Genetic characterisation of fungal disease resistance genes in grapevine using molecular marker technology.

by
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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Genetics in the Faculty of AgriSciences at Stellenbosch University

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December 2014

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated on page III), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

René Veikondis

Date:.2014/11/26.

Declaration by the candidate:

With regard to chapter 3, the nature and scope of my contribution were as follows:

(a) collected samples of all material for phenotypic and genotypic screening; (b) assisted with the preparation and phenotypic scoring of all of the leaf material subjected to fungal inoculations; (c) did the set-up and processing of all samples for electrophoresis on the sequencer at the DNA Sequencing Unit, Stellenbosch University; (d) did all the subsequent data quality and scoring of all of the molecular data generated in the study; e) instructed Mrs D Snyman with regard to changes to the PCR conditions for the optimisation required for the Kishmish Vatkana study and (f) performed all statistical, linkage and QTL analysis.

The following co-authors have contributed to [specify chapter or part of a chapter and page numbers in the dissertation]:

Name	**e-mail address**	**Nature of contribution**
Mrs P Burger	BurgerP@arc.agric.za	Made populations available for study
Mr A Vermeulen	VermeulenA@arc.agric.za	Assisted with phenotypic screening's samples collection and scoring
Mrs D Snyman	cengen@cengen.co.za	DNA extraction and PCR set-up
Mr CJ van Heerden	cjvh@sun.ac.za	Provided information about markers and conditions for relevant populations. Assistance with the interpretation of results.

René Veikondis

Date:.2014/11/26.

Abstract

The aim of this study on grapevine was to genetically characterise, validate and map the reported fungal disease resistance genes of Pölöskei Muskotály (PM), Kishmish Vatkana (KV) and Villard Blanc (VB) in South Africa using QTL analysis. These fungal resistant parents were crossed with other varieties that have desirable fruit qualities in an effort to combine fungal disease resistance with desirable fruit qualities in a single variety. The genetic basis of PM's resistance to downy and powdery mildew has not been investigated before. It does however have VB in its pedigree so the assumption was made that the same QTL/genes present in VB contribute to this resistance. KV's resistance to powdery mildew reportedly originates from the *REN1* gene located on chromosome 13. VB's powdery and downy mildew resistance is conferred by QTL present on chromosome 15 and chromosome 18 respectively and has been reported in numerous studies.

The study populations comprised of 124 F_1 PM x Regal Seedless plants, 16 F_1 PM x G4-3418 plants, 14 F_1 PM x Sunred Seedless plants, 158 F_1 Sunred Seedless x KV plants and 250 F_1 VB x G1-6604 plants. DNA was extracted from the leaves and all plants were screened using microsatellite markers. Phenotypic evaluations of downy and/or powdery mildew resistance were performed on the appropriate populations. The molecular data was used to generate linkage maps and combined with phenotypic data to perform QTL analysis.

From the molecular data generated for the three PM populations it was determined that the F_1 progeny inherited almost exclusively maternal alleles, and could not be used in a mapping study. These populations were eliminated from the study and PM will be used as a pollen donor in future. Molecular data from the Sunred Seedless x KV cross was used to generate a linkage map for chromosome 13 comprising eight markers and spanning 45.6 cM. When combined with the data from two powdery mildew phenotypic screens a QTL peak spanning the *REN1* gene on chromosome 13 of KV was identified. This locus explains between 44.8% and 57.7% of the phenotypic variance observed. The molecular data from the VB x G1-6604 cross was used to generate partial linkage maps for chromosome 15 and 18. Eleven markers were mapped on chromosome 15 spanning 56.4 cM, and ten markers were mapped on chromosome 18 spanning 101.8 cM. When the chromosome 15 linkage map was combined

with the data from two powdery mildew phenotypic screens a QTL associated with powdery mildew resistance was identified on chromosome 15 that explains between 18.9% and 23.9% of the phenotypic variance observed. Likewise a QTL associated with downy mildew resistance was identified on chromosome 18 when the chromosome 18 linkage map was combined with data from two downy mildew phenotypic screens. This QTL explains between 19.1% and 21.2% of the phenotypic variance observed.

This study succeeded in genetically characterising the fungal disease resistance genes of two different sources of grapevine and provided exclusionary information on a third resistance source for future breeding applications.

Opsomming

Die doel van hierdie studie in wingerd was om die genetiese komponent van die swamweerstandsgene van Pölöskei Muskotály (PM), Kishmish Vatkana (KV) and Villard Blanc (VB) in Suid-Afrika te karakteriseer en die teenwoordigheid daarvan te bevestig deur 'n Kwantitatiewe Eienskap Lokus (KEL) benadering te volg. In 'n poging om swamweerstand en goeie vrugeienskappe te kombineer in 'n enkel variëteit is die weerstandige variëteite met vatbare variëteite gekruis wat goeie vrugeienskappe besit. Die genetiese basis van PM se weerstand teen donsskimmel en witroes is nog nie vantevore bestudeer nie. VB is een van sy voorgeslagte en daar is aangeneem dat dieselfde KEL/gene waarskynlik verantwoordelik is vir die weerstand. Dit is gerapporteer dat KV se witroesweerstand afkomstig is van die *REN1* geen op chromosoom 13. Vele publikasies rapporteer VB se weerstand teen witroes en donsskimmel Beide die witroes- en donsskimmelweerstand word oorgedra deur KEL teenwoordig op chromosome 15 en 18 onderskeidelik.

Die populasies gebruik in hierdie studie het bestaan uit 124 F_1 PM x Regal Seedless plante, 16 F_1 PM x G4-3418 plante, 14 F_1 PM x Sunred Seedless, 158 F_1 Sunred Seedless x KV plante en 250 F_1 VB x G1-6604 plante onderskeidelik. Blare is versamel vir DNS isolasie en genotipering met mikrosatellietmerkers. Al drie populasies se weerstand teen donsskimmel en/of witroes is fenotipies geëvalueer. Die molekulêre data is gebruik om genetiese koppelingskaarte op te stel en gekombineer met die fenotipiese data om KEL analise uit te voer.

Die molekulêre data van die drie PM populasies het daarop gedui dat die F_1 nageslag amper uitsluitlik moederlike allele geërf het en kon gevolglik nie gebruik word in die studie nie. Die PM populasies is uitgesluit uit hierdie studie en PM sal voortaan as stuifmeelskenker gebruik word. Molekulêre data van die Sunred Seedless x KV kruising is gebruik om 'n koppelingskaart vir chromosoom 13 op te stel wat 45.6 cM lank is en agt merkers bevat. Die KEL analise van die koppelingskaart en twee fenotipiese datastelle vir witroes het 'n KEL piek geïdentifiseer wat oor die lengte van die *REN1* geen-interval strek. Hierdie lokus is verantwoordelik vir 44.8% tot 57.7% van die fenotipiese variasie wat waargeneem word. Molekulêre data van die VB x G1-6604 kruising is gebruik om gedeeltelike koppelingskaarte

vir chromosome 15 en 18 op te stel. Elf merkers karteer op die chromosoom 15 kaart van 56.4 cM en tien merkers karteer op die chromosoom 18 kaart van 101.8 cM. KEL analise van chromosoom 15 se koppelingskaart en twee witroes fenotipiese datastelle het 'n KEL geïdentifiseer wat 18.9% tot 23.9% van die fenotipiese variasie verduidelik. 'n KEL is ook op chromosoom 18 geïdentifiseer wat 19.1% tot 21.2% van die fenotipiese variasie verduidelik met die gekombineerde analise van chromosoom 18 se koppelingskaart en twee donsskimmel fenotipiese datastelle.

Hierdie studie het die genetiese komponent van die swamweerstandsgene van twee *Vitis* variëteite suksesvol gekarakteriseer en bevestig. Waardevolle telingsinligting oor die derde variëteit is ook onthul.

Acknowledgements

I would like to acknowledge and express my gratitude to the following people and institutions.

My supervisor Dr R Prins for challenging, supporting and advising me, as well as providing me with the many tools and opportunities to learn and grow.

My co-supervisor Prof L Warnich for her insight, support and advice to me.

Costa Veikondis for his endless support. For inspiring and encouraging me throughout.

Phyllis Burger (ARC Infruitec-Nietvoorbij) for creating and maintaining the mapping populations in the greenhouse and her input in my thesis.

Abraham Vermeulen (ARC Infruitec-Nietvoorbij) for assisting with the phenotypic screens and his insight in my thesis.

Debbie Snyman, (CenGen (Pty) Ltd) for technical assistance with the DNA marker work.

Carel van Heerden (Sequencing Unit, Central Analytical Facility, Stellenbosch University) for marker analysis, technical assistance, support and advice.

My colleagues at the Sequencing Unit (Central Analytical Facility, Stellenbosch University) for their support and encouragement.

Deciduous Fruit Producer's Trust (DFPT) and Technology and Human Resources for Industry Program (THRIP) for funding.

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List of symbols and abbreviations

3’	three prime
5’	five prime
%	percentage
°C	degrees Celsius
AFLP	amplified fragment length polymorphism
ARC	Agricultural Research Council
BC	Before Christ
cM	centiMorgan
cm	centimetre
CTAB	cetyltrimethylammonium bromide
DAF	Department of Agriculture and Food, Australia
ddH_2O	double distilled water
DNA	deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	ethylene-diaminetetraacetate
ETOH	ethanol

Fx	filial generation
g	grams
G1	G1-6604
G4	G4-3418
IM	interval mapping
kbp	kilobase pairs
KV	Kishmish Vatkana
LG	linkage group
LOD	logarithm of the odds
MAS	marker-assisted selection
Mich	Michaux
$MgCl_2$	magnesium chloride
MHz	mega hertz
min	minutes
ml	millilitres
mm	millimetres
mM	millimolar
MQM	multiple trait/QTL mapping

NaCl	sodium chloride
NCBI	National Centre for Biotechnology Information
ng	nanogram
NH_4OAc	Ammonium acetate
OIV	Office International de la Vigne et du Vin
P	level of significance/probability
PCR	polymerase chain reaction
PM	Pölöskei Muskotály
QTL	quantitative trait loci
rho	correlation coefficient
RCF	relative centrifugal force
REN1	resistance to *Erysiphe necator* 1
RFLP	restriction fragment length polymorphism
RS	Regal Seedless
Run1	resistance to *Uncinula necator* 1
Rupr	Ruprecht
SCAR	sequence-characterised amplified region markers
SNP	single nucleotide polymorphism
SS	Sunred Seedless

SSR	simple sequence repeat
Subsp	Subspecies
Syn	synonym
TE	Tris ethylenediaminetetraacetic acid buffer
Tm	annealing temperature
Tris	Tris(hydroxymethyl)aminomethane ($C_4H_{11}NO_3$)
Tris-Cl	Tris chloride
µl	micro litres
v	version
VB	Villard Blanc
Vitis vinifera L.	*Vitis vinifera* Linnaeus
VMC	Vitis Microsatellite Consortium
w/v	weight per volume
www	world wide web

Chapter 1

Introduction

Grapevine is a very important agricultural and commercial plant, and one of the most widely grown fruit crops in the world. In 2012, approximately 7.5 million hectares were under grapevine worldwide, with 6% of that being in South Africa. The main producers worldwide are Europe (44%) followed by Asia (28.7%) (Troggio et al. 2008, Zhang et al. 2009, OIV statistical report on World Vitiwiniculture 2013). Grape berries are used commercially in wine making, fresh consumption and drying. There is constant pressure to improve the eating and winemaking quality of the berries and the viticultural aspects of plants from the consumers and producers. The most common and sought after traits for table grapes (fresh consumption) that need to be improved are berry quality, taste, yield, shelf life, disease resistance and seedlessness. Various varieties are available that exhibit one, two, or more of these traits and through traditional breeding they can be combined in one new variety. However, this is a very time consuming and expensive process (Alleweld et al. 1990, Zyprian et al. 2003, Collard et al. 2005, Troggio et al. 2008).

Fungal diseases like downy mildew and powdery mildew are two of the biggest threats to growers as it can lead to major crop loss and even plant loss, if very severe and not controlled (Moreira et al 2011). Chemical control and good vineyard practices have been used to control the outbreak and spread of these fungal infections (Fischer et al. 2004, Barker et al. 2005, Tamm et al. 2006, Gadoury et al. 2012).

Scientists have become involved in this whole process in an effort to decrease the time and money spent on developing new varieties. This is possible because the grapevine genome has been mapped, populated with various molecular markers and the known positions of certain genes and quantitative trait loci (QTL). Some of these mapped genes and QTLs are linked

to/contribute to the plant's response to disease pressure. The presence of molecular markers also makes it possible to identify areas on a genome that is involved with disease resistance by combining phenotypic observations with molecular data (QTL mapping) if no genetic information is available about a specific trait. Once a gene/QTL has been detected and surrounding markers linked to the trait have been identified, the progeny of a traditional crossing experiment can be screened and the progeny that did not inherit the trait in question can be eliminated. This helps to reduce the cost as the breeders spend less on soil preparation, fertilisation, chemical control, erecting trellis systems, planting space, etc. With the identification of new disease resistance genes/QTLs, it becomes an option to combine disease resistance genes from various origins in a single variety. Consequently, the disease resistance will be more durable as it will be challenging for pathogens to overcome all of the various resistance mechanisms of the plant (Eibach et al. 2007).

Chapter 2

Literature review

2.1 General

2.1.1 *Vitis* origin

Grapevine cultivation has been practiced for many centuries across the world with *Vitis vinifera* Linnaeus being the most widely cultivated species. Controversy exists as to the domestication event(s) of *Vitis vinifera* L. One group of scientists hypothesise that there was one domestication event in the Near East, while another group states that there were multiple origins of domestication. Scientists are looking on a molecular level for proof of either of two hypotheses currently published (Arroyo-Garcia et al. 2006, This et al. 2006, Terral et al. 2010).

One hypothesis states that there was one centre of domestication in the Near East between 4000 and 6000 before Christ (BC). Archaeological evidence has been uncovered, in the form of seeds found in Jericho, that grapevine was domesticated more than 5000 years ago and it was most probably from the wild grape classified as *Vitis vinifera* subsp. *sylvestris* C.C. Gmelin. This wild grape was found from north-eastern Afghanistan to the southern borders of the Black Sea and the Caspian Sea (Aradhya et al. 2003). The origin of domesticated grapes is thus believed to be from the Near East from where it has spread to the Mediterranean basin, Far East and New World (Manen et al. 2003, This et al. 2006, Terral et al. 2010, Myles et al. 2011). The second hypothesis states that there was a centre of domestication in the Near East and another in the western Mediterranean region. There are large morphological differences between current eastern and western Eurasian varieties. It is thought to be due to concurrent domestication events in the east and west of *Vitis vinifera* L. subsp *sylvestris* C.C.

Gmelin populations and not migration from the Near East to the west (Grassi et al. 2003, Arroyo-Garcia et al. 2006, Bacilieri et al. 2013, Imazio et al. 2013).

No conclusive evidence has been found in support of either hypothesis as it is possible that the original domestication of *V. vinifera* L. subs. *sylvestris* C.C. Gmelin could have taken place in the Near East with a subsequent westward migration. In Europe this variety could then have been cross-bred with native *V. vinifera* L. subs. *sylvestris* C.C. Gmelin populations, however, this happened after the initial domestication event (Terral et al. 2010, Emanuelli et al. 2013). The process of domestication and cultivation was accelerated because *Vitis* displays a wide variation in morphological characteristic such as adaptability to different climates and soil types, bears edible fruit and the varied uses of the fruit (Alleweldt et al. 1990, Unwin 1996, Barnaud et al. 2006, Troggio et al. 2008).

A great number of different varieties originated from the domestication of wild *Vitis* species, but these numbers increased rapidly when breeders found that *Euvitis* species (species with 38 chromosomes) hybridise easily (Alleweldt et al. 1990, Moreira et al. 2011). These hybrids are made to combine desirable traits from two different species or varieties. These traits, amongst others, can be improved fruit quality, adaptability to environment and disease resistance. Commercial grapevine cultivation is to a large extent driven by consumer demand whether it is seeded versus seedless varieties, shelf life, taste, and colour or berry size. Demand has also risen for grapevine that is naturally more disease resistant as it is more economical for growers and consumers prefer less chemical spraying on crops (Dry et al. 2010).

2.1.2 *Vitis* varieties

Originally varieties were classified into three groups, based on eco-geographical variation: *occidentalis* - small berried wine grapes of Western Europe; *orientalis* - large berried table grapes of West Asia and *pontica* -intermediate berry type from the basin of the Black Sea and Eastern Europe (Aradhya et al. 2003).

Domestication of *Vitis vinifera* L. took place in the Near East and/or western Mediterranean from where it spread to surrounding areas. Its variation and adaptability was however not

sufficient to allow universal cultivation and other wild species were subsequently also domesticated. In America *Vitis labrusca* L. and *Vitis rotundifolia* Michaux were domesticated and in China *Vitis amurensis* Ruprecht (Alleweldt et al. 1990; Aradhya et al. 2003).

The best known *Vitis* species are:

- *Vitis vinifera* L., native to the Eurasian area
- *Vitis aestivalis* Mich., native to eastern North America
- *Vitis rupestris* Scheele, native to North America
- *Vitis riparia* Mich., native to north eastern North America
- *Vitis amurensis* Rupr., native to Siberia and China
- *Vitis rotundifolia* Mich., native to the southern half of North America
- *Vitis labrusca* L., native to north eastern America (Encyclo Wine)

Today there are more than 16 000 identified varieties to be found, however only 7 000 to 8 000 are commercially grown (Alleweldt et al. 1990, Pollefeys et al. 2003). The problem though is that grapevine has a long cultivation history and a wide distribution and this has led to the development of varieties that have many synonyms (same variety grown in different geographical areas that are assigned different names), homonyms (genetically different varieties that have the same names) and curation errors (Myles et al. 2011).

2.1.3 *Vitis* interspecies crosses

Interspecies crosses, commonly referred to as hybrids, are the product of a crossing between any *Vitis* species. Crosses between *Vitis* varieties of the same species can also be found (Encyclo Wine http://www.encyclowine.org/?title=Hybrid_grapes).

In Europe *Vitis* interspecies crosses or hybrids became known after the introduction of the North American insect pest, phylloxera, and pathogens, downy and powdery mildew in the European vineyards in the second half of the 1800s. These non-native pest and diseases had a devastating effect on the European vineyards as the native *Vitis vinifera* grapevines had no

inherent resistance to it. This led to an exploration of the American *Vitis* species and interspecific hybrids to find pest resistant rootstocks or donors of disease resistance genes that could be introduced into the *Vitis vinifera* L. varieties (Unwin 1996, Hoffmann et al. 2008, Gessler et al. 2011). The intensive breeding programmes were mainly geographically centred in and around France and were led by breeders like Kuhlmann, Couderc, Seibel, Seyve and Villard (Alleweldt et al. 1990, Unwin 1996, Hoffmann et al. 2008, Gessler et al. 2011, Encyclo Wine). Wild American species such as *Vitis aestivalis, Vitis rupestris* and *Vitis riparia* with resistance to the introduced pests and diseases, were mainly used to introduce resistance into *Vitis vinifera* L. (interspecific hybrids).

Resistance to the pathogens investigated by Alleweldt et al. (1990) were found in the following species:

Plasmopara viticola (downy mildew) - *Vitis riparia* Mich., *Vitis rupestris* Scheele, *Vitis lincecumii* Buckl., *Vitis labrusca* Mich., *Vitis amurensis* Rupr., *Vitis rotundifolia* Mich.

Erysiphe necator (powdery mildew) - *Vitis aestivalis* Mich., *Vitis cinerea* Engelm., *Vitis riparia* Mich., *Vitis berlandieri* Pl., *Vitis amurensis* Rupr., *Vitis rotudifolia* Mich.

These efforts led to an improvement of varieties in characteristics such as yield, quality, adaptability, disease resistance, seedlessness, berry size and maturation. There is a vast abundance of grapevine varieties because of their asexual propagation and the occurrence of sports which leads to a bank of characteristics to be used in breeding (Alleweldt et al. 1990, Pollefeys et al. 2003). Breeders encountered resistance to these new varieties from growers and consumers as the quality of especially wine produced from these varieties could not be compared to that of pure *Vitis vinifera* L. At the same time rootstock breeding was very successful in combating particularly the phylloxera problem by grafting *Vitis vinifera* L. scions on to these newly created resistant rootstocks (Zhang et al. 2009); this was widely accepted by growers as the fruit and wine quality of the scion was not affected. Fungicides to the most common fungal diseases were also developed.

2.2 Origin and characteristics of parental plants

2.2.1 Pölöskei Muskotály

Pölöskei Muskotály is an interspecies hybrid created by Sándor Szegedi, János Erős and Éva Ésik, from the parents Zalagyöngye and 5917-8, and released in 1967 in Hungary by the Research Institute for Viticulture and Enology in Kecskemét (Kozma 2002b). It has greenish white berries that weigh less than three grams (Image 1), fairly thick skins and a good to high degree of resistance to downy and powdery mildew of the leaves and berries (Kozma 2002b, Pernesz 2004, Pavloušek 2007). Zalagyöngye (syn. Pearl of Zala) was used in many experimental crosses made in Hungary because of its good resistance, early ripening and good taste. There is some controversy about the parentage of Zalagyöngye as some sources consider it a cross of SV12-375 (syn. Villard Blanc) with Csabagyöngye (syn. Pearl of Csaba) and others state that it is a cross of Eger2 (a seed descendent of SV12-375) and Csabagyöngye (Figure 1) (Kozma 2002a, Pavloušek 2007).

When examining the pedigree of Pölöskei Muskotály one can assume that the mildew resistance could have originated from Villard Blanc (see section 2.2.6 for discussion on Villard Blanc resistance), but no supporting publications were found, or any describing the genetics of the resistance.

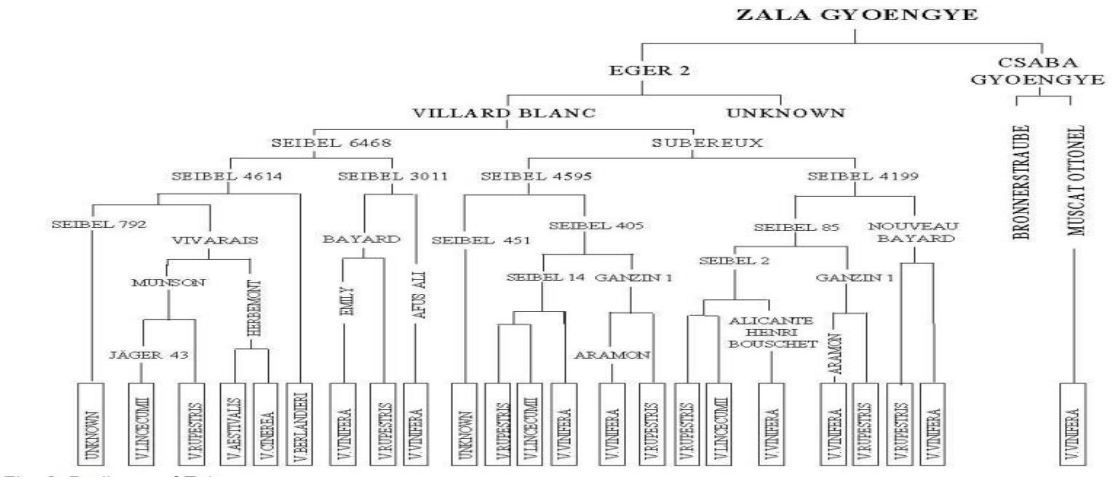

Figure 1 Pedigree of Zalagyöngye (Figure from Study of the Use of the Varieties of Interspecific Vines, EU Final Report, 2003)

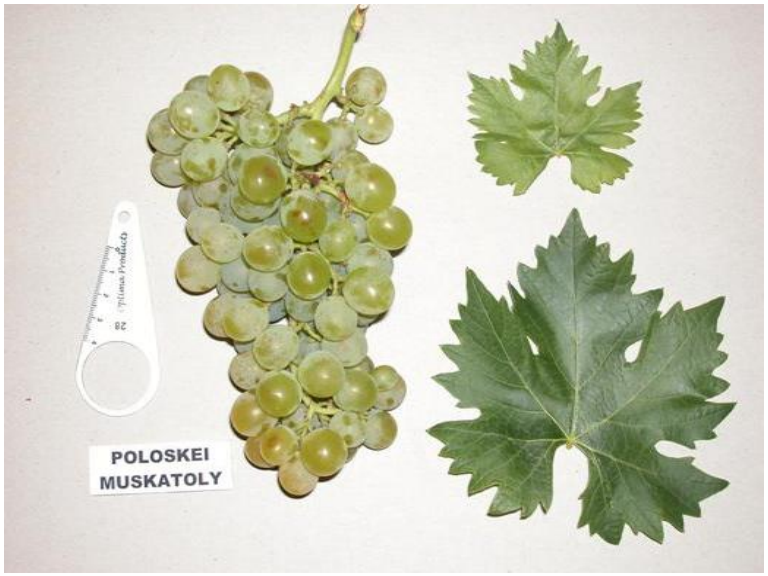

Image 1 Pölöskei Muskotály (photo by P. Burger, ARC Infruitec-Nietvoorbij)

2.2.2 Regal Seedless

Regal Seedless was released by the ARC Infruitec-Nietvoorbij (South Africa) in 1997. It was created by crossing Datal and Centennial Seedless (Figure 2). It has large seedless, oblong berries that are light green to straw coloured and with crunchy texture (Image 2), however it does not have any resistance to the major pathogens (Personal communication P. Burger, ARC Infruitec-Nietvoorbij).

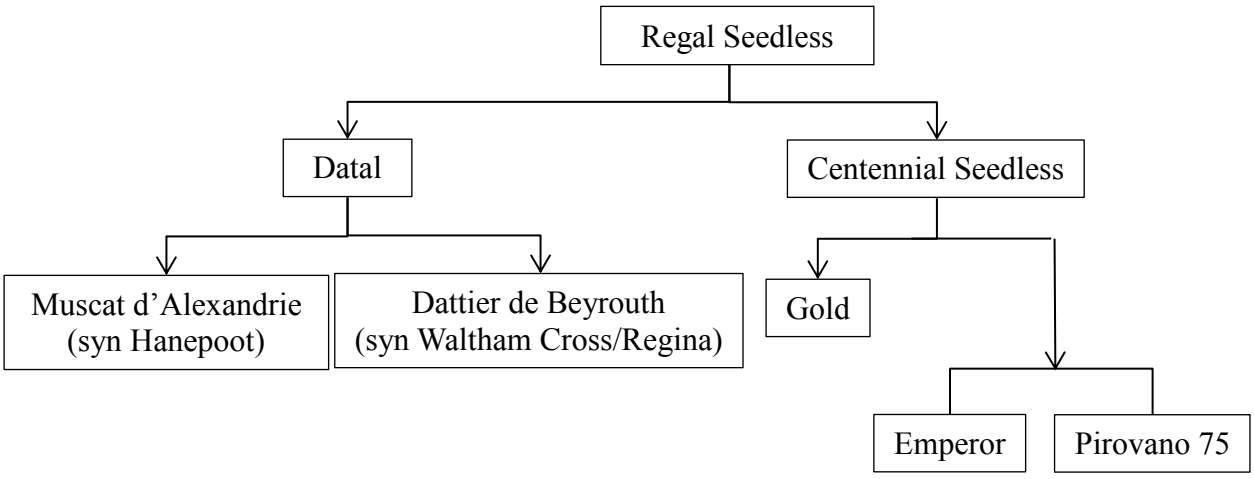

Figure 2 Parentage of Regal Seedless (Personal communication P. Burger, ARC Infruitec-Nietvoorbij, and www.vivc.de)

Image 2 Regal Seedless (photo by P. Burger, ARC Infruitec-Nietvoorbij)

2.2.3 G4-3418

G4-3418 was created in the ARC Infruitec-Nietvoorbij breeding program by crossing Datal and Muscat Seedless (Figure 3). It is late ripening and has large seedless, oblong berries that are light green to straw coloured (Image 3). It does not have resistance to the major pathogens (Personal communication P. Burger, ARC Infruitec-Nietvoorbij).

