RosBREED

COMBINING DISEASE RESISTANCE WITH HORTICULTURAL QUALITY IN NEW ROSACEOUS CULTIVARS

CLEMSON UNIVERSITY

Breeding for Brown Rot (*Monilinia* spp.) Tolerance in Peach

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Abstract

Brown rot, caused by *Monilinia* spp., is one of the most important diseases of stone fruits. The fungus mainly affects the blossoms and fruit, and the resulting disease can lead to significant pre- and postharvest yield losses. Estimated annual cost to the U.S. stakeholders for disease management can reach $170 million. Although some degree of tolerance has been detected in peach landraces ('Bolinha') and interspecific material (almond x peach), most of the commercial cultivars are susceptible. In commercial peach production, the disease can only be controlled by routine fungicide applications, which may cause both environmental and human health concerns. The Clemson University peach breeding program within the RosBREED project aims to understand the genetics behind the peach fruit response to brown rot; with the goal of combining disease tolerance with high fruit quality via DNA-informed breeding. To this end we have phenotyped 26 cultivars/advanced selections and 138 progeny in 9 crosses using 'Bolinha' as source of tolerance. Fruit response to brown rot was assessed in wounded and non-wounded disease assays in 2015 and 2016. Genotypic data, obtained by using 9K peach SNP array, and previously reported QTLs associated with brown rot response in peach fruit, were used to evaluate allelic variability in brown rot associated genomic regions. Phenotypic performance or trait values of these alleles/ haplotypes were discussed. The data presented here provide a foundation for development of predictive DNA information that has a potential for an immediate application in U.S. peach breeding.

Material and Methods

Material: 26 cultivars /advanced selections and 138 progeny from crosses with 'Bolinha' source of resistance were phenotyped for brown rot fruit tolerance/resistance in 2015 and 2016.

Phenotyping: 40 fruits per individual were bagged to protect from chemical sprays and harvested at commercial maturity (Fig. 1.A). 20 unblemished fruits of similar maturity, detected by I_{AD} (Ziosi *et al*., 2008), were used for inoculations. Parallel inoculations, 10 fruits each, wounded and non-wounded, were performed following Martinez-Garcia *et al*. (2013) protocol (Fig. 1.B). Lesion diameter (mm) was recorded after 72h incubation (Fig1.C), and disease severity index (DSI) for each individual was calculated as the product of the average lesion diameter x proportion of lesions greater than 3 mm. (Fig. 1.C & D)

Genotyping: Phenotyped material was genotyped using 9K peach SNP array (Verde *et al.*, 2012).

Haploblocking: Haploblocks/haplotypes were determined for previously reported, brown rot associated QTL regions (Martinez-Garcia *et al*., 2014; Pacheco *et al*., 2015).

