USDA ARS Research in Grape Rootstock Breeding and Genetics

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Abstract

Grape rootstock improvement at the United States Department of Agriculture Agricultural Research Service Grape Genetics Research Unit includes introduction, evaluation and breeding. The primary goal is the enhancement of nematode resistance in rootstocks that provide protection against phylloxera (resistance and tolerance). Phylloxera protection is a requirement of all elite selections in the program. Vineyard trials are in place for regions across the United States and with several varieties. Germplasm collection and evaluation to identify superior novel parents for breeding and introduction are carried out in cooperation with the germplasm repository system and other cooperators; selected individuals from diverse *Vitis* species including *V. cinerea* and *V. cordifolia* as well as related genera are in use in our program.

INTRODUCTION

Resistance to soil borne pests is the primary reason that rootstocks are used in viticulture. Phylloxera protection in particular is a critical rootstock attribute and developing rootstocks that provide protection against phylloxera has been a key goal of rootstock breeding programs. However, other root pests also are important in viticulture and resistant rootstocks are needed against these pests as well.

Root-knot nematodes (*Meloidogyne* species) may infest as much as 65% of California's vineyard acreage, with statewide losses in production estimated at up to 20% (Nicol et al., 1999). Their feeding weakens vines, diminishes productivity, and reduces vine establishment (Ibid.). The use of nematode resistant rootstock varieties, such as Freedom, Harmony, Dog Ridge and Ramsey, is limited by the emergence of aggressive root-knot nematode populations that can feed on and damage these important resistant rootstocks (Nicol et al., 1999; McKenry, 1997; Cain et al., 1984). Apparently, the use of contemporary resistant rootstocks selects for aggressive, resistance-breaking nematode populations. A few rootstock selections have shown promise in trials (McKenry, 1997), but these demonstrate some of the horticultural faults of currently available varieties. These drawbacks include excess vigor induction in scions, nutrient uptake excesses and deficiencies, and poor fruit quality (Hardie and Cirami, 1988; Winkler et al., 1974). The questionable phylloxera resistance of some nematode resistant rootstocks is an issue (Ibid.).

In addition to the diminishing utility of today's rootstocks in nematode control, other management options are decreasing. Stringent regulations phasing out methyl bromide and restricting the use of other chemicals eliminate one nematode management tool, further constrain others, and sharply accentuate the need for resistant rootstocks.

The USDA Agricultural Research Service grape rootstock improvement program, based at the Grape Genetics Research Unit in Geneva, New York, is breeding nematode resistant rootstocks for California and other viticultural regions. This research program works exclusively in grape rootstock breeding, genetics and evaluation. The program's total commitment to grape rootstocks allows us to focus our efforts specifically on their improvement. Introducing rootstocks with nematode resistance superior to the industry standard rootstocks Freedom and Harmony, is the primary objective of our program. While Freedom and Harmony have excellent resistance against many nematode populations, the long term phylloxera resistance of these rootstocks has been questioned due to their complex hybrid ancestry that includes *V. vinifera*. In order to breed superior rootstocks, we are crossing phylloxera resistant and tolerant rootstocks and selections with sources of resistance to aggressive root-knot nematodes.

Our approach to developing improved grape rootstocks, with resistance to aggressive root-knot nematodes, is to screen many seedlings quickly, minimizing time and expense while maximizing the number of seedlings screened. Our seedling screening of nematode resistance permits us to cull substandard seedlings just a few months after they germinate. We greatly reduce the expense and time of field planting and cultivation by screening seedlings before we plant them into the vineyard.

As we identify novel resistance alleles, we compare them to the N allele (found in Freedom, Dog Ridge and 1613C (Lider, 1954)) to determine allelism; this will facilitate resistance pyramiding (which we hope will reduce the incidence of virulent nematode populations). Our cooperation with the USDA ARS Crop Diseases, Pests and Genetics Research Unit (San Joaquin Valley Agricultural Sciences Center) in Parlier, the USDA germplasm repositories in Davis, California and Geneva, New York, Cornell University, and the University of California, Davis Department of Viticulture and Enology gives us ready access to a wide variety of rootstock parents and germplasm, some of which may contain even better resistance alleles.

Our program considers resistance to aggressive root-knot nematodes the top priority in grape rootstock improvement in the United States. Literally dozens of rootstocks are available that provide protection against phylloxera (through tolerance and/or or resistance). Growers select the rootstock that best matches their scion, site and pest pressure. However, rootstocks resistant to the increasingly important aggressive root-knot nematodes are few and even advanced selections are scarce. This underscores the importance of developing rootstocks with resistance to aggressive root-knot nematodes - growers need more and better rootstock options for root-knot nematode control in a genetic background that provides protection against phylloxera.