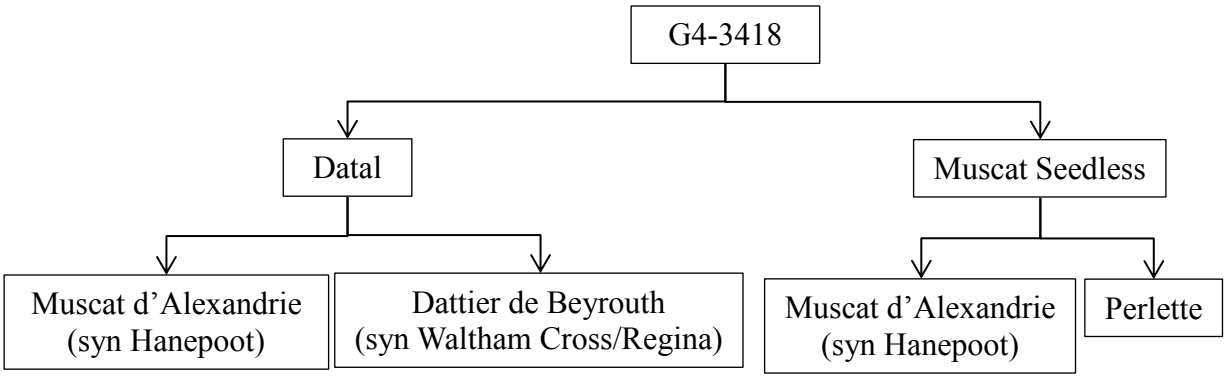

Figure 3 Parentage of G4-3418 (Personal communication P. Burger, ARC-Infruitec-Nietvoorbij)

Image 3 G4-3418 (photo by P. Burger, ARC Infruitec-Nietvoorbij)

2.2.4 Kishmish Vatkana

Kishmish Vatkana is classified as *Vitis vinifera* L. subsp. *vinifera* and is a cultivated grapevine documented from Central Asia since the 1920s. It has very good resistance to powdery mildew (*Erysiphe necator)* that has been linked to a single resistance gene (*REN1*), located on chromosome 13 (Hoffmann et al. 2008). It was found in the Uzbek oasis of Shakhrisabz, just south of Samarqand (Coleman et al. 2009). There is no clarity about the exact origin and parentage but it has a parent-offspring relationship with the seedless table grape Sultanina. The *REN1* gene is not present in Sultanina and must have been transferred from an unknown parent to Kishmish Vatkana. It produces large clusters with anthocyanin pigmented berries that contain the soft remains of aborted seeds (Image 4) (Coleman et al. 2009, www.tilia.zf.medelu.cz).

Kishmish Vatkana did not develop resistance to powdery mildew because of a co-evolution of the pathogen and host plant, but because of stress caused by the pathogen and it is called an abiotic stress adaptation (Kozma et al. 2009). Plants display a secondary line of defence to pathogen infection, the pathogen is allowed to penetrate the plant cells, but the formation of hyphae and conidiophores are restricted. There is also browning of the cell walls and dying off of cells surrounding the infection site. This all prevents the pathogen from developing and spreading and therefore very little damage occurs to the plant. By determining the segregation

ratio of resistant plants to susceptible plants, using phenotypic scores, the *REN1* gene was found to be dominant in Kishmish Vatkana. Microsatellite, or simple sequence repeat (SSR), screening showed that Kishmish Vatkana was heterozygous at the *REN1* locus and it therefore carried the dominant allele for powdery mildew resistance (Hoffmann et al. 2008, Coleman et al. 2009, Kozma et al. 2009).

Hoffmann et al. (2008) placed the location of the *REN1* gene on chromosome 13 in a 7.4 centimorgan (cM) interval (Figure 4). SSR markers spanning a 46.6 cM interval surrounding the position of the gene were used to screen Kishmish Vatkana progeny and it was found that the following markers co-segregated with the trait:

- VMC9H4-2
- VMCNG4E10-1
- UDV-020.

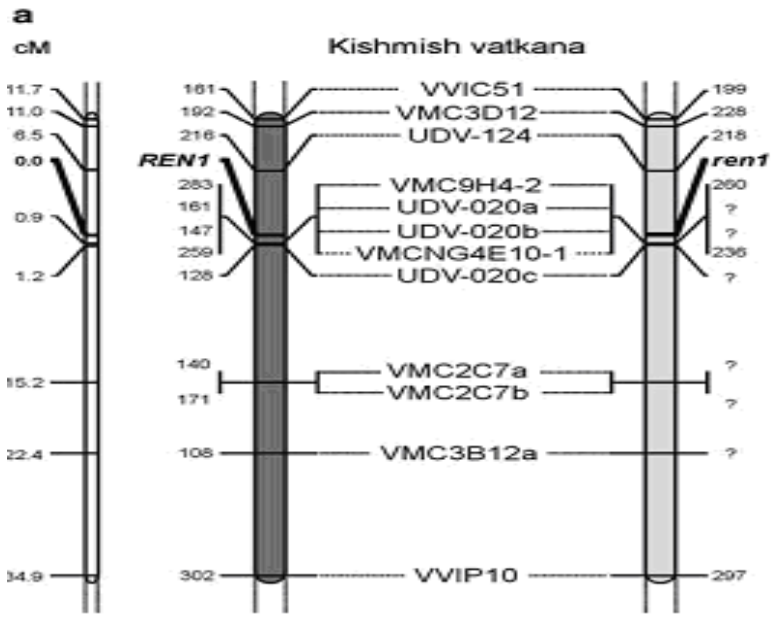

Figure 4 *REN1* locus and surrounding marker positions on distal arm of chromosome 13 (Image from Hoffmann et al. 2008)

Image 4 Kishmish Vatkana (Image from www.tilia.zf.medelu.cz)

2.2.5 Sunred Seedless

Sunred Seedless was released by ARC Infruitec-Nietvoorbij (South Africa) in 1991. It was created by crossing Datal and Ruby Seedless (Figure 5). It has medium sized bunches of seedless, medium sized oval berries with a deep maroon-red colour (Image 5). It does not have any resistance to the major pathogens (Personal communication P. Burger, ARC Infruitec-Nietvoorbij).

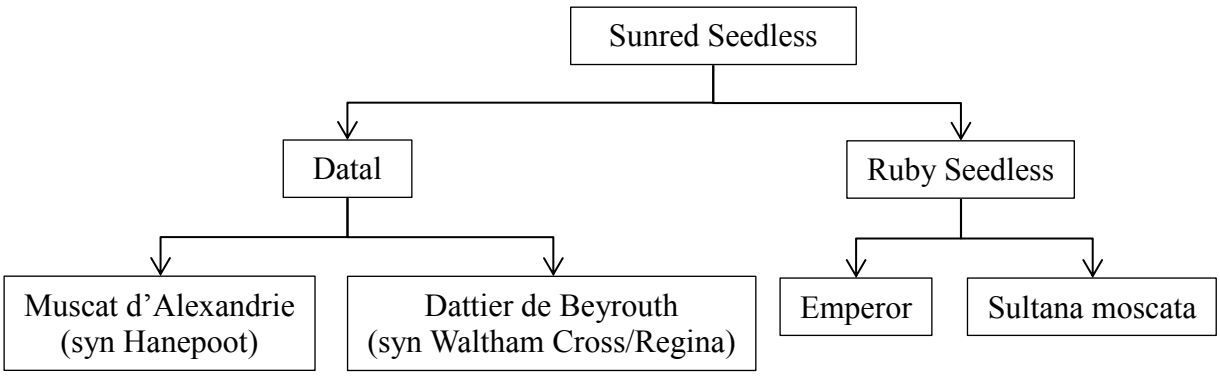

Figure 5 Parentage of Sunred Seedless (Personal communication P. Burger, ARC Infruitec-Nietvoorbij)

Image 5 Sunred Seedless (photo by P. Burger, ARC Infruitec-Nietvoorbij)

2.2.6 Villard Blanc

Villard Blanc is an interspecific crossing of Seibel 6468 and Subereux, created by the breeder Bertille Seyve-Villard (Figure 6) (Akkurt et al. 2007). SV12-375 or Seyve Villard 12-375 are synonyms of Villard Blanc. It is a late ripening, seeded white grape producing medium sized loose clusters of oval berries (Image 6), and is a vigorous grower with resistance to downy and powdery mildew. Villard Blanc has been used extensively in crosses as a resistant parent by various breeders/breeding consortiums (Kozma 2002a, Zyprian et al. 2003, Hajdu et al. 2007, Bellin et al. 2009).

A quantitative trait locus (QTL) associated with downy mildew resistance is located on the distal part of chromosome 18 (Figure 8) while a QTL associated with powdery mildew resistance is located on chromosome 15 (Figure 7) (Zyprian et al. 2003, Akkurt et al. 2007, Bellin et al. 2009).

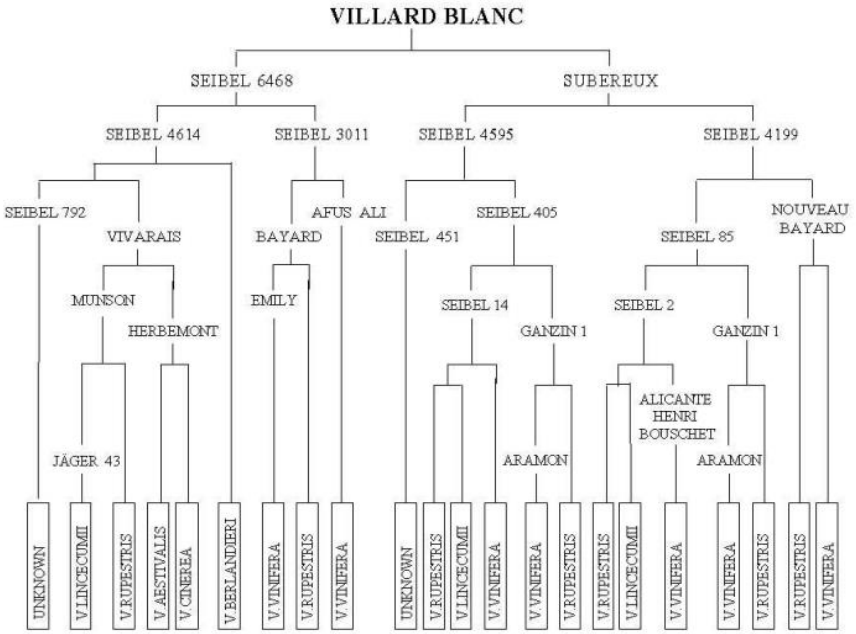

Figure 6 Pedigree of Villard Blanc (Figure from Study of the Use of the Varieties of Interspecific Vines, EU Final Report)

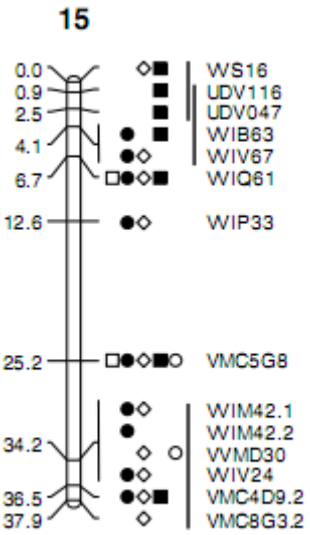

Figure 7 Molecular markers and their positions on chromosome 15 (Doligez et al. 2006)

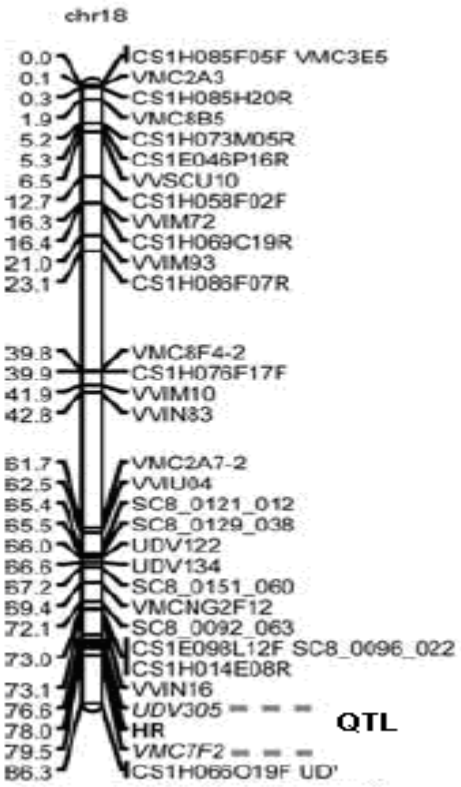

Figure 8 Molecular markers and their positions on chromosome 18. The position of the downy mildew QTL is indicated between markers UDV305 and VMC7F2 (Bellin et al. 2009)

Image 6 Villard Blanc (photo by P. Burger, ARC Infruitec-Nietvoorbij)

2.2.7 G1-6604

G1-6604 was created in the ARC Infruitec-Nietvoorbij's table grape breeding programme by crossing Datal and Dawn seedless (Figure 9). It has large, oblong berries that are light green to straw coloured and has soft rudimentary seeds (Image 7), however it does not have any resistance to the major pathogens (Personal communication P. Burger, ARC Infruitec-Nietvoorbij).

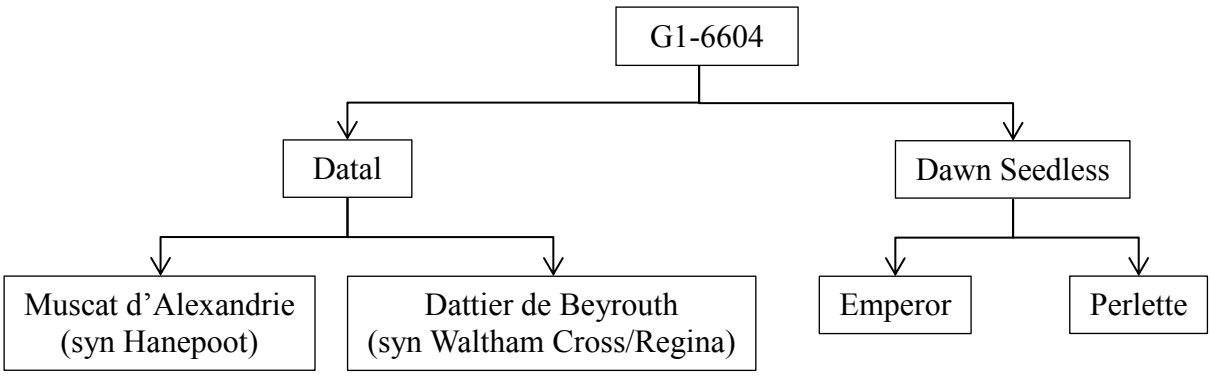

Figure 9 Parentage of G1-6604 (Personal communication P. Burger, ARC Infruitec-Nietvoorbij)

Image 7 G1-6604 (photo by P. Burger, ARC Infruitec-Nietvoorbij)

2.3 Pathogens

2.3.1 Description

Downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator* Syn. *Uncinula necator* and *Oidium tuckeri*) are fungal diseases that infect the photosynthetic tissues like leaves, green stems, inflorescence and berries of almost all species in the *Vitis* genus. It is native to North America and most *Vitis* species that originated there have an inherent resistance to both pathogens. This is because both plant and pathogen have evolved together (Barker et al. 2005, Bellin et al. 2009, Moreira et al. 2011).

2.3.1.1 Downy mildew

Downy mildew is a biotrophic fungus that infects the leaves, shoots and bunches of grapevine. The first noticeable symptom is the appearance of small yellow spots on the upper surface of leaves, called oilspots. These spots have a chocolate halo that disappears as the spot enlarges. Spots can reach a size where it covers the whole leaf or if there are multiple spots on a leaf they will merge to cover the whole leaf surface. For infection to take place leaves need to reach a size where their stomata are active as the fungus enters the plant through its stomata. Very young leaves have very small and widely spaced stomata and this minimizes possible entry points for the fungus. After a warm, humid night the white downy growth, "spores", appear on the underside of these oilspots. Oilspots dry out and become brown, with a yellow border during hot, dry weather. Young shoots that are infected have an oily sheen and turn brown with the appearance of downy growth. These infected sites can die after a period of warm, dry weather. Bunch infection can occur at a very early age and it will develop an oily sheen and turn brown while downy growth will show after a warm, wet period. These infected berries will turn purple, shrink and fall. However, if infection occurred after the berries had reached the size of a pea (resistant to infection), only the stems will be affected as they always stay susceptible. Berry development can be negatively affected though because of a disruption in nutrient delivery (Marais 1981, Magarey et al. 1994, Fourie 2003, Fisher et al. 2007, Gessler et al. 2011).

The disease cycle has four stages. The first stage is the overwintering stage and here oospores are formed in infected tissues during late summer and autumn. They have a thick wall to protect the spores (sexual structures) housed inside. The oospores fall to the ground with leaf fall where they can survive for three to five years. During the second stage, called primary infection the oospores germinate if 10:10:24 conditions are met, rainfall has to be at least 10 mm with temperature being 10°C (or more) for a 24 hour period. Four to eight zoospores are released from a single oospore and these are splashed on to the canopy. In the presence of water and green tissue the zoospores use flagella to swim to stomata where it penetrates the stomata. An incubation period follows and the length is dependent on climatic conditions but is generally between five and ten days. The third stage is the oilspots stage and now the zoospores that have penetrated the stomata start to grow hyphae that grow intra-cellular in the leaf. These areas of growth appear as oily, yellow spots on the upper side of the leaf. The occurrence of these oilspots, due to primary infection, has a very low frequency and is therefore difficult to detect. The last stage is the secondary infection stage and it occurs after warm, humid nights. The fungus forms sporangia producing sporangiophores that grow out of the stomata. The sporangia, containing zoospores, appear as the white down on the underside of the leaf. Sporangia are spread to neighbouring foliage through water (which can be rain, mist or irrigation) and wind. The secondary infection follows the same process as the primary infection to establish the fungus and again release sporangia. The frequency of secondary infection is much higher than with primary infection.

Several cycles of secondary infections can occur in a season if no steps are taken to control it (Marais 1981, Magarey et al. 1994, Fourie 2003, Fisher et al. 2007, Gessler et al. 2011).

2.3.1.2 Powdery mildew

Powdery mildew is a biotrophic fungus that infects the photosynthetic tissues like leaves, green stems, inflorescence and berries of almost all species in the Vitaceae family on a yearly basis. Infections that occur early in the growth season are usually only visible late in the growth season (Halleen 2003). Early leaf infection is difficult to see as it causes a light yellow spot on the upper side of leaves and fungal growth is only visible on close inspection of these spots. With further fungal growth these spots can cover the whole leaf which will take on a

white powdery appearance. Infected shoots show similar small white patches that can grow to cover the whole stem. If the fungus is wiped away the stem shows a brown discoloration where the fungus was present. Severely infected shoots will have stunted growth or die. Bunches are susceptible to infection from before flowering until berries reaches 80 g of sugar per litre of juice. If infection takes places during flowering the flowers will wilt and die. Infection of young berries causes the berries to develop web like patterns before becoming covered in a white powdery growth. Severe infections cause growth retardation, delayed maturity and berry split and larger berries develop brown, hard lesions on the skins. These sites of infection allow access to infections by other pathogens. Berry pedicels that are infected can die and prevent nutrients from reaching the berries (Marais 1981, Magarey et al. 1994, Halleen 2003, Pavlousek 2007, Skinkis 2008, Gadoury et al. 2012).

Powdery mildew infections generally start early in the season but only becomes visible during the warmer, more humid part of the season. The process of infection has two phases an asexual phase and a sexual phase. The asexual phase is visible as the white fungal growth seen on green tissue. The fungus mycelium grows on the plant surface and only penetrates the epidermal cells. Spores formed on the mycelium can be spread through wind to neighbouring leaves/plants. Spore germination occurs during ideal conditions, including temperatures of between 6°C and 33°C and any humidity level except the presence of free water (spores will burst open). Temperatures of 33°C or higher for more than 12 hours can destroy the fungus directly exposed to sunlight. During the growing season the fungus infects developing buds and when new shoots start to form during the next growth season, the shoots and young leaves are already infected. Infected shoots show signs of growth retardation and are called flag shoots. A new cycle of mycelium growth and spore release starts.

During the second or sexual phase the fungus has different mating types, “male” and “female” mycelium. Exchange of genetic material takes place if both types of mycelium come in contact with each other, late in the growth season. The product of mating is cleistothecia (hard, round structures) that contain ascospores. The cleistothecia wash off the leaves and anchor to the bark or falls to the ground during autumn rainfall, where it overwinters. In spring ascospores are released from the cleistothecia; this process is dependent on precipitation and temperature (2.5 mm at a temperature of 10°C or higher). The released

ascospores cause new infections (Marais 1981, Magarey et al. 1994, Halleen 2003, Pavlousek 2007, Skinkis 2008, Gadoury et al. 2012).

2.3.2 Impact of pathogens

Fungal infections of vineyards are a big problem for growers as they incur large costs to prevent and manage infections. A single fungal infection can destroy large crops in a relatively short period of time and as such a preventative spraying strategy is followed to minimize any losses. If a severe outbreak does occur it can lead to huge losses in monetary input, time and income (Fischer et al. 2004, Barker et al. 2005, Tamm et al. 2006, Gadoury et al. 2012).

2.3.2.1 Downy mildew

Downy mildew infections do not usually occur every growth season in a vineyard but it can have devastating effects depending on the severity of the infection as well as the stage in the growth period when it occurs. If the infection occurs early in the growth season there can be multiple secondary infection events that lead to an epidemic in a vineyard (Fourie 2003).

The number of infected leaves on a plant has a direct impact on the level of photosynthesis of a plant and this in turn affects the development of the plant, flowers and bunches because it alters the plant's carbon balance and this negatively affects the plant. A severely affected plant may lose all of its leaves and this exposes bunches to sunburn. The plant will also have a reduction in yield and sugar content of the berries. The effect of the leaf drop may carry through to the following season as the plant's carbon balance is disturbed and not enough carbohydrates needed for new growth at the beginning of the season are assimilated. Young shoots and tendrils that become infected can dry out and die off. This has a negative effect on the formation of leaves, flowers and bunches. Flower clusters, bunches and young berries that are infected can lead to yield loss as there will either be no formation of bunches or the whole bunch can die off. Should an infection occur once the berries reach pea size (five to six mm)

and are resistant, they can still wither and die if the bunch stem is infected and dies (Marais 1981, Magarey et al. 1994, Giuntoli et al. 2000, Fourie 2003, Gessler et al. 2011).

2.3.2.2 Powdery mildew

Powdery mildew in particular is one of the most serious threats as it is not completely dependent on specific temperature and humidity levels for infection and infections occur annually in most vineyards. The timing of the first infection determines the degree of loss experienced as leaves become more resistant when they mature and berries become resistant once they reach a sugar level of 8% per litre of juice (Marais 1981, Halleen 2003).

The effect of a powdery mildew infection in terms of carbon balance, leaf formation, exposure to sunburn and yield is similar to that of downy mildew. Powdery mildew infection during the early fruit formation stage will cause small berries, scarring and berry burst that can lead to sour rot and off-flavours in wine products (Marais 1981, Magarey et al. 1994, Gubler et al. 1999, Halleen 2003, Pavlousek 2007, Skinkis 2008, Dry et al. 2010, Gadoury et al. 2012).

2.3.3 Control measures

There is a lot of pressure on plant breeders to develop resistant varieties because of the public demand for organic and eco-friendly produce. They want a lower impact on the environment and reduce the risk of fungicide use to human health (Barker et al. 2005, Hajdu et al. 2007). An integrated approach of resistant varieties and control of fungicide applications seems to be the way forward.

Fungal management of susceptible varieties can be accomplished by cultural and chemical management. Both are equally important to reduce losses due to fungal infections. Cultural management involves farming practices aimed at reducing the occurrence and spread of the fungus as wind can spread sporangia and this is very difficult to manage (Fourie 2003).

Growers apply weed control, row direction, trellis system, canopy management (ensures adequate air movement in the vineyard and leads to optimum sunlight and spray penetration), irrigation (limit the amount of free moisture) and fertilisation to minimise infections. Chemical management involves the use of systemic and/or contact fungicidal sprays to control fungal growth. Chemical companies release new and more effective fungicides as they become available after a long and expensive evaluation period. Fungicides with a systemic action are very efficient but there is a high level of potential fungal adaptation to these that render them ineffective. Examples of these resistance adaptations are the use of phenylamides against downy mildew and sterol biosynthesis inhibitors against powdery mildew that are no longer as effective as the fungi have developed resistance to these chemicals. The traditional use of copper treatments (downy mildew) combined with the use of sulphur treatments (powdery mildew) are still effective but excessive use will affect the biological activity of soils) and is therefore not desirable because of the negative environmental impact (Boubals 1998, Fourie 2003).

Spraying is a very expensive process as it needs repeated applications (pre and post infection) for prolonged periods to be effective (Barker et al. 2005). Fungicide mixtures with a systemic and contact effect as well as fungicides that control both diseases have been developed. The dosage concentrations, intervals of application and withholding period of fungicide are regulated by the chemical industry and law, as the berries are earmarked for human consumption, but most growers would spray between six and ten times a year.

There are several weather based disease predictor models available to breeders and growers that can be used as warning systems of disease threats, resulting in more effective and cost efficient application of sprays (Magarey et al. 1994, Skinkis 2008).

2.4 Host resistance breeding

2.4.1 Breeding for durable disease resistance

The aim of every grape breeder is to release varieties that are adopted by the growers and consumers because of sought after characteristics. In recent years a demand has developed for grapes that do not only have good growing and fruiting characteristics but also inherent disease resistance. To breed for disease resistance was traditionally a very long and costly process, because of the growing cycle of grapevine and challenges posed by phenotyping methods. The introduction of molecular markers has made it possible to develop various techniques that enable the identification of genes or areas related to disease resistance in grapevine. The ultimate goal of resistance breeding is to combine resistance genes of various origins in a single cultivar, called gene pyramiding, to ensure that pathogens do not easily overcome the resistance and therefore the resistance is termed durable (Lindhout 2002, Boyd 2006, Eibach et al. 2007). Durability was described by Dr Roy Johnson as 'resistance that remains effective when deployed over extensive planting and time, in an environment favourable for the disease' as quoted in Boyd 2006. Extensive research has been performed to determine the loci, genes or QTL for resistance in known resistant varieties and to find molecular markers, such as sequence-characterised amplified region markers (SCARs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs), linked to the resistance locus so that it can easily be tracked in progeny. Once markers linked to a resistance gene or QTL have been identified in a progeny screen only the desired progeny needs to be maintained and this saves the breeder a lot of time and expenses. The selection of progeny by screening for desired traits is called marker-assisted selection (MAS) (Francia et al. 2005). This approach has successfully been applied to the development of SCAR markers linked to powdery mildew resistance (Akkurt 2007) and AFLP markers linked to powdery mildew resistance gene *Run1* (Pauquet et al. 2001).

There are two basic types of disease resistance in plants: (i) monogenic/single gene resistance that generally causes complete resistance and (ii) polygenic/quantitative trait loci (QTL) resistance that is generally incomplete and is caused by multiple genes of partial effect. Single gene resistance is quite easily overcome by pathogens as it often only requires a small evolutionary change on the part of the pathogen that does not have a deleterious effect on it. It

is much more difficult for a pathogen to overcome QTL resistance as it consists of multiple genes that can negatively affect the pathogen and would therefore need multiple evolutionary changes to become resistant (Boyd 2006, Poland et al. 2009).

One of the parental plants used in each of the main crosses used in this study has resistance to powdery and/or downy mildew. The source and position of Pölöskei Muskotály's resistance have not been investigated but is stated in literature that resistance has been observed (Kozma 2002a, Kozma 2002b, Pernesz 2004, Pavloušek 2007). The *REN1* gene, conferring resistance to powdery mildew, is positioned on chromosome 13 of Kishmish Vatkana (Hoffmann et al. 2008). Villard Blanc has resistance to powdery and downy mildew as conferred by a QTL on chromosome 15 (associated with powdery mildew) and one on chromosome 18 (associated with downy mildew) (Akkurt et al. 2007, Bellin et al. 2009, Zyprian et al. 2009).

2.5 Genetic analysis

Grapevine identification and breeding are very difficult and time consuming if a breeder or researcher is solely dependent on phenotypic characteristics for differentiation between varieties. This is because of a large number of factors. There are varieties grown in different geographical areas that are assigned different names (synonyms) or genetically different varieties that have the same names (homonyms). Morphological characteristics are also influenced by the environment, plant growth stage and individual plant biology. In addition, the occurrence of the disease to be screened for is also dependent on the environment and the breeder cannot necessarily evaluate his plots every year for a specific disease phenotype. Grapevine also has a very long reproductive cycle and it can take between seven to 20 years to evaluate results (Hvarleva et al. 2005, Karataş et al. 2007, Vezzuli et al. 2008). The selection of varieties or progeny based on anything but what a breeder or researcher could visually evaluate was not possible at all until the introduction of molecular marker technology (Narain 2010).

Identification and breeding has become a lot easier with the discovery of molecular markers that enable researchers to investigate varieties on a molecular level and in the absence of a pathogen infection. Various markers exist for *Vitis* that can be used for cultivar identification and mapping of desired traits such as restriction fragment length polymorphisms (RFLPs),

AFLPs, single nucleotide polymorphisms (SNPs) and SSRs (Miles et al. 2008). The advantages of SSRs are that they are highly polymorphic, abundant, randomly spread in the genome, co-dominant nature, inherited in a Mendelian manner, robust, neutral, not affected by the environment or developmental stage and reproducible in different laboratories. There are numerous publications available that used SSR markers for the construction of linkage maps and QTL analysis which enables researchers to compare study findings, as was done in part with this study (Sefc et al. 1998, Sanchez-Escribano et al. 1999, Hvarleva et al. 2005). Linkage maps generated using SNP markers are becoming more frequent and as more varieties are sequenced, which is now a possibility since the advent of Next Generation Sequencing, more SSR and SNP markers, as well as various genes, will be discovered (Velasco et al. 2007, Goichoux et al. 2011, Barba et al. 2014).

These advances in molecular science is very beneficial for breeders adopting a molecular approach in their breeding programs as it enables them to identify F_1 progeny that do not have the desired traits at an early stage and eliminate them from the breeding program, thus saving time and money.

