Fine Mapping of Quantitative Trait Loci for Non-host Resistance to Rusts in Barley

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Summary

Nonhost resistance can be defined as immunity, displayed by an entire plant species against all genotypes of a plant pathogen. The genetic basis of (non)host-status of plants is hard to study because it requires interspecific crosses. However, there are some plant species which show a near non-host status. They can provide insights into the genetics and the mechanism of nonhost resistance of a plant species against a specialized plant pathogen.

Barley (*Hordeum vulgare* L.) is such a near-nonhost to several rust fungi of cereals and grasses. The first objective of this study is to perform fine mapping of the resistance to heterologous rusts in SusQ11 near isogenic line and to study the association with *Rphq11*, a QTL on chromosome 2H which has shown previously effective against to the homologous leaf rust (*Puccini hordei*). Phenotyping was performed in 20 near isogenic lines and parental lines (Steptoe and SusPtrit) with six rusts (*P. hordei-bulbosi* Isr (Phb Isr), *P. hordei-murini* (Phm)*, P. hordei-secalini* (Phs), *P. persistans* (Pp), *P. triticina* (Pt) and *P. graminis-lolii* (Pgl)) and genotyping by mean of developing markers based in SNP that are known to be polymorphic between the parental lines and mapped previously in the introgressed area. The results suggest that in this region there are other genes with influence in the resistance to the heterologous rusts but that there is also a clear influence of *Rphq11* in the resistance to Phb Iran and possibly to Phb Isr and Pp. Resistance to Phb (Iran and Israel isolates) and Phs was mapped between 121 and 128 cM, and the resistance to Pp between 120 and 121 cM. More phenotyping experiments are needed to map the resistance to Phm, Pt and Pgl.

Alternatively, *Rnhq* on chromosome 7H was described to confer resistance to four heterologous rust species (*P.triticina, P.hordei-murini, P.graminis-lolii, P.hordei-secalini*). Substitution mapping performed for the *Rnhq* showed three sub-QTLs, each effective to one or two of the above mentioned pathogens. In this study marker development along the introgressed region was done to improve the fine mapping of the QTLs. Several flanking and cosegregating markers with the resistance were developed and they will provide good prospects for future cloning of the underlying genes.

2.Introduction:

2.1 Plant resistance:

2.1.1 Host resistance

Plant host resistance can be divided into two major types of resistance; qualitative and quantitative. We gained substantial understanding of qualitative resistance with the help of research work of Flor (1971). He stated a hypothesis that plants contains single dominant resistance gene (*R* gene) that recognize the complementary avirulence genes (*Avr* genes) of pathogens. This hypothesis is also known as gene for gene hypothesis. Avirulence gene in the pathogen encode a protein that is recognised by complementary *R* genes of the plants. This results in the hypersensitive reaction (HR) and inhibits the growth of the pathogen (incompatible reaction). If the plants do not contain the R genes then pathogen can infect the plants successfully and can grow on the plant (compatible reaction). The modern molecular work is based on the hypothesis of gene-for-gene relationship.

The HR is the result of specific interaction between resistance *R* gene and complementary *Avr* genes of pathogen at the cellular level. If the one of the genes is absent then there is compatible reaction between plant and pathogen, it means plants become susceptible to specific pathogen (Staskawicz, 2001). This gene-for-gene system occurs frequently and very common in biotrophic pathosystems such as rusts, smuts and powdery mildew of cereal crops. The resistance of these system is commonly race-specific and can be easily broken by the introduction of new races of pathogen to the ecosystem.

Breeders have often used the R genes in their resistance breeding programs, but this type of resistance gets easily broken by pathogens and farmers confront the problems of resistance breakdown. Quantitative or polygenic resistance has not been used frequently by the breeders and hence there are many cases of nondurable resistance (Parlevliet,1995).

2.1.2 Nonhost resistance

Even though, nonhost resistance occurs in most of the crop species and more often under desirable and adapted cultivars and germplasm, it has not been considered as a major source of durable resistance. The recurrent selection method can be helpful to improve the selection for quantitative resistance and it could be more durable than host resistance (Ribiero do Vale et al, 2001).

