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THE FORMATION OF ADVENTITIOUS ORGANS. II. THE ORIGIN OF BUDS FORMED ON YOUNG ADVENTITIOUS ROOTS OF POPULUS NIGRA L. 'ITALICA'*

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SUMMARY

Young adventitious roots formed by tissue cultures or stem cuttings of the Lombardy poplar produce buds when treated with kinetin or 6-benzylaminopurine (BA). These buds originate from the superficial layers of the root, often close to the apex. When the buds are in a terminal position the apical tissues have been disturbed by callus formation. The normal activity of the apical root meristem is stopped by cytokinin, and in most cases the cells lose their meristematic appearance.

1. INTRODUCTION

Auxins and cytokinins are known to influence organ formation. Whereas exogenous supplies of indoleacetic acid and other auxins tend to induce root formation, cytokinins are in many cases very effective inducers of bud formation. Both groups of substances are thought to be endogenously produced and to play a role in processes of cell division and organ initiation. Given the stimuli and nutrients needed for organ formation, there must be sensitive cells which are able to respond by mitosis. Moreover the capacity to produce an apex of root or shoot must still be present.

In cuttings, the formation of adventitious organs is ascribed to disturbances of the internal regulation. In many cases of adventitious organ formation under natural or experimental conditions, the reacting cells belong to fully differentiated tissues, for instance the epidermis or pericycle. Cells of these tissues appear to be able to "dedifferentiate" and to resume mitotic activity. In the literature a few cases of adventitious organ formation in a totally different situation are described, namely bud formation in the apical region of the root and root formation at the stem tip. Here, meristematic cells or even cells functioning as initials must be induced to change their activity. Examples are scarce, however, and anatomical data are often lacking. BEIJERINCK (1887) observed the conversion of an adventitious shoot into a root after cutting of the root system of *Rumex acetosella*. EDMONDSON (1925; cited by PRIESTLEY & SWINGLE 1929) was able to reproduce this experiment. Explanted roots of *Neottia* may form terminal shoots, the formation of leaves and a shoot apex

* Dedicated to Professor Karstens on the occasion of his retirement.

being preceded by a type of growth called a protocorm (CHAMPAGNAT 1971). DANKWARDT-LILLIESTRÖM (1957) induced shoot formation at the apex of isolated *Isatis* roots with kinetin. BALLADE (1968) induced bud formation at the apex of very young adventitious roots of *Nasturtium officinale*. Here, most cells differentiate under the influence of the cytokinin application, even the superficial cells of the apex, but in a later stage a new active greening zone is formed, giving rise to one terminal bud.

In our first experiments on organ formation under the influence of cytokinins in poplar callus cultures (VENVERLOO 1973), we found bud formation on the callus, but also on young roots formed earlier on the callus (*fig. 1*). This bud formation took place near the root apex or at some distance from the tip, the budding being preceded by local swelling and the formation of a green callus. To investigate the origin of these buds, an anatomical study of the phenomenon was performed. Because root formation in tissue cultures was infrequent and unpredictable, rooted branch cuttings were used instead of callus cultures.

2. MATERIAL AND METHODS

One-, two-, and three-year-old 25 cm long cuttings taken from Lombardy poplar trees (*Populus nigra* L. '*Italica*') growing in the Leiden Botanic Garden were freed of buds and placed with the lower 7 cm in distilled water in 30 cm long cuvettes in the dark at 23 °C, the cuvettes being plugged with cotton. For sterilization of the surface, the cuttings were immersed in Glorix® (12% sodium hypochlorite solution) for 35 minutes; longer sterilization times were also tried. Segments 1 cm long and bearing one or more 1–50 mm long roots were cut from rooted parts of the branches, mostly aseptically, and transferred to 100 ml erlenmeyer flasks containing various concentrations of cytokinins in bidistilled water. The cytokinins used were kinetin (NBC) and 6-benzylaminopurine (Fluka). The flasks were shaken on a Gyrotory shaker under alternating 12-hour periods of dark and low-intensity light.

The roots to be sectioned were fixed in a FAPA solution containing per litre 500 ml aethanol, 100 ml 40% formalin, 30 ml propionic acid, and 30 ml glacial acetic acid, for at least 24 hours. After fixation, the tissue was embedded in diglycolstearate (Pegosperse 100S, Glyco Chemicals, New York), cut into 15μm-thick sections, stained according to Heidenhains's hematoxylin method (Eisen-haematoxilin I and II, Chroma, Stuttgart) and with astra blue (FM, Chroma; 0.5% in 2% tartaric acid), and then mounted with Eukitt (Kindler, Freiburg).

