A perspective on the importance of managing juvenility in plants: focus on plant improvement and propagation©

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INTRODUCTION

Although I took the opportunity to officially retire from my professorship in horticulture at a major Midwest university, on-going research projects along with increased participation in the projects at Knight Hollow Nursery, Inc. continued to involve me in research activities encompassing both propagation and plant genetic improvement. These activities have also involved discussions with growers and researchers about how to accomplish various goals. In explaining my ideas, I soon became aware that the concept of managing the juvenile/adult phase of development in crops was often not well understood nor its importance well appreciated. Occasionally I ended up taking time to explain my perspective on developmental change in plants and how this would be a major part of the particular project we were discussing. One result of all this "retirement" activity was to include plant juvenility in progress talks I was asked to present. My discussion here today at IPPS is a continuance of this theme.

I am very aware that most of the IPPS audience is thoroughly aware of the importance of managing plant juvenility, so I have skipped over most of the basics. But I do hope that this discussion and the practical example that I highlight will re-emphasize the importance of keeping aware of how plant development influences our everyday progress.

SOME GENERAL THOUGHTS ABOUT JUVENILITY

The concept of juvenility in plants can be quite difficult to discuss since we really do not have a thorough understanding of this part of plant development. For example, if I were given two sections of a plant stem, could I tell which one was more juvenile than the other? Basically the answer is no as we have yet no clear "markers" that I can analyze that will clearly define the juvenile state of these stem pieces. There is ongoing research involving gene expression that hopefully may be able to give us such tools. But for our discussion, what this deficit means is that we are left with circumstantial observations based on plant responses. For example, one of the most reliable markers for juvenile tissue is that it possesses the highest capacity to regenerate missing parts (such as adventitious roots and buds). For the adult phase of development, slower vegetative growth accompanied by the capacity to flower is usually a readily apparent visual marker.

One question I often ask audiences while showing them a flowering potted plant is 'Where is the most juvenile part of this plant?" Intriguingly, this may seem like a simple question, but actually it can be quite complex, again because we do not have clear biological markers. After some thought, three answers are appropriate: the reproductive cells, the roots/rhizomes, and the plant collar. The embryo in seed development can be considered the most juvenile part of a plant; interestingly the seeds form in the most "adult" tissues of a plant (as defined by capacity to flower) and intriguingly undergo complete "rejuvenation." The roots generally do not have a capacity to flower (thus roots are never adult??) and do often retain regenerative capacity (such as root cuttings generating adventitious shoots and adventitious axillary roots). I have always thought that one of the most fascinating morphological parts of a plant is the collar region at the juncture of the root and shoot system. For a plant that developed from a seedling, the collar originated in the highly juvenile embryo and this juvenile trait seems to remain in the collar region throughout the

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life of the plant. The most juvenile shoots of a tree often come from the collar as basal suckers. In the rooting of a stem cutting, maybe all we are doing is in part regenerating a collar region?

EXAMPLE OF THE NEED TO CONTROL JUVENILITY TO ESTABLISH A PROPAGATION PROTOCOL

One of the major projects with which I am now associated involves developing an upper Midwest industry based on growing, processing and marketing American hazelnut, *Corylus americana*. American hazelnut is a native shrub with a center of genetic diversity in the Midwest. Demand for hazelnuts as a component in numerous edible products is high. The combination of these two facts along with a diverse interest of a group of researchers and growers has resulted in the formation of a consortium to develop a new industry (Upper Midwest Hazelnut Development Initiative, http://midwesthazelnuts.org/description.html). All aspects of creating a new industry are being investigated, including sampling and screening native germplasm, perfecting farm management and harvesting protocols, nut processing, and market development. However, one of the major hurdles is not having a commercially reliable clonal propagation protocol. Considerable trials investigating the use of stem cuttings and layerage to clone selections from the wild has been done by cooperators at the University of Minnesota but these efforts so far have not shown a clear route useful at the commercial level. Thus the two universities and KHN were asked to investigate if micropropagation might meet this need. Please note that this work in not complete yet so the observations I present here are just preliminary.

