CLONAL PROPAGATION OF WALNUT ROOTSTOCK GENOTYPES FOR GENETIC IMPROVEMENT

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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 2300 liner sized plantlets for greenhouse screens and 1200 nursery row sized trees for field trials during 2006. These plants were clonally propagated from tissue culture derived microshoots. As a result of devising an *ex vitro* method of rooting microshoots in greenhouse fog chambers, survival of rooted microshoots was improved to 80% for *ex vitro* rooted plantlets as compared to 50% for *in vitro* rooted ones of the same genotypes. *Ex vitro* rooted plantlets grow faster in the greenhouse and are ready for dormancy induction sooner. Rooting of hardwood cuttings of some genotypes is enhanced by using cuttings from stock plants in which bud dormancy has been fulfilled by natural chilling treatment but rooting of cuttings of some genotypes is not consistent from year to year even though bud dormancy has been broken. Trials of bench grafting of root cuttings were carried out but success rate was low. Interestly, some of the best results were obtained with bench grafts stuck directly in the nursery row. These promising results may be related to the storage conditions for root and scion material. Seven field trials involving over 1000 trees of 16 candidate *Phytophthora* and blackline tolerant genotypes were established with farm advisors and campus researchers

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 used three approaches to clonal propagation of candidate rootstock genotypes with nematode, crown gall, *Phytophthora*, or blackline tolerance or resistance:

A. Tissue culture micropropagation with *in vitro* and *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 has had 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 over23 hundred plantlets of 23 genotypes for

replicated disease and pest greenhouse screening tests and for growing on in the nursery row to a size large enough for field trials (Table 1). The general methods for multiplication, rooting, acclimatization and greenhouse growth of *in vitro* tissue culture derived plants has been described in the *Walnut Research Reports for 2001 through 2004*. However, we are continually making improvements and this last year we obtained significant improvement in survival of rooted plantlets by using *ex vitro* rooting of microshoots. For *ex vitro* rooting, microshoots are treated with potassium indolebutyric acid (KIBA) in the culture medium in the laboratory for 2 to 5 days but are stuck *ex vitro* in vermiculite in fog chambers in the greenhouse. This procedure saves laboratory space, time and chemicals as compared to *in vitro* rooting. Table 2 compares the survival of plantlets derived from *in vitro* vs *ex vitro* rooting and shows that *ex vitro* rooted plantlets survived at about 80 % while *in vitro* rooted plants survived at 50% for 40 genotypes. *Ex vitro* rooted plantlets also grew faster and were large enough for dormancy breaking procedures in a shorter time than *in vitro* rooted plantlets. Overall to date, rooting percentage is about the same for *in vitro* and *ex vitro* rooted plantlets but some recent experimental *ex vitro* treatments show potential for substantially increasing rooting percentage. Table 1 shows the inventory by genotype of plantlets produced this last year that are now large enough for greenhouse screens and for planting in the nursery row for producing plants large enough for field trials with farm advisors next year. It also shows the intended use of specific genotypes.

This past year we grew plantlets at three nurseries (Tables 3, 4, and 5). Dormant plantlets were planted in the nursery row in May and will be harvested in late December to mid-January. The harvested plants will be cold stored until planted in field trials with farm advisors during the last half of April and May. The tables show the survival rate and diameter of these dormant, nursery grown trees. There was considerable variation in the survival and size of the trees grown at the three nurseries. The lower survival and smaller size of some genotypes in one nursery was probably due to soil compaction (edge effect of equipment turning) and over watering resulting from irrigation for the water requirement of larger nursery trees adjacent to the experimental planting.

Hardwood cuttings: General methods used for rooting hardwood cuttings of walnut rootstock genotypes have been described in *Walnut Research Reports from 2000 to 2004*. Hardwood cuttings on bottom heated beds are one of the alternative methods to clonally propagate selected rootstock genotypes that do not respond well to tissue culture micropropagation. Hardwood cuttings may also be more cost effective than micropropagation for some selected genotypes such as Vlach and VX211 when the method is optimized. One of the problems with hardwood cutting propagation is year to year variation in success rate. To try to establish the basis of this year to year variation, we performed an experiment to try to confirm an experimental observation from 2004 that Vlach hardwood cuttings rooted better when bud dormancy had been broken vs when bud dormancy is deep, using another genotype. To do this, we collected and stuck cuttings from PX1 (a relatively easy to root genotype like Vlach) on a bottom heated bench after stockplants had received about 400 hours below 45F (January 4) and then again when stockplants had received about 800 hours below 45F (January 31). Cuttings from material collected and stored at 45F from January 4 to January 31 were also stuck on January 31. The rooting results taken eight weeks after sticking on a bottom heated bed at 25 to 30C showed that those cuttings collected and stuck on January 4 (400 hours of chilling) rooted at 59% and those collected and stuck on January 31 (800 hours chilling) rooted at 79% and those collected on

January 4 and stored at 45F before sticking on January 31 rooted at 70%. These results confirm those with Vlach in 2004 and indicate that cuttings from material with lower bud dormancy may root better than cuttings from material that has a deeper dormancy.

