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FINAL REPORT 2020

Winetech Number : PPRI 16/27

1. PROGRAMME & PROJECT LEADER INFORMATION

	Research Organisation leader	Project leader
Title, initials, surname	Prof. J.T. Burger	Prof G Pietersen
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2. PROJECT INFORMATION

Project title	Is poor detection of grapevine leafroll associated virus 3 in South African rootstocks due to host resistance?
Short title	Rootstock resistance to GLRaV-3

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	2017-01	**End date** (mm/yyyy)	2019-12

Key words	grapes, rootstocks, resistance, grapevine leafroll

	CFPA	DFTS	HORTGRO	SATI	WINETECH	ARC	OTHER
TOTALS All years	R 0	R 0	R 0	R 0	R 851700	R 0	R 0

Total cost of entire project	R 851700

3. EXECUTIVE SUMMARY

Objectives and Rationale

In this project we wished to determine whether difficulties in detection of grapevine leafroll associated virus 3 (GLRaV-3) are due to tolerance or immunity of rootstock clones to this virus. By “tolerance” we mean that the virus is able to replicate in the vine but it does not affect the vines phenotype in any way (eg. no symptoms). By “immunity” we mean the virus is incapable of replicating in the vine.

Rationale: To successfully control GLRaV-3 in rootstocks within the Wine Grape Certification Scheme, it is extremely important to ensure that rootstock material supplied to industry is free of this virus. It is therefore imperative 1) to determine whether the observed poor detection of the virus in rootstocks is due to low levels of virus (tolerant host defence response), 2) if so, which rootstock cultivars have this property and 3) whether, in some instances, the virus is absent due to immunity by the host. Proving immunity to GLRaV-3 would allow these rootstocks to be used in industry without any need to test that they are GLRaV-3 free.

Methods

To identify rootstocks in which GLRaV-3 is poorly detected, lignified rootstock sprouts of various cultivars from grafted leafroll symptomatic red cultivar scions are collected, and the respective scion and rootstocks tested for GLRaV-3. Rootstock samples testing negative in spite of the presence of virus in the scion were grafted onto Cabernet franc in order to determine if the virus is present at sub-detectable levels for the techniques used, or whether the rootstocks are immune to GLRaV-3 infection. Tests were also conducted to determine whether GLRaV-3 is able to be transmitted through such rootstocks.

Key Results

It has been confirmed that GLRaV-3 is poorly detected in most individuals of R99, Ruggeri, 101-14, R110, Paulsen, and also US 8/7 relative to the corresponding scion of such plants. We have also shown that transmission of the virus to the commonly utilized rootstocks Richter99, Richter110, 1010-14 and Ramsay can be achieved, but that not all plants became infected. We cannot differentiate whether this is due to inefficient graft transmission of GLRaV-3 (which is known) or differences in susceptibility of specific rootstock individuals. We have also demonstrated that GLRAV-3 can move through a rootstock intergraft both in an acropetal (upward) and basipetal (downward) manner, we are unable to determine whether this is passive via the vascular tissue or whether the virus actively replicated in the interstock.

Key Conclusion of Discussion

We hypothesize that the continued inconsistent results obtained in the industry as well as in experiments performed during this project and the previous one, may be due to genetic differences with regards GLRAV-3 susceptibility in rootstock material and that any given rootstock cultivar is potentially not genetically homogeneous. Our answer to the question “pose at the start of this project “Is poor detection of grapevine leafroll associated Virus 3 in South African rootstocks due to host resistance?” can be answered in the affirmative, but that it differs in individuals of any rootstock.

Take Home message for Industry

Under high infection pressure GLRaV-3 is only detected in some individuals of rootstock cultivars tested thus far. This represents a resistance mechanism to GLRaV-3 which does not appear to be uniform within individuals of the rootstock cultivars. Future studies should identify those individuals with immunity to utilise those as future clonal sources of propagation for each rootstock cultivar. The gene(s) involved in resistance should also be identified and used in breeding or biotechnological programs to introduce these into important wine cultivars for resistance to GLRaV-3 .