3. RESULTS

3.1. Rooting

One of the prerequisites for rooting of poplar cuttings is a culture period in the dark (SHAPIRO 1958). The amount of roots formed varies with the age of the sample and the time of the year. The best results were obtained with cuttings of 2- or 3-year-old shoots in December and January. In the period of active growth,

root formation is low (NANDA & ANAND 1970). In the present study, to prevent complications in the budding experiments, auxins were not used to induce root formation.

Surface sterilization of cuttings with a sodium hypochlorite solution appeared to be insufficient to kill the microorganisms enclosed in periderm and small wound patches. The best results were reached with 1-year-old cuttings with a smooth surface. Cuttings pre-treated for 35 minutes with a hypochlorite solution showed fewer and shorter roots than untreated cuttings. Other disinfectants did not give better results. No treatment giving good surface sterilization without inhibition of rooting was found. Growth of microorganisms was slow, however, because the explants were grown in water or in a cytokinin solution without the addition of sugar or other nutrients. Both sterile and unsterile cultures were used. Cultures showing heavy infections were discarded.

3.2. Structure of the roots

Adventitious roots formed by cuttings sterilized for 35 minutes and kept in the dark for 11–14 days can reach a maximal length of 4 cm, but in most cases growth stops before the roots are 2 cm long. The diameter of the roots varies considerably (0.1–1 mm). The anatomy of roots of different diameter was studied in longitudinal and transverse sections.

The root apex is of the open type (VON GUTTENBERG 1968), i.e. without separate initials for the formation of stele, cortex, epidermis, and calyptra (*fig. 3*). The mean length of the calyptra is about 225 μm. A columella is often present. The apical initials and their direct derivatives are isodiametric; the diameter is normally 2.5 to 7.5 μm and in thick roots up to 20 μm. The first differentiating xylem elements are usually found at a distance of 200–300 μm from the initials; but in thinner roots they are closer (150 μm) and in thick roots much farther away (800 μm). The proximal part of the root is diarch to pentarch, the distal part diarch or triarch. The pericycle consists of one or two layers. A true endodermis is lacking, but there is a layer of cells containing tannins. In the tip zone this "tannin sheath" is not well defined; starting at a distance of 50–100 μm from the apex, the cells of this layer are recognizable by their content of tannins. The cortex almost always consists of 6 to 8 layers, but very occasionally has 9 to 10 layers. The outer cortex cells may reach a diameter of 60 μm. Intercellular spaces are abundant. The epidermal cells are often filled with tannins. At a rather short distance from the apex, the epidermis is ruptured.

No differences in anatomy were found between roots kept under sterile and unsterile conditions. Primordia of lateral roots originated in the pericycle. Young primordia of lateral roots are covered by the "tannin sheath".

3.3. Bud formation

To induce bud formation on the roots, they were transferred to a cytokinin solution. Because experiments with isolated roots were not successful, 1-cm segments of the stem tissue, each bearing one or more roots, were used. The cytokinin solution contained 6-benzylaminopurine or kinetin in a concentration

