THE PHYSIOLOGICAL BASIS OF ADVENTITIOUS ROOT FORMATION

F.T. Davies, Jr.
Dept. of Horticultural Sciences
Texas A&M University
College Station, TX 77843

H.T. Hartmann
Dept. of Pomology
University of California
Davis, CA 95616

Abstract

Poor rooting limits the commercial production of potentially important clones of many woody plant species. Why is it that certain clones are difficult-to-root? And why haven't we made more advances in rooting success? Despite very active research, the primary chemical stimulus(i) to dedifferentiation (the most critical step of adventitious root formation) remains unknown.

This paper explores current research on developmental and physiological aspects of rooting. Adventitious root formation (ARF) is an organized developmental process involving discrete biochemical, physiological and histological events in the induction, initiation, development and elongation of root primordia. Genetic aspects of rooting such as the role of transciption and translation in "competent" cells to dedifferentiate and form root primordia need to be better understood.

Rejuvenation of tissue via tissue culture, hedging and heavy pruning, layering, etiolation, serial cutting propagation, serial grafting, etc., have had some success on phase change in plants and their subsequent rooting potential. Physiological factors in pre-severance conditioning in the selection of cutting material from stockplants are considered.

Possible directions for enhancing future rooting success are addressed.

1. Introduction

The economic importance, potential and problems of clonal regeneration by cuttings as a plant selection tool for breeding, and as a plant propagation tool for reproduction are well documented (Kester, 1983). Many vegetative propagation techniques have been known for over 2000 years, yet rooting of cuttings is still one of the most cost-effective methods of clonally regenerating plants. Poor rooting limits the commercial production of potentially important clones of many woody plant species. Despite years of active research, the primary chemical stimulus(i) to dedifferentiation (the most critical step of adventitious root formation - ARF) remains unknown (Raviv, et al., 1986).

The process of adventitious root formation needs to be studied as a dynamic series of distinct biochemical, physiological and histological events (Davies, et al., 1982) if the science of cutting propagation is to advance. Six areas of current rooting research are addressed in this paper: a) anatomical and histological aspects, b) biochemical and physiological factors, c) adventitious root formation as an organized developmental process, d) status of root developmental sequencing studies, e) physiological factors of "rejuvenation" techniques and pre-severence conditioning of stock plants, and f) future research directions.

2. Anatomical and Histological Factors

Adventitious roots in stem cuttings of many woody plant species have been reported to originate from various tissues. ARF in woody plants generally originate in young secondary phloem, but also arise from vascular rays, cambium or pith (Hartmann and Kester, 1983). Origin of adventitious roots from callus tissue has been associated with difficult-to-root species (Bhella and Roberts, 1975).

Poor rooting in stem cuttings of certain woody species has been correlated with extensive sclerification (Goodin, 1965; Edwards and Thomas, 1980). Beakbane (1969) proposed that thick lignified walls of sclerenchyma tissues were physiological or mechanical barriers to ARF in poor rooting species of _Fagus_, _Prunus_ and _Quercus_. Davies et al. (1982) was unable to find relationships between sclerenchyma density and rooting potential, and reported that differences in rooting capability were related to ease of root initiation rather than restriction of developing root primordia by sclerenchyma. Williams et al. (1984) observed that poor rooting in 16 woody plant species was correlated with suberization of the cortex, rather than inhibition by sclerification. Mature _Griselinia lucida_ had reduced rooting, but the presence of a complete ring of fibers did not present a mechanical barrier to emergence (White and Lovell, 1984); since the initial phase of cambial activation was prevented, they suggested that either the initial stimulus was less effective in mature plants or that target cells were less responsive than those of seedlings, which have very few fibers.

An important area that needs further study is the developmental physiology, histochemistry and histology of the wound healing response (WHR) (Cline and Neely, 1983; Warren Wilson and Grange, 1984). All cuttings are wounded in propagation and the WHR induces cell division and meristematic activity, that may directly or indirectly stimulate adventitious root formation (ARF). A challenge for future studies is to establish the role of specific diffusable morphogens through gradients in tissue regeneration in wounded stems (Wilson and Grange, 1984).

Certainly other contributing factors in the horticultural practices of wounding and stripping bark can be the removal of sclerenchyma and suberized cortical tissue, as well as creating sink areas for metabolites, rooting cofactors, auxin, ethylene evolution, etc.

