

Can transcriptomics shed light on the “old-vine” character of wines?

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Introduction

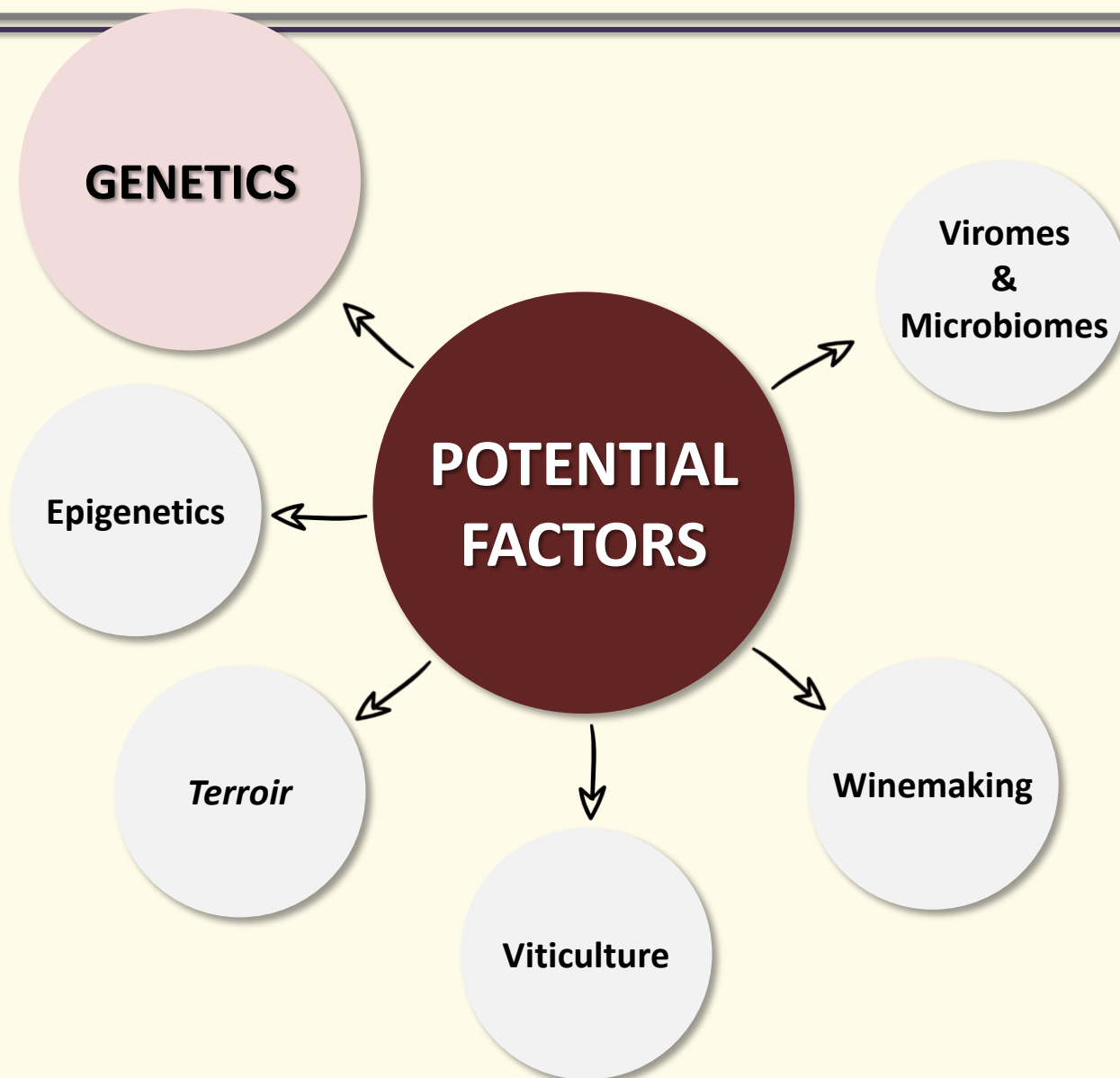
- Vineyards remain profitable despite prolonged exposure to environmental stresses
- Anecdotal evidence - old vines produce wines of greater complexity and depth compared to young vines
- 'Old vine' on wine labels

Old vs. Young vines?

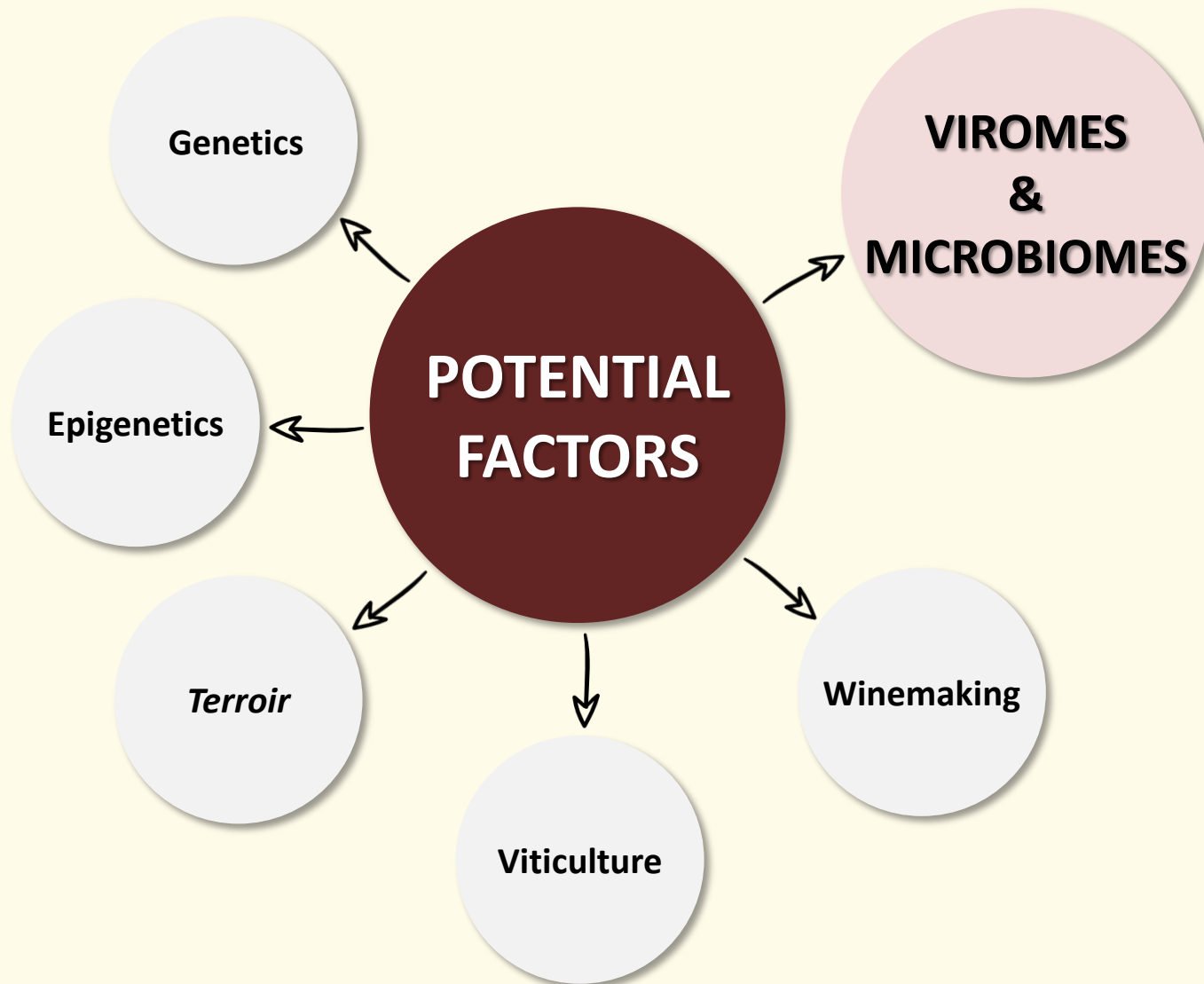
- Geographical definitions
- South African vines:
 - 'old' > 35 years
 - 'young' < 10 years



