SATI	CFPA	HORTGRO	RAISINS SA	WINETECH
tarryn@satgi.co.za Tel: 021 863-0366	inmaak@mweb.co.za Tel: 021 872-1501	anita@hortgro.co.za Tel: 021 870 2958	ferdieb@raisinsa.co.za Tel: 054 495 0283	andraga@winetech.co.za Tel: 021 276 0499

FINAL REPORT 2023

Winetech Number: GenUS GP 20-01

1. PROGRAMME & PROJECT LEADER INFORMATION

	Research Organisation leader	Project leader
Title, initials, surname	Prof Gerhard Pietersen	Prof Gerhard Pietersen
Present position	Professor	Professor
Address	Dept. of Genetics Stellenbosch University Stellenbosch 7600	Dept. of Genetics Stellenbosch University Stellenbosch 7600
Tel. / Cell no.	0826475326	0826475326
E-mail	gerhard@pathsol.co.za	gerhard@pathsol.co.za

2. PROJECT INFORMATION

Project title	Detection and identification of viruses in Nuclear material
Short title	NGS of Nuclear material

Fruit kind(s)	Wine grapes/Table grapes		
Start date (mm/yyyy)	2020-01	**End date** (mm/yyyy)	2022-12

Key words	viruses, nuclear plants, next-generation sequencing, Vitis, grapevines, viroids

	CFPA	RAISIN SA	HORTGRO	SATI	WINETECH	ARC	OTHER
TOTALS All years	R 0	R 0	R 0	R 1044750	R 1044750	R 0	R 320000

Total cost of the entire project	R 2409500

3. EXECUTIVE SUMMARY

Objectives and Rationale

Certification schemes prevent the spread of pathogens via planting material. In the Vine Improvement Association (VIA) Vitis certification scheme of South Africa, only a few viruses are tested for and certified free of, while more than 80+ viruses occur on grapevine. In this project, we determine the total virus and viroid status of nuclear grapevine material in 97 accessions from three Plant Improvement Organisations.

Methods

Nuclear plant accessions from three plant improvement organisations were selected for analysis. Total nucleic acid from these accessions was extracted at multiple time points and from various tissues and pooled. The pooled nucleic acid was subjected to next-generation sequencing, and the presence of viruses and viroids was determined using bioinformatic techniques. The viruses or viroids found were confirmed by specific PCR tests.

Key Results

Only seventeen of the 97 nuclear accessions tested had no detectable viruses or viroids. Amongst the remaining nuclear accessions, five viruses and four viroids or viroid-like entities were identified. The virus infections were rare, being found in only 8 accessions, while viroid infections were common, being found in 76 of the 97 accessions. Viruses observed included Rupestris stem pitting-associated virus (GRSPaV), Grapevine fleck virus (GFkV), Grapevine syrah virus 1 (GSyV-1), Grapevine leafroll-associated virus 4 (GLRaV-4) and Grapevine Rupestris vein feathering virus (GRVFV). Viroids found were hop stunt viroid (HSVd), grapevine yellow speckle viroid-1 (GYSVd-1), grapevine yellow speckle viroid-2 (GYSVd-2), Australian grapevine viroid (AGHVd) and Grapevine hammerhead viroid-like RNA in various combinations.

Conclusion and Discussion / Recommendation

South African vine nuclear material appears remarkably free of grapevine viruses despite only a few of the more than 80 known viruses being tested for locally. This is probably due to the mandatory application of heat therapy and meristem tip culture to create nuclear plants. All remaining and future nuclear plants should be subjected to NGS analysis to confirm their virus/viroid status. Those found to be virus-infected need to be subjected to secondary virus elimination processes. The common presence of viroids suggests that these agents are not eliminated by current elimination processes or spread exceptionally rapidly within the nuclear block facilities. A risk assessment as to the importance of these agents must be made prior to deciding to eliminate these pathogens from nuclear material, and whether to manage these agents.

4. PROBLEM IDENTIFICATION AND MOTIVATION

Problem Identification

Worldwide, certification schemes are used to prevent the spread of pathogens via planting material. While the one-Vitis certification scheme of South Africa requires tests for several specific viruses of grapevines, these do not include tests for over 80 viruses, known to infect grapevines globally, most also found in South Africa. The tests also do not include any of the viroids infecting grapevines globally. It is unknown whether any of these viruses/viroids are present in nuclear plant material and in what incidence they occur. In this proposal, we wish to determine whether nuclear material used within the South African table and wine grape industries is free of all known grapevine viruses/viroids, and if not, to identify those viruses still present in such material. This is a first step towards eliminating or managing them, in accordance with the fundamental objectives of a certification scheme.

Motivation
More than 86 viruses occur on grapevines worldwide. Of these, only grapevine fanleaf virus (GFLV), grapevine leafroll associated viruses 1, 2 and 3 (GLRaV-1, 2, and 3), grapevine fleck virus (GFkV), grapevine virus A (GVA) and grapevine virus B (GVB) must be tested for in nuclear plants in the VIA certification scheme. Next-generation sequencing (NGS) allows the detection of all viruses and viroids within a plant. Viruses in nuclear material are potentially of great concern, even if it results in a small adverse effect, as thousands of progeny plants are derived from such plants, with the majority then being infected. The cumulative effect of a virus on thousands of derived plants is likely economically significant even when the effect per vine is small. This risk is unquantified, as no local nuclear plants have been subjected to virus-wide analysis to the applicant's knowledge, and we do not know which viruses, if any, occur in nuclear material. The NGS system developed can be applied in the certification scheme on a routine basis in future. With decreased margins of profitability within the South African grape growing industry, any industry-wide improvement in the quality and quantity of the final crop will add value.

5. ACCUMULATED PROGRESS TABLE

Objectives	**Milestones (Significant event or stage in a project)**	**Date Achieved**
1. Select 20 Nuclear plant accessions for analysis	Have selected 20 Nuclear plant accessions for analysis	2020-02-28
2. Collect selected nuclear plant material and prepare for analysis.	Have collected selected nuclear plant material and prepared it for analysis.	2020-07-30
3. Do next-generation sequencing and analysis for viruses	Have done next-generation sequencing and analysis for viruses	2020-09-30
4. Establish PCR for viruses found in nuclear material and confirm detection.	Have established PCR for viruses found in nuclear material and confirmed detection.	2021-02-15
5. Select 40 Nuclear plant accessions for analysis	Have selected a further 40 Nuclear plant accessions for analysis	2021-02-28
6. Collect selected nuclear plant material and prepare for analysis.	Have collected selected nuclear plant material and prepared it for analysis.	2021-07-30
7. Do next-generation sequencing and analysis	Have done next-generation sequencing and analysis for viruses	2021-09-30

for viruses		
8. Establish PCR for viruses found in nuclear material and confirm detection.	Have established PCR for viruses found in nuclear material and confirmed detection.	2021-09-15
9. Select 40 Nuclear plant accessions for analysis	Have selected a further 40 Nuclear plant accessions for analysis	2022-02-28
10. Collect selected nuclear plant material and prepare for analysis.	Have collected selected nuclear plant material and prepared it for analysis	2022-07-30
11. Do next-generation sequencing and analysis for viruses	Have done next-generation sequencing and analysis for viruses	2022-09-30
12. Establish PCR for viruses found in nuclear material and confirm detection.	Have established PCR for viruses found in nuclear material and confirmed detection.	2022-12-15
13. Journal publication(s) – final milestone	Have prepared a draft paper for publication	2022-12-30

6. WORKPLAN (MATERIALS AND METHODS)

Select Nuclear plant accessions for analysis.
The three major grape plant improvement organisations (PIO's) in South Africa were invited to select and submit a total of 20 nuclear vine accessions (in total) towards the latter part of the 2019/2020 growing season, 40 during the 2020/2021 growing season and a final 40 during the 2021/2022 growing season. The choice of nuclear accessions to analyse was left to the respective PIO's, but it had been recommended that they select accessions that had contained any virus-like abnormalities amongst the progeny grown under field conditions.

Collect selected nuclear plant material and prepare for analysis.
Petiole and cane material were collected from the PIO's and accessed under codes to provide confidentiality of samples. In the first year of the study, tissue from each accession was collected on multiple occasions throughout the season, and the RNA from each was pooled for NGS. In the two subsequent seasons, each accession was sampled only on two occasions,s and the RNA was pooled from these for NGS. This was done during the last week of February (petiole and leaf tissue) and the last week of June (cane material). The change in collection strategy was the result of a publication appearing in 2021 suggesting that collection on two occasions was adequate (Soltani et al., 2021). RNA was extracted using bag maceration for the petiole and leaf tissue and liquid nitrogen grinding for the cane tissue (White et al., 2008). RNA extractions on any sample on any sampling occasion were repeated multiple times until high-quality RNA was obtained for use in library preparation.

The concentration was determined using a Nanodrop, and preparations with the highest concentrations of RNA within sample replicates were pooled. RNA quality was assessed using a PCR directed at a sequence within the Actin gene of the host plant, which contains an exon (Van den Berg et al., 2004). The total RNA samples extracted were also assessed for RNA integrity (RIN) and quantity on the BioAnalyzer 2100 (Agilent Technologies, Waldbronn, Germany) using the RNA 6000 Nano Chip and reagents according to the protocol, G2938-90037 REV., to ensure they conform to Next Generation Sequencing library preparation requirements.

Next-generation sequencing.
Ribo Depletion on the RNA of each sample was performed with the RiboMinus Plant Kit and RiboMinus Bacteria module (ThermoFisher Scientific) according to the respective manufacturer's protocols; MAN0000777 (Rev. date 08 Dec 2009) and 25-0879 (version A, Rev. date 12th September 2005). The ribosome-depleted RNA was concentrated using the RiboMinus concentration module according to the manufacturer's instructions; MAN0002509; Rev. date: 3rd December 2011. The concentrated, ribosome-depleted RNA was quantified on the Agilent TapeStation using the RNA ScreenTape according to the manufacturer's protocol; G2991-90021, Edition 12/2018.
The Ion Total RNA-Seq Kit v2 (ThermoFisher Scientific) was used to convert the ribo-depleted RNA into a representative cDNA library for strand-specific RNA sequencing on the Ion Torrent™ Proton™ system according to the protocol, MAN0010654 REV C.0.
This library was purified and assessed for yield and fragment size distribution on the Agilent Bioanalyzer 2100 using the High Sensitivity DNA chip and kit (Agilent Technologies) according to the manufacturer's protocol; G2938-90322, Rev D. The libraries were considered of sufficient yield for template preparation and enrichment.
The libraries were diluted to a target concentration of 80pM and pooled in equimolar amounts for template preparation using the Ion 540™ Chef Kit (ThermoFisher Scientific). Twenty-five microliters of diluted, pooled barcoded library were loaded onto the Ion Chef liquid handler for template preparation and enrichment using Ion 540™ Chef Reagents, Solutions and Supplies according to the protocol, MAN0010851 REVF.0. Enriched ion sphere particles were loaded onto an Ion 540™ Chip.
Massively parallel sequencing was performed on the Ion Torrent™ GeneStudio™ S5 Prime System using Sequencing Solutions, Reagents according to the protocol, MAN0010851 REVF.0. All reads were trimmed at CAF to remove barcodes and adaptors.

Bioinformatic analysis for viruses/viroids.
Separate sequence runs of any sample were merged and subjected to bioinformatic analysis using CLC Genomics Software (Qiagen Digital Insights) version 20.0.02. Read data was trimmed for quality parameters.

Reference mapping was performed against the whole genome sequence of all International Taxonomy of Viruses (ICTV) accepted plant viruses, supplemented with grapevine viruses (a standard database in our laboratory), which was updated annually. At the start of the project, this represented 3846 reference sequences of 1848 plant viruses and viroids. Mapping was performed with the following settings: match score = 1; mismatch cost = 2; Linear gap costs, Insertion cost = 3; and Deletion cost = 3. Three stringencies were tested: 1) length fraction = 1, similarity fraction = 0.9 (high stringency), 2) length fraction-0.8, similarity fraction-0.8 (medium stringency), or 3) length fraction = 0.5, similarity fraction = 0,8 (low stringency). In all instances, random mapping of reads was allowed when multiple mapping sites were present.

De novo Assembly was done using both default parameters (automatic word size =23) and optimised parameters for datasets positive for virus infection (word size = 30 and 35) (CLC Genomics Software). Assembled contigs were subjected to BLAST analysis (e-value cut off: 1e-5; query coverage: ≥ 80%) against the NCBI GenBank RefSeq of viruses and viroids using the CLC BLAST function. Contigs were then extracted and subjected to BLASTn analysis at NCBI Genbank to confirm identity.

Confirmatory tests.
Traditional RT-PCR was then used to confirm the presence of the viruses and viroids identified from the sequencing data. A one-step RT-PCR reaction was conducted using 1 µl nucleic acid in a mixture containing 10 µM of each respective forward and reverse primer, 5 X GoTaq® buffer, 100 mM molecular grade DDT, 25 mM $MgCl_2$, 10 mM dNTP mix, 200 U/µl Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT), 40 U/µl Recombinant RNasin® ribonuclease inhibitor and 5 U/µl GoTaq® DNA polymerase (reagents were sourced from either New England BioLabs, Massachusetts, USA or Promega). The remainder of the 25 µl total volume was made up of nuclease-free water. Amplification was done using the following program: one cycle of reverse transcription for 45 min at 37 ˚C, 2 min at 50 ˚C, 5 min at 94 ˚C, and 35 cycles of 30s at 94˚C, 30s at each primer annealing temperature (Table 2), 30 s at 72 ˚C, followed by a terminal extension of 5 min at 72 ˚C. The final elongation step was conducted for 5 min at 72 °C. PCR products were analysed by electrophoresis on a 1-3% agarose gel. PCR products were submitted for bidirectional Sanger sequencing at the Central Analytical Facility at Stellenbosch University. Sequence identity was confirmed using NCBI BLAST.

Phylogenetic relationships for the viral/viroid sequences were determined by aligning the sequences obtained during this study with reference sequences downloaded from NCBI Genbank, using the Maximum Likelihood method (1000 bootstrap replications, General Time Reversible model) implemented in MEGA 7.0 (Kumar 2016). Percentage identity scores between viroid sequences were determined using the Sequence Demarcation Tool (SDTv1.2) (Reuter 2010).

7. RESULTS AND DISCUSSIONS

Of the 97 Nuclear accessions submitted by Plant Improvement Organisations (PIO's) for analysis for their virus and viroid status, 25 were Table grape cultivars, 17 Table/Raisin grape cultivars, 7 Raisin grape cultivars, 13 Rootstock cultivars, and 35 Wine grape cultivars (Table 1). Of these 77 had been subjected to heat therapy and meristem tip culture at the PIO (in two instances to two successive cycles), three had been subjected to only two meristem tip culture cycles, while the remaining 17 nuclear accessions had, not been passed through heat therapy or meristem tip culture cycles locally but may have been subjected to these in their respective breeding programs internationally. The latter were all Raisin, Table or Table/Raisin grape cultivars, and all were from only one of the PIO's.