3. Biochemical and Physiological Factors

Prerequisites for adventitious root formation to occur include: 1) availability and receptivity of parenchyma cells for regenerating *de novo* meristematic regions, 2) various modifications of the rhizocaline complex of phenolics (inhibitors, promoters-rooting cofactors), auxin, enzyme systems (Bouillenne et al., 1955; Fadl and Hartmann, 1967; Hess, 1969; Bassuk and Howard,1981), and 3) substrate needs such as carbohydrate accumulation and partitioning (Haissig, 1984), and changes in nitrogen and amino acids (Suzuki and Kohno, 1983; Hambrick et al., 1987).

Hormonal response through the uptake and distribution of auxin in ARF is well documented (Bridglall and van Staden, 1985; Jarvis and Shaheed, 1986). Root inducing promoters, cofactors and proanthocyanidins continue to be reported (Tognoni and Lorenzi, 1983; Raviv et al., 1986; Vazquez and Gesto, 1986). IBA treatments may control endogenous auxin levels of cuttings either through direct regulation of the IAA oxidase system or indirectly through the transport of auxin protectors (Mato and Vieitez, 1986).

Auxin is frequently not the limiting factor in difficult-to-root species. Haissig (1974) proposed that the lack of ARF in response to auxin may be due to: 1) the lack of essential enzymes (isoenzymes) to synthesize root-inducing auxin-phenol conjugates, 2) presence of enzyme inhibitors, 3) lack of enzyme activators, 4) lack of substrate phenolics, and 5) physical separation of enzyme reactants due to cellular compartmentalization. Hence, predisposition of cells (competent cells) to initiate root primordia is dependent on active enzymes and/or substrate which difficult-to-root cuttings may lack for synthesis of auxin-phenolic conjugates. Controversy exists since auxin-phenol-enzyme complexes and promoter-inhibitor systems of ARF have not been universally observed in plants nor found *in vivo* (Bassuk et al., 1981).

4. The Organizational Process of Rooting

Researchers have recognized different stages of *de novo* ARF. Girouard (1967) and Davies et al. (1982) reported adventitious rooting as a four-stage process: 1) dedifferentiation or remeristemation, 2) initiation or inception as cells begin to devide and form slightly organized groups (root initials), 3) differentiation of root primorida, and 4) primordia elongation. Sircar and Chatterjee (1973) observed five histologically distinct stages of rooting in *Vigna* hypocotyl cuttings, and Smith and Thorpe (1975) reported 3 phases in the differentiation of *Pinus radiata* seedling root primordia: a) preinitiative phase with no histological changes, b) an initiative phase forming a meristematic locus, and c) a post initiative phase with meristemoids differentiating into root primordia. However, only limited information exists in the literature where ARF has been studied as an organized developmental process in economically important woody plants.

5. Developmental Sequencing Studies of ARF

There are considerable advantages of integrating biochemical, physiological and developmental anatomy studies in ARF. Inability of woody cuttings to form adventitious roots (which is an organized developmental process) may be attributed to inability of parenchymatous cells to undergo transformation into primordia for physiological reasons.

Researchers have approached ARF as a developmental process involving sequences of histological phenomena with each stage having different requirements for growth substances (auxin, cytokinins, GA, etc.). Thus, time separation of adventitious rooting stages has made possible the study of concurrent physiological events (Ericksen and Mohammed, 1974; Smith and Thorpe, 1975; Davies and Joiner, 1980; Bollmark and Eliasson, 1986).

Developmental studies have also been conducted to show the importance of transcription and translation and subsequent RNA and protein metabolism during the initiation and development of root primordia (Oppenoorth, 1979; Tripepi, et al., 1983: Davies, 1984; Jarvis, et al., 1985). However, it is still unclear how RNA metabolism is altered within those cells that eventually give rise to root initials. Gross changes of RNA in stem tissues may not indicate the nature of changes in those relatively few number of cells undergoing dedifferentiation. Hence, there is a need to extend studies to include micro-autoradiography, histochemical and biochemical studies on a micro tissue level.

