Host-Parasite Interactions between Avocado Rootstocks and *Phytophthora cinnamomi*

New Project; Year 1 of 1

Project leaders: C. Thomas Chao (909) 787-3441
e-mail: chih-cheng.chao@ucr.edu
Department of Botany and Plant Sciences, University of California Riverside
And
J. Menge (909) 787-4130
e-mail: john.menge@ucr.edu
Department of Plant Pathology, University of California Riverside

Cooperating personnel: R. Cao

Benefit to the Industry

This project will provide information on the heritability of resistance of avocado rootstocks against PRR. This information will indicate how strong is the resistance. If the resistance is strongly inherited, then smaller number of seedlings are needed for selection of resistant cultivars. If the heritability is low, then large number of seedlings are needed for the selection. The study can also estimate the mechanism of resistance in avocado rootstocks that will suggest different approaches for future rootstock breeding and selection.

The study of the diversity of PRR isolates in California will show us how different or similar the PRR isolates are. If the PRR isolates are very similar, then few isolates that represent all PRR groups can be used in future rootstocks screening to ensure the representation of all possible PRR isolates. If the PRR isolates are very diverse, then more isolates should be used for future rootstocks screening experiments. In combination with the study of the pathogenicity of PRR isolates on individual rootstock, if the diversity and pathogenicity are highly correlated, then diversity might be used as indexes for pathogenicity. In the future, PRR isolates can be characterized into different diversity based on quick AFLP analysis and their pathogenicity on different rootstocks can be inferred. PRR isolates from the future isolation can be easily compared with current PRR isolate collection. If the new isolates have similar AFLP profiles, it is most likely that their pathogenicity on different rootstocks will be similar to known isolates. If any new PRR isolates have different AFLP profiles, then it is necessary to test their pathogenicity on a set of rootstocks to ensure that potentially they will not overcome all available resistance. All the information will greatly influence the rootstocks breeding program. Do we need to breed resistant rootstocks against very similar PRR isolates or very diverse PRR isolates? If the PRR isolates are very different in their AFLP profiles and pathogenicity, it might suggest that the PRR populations could change quickly. If PRR populations can change quickly, should we develop a single highly resistant rootstock for the whole industry or should we develop different rootstocks with different resistance genes? Multiple resistant rootstocks with different resistance genes will impose less selection pressure on the PRR populations that in term can ensure the durability of the rootstocks.

The study of resistance of individual rootstock against different PRR isolates would show us how many different resistance genes we are dealing with. Are the resistance genes in one rootstock the same as those in another rootstock? If they are different, then appropriate crosses should be made between the two resistant rootstocks to breed rootstocks with higher resistance. This study will indicate which crosses should be made. We will also have the proper set of PRR isolates (based on parental rootstocks inoculation information) to test progeny for their resistance.

Objectives

The overall objective of this research is to study the interaction between avocado rootstocks and Phytophthora root rot isolates in California. There are three specific objectives for this part of the study. An additional objective studying the Phytophthora collar rot isolates in California was added for 2000 - 2001 proposal.

- Study the inheritance of the control of resistance in avocado rootstocks against PRR in collaboration with the avocado rootstock breeding program of Dr. John Menge.
- Study the diversity of PRR isolates from California using Amplified Fragment Length Polymorphism (AFLP) markers and to separate the PRR isolates into different diversity groups.
- Inoculate PRR isolates from different diversity groups on individual rootstock and to determine the pathogenicity of individual PRR isolate and the resistance of individual rootstock.
- Study the diversity of Phytophthora collar rot isolates in California using the AFLP markers (new objective for 2000 - 2001).

Summary of Proposed Research

Objective (1): The following questions were asked in the 1999-2000 proposal:
What is the heritability of resistance against PRR?
How high or low is the heritability?
What type of genetic mechanisms might control the resistance in avocado rootstocks, additive or dominance?