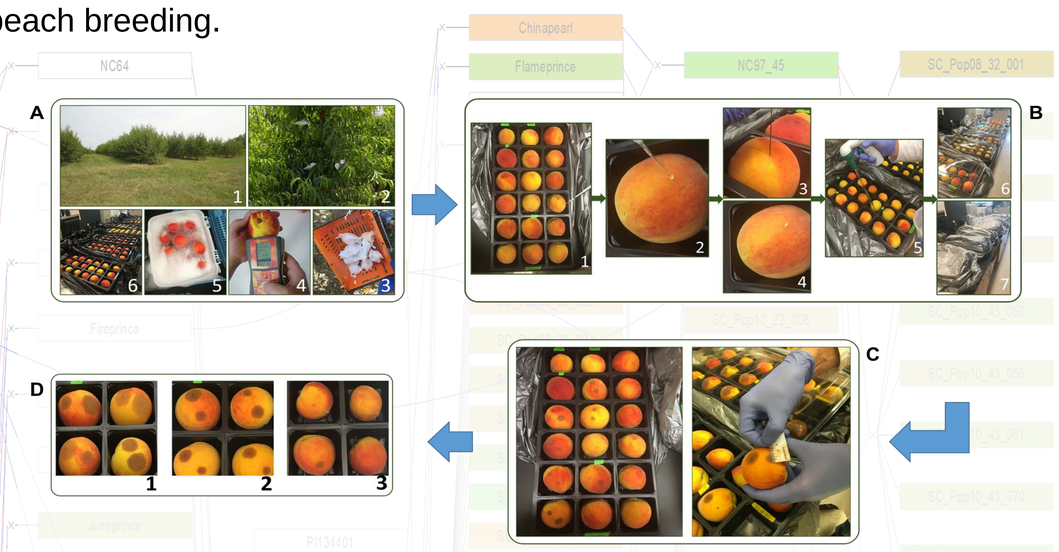

Fig. 1. Phenotyping. (A) Fruit sampling and sterilization: A1, germplasm block; A2, bagged fruit to prevent pesticide deposit; A3, harvest; A4, maturity assessment (Ziosi *et al*., 2008); A5, surface sterilization; A6, ready for inoculation. **(B) Fruit inoculation and incubation**. B1-4, parallel inoculation of wounded (B3) and non-wounded fruit (B4); B5, maintaining humidity; B6-7, 72h incubation. **(C) Disease severity index (DSI) assessment. (D) DSI observed in pedigreed germplasm.** D1, high >25; D2, moderate 10-25 D3, low <10.

Results

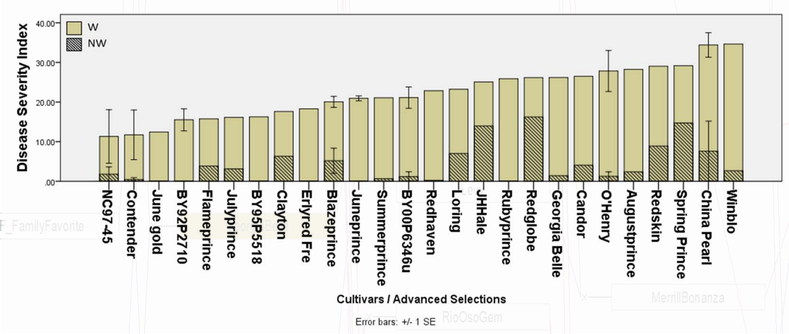

Fig. 2. Disease severity index observed in wounded (W) and non-wounded (NW) fruits of peach cultivars and advanced selections

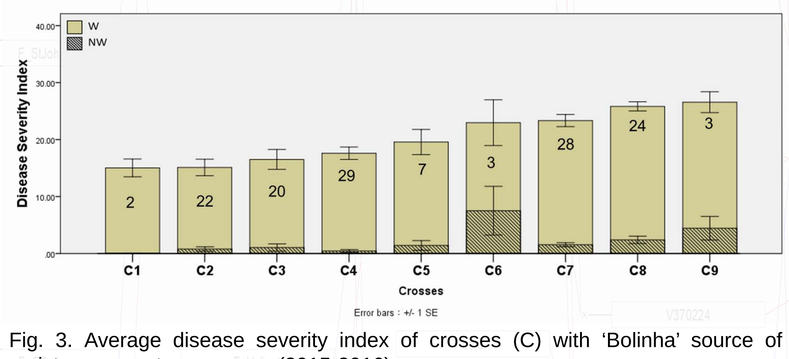

Fig. 3. Average disease severity index of crosses (C) with 'Bolinha' source of resistance over two seasons (2015-2016)

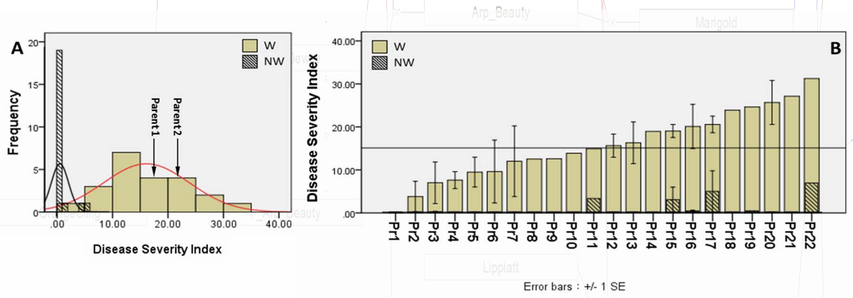

Fig. 4. Disease severity index distribution (A) and brown rot response (B) in cross 2 (C2). Pr – progeny.

