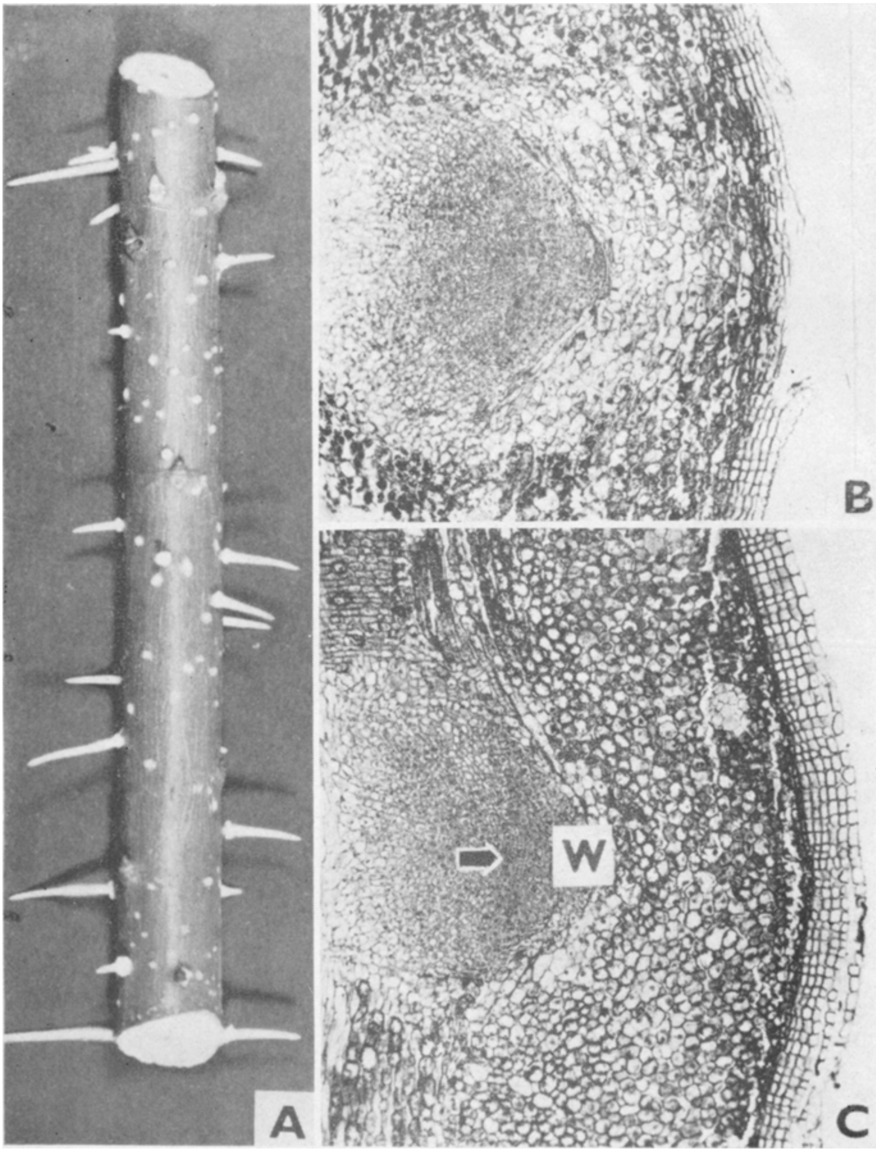

Fig. 1. The poplar stem cutting one week after rhizogenesis induction. The activation of root primordia is asynchronous (A). The peripheral part of the poplar stem in transverse (B) and radial (C) sections with a root primordium 24 h after induction of rhizogenesis. The site of root apical meristem organization is marked by an arrow. W — "Wurzeltasche". B, C × 70.