2.5.1 Linkage map construction

Linkage maps are valuable tools for researchers as they provide information on the position of molecular markers on the genome of an organism as well as the relative distances between these markers (Collard et al. 2005, Van Os et al. 2005). This information is then useful to make a decision about the merit of using these markers in MAS approaches.

Linkage maps are constructed by calculating recombination events between markers. During meiosis recombination can occur at intervals between the two parental chromosomes. This leads to a progeny with a combination of parental and recombinant genotypes, including molecular markers and genes. If genes and/or markers occur closely together on a chromosome there will be no or few recombination events between them and they will be inherited as a unit. The further apart they are the more recombination events can take place between them. The recombination frequency between markers can be calculated and this gives an indication of the order and distance between the markers in centiMorgan (cM) (Collard et al. 2005). One cM is defined as a distance where 1% of crossovers can occur and it can vary between 10 – 1000 kbp, depending on the genome size of the species (Narain 2010).

This concept that the frequency of crossing over between two genes/markers gives an indication of the distance between them on a map, thus linking a unit to a cross-over percentage, was introduced by Sturtevant in 1913 (Van Os et al. 2005).

In order to construct a linkage map a researcher needs to follow the following steps:

- Create a segregating mapping population where the parents differ for the trait(s) of interest.
- Genotype polymorphic molecular markers in the parents and progeny.
- Perform linkage analysis of the markers using statistical programs.

The number of individuals in a mapping population affects the accuracy and resolution obtained during linkage mapping (Bernardo 2004, Miles et al. 2008). This is because the markers might not be spread evenly across a genome, but could cluster in areas. Recombination frequency is also not equal across chromosomes but so called "hotspots" occur where there are higher occurrences of recombination. It is therefore beneficial to have populations with more than 50 individuals to generate a reliable linkage map (Collard et al. 2005).

Linkage maps for all 19 chromosomes of *Vitis vinifera* have been generated and are populated with various types of molecular markers and genes. These maps are available to all researchers to compare their data/linkage maps to or to add new markers (Grando et al. 2003, Fischer et al. 2004, Di Gaspero et al. 2007).

2.5.2 QTL analysis

There are quite a few agricultural traits, important to breeders, which are not controlled by single genes but instead by many genes. These genes can be spaced throughout the genome of the plant and each contributes partly to the overall trait and is often influenced by the environment. The areas where these genes occur are called quantitative trait loci (QTL). By performing QTL analysis a researcher can identify the position of a gene or a QTL on a

linkage map constructed for a plant. This is done by combining phenotypic data and linkage maps (Kearsey 1998, Collard et al. 2005).

QTL analysis is performed by computer software programs designed to do the statistical calculations. There are three basic methods that are commonly used: single-marker analysis, simple interval mapping and composite interval mapping. The output from these statistical programs will indicate the most likely position of the QTL on the linkage map and also indicate how much of the variation observed can be explained by the QTL (Collard et al. 2005).

The genetic information contained in marker data for markers surrounding a QTL gives interval mapping statistical power. The likely position of a segregating QTL in a genome is determined to generate a QTL likelihood map while the likelihood of a locus with no effect is also determined simultaneously. These two likelihood values are compared and expressed as a logarithm of the odds (LOD) score that is used as a value for detecting QTLs of any significance. The higher a LOD score is at a specific area/point on a linkage map, the more likely it is that it is the position of a QTL, given that a predetermined threshold value is exceeded (Van Ooijen 2004).

If the phenotypic data obtained is plotted on a graph and there is a significant difference between means of the groups and a molecular marker it can be assumed that that marker is linked to a QTL for the trait being investigated. The difference in mean values between groups is expressed as a P value. A P value of less than 0.05 ($P<0.05$) means that there is a significant difference between the groups and thus the chances are better that a marker is linked to the QTL. A P value of more than 0.05 ($P>0.05$) means that there is no significant difference between the groups and it is assumed that a marker is not linked to a QTL (Kearsey 1998, Collard et al. 2005).

2.6 Objectives

The aim of this study was to genetically characterise the fungal disease resistance genes of three different resistance sources of grapevine using molecular marker technology. Different disease resistant varieties were crossed with good fruit quality varieties and the resulting F_1 progenies were investigated for their inherited disease resistance. This study would lay a foundation for the breeder to eliminate F_1 progeny plants, which did not inherit the desired disease resistance, very early in the breeding schedule. This could result in a significant reduction in the resources spent on carrying unsuitable plants through the breeding cycle.

At the start of the study no information was available about the map position of the fungal resistance of Pölöskei Muskotály (PM). Firstly, the project aimed to study the genetic basis of the fungal resistance of PM in the PM x Regal Seedless (RS) and PM x G4-3418 (G4) crosses respectively by using the following approach:

- Identify markers that are polymorphic between the parental lines, PM vs. RS and G4.
- Screen the mapping population with the polymorphic markers to construct linkage maps of all nineteen linkage groups (LGs).
- Determine the phenotype of each individual in the respective F_1 mapping populations to evaluate its resistance to downy and powdery mildew.
- Determine the number of resistance components/loci and their map locations through QTL analysis.

Hoffman et al. (2008) mapped the *REN1* gene in Kishmish Vatkana (KV) to chromosome 13. As a second focus, the project aimed to validate markers in the region of the *REN1* gene and to determine the efficiency of the resistance in South Africa in a Sunred Seedless (SS) x KV cross. This would be done by using the following approach:

- Identify known markers that are polymorphic between the parental lines, SS and KV.
- Screen the F_1 mapping population with the polymorphic markers to construct a partial linkage map for chromosome 13.
- Determine the phenotype of each individual in the mapping population to evaluate its resistance to powdery mildew.
- Confirm the presence of a resistance component and its map location through QTL analysis.

Villard Blanc (VB) is widely documented as a cultivar with strong resistance to fungal disease. It has been used in various breeder crosses to transfer resistance to progeny plants. QTLs and resistance loci to powdery and downy mildew were mapped on chromosome 15 and chromosome 18 (Bellin et al. 2009, Zyprian et al. 2009). Lastly, the project thus aimed to validate markers in these chromosome regions and determine the efficiency of the resistance in the VB x G1-6604 (G1) cross by using the following approach:

- Identify markers on chromosome 15 and chromosome 18 that are polymorphic between the parental lines, VB and G1.
- Screen the F_1 mapping population with the polymorphic markers to construct partial linkage maps of chromosome 15 and chromosome 18.
- Determine the phenotype of each individual in the mapping population to evaluate its resistance to downy and powdery mildew.
- Confirm the presence of resistance loci and map their locations through QTL analysis.

In summary there were three objectives in the study. Firstly to study the genetic basis of PM's fungal disease resistance, to construct linkage maps for all 19 chromosomes and determine the number and location of resistance components/loci. Secondly SSR markers known to be linked to the *REN1* gene were validated in a local KV F_1 population, to construct a linkage map of chromosome 13 and perform QTL analysis. Lastly markers located on chromosome 15 and 18 of VB were validated and partial linkage maps constructed. QTL analysis was done to identify possible QTLs contributing to fungal disease resistance.

Chapter 3

Materials and Methods

3.1 Assistance by co-workers

P. Burger (plant breeder, ARC Infruitec-Nietvoorbij) created all the mapping populations and made them available for use in this study. The management of these populations with regards to their nutritional needs and maintenance in the hot house and plant tunnels was done by her.

D Snyman (CenGen, Worchester) provided technical assistance by performing all DNA extractions and PCR reaction set-ups according to instructions provided after evaluating molecular data generated. Fourteen of the eighteen PCR multiplexe marker combinations employed in this study were developed by Mr CJ van Heerden (PhD thesis to be submitted). They are indicated in each section where they were used.

A Vermeulen (Plant Pathologist, ARC Infruitec-Nietvoorbij) assisted with the collection of samples for phenotypic scoring, infection of samples and the phenotypic scoring of samples.

3.2 Strategy of study

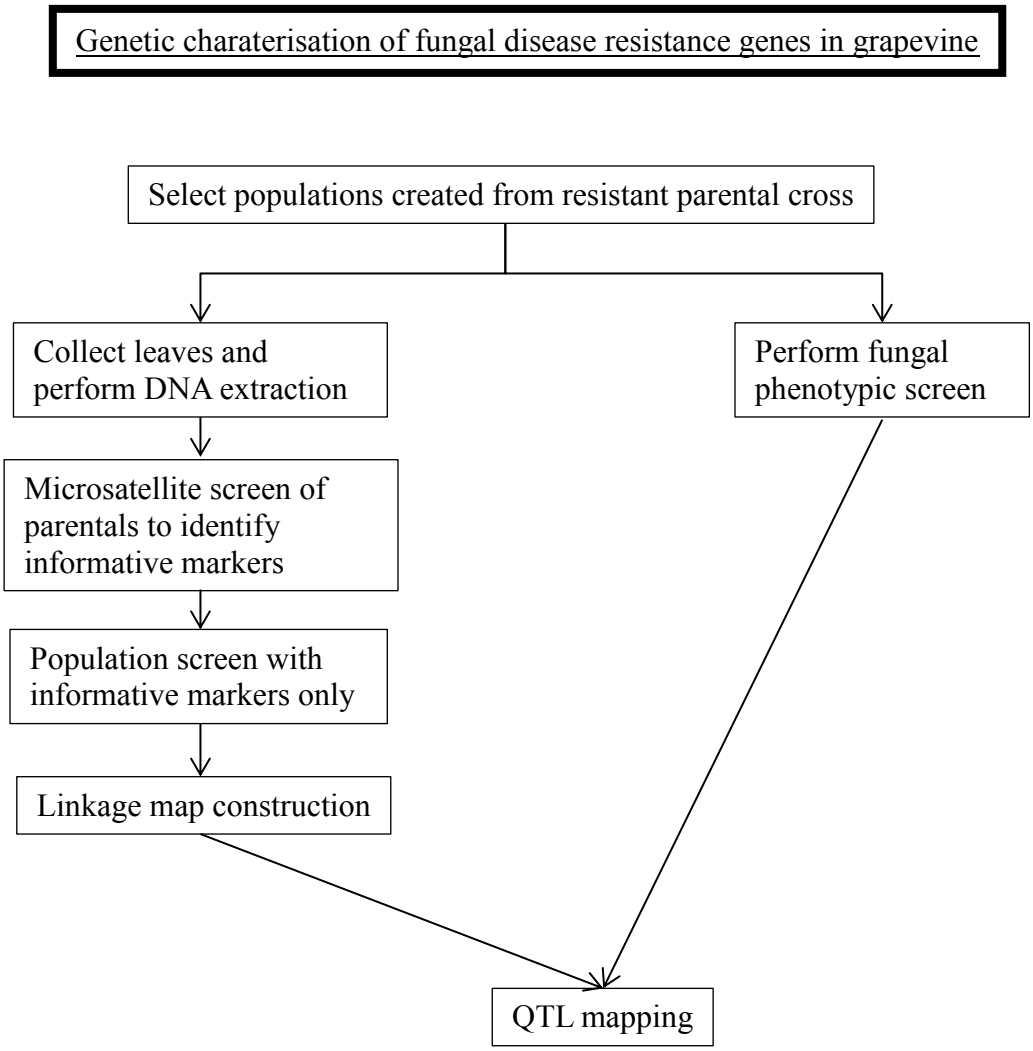

Figure 10 Schematic representation of the study flow

3.3 Plant material

Various plant populations (Table 1) were considered and/or used in this study in order to achieve the objectives set out (Figure 10). The populations were created at the ARC Infruitec-Nietvoorbij, Stellenbosch by crossing parental plants known to have desirable traits (fruit quality and fungal disease resistance) and using the resulting F_1 populations for QTL mapping. The parental plants with the fungal disease resistance were:

- Pölöskei Muskotály – downy and powdery mildew resistance.
- Kishmish Vatkana – powdery mildew resistance.
- Villard Blanc – downy and powdery mildew resistance.

The fungal disease resistance information available on the parental plants had not been validated in South African populations although it was not expected to differ from findings by other researchers/research groups.

PM x RS and PM x SS crosses were made in 2007 and 2008. The seeds were extracted from the berries and stratified before germination in September 2008 in order to establish plants that were used for the molecular and inoculation studies in 2009. The PM x G4 cross was made in 2006 and seeds were extracted from the berries and stratified before germination in September 2007 in order to establish plants that were used for the molecular and inoculation studies in 2009. Young leaves that had fully opened were collected from the resulting PM x RS F_1 population in April 2009 and DNA was extracted. Leaves from the PM x G4 and PM x SS F_1 populations were collected in June 2009 and DNA was extracted.

A SS x KV cross was made in 2008 and plants were developed by embryo rescue techniques in order to establish plants that were used for the molecular and inoculation studies in 2009/2010. Young leaves that had fully opened were collected from the resulting F_1 population in October 2009 and DNA was extracted.

A VB x G1 cross was made in 2008. Extracted seeds were stratified and germinated in 2009 in order to establish plants that were used for the molecular and inoculation studies in 2010. Fully opened young leaves were collected from the resulting VB x G1 F_1 population in April and May 2010 and DNA was extracted.

Table 1 Number of offspring per population used in the study.

Population	Number of plants
Pölöskei Muskotály (PM) x Regal Seedless (RS)	124 F_1 plants
Pölöskei Muskotály (PM) x G4-3418 (G4)	16 F_1 plants
Pölöskei Muskotály (PM) x Sunred Seedless (SS)	14 F_1 plants
Sunred Seedless (SS) x Kishmish Vatkana (KV)	158 F_1 plants
Villard Blanc (VB) x G1-6604 (G1)	250 F_1 plants

3.4 Phenotypic screening

3.4.1 Downy mildew

The downy mildew screen was only performed on the F_1: VB x G1 population using a leaf disc assay (Brown et al. 1999). Two leaves were collected between the fourth and sixth nodes from each plant and taken to the laboratory in numbered paper envelopes. The leaves were rinsed in a 10% bleach solution prior to handling in the laboratory to remove all pathogens. Six leaf discs of one cm diameter were excised from each leaf and floated, upper side up, on water in a Petri dish. The six discs per leaf were divided between two Petri dishes so that duplicates were generated to confirm phenotypic scores. Each leaf disc was inoculated with a 60 µl drop of downy mildew suspension. The downy mildew suspension's concentration was calculated as 1.325×10^5 sporangia/ml by the resident plant pathologist after examining the suspension under a microscope. The Petri dishes were left uncovered for six hours to allow the drop to dry on the leaves before the lids were put on. The leaves were then left for five to seven days in a laboratory where a natural light cycle and constant temperature (24°C) was maintained.

When downy mildew growth showed on the discs they were scored manually using a microscope to determine the level of development of the pathogen. Scoring was done according to criteria of the "Office International de la Vigne et du Vin" (OIV score) for leaf discs (Table 2) (Organisation Internationale de la Vigne et du Vin (OIV) 2009).

Table 2 Degree of downy mildew resistance based on the OIV452-1 index (Organisation Internationale de la Vigne et du Vin (OIV) (2009, Bellin et al. 2009).

Class[a]	**OIV descriptor for resistance**	**Sporulation**[b]
1	Very little	Sporagiophores densely cover the whole disc area.
3	Little	Predominant patches of dense sporulation.
5	Medium	Patches of sparse sporulation equally intermixed with asymptomatic areas.
7	High	Small spots with sparse sporangiophores.
9	Very high	Absence of sporangiophores.

[a] Classification of disease resistance based on fungal sporulation levels

[b] Fungal sporulation levels

OIV scores between five and nine were considered to be resistant to downy mildew while scores of one and three were considered to be susceptible to downy mildew. The obtained scores were converted to a single tab delimited file, suitable for subsequent QTL mapping analysis and missing values were replaced by an asterisk (*).

3.4.2 Powdery mildew

The powdery mildew screen was performed on a whole leaf and the whole plant for the F_1: SS x KV and F_1: VB x G1 populations.

For the whole leaf score two leaves were collected between the fourth and sixth nodes from each plant and taken to the laboratory in numbered paper envelopes. Pathogens were removed by rinsing the leaves in a 10% bleach solution prior to handling in the laboratory. The leaves were floated on water in a Petri dish and each leaf was inoculated with a few drops of powdery mildew suspension. The lids were put on the Petri dishes after they were left uncovered for six hours to allow the drop to dry on the leaves. The leaves were then left for five to seven days in a laboratory where a natural light cycle and constant temperature (24°C) was maintained.

When powdery mildew growth showed on the leaves they were scored manually using a microscope to determine the level of development of the pathogen. Scoring was done according to criteria of the "Office International de la Vigne et du Vin" (OIV score) for whole leaves and whole plants (Table 3) (Organisation Internationale de la Vigne et du Vin (OIV) (2009).

Table 3 Degree of powdery mildew resistance based on the OIV455 index (Organisation Internationale de la Vigne et du Vin (OIV) (2009, Pavloušek 2007).

Class[a]	**OIV descriptor for resistance**	**Infection**[b]
1	Very low	Very strong leaf infection, almost all leaves are attacked.
3	Low	Leaves are almost covered with mycelium, most of the leaves are infected.
5	Medium	Infected leaves have small rounded spots, medium level of leaf infection.
7	High	Bright spots on leaves and small necrotic spots, single leaves infected.
9	Very high or total	Absence of disease symptoms.

[a] Classification of disease resistance based on fungal infection levels

[b] Fungal infection levels

For the whole plant score infected plants were randomly inserted between the F_1 population in the breeding tunnels. After two weeks the plants were inspected to evaluate if the pathogen infection had spread effectively. Once it was determined that the infection was well established in the population the whole plant was scored as well as two leaves between the fourth and sixth nodes from each plant. The scoring was done manually according to the OIV 455 values in Table 3. OIV scores between five and nine were considered to be resistant to powdery mildew, while scores of one and three were considered to be susceptible to powdery mildew. The obtained scores were converted to a single tab delimited file, suitable for subsequent QTL mapping analysis and missing values were replaced by an asterisk (*).

3.4.3 Correlation of phenotypic scores

The obtained scores were converted to comma separated files that were imported into the software program R (R Development Core Team 2011) to evaluate the correlation between the scores (*rho* value) as well as the significance of the correlation (P values), if found. Two statistical tests, available within R, were applied to the datasets. The Pearsons test assumes that the relationship between the datasets is linear while the Spearman test does not have any assumption about the frequency distribution of all variables or a linear relationship between datasets.

To interpret the *rho* values obtained from both tests the following guidelines were used:

- values between 0.9 to 1 - the correlation was very strong
- values between 0.7 and 0.89 - correlation was strong
- values between 0.5 and 0.69 - correlation was moderate
- values between 0.3 and 0.49 - correlation was moderate to low
- values between 0.16 and 0.29 - correlation was weak to low
- values below .16 - correlation was too low to be meaningful.

3.5 Molecular techniques

3.5.1 DNA Extraction

A cetyltrimethylammonium bromide (CTAB) extraction method (Doyle et al. 1990) was used to obtain good quality DNA from leaf material of the various populations (Table 1).

Leaves were collected from labelled specimens shortly after bud break, during the time of active shoot elongation from 2008 to 2010. Care was taken to only pick leaves that were fully open but as close as possible to the shoot tip. They were placed in labelled plastic bags and transported in a cooler box to the laboratory for extraction. There was some difficulty in getting leaves from the F_1: PM x RS and F_1: PM x G4 populations as the plants grew very

poorly (Image 8). No pathogen or pests were identified as the cause of this weak growth. Soil and plant samples from the F_1: PM x RS population was sent to a soil testing facility for analysis to determine if toxicity of a nutrient(s) could cause the slow growth. Boron levels were elevated in the plants, but they were not at toxic levels.

Image 8 PM x RS offspring late in growth season (photo by P. Burger, ARC Infruitec-Nietvoorbij)

A one cm piece of leaf was cut and transferred to labelled 2.2 ml Eppendorf safe lock tubes (Eppendorf, RSA). Cleaning of the punch and tweezers used was done between samples to prevent any transfer of leaf material between different samples. Two metal beads with a diameter of three mm were also placed in the tube with the leaf material. A CTAB DNA extraction was performed. The 2.2 ml tubes, with the leaf material and metal balls, were placed in a Qiagen TissueLyser (Retsch GmbH, Germany) and shaken at 30 MHz for two to four min, until the leaf material was finely ground. 750 µl of 2% CTAB extraction buffer [100 mM Tris (pH 8.0); 20 mM ethylene-diaminetetraacetate (EDTA); 1.4 mM NaCl; 10% (w/v) CTAB; with the addition of 0.2% ß-mercaptoethanol] was added and kept at 65 ºC for sixty min while mixing every ten min. 500 µl of 24 Chloroform:1 Isoamylalcol was added, mixed well and then centrifuged at 12 000 RCF for five min. The upper phase was transferred to a 1.5 ml microtube and 500 µl Isopropanol was added before leaving it at room temperature for twenty min. The sample was centrifuged at 12 000 RCF for five min and supernatant discarded. 500 µl of 70% EtOH was added and then left at room temperature for twenty min

before it was centrifuged at 12 000 RCF for five min. The supernatant was discarded and the pellet left to air dry. Once the pellet was dry 200 µl of TE [10 mM Tris-Cl (pH 8.0); 1 mM EDTA (pH 8.0)] was added and then left at 4ºC overnight.

The following morning 20 µl of ammonium acetate (7.5M NH4OAc) and 200 µl of 24 Chloroform:1 Isoamylalcol was added before spinning the tubes in a centrifuge at 12 000 RCF for five min. The supernatant was transferred to a 1.5 ml microtube and 500 µl of 100% EtOH added before it was left for two hours at -20ºC. Centrifugation at 12 000 RCF for fifteen min performed and the supernatant discarded. 500 µl of 70% EtOH was added and centrifuged at 12 000 RCF for ten min after which the supernatant was discarded again. 500 µl of 70% EtOH was added and centrifuged at 12 000 RCF for ten min after which the supernatant was discarded before the pellet was left to air dry. 50 µl of TE [10 mM Tris-Cl (pH 8.0); 1 mM EDTA (pH 8.0)] was added when the pellet was dry.

The DNA concentration was determined with a spectrophotometer (NanoDrop® ND-100, Nanodrop Technologies Inc., Wilmington, Delaware, USA) and a dilution of 30 ng/ul was prepared for PCR applications. The rest of the extracted DNA was stored at -20ºC.

3.5.2 Amplification

The markers chosen for each objective varied depending on whether a genome scan where all/selected linkage groups are covered was required or whether a specific gene/QTL region was targeted. Molecular markers were identified and primer sets, with the forward primer (5'-3') fluorescently labelled, were ordered from Life Technologies, South Africa. Care was taken during this process to ensure that markers were labelled in such a way that it could be combined in multiplex PCR reactions.

For all the PCR reactions the set-up in Table 4 was used with the addition of the appropriate multiplex primers, either as determined by Mr CJ van Heerden (for PM and VB populations) (PhD thesis to be submitted) or in this study (KV population). All markers were searched for on the National Centre for Biotechnology Information (NCBI) Map Viewer webpage (www.NCBI.nlm.nih.gov) and their presence on the *Vitis* genome confirmed as well as their

chromosome position. The unknown primer sequences for the chromosome 13 markers, to be used in the SS x KV study, were also obtained in this way. The majority of the markers used were originally developed by:

- The Vitis Microsatellite Consortium – VMC
- Bowers et al. (1996 and 1999) – VVMD
- Sefc et al. (1998) – VrZAG
- Merdinoglu et al. (2005) – VVI (Grando et al. 2003, Moreira et al. 2011).

Table 4 PCR multiplex reaction set up with consumables used and quantities of each.

PCR multiplex set up	**Stock**	**Final [ ]**	**Volume**
Buffer (Anatech, RSA)	10x	1x	1 µl
dNTP (Kapa Biosystems, RSA)	5 mM	5 mM	0.4 µl
25 mM $MgCl_2$ (Anatech, RSA)	25 mM	1.8 mM	0.72 µl
Supertherm Taq (Anatech, RSA)	5 U/µl	0.75 U	0.15 µl
Multiplex appropriate primers	10 pmol/µl	0.3 pmol	0.2 µl of each forward and reverse primer
DNA	30 ng/µl		1 µl
ddH_2O			Add sufficient water to make up the total volume of 10 µl
Total			10 µl

Samples were amplified using the GeneAmp PCR system 9700 and the Veriti 96-well thermo cycler (Life Technologies, RSA). All amplification cycle reactions were performed at an initial denaturation of 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing for 10 sec and extension at 72°C for 15 sec. All cycles were concluded with a final extension step at 72°C for 30 min before cooling down to a 4 ºC holding step. The annealing temperature was appropriate to each multiplex and is indicated in Tables 5, 6, 7 and 8 respectively.

3.5.2.1 Pölöskei Muskotály

Five multiplex PCR reactions (27 SSRs) were used to screen the parental plants Pölöskei Muskotály and Regal Seedless. The F_1: PM x RS population was subsequently screened with the twenty SSR markers in multiplex 1, 3, 4 and 11 (Table 3). One multiplex PCR reaction (seven SSRs) was used to screen the F_1: PM x G4 and F_1: PM x SS populations, the screened markers are listed in Table 5 as Multiplex 1. This was done as the first step in a genome scan to identify polymorphic markers for use in linkage map construction (Table 3). The markers were selected by Mr CJ van Heerden (PhD thesis to be submitted).

Table 5 SSR markers used in PM x RS, PM x G4 and PM x SS population screens.

Multiplex[a]	Marker[b]	Forward primer sequence (5'–3')	Reverse primer sequence (3'-5')	Source	LG[c]	Label colour[d]	Tm
Multiplex 1	VrZAG21	tcattcactcactgcattcatcggc	ggggctactccaaagtcagttcttg	Sefc et al. (1998)	4	NED	
	VrZAG25	ctccacttcacatcacatggcatgc	cggccaacatttactcatctctccc	Sefc et al. (1998)	10	FAM	
	VrZAG47(VVMD27)	ggtctgaatacatccgtaagtatat	acggtgtgctctcattgtcattgac	Sefc et al. (1998)	5	FAM	
	VrZAG62	ggtgaaatgggcaccgaacacacgc	ccatgtctctcctcagcttctcagc	Sefc et al. (1998)	7	VIC	52ºC
	VrZAG79	agattgtggaggagggaacaaaccg	tgcccccattttcaaactcccttcc	Sefc et al. (1998)	5	VIC	
	VrZAG83	ggcggaggcggtagatgagagggcg	acgcaacggctagtaaatacaacgg	Sefc et al. (1998)	4	PET	
	VVMD7	agagttgcggagaacaggat	cgaaccttcacacgcttgat	NCBI	7	PET	
Multiplex 3	UDV-108	tgtagggttccaaagttcagg	gccttttatatgtggtggagca	NCBI	18	FAM	
	VMC8B5	aaaggagacatctgcatcat	gccttgatcttccttctaat	Vitis Microsatellite Consortium	18	NED	52ºC
	VVIM93	caacgtttattgtaagagcctc	gcttagcttgctagaaacttga	Merdinoglu et al. (2005)	18	PET	
	VVMD17	tgactcgccaaaatctgacg	gcacacatatcatcaccacacgg	Bowers et al. (1999)	18	VIC	
Multiplex 4	VMC7F2	aagatgacaatagcgagagagaa	gaagaaagtttgcagtttatggtg	Vitis Microsatellite Consortium	18	PET	
	VVIT68	gggttgtttcgtgtattgtatg	gtgaatgaacaaagtgggaaag	Merdinoglu et al. (2005)	5	FAM	52ºC
	VVIU04	ccatgtgaacccaggacatac	ccctgaccacagaagctaaac	Merdinoglu et al. (2005)	18	FAM	
	VVIV16	acaaaagcggaaacgatcgaat	gagaagacctatttttcctgtgg	Merdinoglu et al. (2005)	18	VIC	

Table 5 continued

Multiplex[a]	Marker[b]	Forward primer sequence (5'–3')	Reverse primer sequence (3'-5')	Source	LG[c]	Label colour[d]	Tm
Multiplex 11	VMC2A3	attgaaactccggaagcttagg	gcttcgtgtagaagcttcacaggt	Vitis Microsatellite Consortium	18	PET	
	VMC2B1.1	ggcacatgagcgattacatttc	gtgagctttgtgtgcacattttc	Vitis Microsatellite Consortium	18	NED	
	VMC3E5	gatttgtctttacaaggcgttc	gccaggagacttgctttgtattt	Vitis Microsatellite Consortium	18	FAM	52ºC
	VMC8F4.2	gcgtaaagcatattcaagcatt	gaagttagcgcagatgaaagat	Vitis Microsatellite Consortium	18	VIC	
	VVIN16-CJVH	cccgcccttcctatttgta	gaagccaatgaaagaagaattaaca	Van Heeden C PhD thesis	18	FAM	
Multiplex 24	VMC1A5	tcacacaattctcccatgaaatag	gaacaagttggcatgttggtta	Vitis Microsatellite Consortium	3	FAM	
	VVMD8-cjvh	ccagtgtgggtcacttgtgt	ggatcacctacagacagtccaa	Van Heeden C PhD thesis	11	PET	
	VVC62	tgggattaacacggacttctt	gtggctaagctagccctgta	NCBI	14	NED	
	VVIN74-cjvh2	tggcataactttgatgggtaaa	gtcacccttgtttcactccagta	Van Heeden C PhD thesis	19	PET	57ºC
	UDV047	tgtatgataatccataatgtgc	gtaggcatgcttgacttattc	NCBI	15	VIC	
	VMC1G3.2-cjvh	tcatcgctttccaaacataat	gacttagcttcagaagaaaataga	Van Heeden C PhD thesis	12	NED	
	VMC2H4	accaggtgtgcctataagaatc	gtctctggaacatccaatcaac	Vitis Microsatellite Consortium	12	VIC	

[a] Multiplex reaction number

[b] SSR marker name

[c] Linkage group that SSR marker is positioned on

[d] Fluorescent label colour

3.5.2.2 Kishmish Vatkana

Four multiplex PCR reactions (13 SSRs) spanning the *REN1* locus (Hoffmann et al. 2008) were used to screen the F_1: SS x KV population. The nine SSRs listed by Hoffmann et al. (2008) were used as well as an additional four SSRs identified on chromosome 13 by Mr CJ van Heerden as part of his project. The markers selected had to be optimised for marker combinations per multiplex as well as annealing temperature. Various marker combinations were tested at annealing temperatures ranging between 56ºC and 63ºC. For the optimised reaction set-up all multiplex PCR reactions were performed separately, but the products were pooled in equal volumes for multiplex 1 and 2 as well as multiplex 3 and 4 (Table 6). The reason for this was that the annealing temperatures differed but the markers could be electrophoresed together to save costs.