Difference in wine quality?

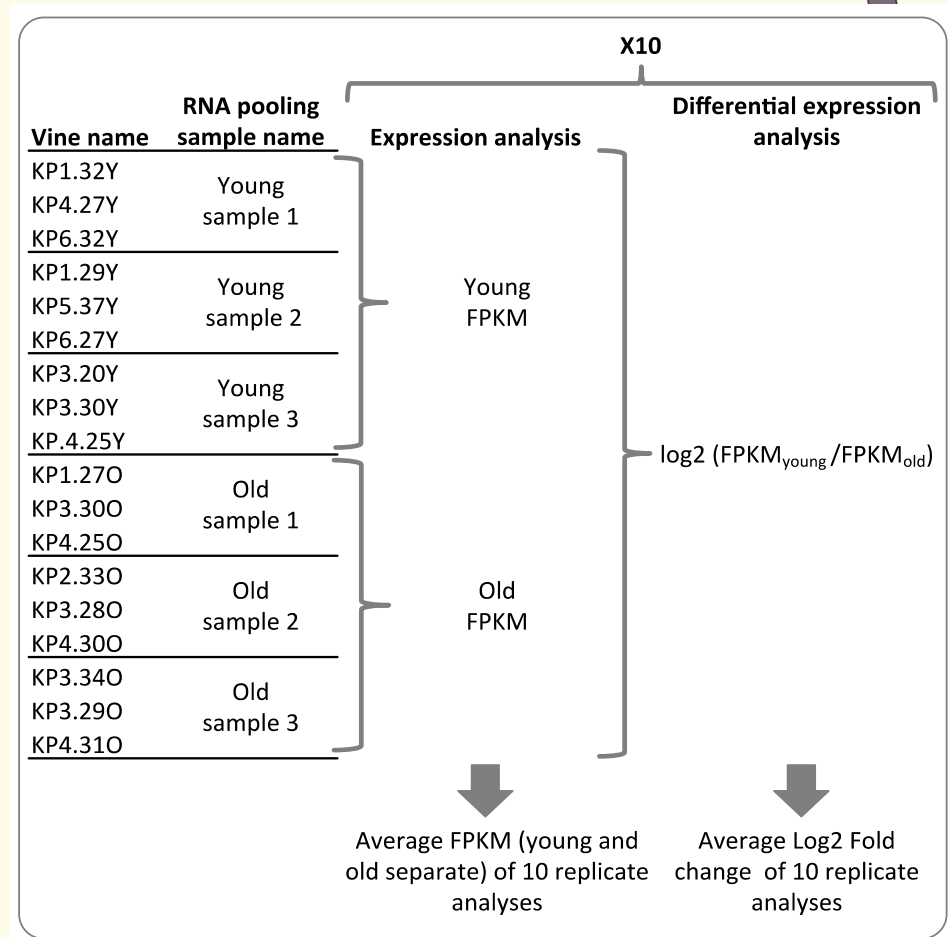


Difference in wine quality?