To have a practical micropropagation protocol, at least four stages must be met: isolation of tissue in a sterile environment, stabilization of tissue for growth in microculture, production of high quality microshoots that will provide microcuttings, and microcutting rooting and acclimation to greenhouse environments.

For isolation, the first source of plants was field plantings of native swarms that had been selected over several years of observation for nut productivity. Note in Figure 1 that these plants were showing flower buds and thus most of the shoots were adult. Even non-flowering shoots from these plants were difficult to sterilize and did not perform in microculture (Figure 2). Thus several approaches at rejuvenation were attempted. Divisions of field plants were taken and grown in pots in a greenhouse. Tissue samples taken from growth of flowering shoots again were not successful in microculture. However, when suckers from rhizomes or the collar region (Figure 3) were sampled, more successful sterilization and establishment in microculture was achieved (Figures 2 and 4). More than 50% of the 18 clones selected for propagation were successfully isolated in microcultrure.

Figure 1. An American hazelnut plant in a field of individual selected plants that were obtained from wild swarms for their general productivity. Note the shrub growth form and the flowering of the two year old stems.

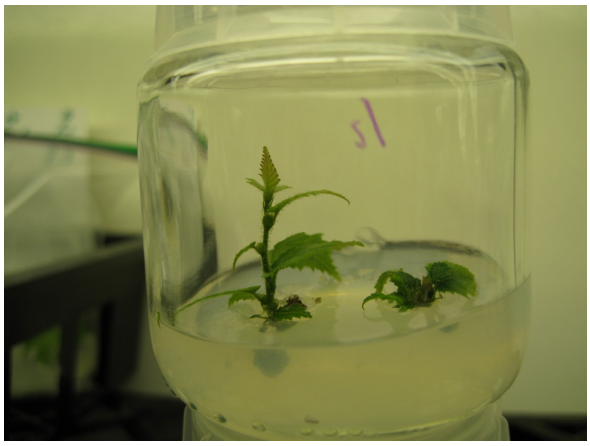

Figure 2. New stem pieces isolated from a highly adult hazelnut stock plant (right) and a non-flowering (juvenile) stock plant sucker.

Figure 3. A division of a field American hazelnut plant potted and growing in a greenhouse. Shoot on the right is from a one year old stem and will set flowers in the fall; shoot on the left is a non-flowering (juvenile) shoot originating from a rhizome or collar bud.

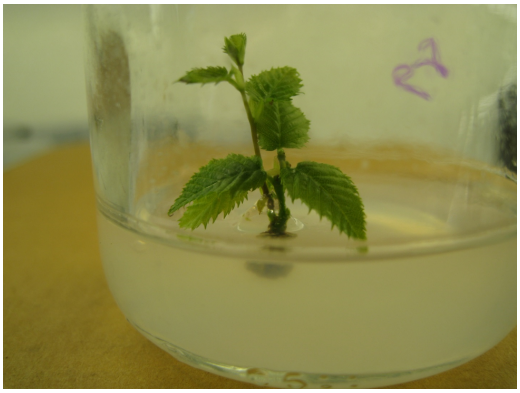

Figure 4. A subcultured microshoot where the original shoot (right) has stopped growing and a new, more vigorous and continuously growing (juvenile?) shoot has emerged.

Continued and more vigorous microshoot growth of tissue was successful in establishing (stabilizing) growing shoot cultures (Figure 5). During 3 to 6 months of subculturing growing microshoots, the emergence of basal shoots (Figure 4) was often evident; such shoots continued more active growth on subsequent subcultures than did the subcultures of the original shoot from which they emerged. This vigorous microshoot growth from the base of established shoots visually resembled the emergence and growth of shoot suckers with greenhouse grown stock plants. With 8 clones, microshoots suitable for use as microcuttings (Figure 5) were obtained.

