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Latent Root Primordia in Poplar Stems

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Abstract. Root primordia initiate in poplar stems in the secondary growing parts, that is in the parts where the elongation growth is terminated and the leaves are mature. Their initiation is connected with the occurrence of unusual biseriate, rarely multiseriate rays. A small cell group in the secondary phloem is initiated by cell division of the ray. It gradually enlarges by continuing cell division, by the addition of cells adjacent to the cell group and by cambial activity. Thus, a hemispherical root primordium is formed, for which a permanent occurrence of reserve lipids is characteristic. In stems several years old the intraprimordial mitotic activity is rhythmically renewed together with the cambium function renewal. Latent root primordia slightly enlarge with the passing years, whereas mainly the cells localized in their centre divide. Further organization and root histogenesis was not observed either in older root primordia. Adjacent to root primordia, cambial initials produce the secondary xylem elements increasingly. Xylem protuberances are thus formed under root primordia. Primordia initiation is most frequent within the first year of stem development, though they can also initiate in later years.

Initiation and development of stem adventitious roots represent a continuous process in some species whereas in others the process consists of two distinct phases. Root primordia originate in stems within the first phase and they can persist for years in a latent stage. Their further formation occurs at a relatively high rate under conditions which induce rhizogenesis within the second phase. The primordia localization in poplars is not morphologically exactly limited. This makes the study of their initiation and development more difficult.

MATERIAL AND METHODS

The material used for this investigation was the fast growing poplar (*Populus euroamericana* DODE 'I 214'.

Root Primordia in One-year-oldStem

The stem segments at various degrees of development and the length of 0.5 cm were fixed in Nawaschin's solution in vacuum and after dehydration by graded ethanol series they were embedded through methylsalicylate in

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paraffin. Since the stem is formed of a heterogeneous tissue complex the preparation of suitable paraffin sections caused difficulties. The embedded material was softened in vacuum for 2—3 days in water. One side of the paraffin block was pared away to expose the tissues before immersion. The microtome sections obtained of the thickness 7—10 μm were stained with Heidenhain's hematoxylin, with a mixture of malachite green and acid fuchsine (NEČESANÝ 1955) or in tannic acid and iron chloride after Foster (GRAY 1954). In some cases the cell content was removed by Javelle's water (GERLACH 1969). Part of the material was cut by microtome after softening in glycerinalcohol without embedding in paraffin.

Root Primordia in Older Stems

Primordia localization was determined by the aid of tangential sections of the secondary xylem. This was enabled by xylem sectors adjacent to the primordia which differed from the rest of secondary xylem in their structure. A block was cut out at a corresponding radius from the stem. It contained besides root primordium with surrounding secondary phloem cells, cambial cells and a part of xylem cells. The material was processed in the same manner as in the case of one year old stems.

Seasonal Changes in Root Primordia

In order to study the seasonal changes in root primordia one-year-old shoots of two-year-old trees were used. Intraprimordial and cambial mitotic activity was followed in material sampled at monthly intervals from March to October. The material preparation was the same as in the previous cases. Sections were stained with Heidenhain's hematoxyline. The lipid presence was investigated by Sudan III, or by oily red (PEARSE 1960) using thin hand-cut sections of root primordia at monthly intervals throughout the whole year. The control was stained similarly after extracting lipids from sections by acetone (6 hours at room temperature).

For the photomicrodocumentation Reichert Zetopan was used.

RESULTS

The position of the root primordia can be determined after stem decortication. Primordia are found in the peeled-off bark appearing as meristematic "dimples", whereas at the places adjacent to the decorticated xylem small protrusions can be seen. Rooting of the stem cuttings in the optimum period when the roots are formed practically from all primordia represents another possibility of determining root primordia position. Root primordia are originated in nodes and internodes individually without forming a regular pattern; less frequent are groups of two or more primordia. Their occurrence is not bound to that of lenticels as was also observed by BRAUN (1963). The acropetal course of root primordia initiation in the stem determines the gradual decrease in age and size of root primordia in the direction towards the top. The primordia are not yet formed in the youngest nodes of developing shoots even at the end of vegetation.

