Development of Next Generation Rootstocks for California Vineyards

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Project Summary/Abstract

Briefly describe the long-term objectives for achieving the stated goals of the project.

The vast majority of rootstocks being used in California vineyards were developed for European climates and soils about 100 years ago. There are only a few rootstocks bred in California. Freedom and Harmony were released from the USDA-Fresno in the 1960s and have been widely used on nematode-prone soils in the San Joaquin Valley. The rootstock 039-16 was released from UC Davis in 1989 to control fanleaf degeneration and has been used in the North Coast and Lodi. More recently, rootstocks were released from UC Riverside (RS3 and RS9)" and UC Davis (GRN-1, -2, -3, -4 and -5) for use on soils with multiple and aggressive nematode species, some of which are capable of damaging Freedom and Harmony. The GRN series were also tested for resistance to a combined inoculum of aggressive root-knot nematode strains plus dagger nematode, *Xiphinema index,* and at warm soils temperatures where resistance to root-knot nematode often breaks down. The GRN series resist these combined inoculum and conditions and were then tested for resistance to citrus, lesion and ring nematodes and for phylloxera resistance; all resist phylloxera and lesion, several resist citrus, and GRN-1 resists all of these pests. Rootstock breeding for broad and durable nematode resistance must continue and focus on resistance to complexes of nematode species, in addition to improving ease of propagation and nursery production. However, there are many other soil-based problems that confront California viticulture and rootstock breeding must be directed at these problems. These issues include drought tolerance; salt and nutrient tolerance; resistance to the grapevine fanleaf *virus!Xiphinema index* (dagger nematode) disease complex; tolerance to viruses that impact ripening and induce union failure (leafroll, corky bark, the rugose wood virus complex, etc.); rootstock with resistance to Pierce's disease for use with new PD resistant fruiting varieties; rootstocks with better adaptation to California's soils and climates; and maintaining the highest levels of phylloxera resistance.

The following proposal presents an outline of a 10-year program designed to improve grapevine rootstocks for resistance to pests and pathogens and to better tolerate abiotic stress. A primary goal is to update screening techniques with the ability to test new germplasm at faster pace and develop molecular markers for all traits in order to employ molecular breeding to expedite the classical breeding of rootstocks. These markers will be used to accelerate seedling screening and would enable us to rapidly stack (combine) multiple traits in one breeding population.

Rootstock breeding in the Walker lab is addressing many of above .issues with joint funding from the California Grape Rootstock Improvement Commission, the CDFA Nursery Improvement Advisory Board and the California Table Grape Commission. The AVF initiated funding for the program in the early 1990s and has funded efforts to breed fanleaf resistant rootstocks over the last six years. In order to make sustained and significant progress on all of the above issues, secure multi-year funding will be needed.

Objectives This research proposal is aimed at achieving the following objectives:

1. Drought and Salt Tolerance

Evaluate the salt and drought tolerance of Vitis species from the southwest U.S. Salt tolerance - exclusion vs. avoidance strategies

Uncoupling drought tolerance from an expansive root architecture

2. Pest/Disease

Nematodes - dagger, ring, root-knot, citrus, lesion, nematode complexes Phylloxera - nodosity feeding
Pierce's disease
Fungi - young vine decline, armillaria, phytophthora

3. Control of Growth and Phenology

Root architecture - drought and salt tolerance

Control of vigor and phenology (budbreak, ripening, root senescence)

4. Virus Tolerance/Resistance

Fanleaftolerance- disruption of symptoms

Tolerance to red leaf and virus-induced incompatibility

Scope of Work

escribe the goals and specific objectives of the proposed project and summarize the expected outcomes. f applicable, describe the overall strategy, methodology, and analyses to be used. nclude ho the data ill be collected, analyzed, and interpreted as ell as any resource sharing plans as appropriate. iscuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the goals and objectives.

Genetic resources, available information and proposed plan to accomplish Objectives:

1) Drought and Salt Tolerance

rought tolerance - Population growth and political pressure will make water less available for viticulture in the future, whether or not climate change impacts its availability. Most grape species grow in or near water, or in areas with abundant summer rainfall. Those that grow in arid areas survive with a large expansive root system. We have been examining root architecture and are developing strong correlations between known rootstock drought tolerance and rooting depth. I have collected *Vitis* species throughout the southwestern US over the last 10 years for use in breeding. We have developed multiple breeding populations and molecular genetic resources a genetic map from a Ramsey (very expansive root system) x Riparia Gloire (weak shallow root system) population of 185 individuals, and a 101-14 x l lOR population of about 150 individuals that should allow us to develop a better genetic understanding of drought tolerance. We are continuously testing new germplasm that shows deep rooting characteristics in early stages of propagation. These studies may also establish whether drought tolerance can be uncoupled from rooting depth so that drought tolerance can be available within levels of rootstock vigor. Many other crosses have been made to better adapt rootstocks to California's growing conditions.