Table 6 SSR markers used in SS x KV population screen.

Multiplex[a]	Marker[b]	Forward primer sequence (5'–3')	Reverse primer sequence (3'-5')	Source	LG[c]	Label colour[d]	Tm
Multiplex 1	UDV020	tgttggtgtgtgtttgtacgtg	tgttggcctgatgttgagag	NCBI	13	FAM	57ºC
	UDV038	cccaagatgaaaaccaagaga	gaaataaggccttgtaccacttg	NCBI	13	VIC	
	VMC3D12	cgacatgatccgagtctacc	ggtctcccatctccatcac	Vitis Microsatellite Consortium	13	NED	
Multiplex 2	UDV124	gcatcttcttcttcccaacc	gagtgcatttgtcaaagtcgtg	NCBI	13	PET	56ºC
	UDV129	aagctaaggtcttatggcatctg	tttctagatgctgacttctcaagtg	NCBI	13	VIC	
	VMC3D8	aaaccaaacggaaaaat	accttccctttcaatca	Vitis Microsatellite Consortium	13	PET	
	VMC9H4-2	cacatcattcattgatgaggct	gcagttgatgcaaaacaacagt	Vitis Microsatellite Consortium	13	PET	
	VVIP10	tgccttgacattgttttcatcc	gaaactgggctgttattgttga	Merdinoglu et al. (2005)	13	FAM	
Multiplex 3	VVIC51	ctttgaagcacaaaatcgagct	accaaagggaagcaaaagaaaa	Merdinoglu et al. (2005)	13	NED	59ºC
	VMCNG4E10.1	aatgcagcagcgccagatg	gcaggctgctgctgtttg	Vitis Microsatellite Consortium	13	VIC	
	VMC2C7	tgggatgatgattattgggatg	ataaggcaggttgattcaagga	Vitis Microsatellite Consortium	13	FAM	
	VMC3B12a	ataaggcaggttgattcaagga	catcacaggttgattcgacact	Vitis Microsatellite Consortium	13	PET	
Multiplex 4	VMC2A9	acaaccacccaatgctacaa	gctgcaggtttggaagatta	Vitis Microsatellite Consortium	13	VIC	63ºC

[a] Multiplex reaction number

[b] SSR marker name

[c] Linkage group that SSR marker is positioned on

[d] Fluorescent label colour

3.5.2.3 Villard Blanc

Regent is a close relative to Villard Blanc as they share ancestry and we believe that they have the same QTLs that confer resistance. The selected SSR markers were positioned around the chromosome regions on chromosomes 15 and 18 where the QTLs for powdery and downy mildew were identified in Regent. Twenty-three SSRs were previously identified for chromosome 15 and chromosome 18 of Regent, by Mr CJ van Heerden (PhD thesis to be submitted). The two parental varieties were screened with the identified markers and the informativeness of each marker was evaluated before they were used to screen the F_1: VB x G1 population.

Three multiplex PCR reactions, containing eleven SSRs, were used to screen chromosome 15 and four multiplex PCR reactions, containing twelve SSRs, were used to screen chromosome 18 (Table 7 and Table 8).

All of the chromosome 18 multiplex PCRs were done separately but the products from multiplexes 2, 2-1 and 2-2 were pooled in equal ratios. The reason for this was that the annealing temperatures differ but the markers could be electrophoresed together to save costs.

Table 7 SSR markers used for chromosome 15 in the VB x G1 population screen.

Multiplex[a]	Marker[b]	Forward primer sequence (5'–3')	Reverse primer sequence (3'-5')	Source	LG[c]	Label colour[d]	Tm
Multiplex 1_3	VVIB63	agtccaacactgcacagataa	gcgagagaaatgtggaggagta	Merdinoglu et al. (2005)	15	NED	58ºC
	VMC8G3.2	gggcggagatttaacagtca	gcattgtgccattggatcttg	Vitis Microsatellite Consortium	15	VIC	
	VVIM42b	taccagcactggcaataaca	gtggaaacagccatgttcata	Merdinoglu et al. (2005)	15	PET	
	VVIM42a	tgacatcctcaacgaggtaag	gattggacttctcccctaaga	Merdinoglu et al. (2005)	15	FAM	
	VVIQ61	tgtaactgctaatctttctggg	ggaacaatgctggataagatga	Merdinoglu et al. (2005)	15	VIC	
	VVIP33	aaacaatgctgttaacctggat	gagggggtgtttagtaatttcaa	Merdinoglu et al. (2005)	15	NED	
Multiplex 2_4	UDV047	tgtatgataatccataatgtgc	gtaggcatgcttgacttattc	NCBI	15	VIC	56ºC
	VMC5G8	gcacatgcacatcttgtttc	gcctcctatgccctttgtgta	Vitis Microsatellite Consortium	15	NED	
	UDV116	caccacttcttcaagtcccact	gaagatttcatgcaccctaatga	NCBI	15	FAM	
Multiplex 3_5	VVIV24	gactaaaaaccaaagctactgt	gagcacgcatttcatctgaattt	Merdinoglu et al. (2005)	15	NED	55ºC
	VChr15a	caatcccaacagttccatga	cgttttctccttcggacaag	NCBI	15	FAM	

[a] Multiplex reaction number

[b] SSR marker name

[c] Linkage group that SSR marker is positioned on

[d] Fluorescent label colour

Table 8 SSR markers used for chromosome 18 in the VB x G1 population screen.

Multiplex[a]	Marker[b]	Forward primer sequence (5'–3')	Reverse primer sequence (3'-5')	Source	LG[c]	Label colour[d]	Tm
Multiplex 1	UDV134	ttccatggtgaatgcattagt	gggattacttggtcgtattttatgt	NCBI	18	VIC	57ºC
	VMC6F11	acaactttgtgctgccactacc	agccagagttactatgctgcca	Vitis Microsatellite Consortium	18	PET	
	UDV108	tgtagggttccaaagttcagg	gccttttatatgtggtggagca	NCBI	18	NED	
	VMC7F2	aagatgacaatagcgagagagaa	gaagaaagtttgcagtttatggtg	Vitis Microsatellite Consortium	18	PET	
Multiplex 2	VVIP08	gaataagagaggggcaatacta	gaggaacaagaagcttgaagact	Merdinoglu et al. (2005)	18	FAM	50ºC
	VVIR09	aagtgtgtttgactccagaaaa	actgatcaaacttctctagaga	Merdinoglu et al. (2005).	18	NED	
Multiplex 2-1	VVMD17	tgactcgccaaaatctgacg	gcacacatatcatcaccacacgg	Bowers et al. (1999)	18	VIC	62ºC
Multiplex 2-2	VVIN16-cjvh	cccgcccttcctatttgta	gaagccaatgaaagaagaattaaca	Van Heeden C PhD thesis	18	FAM	51ºC
Multiplex 3	VMC8B5	aaaggagacatctgcatcat	gccttgatcttccttctaat	Vitis Microsatellite Consortium	18	NED	52ºC
	VVIM93	caacgtttattgtaagagcctc	gcttagcttgctagaaacttga	Merdinoglu et al. (2005)	18	PET	
Multiplex 4	VMC3E5	gatttgtctttacaaggcgttc	gccaggagacttgctttgtattt	Vitis Microsatellite Consortium	18	FAM	52ºC
	VMC8F4-2	gcgtaaagcatattcaagcatt	gaagttagcgcagatgaaagat	Vitis Microsatellite Consortium	18	VIC	
	VMC2A3	attgaaactccggaagcttagg	gcttcgtgtagaagcttcacaggt	Vitis Microsatellite Consortium	18	PET	

[a] Multiplex reaction number

[b] SSR marker name

[c] Linkage group that SSR marker is positioned on

[d] Fluorescent label colour

3.5.3 Capturing genotype data

The amplified PCR products were electrophoresed at the Central DNA Sequencing Facility at Stellenbosch University (www.sun.ac.za/saf). PCR purification was done using a NucleoSpin® 96 Extract II PCR purification Kit (Machery-Nagel kit, Separations) on a Tecan Evo 150 Liquid Handler (Diagnostic Products, RSA) according to the manufacturer's protocol. Two µl of each of the cleaned product was mixed with 0.2 µl GeneScan™- 500 LIZ® size standard (Life Technologies, RSA) and 9.5 µl of HiDi formamide (Life Technologies, RSA). The samples were denatured at 95 ºC for five minutes and placed on ice for five minutes. The samples were electrophoresed on an Applied Biosystems 3730*xl* Genetic Analyser using the default Fragment analysis run parameters, supplied by Life Technologies (RSA). The data was collected with Data Collection version 3 software (Life Technologies, RSA).

The data files generated by the Data Collection software were imported in GeneMapper v3.7 (Life Technologies, RSA) and analysed. Panels and bins were created for each multiplex to simplify the analysis. Each possible allele for a marker, identified in the parental samples, was marked with a bin, as this was also a quick method to highlight null alleles and mutations in a marker. The software summarised the data for each plant and this table was then exported as tab-delimited files for further calculations. These tab-delimited files were converted to Excel (Microsoft Office) files to summarise the data and to have it in a format that was compatible with the software that was used for the linkage map and QTL map construction.

3.6 Linkage map construction

3.6.1 TMAP and JoinMap (version 4.1)

Linkage analysis of the generated SSR markers for each population was performed using TMAP (Cartwright et al. 2007) and JoinMap (version 4.1) (Van Ooijen 2006, 2011) software programs. The TMAP software calculated the grouping of markers into linkage groups and determined the phasing of each marker. Maps were generated to indicate the order of markers and the distance between them. In JoinMap the LOD score was calculated to determine

possible linkage between markers and maps were generated to indicate the order of the markers and the distance between them. The distances between markers are given in cM and the Kosambi mapping function was chosen for the calculation. The Kosambi mapping function assumes that recombination events influence the occurrence of adjacent recombination events (Collard et al. 2005).

The output files from GeneMapper were converted to the specific input formats required by TMAP and JoinMap and all individuals originating from self-pollinating or clonal events were removed. The two allele calls for each marker was combined but separated by a double colon (:) to create the TMAP input file. Missing values were indicated using a dash (-). These converted values as well as the marker names were pasted in a Notepad format that could be imported into TMAP. To create the input format required by JoinMap allele calls were converted to a two character coding system as specified in the JoinMap Manual. The four parental alleles were coded according to the JoinMap specified coding system and applied to the F_1 alleles (Table 9). Missing values were indicated using a dash (-). A Notepad format file was again created that could be imported into JoinMap.

Table 9 Coding system for parental alleles to be JoinMap compatible (Van Ooijen 2006).

Code	**Description**
abxcd	Four alleles, locus heterozygous in both parents
efxeg	Three alleles, locus heterozygous in both parents
hkxhk	Two alleles, locus heterozygous in both parents
lmxll	Locus heterozygous in first parent
nnxnp	Locus heterozygous in second parent

The result of the TMAP and JoinMap analyses are linkage groups shown with all linked markers grouped together in the order that they occur on the genome. These linkage groups are representative of chromosomes or chromosome segments (Collard et al. 2005).

3.7 QTL Analysis

3.7.1 MapQTL

MapQTL version 6 (Van Ooijen 2009) was used to combine the marker information, phenotypic scores and map positions (from JoinMap) and to obtain a map where the position of a QTL or gene in a genome was indicated. This data was subjected to various statistical calculations to ensure that the most accurate position of a QTL was reflected.

During Interval Mapping (IM) a QTL likelihood map was constructed showing the position of a QTL linked to the pathogen resistance investigated. A permutation test, running 1000 permutations, was performed to determine the minimum LOD value at which a marker contributes to the observed resistance. Cofactors segregating with the QTL were determined and multiple QTL mapping (MQM) was performed. This calculation allowed for the detection of other QTLs in the genome/area that could have a contributing effect. It also confirmed the presence of the QTL found during the IM step.

Chapter 4

Results

In this study, the fungal disease resistance genes/QTLs from three resistance sources, namely Pölöskei Muskotály, Kishmish Vatkana and Villard Blanc were characterised. Phenotypic characterisations for powdery and downy mildew were performed on F_1 populations, created by P. Burger. Molecular marker data was combined with the phenotypic data in order to compile the relevant linkage maps and perform QTL mapping.

4.1 Characterisation of Pölöskei Muskotály

4.1.1 Phenotyping

No phenotypic evaluations were performed on the populations derived from PM (F_1: PM x RS, F_1: PM x G4 and F_1: PM x SS) as the poor plant growth and information gathered from the molecular analysis (section 4.1.2) led to the conclusion that all three populations were not true F_1 populations, but resulted mostly from self-pollination and were thus not suitable for the purpose of this study.

4.1.2 Molecular analysis

4.1.2.1 Identification of informative markers

4.1.2.1.1 Pölöskei Muskotály (PM) and Regal Seedless (RS)

Pölöskei Muskotály (PM) and Regal Seedless (RS) were typed for 27 SSR markers representing 11 chromosomes in the genome. Twelve of these markers are situated on chromosome 18, the linkage group responsible for downy mildew resistance in Regent and Villard Blanc (Fischer et al. 2004, Bellin et al. 2009, Van Heerden et al. 2014). This was a therefore a region of particular interest as PM is a descendant of Villard Blanc. Nine of the markers were heterozygous in both parents (eg. VrZAG25, VVMD7 and UDV108) and thus totally informative. One marker was homozygous in each parent, but for different alleles in the parents (VrZAG21), while the remainder of the markers shared some common alleles between the two parents. In this last group of markers both of the parents could be heterozygous but there was a common allele between the parental genotypes (eg. VrZAG47 and VrZAG62) or one parent was homozygous while the other parent was heterozygous, again with a shared allele between the two parental genotypes (eg. VMC8B5 and VVIV16). These markers were all considered to be informative, but to a lesser extent, and therefore useful for linkage map construction. One marker (VVIU04) only amplified for RS but not for PM. (Table 10)

Table 10 Parental genotypes for PM and RS. Numbers indicate allele sizes.

Multiplex 1	VrZAG21	VrZAG21	VrZAG25	VrZAG25	VrZAG47	VrZAG47	VrZAG62	VrZAG62	VrZAG79	VrZAG79	VrZAG83	VrZAG83	VVMD7	VVMD7
Poloskei Muskotaly	199	199	225	233	156	158	180	188	252	252	193	197	237	243
Regal Seedless	190	190	236	238	156	161	186	188	248	252	191	197	239	249

Multiplex 3	UDV108	UDV108	VMC8B5	VMC8B5	VVIM93	VVIM93	VVMD17	VVMD17
Poloskei Muskotaly	214	236	142	157	114	127	219	221
Regal Seedless	237	239	157	157	114	129	220	221

Multiplex 4	VMC7F2	VMC7F2	VVIT68	VVIT68	VVIU04	VVIU04	VVIV16	VVIV16
Poloskei Muskotaly	207	211	255	259			104	124
Regal Seedless	200	201	257	261	103	172	104	104

Multiplex 11	VVIN16-cjvh	VVIN16-cjvh	VMC2A3	VMC2A3	VMC2B1.1	VMC2B1.1	VMC3E5	VMC3E5	VMC8F4.2	VMC8F4.2
Poloskei Muskotaly	245	247	164	186	83	105	110	114	94	108
Regal Seedless	247	253	154	176	93	95	110	110	94	97

Multiplex 24	UDV047	UDV047	VMC1A5	VMC1A5	VMC1G3.2	VMC1G3.2	VMC2H4	VMC2H4	VVIN74-cjvh2	VVIN74-cjvh2	VVMD8-cjvh	VVMD8-cjvh	VVC62	VVC62
Poloskei Muskotaly	157	157	176	182	187	195	203	209	188	196	223	264	225	250
Regal Seedless	120	124	182	194	170	197	216	226	198	198	224	224	232	250

Before the parental screen for multiplex 24 had been completed it was decided to screen the F_1: PM x RS population (124 individuals) using multiplex 1, 3, 4 and 11 (Table 3) in an effort to start linkage group construction. Close inspection of the alleles amplified revealed that 78 of the 124 F_1 plants had the same alleles as the maternal plant (PM) for almost all the markers. In some instances only a single allele from the maternal plant was present while in other instances both alleles from the maternal plant was inherited (Figure 11). In some instances the

marker could not be classified as a maternal profile only because the parental plants shared alleles. The majority of the F_1: PM x RS population thus originated through self-pollination of PM and linkage analysis could not be performed.

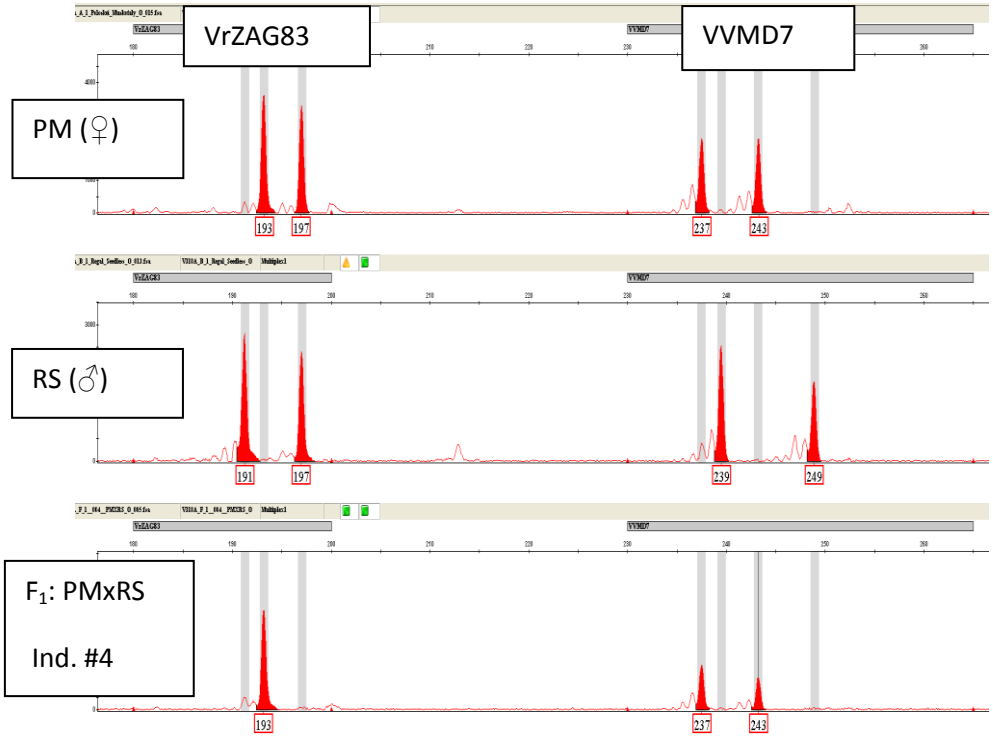

Figure 11 Electropherogram for SSR marker VVMD7 illustrating the inheritance of only maternal alleles in the F_1 individual #4 believed to be derived from a PM x RS cross.

4.1.2.1.2 Pölöskei Muskotály (PM) and G4-3418 (G4)

After finding a high percentage of individuals showing inheritance of only maternal alleles in the F_1: PM x RS population, DNA analysis with multiplex 1, consisting of seven SSR markers, on the parental plants and a subset of the F_1: PM x G4 population (16 plants), revealed a similar situation where fourteen plants had only maternal alleles.

PM and G4 shared at least one allele in five of the seven SSR markers and the profiles could therefore not be classified as exclusively maternal profiles. However, the two markers VrZAG21 and VrZAG47 clearly amplified only PM alleles in 14 of the 16 individuals typed

and no paternal alleles were amplified (Table 11). Furthermore, for markers VrZAG79, VrZAG83 and VVMD7 none of the alleles unique to G4 were found in the 14 F_1 individuals. All these factors pointed towards the same self-pollination problem by PM as had been found for the F_1: PM x RS population.

Apart from self-pollination it seems that in cases where cross pollination did occur, another parent may have been involved. Plants #12 and #13 were possibly derived from a different cross as they had some unique marker alleles not characteristic of either parent. Although most of the alleles present are the same as that of the parental plants, non-parental alleles are present for markers VrZAG25 (allele 236), VrZAG47 (allele 165), VrZAG62 (allele 194), VrZAG79 (allele 255), VrZAG83 (allele 187 and allele 201) and VVMD7 (allele 260).

Table 11 Allele comparisons for F_1: PM x G4.

Marker	VrZAG21	VrZAG21	VrZAG25	VrZAG25	VrZAG47	VrZAG47	VrZAG62	VrZAG62	VrZAG79	VrZAG79	VrZAG83	VrZAG83	VVMD7	VVMD7
PM[a]	197	197	224[d]	232	153	155	177	185	251	251	191	195	236	242
G4[b]	187	187	224	224	159	168	185	185	247	251	189	195	238	242
#001PM x G4	197	197	224	224	155	155	177	185	251	251	191	195	236	242
#002PM x G4	197	197	224	232	155	155	177	185	251	251	195	195	236	242
#003PM x G4	197	197	232	232	155	155	177	185	251	251	191	195	236	242
#004PM x G4	197	197	224	232	155	155	177	185	251	251	191	195	236	242
#005PM x G4	197	197	224	232	155	155	177	185	251	251	191	195	236	242
#006PM x G4	197	197	224	232	155	155	185	185	251	251	191	195	242	242
#007PM x G4	197	197	224	232	155	155	177	185	251	251	191	195	236	242
#008PM x G4	197	197	232	232	153	153	177	185	251	251	191	195	236	242
#009PM x G4	197	197	232	232	153	153	177	185	251	251	191	191	236	242
#010PM x G4	197	197	232	232	155	155	177	185	251	251	195	195	236	242
#011PM x G4	197	197	232	232	155	155	177	185	251	251	195	195	236	242
#012PM x G4	197	197	232	232	155	155	177	185	251	255	191	201	236	238
#013PM x G4	197	197	232	236[c]	153	165	177	194	247	251	187	191	236	260
#014PM x G4	197	197	232	232	153	153	177	177	251	251	191	195	236	236
#015PM x G4	197	197	232	232	155	155	177	177	251	251	191	191	236	236
#016PM x G4	197	197	232	232	155	155	177	185	251	251	191	191	236	242

[a]Alleles present in PM shaded green

[b]Alleles present in G4 shaded yellow

[c]Unique alleles not shaded

[d] PM and G4 common alleles shaded grey

4.1.2.1.3 Pölöskei Muskotály (PM) and Sunred Seedless (SS)

The results from the previous two populations where PM was used as a maternal parent eliminated these populations for use in a mapping study. A third population, where PM was also used as a maternal parent, was considered as a replacement population. DNA analysis with multiplex 1, consisting of seven SSR markers, on the parental plants and a subset of the F_1: PM x SS population (14 plants), revealed that seven plants were the product of self-pollination. The only plant that had inherited exclusively maternal alleles was plant #003 while the six other plants had alleles that could only be inherited from the PM parent as well as alleles that were common between PM and G4 for markers VrZAG25 and VrZAG83. Plant #002 and #004 displayed PM markers for all of the SSR markers except VrZAG25 and VrZAG83 where they displayed the common alleles. Plants #006 and #007 displayed PM markers for all of the SSR markers except VrZAG25 where they displayed the common alleles, while plants #008 and #014 displayed the common allele for VrZAG83 while the rest of the alleles originated form PM. In these six plants it stands to reason that the plant was a product of self-pollination if five/six of the markers originated from PM and the questionable markers display alleles that are common between the two parents (Table 12). The results indicated that 50% of the individuals in this population were also the result of self-pollination by PM, similar to the F_1: PM x RS population.

The PM populations were then excluded from the study as the information gained from the marker analysis showed that it consisted predominantly of self-pollinated individuals and could thus not be used in a linkage mapping study.

Table 12 Allele comparison for F_1: PM x SS.

Marker	VrZAG21	VrZAG21	VrZAG25	VrZAG25	VrZAG47	VrZAG47	VrZAG62	VrZAG62	VrZAG79	VrZAG79	VrZAG83	VrZAG83	VVMD7	VVMD7
Pölöskei Muskotály	199	199	225[c]	233	156	158	180	188	252	252	193	197	237	243
Sunred Seedless[b]	205	213	225	225	161	170	186	203	248	256	191	197	249	249
PM x SS_#001	199	213	225	233	156	170	180	186	248	252	191	197	237	249
PM x SS_#002	199	199	225	225	158	158	188	188	252	252	193	197	243	243
PM x SS_#003	199	199	233	233	156	158	180	180	252	252	193	193	237	237
PM x SS_#004	199	199	225	233	156	158	188	188	252	252	197	197	243	243
PM x SS_#005	199	205	225	233	158	170	188	203	252	256	191	193	243	249
PM x SS_#006	199	199	225	225	156	158	180	188	252	252	193	193	237	243
PM x SS_#007	199	199	225	233	156	158	180	188	252	252	193	193	237	243
PM x SS_#008	199	199	233	233	156	158	180	188	252	252	193	197	237	243
PM x SS_#009	199	213	225	233	158	161	180	186	248	252	191	197	237	249
PM x SS_#010	199	205	225	225	156	170	180	186	252	256	191	197	237	249
PM x SS_#011	199	213	225	233	158	158	180	186	252	256	191	193	237	249
PM x SS_#012	199	205	225	233	156	170	186	188	252	256	191	193	243	249
PM x SS_#013	199	213	225	233	158	170	188	203	252	256	197	197	243	249
PM x SS_#014	199	199	233	233	156	158	180	188	252	252	193	197	237	243

[a]Alleles present in PM shaded green

[b]Alleles present in G4 shaded yellow

[c]Common alleles present in PM and SS shaded grey

4.2 Characterisation of Kishmish Vatkana

4.2.1 Phenotyping

A powdery mildew screen was performed on the F_1: SS x KV population on three dates spanning two growth seasons. The scores were done on whole leaves (23/11/2009) and whole plants (30/11/2009 and 17/02/2011). The whole leaf score (23/11/2009) showed an uneven distribution pattern over the OIV score range with the majority of the population showing high resistance to downy mildew (Figure 12). The parental control leaves that were included in the screen showed very high resistance (OIV 9) for KV and medium resistance (OIV 5) for SS. The large proportion of plants displaying a high level of resistance and the susceptible parent displaying medium resistance to powdery mildew led to the conclusion that the score was inaccurate as a segregation ratio of 1:1 is expected from a single dominant gene segregating in a F_1 population. These scores display a 4 (resistant):1 (susceptible) ratio and was not considered for further analysis. The scores produced from the whole plant screens

performed in two different seasons (30/11/2009 and 17/02/2011) had a more even distribution of the OIV scores (Figure 13 and Figure 14). The parental control plants that were included in the screen showed very high resistance (OIV 9) for KV and very low and low resistance (OIV three and OIV 1) for SS (refer to Appendix 1.1 for phenotypic scores).

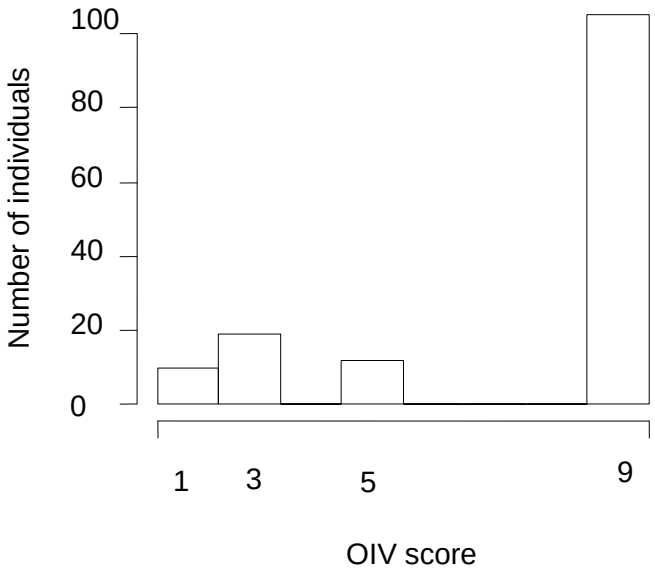

Figure 12 OIV score distribution of individuals of the F_1: SS x KV population for the whole leaf powdery mildew score 23/11/2009.