With regard to the parent stem differentiation, the root primordia start to develop in secondary growing parts, that is in the parts with stopped internodal elongation. The cross-section of a young poplar stem is a pentagon whose corners are formed by stem ribs (Fig. 1A). Due to the secondary thickening its cross-section changes to a rounder one and the rib position is demonstrated by the pentagonal pith only. Root primordia initiate in the place of original ribs in connection with the course of leaf traces. Their initiation proceeds acropetally according to the stem development though with local deviations. The deviations are conditioned by physiological gradients taking place inside the stem and in some cases causing the initiation of additional root primordia.

As we have further observed, the origin of root primordia is bound to the occurrence of biseriate, rarely multiseriate rays. Cell division in this unusual ray results in the formation of a small cell group in the secondary phloem (Fig. 1B), gradually increasing by continuous cell division, in the forming primordium by the cambial activity and by the addition of cells adjacent to the group. A hemispherical primordium is thus formed. Finally, the intra-primordial cell division is predominant and is concentrated most of all in the central region of the root primordium. Further organization in root primordium does not take place (Fig. 2A, B). Rich occurrence of reserve lipids is a typical characteristic of the primordial cells, whereas the main reserve substance of the parenchyma cells of adjacent phloem tissue is starch (LUXOVÁ *et al.* 1980). Lipid occurrence is not a seasonal feature. So far as rhizogenesis is not induced, the primordial cells are permanently filled with lipid droplets.

Cambial initials produce the secondary xylem elements to an increased degree in a part adjacent to the developing root primordium. Thus a protuberance under the primordium on the xylem core is formed and the primordium gets closer to the stem surface. Cells of the peripheral tissues, that is the phloemic and cortical cells which adjoin the enlarging primordium, are forced to change their shape.

With the onset of the rest period root primordia pass temporarily into dormancy. The intraprimordial mitotic activity is renewed approximately together with the seasonal pattern of cambial activity. The most actively dividing cells are those in the centre of the primordium which becomes larger in the radial and tangential directions. When, *e.g.*, the diameter of the biggest primordia in a one-year-old stem is about 700 μm, that of the six-year-old primordia is about 1200 μm. Primordia enlargement is limited by the adjacent peripheral tissues of the stem. In some cases the lack of space does not apply only to adjacent stem tissues which are pushed by the enlarging primordium to the sides and towards the periphery. Also, the primordial cells can collapse, or the results of pressure can be expressed by locally rectified cell division. However, in older primordium the root histogenesis does not take place either (Fig. 3A).

Xylem, formed by cambium under the root primordium, differs from the rest of the secondary xylem in more ways: these are the presence of unusual rays, irregular ray density, limited number of vessel elements and overall smaller cell size. Formation of xylem protuberances enlarging with passing years (Fig. 3B), is due to the increased cambial activity and not to the cell enlargement. Also, the cell orientation here is changed. The cells bend with respect to the longitudinal axis of the stem. The mentioned structural deviations within the xylem sector under the root primordium enable the determination of the position of root primordium without damaging it.

Simultaneously it is possible to determine the time of its initiation. Even though the primordial initiation is most frequent within the first year of the stem development, new primordia can initiate in later years too.