In cooperation with Dr. Jonh Menge's avocado rootstock breeding program, the results of rootstock evaluation from 1992 - 1999 was analyzed. A total of 24,427 open pollinated progeny from 22 rootstocks were used for the analysis. The result of half-sib analysis of open pollinated avocado rootstock populations for resistance against PRR is listed in Table 1. The narrow sense heritability estimate based on analysis of all seedlings from 1992-1999 is 0.21. The narrow sense heritability estimates for 1992-93, 1993-94, 1994-95, 1995-96, 1996-97, 1997-98, 1998-99 are 0.14, 0.33, 0.44, 0.15, 0.13, 0.73, and 0.67, respectively. The difference in the estimates may due to different OP families were used for analysis and difference in isolates used for the inoculation in different years. Materials with higher PRR resistance such as Spencer and UC2001 have been incorporated into the analysis in the past 2 years. The incorporation of these higher resistant materials can increase the narrow sense heritability estimates. These estimates are similar to the estimates of other traits of avocado in some year and higher in some other year (Lavi et al., 1993). These estimates are lower than the narrow sense heritability estimates, 0.74-0.85, of

resistance in Jarrah trees against *P. cinnamomi* (Stukely and Crane, 1994) and 0.79 of resistance in Radiata pines against *P. cinnamomi* (Butcher and Stukely, 1997).

Additive genetic variance is the major component of the total genetic variance. This result suggests that the best approach to increase the resistance in avocado rootstocks against PRR through is to screen as many progeny as possible. The estimates of narrow sense heritability are most likely to be biased upward due to the population structures (Squillace, 1974; St Clair and Adams, 1991). All OP progenies came from isolated blocks of avocado rootstocks. A portion of the OP progenies may share the same pollen sources, and they could be full-sib progenies instead of half-sib progenies. Therefore, the real narrow sense heritability should be lower than the estimates.

The lack of information on the genetic control of resistance in avocado rootstocks against PRR could be due to several reasons: the long life cycle of avocado trees, the difficulties of making controlled crosses, and the lack of information about the pathogen populations and their pathogenicity on individual rootstocks. The difficulties in making crosses make the use of OP families for genetic analysis the easiest approach. Screening and evaluation of progeny in the greenhouse and at early seedling stage does not allow us to evaluate the genetic x environment interaction and its effect on the expression of the resistance in the field. The lack of estimation of the genetic x environment interaction and lack of information of the genetic structures of the pathogen populations may account the difficulties of developing avocado rootstocks with very high level of resistance against Phytophthora root rot in the field. Isolates of *P. cinnamomi* have been reported to have different level of pathogenicity on chestnut, northern red oak, pine, and eucalyptus (Robin and Desprez-Loustau, 1998). Progress, however, is being made in the past few decades. With more understanding of the genetics of resistance in the rootstocks and the genetics of the pathogen populations, we should be able to breed avocado rootstocks with high resistance to Phytophthora root rot in the future.

Objective (2): Study the diversity of PRR isolates from California using Amplified Fragment Length Polymorphism (AFLP) markers and separate the PRR isolates into different diversity groups.

As of 9/15/2000, the DNA of 26 isolates of *P. cinnamomi* and *P. citricola* have been isolated (listed in Table 2). Dr. Menge is continuing the survey of Phytophthora root rot of avocado and more samples will be used for this study. The development of AFLP protocol for *Phytophthora* spp. is much more difficult task than AFLP protocol for plant species. Progress has, however, been made in the last 6 months. Initially, we used silver staining for visualization of AFLP markers. We switched to LI-COR IR2 automated sequencers two months ago for more detail and better analysis. The AFLP profiles of 17 isolates are shown in Figure 1. The AFLP protocol includes the following steps: restriction digestion of genomic DNA, ligation to adapters to genomic DNA, pre-amplification reactions, and selective-amplification. The PCR products are then separated on 6.5% polyacrylamide KB^{Plus} gel with ammonium persulfate (APS) and TEMED. Both IRD800 and IRD700 markers were loaded for fluorescent labeling. The relationship among of 17 Phytophthora isolates based on the preliminary data of polymorphism of AFLP markers by UPGMA analysis is shown in Figure 2.

Objective (3): Inoculate PRR isolates from different diversity groups on individual rootstock, and determines the pathogenicity of individual PRR isolate and the resistance of individual rootstock.

One hundred trees of Thomas, Duke 7, and Toro Canyon rootstocks, 300 total, have been ordered through Brokaw Nursery. These trees will be transferred to UCR for future inoculation experiments. Additional avocado rootstocks from other regions, such as VC241, VC245, VC256 (Israel), UC2014 (W-14 South Africa), Evstro (South Africa), Gordon (South Africa), Dusa (South Africa), Latas (South Africa), Poly-N (polyploid from UCLA), or PP4 (Zentmyer, maternal parent Barr Duke) will be propagated in the next season for future inoculation experiments. Currently, there is no enough budwood for all the propagation need.