Table 1. Haploblocks/haplotypes detected in QTLs associated with brown rot response in peach fruit. QTL1.1 and QTL1.2 were detected in peach x almond progeny (Martinez-Garcia *et al.,* 2013); SK_if_2009 and FL_rd_2009, skin and flesh associated QTLs, respectively, were detected in 'Contender' x 'Elegant Lady' progeny (Pacheco *et al.,* 2014); H - haploblock

Linkage group	QTL / Haploblock		Haploblock region (Mb)	Flanking SNPs	Number of SNPs	Number of alleles / Haplotypes
LG1	QTL1.1	H1	1.78-1.88	SNP_IGA_5258 SNP_IGA_5726	4	3
		H2	6.95-7.99	SNP_IGA_19818 SNP_IGA_22766	3	6
		H3	8.23-8.31	SNP_IGA_23251 snp_1_7856380	3	4
		H4	9.26-9.71	SNP_IGA_25403 SNP_IGA_26500	5	5
		H5	10.39-10.63	SNP_IGA_28112 SNP_IGA_28465	5	4
	QTL1.2	H1	26.92-27.06	SNP_IGA_88104 SNP_IGA_88772	5	5
		H2	30.86-32.14	SNP_IGA_99110 SNP_IGA_101065	4	5
LG2	SK_if_2009		21.89-22.47	SNP_IGA_274142 SNP_IGA_276426	10	7
LG3	FL_rd_2009		9.28-9.8	SNP_IGA_320761 SNP_IGA_321596	5	5

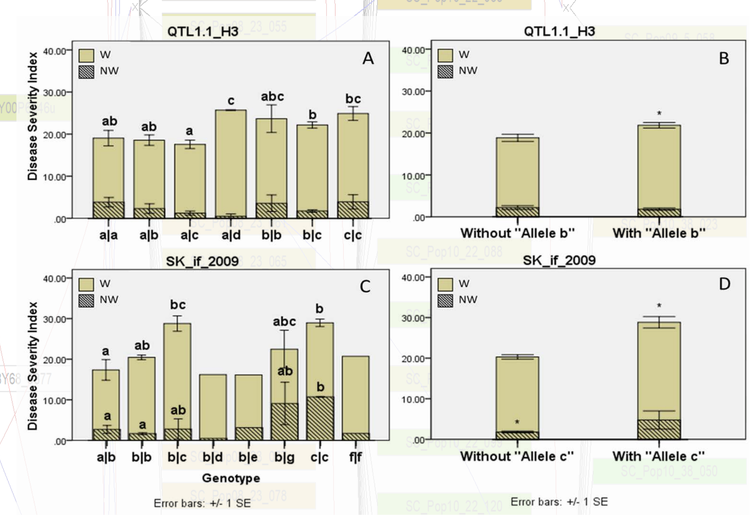

Fig. 5. Trait values of brown rot associated genotypes (A and C) and haplotypes/alleles (B and D) detected in peach. A-B, QTL on chromosome 1 (Martinez-Garcia *et al*., 2013); C-D, skin associated QTL detected on chromosome 2 (Pacheco *et al*., 2014). W – wounded; NW – non-wounded assay.

- **Phenotypic data revealed different responses to brown rot infection of wounded and non-wounded fruit in peach pedigreed germplasm (Fig. 2 & 3).**
- **Wounding increased infection rate and disease severity index (DSI) in the analyzed material (Fig. 2 & 3).**
- **Crosses showed transgressive segregation for brown rot DSI, with some progeny having very low DSI in both wounded and non-wounded fruit (Fig. 4) .**
- **Analyzed peach germplasm exhibited sufficient brown rot tolerance / resistance variability for QTL discovery and is suitable for pedigree based analysis (PBA).**
- **Analysis of the brown rot associated regions in peach genome revealed 5 and 2 haploblocks in two QTLs reported on chromosome 1 (Martinez-Garcia *et al*., 2013), and one haploblock for each of the QTLs reported on chromosomes 2 and 3 (Pacheco *et al*., 2014), with number of haplotypes / alleles ranging from 3 – 7 (Table 1).**
- **Non-wounded treatment elicited similar response among genotypes in QTL1.1 region, while wounded treatment showed significant differences in brown rot response (Fig. 5A). Detailed analysis on effect of each haplotype/allele revealed presence of allele 'b' causing significantly higher DSI in wounded treatment (Fig. 5B).**
- **SK_if_2009 QTL genotypes exhibited significantly different responses to brown rot infection in both treatments (Fig. 5C). Analysis of individual haplotype/allele effect, revealed significantly lower DSI in both treatments when allele 'c' is absent (Fig. 5D).**
- **Further analyses, to uncover additional regions in peach genome associated with brown rot DSI and to elucidate trait values of brown rot associated haplotypes are needed.**

WHAT'S NEXT?

→ **Phenotype 'Contender' derived crosses and re-run analysis of haplotype trait values.**

→ **Detect additional QTLs via PBA; (re-)define haploblocks / haplotypes, and trait value for each haplotype in the brown rot associated regions.**

→ **Develop and validate DNA test for brown rot tolerance in unrelated peach material.**

References

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