MICROPROPAGATION POTENTIAL FOR THE AMERICAN HAZELNUT

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August 14th, 2012

One of the major challenges in the development of the American hazelnut as a new crop is the difficulty in clonally propagating selected individuals. In most fruit and nut crops, selected individuals are grafted to produce new plants that are genetically identical. This is not feasible with the American hazelnut because grafting is difficult and the seedling rootstocks produce new stems that will bypass the desired individual anyway. Rooted cuttings can be utilized in many woody species, but trials with the American hazelnut have been variable, usually with a low percentage rooting and poor survival of those cuttings that do root (Fig. 1).

Fig. 1. One rooting test with American hazelnut that yielded only 10% satisfactory rooting.

Micropropagation is the multiplication and recovery of whole plants using an in vitro, laboratory-based system. Micropropagation has been used to facilitate the propagation of many recalcitrant plants, and in the program of Professor Brent McCown at the Horticulture Department of the University of Wisconsin - Madison, we have been working with this type of woody plant biotechnology for over thirty years. As participants in the Upper Midwest Hazelnut Development Initiative, we are applying this expertise to the micropropagation of the American hazelnut.

Why do micropropagation? There are many advantages and very few drawbacks to micropropagation. With micropropagation we can:

- Propagate anytime, independent of weather, climate and location.
- Propagate much faster, achieving exponential increase once cultures are established.
- Propagate free of pests and diseases.
- Produce extremely uniform plants.
- Produce plants that grow and establish faster than those obtained from conventional cuttings.

However, micropropagation is not always successful and it can be quite expensive due to the costs of specialized equipment, supplies and personnel required. Research is necessary to overcome these difficulties.

Our specific goals in micropropagation research with the American hazelnut at this point in time are: 1) to perform clonal propagation to allow horticultural research (mulching, fertilizing, pruning, etc.) uncompromised by the genetic variation present with seedling plants, and 2) to facilitate the selection and development of elite individuals for enhanced yield and ease-of-culture through replicated clonal trials. This article describes in text and pictures our initial efforts towards micropropagating the American hazelnut, from selected parent plants to hundreds of genetically identical, field-ready plants.

The process started with the selection of high nut producing plants from the wild, performed by cooperators Jason Fischbach in Northern Wisconsin and Michael Demchik in Central Wisconsin. Collar (basal, underground stem) divisions were dug from selected plants and established in the greenhouse. These were cut back to force new growth from the juvenile, underground collar region (Fig. 2). Because bacteria and fungi grow so fast and are undesirable anyway, micropropagation requires sterilized culture vessels and medium, as well as a sterile transfer cabinet and sterilizable tools to handle the tissue (Fig. 3). In addition, the starting plant material must be isolated from contaminating microorganisms using dilute bleach or similar chemicals to surface sterilize the tissue. Successful isolates (stem pieces with no contaminates) developed new growth from lateral buds and when tall enough, the tips were cut and transferred to fresh medium (Fig. 4). The bases were also transferred, yielding new stems; repetition of this process resulted in rapid multiplication (Fig. 5). So far the process has worked well, but we have not yet optimized the multiplication phase as we considered it more important to test rooting, plant development and field performance first. However, optimization is important for the improvement of micropropagation efficiency and thus final per plant cost.

Once enough microcuttings were available, rooting was tested in the lab and found to be far more successful to that of conventional cuttings (Fig. 6). The quality of roots produced was excellent and new growth on the resulting rapidly-growing plantlets was readily acclimated to ambient conditions by gradual exposure to open air (Fig. 7). Subsequently, a larger set of cuttings were rooted, acclimated and established in the greenhouse. These were potted into 2 5/8 x 2 5/8 x 5 inch tree bands and after further growth were acclimated to outside conditions, initially under shade cloth (Fig. 8). These plants had excellent root mass and over time the buds on the lower stems began to swell (Fig. 9). This was significant as we wanted to maintain viable vegetative buds that would be buried with subsequent re-potting. The idea was to produce an underground vegetative axis for the establishment of a collar-like region similar to seedlings. This is important for the later production of new stems which may be critical for nut productivity over time. Therefore, a large part of the lower stems were buried when the plants were re-potted into 4 x 4 x 10 inch tree bands. The larger tree bands and heavy fertilization resulted in extremely uniform, rapidly growing plants (Figs. 10 and 11). Currently the plants are very vigorous and are quickly approaching a size suitable for field planting (Fig. 12). These plants will be used for field-establishment trials using fall plantings of dormant plants and spring plantings of actively growing plants.

The plants have started to branch and it is encouraging to see branching from buried buds (Fig. 13) for the reasons stated above. We have produced a sufficient number of plants to also start to address goal 1 listed above: in 2013 we will be begin to examine the effects of different levels of mulch and different levels of fertilizer on plant establishment and growth after planting.