Nonhost resistance is the resistance displayed by all the members of the plant species against a specific pathogen species (Heath 1981). This is the most common type of resistance and very few plant pathogens can successfully infect the specific plant species (Atenza, Jafary et al. 2004). Heterologous pathogens are the pathogens that are involved in the nonhost resistance. Neu et al. (2003) performed a molecular analysis of the interaction of *Hordeum vulgare- Puccinia triticina* and they found four genes which expressed differentially in this interaction. However, with this study it was not possible to understand the genetics behind the nonhost resistance. Nonhost resistance for the plant species that are taxonomically different can be based on the morphological properties (such as hairy leaves) (Heath 2000). For the plant species that are closely related, nonhost resistance could be based on the perception of the pathogen by the plants, the mechanism could be involved in recognising PAMP (pathogen associated molecular pattern) signals or involvement of *R*-genes for hypersensitivity (Zipfel and Felix 2005).

If the resistance can remain effective for many years over a large geographical area, then it can be considered as a durable resistance (Johnson, 1984). Specialized plant pathogens can easily affect the host plant species, but when they are introduced to non-host, but closely related to their host species, pathogens failed to infect that non-host plant species. Hence it is interesting to find out the genetics of the nonhost resistance, which could lead to the broad-spectrum and durable resistance.

2.1.3 The Barley- leaf rust model to study nonhost resistance

Jan Parlevliet started the research on barley to the leaf rust (*Puccinia hordei*) rusts at the department of Plant Breeding in Wageningen in early 70's. Then, this research was continued by Rients Niks and he also started the research line about nonhost resistance to rusts and powdery mildews in barley. To study the genetics of resistance, the plant species was needed which display the status of intermediate host (near nonhost) to heterologous rust species. But it is difficult to differentiate between host and nonhost status and is not always clear (Heath1985; Niks1987). The intermediate host status (near nonhost) was proposed, in this classification only few accessions show moderate susceptibility to heterologous pathogens (Niks 1987). Barley (*Hordeum vulgare L.*) display a near nonhost status against some heterologous pathogens, for example, wheat leaf rust fungus (*Puccinia triticina* Ericks.) and the wall barley leaf rust (*P. hordei-murini*). (Niks et al. 1996). Hence the barley is considered as a useful model crop to study the genetics and mechanism of nonhost resistance.

Atienza et al (2004) suggested that nonhost resistance of barley is because of additional effect of rust species specific genes and genes effective for heterologous rusts as well. SusPtrit is a experimental line developed by Atienza et al (2004) to study the genetics of nonhost resistance whic is susceptible to *P. triticina* and other rusts like *P. hordei-murini*, *P. hordei-secalini*, *P. persistans*, *P. hordei-bulbosi* or *P. graminis-lolii*. *P. triticina* is a host rust species for Wheat. At this moment the research group has performed mapping for host and nohost resistance to rusts and powdery mildews using eight mapping populations. Four of them include SusPtrit as parental line (VadaxSusPtrit, Cebada CapaxSusPtrit, Golden PromisexSusPtrit and L94xSusPtrit). Putting all together the information of the mapping studies more than 100 QTLs have been mapped (see table 2.1). The main conclusions of this works for nonhost resistance are that:

- Resistance is mostly nonhypersensitive
- It is mostly polygenic
- Resistance levels to heterologous rusts are moderately correlated
- QTLs have different and overlapping specificities
- Per mapping population different QTLs

Table 2.1. Host and nonhost resistance QTLs

MAPPING POP.	Host resistance			H + Nh rust	Non-host Resistance			TOTAL
	Mildew	Rusts	Blast		Mildews	Rusts	Nh_R + Nh_M	
VxS	7	15	1	1	1	19	0	44
CCxS	4	4	0	0	0	12	1	21
GPxS	0	1	0	0	1	16	0	18
OWB	2	4	0	0	0	6	0	12
SxM	5	4	0	0	0	0	0	9
L94xV	0	0	0	0	0	3	0	3
L94xS	0	0	0	0	0	4	0	4
TOTAL	18	28	1	1	2	60	1	111