6. Physiological Factors of Rejuvenation and Pre-Severance Conditioning.

In cutting propagation of difficult-to-root woody plant species, ease of ARF declines with age of parent stock, which is an important problem because desirable characteristics are frequently not expressed until after a plant has reached maturity. Horticultural and forestry practices can rejuvenate physiologically mature stockplants, cuttings, explants, etc., and frequently improve rooting success (Kester, 1976; Zimmerman, 1976). Yet our knowledge of physiological and biochemical factors between juvenile and mature material and in preconditioning of stock plants is incomplete.

The physiological state of the propagule (ramet) can be improved by pre-severance conditioning of the stockplant (ortet) by: a) hedging and heavy pruning of stockplants, b) serial (multiple) graftage of mature difficult-to-root clones onto juvenile stock plants, c) serial cutting propagation (Morgan, et al. 1980; St. Clair, 1985), d) ring barking, girdling, etiolation and blanching (Delargy and Wright, 1978, 1979; Maynard and Bassuk, 1987) e) carbohydrate/nitrogen accumulation and partitioning (Haissig, 1984; Hambrick et al., 1987), f) selection of juvenile material from adventitious shoots arising on roots or from sphaeroblasts, g) reversion through application of sprays, pastes or injections of growth substances (GA, growth retardants, cofactors, etc.) and h) environmental manipulation (temperature, photoperiod). Essentially stock plants are being physiologically preconditioned by mechanical, chemical and environmental means so that "competent cells" (Haissig, 1974; Tripepi et al., 1983) in propagules can dedifferentiate and develop into meristematic regions of adventitious root primordia.

Rejuvenation of tissue _in vitro_ has tremendous potential to increase rooting of tissue culture derived cuttings (Kester, 1976; Mullins et al., 1979; Lyrene, 1981). Reversion from mature to juvenile characteristics may be a general phenomemon in plant tissue culture. The cause of juvenile reversion in blueberry shoot-tip cultures (Lyrene, 1981) is unknown, but a comparison between treatments that cause juvenile reversion (zygote formation, formation of apomictic embryos and adventitious bud formation from roots or stems) and ineffective treatments (sprouting of physiologically mature buds on grafts or cuttings) indicate that effective treatments all involve initiation of shoots from single cells (Broertjes and Van Harten, 1978). Part of the molecular basis in phase change in _Hedera helix_ may involve alteration in the rate of transcription of certain genes in the apices of the mature form (Rogler and Dahmus, 1974; Domoney and Timmins, 1980).

The juvenile condition can also be restored from mature plants by asexual embryos, which is the topic of the speaker on artificial seeds (Durzan, 1987).

7. Future Research Directions

To better understand the physiological basis of ARF future research should be focused on the following five research areas:

1) Confine biochemical studies to the small unit of tissue involved in the ARF process (root initials, primordia and adjacent cells), since much of the research to date has been done with gross stem tissue of which ARF comprises only a small mass of tissue.

2) Coordinate the sequential series of developmental events in ARF with gene action, biochemical, cytochemical and developmental anatomy changes.

3) A greater understanding of the wound healing response and phenolic metabolism and their relationship to ARF is needed, particularly since all cuttings are initially wounded.

4) The physiological factors of pre-severance conditioning and rejuvenation techniques on the propensity of cuttings to root needs to be better understood.

5) Enhancing ARF by genetic manipulation through incorporation of plasmid systems, etc. to increase auxin, rooting cofactors and other metabolite production in host plants (Becard, et al., 1987; Patena, et al., 1987).

References

Beakbane, A.B., 1969. Relationship between structure and adventitious rooting. Proc. Inter. Plant Prop. Soc. 19:192-201.

Becard, G., Fortin, J.A., and Piche, Y., 1987. Phases of VAM development on Ri T-DNA transformed roots. Procd. North Amer. Conf. on Mycorrhizae. Gainesville, FL

Bhella, H.S., and Roberts, A.N., 1975. Seasonal changes in origin and rate of development of root initials of Douglas-fir stem cuttings. J. Amer. Soc. Hort. Sci. 100:643-646.

Bollmark, M., and Eliasson, L., 1986. Effects of exogenous cytokinins on root formation in pea cuttings. Physiol. Plant. 68:662-666.

Bouillene, R., and Bouillene-Walrand, M., 1955. Auxines et bouturage. Rep. 14th Intl. Hort. Congr. 1:231-238.

