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Current and Future Perspectives Regarding Avocado Rootstock Breeding at UCR

Abstract

Phytophthora cinnamomi, the causal agent of Phytophthora Root Rot (PRR) of avocado, is the most serious disease of avocado worldwide. The use of resistant rootstocks to control PRR is an ultimate goal for the rootstock breeding program at UCR, which is one of the most advanced in the world. The program has been important to the avocado industry by testing and or developing most of the PRR tolerant rootstocks in current use today. Avocado yield, tree growth, and fruit quality will all increase dramatically if PRR is controlled effectively. More importantly, profitability will increase for individual growers. By integrating our current historical approach to rootstock breeding with newer molecular methodologies, we will be able to advance rootstock development by eliminating selfed progeny from the program, maximize specific genetic crosses by setting up new breeding blocks

after determining parental relationships within blocks, determine the genetic relationships among the various rootstocks, and potentially find markers associated with PRR resistance and or other important agronomic traits so that marker assisted selection will be used in avocado rootstock development in the future.

Introduction

Avocado (*Persea americana* Miller) is a significant and nutritious fruit crop grown in both the tropical and subtropical regions in many parts of the world. World production of avocados in 2004 was estimated at approximately 3.5 million tons with the world leader being Mexico followed by Indonesia, United States, Columbia, Brazil, Chile, Dominican Republic, Peru, China, and Ethiopia with exports alone valued at $605.74 million (Evans and Nalampang 2006). Within the United States, California dominates avocado production (~90%) and in the 2007-2008 season the crop was valued at $327,141,689 (CAC annual report, www.avocado.org).

Three botanical races of *P. americana* have been domesticated from their putative centers of origin; the Mexican race (*P. americana* var. *drymifolia*), the Guatemalan race (*P. americana* var. *guatemalensis*), and the West Indian race (*P. americana* var. *americana*). Each race possesses distinct agronomic characteristics such as productivity, general shape, taste and color of fruit, timing and length of fruit set, cold hardiness, disease resistance, and salinity tolerance being amongst the most important traits within a race. However, cross fertility amongst and within the botanical races has led to extensive genetic variability within the 'species' and many extant cultivars are racial hybrids, thus possessing variable characteristics. From a breeding prospective this is a good situation, especially when developing rootstocks with desirable characteristics since both the scion and rootstock are routinely clonally propagated.

Avocado Root Rot and Rootstock Breeding

With respect to rootstock breeding, the most important agronomic trait is resistance or tolerance to *Phytophthora cinnamomi*, the most serious disease of avocado worldwide.

The disease has actually eliminated commercial production in many areas in Latin America and is the major limiting factor of production in Australia, South Africa, and California (Ploetz et al. 2002). The use of resistant rootstocks to control Phytophthora Root Rot (PRR) of avocado has long been proposed as the ultimate method for controlling the disease, which affects 60-75% of California groves and can cause estimated losses of over $30 million per year. The UCR program for selecting and breeding PRR resistant rootstocks is one of the most advanced in the world and has been important to the avocado industry by testing and or developing most of the rootstocks in current use.

Zentmyer (1957, 1963, 1980) was the first proponent of using rootstocks to control the disease. Zentmyer, with the help of G. Schieber of Guatemala, amassed a large collection of wild avocados from around the world in the 1950s and 1960s. By 1995, nearly 80 species of avocado ranging from the southern United States to Chile and the Caribbean Islands had been collected and screened for resistance to PRR. Many species such as *Persea alba, P. caerula, P. chrysophylla, P. schiedeana, P. donnell-smithi, P. borbonia, P. pachypoda, P. liebmani,* and *P. cinerascens* were found to exhibit a very high degree of resistance to root rot. Unfortunately, none of these rootstocks was graft compatible with the commercial *P. americana* and all attempts to use interstocks with these rootstocks failed. Zentmyer also screened a large variety of *P. americana* types and discovered the 'Duke' cultivar which was brought from Mexico to Oroville California in 1912 (Zentmyer, 1963). Two seedlings from this cultivar, the 'Duke 6' and 'Duke 7', were found to be partially resistant to PRR, and in 1975 'Duke 7' became the first commercial rootstock that was tolerant to *P. cinnamomi*. It was highly successful and has been used worldwide to combat PRR (Zentmyer, 1980). With the success of Zentmyer's program, similar screening programs also became established in Israel (Ben-Ya'acov and Michleson, 1995), South Africa (Kremer-Köhne et. al., 2001), Canary Islands (Gallo-Llobet, 1992), Florida (Ploetz et al., 2002) and Australia (Sedgley and Alexander, 1983).