2.2 Background of the current *Rphq11* research

Marcel et al (2007a) identified four different QTLs in the mapping population of Steptoe X Morex, in this *Rphq11* had the largest explained phenotypic variance (43.1%). The resistant allele of the QTL *Rphq11* comes from Steptoe. In that study, the mapping experiments were performed and recombinants were identified in the F_4 generation of above mentioned parental lines (Steptoe X Morex). After that, Steptoe X SusPtrit crosses were made to introduce the resistance QTL in a susceptible background to create a near isogenic line (NIL) which facilitate the fine mapping. The name of this NIL is SusQ11.

Fine mapping of *Rphq11* was performed by Lorriaux (2007), Yeo (2008) and Yeo et al (unpublished). Several molecular markers were developed in these studies in order to do marker saturation of the area of interest. Substitution mapping performed by Yeo et al. (unpublished) revealed the genetic position of *Rphq11*. *Rphq11* is located on chromosome 2H at the distance of 91.28cM according to a recently cosensus map developed at Niks'group, and the peak marker is WBE144 (BOPA2_12_10969). During the phenotyping experiments conducted by Yeo et al. (2008, unpublished) it was observed that SusQ11 also provides partial resistance against heterologous rusts species such as *P. hordei-secalini, P. triticina and P. hordei-bulbosi* (Table 2.2). The initial hypothesis was that Rphq11 provides partial resistance to *P. hordei* and heterologous rusts. However, Yeo et al (unpublished) found discrepancies between the resistance patterns in the recombinants between *P. hordei* and *P. hordei-bulbosi* (Iran isolate) which make evident the possible interaction of other genes with *Rphq11*. Another possibility is the presence of other genes for nonhost resistance in the introgressed area of Steptoe in SusPtrit to create SusQ11.

2.3 Background of the current *Rnhq* research

Qi et al. (1995) created a dense linkage map using the recombinant inbred lines (RIL) population derived from the cross between cultivers Vada and L94. Initially, this population was created to study the partial resistance against barley leaf rust (*Puccinia hordei*). Qi and Niks et al.(1998) found six QTLs for partial resistance, also this RIL population was used to screen against heterologous rust species *Puccinia triticina* and *Puccinia hordei-murini* at the seedling stage (Niks, Fernandez et al. 2000). During this screening, a QTL was discovered which was effective against *P. hordei-murini* and also provides resistance against *P. triticina*. The donor parent for this QTL is Vada. This *Rnhq* (nonhost) QTLwas mapped on the long arm of Chromosome 1 (7H) and it was also observed that it does not provide resistance against host rust pathogen (*P. hordei*). Niks et al. (unpublished) continued the work on nonhost QTL (*Rnhq*) by creating near isogenic lines (NILs) with L94 background and later with SusPtrit backgorund. The resistance/susceptibility patterns of these NILs to *P. hordei* and some heterologous rusts is presented in table 2.1. After that, fine mapping of *Rnhq* was performed by van Dijk (2007) and also other researches of Nik's group (data not published yet) using the resistant NIL L94-*Rnhq* and susceptible L94 as a parents. SKT1 marker was detected as the peak marker at a position around 86cM.

Table 2.2. NILs with different resistance QTLs inoculated with P. hordei and several heterologous rusts. Data for *P. hordei* is presented in terms of relative latency period and for the other rusts in relative infection frequency