4. PROBLEM IDENTIFICATION AND MOTIVATION

Problem Identification

In Vitis rootstocks, grapevine leafroll associated virus 3 (GLRaV-3) does not produce symptoms and is generally poorly detected by ELISA and PCR. In a previous study (PPRI 13/31) we had confirmed that poor detection of GLRaV-3 by PCR occurs in R99 rootstocks, as opposed to the corresponding scion of any specific grafted vine where it is efficiently detected. We had confirmed that this difference in detection was not due to the sampling of rapidly growing, young rootstock material as opposed to mature scion material, as, with our sampling strategy, we had only collected and tested lignified rootstock cane tissue. We had also shown that poor detection was not due to an inability to detect diverging GLRaV-3 variants selected only by the rootstock. Generally, the rootstock and corresponding scion of a vine contained the same variants of GLRaV-3, with a few exceptions. Due to the difficulties in obtaining lignified rootstock material from known infected grapevine plants we did most of the studies in the previous study on R99. It is important we expand this to the other important rootstock cultivars and their clones used in South Africa.

Motivation

To successfully control GLRaV-3 in rootstocks within the Wine Grape Certification Scheme, it is extremely important to ensure that rootstock material supplied to industry is free of this virus. It is therefore imperative 1) to determine whether the observed poor detection of the virus in rootstocks is due to low levels of virus (tolerant host defence response), 2) if so, which rootstock cultivars have this property and 3) whether, in some instances, the virus is absent due to immunity by the host. Proving immunity to GLRaV-3 would allow these rootstocks to be used in industry without any need to test that they are GLRaV-3 free.

5. ACCUMULATED PROGRESS TABLE

Objectives	Milestones (Significant event or stage in a project)	Date Achieved
Determine if rootstocks clones (other than Richter 99 which has been tested previously) obtained from known GLRaV-3 infected commercial vines are infected.	1.1 Identify severely leafroll infected old motherblock vineyards in industry with specific rootstock clones	2018-02-05
	1.2 Within these vineyards, identify individual plants with rootstock suckers. Mark vines and arrange with growers to avoid removal of sucker shoots.	2018-02-05
	1.3 Collect both scion and rootstock samples from single vines annually	2018-05-21
	1.4 Determine GLRaV-3 presence by PCR in rootstock and scion, analyse data.	2017-07-03

	1.5 Graft C. franc onto harvested rootstock material	2018-07-09
	1.6 Monitor C. franc vines for leafroll and test for GLRaV-3 by PCR	2019-07-01
2. Determine tolerance/immunity of commercially important rootstock clones	2.1 Have Cabernet franc grafted onto each of the commonly utilised rootstock clones.	2017-06-05
	2.2 Allow to callus and root	2017-07-05
	2.3 Plant out in rock-wool or sterilized soil in pots in insect-free greenhouse	2017-08-04
	2.4 Do chip-budding of pure GLRaV-3 variant-infected sources onto the rootstock of each grafted vine	2018-07-05
	2.5 Monitor cabernet franc scion for leafroll symptoms and confirm infection by PCR tests	2019-06-05
3. Determine if GLRaV-3 transmission of virus is possible through an inter-graft of rootstock cultivars.	3.1 Cut scion cane material of pure sources of GLRaV-3 (variants 2 and 6) and make rooted cuttings of these.	2017-10-02
	3.2 Have Cabernet franc grafted onto each of the commonly utilised rootstock clones.	2017-05-05
	3.3 Allow to callus and root	2017-07-10
	3.4 Graft the above scion rootstock onto a rooted GLRaV-3 infected source (scion material on own roots)	2018-07-16
	3.5 Monitor C. franc for symptoms and test plant components by ELISA/PCR	2019-07-29

6. WORKPLAN (MATERIALS AND METHODS)

Determine if rootstocks obtained from known GLRaV-3 infected commercial vines (scions) are also infected with GLRaV-3.

In the 2016/2017 and 2017/18 growth season, using Vititec database records we identified former motherblocks of red-berried cultivars, written off due to leafroll infection. Amongst these motherblocks we selected those with known clones of rootstocks (Jacquez, 101-14, Richter99, Richter110, Ramsey, Paulsen 1103, Ruggeri I40, 143B, US 8-7, SO4). We monitored these vineyards in Autumn of the two respective seasons to identify 10-15 individual vines, showing clear leafroll symptoms on the scion but with sizable, lignified rootstock shoots. As expected very few such instances were observed in all vineyards. Lignified rootstock shoots and scion cane/petiole material were collected from each individual vine. Both samples per vine were tested for GLRaV-3 with PCR (Goszczynski, 2014). Where rootstock material was still available after use in PCR these were grafted on virus-free Cabernet franc material, callused, rooted and grown in insect-free greenhouses in sterilised soil. These were monitored for symptoms and tested for GLRaV-3 by PCR.