In order to use resistant rootstocks, they must be clonally propagated so they contain the same genetic identity as the parent plant. Heritability of resistance traits in avocado is generally less than 1% (Coffey, 1992). Therefore, seedlings produced from seeds gathered from resistant or tolerant trees usually show little resistance, which is why 1,000's of seeds must be screened annually. In most cases the mechanisms for resistance are not yet fully understood. However, three mechanisms are thought to reduce PRR in *P. americana*; 1) reduced root exudates to attract *P. cinnamomi* zoospores (Botha and Kotzé, 1989), 2) rapid root regeneration (Gabor and Coffey, 1990), and 3) root systems that grow deeper in the soil which are thought to 'escape' inoculum of *P. cinnamomi* since most avocado cultivars have shallow root systems (B. Faber per com). The chemical borbonol is also thought to be a potent antifungal agent in tissues of resistant species of *Persea* but the susceptible cultivar 'Topa Topa' also produces this compound so the role of this chemical is not fully understood (Zaki et al., 1980). Most of these mechanisms probably act in tandem and are likely controlled by several genes, making it difficult to manipulate the resistance 'gene(s)' using modern genetic techniques. None of the rootstocks identified so far is able to withstand infections by *P. cinnamomi* when inoculum levels are extremely high which is why several other methods of control must be used in conjunction with tolerant rootstocks in order to control the disease effectively under these conditions. However, the ultimate goal of any program is to eventually find a rootstock that is truly resistant to *P. cinnamomi*.

Another potential problem related to breeding is that rootstocks may not perform well under all avocado growing conditions, and some like 'Thomas' and 'G755' may not yield as well as other rootstocks when *Phytophthora* is not present (Menge, per com). Salinity, cold, climate, nutrient uptake and other diseases further confuse the issues and makes breeding rootstocks difficult and no rootstock produced thus far will perform well under all conditions. This is why Australia prefers Guatemalan varieties, Israel prefers West Indian varieties and California uses Mexican varieties (Ben Ya'acov and Michleson, 1995) or racial hybrids are used. However, it appears that when a well-adapted rootstock is used in conjunction with other horticultural and chemical control methods, the trees can survive in the presence of *P. cinnamomi* but the control measures are costly (Menge et al., 1992). Now that characteristics of specific rootstocks are becoming evident from a large existing gene pool, the era of incorporating various beneficial characteristics into rootstocks through the breeding process is possible, and it may be the best way to improve yield and tree performance of a given cultivar like 'Hass' (Ben Ya'acov and Michleson, 1995).

Avocado Flowering System and its Implications for Breeding

Persea americana possesses a unique flowering mechanism termed diurnally synchronous protogynous dichogamy where male and female structures of the flowers function at different time periods (Davenport, 1986). Each cultivar or selection can be classified into one of two flowering types, termed Type A or Type B. The flowers of Type A cultivars open in the morning and function as females. They then close and reopen the next day in the afternoon and function as males which corresponds to an approximate 36 hour cycle. Type B cultivars exhibit the exact opposite behavior but in a 24 hour cycle and not 36 hour cycle. This system is thought to have evolved to promote outcrossing within the species as discussed below (Borrone et al., 2008). However, whether self or cross-pollination events occur within a single avocado tree, only extremely small percentage of flowers, generally less than 0.1%, will actually set fruit which complicates breeding matters. Additionally, hand pollination is impractical for a breeding program due to the high cost and low fruit set in return. For example, it has been estimated that for every thousand flowers hand pollinated, only one flower on average may produce a mature fruit (Torres et al. 1986). However, Alcaraz and Hormaza in this issue of the Yearbook report on a much higher success rate of hand pollination than most previously published studies.

