

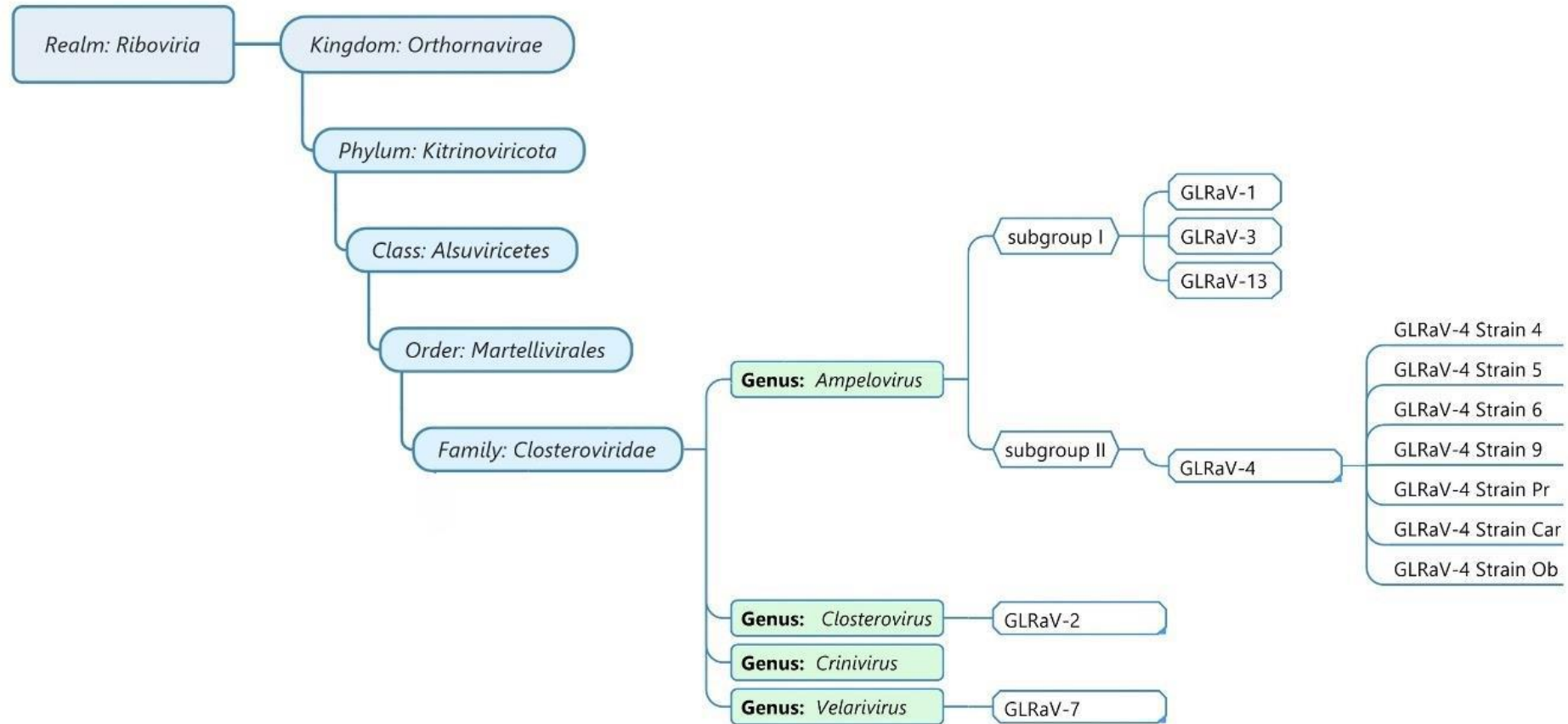
Virus infection dysregulates table grape ripening through genome-wide reprogramming of RNA and miRNA

