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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

plant is grown, have a profound effect on subsequent propagation. Such effects may include production of larger or more cuttings, and improved root regeneration by cuttings taken from such stock plants. Nutrition, light, temperature, genotype, plant growth regulator applications, physical manipulations, and season have all been reported to influence propagation efforts.

LITERATURE REVIEW

There are numerous references linking stock plant nutrition with rooting of cuttings. The classic work of Kraus and Kraybill (26) is a notable example in which they demonstrated that carbohydrate-nitrogen ratio affected rooting of tomato cuttings. Other nutrient levels including boron (41) have also been considered important factors in the rooting of cuttings.

Reduced light intensities have been reported to enhance rooting of *Forsythia* and *Weigela* (27) and *Dahlia* (4). Extreme light reduction leading to etiolation also has frequently been reported to improve rooting (14,25). Several workers have reported that reduced stock plant light intensities can lead to better rooting (1,24,30). An increased level of endogenous auxin is generally thought to occur under reduced light, thus enhancing rooting (29).

Whalley (42) has reviewed literature on photoperiod effects on rooting of various ornamentals. Bhella and Roberts (3) reported that rooting of Douglas fir cuttings was increased by long days (LD). Others have also shown an effect of stock plant photoperiod on rooting of cuttings (2, 21). Night interruption or day extension with supplemental lighting has been utilized to provide a continuous supply of cuttings and explants for culture *in vitro* (19,35,36). Light quality has also been shown to affect cutting production and rooting (19). Red light increased axillary bud activity while far-red caused better rooting, similar to reported effects for *in vitro* culture of petunia leaf segments (34). In the latter work, a 30-min. day extension with red light nearly tripled microshoot production. Stock plant temperature regime, growth retardant treatments, cultivar, and girdling have all been implicated as possible factors influencing subsequent rooting of cuttings (17,37,38).

A greater yield of haploid plants resulted from *in vitro* culture of anthers from tobacco plants grown under short days of high light intensity (9), but Hughes, *et al.* (23) produced high yields of protoplasts from barley plants grown under short days with low light intensities. Begonia leaf cultures were positively affected by long days applied to the stock plants (18), and Paterson and Rost (31) showed that jade plant (*Cras-*

sula argentea) leaves regenerated better if stock plants had been held in the dark or under short days. Cheng and Smith (6) for tobacco tissue cultures, and Read, *et al.* (34) for petunia leaf segment cultures, demonstrated dramatic differences in productivity among different genomes or cultivars.

Although the aforementioned reports and others exist in the literature, early literature on the influence of the stock plant on micropropagation is still somewhat limited. It seemed apparent that an organized approach to stock plant investigations was required, since the stock plant clearly has a profound influence on propagation success. For the past several years we have therefore concentrated our research on the stock plant as a means of modifying in vitro responses and increasing micropropagation success. This specialization may be grouped into four categories:

1. Treatments applied to the intact stock plant.
2. Treatments applied to the detached explant prior to culture.
3. Treatments applied to the tissue while subjected to continuous or repeated culture. This, in essence, treats the tissue as a miniature stock plant, or "microstock".
4. Treatments applied to cut deciduous branches in a "forcing solution".

Intact Stock Plant Treatments. Conventional greenhouse or growth chamber methods (13,15,36) were employed for growing the stock plants and *in vitro* culture techniques were those described by Read *et al.* (36), Gavinlertvatana *et al.* (16), and Economou and Read (13). Nutrition of the stock plant was shown to profoundly influence shoot proliferation in tomatoes (36). However, in *Salix* (15), little difference was seen in *in vitro* shoot production, even though macrocuttings were affected by levels of N, P, and K provided to the same stock plants as those used for in vitro tests. More strikingly different shoot multiplication *in vitro* was observed among the eleven *Salix* clones tested. This was consistent with observations with *Petunia* (34), *Alnus* (35), and azalea (10) in which shoot production varied greatly among cultivars and clones.

Treatment of the stock plant with growth regulating chemicals can also strongly affect shoot and callus formation *in vitro*. Chlormequat sprays applied to tomato stock plants led to greater shoot numbers produced by culture of leaf segments taken from such stock plants (36). This is consistent with results of de Lange and de Bruijne (7). Increases in callus production of *Dahlia* resulted when leaf segments from plants sprayed with daminozide were cultured *in vitro* (16). An inter-

action with stock plant photoperiod was also observed, with short day treatments combining with daminozide to cause greater callus formation and ethylene production in the flask atmosphere. More recently we have found that 2000 ppm daminozide sprays applied to *Petunia* stock plants 8 days prior to leaf segment culture resulted in greater shoot proliferation than for leaf segments from water-sprayed stock plants. Further research with this approach is required, since rates of application and time elapsed after chemical application can modify explant response. As with cuttings, physiological stage can also have an effect on *in vitro* performance, since shoot proliferation is less from leaf explants taken from plants in the flowering stage than those in an actively growing vegetative state (36).

