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

Continuing Project, Year 3 of 3, 2001 -2002

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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:

(1) Study the inheritance of the control of resistance in avocado rootstocks against Phytophthora Root Rot (PRR).
(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.

(3) Inoculate PRR isolates from different diversity groups on individual rootstocks, and determines the pathogenicity of individual PRR isolates and the resistance of individual rootstocks.
(4) Study the diversity of Phytophthora collar rot isolates in California using the AFLP markers. The AFLP marker study will determine the diversity of Phytophthora collar rot isolates and the relationship between different isolates throughout California.

Summary:

Objective (1):

Study the inheritance of the control of resistance in avocado rootstocks against Phytophthora Root Rot (PRR).

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 (Chao et al., 1999). 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.

A total of 26 isolates of *P. cinnamomi* and *P. citricola* have been isolated (listed in Table 2). The AFLP analyses of *P. cinnamomi* were accomplished for almost all the *P. cinnamomi* isolates within the UCR *Phytophthora* collections. We have screened 40 combinations of primer sets for the AFLP markers on two isolates of *P. cinnamomi*. Not all the primer set combination worked on all these two isolates. Some primer set combinations worked on one isolate, but they do not work well on other one. A total of 14 primer set combinations had been identified to work on both the *P. cinnamomi* isolates. Four out of those 14 primer set combinations worked on all *P. cinnamomi* isolates tested. We applied these 4 primer sets on all our *P. cinnamomi* isolates. The genetic relationship between different *P. cinnamomi* based on the UPGMA analyses of the AFLP polymorphisms are shown in Figure 1.

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.

We are continuing the inoculation experiments with selected isolates of *P. cinnamomi* based on the results from the AFLP marker study.

Objective (4).

Study the diversity of Phytophthora collar rot isolates in California using the AFLP markers.

The AFLP marker study will determine the diversity of Phytophthora collar rot isolates and the relationship between different isolates throughout California.

We finished the AFLP analyses of the 10 *Phytophthora citricola* isolates listed above. Isolates M214 is most distant from all other isolates based on the polymorphism. We were able to differentiate 8 isolates except M215 and M216. The dendogram of genetic similarity of *P. citricola* isolates is shown in Figure 2. Isolates M214, M215, M216, M219, and M220 were isolates from the same location (Table 2), however, only M215 and M216 are identical. These two isolates are closely related to isolate M265, an isolate from South Coast Research and Extension Center. Overall, the isolates are very diverse based on the AFLP polymorphisms.

Last year, we also finished a study of 39 avocado scions and rootstocks using AFLP markers and the results were very interesting. Among the 12 VC rootstocks we tested, we were able to identify 6 were hybrid origin, possibly West Indian x Mexican (VC26, VC28, VC51, VC66, VC802, and VC803), not pure West Indian. These 6 hybrid VC rootstocks may have more cold resistance and Phytophthora root rot resistance than pure West Indian rootstocks and may be more suitable for California production. We also identified two rootstocks, Dusa and Evastro from South Africa, to be hybrid origin instead pure Mexican.

Table 1. ANOVA, Type III expected mean squares, estimates of narrow sense heritability (h_{ns}^2), 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,
	Error	4261	42.15	$*^2_e$		Toro Canyon, Thomas, UC2011, UC2020, UC2054.
95-96	Female	11	188.67**	$*^2_e + 169.22 *^2_F$	0.15	D9, Evstro, G1038, G3-71, G6, KiddDuke, PolyN,
	Error	2156	24.60	$*^2_e$		Rollie, Toro Canyon, Thomas, UC2001.
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,
	Error	2361	78.14	$*^2_e$		Toro Canyon, Thomas, UC2001.
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)**
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)*
M283	*P. cinnamomi – avocado roots, Ventura, 6/95 (hyphal tip)*
M295	*P. cinnamomi –Vanoni Ranch, Somis, CA, Ventura Co., Jim Downer, 11/96*
#12	*P. cinnamomi – Vanoni, Tree 12, Ventura, 6-25-99*
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
M214	*P. citricola – avocado bark, Ventava, CA., Aziz Alizadeh 6/91*
M215	*P. citricola – avocado secondary root, Ventava, CA., Aziz, Alizadeh, 6/91*
M216	*P. citricola – avocado bark, Ventava, CA., Aziz, Alizadeh, 6/91.*
M218	*P. citricola – avocado bark, Ventava, Aziz Alizadeh, 6/91.*
M219	*P. citricola – avocado wood (root), Ventava, CA., Aziz Alizadeh, 6/91.*
M220	*P. citricola – avocado secondary root (main), Ventava, CA., Aziz Alizadeh, 6/91.*
M266	*P. citricola – avocado canker, South Coast field station, T13, 12/94.*
M265	*P. citricola – avocado canker, South Coast field station, T5, 12/94.*
M285	*P. citricola – avocado trunk canker, Keeavo grove, Fallbrook, CA., 9/95.*
M318	*P. citricola*
M319	*P. citricola*

Figure 1. UPGMA analysis of Phytophthora cinnamomi isolates based on polymorphism of AFLP markers.