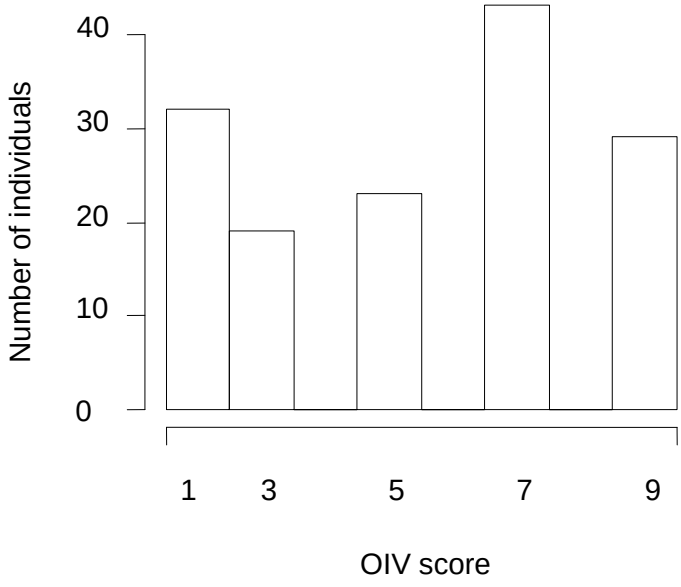

Figure 13 OIV score distribution of individuals of the F_1: SS x KV population for the whole plant powdery mildew score 30/11/2009.

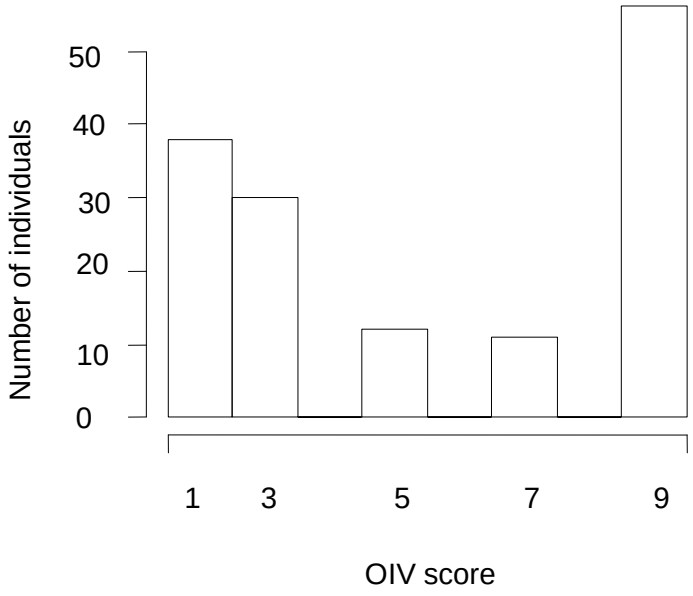

Figure 14 OIV score distribution of individuals of the F_1: SS x KV population for the whole plant powdery mildew score 17/02/2011.

Pearson and Spearman correlations between the scores were done using the software program R. The Pearson *rho* values (level of correlation between scores) indicated that there was a moderate correlation between the two whole plant scores, 30/11/2009 and the 17/02/2011. The Pearson levels of significance were all above a P = 0.001 value (Table 13). The Spearman *rho* values and P values confirmed the levels of correlation and significance seen with the Pearson calculations.

Table 13 Pearson correlations (*rho* value) between powdery mildew scores performed on F_1: SS x KV population.

***rho* value**[a]	**F_1: SS x KV 17/02/2011**	**F_1: SS x KV 30/11/2009**
F_1: SS x KV 17/02/2011	1	0.5612963
F_1: SS x KV 30/11/2009	0.5612963	1

P value[b]	**F_1: SS x KV 17/02/2011**	**F_1: SS x KV 30/11/2009**
F_1: SS x KV 17/02/2011	1	2.08E-13
F_1: SS x KV 30/11/2009	2.076E-13	1

[a]Pearson level of correlation between scores
[b]Significance of correlation

4.2.2 Molecular analysis

4.2.2.1 Identification of informative markers

Sunred Seedless (SS) and Kishmish Vatkana (KV) were screened with 13 SSR markers located on chromosome 13. Of the 13 markers tested, 12 were informative and one (VMC3D8) would not amplify in either parental sample. The F_1: SS x KV population (158 plants) was genotyped for the 12 SSR markers. The majority of the markers produced SSR profiles that were easily scorable as the individuals were either homozygous (single allele) or heterozygous (two alleles). Five markers (UDV020, UDV038, VMC2C7, VMC2A9 and VMC3B12) had multiple binding sites for the primers and this produced complex SSR profiles for certain individuals (Figure 15). For all of these distinct alleles, in complex profiles, sizes were assigned.

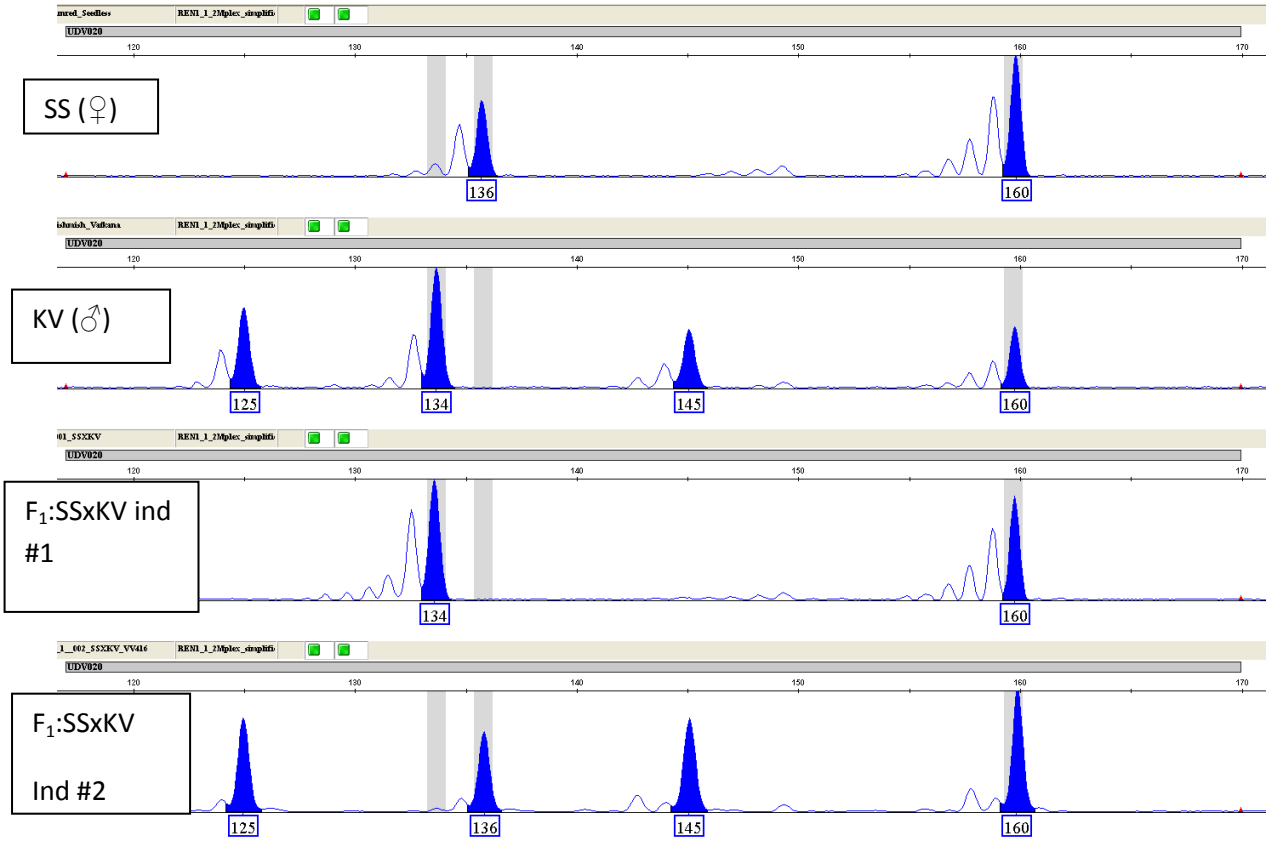

Figure 15 SSR marker UDV020 displaying multiple alleles in KV parent and their inheritance in individual #1 and #2. Alleles 125 and 145 were always present when allele 160 was inherited from KV.

Allele calls were exported from GeneMapper analysis software as tab delimited files and converted to Excel (Microsoft Office) files.

4.2.2.2 Linkage map construction

4.2.2.2.1 TMAP and JoinMap (version 4.1)

Before the markers that had multiple peaks could be imported attempts were made to simplify the allele calls as the complex multiple calls could not be accommodated in TMAP and JoinMap V4.1. These software programs only allow for both parents and progeny to have two allele calls per marker. For TMAP the combined allele calls for the parental plants should be in a <<parent1 allele 1: parent1 allele 2 x parent2 allele 1: parent2 allele 2>> format and the progeny as progeny allele1: progeny allele 2. JoinMap V4.1's coding system (Table 10) also only allows for two alleles per marker.

Of the 12 markers used to screen the population only eight could be imported into TMAP and JoinMap V4.1 and then used to generate a linkage map. The five markers mentioned earlier, that had multiple binding sites proved to be problematic when attempts were made to simplify the inherited allele calls to be suitable for importation into TMAP and JoinMap. Only one marker, UDV020, could be simplified to the extent that it could be imported into TMAP and JoinMap as it was noted that alleles 125 and 145 were always present if the 160 allele was inherited from KV. These allele calls were therefore removed from all progeny that inherited the 160 allele from KV and the dataset could be imported (Figure 15). The allele calls for the four remaining markers (UDV038, VMC2C7, VMC2A9 and VMC3B12) were removed from the dataset.

TMAP calculated the position of the eight markers on chromosome 13 as well as the distance between the markers to create a consensus map for the linkage group. The map was split into the two parental maps (maternal and paternal) using the 'split' option in the 'BuilderSplit' programme and an error rate for the statistical placement of the markers on the chromosome was calculated (Table 14).

The SS (maternal map) generated was 33.7 cM long and the largest intermarker gap was between UDV020 and VVIP10, 16.3 cM. The software was very confident in the marker placements as the error rates were all 0 except for UDV020 and UDV124. The KV (paternal map) was 37.8 cM long and the largest intermarker gap was again between UDV020 and VVIP10, 24.2 cM. The software was confident in the marker placements as all but the same two markers as before had error rates of 0.

Table 14 Position of SSR markers on chromosome 13 for Sunred Seedless (SS) and Kishmish Vatkana (KV) according to TMAP.

SSR marker	Marker position on SS (cM)[a]	Marker position on KV (cM)[a]	Distance between markers on SS (cM)[a]	Distance between markers on KV (cM)[a]	Error rate for marker placement on SS (%)[b]	Error rate for marker placement on KV (%)[b]
UDV129	0	0	0	0	0	0
VMC3D12	1.4	2.7	1.4	2.7	0	0
VVIC51	1.4	2.7	0	0	0	0
UDV124	6.6	7	5.2	4.3	0.2	0.5
VMC9H4_2	16.7	12.7	10.1	5.7	0	0
VMCNG4E10-1	16.7	12.7	0	0	0	0
UDV020	17.4	13.6	0.7	0.9	0.7	3.2
VVIP10	33.7	37.8	16.3	24.2	0	0

[a]Distance between SSR markers in centimorgan (cM)

[b]Error rates for placement on chromosome as percentage

The converted genotype table was imported into JoinMap V4.1 and the software calculated the position of the markers on the chromosome as well as the distance between the markers to create a consensus map for the linkage group (1 in Figure 16). The parental maps for the F_1: SS x KV population was calculated using the two-way-pseudo-testcross method, 1_P1 represents the SS maternal map and 1_P2 represents the KV paternal map (Figure 16). The 1_P1 (SS) map is 39.4 cM long and the largest intermarker gap is 19.6 cM where the 1_P2 (KV) map is 51.8 cM long and the largest intermarker gap is 33.3 cM. The combined map 1 (F_1: SS x KV) is 45.6 cM long and the largest intermarker gap is 26.5 cM.

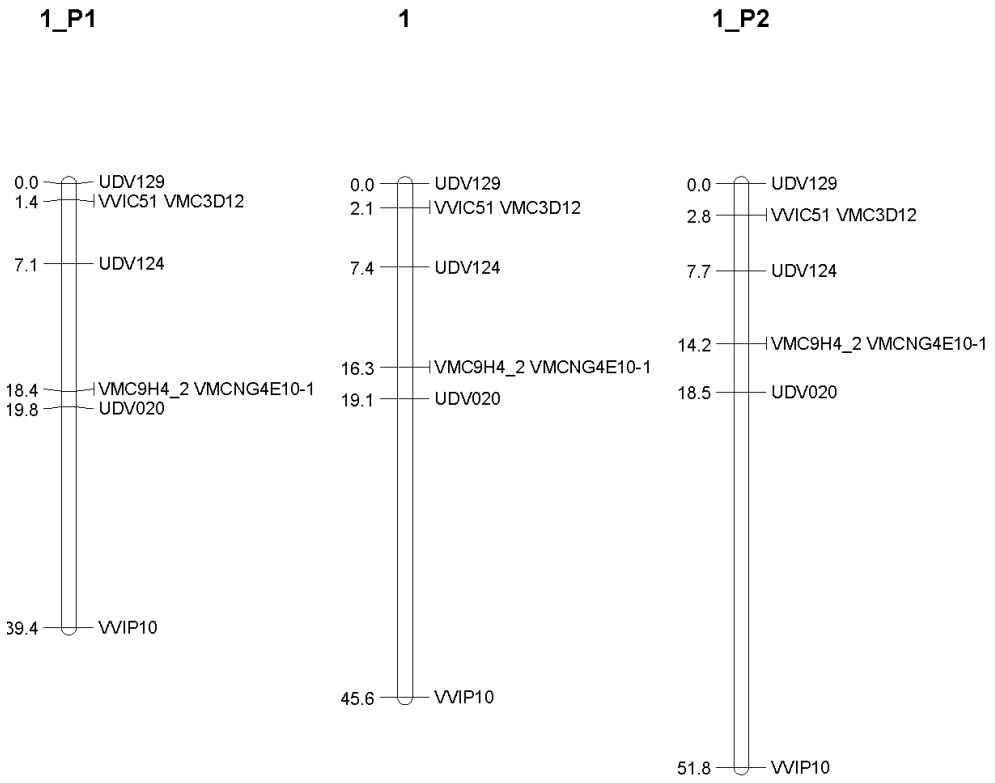

Figure 16 SSR marker positions on chromosome 13 for the parental plants and the F_1: SS x KV population. Sunred Seedless is represented by 1_P1, Kishmish Vatkana is represented by 1_P2 and the F_1: SS x KV population by 1. Map distances are in cM.

4.2.2.3 QTL Analysis

The converted genotype table en phenotypic scores were imported into MapQTL and subjected to statistical analysis to determine if a gene/QTL were present that was linked to powdery mildew resistance. The F_1: SS x KV population was examined as well as the two parentals, Sunred Seedless (SS) and Kishmish Vatkana (KV).

The Kruskal-Wallis calculations performed indicated that the markers (used in the study) were linked to the powdery mildew resistance with a high level of significance ($P < 0.005$) for KV and no linked markers were indicated for SS. In the F_1: SS x KV population the inherited markers showed the same high level of significant linkage to the resistance QTL (Table 15).

Table 15 Kruskal-Wallis indication of markers significantly linked to the powdery mildew resistance on chromosome 13.

	Linkage group	SSR Marker	Significance (P value)[a] 30/11/2009	Significance (P value)[a] 17/02/2011
F_1: SS x KV	LG13	UDV129	*******	*******
	LG13	VVIC51	*******	*******
	LG13	VMC3D12	*******	*******
	LG13	UDV124	*******	*******
	LG13	VMC9H4_2	*******	*******
	LG13	VMCNG4E10-1	*******	*******
	LG13	UDV020	*******	*******
	LG13	VVIP10	*****	******

[a]Level of significance, ***** P=0.001, ****** P=0.0005, ******* P=0.0001

Interval mapping with the phenotypic scores 30/11/2009 and 17/02/2011, indicated that the entire chromosome region of 45.6 cM was significant. The LOD score threshold of 2.6 was determined after running a permutation test (1000 iterations) and selecting the value linked to the linkage group relative cumulative score of 0.95 (P = 0.05). This QTL region explains up to 44.8% (LOD 18.86) and 57.7% (LOD 27.48) of the phenotypic variance observed for the two scores respectively (Table 16, Figure 17).

Table 16 The location, significance and confidence interval of QTL identified by Interval Mapping in F_1: SS x KV progeny for powdery mildew resistance.

LG[a]	QTL confidence interval	Nearest markers		30/11/2009	17/02/2011
13	UDV124 - UDV020	VMC9H4_2, VMCNG4E10-1	Max LOD	18.86	27.48
			% Var[b]	44.8	57.7
			LOD threshold - LG13	2.6	2.6

[a]Linkage group

[b]Percentage phenotypic variance explained

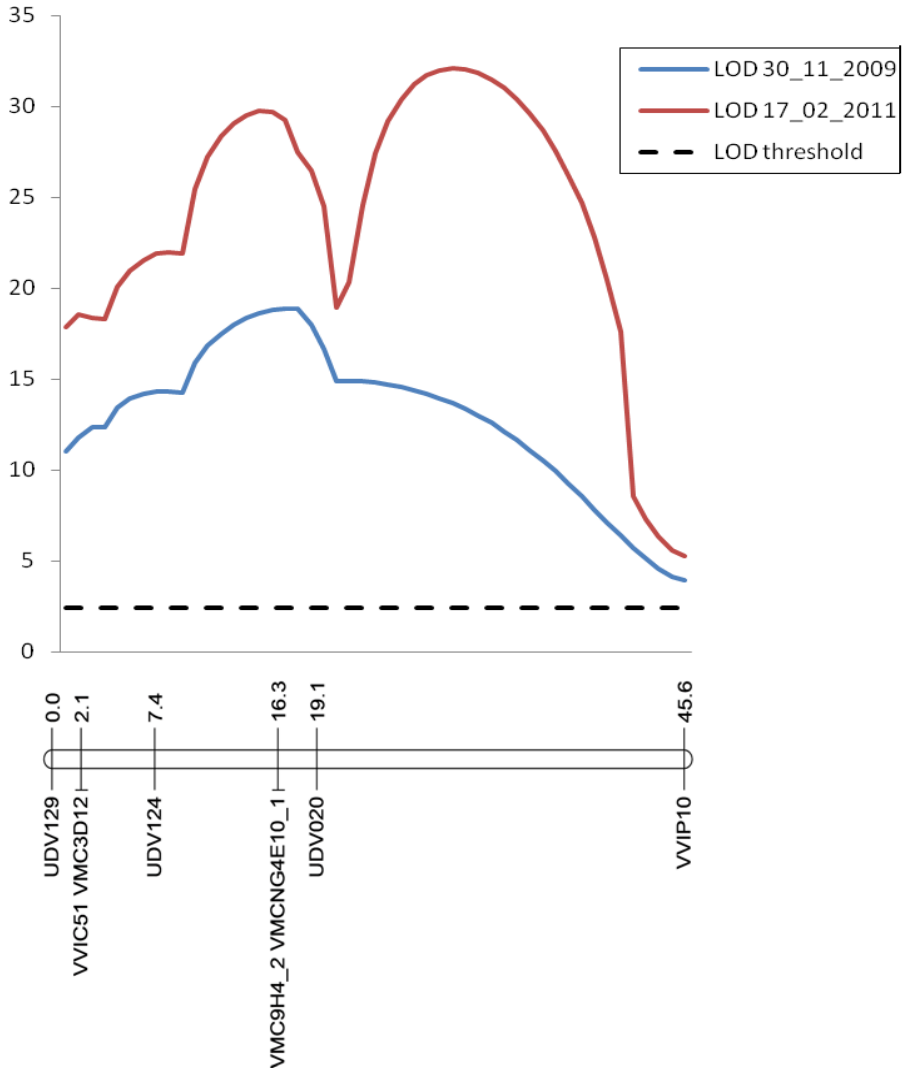

Figure 17 QTL for powdery mildew resistance on chromosome 13, calculated with Interval mapping. The LOD threshold is 2.6.

To improve the definition of the QTL region obtained with IM, automatic co-factor selection was performed and VMC9H4-2 was identified as a co-factor for the QTL region. A multiple QTL model (MQM) calculation was then done, incorporating the selected co-factor and for each of the two phenotypic scores (Figure 18). The two scores (30/11/2009 and 17/02/2011) produced an improved QTL peak, which was now clearly placed between SSR markers UDV124 and UDV020. The percentage of variance explained was between 44.8% (LOD 18.86) and 57.7% (LOD 27.48) for this area (Table 17, Figure 18).

Table 17 The location, significance and confidence interval of QTL identified by MQM in F_1: SS x KV progeny for powdery mildew resistance.

LG[a]	QTL confidence interval	Nearest marker		30/11/2009	17/02/2011
13	UDV124 – UDV020	VMC9H4_2[c]	Max LOD	18.86	27.48
			% Var[b]	44.8	57.7
			LOD threshold - LG13	2.6	2.6

[a]Linkage group

[b]Percentage phenotypic variance explained

[c]Identified with co-factor selection

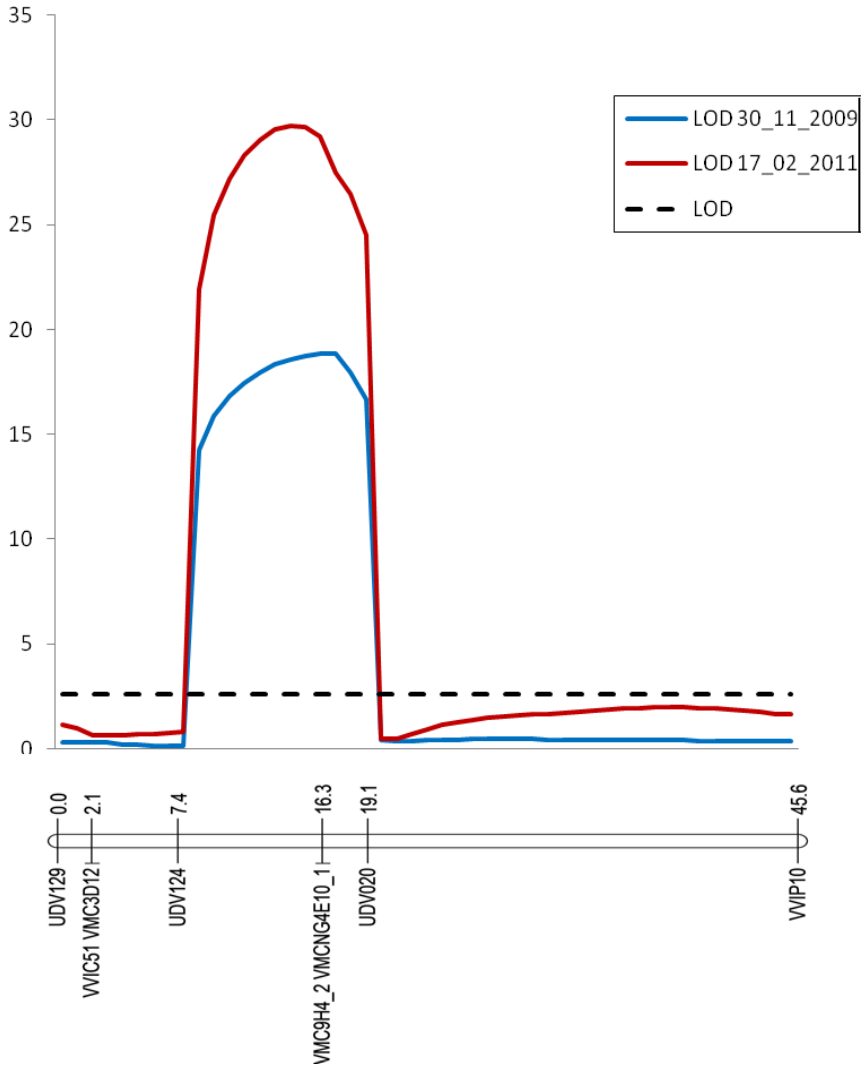

Figure 18 QTL for powdery mildew resistance on chromosome 13, calculated with MQM mapping. The LOD threshold is 2.6.

In an effort to identify the specific KV alleles that are linked to the fungal resistance the estimated average of the quantitative trait's distribution associated with all allele combinations (mu) was looked at (mu_*ac*, mu_*ad*, mu_*bc*, mu_*bd*) for the markers linked significantly to the QTL (Table 18). Even though marker UDV020 appears to be the nearest marker on the one side of the QTL confidence interval it only has a LOD of 0.42 and is therefore not significantly linked to the QTL. For marker UDV124 the *a* allele is 221 (from SS), *b* is 232 (from SS), *c* is 219 (from KV) and *d* is 221 (from KV). For marker VMC9H4_2 the *a* allele is 288 (from SS), *b* is 327 (from SS), *c* is 273 (from KV) and *d* is 296 (from KV). In both these instances the averages for the *ad* and *bd* allele combinations are much higher than the values for the *ac* and *bc* allele combinations. This indicates that the *d* allele is associated with the resistance and for UDV124 that is 221 (from KV) and for VMC9H4_2 that is 296 (from KV).

Table 18 Average distribution of allele combinations of SSR markers associated with the powdery mildew resistance QTL on chromosome 13.

Trait	Locus	Alleles	LOD	mu_*ac* {00}[a]	mu_*ad* {00}[a]	mu_*bc* {00}[a]	mu_*bd* {00}[a]
30/11/ 2009	UDV124	221:232 x 219:221	14.28	2.40	4.03	2.13	3.95
	VMC9H4_2	288:327 x 273:296	18.86	2.29	4.19	2.08	4.00
17/02/ 2011	UDV124	221:232 x 219:221	21.92	1.97	4.48	1.95	4.18
	VMC9H4_2	288:327 x 273:296	27.48	1.73	4.62	2.05	4.21

[a]Estimated average of the quantitative trait's distribution associated with an allele combination

4.3 Characterisation of Villard Blanc

4.3.1 Phenotyping

A powdery mildew screen was performed on whole leaves (10/02/2012) and on the whole plant (15/02/2012). The scores showed a normal distribution pattern over the OIV score range with the largest proportion of the population showing a medium resistance to powdery mildew (Figure 19 and Figure 20). The parental control leaves and plants that were included in the screen showed very high resistance (OIV nine) for VB and high resistance (OIV seven)

for G1 (refer to Appendix 1.2). The fungal disease resistance investigated is conferred by a QTL and it was expected to see a broad range of resistance scores as a QTL only transfers partial resistance to disease.