The realization that avocado possessed this unique flowering mechanism resulted in researchers exploring the possibility of increasing yield by inter-planting cultivars with the opposite flowering type within a grove. However, results from different studies have been debatable as discussed by Davenport (1986). For example, Robinson (1933) noted that some Florida growers were obtaining good fruit set in solid plantings of some cultivars yet inter-plantings were still being recommended in the late 1930's (Davis, 1939). Around this same time period in California, many growers continued to use single cultivars planted into blocks because of the work of Clark (1923, 1924) and Clark and Clark (1926) who demonstrated that caged trees set as much fruit as those that were left to be open pollinated by flying insects. However, in the late 1950's, Bergh and Gustafson (1958) suggested that cross-pollination may be more important than previously thought to increasing fruit set in California. However, even today there is no definitive evidence that inter-plantings are the best choice and some California growers still do not use this practice while others still do (Mary Lu Arpaia, per com). Conflicting results within and between studies is likely due to the significant differences between cultivars with respect to floral initiation, behavior, and pollen-tube growth responses that are likely tied to micro and macro-environmental sensitivities (e.g., Borrone et al., 2008). However, research still continues to this day on this topic. For example, Alcaraz and Hormaza (2009) found that the use of three type B cultivars, 'Fuerte', 'Nobel', and 'Marvel', inter-planted among 'Hass', increased the chances of fruit set by increasing the overlap in the flowering cycle between 'Hass' and the other cultivars since different flowering phenology was observed between the type-B cultivars.

The Use of Molecular Markers in Studying Pollination Biology and Breeding in Avocado

The development of molecular methods in the study of avocado pollination biology has not resolved the debate concerning the merits of inter-plantings but has been used almost exclusively to determine the levels of inbreeding versus outcrossing. The first molecular marker system used in avocado was isozymes (Torres et al., 1978). Torres and Bergh (1978) evaluated the isozyme patterns from seedlings of 'Pinkerton' planted in a solid block but unprotected from pollination by other nearby cultivars. They found that 'Pinkerton' seedlings, resulting from self-pollination, accounted for 2 to 69% of the total seedlings. Degani and Gazit (1984) examined seedlings from caged pairs of six cultivars and found that the percentage of seedlings that were a product of self pollination ranged from 8 to 93%, four of the six cultivars demonstrated a tendency to self-pollinate, and there was no apparent correlation between percentage selfing and fruit set. Studies using isozymes have also determined that some cultivars are better pollinizers than others. For example, Sulaiman et al. (2004) found that a minimum of 46% and a maximum of 85% of embryos from the variety 'Gwen' were pollinated by the variety 'Ryan' in Australia. This was true even in cases where a 'Ryan' donor was up to 50 meters away from a 'Gwen' tree that was surrounded by other varieties.

Several additional early studies using isozymes and randomly amplified polymorphic DNA markers - RAPDs (Kobayashi et al., 2000) found similar results; some level of outcrossing was always found, but the rates varied according to location, design of the orchard, developmental stage of the fruit sampled, and the cultivars investigated (e.g., Borrone et al., 2008). However, most of the earlier studies were based on marker systems with limited amounts of polymorphisms that limited the level of genotypic resolution and statistical power. More recently, more powerful microsatellite markers have been developed for avocado that is increasing our ability to investigate the breeding system within avocado more thoroughly (Ashworth and Clegg, 2003; Ashworth et al., 2004; Borrone et al., 2007). For example, Ashworth et al. (2007) found that almost all progeny (203 of 204) from a 'Gwen' mother tree were the result of outcrossing in California and Borrone et al. (2008) found that 74% and 96% progeny from 'Simmonds' and 'Tonnage', respectively, were the result of cross-pollination in a block of avocado trees grown in Florida. The latter study also suggests that controversies in Florida during the 1930's concerning inter-plantings versus solid block plantings may have not been well founded and highlights the power of newer molecular techniques to study pollination biology. There are now over 300 microsatellite loci available for avocado (R. Schnell, per com) to investigate genetic diversity and genetic relationships among avocado rootstocks. These molecular techniques will be powerful tools to more fully understand the avocado breeding system, explore genetic relationships among avocado rootstocks/scions, and will inevitably aid in improving avocado breeding in the future.

Overview of the Current UCR Avocado Rootstock Breeding Program

A summary of results for the current rootstock research program funded by the California Avocado Commission over the past 20 years will be published in the 2010 Yearbook since the original project proposal, submitted by Dr. John Menge, is ending next year. For the purposes of this paper, my goal is to provide some overall perspectives about the history or the program, some of the current progress, and how the program can advance in the future.