MATERIALS AND METHODS

In order to breed improved rootstocks with resistance against aggressive root-knot nematode populations, we identify sources of nematode resistance and hybridize them with elite rootstocks. Many of the sources of nematode resistance that we have identified are wild species that are difficult to root and graft and have undesirable horticultural characteristics, such as small diameter cuttings or frequent branching (leading to brushiness). We seek to combine the resistance to nematodes with the desirable horticultural features that are found in elite rootstocks while breeding in a phylloxera resistant or tolerant background.

We identify suitable sources of nematode resistance through direct evaluation of the parental material and through progeny testing. The United States National Grape collection, held in the National Plant Germplasm System repositories at Davis, California and Geneva, New York, is an excellent source of diverse North American *Vitis* germplasm. *Vitis* species (and their hybrids) from eastern North America are the basis of our rootstock breeding program because of the recognized protection that these species provide against damage from root-feeding phylloxera. Since phylloxera is the main reason that rootstocks are used in viticulture, it is critical that our new nematode resistant rootstocks also should provide protection against phylloxera.

Our resistance evaluations are not limited solely to *Vitis*. Related genera in the Vitaceae may be sources of nematode resistance if techniques to overcome the barriers to their use in breeding can be overcome. We have tested seedling populations of *Ampelopsis arborea* and *Cissus antarctica*, the latter a member of a group of *Cissus* species that are considered closely affiliated to *Vitis* (Rossetto et al., 2002), and we are cooperating with several institutions to develop somatic hybridization technology that may provide access to more distantly related germplasm for use in grape rootstock improvement.

We evaluate the nematode resistance of grape rootstock seedlings using the technique developed by Cousins and Walker (2001). We start the seeds in Petri dishes and then plant the seeds into a sandy soil mix in individual 2.5 inch square pots once they germinate. We estimate about 60% seed germination overall. Once seedlings have two true leaves we inoculate them with root-knot nematode juveniles. This stage is reached about four weeks after the seeds are placed in the Petri dishes to germinate. We inoculate seedlings with 1500 infectious root-knot nematodes (mobile juveniles, stage J2). The root-knot nematode populations we use in screening include aggressive populations that can reproduce on Freedom and Harmony. We use seedlings of nematode susceptible and resistant rootstocks as controls.

We evaluate nematode resistance six weeks after inoculation. We select resistant seedlings using a tiered system. If a seedling shows galled roots when we remove the pot, we discard the seedling - we do not need to expend additional effort on an obviously susceptible plant. We discard seedlings showing visible galls once we have washed the soil from the roots. We estimate that we discard about 90% of the plants by this point. We then stain those root systems that do not have galls in an eosin-Y solution (0.25 g/L eosin-Y in water for one hour). This solution colors root-knot nematode egg masses bright red and renders the egg masses easily visible. We eliminate the seedling if even one egg mass is visible upon inspection. We select only seedlings that pass each of these three levels of screening for propagation and further testing. We propagate selected seedlings using green cuttings in a mist bed. For genetic testing, we count all of the egg masses on each seedling root system. In germplasm evaluation to identify candidate parents for rootstock breeding, we test replicated rooted cuttings, again counting all of the egg masses to determine nematode reproduction.

Seedlings that demonstrate resistance to root-knot nematodes and are candidates for expressing other suitable horticultural traits are planted in a grow-out block. Our experience indicates that about 1% of the seedlings (taken across all populations) are resistant to aggressive root-knot nematodes and suitable for advancement to the field. As the vines mature, we assess them for horticultural qualities at the earliest opportunity. We are interested in the ease of rooting and grafting, lateral bud growth (brushiness), and other characteristics important in nursery production. Demonstrable phylloxera resistance or tolerance is a requirement for all selections, but genotypes need not show universal pest resistance - our main goal is improved resistance against aggressive root-knot nematodes.

After nematode resistant vines are selected, they are planted in a vineyard in California. We have contracted with a nursery that cultivates the vines for us. Planting the vines in California is critical to the success of this project. Our selections grow much faster in California than in New York due to the warmer climate and longer growing season. We suspect that many of the selections would not be cold hardy in New York since one of their parents is a cold-tender wild species. For example, *V. mustangensis* is an excellent source of resistance to aggressive root-knot nematodes, but this wild grape native to Texas is not cold hardy in central New York. For rootstock trials in California, cuttings grown in California are subject to fewer phytosanitary regulations than if they had been grown in New York or other states - this makes it easier for rootstock trials to include our selections.