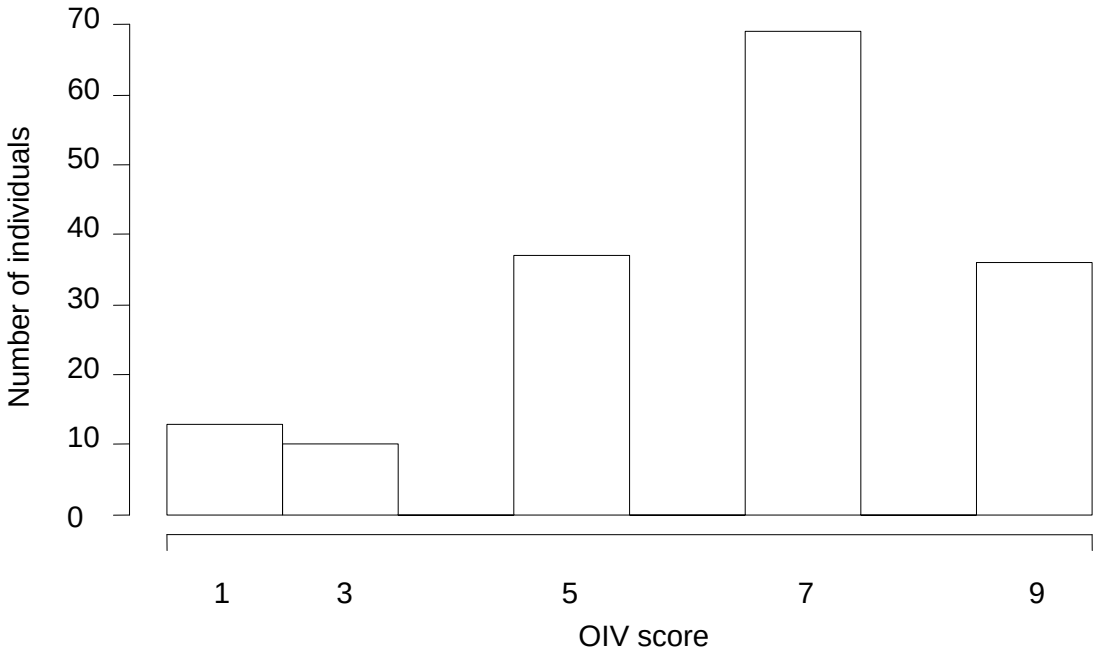

Figure 19 OIV score distribution of individuals of the F_1: VB x G1 population for the whole leaf powdery mildew score 10/02/2010

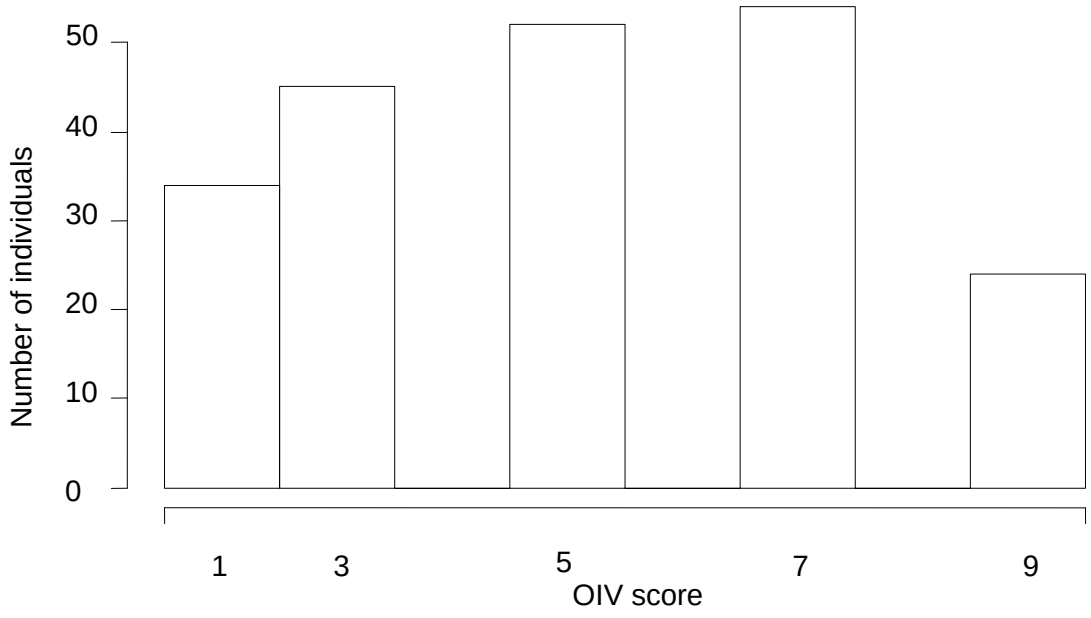

Figure 20 OIV score distribution of individuals of the F_1: VB x G1 population for the whole plant powdery mildew score 15/02/2010

Pearson and Spearman correlation test between the scores were done using the software program R. The Pearson and Spearman were very similar and their *rho* values (level of correlation between scores) indicated that there was a strong correlation between the 10/02/2010 score and the 15/02/2010 score. The Pearson levels of significance were all above a P = 0.001 value (Table 19) and was confirmed by the Spearman levels of significance.

Table 19 Pearson correlations (*rho* value) between powdery mildew scores performed on F_1: VB x G1 population.

***rho* value**[a]	**F_1: VB x G1 15/02/2010**	**F_1: VB x G1 10/02/2010**
F_1: VB x G1 15/02/2010	1	0.8047168
F_1: VB x G1 10/02/2010	0.8047168	1

P value[b]	**F_1: VB x G1 15/02/2010**	**F_1: VB x G1 10/02/2010**
F_1: VB x G1 15/02/2010	1	2.20E-16
F_1: VB x G1 10/02/2010	2.20E-16	1

[a]Pearson level of correlation between scores

[b]Significance of correlation

A downy mildew screen was performed on the same leaf discs at two different scoring dates (05/12/2010 and 09/12/2010). The scores showed an uneven distribution pattern over the OIV score range with a large majority of the population showing high resistance to downy mildew (Figure 21 and Figure 22). There was quite a number of missing values present as some of the leaf discs died during the experiment. The parental control plants that were included in the screen showed high resistance (OIV seven) for VB and very low and low resistance (OIV three and OIV one) for G1. There were also no individuals found exhibiting total resistance to downy mildew even after rescoring the population four days after the initial score was performed (refer to Appendix 1.3). A broad range of resistance scores was expected as this fungal resistance is also conferred by a QTL.

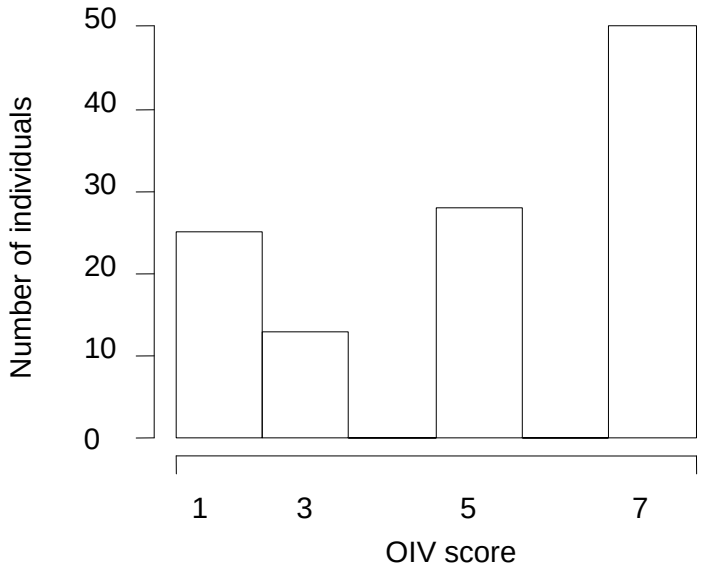

Figure 21 OIV score distribution of individuals of the F_1: VB x G1 population for the leaf disc downy mildew score 05/12/2010

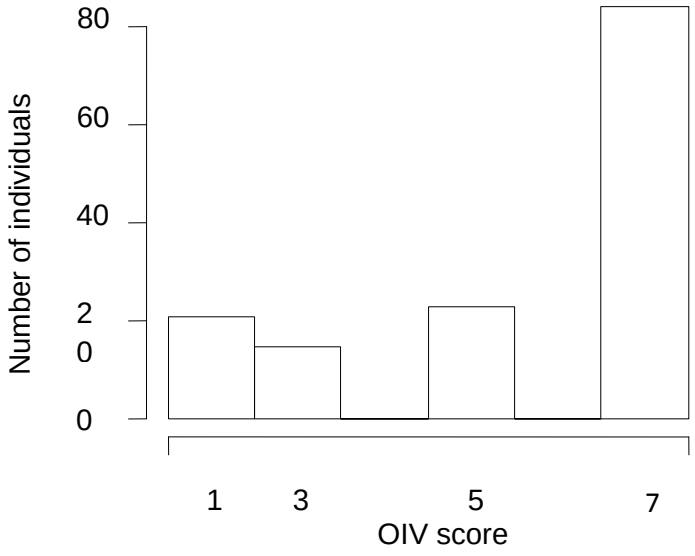

Figure 22 OIV score distribution of individuals of the F_1: VB x G1 population for the leaf disc downy mildew score 09/12/2010

Pearson and Spearman correlation tests between the scores were done using the software program R. The Pearson and Spearman were very similar and their *rho* values (level of correlation between scores) indicated that there was a strong correlation between the leaf disc scores done on the 05/12/2010 and the 09/12/2010. The Pearson levels of significance were all above a P = 0.001 value (Table 20) and was confirmed by the Spearman levels of significance.

Table 20 Pearson correlations (*rho* values) between downy mildew scores performed on F_1: VB x G1 population.

***rho* value**[a]	**F_1: VB x G1 leaf disc 09/12/2010**	**F_1: VB x G1 leaf disc 05/12/2010**
F_1: VB x G1 leaf disc 09/12/2010	1	0.804717
F_1: VB x G1 leaf disc 05/12/2010	0.804717	1

P value[b]	**F_1: VB x G1 leaf disc 09/12/2010**	**F_1: VB x G1 leaf disc 05/12/2010**
F_1: VB x G1 leaf disc 09/12/2010	1	2.20E-16
F_1: VB x G1 leaf disc 05/12/2010	2.20E-16	1

[a]Pearson level of correlation between scores

[b]Significance of correlation

4.3.2 Molecular analysis

4.3.2.1 Identification of informative markers

Villard Blanc (VB) and G1-6604 (G1) were typed for 14 SSR markers on chromosome 15 and 13 SSR markers on chromosome 18. Of the 14 markers used to screen chromosome 15 three markers (VVS16, VVIV67 and VChr15b) would not amplify but the remaining 11 SSR markers were informative and used to screen the F_1: VB x G1 population (250 plants). Of the 13 markers used to screen chromosome 18 one marker (VVIR09) would not amplify. The remaining 12 SSR markers were informative and used to screen the F_1: VB x G1 population (250 plants).

The majority of the markers produced simple scorable SSR profiles, but one marker on chromosome 18 (VMC6F11) had multiple binding sites and produced complex SSR profiles. VB had three peaks and G1 had five peaks of which only one overlapped with an allele found in VB. Both parental plants also had null alleles present. All of these alleles were inherited in various combinations in the F_1 progeny. Sizes were assigned to all the peaks, irrespective of the number of peaks per sample. Five alleles (81, 90, 94, 96 and 114) occurred repeatedly in the progeny plants and were eliminated from the dataset which left alleles 98, 114 and the

respective null alleles that inherited in various combinations in the progeny and were used for linkage map construction and QTL mapping.

Allele calls were exported from GeneMapper analysis software as tab delimited files and converted to Excel (Microsoft Office) files.

4.3.2.2 Linkage map construction

4.3.2.2.1 TMAP and JoinMap (version 4.1)

TMAP calculated the position of the markers on both linkage group 15 and 18 as well as the distance between the markers to create consensus maps for each linkage group. The maps were split into the two parental maps (maternal and paternal) using the 'split' option in the 'BuilderSplit' programme and an error rate for the statistical placement of the markers on the chromosome was calculated.

For chromosome 15 all 11 markers used in the screen could be imported into TMAP and JoinMap. The VB (maternal map) generated was 34.3 cM long and the largest intermarker gap was between VVIM42b/VMC8G3.2/VVIM42a (grouped at the same position) and VVIV24, 11.9 cM. The software was very confident in the marker placements as the error rates were all 0 except for VMC8G3.2 that was 0.1023. The G1 (paternal map) was 58.3 cM long and the largest intermarker gaps were between VMC8G3.2 and VVIM42a, 11.9 cM as well as between VVIM42a and VVIV24, 11.9 cM. The software was very confident in the marker placements as the error rates were all 0 except for VMC8G3.2 that was 0.1023 (Table 21).

Table 21 Position of SSR markers on chromosome 15 for Villard Blanc (VB) and G1-6604 (G1) according to TMAP.

SSR marker	Marker position on VB (cM)[a]	Marker position on G1 (cM)[a]	Distance between markers on VB (cM)[a]	Distance between markers on G1 (cM)[a]	Error rate for marker placement on VB (%)[b]	Error rate for marker placement on G1 (%)[b]
UDV116	0	0	0	0	0	0
UDV047	6.5	5.4	6.5	5.4	0	0
VVIB63	7.5	10.9	0.9	5.4	0	0
VVIQ61	7.9	14.2	0.5	3.4	0	0
VVIP33	11.2	17.6	3.3	3.4	0	0
VMC5G8	20.6	24.6	9.4	7	0	0
VVIM42b	22.5	28	1.9	3.3	0	0
VMC8G3.2	22.5	31.3	0	3.3	0.1023	0.1023
VVIM42a	22.5	34.6	0	3.3	0	0
VVIV24	22.5	46.5	0	11.9	0	0
VChr15a	34.3	58.3	11.9	11.9	0	0

[a]Distance between SSR markers in centimorgan (cM)

[b]Error rates for placement on chromosome as percentage

Before the chromosome 18 markers were processed an attempt was made to simplify the marker that had multiple peaks (VMC6F11) as the complex multiple calls could not be accommodated in TMAP and JoinMap V4.1. Five alleles (81, 90, 94, 96 and 114) occurred repeatedly in the progeny plants and were eliminated from the dataset which left alleles 98, 114 and the respective null alleles that inherited in various combinations in the progeny and were used for linkage map construction. Two other markers UDV108 and VMC3E5 were discarded as both parents were homozygous and therefore they were not informative. Of the 12 markers used to screen the population only 10 could be imported into TMAP and JoinMap V4.1 and used to generate a linkage map.

For chromosome 18 the VB (maternal map) generated was 85.8 cM long and the largest intermarker gap was between VMC2A3 and VMC8B5, 17.7 cM. The software was very confident in the marker placements as the error rates were all 0 except for VVIP08 that was 0.008 and VVMD17 and VVIN16-cjvh that was 0.0092. The G1 (paternal map) was 89.1 cM long and the largest intermarker gap was between VMC2A3 and VMC8G3.2, 13.8 cM. The

software was very confident in the marker placements as the error rates were all 0 except for VVIP08 that was 0.008 and VMC6F11 that was 0.0092 (Table 22).

Table 22 Position of SSR markers on chromosome 18 for Villard Blanc (VB) and G1-6604 (G1) according to TMAP.

SSR marker	Marker position on VB (cM)[a]	Marker position on G1 (cM)[a]	Distance between markers on VB (cM)[a]	Distance between markers on G1 (cM)[a]	Error rate for marker placement on VB (%)[b]	Error rate for marker placement on G1 (%)[b]
VMC2A3	0	0	0	0	0	0
VMC8B5	6.1	13.8	6.1	13.8	0	0
VVIM93	23.7	27.5	17.7	13.8	0	0
VMC8F4-2	32.1	38.3	8.3	10.7	0	0
VVIP08	41.6	49.5	9.6	11.3	0.008	0.008
UDV134	49.3	60.8	7.6	11.3	0	0
VVMD17	66.4	72	17.1	11.2	0	0
VVIN16-cjvh	83.5	80.3	17.1	8.3	0.0092	0
VMC6F11	83.5	80.3	0	0	0	0.0092
VMC7F2	85.8	89.1	2.3	8.8	0	0

[a]Distance between SSR markers in centimorgan (cM)

[b]Error rates for placement on chromosome as percentage

The converted genotype tables were imported into JoinMap V4.1 and the software calculated the position of the markers on the linkage groups as well as the distance between the markers to create a consensus map for linkage groups 15 and 18. The linkage group 15 parental maps for the F_1: VB x G1 population was calculated using the two-way-pseudo-testcross method, 1_P1 represents the VB maternal map and 1_P2 represents the G1 paternal map. The 1_P1 (VB) map is 35.4 cM long and the largest intermarker gap is 11.5 cM where the 1_P2 (G1) map is 62.6 cM long and the largest intermarker gap is 26.4 cM. The combined map 1 (F_1: VB x G1) is 56.4 cM long and the largest intermarker gap is 14.9 cM. Not all of the markers imported mapped on the respective parental maps but was present on the combined map. Marker VChr15a did not map on the VB maternal map and markers UDV047, VVIQ61, VVIV24, VVIM42b and VMC8G3.2 did not map on the G1 paternal map (Figure 23).

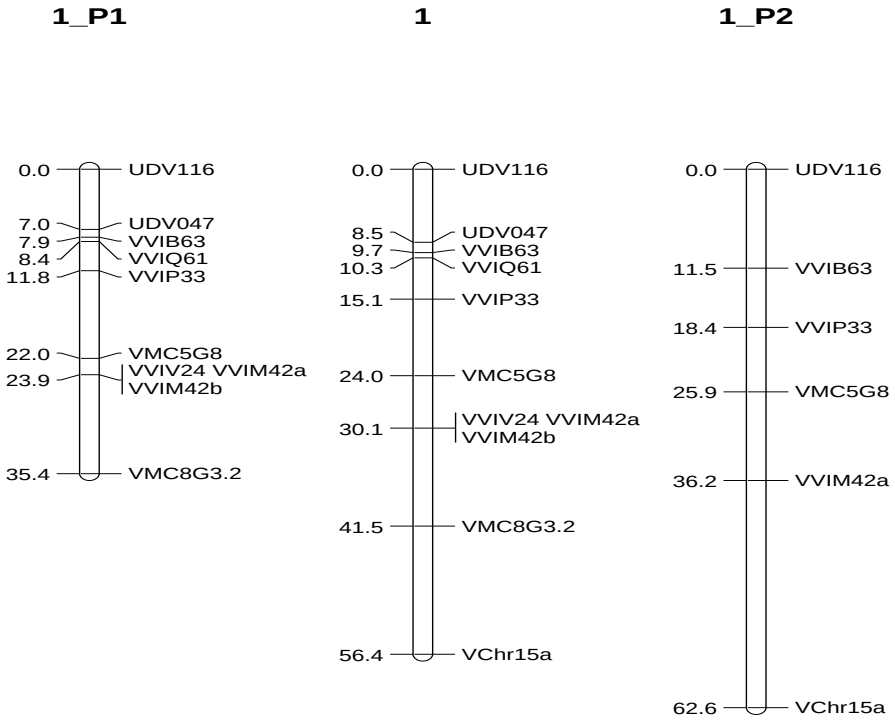

Figure 23 SSR marker positions on chromosome 15 for the parental plants and the F_1: VB x G1 population. Villard Blanc is represented by 1_P1; G1-6604 is represented by 1_P2 and the F_1: VB x G1 population by 1. Map distances are in cM.

The linkage group 18 parental maps for the F_1: VB x G1 population were calculated using the two-way-pseudo-testcross method, 1_P1 represents the VB maternal map and 1_P2 represents the G1 paternal map. The 1_P1 (VB) map is 102.0 cM long and the largest intermarker gap is 39.9 cM where the 1_P2 (G1) map is 101.6 cM long and the largest intermarker gap is 32.2 cM. The combined map 1 (F_1: VB x G1) is 101.8 cM long and the largest intermarker gap is 23.1 cM. Not all of the markers imported mapped on the respective parental maps but were present on the combined map. Marker VVMD17 did not map on the VB maternal map and markers VMC8B5 and VVIP08 did not map on the G1 paternal map (Figure 24).

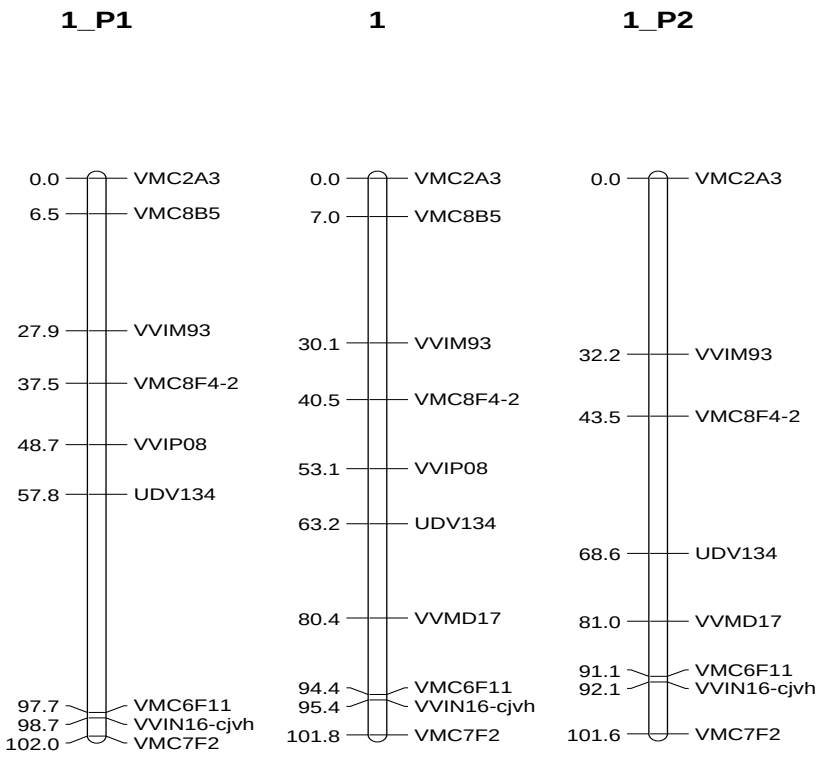

Figure 24 SSR marker positions on chromosome 18 for the parental plants and the F_1: VB x G1 population. Villard Blanc is represented by 1_P1; G1-6604 is represented by 1_P2 and the F_1: VB x G1 population by 1. Map distances are in cM.

1.3.2.3 QTL Analysis

The converted genotype tables en phenotypic scores were imported into MapQTL and subjected to statistical analysis to determine if QTLs were present that were linked to downy and powdery mildew resistance. The F_1: VB x G1 population was examined as well as the two parentals, Villard Blanc (VB) and G1-6604 (G1).

The Kruskal-Wallis calculations performed indicated that most of the markers on chromosome 15 of VB were linked to the powdery mildew resistance with a high level of significance ($P < 0.005$). Four markers (VMC5G8, VVIM42a, VVIV24 and VVIM42b) were linked as well but with a lower order of significance, P=0.01 and P=0.05. One marker VMC8G3.2 was not linked to the QTL. In the F_1: VB x G1 population the inherited markers showed the same high level of significant linkage to the resistance QTL from marker

UDV116 to VVIP33. Three other markers were linked as well but with a lower level of significance, VMC5G8 to VVIM42a. Two markers, VMC8G3.2 and VChr15a, displayed no significant linkage to the QTL (Table 23).

Table 23 Kruskal-Wallis indication of markers significantly linked to the powdery mildew QTL on chromosome 15.

	Linkage group	**SSR Marker**	**Significance (P value)[a]**
F_1: VB x G1	LG15	UDV116	*******
	LG15	UDV047	*******
	LG15	VVIB63	*******
	LG15	VVIQ61	*******
	LG15	VVIP33	*******
	LG15	VMC5G8	**
	LG15	VVIV24	**
	LG15	VVIM42a	***
	LG15	VMC8G3.2	-
	LG15	VChr15a	-

[a]Level of significance, *P= 0.1, ** P= 0.05, *** P= 0.01, ******* P= 0.0001

The Kruskal-Wallis calculations performed indicated that most of the markers on chromosome 18 of VB were linked to the downy mildew resistance with a high level of significance ($P < 0.0001$). Three markers (VMC8F4-2, VVIP08 and UDV134) were linked as well but with a lower order of significance. Three markers (VVIM93, VMC8B5 and VMC2A3) were not linked to the QTL. In the F_1: VB x G1 population the inherited markers showed the same high level of significant linkage to the resistance QTL for markers VMC7F2, VMC6F11 and VVIN16-cjvh. Two other markers were linked as well but with a lower level of significance, UDV134 and VVIP08. The rest of the markers displayed no significant linkage to the QTL (Table 24).

Table 24 Kruskal-Wallis indication of markers significantly linked to the downy mildew QTL on chromosome 18.

	Linkage group	**SSR Marker**	**Significance (P value)[a]**
F_1: VB x G1	LG18	VMC2A3	-
	LG18	VMC8B5	-
	LG18	VVIM93	-
	LG18	VMC8F4-2	-
	LG18	VVIP08	**
	LG18	UDV134	**
	LG18	VVMD17	-
	LG18	VVIN16-cjvh	*******
	LG18	VMC6F11	*******
	LG18	VMC7F2	*******

[a]Level of significance, *P= 0.1, ** P= 0.05, **** P= 0.005, ******* P= 0.0001

Interval mapping of chromosome 15 with the powdery mildew phenotypic scores 10/02/2010 and 15/02/2010, indicated the presence of a QTL between SSR markers UDV116 and VMC8G3.2 (41.5 cM) that was significantly contributing to the observed powdery mildew resistance. A LOD score threshold of 2.5 was determined after running a permutation test with 1000 iterations and selecting the value linked to a linkage group relative score of 0.95 (P = 0.05). This QTL region explains up to 18.9% (LOD 7.47) and 23.9% (LOD 12.29) of the phenotypic variance observed for the two scores respectively (Table 25, Figure 25).

Table 25 The location, significance and confidence interval of QTL identified by IM in F_1: VB x G1 progeny for powdery mildew resistance.

LG[a]	**QTL confidence interval**	**Nearest marker**		**10/02/2010**	**15/02/2010**
15	UDV116 – VMC5G8	UDV116	Max LOD	7.47	12.26
			% Var[b]	18.9	23.9
			LOD threshold - LG15	2.5	2.5

[a]Linkage group

[b]Percentage phenotypic variance explained

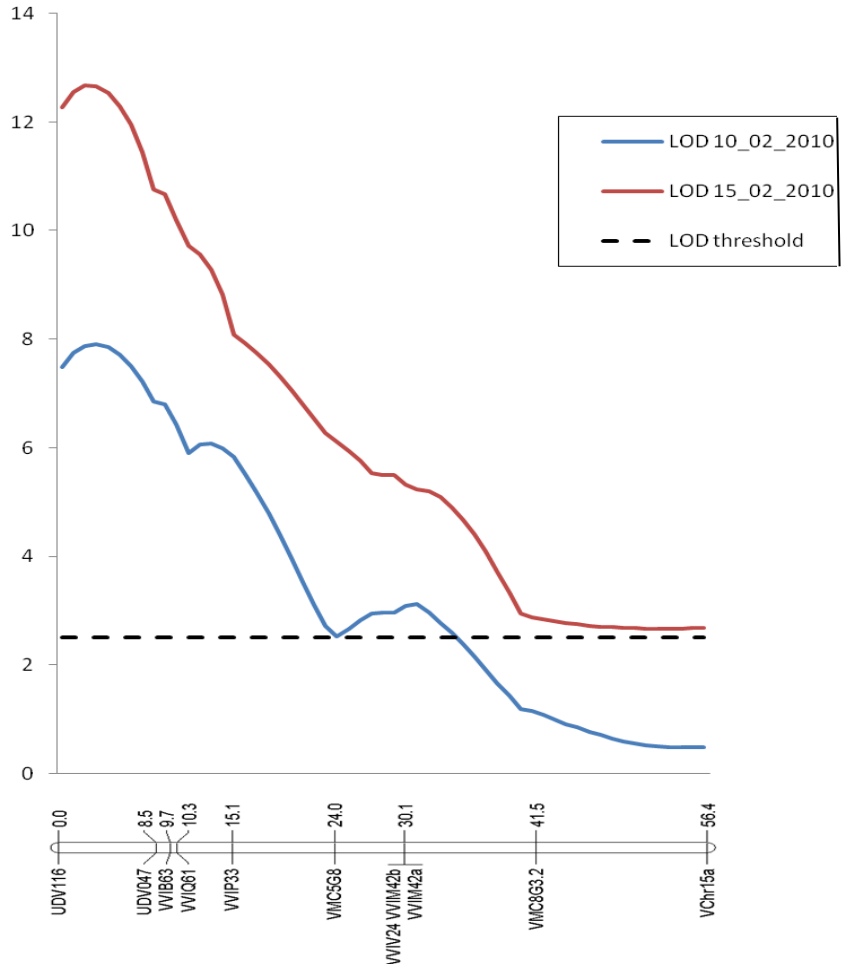

Figure 25 QTL for powdery mildew resistance on chromosome 15, calculated with Interval mapping. The LOD threshold is 2.5.

Interval mapping of chromosome 18 with the downy mildew phenotypic scores 05/12/2010 and 09/12/2010, indicated the presence of a QTL between SSR markers UDV134 and VMC7F2 (38.6 cM) was significantly linked to the observed resistance. A LOD score threshold of 2.5 was determined after running a permutation test with 1000 iterations and selecting the value linked to a linkage group relative score of 0.95 (P = 0.05). This QTL region explains up to 19.1% (LOD 5.34) and 21.2% (LOD 7.39) of the phenotypic variance observed for the two scores respectively (Table 26, Figure 26).

Table 26 The location, significance and confidence interval of QTL identified by IM in F_1: VB x G1 progeny for downy mildew resistance.

LG[a]	QTL confidence interval	Nearest markers		05/12/2010	09/12/2010
18	VVMD17 – VMC7F2	VVIN16-cjvh, VMC6F11	Max LOD	5.34	7.39
			% Var[b]	19.1	21.2
			LOD threshold - LG18	2.5	2.5

[a]Linkage group

[b]Percentage phenotypic variance explained

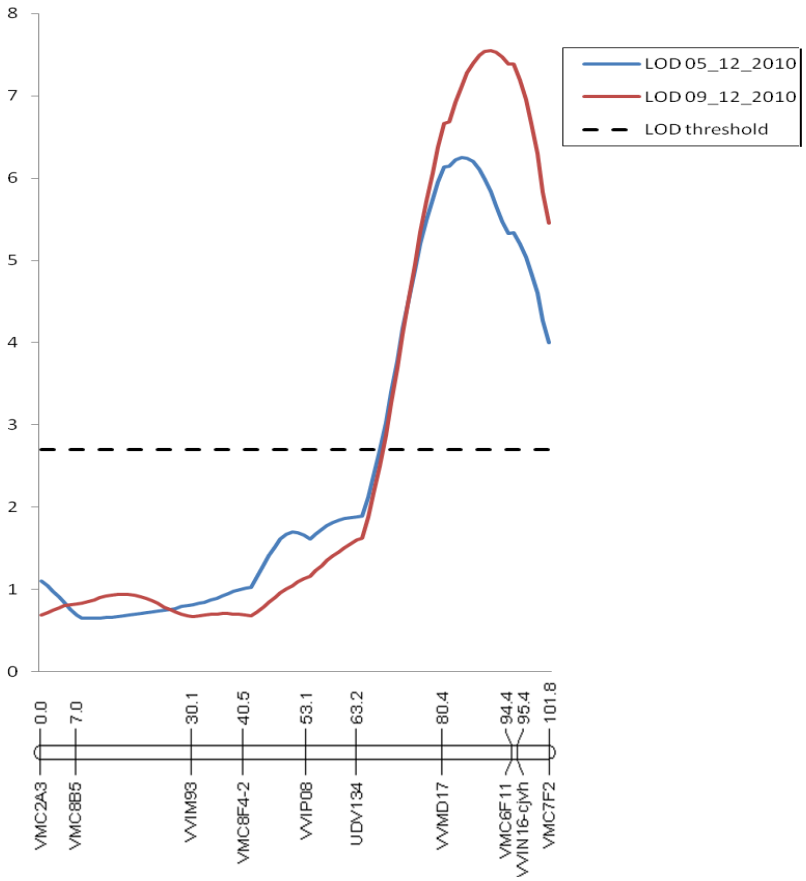

Figure 26 QTL for downy mildew resistance on chromosome 18, calculated with Interval mapping. The LOD threshold is 2.5.

