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

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ABSTRACT

Three year data on our efforts to understand genetics of resistance to brown rot (BR) caused by *Monilinia fructicola* has been finished. A new number of wild peach accessions and old cultivars with high level of resistance to BR have been detected suggesting that these may be untapped sources of resistance to the fungus. Analysis of cultivars' reaction to wounded and nonwounded fruit inoculations indicated that lesion size of BR was under genetic control. Results demonstrated that similar as well as different resistance mechanisms may be present for wounded vs. nonwounded fruit inoculations. We conducted three years of inoculation experiments on a progeny population that will help for our disease resistance markers development. We have developed a detailed fruit quality and ripening gene linkage map for Prunus which is required to select disease reliable marker assistance markers. Molecular analyses of resistance/susceptibility segregation in this progeny population, relationships between resistance/susceptibility and fruit quality traits among cultivar subsets, and linkage and QTL (quantitative trait loci) mapping are underway as steps to develop markers for disease resistance.

INTRODUCTION

Two major postharvest diseases of stone fruits are brown 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 (Ogawa

et al, 1985; Cantoni et al., 1996), improved levels of resistance have been identified in some cultivars such as 'Bolinha', (Feliciano et al., 1987; Bostock et al., 1994; 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). The processing canning peach breeding program of UC Davis is incorporating the epidermis-based resistance to brown rot into improved cultivars through a recurrent selection program (Gradziel et al., 2003). 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.

The main goal of our group is to develop predictive molecular tools that peach and nectarine breeders can use to quickly develop disease resistant superior cultivars such that there will be less reliance on chemical fungicide usage. The specific objectives of this research are: 1. Determine the genetic control of resistance to brown and sour rot in peach cultivars and two cling peach progeny populations, and 2. Develop scaffold linkage maps with these populations and localize genomic regions controlling resistance with tightly linked molecular markers.

Materials and Methods

1. Plant Material

Fruit were collected at commercial maturity from fields at the Kearney Agricultural Center (KAC), Parlier, UC Davis/USDA Germplasm Repository, and from organic growers. Fruit were either transported from KAC to Davis for brown rot inoculations or from Davis to KAC for sour rot inoculations. Materials sampled included canning peach and fresh market cultivars, peach and nectarine cultivars, canning peach breeding lines, segregating progeny of two mapping populations – Pop-BR1 ('Dr. Davis' × F8,1-42) and Pop-BR2 ('Loadel' × UCD96,4-55), old peach cultivars and related wild accessions. Many cultivars were obtained from multiple sources.

2. Inoculations and Lesion Size Measurements

All brown rot inoculations were conducted at the Bostock Lab, Plant Pathology Department, UC Davis and all sour rot inoculations were conducted at the Michailides lab, KAC, Parlier. Prior to inoculation, fruit flesh color was measured with the nondestructive impact firmness sensor as an indicator of maturity (Slaughter et al., 2006). Fruit were surface sterilized by allowing them to sit for 30 seconds in a 10% bleach solution. They were rinsed twice by dipping them in separate buckets of clean water, and then allowed to dry on paper towels. Crispers were prepared by washing with hot soapy water and rinsing with 95% ethanol, and air drying. The bottom of the crisper was covered with 1/8 to 1/4 of an inch of water, and lined with a crisper liner. Fruit were placed in crispers with

the smooth, flat side up. Inoculum of Monilinia fructicola (brown rot) and Geotrichum candidum (sour rot) spore was prepared with 25,000 spores/ml concentration. Inoculation was done by pipetting a 10 µl drop of spores onto the fruit. Controls are prepared in the same way, except sterile water was used instead of spores. Wounded inoculation was achieved by wounding the peach fruit surface with a flamed metal tool with a sharp point, and inoculating with the spores. Only wounded inoculation was carried out for sour rot. After inoculation closed crispers were covered with two layers of damp cheesecloth and allowed to sit for 15 hours. The inoculum drops were then removed by wicking away with a Kimwipe, and the crisper lids were replaced. Three days after inoculation the lesion diameters were measured with a ruler.

3. Molecular Analysis (ongoing)

Survey of polymorphism between parents of Pop-BR1 was conducted among publicly available Prunus SSR markers and EST SSR markers from our Chillpeach database located at: http://bioinfo.ibmcp.upv.es/genomics/ChillPeachDB/login.php). Most of these SSRs have been mapped to the reference T×E and Pop-DG linkage maps. Resistance gene analog degenerate primers developed for Rosaceae (Samuelian et al., 2008) were also tested on the parents and progeny of Pop-BR1. A scaffold map was developed for Pop-BR1 and used for preliminary quantitative trait analysis (QTL) of resistance to BR. Survey of polymorphism was also conducted for candidate genes. Bin mapping of candidate genes to the Prunus T×E reference map was attempted. Further molecular marker and QTL analyses are ongoing.