To successfully breed grapevine rootstocks that maintain productivity, using less available water will require the development of a greenhouse assay and/or physiological indicator that successfully predicts the field performance of a genotype under long-term field conditions. Such an assay and/or indicator do not currently exist. We have initiated studies ofroot anatomy to identify features present in drought resistant genotypes that might be used in germplasm screening. Several greenhouse assays will be tested, and the results compared with a long-term field trial ofRiparia Gloire, 420A, Teleki SC, 101-14, 1 lOR, 140Ru and Ramsey at UC Davis. Greenhouse assays will target features that could aid in drought avoidance by the establishment of larger, downwardly angled root systems that may enable rootstocks to tap into soil water unavailable to smaller, laterally spreading root systems. Because drought and saline soils often occur together, variables such as root biomass, rooting depth, root architecture, rooting angles and root: shoot ratios will be measured both under drought alone and drought coupled with salinity stress. Root anatomical features will also be determined from grapevine genotypes subjected to drought, salt stress, and both, and include features such as xylem vessel diameter and water column cavitation repair. Wild *Vitis* germplasm will also be screened using factors found to be most important for drought resistance, and crosses performed using the most promising germplasm. The long-term field trial will measure physiological factors such as leaf water potential and canopy development, water use patterns in the soil, and cropping indices such as yield and fruit quality. Field treatments will vary the applied water as a% ofETo (0, 20 and 75) and will include an analysis ofdry farming and buried drip irrigation.

As mentioned above, root architecture plays a key role in drought tolerance and is likely involved in salt tolerance. As expected there is a strong association between the depth of a root system and the vigor a rootstock induces in a scion. We have found that the root angles of a one-year old dormant rooting or a 4-week-old herbaceous cutting rooted under mist also correlate well to known field vigor. This discovery has allowed us to propagate young rootstock selections and sort them into shallow, medium and deep rooting groups so that we can address a broad range ofrootstock needs.

alt tolerance - We have developed, a rapid potted-vine system to screen for chloride exclusion that duplicates what has been discovered from long-term field studies in Australia. We have used this assay to test a wide range of germplasm that I have collected in multiple collection trips and have identified new accessions that are promising for breeding salt tolerance. We also tested subsets of different mapvmg populations (Ramsey x Riparia Gloire, 'F2-7' x V. rupestris 'St. George' and V. vinifera 'F2-35' x V. berlandieri '9031) to look for possible segregation of the trait. The results indicate that valuable genetic loci are present in these hybrid populations, but that they were not yet segregating to a degree necessary to develop molecular markers. High-performing individuals from both populations were crossed in Spring 2011 to V. vinifera to produce new breeding populations.

The potted vine system is also being used to test a wide range of rootstocks and my southwestern grape species collections (including *V. girdiana* from a salt flat north of Las Vegas, NV). These studies were initiated in 2010 and five screens using different southwestern accessions have been completed to date. Based on the results of these screens, twenty crosses were made in Spring 2011, all of which involved very strong chloride excluders paired with very weak chloride excluders. These crosses should provide an abundance of segregating hybrid germplasm from which to develop molecular markers, and individual progeny that did not involve *V. vinifera* as a parent might be directly useful as rootstocks. One example of these twenty crosses, *V. acerifolia* x *V. rupestris* 'Pump Station,' is listed in the table below.

Additional field collections from the southwestern U.S. will be made in subsequent years, as will additional greenhouse screens of this material. Our screening system can also be used to study other nutrient problems such as magnesium and boron toxicity.

Table 1. Outline of genetic resources, mapping populations, and screening techniques available for mapping tolerance to salinity and drought stress in grapevine rootstocks. We have a completed genetic map for the Ramsey x Riparia Gloire population (Lowe artd Walker 2006. Theor. Appl. Genet. 112:1582-1592).

Stress factor	Genetic background	Population availability	Screening technique
Soil Salinity	1) Ramsey x Riparia Gloire	Yes	Yes
	2) *V. vinifera* x (*V. vinifera* x *V. rupestris* 'St. George')	Crossed in spring 2011	Yes
	3) *V. vinifera* x (*V. vinifera* x *V. berlandieri* '9031')	Crossed in spring 2011	Yes
	4) *V. acerifolia* x *V. rupestris* 'Pump Station'	Crossed in spring 2011	Yes
Drought	1) Ramsey x Riparia	Yes	No
	2) Widely-used CA rootstocks	Yes	No

2) Pest/Disease Resistance

Grape rootstock breeding requires: germplasm - accessions of grape species with the traits of interest; a means to evaluate the trait; seedling populations to select from and to study the inheritance of traits; and genetic maps of these populations so that DNA markers can be linked to the traits and then be used to accelerate and enable the selection of the optimum progeny, and allow the study of gene function and durability. Table 2 summarizes the available genetic resources for different pathogens.