In the first season of collection, RNA was extracted from each accession on multiple occasions and multiple tissues during the season, to account for any seasonal changes in virus titre or tissue tropism. These were then pooled for library preparation. However, in the second and third seasons, only two periods of collection and extraction were conducted. On any occasion of sample collection, RNA extractions were performed multiple times if needed until RNA of sufficiently high quality and concentration was obtained to pool for NGS library preparation (Figure 1). It was generally striven to obtain RNA integrity values (RIN values) from any RNA preparation of more than 6. In a few instances (Figure 2), this could not be achieved as plant tissue was exhausted material through the multiple extractions and in four instances, library preparations had to be made despite low RIN values.

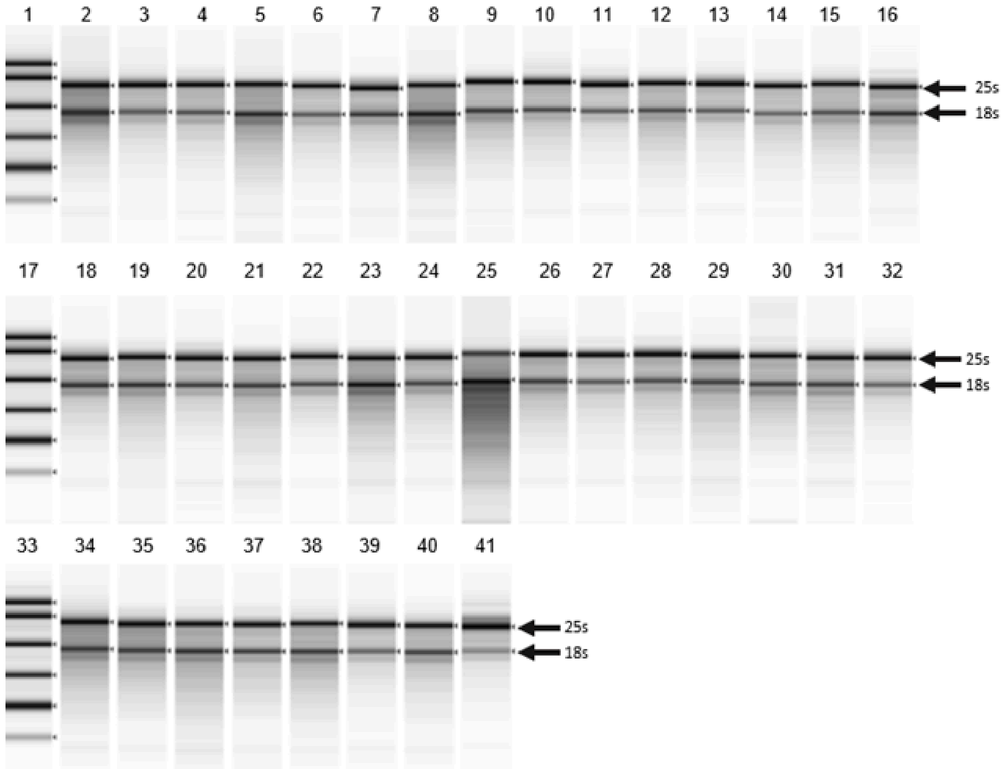

Figure 1: Electrophoresis gel of the RNA extraction preparation of grapevine nuclear samples purified in 2022 for use in next-generation sequencing. Lanes 1, 17 and 33 represent a known molecular marker, while the remaining lanes represent individual samples obtained from each of the three plant improvement organisations taking part in this project. The quality of ribosomal RNA (of 25S or 18S) was used as a proxy for viral RNA quality.

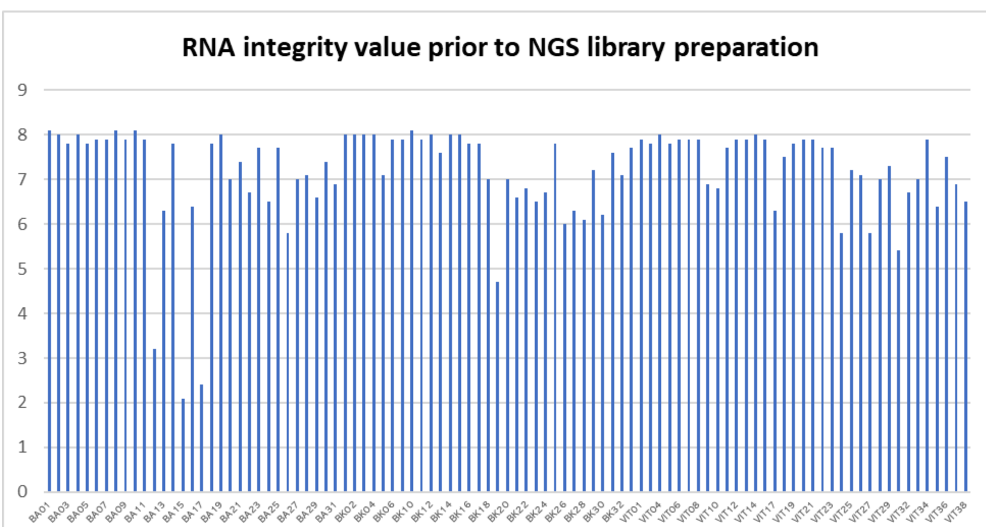

Figure 2: Graph of RNA integrity values obtained for grapevine nuclear samples utilised in next-generation sequencing library preparation.

The concentration of RNA obtained varied significantly between various nuclear grapevine sample extractions (Figure 3) but yielded volumes of between 10ul and 75ul. Therefore, the total amount of RNA obtained in all instances was greater than 1500 ng RNA, with only one sample having less than that at 840 ng total RNA. Appropriate volumes of RNA were provided for library preparation.

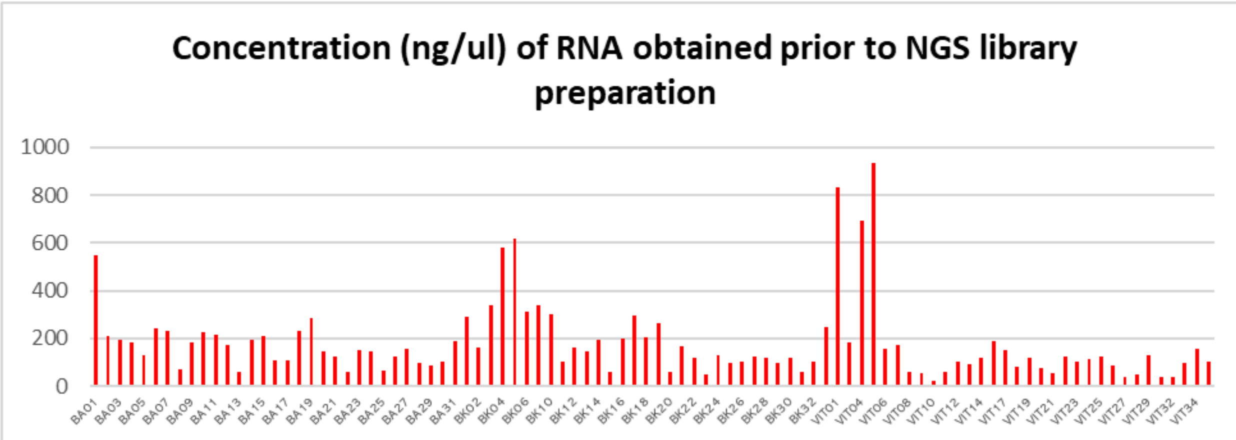

Figure 3: Graph of RNA concentration (ng/ul) of nuclear grapevine samples submitted for next-generation sequencing library preparations.

Most samples were subjected to multiple sequencing runs to obtain the required 40 million reads. The combined number of raw reads obtained per accession from multiple Ion Torrent RNA sequence runs ranged from 30 785 717 to 61 267 502 million with an average of 43 374 337 million reads across the 97 sample libraries (Supplementary data Table 1). After assembly using CLC genomics workbench using default parameters, an average of 17 706 contigs were obtained along with an average N50 of 401. Using alternative word sizes, the assembly was repeated for datasets which contained virus contigs and word size 35 was shown to increase contig length and coverage of viruses identified.

Reference mapping at various stringencies to over 1800 known virus species, as well as BLAST analysis of *de novo* assembled contigs, revealed that 17 of the 97 nuclear accessions had no detectable viruses or viroids (Table 1). Amongst the remaining nuclear accessions, five viruses and four viroids or viroid-like entities were identified. The virus infections were rare, being found in only 8 accessions, while viroid infections were common, being found in 76 of the 97 accessions. The presence of each viroid and virus was confirmed using RT-PCR and Sanger sequencing of the resulting amplicons. Viruses observed included Rupestris stem pitting-associated virus (GRSPaV), Grapevine fleck virus (GFkV), Grapevine syrah virus 1 (GSyV-1), Grapevine leafroll-associated virus 4 (GLRaV-4) and Grapevine Rupestris vein feathering virus (GRVFV).

				Viruses/viroids detected									
				Viruses					Viroids				
Accession	Type of grape	Thermotherapy	Meristem tip culture	Grapevine fleck virus	Grapevine rupestris stem pitting associated virus	Grapevine syrah virus 1	Grapevine leaf roll associated virus 4	Grapevine Ruspestris vien feathering virus	Grapevine yellow speckle viroid 1	Grapevine yellow speckle viroid 2	Hop stunt viroid	Australian grapevine viroid	Grapevine hammerhead viroid-like RNA
BK01	Raisin	√	√						+	-	-	-	-
BK02	Raisin	√	√						+	-	-	-	-
BK15	Raisin	x	x						+	+	+	+	-
BK16	Raisin	x	x						+	-	+	-	-
BK24	Raisin	x	x						-	-	+	-	-
BK28	Raisin	x	x						-	-	+	-	-
BK29	Raisin	x	x				+	+	+	+	+	+	-
VIT25	Rootstock	√	√						-	-	+	-	-
VIT26	Rootstock	√	√						-	-	+	-	-
VIT27	Rootstock	√	√						-	-	+	-	-
VIT28	Rootstock	√	√						-	-	-	-	-
VIT29	Rootstock	√	√						+	-	+	-	-
VIT30	Rootstock	√	√						-	-	+	-	-
VIT32	Rootstock	√	√						+	+	+	-	-
VIT33	Rootstock	√	√						+	+	+	-	-
VIT34	Rootstock	√	√						-	-	-	-	-
VIT35	Rootstock	√	√						-	-	+	-	-
VIT36	Rootstock	√	√						-	-	-	-	-
VIT37	Rootstock	√	√						-	-	-	-	-
VIT38	Rootstock	√	√						-	-	-	-	-
BA14	Table	√	√						+	-	-	-	-
BA15	Table	√	√						+	-	-	-	-
BA16	Table	√	√						+	+	+	+	-
BA17	Table	√	√						+	-	+	-	-
BA30	Table	√	√						+	+	+	-	-
BK03	Table	√	√						-	-	-	-	-
BK04	Table	√	√						-	-	+	-	-
BK10	Table	√	√						-	-	+	-	-
BK11	Table	√	√						+	+	+	-	-
BK12	Table	√	√						+	-	+	-	-
BK13	Table	√	√						-	-	+	-	-
Vit06	Table	√	√						-	+	+	-	-
Vit07	Table	√	√						+	-	-	-	-
Vit17	Table	√	√						+	-	+	-	-
Vit18	Table	√	√						+	+	+	-	-
Vit19	Table	√	√						+	-	-	-	-
Vit20	Table	√	√						-	-	-	-	-
Vit21	Table	√	√						+	+	+	-	-
Vit22	Table	√	√						+	-	-	-	-
Vit23	Table	√	√						-	-	-	-	-
VIT24	Table	√	√						-	-	-	-	-
BA07	Table	x	2X						-	-	-	-	-
BA18	Table	x	2X						+	-	+	-	-
BA19	Table	x	2X						-	-	-	-	-
BK25	Table	x	x						+	-	+	-	-
BK06	Table and Raisin	√	√						-	-	-	-	-
BK07	Table and Raisin	√	√						-	-	+	-	-
BK14	Table and Raisin	√	√						+	-	+	-	-
BK20	Table and Raisin	√	√						-	-	-	-	-
BK31	Table and Raisin	√	√						-	-	+	-	-
BK33	Table and Raisin	√	√						+	-	+	-	-
BK05	Table and Raisin	x	x						-	+	+	-	-
BK17	Table and Raisin	x	x						-	-	+	-	-
BK18	Table and Raisin	x	x		+				-	-	+	-	-
BK19	Table and Raisin	x	x						+	-	+	-	-
BK21	Table and Raisin	x	x						+	-	+	-	-
BK22	Table and Raisin	x	x						-	-	+	-	-
BK23	Table and Raisin	x	x		+				-	-	+	-	-
BK26	Table and Raisin	x	x						+	-	-	-	-
BK27	Table and Raisin	x	x						+	-	+	-	-
BK30	Table and Raisin	x	x						-	-	+	-	-
BK32	Table and Raisin	x	x						+	-	+	-	-
BA01	Wine	√	√						+	-	+	-	-
BA03	Wine	√	√						-	+	+	-	-
BA05	Wine	√	√						-	-	-	-	-
BA06	Wine	√	√						-	-	+	-	-
BA08	Wine	√	√	+					+	+	+	-	-
BA09	Wine	√	√						+	+	+	-	+
BA10	Wine	√	√						-	-	-	-	-
BA11	Wine	√	√						-	-	-	-	-
BA12	Wine	√	√						+	-	+	-	-
BA13	Wine	√	√						+	-	-	-	-
BA20	Wine	√	√						+	-	-	-	-
BA21	Wine	√	√						-	-	-	-	-
BA22	Wine	√	√		+	+			-	-	+	-	-
BA23	Wine	√	√						+	+	+	-	-
BA24	Wine	√	√		+				+	+	+	-	+
BA25	Wine	√	√						+	-	+	-	-
BA26	Wine	√	√		+				+	-	+	-	-
BA27	Wine	√	√						+	-	+	-	-
BA28	Wine	√	√						+	-	+	-	-
BA29	Wine	√	√		+				+	-	+	-	-
BA31	Wine	√	√						-	-	-	-	-
Vit01	Wine	√	√						-	-	+	-	-
Vit02	Wine	√	√						+	-	-	-	-
Vit04	Wine	√	√						+	+	+	-	-
Vit05	Wine	√	√						-	+	+	-	-
Vit08	Wine	√	√						+	+	+	-	-
Vit09	Wine	√	√						+	-	+	-	-
Vit10	Wine	√	√						+	-	+	-	-
Vit11	Wine	√	√						+	-	-	-	-
Vit12	Wine	√	√						-	-	-	-	-
Vit13	Wine	√	√						+	-	+	-	-
Vit14	Wine	√	√						+	-	+	-	-
Vit15	Wine	√	√						-	-	-	-	-
BA02	Wine	2X	√						+	-	+	-	-
BA04	Wine	2X	√						-	-	-	-	-

Table 1: Viruses and Viroids observed in grapevine nuclear accession following next-generation sequencing analysis using de novo assembly and reference mapping (For an enlarged version, see supplementary data Table 8).