4. Statistical Analysis

Analysis of variance (ANOVA) was conducted on the lesion size data using the GLM procedure of SAS. Relationships between resistances to brown rot wounded and nonwounded inoculations were assessed by linear correlations. Linkage mapping was conducted with the use of statistical software JoinMap® 4 (Van Ooijen 2006) and QTL analysis was accomplished with the use of non-parametric Kruskal-Wallis test and interval mapping procedure of MapQTL® 5.0 software (Van Ooijen 2005).

RESULTS

1. Resistance Validation

Inoculation of our 24 cultivars, selected based on the 2008 brown rot resistance results, reveals consistency in resistance to brown rot in the three years. Fruit collection was made from fungicide-free sources and many cultivars inoculated with brown rot were obtained from two or more sources, making a total of 123 entries. BR inoculations were repeated in 2008 for a subset of these 24 cultivars selected to represent the range of cultivar reactions to inoculations in 2008. Also for BR, a total of 204 progeny of 'Loadel' × 'UCD96,4-55' (82 progeny) and 'Dr. Davis' × 'F8,1-42' (122 progeny) cling peach populations were inoculated for the last year to assess segregation for resistance to the fungus. In addition 12 old cultivars and wild accessions were inoculated with brown rot for discovery of new resistance sources.

2. Disease Resistance Segregation on Population Progeny

Brown rot resistance for wounded and non-wounded inoculations was detected on selected new genotypes (Fig. 1 and 2) suggesting that these genotypes should be considered as sources of resistance to brown rot in breeding programs.

Putative QTLs and Candidate Genes A few putative QTLs have been detected for resistance. The knowledge of inheritance of resistance will lay the groundwork for molecular analysis of the resistance factors which in turn will translate to the discovery of molecular markers that can be used in marker-assisted selections to fast-track the development of brown rot-resistant peach and nectarine cultivars. Candidate genes that are linked to BR resistance QTLs are being used to assess the potential markers. A total of 230 SSRs and 37 candidate genes primer pairs were screened for polymorphism using the parents and progeny subsets of Pop-BR1 out of which 52 SSR and two CGs were polymorphic. The polymorphic SSRs generated 59 SSR markers. In addition, eight RGA markers were generated. The total number of markers available for linkage analysis was 69 (59 SSRs, 8 RGAs and 2 CGs). A scaffold linkage map was constructed from this data consisting of 31 markers spread over 12 linkage groups of two to five markers each. These were organized into seven linkage groups corresponding to the T×E reference map using common SSR markers, with the locations of putative QTLs conferring resistance to BR. Putative QTLs were detected by non-parametric Kruskal-Wallis (KW) test and interval mapping, respectively. KW test detected three. One QTL was stable for each inoculation method across the three years. Interval mapping analysis detected one QTL for wounded inoculation in 2007 on linkage group G1 controlling up to 52% of observed variation. One QTL was detected on the same linkage group for wounded inoculation in 2008 controlling about 24% of observed variation. The proximity of these two QTLs suggests that they may be controlled by the same gene. Marker saturation of this region will aid QTL position refinement. Two candidate genes in the cutin and lignin biosynthesis pathway mapped to regions on the T×E Prunus reference map corresponding to locations of two putative QTLs detected for BR resistance on Pop-BR1 (result not shown). Further work is needed to map these genes directly to Pop-BR1 and validate their relationship with the QTLs.

POP-DG LINKAGE MAP

This fruit quality gene linkage map of Prunus containing genes putatively involved in the determination of fruit texture, pigmentation, flavor, and chilling injury resistance. We have developed a detailed fruit quality and ripening gene map for Prunus. The fruit quality gene map contains 133 candidate genes (CGs) implicated in fruit ripening, softening, flavor, and pigmentation, and chilling injury resistance. The Pop-DG intraspecific peach linkage map contained a total of 211 markers (208 molecular and three morphological) distributed over eight linkage groups corresponding to the haploid chromosome number of peach. The map covered 818.2 cM of the peach genome with an average of 4.0 cM interval between markers. The markers on Pop-DG map consisted of three Mendelian trait loci, 24 CGs, 79 SSRs, 40 RAFs, 23 SRAPs, 14 IMAs, and 28 CG accessory markers associated with CGs. Of the 79 SSR markers on Pop-DG, 39 were shared with the published Prunus T×E reference map. These common markers enabled the determination of linkage group orientation and assignment of linkage group numbers for the Pop-DG map. Shared markers were co-linear between Pop-DG and T×E except in three cases. Marker positions for BPPCT024, BPPCT030, and pchgms1 were inverted at the lower end of linkage group G2 of Pop-DG compared to G2 of T×E, positions of BPPCT021 and UDP96-008 were inverted in the middle of G3 of

