CLONAL PROPAGATION OF WALNUT ROOTSTOCK GENOTYPES FOR GENETIC IMPROVEMENT 2006

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ABSTRACT

We continued to clonally propagate candidate pest and disease resistant or tolerant genotypes for greenhouse screening with liner sized plantlets and for field trials with bareroot nursery row grown trees. We produced over 2700 liner sized plantlets of 27 genotypes for greenhouse screens and growing in the nursery row to a size large enough for grafting and use in orchard trials. We also produced over 1300 plantlets of 48 lines transformed for resistance to crown gall for 1) greenhouse test for susceptibility to gall formation 2) translocation of macromolecules across the graft union 3) a small field plot for testing horticultural characteristics. These plants were clonally propagated from tissue culture derived microshoots. As a result of optimizing an *ex vitro* method of rooting microshoots in greenhouse fog chambers, rooting percent of microshoots was improved to 55% and survival of rooted microshoots was improved to 82%.This is now a very efficient propagation system where rooted plantlets grow faster in the greenhouse and are ready for dormancy induction sooner. We devised a root bench grafting system which can be used to produce a nut variety grafted nursery tree in one growing season. It provides temperature control at the graft union and at the base of the root so a grafted plantlet can be produced in three weeks. An orchard trial was established using nursery trees with Chandler grafted onto *Phytophthora* resistant, clonally propagated AZ2 and RX1 genotypes. RX1 transplanted successfully at a very high percentage and AZ2 transplanted very poorly with a 90% failure rate. Propagating material of the most promising rootstock genotypes has been distributed to several nurseries so they can begin they process of commercialization and orchard testing.

GOAL AND OBJECTIVES

The goal of this project is to provide the California walnut industry with new clonal rootstocks selected or designed to combat the most threatening pests and diseases. The overall objective is to devise clonal methods of propagation for candidate genotypes and provide clonal plantlets so that they can be evaluated in greenhouse and field replicated disease and pest challenge tests.

PROCEDURES AND RESULTS

Propagation

We have continued to use three approaches to clonally propagation candidate rootstock genotypes with nematode, crown gall, *Phytophthora*, or blackline tolerance or re sistance:

A. Tissue culture micropropagation with *ex vitro* rooting of microshoots.
B. Dormant hardwood cuttings on bottom heated beds.
C. Bench grafted root cuttings during the dormant season.

Tissue culture micropropagation: This approach continued to have the greatest emphasis and effort during the past year because it is the fastest and most reliable method of clonal propagation at this time. During the past year we have produced over 2700 plantlets of 27 genotypes for replicated disease and pest greenhouse screening tests and for growing on in the nursery row to a size large enough for grafting and use in orchard trials (Table 1). In addition we produced over 1300 plantlets of 48 lines transformed for putative resistance to crown gall (and non-transformed control lines) for use 1) in greenhouse screening tests for gall formation, 2) translocation experiments to test for movement of macromolecules from the rootstock across the graft union to the scion and 3) a small field trial to determine horticultural characteristics (Table 2). All of the tissue culture derived microshoots are rooted *ex vitro* because *ex vitro* rooted plantlets survive better and grow more vigorously than *in vitro* rooted plantlets. For *ex vitro* rooting, microshoots are treated with a rooting hormone in the laboratory but are stuck *ex vitro* in fog chambers in the greenhouse. This year we have optimized the *ex vitro* rooting process by performing experiments to compare the effects of various parameters on rooting percentage. These experiments showed that treatment with 10mg/l of the rooting hormone potassium indolebutyric acid (KIBA) for four days in the lab gives optimal rooting numbers and rooting percentage. Overall, for the many genotypes and lines that were rooted, the rooting percentage was 55% (Table 2) and combined with an overall survival rate of 82% of rooted microshoots (Table 1), the *ex vitro* rooting method makes a very efficient method of clonally propagating walnut rootstock genotypes.

This past year we grew plantlets at one additional one nursery. Dormant plantlets were planted in the nursery row in late January. Survival was very high and by early September most plants were large enough for fall budding. Eight of the most promising disease and pest resistant genotypes comprising about 450 trees were budded with Chandler scions and after growth during 2007 should be ready to plant in orchard trials in 2008 (Table 4).