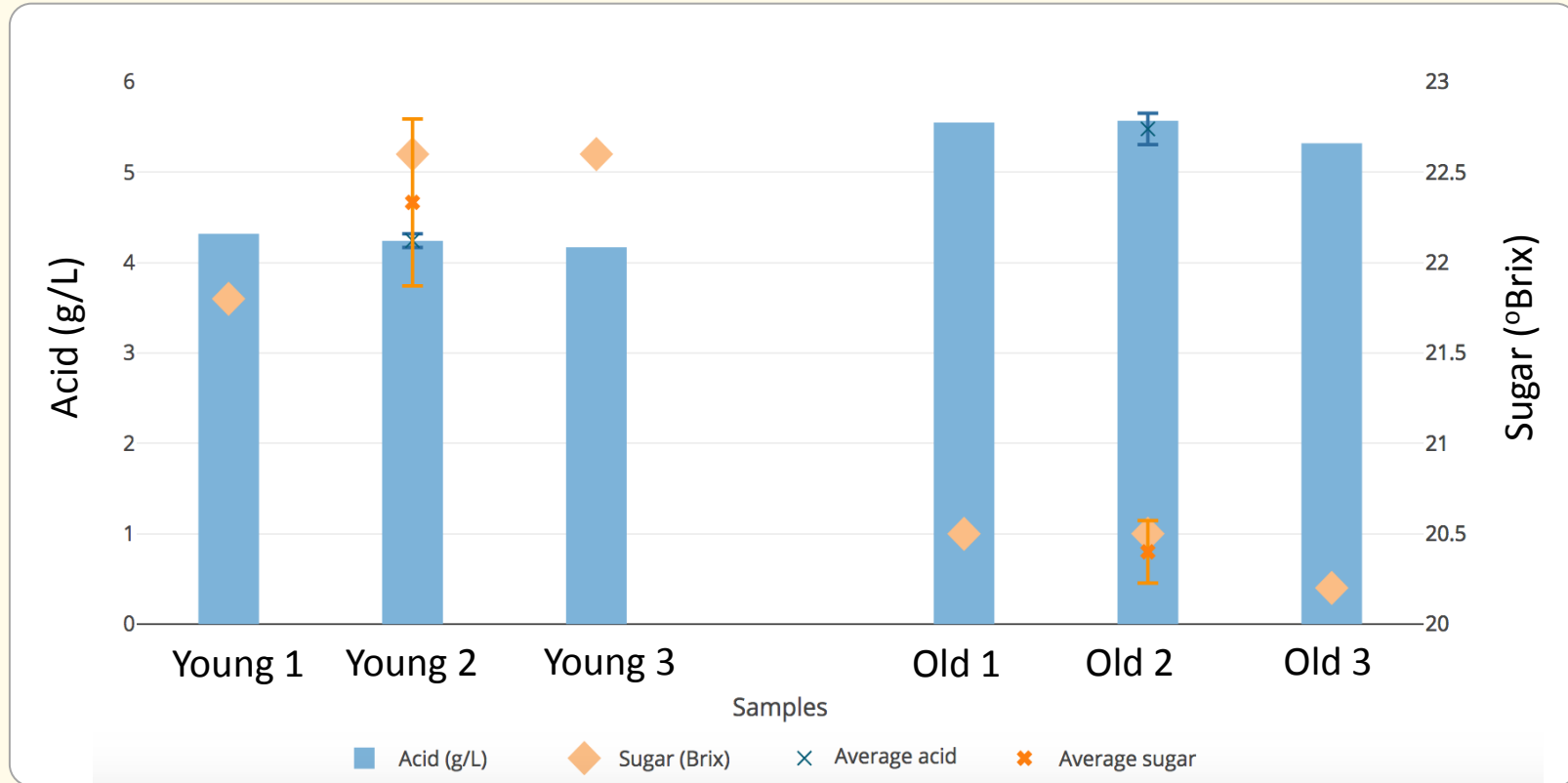


Materials & Methods

- Pinotage
 - (Pinot noir x Cinsaut - 1925)
 - 8% of total plantings in SA
 - Genome sequence (Poster 16)
 - Genome editing (Poster 12)
- Samples from a commercial Pinotage young and old inter-planted vineyard in Stellenbosch
- Berry and leaf material sampled from nine young and nine old vines at harvest time in Jan 2016
- RNA from 18 vines separately extracted, pooled to yield three young and three old plant biological replicates
- Sequencing library prep and RNA-Seq on an Illumina platform was outsourced to a commercial service provider



Sugar & Acid concentration in grape juice

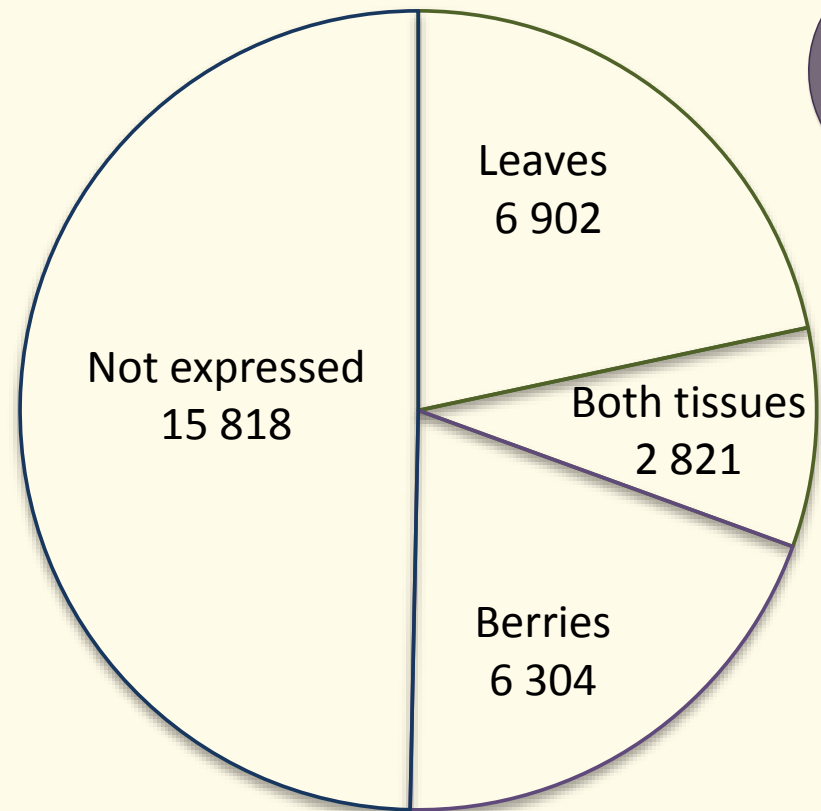


- Juice from 18 vines pooled the same as for RNA
- Titratable acid and sugar measured
- Young: sugar 22.33°Brix and acid 4.24g/L
- Old: sugar 20.4°Brix and acid 5.48g/L



Genes expressed in leaves & berries

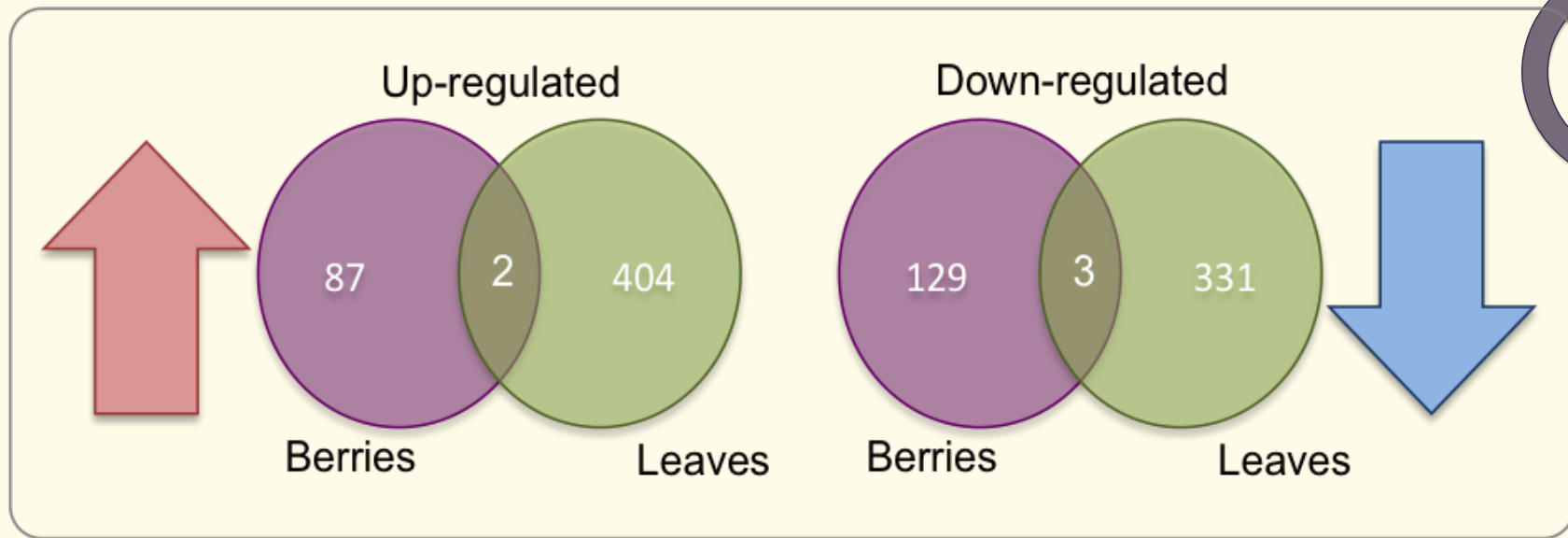
- Pinot noir has 31 845 annotated genes
- 16 027 of these are expressed in Pinotage
- 598 more genes expressed in leaves than in berries



Loci in V2.1 CRIBI annotation (FPKM ≥ 10)



Differentially Expressed Genes (DEGs)