Determine tolerance/immunity of commercially important rootstock clones
Cabernet franc was grafted onto each of the commonly utilised rootstock cultivars and their clones (20 replicates). These were allowed to callus and root and were then planted out in rock-wool or sterilized soil in pots in insect-free greenhouses. A pure source of GLRaV-3 (no other viruses found using NGS) of GLRaV-3 variants II and VI, were chip-budded onto the rootstock portion of 7 replicates of each rootstock/scion. The Cabernet franc scion was monitored for leafroll symptoms over two seasons and infection in the scion confirmed by GLRaV-3 (Goszczynski, 2014) PCR tests.

Determine if transmission of virus is possible through an inter-graft of rootstock cultivars.
Scion cane material of pure sources of GLRaV-3 (variants II and VI) were cut and rooted cuttings made of these. In parallel Cabernet franc was grafted onto each of the commonly utilised rootstock clones and also allowed to callus and root. Once both those components were ready the above scion/rootstock was cut and grafted onto the rooted GLRaV-3 infected source (scion material on own roots), or the GLRaV-3 infected material was grafted onto an inverted rootstock scion combination. These were planted out in sterilized soil in pots in insect-free greenhouse, and in all instances the Cabernet franc scion was monitored for leafroll symptoms and infection confirmed by PCR tests

7. RESULTS AND DISCUSSIONS

Determine if rootstocks obtained from known GLRaV-3 infected commercial vines (Scions) are infected with GLRaV-3.
A total of 42 and 18 vines samples (rootstock and corresponding scion from the same vine) were collected in May 2017 and May 2018, respectively The rootstocks collected represent those clones most often used in the South African wine industry and included R110, US 8-7, Ruggeri 140, 101-14 Mgt, and Paulsen 1103. R99 was excluded as this rootstock had been assessed in a previous study (Harris, 2017). Sampling criteria required scions to have clear leafroll symptoms and sizeable, lignified rootstock suckers growing from the stems. However, diligent wine farmers usually prune rootstocks, thus locating suckering vines of any description (even small unlignified suckers) was challenging.

The 60 vines were tested for GLRaV-3 by PCR with the majority of the rootstocks from these vines having had a negative GLRaV-3 status compared to corresponding scion (Table 1). GLRaV-3 was detected in nine out of 60 (15%) rootstock samples and in 50 out of 60 (83%) scions. When positive, rootstock samples yielded lower amplicon concentrations (observed as faint bands in electrophoresis) than corresponding scions, despite the generally lower total RNA concentrations of scion used as templates in the PCRs. This would suggest that the concentration of initial RNA template used was of little importance.

Table 1: Grapevine leafroll associated virus 3 positive samples per rootstock/scion combination_

Rootstock/scion combination		Roostocks GLRaV-3	Scions GLRaV-3	N
Rootstock	Scion	positive	positive	
101-14 Mgt	Merlot	0	7	10
US 8-7	Touriga Nacional (TN) 1A	1	10	14
Ruggeri 140	Merlot	0	10	10
R110	Merlot	1	4	5
R110	Cabernet Sauvignon	0	3	3
R110	Shiraz	0	2	2
R110	Touriga Nacional (TN) 1A	0	2	4
Paulsen	Merlot	3	7	7
Paulsen	Ruby Cabernet	3	3	3
Ramsey	Crimson	1	1	2
Total		9	49	60

N=Total amount of vines tested

C. franc scions were grafted onto rootstock material collected 2017 and 2018 where sufficient material was left after RNA extraction. The rootstocks collected in 2017 tested negative for GLRaV-3 (when subjected to PCR) and in order to confirm these results plant material was transported to Vititec for grafting. Unfortunately, these grafts were unsuccessful and only one vine, C. franc grafted onto Ramsey (Accession: 17-7101), took. The GLRaV-3 specific PCR revealed that the C. franc indicator vine was negative for the virus following grafting.