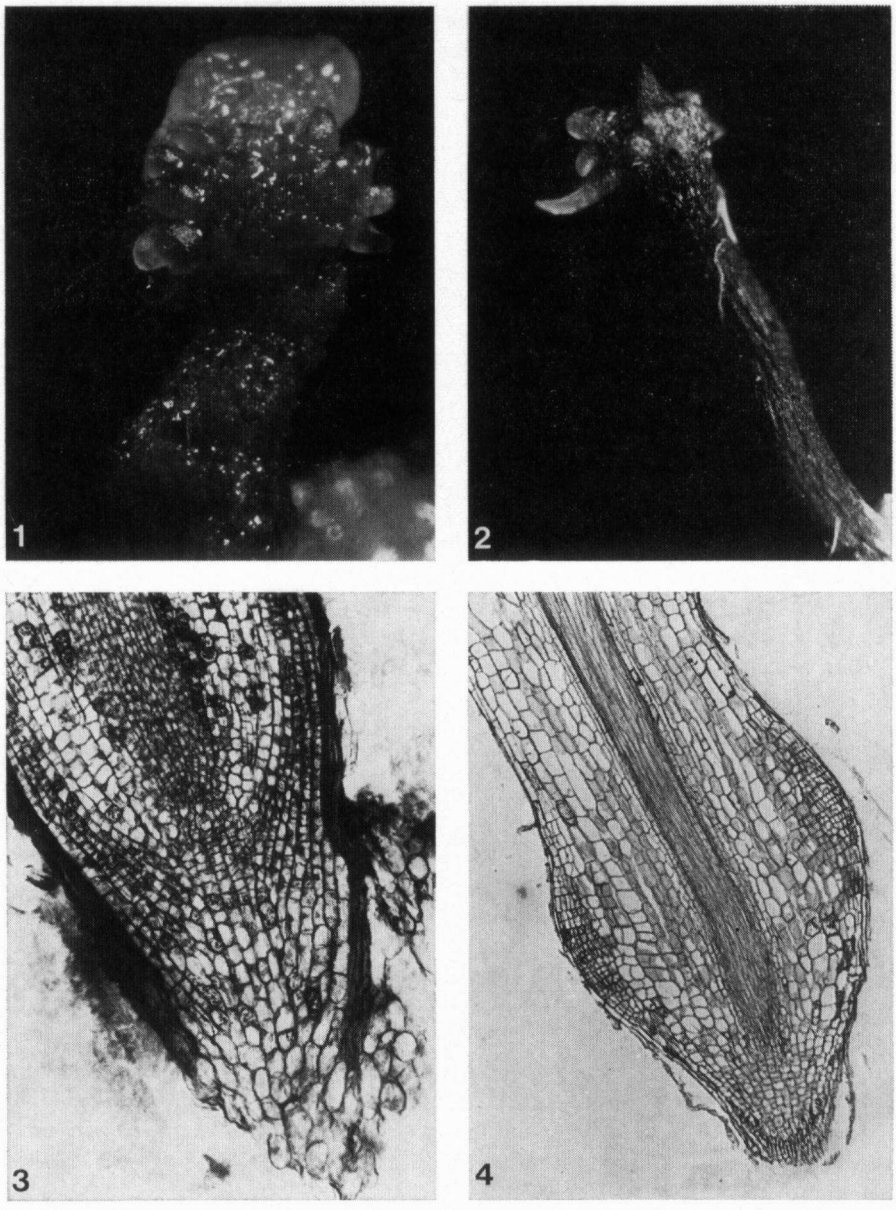


Fig. 1. Ring of leaf primordia formed under the influence of 6-benzylaminopurine (BA) on a root in a callus culture. The tip has produced a light green callus. × 16. Fig. 2. Kinetin-treated root on a branch segment. Growth at the tip has subsided; buds are found on a green callus in a subterminal position. × 16. Fig. 3. Root tip of a 4.5-cm-long control root in water. The root cap, with columella, is well developed. × 145. Fig. 4. Root treated with BA. The situation at the apex has changed. A zone of division surrounds the tip about 0.4 mm from the apex. × 54.