Once selections have been further screened for horticultural attributes, cuttings are made available to researchers, nurseries and others so that these selections can be included in rootstock trials. In cooperation with the University of California, Cornell University, and grapegrowers, we are evaluating rootstocks and rootstock selections. In addition to pest resistance and tolerance, vine size and fruit yield and quality are of primary interest in our rootstock performance trials.

RESULTS AND DISCUSSION

Through direct nematode resistance evaluation of grape accessions and through progeny testing of grape accessions we have identified candidate parents for breeding nematode resistant and phylloxera protective rootstocks. Tables 1 and 2 show the results of our direct nematode resistance evaluation of accessions from the U.S. national grape collection repositories. Those accessions which permit no nematode resistance are candidates for direct use as rootstocks or as parents in rootstock breeding. Note that the performance of related material is not necessarily similar - some accessions which share species ancestry have very different nematode resistance, which emphasizes the importance of evaluating individual accessions.

Within the North American *Vitis* species, our efforts have focused primarily on the species affiliated with *V. aestivalis*, *V. mustangensis*, *V. cordifolia*, and *V. cinerea* as sources of nematode resistance. Of these *V. mustangensis* alone has had a traditional role in rootstock breeding, through *V. × champinii*, the primary hybrid of *V. mustangensis* and *V. rupestris*. We have identified and deployed four additional sources of resistance to aggressive root-knot nematodes and we are using these sources extensively in our program (Boyden and Cousins, 2003; Cousins and Lauver, 2003; Cousins et al., 2003). Screening in 2002 confirmed our incorporation of resistance from the Muscadine grape *V. rotundifolia* into one of our exceptionally resistant selections and we continue to use *V. rotundifolia* and its hybrids in breeding. The 2005 vineyard plantings included many selections with *V. cinerea* parentage; this species, also in the background of the rootstock Boerner, appears promising as a source of nematode resistance. *Vitis cordifolia* has been an excellent source of nematode resistance, but its primary hybrids often show the poor rooting ability of *V. cordifolia*.

The diversity of nematode resistance sources and rootstock parents is demonstrated in the nematode resistant selections that have been planted to the vineyard. Tables 3 and 4 list nematode resistant selections planted out in 2004 and 2005, respectively. Field planting began in 2003. These selections were screened for nematode resistance, found to permit no nematode reproduction, and then were propagated as green cuttings from the original seedling. Many of the resistant selections could derive nematode resistance from both parents. We suspect that good rooting ability is derived mostly from *V. riparia* and *V. rupestris* in our populations, as many of the germplasm or nematode resistant parents are difficult to root.

Vineyard trials of our rootstock selections grafted to Syrah began in 2005 with 16 selections. Three plots were planted, all in the San Joaquin Valley in south central California. In this warm climate production region, root-knot nematodes are the most important soil pest, although phylloxera also is found in the district. We are evaluating rootstocks and rootstock selections in table, juice, raisin, and wine grape production in California and New York.

Our research into nematode resistance in other genera in the Vitaceae led to our identification of resistance in *Ampelopsis arborea*. All seedlings in a test population of this species demonstrated complete resistance to our aggressive root-knot nematodes. However, there are no reports of successful sexual hybridization between any *Ampelopsis* species and any *Vitis* species. While somatic hybridization between *Vitis* and *Bupleurum* (Umbelliferae) has been reported (Song et al., 1999), the use of somatic hybridization in grape breeding is still an emerging technology that will need further refinement before it is a regular method. We found no complete resistant to root-knot nematodes in the population of *Cissus antarctica* that we evaluated, although there was variation in the population for nematode resistance level.

The USDA Agricultural Research Service has been breeding grape rootstocks for more than fifty years. Two important nematode resistant rootstocks, Harmony and Freedom, were developed by the USDA ARS. We are building on past success to breed improved grape rootstocks with enhanced nematode resistance and durable phylloxera protection. Through germplasm evaluation and parental selection, thorough pest and disease resistance and vineyard characterization, and horticultural testing we will develop superior rootstocks.

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Tables

Table 1. Resistance of grape germplasm accessions against aggressive root-knot nematodes.

Accession	Parentage	Mean Nematode Egg Masses
GVIT 699	*V. doaniana* × *V. riparia*	0
GVIT 703	*V. cordifolia* × *V. riparia*	0
GVIT 728	*V. longii* × *V. riparia*	0.2
GVIT 734	*V. riparia* × *V. mustangensis*	1.4
GVIT 726	*V. labrusca* × *V. riparia*	1.5
GVIT 158	*V. acerifolia*	2
GVIT 780	*V. acerifolia*	2.8
GVIT 702	*V. acerifolia*	3
GVIT 733	*V. solonis* × *V. riparia*	3.3
GVIT 154	*V. acerifolia*	3.5
NE36A	*V. riparia* hybrid	9.2
USDA 125	putative *V. cordifolia* × *V. riparia*	27.2
GVIT 736	*V. labrusca* × *V. flexuosa*	48.6
GVIT 735	*V. solonis* × *V. rupestris*	87.6
Control		
1202C	*V. vinifera* × *V. rupestris*	34.5

Table 2. Resistance of grape germplasm accessions against aggressive root-knot nematodes.