All material that comes through the rootstock-breeding program at UCR is first tested under greenhouse conditions for an initial screening of approximately two years. Selected avocados from our various breeding blocks (described below) are collected and seeds are planted into vermiculite beds (36x36x15cm) with 36 seeds to a bed. At the 5th or 6th week the "mother" seeds are removed from the plants so that the plants cannot rely on nutrient reserves from the seeds once the plants are inoculated with *P. cinnamomi*. The seedlings are allowed to grow for a total of 8 to 9 weeks prior to the first inoculation with *P. cinnamomi* colonized millet seeds and the inoculum levels used throughout the screening process are very high so that weak plants are screened out quickly. Around the 16th week the seedlings are removed from the vermiculite and visually rated for percentage of healthy roots remaining. Seedlings that have 70% or more healthy roots are retained for further testing. Seedlings that make it through the first round of selection are then transplanted into half gallon pots using an inoculated 50/50 mix of vermiculite and special UC mix (0.38 m^3 #30 silica, 0.38m^3, Peat Moss, 1.70 kg Dolomite, 0.11kg KNO_3, 1.1 kg Phosphate). Each plant is labeled with the parent tree and field location where the seed was collected. The plants are grown for an additional 8 to 9 weeks before the second evaluation. Dead and weak plants are discarded while the strong plants are re-inoculated and transplanted into one gallon pots. This process is repeated every 6 to 8 weeks until usually 10 or fewer plants remain at the end of the year, each time adding *P. cinnamomi* inoculum to the soil. The final plants are tagged with a metal tag and given an advanced selection number. Since 1989, over 55,000 seeds have been screened using this method resulting in more 90 selections that have been given advanced numbers using this approach (Table 1).

The selected advanced lines are then allowed to grow in the greenhouse until enough budwood is available to create a healthy non-inoculated mother tree grafted to a seedling. Two trees from each selection are finally planted into a field plot at the UC South Coast Research and Extension Center in Irvine, California, (SCREC) and are grown for 2 to 3 years until enough budwood can be collected without causing damage to the tree. Budsticks are collected in late winter to early spring and given to commercial avocado nurseries to produce clonal rootstocks that are budded with a 'Hass' scion for further field testing.

Current Breeding Blocks

Our main germplasm collection of rootstocks is grown at the SCREC as previously mentioned. This collection consists of a large bock of trees containing two trees of each advanced selection and represents a single large breeding block from which we annually collect and screen seedlings for PRR. This germplasm collection currently has over 300 different cultivars/selections and all of our approximately 100 advanced selections that have resulted from the current program, which began in the early 1990's. On the UCR campus, we currently have 9 breeding blocks which are consistently producing fruit and we have planted one new block last year with some of our best current PRR tolerant selections which will not produce fruit for 3-4 years (Table 2). Similar breeding blocks with specific selections will be planted on the UCR campus this coming year since we now have a better understanding of the genetic diversity of our collection, as described below. These new breeding blocks will have some of our best advanced selections as well as specific selections that tend to produce fruit better than others, which will provide more germplasm for the future to screen.

The Future of Rootstock Breeding at UCR

A schematic diagram for the historical and proposed future research approach is outlined in Figure 1. By integrating the historical approach to rootstock breeding with newer molecular methodologies, we will be able to advance the program by 1) eliminating selfed progeny from the program, 2) maximize genetic crosses by setting up new breeding blocks after determining parental relationships within blocks, 3) determine the overall genetic relationships among the various rootstocks, and 4) to potentially find markers associated with PRR resistance and or other important agronomic traits so that marker assisted selection will eventually be used in avocado rootstock development. Marker assisted selection is also a future goal for the scion breeding program run by Dr. Mary Lu Arpaia and Dr. Harley Smith (UC Riverside) in collaboration with Dr. Michael Clegg (UC Irvine).