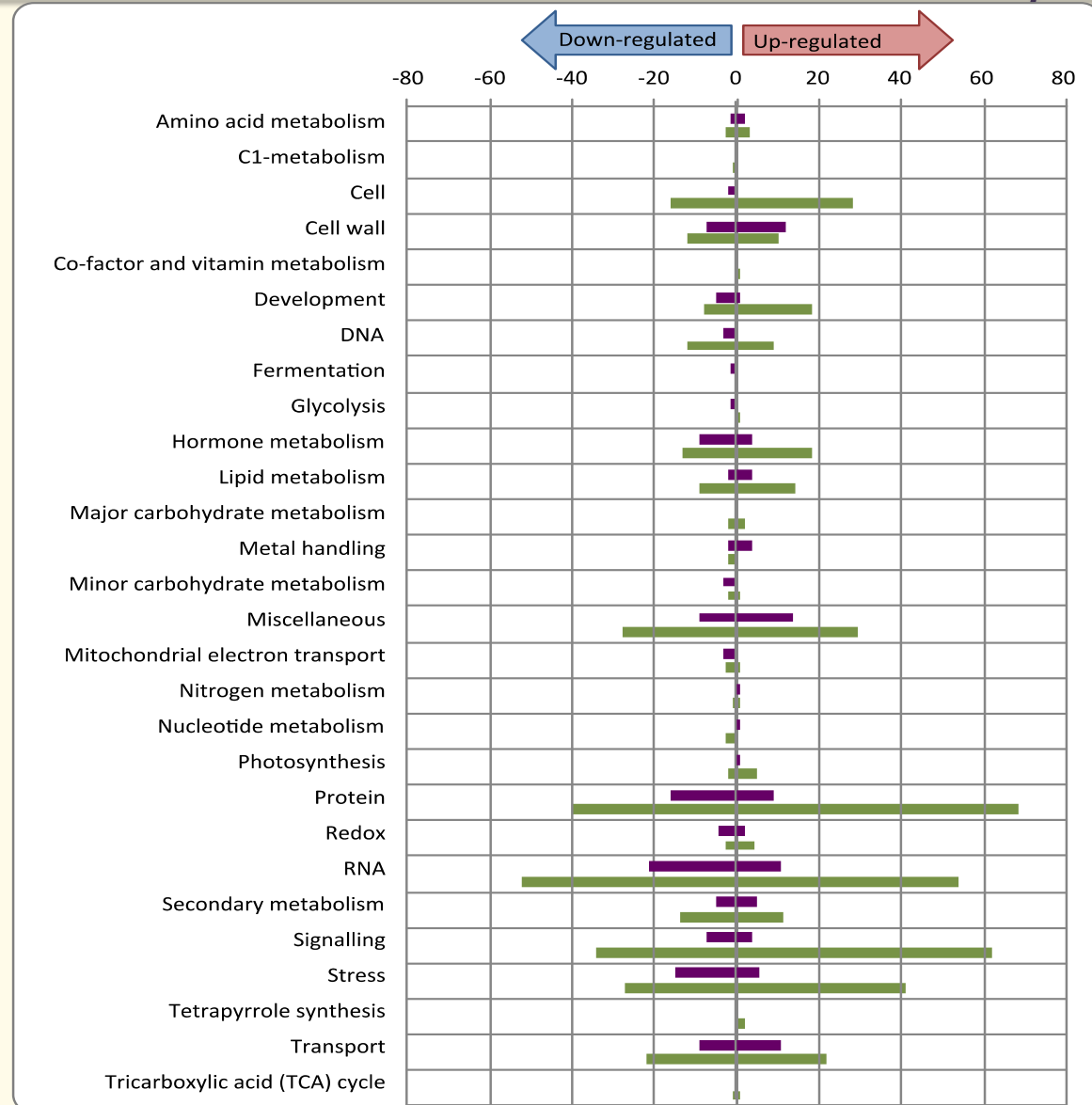


- Of the 16 027 total genes expressed in berries and leaves, 956 genes were differentially expressed between young and old vines.
- Five DEGs were present in both berries and leaves.



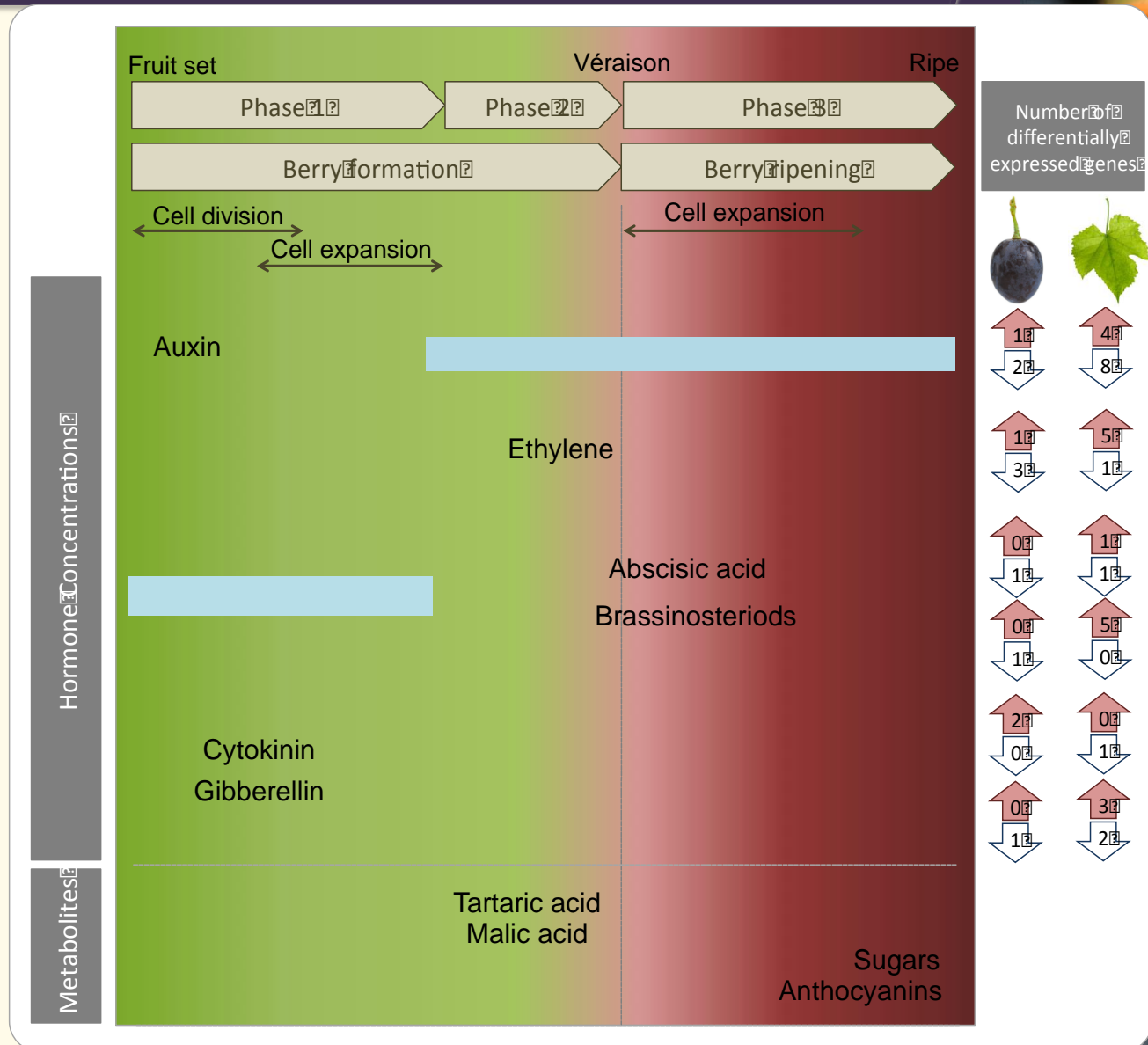
What are these DEGs?

