

Molecular epidemiology and physiology of Shiraz Disease in South Australia



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Background: Shiraz Disease (SD) in Australia

- First reported from South Africa in 1985.
- First discovered in a South Australian vineyard in 2000.
- Symptoms of SD: restricted spring growth, uneven lignification of shoots, bright red leaves remain during winter.
- Key pathogens of SD: Grapevine virus A (GVA), maybe some Grapevine leafroll associated viruses (GLRaVs).
- Epidemiology and natural spread : propagating and grafting virus-infected grapevine materials. Transmission by mealybugs and scale insects.
- Negatively impacts on berry ripening and quality. Economic impact on Shiraz in Australia approx. \$200,000 per ha of virus infected vines.



Non-SD (Healthy)



SD (Diseased)

Research questions and methods

Q1: Which viruses, in addition to GVA, are present in SD infected vines?	Q2: Diversity of GVA variants in South Australian vineyards?	Q3: How does SD affect Shiraz vine physiology and berry quality?
Reverse transcription polymerase chain reaction (RT-PCR) , Metagenomics Next-Generation sequencing (NGS)	Amplicon NGS	Monitoring SD vine and non-SD vine using Viticanopy app, AP4 Leaf Porometer, SPAD chlorophyll meter, Near-infrared spectroscopy(NIR)

Results

Metagenomics NGS data: Optimisation of extraction methods

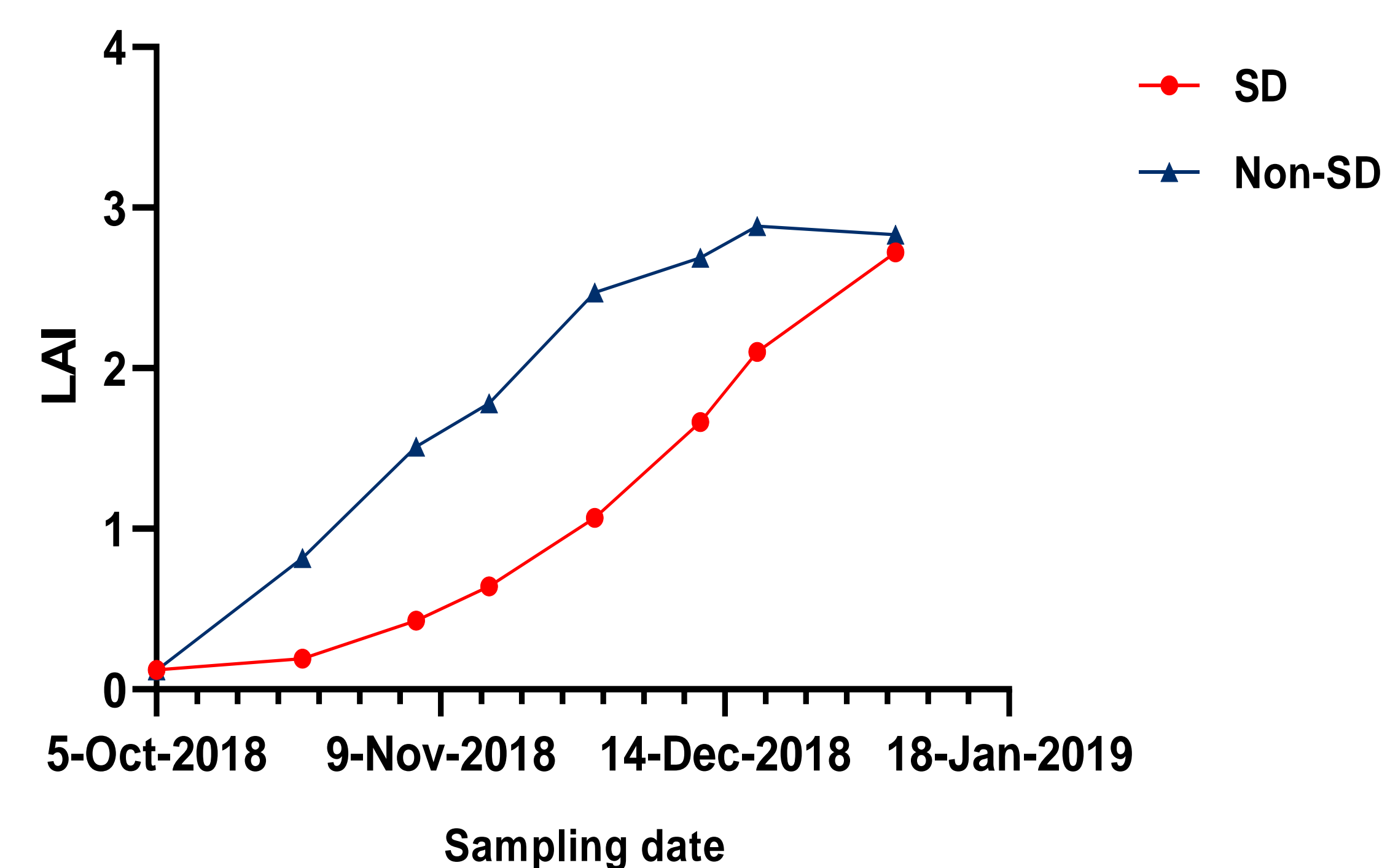
Sample ID	Extract Format	Total raw reads	Virus detected	Viral reads
Sample 1	Total nucleic acid extraction	1,011,990	GRSPaV, GLRaV-1, GVA	0.01%
Sample 1	Double-stranded RNA	985,459	GRSPaV, GLRaV-1, GVA	0.13%
Sample 1	dsRNA+ ribosomal RNA depletion	1,594,388*	GRSPaV, GLRaV-1, GVA*	*22.18%
Sample 2	Total nucleic acid extraction	1,487,659	GRSPaV	0.03%
Sample 2	Double-stranded RNA	385,256	GRSPaV, GLRaV-4/6, GLRaV-4/9, GVA*	0.27%
Sample 2	dsRNA+ dsRNA+ ribosomal RNA depletion	2,446,079*	GRSPaV, GLRaV-4/6, GLRaV-4/9, GVA*	*72.24%

Sequencing platform: Illumina MiSeq sequencer with 300bp read length

Module used for data analysis: Trim Galore (0.4.2), SPAdes (3.12.0), BLAST+ (2.6.0)

Virus species: Grapevine leafroll-associated viruses 1 (GLRaV-1), Grapevine leafroll-associated viruses 4 strain 6, strain 9 (GLRaV-4/6, 4-9), Grapevine rupestris stem pitting associated virus (GRSPaV)

Leaf area index during canopy development at the Langhorne Creek site



Leaf Area Index (LAI) measured using the Viticanopy App: 15 SD vines and 15 Non-SD vines were selected based on symptom observation and RT-PCR test results.

Conclusions

- The complete genome sequence of the first Australian isolate of GVA and some other grapevine viruses was obtained by Metagenomics NGS.
- Ribosomal RNA depletion is needed in order to optimize the Metagenomics NGS results; hence, more viral reads obtained and more viruses detected.
- The Viticanopy results show SD vines are lower in vigour, may have altered photosynthesis compared to non-SD vines. SD vines also have delayed flowering time and berry development compared to non-SD vines (data not shown).

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