Development of Predictive Tools for Brown and Sour Rot Resistance in Peaches and Nectarines

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Abstract

Brown rot (BR) caused by *Monilinia fructicola* is a serious pre- and postharvest disease of peach and nectarine. In 2008, 16 cultivars were selected from 2007 data covering the range of reactions to BR. This cultivar subset was challenged with BR and fruit quality traits data were also obtained. In both years, a total of 204 individuals of two peach progeny populations were evaluated for genetic analysis of resistance. Analyses of resistance/susceptibility segregation in the two progeny populations, relationships between resistance/susceptibility and fruit quality traits among the 18 cultivar subsets, and linkage and QTL (quantitative trait loci) mapping are underway. A partial scaffold map has been constructed for one of the populations. Data analysis showed potential genomic regions conferring resistance to both wounded and non-wounded BR inoculations. In this research, analysis of cultivars' reaction to wounded and non-wounded fruit inoculations, showed that lesion size of BR and sour rot (SR) was under genetic control. Results showed that similar as well as different resistance mechanisms may be present for wounded vs. non-wounded fruit inoculations. Host resistance also varied between SR and BR as t-test analysis showed significant differences between the reactions of the cultivars to both fungi. A number of wild peach accessions and old cultivars showed a high level of resistance to BR suggesting that these may be untapped sources of resistance to the fungus. Association between resistance and molecular markers will be elucidated and informative markers converted to predictive tools that are applicable in marker-assisted breeding of superior peach and nectarine cultivars. This season's data justify pursuing the molecular genetic approach as resistance was determined in our tested populations.

Introduction

Two major postharvest diseases of stone fruits are brown rot and sour rot caused by *Monilinia fructicola* (G. Wint.) Honey, and *Geotrichum candidum* Link (Adaskaveg et al., 2005; Biggs and Northover, 1985; Byrde and Willetts, 1977; Michailides et al., 2004). Effective control of these pathogens and other postharvest diseases is by routine

application of chemical fungicides (Adaskaveg et al., 2005; Margosan et al., 1997) particularly if fruit is to be stored and/or shipped long distances. However, there is increasing concern about the environmental effects and safety of chemical fungicides, and the development of fungicide-resistant postharvest fungal pathogens has been reported (Hong et al., 1998). Regulatory agencies have reacted to public pressure and introduced comprehensive legislation to reduce pesticide use (Irtwange, 2006; Karabulut and Baykal, 2003). Host resistance to plant pathogens is perhaps the most cost effective and environmentally safe strategy for disease management. Although commercial cultivars are generally susceptible to brown rot (Cantoni et al., 1996; Ogawa et al, 1985), improved levels of resistance have been identified in some cultivars such as 'Bolinha', (Bostock et al., 1994; Feliciano et al., 1987; Gradziel et al., 2003), and two breeding lines in the breeding program of Dr. S. P. Gonzalez, Universidad Autonoma de Queretaro, Mexico. Research efforts are ongoing to breed peach cultivars resistant to brown rot. The cling-peach breeding program of Dr. Tom Gradziel (UC Davis) has incorporated sources of resistance from almond into several breeding lines (Gradziel, 2002; Gradziel et al., 2003). Bostock et al. (1999) reported that chlorogenic and caffeic acids are major phenolic acids in the epidermis and subtending cell layers of peach fruit and that their concentrations are especially high in immature fruit with a high level of resistance to brown rot and decline as fruit mature with a corresponding increase in disease susceptibility (also see Lee and Bostock, 2006). Augmenting traditional breeding practices with more modern molecular mapping technologies will better equip the breeder to meet the challenge of breeding sustainable resistance. The detection of sour rot caused by *G. candidum* in peach and nectarine is relatively new (Michailides et al., 2004). In the program of Drs. Michailides and Bostock, several peach and nectarine cultivars have been observed to possess high levels of resistance to this pathogen.

A project funded by the USDA-CSREES NRI (NIFA) combines high density SNP linkage mapping with improved quantitative trait evaluations for the development of markers-assisted breeding (MAB) tools for peach is complementing these efforts.

Objectives

- Determine the genetic control of resistance to brown and sour rot in fresh peach cultivars and two cling peach progeny populations
- Develop linkage maps for Pop-BR1 and Pop-BR2, and localize genomic regions controlling resistance to brown rot and sour rot and identify molecular markers associated with resistance
- The long-term goal of our program is to develop disease resistant peach and nectarine cultivars, thereby reducing postharvest chemical fungicide usage

Results

Three peach genomes were sequenced using Roche 454 and Illumina/Solexa technologies to obtain long contigs. The sequences were aligned to the 'Lovell' peach genome released April 01, 2010 by the International Peach Genome Initiative (IPGI). 'Dr. Davis', 'F8, 1-42' and 'Georgia Belle' were sequenced to identify SNPs for two breeding populations, Pop DF ('Dr. Davis' x 'F8, 1-42') with 119 progeny and Pop DG ('Dr. Davis' x 'Georgia Belle') a diverse germplasm with 118 progeny. Significant variability was observed in forces to measure flesh integrity, stone adhesion, antioxidants and flesh browning potential.

Roche 454 sequencing produced 980,000 total reads with 236 Mb sequence for 'Dr. Davis' and 735,000 total reads with 172 Mb sequence for 'F8, 1-42'. 84 bp x 84 bp paired end Illumina/Solexa sequences yielded 25.5, 21.4, 25.5 million sequences for 'Dr. Davis', 'F8, 1-42' and 'Georgia Belle', respectively. BWA/SAMTOOLS were used for alignment of raw reads and SNP detection, with custom PERL scripts for SNP filtering. Velvet's Columbus module was used for sequence assembly. Comparison of aligned and overlapping sequences from both Roche 454 and Illumina-Solexa resulted in the selection of 6654 high quality SNPs for 'Dr. Davis' vs. 'F8, 1-42' and 'Georgia Belle', distributed on eight major peach genome scaffolds as defined from the 'Lovell' assembly. The eight scaffolds contained about 215-225 Mb of peach genomic sequences with one SNP/ ~40,000 bases. Populations DF and DG were scored for 1536 SNPs, evenly distributed across the eight peach scaffolds, with the Illumina 'Golden Gate' assay.

Additional QTL affecting disease resistance quality traits are being mapped on a linkage map using our 1536 SNPs and 80 SSR markers and will be aligned to PopDG, Pop DF (in preparation) and T x E linkage map. Markers tightly linked to the QTLs will be converted to marker assistance selection (MAB) tools.

Deliverables

We delivered a peach partial genome sequence of 'Dr. Davis', 'F8, 1-42' and 'Georgia Belle' to the NCBI that will enhance Prunus genomic database and assist in eventual full genome sequence of peach.

We submitted 6,657 SNPs to NCBI from parents of our populations that we have phenotyped in previous years and high density SNP linkage maps through 454 shotgun genome sequencing and Illumina Golden Gate SNP genotyping.

We evaluated phenotype disease resistance of exotic segregating populations designed to introgress quality traits from less-domesticated germplasm.

A high throughput SNP genotyping platform for marker-assisted breeding (MAB) of peach was developed.

Final Comments

These accomplishments will accelerate the development of predictive molecular tools (MAB) that peach and nectarine breeders can use to quickly develop disease resistant superior cultivars or growers can use to predict cultivar disease susceptibility, such that there will be less reliance on chemical fungicide usage.