NEW PLANT GROWTH REGULATORS FOR CUTTINGS AND FOR TISSUE CULTURE

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INTRODUCTION and LITERATURE REVIEW

For centuries propagators have sought ways to enhance the rooting of cuttings that rooted with difficulty, to cause non-rooting cuttings to root, to hasten healing of grafts and, in general, to cause ease and speed of propagability to be improved. In the early 1930's, a marvelous breakthrough occurred for cutting propagation when Thimann and Went (19) and their coworkers (18) discovered that a root-promoting substance, indoleacetic acid (IAA) was found in many higher plants. This substance, dubbed "*auxin*" or IAA, when applied to the base of cuttings could be used to hasten the rooting of cuttings of many species and cause rooting in others previously difficult or impossible to root. Even better rooting results were found for analogs of IAA, such as indolebutyric acid (IBA) and naphthaleneacetic acid (NAA). Today, these latter compounds are the active principles in numerous commercially available rooting compounds. Because IAA breaks down in light and IBA and NAA are essentially non-labile in light, IBA and NAA are more commonly included in such formulations.

Although much research was conducted in ensuing years, no additional economically feasible chemical treatments were found until recently. Hess' work with *rooting cofactors* is perhaps the most notable of such attempts to find additional root *stimulating compounds* (6). He successfully demonstrated that extracts of juvenile *Hedera helix* (English ivy) could interact synergistically with IAA to increase rooting in the mung bean rooting bioassay. However, no commercial applications utilizing rooting cofactors have taken place, although the principles demonstrated by the work of Hess and others should stimulate further investigations along this line of research.

Gibberellins, first discovered in 1939 in Japan, are now known to be a large group of naturally occurring compounds that cause internode elongation and numerous other plant responses. Their discovery led to a flurry of research with these compounds in the 1950's and later, including attempts to utilize them in improving propagation success. However, gibberellins had little effect on rooting of cuttings, or frequently were even inhibitory (1,13).

The work of Folke Skoog and associates in Wisconsin led

to the discovery of another important class of active plant growth regulating chemicals, the *cytokinins* (16). Cytokinins are involved in division, growth, and differentiation of plant cells. In tissue culture systems, such as callus derived from tobacco pith, it has been demonstrated that cytokinins function in balance with auxin to induce shoots or buds. This knowledge has greatly enhanced our ability to propagate numerous species through tissue culture (*micropropagation*). This subject will be addressed further in the discussion of the application of new compounds to *in vitro* culture.

Certain *fungicides* have also been demonstrated to enhance rooting of cuttings. In some cases, as reported for cuttings treated with captan, enhancement of rooting was in excess of the response expected if the fungicide had been merely controlling pathogens (20). Benomyl, another fungicide, has also been suggested as a possible enhancer of shoot multiplication in tissue culture systems, since it is reported to have cytokinin-like qualities.

Research in our laboratory in the 1960's and 1970's illustrated that compounds generally employed as *growth retardants*, notably daminozide (SADH, B-Nine) and chlormequat (CCC, Cycocel) can profoundly influence propagation success. Tuberous root formation in dahlia was greatly increased under normally non-inductive photoperiods (long-days) by whole-plant sprays of 2500 to 5000 ppm daminozide or 1000 to 2500 ppm of chlormequat (9). Cuttings taken from such daminozide-sprayed plants rooted more readily than did those from the control plants, but rooting was depressed for cuttings taken from plants sprayed with chlormequat. Cuttings of several herbaceous species were also found to root more quickly than non-treated cuttings when the cutting bases were dipped for short periods of time (15 to 60 seconds) in 1000 to 5000 ppm daminozide solutions (12). Such daminozide-induced rooting was consistent for chrysanthemum, carnation, dahlia, poinsettia, geranium (*Pelargonium*), and other herbaceous species (6,12,13). It was also effective for cuttings of several woody species, especially *Juniperus* spp.