Table 1. ANOVA, Type III expected mean squares, estimates of narrow sense heritability (hns2), stander error of estimates (SE), and OP families of avocado rootstocks used for Phytophthora root rot inoculation.

Year	Sources	df	Mean squares	Expected M.S.	h_{ns}^2	OP families
92-99	Female	21	4371.32**	$*^2_e + 942.6\ *^2_F$	0.21	All families.
	Error	24405	81.47	$*^2_e$		
92-93	Female	5	1272.99**	$*^2_e + 425.83\ *^2_F$	0.14	BarrKuke, D9, Toro Canyon, Thomas, UC2001.
	Error	3978	80.22	$*^2_e$		
93-94	Female	6	4569.36**	$*^2_e + 735.07\ *^2_F$	0.33	BarrDuke, Borchard, D9, G6, Toro Canyon, Thomas.
	Error	5597	68.84	$*^2_e$		
94-95	Female	11	1814.40**	$*^2_e + 341.6\ *^2_F$	0.44	BarrDuke, D9, Duke7, G1024, G1033, G6, Toro Canyon, Thomas, UC2011, UC2020, UC2054.
	Error	4261	42.15	$*^2_e$		
95-96	Female	11	188.67**	$*^2_e + 169.22\ *^2_F$	0.15	D9, Evstro, G1038, G3-71, G6, KiddDuke, PolyN, Rollie, Toro Canyon, Thomas, UC2001.
	Error	2156	24.60	$*^2_e$		
96-97	Female	6	1153.21**	$*^2_e + 540.37\ *^2_F$	0.13	BarrDuke, D9, G6, Toro Canyon, Thomas, UC2001.
	Error	3529	60.77	$*^2_e$		
97-98	Female	10	783.03**	$*^2_e + 212.75\ *^2_F$	0.73	BarrDuke, CR171, D9, Duke7, G6, G810, Spencer, Toro Canyon, Thomas, UC2001.
	Error	2361	78.14	$*^2_e$		
98-99	Female	7	11605.97**	$*^2_e + 330.19\ *^2_F$	0.67	BarrDuke, D9, Duke7, G6, Spencer, Thomas, UC2001.
	Error	2489	170.78	$*^2_e$		

(**=P,0.01)

Table 2. Strain number and description of Phytophthora cinnamomi and *P. citricola* used in the experiment.

Strain #	**Description (or original records)**
M221	*P. cinnamomi - avocado main root (bark), Ventava, Azia Alizadeh, 6/91*
M253	*P. cinnamomi - avocado roots, San Diego Co., Nursery, Gary King, 4/93*
M254	*P. cinnamomi - avocado roots, A2 Mating type, San Diego Co., nursery, Gary King, 4/93*
M262	*P. cinnamomi - avocado roots, San Luis Rey, S.D.Co., 10/94*
M280	*P. cinnamomi - avocado roots, Ventura, 6/95 (hyphal tip)*
M281	*P. cinnamomi - avocado roots, Ventura, 6/95 (hyphal tip)*
M282	*P. cinnamomi - avocado roots, Ventura, 6/95 (hyphal tip)*
M283	*P. cinnamomi - avocado roots, Ventura, 6/95 (hyphal tip)*
M293	*P. cinnamomi - avocado roots, ProAg, Don Lee Ranch, Somis, Ventura Co., 5/96*
M295	*P. cinnamomi - Vanoni Ranch, Somis, CA, Ventura Co., Jim Downer, 11/96*
M316	*P. cinnamomi - avocado roots, Cavaletto Ranch, San Luis Obispo, 4-2000*
#1	*P. cinnamomi - Powell Ranch Tree 1 - S.D.Co., 4-17-00*
#12	*P. cinnamomi - Vanoni, Tree 12, Ventura, 6-25-99*
GB629a	*P. cinnamomi* - Walnut, Los Molinos, CA, Leachman, Soil, 9/6/96
GB647	*P. cinnamomi* - Walnut, Merced, CA, Crane, Roots, 9/19/96
GB661	*P. cinnamomi* - Walnut, Merced, CA, Crane, Soil, 12/27/96
GB1539	*P. cinnamomi* - Walnut, Modesto, CA, Boone, Roots, 1/28/98
GB1543	*P. cinnamomi* - Walnut, Modesto, CA, Heinrich, Roots, 1/28/98
GB1758a	*P. cinnamomi* - Walnut, Belota, CA, Eilers, Soil, 1/6/98
GB2102	*P. cinnamomi* - Blueberry, Roots, 6/4/98
GB2448	*P. cinnamomi* - Oak, Dendrotech, Bob Gross, Soil, 2/1/99
GB2905	*P. cinnamomi* - Walnut, Stockton, CA, Origone, Roots, 9/30/99
M218	*P. citricola - avocado bark, Ventava, Aziz Alizadeh, 6/91*
M317	*P. citricola - Moro BLK D roots, 4-6-00*
M318	*P. citricola - Cavaletto Nipomo roots, 4-6-00*
M319	*P. citricola - Nipomo BLK 3 roots, 4-6-00*