To move the rootstock breeding program forward in the future, it will be important to know if one or more of our tolerant rootstocks are preferentially the pollen donor(s) so breeding blocks can be set up to maximize genetic exchange among all of the best tolerant rootstock varieties/selections and to eliminate duplicate accessions which could have resulted from selfing. We have recently finished genotyping over 80 advanced selections using molecular techniques and have found that none of our advanced selections were the result of selfing and there is a great deal of genetic diversity among our rootstocks. Figure 2 shows a subset of this data as an example. Now that the genetic diversity among many of our advanced lines is known, we can use this knowledge to make decisions regarding which varieties to field test and it will also enable us to set up breeding blocks with diverse rootstocks in order to try and pyramid resistance within newer accessions. For example, if only 3 rootstocks could be chosen to field test, it would make more sense to choose rootstocks that are distributed throughout the phylogeny (more genetically diverse) such as ‘Latas’, ‘Dusa’, and PP24 as opposed to choosing very closely related advanced lines such as PP19, PP61, PP84 (Fig. 2). This same logic will also be followed when setting up new breeding blocks because there is likely a higher probability that different mechanisms of resistance/tolerance would be inherited into new accessions if the parental genotypes are diverse rather than very closely related. For example, there is likely a high probability that PP4 (Zentmyer) and PP24 (Steddom) share a similar mechanism for resistance since they are more closely related to one another than to any of the other advanced selections tested to date (Fig. 2). In contrast, the mechanism of resistance found in 'Latas' is likely different than that found in more distantly related varieties. However, even after decades of research, very little is known about the molecular mechanisms that infer resistance against *P. cinnamomi* (Cahill et al., 2008). Moreover, no gene-for-gene interactions have been determined for *P. cinnamomi* in any of the hundreds of hosts of this pathogen. Therefore, resistance appears to be polygenic and likely very complex so that at this point in time, the best approach is to integrate traditional breeding with newer technologies to advance rootstock breeding. However, future research also needs to be directed specifically at trying to understand the resistance mechanisms in avocado against *P. cinnamomi* at the cellular and genetic level.

As outlined in Figure 1, one of the key features of the current program at UCR is to consistently select the best varieties that show tolerance to root rot and continually plant them into breeding blocks, which are continually evaluated and replaced as better rootstocks are found. The objective is to then select progeny from these blocks and screen them for PRR resistance under greenhouse conditions for up to two years as described above. Rootstocks that make it through this process are then saved as clonal germplasm and eventually field tested with a 'Hass' scion grafted to the clonal rootstock material. Currently, there are over 90 advanced lines but only around half have been field tested to any capacity. Many more selections still need to be field-tested and one of the limiting factors for the current program is finding enough avocado grower collaborators with suitable land to test these rootstocks. Therefore, I encourage any potential collaborators to contact me directly if you would like to participate in field-testing these advanced selections. In order to participate, we require enough space to plant approximately 200 trees on root rot infested soil and we will need to be able to collect yield data for at least 5 years.

The overall breeding strategy of this program appears to be working well because more and more advanced lines are making it past the first round of selection compared to earlier in the program. For example, 618 advanced lines made it through the first round of selection from 2004-2008 whereas from 1999 to 2003, only 242 advanced lines made it though. However, after two more years of greenhouse screening/inoculations, only a subset of plants make it to the final selection and are given advanced selection numbers. It should also be noted that that we have been more highly selective over the past five years and have only been keeping the best plants. Moreover, we have also been increasing the number of maternal parents that finally contribute to the final advanced selections, further demonstrating that this approach is working (Table 2). Finally, three varieties developed from this program, 'Zentmyer', 'Uzi', and 'Steddom', that have superior tolerance to PRR compared to any other rootstock that UCR has released are currently being scheduled for release by the University. Under hazardous PRR conditions, these rootstocks on average yield two times that of the standard older tolerant variety 'Thomas' (Table 3). A manuscript describing these rootstocks is currently being drafted. We also have some additional selections that are showing great promise as well (Fig. 3). However, as previously mentioned, many more selections still need to be field-tested that have made it through our greenhouse screening process.

Conclusions

Controlling avocado root rot meets one of the most important avocado industry priorities since the disease is often the limiting factor in avocado production worldwide. If PRR is controlled, avocado yields, growth, and fruit quality will all increase dramatically. More importantly, profitability will increase for individual growers. Despite intensive efforts to control PRR by resistant rootstocks, irrigation management, soil sterilization, fungicides, amendments, cultural practices, biological control and many other methods, PRR is still a significant problem and causes tens of millions of dollars in annul losses worldwide. Because more growers are using tolerant rootstocks and proper control measures, damage is no longer as noticeable as it was in the 1980's. However, the disease has become more insidious because it reduces yield and profit continuously in a grove. An integrated approach toward controlling PRR is the best means of reducing this disease but the ultimate long-term objective is to find a truly PRR resistant rootstock for avocado. Research has proceeded for many years (since the late 1940's) using traditional approaches and we just now have the molecular tools necessary to leap forward in our understanding of avocado genetics and how genomic diversity is associated with PRR and other important agronomic traits. These newer approaches, along with the current traditional approaches, will certainly play a major role in advancing our breeding efforts. In the long run, this will lead to better disease control and higher profitability to the avocado grower.