Accession	Parentage	Mean Nematode Egg Masses
Rem 34-77	*V. cordifolia*	0
GVIT 239	*V. palmata*	0
Cache 8	*V. palmata*	0
Illinois 473-1	*V. cordifolia* × *V. rupestris*	0
Illinois 885-1	*V. cinerea* × *V. rupestris*	0
J-167-048	*Vitis* species	0
Ru-66-10	*V. palmata*	1.5
Illinois 547-1	*V. rupestris* × *V. cinerea*	2
J-167-045	*Vitis* species	3.8
Illinois 547-X	*V. rupestris* × *V. cinerea*	5.5
Illinois 547 × 5A	*V. rupestris* × *V. cinerea*	7
Illinois 547-2	*V. rupestris* × *V. cinerea*	15.3
Rem 40-77	*V. cordifolia*	28.5
Illinois 547-3	*V. rupestris* × *V. cinerea*	29
Controls		
1202C	*V. vinifera* × *V. rupestris*	58
41B	*V. vinifera* × *V. berlandieri*	73.3

Table 3. Nematode resistant selections planted in 2004.

Population	Nematode resistant selections planted in vineyard
(*Vitis mustangensis* × *V. rupestris*) × Riparia Gloire	11
(*V. mustangensis* × *V. rupestris*) × 1616C	5
101-14 Mgt × (*V. mustangensis* × *V. rupestris*)	7
101-14 Mgt × *V. bloodworthiana*	1
V. mustangensis × 1616C	1
106-8 Mgt × (*V. rufotomentosa* × ((*V. acerifolia* x (*V. mustangensis* × *V. rupestris*))	1
Cosmo 10 × *V. cordifolia*	15
V. riparia × (*V. mustangensis* × *V. rupestris*)	1
V. riparia × (*V. mustangensis* × *V. riparia*)	1
V. riparia × (*V. mustangensis* × *V. riparia*)	2
V. riparia × (*V. mustangensis* × *V. riparia*)	1
V. mustangensis × 3309C	3
V. rotundifolia hybrid × Schwarzmann	5
101-14 Mgt × *V. mustangensis*	1
Kober 5BB-6 × *V. mustangensis*	3
V. rotundifolia hybrid × 1616C	1
Kober 5BB-6 × (*V. mustangensis* × *V. rupestris*)	1
Teleki 5A × *V. mustangensis*	4
Teleki 5BB × (*V. mustangensis* × *V. rupestris*)	3
V. nesbittiana × 1616C	17
V. nesbittiana × Riparia Gloire	2
Kober 5BB-6 × (*V.* × *champinii* × *V. rufotomentosa*)	1
Kober 5BB-6 × (*V.* × *champinii* × *V. rufotomentosa*)	1
Teleki 5BB × (*V.* × *champinii* × *V. rufotomentosa*)	1
Total selections planted to vineyard 2004:	**89**

Table 4. Nematode resistant selections planted in 2005.

Population	Nematode resistant selections planted in vineyard
(*V. cinerea* × *V. riparia*) × SO4	8
Florilush × 1103P	2
Teleki 5A × (*V. aestivalis* × SO4)	1
V. × *slavinii* × (*V. aestivalis* × SO4)	1
V. × *slavinii* × 5C	1
V. mustangensis × (*V. mustangensis* × *V. rupestris*)	2
101-14 Mgt × (*V.* × *doaniana* × *V. mustangensis*)	2
101-14 Mgt × (*V. mustangensis* × *V. rupestris*)	3
(*V. cordifolia* × *V. rupestris*) × SO4	3
(*V. cordifolia* × *V. rupestris*) × (*V. mustangensis* × *V. rupestris*)	1
Cosmo 2 × (*V. aestivalis* × SO4)	1
(*V. longii* × *V. mustangensis*) × (*V. mustangensis* × *V. rupestris*)	2
(*V. longii* × *V. mustangensis*) × (*V. mustangensis* × *V. rupestris*)	2
(*V. rufotomentosa* × *V. rupestris*) × (*V. champinii* × *V. rufotomentosa*)	1
Cosmo 10 × (*V. cinerea* × *V. riparia*)	3
Cosmo 10 × (*V. cinerea* × *V. rupestris*)	3
V. cordifolia × SO4	1
Total selections planted to vineyard 2005:	**37**