Stock Plant Etiolation

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The discovery and use of auxins in rooting and the development of mist propagation are unequivocal milestones in the history of propagation due to their broad applicability and effectiveness. Stock plant etiolation may prove to be in a similar class with these techniques. The practical use of stock plant etiolation to improve rooting in cuttings has largely been spurred on in the past 10 years by the successes achieved by Howard and others at the East Malling Research Station, United Kingdom (9). For all the renewed interest, however, the practice of withholding light to improve propagation is probably an ancient one, having been employed every time a stool bed or layer was made or even a cutting inserted into opaque media.

What constitutes stock plant etiolation in propagation? Etiolation simply is the growing of plants in the absence of light. However, as the term has come to be used among propagators, it also refers to growing plants in heavy shade. Stock plant etiolation generally refers to the initiation of new stock plant growth in the dark. Typically, a dormant stock plant is covered by a light-tight barrier and the new growth is made in darkness. Shading is gradually reduced after the shoots reach ≈5–10 cm in length and the shoot allowed to turn green. An opaque adhesive band often is wrapped around the base of the new shoot (the future cutting base), at the time of shade removal, thereby retaining its etiolated condition while the rest of the shoot turns green in the light. The shoot is removed just below the banded section after several weeks of regreening. The band is removed and the cutting inserted into the rooting bench in the normal manner (12). A related practice called blanching refers to light-grown plants that subsequently are shaded. With this treatment, the shoot accomplishes its initial growth in the light and then has a portion of its stem banded with an opaque material such as black adhesive tape for several weeks before the cutting is made. Shading, which simply refers to any stock-plant growth under reduced light conditions, has also been used as a successful propagation pretreatment in a number of genera. A recent review has revealed more than 28 genera that have successfully responded to either etiolation, shading, or blanching as pretreatments to cutting propagation (13). Despite the often dramatic successes of these various techniques, the mechanism whereby etiolation works is still poorly understood, although several researchers have gathered an impressive amount of correlative and circumstantial evidence.

In 1922, Reid (15) successfully rooted cuttings of *Camphora* after stock plant etiolation. She also undertook the first anatomical study of etiolated tissues in the rooting zone and reported findings that were to be repeated many times in the future. Etiolated stem tissue was less lignified than light-grown tissue, had decreased cell wall thickness, and increased protoplasmic content in the cells. She showed that lignification was a developmental process that lagged behind tip growth. Etiolation delayed lignification so that etiolated shoots were lignified to the sixth node while light-grown shoots were lignified to the third node. This decrease in lignification is not caused by stem growth, which causes gaps in normal tissue. Thus, the anatomical explanation of etiolation's effect began with the idea that a reduction in the mechanical properties of the stem were responsible for the ease of rooting caused by stock plant etiolation. These findings also have been reported by others (5, 8).

Gardner in 1936 (6) was the first to use black adhesive tape to blanch 'McIntosh' apple stems, which led to high rooting levels in this difficult-to-root species. He also reported the additive effect of girdling along with etiolation and the observation that blanched shoots contained more undifferentiated tissues, perhaps leading to easier root initiation. He hypothesized that the girdle was increasing some translocated substance in the etiolated zone, which worked with the anatomical changes to increase the ease of rooting.

Not until the 1960s did work on etiolation become popular again. Frolich's work in 1961 (5) with etiolated avocado shoots showed that roots were produced only in that part of the stem that had been etiolated. Nonetiolated sections above and below that section produced no roots. Again, he cited localized anatomical changes in the stem tissue as the likely reason of this effect, although localized biochemical changes were not ruled out. He also showed that there was a continuum between the total duration of light at a given intensity and the reduction in rooting; the longer a mung bean's exposure to light of any spectral quality the poorer was its rooting. This was the first evidence that total darkness was not necessary to achieve some level of increased rooting and that the promotional effect was correlated with light intensity.