Wisam Hazim Salo

Doctor of Philosophy of The University of Western Australia

School of Molecular/ Genomics and Bioinformatics

Sciences 2022



- **The effect of virus infection
and viral load on berry
quality**

The variation in berry quality of the Three Clone

Control



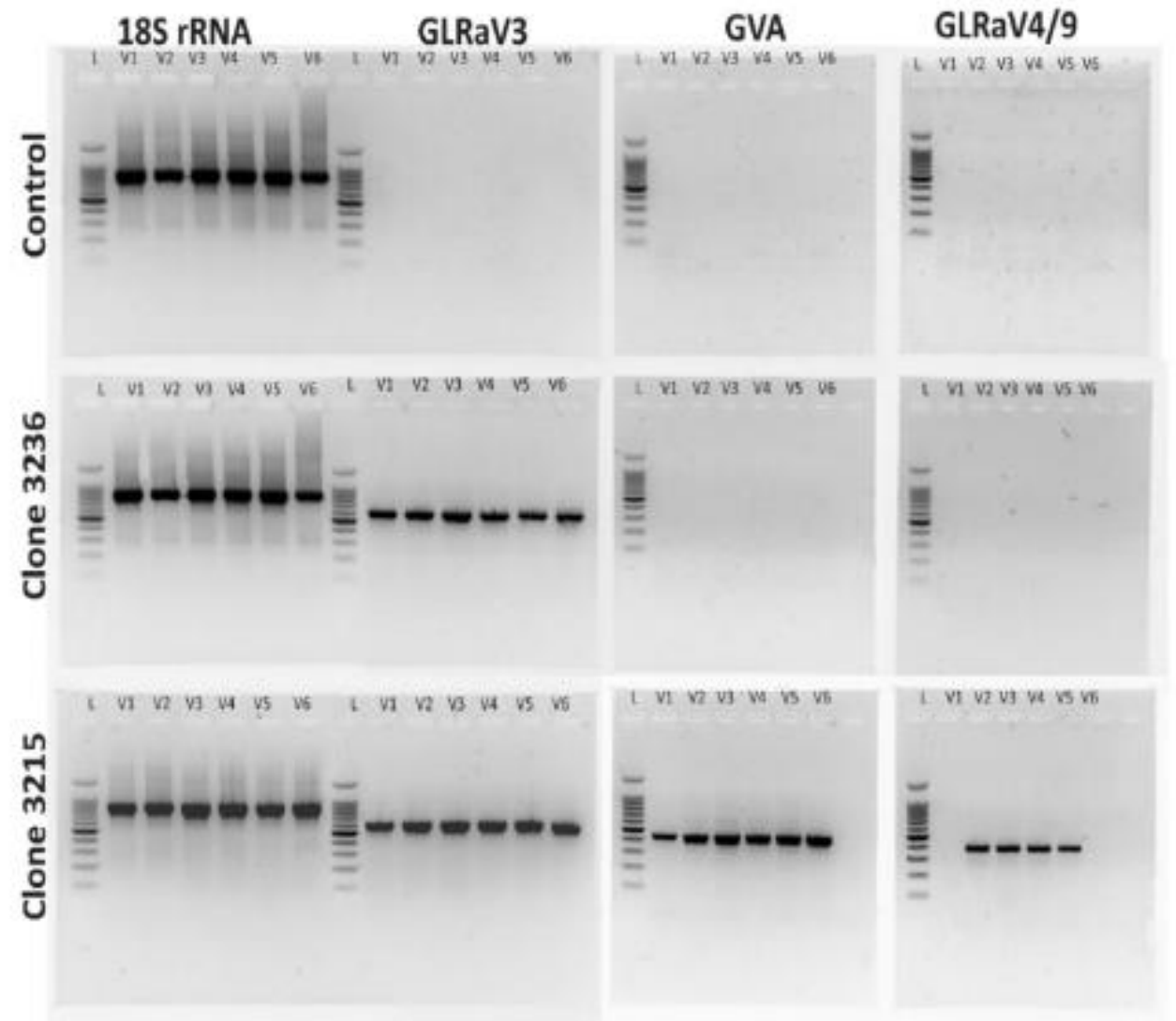
Single virus infection



Mixed viral infection



RT-PCR Detection of the Viruses



MANOVA analysis

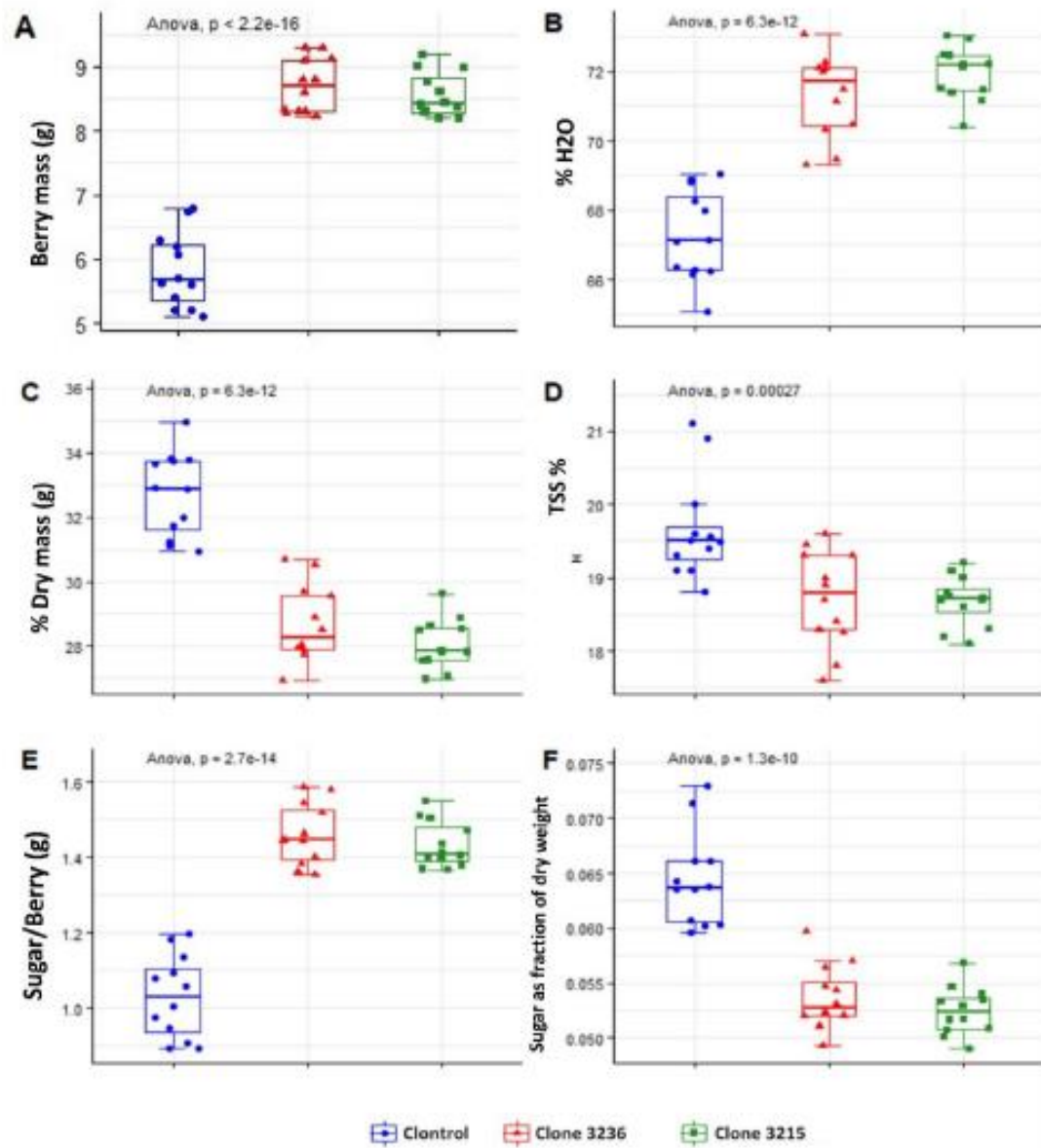
Chapter 3

Table 3.4. The variation of the berry quality of the infected clones compares with the control. Means, standard deviations, Fisher value and the p Value were calculated using MANOVA, where each clone comprise 6 replicates ($n=6$).

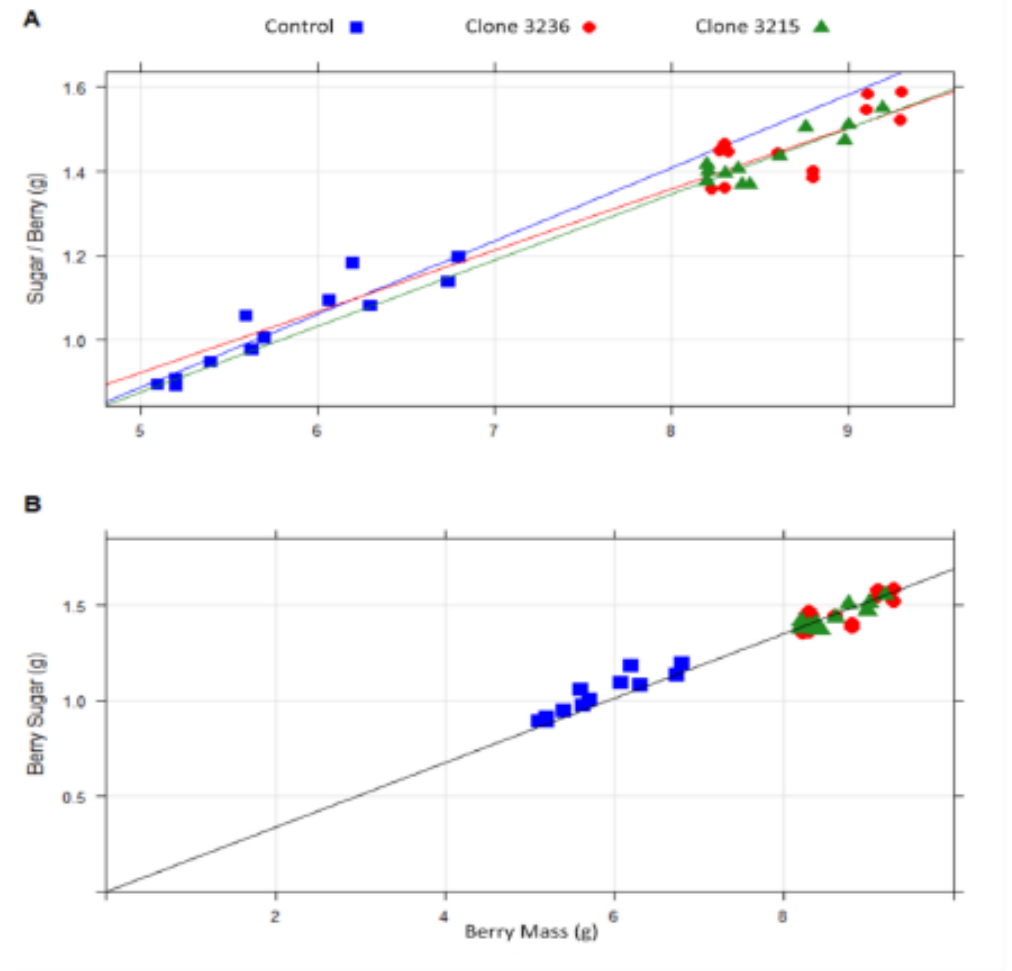
Berry quality	Clones								Seasons					
	Control		Clone 3236		Clone 3215		F		2017		2018		F	
	M	SD	M	SD	M	SD			M	SD	M	SD		
Mass (g)	5.83	0.59	8.70	0.42	8.56	0.35	175.23	***	7.74	1.22	7.66	1.62	0.31	
°Brix	19.66	0.70	18.72	0.65	18.68	0.34	11.05	***	18.84	0.67	19.19	0.76	3.39	.
Titrateable acidity (%)	0.51	0.02	0.57	0.02	0.59	0.05	33.04	***	0.58	0.06	0.54	0.03	18.50	***
Dry Mass (%)	32.73	1.30	28.70	1.19	28.06	0.78	62.24	***	29.95	2.30	29.71	2.47	0.41	
H ₂ O (%)	67.27	0.15	71.3	0.09	71.94	0.12	62.24	***	70.05	2.30	70.29	2.47	0.41	
Anthocyanin/ berry (mg)	1.72	0.19	0.78	0.20	0.58	0.26	86.22	***	1.06	0.56	1.00	0.55	0.44	
phenol/berry (aU)	1.15	0.27	1.01	0.31	0.82	0.25	4.54	*	1.02	0.32	0.97	0.29	0.30	

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.1$

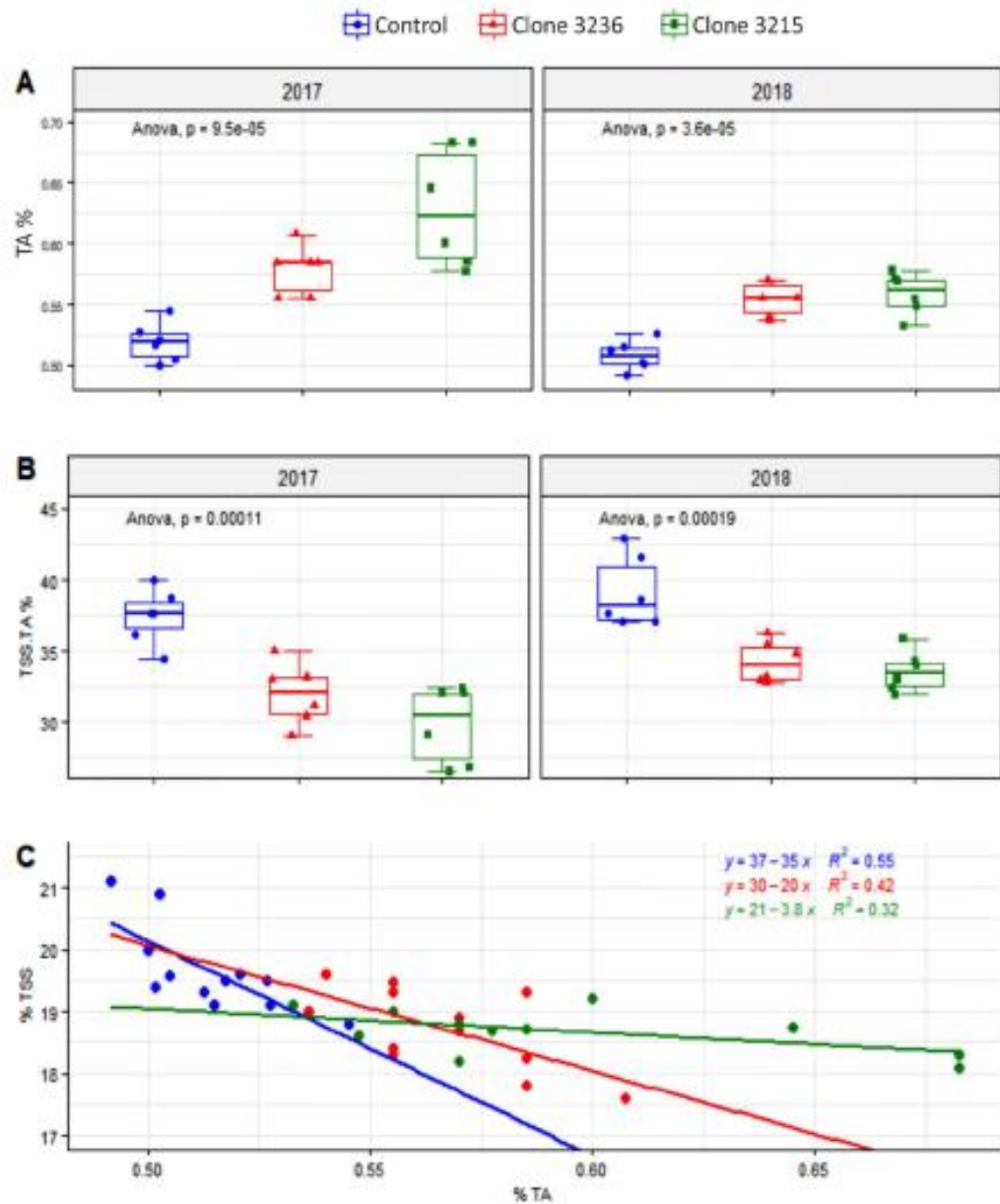
M: Mean, SD: Standard Deviation, F : Fisher Value