- Functional bins are an indication of roles in metabolism
- DEGs involved in fruit ripening as an indicator of wine quality
- Focus on genes involved in “ripening” hormone signalling and biochemical changes associated with berry ripening



Berry ripening

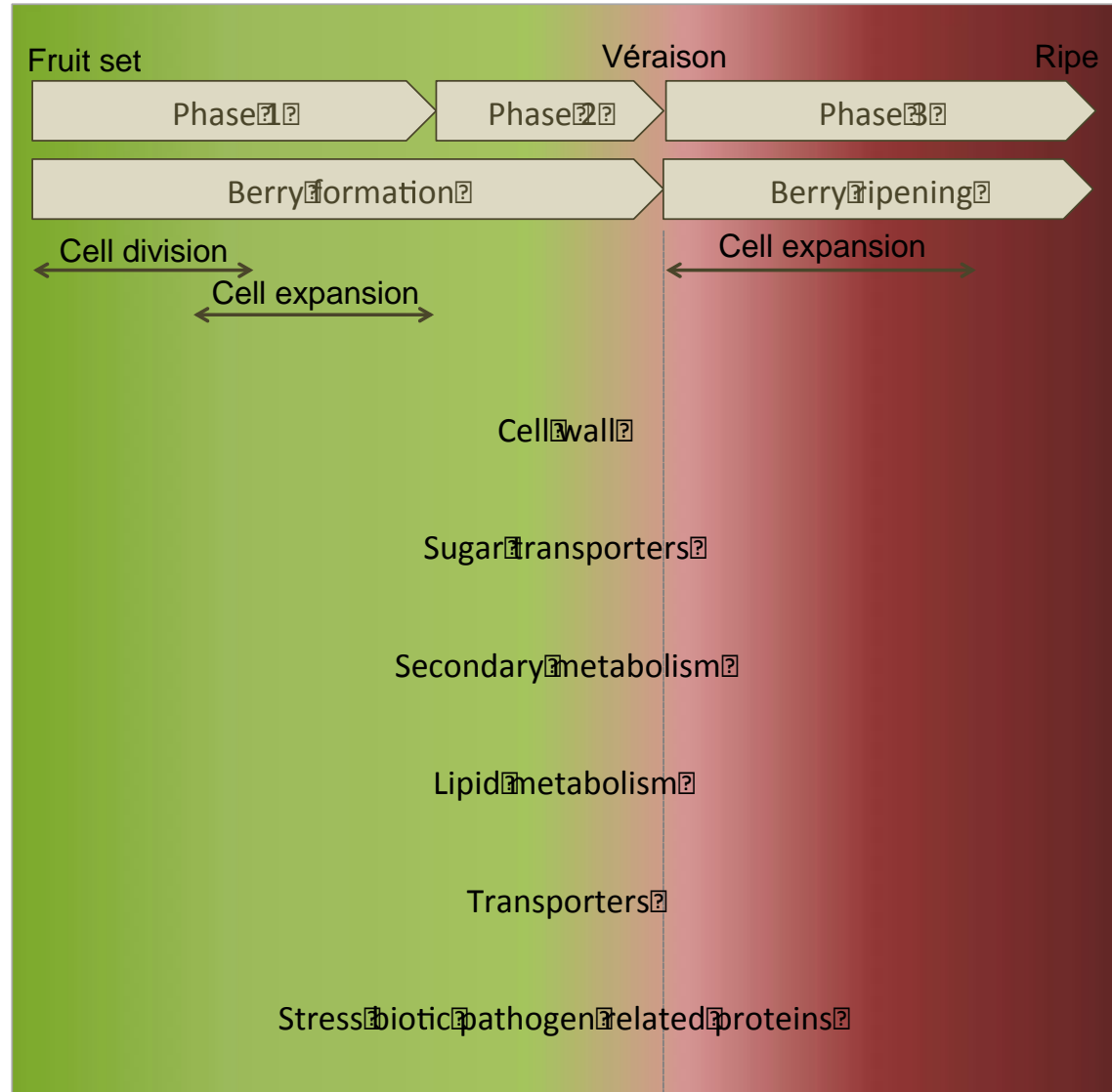
- “ripening” hormones
- 46 DEGs involved in hormone metabolism and signal transduction identified
- Mostly associated with **auxin** and **ethylene** metabolism