Blackline tolerant genotypes WIP5 (87-32-1) and WIP6 (87-50-1) do not respond well to micropropagation. However, in some years these two genotypes have rooted well from hardwood cuttings but not in other years. Because we had results with Vlach that suggested (now confirmed with PX1) that hardwood cuttings root best when bud dormancy is low, we took cuttings of these two genotypes on February 5 when the bud dormancy chilling requirement had been fulfilled. Eight weeks after sticking, WIP5 rooted at 76% but WIP6 rooted at only 8%. VX 211, a vigorous paradox genotype that usually has a very good rooting percentage, rooted at only 40% when stuck at the same time. These results suggest that the dormancy status of cuttings is not the factor most limiting to rooting and probable not related to year to year variation in rooting percentages of individual genotypes.

Twenty-eight clonal plants of *J. ailantifolia x J. regia* (DAR) selected for vigor under adverse soil conditions were rooted from hardwood cuttings and grown in containers for use to establish a stock block of this genotype. Hardwood cuttings rooted at a moderate success rate of 31%. Two blackline tolerant genotypes (85-117-20 and 85-117-21) which had not previously been clonally propagated were rooted from hardwood cuttings to establish a stock block of these genotypes. Genotype 85-117-20 rooted at 63% to give 10 plants and 85-117-21 rooted at 24% to give eight plants.

Bench Grafting Root Cuttings: Two large experiments (and several small ones) were carried out with roots harvested from one year old nursery trees in mid-January and cold stored at 45F in plastic bags until used for grafting. Roots from 15 different genotypes were used and the scions were usually Chandler but some Howard and seedling paradox were used in one experiment. Both machine (wedge) and hand whip and whip and tongue grafts were used. Graft unions were wrapped with plastic tape or masking tape with or without being painted with asphalt emulsion. One experiment was initiated on February 28 and the other on April 28. The February 28 experiment used two environments to try to callus the graft union, a bottom heated moist peat moss at 30C with the graft stuck with the union mostly above the peat and a high humidity (100%) grape callusing room at 30C. In the grape callusing room, some graft unions were buried in the peat and some were stuck with the union mostly above the peat as with the bottom heated environment. Some of the root pieces formed new adventitious roots in both environments. Only a small percentage of the graft unions callused and a majority of these were wrapped with masking tape and in the high humidity grape callusing room. A small number of these developed into grafted plants. The April 28 experiment used five environments, including shavings with and without bottom heat at 30C and vermiculite with and without bottom heat at 30C and sticking in the nursery row. There was no callusing of unions or rooting of roots in the shavings or vermiculite environments but a small percentage of the hand made bench grafts wrapped with masking tape and painted with asphalt emulsion involving paradox seedlings on paradox seedling roots stuck in the nursery row, callused, rooted and grew into grafted plants. This small success under harsh nursery row conditions in May could be related to the way the roots used for grafting were stored in that, in this case the roots were obtained at the time of grafting from intact bare root trees that had been stored at 34-36F. In all other cases the roots were stored detached in plastic bags at 45F. The importance of root storage conditions will be investigated in the coming year.

Field Trials

We had over 1200 plants of 17 genotypes available for field trials in 2005. These had been grown at nursery 1, dug in January and placed in cold storage. Another set of about 300 plants of 3 genotypes was grown at Nursery 2 and was left in place for grafting in 2005. Farm Advisors were informed of the rootstocks available and their attributes. Five farm advisors designed and planted rootstock trials in replant situations (Table 6). In addition Greg Browne established a plot for artificial inoculations with *Phytophthora* and Bruce Lampinen also established a trial. Each Farm Advisor has prepared a separate report, oral or written in *Walnut Research Reports*. The rootstocks were planted in specific field situations to address either *Phytophthor*a problems or blackline problems. In all 7 field trials involving over 1000 plants of 16 candidate genotypes were established. In most cases survival was excellent, but growth was variable.

At Nursery 2 the grafting take of one-year-old clonal trees was very high. Some trees did not get grafted because they were too small. Of the 259 grafted trees of families AZ2, RX1 and AX1, the majority are over 7/8 inches and very similar to the nursery-grafted paradox seedling rootstocks near by. More than half of the RX1 were = 1 inch in diameter making it the most vigorous family.