Of the viruses found, the most abundant was GRSPaV, identified in six of the 97 (0.06%) samples, while the other viruses were each just found in a single accession. The number of contigs obtained with de novo assembly using word size = 35, retrieved for each of the viruses identified, and the length of the partially reconstructed genome of each virus, as well as the results of genome reconstruction using reference mapping analysis, are shown in Supplementary Tables 2 and 3, respectively.

All virus and viroid infections observed in the NGS data were confirmed with reverse transcriptase PCR and Sanger sequencing. Primers used in these confirmatory tests are present in Table 2. Phylogenetic analysis of the Sanger sequence data served as a further confirmation and indication of viral strain identity. As the aim was just to confirm the infection, phylogenetic analysis was not conducted on the whole genome but was only based on the PCR amplified region.

Table 2: Primers utilised in reverse transcriptase polymerase chain reaction (RT-PCR) tests to confirm the next-generation sequencing results.

Species	Primer Sequence	Band size	Annealing Temp	Reference
GRSPaV	CPORF_R8620 5'-ATTAGTACGGTATTCCAGCG-3' CPORF_7709F 5'-TGAAGGCTTTAGGGRTTAG-3'	928	58°C	(Mostert et al., 2018)
GLRaV-4	LRAmpF 5'-ATTTAGGTAATGTWGTRGCTAC-3' LRAmpR 5'-TATCCTCAGWGAGGAARCGG-3'	485	46°C	(Abou Ghanem-Sabanadzovic et al. 2012)
GSyV-1	GVQCP-F 5'-TCCCAGCTTCAGGGTGAATT -3' GVQCP-R 5'-GCATTGCTGCGCATTGGAGG -3	720	52°C	(Engel et al., 2010)
GFkV	GFkV_3_F 5'-AGCCTGCTGTCTCTAGCT-3' GFkV_3_R 5'-CACCTGTGGCTTCTACCG-3'	500	49°C	(Usher, per com)
GRVFV	GRVFV-SAF215 5'TACAAGGTGAATTGCTCCACAC-3' GRVFV-SAR1025 5'-TCATTGGCGATGCGTTCG -3'	320	50°C	(Lesilane, per com)
HSVd	HSVdI - GCGTCTCATCGGAAGAGCC-3' HSVdII 5'-GACCGGTGGCATCACCTCT-3'	296	56°C	(Kofalvi et al. 1997)
GYSVd-1	AS 5'-GCGGGGGTTCCGGGGATTGC-3' S 5'-TAAGAGGTCTCCGGATCTTCTTGC-3'	367	55°C	(Polivka et al., 1996)
GYSVd-2	P1 5' ACTAGTACTTTCTTCTATCTCCCGAAGC-3' P2 5'-ACTAGTCCGAGGACCTTTTCTAGCGCTC-3	370	60°C	(Jiang et al., 2009)
AGVd	AS 5'-GTCGACGACGAGTCGCCAGGTGAG-3' S 5'-GTCGACGAAGGGTCCTCAGCAGAG-3'	370	64°C	(Wah & Symons, 1997)
GHVd	Vir-F2 5'GTGTGGTGCTCCTGACGAGTCCA 3' Vir-R2 5' CTAGCCTAGGAGCGAATCTGCCA 3'	320	60°C	(Candresse et al., 2017)

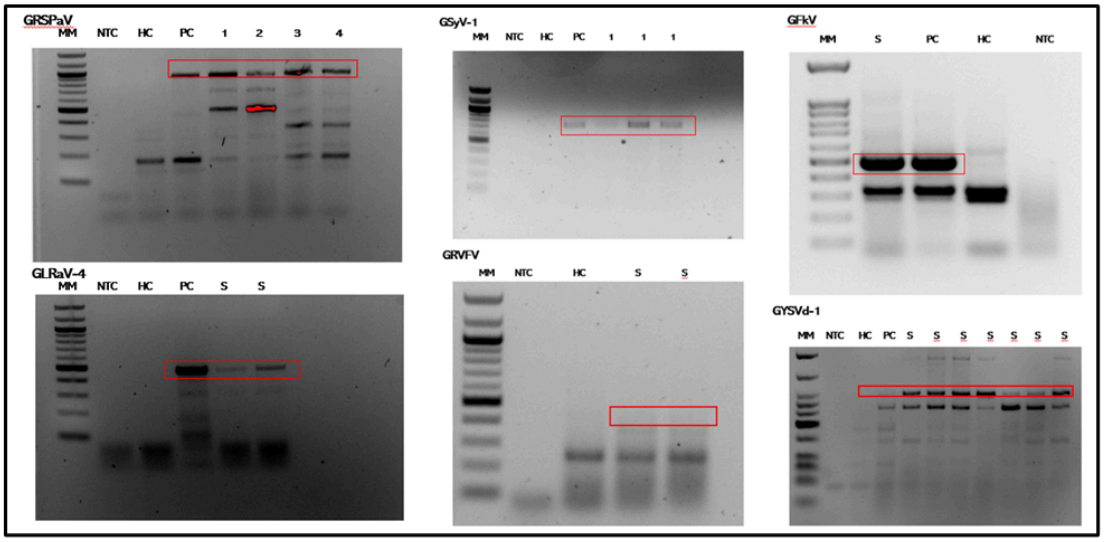

Figure 4: Confirmation of GRSPaV, GLRaV-4, GSyV-1, GRVFV, GFkV and GYSVd-1 found during next-generation sequencing analysis of nuclear grapevine samples by PCR and Sanger sequencing. PCR's utilised were not necessarily optimized and only the regions in red blocks were cut out, purified, and submitted for Sanger sequencing. Samples are designated either with a number or just by "S".

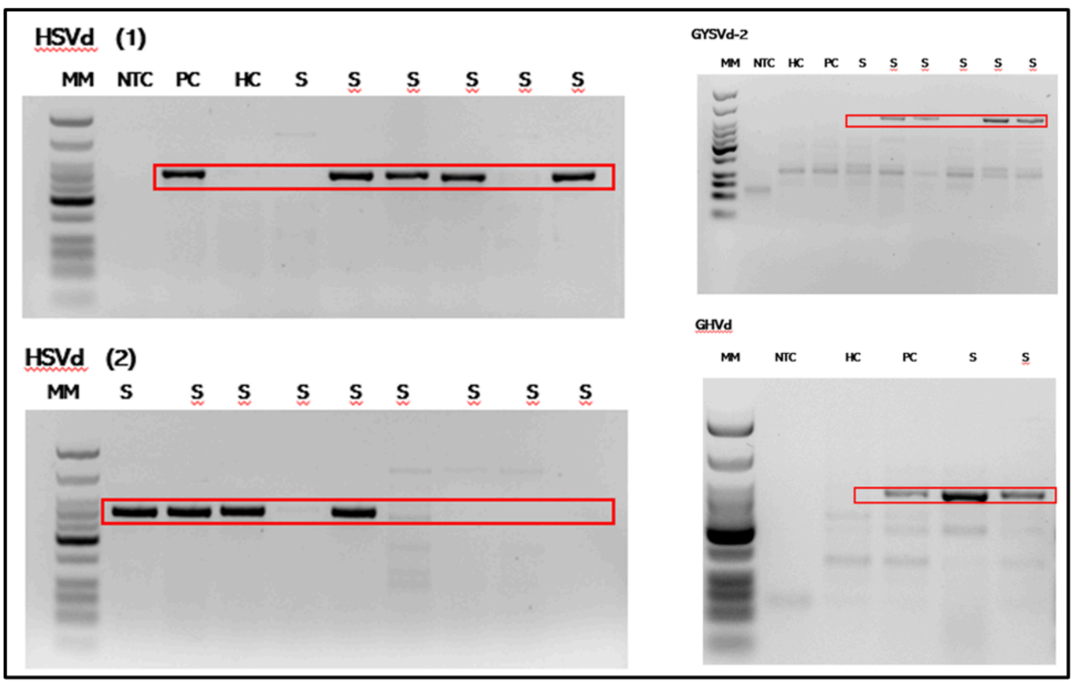

Figure 5: Confirmation of HSVd, GYSVd-2 and GHVd found during next-generation sequencing analysis of nuclear grapevine samples by PCR and Sanger sequencing. PCR's utilised were not necessarily optimized and only the regions in red blocks were cut out, purified, and submitted for Sanger sequencing. Samples designated by an "S".

Phylogenetic analysis of the coat protein region of the GRSPaV isolates reveals that four isolates belong to group I and two of the isolates belong to group IId (Figure 6). GSyV-1 was identified in one sample, and phylogenetic analyses of the coat protein region revealed that this isolate is most like GSyV-1 strain Cultivated-TN1 (MN716774) and appears to group into the European clade (Figure 7). GFkV was identified in one sample and shares similarity with GFkV isolate A1567c (ON567253). GLRaV-4 was identified in one sample, and sequence comparison revealed that it is most like GLRaV-4 isolate LR106 (FJ467503), which belongs to the GLRaV-4 group.

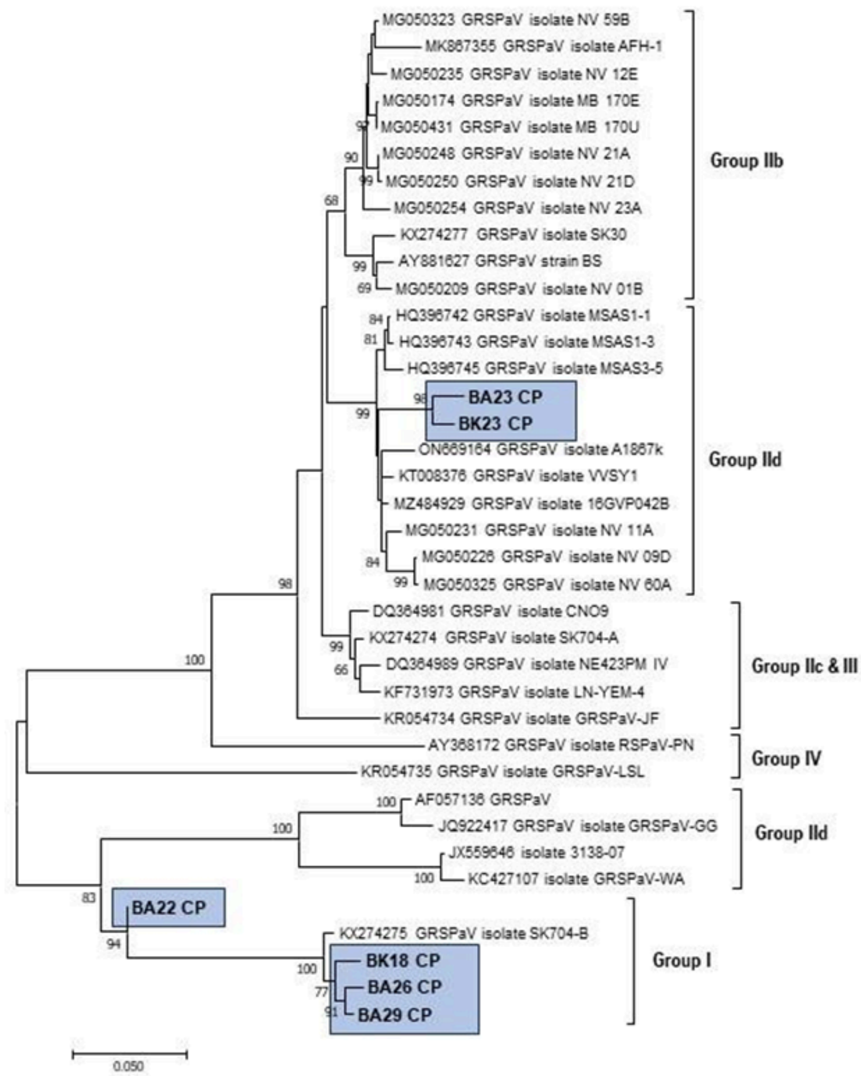

Figure 6: Phylogenetic tree based on the alignment of nucleotide sequences of grapevine rupestris stem pitting-associated virus (GRSPaV) coat protein sequences obtained in this study and from NCBI Genbank. The tree was constructed in MEGA 7 0 using Maximum Likelihood analysis at 1000 bootstraps. The blue squares denote the South African GRSPaV sources from this study.

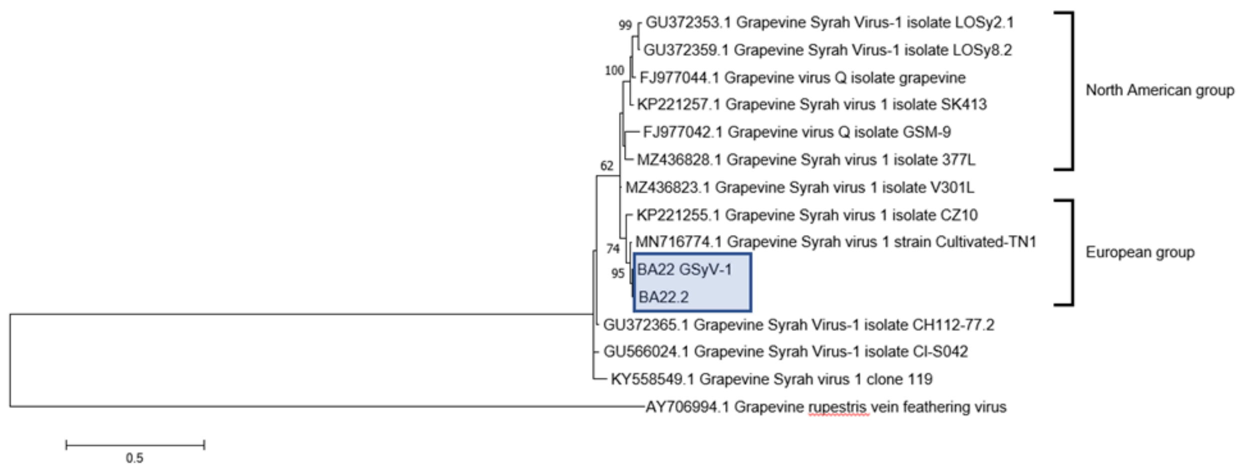

Figure 7: Phylogenetic tree based on the alignment of nucleotide sequences of Grapevine Syrah virus-1 (GSyV-1) coat protein sequences obtained in this study and from NCBI Genbank. The tree was constructed in MEGA 7 0 using Maximum Likelihood analysis at 1000 bootstraps. The blue square denotes the South African GSyV-1 source from this study.

Viroids were the most frequently identified viral-like agent within the grape nuclear accessions, being present in 76 of the 97 (75.2%) samples, with four of the seven known grapevine viroid species and one putative viroid species being identified (Table 1). Of the four viroids identified, HSVd is the most abundant being present in 63 of the 97 (65.4%) samples followed by GYSVd-1 being present in 52 of the 97 (53.6%) samples, the GYSVd-2 being present in 17 of the 97 (17.6%) samples, with AGVd being the least abundant, being present in 3 of the 97 (0.03%) samples. While GHVd was identified in 2 of the 97 samples (0.02%) (Table 1). CEVd, JGVd, and GLVd were not identified in grapevine nuclear material.