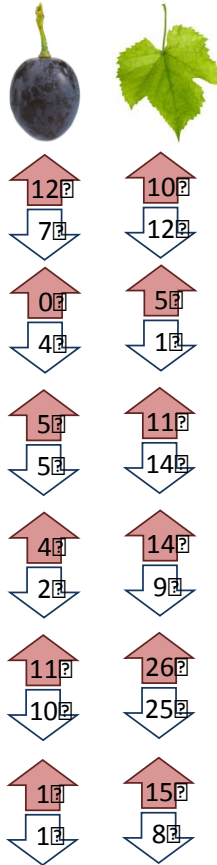
Biochemical changes

- 203 DEGs involved in biochemical changes

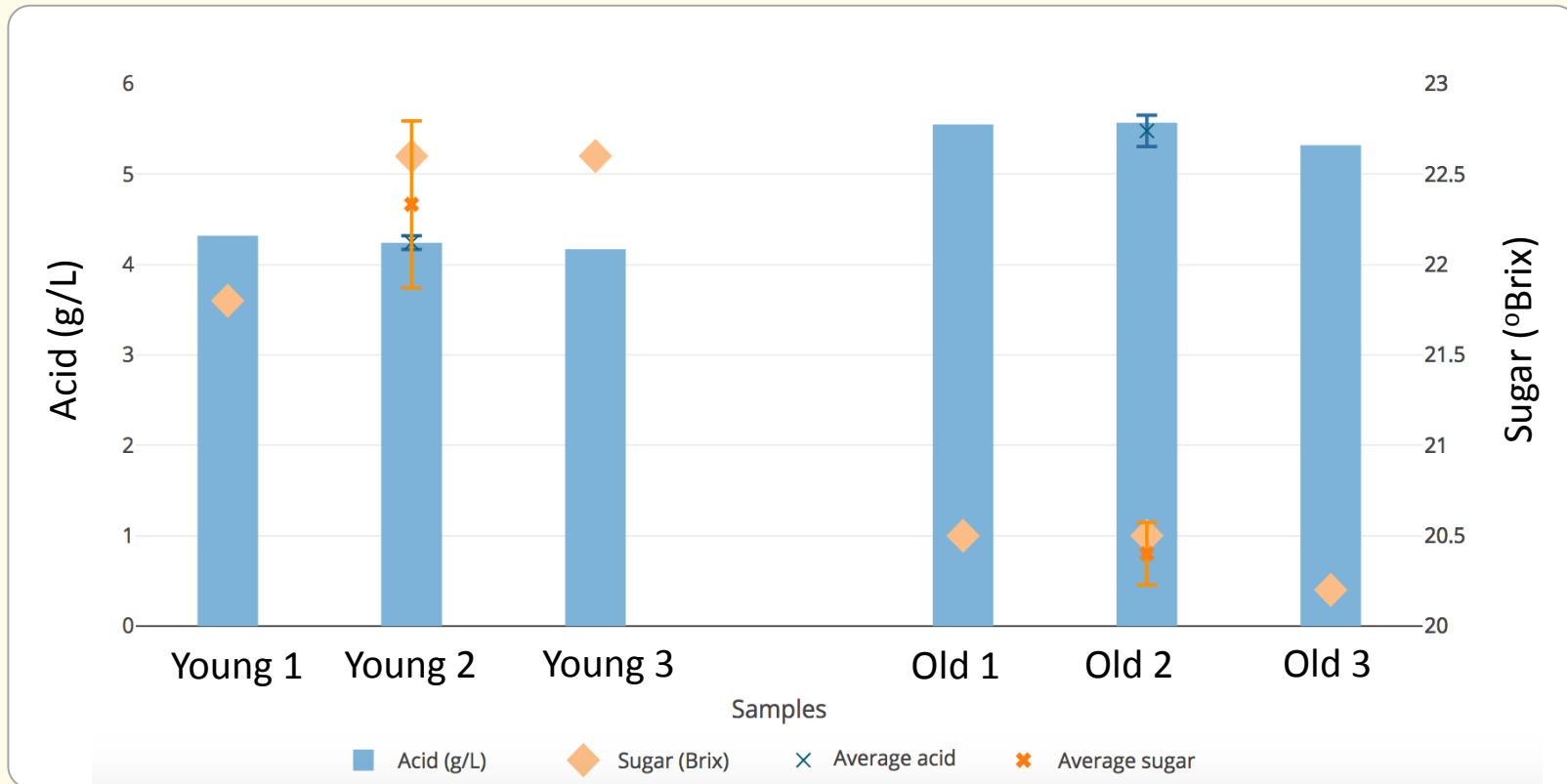
Biochemical changes during fruit ripening



Number of differentially expressed genes

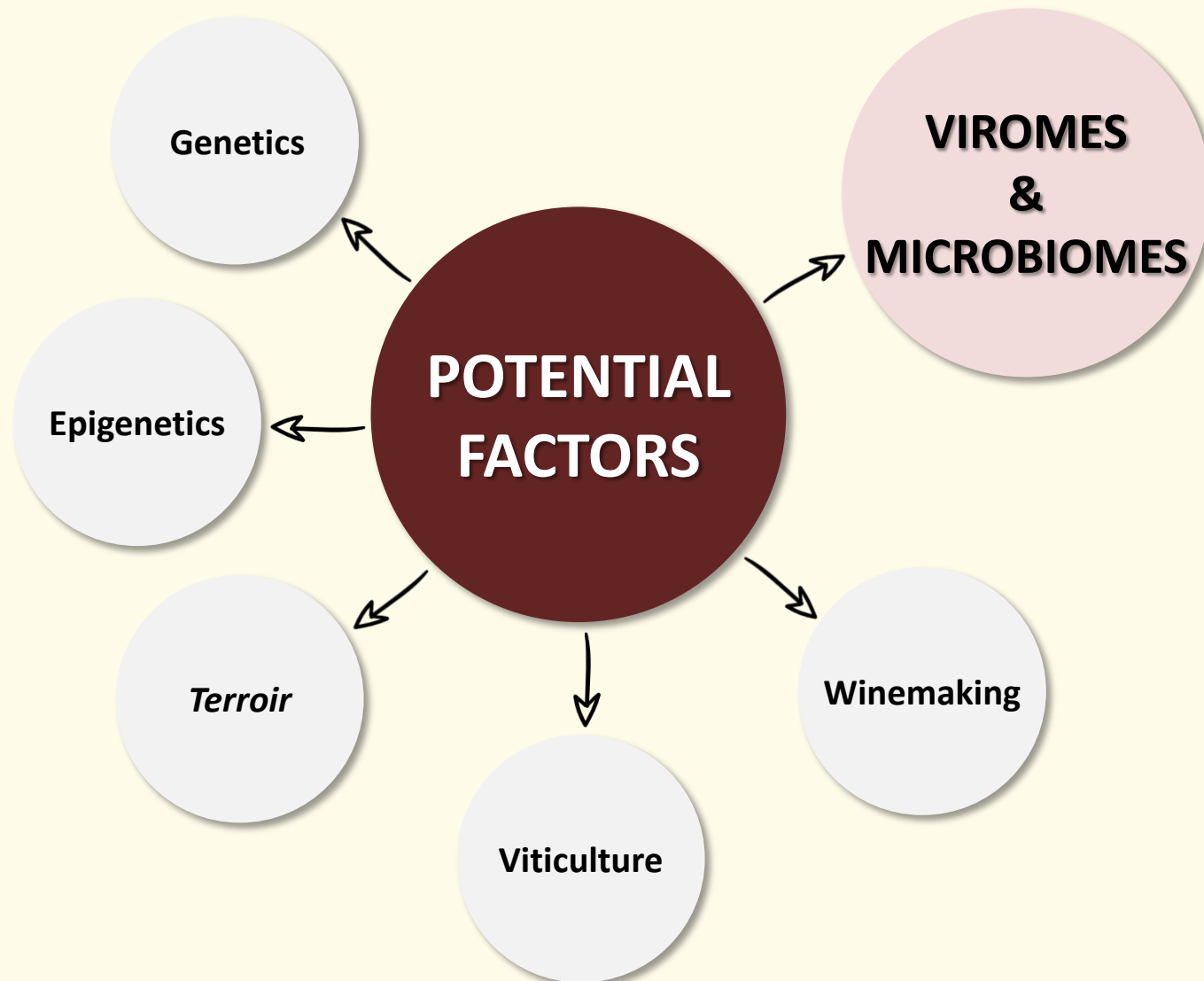


Sugar & Acid concentration in Grape Juice



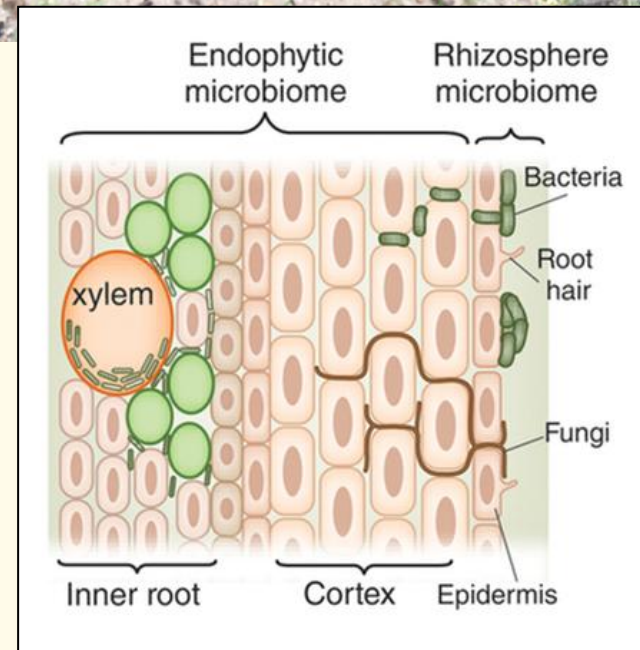
- Data suggest that young vines have progressed further along the ripening pathway.

