DEVELOPMENT OF PHYTOPHTHORA ROOT ROT-RESISTANT AVOCADO ROOTSTOCKS FOR THE CARIBBEAN

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Abstract. Phytophthora root rot (PRR), caused by Phytophthora cinnamomi Rands, poses the greatest limitation to avocado production worldwide. Through a collaborative effort between researchers at the USDA-ARS facility in Miami and the University of Florida in Homestead, PRR-resistant avocado (Persea americana) rootstocks are being developed for the Caribbean. Avocado breeding presents several challenges. These include a long regeneration time (4-15 years) and the low efficiency of controlled pollinations. To overcome these challenges we will: i) increase the number of accessions from the Caribbean in breeding blocks; ii) screen large numbers of half-sib and full-sib seedling progeny in controlled greenhouse and field trials; iii) genetically characterize parents of PRR-tolerant and susceptible progeny with molecular markers and iv) with the identified markers, develop marker-assisted selection protocols for resistance.

Avocado, Persea americana Miller, is an important tropical and subtropical fruit crop. Global production exceeds 2.4 million metric tons (MMT) per year, much of which is grown in the Caribbean Basin (FAO, 2001). Three varieties/races of this species include commercial cultivars: the Mexican, P. americana var. drymifolia; the Guatemalan, P. americana var. guatemalenis; and the West Indian, or Antillean, P. americana var. americana (Bergh, 1976; Bergh and Ellstrand, 1986). The West Indian variety and hybrids that include West Indian parentage are best adapted to the humid sub-tropical and tropical climates that are found in the Caribbean. The Subtropical Horticultural Research Station in Miami, Florida holds one of the largest collections of West Indian avocados. The diverse germplasm housed in this collection is being used to breed avocados for the Caribbean region.

Phytophthora Root Rot

Phytophthora root rot (PRR), caused by Phytophthora cinnamomi Rands, is the most important constraint to avocado production worldwide (Menge and Ploetz, 2003). PRR significantly impacts production throughout the Caribbean and has eliminated avocado as an important crop in many areas. It has also decimated important repositories of avocado germplasm in the region, such as those that were present at the USDA-ARS station in Isabella, Puerto Rico, and the Pan American School of Agriculture in Zamorano, Honduras.

The genus Phytophthora includes some of the world’s most destructive plant pathogens (Erwin and Ribeiro, 1996). One of the most important members of the genus is P. cinnamomi. It is found in over 70 countries and affects over 1000 plant species (Erwin and Ribeiro, 1996; Menge and Ploetz, 2003; Zentmyer, 1980). On avocado, P. cinnamomi causes trunk cankers and, more importantly, kills feeder roots, reducing fruit yield and killing mature trees (Menge and Ploetz, 2003).

Phytophthora cinnamomi spreads rapidly and is a persistent soilborne pathogen. Its prevalence in so many systems has been attributed to its ability to adapt to new environments, broad host range, saprophytic capabilities, and the production of spore types that serve survival and dispersal functions (Judelson and Blanco, 2005). Consequently, this pathogen is present in virtually all regions where avocado is grown.

Phytophthora cinnamomi’s ability to cope with diverse environments is a key to its success. Genetic variation that enables the pathogen to adapt to different circumstances is generated via mitotic recombination. Zoospore production, mobility and their infection of the host is favored in wet soils, whereas chlamydomospores and encysted zoospores facilitate its survival in dry soils (Menge and Ploetz, 2003). The management of P. cinnamomi-induced disease must account for the above attributes as well as the following shortcomings.

The most effective chemical control measures, metalaxyl and mefanoxam (respectively, Ridomil and Ridomil Gold), fosetyl-Al (Aliette) and various phosphonates, reduce disease over short periods (months) but do not eliminate P. cinnamomi. Their continued application for long-term control can lead to the selection of resistant populations of the pathogen which become increasingly prevalent with increased use (Coffey and Bower, 1984; Cohen and Coffey, 1986). Furthermore, fosetyl-Al and the phosphonates are most effective in P-limiting soils that are unlike those in South Florida. The success of these chemical measures is limited by all of these factors.

Organic and biological strategies also face limitations. Mulches and biocontrol organisms have been used against PRR, but require specific conditions to function optimally. To date, they have been only moderately effective. Unfortunately, flooding events that result from hurricanes and changes in water management in South Florida have dramatically tipped the balance in the pathogen’s favor and further reduced the opportunities for the success of these strategies (Ploetz et al., 2002). Given the plasticity of P. cinnamomi and the limitations of the above strategies there is a clear need for better options to manage this disease (Menge and Ploetz, 2003). This paper
describes work to produce PRR-tolerant avocado rootstocks that could be used in the Caribbean Basin.