We have started to work on the second stated goal: we now have several selections in vitro that should be ready for replicated performance trials in 2013 or 2014. We are certain that micropropagation will play a vital role in the future improvement of the American hazelnut for a new orchard crop for the Upper Midwest. Eventually other kinds of propagation using micropropagated plants as stock plants may prove to be less expensive and research aimed at such secondary propagation is planned.

The pictures below offer a clear view of the process and the results we have obtained so far. If there are any comments or questions regarding micropropagation of the American hazelnut, please contact Eric Zeldin by email at elzeldin@wisc.edu.

Fig. 2. Micropropagation starts with a high nut producing selection from the wild established in the greenhouse. Pruning of the stems removes apical dominance and new, more juvenile stems erupt from the underground collar region (right picture). Greenhouse stems are cleaner than those from the field (fewer bacteria and fungal spores) and juvenile tissue usually yields the bests results for micropropagation.

Fig. 3. A sterile transfer cabinet used to perform sterile transfers. Stems from hazelnuts cultures (left in picture) will be cut and placed on new media (center) using surgical steel quality sterilized tools (right).

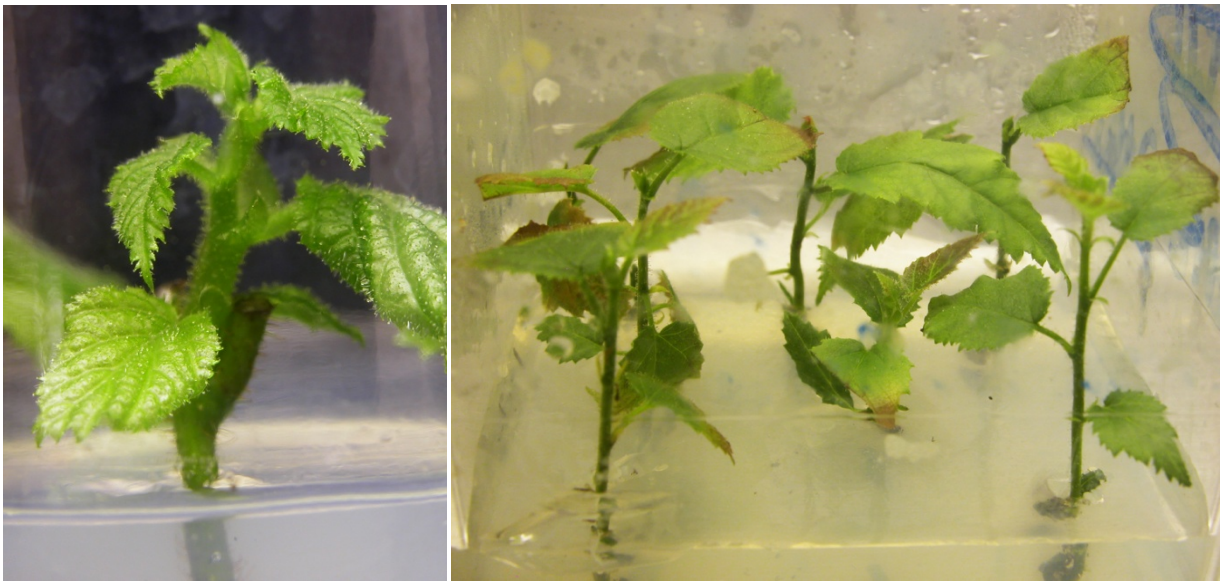

Fig. 4. A hazelnut isolate exhibiting new growth (left picture) and a group of new stems produced in vitro (right picture).

Fig. 5. Hazelnut cultures in the culture room. The first three trays (left picture) contain about 500 stems genetically identical to the source plant they were originally isolated from. A closer view (right picture) shows a culture with microstems ready for cutting and rooting. Further optimization of the multiplication phase will result in faster and cheaper multiplication.