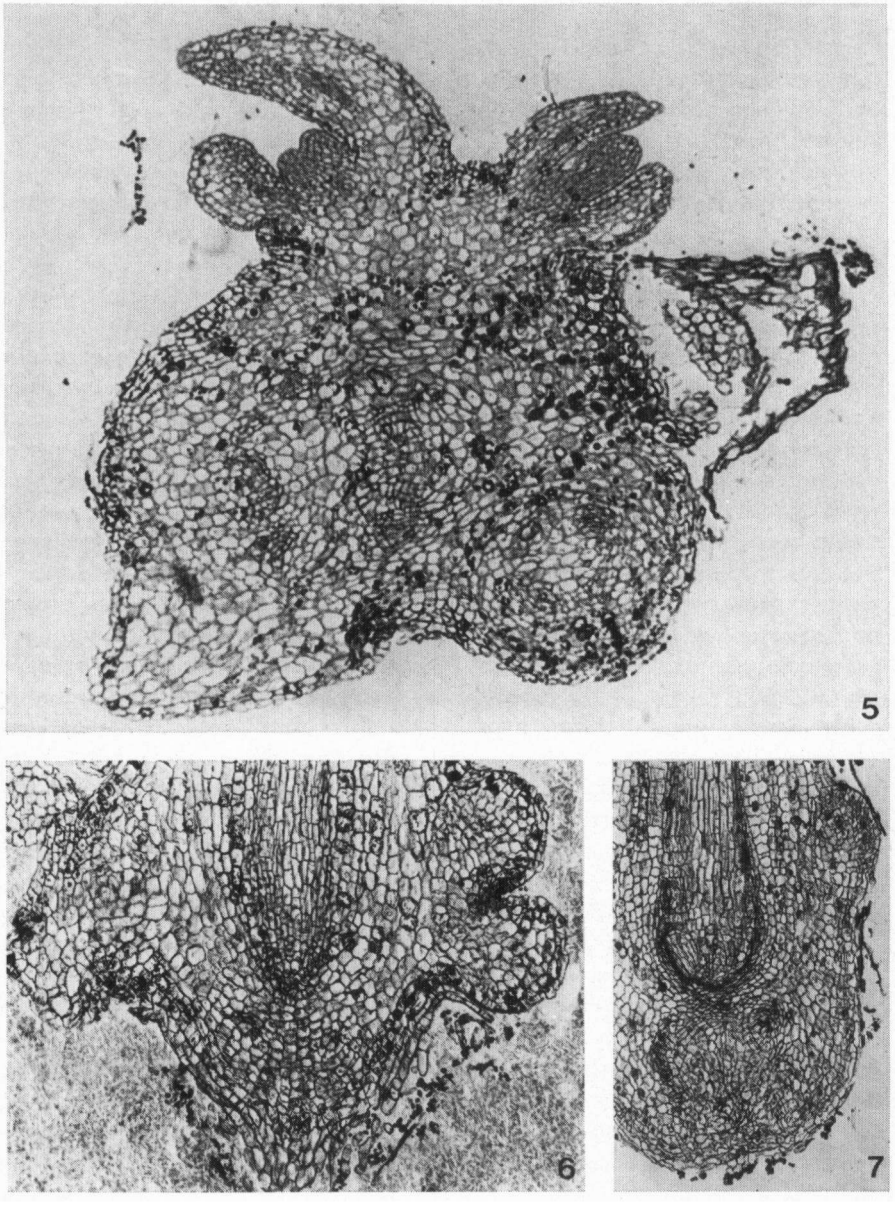
of 0.05, 0.2, or 1.0 mg/l. In these experiments 116 explants were transferred to a cytokinin solution and 36 control explants to water. A total of 142 roots were treated with cytokinins. After about five weeks, 6% of the roots had developed buds or small leaves at a short distance from the tip, 3% had buds at a greater distance from the tip, and 22% showed a swelling but no buds; 68% had no buds and no swelling. The roots treated with water showed neither budding or swelling. Bud formation was more frequent on the stem segments: 38% formed one or more buds or leaves. Some of the water-treated segments also showed bud formation (14%).

Bud formation was found on roots of 3–30 mm length. Short roots showed budding near the tip; on longer roots bud formation occurred at other places on the root surface as well. Since the roots were used in successive experiments with different cytokinins or different cytokinin concentrations, no conclusion can be drawn about the best concentration for bud induction. All of the concentrations of BA and kinetin used were effective in inducing swelling of the root tip; and all but the highest BA concentration induced some budding on the roots.

Bud formation near the apex is always preceded by swelling, but swelling is not always followed by bud formation. When the whole root tip is deformed, a club-shaped or irregular structure arises. Often, a pointed apex is still present and the swelling is found a short distance from the tip (*fig. 4*). Anatomical investigation shows all swellings to be correlated with local cell divisions or profuse callus formation. In general, early stages of the cell divisions induced by cytokinins are found in the peripheral zones of the root tip (*figs. 4* and *6*). Cells of the epidermis and the outer cortical layers have undergone periclinal and some anticlinal divisions. Often, as in the case of the root in *fig. 4*, a ring of dividing tissue has been formed at some distance from the root rip. Later stages with profuse callus formation at this site were found, sometimes with differentiation of buds.

The structure of the root apex seldom remains unaltered after cytokinin treatment. In most cases the apical meristems seem to have lost their function. Either mitotic activity has ceased or callus has been formed by divisions in an abnormal pattern. Differentiation has continued after the cessation of mitotic activity. Differentiated xylem elements of the stele are found at a short distance from the apex. Both the apical cells themselves and their derivatives have increased in dimension and differentiated into parenchyma cells or isodiametric tracheids. When irregular proliferation of the apical zone has taken place, the resulting callus may contain groups of tracheids. Buds may be formed at the surface (*fig. 5*).