Nils	1.2.1	Co.4	Uppsala	Phs French	Phs Wage	Phs Gro	Pt flam	Pt BWR96258	Pt INRA	Phb Iran	Phb Israel
	RLP(3 reps)	RLP (4 reps)	RLP (2 Rep)	RIF (2 rep)	RIF (2rep)	RIF (2 rep)	RIF (3 reps)	RIF (2 reps)	RIF (2 rep)	RIF (2 reps)	RIF (2 reps)
SusPtrit	100	100	100	100	100	100	100	100	100	100	100
Su-Rphq2	104	100	103	139	88	133	112	118	132	121	113
Su-Rphq3	104	101	105	122	108	149	57	61	78	85	88
Su-Rphq11	104	102	106	56	44	57	92	40	58	2	43
Su-Rphq16	107	106	107	163	109	119	96	105	93	91	98
Su-Rnhq.L	99	99	100	77	70	75	85	157	129	87	57
Su-Rnhq.v	100	102	104	73	66	79	86	113	98	71	54
L94	100	100	100	100	100	100	100	100	100	100	*
L94-Rphq2	106	106	105	24	96	63	87	60	105	79	*
L94-Rphq3	103	105	104	19	51	36	34	37	33	88	*
L94-Rnhq	*	100	100	68	15	18	76	48	96	87	*
L94-Q4			98	174		142	113		222		

Nils	Pgl	Phm	Pp	Pgt
	RIF (2 reps)	RIF (2 reps)	RIF (2 reps)	RFF (2 reps)
SusPtrit	100	100	100	100
Su-Rphq2	108	103	140	102
Su-Rphq3	31	80	114	91
Su-Rphq11	32	44	54	66
Su-Rphq16	102	113	189	88
Su-Rnhq.L	57	93	75	116
Su-Rnhq.v	54	87	65	123
L94	100	100	HR	100
L94-Rphq2	115	72	HR	115
L94-Rphq3	31	80	HR	49
L94-Rnhq	3	28	HR	107
L94-Q4		135		

Recently, the same homo-recombinats used before and the parental lines were genotyped with a SNP array of 7900 loci (9K Infinium i-select array).It was found 58 markers segregating in that material. It was possible to give a position to many of the 7900 SNP loci by the information provided by the company of the array and some publications but also for a consensus map developed at Niks' group based in three mapping populations (VadaxSusPtrit; Cebada CapaxSusPtrit and Golden PromisexSusPtrit). The size of the introgression from Vada in L94 was estimated in around 36cM. Substitution mapping was performed with the genotyping and phenotyping data and it was found that *Rnhq* was divided in 3sub-QTLs with different specificities. One QTL was found effective to *P. hordei-murini* Rhenen and *P. hordei-secalini* Wageningen (*Rnhq-Phm/Phs*) in position 63cM, another one in 86cM for resistance to *P. triticina* Swiss (*Rnhq-Pt*) and finally a third one at 94-99 cM for resistance to *P. graminis-lolii*. Now the research is focussed in working with the SNP markers in those areas in order to developed markers flanking and cosegregating with this QTLs to improve the fine mapping.

2.4 Research Questions and objectives of this thesis

The main objective of this thesis was to fine map the resistance to heterologous rusts in SusQ11 genotype. The fine mapping of this *Rphq11*region will help to find if there is association between host and nonhost resistance. The first step was the marker development at every 5cM interval on chromosome 2H around *Rphq11* region. The second step was to phenotype the homo-recombinants with six heterologous rusts, to perform substitution

mapping and to identify the location of resistance effective for heterologous rusts using high resolution mapping. Secondly, teh study was focussed in another QTL, *Rnhq,* with the main objective of developing molecular markers based in SNPs to improve the fine mapping of the three sub-QTLs around *Rnhq* region.

The research Question are listed below; separately for each QTL of interest in this study
General Research questions: **Rphq11**
Q. Is there any association between host and nonhost resistance? (shared genes?)
Q. Is *Rphq11* involved in nonhost resistance?

General Research question: **Rnhq**
Q. Is the resistance to heterologous rusts due to the same genes or different sets of genes for each rust?

2.1 Materials & Methods :

2.1.1Fine mapping of Rphq11

2.1.2 Plant materials:

A near isogenic line (NIL) previously developed in SusPtrit background with an introgression from Steptoe cultivar with the resistance QTL *Rphq11* was used for this study (SusQ11). The size of the introgression from Steptoe in the NIL was estimated about 56.5 cM (from 80 to 146.5cM in 2H chromosome) according to a consensus map (Own data not published yet). SusPtrit wass crossed with SusQ11 in order to produce homorecombinants with different fragments of the Steptoe genome to perform substitution mapping. This was done initially by Yeo et al (own data not published) to fine map *Rphq11*. In the present study, a total of 21 of these recombinants, SusQ11, SusPtrit and Steptoe were used in the phenotyping and genotyping experiments..