The C. franc/rootstocks collected 2018 grafts were successfully grafted Table 2). The R110 rootstocks previously tested negative for GLRaV-3 (when subjected to PCR, as previously mentioned), however, 3 out of 4 (75%) of the C. franc scions grafted onto these rootstocks tested positive for GLRaV-3 (Table 8). C. franc scions grafted onto GLRaV-3 negative Paulsen 1103 rootstocks had 1 out of 3 (33%) GLRaV-3 positives. This would suggest that either the RT-PCR employed was not sensitive enough to detect the virus in these rootstock samples or that the rootstocks themselves have a defence mechanism against the virus, which resulted in decreased virus populations that were low enough to go undetected by conventional PCR but high enough to be transmitted to grafted scion material.

Table 2: Grapevine leafroll associated virus 3 positive scion indicator samples per graft using Paulsen and Richter 110 rootstocks collected in 2018

Rootstock	Scion	C. franc GLRaV-3 positive	N
Paulsen (18-4005, 18-4007) (-)		1	3
Paulsen (18-4011, 18-4015, 18-4017, and 18-4019) (+)		5	8
R110 (18-4035) (-)	Cabernet	0	1
R110 (18-4037) (-)	franc	3	3
Cabernet franc GLRaV-3 positive control		2	2

(-)=GLRaV-3 negative; (+)=GLRaV-3 positive, N=Total amount of vines tested

Determine tolerance/immunity of commercially important rootstock clones

Cabernet franc scion grafted onto commonly used rootstock cultivars R110, R99, 101-14 and Ramsey were obtained from Vititec. These were shipped to Pretoria and planted out in sterilized soil in pots in the insect-free greenhouse. Three sources of GLRaV-3 (17-7082; 17-7084; and 17-7093), determined by next-generation sequencing to be pure sources of variant VI, were also grown in pots in the adjacent closed-off section of the greenhouse at UP. GLRaV-3 source 17-7082 was used to graft-inoculate on the rootstock portion of replicates of the above-mentioned combinations. Eleven R110 combinations were graft-inoculated, six of 101-14, seven of R99 and four of Ramsey. For each combination, a healthy control was retained. The inoculated vines are maintained in the greenhouse and monitored for symptoms on the

C. franc leaves. The Cabernet franc components were tested for GLRaV-3 by PCR. None of the healthy controls were infected. Nine of eleven Richter110 were infected, 3 of 6 101-14 vines, 4 of 7 Richter99 vines and all four Ramsays. From this it is clear that none of the rootstock sources obtained can be considered immune to GLRaV-3 with each one having individuals that were infected by GLRaV-3. In those instances where the C. franc component of the inoculated plants were negative one cannot differentiate between whether the graft inoculation did not work or whether the rootstocks are immune (virus does not replicate).

Determine if transmission of virus is possible through an intergraft of a rootstock cultivar

Five replicates of omega grafted canes were made where two different GLRaV-3 sources were separated from a known, susceptible scion indicator cultivar (Cabernet franc) by various rootstock cultivar inter-grafts (Figure 1). This was done to determine whether GLRaV-3 could be transmitted to the indicator via the rootstock. Grafting was done by M. Dirk Visser of Vititec, and the GLRaV-3 sources (18-0061 and 18-0064) were both characterised by Dr. R. Bester at SU. 18-0061 contained GLRaV-3 variant I, II, and VI and was also infected with GVA whereas 18-0064 only contained GLRaV-3 variant II. Following callusing, and rooting at Vititec, plants were assessed for root and shoot development and planted into pots. These were transferred to a greenhouse at Stellenbosch University where they were treated with imidachloprid and maintained throughout the season.

Rootstock	Intergraft	Scion	Replicates
GLRaV-3 var II, III, VI and Viti/Foveavirus (18-0061)	AA	C. franc	5
	CF		5
	PS		5
	RQ		5
	RY		5
	SC		5
	UC		5
GLRaV-3 var II virus (18-0064)	AA	C. franc	5
	CF		5
	PS		5
	RQ		5
	RY		5
	SC		5
	UC		5
C. franc (longer with bud)	AA	GLRaV-3 var II, II, VI and Viti/Foveavirus (18-0061)	5
	CF		5
	PS		5
	RQ		5
	RY		5
	SC		5
	UC		5
C. franc (longer with bud)	AA	GLRaV-3 var II (18-0064)	5
	CF		5
	PS		5
	RQ		5
	RY		5
	SC		5
	UC		5
AA	none	CF	5
RQ			5
RY			5
SC			5
UC			5
PS			5