We have provided six trees each growing in 1.5 gallon pots of 20 transgenic lines that are putatively crown gall resistant along with three control lines for greenhouse inoculation and screening for gall formation by Dan Kluepfel's laboratory. We have also provided 25 plantlets each of five of our most promising genotypes (VX211, Vlach, RX1, AZ025, WIP3) and Px1 as a susceptible control for Janine Hasey's experiments on crown gall susceptibility of clonally propagated plants in comparison to seedling plants.

Because of some concern about the root architecture of tissue culture *in vitro* propagated and container grown plantlets, we have initiated some experiments on root pruning at the time of containerizing *ex vitro* rooted microshoots. It is too soon to know the effect of root pruning on root architecture but we have found that the roots of newly rooted microshoots can be pruned very severely to short ¼ inch stubs without affecting the subsequent survival and growth of the rooted pruned plantlets in comparison to the unpruned controls. The rooted pruned plants are very much easier and faster to plant in small containers.

Hardwood cuttings: Work on rooting hardwood cuttings was limited to producing clonal plants for a Mike McKenry nematode susceptibility experiment and producing clonal plants for Joe Grant of two wingnut genotypes that may be Chandler graft compatible. We produced about 10 plants each of UZ229, Vlach and VX211 for Mike McKenry and about 20 plants each of two genotypes for Joe Grant.

Bench Grafting Root Cuttings: We performed two experiments on root grafting this past year. One was done in mid-February with freshly harvested roots of AX1 and one was done in mid-April with roots of AZ2 and RX1 that had been stored at 34-36 F since harvest of the bareroot grafted trees. Chandler scion material that had been stored at 34-36 F was whip and tongue grafted to the root pieces. Root and scion pieces were about 6 inches long and ½ to ¾ inch in diameter. The base of the root piece of dipped in 8000 PPM KIBA solution and the graft union was wrapped with masking tape and painted with asphalt emulsion. The completed grafts were covered with wet wood shavings. The main factor that was varied was the method of controlling the temperature at the graft union and the base of the root piece. The temperature used was 82F (28C). The three methods used to control temperature are dual suspended heating cable, hot pipe on a heated bed and callusing room. Table 3 shows the combined results for the two experiments. All three methods of controlling temperature worked well for callusing the graft union with a 75 to 100% success rate for the three genotypes. However, rooting of the root piece had a somewhat lower and more variable success rate. AZ2 consistently rooted poorly in all three temperature control systems whereas the other two genotypes rooted well in at least two of the heating systems. Using any one of these temperature control systems a grafted plantlet can be produced in three weeks. These results indicate that all three temperature control systems can be used successfully for grafting and rooting root cuttings. However, the least convenient and the most cumbersome to use is the hot pipe system. The plants resulting from grafted root cuttings were transplanted into 1.5 gallon containers in the greenhouse where nearly 100% survived and grew vigorously. These containerized plants will be used in an orchard trial to be planted in 2007. These results show the potential for using root grafting with nut variety scions for producing an orchard planting size tree in one growing season.

Field Trials

One new orchard trial using nursery grafted, clonally propagated roostocks was initiated in the past year in Butte County. This is our first orchard trial using nut variety grafted nursery trees with clonally propagated rootstocks selected for *Phytophthora* resistance. The two rootstocks used were AZ2 and RX1 with Chandler as the scion nut variety. The trial was established using about 80 trees of each clonal rootstock and seedling paradox as controls. The results for transplant survival were very disappointingly poor for the AZ2 rootstock trees with about 90% failure and poor initial growth of the few surviving trees. However, RX1 rootstock trees transplanted very well giving nearly 100% survival and excellent initial growth. This trial demonstrates the importance of evaluating for horticultural characteristics as well as disease resistance characteristics under more than one condition (AZ2 had transplanted well in a re-plant situation in San Joaquin County). The poor bareroot transplantability of AZ2 apparently eliminates AZ2 as a potential commercial rootstock although it is possible that it might be suitable as a container grown tree. Its poor bareroot, root regeneration capability was also indicated in the root grafting experiment discussed above where it regenerated roots poorly even when treated with 8000 PPM KIBA.

Nursery Propagation and Commercialization

One of our objectives is to provide plantlets and other propagating materials of the most promising genotypes to commercial nurseries so they can begin to learn how to clonally propagate them and fit them into their commercial production system. Having the nurseries propagate these materials will make it easier to get orchard trials established so we can find out how they perform in an orchard situation. We have provided propagating material to five nurseries at this time and are willing to provide material to any nursery that desires to have some.