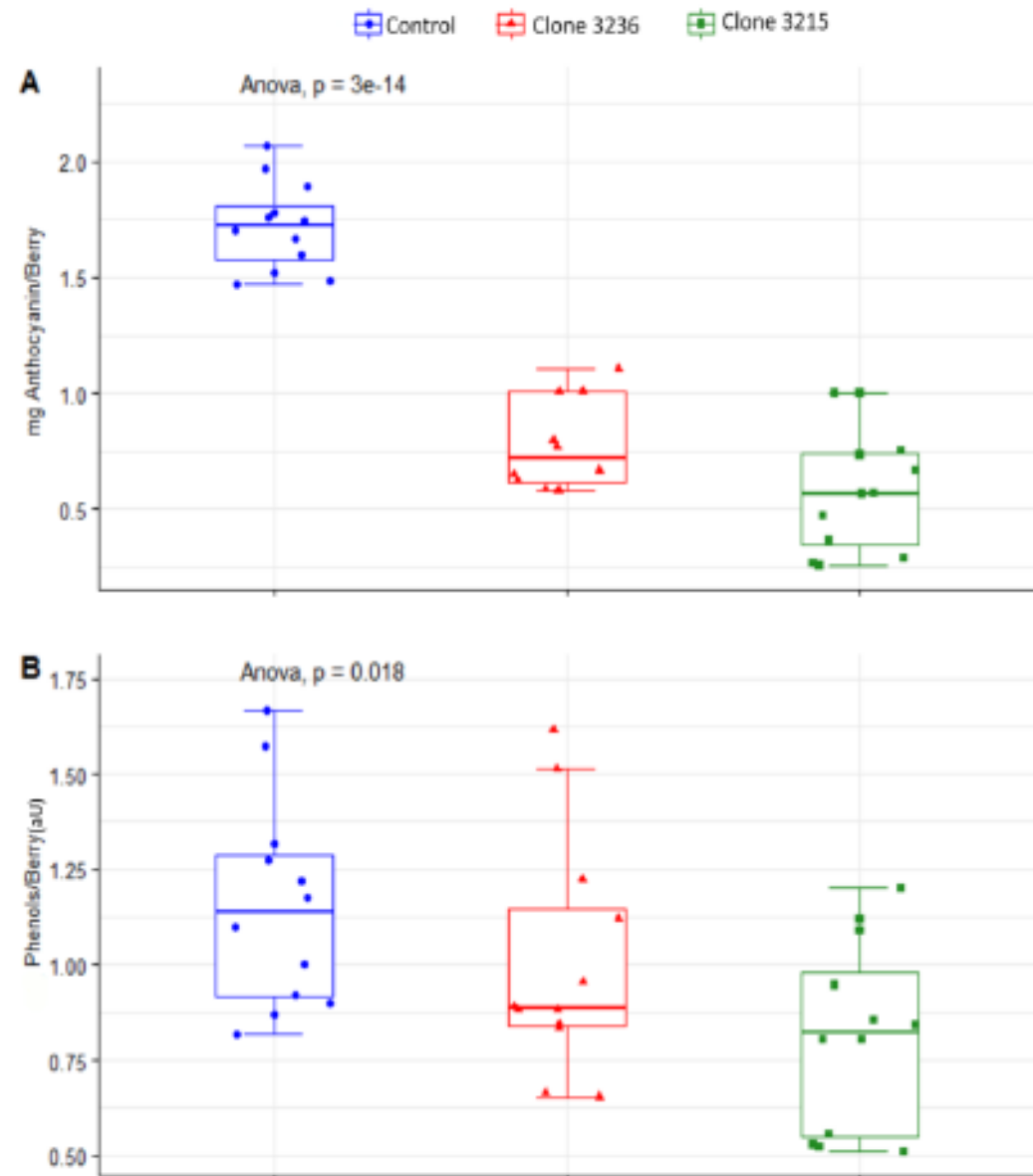
ANOVA analysis for the berry quality



Linear regression for the sugar concentration

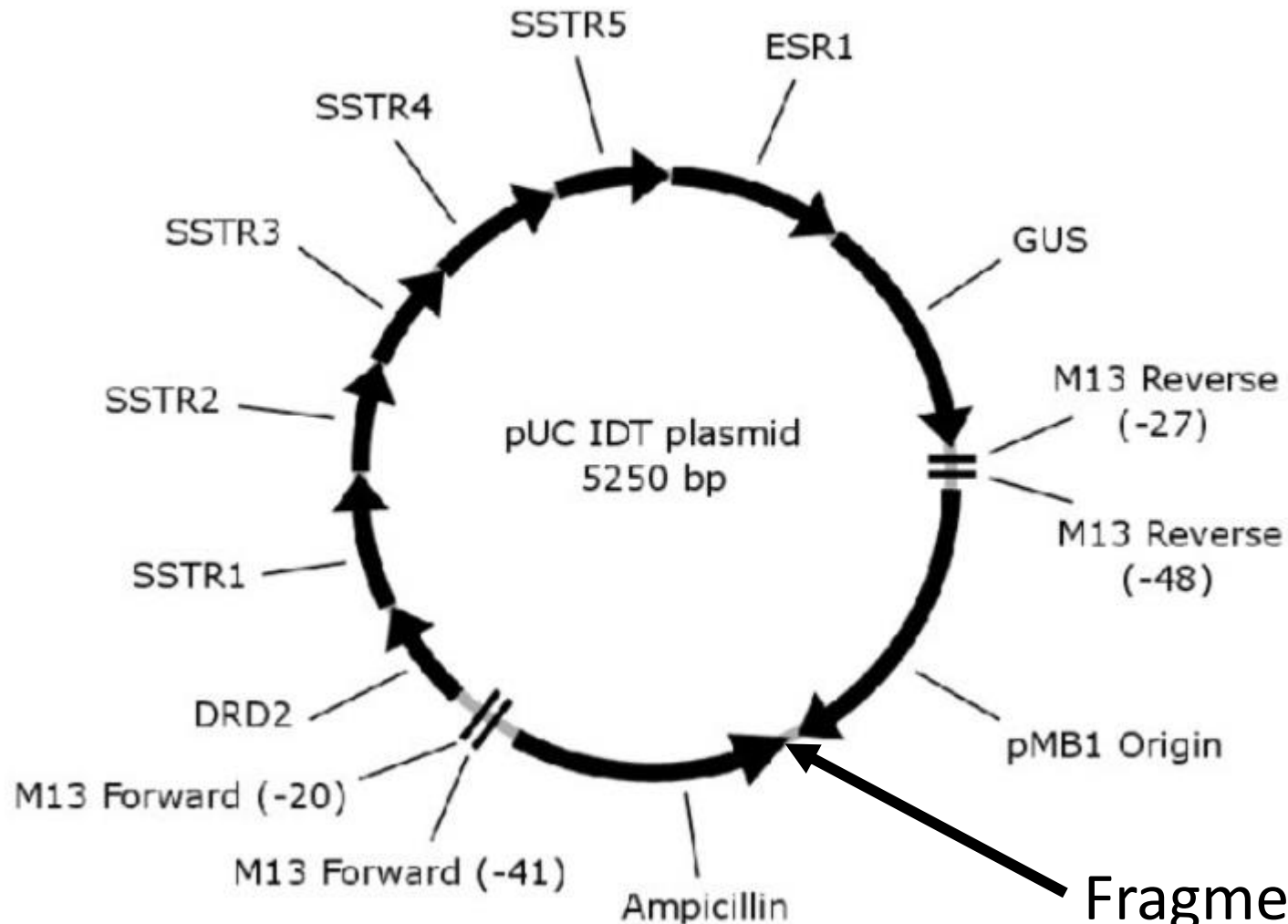


ANOVA analysis and linear regression for TA



ANOVA analysis anthocyanin and phenols

Vector name	Vector size	Selection marker	Application	Sequence
pUCIDT (Amp)	2752	Ampicillin	Cloning	pUCIDT (Amp).txt