This year about 1200 rootstocks will be available for field trials. These are smaller than those of last year and will need at least another season's growth before they are graftable. We may move the majority to another nursery row for the next growing season.

TABLE 1 INVENTORY OF CLONAL WALNUT ROOTSTOCK FOR FIELD TRIALS AND TESTING

The first column shows ungrafted bareroot rootstocks from Nursery 3 ready for field planting now; the second column shows dormant liners ready to be planted in a nursery; and the third column shows dormant liners available for greenhouse retesting for crown gall (Kluepfel) or *Phytophthora* (Browne). Nematode retests are already underway (McKenry).

CLONE	For Field Trials 2006	To Plant in Nursery 2006	Pest and Disease Tests 2006	NOTES
Crown gall				
AZ025	39	193	60	Kluepfel for retesting. Also test for *Phytophthora*
Gene silenced controls and constructs n=>40 in two backgrounds will be tested in the lab and given to Kluepfel for retesting.n=193				
Nematodes				
VX211	53	1	60	Moderately tolerant to *P. citricola*. Selected for tolerance to nematodes. Most promising.
Phytophthora				
AZ2*		18		Moderately tolerant to *P. citricola.*
AZ3*	1	8		Moderately tolerant to *P. citricola*
JX2	31	26		Moderately tolerant to *P. citricola*
RX1	99	132	60	Moderately tolerant to *P. citricola.*
AX1	86	144	60	Susceptible to *P. citricola.* Control
GZ1	10	12		Susceptible to *P. citricola.* Control
Px1	91	127	60	Susceptible to *P. citricola.* Control
AZ1*	24	7		In process of being retested for *P. citricola*
CW1	4	372	60	In process of being retested for *P. citricola*
UX1	41	11		In process of being retested for *P. citricola*
GZ2	49	39	60	In process of being retested for *P. citricola*
GZ3		132	60	In process of being retested for *P. citricola*
MW1			43	In process of being retested for *P. citricola*
UX2	54	23	60	In process of being retested for *P. citricola*
Burbank		13	60	In process of being tested for *P. citricola*
RR1			39	In process of being tested for *P. citricola*
RR4			53	In process of being tested for *P. citricola*
Blackline tolerant				
WIP3	331	119		Tolerant to CLRV. Susceptible to *P. citricola*
WIP2	135	11		Tolerant to CLRV
WIP6	1	15		
Vigorous control				
Vlach	154	154	60	
TOTALS	1204	1557	795	

* Nursery source. May have ownership issues since not all nursery agreements have been turned in to Tech Transfer Center.

TABLE 2. SURVIVAL OF IN VITRO AND EX VITRO ROOTED SHOOTS -2005

	In Vitro		*Ex Vitro*	
Genotype	**% survival**	**# alive**	**% survival**	**# alive**
84-121	40	4	87	13
AX1	55	84	89	72
AZ025	53	101	79	185
AZ2	83	10	100	2
AZ3	40	6	75	3
Burbank	86	36	94	47
CW1	57	207	88	129
DAR	15	4	63	5
GA 2404 2G11	7	1	100	3
GA 2404 2G7	25	6	100	1
GA 2404 2H5	100	1	33	1
GA 2601 2D12	25	1	0	0
GA 2601 2F5	0	0	100	2
GZ1	44	10	100	5
GZ2	60	50	78	55
GZ3	65	102	81	96
J1 12a	100	5	67	2
J1 14a	25	1	71	5
J1 17a	0	0	67	2
J1 19a	0	0	100	12
J1 3a	64	9	0	0
J1 6a	0	0	20	1
JX2	50	16	100	14
MCG 42-1-1	33	53	78	99
MCG 85-6-2	69	11	71	5
MW1	24	22	65	20
Px1	24	40	88	116
Px1B	16	23	43	3
Rol abc 2-2-1	67	2	0	0
RR4	66	44	80	36
RX1	85	115	84	43
UX1	37	15	38	3
UX2	49	47	87	26
UZ1	63	5	100	9
Vlach	59	82	88	106
VX211	72	31	77	49
W17	13	6	16	5
WIP2	21	5	20	1
WIP3	63	94	76	29
WIP6	38	6	63	10
Total		**1255**		**1215**

TABLE 2 CONTINUED. SUMMARY

Total Rooted (n)	4050
Total Survival (n)	2470
Overall Survival	61%
Total *in vitro* rooted (n)	2534
In vitro rooted survival (n)	1255
Survival of *in vitro* rooted	50%
Total *ex vitro* rooted (n)	1516
Ex vitro rooted survival (n)	1215
Survival of *ex vitro* rooted	80%