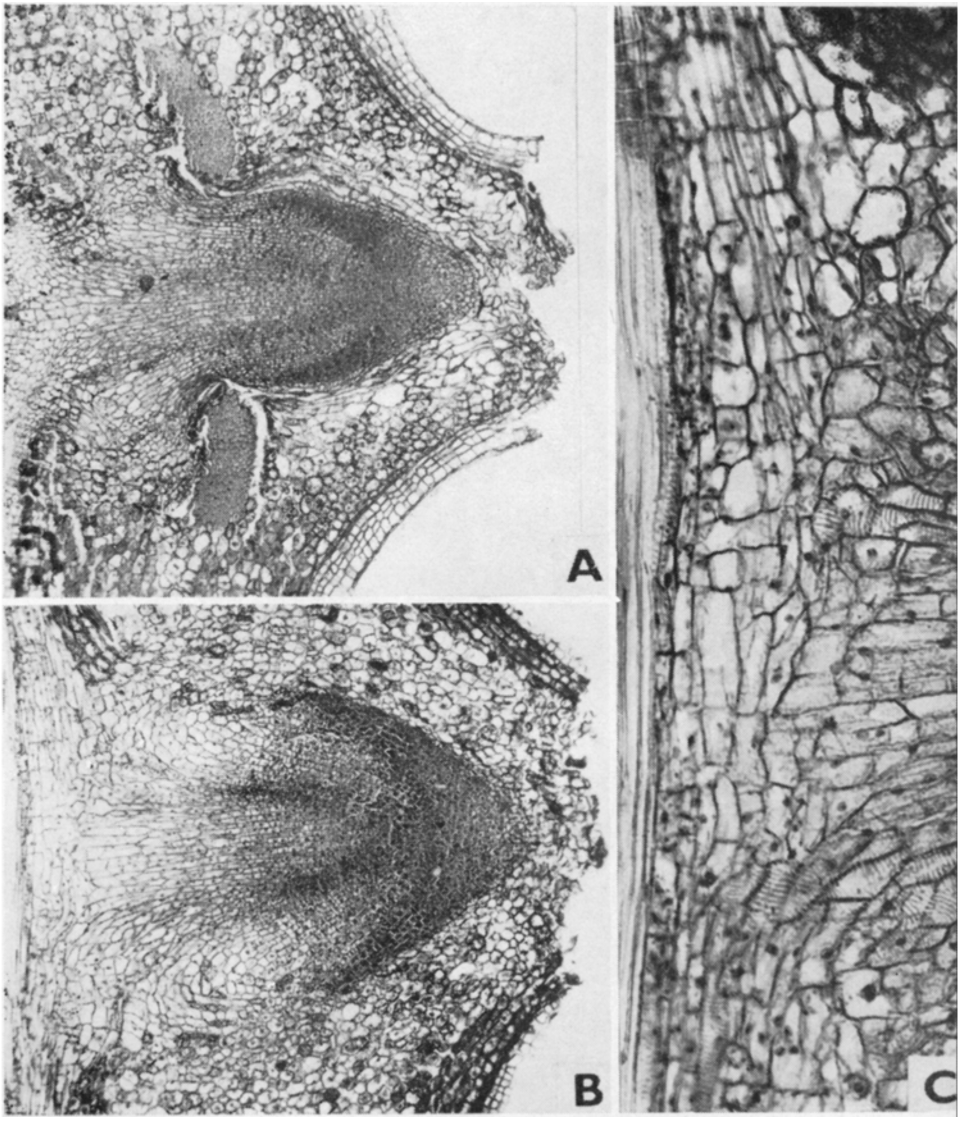

Fig. 2. The root primordium 72 h after induction of rhizogenesis in transverse (A) and radial (B) sections of the stem. Connecting tracheids can be seen in the proximal region of the root primordium (C). A, B × 55; C × 160.

