GENETIC VARIATION WITHIN THE TRANSLATOME OF PLUM POX VIRUS IN RESPONSE TO LEAF DEVELOPMENT AND VERNALIZATION

YVETTE B. TAMUKONG[1,4], Tamara D. Collum[2,3], Andrew L. Stone[3], Diana J. Sherman[3], Madhu Kappagantu[4], Elizabeth E. Rogers[3], Christopher Dardick[2] and James N. Culver[1,4]

[1]Institute for Bioscience and Biotechnology Research, College Park, MD, [2]USDA-ARS, Appalachian Fruit Research Laboratory, Kearneysville, WV, [3]USDA-ARS Foreign Diseases and Weed Sciences Research Unit, Fort Detrick, MD, [4]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD

Introduction

Plum pox virus (PPV), existing in the form of quasispecies, is a worldwide threat to stone fruit production causing economic losses 10 billion dollars. Selection on the quasispecies may lead to the emergence of more virulent pathogens. Previous studies have identified ranges of genetic variants within infected plum trees with the potential to affect disease.

Aim

- Investigate the dynamics of PPV sequence variants affecting infection over two growth seasons.
- Compare variant population between developmental timepoints and between tissues

Methods

- Generated transgenic plum lines FLAG-tagged ribosomal protein L18 (RPL18) from either the phloem specific SUC2 or 35S promoters (Fig. 1).
- Infected lines with PPV by graft and aphid inoculation.
- Over 2 growth periods leaf tissues were collected from infected plum lines and translatome mRNA TRAP purified at 2, 4, 6, and 12 weeks developmental age as well as from pre- and post- vernalization buds for high throughput sequencing analysis (Fig. 2).

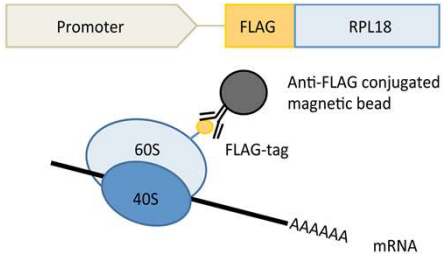

FIG. 1: FLAG-tagged ribosomal protein L18 (RPL18) was used to immuno-purify mRNA-ribosome complexes. This is the Translating ribosome affinity purification (TRAP). Ribosomal mRNA is generally being translated and thus PPV reads from this pool will likely represents viral genomes that are contributing to the infection process.

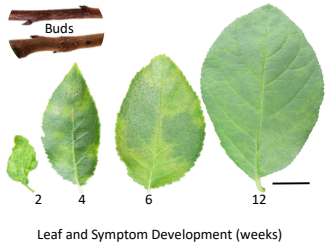

FIG. 2: Representative photographs of plum pox virus (PPV) infected leaves collected at 2, 4, 6 and 12 weeks as well as buds used for translatome analysis. Bar represents 1 cm.

- Translatome mRNA (mRNA complexed with FLAG-tagged ribosomes) were TRAP purified from all 59 samples and sent for high throughput sequencing.
- 59 output libraries, composed of 151 nucleotide paired-end reads, were analyzed using CLC Genomics Workbench v. 10.0.1 (Qiagen, Valencia, CA, U.S.A.). See Table 1 for analysis pipeline.

Table 1: Analysis pipeline. Preliminary Quality control, mapping of RNA Sequences, variant calling.

Software:	CLC genomics Workbench (, Valencia, CA, U.S.A.)
Starting material:	FastQ files
Goal	Setting
Trim reads	TruSeq LT trim adaptor list
	Ambiguous nucleotides ≥ 2, Quality ≥ 20%
Map reads to reference	PPV-D isolate PENN7 Ref Genome (NCBI EF640935)
	Match score 1, Mismatch cost 2, Linear gap cost, Insertion cost 3, Deletion cost 3
	Length fraction 0.5, Similarity fraction 0.8
	No Global alignment
	Auto-detect paired distances Yes
	Ignore Non-specific matches
Variant calling	Low Frequency Variant Detection tool
	Minimum frequency: 0.5
	Coverage ≥ 10, Count ≥ 2
	Forward/reverse balance between 0.25 and 0.75
Amino Acid Changes	Amino Acid Changes tool
	PPV-D isolate PENN7 CDS track

Table 2. Tissue distribution of translatome identified PPV variants within four frequency groups. [1]Classificaiton of variants based on frequency and presence within individual trees.