Difference in wine quality?



Introduction

- 70+ grapevine-infecting virus- & virus-like agents
 - 65 viruses
 - GLRaV-3 is the most important
- Fungal & bacterial **pathogens** and **endophytes**



Root microbiome (Hirsch & Mauchline, 2012)



Materials & Methods



4 old & 4 young Pinotage vines



Nucleic acid extraction



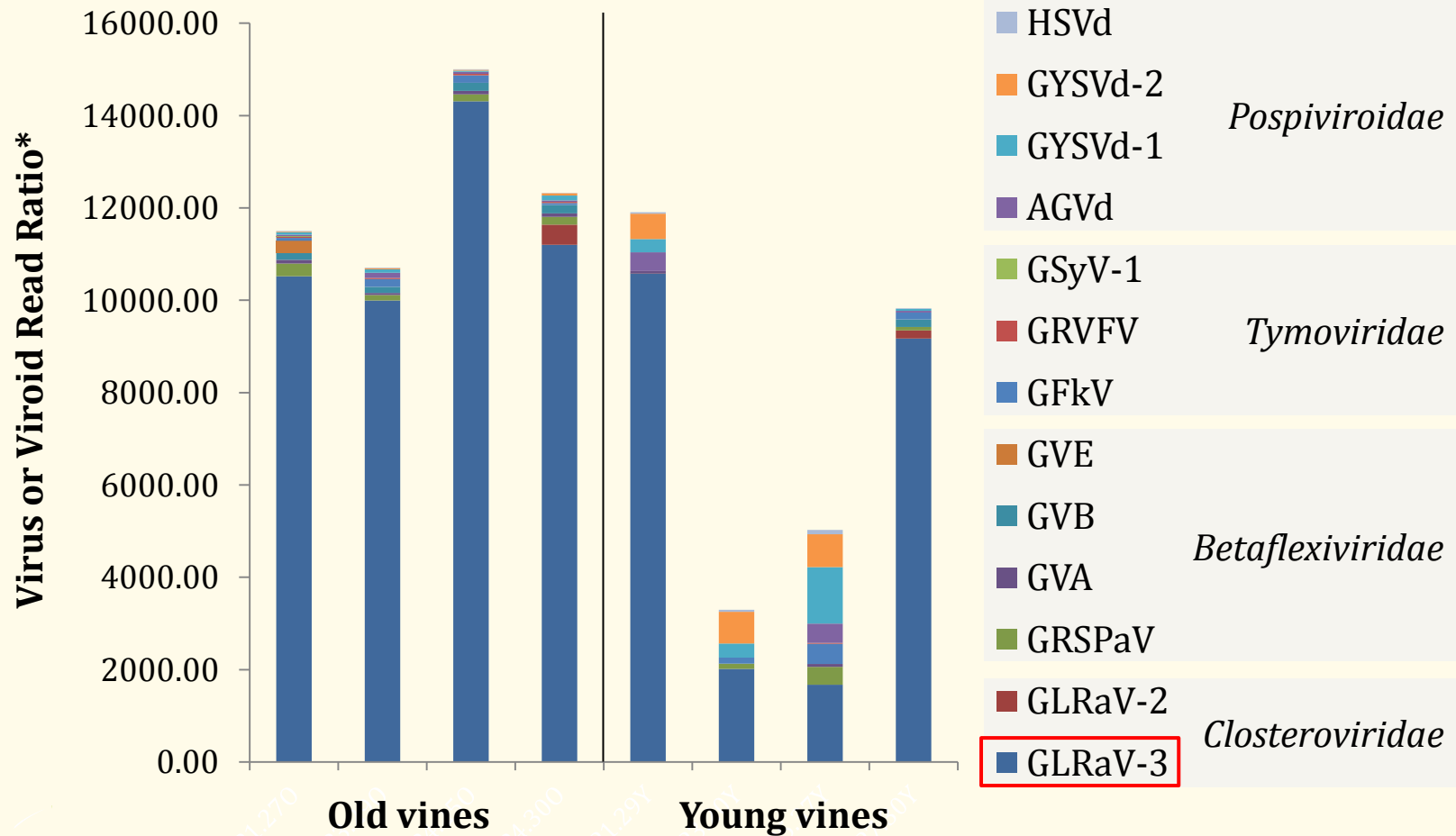
Next-generation sequencing



Bioinformatic analyses



Results - Viral diversity



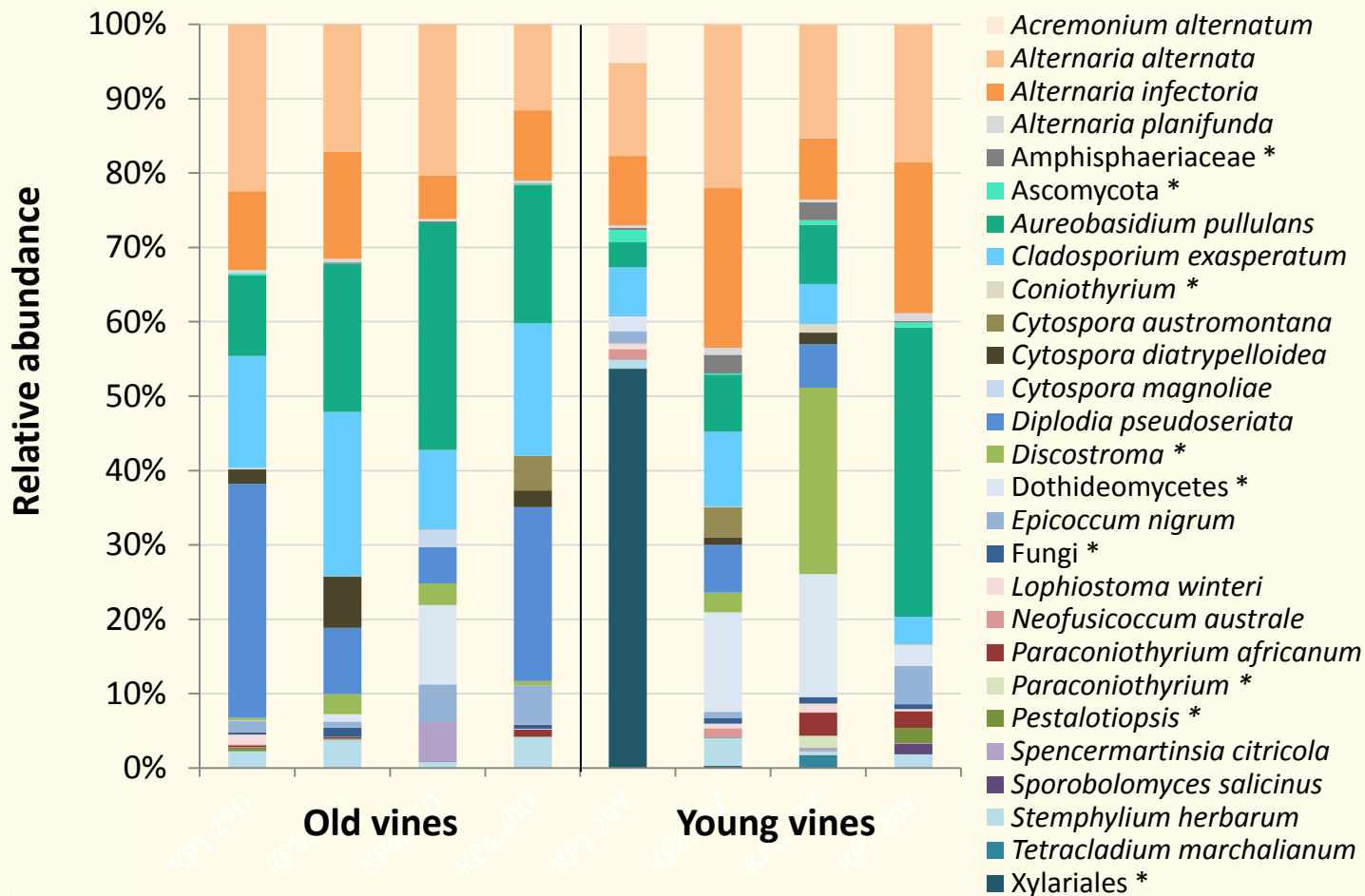
*Virus or Viroid Read Ratio = read count [contigs of species] / reference genome length * read count [total assembled contigs] * 1E+03 * 1E+06