Brindglall, S.S., and Van Staden, J., 1985. Effect of auxin on rooting and endogenous cytokinin levels in leaf cuttings of Phaseolus vulgaris L. J. Plant Physiol. 117:287-292.

Broertjes, C., and Van Harten, A.M., 1978. Application of mature breeding methods in the improvement of vegetatively propagated crops. Elsevier, N.Y.

Cline, M.N., and Neely, D., 1983. The histology and histochemistry of the wound healing process in geranium cuttings. J. Amer. Soc. Hort. Sci. 108:452-496.

Davies, F.T. Jr. and Joiner, J.N., 1980. Growth regulator effects on adventitious root formation in leaf bud cuttings of juvenile and mature Ficus pumila. J. Amer. Soc. Hort. Sci. 100:643-646.

Davies, F.T. Jr., 1984. Shoot RNA, cambial activity and indolebutyric acid effectivity in seasonal rooting of juvenile and mature Ficus pumila cuttings. Physiol. Plant. 62:571-575.

Davies, F.T. Jr., Lazarte, J.E. and Joiner, J.N., 1982. Initiation and development of roots in juvenile and mature leaf bud cuttings of Ficus pumila L. Amer. J. Bot. 69:804-811.

Delargy, J.A., and Wright, C.E., 1978. Root formation of apple in relation to ringbarking and to etiolation. New Phytol. 81:117-127.

Delargy, J.A. and Wright, C.E., 1979. Root formation in cuttings of apple in relation to auxin application and to etiolation. New Phytol. 82:341-347.

Domoney, C., and Timmins, J.N., 1980. Ribosomal RNA gene redundancy in juvenile and mature ivy (Hedera helix). J. Exp. Bot. 31:1093-1100.

Durzan, D.J., 1987. Rooting in woody perennials: problems and opportunities with artificial seeds. Procd. Int. Sympos. Veg. Prop. Woody Sps. Pisa, Italy.

Edwards, R.A., and Thomas, M.B., 1980. Observations on physical barriers to root formation in cuttings. Plant Prop. 26:6-8.

Ericksen, E.N., and Mohammed, S., 1974. Root formation in pea cuttings II. Influence of indole-3-acetic acid at different developmental stages. Physiol. Plant. 32:158-162.

Fadl, M.S., and Hartmann, H.T., 1967. Relationship between seasonal changes in endogenous promotors and inhibitors in pear buds and cutting bases and rooting of pear hardwood cuttings. Procd. Amer. Soc. Hort. Sci. 91:96-112.

Girouard, R.M., 1967. Initiation and development of adventitious roots in stem cuttings of Hedera helix: anatomical studies of the juvenile growth phase. Can. J. Bot. 45:1877-1881.

Goodin, J.P., 1965. Anatomical changes associated with juvenile to mature growth phase transition in Hedera. Nature 208:504-505.

Haissig, B.E., 1974. Influence of auxins and auxin synergists on adventitious root primordium initiation and development. N.Z. J. For. Sci. 4:299-310.

Haissig, B.E., 1984. Carbohydrate accumulation and partitioning in Pinus banksiana seedlings and seedling cuttings. Physiol. Plant. 61:13-19.

Hambrick, C.E. III., Davies, F.T. Jr., and Pemberton,H.B., 1987. Carbohydrate/Nitrogen levels and seasonal rooting of Rosa multiflora 'Brooks 56' hardwood cuttings. J. Amer. Soc. Hort. Sci. 113: In Press.

Hartmann, H.T., and Kester, D.E., 1983. Plant Propagation: Principles and Practices. 4th ed. Englewood Cliffs, N.J.; Prentice Hall.

Hess, C.E., 1969. Internal and external factors regulating root initiation, In: W.J. Whittington, (ed.) Root Growth. Butterworths, London.

Jarvis, B.C., and Shaheed, A.I., 1986. Adventitious root formation in relation to the uptake and distribution of supplied auxin. New Phytol. 103: 23-31.

Jarvis, B.C., Yasmin, S., and Coleman, M.T., 1985. RNA and protein metabolism during adventitious root formation in stem cuttings of Phaseolus aureus. Physiol. Plant. 64:53-59.

