'Silencing of host *ParPMC* genes confers resistance to *Plum Pox* Virus (PPV) in apricot (*Prunus armeniaca* L.)'

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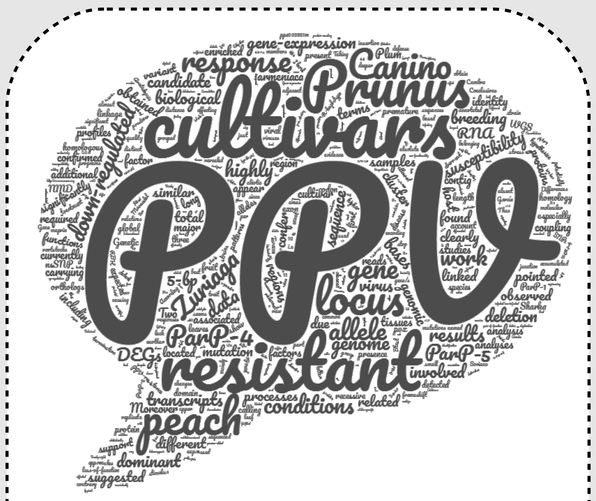
Background:
Plum pox virus (PPV), causing Sharka disease, is one of the main limiting factors for *Prunus* production worldwide. In apricot (*Prunus armeniaca* L.) the major PPV resistance locus (*PPVres*), comprising ~ 196 kb, has been mapped to the upper part of linkage group 1. Within the *PPVres*, 68 genomic variants linked in coupling to PPV resistance were identified within 23 predicted transcripts according to peach genome annotation. Taking into account the predicted functions inferred from sequence homology, some members of a cluster of meprin and TRAF-C homology domain (MATHd)-containing genes were pointed as PPV resistance candidate genes.

Results:
Here, we have characterized the global apricot transcriptome response to PPV-D infection. None of the *PPVres* locus genes was differentially expressed between infected/non-infected tissues, suggesting that PPV-D presence does not modulate their expression. However, six *PPVres* locus genes (*ParP-1* to *ParP-6*) appeared differentially expressed in resistant/susceptible cultivars (Figure 1). Two of them (*ParP-3* and *ParP-4*), that encode MATHd proteins, appear clearly down-regulated in resistant cultivars, as confirmed by qRT-PCR (Figure 2). Concurrently, variant calling was performed using whole-genome sequencing data of 24 apricot cultivars (10 PPV-resistant and 14 PPV-susceptible) and 2 wild relatives (PPV-susceptible) (Figure 3). *ParP-3* and *ParP-4*, named as *Prunus armeniaca PPVres* MATHd-containing genes (*ParPMC1* and *ParPMC2*, respectively), are the only 2 genes having allelic variants linked in coupling to PPV resistance. *ParPMC1* has 1 nsSNP, while *ParPMC2* has 15 variants, including a 5-bp deletion within the second exon that produces a frameshift mutation. *ParPMC1* and *ParPMC2* are highly homologous (87.5% identity) and adjacent in the MATHd genes cluster suggesting they are paralogs originated from a tandem duplication (Figure 4). Cultivars carrying the *ParPMC2* resistant (mutated) allele show lack of expression in both *ParPMC2* and especially *ParPMC1*.

Conclusions: Accordingly, we hypothesize that *ParPMC2* is a pseudogene that mediates down-regulation of its functional paralog *ParPMC1* by silencing. As a whole, results strongly support *ParPMC1* and/or *ParPMC2* as host susceptibility genes required for PPV infection which silencing may confer PPV resistance trait. This finding may facilitate resistance breeding by marker-assisted selection and pave the way for gene edition approaches in *Prunus*.