DISCUSSION

Root primordia within the secondary growing stems are of various origin. Overall, the endogenous origin is predominant: primordia can originate from ray parenchyma, from interfascicular, less often from fascicular cambium, from phloem parenchyma, from parenchymatous interruptions in the secondary xylem which are formed due to the presence of leaf-gaps or buds, from peripheral parenchyma tissue of the secondary cortex, possibly from phellogen (FAHN 1967, GUTTENBERG 1968). Some of the mentioned possibilities can combined, too. Another cause of differences is the root primordia initiation in younger and older stems in connection with the changes concerning the arrangement of their tissues (PRIESTLEY and SWINGLE 1929, BRAUN 1963). Primordia initiation in the poplar stem is associated with the arrangement of leaf traces which was concluded for the first time also for other species by VAN DER LEK (1924). LARSON and ISEBRANDS (1971, 1974) investigated in detail vascularization in poplar shoots. Their interpretation of the development of the vascular system in the shoot regulated by the leaves is very important for an understanding of the principles of root primordia initiation. Identification of the root initials in species where primordia do not initiate in definite positions is difficult or impossible. As mentioned by HAISSIG (1970), exactly where in a tissue the initials will arise is usually a matter for speculation. We have used as a guide for the determination of the position of poplar root primordia the occurrence of biseriate or multiseriate rays. Xylem of genus *Populus* (with the exception of *Populus alba* L., HERMANN 1922) has been characterized by the occurrence of uniseriate rays (METCALFE and CHALK 1957). We have sporadically observed unusual bi- and multiseriate rays. They are regularly associated with root primordia but their presence does not always need condition their initiation. It is possible that these unusual rays represent potential *loci* of root primordia initiation. The primordia will be formed at those places under more favourable conditions. Observation of the variable primordia size would point towards such phenomena in some stems. Authors who studied the occurrence of root primordia in this genus did not mention any relationship between the root primordia initiation and the presence of unusual rays. According to VAN DER LEK (1924) the root primordia initiate as a continuation of medullary rays. BRAUN (1963) notices the occurrence of biseriate rays within the xylem protuberances opposite root primordia, though he does not connect their presence with root initiation. Neither did we encounter in other papers dealing with root primordia initiation in some angiosperms any relating root primordia initiation to unusual ray occurrence (CARLSON 1933, 1938, 1950, FAVRE and MÉDARD 1969, HAISSIG 1970). As far as the gymnosperms are concerned, BANNAN (1941, 1942) does mention this relationship, though not as a general phenomenon, but as a characteristic of some analysed species — actually of *Thuja occidentalis* L., *Juniperus horizontalis* MOENCH, *Juniperus communis* var. *depressa* PURSH and *Juniperus virginiana* L. Stem adventitious roots initiate here in cambial of phloemic part of rays which differ from the rest of rays in a more extensive thickness, just as in the case of poplar.

The relationship between the occurrence of the biseriate rays and the initiation of root primordia leads to the assumption of locally more intensive radial transport of metabolites and thus to the induction of root primordia. With regard to induction, it is obviously important to put a stop to internodal elongation of the parent stem. In the case of poplar, the stop of internodal elongation is identical in time with the stop of leaf lamina expansion (LARSON and ISEBRANDS 1971). As a young poplar leaf expands and matures, its role changes from that of importer to exporter of photosynthesis products (LARSON and DICKSON 1973).

The beginning of root primordia initiation is generally similar. The future initials remeristematize and simultaneously expand with concomitant swelling of their nuclei. The cells are filled with dense cytoplasm. A small group of meristematic cells is formed by their division and enlarges by incorporation of adjacent tissues as well (CARLSON 1933, CONNARD and ZIMMERMANN 1931, SMITH 1936). No further organization of the latent root primordium takes place in poplar, neither are any histogens produced. VAN DER LEK (1924) named this type of root primordium meristematic. However, according to KUBICA (1980), endoreduplication takes place in some cells of poplar root primordia.

As far as the organ specificity of primordia is concerned, they are generally classified as "root primordia". Under the conditions stimulating rhizogenesis they can quickly give rise to adventitious roots. However, root determination is realized at the beginning of rhizogenesis only. In fact, under favourable conditions either roots or shoots can develop from the so-called root" primordia of poplar (LUX and LUXOVÁ, unpublished). This is also true of older primordia which are still not organ determined. According to BRAUN (1963) primordia are preserved in the stems until the outer bark formation. The vascular cambium then again produces the phloemic elements under root primordia. The primordia get to the periphery and become part of the bark by which they are separated from the stem. The mentioned so-called primary root primordia are replaced by the secondary root primordia which initiate in dilated bark regions and are similar to the primary root primordia.

REFERENCES

BANNAN, M. V.: Vascular rays and adventitious root formation in *Thuja occidentalis* L. — Amer. J. Bot. **28** : 457—463, 1941.

BANNAN, M. W.: Notes on the origin of adventitious roots in the native Ontario conifers. — Amer. J. Bot. **29** : 593—598, 1942.

BRAUN, H. J.: Wurzelkeime in jungen und älteren Sprossachsen von *Populus*. — Z. Bot. **51** : 441 to 451, 1963.

CARLSON, M. C.: Comparative anatomical studies of Dorothy Perkins and American Pillar Roses. I. Anatomy of canes. II. Origin and development of adventitious roots in cuttings. — Contr. Boyce Thompson Inst. **5** : 313—330, 1933.

CARLSON, M. C.: The formation of nodal adventitious roots in *Salix cordata*. — Amer. J. Bot. **25** : 721—725, 1938.

CARLSON, M. C.: Nodal adventitious roots in willow stems of different ages. — Amer. J. Bot. **37** : 555—561, 1950.