To improve the definition of the QTL region obtained with IM on chromosome 15, automatic co-factor selection was performed. UDV116 and VMC5G8 were identified as co-factors for the QTL region. A multiple QTL model (MQM) calculation was then done, incorporating the selected co-factors and for each of the two phenotypic scores (Figure 27). The two scores

(10/02/2010 and 15/02/2010) produced an improved QTL peak, which was now clearly placed between SSR markers UDV116 and UDV047. The percentage of variance explained was between 18.9% (LOD 7.47) and 23.9% (LOD 12.26) for this area (Table 27, Figure 27).

Table 27 The location, significance and confidence interval of QTL identified by MQM in F_1: VB x G1 progeny for powdery mildew resistance.

LG[a]	QTL confidence interval	Nearest markers		10/02/2010	15/02/2010
15	UDV116 – UDV047	UDV116[c], VMC5G8[c]	Max LOD % Var[b] LOD threshold - LG15	7.47 18.9 2.5	12.26 23.9 2.5

[a]Linkage group

[b]Percentage phenotypic variance explained

[c]Identified with co-factor selection

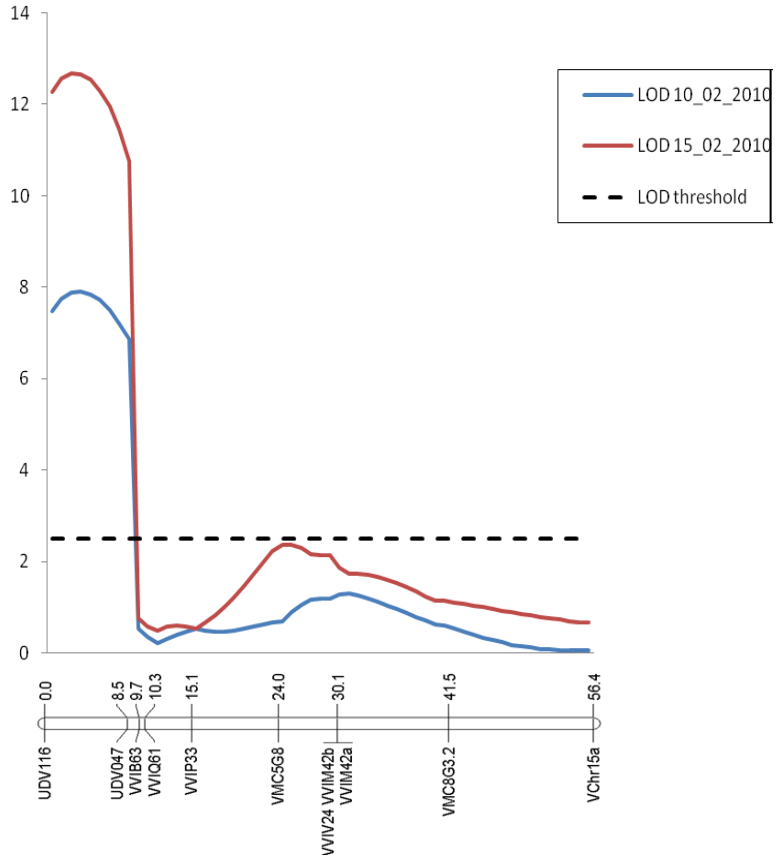

Figure 27 QTL for powdery mildew resistance on chromosome 15, calculated with MQM mapping. The LOD threshold is 2.5.

To improve the definition of the QTL region obtained with IM on chromosome 18, automatic co-factor selection was performed. VMC6F11 was identified as a co-factor for the QTL region. A multiple QTL model (MQM) calculation was then done, incorporating the selected co-factor and for each of the two phenotypic scores (Figure 28). The two scores (05/12/2010 and 09/12/2010) produced an improved QTL peak, which was now clearly placed between SSR markers VVIN16-cjvh and VMC7F2. The percentage of variance explained was between 19.1% (LOD 5.33) and 21.2% (LOD 7.39) for this area (Table 28, Figure 28).

Table 28 The location, significance and confidence interval of QTL identified by MQM in F_1: VB x G1 progeny for downy mildew resistance.

LG[a]	**QTL confidence interval**	**Nearest markers**		**05/12/2010**	**09/12/2010**
18	VVIN16-cjvh – VMC7F2	VMC6F11[c]	Max LOD	5.33	7.39
			% Var[b]	19.1	21.2
			LOD threshold - LG18	2.5	2.5

[a]Linkage group

[b]Percentage phenotypic variance explained

[c]Identified with co-factor selection

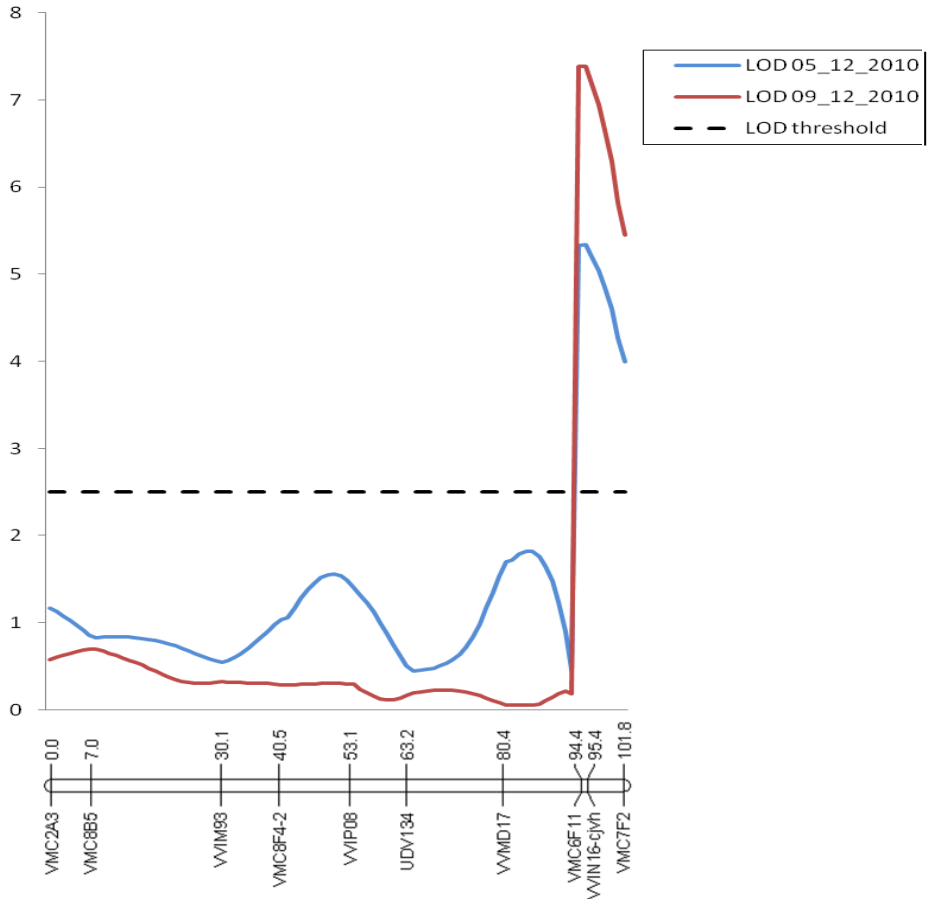

Figure 28 QTL for downy mildew resistance on chromosome 18, calculated with MQM mapping. The LOD threshold is 2.5.

In an effort to identify the specific VB alleles that are linked to the fungal resistance the estimated average of the quantitative trait's distribution associated with all allele combinations (mu) was looked at (mu_*ac*, mu_*ad*, mu_*bc*, mu_*bd*) for the markers linked significantly to the QTL. SSR marker UDV116 is linked to powdery mildew resistance on chromosome 15 and the *a* allele is 348 (from VB), the *b* allele is 357 (from VB), the *c* allele is 127 (from G1) and the *d* allele is 130 (from G1). The average for the *ac* and *ad* allele combinations are much higher that the values for the *bc* and *bd* allele combinations. This indicates that the resistance is associated with the *a* allele of UDV116 and that is allele 348 from VB (Table 29).

Table 29 Average distribution of allele combinations of SSR marker UDV116 associated with the powdery mildew resistance QTL on chromosome 15.

Trait	Locus	Alleles	LOD	mu_*ac* {00}[a]	mu_*ad* {00}[a]	mu_*bc* {00}[a]	mu_*bd* {00}[a]
10/02/2010	UDV116	348:357 x 127:130	7.47	4.00	4.20	3.03	3.19
15/02/2010	UDV116	348:357 x 127:130	12.26	3.53	3.57	2.35	2.30

[a]Estimated average of the quantitative trait's distribution associated with an allele combination.

SSR markers VVIN16-cjvh, VMC6F11 and VMC7F2 were linked to downy mildew resistance on chromosome 18. For VVIN16-cjvh the *a* allele is 245 (from VB), the *b* allele is 247 (from VB), the *c* allele is 247 (from G1) and the *d* allele is 253 (from G1).For VMC6F11 the *a* allele is 114 (from VB), the *b* allele is null (from VB), the *c* allele is 98 (from G1) and the *d* allele is null (from G1). For VMC7F2 the *a* allele is 196 (from VB), the *b* allele is 211 (from VB), the *c* allele is 200 (from G1) and the *d* allele is 207 (from G1). The average for the *bc* and *bd* allele combinations are much higher that the values for the *ac* and *ad* allele combinations. This indicates that the resistance is carried on the *b* allele of VVIN16-cjvh (247 from VB), the *b* allele from VMC6F11 (null from VB) and the *b* allele from VMC7F2 (211 from VB) (Table 30).

Table 30 Average distribution of allele combinations of SSR markers associated with the downy mildew resistance QTL on chromosome 18.

Trait	Locus	Alleles	LOD	mu_*ac* {00}[a]	mu_*ad* {00}[a]	mu_*bc* {00}[a]	mu_*bd* {00}[a]
05/12/2010	VVIN16-cjvh	245:247 x 247:253	5.33	2.48	2.42	3.48	3.50
05/12/2010	VMC6F11	114:null x 98:null	5.34	2.48	2.42	3.46	3.52
05/12/2010	VMC7F2	196:211 x 200:207	4	2.52	2.47	3.48	3.27
9/12/2010	VVIN16-cjvh	245:247 x 247:253	7.39	2.58	2.76	3.70	3.69
9/12/2010	VMC6F11	114:null x 98:null	7.39	2.58	2.76	3.69	3.70
9/12/2010	VMC7F2	196:211 x 200:207	5.45	2.64	2.85	3.69	3.52

[a]Estimated average of the quantitative trait's distribution associated with an allele combination.

Chapter 5

Discussion

Fungal disease and the control thereof is a concern for breeders and growers of grapevine alike. Efforts are made to develop varieties that have fungal disease resistance genes/QTLs from multiple sources in an effort to obtain durable resistance that is beneficial to all parties involved. In this study, the fungal disease resistance genes/QTLs from three resistance sources, namely Pölöskei Muskotály, Kishmish Vatkana and Villard Blanc were investigated on a molecular basis. Molecular marker data was combined with the phenotypic data in order to compile the relevant linkage maps and perform QTL mapping. It was decided to use SSR markers for this study as there is an abundance of literature available on the use of various SSR markers to generate the linkage maps and QTL maps published. These SSR linkage maps are also populated with AFLP, SCAR and SNP markers but to a lesser extent.

All of the genotypic data generated during the course of this study was done by multiplexing SSR markers from the PCR step, when possible, in an effort to make the process as cost efficient as possible. This is important as the molecular cost incurred has an impact on breeders if they incorporate this approach into breeding programs. Care was taken during the selection of markers and the fluorescent labels used so that as many markers as possible could be amplified in a single multiplex. The multiplex PCR set-up was done using a standard set of reagent volumes but optimising the reaction at a specific temperature rather than adapting individual primer volumes to achieve good amplification of the markers. This was successful for almost all of the markers used and there were only five markers out of a total of sixty seven markers that would not amplify. Some markers produced complex amplification patterns that rendered them useless for mapping as it could not be incorporated into the statistical programs used for the mapping studies but this can be overcome by redesigning the primers to be more specific.

5.1 Pölöskei Muskotály

Three PM-derived F_1 populations were investigated during this study. It was problematic to obtain leaves for DNA extraction from these populations as the F_1 plants exhibited very poor growth. Parental plants were screened with 27 SSR markers and they displayed various levels of informativeness but they could all be used for linkage mapping and QTL analysis. The first F_1 population to be investigated, PMxRS, was screened with 20 SSR markers and it showed that 62% of the population was the result of self-pollination of PM. Examination of marker alleles of heterozygous F_1s, revealed that they were either heterozygous (like PM) or homozygous for either one of the alleles occurring in PM (Figure 11). This meant that less than half of the population could be used for the study and it was decided to investigate two other populations that were available. The two alternative PM populations (F_1: PM x G4 and F_1: PM x SS) , which were tested with a smaller set of SSR markers, showed the same high level of self-pollination events as in the F_1: PM x RS population. 87% and 50% of the respective F_1: PM x G4 (Table 11) and F_1: PM x SS (Table 12) individuals was the result of self-pollination events.

Two plants in the F_1: PM x G4 population seemed to be the result of cross pollination but on closer inspection it appeared that another parent may have been involved. Plants #12 and #13 were possibly derived from a different cross as they had some unique marker alleles not characteristic of either parent. An alternative explanation for the unique marker alleles present in plant #12 and #13 is the presence of transposable elements in the grapevine genome that could have modified the length of the repeat in question as 21% of the grapevine genome is made up of transposable elements (Velasco et al. 2007).

These genotypic findings explained the poor growth vigour of the F_1 PM-derived populations as cleistogamy or self-pollination occurs in grapevines before the flowers open; although they are mostly cross-pollinators (Harst et al. 2009). Previous studies (Filler et al. 1994, Marais 1981, Aradhya et al. 2003) have shown that self-pollination in grapevine can lead to inbreeding depression.

The results obtained from the molecular analysis made it clear that none of the three PM-derived populations were indeed the result of true crosses. The Pölöskei Muskotály

populations were thus eliminated from the study. As Villard Blanc, a known fungal resistant variety (Kozma 2002a, Zyprian et al. 2003, Hajdu et al. 2007, Bellin et al. 2009) is part of PM's pedigree, it was expected that we would detect the same QTL in this study. Unfortunately this aim could thus not be achieved. Most of the information on PM is in either Russian or Hungarian so it was unknown that PM is probably a cleistogamous cultivar (personal communication P. Burger 2009) and could not be used as a maternal plant during crossing experiments. However, the discovery is a significant finding, as the breeder will in future use PM as pollen parent instead.

5.2 Kishmish Vatkana

Hoffmann et al. (2008) reported that powdery mildew resistance was conferred by a dominant allele of the *REN1* gene located on chromosome 13 of Kishmish Vatkana and that a segregation pattern of 1 (resistant):1 (susceptible) is expected for the trait. Bulk segregant analysis was performed on a F_1 population in order to identify the region associated with the resistance and it was found on a specific area on chromosome 13. A linkage map was generated using only markers on the identified chromosome. In this study a linkage map of only chromosome 13, for a local F_1: SS x KV population, was generated and a QTL approach was followed to map the powdery mildew resistance on it due to the quantitative nature of the phenotypic score which do not allow the clear distinction of resistant and susceptible classes.

In order to determine the ratio of resistant plants: susceptible plants, phenotypic scores of five, seven and nine (OIV scores) were counted as resistant plants and scores of one and three (OIV scores) were considered to be susceptible. The 23/11/2009 score showed that 79% of the F_1: SS x KV progeny displayed medium to high levels of resistance and the two Sunred Seedless (susceptible parent) control plants were scored as five (medium resistance) which is higher than expected. The resistance in the susceptible control plants and progeny population is very high and could be due to ineffective inoculation events or an environment that did not promote the growth of the pathogen (temperature and humidity). It was decided to discard these results as they were not considered to be accurate. The 30/11/2009 score had a more even distribution of the OIV scores and 63% of the individuals displayed medium to high levels of resistance while for the 17/02/2011 score 53% of the individuals displayed medium to high levels of resistance. Both of these scores come close to a 1:1 segregation pattern. The

control plants for these two screens were scored with a high resistance for the Kishmish Vatkana parent and a low to very low resistance for the Sunred Seedless parent, as expected. The more even distribution patterns could be due to the fact that the environment in the plant tunnels more closely represents the natural environment in terms of factors involved in pathogen spread and growth. Correlation calculations showed that there was a moderate correlation between the 30/11/2009 and 17/02/2011 scores. The whole leaf scoring process is very labour intensive and did not yield good results in this study. This factor could prove to be problematic if the population size is very big, as needed for fine mapping. The process of performing phenotypic screens needs to be managed more efficiently to produce results that are accurate as it is crucial for accurate results. Alternative methods for more accurate, less time consuming scoring should be investigated like the semi-automated procedure described by Peresotti et al. (2011).

Molecular marker analysis was successful for 12 of the 13 selected SSR markers. Eight of the markers were the same as used by Hoffmann et al. (2008) and the additional markers were added in an effort to improve the linkage map density and to pinpoint the location of the QTL more precisely. Two markers (UDV-020 and VMC2C7) identified by Hoffmann et al (2008), and three additional markers (UDV038, VMC2A9 and VMC3B12-cjvh) produced multiple alleles. The scoring of these markers proved to be more problematic than initially thought as they could not all be simplified by eliminating alleles that occurred in all the progeny (Figure 15). Only one of the complex SSR markers, UDV020, could be used for mapping purposes. The area around the *REN1* gene is highly repetitive (Coleman et al. 2009) and may explain this phenomenon. For future use of these markers it might help to redesign them so that they produce less complex profiles.

Linkage mapping of chromosome 13 was performed to generate Sunred Seedless and Kishmish Vatkana parental maps and an SS x KV combined map. The linkage maps generated for Sunred Seedless with TMAP and JoinMap, with respective lengths of 33.7 cM and 39.4 cM, were fairly similar. In contrast the Kishmish Vatkana linkage map, 37.8 cM (TMAP) and 51.8 cM (JoinMap), showed quite a significant difference in length between the two maps and it is most likely due to the difference in the way that the software packages calculate map distances. TMAP is better able to handle incomplete datasets as well as markers that are not completely informative (Cartwright et al. 2007). The SS x KV combined map is 45.6 cM long and this is very similar to the published length of 46.6 cM (Hoffmann et al.

2008) but 10 cM longer than the Coleman et al. (2009) published map, 35.8 cM. The marker order is the same but the positions of the markers are slightly different. There is a 26.5 cM gap between markers UDV020 and VVIP10 as the two markers that were supposed to map in this position had multiple alleles that could not be used for mapping. Both of these markers were also less informative as both were heterozygous but shared a common allele.

A QTL approach proved to be successful in mapping powdery mildew resistance in a local SS x KV population to the same area as the single dominant *REN1* powdery mildew resistance gene reported by Hoffmann et al. (2008). Kruskal-Wallis (single marker regression) analysis revealed that all of the markers on chromosome 13 were associated with resistance to powdery mildew with $P<0.005$. When using the 17/02/2011 phenotypic score IM showed that a large region between markers UDV124 and VVIP10 was significant. MQM mapping was performed to define the area better. While all of the markers in this area had LOD scores above the LOD threshold in the IM map it dropped below the LOD threshold for the MQM map in the area between markers UDV020 and VVIP10. The area of significance was narrowed down to between markers UDV-124 and UDV-020. This is because the mapping was done with co-factor selection that is much more stringent in the placement of the QTL and the contributing effect of the markers (MapQTL6 manual) and therefore more trustworthy. The broad significant peak shown between markers UDV124 and VVIP10 with the 17/02/2011 phenotypic score was most likely not an accurate reflection of the situation and possibly due to errors in the phenotypic data. IM and MQM mapping using the 30/11/2009 phenotypic data showed an area of significance between markers UDV-124 and UDV-020. The two markers VMC9H4-2 and VMCNG4E10-1 are the most closely linked markers to the QTL followed by UDV-124 and UDV-020 as flanking markers. These findings confirm the results published by Hoffmann et al. (2008) and Coleman et al. (2009).

The effectiveness of the powdery mildew resistance *REN1* gene was confirmed in the local South African population and closely linked markers, with the resistance associated alleles, were identified that can in future be used for MAS breeding purposes and the pyramiding of resistance genes.

5.3 Villard Blanc

5.3.1 Chromosome 15

Two powdery mildew phenotypic screens were performed on the F_1: VB x G1 population and in order to determine the ratio of resistant plants: susceptible plants phenotypic scores of five, seven and nine (OIV scores) were counted as resistant plants and scores of one and three (OIV scores) were considered to be susceptible. For the 10/02/2010 score 80% of the F_1: VB x G1 population displayed medium to very high levels of resistance and for the 15/02/2010 score 60% displayed levels of medium to very high resistance. The 10/02/2010 score shows a fairly high percentage of resistance and it could be due to ineffective inoculation events or an environment that did not promote the growth of the pathogen. However, the phenotypic scores for the VB and G1 control plants were very high (OIV 9) and high (OIV 7) respectively for both scores. These high scores make it plausible that there could be a high incidence of resistance and that there was no problem during the phenotypic scoring process. It was decided to continue with these scores for the QTL mapping. The distribution of the scores across the OIV score range for the 15/02/2010 score is in the expected bell shape curve with a more even distribution across the whole range of scores. Correlation calculations showed that there was a strong correlation between these two with a very high level of significance ($P < 0.001$).

Molecular marker analysis was successful for 11 of the 14 selected SSR markers for chromosome 15. Two markers (VVIB63 and UDV047) were identified that carried null alleles but these were scored as alleles as a clear inheritance pattern was observed in the F_1 progeny.

Linkage mapping of chromosome 15 was performed to generate Villard Blanc and G1-6604 parental maps and a VB x G1 combined map. The linkage maps generated for Villard Blanc with TMAP and JoinMap, with respective lengths of 34.3 cM and 35.4 cM, were fairly similar. The order and placement of two markers mapped by TMAP is slightly different to that generated by JoinMap. This could be due to the fact that TMAP is better able to handle incomplete datasets as well as markers that are not completely informative (Cartwright et al.

2007). Marker VMC8G3.2 is mapped at 22.5 cM by with TMAP and 35.4 cM with JoinMap while marker VChr15a is not mapped at all by JoinMap. Both of these markers are not very informative (VMC8G3.2: 220 226 x 226 226 and VChr15a: 148 148 x 139 152). The G1-6604 linkage map was 58.3 cM (TMAP) and 62.6 cM (JoinMap) which is also fairly similar and the order and placement of the markers are similar except that five markers were not mapped by JoinMap (UDV047, VVIQ61, VVIM42b, VMC8G3.2, VVIV24). This was most likely due to the fact that these markers were homozygous in G1 and therefore not very informative. The VB x G1 combined map was 56.4 cM long and this is very similar to the published map length. Adam-Blondon et al. (2004) published a partial map for chromosome 15 from marker VVIB63 to VVIV24 and it is 23.2 cM and the map generated in this study was 20.4 cM. Doligez et al. (2006) published a linkage map for chromosome 15 built from five different grapevine varieties from marker UDV116 to VMC8G3.2 and it is 37 cM and the map from this study is 41.5 cM. Di Gaspero et al. (2007) published a complete map for chromosome 15 that has a total length of 75.7 cM but the length between markers UDV116 and VMC8G3.2 is 49.5 cM and the map from this study is 41.5 cM. In all three publications there are shared markers between the different maps and markers that correspond to the ones used in this study. The general marker order is the same as for our combined linkage map and the slight variation in lengths between the different maps is possibly explained by the presence/absence of markers and how informative the markers were for the specific varieties used in each study.

QTL analysis was performed using the genotypic and phenotypic datasets for chromosome 15. Kruskal-Wallis (single marker regression) analysis revealed several markers on chromosome 15 to be associated with resistance to powdery mildew with $P<0.005$. These associated markers were spanning a 15 cM stretch from UDV116 to VVIP33 on chromosome 15. The position of a QTL linked to powdery mildew resistance, as calculated with IM, was also placed between UDV116 and VVIP33. All of the markers shown by the Kruskal-Wallis calculation to have a significant linkage to the QTL had a LOD score above the threshold. MQM mapping was performed to confirm the area of significance found with IM and a similar area of significance was identified except that the QTL was located between UDV116 and VVIB63. The addition of co-factor selection has effectively narrowed the range on the map where the QTL is positioned. The QTL identified for powdery mildew resistance spans a length of 9.7 cM and the most closely linked markers are UDV116, UDV047 and VVIB63. This information can be used in future for MAS breeding purposes and the pyramiding of

resistance genes. Akkurt et al. (2007) reported powdery mildew resistance on chromosome 15 of Villard Blanc in a Gf.Ga-47-42 x Villard Blanc cross and developed SCAR markers associated with the resistance QTL. These associated SCAR markers occur in the area between markers UDV116 and VVIP33 which is the same QTL area identified by this study using a different Villard Blanc derived population.

5.3.2 Chromosome 18

Two downy mildew phenotypic screens were performed on the F_1: VB x G1 population. A number of plants could not be included in the screen as the plants had died or the ends of young shoots were damaged during the movement of plants from one plant tunnel to another. None of the leaf discs displayed total resistance to the downy mildew infection but this can be expected from resistance conferred by a QTL as it confers a partial degree of resistance and is also affected by the environment. The same leaf discs were used for the two screens, five days apart, as the initial evaluation (05/12/2010) showed that the infection had not taken place in quite a number of discs. At the time it was postulated that the laboratory environment where the screen took place was not constant enough. Steps were taken to remedy this and a second score was done (09/12/2010) that was more successful. If all the plants that were scored five and seven (OIV scores) were combined as resistant plants for the 05/12/2010 score, 66% of the F_1: VB x G1 population displayed medium to high levels of resistance and for the 09/12/2010 score 71% displayed levels of medium to high resistance. This is once again a fairly high percentage and could be due to ineffective inoculation events or an environment that did not promote the growth of the pathogen, but the control plants for these two screens were scored with a high resistance for the VB parent and a low to very low resistance for the G1 parent. Correlation calculations showed that there was a strong correlation between the two scores performed with a very high level of significance. This is to be expected from scores taken in such a short time span and a second season phenotypic screen would have been more desirable.

Molecular marker analysis was successful for 12 of the 13 selected SSR markers for chromosome 18 but two markers (UDV108 and VMC3E5) were not informative in our population and were thus not used for linkage mapping and QTL analysis.

Linkage mapping of chromosome 18 was performed to generate Villard Blanc and G1-6604 parental maps and a VB x G1 combined map. The linkage maps generated for Villard Blanc with TMAP and JoinMap, with respective lengths of 85.8 cM and 102.0 cM, had the same marker order, but a significant difference in length. Marker VVMD17 was not mapped for Villard Blanc, but this is most likely due to the fact that it was homozygous (VVMD17 alleles: 220 220 x 220 221). The G1-6604 TMAP and JoinMap linkage maps were 89.1 cM and 101.6 cM respectively and had the same marker order. Markers VMC8B5 and VVIP08 were not mapped for G1-6604 as they were also homozygous. These two parental maps showed quite a significant difference in length between the two maps and it is most likely due to the difference in the way that the software packages calculate map distances. Seventy per cent of the markers on chromosome 18 shared alleles between the parental genotypes and were thus less informative. TMAP employs a multipoint-likelihood maximisation method and is more robust in dealing with incomplete datasets and less informative markers (Cartwright et al. 2007). JoinMap (v4.1) also employs a multipoint-likelihood maximisation method but specifically for cross pollinating populations and thus produces slightly longer maps but with marker orders comparable to that generated by TMAP. Other possible explanations for a difference in map lengths could be missing data and genotyping errors. The dataset for this population had a very low percentage of missing values so it is not likely that this is influencing the lengths of the generated maps. Genotyping errors are always a risk when dealing with large datasets so care was taken to prevent this by using the bins and panels generated in GeneMapper to aid in the analysis of this dataset but it cannot be definitely eliminated as a possible contributing factor to the difference in map lengths observed. The combined map for F_1: SS x KV is 101.8 cM long and this is very similar to the published map length. Adam-Blondon et al. (2004) published a linkage map containing amongst others seven of the markers used in this study with a length of 95.1 cM. Doligez et al. (2006) published a complete linkage map for chromosome 18 that has a total length of 131.5 cM but the length between markers VMC2A3 and VMC7F2 is 102.3 cM. Di Gaspero et al. (2007) published a complete linkage map for chromosome 18 as well that has a total length of 167.2 cM but the distance between markers VMC2A3 and VMC6F11 is 98.3 cM and the map from this study is 95.4 cM. In all three publications the marker order is the same as for our combined linkage map. The slight variation in lengths between the different maps is possibly explained by how informative the markers were for the specific varieties used in each study.