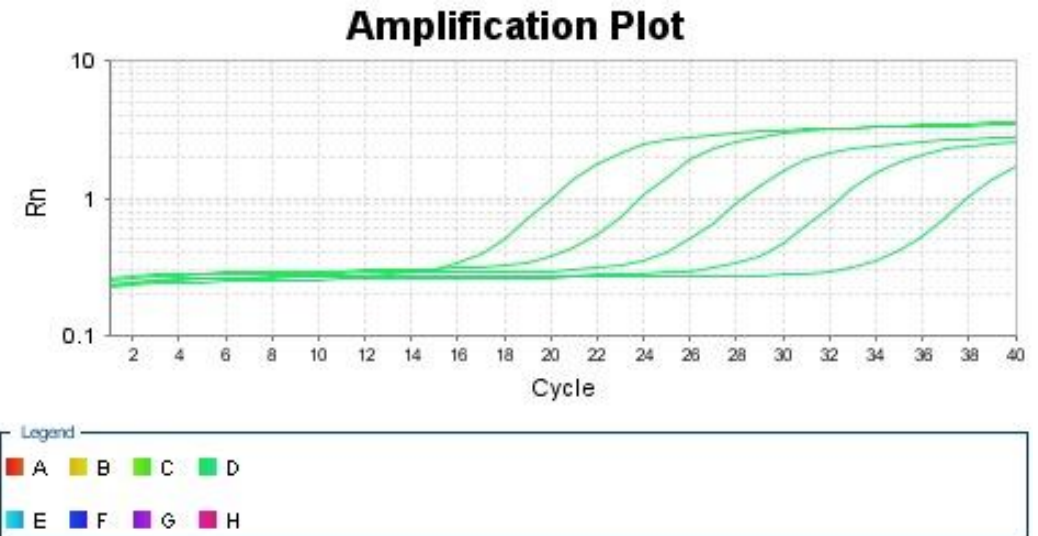
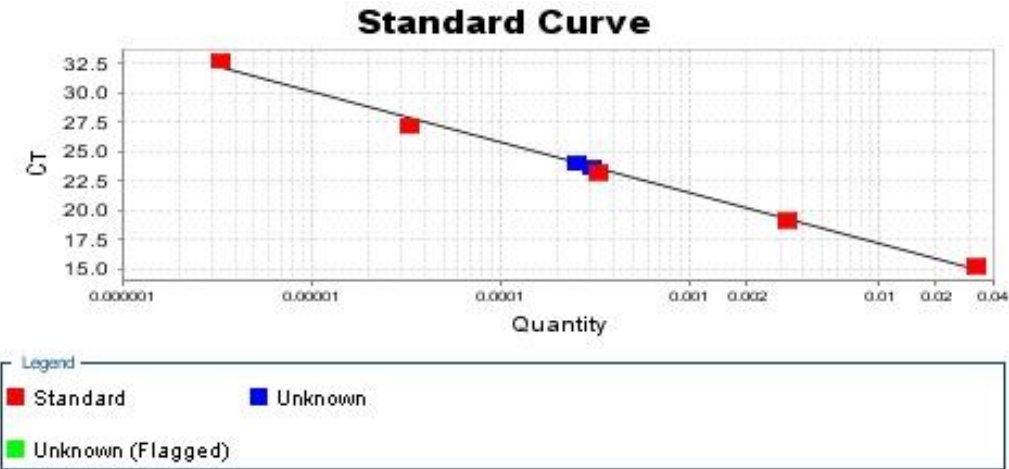
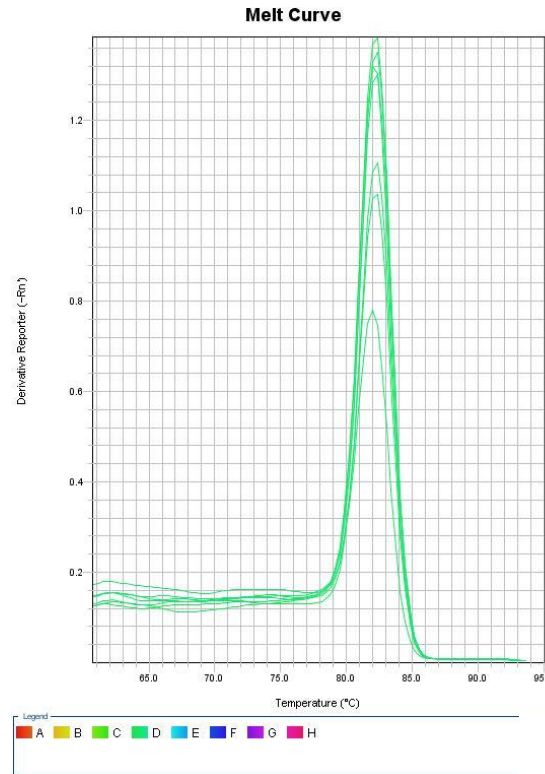


Vector design as
a standard for
absolute qPCR

Fragment insertion site

The absolute qPCR

- The viral copy number were calculated using the absolute qPCR approach and depending the accuracy of the standard curve



Viral load relation to berry weight and anthocyanin

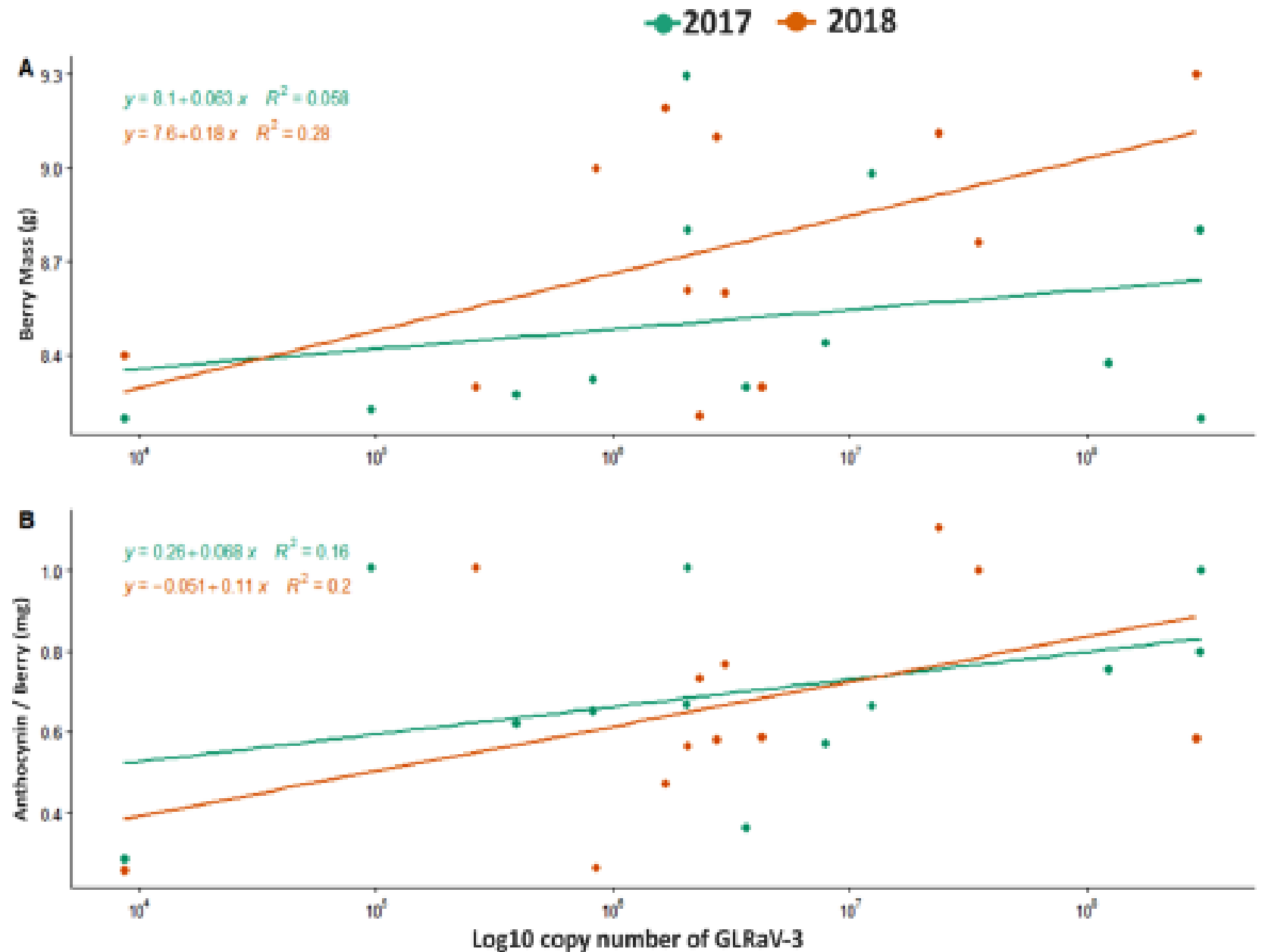


Table 3.5. The summary of the ANCOVA test including a season as the primary variate and the GLRaV-3 load for both infected clones as a covariate against the berry quality parameters

GLRaV-3										
	Mass		°Brix		Titratable acidity		Dry Mass		Anthocyanin	
	M	p	M	p	M	p	M	p	M	p
(Intercept)	8.48	<0.001	18.55	<0.001	0.61	<0.001	28.41	<0.001	0.66	<0.001
GLRaV-3	0	0.425	0	0.949	0	0.604	0	0.197	0	0.314
Seasons [2018]	0.24	0.136	0.3	0.17	-0.05	0.002***	-0.33	0.441	-0.02	0.844
Observations	24		24		24		24		24	
R ² / R ² adjusted	0.115 / 0.030		0.091 / 0.005		0.371 / 0.311		0.118 / 0.034		0.054 / -0.036	
GLRaV-4 strain 9										
	Mass		°Brix		Titratable acidity		Dry Mass		Anthocyanin	
	M	p	M	p	M	p	M	p	M	p
(Intercept)	8.33	<0.001	18.78	<0.001	0.62	<0.001	28.64	<0.001	0.73	<0.001
GLRaV-4	0	0.375	0	0.132	0	0.263	0	0.067	0	0.124
Season [2018]	0.27	0.197	0.12	0.535	-0.07	0.007***	-0.32	0.436	-0.05	0.757
Observations	12		12		12		12		12	
R ² / R ² adjusted	0.247 / 0.079		0.254 / 0.088		0.597 / 0.508		0.365 / 0.224		0.253 / 0.086	
GVA										
	Mass		°Brix		Titratable acidity		Dry Mass		Anthocyanin	
	M	p	M	p	M	p	M	p	M	p
(Intercept)	8.46	<0.001	18.43	<0.001	0.64	<0.001	27.72	<0.001	0.45	0.005
GVA	0	0.709	0	0.071	0	0.355	0	0.03	0	0.062
Season [2018]	0.24	0.327	0.3	0.167	-0.08	0.007***	0.15	0.731	0.1	0.54
Observations	12		12		12		12		12	
R ² / R ² adjusted	0.187 / 0.007		0.336 / 0.189		0.578 / 0.485		0.460 / 0.340		0.344 / 0.198	

***p < 0.001, **p < 0.01, *p < 0.05, p < 0.1, M: Mean, p: p value

The effect of grapevine leafroll-associated virus infection on the berry transcriptome.