Sequence analysis and comparisons revealed that the HSVd isolates fall into three groups (Figure 8), with the majority of HSVd isolates obtained in this study being most like HSVd isolate PGH-2 (GenBank accession KR909028) with high levels of sequence identity (96.3-98.7%), the second group being most like HSVd isolate A1541t (GenBank accession ON669211), and third and smallest group being most like HSVd isolate SLD-Y23 (GenBank accession KY270463) (Figure 8, Supplementary data table 4).

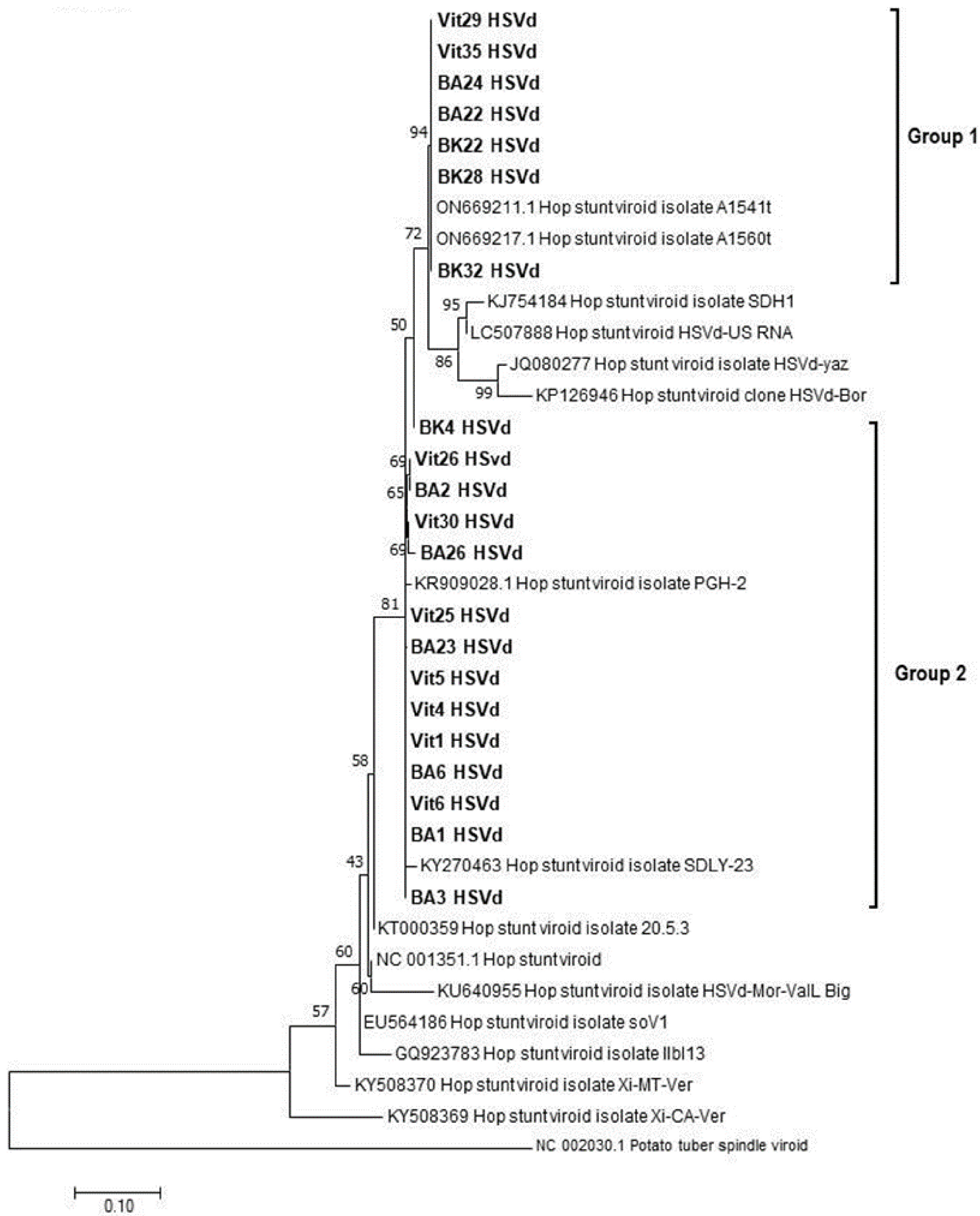

Figure 8. Phylogenetic tree based on the alignment of nucleotide sequences of HSVd isolates obtained in this study (Bold) and from NCBI Genbank. The group designations are indicated to the right of the tree. The tree was constructed in MEGA 7 0 using Maximum Likelihood analysis at 1000 bootstraps. Potato Tuber Spindle Viroid was used as an outgroup.

Sequence analysis revealed that each of the four known variants of GYSVd-1 is present. This can be observed in the phylogenetic analyses (Figure 9), as well as sequence comparisons between the four types of GYSVd-1 with isolates sharing similarities ranging between 81-99% (Supplementary data Table 5). Mixed infections of the four GYSVd-1 types were confirmed through reference mapping, BLASTn analysis, RT-PCR and Sanger sequencing.

Figure 9: Phylogenetic tree based on the alignment of nucleotide sequences of GYSVd-1 isolates obtained in this study (Bold) and from NCBI Genbank. Group designation is provided to the right of the tree. The tree was constructed in MEGA 7 0 using Maximum Likelihood analysis at 1000 bootstraps. Pepper Chat Fruit Viroid was used as an outgroup.

Sequence analysis revealed that the isolates of GYSVd-2 observed in this study (Supplementary data Table 6) were like most reported GYSVd-2 sources, ranging from 99-100% similarity in nucleotide identity (Figure 10). Low levels of diversity exist amongst the GYSVd-2 isolates (Figure 10).

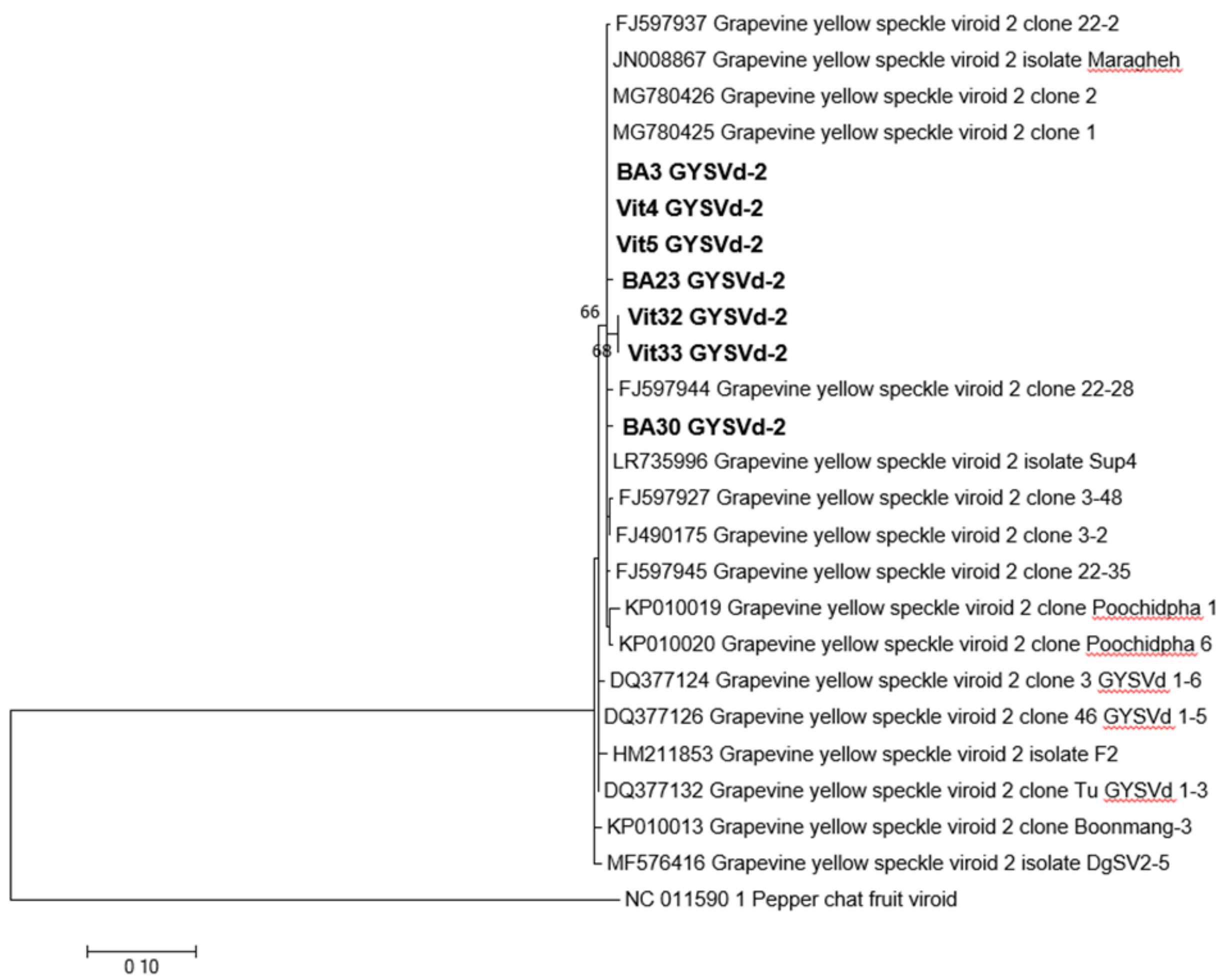

Figure 10: Phylogenetic tree based on the alignment of nucleotide sequences of Grapevine yellow speckle viroid-2 (GYSVd-2) sources obtained in this study (Bold) and from NCBI Genbank. The tree was constructed in MEGA 7 0 using Maximum Likelihood analysis at 1000 bootstraps. Pepper Chat Fruit Viroid was used as an outgroup.

Sequence analysis reveals that a small amount of variation exists in AGVd isolates found during this study, as with known sources of this viroid (Figure 11, Supplementary data Table 7).

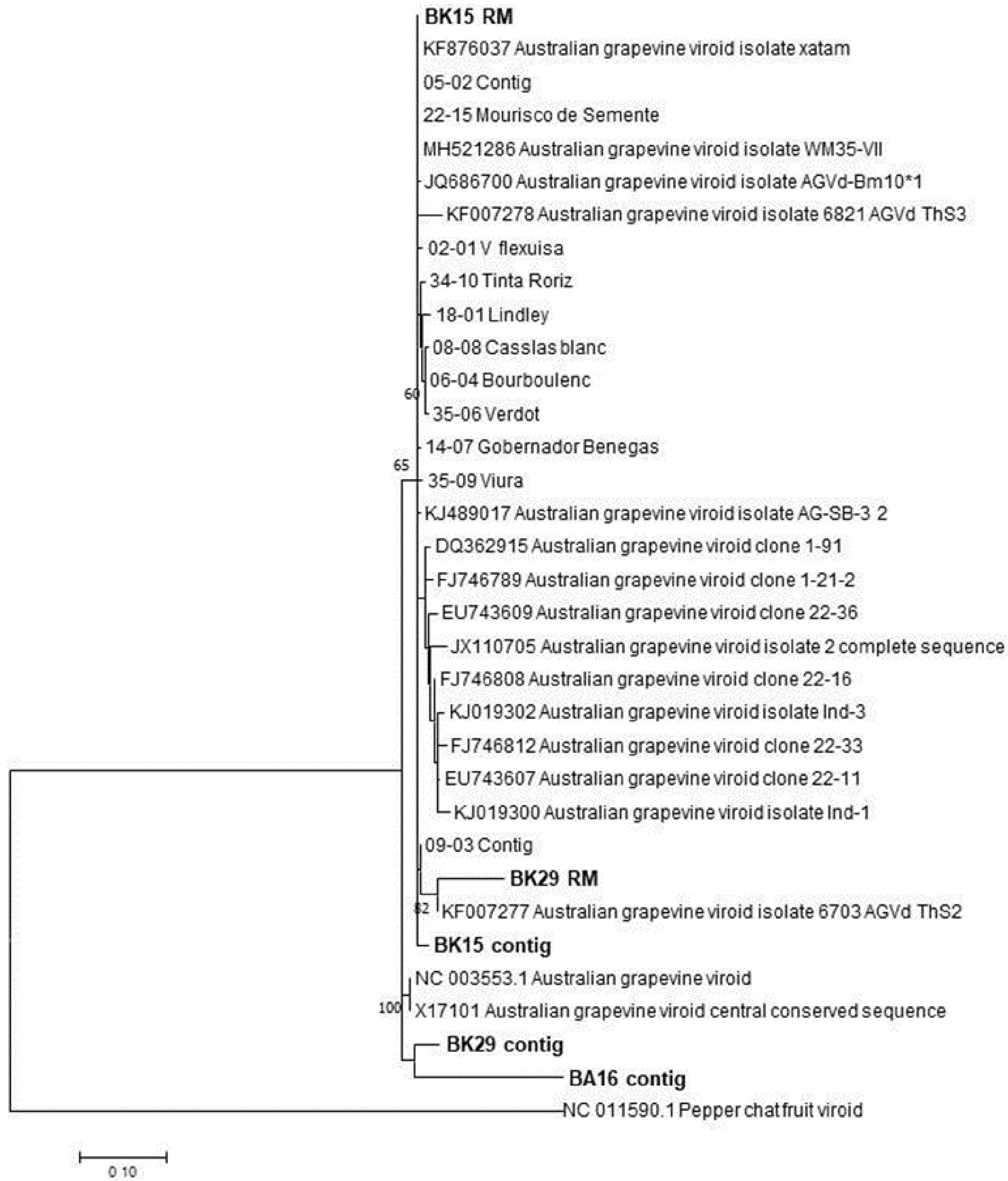

Figure 11: Phylogenetic tree based on the alignment of nucleotide sequences of AGVd isolates obtained in this study (Bold) and from NCBI Genbank. The tree was constructed in MEGA 7 0 using Maximum Likelihood analysis at 1000 bootstraps. Pepper Chat Fruit Viroid was used as an outgroup. (RM = sequence obtained through reference mapping, contig = sequence obtained through BLAST of assembled contigs).

8. CONCLUSIONS AND RECOMMENDATIONS

Grapevine nuclear accessions within the South African Vitis Certification Scheme are routinely only tested for very few of the known viruses of grapevines. Despite this, the current study has revealed that nuclear material is free of most of the known viruses of grapevines. This is probably due to the incisive mandatory implementation of heat therapy and meristem tip culture for at least four decades (for wine grapes) in South Africa, resulting in the fact that most nuclear plants in use here have been subjected to these processes. The presence in the material sampled, classified as "Nuclear" that has not been subjected to these virus-elimination processes, is anomalous, and it is possible that this material has been introduced to the certification scheme prior to implementing these processes. It is also possible that this material was submitted to this study for exactly this reason by the PIO. Of the viruses found within the nuclear material, only GRSPaV and GFkV are viruses for which tests within certified material are required. In both these instances, these viruses have not been routinely tested for by ELISA locally due to the previous absence of large-scale tests, but selected nuclear samples were subjected on a voluntary basis to PCR tests conducted at the formerly Waite Institute, Australia. It is possible that the accession found infected by these two viruses has not been tested at Waite or that the primer systems used at the time were unable to detect these specific variants. Routine ELISA testing for GFkV has been recommended within the Vine Improvement Association (VIA) scheme following a recent SATI and Winetech-funded project in which international ELISA kits to this virus were validated for local use. The remaining viruses are not generally tested for within the certification scheme, and their presence is more understandable. It is recommended that these specific nuclear accessions be subjected to a further round of heat therapy and meristem tip culture to eliminate GRSPaV, GFkV, GRVFV, GSyV-1 and GLRaV-4 from the nuclear material. Tests for these viruses at the foundation- or mother-block levels need only be considered if any of these viruses are shown to spread naturally in South Africa, hence would re-infected certified material, and are of economic importance. The presence of progeny vines of these nuclear accessions within the field makes such studies feasible. The value of testing grapevine nuclear material by NGS has also been demonstrated during this project, and it is recommended that this be incorporated into the South African One Vitis certification scheme as a mandatory test on all nuclear material (both retrospectively and in future).