Maintenance behavior[1]	Shared, Found in both pSUC2 and p35S	Phloem (pSUC2) Only	Whole Tissue (p35S) Only
Group 1 Variants with >95% frequency in all trees	15	0	0
Group 2 Variants that vary frequency between samples	28	14	2
Group 3 Variants with consistently low frequencies	61	21	12
Group 4 Variants that appear nonconsecutively in one or two samples	179	1255	1160

Results

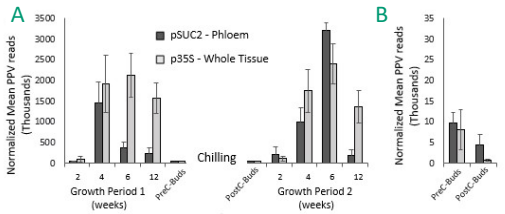

Fig. 3: PPV levels in plum whole-leaf and phloem translatomes. **A** PPV translatome associated leaf and bud RNA collected over two growth periods. **B** Expanded view of bud translatome RNA collected for one chilling period. Bars represent the mean of three independent trees ± standard error, except for the pSUC2 two-week time point for growth period two, which represents the mean of two biological replicates.

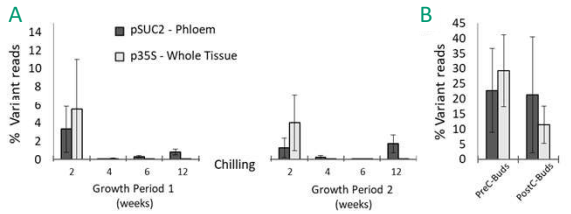

Fig. 4: **A** PPV variant diversity in plum whole-leaf and phloem translatome RNA. Variants include nucleotide polymorphisms, indels and replacements with SNPs making up the majority, (85%). Diversity of PPV populations are a percent of normalized PPV reads containing variants in collected plum tissues over two growth periods. **B** PPV variant diversity in plum bud translatome RNA collected for one chilling period. Bars represent the mean of three biological replicates ± standard error, except for the pSUC2 at 2GP2 time point which is the mean of two biological replicates.

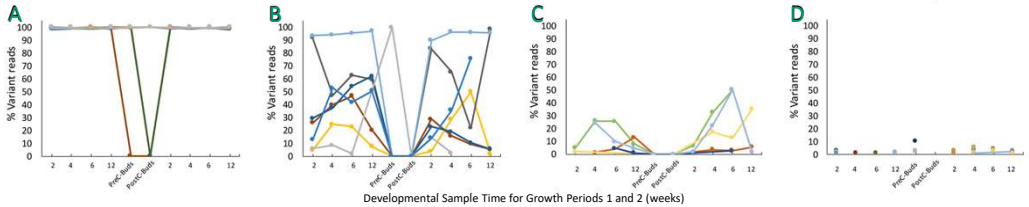

Fig. 5: Maintenance of individual variants over sampling times. The percent frequency of individual variants at each sample time is shown for the four identified variant groups. **A** Group 1 variants appearing at a high frequencies at most time points. **B** Group 2 variants displaying variable frequencies. **C** Group 3, variants consistently showing frequencies <50%. **D** Group 4 variants showing no consistent maintenance pattern. Graphs are representative of variants identified in one PPV infected translatome tree.

Discussion

- Overall infection levels generally displayed rapid increases in leaf infection between 2 and 4 or 4 and 6 weeks with dramatically lower infection levels in the pre- and post-vernalization buds. (Fig. 3)
- In older 12-week leaves, the level of PPV associated with phloem translatome is significantly reduced compared to that in the whole tissue translatome for both growth periods (Fig. 3), indicating tissue specific variation in virus levels.
- Despite having the lowest PPV levels, the number variant containing reads, measured as a percent of the total PPV translatome, was greater in diversity within bud tissue and to lesser extent within newly emerging two-week-old leaf tissues than in any of the other leaf samples (Fig. 4). Low PPV translatome levels in these tissues likely limit the production of viral proteins associated with super-infection exclusion and limit its effectiveness in repressing mutant virus accumulation or maintenance.
- The total set of 2747 could be characterized into 4 distinct groups based on population frequency and maintenance overtime (Fig. 5; Table 2). None of the high frequency variants, Group 1, are present in either tissue specific set but we do find that there are more group 2 and 3 variants that are specific just to the phloem (Fig. 5; Table 2).

Conclusions

- TRAP is such a system that allows for tissue specific observation.
- Tissue and developmental stage impact the levels and diversity of PPV.
- Buds are likely a unique environment for the maintenance of variants from one growing season to the next.
- Individual variants could be placed into four distinct groups based on their frequency and maintenance during the sampling period.
- There may exist phloem specific PPV variants with yet to be discovered advantages.

Ongoing and Future Efforts

- Investigate the role of roots and buds in the seasonal maintenance of PPV variants .
- Assess host gene regulatory changes and their impact on PPV in buds and roots pre-, peri- and post- dormancy.

Reference

Silvie Van den Hoecke, et al. "Analysis of the Genetic Diversity of Influenza a Viruses Using Next-Generation DNA Sequencing." BMC Genomics, vol. 16, 2015, pp. 79–79.

Tamukong, Y. B. et al. (2020) "Dynamic Changes Impact the Plum Pox Virus Population Structure during Leaf and Bud Development," Virology. doi: 10.1016/j.virol.2020.06.014.

Acknowledgements

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