QTL analysis was performed using the genotypic and phenotypic datasets for chromosome 18. Kruskal-Wallis (single marker regression) analysis revealed several markers on chromosome 18 to be associated with resistance to downy mildew with $P<0.005$. These associated markers were from the region flanked by markers VVIN16-cjvh and VMC7F2 and it spans a seven cM stretch at the distal end of chromosome 18. The position of a QTL linked to downy mildew resistance, as calculated with IM, was placed between SSR markers VVMD17 and VMC7F2. All of the markers shown by the Kruskal-Wallis calculation to have a significant linkage to the QTL had a LOD score above the threshold. MQM mapping was performed to confirm the area of significance identified by IM and results similar to that of IM was seen except that the QTL was located between markers VVIN16 and VMC7F2. The addition of co-factor selection has effectively narrowed the range on the map where the QTL is positioned.

A QTL for downy mildew resistance was reported on chromosome 18 by Eibach et al. (2007), Zyprian et al. (2009) and Bellin et al (2009). Zyprian et al. (2009) reported the presence of a QTL on chromosome 18 in Villard Blanc associated with downy mildew resistance, but very little molecular information was provided. They referred to a publication by Eibach et al. (2007) on Regent resistance to downy mildew for associated markers, the markers were VMCNG-2f12, UDV130 and UDV108. The first two markers were not used in this study and can be considered for future use. UDV108 was genotyped and then discarded as the parental plants were both homozygous in our population. The QTL position reported by Bellin et al. (2009) was between the SSR markers VMC7F2 and VVIN16 which is the same QTL found in this study. Flanking markers and resistance carrying alleles were identified and this can be used for MAS breeding purposes and the pyramiding of resistance genes in future breeding exercises.

Chapter 6

Conclusion

This study achieved the objectives set out at the start for two of the three aims specified. The first objective of creating a linkage map for PM and subsequent mapping of the fungal disease resistance could not be achieved as three attempts proved it impossible to generate a mapping population when using PM as maternal parent. The majority of the PM F_1 populations investigated inherited only maternal alleles and could not be used for linkage mapping or QTL analysis as they were not segregating populations. However, the knowledge of PM's cleistogamous nature is valuable to the breeding program who will in future attempt to use it as a male parent only. This is a very good example of how molecular studies can assist breeders to save time and spare costs involved in developing and maintaining populations that do not have desired traits without having to wait until the plants are fully grown.

In the second objective the presence and effectiveness in SA of the *REN1* powdery mildew resistance gene of KV was proven. We confirmed its presence in the local breeding population and showed that it can be inherited by progeny created for breeding and developmental purposes. An indication of its level of resistance and the frequency of inheritance was demonstrated by the scores obtained during the powdery mildew phenotypic screens performed. It also mapped to the reported map position on LG13 and closely associated markers were identified for future MAS efforts. F_1: SS X KV *REN1*-carrying seedlings were identified for the breeder to be used in the breeding program.

As a third objective the presence and position of the QTLs for powdery and downy mildew resistance were confirmed on chromosomes 15 and 18 of the Villard Blanc (VB) population and the disease associated alleles were identified. The powdery and downy mildew resistant genotypes of the F_1: VB X G1 seedlings were pointed out to the breeder. An indication of its

level of resistance and the frequency of inheritance was demonstrated by the scores obtained during the powdery mildew phenotypic screens performed.

Through the knowledge generated in this study, the breeding program can now discard plants not carrying the desired genes/QTL very early in the breeding cycle by screening for disease associated alleles and thus using these markers, closely linked to the traits of interest, as an integral part of the breeding program in any future crosses. This will reduce the time and cost involved in development of desirable varieties. Breeding efforts can begin to combine the *REN1* powdery mildew resistance gene and the Villard Blanc powdery mildew (chromosome 15) and downy mildew (chromosome 18) resistance QTLs into a single variety in an effort to pyramid resistance to these fungal diseases for more durable resistance. Other sources of resistance can be investigated in this manner to obtain other resistance genes/QTLs that can be used for gene pyramiding, thereby increasing the durability of fungal disease resistance. One such new source of resistance is the *REN5* gene identified in a *Muscadinia rotundifolia* variety that transfers resistance to powdery mildew (Blanc et al. 2012). Two new genes conferring resistance to powdery and downy mildew were also identified in another *Muscadinia rotundifolia* variety and named MrRUN1 (*Muscadinia rotundifolia* resistance to *Uncinula necator*) and MrRPV1 (*Muscadinia rotundifolia* resistance to *Plasmopara viticola*) (Feechan et al. 2013). A third locus, named *Run2*, was identified in another *Muscadinia rotundifolia* variety that confers resistance to powdery mildew (Riaz et al. 2011). It is evident that *Muscadinia rotundifolia* varieties could be valuable sources of resistance and should be investigated. Pölöskei Muskotály's resistance to powdery and downy mildew can be investigated again once populations are available that were made by using Pölöskei Muskotály as a pollen parent.

A couple of limitations were identified during the course of the study. The first one was observed during the investigation of Pölöskei Muskotály as a resistant parent. Not enough information was available as to the nature of Pölöskei Muskotály and how it should be used in breeding experiments. Due to this fact it was in error used as a maternal plant and this lead to self-pollination events in the majority of the F_1 progenies rendering them useless for mapping purposes. As this information was not available beforehand there is not much that can be done to prevent this from happening in future investigations but care should be taken to gather as much information as possible at the start of similar studies. A second limiting factor became

clear during the phenotypic screens performed on the various populations. The leaf disc assays were very time consuming and it is crucial that the laboratory environment is controlled precisely to be as close as possible to that of a natural environment with regards to especially temperature, light and humidity. The whole leaf and whole plant assays used for some of the populations generally seemed to give a more accurate reflection of the degree of resistance but this is also very time consuming. Less labour intensive and more accurate ways to score the fungal growth consistently would improve the quality of the QTL maps generated. A semi-automatic, non-destructive method can be investigated as reported by Peressotti et al. (2011).

Although SSRs are predominantly used in linkage mapping and QTL studies lately the trend has been shifting to the use of SNPs as it is a cheaper method for large scale screening exercises. SNP technology has advanced in recent years and it is now possible to run very large SNP assays at a fraction of the cost of a SSR assay. New SNPs are also continuously being published and this helps to populate the existing linkage maps with more markers at a researcher's disposal (Guichoux et al. 2011, Barba et al. 2014).

With the availability of Next Generation Sequencing, Bioinformatics and the subsequent sequencing of the first *Vitis* genome (Velasco et al. 2007) a "whole new world" has opened up for research of grapevine. The data obtained from genome sequencing can be used to search for new molecular markers to complement the existing array of microsatellite or SNP markers. It will be possible to find genes that confer disease resistance and not only QTLs. It can be a costly process, but as technology improves the cost of using these machines have decreased dramatically.

By looking at ways to overcome the limitations experienced during this study and incorporating some or all of the future perspectives mentioned earlier traditional grapevine breeding can benefit immensely from modern molecular techniques.

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Electronic resources

2nd Edition of the OIV descriptor list for grape varieties and *Vitis* species 2009

http://www.oiv.int/oiv/info/enplubicationoiv#grape

Last accessed 08/2014

Encyclo Wine

http://www.encyclowine.org/?title=Hybrid_grapes

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Kishmish Vatkana Image

http://tilia.zf.mendelu.cz/ustavy/556/ustav_556/atlas_bezsem_reva/E/Kereses.htm

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NCBI:

www.NCBI.nlm.nih.gov

Last accessed 08/2014

Vitis *International Variety Catalogue*

www.vivc.de

Last accessed 08/2014

Appendix 1

Phenotypic scores

1.1 SS x KV powdery mildew phenotypic score

Plant number	OIV score		
	23/11/2009	30/11/2009	17/02/2011
Kishmish Vatkana 1	9	9	9
Kishmish Vatkana 1	9	9	9
Sunred Seedless 1	5	3	3
Sunred Seedless 1	5	3	1
SS x KV 001	9	3	1
SS x KV 002	9	7	9
SS x KV 003	9	5	9
SS x KV 004	9	7	3
SS x KV 005	1	3	1
SS x KV 006	9	7	9
SS x KV 007	5	5	3
SS x KV 008	9	5	9
SS x KV 009	9	1	9
SS x KV 010	9	3	1
SS x KV 011	9	1	3
SS x KV 012	9	9	9
SS x KV 013	3	1	1
SS x KV 014	9	7	9
SS x KV 015	9	9	9
SS x KV 016	9	5	9
SS x KV 017	9	9	9
SS x KV 018	9	5	9
SS x KV 019	1	7	3
SS x KV 020	9	3	1
SS x KV 021	9	1	1
SS x KV 022	9	7	9
SS x KV 023	9	9	9
SS x KV 024	3	1	1
SS x KV 025	9	9	7
SS x KV 026	9	7	9
SS x KV 027	9	3	9
SS x KV 028	9	7	1

SS x KV 029	9	5	9
SS x KV 030	9	1	1
SS x KV 031	9	5	9
SS x KV 032	1	5	5
SS x KV 033	9	9	9
SS x KV 034	1	1	3
SS x KV 035	1	5	3
SS x KV 036	9	5	9
SS x KV 037	3	3	1
SS x KV 038	9	9	7
SS x KV 039	9	9	9
SS x KV 040	9	9	9
SS x KV 041	9	5	9
SS x KV 042	3	3	3
SS x KV 043	9	7	9
SS x KV 044	9	5	9
SS x KV 045	3	7	3
SS x KV 046	9	5	1
SS x KV 047	5	7	1
SS x KV 048	9	5	9
SS x KV 049	9	7	9
SS x KV 050	3	1	3
SS x KV 051	5	1	3
SS x KV 052	9	3	3
SS x KV 053	9	9	9
SS x KV 054	*	7	9
SS x KV 055	9	1	5
SS x KV 056	9	7	9
SS x KV 057	9	7	9
SS x KV 058	9	1	3
SS x KV 059	3	3	3
SS x KV 060	9	7	9
SS x KV 061	9	7	9
SS x KV 062	9	9	9
SS x KV 063	3	1	3
SS x KV 064	3	1	1
SS x KV 065	9	3	9
SS x KV 066	3	7	1
SS x KV 067	9	9	9
SS x KV 068	3	1	1
SS x KV 069	1	1	1
SS x KV 070	9	7	9
SS x KV 071	9	7	9
SS x KV 072	9	7	3

SS x KV 073	3	5	9
SS x KV 074	9	7	9
SS x KV 075	1	1	1
SS x KV 076	9	5	9
SS x KV 077	9	9	9
SS x KV 078	9	7	1
SS x KV 079	9	5	3
SS x KV 080	5	3	1
SS x KV 081	9	9	5
SS x KV 082	9	3	3
SS x KV 083	9	7	5
SS x KV 084	9	7	9
SS x KV 085	1	3	3
SS x KV 086	5	1	1
SS x KV 087	9	7	3
SS x KV 088	9	1	1
SS x KV 089	9	7	9
SS x KV 090	9	1	1
SS x KV 091	9	1	9
SS x KV 092	1	1	1
SS x KV 093	3	1	1
SS x KV 094	3	*	3
SS x KV 095	9	1	1
SS x KV 096	9	7	3
SS x KV 097	9	7	1
SS x KV 098	5	9	7
SS x KV 099	9	9	9
SS x KV 100	9	5	9
SS x KV 101	5	1	1
SS x KV 102	9	7	3
SS x KV 103	9	9	9
SS x KV 104	9	7	5
SS x KV 105	5	3	3
SS x KV 106	9	1	1
SS x KV 107	3	1	3
SS x KV 108	9	9	7
SS x KV 109	9	9	9
SS x KV 110	9	7	3
SS x KV 111	3	5	1
SS x KV 112	9	9	1
SS x KV 113	9	7	9
SS x KV 114	3	*	1
SS x KV 115	9	7	1
SS x KV 116	3	3	3

SS x KV 117	9	7	7
SS x KV 118	9	9	9
SS x KV 119	9	9	3
SS x KV 120	9	7	9
SS x KV 121	9	3	1
SS x KV 122	*	7	7
SS x KV 123	5	1	1
SS x KV 124	9	7	1
SS x KV 125	9	9	5
SS x KV 126	5	5	5
SS x KV 127	5	1	5
SS x KV 128	9	7	9
SS x KV 129	9	9	5
SS x KV 130	9	5	3
SS x KV 131	9	3	1
SS x KV 132	9	5	3
SS x KV 133	9	9	1
SS x KV 134	5	7	1
SS x KV 135	9	9	7
SS x KV 136	9	7	7
SS x KV 137	9	3	1
SS x KV 138	9	3	3
SS x KV 139	9	7	5
SS x KV 140	9	9	5
SS x KV 141	9	9	9
SS x KV 142	9	7	9
SS x KV 143	9	9	7
SS x KV 144	5	1	1
SS x KV 145	9	9	7
SS x KV 146	9	5	3
SS x KV 147	3	3	1
SS x KV 148	9	9	9
SS x KV 149	1	1	7
SS x KV 150	9	3	3
SS x KV 151	3	5	1
SS x KV 152	9	7	*
SS x KV 153	3	1	1
SS x KV 154	1	7	9
SS x KV 155	5	1	7
SS x KV 156	9	5	9
SS x KV 157	9	3	5
SS x KV 158	9	1	1

1.2 VB x G1 powdery mildew phenotypic score

Plant number	OIV score	
	10/02/2012	15/02/2012
Villard Blanc 1	9	9
Villard Blanc 2	9	9
Villard Blanc 3	9	9
G1-6604	7	7
VB x G1 001	5	3
VB x G1 002	*	3
VB x G1 003	7	7
VB x G1 004	5	3
VB x G1 005	*	*
VB x G1 006	*	9
VB x G1 007	7	9
VB x G1 008	7	7
VB x G1 009	*	*
VB x G1 010	9	7
VB x G1 011	7	7
VB x G1 012	*	7
VB x G1 013	1	1
VB x G1 014	7	5
VB x G1 015	7	5
VB x G1 016	9	7
VB x G1 017	7	5
VB x G1 018	7	7
VB x G1 019	7	3
VB x G1 020	9	7
VB x G1 021	5	3
VB x G1 022	5	3
VB x G1 023	9	7
VB x G1 024	9	9
VB x G1 025	7	7
VB x G1 026	7	9
VB x G1 027	*	*
VB x G1 028	9	9
VB x G1 029	7	5
VB x G1 030	7	7
VB x G1 031	3	3
VB x G1 032	5	5
VB x G1 033	5	3
VB x G1 034	*	9
VB x G1 035	5	5
VB x G1 036	7	5
VB x G1 037	9	7

VB x G1 038	*	1
VB x G1 039	9	7
VB x G1 040	9	9
VB x G1 041	5	5
VB x G1 042	7	7
VB x G1 043	7	7
VB x G1 044	7	5
VB x G1 045	9	5
VB x G1 046	1	1
VB x G1 047	1	1
VB x G1 048	7	3
VB x G1 049	7	5
VB x G1 050	5	3
VB x G1 051	1	1
VB x G1 052	9	9
VB x G1 053	*	5
VB x G1 054	5	3
VB x G1 055	5	5
VB x G1 056	*	1
VB x G1 057	5	1
VB x G1 058	*	1
VB x G1 059	7	3
VB x G1 060	5	1
VB x G1 061	7	7
VB x G1 062	*	3
VB x G1 063	5	1
VB x G1 064	*	1
VB x G1 065	*	7
VB x G1 066	7	5
VB x G1 067	3	1
VB x G1 068	*	1
VB x G1 069	7	7
VB x G1 070	*	3
VB x G1 071	1	1
VB x G1 072	5	1
VB x G1 073	*	5
VB x G1 074	*	1
VB x G1 075	*	1
VB x G1 076	5	5
VB x G1 077	7	7
VB x G1 078	5	3
VB x G1 079	9	5
VB x G1 080	9	9
VB x G1 081	7	7

VB x G1 082	*	3
VB x G1 083	9	9
VB x G1 084	1	1
VB x G1 085	5	5
VB x G1 086	9	9
VB x G1 087	*	3
VB x G1 088	5	3
VB x G1 089	*	3
VB x G1 090	*	*
VB x G1 091	7	5
VB x G1 092	*	7
VB x G1 093	5	3
VB x G1 094	*	5
VB x G1 095	*	1
VB x G1 096	*	9
VB x G1 097	*	3
VB x G1 098	7	7
VB x G1 099	3	1
VB x G1 100	1	1
VB x G1 101	9	9
VB x G1 102	7	7
VB x G1 103	*	3
VB x G1 104	7	9
VB x G1 105	*	*
VB x G1 106	9	7
VB x G1 107	*	7
VB x G1 108	*	*
VB x G1 109	5	3
VB x G1 110	3	3
VB x G1 111	7	5
VB x G1 112	*	5
VB x G1 113	7	3
VB x G1 114	7	7
VB x G1 115	5	3
VB x G1 116	7	5
VB x G1 117	1	1
VB x G1 118	*	9
VB x G1 119	7	7
VB x G1 120	7	5
VB x G1 121	9	5
VB x G1 122	7	7
VB x G1 123	5	3
VB x G1 124	9	9
VB x G1 125	5	3

VB x G1 126	9	7
VB x G1 127	9	7
VB x G1 128	7	9
VB x G1 129	5	5
VB x G1 130	5	5
VB x G1 131	7	7
VB x G1 132	7	7
VB x G1 133	7	5
VB x G1 134	7	7
VB x G1 135	7	5
VB x G1 136	7	5
VB x G1 137	7	7
VB x G1 138	7	5
VB x G1 139	*	*
VB x G1 140	1	1
VB x G1 141	7	5
VB x G1 142	9	7
VB x G1 143	3	3
VB x G1 144	5	3
VB x G1 145	*	3
VB x G1 146	5	5
VB x G1 147	5	3
VB x G1 148	7	5
VB x G1 149	5	5
VB x G1 150	9	5
VB x G1 151	*	*
VB x G1 152	7	5
VB x G1 153	9	7
VB x G1 154	5	5
VB x G1 155	7	5
VB x G1 156	7	7
VB x G1 157	3	1
VB x G1 158	*	1
VB x G1 159	9	5
VB x G1 160	*	*
VB x G1 161	*	9
VB x G1 162	*	*
VB x G1 163	1	1
VB x G1 164	9	7
VB x G1 165	*	3
VB x G1 166	9	7
VB x G1 167	1	1
VB x G1 168	5	3
VB x G1 169	*	1

VB x G1 170	5	3
VB x G1 171	*	3
VB x G1 172	1	1
VB x G1 173	9	7
VB x G1 174	7	7
VB x G1 175	5	3
VB x G1 176	9	9
VB x G1 177	9	9
VB x G1 178	3	1
VB x G1 179	*	7
VB x G1 180	3	3
VB x G1 181	3	3
VB x G1 182	*	3
VB x G1 183	*	5
VB x G1 184	*	*
VB x G1 185	3	3
VB x G1 186	1	1
VB x G1 187	5	5
VB x G1 188	*	7
VB x G1 189	9	5
VB x G1 190	7	5
VB x G1 191	*	1
VB x G1 192	7	7
VB x G1 193	*	*
VB x G1 194	7	5
VB x G1 195	9	9
VB x G1 196	9	7
VB x G1 197	7	3
VB x G1 198	9	9
VB x G1 199	1	1
VB x G1 200	5	5
VB x G1 201	*	7
VB x G1 202	9	9
VB x G1 203	*	3
VB x G1 204	9	9
VB x G1 205	9	9
VB x G1 206	*	*
VB x G1 207	5	3
VB x G1 208	7	7
VB x G1 209	9	9
VB x G1 210	7	5
VB x G1 211	*	*
VB x G1 212	*	1
VB x G1 213	7	7

VB x G1 214	9	5
VB x G1 215	9	7
VB x G1 216	7	7
VB x G1 217	9	9
VB x G1 218	9	9
VB x G1 219	7	9
VB x G1 220	7	3
VB x G1 221	7	7
VB x G1 222	7	7
VB x G1 223	9	7
VB x G1 224	7	5
VB x G1 225	7	7
VB x G1 226	9	7
VB x G1 227	7	5
VB x G1 228	7	5
VB x G1 229	7	7
VB x G1 230	*	9
VB x G1 231	7	7
VB x G1 232	*	1
VB x G1 233	9	9
VB x G1 234	9	7
VB x G1 235	*	5
VB x G1 236	7	5
VB x G1 237	7	7
VB x G1 238	7	5
VB x G1 239	7	7
VB x G1 240	9	9
VB x G1 241	7	7
VB x G1 242	9	9
VB x G1 243	9	9
VB x G1 244	*	3
VB x G1 245	9	5
VB x G1 246	7	7
VB x G1 247	7	1
VB x G1 248	9	9
VB x G1 249	*	9
VB x G1 250	*	7

1.3 VB x G1 downy mildew phenotypic score

Plant number	OIV score	
	05/12/2010	09/12/2010
Villard Blanc 1	7	7
G1-6604 1	3	3
Villard Blanc 2	7	7
G1-6604 2	3	1
VB x G1 001	1	3
VB x G1 002	7	7
VB x G1 003	5	7
VB x G1 004	7	7
VB x G1 005	*	*
VB x G1 006	*	*
VB x G1 007	7	*
VB x G1 008	*	*
VB x G1 009	*	*
VB x G1 010	3	5
VB x G1 011	*	*
VB x G1 012	*	*
VB x G1 013	5	5
VB x G1 014	*	*
VB x G1 015	5	5
VB x G1 016	*	*
VB x G1 017	3	1
VB x G1 018	*	*
VB x G1 019	7	7
VB x G1 020	5	5
VB x G1 021	3	7
VB x G1 022	5	5
VB x G1 023	*	*
VB x G1 024	*	*
VB x G1 025	7	7
VB x G1 026	7	7
VB x G1 027	*	*
VB x G1 028	*	*
VB x G1 029	*	*
VB x G1 030	*	*
VB x G1 031	7	7
VB x G1 032	*	7
VB x G1 033	5	5
VB x G1 034	*	*
VB x G1 035	3	3
VB x G1 036	*	*
VB x G1 037	7	7

VB x G1 038	7	7
VB x G1 039	*	1
VB x G1 040	*	7
VB x G1 041	7	7
VB x G1 042	*	*
VB x G1 043	*	7
VB x G1 044	7	7
VB x G1 045	*	*
VB x G1 046	*	*
VB x G1 047	1	3
VB x G1 048	1	1
VB x G1 049	*	*
VB x G1 050	*	*
VB x G1 051	7	7
VB x G1 052	*	*
VB x G1 053	*	7
VB x G1 054	7	7
VB x G1 055	7	7
VB x G1 056	*	7
VB x G1 057	*	*
VB x G1 058	7	7
VB x G1 059	*	7
VB x G1 060	*	*
VB x G1 061	*	*
VB x G1 062	7	7
VB x G1 063	7	7
VB x G1 064	*	7
VB x G1 065	*	*
VB x G1 066	1	1
VB x G1 067	7	5
VB x G1 068	5	3
VB x G1 069	*	7
VB x G1 070	*	7
VB x G1 071	*	*
VB x G1 072	1	1
VB x G1 073	5	7
VB x G1 074	*	7
VB x G1 075	5	7
VB x G1 076	3	5
VB x G1 077	*	*
VB x G1 078	*	*
VB x G1 079	1	3
VB x G1 080	*	*
VB x G1 081	3	7

VB x G1 082	7	7
VB x G1 083	*	*
VB x G1 084	*	*
VB x G1 085	*	*
VB x G1 086	*	*
VB x G1 087	7	7
VB x G1 088	*	7
VB x G1 089	5	7
VB x G1 090	*	*
VB x G1 091	5	7
VB x G1 092	7	7
VB x G1 093	7	7
VB x G1 094	*	7
VB x G1 095	5	5
VB x G1 096	*	*
VB x G1 097	5	1
VB x G1 098	*	*
VB x G1 099	7	7
VB x G1 100	*	*
VB x G1 101	*	*
VB x G1 102	5	5
VB x G1 103	*	*
VB x G1 104	*	7
VB x G1 105	*	*
VB x G1 106	*	*
VB x G1 107	*	*
VB x G1 108	*	*
VB x G1 109	5	5
VB x G1 110	1	1
VB x G1 111	*	*
VB x G1 112	7	7
VB x G1 113	7	7
VB x G1 114	*	*
VB x G1 115	1	5
VB x G1 116	1	3
VB x G1 117	5	5
VB x G1 118	*	*
VB x G1 119	1	3
VB x G1 120	3	3
VB x G1 121	1	5
VB x G1 122	1	1
VB x G1 123	1	5
VB x G1 124	7	7
VB x G1 125	7	7

VB x G1 126	*	*
VB x G1 127	*	*
VB x G1 128	7	5
VB x G1 129	*	*
VB x G1 130	*	7
VB x G1 131	5	5
VB x G1 132	7	7
VB x G1 133	*	*
VB x G1 134	7	7
VB x G1 135	7	7
VB x G1 136	7	7
VB x G1 137	*	7
VB x G1 138	*	*
VB x G1 139	7	7
VB x G1 140	7	7
VB x G1 141	3	3
VB x G1 142	*	*
VB x G1 143	*	*
VB x G1 144	*	7
VB x G1 145	5	7
VB x G1 146	7	7
VB x G1 147	*	*
VB x G1 148	7	7
VB x G1 149	7	7
VB x G1 150	*	7
VB x G1 151	*	*
VB x G1 152	*	*
VB x G1 153	*	*
VB x G1 154	7	5
VB x G1 155	*	7
VB x G1 156	*	*
VB x G1 157	5	5
VB x G1 158	7	7
VB x G1 159	7	7
VB x G1 160	5	5
VB x G1 161	5	1
VB x G1 162	7	*
VB x G1 163	1	3
VB x G1 164	1	3
VB x G1 165	*	7
VB x G1 166	7	7
VB x G1 167	3	3
VB x G1 168	1	1
VB x G1 169	7	7

VB x G1 170	*	*
VB x G1 171	3	3
VB x G1 172	7	7
VB x G1 173	7	7
VB x G1 174	5	7
VB x G1 175	*	*
VB x G1 176	*	*
VB x G1 177	7	7
VB x G1 178	*	7
VB x G1 179	*	*
VB x G1 180	3	3
VB x G1 181	7	7
VB x G1 182	7	1
VB x G1 183	*	*
VB x G1 184	*	*
VB x G1 185	1	1
VB x G1 186	3	3
VB x G1 187	1	1
VB x G1 188	1	1
VB x G1 189	*	*
VB x G1 190	*	*
VB x G1 191	1	1
VB x G1 192	5	5
VB x G1 193	7	7
VB x G1 194	7	7
VB x G1 195	*	*
VB x G1 196	*	*
VB x G1 197	5	7
VB x G1 198	5	7
VB x G1 199	*	7
VB x G1 200	*	*
VB x G1 201	*	7
VB x G1 202	7	7
VB x G1 203	1	1
VB x G1 204	7	*
VB x G1 205	1	1
VB x G1 206	7	7
VB x G1 207	7	7
VB x G1 208	7	7
VB x G1 209	*	7
VB x G1 210	7	7
VB x G1 211	*	*
VB x G1 212	*	7
VB x G1 213	*	*

VB x G1 214	*	7
VB x G1 215	1	1
VB x G1 216	5	7
VB x G1 217	*	*
VB x G1 218	*	7
VB x G1 219	7	*
VB x G1 220	1	1
VB x G1 221	*	*
VB x G1 222	*	7
VB x G1 223	1	1
VB x G1 224	5	5
VB x G1 225	*	*
VB x G1 226	*	*
VB x G1 227	*	*
VB x G1 228	*	7
VB x G1 229	*	*
VB x G1 230	*	7
VB x G1 231	3	1
VB x G1 232	7	7
VB x G1 233	*	7
VB x G1 234	*	7
VB x G1 235	*	7
VB x G1 236	*	*
VB x G1 237	*	7
VB x G1 238	7	*
VB x G1 239	*	*
VB x G1 240	*	*
VB x G1 241	*	*
VB x G1 242	*	7
VB x G1 243	5	5
VB x G1 244	*	7
VB x G1 245	*	7
VB x G1 246	7	7
VB x G1 247	*	7
VB x G1 248	7	7
VB x G1 249	*	7
VB x G1 250	*	7

Appendix 2

Conference presentations

2.1 Poster presentations

Veikondis R, Prins R, Van Heerden CJ, Burger P. A practical application of genotyping in table grape breeding. 8th Southern African Plant Breeders' Association Symposium March 2010.

2.2 Oral presentations

Veikondis R, Van Heerden CJ, Burger P, Prins R. Genetic characterisation of fungal disease resistance genes in grapevine using molecular marker technology. South African Society of Enology and Viticulture Conference September 2010. Lord Charles Hotel, Somerset West.