The common presence of viroids within the nuclear material suggests that heat therapy and meristem tip culture do not successfully eliminate these pathogens, or that they are spreading uncontrolled within nuclear plant facilities. None of the viroids is included in the certification scheme. A decision on whether to manage these agents would require developing a method to eliminate these pathogens from nuclear material, possibly in parallel with heat therapy and meristem tip culture, along with determining whether they result in economic consequences within the planting material to warrant their management. Project proposals to address these two issues have declined during the past two years, but require careful reconsideration.

9. PLANNED OUTPUTS

a) TECHNOLOGY DEVELOPMENT, PRODUCTS AND PATENTS

An NGS analysis pipeline has been established within our laboratory for the general testing of viruses of grapevines. This can serve as the basis within the South African One-Vitis Certification Scheme for future tests for viruses within nuclear accessions. Viruses present in specific nuclear accessions were identified, and recommendations for their elimination were provided, in order to ensure the continued high regard in which the South African Vitis certification scheme is held and to prevent potential economic consequences associated with propagating thousands of progenies of such infected vines. The problem of the near-ubiquitous occurrence of viroids within nuclear material, and by extension the entire grapevine growing industry in South Africa, has been identified.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

The near-ubiquitous occurrence of viroids within grapevine nuclear material in South Africa must be communicated with great sensitivity to the industry. While this problem is probably global in grape production, and most of these viroids are found wherever grapes are grown, this represents the first (to our knowledge) report of the widespread occurrence of these in such high-level planting material. While all these viruses and viroids are reported from most countries, it is unlikely to have phytosanitary implications. There should be no negative consequences in publishing this study in scientific journals, and it is likely to result in these tests being done internationally, too. However, growers and purchasers of the viroid-infected planting material locally need to be informed, but with the necessary caveats regarding the state of the art of detection and viroid elimination and unknowns regarding the economic importance of these pathogens. This issue cannot just be ignored, and the necessary steps to address this are required. South Africa would be taking the lead in this process as befits the high regard in which our planting material is held internationally, and the leading role South Africa plays in the provision of healthy grapevine material. It is recommended that this report be provided to the Technical Committee of PlantSA Vine Improvement Association (VIA) for discussion and action, but that it not be published in popular form, but rather communicated on grower's days, where the necessary qualifications to the results can be explained in detail, and questions fielded.

c) HUMAN RESOURCES DEVELOPMENT / TRAINING (STUDENTS)

Student Name and Surname	**Student Nationality**	**Degree (e.g., Hons, MSc)**	**Level of studies in the final year of the project**	**Total Bursary Cost for Industry for the entire project**
Honours				
Masters				
PhD				
Seamus W Morgan	South African	PhD	Almost finished	R 0
Postdocs				

d) LIKELY PUBLICATIONS (POPULAR, PRESS RELEASES, SCIENTIFIC)

A manuscript has been published on the work preceding this study, in which NGS was used to determine the viroid status of the grapevine in the local germplasm collection. A publication on this study is planned (in the draft phase) but will first be discussed with the industry and funding bodies before submission for publication.

e) PRESENTATIONS/PAPERS THAT COULD BE DELIVERED

Seamus W. Morgan, S.W., Read, D.A., Burger, J.T, and Pietersen, G., 2022. Grapevine

Infecting viroid diversity in South Africa. Poster delivered at the 52nd Congress of the SASSP, 1-3 August.2022, Pretoria,

10. PROJECT OUTCOME AND IMPACT

New Knowledge	Benefits of the Supply Chain	Direct Grower Application	Direct Packhouse/Winery/ Cellar Application	Other
		X		

Other is:

The Value of the project to the industry

The near-ubiquitous presence of viroids of grapevine in nuclear material is likely to affect the vine clone performance without being observed in the absence of viroid-free vines. The PIO's managing the individual vines with virus infection (8) need to examine the reason for their presence in nuclear material.

11. PERSONS PARTICIPATING IN THE PROJECT:

INITIALS AND SURNAME	HIGHEST QUALIFICATION	RACE (B, C, I, W)	GENDER (M, F, X)	INSTITUTE DEPARTMENT	POSITION	TOTAL COST TO PROJECT
RESEARCH PERSONNEL						R 850000
Seamus Morgan	MSc	W	M	SU Genetics	Student	R 0
Gerhard Pietersen	PhD	W	M	SU Genetics	PL	R 850000
SUPPORT PERSONNEL						R 0

POSITION: Co = Co-worker (another researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research Assistant
TA = Technical assistant/ technician

12. TOTAL COST OF PROJECT

TOTAL ANNUAL COSTS (ALL YEARS)	CFPA	RAISINS SA	HORTGRO	SATI	WINETECH	ARC	OTHER	TOTAL
2018 and before	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
2019	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
2020	R 0	R 0	R 0	R 295000	R 295000	R 0	R 100000	R 690000
2021	R 0	R 0	R 0	R 351250	R 351250	R 0	R 110000	R 812500
2022	R 0	R 0	R 0	R 398500	R 398500	R 0	R 110000	R 907000
2023	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
TOTAL	R 0	R 0	R 0	R 1044750	R 1044750	R 0	R 320000	R 2409500

Supplementary data

Table 1: Next-generation sequencing metrics for grapevine nuclear samples.

	Before trimming		After trimming			de novo Assembly
Sample	**Number of reads**	**Average read length (nt)**	**Number of reads/run**	**Total reads per sample**	**Averagereadlength**	**Number of contigs**
Vit01	**12 427 142**	**150,09**	**12427142**		**149**	
	7 891 880	**147,81**	**7890083**		**146,45**	
	7 959 750	**149,64**	**7958525**		**148,41**	
	13 928 467	**152,1**	**13928467**		**151,08**	
	13 737 081	**149,84**	**13737081**	**55944320**	**148,81**	**21168**
Vit02	**10 329 801**	**139,79**	**10329801**		**138,7**	
	11 403 766	**138,55**	**11403766**		**137,52**	
	6 733 489	**137,38**	**6732449**		**136,1**	
	6 555 286	**138,85**	**6554596**		**137,66**	
	11 580 005	**140,2**	**11580005**	**46602347**	**139,12**	**17728**
Vit04	**10 777 814**	**152,9**	**10777814**		**151,94**	

	11 993 103	**152,52**	**11993103**		**151,61**	
	6 789 197	**151,72**	**6788701**		**150,53**	
	6 792 769	**152,74**	**6792431**		**151,68**	
	12 599 350	**152,73**	**12599350**	**48952233**	**151,77**	**20070**
Vit05	**13 772 638**	**141,17**	**13772638**		**140,21**	
	14 834 742	**140,85**	**14834742**		**139,95**	
	8 445 900	**139,97**	**8445114**		**138,8**	
	8 346 026	**141,39**	**8345521**		**140,32**	
	14 235 372	**143,44**	**14235372**	**59634678**	**142,51**	**22308**
Vit06	**11 318 637**	**145,01**	**11318637**		**143,94**	
	12 357 588	**144,23**	**12357588**		**143,21**	
	7 460 707	**142,93**	**7459863**		**141,65**	
	7 250 025	**143,86**	**7249425**		**142,67**	
	12 627 009	**144,76**	**12627009**	**51013966**	**143,68**	**24745**
Vit07	**12 581 857**	**143,85**	**12581857**		**142,84**	
	13 856 825	**143,01**	**13856825**		**142,05**	
	8 690 081	**139,59**	**8688654**		**138,32**	
	8 932 521	**140,47**	**8931543**		**139,29**	

	14 667 744	**142,95**	**14667744**	**58729028**	**141,94**	**30302**
BK01	**11 003 865**	**147,24**	**11003865**		**146,27**	
	12 883 124	**147,16**	**12883124**		**146,34**	
	7 532 609	**143,42**	**7531178**		**142,15**	
	7 495 350	**142,99**	**7494165**		**141,77**	
	12 466 074	**146,03**	**12466074**	**51381022**	**144,91**	**18090**
BK02	**9 479 131**	**142,02**	**9479131**		**141**	
	11 265 327	**142,05**	**11265327**		**141,18**	
	6 700 137	**138,36**	**6698896**		**137,02**	
	6 765 731	**137,59**	**6764517**		**136,3**	
	10 963 271	**140,96**	**10963271**	**45173597**	**139,76**	**18313**
BK03	**12 305 767**	**144,91**	**12305767**		**143,83**	
	14 113 245	**145,6**	**14113245**		**144,68**	
	8 078 346	**140,34**	**8075678**		**138,93**	
	8 021 299	**139,72**	**8018948**		**138,38**	
	13 598 568	**145,36**	**13598568**	**56117225**	**144,22**	**15729**
BK04	**9 019 087**	**139,75**	**9019087**		**138,91**	
	11 635 371	**138,94**	**11635371**		**138,26**	

	6 830 760	135,76	6830310		134,68	
	6 857 209	134,55	6856723		133,5	
	11 798 030	137,23	11798030	46140457	136,14	37082
BK05	10 460 189	147,48	10460189		146,58	
	12 144 205	147,33	12144205		146,57	
	7 180 918	143,6	7179869		142,41	
	7 170 831	142,95	7169870		141,82	
	11 845 879	146,29	11845879	48802022	145,28	20764
BK06	7 975 122	121,06	7975122		120,2	
	9 932 012	119,41	9932012		118,75	
	6 249 678	116,38	6248875		115,31	
	6 545 854	115,22	6544959		114,18	
	9 839 184	118,65	9839184	40541850	117,57	35822
BK07	9 726 385	137,72	9726385		136,73	
	11 788 191	136,59	11788191		135,76	
	6 887 399	134,07	6886810		132,9	
	7 009 785	132,85	7009258		131,73	
	11 574 837	135,44	11574837	46986597	134,25	27853

BA01	**9 530 162**	**148,71**	**9530162**		**147,63**	
	10 349 360	**148,54**	**10349360**		**147,54**	
	6 203 576	**145,06**	**6 202 245**		**143,66**	
	6 478 835	**146,22**	**6 477 878**		**144,91**	
	10 903 240	**148,62**	**10 903 240**	**43465173**	**147,57**	**12098**
BA02	**13 475 878**	**149,34**	**13 475 878**		**148,27**	
	14 751 765	**149,26**	**14 751 765**		**148,26**	
	8 551 522	**144,62**	**8 548 564**		**143,27**	
	8 918 326	**146,08**	**8 916 089**		**144,81**	
	15 570 011	**149,65**	**15 570 011**	**61267502**	**148,65**	**22156**
BA03	**9 629 506**	**142,75**	**9 629 506**		**141,78**	
	10 734 426	**141,63**	**10 734 426**		**140,71**	
	6 462 798	**138,14**	**6 462 046**		**136,94**	
	6 684 289	**139,38**	**6 683 783**		**138,27**	
	11 651 391	**141,58**	**11 651 391**	**45162410**	**140,59**	**28016**
BA04	**11 349 527**	**143,09**	**11 349 527**		**142,09**	
	12 471 855	**142,55**	**12 471 855**		**141,62**	
	7 581 073	**139,23**	**7 579 418**		**137,97**	

	7 746 768	**140,23**	**7 745 596**		**139,08**	
	12 815 253	**142,99**	**12 815 253**	**51964476**	**142,01**	**25319**
BA05	**8 479 320**	**127,81**	**8 479 320**		**126,95**	
	5 780 986	**124,82**	**5 780 106**		**123,66**	
	7 499 966	**129,03**	**7 499 966**		**128,09**	
	6 044 887	**124,96**	**6 044 209**		**123,87**	
	9 394 767	**127**	**9 394 767**	**37199926**	**126**	**30524**
BA06	**8 943 797**	**142,82**	**8 943 797**		**141,9**	
	9 878 639	**142,05**	**9 878 639**		**141,17**	
	5 911 921	**139,09**	**5 911 294**		**137,96**	
	6 145 761	**140,01**	**6 145 310**		**138,98**	
	10 558 031	**142,11**	**10 558 031**	**41438149**	**141,19**	**24043**
BA07	**10 685 766**	**146,03**	**10 685 766**		**145,07**	
	12 348 246	**145,94**	**12 348 246**		**145,11**	
	7 196 972	**143,97**	**7 196 327**		**142,77**	
	7 100 260	**143,3**	**7 099 693**		**142,16**	
	11 780 135	**144,81**	**11 780 135**	**49111379**	**143,69**	**17944**
Vit08	**6 152 907**	**122,45**	**6 148 350**		**121,13**	

	5 581 298	**123,73**	**5 576 714**		**122,41**	
	5 442 519	**122,95**	**5 438 064**		**121,61**	
	5 392 851	**123,34**	**5 388 759**		**122,09**	
	4 947 085	**123,33**	**4 943 047**		**122,02**	
	5 711 800	**123,38**	**5 708 171**		**122,19**	
	5 684 524	**122,19**	**5 680 515**		**120,99**	
	5 914 218	**122,45**	**5 910 478**	**44827202**	**121,26**	**20693**
Vit09	**5 944 354**	**113,28**	**5 942 773**		**112,37**	
	4 604 271	**112,71**	**4 602 720**		**111,65**	
	5 878 316	**113,74**	**5 876 036**		**112,67**	
	5 760 716	**113,17**	**5 758 540**		**112,08**	
	5 649 888	**113,3**	**5 648 091**		**112,3**	
	5 174 639	**113,2**	**5 172 716**		**112,15**	
	5 933 105	**113,36**	**5 931 480**		**112,43**	
	5 780 361	**112,76**	**5 778 674**	**44725650**	**111,83**	**17732**
Vit10	**4 210 987**	**104,69**	**4 210 320**		**103,78**	
	5 788 798	**105,39**	**5 787 632**		**104,45**	
	5 708 738	**104,99**	**5 707 582**		**104,04**	