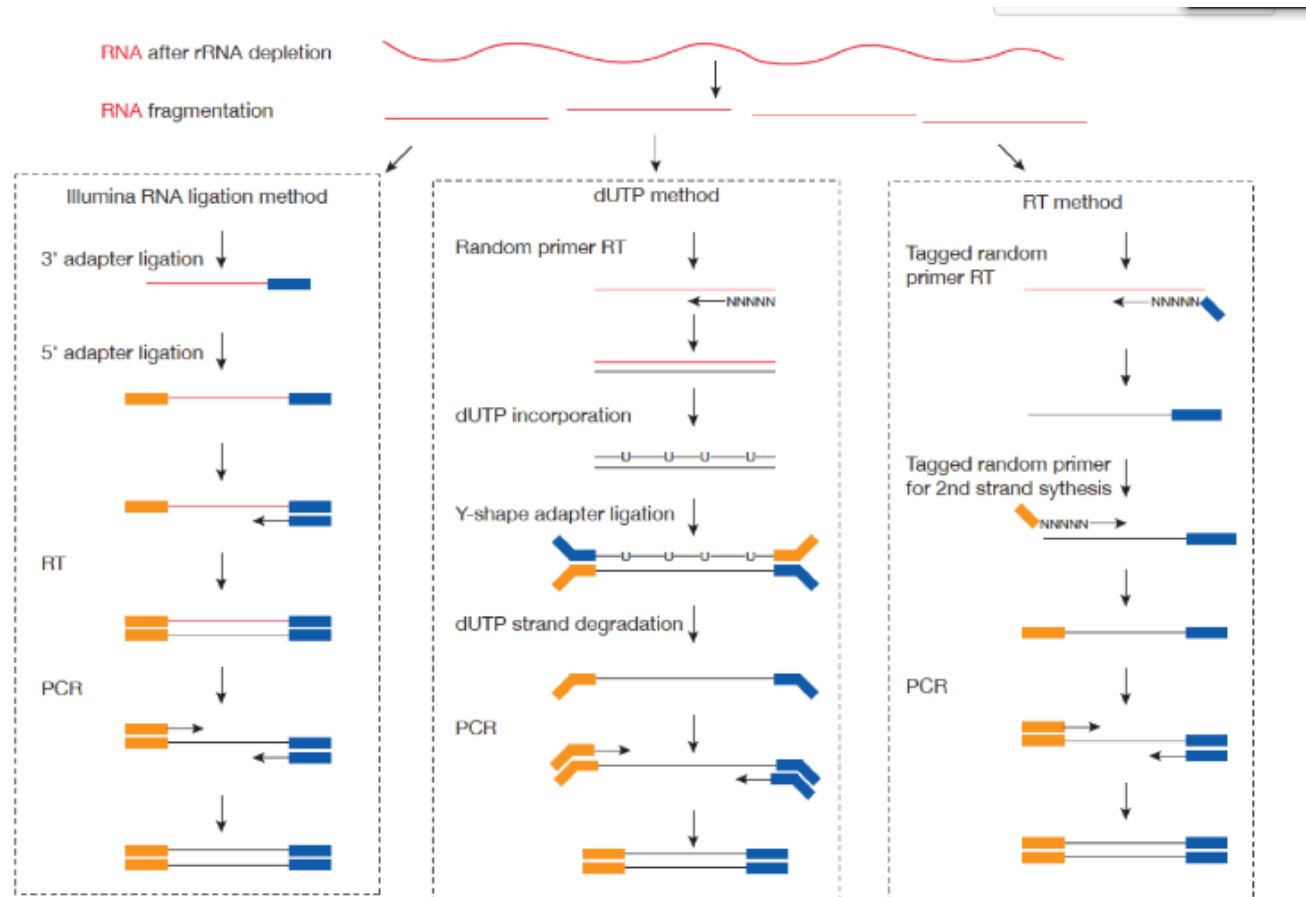
- **NGS RNA-Seq for the total RNA from the berry tissue**



The library preparation

The library prepared using Ribo Zero to remove the rRNA

Then a prior end library has been built using Illumina total RNA-TrueSeq protocol



DATA ANALYSIS

QC

- Quality control using FASTQC
- Trimming the adaptors and the primers

Mapping

- Mapping using HISA2
- To a reference genome

Counting

- Counting the gene expression using HTseq

DGE

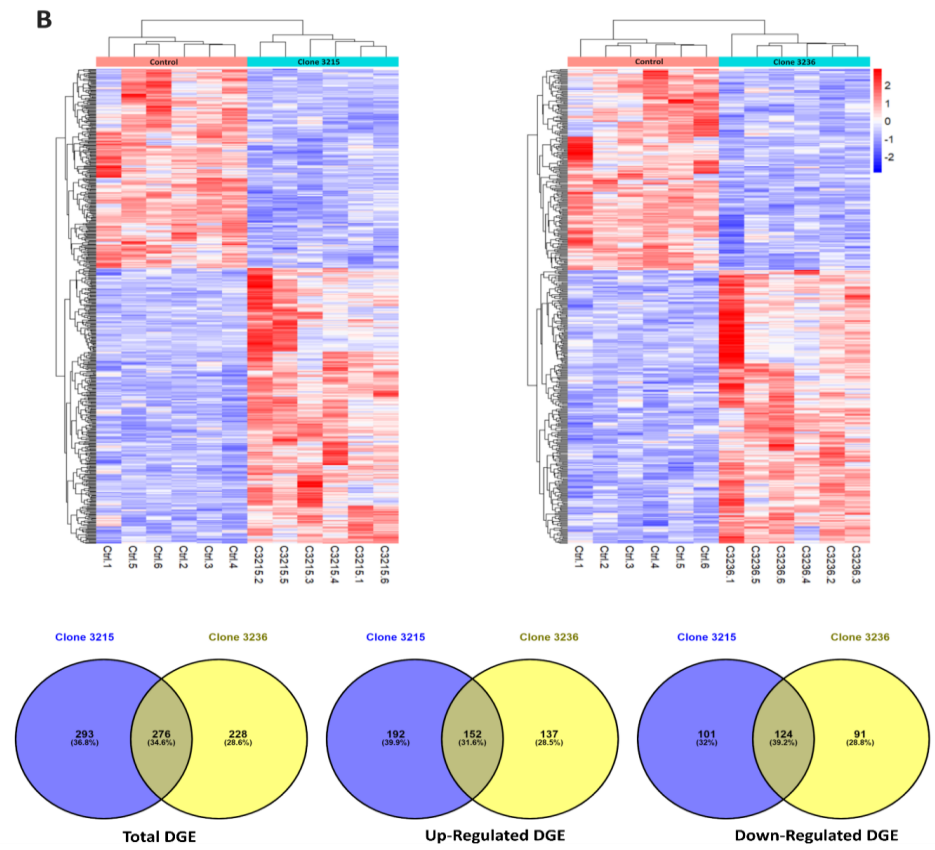
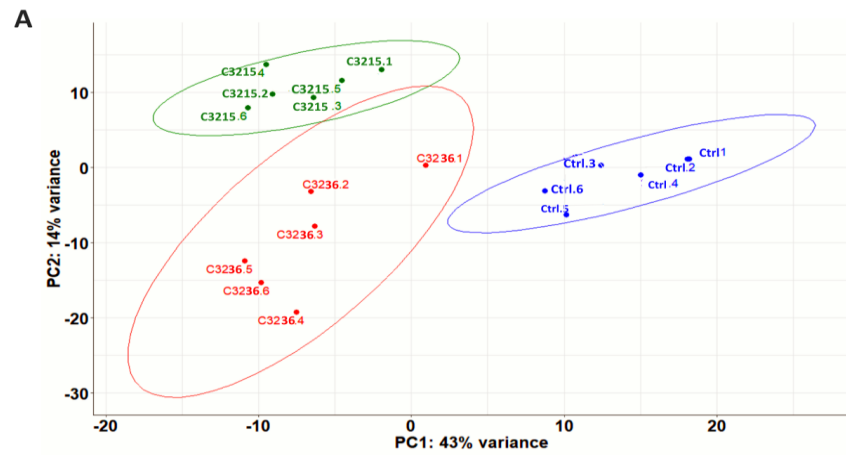
- Differential Gene Expression using DESeq2 Package from “R” Language