Table 2. Outline of availability of genetic resources, mapping populations, screening techniques and markers in order to tag resistance regions to assist molecular rootstock breeding.

Pathogen/Disease	Grape species	Population availability	Screening technique	Markers
Dagger nematode (*X. index*)	b42-26 (Complex hybrid)	Yes	Yes	Yes
	b40-14 (*V. arizonica*)	Yes	Yes	Yes
	Trayshed (*M. rotundifolia*)	Yes	Yes	No
Nematodes: root-knot, citrus, lesion, ring	Trayshed (*M. rotundifolia*) *Vitis* species	Yes	Yes	No
Phylloxera	*V. berlandieri* (selection 9031)	Yes	Yes	No
	V. arizonica b42-26	Yes	Yes	No

	V. cinerea (selection 9007)	Yes	Yes	No
	V. rupestris (Ganzin)	Yes	Yes	No
Young vine decline, armillaria, phytophthora	Germplasm discovery needed	No	Collaboration with Kendra Baumgartner	No
Fanleaf degeneration	Trayshed (*M. rotundifolia*)	Yes	Developing	No
Red leaf and virus induced incompatibility	Germplasm discovery needed (*V. rupestris*, *M. rotundifolia*)	Developing	Developing	No
Pierce's disease	Multiple backgrounds (b43-17, b40-14, b42-26)	Yes	Yes	Yes

Nematodes: We have effective screens for multiple root-knot species; *X index* the dagger nematode; and citrus, lesion and ring nematodes. We have genetic markers for *X index* from two different resistance sources *(V. arizonica/girdiana* b42-26 and *V. arizonica* b40..:14) and have characterized the resistance gene from b42-26, *XiRJ* (Hwang et al. 2010 Theor. Appl. Genet. 121:780-799). We are making headways to characterize the root-knot nematode resistance from the GRN rootstock series and the mapping populations are being constructed and tested in order to combine resistances more rapidly. We have good resistance to ring nematode in GRN-1, 039-16 and in all tested members ofour 101-14 x Trayshed population. However, we need to find this trait in an easy to root background. We have streamlined the assay for root-knot nematodes and developed the capacity to test large pool of germplasm and breeding populations at faster pace with reliability.

Phylloxera: Although rootstocks were developed to control phylloxera over one hundred years ago, we still do not fully understand the mechanisms or genetic control of this resistance. From the past 20 years of phylloxera work with Jeffrey Granett, we have determined that biotype B phylloxera was in fact many strains; all capable of damaging AXR#1 rootstock and that the genetic diversity of phylloxera in California is higher than was earlier presumed. We have also determined that resistance to tuberosity development (the damaging galls on mature roots) is genetically very different than resistance to nodosity feeding (generally non-damaging galls on root tips) as derived from *rupestris.* We have developed multiple mapping populations and screening techniques and are currently studying the genetic control of resistance in *riparia* and *berlandieri* at the nodosity level and evaluating nodosity-based strains adapted to 101-14, Freedom and St. George. The genetic map will be developed based on a limited mapping strategy to tag resistance. The overall goal is to better understand root-tip feeding and to ensure new rootstocks have the highest and broadest level of resistance to this sort of feeding, and to develop genetic markers associated with this resistance to enable efficient breeding. As phylloxera is evolving and increasing the risk of emergence of new aggressive types, there is always need to identify germplasm that resist feeding damage to different types of phylloxera. To fulfill this objective, we are maintaining phylloxera lines to test new germplasm to identify resistant accessions that could be incorporated into the breeding program.

Pierce 's disease: Over the past five years, we have been developing PD resistant rootstocks with our PD resistance gene, *PdRJ* (Riaz et al. 2008, 2009), for use with our PD resistant fruiting varieties. These rootstocks will be used to prevent death of the vine if the very low levels of PD bacteria in these scions migrated downwards into a susceptible rootstock. We have crossed *V. arizonica/candicans* b43-17 (source of *PdRJ)* with several GRN rootstock selections and screened these populations for PD resistance. The most resistant have been crossed to commercial rootstocks with a range of horticultural traits from better rooting to vigor control, better intemode length, and limited lateral shoot production. These rootstocks will be used with PD resistant wine, table and raisin grapes. The fruiting scion varieties are now 97% *vinifera,* prevent disease, and greatly suppress the multiplication and movement of PD bacteria.

Fungal diseases: There is not much information on grape species with resistance to young vine decline organisms, armillaria, and phytophthora. We intend to cooperate with Kendra Baumgartner (and hopefully benefit from information generated from a developing SCRI proposal) to establish screening techniques in order to test germplasm for resistance. Once resistant material is identified crosses will be made to integrate fungal resistance into GRN and other elite rootstock backgrounds and to develop mapping populations.