	5 579 978	**105,15**	**5 579 067**		**104,29**	
	5 060 519	**104,81**	**5 059 515**		**103,88**	
	5 844 927	**105,1**	**5 844 113**		**104,31**	
	5 670 906	**104,65**	**5 670 000**		**103,85**	
	5 847 594	**105,17**	**5 846 733**	**43712447**	**104,4**	**17051**
Vit11	**4 943 108**	**113,86**	**4 941 964**		**112,84**	
	5 539 282	**114,89**	**5 537 669**		**113,84**	
	5 442 770	**114,32**	**5 441 244**		**113,26**	
	5 290 996	**114,78**	**5 289 774**		**113,81**	
	4 779 843	**114,64**	**4 778 473**		**113,61**	
	5 509 000	**114,68**	**5 508 007**		**113,78**	
	5 360 435	**114,14**	**5 359 408**		**113,23**	
	5 582 586	**114,42**	**5 581 589**	**42448020**	**113,53**	**20332**
Vit12	**5 261 149**	**114,2**	**5 259 825**		**113,19**	
	5 552 871	**115,12**	**5 551 124**		**114,08**	
	5 427 127	**114,56**	**5 425 467**		**113,51**	
	5 293 583	**114,94**	**5 292 095**		**113,99**	
	4 719 843	**115**	**4 718 352**		**113,98**	

	5 516 452	114,92	5 515 227		114,03	
	5 322 856	114,44	5 321 522		113,55	
	5 549 584	114,87	5 548 321	42643465	113,99	21463
Vit13	6 300 206	115,67	6 296 355		114,41	
	5 458 667	116,62	5 454 576		115,33	
	5 325 817	116,17	5 322 014		114,88	
	5 101 550	116,6	5 098 335		115,4	
	4 592 996	116,55	4 589 745		115,29	
	5 318 040	116,87	5 315 382		115,74	
	5 173 983	116,27	5 171 213		115,13	
	5 425 347	116,55	5 422 644	42696606	115,43	15492
Vit14	5 615 355	111,93	5 613 886		110,98	
	4 561 441	111,18	4 560 107		110,07	
	5 635 959	112,11	5 633 799		110,97	
	5 554 768	111,56	5 552 633		110,41	
	5 320 591	112,09	5 318 962		111,04	
	4 831 315	111,92	4 829 589		110,81	
	5 591 604	112,08	5 590 234		111,1	

	5 364 944	**111,71**	**5 363 470**	**42475977**	**110,74**	**29097**
Vit15	**5 512 458**	**113,53**	**5 509 333**		**112,45**	
	5 871 106	**112,62**	**5 866 890**		**111,37**	
	5 591 491	**113,56**	**5 586 843**		**112,3**	
	5 458 024	**112,92**	**5 453 442**		**111,66**	
	5 234 700	**113,57**	**5 230 941**		**112,4**	
	4 725 439	**113,57**	**4 721 653**		**112,34**	
	5 452 484	**113,82**	**5 449 310**		**112,71**	
	5 272 766	**113,32**	**5 269 467**	**43118468**	**112,21**	**23363**
Vit17	**4 758 119**	**117,09**	**4 757 137**		**116,04**	
	5 408 090	**118,03**	**5 406 665**		**116,95**	
	5 324 257	**117,58**	**5 322 941**		**116,49**	
	5 063 573	**118,1**	**5 062 550**		**117,12**	
	4 672 312	**118,13**	**4 671 179**		**117,08**	
	5 321 186	**118,13**	**5 320 237**		**117,21**	
	5 117 456	**117,79**	**5 116 510**		**116,86**	
	5 316 416	**117,96**	**5 315 497**	**40981409**	**117,05**	**21055**
Vit18	**5 600 825**	**115,2**	**5 598 317**		**114,01**	

	5 671 944	**115,92**	**5 668 794**		**114,7**	
	5 538 121	**115,42**	**5 535 085**		**114,2**	
	5 275 918	**116,17**	**5 273 433**		**115,05**	
	4 708 294	**116,19**	**4 705 914**		**115**	
	5 460 935	**116,38**	**5 459 023**		**115,32**	
	5 238 456	**116**	**5 236 407**		**114,94**	
	5 506 233	**116,16**	**5 504 294**	**43000726**	**115,13**	**16399**
Vit19	**4 744 043**	**110,58**	**4 741 822**		**109,48**	
	5 788 850	**111,35**	**5 785 486**		**110,2**	
	5 685 091	**110,8**	**5 681 927**		**109,65**	
	5 439 416	**111,27**	**5 436 876**		**110,21**	
	4 967 312	**111,01**	**4 964 784**		**109,88**	
	5 681 007	**111,43**	**5 678 863**		**110,45**	
	5 483 069	**110,95**	**5 480 814**		**109,97**	
	5 719 744	**111,19**	**5 717 594**	**43488166**	**110,23**	**23394**
Vit20	**7 006 237**	**122,78**	**7 003 268**		**121,51**	
	5 558 828	**123,95**	**5 556 131**		**122,66**	
	5 460 908	**123,27**	**5 458 250**		**121,96**	

	5 273 974	**123,92**	**5 271 738**		**122,71**	
	4 793 245	**123,7**	**4 790 910**		**122,44**	
	5 539 107	**123,72**	**5 537 126**		**122,59**	
	5 452 395	**122,41**	**5 450 162**		**121,27**	
	5 695 058	**122,5**	**5 692 973**	**44779752**	**121,38**	**22269**
Vit21	**5 172 881**	**115,28**	**5 171 564**		**114,32**	
	5 484 750	**114,4**	**5 483 092**		**113,29**	
	5 320 657	**115,16**	**5 318 680**		**114**	
	5 222 834	**114,66**	**5 220 914**		**113,5**	
	4 952 431	**115,34**	**4 950 996**		**114,29**	
	4 503 196	**115,13**	**4 501 670**		**114**	
	5 191 624	**115,44**	**5 190 339**		**114,46**	
	4 964 245	**115,1**	**4 962 901**	**40812619**	**114,14**	**25148**
Vit22	**4 798 286**	**113,65**	**4 797 232**		**112,63**	
	5 665 425	**114,56**	**5 663 868**		**113,49**	
	5 678 528	**113,92**	**5 677 025**		**112,86**	
	5 269 866	**114,56**	**5 268 667**		**113,59**	
	4 992 974	**114,28**	**4 991 696**		**113,23**	

	5 621 845	**114,49**	**5 620 851**		**113,58**	
	5 372 818	**114,17**	**5 371 797**		**113,27**	
	5 481 130	**114,5**	**5 480 143**	**42880872**	**113,62**	**29953**
Vit23	**4 828 772**	**110,54**	**4 827 396**		**109,5**	
	5 505 448	**111,31**	**5 503 542**		**110,21**	
	5 414 720	**110,86**	**5 412 899**		**109,76**	
	5 171 754	**111,4**	**5 170 358**		**110,41**	
	4 666 258	**111,15**	**4 664 766**		**110,06**	
	5 399 570	**111,44**	**5 398 378**		**110,53**	
	5 202 544	**111**	**5 201 301**		**110,09**	
	5 411 798	**111,34**	**5 410 630**	**41600864**	**110,45**	**25424**
BA08	**5 959 556**	**115,47**	**5 956 001**		**114,22**	
	5 598 280	**116,42**	**5 594 197**		**115,15**	
	5 505 783	**115,8**	**5 501 850**		**114,52**	
	5 259 713	**116,17**	**5 256 587**		**114,98**	
	4 817 236	**116,15**	**4 813 791**		**114,87**	
	5 532 992	**116,38**	**5 530 156**		**115,25**	
	5 397 321	**115,62**	**5 394 213**		**114,49**	

	5 681 720	**115,87**	**5 678 791**	**43752601**	**114,75**	**12960**
BA09	**5 139 156**	**107,33**	**5 136 548**		**106,39**	
	5 079 874	**107,28**	**5 077 461**		**106,3**	
	5 143 892	**107,42**	**5 141 321**		**106,45**	
	4 966 060	**106,95**	**4 963 259**		**105,87**	
	4 438 774	**106,64**	**4 436 061**		**105,53**	
	4 816 872	**106,93**	**4 814 199**		**105,84**	
	4 229 529	**106,86**	**4 227 171**		**105,71**	
	4 078 369	**105,69**	**4 076 110**	**37892526**	**104,57**	**17113**
BA10	**4 853 175**	**127,53**	**4 851 522**		**126,53**	
	5 226 893	**127,72**	**5 225 210**		**126,7**	
	5 180 766	**127,73**	**5 178 942**		**126,74**	
	5 033 843	**127,29**	**5 031 924**		**126,18**	
	4 548 982	**127,14**	**4 546 840**		**126,01**	
	4 892 313	**127,31**	**4 890 357**		**126,2**	
	4 323 370	**127,31**	**4 321 573**		**126,14**	
	4 121 542	**126,03**	**4 119 824**	**38180884**	**124,89**	**16867**
BA11	**4 831 086**	**109,06**	**4 829 988**		**108,19**	

	5 275 608	**109,01**	**5 274 417**		**108,11**	
	5 230 706	**109,08**	**5 229 399**		**108,19**	
	5 012 452	**108,65**	**5 011 049**		**107,65**	
	4 426 152	**108,2**	**4 424 736**		**107,17**	
	4 819 776	**108,69**	**4 818 483**		**107,68**	
	4 231 937	**108,59**	**4 230 719**		**107,53**	
	4 090 351	**107,12**	**4 089 014**	**37918068**	**106,08**	**12055**
BA12	**6 027 277**	**133,13**	**6 025 952**		**132,05**	
	5 360 750	**133,1**	**5 359 620**		**131,98**	
	5 299 654	**132,99**	**5 298 279**		**131,89**	
	5 088 204	**132,57**	**5 086 715**		**131,35**	
	4 482 831	**132,46**	**4 481 121**		**131,24**	
	4 893 985	**132,78**	**4 892 428**		**131,56**	
	4 331 032	**132,64**	**4 329 499**		**131,37**	
	4 071 659	**131,14**	**4 070 094**	**39555392**	**129,89**	**14458**
BA13	**5 512 891**	**113,28**	**5 510 776**		**112,33**	
	5 033 371	**113,29**	**5 031 460**		**112,32**	
	4 934 585	**113,33**	**4 932 635**		**112,38**	

	4 730 447	**113**	**4 728 388**		**111,93**	
	4 169 206	**112,83**	**4 167 124**		**111,74**	
	4 540 625	**113,12**	**4 538 574**		**112,05**	
	3 976 375	**113,14**	**3 974 576**		**112,01**	
	3 785 137	**111,91**	**3 783 308**	**36682637**	**110,82**	**12734**
BA14	**5 361 416**	**110,22**	**5 357 307**		**109,06**	
	5 184 074	**110,39**	**5 180 456**		**109,2**	
	5 123 635	**110,31**	**5 119 746**		**109,12**	
	4 922 454	**109,84**	**4 918 263**		**108,55**	
	4 328 560	**109,63**	**4 324 553**		**108,31**	
	4 731 371	**109,86**	**4 727 485**		**108,57**	
	4 155 224	**109,79**	**4 151 711**		**108,44**	
	3 953 751	**108,47**	**3 950 312**	**37760485**	**107,15**	**12844**
BA15	**5 738 118**	**106,64**	**5 734 579**		**105,56**	
	5 373 877	**106,37**	**5 370 608**		**105,24**	
	5 313 824	**106,37**	**5 310 312**		**105,23**	
	5 118 823	**105,83**	**5 115 201**		**104,57**	
	4 515 034	**105,39**	**4 511 242**		**104,1**	

	4 932 642	**105,76**	**4 929 049**		**104,47**	
	4 317 320	**105,47**	**4 313 969**		**104,1**	
	4 158 148	**104,29**	**4 154 805**	**39467786**	**102,97**	**15725**
BA16	**5 925 187**	**99,4**	**5 919 706**		**98,32**	
	5 264 766	**99,52**	**5 260 390**		**98,42**	
	5 158 681	**99,69**	**5 154 117**		**98,59**	
	4 981 482	**99,15**	**4 976 615**		**97,93**	
	4 405 914	**99,02**	**4 401 217**		**97,77**	
	4 794 944	**99,12**	**4 790 288**		**97,88**	
	4 213 604	**99,02**	**4 209 316**		**97,72**	
	4 052 918	**98,12**	**4 048 763**	**38797496**	**96,87**	**16343**
BA17	**5 717 761**	**124,19**	**5 714 857**		**122,95**	
	5 306 880	**124,4**	**5 304 462**		**123,13**	
	5 229 389	**124,11**	**5 226 716**		**122,85**	
	5 090 016	**123,75**	**5 087 148**		**122,37**	
	4 596 276	**123,49**	**4 593 199**		**122,08**	
	4 946 851	**123,77**	**4 944 138**		**122,38**	
	4 373 842	**123,64**	**4 371 377**		**122,18**	

	4 178 250	**122,18**	**4 175 733**	**39439265**	**120,77**	**15323**
BA18	**5 604 703**	**112,11**	**5 601 188**		**111,01**	
	5 332 372	**112,32**	**5 329 362**		**111,19**	
	5 230 222	**112,34**	**5 226 969**		**111,23**	
	5 017 473	**111,97**	**5 014 120**		**110,74**	
	4 380 260	**111,91**	**4 376 837**		**110,65**	
	4 789 734	**112,15**	**4 786 545**		**110,91**	
	4 222 529	**112,05**	**4 219 535**		**110,74**	
	3 996 269	**110,88**	**3 993 349**	**38573562**	**109,61**	**15657**
BA19	**4 368 821**	**110,46**	**4 365 383**		**109,35**	
	5 386 018	**110,28**	**5 382 005**		**109,13**	
	5 264 019	**110,31**	**5 259 796**		**109,17**	
	5 094 231	**109,72**	**5 089 802**		**108,45**	
	4 521 504	**109,4**	**4 517 036**		**108,09**	
	4 916 629	**109,69**	**4 912 147**		**108,4**	
	4 323 750	**109,47**	**4 319 681**		**108,1**	
	4 137 853	**108,17**	**4 133 865**	**38012825**	**106,86**	**14146**
BK10	**4 907 304**	**117,95**	**4 904 993**		**116,89**	

	5 290 049	**118,11**	**5 287 652**		**117,01**	
	5 182 731	**118**	**5 180 155**		**116,92**	
	5 007 241	**117,65**	**5 004 618**		**116,46**	
	4 423 582	**117,65**	**4 420 781**		**116,45**	
	4 805 710	**117,79**	**4 803 228**		**116,6**	
	4 223 018	**117,82**	**4 220 739**		**116,58**	
	4 031 224	**116,59**	**4 028 894**	**37870859**	**115,39**	**11188**
BK11	**5 683 139**	**109,15**	**5 679 401**		**108,09**	
	5 205 633	**109,33**	**5 202 607**		**108,22**	
	5 112 420	**109,24**	**5 109 122**		**108,14**	
	4 957 218	**108,77**	**4 953 585**		**107,53**	
	4 425 414	**108,47**	**4 421 703**		**107,19**	
	4 787 565	**108,68**	**4 784 098**		**107,41**	
	4 209 723	**108,51**	**4 206 468**		**107,16**	
	4 047 710	**107,38**	**4 044 614**	**38428822**	**106,08**	**16910**
BK12	**4 221 732**	**111,79**	**4 220 050**		**110,76**	
	5 285 634	**111,59**	**5 283 506**		**110,52**	
	5 215 623	**111,71**	**5 213 258**		**110,64**	