Explant Pre-culture Treatments. A practical method of applying "stock plant" treatments is by utilizing the explant as the subject to be treated rather than the intact stock plant. *Petunia* leaf segments have been treated with cytokinins prior to culture on a cytokinin-free medium, resulting in shoot proliferation similar to that achieved when the cytokinin is incorporated into the medium (12,13,36). Dipping the entire leaf or the leaf segment for 3 minutes in 400 ppm benzyladenine (BA) was most effective. Further research in which *in vitro*-derived microshoots were dipped in $N^6(\Delta^2$-isopentenyl)-adenine (2iP) or N-(2-chloro-4-pyridyl)-N^1-phenylurea (4PU-Cl) caused shoot proliferation similar to that reported with 2iP incorporated in the proliferation medium (10,11). This method of growth regulator treatment offers promise for further gains in micropropagation efficiency. Rates and timing require further study, as well as consideration of this method for utilizing compounds which are expensive or difficult to readily incorporate into the medium. It is also feasible to employ this technique as a means of micronutrient, growth substance, or other chemical pulsing to briefly stimulate the tissue. These approaches are currently under investigation in our laboratory.

Treatments Applied to the Culture Tissue (Microstock). Hughes (22) has reviewed the exogenous factors affecting growth and morphogenesis in plant tissue culture systems, but little emphasis has been placed on treating the culture tissue as a miniature stock plant, or microstock. However, because of the efficiencies of space utilization, study of this approach is deemed useful, since the elimination of large stock plant inventories would be possible and light intensity, photoperiod, light quality, and temperature can be readily manipulated. These factors can often be easily modified to improve micropropagation success, as well as to enable studies of their physiological effects.

Low light intensities (10 or 30 Em^{-2} sec^{-1}) applied to microstock cultures of azalea increased number of cuttings produced *in vitro* and improved their rootability when compared with microstocks cultured under higher light intensities (10,32). A possible reason for the improved root formation of cuttings from low light may be related to the presence of a greater level of root-promoting substances, such as indoleacetic acid (IAA), since IAA is known to be reduced under high light levels (29). Rooting of microcuttings was also promoted when microstocks were cultured for 2 weeks under red (R) light following 2 weeks of far-red (FR) prior to placing the microcuttings in a rooting medium (10,32). Photoperiodic effects have also been studied in our laboratory, but no consistent relationships have been established, in spite of the earlier mentioned reports (9,23,31). Similarly, no distinct temperature influences have been delineated, although like Heide (18), we have seen an apparent interaction of temperature with other culture environmental factors.

Another area of concern in the study of microstocks is the problems inherent in the typical culture environment, particularly as they influence leaf anatomy and subsequent plantlet establishment. Sutter and Langhans (39) and others (5,8) have indicated that epicuticular wax formation is lacking or greatly reduced, and that leaf anatomy and stomatal behavior are abnormal. These factors result in difficulty in acclimating plantlets to ambient conditions of low humidity and high light normally encountered in the greenhouse or field. Methods usually suggested for circumventing this difficulty most often involve gradually reducing relative humidity and providing heavy shade at first to keep light levels low. These can then be gradually increased by sequentially reducing the amount of shading material used. However, our work (33) illustrates that a greatly reduced light level is not requisite for successful direct rooting of microcuttings in a controlled environment rooting facility (CERF) in the greenhouse. The CERF provided high relative humidity, but much higher light levels than in the systems usually employed for direct rooting or acclimation. This higher light intensity probably contributed to the fact that growth rate of hardy deciduous azalea (*Rhododendron* spp.) microcuttings was superior to microcuttings rooted in more conventional low light systems. Also, acclimation to greenhouse conditions was achieved up to 2 weeks faster. Such rapid acclimation is highly desirable and argues for further study to determine optimum combinations of humidity and light for the most acceptable rooting and acclimation of *in vitro*-derived plants.

Forcing Solution Treatments. Research with numerous de-

ciduous tree and shrub species has demonstrated that new softwood growth can be "forced" from buds of quiescent stems (after "dormancy" or cold requirement has been met). Such softwood growth can sometimes be rooted as softwood cuttings, but it is more frequently an excellent source of clean explant material for tissue culture use. The optimum forcing solution for chestnut (*Castanea* spp.) and several other woody species is similar to those used as floral preservatives, i.e. it contains 200 ppm 8-hydroxyquinoline citrate and 2% sucrose. We have subsequently demonstrated that such forcing solutions can be effectively used as "delivery systems" for plant growth regulating chemicals such as gibberellins and cytokinins. GA_3 applied in this fashion has been used to stimulate extension or elongation of the softwood shoots, although it may later lead to retardation of *in vitro* development. Conversely, cytokinins such as benzyladenine can be metered into the tissues via the "forcing solution" and subsequently may increase shoot production *in vitro*.

DISCUSSION/FUTURE RESEARCH

Changes in endogenous hormone levels resulting from stock plant treatments need to be further documented. Limited studies with leaf segment culture of *Petunia* stock plant treatments of R and FR light from germination to explant excision have indicated a correlation between endogenous hormone levels and such light treatments (36). It is therefore logical that further investigation of endogenous hormonal changes resulting from stock plant treatments is required. Repeated culture of azaleas *in vitro* has led to a state of "habituation" with improved microshoot production and rooting (11), but recent work with *Alnus* has shown opposite results if cytokinin is discontinued as a culture medium constituent (28). In addition, recent work with leatherleaf fern (*Rumohra adiantiformis*) suggests that anatomical studies may lead to a better understanding of *in vitro* responses to stock plant treatments. From these initial studies, it seems clear that stock plants offer a convenient vehicle to expand our knowledge and effectiveness in propagation, as well as facilitating more effective physiological research.

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