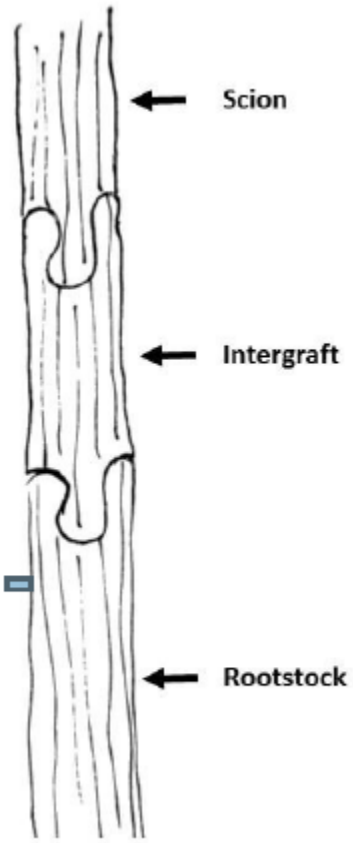

Figure 1: Layout of grafting experiment to determine the transmission of GLRaV-3 from an infected source to a C. franc indicator via a rootstock inter-graft.

Not all grafted replicates survived or sprouted, but at least three replicates of all rootstock treatments had successful graft take and C. franc cane production. In a few instances the three replicates were obtained by combining the results of both virus sources. Plants were monitored for symptoms monthly and petiole material of the C. franc canes collected in April, 2019 for RNA extraction and tests for GLRaV-3 by PCR. Symptoms were very difficult to differentiate from a general red-bronzing symptom caused by excessive nutrients in the greenhouse irrigation system. Table 3 Summarises the results obtained by testing with GLRaV-3 PCR.

Table 3: Percentage of Cabernet franc indicator canes to which GLRaV-3 was transmitted while separated by various rootstock inter-graphs from two different GLRaV-3 sources (of variants I, II and VI).

Scion	Inter-graft	Root	Percentage of CF indicators positive
CF	AA	GLRaV-3	100% (n = 5)
GLRaV-3	AA	CF	33% (n = 6)
CF	AA		0% (n = 2)
CF	CF	GLRaV-3	44% (n = 9)
GLRaV-3	CF	CF	40% (n = 5)
CF	CF		0% (n = 2)
CF	PS	GLRaV-3	57% (n = 7)
GLRaV-3	PS	CF	100% (n = 3)
CF	PS		0% (n = 2)
CF	RQ	GLRaV-3	75% (n = 8)
GLRaV-3	RQ	CF	50% (n = 6)
CF	RQ		0% (n = 1)
CF	RY	GLRaV-3	60% (n = 5)
GLRaV-3	RY	CF	60% (n = 5)
CF	RY		0% (n = 2)
CF	SC	GLRaV-3	75% (n = 8)
GLRaV-3	SC	CF	100% (n = 5)
CF	SC		0% (n = 2)
CF	UC	GLRaV-3	78% (n = 9)
GLRaV-3	UC	CF	80% (n = 5)
CF	UC		0% (n = 1)

CF = Cabernet franc; AA = 101-14; PS = Paulsen 1103; RQ = Richter 110; RY = R99; SC = ; UC = US
8/7; GLRaV = combined results of GLRaV-3 source 18-0061 and 18-0064 (GLRaV-3 var I, II and VI and
GVA).

From the results it is clear that GLRaV-3 is capable of being transmitted both upwards and downwards to the C. franc indicator cane via all of the rootstock cultivars. However once again this did not occur through all individual rootstock inter-grafts. Unfortunately, these results do not

allow us to determine whether such transmission via the phloem is just passive, with the virus not replicating and just moving along with the phloem contents, or whether the virus actively replicates in the rootstock component. We cannot consistently detect GLRaV-3 in any of the rootstock cultivars from field collected GLRaV-3 infected plants (Objective 1) and don't know if this is 1) due to varying low virus titers in rootstocks, 2) or due to different genetic variants of the rootstocks. Hence, should the latter hypothesis be correct, we cannot discount that in this experiment that the specific rootstocks utilised in the grafting experiments may be some of the genetically more susceptible variants. Care will have to be taken to establish clonal vines of a characterised phenotype in any future studies on rootstocks.