Challenges of Avocado Breeding

The genetic improvement of avocado faces several challenges. These include:

i) limited tolerance to PRR;
ii) prolonged juvenility and low reproductive success;
iii) the high cost of germplasm conservation; and
iv) the sensitivity of some germplasm to climatic and edaphic change.

Since avocado and *P. cinnamomi* originated in different areas of the world (i.e., did not coevolve), no evolutionary selection for resistance has occurred in the crop. Depending on the genotype, seedlings take between 5 and 15 years to mature. Only 1% of the flowers that are produced result in fruit, and these may result from cross- or self-pollination (Davenport et al., 1994). Thus, the production of hybrids is problematic.

Despite these challenges, PRR-tolerant rootstocks have been produced in California. This success resulted from decades of germplasm collection and selection by Drs. George Zentmyer and John Menge. Unfortunately, these rootstocks are poorly suited for tropical climates and calcareous soils. Moreover, these efforts have been primarily empirical and are extremely time-consuming.

The efficacy of rootstock improvement efforts could be increased if molecular markers for PRR tolerance were available. In crop breeding, the use of markers that are associated with desirable traits is broadly termed marker-assisted selection (MAS). Marker-assisted selection is particularly useful with crops that have long regeneration times and it has become the cardinal focus of modern tree breeding (Paterson, 1998). Unfortunately, information that has come from much of the MAS work is not applicable to avocado since it belongs to an early lineage of plants that precedes the eudicots, the group in which most crop plants belong and on which most MAS work is not applicable (Zanis et al., 2002). Fortunately, information that has come from much of the MAS work is not applicable to avocado since it belongs to an early lineage of plants that precedes the eudicots, the group in which most crop plants belong and on which most MAS work has been conducted (Zanis et al., 2002). There is a critical need to increase the number of markers that are available for avocado and our general knowledge of the genetics of this crop.

A Globally Important Repository and Distributor of Avocado Germplasm

West Indian avocados were first documented in Miami in 1850, but may have been grown in Florida earlier in the century (Knight, 2002). These ‘naturalized’ individuals were found in hardwood hammocks and were presumably of Cuban origin (Knight, 2002). Selection efforts involving these avocados resulted in the development of the ‘Pollock’ and ‘Trapp’ cultivars. These were the first clonally propagated commercial avocado cultivars and are still grown in South Florida today (Degner et al., 1994).

Concomitant with these early efforts to improve avocado was the establishment of the Miami Research Station in 1898 as a plant introduction center. Initially located in what is now an urban banking district, this USDA station moved to its current location in 1914 (McGuire et al., 1999). Dr. David Fairchild, a well-known botanist and plant collector, helped to secure the 197-acre site on an old army aviation training center, Chapman Field. He and Wilson Popenoe, another accomplished plant explorer, played crucial roles in establishing the Miami USDA-ARS avocado collection (hereafter, the Miami collection) (Rosengarten, 1991). Since their time, contributions to the repository have been made by commercial nurseries, local growers and researchers at institutions throughout the avocado-growing regions of the world.

The Miami collection now maintains 265 avocado accessions from the three commercial races of avocado, as well as important avocado relatives (Table 1). It is one of the largest and most diverse collections of cultivars in the world (Fig. 1). Other major collections include the Campus de Jaboticabal in Jaboticabal, Brazil with over 300 accessions and the Direc- cion de Investigaciones de Citricos y Otros Frutales in Havana, Cuba with close to 280 accessions (International Plant Genetic Resources Institute 2000-2004).

Maintaining avocado germplasm is costly. Seed cannot be stored for long before they lose viability, and seedling trees do not bear fruit for a decade or more (Debouck and Libreros Feria, 1993). In situ collections require extended commitments of time and space, and some *Persea* taxa that are important in breeding, such as *P. scheideana*, are sensitive to edaphic and climatic conditions (Ben-Ya'Acov et al., 1992). For instance, some *Persea* accessions do not set fruit at the University of California South Coast Research Extension Center (Dr. Mary-Lu Arpaia, personal communication). The Miami collection is located in one of the few areas in the United States where avocados that are adapted to tropical and subtropical climates can be maintained.

The conservation and distribution of germplasm in the Miami collection have undoubtedly contributed to the increases in global avocado production (Knight, 2002). Accessions have been distributed to more than 40 countries, and a web-based service, the germplasm resource information network (GRIN), has increased the accessibility of the collection for growers and scientists (USDA-ARS National Plant Germplasm System, 1980). Germplasm exchange with these recipients and other repositories will continue to increase the diversity and value of the Miami collection.

