THE DEVELOPMENT OF ROOTS ARISING FROM CALLUS TISSUES IN YOUNG SEEDLING CUTTINGS OF PINE

—A PRELIMINARY REPORT—

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The genus Pinus is well known as one of the most difficult plants to propagate vegetatively, and we can not refer to a few successful report on it so far as authors know. Especially in Japan, authors have heard that many trials have failed to propagate akamatu (P. densiflora) by cuttings. These difficulties notwithstanding, the successful vegetative propagation of this plant is greatly desired, because the akamatu is one of the most important forest trees in Japan, and the inherited characters of it are said to be in great variation, so that the clonal selection should be the first problem of akamatu breeding.

Generally, cuttings of young seedlings are easy to root, though the plant is difficult to propagate vegetatively in the usual way. In several pines (P. silvestris, P. strobus, P. resinosa, P. taeda) the same phenomenon was reported by GARDNER (1929), Authors also obtained good results, testing the one year old seedling cuttings of modoomatu (so called natural hybrid of P. densiflora × P. Thunbergii), of which 40 per cent rooted, 49 per cent callused, 2 per cent non-callused but living, and only 7 per cent died, during the period from March to June 1947 (Unpublished). Authors considered that this point may become the clue to obtaining technically, the success in vegetative propagation of pine, and made the present test. Though satisfactory results have not been obtained owing to some defectiveness and omission, the outlines of the present results are here reported, and the authors are going to examine the problem more thoroughly.

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Materials and Methods. The epicotylic shoots were used as test materials, which were cut from young seedlings of akamatu sown in pots in early February and early March 1947. Owing to deficiency of the material, young shoots of modoomatu were added to it, which were grown from trimmed epicotyls of one year old seedlings.

On July 16, those of about two to three centimeters long were cut at the point immediately above the cotyledons using a sharp razor at nearly right angle. The

*Family names are set ahead according to their mother tongue.
cuttings, of which the leaves of proximal halves were detached by scissors, were planted in pots, filled with sand as propagating media and placed in greenhouse. Irrigation was made automatically by means of ripped cotton cloths from a water-filled tap.

Average temperature of air and the media at 10 a.m. were 38.5 °C and 29.5 °C during the test period.

Every day, after the planting, two modoomatu cuttings were picked up for the purpose of investigating the developments of callus tissue. Akamatu cuttings were tested to observe the root development, on every three days beginning from August 2, and on August 14 and 15, all the rest of the cuttings were examined because already a rooted cutting was discovered.

Free hand longitudinal sections were mainly used, stained in safranin and Delafield's hematoxylin.

The Structure of Epicotyllic shoots of pine. The tested shoots of akamatu and modoomatu showed no structural differences as far as the authors could see. They appear in cross sections as follows (fig. 1).

Epidermis is a layer of small cells, having cuticula somewhat remarkable. Cortex consists of about ten layers of cells rather irregularly arranged, in which man can recognize the evident nuclei. Resin canals are found four to six in number, and sometimes they have been in the process of differentiation.

Primary vascular system is formed by about 10 bundles unequal in their size, and one or two leaf traces can be seen in every section. In a primary bundle, protoxylem is seen as three or four layers, each of them consisting of one or two tracheids. On the outside of the protoxylem, there is the metaxylem four or five layers thick. Primary phloem is recognized as four to six layers at the outermost position of a bundle.

Secondary vascular systems are found in such shoots that have been used for this test, to be each about four to six layers of cells in xylem and phloem; they make up the vascular cylinder, continuous except at the leaf gaps. Primary and secondary rays, sometimes resin canals also, are visible among them.
Pith is so small in its diameter that it is only about 1/6 of the stem, and the cells of it are smaller than the cortical cells, especially in the outer layers near the primary xylem.

**Development of Callus.** At first, observations were made for a period of 9 days, using modoomatu, to determine the original tissues from which the callus develop. Results were not clear owing to the great individual variabilities, but it is considered to be as follows:

For about two days after cuttings were made, no significant change could be seen in them, only some coagulations of resin was visible on the cut surface. First sign of meristematic activity appeared in longitudinal sections a little above the cut base, 3 days after cuttings were made. Namely, some cells in phloem and cambium became larger, containing dense cytoplasma and large nuclei, and the arrangement of them became somewhat irregular. The membrane of the cells exposed directly to the cut surface became rather brownish, probably by their suberization.

Later, for a period between 4th and 7th day, there were few noticeable changes, only that such groups of cells seemed to increase their number, and that the cortical cells near the phloem became equally active. A cutting picked up on the 5th day appeared to develop its callus already, probably having developed from cells nearer to the cut surface. Pith also took a part in the callus formation, probably a little later.