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Maternal avocado varieites/selections														
Duke 7	Thomas	G6	Spencer	Duke 9	UC 2001	Barrduke	Toro Caynon	PP40	PP 29	PP 36	PP 4	PP 80	PP 81	VC 7
PP 92	PP 4	PP 14	PP 48	PP 5	PP 19	PP 15?	PP 24	PP 95	GD 2	GD 8	GD 18	GD 19	GD 22	GD 24
PP 96	PP 15?	PP 22	PP 49	PP 21	PP 34		PP 40	GD 1				GD 20		
GD 7	PP 18	PP 25	PP 50	PP 26	PP 35			GD 3				GD 21		
GD 12	PP 55	PP 29	PP 51	PP 28	PP 36			GD 4						
GD 13	PP 68	PP 60	PP 52	PP 33	PP 43			GD 10						
GD 14	PP 69	PP 83	PP 88	PP 37	PP 44									
GD 23	GD 9	PP 89		PP 41	PP 45?									
	GD 17	PP 94		PP 42	PP 57									
				PP 45?	PP 58									
				PP 53	PP 61									
				PP 54	PP 63									
				PP 56	PP 70									
				PP 59	PP 71									
				PP 62	PP 72									
				PP 67	PP 73									
				PP 79	PP 80									
				PP 93	PP 81									
				GD 15	PP 82									
				GD 16	PP 84									
					PP 85									
					PP 87									
					PP 90									
					PP 91									
					GD 6									

Table 1. Current advanced selections within the rootstock breeding program listed by the maternal avocado/selection that they were derived from. PP selections were accumulated by Dr. John Menge whereas GD selections have been selected within the past five years. Note that many of the newer varieties have been derived from more diverse rootstock selections. A '?' denotes discrepancies based on field notes.

Block	Year Planted	Cultivar/Selection	No. of trees
#1	2001	Dusa, D9, D7, Latas, PP4, UC 2001, PP86	12 trees
#2	1999	PP4, Toro Canyon, Thomas, Spencer	11 trees
#3	1998	PP4, Thomas, Barrduke, VC 256	12 trees
#4	2001	PP#s 1, 2, 4, 5, 15, 16, 19, 21, 26, 29, 36, 40, 41, 42, 50, 52, 54, 55, 56, 57, 58, 59, 60, 62, 63, 85	28 trees
#5	2005	Wilg,VC241, PP21, PP37, PP41, Wurtz, PP67 ,PP85, PP91	13 trees
#6	2005	VC#S 7, 15, 26, 28, 40, 51, 65, 66, 803, 804, 817, G335, G1033, PP87, PP91, Anaheim	18 trees
#7	1991	Thomas, UC2001, D7, D9, G6, Barrduke	22 trees
#8	2003	Toro Caynon, Dusa, Latas, VC207, VC218,VC801, PP87	12 trees
#9	2003	Dusa, Latas, Toro Caynon,VC218, VC207, PP86	12 trees
#10	2009	PP4, PP14, PP24, PP35	12 trees

Table 2. Avocado breeding blocks on the UCR campus.

	Yield (kg/tree)			Canopy volume (m^3)		
	3yr	4yr	5yr	3yr	4yr	5yr
Thomas	12.84	8.46	1.64	9.44	11.40	21.32
Dusa	17.55	38.59	53.23	11.58	15.06	44.85
Zentmyer	17.41	8.80	51.77	12.60	11.61	44.54
Uzi	18.66	31.77	43.68	13.05	18.94	37.89
Steddom	20.77	28.86	41.00	10.77	13.54	35.59

Table 3. Average yield and canopy volume of 'Hass' scions grafted to clonal rootstocks from root rot trials conducted in the northern and southern avocado production zones of California.