Bud formation is always preceded by callus formation, and buds often occur in groups on the callus. They usually consist of two or more leaves and a shoot apex. In most cases the growth of these buds stops at an early stage; in rare cases they give rise to shoots with stems of some length.



Figs. 5–7. Kinetin-treated roots. Fig. 5. Transverse section taken 0.1 mm from the end. The tissues of the apex have proliferated, and buds have formed from the tip callus. × 80. Fig. 6. Longitudinal section. Callus and organ formation are found on the flanks of the root tip. × 97. Fig. 7. Irregular proliferation of root cap and apical zone. A pattern of periclinal divisions is seen in the superficial layers on the flank. × 85.

4. DISCUSSION

After treatment with cytokinins, buds may be formed at different sites on the surface of young poplar roots. No case of direct transformation of a root apex into a shoot apex was observed. Buds do occur in a more or less terminal position, but always accompanied by profuse callusing of the apical zone. There seems to be a preference for meristem formation and budding in a subterminal position, which was also observed in roots on callus cultures transferred to an agar medium containing a cytokinin. This predilection may be correlated with the stage of differentiation of epidermis and cortex along the root. Another factor of importance is the presence of a root cap. The root cap is well developed when the root emerges from the stem. Experiments with very young roots lacking a root cap are not feasible in poplar explants, which form roots endogenously. In experiments with *Nasturtium* (BALLADE 1970) only very small roots (up to 0.4 mm), still without a cap, gave rise to a terminal bud. As in the poplar experiments, the normal activity of the apical root meristems was stopped by cytokinin, but in *Nasturtium* one or two cell layers of the apex, including the apical initials, took part in the formation of a bud, and extensive callusing was not observed.

Bud formation on stem segments of the Lombardy poplar is not dependent on exogenously supplied cytokinins. In the present experiments bud formation on the stem was observed, presumably under the influence of endogenous cytokinins, in the explants in water without a cytokinin.

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REFERENCES

BALLADE, P. (1968): Caulogenèse apicale sur les jeunes racines axillaires du cresson (Nasturtium officinale R.Br.). *Soc. Bot. Fr., Mém.* **115**: 250–258.

— (1970): Précisions nouvelles sur la caulogenèse apicale des racines axillaires du cresson (Nasturtium officinale R.Br.). *Planta* **92**: 138–145.

BEIJERINCK, M. W. (1887): Beobachtungen und Betrachtungen über Wurzelknospen und Nebenwurzeln. *Verh. Kon. Akad. Wet. Amsterdam* **25**: 1–146.

CHAMPAGNAT, M. (1971): Recherches sur la multiplication végétative de Neottia nidus-avis Rich. *Ann. Sci. nat. Bot.*, 12e sér. **12**: 209–248.

DANKWARDT-LILLIESTRÖM, C. (1957): Kinetin induced shoot formation from isolated roots of Isatis tinctoria. *Physiol. Plant* **10**: 794–797.

GUTTENBERG, H. VON (1968): Der primäre Bau der Angiospermenwurzel. In: *Handbuch der Pflanzenanatomie* (ed. K. LINSBAUER, G. TISCHLER & A. PASCHER), Band **8, 5**. Borntraeger, Berlin.

NANDA, K. K. & V. K. ANAND (1970): Seasonal changes in auxin effects on rooting of stem cuttings of Populus nigra and its relationship with mobilization of starch. *Physiol. Plant.* **23**: 99–107.

PRIESTLEY, J. H. & C. F. SWINGLE (1929): Vegetative propagation from the standpoint of plant anatomy. *U.S. Dept. Agric. Washington D.C., Techn. Bull.* **151**.

SHAPIRO, S. (1958): The role of light in the growth of root primordia in the stem of the Lombardy poplar. In: K. V. THIMANN, *The physiology of forest trees*: 445–465. Ronald Press, New York.

VENVERLOO, C. J. (1973): The formation of adventitious organs. I. Cytokinin induced formation of leaves and shoots in callus cultures of Populus nigra L. 'Italica'. *Acta Bot. Neerl.* **22**: 390–398.