The work reported by Herman and Hess in 1963 (8) presented both anatomical and biochemical evidence for the etiolation effect. Using *Phaseolus vulgaris* L. and *Hibiscus rosa-sinensis* L., they showed that etiolation greatly increased a tissue's sensitivity to exogenously applied auxin; however; they also found that endogenous auxin was in no greater supply in etiolated than in light-grown tissue. Anatomically, they cited a reduction in the mechanical strengthening tissues, decreased lignification, and reduced cell differentiation (among other observations) as enhancing potential root primordia formation. In some less-convincing bioassay work, they proposed that there was increased auxin cofactor content in etiolated stems, which increased the efficacy of applied auxin. However, the crude extraction procedure and lack of dilution series made this conclusion doubtful.

Konishi and Galston (11) showed that phenolic inhibitors of 1*H*-indole-3-acetic acid (IAA) oxidase in *Ipomaea purpurea* Lam. were more abundant in etiolated tissues; however, there was no correlation with rooting carried out with this work. The etiolation-induced IAA oxidase inhibition hypothesis is an attractive one, although it assumes that the actual limiting factor in root initiation is auxin. It has been shown, however, that even high exogenously applied auxin levels cannot compensate for the poor rootability of many difficult-to-root species, whereas etiolation can (12). Moreover, Gorter (7) showed that cofactors of auxin oxidase also can act as root promoters.

Delargy and Wright (3) produced compelling evidence that a substance produced by apple leaves in the light was being translocated proximally to an etiolated zone, where it enhanced the etiolation effect. Ringbarking proximally to an etiolated zone enhanced rooting, whereas ringbarking distal to the etiolated zone nullified the enhanced effect. In further work (4) they showed that, as the length of etiolated stem increased from 2.5 to 5.0 cm, rooting percentage increased, implying that there was an additive effect of the "etiolation factors" on a plant's capacity to root. However, this effect may have been due to increased area for root primordia formation. They also showed that etiolation and taping of apple shoots were not enhanced by adding auxin but rooted at high percentages (70%) regardless. Blanching of these shoots was about half as effective as etiolation.

Work by Kawase and Matsui (10) in 1980 showed that disbudding of the terminal nullified the effect of etiolation in *Phaseolus vulgaris*, although the presence of a bud could be substituted for by the addition of 30% 1*H*-indole-3-butyric acid (IBA) in lanolin on the wound surface. Additionally, they have shown the only evidence of the possible transmissibility of a factor in etiolated tissue when they observed that by increasing the number of bands on *Phaseolus* spaced 0.5 cm apart on the stem, they found the larger number of primordia in the more proximal segments.

Recent work by Al Barazi and Schwabe (1) with *Pistacia vera* L. showed that IAA oxidase activity was not enhanced by etiolation, whereas polyphenol oxidase (PPO) was increased by 100%. PPO has been implicated as a factor causing the increased rooting of apple cuttings (2).

This sampling of papers on the mechanism of etiolation points out the paucity of information in the field, although some generalizations can be made. Etiolation greatly enhances a stem's sensitivity to auxin. Translocated factors produced distal to an etiolated segment also enhance the etiolation effect. Etiolation induces anatomical changes in stem tissue that may increase initiation of root primordia, primarily due to undifferentiated parenchymatous cells and lack of mechanical barriers. Etiolation has also been associated with changes in phenolic substances that may act as auxin cofactors or inhibitors of IAA oxidase, but, of all the work done on etiolation, this is the least substantiated.

It remains to creatively use the inherent capability to manipulate the etiolation system to narrow some of the confusions that exist. For instance, can etiolated stem tissue be sequentially extracted over its period of regreening and the subsequent loss of rooting capability in the etiolated tissue correlated with the loss of a chemical root promoter tested on a bioassay? Conversely, can green tissue be extracted as it is sequentially blanched to see the gradual appearance of a root promoter or other basic metabolite in a bioassay that would correlate with increased rooting in the blanched tissue?

These and many other questions need to be answered and up-to-date techniques used if we are to make further progress in elucidating the mechanism of etiolation's effectiveness. At the same time, further work continues to be done to refine the practicality of the technique itself (9, 12, 14) so that its acknowledged effectiveness can be made useful to propagators with difficult-to-root plant materials.

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