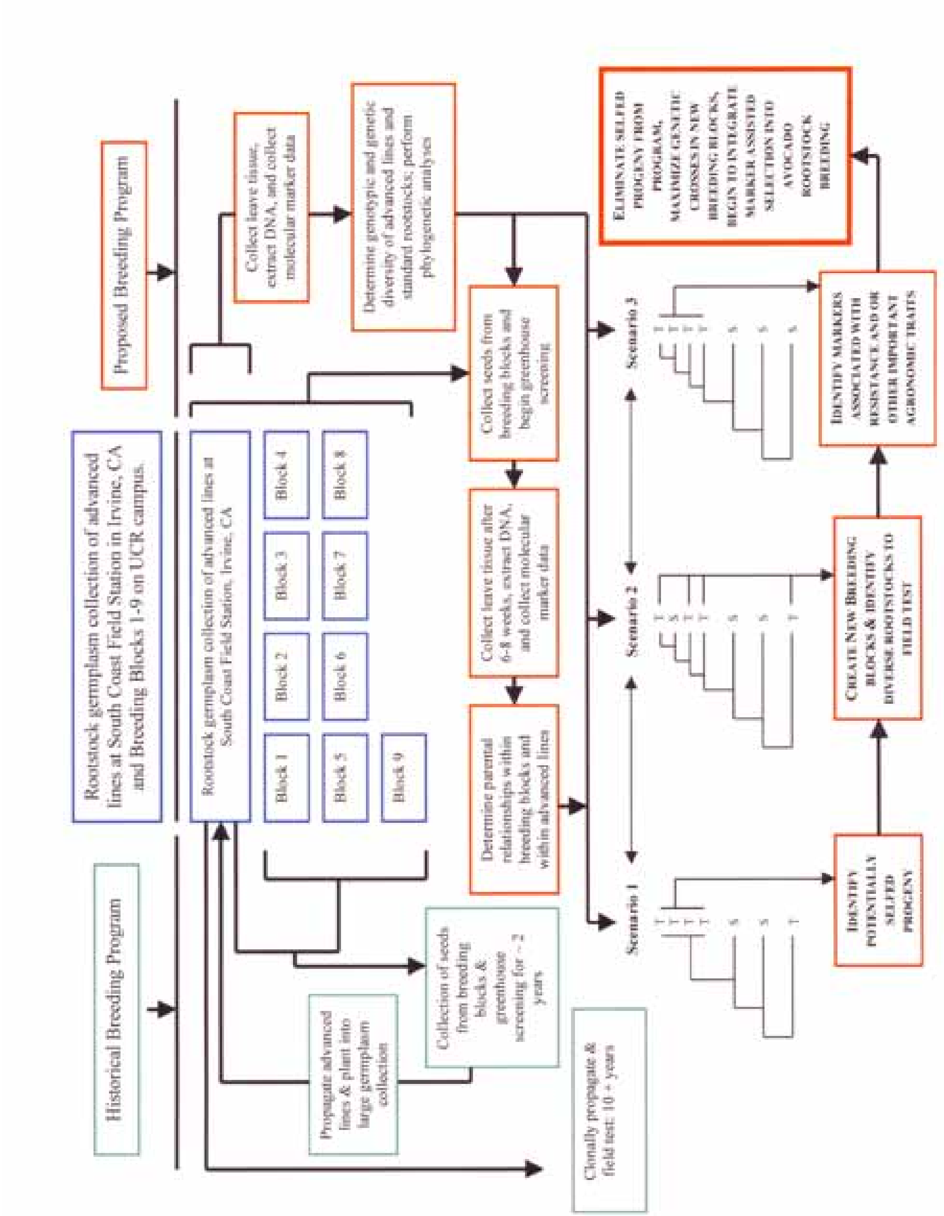

Figure 1. Schematic diagram of our current and proposed breeding program. T = PRR tolerant and S = PRR susceptible selections in the depicted hypothetical phylogenetic trees. Hypothetical selections that are more closely related to each other are clustered together on short 'branches' compared to more distantly related selections that are farther apart on the tree and separated by longer 'branches'.