Results and Discussion – Viral diversity

- **Greater viral diversity in old vines:**
 - 6 - 8 viruses across old vines (27 viruses, 31 variants detected)
 - 2 - 5 viruses across young vines (15 viruses, 16 variants detected)
- Possible divergent variant of GLRaV-3
- GSyV-1
 - not previously detected in South African vines
 - confirmed by RT-PCR & Sanger sequencing
- Five mycoviral families detected
 - *Chrysoviridae*, *Endornaviridae*, *Narnaviridae*, *Partitiviridae* and *Totiviridae*
- A contig detected that aligned to a grapevine hammerhead viroid-like RNA sequence
 - unproven viroid nature (viroid-like RNA entity)
- Results confirmed by RT-PCR detection assays



Fungal Diversity



Relative abundance of fungal species $\geq 1\%$ in at least one of eight samples

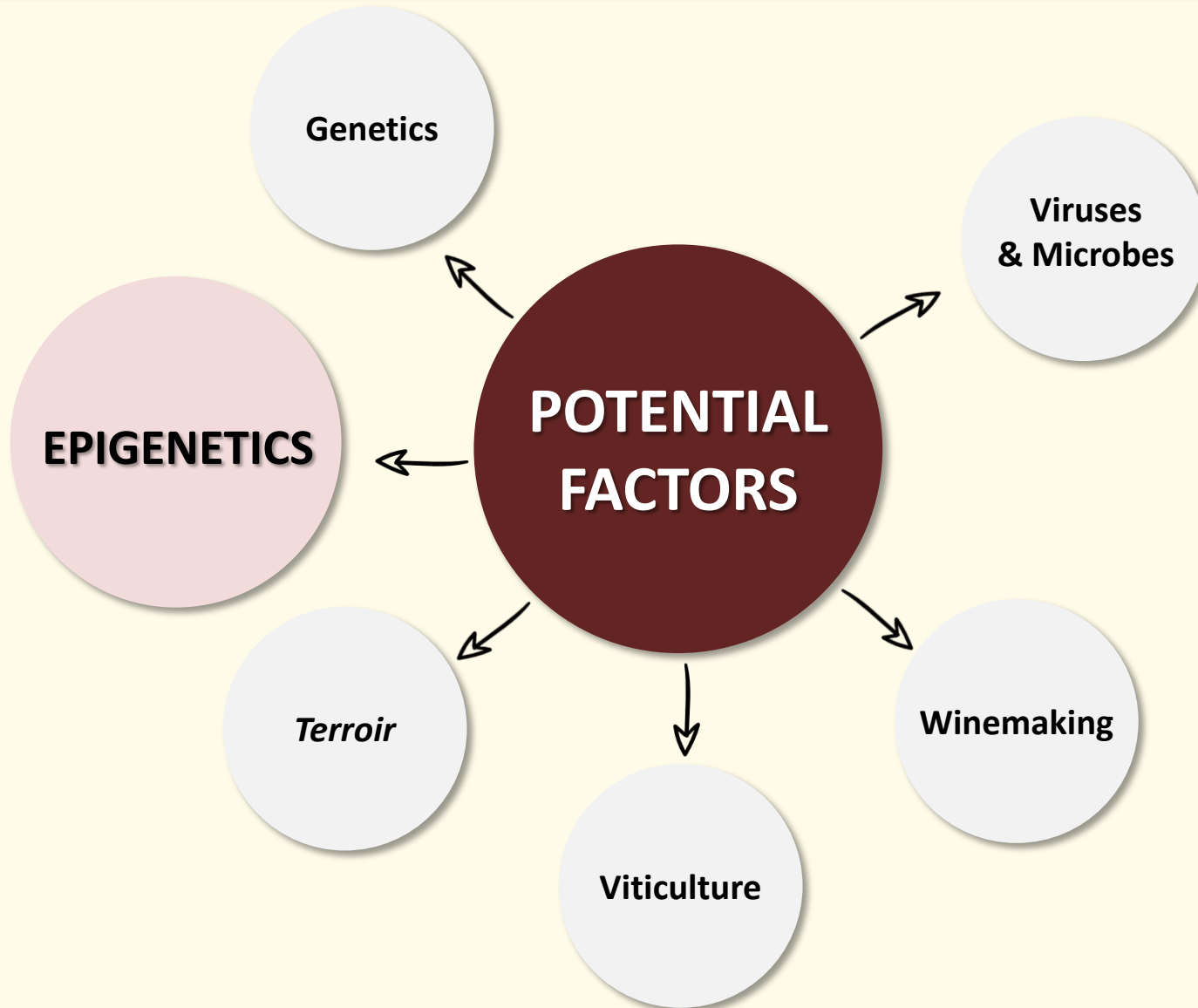
*Unidentified within taxonomic group

Conclusion

- First molecular genetics look into the unique character of old Pinotage vines
 - Suggest that berries of old vines take longer to ripen
 - More diverse virus community in old vines
 - Greater fungal diversity in young vines
- Can gene expression shed light on the “old-vine” character of wines?
 - No specific trends observed between young & old vines



So, what next?



Acknowledgements



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