Previous Rootstock Work in South Florida

Efforts to identify PRR-tolerant avocados have utilized the genetic diversity in the Miami collection. Using 14 molecular markers, germplasm in the collection was estimated to have

<table>
<thead>
<tr>
<th>Botanical race</th>
<th>Number of accessions</th>
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<tbody>
<tr>
<td>West Indian</td>
<td>68</td>
</tr>
<tr>
<td>Guatemalan</td>
<td>35</td>
</tr>
<tr>
<td>Mexican</td>
<td>31</td>
</tr>
<tr>
<td>West Indian × Guatemalan</td>
<td>32</td>
</tr>
<tr>
<td>West Indian × Mexican</td>
<td>8</td>
</tr>
<tr>
<td>Guatemalan × Mexican</td>
<td>30</td>
</tr>
<tr>
<td>Complex hybrids</td>
<td>31</td>
</tr>
<tr>
<td><em>Persea</em> spp.</td>
<td>3</td>
</tr>
<tr>
<td>Unclassified <em>Persea</em></td>
<td>7</td>
</tr>
<tr>
<td>Total number of accessions</td>
<td>265</td>
</tr>
</tbody>
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an unbiased genetic diversity of 0.83 (Schnell et al., 2003). Our confidence that PRR-tolerant rootstocks will ultimately be produced with this germplasm is bolstered by this high genetic diversity, the fact that few reproductive barriers exist within and among the P. americana varieties and P. scheideana (a PRR-tolerant species that hybridizes naturally with P. americana), and that heritability for PRR tolerance was 0.45 in previous work (Ploetz et al., 2002).

To date, almost 4,000 half-sib progeny (open-pollinated seedlings) from over 60 accessions in the Miami collection have been evaluated for tolerance to PRR (Fig. 2). West Indian and West Indian × Guatemalan hybrid progeny were most tolerant, and the parents of superior progeny included ‘Arguelo,’ ‘Catalina,’ ‘Dade,’ ‘Family,’ ‘Girrardin,’ ‘Hiwassee,’ ‘Maxima,’ ‘Monroe,’ ‘Simmonds,’ ‘Pollock,’ and ‘Tensen.’ From this work two root rot resistant accessions have been identified and characterized with existing molecular markers (Ashworth et al., 2003; Sharon et al., 1997). Further work to ascertain if these selections are commercially viable is underway.

**Combining Conventional and Molecular Breeding Techniques**

The commercial viability of selections is determined in nursery and field trials. PRR-tolerant seedlings are cloned and, in replicated nursery trials, are rated for resistance vs tolerant and susceptible commercial rootstocks, respectively, ‘Dusa’ and ‘Waldin.’ Tolerant clones are then grafted with ‘Simmonds’ scions and screened in P. cinnamomi-infested plots at the USDA-ARS facility in Miami. Annual measures of survival, stem diameter, canopy volume and, ultimately, yield, are used to assess the performance of the later selections in the field.

Previously identified elite parents are used to develop additional selections, either by screening additional open-pollinated progeny from these parents, or by producing hybrids among these parents. For the later objective, scions with complementary flowering (i.e., with A and B phenotypes and the same seasonality) are used in both high-density plantings and grafted onto individual trees in the field.

Molecular markers will be used to determine if the above crossing efforts are successful and in analyses that associate markers with PRR tolerance. The microsatellite markers, or simple sequence repeats (SSR), that will be used are both cost effective and efficient for parentage analysis, linkage mapping and association studies. Association analysis is a population-based technique used to identify marker-trait associations. This technique was developed for human genetics but has been successfully applied in plants (Schnell, 2005). The advantage of association analysis is that extensive familial relationships do not have to be developed. Thus, the open-pollinated populations that are available from the Miami collection can be used. If significant relationships between microsatellite alleles and PRR tolerance are detected, these markers could be used to screen for tolerance rather than the current, time-consuming tests.

**Long-term Program Goals**

Association analyses the first step in the development of a marker-assisted selection program (Fig. 3). Marker-assisted selection is well-suited to situations where the genetic basis for a desired trait is complex (Paterson, 1998); PRR tolerance in avocado is hypothesized to be such a trait (John Menge personal communication). As a polygenic trait, PRR tolerance would not segregate among individuals in discrete groups but rather as a continuum. This is the case with many crop traits valuable to agriculture. Such complex traits can be identified...
as individual quantitative traits loci (QTLs) that may be manipulated in breeding (Paterson, 1998). The program goal is to identify QTLs that are associated with PRR tolerance. They would enable the rapid identification of parents and progeny that possess tolerance. Until then, traditional screening methods will be employed.

Literature Cited