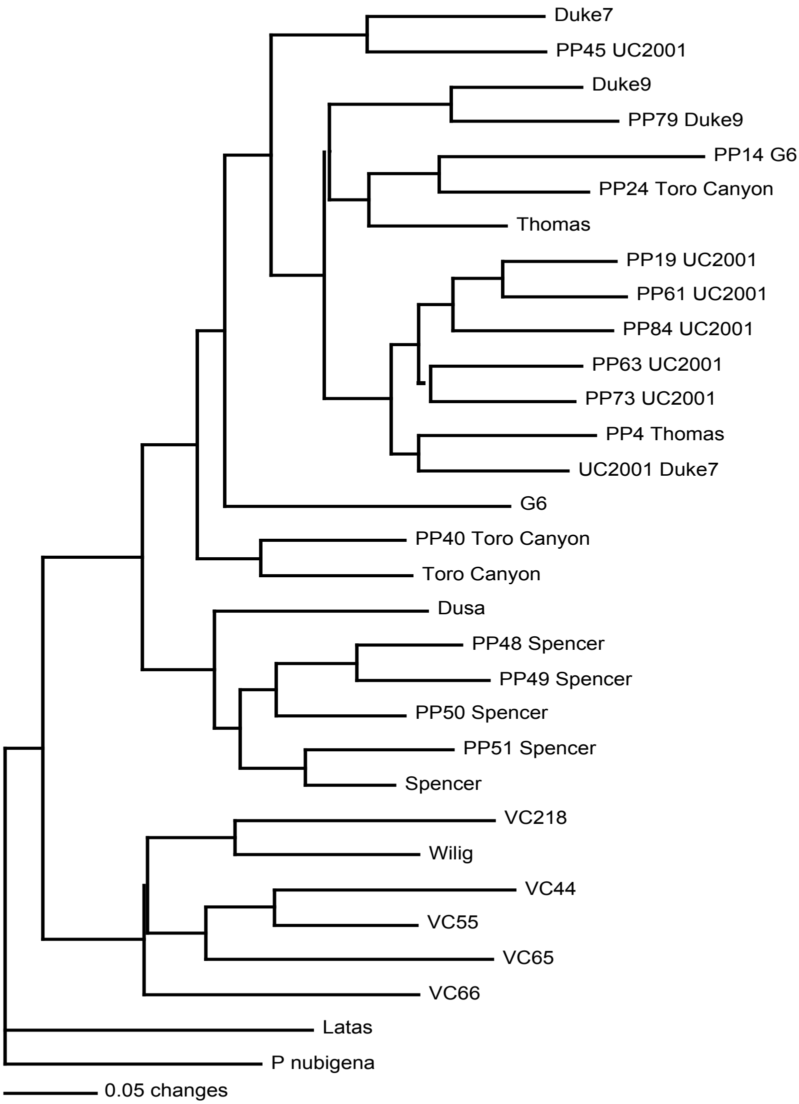

Figure 2. Neighbor joining phylogeny based on 58 polymorphic molecular markers (AFLP) from some representative rootstock cultivars/selections in the UCR program. The tree is rooted with *P. nubigena*, a close relative of *P. americana.* First name indicates the specific cultivar and the second name indicates the maternal mother tree if known. PP numbers are from our advanced tolerant lines based on ~ 2yrs of greenhouse screening. PP4 (Zentmyer), 14 (Uzi) and 24 (Steddom) are three PRR tolerant rootstocks that are in the process of being released and PP45 & 40 are two new rootstocks, 'Eddie' and 'Brandon', showing great promise as shown in Figure 3. Note that many clades are clustering by maternal parent. VC lines are known West Indian cultivars whereas the rest are Mexican or Mexican-Guatemalan hybrids.

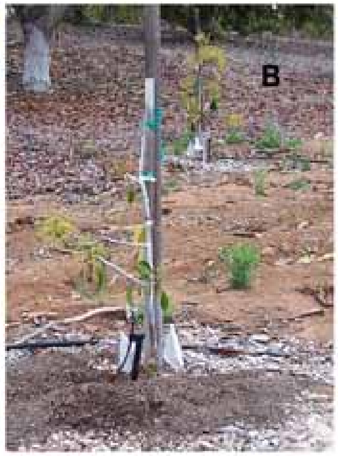

Figure 1. A) Eddie, a new UCR rootstock (left) grafted with Hass and Dusa rootstock (right), a selection from South Africa and tested by the UCR program, grafted with Hass growing under heavy root rot conditions. B) Thomas rootstock, a previously UCR released tolerant cultivar from the 80s, grafted with Hass showing significant symptoms of avocado root rot. C) Brandon, a new UCR rootstock grafted to Hass growing under heavy root rot conditions.