GO

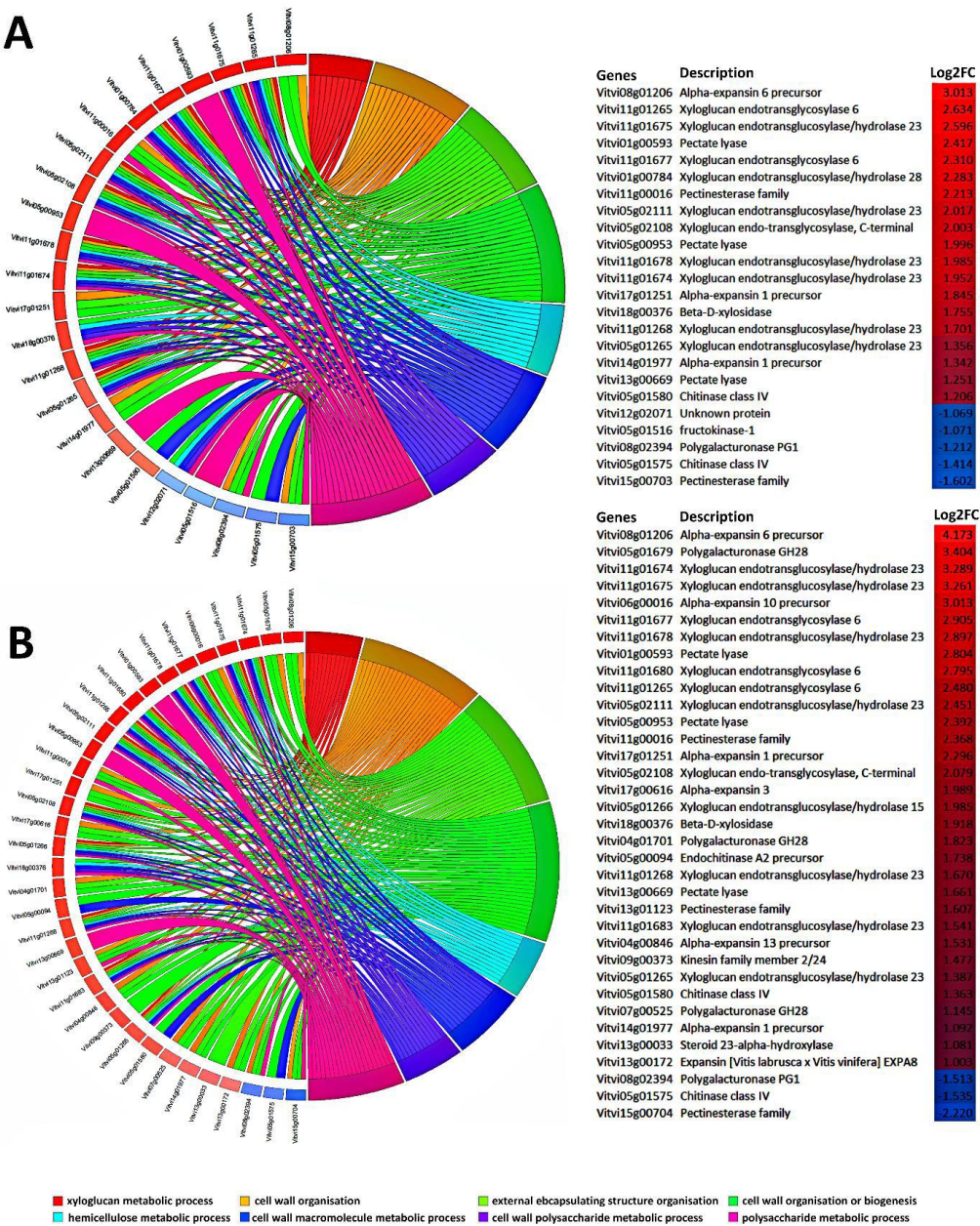
- Gene ontology using gProfiler package from “R” languages
- Plotted using GOPLLOT package from “R” languages

Pathway

- Pathway analysis using pathview package from “R” languages

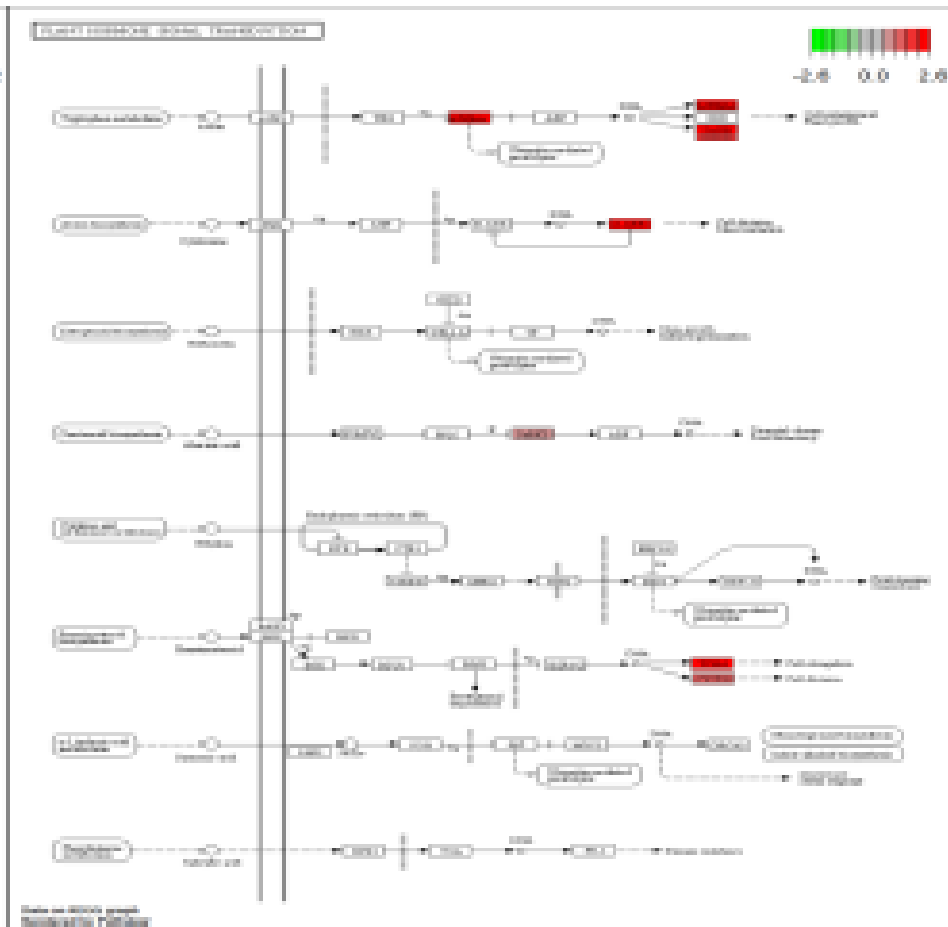
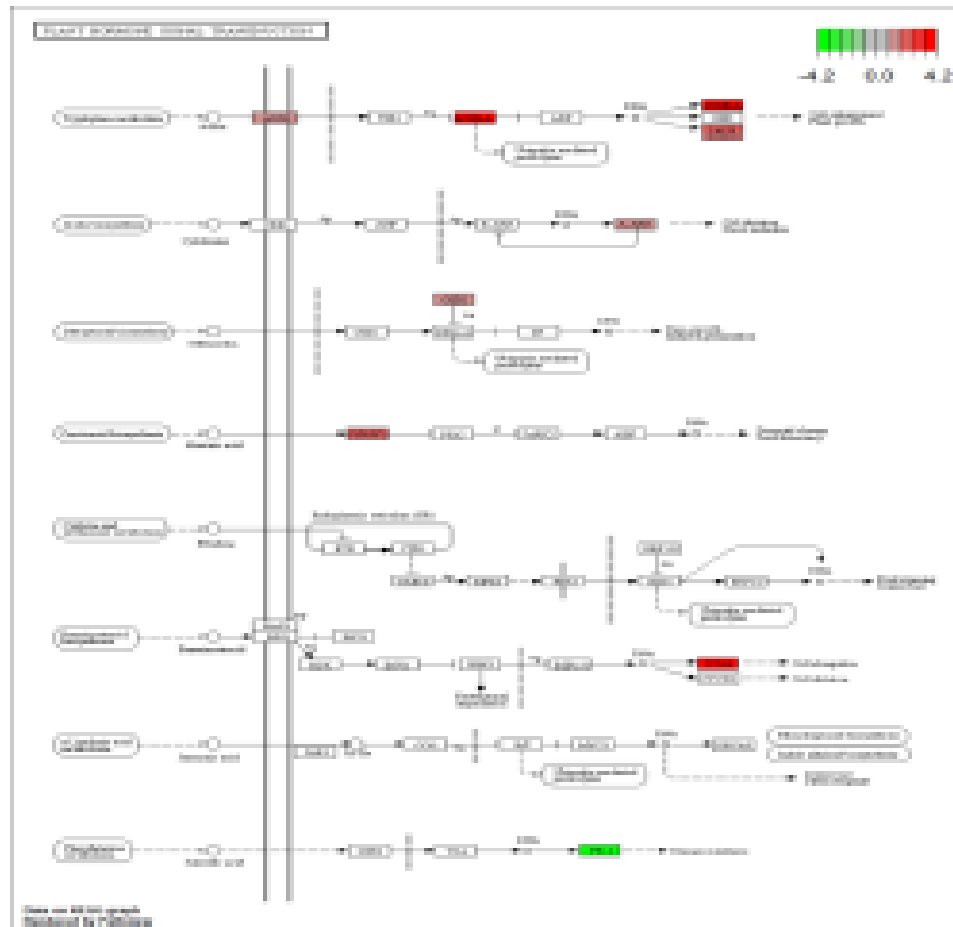