	5 099 244	**111,17**	**5 096 376**		**109,98**	
	4 602 263	**110,73**	**4 599 189**		**109,52**	
	4 966 884	**111,08**	**4 964 136**		**109,88**	
	4 354 834	**110,79**	**4 352 144**		**109,52**	
	4 222 460	**109,32**	**4 219 631**	**37968674**	**108,08**	**13305**
BK13	**4 835 904**	**107,85**	**4 834 071**		**106,87**	
	5 065 717	**107,7**	**5 064 014**		**106,67**	
	4 967 544	**107,75**	**4 965 637**		**106,71**	
	4 797 822	**107,14**	**4 795 465**		**105,97**	
	4 197 501	**106,87**	**4 195 156**		**105,64**	
	4 593 578	**107,11**	**4 591 303**		**105,91**	
	4 022 532	**106,89**	**4 020 389**		**105,61**	
	3 849 284	**105,73**	**3 847 152**	**36329882**	**104,5**	**14192**
BK14	**5 595 295**	**105,21**	**5 592 233**		**104,19**	
	5 225 721	**105,05**	**5 222 932**		**104**	
	5 099 380	**105,2**	**5 096 294**		**104,16**	
	4 922 976	**104,67**	**4 919 577**		**103,5**	
	4 349 984	**104,45**	**4 346 509**		**103,22**	

	4 728 266	**104,72**	**4 724 795**		**103,51**	
	4 158 774	**104,45**	**4 155 495**		**103,17**	
	3 999 057	**103,29**	**3 995 768**	**38079453**	**102,04**	**15084**
BK15	**8 586 713**	**90,84**	**8 564 489**		**89,23**	
	9 168 005	**86,09**	**9 152 935**		**84,64**	
	9 707 261	**82,55**	**9 692 492**		**81,01**	
	8 593 882	**86,9**	**8 582 133**	**36055861**	**85,49**	**10579**
BK16	**5 845 060**	**103,86**	**5 837 966**		**102,58**	
	7 354 867	**101,94**	**7 349 985**		**100,84**	
	9 729 244	**98,13**	**9 723 108**		**96,94**	
	10 972 494	**100,64**	**10 966 632**	**33901665**	**99,58**	**16441**
BK17	**8 361 865**	**125,03**	**8 358 911**		**123,94**	
	9 328 716	**124,26**	**9 325 154**		**123,06**	
	14 692 417	**124,59**	**14 686 690**	**32383187**	**123,5**	**19637**
BK18	**5 347 141**	**88,88**	**5 340 211**		**87,52**	
	7 456 501	**84,49**	**7 451 553**		**83,3**	
	9 621 345	**81,13**	**9 614 775**		**79,88**	
	10 473 950	**84,7**	**10 468 560**	**32898937**	**83,58**	**11843**

BK19	9 580 917	85,36	9 570 577		83,95	
	5 604 121	89,19	5 590 190		87,49	
	9 539 895	83,91	9 528 102		82,47	
	9 647 006	80,53	9 634 891	34371939	79	9332
BK20	8 818 408	95,99	8 790 718		94,21	
	16 202 985	90,85	16 170 456		89,23	
	8 657 275	89,29	8 642 974		87,67	
	6 044 568	94,45	6 036 279	39723236	92,97	11870
BK21	10 935 532	76,9	10 929 156		75,96	
	4 497 588	78,99	4 491 803		77,84	
	8 519 518	79,21	8 512 668		78,2	
	6 833 079	76,02	6 828 649	30785717	74,97	8448
Vit24	30 830 622	84,01	30 775 301		82,53	
	9 761 235	84,24	9 752 696	40591857	82,92	8484
Vit25	33 103 205	103,53	33 061 938		102,12	
	9 910 497	105,08	9 904 527	43013702	103,77	20357
Vit26	32 674 646	106,87	32 643 333		105,39	
	9 498 729	105,42	9 493 744	42173375	104,07	12674

Vit27	**33 555 178**	**106,61**	**33 520 718**		**105,11**	
	8 381 323	**106,83**	**8 376 772**	**41936501**	**105,51**	**10290**
Vit28	**26 084 261**	**116,56**	**26 071 374**		**115,31**	
	19 779 267	**114,2**	**19 764 967**	**45863528**	**112,98**	**17615**
Vit29	**23 799 267**	**106,95**	**23 779 038**		**105,64**	
	22 984 889	**103,33**	**22 954 205**	**46784156**	**101,95**	**22546**
Vit30	**24 494 559**	**93,49**	**24 464 526**		**92,3**	
	21 296 901	**96,38**	**21 282 584**	**45791460**	**95,23**	**4894**
Vit32	**11 641 735**	**104,56**	**11 633 980**		**102,77**	
	27 657 218	**104,92**	**27 625 242**	**39298953**	**103,39**	**12028**
Vit33	**25 552 643**	**97,49**	**25 545 804**		**96,09**	
	18 004 124	**96,83**	**17 998 231**	**43556767**	**95,74**	**6011**
Vit34	**19 992 974**	**103,44**	**19 978 995**		**102,14**	
	22 701 955	**103,36**	**22 690 656**	**42694929**	**101,75**	**15949**
Vit35	**23 185 836**	**93,51**	**23 165 157**		**92,18**	
	19 218 902	**93,18**	**19 197 826**	**42404738**	**91,86**	**10322**
Vit36	**24 293 700**	**100,43**	**24 276 444**		**99,19**	
	23 749 700	**101,47**	**23 737 743**	**48043400**	**100,28**	**18708**

Vit37	**22 801 768**	**111,85**	**22 787 320**		**110,41**	
	22 987 041	**110,78**	**22 966 403**	**45788809**	**109,36**	**18188**
Vit38	**22 079 648**	**95,87**	**22 061 851**		**94,46**	
	24 230 624	**98,87**	**24 215 406**	**46310272**	**97,56**	**7718**
BK22	**20 578 417**	**93,67**	**20 562 777**		**92,44**	
	22 502 500	**94,43**	**22 485 299**	**43080917**	**93,15**	**13364**
BK23	**16 097 110**	**96,2**	**16 088 141**		**94,89**	
	28 993 071	**94,8**	**28 972 731**	**45090181**	**93,55**	**9334**
BK24	**15 270 612**	**100,9**	**15 258 751**		**99,47**	
	28 058 639	**101,71**	**28 033 992**	**43329251**	**100,18**	**18941**
BK25	**26 380 699**	**85,88**	**26 352 246**		**84,48**	
	11 829 951	**86,28**	**11 819 051**	**38210650**	**84,92**	**22606**
BK26	**18 436 421**	**106,02**	**18 423 602**		**104,74**	
	19 770 722	**105,14**	**19 755 871**	**38207143**	**103,81**	**13925**
BK27	**13 501 261**	**111,75**	**13 483 241**		**110,1**	
	25 874 784	**113,09**	**25 841 237**	**39376045**	**111,48**	**16374**
BK28	**23 550 481**	**96,84**	**23 537 245**		**95,62**	
	21 475 692	**94,96**	**21 461 549**	**45026173**	**93,73**	**19149**

BK29	**21 750 497**	**101,32**	**21 741 507**		**100,21**	
	22 728 584	**103,29**	**22 721 715**	**44479081**	**102,19**	**24750**
BK30	**27 039 671**	**101,52**	**27 030 706**		**100,34**	
	19 053 981	**99,46**	**19 045 790**	**46093652**	**98,26**	**11560**
BK31	**21 082 969**	**107,95**	**21 071 680**		**106,67**	
	28 319 941	**107,36**	**28 307 143**	**49402910**	**106,02**	**13615**
BK32	**26 788 870**	**81,86**	**26 780 956**		**81,12**	
	16 732 862	**81,83**	**16 730 279**	**43521732**	**81,05**	**10263**
BK33	**22 702 181**	**106,37**	**22 683 317**		**105,14**	
	25 718 390	**105,84**	**25 701 127**	**48420571**	**104,52**	**17246**
BA20	**29 762 765**	**111,21**	**29 747 030**		**110,02**	
	23 213 130	**107,97**	**23 197 953**	**52975895**	**106,7**	**26595**
BA21	**24 432 885**	**107,84**	**24 416 822**		**106,56**	
	20 752 593	**105,39**	**20 736 232**	**45185478**	**104**	**9665**
BA22	**22 412 376**	**113,37**	**22 382 943**		**111,73**	
	20 747 433	**111,01**	**20 716 464**	**43159809**	**109,26**	**15658**
BA23	**23 947 899**	**125,38**	**23 926 711**		**123,71**	
	18 740 041	**128,7**	**18 724 241**	**42687940**	**127,12**	**24569**

BA24	**23 839 063**	**118,91**	**23 824 110**		**117,42**	
	19 198 179	**117,87**	**19 185 491**	**43037242**	**116,3**	**15495**
BA25	**25 152 483**	**111,47**	**25 131 445**		**109,89**	
	17 856 767	**111,09**	**17 844 170**	**43009250**	**109,55**	**17671**
BA26	**18 162 957**	**112,57**	**18 149 072**		**111,18**	
	22 556 374	**111,96**	**22 539 860**	**40719331**	**110,59**	**11112**
BA27	**20 991 875**	**109,53**	**20 957 895**		**107,81**	
	21 231 061	**109,99**	**21 201 668**	**42222936**	**108,36**	**12098**
BA28	**23 394 567**	**114,17**	**23 382 017**		**112,96**	
	52 769 746	**113,46**	**52 736 044**	**76164313**	**112,22**	**24861**
BA29	**21 880 435**	**123,53**	**21 855 995**		**121,72**	
	16 563 651	**120,64**	**16 536 088**	**38444086**	**118,78**	**16701**
BA30	**21 895 405**	**101,18**	**21 880 281**		**99,71**	
	15 895 855	**103,1**	**15 889 465**	**37791260**	**101,7**	**19549**
BA31	**17 002 311**	**97,94**	**16 976 103**		**96,23**	
	21 767 731	**100,71**	**21 745 097**	**38770042**	**99,07**	**12673**

Table 2: Viral genomes obtained following BLAST analysis and reconstruction of contigs produced from de novo assembly (word size 35, bubble size 50)

Sample	Virus	Consensus Length	Contigs	Coverage	Reference	reference length	Percentage genome reconstructed
BK18	GRSPaV	5655	14	0,65	MG938344	8729	65
BK23	GRSPaV	5635	22	0,76	MG925336	8724	65
BA22	GRSPaV	8396	51	2,2	MG938347	8725	96
BA24	GRSPaV	8534	14	1,55	MG938303	8706	98
BA26	GRSPaV	7711	50	1,71	MG938344	8729	88
BA29	GRSPaV	7602	43	1,49	MG925336	8729	87
BK29	GLRaV-4	13281	15	1,16	KY821095	13857	96
BK29	GRVFV	2977	10	0,52	MZ451107	6617	45
BA8	GFkV	5032	8	0,7	ON567253	7485	67
BA22	GSyV	3183	11	0,58	MZ440716	6466	49

Table 3: Reference mapping results of samples containing viruses at length fractions of 0.9 and a similarity fraction of 0.8.

Sample	Virus	Consensus length	Total read count	Coverage	Reference	Reference length	Percentage genome reconstructed
BK18	GRSPaV	8614	695	6,39	MG938344	8729	99
BK23	GRSPaV	8262	774	6,95	MG925336	8724	95
BK29	GLRaV-4	13859	21521	154,91	KY821095	13857	100
BK29	GRVFV	6690	1445	19,17	MZ451107	6738	99
BA8	GFkV	6982	19043	216,26	ON567253	7485	93
BA22	GSyV	5964	348	5,24	MZ440716	6466	92
BA22	GRSPaV	8450	470	5,97	MG938347	8725	97
BA24	GRSPaV	7538	683	8,98	MG925334	8726	86
BA26	GRSPaV	8262	795	9,99	MG938344	8729	95
BA29	GRSPaV	7956	305	4,3	MG938344	8729	91

Table 4: Reference mapping results of samples containing Hop stunt viroid (HSVd) at length fractions of 0.9 and similarity fraction of 0.8.

Sample	Consensus length	Total read count	Average coverage	HSVd Reference sequence	Reference length	Percentage of genome mapped
BA1	288	69	26,46	NC001351	302	95
BA2	294	16	7,05	NC001351	302	97
BA3	295	48	16,38	NC001351	302	98
BA6	297	213	77,68	NC001351	302	98
BA8	302	51	17,1	GVICG	296	102
BA9	298	109	37,95	X06873	298	100
BA12	299	28	10,08	Y14050	297	101
BA17	299	53	19,64	Y14050	297	101
	297	63	24,81	GVICG	296	100
BA18	297	162	53,47	Y14050	297	100
	295	155	51,11	GVICG	296	100
BA22	299	176	63,53	X06873	298	100
BA23	303	141	41,19	X06873	298	102
	298	354	129,27	Y14050	297	100
	296	369	123,04	GVICG	296	100
BA24	299	663	244,81	X06873	298	100
	296	97	26,64	Y14050	297	100
	296	51	10,61	GVICG	296	100
BA25	297	171	55,11	Y14050	297	100
	297	231	76,41	GVICG	296	100
BA26	304	52	15,15	GVICG	296	103
	297	50	15,87	Y14050	297	100
BA27	303	26	7,43	X06873	298	102
	301	41	12,77	Y14050	297	101
	301	47	11,82	GVICG	296	102
BA28	297	38	12,88	Y14050	297	100
	295	37	10,64	GVICG	296	100
BA29	315	33	11,69	Y14050	297	106
	297	42	13,48	GVICG	296	100
Vit1	297	248	97,98	Y14050	297	100
	296	182	66,85	GVICG	296	100
Vit8	300	56	18,19	X06873	298	101
	296	155	55,72	Y14050	297	100
	295	119	40,29	GVICG	296	100
Vit9	296	157	52,05	Y14050	297	100
	295	184	60,36	GVICG	296	100
Vit10	296	113	37,15	Y14050	297	100
	290	120	38,32	GVICG	296	98
Vit13	301	90	31,27	X06873	298	101
	297	52	17,84	GVICG	296	100
	296	64	22,33	Y14050	297	100
Vit14	296	175	58,94	Y14050	297	100
	296	197	62,96	GVICG	296	100
Vit17	295	57	20,36	Y14050	297	99
	291	47	15,23	GVICG	296	98
Vit18	304	101	32,37	GVICG	296	103
	296	82	28,45	Y14050	297	100
Vit21	303	21	7,16	Y14050	297	102
	301	44	15,98	X06873	298	101
Vit22	303	66	22,66	X06873	298	102
	301	140	40,94	GVICG	296	102
	296	127	44,23	Y14050	297	100
Vit25	297	259	85,74	Y14050	297	100
	296	309	99,31	GVICG	296	100
Vit26	297	26	8,73	Y14050	297	100
	292	19	6,44	X06873	298	98
Vit27	297	38	12,59	Y14050	297	100
	293	49	14,79	GVICG	296	99
Vit29	298	305	105,6	X06873	298	100
Vit30	298	198	57,23	GVICG	296	101
	297	179	51,63	Y14050	297	100
Vit32	297	147	45,73	Y14050	297	100
	295	158	49	GVICG	296	100
Vit33	297	87	23,96	X06873	298	100
	296	82	24,41	Y14050	297	100
	296	93	25,04	GVICG	296	100
Vit35	298	280	88,2	X06873	298	100
BK4	298	38	13,7	GVICG	296	101
	298	159	59,44	X06873	298	100
	297	48	16,38	Y14050	297	100
BK5	298	385	145,28	X06873	298	100
BK7	298	404	153,99	X06873	298	100
BK10	299	77	28,48	X06873	298	100
BK11	297	31	9,87	X06873	298	100
	296	63	21,91	Y14050	297	100
	296	53	15,72	GVICG	296	100
BK12	300	28	8,78	X06873	298	101
	296	73	27,05	Y14050	297	100
BK13	302	113	32,4	GVICG	296	102
	297	89	27,62	Y14050	297	100
	294	40	13,18	X06873	298	99
BK14	299	33	5,71	GVICG	296	101
	298	486	162,14	X06873	298	100
	294	80	24,73	Y14050	297	99
BK15	299	117	35,97	X06873	298	100
BK16	296	94	28,48	Y14050	297	100
	294	32	9,5	X06873	298	99
	281	83	21,35	GVICG	296	95
BK17	299	263	103,84	X06873	298	100
BK18	297	146	36,2	GVICG	296	100
	297	109	29,55	Y14050	297	100
BK19	297	30	4,45	Y14050	297	100
	297	57	14,32	X06873	298	100
BK21	298	70	12,93	X06873	298	100
BK22	299	339	103,86	X06873	298	100
BK23	298	138	41,57	X06873	298	100
BK24	299	226	70,13	X06873	298	100
BK26	298	374	123,91	X06873	298	100
BK27	297	307	105,28	Y14050	297	100
	296	290	91,98	GVICG	296	100
BK28	298	235	80,33	X06873	298	100
BK29	299	549	184,74	X06873	298	100
	296	147	42,03	Y14050	297	100
BK30	298	128	37,62	Y14050	297	100
	296	166	47,96	GVICG	296	100
	290	70	18,38	X06873	298	97
BK31	299	232	74,77	X06873	298	100
BK32	304	349	61,51	Y14050	297	102
	299	955	248,95	X06873	298	100
	271	204	31,76	GVICG	296	92
BK33	299	868	297,07	X06873	298	100
	280	81	16,78	GVICG	296	95

