

## Use of Root Promoting Substances and Procedures: Why and How?<sup>©</sup>

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### PHYSIOLOGICAL BACKGROUND

A review of the basic plant physiological events occurring when a cutting is forming roots shows that first a wounding of the stem or leaf must take place. This wounding initiates among other an increased production of ethylene. During the last 70 years the activity of ethylene in the root initiation processes has been controversial and numerous contradictory reports have been published: ethylene enhances root formation, has no effect, or inhibits. Many of the differing results can be ascribed to methodological shortcomings: time of administration, controls, and presence or absence of preformed root initials in the plant material under investigation. It is now definitely proven, that ethylene is necessary, albeit in very minute quantities for the initial atypical cell divisions, which are a prerequisite for root initiation in cuttings (Boot et al., 2003). The effects of ethylene are of very short duration; higher concentrations and long time exposures are inhibitory. The probable mode of action for ethylene is to change the competence of some cells for receiving the auxin signal(s) (de Klerk, 2003).

It is thus still accepted facts that auxin is involved in the early events of root initiation; it may be IAA as well as IBA or any of the synthetic auxins or auxin-promoting substances. Indolebutyric acid has been shown to be a naturally occurring auxin by Ludwig-Müller and Epstein (1991); it is also evident that IBA aside from its function as "slow-release" auxin has a more direct role in root initiation processes (Bartel et al., 2001). This latter reference by the way is a very informative paper with details of IAA and IBA synthesis and breakdown as well as conjugates of these. During recent years a multitude of research papers have appeared describing molecular biological experiments revealing the first steps in root initiation. A number of gene sequences (>25) are activated by ethylene and or auxin (Kepinsky et al., 2003).

The influence of ethylene on root initiation has also been under investigation by methods applying compounds which block ethylene reception in cuttings. Both the old ethylene receptor blocker STS and the newer ones (1-MCP and related compounds) inhibit rooting in cuttings of several species (Thinggaard et al., 2003). Cuttings from genetically modified plants that have been rendered more or less insensitive to ethylene by insertion of the gene *etr 1-1* in order to secure better longevity of flowers exhibit poor root formation unless the gene expression is strictly limited to floral organs (Clark et al., 1999).

During recent years a number of other active compounds or principles have appeared to play a role at least in some plant systems. Endogenous compounds such as brassinosteroids, polyamines, chrysanthemic acid, and jasmonate have been implicated in a number of processes and functions in plants such as senescence, cell division, flowering, and rooting.

## OTHER ACTIVE SUBSTANCES

**Brassinosteroids.** Brassinosteroids have been isolated first from rape seed pollen, but have since been found in many other plants and organs. They are supposedly involved in a number of plant processes among these root formation in cuttings (Pelacho et al., 1997). They have, however, also been shown to inhibit root growth so in some ways they are similar to auxins (Schumacher and Chory, 2000).

**Polyamines.** The polyamines have strange names and most of them smell horribly. The effects depend on number of amine groups in the molecule. Putrescine inhibits rooting while spermidine promotes both rooting and root growth. The compound MBGB [methylglyoxal-bis-(guanylylhydrazone)] which inhibits the formation of spermidine also inhibits rooting even the IBA induced (Jarvis et al., 1983). It is further implied that the conversion of putrescine to gamma amino butyric acid is the reason for the activity in relation to root initiation (Hausman et al., 1997). It has even been suggested that the concentration of certain polyamines in a cutting would indicate its ability for rooting (Rugini et al., 1997).

**Chrysanthemic acid.** Chrysanthemic acid and derivatives of this acid can be extracted from *Chrysanthemum* species and it is used in some “natural” insecticides. According to Zhao et al. (1990) +/- cis chrysatemate in low concentrations (mM) promotes the rooting of cuttings of several species.

**Phenols.** For several years, phenols have been proclaimed as more or less active root-promoting substances. Jasmonic acid is one of these and it originates as the name suggests from jasmine and it has lately been a very widely used compound in experiments with genetic manipulation (Pelarco et al., 1997). Several new derivatives of diphenyl urea have also proven to promote rooting in apple rootstock cuttings. This was evident both for derivatives that showed “normal auxin activity” and for those, which did not (Ricci et al., 2003).