Chord analysis for differentially Expressed Genes in the Cell wall

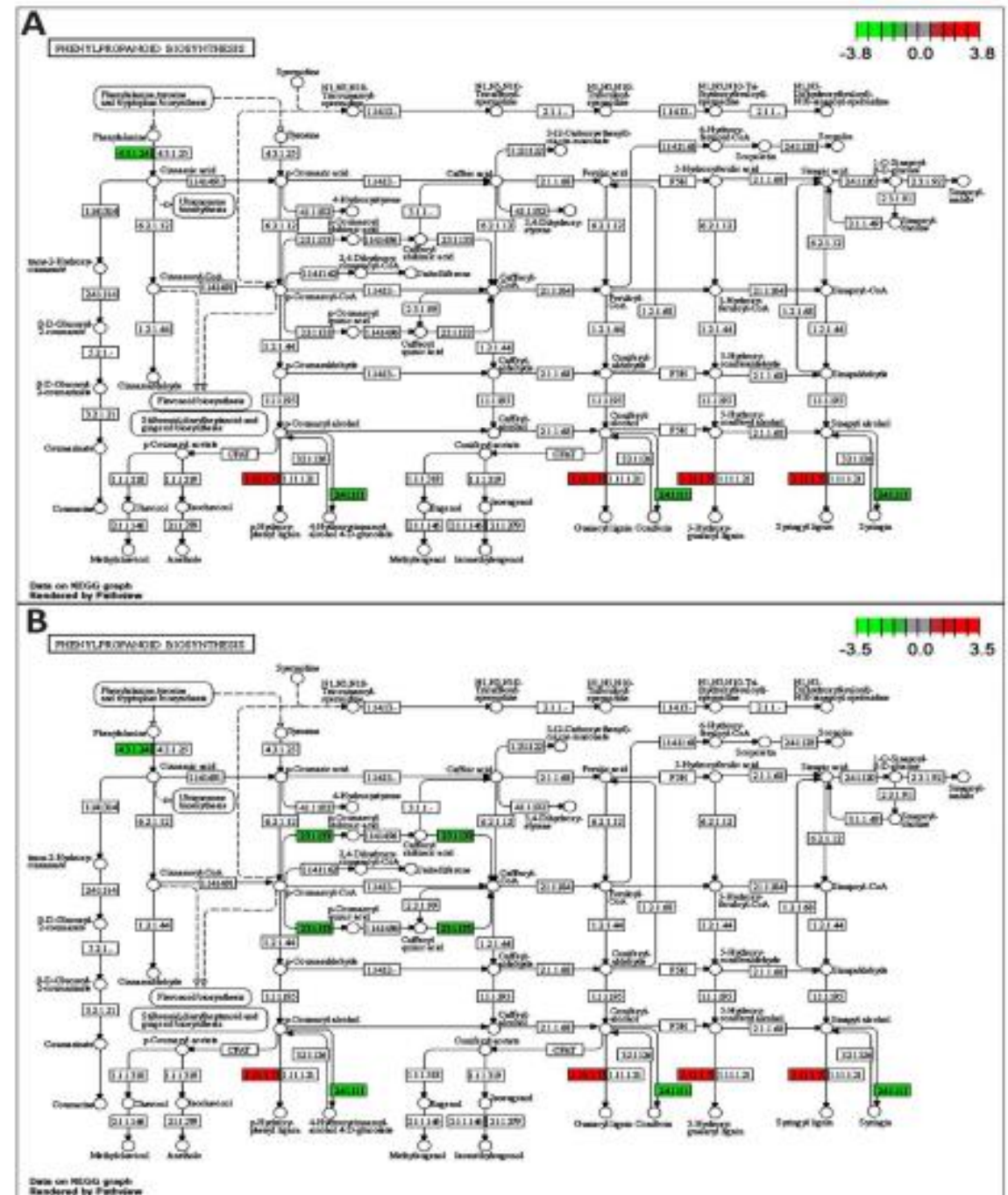


Hormonal pathway analysis

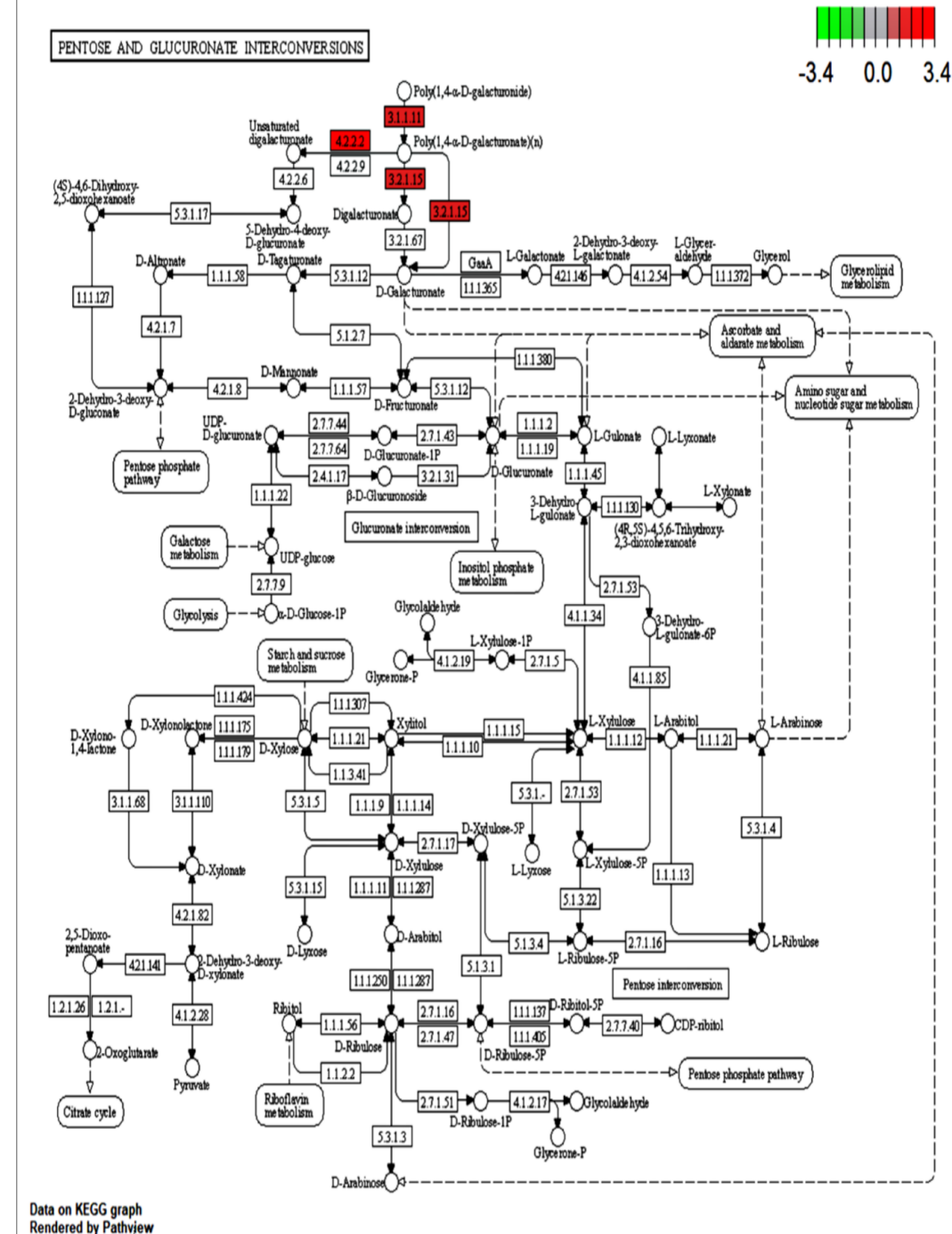
Chapter 4



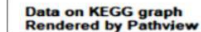
The phenylpropanoid biosynthesis pathway down-regulation



The pentose and glucuronate interconversion pathways up-regulation

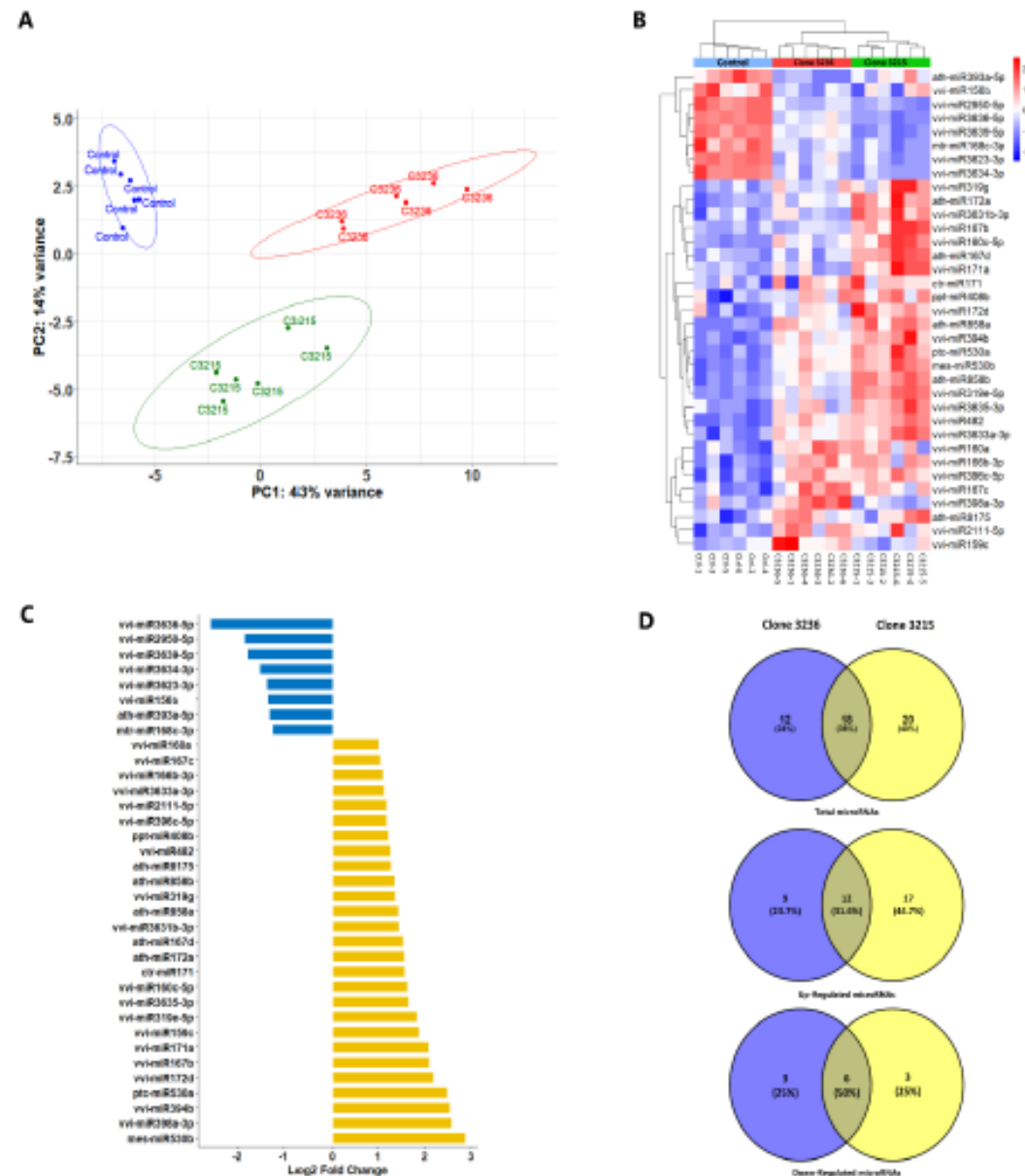


3215



**The effect of grapevine leafroll-associated virus
infection on the expression of miRNAs in cv.
Crimson Seedless table grape berries**