Table 5: Reference mapping results of samples containing Grapevine yellow speckle viroid-1 (GYSVd-1) at length fractions of 0.9 and similarity fraction of 0.8.

Sample	Consensus length	Total read count	Average coverage	GYSVd-1 Reference sequence	Reference length	Percentage of genome
BA1	372	13	3,2	NC_001920.1	366	102
BA9	366	33	8,62	NC_001920	366	100
BA12	388	8	3,16	DQ371469	365	106
BA13	369	25	6,42	EU682453	367	101
BA14	380	4	1,13	DQ371469	365	104
BA15	372	28	5,11	DQ371469	365	102
	366	41	8,75	NC_001920	366	100
	362	29	4,81	AF059712	366	99
BA17	358	14	3,12	NC_001920	366	98
	357	15	1,98	DQ371469	365	98
BA18	366	33	10,96	NC_001920	366	100
	354	33	9,02	DQ371469	365	97
	343	30	8,62	AF059712	366	94
BA20	366	74	18,46	NC_001920	366	100
	365	70	18,13	DQ371469	365	100
BA23	366	66	17,83	EU682453	367	100
	365	41	8,04	JF746190	367	99
BA24	368	61	12,95	EU682453	367	100
	368	18	3,58	DQ371476	367	100
	353	62	13,89	JF746190	367	96
BA25	366	28	9,03	EU682453	367	100
BA27	366	33	7,96	NC_001920	366	100
	350	23	6,62	AF059712	366	96
	341	19	5,67	DQ371469	365	93
BA28	366	58	16,69	NC_001920	366	100
	365	59	18,39	DQ371469	365	100
	357	57	18,64	AF059712	366	98
	349	14	2,65	JF746190	367	95
BA29	342	10	2,81	HQ222363	367	93
BA30	366	111	26,1	NC_001920	366	100
	365	67	13,46	DQ371469	365	100
	343	74	16,63	AF059712	366	94
Vit8	366	53	15,8	NC_001920	366	100
	362	37	10,2	DQ371469	365	99
	341	37	9,17	AF059712	366	93
Vit9	366	73	19,74	EU682453	367	100
Vit10	367	95	21,87	EU682453	367	100
	362	102	31,25	JF746190	367	99
Vit11	366	4	1,9	JF746190	367	100
Vit13	357	11	2,56	EU682453	367	97
Vit14	362	102	26,6	AB028466	368	98
Vit18	366	53	17,69	NC_001920	366	100
	365	43	13,66	DQ371469	365	100
	357	32	8,25	AF059712	366	98
Vit19	366	98	26,67	NC_001920	366	100
	365	57	17,06	DQ371469	365	100
	358	70	20,6	AF059712	366	98
Vit21	375	5	1,86	NC_001920	366	102
Vit22	362	48	16	JF746190	367	99
Vit26	366	34	8,54	NC_001920	366	100
	365	27	6,85	DQ371469	365	100
Vit29	369	193	45,05	EU682453	367	101
	367	162	45,58	JF746190	367	100
Vit32	361	17	4,27	EU682453	367	98
BK1	369	18	4,54	NC_001920	366	101
BK2	366	25	7,84	NC_001920	366	100
BK11	366	43	9,41	NC_001920	366	100
	365	35	8,52	DQ371469	365	100
	364	28	5,46	AF059712	366	99
BK12	366	50	13,66	NC_001920	366	100
	365	52	13,04	DQ371469	365	100
	346	40	8,83	AF059712	366	95
BK14	365	69	16,42	DQ371469	365	100
	365	91	24,93	NC_001920	366	100
	362	87	24,19	AF059712	366	99
BK15	366	34	4,6	NC_001920	366	100
	351	50	6,12	DQ371469	365	96
BK16	366	42	8,05	DQ371469	365	100
	366	60	13,46	NC_001920	366	100
	345	41	8,69	AF059712	366	94
BK19	337	35	3,83	NC_001920	366	92
BK21	366	45	6	DQ371469	365	100
	366	55	9,4	NC_001920	366	100
	332	43	5,53	AF059712	366	91
BK25	366	236	51,48	NC_001920	366	100
	365	125	24,16	DQ371469	365	100
	360	193	38,33	AF059712	366	98
BK26	366	103	23,75	NC_001920	366	100
	365	63	13,79	DQ371469	365	100
	358	70	15,16	AF059712	366	98
BK27	366	74	17,87	NC_001920	366	100
	350	40	8,3	AF059712	366	96
BK29	366	181	43,33	NC_001920	366	100
	365	142	32,57	AF059712	366	100
	365	107	25,6	DQ371469	365	100
	357	80	18,73	DQ371476	367	97
BK32	366	203	28,25	AF059712	366	100
	366	249	41,3	NC_001920	366	100
	365	154	21,14	DQ371469	365	100
BK33	366	291	68,93	NC_001920	366	100
	365	235	51,13	AF059712	366	100
	365	218	48,33	DQ371469	365	100

Table 6: Reference mapping results of samples containing Grapevine yellow speckle viroid-2 (GYSVd-2) at length fractions of 0.9 and similarity fraction of 0.8.

Sample	Consensus length	Total read count	Average coverage	GYSVd-2 Reference sequence	Reference length	Percentage of genome
BA3	362	10	3,12	JN008867	363	100
BA9	359	19	5,13	MG780426	375	96
BA15	366	27	5,49	MG780425	375	98
BA23	384	45	9,25	MG780425	375	102
	379	35	7,27	MG780426	375	101
	371	51	11,53	NC_003612	363	102
BA24	379	40	8,95	MG780426	375	101
	369	37	6,59	MG780425	375	98
	363	62	15,7	NC_003612	363	100
	346	30	4,9	FJ597939	363	95
	343	56	12,81	GYSSVXXA	363	94
BA30	369	28	7,02	FJ597939	363	102
	345	24	4,49	MG780425	375	92
Vit8	370	12	2,46	MG780425	375	99
	361	7	1,72	FJ597939	363	99
Vit18	373	43	9,95	MG780425	375	99
	371	32	8,38	MG780426	375	99
	364	47	13,31	NC_003612	363	100
	346	38	8,56	FJ597940	363	95
Vit32	363	7	1,7	MG780425	375	97
Vit33	375	57	11,51	MG780426	375	100
	369	46	7,07	FJ597939	363	102
	368	58	10,67	FJ597940	363	101
	368	56	11,13	NC_003612	363	101
BK5	265	9	2,37	NC_003612	363	73
BK11	368	17	4,18	FJ597939	363	101
	354	15	3,9	MG780425	375	94
BK15	303	11	1,77	NC_003612	363	83
BK29	366	80	17,33	FJ597940	363	101
	385	73	15,2	MG780425	375	103
	369	75	14,44	NC_003612	363	102
	368	58	12,62	FJ597939	363	101

Table 7: Reference mapping results of samples containing Australian grapevine viroid (AGVd) at length fractions of 0.9 and similarity fraction of 0.8.

Sample	Consensus length	Total read count	Average coverage	Reference sequence	Reference length	Percentaqe of genome
BA16	225	2	0,55	FJ746748	366	61
BK15	237	3	0,76	X17101	369	64
BK29	367	34	11,03	DQ362908	369	99

Table 8: Enlarged version of text Table 1.

				Viruses/viroids detected									
				Viruses					Viroids				
Accession	Type of grape	Thermotherapy	Meristem tip culture	Grapevine fleck virus	Grapevine rupestris stem pitting associated virus	Grapevine syrah virus 1	Grapevine leaf roll associated virus 4	Grapevine Ruspestris vien feathering virus	Grapevine yellow speckle viroid 1	Grapevine yellow speckle viroid 2	Hop stunt viroid	Australian grapevine viroid	Grapevine hammerhead viroid-like RNA
BK01	Raisin	√	√						+	-	-	-	-
BK02	Raisin	√	√						+	-	-	-	-
BK15	Raisin	x	x						+	+	+	+	-
BK16	Raisin	x	x						+	-	+	-	-
BK24	Raisin	x	x						-	-	+	-	-
BK28	Raisin	x	x						-	-	+	-	-
BK29	Raisin	x	x				+	+	+	+	+	+	-
VIT25	Rootstock	√	√						-	-	+	-	-

VIT26	Rootstock	√	√						-	-	+	-	-
VIT27	Rootstock	√	√						-	-	+	-	-
VIT28	Rootstock	√	√						-	-	-	-	-
VIT29	Rootstock	√	√						+	-	+	-	-
VIT30	Rootstock	√	√						-	-	+	-	-
VIT32	Rootstock	√	√						+	+	+	-	-
VIT33	Rootstock	√	√						+	+	+	-	-
VIT34	Rootstock	√	√						-	-	-	-	-
VIT35	Rootstock	√	√						-	-	+	-	-
VIT36	Rootstock	√	√						-	-	-	-	-
VIT37	Rootstock	√	√						-	-	-	-	-
VIT38	Rootstock	√	√						-	-	-	-	-
BA14	Table	√	√						+	-	-	-	-
BA15	Table	√	√						+	-	-	-	-
BA16	Table	√	√						+	+	+	+	-
BA17	Table	√	√						+	-	+	-	-
BA30	Table	√	√						+	+	+	-	-
BK03	Table	√	√						-	-	-	-	-
BK04	Table	√	√						-	-	+	-	-
BK10	Table	√	√						-	-	+	-	-
BK11	Table	√	√						+	+	+	-	-
BK12	Table	√	√						+	-	+	-	-
BK13	Table	√	√						-	-	+	-	-
Vit06	Table	√	√						-	+	+	-	-
Vit07	Table	√	√						+	-	-	-	-
Vit17	Table	√	√						+	-	+	-	-
Vit18	Table	√	√						+	+	+	-	-
Vit19	Table	√	√						+	-	-	-	-
Vit20	Table	√	√						-	-	-	-	-
Vit21	Table	√	√						+	+	+	-	-
Vit22	Table	√	√						+	-	-	-	-
Vit23	Table	√	√						-	-	-	-	-
VIT24	Table	√	√						-	-	-	-	-
BA07	Table	x	2X						-	-	-	-	-
BA18	Table	x	2X						+	-	+	-	-
BA19	Table	x	2X						-	-	-	-	-
BK25	Table	x	x						+	-	+	-	-
BK06	Table and Raisin	√	√						-	-	-	-	-
BK07	Table and Raisin	√	√						-	-	+	-	-
BK14	Table and Raisin	√	√						+	-	+	-	-
BK20	Table and Raisin	√	√						-	-	-	-	-
BK31	Table and Raisin	√	√						-	-	+	-	-
BK33	Table and Raisin	√	√						+	-	+	-	-

BK05	Table and Raisin	x	x						-	+	+	-	-
BK17	Table and Raisin	x	x						-	-	+	-	-
BK18	Table and Raisin	x	x		+				-	-	+	-	-
BK19	Table and Raisin	x	x						+	-	+	-	-
BK21	Table and Raisin	x	x						+	-	+	-	-
BK22	Table and Raisin	x	x						-	-	+	-	-
BK23	Table and Raisin	x	x		+				-	-	+	-	-
BK26	Table and Raisin	x	x						+	-	-	-	-
BK27	Table and Raisin	x	x						+	-	+	-	-
BK30	Table and Raisin	x	x						-	-	+	-	-
BK32	Table and Raisin	x	x						+	-	+	-	-
BA01	Wine	√	√						+	-	+	-	-
BA03	Wine	√	√						-	+	+	-	-
BA05	Wine	√	√						-	-	-	-	-
BA06	Wine	√	√						-	-	+	-	-
BA08	Wine	√	√	+					+	+	+	-	-
BA09	Wine	√	√						+	+	+	-	+
BA10	Wine	√	√						-	-	-	-	-
BA11	Wine	√	√						-	-	-	-	-
BA12	Wine	√	√						+	-	+	-	-
BA13	Wine	√	√						+	-	-	-	-
BA20	Wine	√	√						+	-	-	-	-
BA21	Wine	√	√						-	-	-	-	-
BA22	Wine	√	√		+	+			-	-	+	-	-
BA23	Wine	√	√						+	+	+	-	-
BA24	Wine	√	√		+				+	+	+	-	+
BA25	Wine	√	√						+	-	+	-	-
BA26	Wine	√	√		+				+	-	+	-	-
BA27	Wine	√	√						+	-	+	-	-
BA28	Wine	√	√						+	-	+	-	-
BA29	Wine	√	√		+				+	-	+	-	-
BA31	Wine	√	√						-	-	-	-	-
Vit01	Wine	√	√						-	-	+	-	-
Vit02	Wine	√	√						+	-	-	-	-
Vit04	Wine	√	√						+	+	+	-	-
Vit05	Wine	√	√						-	+	+	-	-
Vit08	Wine	√	√						+	+	+	-	-
Vit09	Wine	√	√						+	-	+	-	-
Vit10	Wine	√	√						+	-	+	-	-
Vit11	Wine	√	√						+	-	-	-	-
Vit12	Wine	√	√						-	-	-	-	-
Vit13	Wine	√	√						+	-	+	-	-
Vit14	Wine	√	√						+	-	+	-	-