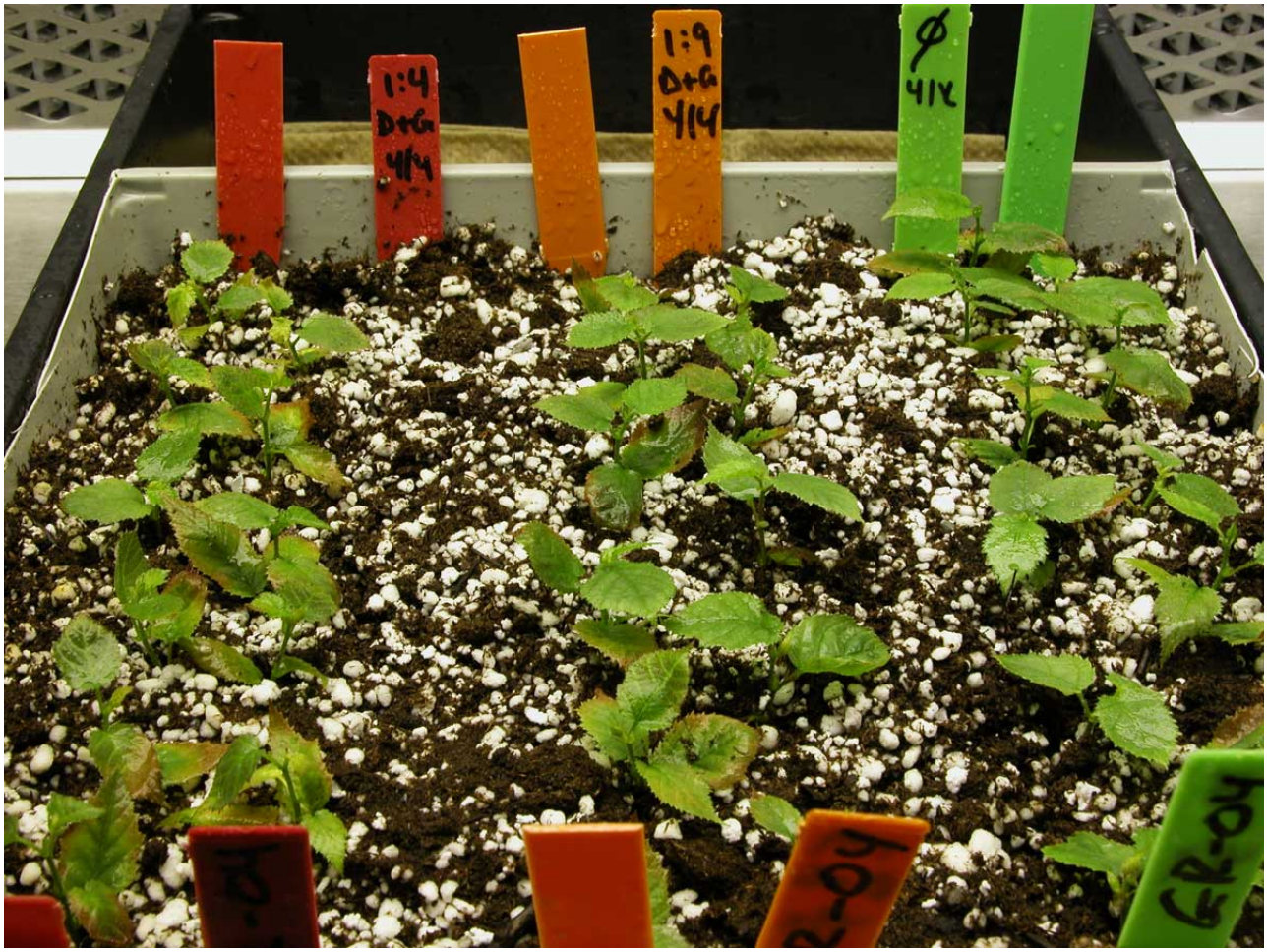

Fig. 6. Microcutting rooting trial. Two to three inch microcuttings were dipped in rooting hormone and placed in propagation mix (peat/perlite) in a 1020 tray covered with a moisture retaining plastic dome. Greater than 95% rooting has been achieved using microcuttings and rooting hormone treatments.

Fig. 7. Rooted microcutting with roots exposed (left picture) and young, greenhouse acclimated plants derived from microcuttings (right picture). Good quality rooting is essential for the tissue to acclimate from protected, in vitro culture to exposed ambient conditions.

Fig. 8. Greenhouse acclimated plantlets were repotted into 2 5/8 x 2 5/8 x 5 inch tree bands using commercial grower's mix and subsequently acclimated to outside conditions in a coldframe.

Fig. 9. A plant from Fig. 8 with the tree band removed to show root development (left picture) and a detail of the lower stem of another plant to show lateral bud swelling (right picture). These buds will be buried after re-potting.

Fig. 10. Re-potting of micropropagated plants to larger, 4 x 4 x 10 inch tree bands (left picture) and a top view of the re-potted plants showing a high degree of uniformity (right picture).

Fig. 11. A side view of the micropopagated plants in large tree bands after further growth.

Fig. 12. A single plant from the picture in Fig. 11, demonstrates the strong, vigorous growth achieved to date, with another month or so before dormancy sets in. These micropropagated American hazelnut plants will be tested both for fall 2012 (dormant) and spring 2013 (active growth) planting to determine which planting time yields the best field establishment.

Fig. 13. Many of the micropropagated plants are beginning to branch at this time (left picture) and many of these branches are coming from buried nodes (right picture). The development of new stems from buried nodes may be a critical factor for micropropagated American hazelnuts, as the re-establishment of a collar-like region may be required for continual production of new stems and nut producing capability.