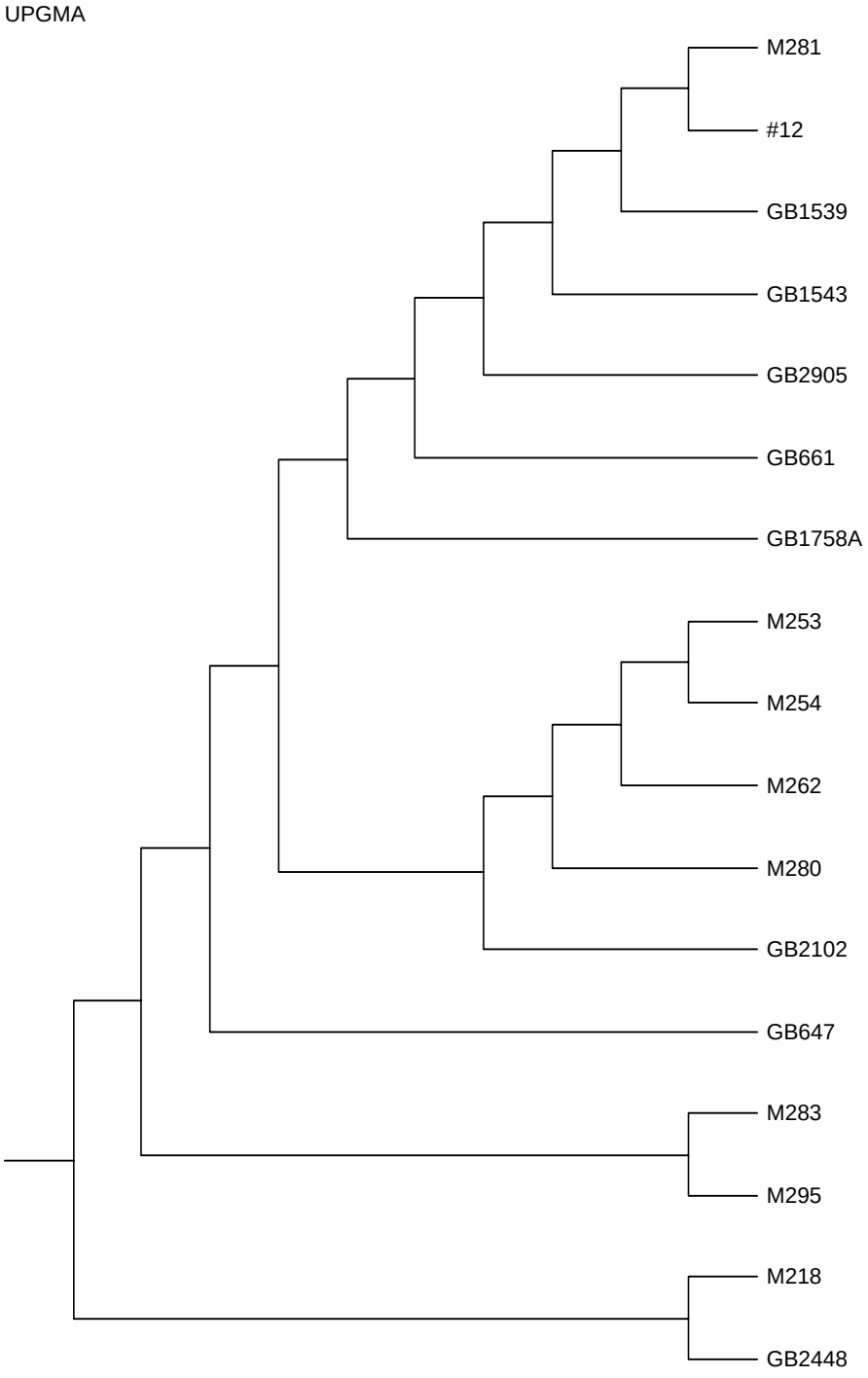

Figure 2. UPGMA analysis of Phytophthora isolates based on polymorphism of AFLP markers.