As for the manner in which the callus developed over the cut surface from these meristems which appeared above, the authors had limited observations concerning the facts. The sections of the 8th day cutting showed the parenchymatous callus developing over the cut surface, from cambium, phloem and inner parts of cortex covering the xylem and nearly the pith (fig. 2). Next day, the sections showed that the callus had already developed rather remarkably, and man could recognize the differentiation of tracheids, which seemed to be developing in connection with the newly formed xylem tissue in the stem itself.

Further development of callus was not made evident because the

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Fig. 2. A longitudinal section of the 8th day cutting. Callus are developing from cambium, phloem and inner cortex (x 70).
authors stopped the observation, but in the 17th day cuttings of akamatu, well developed callus, of which the surface was covered by cork layers, was found covering the whole cut end. And, significantly, developed wound woods were found in the callus, which were accompanied with some layers of long cells continuous to the cambium and phloem in the stem. So these layers were considered to be cambium and partly differentiated phloem in callus tissue. Between the cork and phloem, there were parenchymatous semi-meristematic tissue.

Adventitious Roots from Callus Tissue. Though the cuttings picked up during the 17th to 26th days, had neither developed the root nor contained the plainly visible meristematic groups of cells considered to be root primordia; but a cutting taken on the 29th day showed unexpectedly a root developing already about three millimeters in length. Then, all the rest of the cuttings were taken, among them being some other rooted cuttings and some non-rooted but having protuberances on the callus surfaces. All these cuttings were sectioned and examined; and the following observations were made.

These roots were all arising from callus tissues only, and root primordium-like meristems were never discovered in the stems themselves.

The tissues of roots, which were clearly recognized as roots, appeared not from the inner parts of callus, but from near the surface of it. In other words, no breaking off of callus tissue, introduced by root passage, could be seen, and the cortex and the epidermis of roots were continuous to the parenchymatous tissues under its cork layers. Vascular connections between the roots and the callus xylem were made by tracheids, which seemed to be differentiated from callus parenchyma (fig. 3). No root primordium was found in these, which were seen as simple callus in appearance.

Protuberances of callus could be classified to some degree, varying from ones that consisted of simple parenchymatous cells similar to the other parts of the callus, to others in which the apical cells made up the complete meristematic tissue, resembling root apexes. And these meristematic cells did not form any definite group clearly distinguishable from the other parts, but gradually decreased their activities and were con-
tinuous to the normal callus parenchyma (fig. 4).

Drawing a conclusion, the authors are of the opinion that the adventitious roots arising from callus tissues of akamatu seedling cuttings are exogenous in their origin.

Discussion. Though many authors have reported their observations concerning the origins and developments of adventitious roots in many species of plant, many of these are the rooting from own tissues of cut materials. Namely, the developments of preformed root primordia in stems, or of newly initiated ones in normal tissues after cuttings were made, were mainly observed, and about the developments of roots from callus tissues, when the problem was defined to stem cuttings only, the authors could refer only to the following articles: by Bannan (1942) in layers of Larix, by Sato K. (1930) in Cryptomeria cuttings, by Simón (1908) in Populus cuttings and by Toda (1948) in Evonymus cuttings. All of these are lacking in detailed observation. But according to Simón's text figure, the root is arising from the inner part and developing through the outer layers of the callus, which is therefore broken off. Sato K. and Toda also illustrated that the primordium arose in callus tissues. In other words, these roots are seen to be endogenous. Moreover the roots from callus, which have developed from other organs than stems, such as from root cuttings of Crambe (Priestley and Swingle, 1929), from leaf cuttings of Ipomoea (Yasui, 1946) and Begonia (Hansen, 1881), seem to be endogenous as far as judged from their descriptions.

Exogenous roots have been observed in Family Crusiferae by Hansen (1881), Lemaire (1886) and Wilson (1927), and stated to be characteristic to this family by these authors and Priestley and Swingle (1929). Later, Howe (1931) stated that the adventitious roots of Cerealopteris are exogenous, though he was not the first investigator of this phenomenon; he has cited the KNYS's article (1875). Recently Bannan (1942) studied the rooting from coniferous layers anatomically, and from these observations, rooting from dormant buds of Abies and Picea were considered to be exogenous, though he did not give such conclusion at all.

If the authors' observations are correct, it is very interesting to note that a new example of exogenous root has been found, other than in Family Crusiferae or fern. Authors are going to examine the more detailed phenomenon closely.
Summary

The developments of callus and adventitious roots were studied in cuttings of epicotyl shoots of akamatu (Pinus densiflora) seedlings, and the new shoots of modoomatu (so-called natural hybrid of P. densiflora × P. Thunbergii), which have developed from decapitated epicotyls of one year old seedlings.

At first, callus develop from the cambium and the phloem, and then, from the cortex near the phloem, and from the pith. In callus, irregular xylem tissues, which are accompanied by cambium and phloem to its outer surface, develop in connection with the newly formed xylem of the stem.

Roots appear at first as the protuberances of callus, and seem to be exogenous in their origin. More detailed developing processes should be examined.

Literature cited