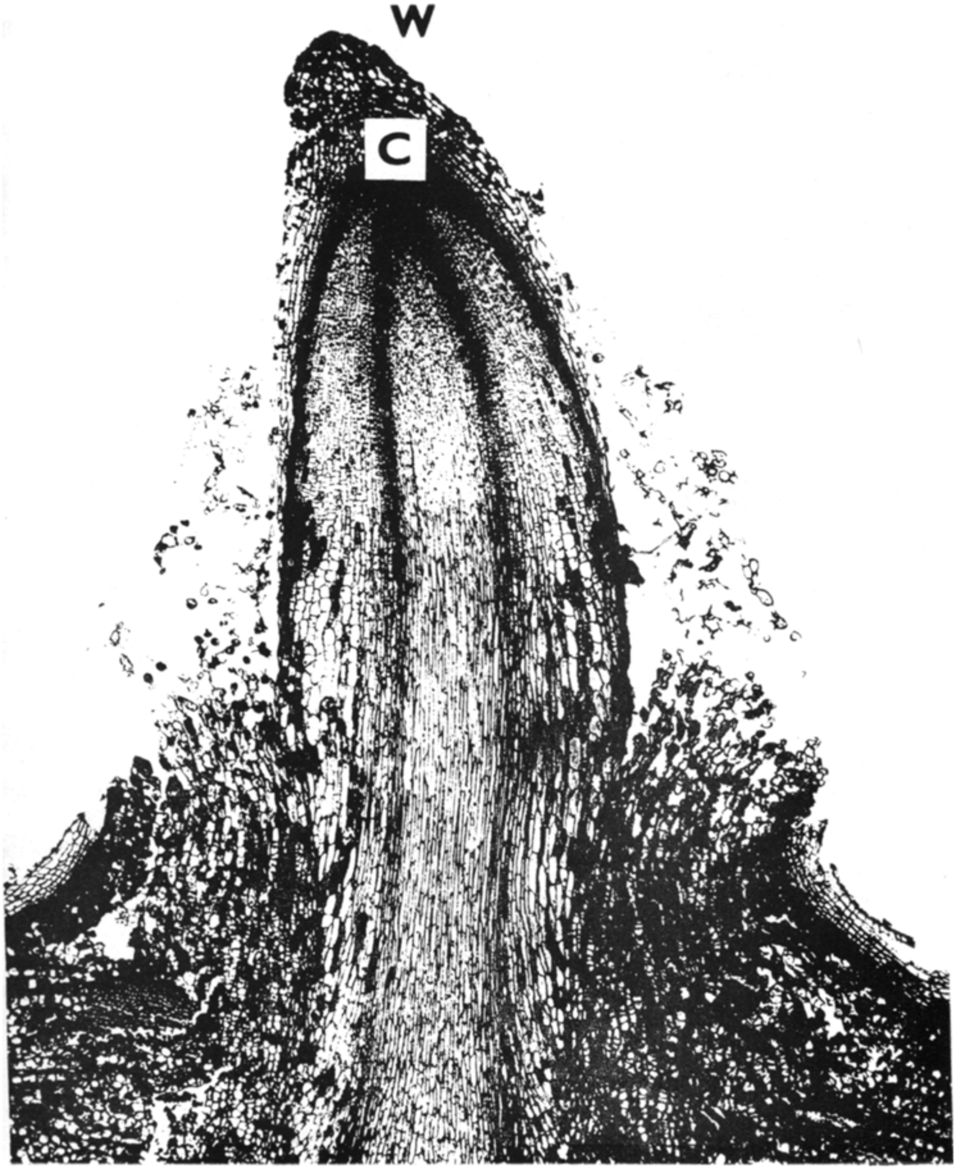

Fig. 3. Longitudinal section of an emergent root 96 h after induction of rhizogenesis. C — root cap; W — "Wurzeltasche". ×40.

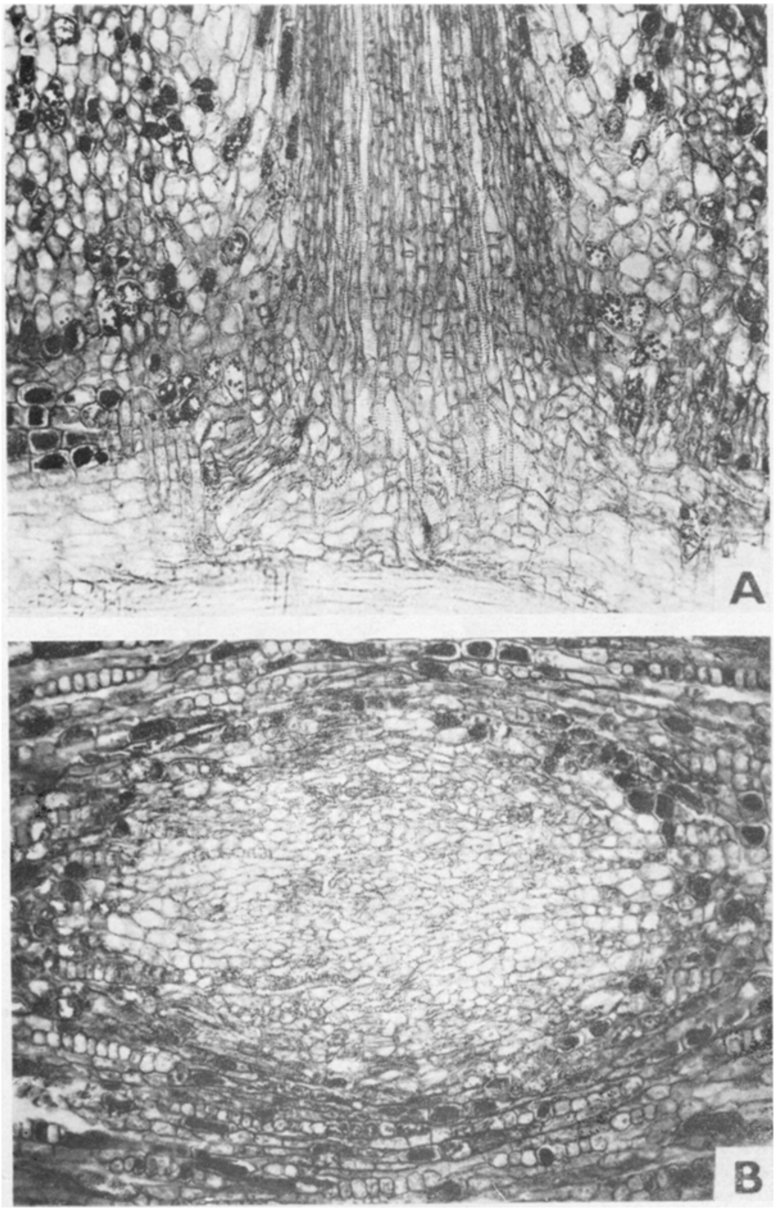

Fig. 4. The basal connecting region (A) and the transection of the connecting disc of the root, which was approximately at the same degree of development (B). A, B × 100.