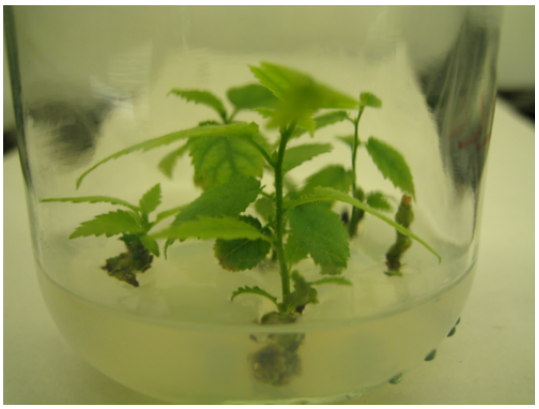

Figure 5. A stabilized shoot culture of an American hazelnut selection. The larger shoots are appropriate for use as a microcutting.

For rooting/acclimation, 1-month-old microshoots were harvested from the shoot cultures, and treated with water-soluble IBA (1000 ppm) dips before sticking in soilless mix. Microcuttings were exposed to 18 h of fluorescent lighting in 1020 flats covered with clear plastic domes. Rooting and or callusing was evident as new leaf regrowth became apparent. Such cuttings were acclimated by slowly removing the plastic dome under the rooting environment. Surviving microplants were potted and moved to the greenhouse.

Unfortunately, the losses incurred during rooting, acclimation, and greenhouse culture were high, with less than 20% of the original microcuttings surviving as rapidly-growing liners. Although roots often formed on 30-50% of the microcuttings, this was usually associated with prominent callusing (Figure 6). When such microplants were moved to more stressful environments (greenhouse), over 90% of the microplants stopped growing and gradually succumbed.

Figure 6. A newly rooted American hazelnut cutting showing significant callus ball at base.

With the general lack of success in both microcutting and the earlier stem cutting trials, at this point we became curious about the innate capability of American hazelnut cuttings to regenerate adventitious roots. To explore this question, we grew seedlings of selected swarms of wild hazelnuts and harvested softwood stem cuttings. After treating with 1000 ppm of soluble IBA, 50 to 100% of the cuttings rooted and the resulting potted plants continued vigorous growth (Figure 7). Similar cuttings taken from more established stock plants in the greenhouse were largely unsuccessful and usually only produced massive callus balls at the base of the stem. Interestingly, similar softwood cuttings taken from rapidly growing plants originating from micropropagation also showed a high capacity to root and successfully acclimate (Figure 8).

Figure 7. Cuttings of American hazelnut during acclimation and early growth in a greenhouse. Cuttings on right are from young seedling stock plants and on left from microcuttings. Note the non-uniformity and deterioration of many of the microcutting-generated plants.

Figure 8. Young plants of American hazelnut from two different sources. Right is a microcutting and left is a cutting from a microcutting-generated stock plant similar to the one on the left. Size of plants is just an indication of differing ages.

DISCUSSION AND CONCLUSION

These early attempts to propagate American hazelnut native germplasm selections using cuttings were frustrating but enlightening:

- Working only with juvenile tissues was critical. Seedling cuttings demonstrated a high capability to root that was not evident in softwood cuttings from more adult

stock plants.

- Establishment of successful microshoots in culture only reliably occurred using source tissues from juvenile growth (such as suckers).
- As has been observed with other micropropagation protocols, continued subculturing of actively growing shoots in microculture seems to lead to further rejuvenation which has been hypothesized as a major part of the "stabilization" phase of establishing a micropropagation protocol.

So where do we go from here with the cloning of American hazelnut via micropropagation? One approach that is being explored is the combination of micropropagation and stem cutting propagation. Although micropropagation has so far not proven commercially successful, it does generate useful and apparently highly juvenile stock plants from which stem cuttings with a high capacity to root can be obtained. Our approach may be to annually generate juvenile stock plants via micropropagation and use these to produce multiple generations of softwood cuttings (Figure 7).

SUMMARY

With our initial trials of generating a cloning technology for American hazelnut germplasm, the general recalcitrance of this species to regenerate roots was evident. The importance of maintaining a juvenile state of the stock used for either cuttings or micropropagation seems critical. Fortunately maintenance of the juvenile state by use of micropropagated stock plants offers an approach to overcome this limitation to the development of this industry.