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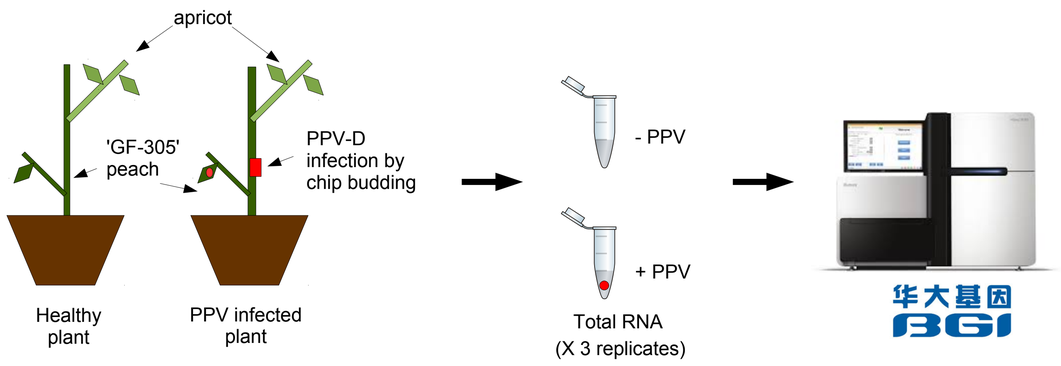
RNAseq

3 cultivars:
- **Resistant: Goldrich and Stella**
- **Susceptible: Canino**

→ **2 treatments:**
- **Non-infected**
- **Infected**

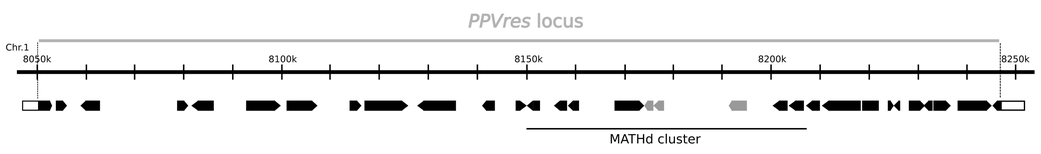
→ **BioProject PRJNA387702**

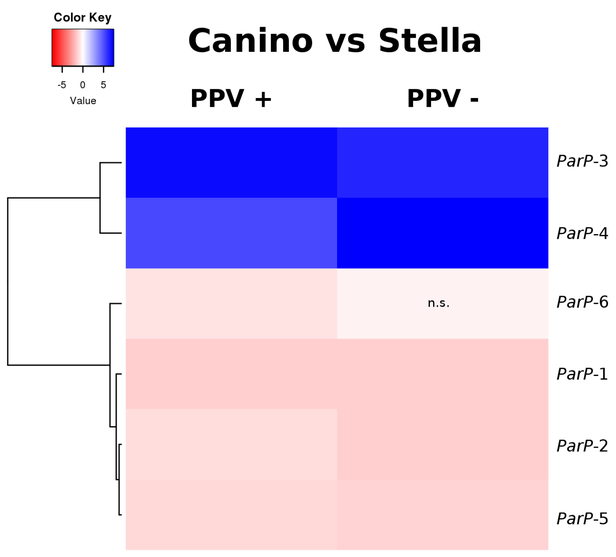


***De novo* transcriptome assembly & annotation**

Differential expression analysis

Transcriptome profiles differ between resistant/susceptible apricot cultivars

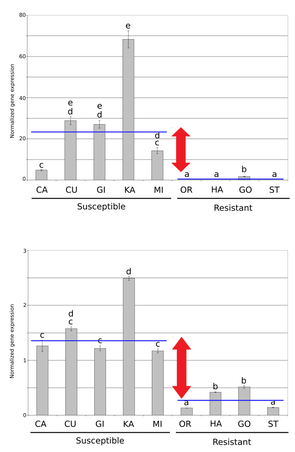




(Figure 1)

Susceptible > Resistant

Confirmed by qPCR using more cultivars:



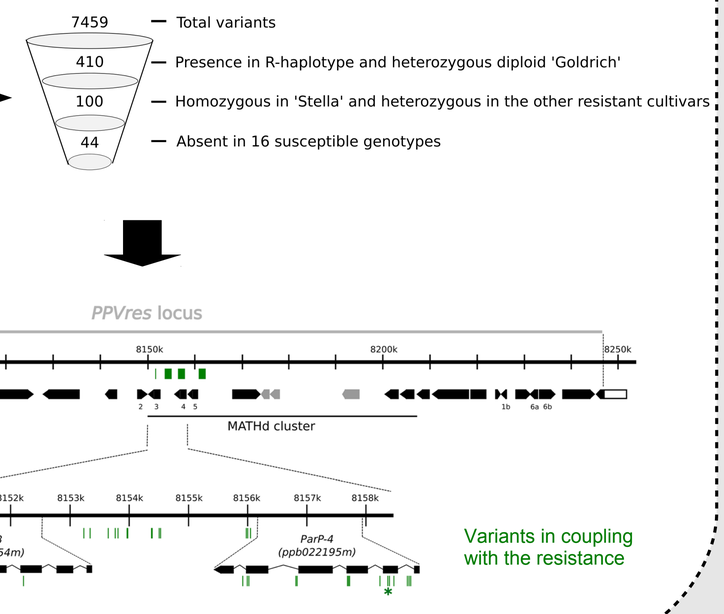
(Figure 2)

Whole-genome sequencing

WGS mapping, variant calling and filtering

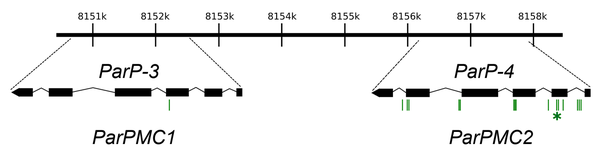
24 cultivars:
- 10 PPV resistant
- 14 PPV susceptible

2 wild related species (susceptible)
6 BAC clones (R-haplotipe Goldrich)



(Figure 3)

Variants in coupling with the resistance

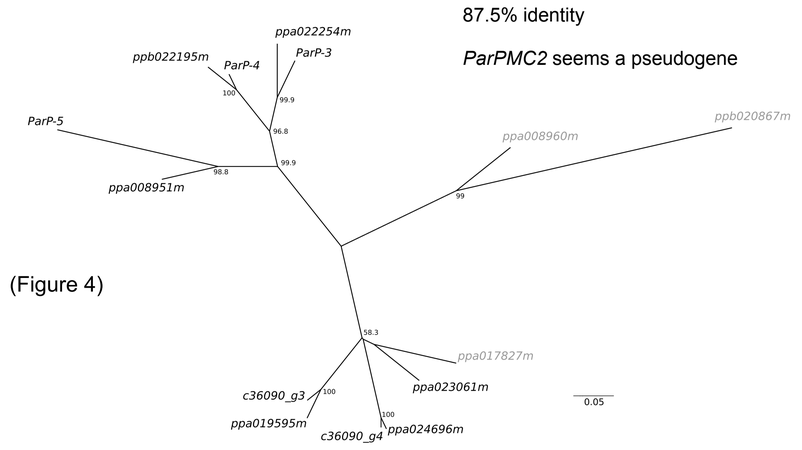


***Prunus armeniaca PPVres* MATHd-containing genes (*ParPMC*)**
Host susceptibility genes required for PPV infection

Paralogs

87.5% identity

ParPMC2 seems a pseudogene



(Figure 4)

Down-regulation of *ParPMC1* and *ParPMC2* gene expression is the differential factor between PPV resistant and susceptible apricot cultivars regarding the *PPVres* locus. **How?**

1) Mutations in the promoter regions of the PPV resistant alleles may be directly affecting their expression, but half-gene dosage can not account for the observed differences between resistant and susceptible cultivars.

2) *ParPMC1* and *ParPMC2* gene expression may also be prevented by **gene-silencing**. How? Non-mediated decay? Pseudogene-silencing his protein-coding cousin?

More analyses are in progress to elucidate this mechanism

Acknowledgments

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