Micropropagation of Walnut Rootstocks©

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INTRODUCTION

Walnut is a popular crop with 5 million trees annually planted in California. Paradox walnut [*Juglans hindsii* × *J. regia* (Northern California black walnut × English walnut)] is a popular rootstock due to its high vigor. Nurseries often grow Northern California black walnut and a pollinating English cultivar side-by-side to produce paradox walnut. One disadvantage of this un-controlled open pollination is that its success varies from year to year and in some years the success rate is as low as 25%. This leads to a shortage of paradox walnut trees for walnut growers. Another disadvantage is genetic variability from one paradox walnut seedling to another that leads to tree-to-tree differences (disease resistance, vigor, etc.) in the orchard.

Clonal propagation can solve the aforementioned disadvantages of paradox walnut seedlings. However, a major hurdle in adopting clonal propagation for walnuts is that paradox walnut cuttings are much more difficult to root than rootstocks of other fruit and nut crops. The success rate of rooting of hardwood walnut cuttings has been so low that nurseries have not adopted this practice. On the other hand, success in rooting cuttings grown by tissue culture has dramatically increased and is likely to revolutionize the availability of rootstocks that were never available before.

MICROPROPAGATION

New Rootstocks for Micropropagation. University of California, Davis has recently released two new paradox walnut rootstocks, RX-1 and VX211. Rootstock RX-1 provides resistance to *Phytophthora* and VX-211 provides resistance to nematodes. As one can imagine, this genetic resistance is good for the life of the tree in the orchard and provides protection even if the pathogens get introduced in the orchard at a later stage. Rootstock breeding is an active program at UC Davis and a release of more desirable rootstocks are expected. These rootstocks have been released to some laboratories that specialize in micropropagation. Accordingly, the use of clonal rootstock is expected to increase and meet the demand of the California's planting needs.

Micropropagation at Micro Paradox Inc. Although, in principal, walnut micropropagation is similar to other crops, it requires more and longer tissue culture stages, larger facilities, and more tissue culture staff to produce the same number of plants than for other species. The following is a brief explanation of the various stages involved.

Establishment. Establishment of new varieties in culture is difficult for walnuts due to the presence of endogenous bacteria and phenolic substances in the tissue that interfere with culture establishment. Nodal sections from actively growing shoots are surface-sterilized with 1% sodium hypochlorite and planted on DKW medium (Driver and Kuniyuki, 1984) with ½X salts. The tissue is frequently (daily) transferred to new media until no browning of media due to phenolic exudates is detected. The culture is then transferred to full-strength DKW medium and considered adapted for micropropagation after 3 subcultures.

Multiplication. This phase is relatively easy but a very important phase for the success of the project. Growing healthy cuttings insures a balanced root system later. Nodal sections of cuttings are cultured on a DKW multiplication medium. The cultures are maintained in the light for 12 h at 72–75 °F followed by 12 h in the dark at 75 °F. Cultures are visually examined for any contamination and clean cultures are sub-cultured every 3 weeks. On average, a 3X multiplication rate is achieved. Starting with 100 cuttings, 1.4 million cuttings can be produced in 6 months (Table 1).

Table 1. Number of cuttings that can be produced from a starting number of 10 and 100 cuttings (multiplication rate of 3X in 21 days).

Day	Start	21 days
Day 1	10	100
Month 1	49	490
Month 2	240	2,401
Month 3	1,176	11,765
Month 4	5,765	57,648
Month 5	28,248	282,475
Month 6	138,413	1,384,129

Root Induction and Rooting. The cuttings from the multiplication phase are harvested and planted on a DKW root-induction medium. This induction phase is conducted in the dark for most taxa. The cuttings are then transferred to a rooting medium to develop roots.

Greenhouse Acclimatization. The rooted cuttings are transplanted in Anderson pots containing Sunshine mix #4 potting mix. High humidity (>95%) is maintained. The humidity is then reduced to 80% for approximately 1 week and then to normal greenhouse environment. After 2 weeks, plants are moved to a shade house for hardening. During the entire growing process in the greenhouse and shade house, air pruning of the root system is promoted by open-bottom pot design and open-mesh bench design.

Grading and Sorting. Usually, plant growth and height is variable during acclimatization. Plants with bigger leaves restrict growth of smaller plants by shading. Therefore, we frequently grade plants and sort them into two categories: big and small. The smaller plants are then moved to another area to allow their optimal development.

Identity Tracking by DNA Fingerprinting. Since plants go through a lot of handling during various micropropagation phases there is a possibility of mislabeling. Therefore, we test leaf samples to confirm their identity by DNA fingerprinting in our on-site genetic laboratory.

Superior Root Architecture. A key feature of our plants is their superior root architecture. Our plants have 5–8 roots starting from the base of the cutting that span out at a 45° angle (Fig. 1). This balanced root system provides better anchoring upon planting in the soil and the trees do not get blown over by the wind. Roots of our plants do not show defects such as curling and circling due to our practice of air pruning.

Field Experience. During the last 2 years, we have sold 200,000 trees to various nurseries in California. Feedback from our clients indicates that the micropropagated trees are very uniform, show the promised vigor, and have high stands (Fig. 2). All major nurseries agree that dormant micro-trees from our micropropagation program, when planted in February, are vigorous enough to be easily June-budded. This is preferred by nurseries as their production cycle is reduced from 2 years to 1 year resulting in reduced growing costs. We are further partnering with nurseries to study if

Figure 1. Root structure of dormant trees produced by micropropagation.

Figure 2. Growth of micropropagated VX-211 rootstock at Burchell Nursery near Oakdale, California.

September planting of actively growing trees can enhance their root development in winter before dormancy and result in stronger plants for June budding.

LITERATURE CITED

Driver, J., and **A.H. Kuniyuki.** 1984. In vitro propagation of Paradox walnut rootstock. HortScience 19:507–509.

QUESTIONS AND ANSWERS

Mike Bone: What material are you using for explants?

Parm Randhawa: We used clean and sterile cultures we obtained from UC Davis.

Mike Bone: How are you sterilizing your starting material?

Parm Randhawa: We use a soapy water wash followed by bleach when we start cultures from field-grown material.