Pop-DG compared to G3 of T×E, and positions of BPPCT026 and CPPCT004 were inverted towards the upper end of G5 of Pop-DG compared to G5 of T×E. One SSR marker (BPPCT036) that was originally placed on linkage group G4 of T×E [7] mapped to the distal end of G1 in Pop-DG. To resolve this discrepancy, BPPCT036 was tested on the T×E bin set which confirmed its true location in bin 1:73, corresponding to its position on the Pop-DG linkage map. The Pop-DG peach map is almost entirely co-linear with the Prunus reference T×E map such that locations of markers and quantitative trait loci (QTLs) located on Pop-DG can be readily cross-referenced to T×E and other Prunus maps aligned to T×E. Similarly, markers and QTLs in other Prunus maps (and other Rosaceae crop maps as comparative genomics advances in this family) can be compared to the Prunus fruit quality gene map to identify genes controlling fruit ripening and sensory quality.

As peach fruit development, growth, ripening, and senescence include major biochemical and sensory changes in texture, disease resistance, color, and flavor, the genetic dissection of these complex processes has important applications in crop improvement, to facilitate maximizing and maintaining stone fruit quality from production and processing through to marketing and consumption. The candidate gene approach combined with bin-mapping and availability of a community-recognized reference genetic map is a required step for an efficient way of locating genes related to disease resistance in a target genome. This fruit quality gene map of Prunus containing genes putatively involved in the determination of fruit texture, pigmentation, flavor, and chilling injury resistance.

Discussions

The reactions of various genotypes of peach and nectarine to brown rot and sour rot inoculations indicated that there is genetic resistance to these postharvest fungi. Some established cultivars showed very good resistance to the fungi under the experimental conditions used in this study. This showed that perhaps postharvest fungicide applications can be reduced or cancelled for these cultivars. Because lesion sizes were larger for wounded inoculations across the board compared to nonwounded inoculations, care should be taken during harvest to minimize physical injury to the fruit to avoid cracks on the skin through which the fungi can gain entrance. Organic growers may find the information generated in this study helpful in selecting cultivars for their production. A weak, but significant linear relationship was observed between wounded and nonwounded BR inoculation methods. However, several cultivars and progeny that displayed resistance to nonwounded inoculation were susceptible to wound inoculation. This indicated that similar as well as different resistance mechanisms may be present for wounded vs. nonwounded fruit. Host resistance also varied between sour rot and brown rot 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 brown rot (results not shown) suggesting that these may be untapped sources of resistance to the fungus.

Future Plans

We will continue with the molecular marker analysis of resistance to both fungi. The linkage map developed for Pop-BR1 will be expanded and a similar map will be constructed for Pop-BR2. Detailed QTL analyses will be conducted on the expanded maps to validate putative QTLs discovered so far and to detect additional QTLs. Markers closely linked to the resistance QTLs will be identified for use in breeding programs subset.