Kester, D.E., 1976. The relationship of juvenility to plant propagation. Procd. Int. Plant Prop. Soc. 26:71-84.

Kester, D.E., 1983. The clone in horticulture. HortScience 18:831-837.

Lyrene, P.M., 1981. Juvenility and production of fast-rooting cuttings from blueberry shoot cultures. J. Amer. Soc. Hort. Sci. 106:396-398.

Mato, M.C., and Vieitez, A.M., 1986. Changes in auxin protectors and IAA oxidase during the rooting of chestnut shoots in vitro. Physiol. Plant. 66:491-494.

Maynard, B.K. and Bassuk, N.L., 1987. Stockplant etiolation and blanching of wood plants prior to cutting propagation. J. Amer. Soc. Hort. Sci. 112:273-276.

Morgan, D.L., McWilliams, E.L. and Parr, W.C., 1980. Maintaining juvenility in live oak. HortScience 15:493-494.

Mullins, M.G., Nair, Y., and Sampet, P., 1979. Rejuvenation in vitro: Induction of juvenile characters in an adult clone of Vitis vinifera L. Ann. Bot. 44:623-627.

Oppenoorth, J.M., 1979. Influence of cycloheximide and actinomycin D on initiation and early development of adventitious roots. Physiol. Plant. 47:134-138.

Patena, L., Sutter, E., and Dandekar, A., 1987. Root induction by Agrobacterium rhizogenes in difficult-to-root woody species. Procd. Int. Sympos. Veg. Prop. Woody Sps. Pisa, Italy.

Raviv, M., Becker, D., and Sahali, Y., 1986. The chemical identification of root promoters extracted from avocado tissues. Plant Growth Regul. 4:371-374.

Rogler, C.E., and Dahmus, M.E., 1974. Gibberellic acid-induced phase change in Hedera helix as studied by deoxyribonucleic acid-ribonucleic acid hybridization. Plant Physiol. 54:88-94.

Sircar, P.K., and Chatterjee, S.K., 1973. Physiological and biochemical control of meristemization and adventitious root formation in Vigna hypocotyl cuttings. Plant Prop. 19:16-17.

Smith, D.R., and Thorpe, T.A., 1975. Root initiation in cuttings of Pinus radiata seedlings. I. Developmental sequence. J. Exp. Bot. 26:184-192.

Smith, D.R., and Thorpe, T.A., 1975. Root initiation in cuttings of Pinus radiata seedlings. II. Growth regulator interactions. J. Exp. Bot. 26:193-202.

St. Clair, J.B., Kleinschmit, J. and Svolba, J., 1985. Juvenility and serial vegetative propagation of norway spruce clones (Picea abies Karst). Silvia Genet. 34:42-48.

Suzuki, T. and Kohno, K., 1983. Changes in nitrogen levels and free amino acids in rooting cuttings of mulberry (Morus alba). Physiol. Plant. 59:455-460.

Tognoni, F., and Lorenzi, R., 1983. Identification of root-promoting substances from Picea glauca. HortScience 18:893-894.

Tripepi, R.R., Heuser, C.W., and Shannon, J.C., 1983. Incorporation of tritiated thymidine and uridine into adventitious-root initial cells of Vigna radiata. J. Amer. Soc. Hort. Sci. 108:469-474.

Vázquez, A. and Gesto, D.V., 1986. Rooting, endogenous root-inducing cofactors and proanthocyanidins in chestnut. Biol. Plant. 28:303-306.

Warren Wilson, J., and Grange, R.I., 1984. Regeneration of tissues in wounded stems: a quantitative study. Annal. Bot. 53:515-525.

Weigel, V., Horn, W., and Hock, B., 1984. Endogenous auxin levels in terminal stem cuttings of Chrysanthemum morifolium during adventitious rooting. Physiol. Plant. 61:422-428.

White, J., and Lovell, P.H., 1984. The anatomy of root initiation in cuttings of Griselinia littoralis and Griselinia lucida. Annal. Bot. 54:7-20.

Williams, R.R., Taji, A.M. and Bolton, J.A., 1984. Suberization and adventitious rooting in Australian plants. Aust. J. Bot. 32:363-366.

Zimmerman, R.H. (ed.), 1976. Symposium on juvenility in woody perennials. Acta Hort. 56:1-317.