2.1.3 Phenotyping:

Because previous experiments indicated that SusQ11 is partial resistant to six heterologous rusts (Table 1) (Yeo et al., unpublished), they were used to phenotype the homorerecombinants and the parental lines. The rust fungi pathogens were maintained on their respective host (Table. 1). For inoculation fresh urediniospores collected in the 24 hours before to the inoculation were used. Inoculation for all six heterologous rusts was performed the same day in a classical settling tower for rust inoculation. This experiment included a single replication with four plants per genotype because there were not enough seeds of all the homorecombinants for Rphq11 to carry out more replicas.

Table 1: The rust fungi and the respective host used for inoculation in the phenotyping experiment for *Rphq11*

Rust fungi	Rust fungi short name	Host species	Host common name
P. hordei secalini French	*Phs*	*Hordeum secalinum*	Meadow barley
P. hordei murini Rhenen	*Phm*	*Hordeum murinum*	Wall barley
P. persistens	*Pp*	*Agropyron repens*	Couch grass
P. triticina Swiss	*Pt*	*Triticum aestivum*	Common wheat
P. graminis lolli	*Pgl*	*Lolium perenne*	Perennial Ryegrass
P. hordei bulbosi Israel	*Phb*	*Hordeum bulbosum*	Bulbous barley

Boxes of size 37*39 cm were used for the sowing of the seeds and to perform the inoculation experiment. For every rust 2 boxes were used and in each box the parental lines (Steptoe and Susptrit) and the NIL, SusQ11, were sown as a reference. 11 recombinants were sown in each box distributed ramdomly. In total, 12 boxes were used to perform this experiment with around 52 plants per box. The seeds were sown in two rows including at least four or five seeds of each recombinant and parental lines. Inoculations were carried out 10 days after the sowing. The first leaf of each plant was pinned with the adaxial side facing upwards while the other leaves were removed. In each box the glass slid was placed in order to to check if the urediniospores would germinate.

Figure 2.1 Pictures of plants in the boxes, Left side picture show two boxes inoculated with *P. hordei secalini* and right hand side picture show one box treated with *P. graminis lolli*.

Urediniospores were mixed with approximately a ten-fold greater volume of lycopodium spores to homogenise the distribution of the inoculum. Hence, 2.5 mg of each rusts urediniospores were mixed with 25 mg of lycopodium powder and blown over the plants in a settling tower. It has a rotating base and this facilitates the uniform distribution of urediniospores. After that the boxes were kept for approximately five minutes in the settling tower to settle down the urediniospores, and then the boxes were removed from the settling tower. To avoid the contamination during this inoculation procedure the other boxes which were being used for inoculation with another heterologous rusts were kept outside the settling tower room.

Figure 2.2 Picture of settling tower used for inoculation.

After inoculation, boxes were placed in a humidity chamber to incubate the urenidiospores overnight at 100% relative humidity and were transferred to a greenhouse compartment the next day.

Finally, evaluation of this phenotyping experiment was performed 12 days after inoculation by counting the number of pustules per area of leaf inoculated with respective pathogen, i.e. infection frequency.

2.1.4 Marker development & primer design:

The molecular analysis was done in 11 steps and the scheme of this molecular analysis shown below in figure 2.3.