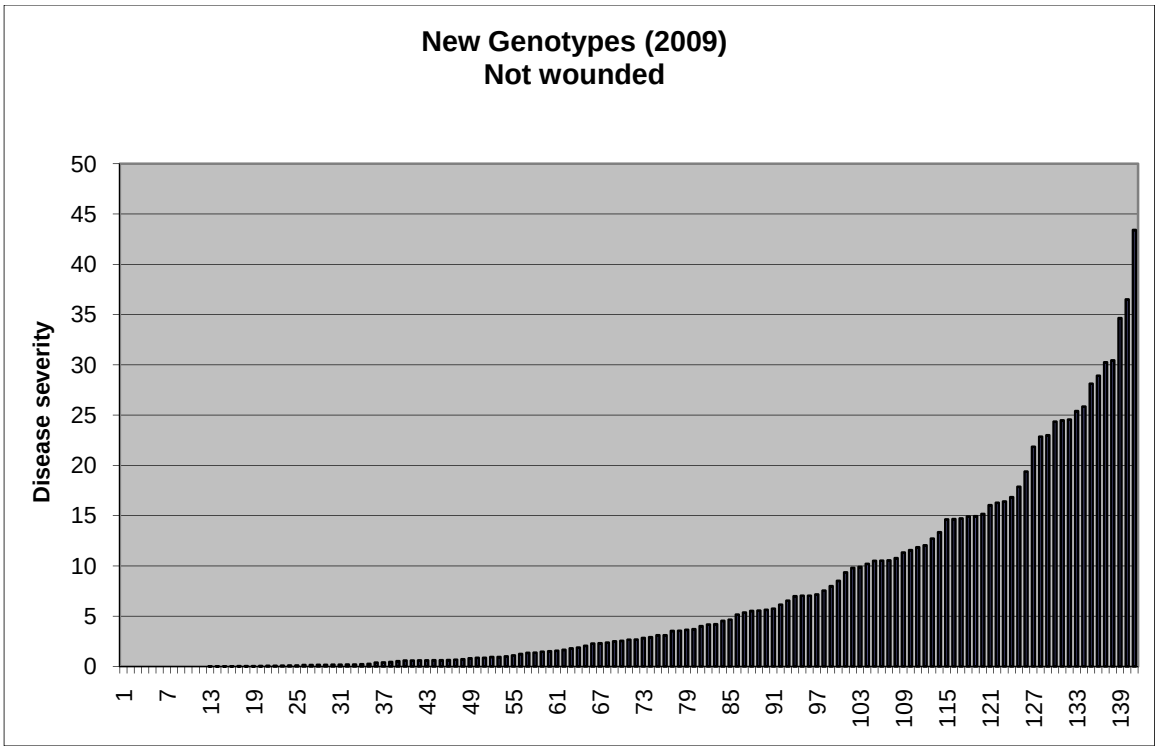

Figure 1. Frequency distributions of new peach genotypes showing segregation of resistance to brown rot nonwounded inoculations.

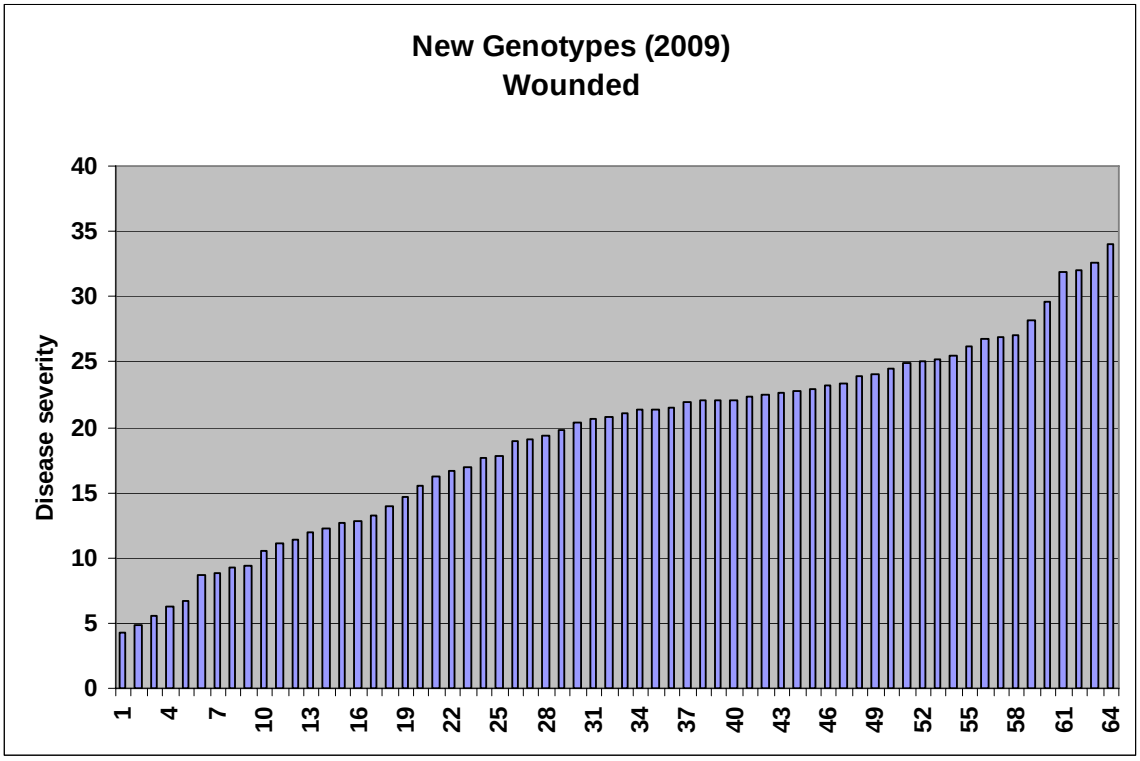

Figure 2. Frequency distributions of new peach genotypes showing segregation of resistance to brown rot wounded inoculations.

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