Chlormequat, on the other hand, inhibited formation of adventitious roots to a level less than that produced by the non-treated cuttings. In spite of the observation by Read and Bryan (8) that daminozide sprays could alleviate chlorosis caused by chlormequat, stimulation of rooting by chlormequat treatment has been inconsistent, suggesting that further research is required.

A *controlled-release* method of delivery of growth regulators was explored in the early 1970's, in which a polymer-

encapsulated formulation of chlormequat was incorporated into the growing medium for height control and into the rooting medium for stimulation of early rooting (10,11). However, when geranium cuttings were rooted in a medium containing controlled-release chlormequat, subsequent root development was greatly retarded. Carpenter and Carlson (2) also noted poinsettia root stimulation when incorporating chlormequat in a potting medium for stock plants, but Shanks (15) experienced mixed results when rooting cuttings from stock plants that had been sprayed with ethephon (Ethrel).

From the foregoing research reports (and the work of numerous other researchers), it becomes readily apparent that opportunities abound for further research with new growth regulating chemicals and new approaches with known chemicals.

NEW CHEMICALS

Several new chemicals have been developed by chemical companies and research laboratories in recent years. This report will focus briefly on three of them: triacontenol, conjugated auxins, and substituted phenyl urea derivatives.

MATERIALS AND METHODS

Tissue cultures of hardy deciduous azaleas from the University of Minnesota woody ornamental breeding program (led by Dr. Harold Pellett), and *Typha glauca* callus cultures were employed as the test units. Methods for producing the azalea cultures (U. of Minnesota Accession 800112) were those described by Economou and Read (3) and Fellman (4), while the *Typha* callus method was described by Zimmermann (21). The tissues cultured by these methods were subsequently placed on the appropriate test media, in which the chemicals being evaluated had been incorporated at various levels.

RESULTS AND DISCUSSION

Triacontanol. Table 1 shows the mean number of shoots produced by a hybrid deciduous azalea cultured on Economou and Read medium containing various levels of triacontanol. In contrast to reports by Ries' group (14), no significant differences were found in shoot or root formation. However, as also found with two other experiments, there appeared to be a trend toward an increase in root production, but this increase was considerably less than one would anticipate had an auxin been used (e.g. NAA, IAA). It is apparent that further studies are required to clarify potential uses for triacontanol in propagation schemes.

Table 1. Effect of different triacontanol concentrations on root and shoot formation from azalea accession 800112 after 4 weeks culture *in vitro*.

Triacontanol concentration (mg/liter)	Mean number of: shoots	roots
0.001	0	0.67
0.01	0	1.67
0.05	0	1.35
0.1	0	1.33
1.0	0	2.01
Control (0 level)	0	1.20

IAA-Conjugates. IAA-conjugate (D,L-alanine)[1] was tested on a *Typha* callus bioassay, with the expectation that because it was in a conjugated form it would resist degradation and thus remain more active. However, the mean callus rating (Table 2) was similar to that for NAA, but inferior to the stronger auxin-like compounds: picloram and 2,4-D. Note that more roots were produced by NAA than by the IAA-conjugate, but no roots were produced by the best callus stimulating treatments. These preliminary findings are considered inconclusive at this time, but suggest a level of activity worthy of further investigation.

Table 2. Callus and root production on *Typha glauca* female spike segments cultured *in vitro* on media containing different growth regulating *chemicals*.

Plant growth regulator	Mean callus[z] rating	No. explants[y] producing roots
10 mg/l 2,4-D	1.65	0
10 mg/l picloram	1.91	0
10 mg/l NAA	1.00	16
10 mg/l IAA conjugate	1.00	4

[z] rated on a 4 point scale, where 1 = no callus, 4 = excellent callus.
[y] 23 explants per treatment.