Figure 1. AFLP marker profiles of 17 Phytophthora isolates, with primer set E-TA + M-T.

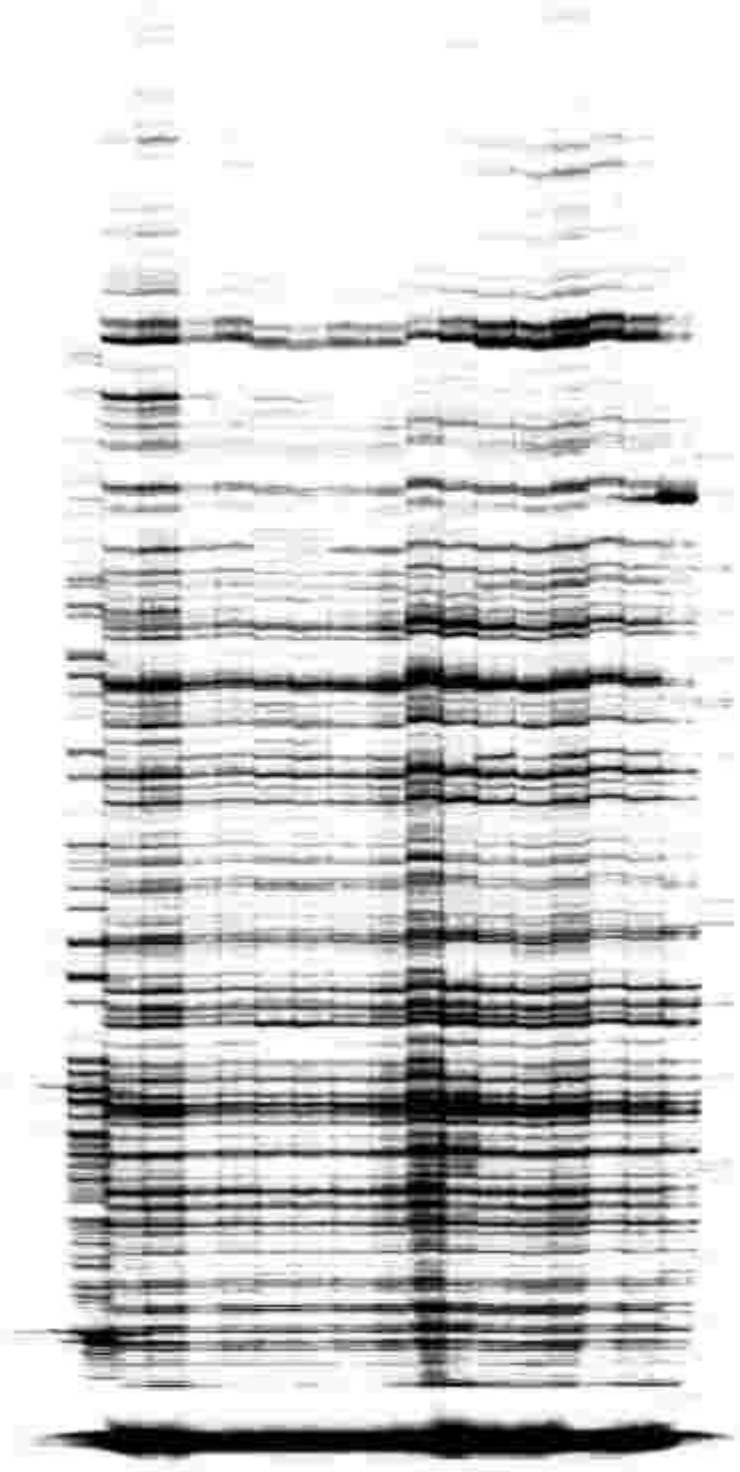

Figure 2. Preliminary UPGMA analysis of Phytophthora isolates based on polymorphism of AFLP markers.