**Other Compounds.** Thus, it appears that we have candidates enough for the title “root promoters or elicitors” but these have not been available as practical tools for the industry.

This, however, should be possible for the concept “slow-release auxins” and elicitors (van der Krieken et al., 1997). These authors have combined IAA or IBA to BSA (bovine serum albumin) through either a carboxyl or an amide binding. A total of 35 auxin molecules can be attached to each BSA molecule. It is possible for plant tissues to absorb such large conglomerates and later more or less specific enzymes will release the auxins slowly. Other conjugates including some of the above-mentioned elicitors have been tested with even better results than BSA. These include IAA-hexanoic acid, IAA-polyamine, and IBA-amino acids. The combination of auxin and elicitors was able to increase rooting in difficult-to-root apple rootstock cuttings (in vitro) up to several hundred percent. It is possible that the sugar conjugates can be taken up into the long distance transport tissues of the cutting while the amide conjugates probably have a more local effect near treatment sites (Bartel et al., 2001).

## GENETIC MANIPULATION

Genetic manipulation of plants is also a hot issue for ornamentals and woody horticultural plants. In addition to the earlier described experiments with ethylene, many labs have been engaged in transforming difficult-to-root plants with genes

presumably giving rise to easier rooting cultivars (Welander, 1997). Some of the genes isolated or their effects on auxin synthesis or transport are available for research purposes from the Salk institute (<[www.salk.edu](http://www.salk.edu)>). Other constructs have long been known since they are naturally occurring in *Agrobacterium rhizogens* (rolA and B a.o.) and they have been incorporated in a number of plants with better rooting as a result, for example, the Dutch experiments with Rol genes transferred to root stock clones of roses meant for cut-rose production in greenhouses (Visser et al., 2003). Here a considerably better rooting resulted and when the grafted plants later were grown in the greenhouse a 23% higher yield of cut flowers were obtained due to the better roots. Other desirable effects from introducing these genes have been shown in *Limonium* where a reduced height was obtained (Mercuri et al., 2000). Since these genes are naturally found in *Agrobacterium*, it is logical to expect that an addition of the bacterium to cuttings would increase rooting. For further study of these possibilities.

Mycorrhizal fungi have also been suggested as means for better rooting (Nelson 1987, Bansal and Mukerji, 1993), and producers of mycorrhiza usually claim this as one of many positive aspects of their products.