Differential miRNA EXPrESSION Analysis



miR	sequence	Len	p value	padj	Average number of reads			
					log2FC	Ctrl	Cln 3236	Cln 3215
ath-miR172a	AGAAUCUUGAUGAUGCUGCAU	21	4.76E-05	3.65E-04	1.56	11.36	20.73	48.01
vvi-miR3636-5p	UCGGUUUGCUUCUUUGAUAGAUUC	24	3.63E-18	2.26E-16	-2.57	453.73	106.9	44.91
vvi-miR2950-5p	UUCCAUCUCUUGCACACUGG	20	6.53E-23	1.09E-20	-1.84	238.27	77.89	54.75
vvi-miR3639-5p	UUGACUUCUGAAAGGCUAAA	20	1.20E-10	2.84E-09	-1.78	190.32	77.02	33.31
vvi-miR3634-3p	UUUCCGACUCGCACUCAUGCCGU	23	1.02E-18	8.47E-17	-1.51	8462.17	3284.46	2654.21
vvi-miR3623-3p	UGGUGCUUGGACGAAUUUGCUA	22	5.21E-16	2.60E-14	-1.37	6153.65	2661.83	2114.85
vvi-miR156b	GCUCUCUAGACUUCUGUCAUC	21	2.05E-04	1.33E-03	-1.35	340.87	114.78	153.04
ath-miR393a-5p	UCCAAAGGGAUCGCAUUGAUCC	22	8.07E-05	5.93E-04	-1.31	31.27	7.97	17.7
mtr-miR168c-3p	CCCGCCUUGCAUCAACUGAAU	21	1.10E-12	4.22E-11	-1.24	1181.01	557.87	443.29
vvi-miR160a	UGCCUGGCUCCUGAAUGCCA	21	2.95E-04	1.82E-03	1.01	10.42	22.65	18.47
vvi-miR167c	UGAAGCUGCCAGCAUGAUCUC	21	7.29E-04	4.04E-03	1.04	8.03	17.87	14.43
vvi-miR166b-3p	UCGGACCAGGCUUCAUUCUC	21	2.00E-09	3.32E-08	1.1	984.35	2089.54	2128.22
vvi-miR3633a-3p	UUCCUAUACCACCCAUUCCCUA	22	2.33E-08	2.98E-07	1.12	564.36	1015.65	1433.51
vvi-miR2111-5p	UAAUCUGCAUCCUGAGGUCU	20	7.82E-05	5.83E-04	1.18	14.95	37.52	31.61
vvi-miR396c-5p	UUCCACAGCUUUCUUGAACUG	21	9.60E-12	3.19E-10	1.18	113.83	254.26	266.06
ppt-miR408b	UGCACUGCCUCUCCCCUGGCU	21	5.34E-04	3.06E-03	1.21	9.12	18.62	23.66
vvi-miR482	UCUUUCCUACUCCUCCAUUCC	22	4.09E-23	1.02E-20	1.27	1804.21	3917	4757.42
ath-miR8175	GAUCCCCGGCAACGGCGCCA	20	3.06E-03	1.37E-02	1.27	4.92	12.41	11.90
ath-miR858b	UUCGUUGUCUGUUCGACCUUG	21	5.22E-10	1.10E-08	1.35	1832.43	3337.6	6028.69
vvi-miR319g	UUGGACUGAAGGGAGCUCCA	21	1.53E-03	7.58E-03	1.37	11.23	19.56	39.72
ath-miR858a	UUUCGUUGUCUGUUCGACCUU	21	2.51E-09	3.91E-08	1.43	23.59	52.56	76.30
vvi-miR3631b-3p	UGUUGGAUGAUGUCAUAAGU	21	3.40E-03	1.50E-02	1.44	5.19	7.74	21.63
ath-miR167d	UGAAGCUGCCAGCAUGAUCUGG	22	9.68E-04	5.03E-03	1.54	10.42	13.71	47.11
ctr-miR171	UUGAGCCGCGUCAUAUUCUCC	21	5.95E-03	2.52E-02	1.56	4.00	10.61	12.42
vvi-miR160c-5p	UGCCUGGCUCCUGUAUGCCA	21	2.27E-06	1.86E-05	1.63	22.59	43.24	97.00
vvi-miR3635-3p	AUUAUGUCCACACAUGCCUC	21	1.04E-04	7.32E-04	1.65	4.61	10.03	17.90
vvi-miR319e-5p	UUUGGACUGAAGGGAGCUCCU	21	2.68E-08	3.27E-07	1.83	9683.92	18265.83	50760.86
vvi-miR159c	UUUGGUUUGAAGGGAGCUCU	20	2.12E-03	1.00E-02	1.88	102.04	636.43	116.31
vvi-miR171a	UGAUUGAGCCGUGCCAUAUC	21	3.79E-04	2.25E-03	2.09	8.09	12.14	56.02
vvi-miR167b	UGAAGCUGCCAGCAUGAUCUA	21	1.39E-04	9.53E-04	2.1	32.46	39.89	238.7
vvi-miR172d	UGAGAAUCUUGAUGAUGCUGC	21	8.53E-04	4.68E-03	2.19	2.26	4.97	12.13
vvi-miR394b	UUGGCAUUCUGUCCACCUCC	20	8.85E-10	1.70E-08	2.54	7.49	29.01	58.37
vvi-miR398a-3p	UGUGUUCUCAGGUCACCCCUU	21	8.73E-03	3.51E-02	2.58	25.11	283.63	16.44
mes-miR530b	UGCAUUUGCACCUGCACCUGA	21	1.85E-18	1.32E-16	2.87	151.9	708.93	1514.2

Len: Length of the miR

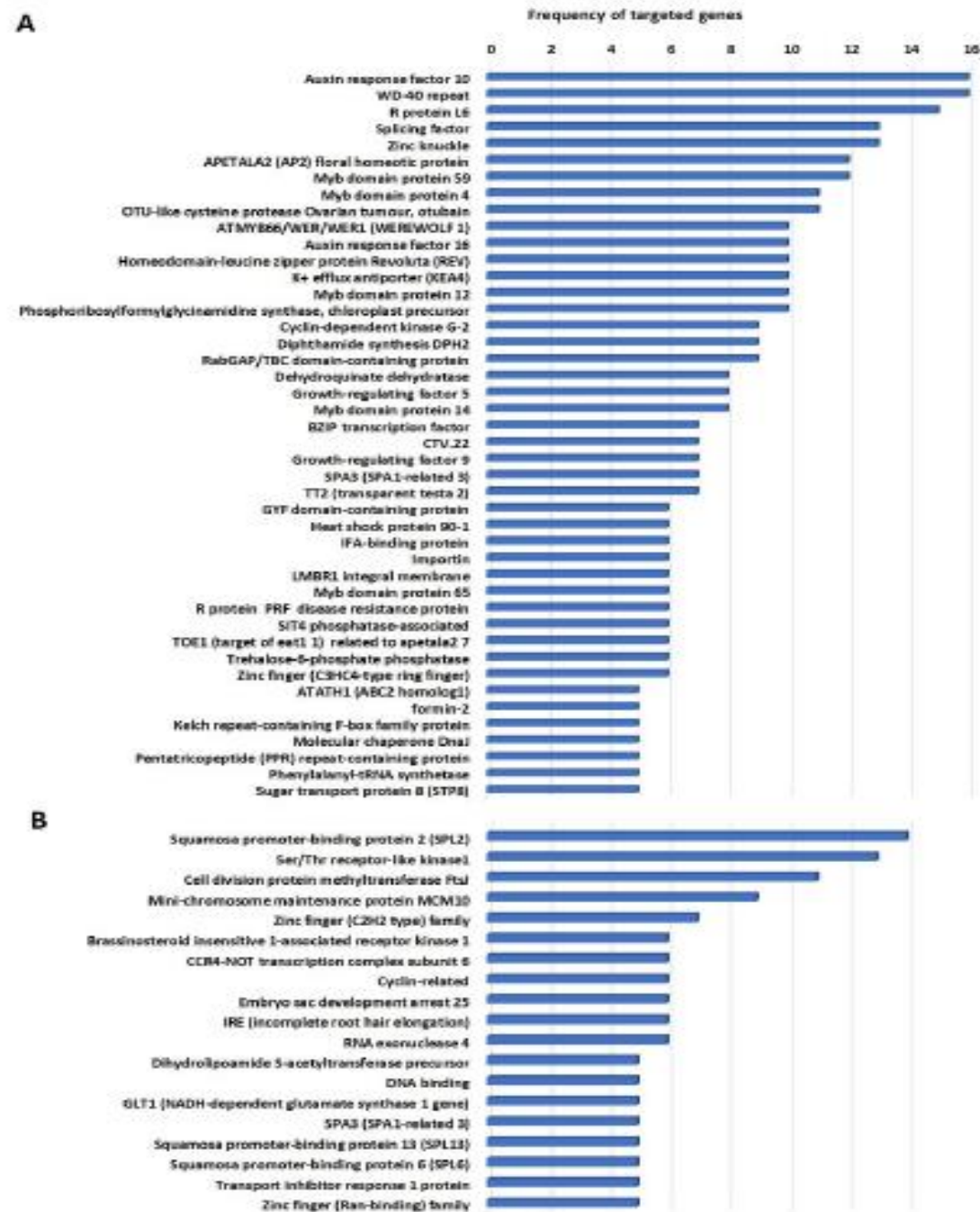
Padj: the adjusted *p* value generated by calculating the false discovery rate FDR

log2FC: the binary logarithm of the fold change

Ctrl: control

Cln: clone

Highly frequent targeted genes



The Gene Ontology analysis

A



B