8. CONCLUSIONS AND RECOMMENDATIONS

GLRaV-3 is poorly detected in all rootstocks tested relative to the corresponding scions. In some of these instances where rootstocks from individuals tested negative by PCR, virus was present as evidenced by graft transmission of those rootstocks to a C. franc indicator. This suggests a PCR sub-detectable level of virus titers. GLRaV-3 was demonstrated capable of infecting some individuals of all rootstock cultivars assessed (Richter99, Richter110, 101-14, and Ramsay) hence no rootstock wide immunity exists (virus doesn't replicate in all of the rootstock individuals). At the same time however not all replicates inoculated became infected, and while this may suggest that some of the rootstock individuals used as inoculum are immune to GLRaV-3 (it does not replicate in the vine) we cannot, based on the relatively low replicates we were able to obtain, discount whether this is due to inefficient viral transmission or genetic differences amongst individual rootstocks.

Data generated thus far suggests that commonly utilised rootstocks do contain some form of resistance to GLRaV-3. They do not display symptoms of disease, virus titers appear to be low or the virus is not uniformly distributed in the rootstock resulting in poor detection by PCR. However this resistance does not appear uniformly within individuals of any rootstock cultivar, with virus being detected in some but not others. We propose that for the certification scheme an individual be identified of each rootstock type which is immune to GLRaV-3 (virus not detected in the rootstock by PCR or graft transmission in spite of a high infection pressure) and that this individual be clonally propagated to serve as the industry standard. Furthermore we propose that the gene(s) involved in GLRaV-3 be identified for use in breeding programs or via molecular approaches to obtain GLRaV-3 resistant noble cultivars.

9. PLANNED OUTPUTS

a) TECHNOLOGY DEVELOPMENT, PRODUCTS AND PATENTS

None

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Popular article, peer-reviewed publication

c) HUMAN RESOURCES DEVELOPMENT / TRAINING (STUDENTS)

Student Name and Surname	Student Nationality	Degree (eg Hons, MSc)	Level of studies in final year of project	Total Bursary Cost for Industry for entire project
Honours				
Masters				
Shaina Facey	South African	MSc.	Finished	R 255000
PhD				
Postdocs				

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

Resistance to grapevine leafroll associated virus type 3 (GLRaV-3) in various commonly utilised Vitis rootstocks. Facey, S., Harris, M., and Pietersen, G.

e) PRESENTATIONS/PAPERS THAT COULD BE DELIVERED

Resistance to grapevine leafroll associated virus type 3 (GLRaV-3) in various commonly utilised Vitis
rootstocks. Facey, S., Harris, M., and Pietersen, G.

10. PROJECT OUTCOME AND IMPACT

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

Other is:

The Value of the project to industry

The project has confirmed the poor detection of GLRaV-3 in individuals of ALL rootstocks commonly utilised in the wine Industry. We hypothesize that the continued inconsistent results obtained in the industry as well as in experiments performed during this project and the previous one, may be due to genetic differences with regards GLRAV-3 susceptibility in rootstock material and that any given rootstock cultivar is potentially not genetically homogeneous. Our answer to the question “pose at the start of this project “Is poor detection of grapevine leafroll associated Virus 3 in South African rootstocks due to host resistance?” can therefore be answered in the affirmative, but that it differs in individuals of any rootstock. Future studies should identify those individuals with immunity to utilise those as future clonal sources of propagation for each rootstock cultivar. The gene(s) involved in resistance should also be identified and used in breeding or biotechnological programs to introduce these into important wine cultivars for resistance to GLRaV-3 .

11. PERSONS PARTICIPATING IN THE PROJECT:

INITIALS AND SURNAME	HIGHEST QUALIFICATION	RACE (M,W)	GENDER (M,F)	INSTITUTE DEPARTM	POSITION	TOTAL COST TO PROJECT
RESEARCH PERSONNEL						R 180000
G. Pietersen	PhD	W	M	UP Microbiology	Extra-ordinary Professor	R 180000
SUPPORT PERSONNEL						R 0

POSITION: Co = Co-worker (other 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	Raisin SA	HORTGRO	SATI	WINETECH	ARC	OTHER	TOTAL
2015	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
2016	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
2017	R 0	R 0	R 0	R 0	R 296700	R 0	R 0	R 296700
2018	R 0	R 0	R 0	R 0	R 225000	R 0	R 0	R 225000
2019	R 0	R 0	R 0	R 0	R 330000	R 0	R 0	R 330000
2020	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
TOTAL	R 0	R 0	R 0	R 0	R 851700	R 0	R 0	R 851700