#### LITERATURE CITED

- Bartel B., S. LeClaire, M. Magidin, and B.K. Zolman.** 2001. Inputs to the active indole-3-acetic acid pool: De novo synthesis, conjugate hydrolysis and indole-3-butyric acid  $\beta$ -oxidation. *J. Plant Growth Reg.* 20:198–216. <<http://www.bcm.tmc.edu/cnr/faculty/pdfs/leclere.REVIEW.pdf>> (27 Oct. 2004).
- Boot, K.J.M.; L. Goosen-deRoo, H.P. Spaink, and J.W. Kijne.** 2000. Adventitious root formation in stem segments of tobacco. *Adventitious Root Formation: Third International Symposium on Adventitious Root Formation*, 27 June – 1 July 2000 Veldhoven, The Netherlands.
- Clark, D.G., E.K. Gubrium, H.J. Klee, J.E. Barrett, and T.A. Nell.** 1999. Root formation in ethylene insensitive plants. *Plant Physiol.* 121:53–59.
- Damiano, C. and A. Monticelli.** 1999. In vitro fruit trees rooting by *Agrobacterium rhizogenes* wild type infection. *EJB Electronic J. Biotechnol.* <<http://www.inea.it/isf/index.html>>.
- de Klerk, G-J.** 2000. Multiple effects of ethylene during rooting of micropropagules. *Adventitious root formation: Third international symposium on adventitious root formation*, 27 June – 1 July 2000 Veldhoven, The Netherlands.
- Hausman, J-F., C. Kevers, D. Evers, and T. Gaspar.** 1997. Conversion of putrescine to  $\gamma$ -aminobutyric acid, an essential pathway for root formation by poplar shoots in vitro, pp 133–139. In: A. Altman and Y. Waisel, (Eds.), *Biology of root formation and development*, Plenum Press, New York, New York.
- Jarvis, B.C., P.R.M. Shannon, and S. Yasmin.** 1983. Involvement of polyamines with adventitious root development in stem cuttings of mung bean. *Plant and Cell Physiol.* 24:677–683.
- Kepinsky, S., D. Rouse, M. Tanimoto, and O. Leyser.** 2000. Stability of the *Arabidopsis* AXR3/IAA17 protein is regulated by auxin. *Adventitious root formation: Third international symposium on adventitious root formation*, 27 June – 1 July 2000 Veldhoven, The Netherlands.
- Krieken, W. van der, J. Kodde, M.H.M. Visser, D. Tsardakas, A. Blaakmeer, K. de Groot, and L. Legstra.** 1997. Increased induction of adventitious rooting by slow release auxins and elicitors, pp 95–104. In: A. Altman and Y. Waisel, (Eds.), *Biology of root formation and development*, Plenum Press, New York, New York.
- Ludwig-Müller, J., and E. Epstein.** 1991. Occurrence and in vivo biosynthesis of indole-3-butyric acid in corn (*Zea mays* L.). *Plant Physiol.* 97: 765–770.
- Mercuri, A., S. Bruna, I. de Benedetti, I. G. Burchi, and D. Schiva.** 2000. *Agrobacterium*-mediated transformation of *limonium* spp.: modification of plant architecture. *Atti V Giornate Scientifiche S.O.I.* 2000:37–38.

- Nelson, S.D.** 1987. Rooting and subsequent growth of woody ornamentals softwood cuttings treated with endomychorrhizal inoculum. *J. Amer. Soc. Hort. Sci.* 112:263–266.
- Pelacho, A.M., J. Pérez-Catalan,** and **L. Martin-Closas.** 1997. Root development in in vitro potato explants as affected by jasmonic acid, pp141–145. In: A. Altman and Y. Waisel, (Eds.), *Biology of root formation and development*, Plenum Press, New York, New York.
- Ricci, A., A. Carra, L. Dramis, E. Caboni,** and **C. Branca.** 2000. Adventitious roots induced by new diphenylurea derivatives. *Adventitious Root Formation: Third International Symposium on Adventitious Root Formation*, 27 June – 1 July 2000 Veldhoven, The Netherlands.
- Rugini, E., G. di Francesco, M. Muganu, S. Castolfi,** and **G. Caricato.** 1997. The effect of polyamines and hydrogen peroxide on root formation in olive and the role of polyamines as an early marker for rooting ability, pp 65–73. In: A. Altman and Y. Waisel, (Eds.), *Biology of root formation and development*, Plenum Press, New York, New York.
- Schumacher, K.** and **J. Chory.** 2000. Brassinosteroid signal transduction: Still casting the actors. *Curr. Opin. Plant Biol.* 3:79–84
- Thinggaard, M., R. Müller, M. Serek, E. Sisler,** and **A. Skytt Andersen.** 2000. Rooting of *Codiaeum* Cuttings. *Proceedings of III Symposium on Adventitious Root Formation*, 2000, Veldhoven, The Netherlands.
- Visser, P.B., L.C.P., Keizer, T.P.M. Van der Salm,** and **J. De Jong.** 2000. Introduction of ROL A,B,C genes in rootstocks enhances rose production. *Adventitious root formation: Third international symposium on adventitious root formation*, 27 June – 1 July 2000 Veldhoven, The Netherlands.
- Welander, M., N. Pawlicki, A. Holefors,** and **F. Wilson.** 1998. Genetic transformation of apple rootstock M26 with the rolB gene and its influence on rooting. *J. Plant Physiol.* 53:371–380.
- Ueda, J.** 1989. Promotive effect of capillarol and related compounds on root growth. *Physiol. Plant.* 76:42–46.