3) Rootstock Influence on Phenology - The *Vitis* species are adapted to seasonal change based on where they originated. *Vitis' riparia* and *riparia-based* rootstocks have a shorter cycle of growth and lose their leaves earlier as a result of the northerly climate and short growing season in which they evolved. Conversely, species like *V. berlandieri,* which evolved in southern latitudes, have a longer cycle of growth and go dormant later. This phenology seems to apply to *vinifera/rootstock* combinations and promotes or delays scion development as a result of root activity. This is a very important trait given California's dry climate, and as a means of adapting to earlier or later harvest. I have been selecting species selections at both ends of the dormancy range and will use these to better understand this character and breed new rootstocks adapted to California's varied climate and grower needs. For example, a late season 420A-like rootstock that roots and grafts well in the nursery or a very early maturity *riparia-like* rootstock that promotes stronger vigor and higher yields for San Joaquin Valley growers.

While the above projects focus on specific viticultural problems that rootstocks may be capable of addressing, my objective is to discover, identify and, through classical breeding techniques, bundle gene complexes that will provide resistance to a broad array of soil-borne problems and adaptation. to California's climates and soils. This strategy should engender the development of a series of next generation grape rootstocks that will assist viticulturists in planting long-lived disease, virus and soil borne pest-resistant vineyards. To achieve this objective, a multiyear source of funds will be required to develop and retain a stable research group dedicated to this process. I welcome the assistance of the AVF Board in providing direction and guidance toward the creation of rootstocks that will allow the California grape and wine industry to prosper.

4) Virus Tolerance/Resistance

At present, only one rootstock exists for control of fanleaf degeneration, the *V. vinifera* x *M rotundifolia* hybrid 039-16. However, it is not resistant to root-knot nematode, difficult to propagate and has questionable phylloxera resistance because of its *V. vinifera* parentage. GRN-1 may also possess the ability to tolerate fanleaf infection, but it will take years of field trials to be convinced. The 101-14 x Trayshed mapping population mentioned in Table 2 is uniquely suited to the development of new fanleaf tolerant rootstocks, with better nematode resistance, strong phylloxera resistance and the possibility of better propagation qualities.

Please see my other A VF submission "Molecular genetic support to optimize the breeding of fanleaf resistant rootstocks" for details on breeding for *M rotundifolia-based* tolerance to fanleaf; the use of genetic markers tightly linked to the *X index* resistance gene, *XiRJ;* and the verification *XiRJ*'s role in dagger nematode resistance.

Many viruses cause graft incompatibility when infected scions are grafted on some rootstocks, and these unstable rootstock/clone/virus combinations are being empirically discovered as vineyards fail. St. George rootstock appears to be relatively tolerant of this group of viruses. Development of effective screening procedure is needed before a wider range of germplasm. We have created populations to study this virus/rootstock interaction and to develop virus-tolerant rootstocks that are more suitable than St. George, which is susceptible to nematodes and overly vigorous on some soils. However, reliable screening techniques are needed to establish to test germplasm. We have initiated the first efforts to develop a rapid tissue culture based screen that relies on observation of the cambium interface to see whether we can detect the early onset of cambium/phloem damage.

ESTIMATED SUCCESS IN **ACCOMPLISHING OBJECTIVES** IN **TIME FRAME:** Rootstock breeding is a continuous process. Our breeding objectives include development of new rootstocks with: resistance to aggressive root-knot nematodes strains; resistance to nematode complexes; resistance to grapevine fanleaf virus and its dagger nematode vector, *X index;* tolerance salinity and drought; ability to control scion growth and fruit maturation; tolerance to viruses that affect ripening and graft unions; resistance to PD; and phylloxera resistance in all backgrounds. The first rootstock cultivars have been certified and were released to nurseries in Spring 2008. They have resistance to aggressive strains of root-knot and dagger nematodes, both singly and in combined tests, and maintain resistance at high soil temperatures (3 0C, where traditional sources of resistance fail). These selections also resist citrus and lesion nematode and one resists ring nematodes.

Resistance or adaptation to these problems exists within the *Vitis* species and many of the crosses we have made will combine several of the various characters into one background. Resistance to most pests and diseases of grape exists in grape species. The constraints to selecting out rootstocks from crosses with these species are adequate funding and appropriate screening techniques.

We have had excellent success developing many genetic maps for resistance to PD, *X index,* phylloxera and powdery mildew, and have more mapping experience across multiple traits than any lab in the world. We are now in position to use our mapping knowledge to greatly expedite the development of genetic maps and markers, as evidenced by our recent development of markers for several fonns of powdery mildew resistance (Riaz et al. 2011).