TABLE 3. ROOTSTOCKS GROWN AT NURSERY 1. 2005

	Planted	**Survived**	**Diameter (mm)**					
			Over 10 mm	**Over15 mm**				
Clone	**N**	**N (%)**	**N (%)**	**N (%)**	**Mean**	**SD**	**Range**	**CV**
VX211	20	20 (100)	20 (100)	20 (100)	32	7.0	18-47	22
AZ2	20	17 (85)	17 (85)	11 (55)	19	1.5	11-29	8
Totals	40	37 (93)	37 (93)	31 (77)				

TABLE 4. ROOTSTOCKS GROWN AT NURSERY 2. 2005

	Planted	**Survived**	**Diameter (mm)**					
			Over 10 mm	**Over 15 mm**				
Clone	**N**	**N (%)**	**N (%)**	**N (%)**	**Mean**	**SD**	**Range**	**CV**
VX211 (hw)	16	15 (94)	15 (94)	15 (94)	22	2.9	17-26	13
AZ2	20	14 (70)	7 (35)	3 (15)	11	4.8	5-21	43
AX2	20	16 (80)	15 (75)	11 (55)	17	4.7	9-25	28
RX1	59	53 (90)	48 (81)	13 (22)	13	2.8	5-18	22
UX022	98	88 (90)	71 (72)	23 (23)	12	3.3	4-22	27
GZ2	93	67 (72)	59 (63)	30 (32)	14	3.5	5-21	25
WIP3	98	95 (97)	88 (90)	48 (49)	14	3.2	5-24	23
Totals	404	348 (86)	303 (75)	143 (35)				

(hw) hardwood cuttings; all others liners. Planted 4/13/05

TABLE 5. ROOTSTOCKS GROWN AT NURSERY 3. 2005

	Planted	Survived	Diameter (mm)					
			Over 10 mm	Over15 mm				
Clone	N	N (%)	N (%)	N (%)	Mean	SD	Range	CV
Nematodes								
VX211	70	67 (96)	53 (79)	12 (18)	12	2.9	5-19	24
Phytophthora								
AZ3	6	6 (100)	1 (17)	0	8	2.1	6-11	26
NZ1	9	8 (89)	2 (25)	0	8	2.0	5-10	25
JX2	135	63 (47)	31 (49)	1 (2)	9	2.8	4-15	31
RX1	290	284 (98)	99 (35)	0	9	1.9	2-14	21
AX1	148	143 (97)	86 (60)	18 (13)	11	2.9	5-19	26
GZ1	19	13 (68)	10 (77)	3 (23)	12	3.8	4-18	32
Px1	844	731 (87)	91 (12)	2 (<1)	6	2.5	2-15	42
AZ1	28	28 (100)	24 (86)	1 (4)	11	2.2	7-17	20
UX1	55	49 (89)	41 (84)	11 (22)	12	2.7	6-18	22
GZ2	208	178 (86)	49 (28)	2 (1)	8	2.5	2-16	31
UX2	84	68 (81)	54 (79)	13 (19)	12	2.9	7-20	24
CW1	44	40 (91)	4 (10)	0	6	2.1	3-12	35
Blackline								
WIP3	619	547 (88)	331 (61)	123 (22)	11	4.1	3-22	37
WIP2	249	235 (94)	135 (57)	20 (1)	10	2.9	3-23	29
WIP6	11	9 (82)	1 (11)	0	6	1.7	4-10	28
Crown gall								
AZ025	52	47 (90)	39 (83)	15 (32)	13	3.0	7-19	23
Other								
84-121	10	9 (90)	1 (11)	0	7	2.4	5-12	34
Vlach	168	164 (98)	154 (92)	74 (45)	14	3.0	4-21	21
Totals	3049	2689 (88)	1206 (40)	295 (10)				

TABLE 6. FIELD TRIALS OF SELECTED CLONAL ROOTSTOCKS ESTABLISHED IN 2005.

Investigator	Grower	Problem	Genotypes N	Rootstocks per genotype N	Total N	Survival
Kathy Kelley	Crane	Phytophthora	12	25	330	Poor
Joe Grant	Dondero	Phytophthora	5	40	200	Excellent
Bill Coates	Bonturi	Blackline	2	10	20	
Bruce Lampinen	Lester	Water	3	22	66	Excellent
Janine Hasey	Doublenut	Phytophthora	4	12	48	Excellent
Janet Caprile	Tennant	Blackline	3	6	18	Excellent
Greg Browne	Pl. Path.	Phytophthora	11	30	330	Excellent