Rooting Cofactors: Past, Present, and Future©

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Research to find substances that may be responsible for root initiation has been underway for over 100 years. The presence of leaves and buds have been shown to have a promotive effect on root initiation. If leaves and/or buds are removed or if the stem is girdled, rooting decreases. The effect of girdling the stem indicates that the root-promoting substances are transported in the phloem. Sugars and nitrogenous substances certainly play a role and the beneficial effects of leaves can, in part at least, be replaced by supplying leafless cuttings sugars and amino acids.

The discovery of auxin, indole-3-acetic acid (IAA), in the 1930s and its ability to stimulate root initiation demonstrated that a hormonal substance or rhizocaline was involved in the process of root initiation. The synthesis of compounds, such as, indolebutyric acid (IBA) and naphthaleneacetic acid (NAA), provided plant propagators with compounds that could increase the range of plants propagated by cuttings, shorten the rooting period, and increase the number of roots per cutting.

However, many plants remain difficult to root and that stimulated plant scientists to look for other substances that could regulate root initiation. Another explanation why cuttings are difficult to root is the presence of inhibitors that block root initiation. A scientist working in Israel in the 1950s showed that if difficult-to-root grape cuttings were soaked in water, they became easier to root. If easy-to-root grape cuttings were soaked in the water that had been used to leach difficult-to-root cuttings, they became difficult-to-root. The conclusion was that a water-soluble inhibitor was leached out of the difficult-to-root cuttings and when the inhibitor was supplied to the easy-to-root cuttings, they became difficult to root.

I was a graduate student at Cornell University at the time and was working on my Ph.D. under the direction of J.P. Nitsch. We used *Hedera helix*, the English ivy, as the experimental material. It has a juvenile form that is easy to root and a mature form that is difficult to root. Both forms can be found on the same plant and are very different morphologically, so it is easy to distinguish one form from the other. The fact that both forms could be found on the same plant gave assurance that the differences in rooting were not due to differences in genetic composition. The juvenile form rooted very easily and responded dramatically to the use of root-promoting substances, such as, NAA. The mature form showed little response. This provided evidence that either another substance or substances were required for root initiation or there was an inhibitor present that blocked rooting. Soaking the mature cuttings did not enhance rooting. The results could be interpreted that an inhibitor was not involved or that it was not readily leached from the intact mature tissue. We next looked at the growth-promoting and growth-inhibiting substances that could be extracted from dried juvenile and mature tissues with methyl alcohol, the solvent of choice for extracting plant growth substances.

The extracts were separated using paper chromatography and the presence of growth promoting and growth-inhibiting substances was determined by a bioassay developed by Dr. Nitsch. The bioassay was based on the elongation of cells in the coleoptile of young oat seedlings. We found a number of growth-promoting and

growth-inhibiting substances in the extracts. There were seasonal differences with larger amounts of promoting substances present when the plants were actively growing and larger amounts of inhibitors present when the plants were dormant. However, the differences in the amounts of growth-promoting and growth-inhibiting substances were not large enough to explain the difference in rooting response between the juvenile and mature phase.

At this point we developed a new bioassay based on root initiation in cuttings of mung bean, *Phaseolus radiata* (syn. *P. aureus*). The seedlings grew rapidly, were small enough to place 10 cuttings in a vial along with a section of the chromatogram, and rooting took place in 5 to 6 days. When methanol extracts were evaluated in combination with IAA, we found a substantial increase in rooting using extracts from juvenile tissue and little increase with extracts from mature tissue. When the juvenile extract was separated using paper chromatography, four root-promoting substances were found in the juvenile tissue and were labeled cofactors 1 through 4 based on their position on the paper chromatogram. Similar results were obtained using extracts from an easy-to-root red *Hibiscus rosa-sinensis*. The substances worked synergistically with IAA. One of the substances was identified as isochlorogenic acid and using this information, other phenolic substances that promoted root initiation in the presence of IAA were identified. The greatest biological activity was found when the hydroxyl groups on the benzene ring were adjacent to one another as in the compound catechol. We also found that a possible mode of action of catechol was to slow the metabolism of IAA. These and related findings led us to postulate that root initiation was based on the interaction between IAA and other rooting cofactors. If carbohydrates and amino acids were not limiting, the greater number and amount of cofactors present, the easier a cutting would be to root. Subsequently, we partially identified the fourth cofactor as being an oxygenated terpenoid compounds and cofactor one as a lipid-like substance containing alcohol and nitrile groups. Using the mung bean bioassay, other researchers found rooting cofactors in other easy to root plants such as willow and easy to root selections of apple and pear.

When Wes Hackett was at the University of Minnesota he developed a rooting bioassay using the aseptically cultured shoot apices of juvenile and mature forms of *H. helix*. He found that fractionated methanol extracts of juvenile ivy tissue promoted rooting of the juvenile shoot apices and that a combination of catechol and IAA stimulated rooting in a manner similar to NAA. He also concluded that catechol was protecting the IAA. However, when the fractionated extracts were applied to mature ivy shoot apices, rooting was not improved, bringing into question the validity of using the mung bean bioassay to find naturally occurring root-promoting substances. He was able to get a synergistic response from IAA and catechol in the mature shoot apices when they were cultured under low light intensity or in darkness.

I have also worked on the effects of etiolation, growing a shoot in darkness, upon root initiation. Etiolation can improve the rooting response of both easy and difficult-to-root plants. However, an extractable root-promoting factor produced under low light or darkness has not been found. The fact remains that we are still searching for the explanation of why some cuttings are hard to root and why it is that the easy to root cuttings show the greatest response to root promoting substances such as IAA, IBA, and NAA.

The relatively new tools of molecular biology or what is now being called

"genomics" are providing new approaches to the problem. Researchers are trying to determine what genes are activated during the process of root induction, initiation, and development. At any one time a relatively small percentage of the genes within a plant cell are activated. Although the genetic make up of the juvenile and mature ivy shoots taken from the same plant are identical, some genes are "turned on" or expressed while others are "turned off". For example, in the juvenile stage, the genes regulating anthocyanin synthesis, lobed leaves, and perhaps rooting potential are "turned on". In the mature phase, these genes are "turned off" and other genes such as the genes that regulate flower initiation and an upright form of growth are turned on. Using this information researchers are looking for the first genes to be "turned on" immediately after IAA is supplied to the cuttings. Others are looking at mutants that may differ from normal plants by a single gene. For example, mutants have been identified in the weed *Arabidopsis thaliana*, which have prolific root formation. The mutant is known as "rooty", and is a recessive trait located on chromosome 2. Another mutant does not root at all in response to external auxin application. There is a major international project to map all of the genes of the plant *Arabidopsis*, much like the human genome project. Someday, the tools of molecular biology or genetic engineering may make it possible to transform a plant to make it easy-to-root. But for now, the bottom line is that difficult-to-root cuttings still remain a challenge for the propagator whether in business or in the laboratory.