Substituted Phenyl Ureas. Perhaps the most promising new group of chemicals for propagators to consider, N-(2-chloro-4-pyridyl)-N[1]-phenylurea derivatives (4PUs), have been reported to have cytokinin-like properties by Takahashi, *et al*, (16) when employed in the tobacco callus bioassay. They indicated that 4PU-30 (or 4PU-Cl), which has a chlorine in the 2-position of the pyridyl ring, had activity 100 times that of benzyladenine. Since little research had been reported on the effects of 4PU-Cl for propagation purposes, we decided to in-

[1] Supplied by Norman Good, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan.

vestigate its shoot-forming potential, using *Typha* callus cultures and hardy deciduous azalea microcutting explants. In the *Typha* callus bioassays, 4PU-1080, which has two chlorine substituents on the pyridyl ring, was also tested.

Typha Callus Experiments. Rates of 100 mg/l of both 4PU-Cl and 4PU-1080 caused death of the explants. However, 1 mg/l 4PU-Cl caused prolific production of green roots by calli derived from a medium containing picloram. This was similar to the response caused by BA at 5 mg/l. However, 1 mg/l 4PU-1080 stimulated an even more prolific production of green roots than the other treatments. In no case were buds or shoots produced in the 8 week-duration of these experiments.

A much greater responsiveness to 4PU compounds was observed for the hardy deciduous azalea tissue cultures (4). Table 3 illustrates that relatively low concentrations of 4PU-Cl (0.05M) resulted in dramatic increases in shoot numbers. This response was consistent between trials involving media containing 0.6M IAA, or no auxin in the medium. In other experiments comparing the influence of different cytokinins on azalea shoot production *in vitro*, significantly greater shoot numbers were produced by azalea cultures with 0.05M 4PU-Cl than by cultures containing 0.05M zeatin or 2iP. Both zeatin and 2iP are commonly used for azalea shoot proliferation, but at higher concentrations. This would suggest that 4PU-Cl may have a stronger cytokinin-like activity than zeatin and 2iP. In addition, tiny bud-like protuberances appeared on the leaves of the microshoots produced on media containing 0.5M 4PU-Cl after 10 weeks in culture, further suggesting a strong cytokinin effect. Anatomical studies showed that these structures had a somewhat bud-like character and developed from trichomes situated over vascular tissue. Although they did not grow into shoots, they did acquire a meristematic dome-like structure and one or more leaf primordia.

Table 3. Production of shoots by azalea accession 800112 after culture for 10 weeks on media containing different 4PU-Cl concentrations. Means are for 20 cultures per treatment.

4PU-Cl Concentration (M)	Mean No. of Shoots per culture
0	1.0 ab[1]
0.0005	1.0 ab
0.005	1.4 b
0.05	2.3 b
0.5	5.1 c
5.0	0.2 a
50.0	0.1 a

[1] Values followed by the same letter are not significantly different at the 5% level.

Additional studies are required to determine optimum levels for 4PU derivatives used in tissue culture of azaleas and to stimulate and monitor development of the bud-like protuberances. Applications of these compounds for other species and other propagation methods (e.g. cuttings) should be investigated, since they are obviously extremely active plant growth regulating chemicals. As more is learned about the physiological effects of these and other compounds and how they interact with known growth regulators such as auxins, gibberellins, and cytokinins, additional strides in the world of plant propagation are highly probable.

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PROGRAMMING STOCK PLANTS FOR PROPAGATION SUCCESS

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Abstract. Our research has demonstrated that the stock plant (mother or source plant) has profound influence on subsequent success of explants cultured *in vitro*. Extremely different *in vitro* performance results from different levels of mineral nutrition, plant growth regulator applications, light quality, and photoperiod treatments of the stock plant. Cultivar differences have been demonstrated also, even for species which are easy to culture. Further, preculture treatments of the explant with cytokinins can increase microshoot yield equivalent to that produced by incorporating the same cytokinin into the medium. When established cultures are treated as stock material (microstocks), light intensity and light quality can be manipulated to improve number of microshoots produced and the subsequent rootability of such microshoots. Forcing solutions have also shown promise as a delivery system for incorporating plant growth regulators into softwood growth of forced deciduous woody species. Pertinent literature is reviewed and possible relationships to endogenous hormone levels are discussed.

Propagators and researchers have known for some time that treatments applied to the stock plant (mother plant, source plant), and to the environment under which the stock