CONNARD, M. H., ZIMMERMAN, P. W.: Origin of adventitious roots in *Portulaca oleracea* L. — Contrib. Boyce Thompson Inst. **3** : 337—346, 1931.

FAHN, A.: Plant Anatomy. — Pergamon Press, Oxford 1967.

FAVRE, J.-M., MÉDARD, R.: Ontogenie des racines adventives chez la vigne (*Vitis vinifera* L.) cultivée *in vitro*. — Rev. gén. Bot. **76** : 455—467, 1969.
GERLACH, D.: Botanische Mikrotechnik. — Thieme Verlag, Stuttgart 1969.
GRAY, P.: The Microtomist's Formulary and Guide. — Constable and Company, London 1954.
GUTTENBERG, H. v.: Der primäre Bau der Angiospermenwurzel. Handbuch der Pflanzenanatomie Bd. VIII., Teil 5. — Gebrüder Borntraeger, Berlin—Stuttgart 1968.
HAISSIG, B. E.: Performed adventitious root initiation in brittle willows grown in a controlled environment. — Can. J. Bot. **48** : 2309—2312, 1970.
HERMANN, H.: Vergleichende Holzanatomie der Pappeln und Baumweiden. — Bot. Arch. **2** : 35 to 56, 79—112, 1922.
KUBICA, Š.: Investigation of the relative DNA content in stem root primordia of the poplar in the course of a year. — Biológia (Bratislava) **35** : 711—716, 1980.
LARSON, P. R., DICKSON, R. E.: Distribution of imported ^{14}C in developing leaves of eastern cottonwood according to phyllotaxy. — Planta **111** : 95—112, 1973.
LARSON, P. R., ISEBRANDS, J. G.: The plastochron index as applied to developmental studies of cottonwood. — Can. J. For. Res. **1** : 1—11, 1971.
LARSON, P. R., ISEBRANDS, J. G.: Anatomy of the primary-secondary transition zone in stems of *Populus deltoides*. — Wood Sci. Techn. **8** : 11—26, 1974.
LUXOVÁ, M., LUX, A., ČIAMPOROVÁ, M.: Reserve lipids in stem root primordia of poplar. — J. exp. Bot. **31** : 859—862, 1980.
METCALFE, C. R., CHALK, L.: Anatomy of the Dicotyledons. Vol. II. — Clarendon Press, Oxford 1957.
NEČESANÝ, V.: [Anatomy of reaction wood of hardwoods and submicroscopic morphology of its cell walls]. In Czech. — Přírodov. Sbor. ostrav. Kraje **16** : 184—202, 1955.
PEARSE, A. G. E.: Histochemistry Theoretical and Applied. Second edition. — J. and A. Churchill, London 1960.
PRIESTLEY, J. H., SWINGLE, CH. F.: Vegetative propagation from the standpoint of plant anatomy. — Tech. Bul. No. 151, U.S. Dept. of Agriculture, Washington 1929.
SMITH, A. I.: Adventitious roots in stem cuttings of *Begonia maculata* and *B. semperflorens*. — Amer. J. Bot. **23** : 511—515, 1936.
VAN DER LEK, H. A. A.: [Root forming in woody shots.] In Dutch. — Meded Landbouwhogesch. Wageningen **28** : 1—230, 1924.

Figures at the end of the issue.

BOOK REVIEW

WALTER, H.: VEGETATION OF THE EARTH AND ECOLOGICAL SYSTEMS OF THE GEO-BIOSPHERE. Second Edition. — Springer Verlag, Berlin—Heidelberg, New York, 1979. 274 pp., 124 figs. DM 28,—; US $ 15.40.

The popular handbook of professor H. Walter on the vegetation conditions in the individual zones of the Earth has again appeared in English. It is a translation of the third German edition of 1977. Compared with the first English edition of 1973, the book is considerably reworked and contains expanded chapters on ecological data, particularly from production biology, on the distribution of the phytomass and on the primary production in the biosphere. The author introduces some special terms such as zonobiome for the zonal vegetation, orobiome for a height stage and pedobiome for azonal soil-dependent vegetation.

A detailed review of the first edition was published in Biol. Plant 17 : 30, 1975.

The advantage of the book is not only a high quality of its content (the ecological concept of vegetation units in relation to the environment — the type of climate) but a clear and attractive presentation. It is to be recommended to anyone interested in plants and not only to those involved in phytogeography or plant ecology.

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