Previously to this study, SusPtrit, SusQ11 and Steptoe were genotyped with the 9K i-select Infinium array (around 7.500 SNP loci). It was found 2642 polymorphic loci between SusPtrit and Steptoe, and 186 between SusPtrit and SusQ11. Those 186 loci were placed in 2H between 88.7 and 146.5cM according to data of our consensus map. Therefore, the introgression of Steptoe containing *Rphq11* is estimated in 57.7cM and contains 186 SNP loci. The previous study done by Yeo et al. (unpublished data) about the fine mapping of *Rphq11* was focused in the area between 88 and 119cM. For the fine mapping of the resistance to the heterologous rusts we decided to cover the region between 105 and 146.5cM because this is the most likely to map the resistance to the heterologous rusts. 20 SNP loci were selected in intervals of 5cM approximately in this area (105-146.5cM).
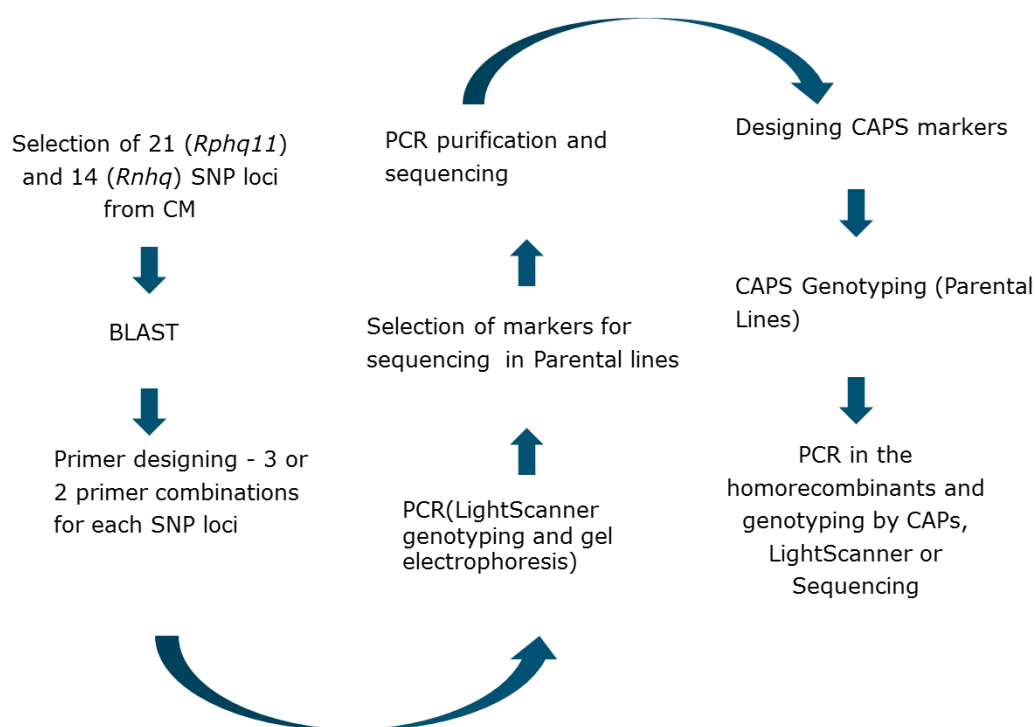
Figure2.3 Scheme of molecular analysis

The SNP loci selected for *Rphq11* are listed below in the Table 2.2. The sequences of these SNP loci used in the array was blasted (MEGABLAST) in NCBI database to find the highly similar homologous in barley if possible, and if not in rice, *Brachypodium* or wheat. The location of the SNPs described in the array were identified in the sequences found with high homology in order to define the region for designing primers. Subsequently, primers pairs were developed keeping in mind that SNP should be more or less in the middle of the forward and reverse primer segments. These primers were designed using the programs Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and NetPrimer (http://www.premierbiosoft.com/netprimer/index.html). For every SNP marker listed below, three primer pairs were designed. Where it was not possible to design three primers, two primer pairs were done. Total 61 primer combinations were designed(appendices). After that, PCRs in the parental lines and SusQ11 were done to see whether the primer pairs produce a clear amplicons.

Table 2.2 List of the SNP loci selected from the SNP database with their position on Chromosome 2H and the accession number used for designing primers for QTL *Rphq11*.

SNP Loci Name	Chromosome	Position	Accession
SCRI_RS_135248	2H	105.72	AK367668.1
BOPA2_12_30555	2H	110.03	AK249620.1
BOPA1_ABC13569-1-1-107	2H	111.18	AK252242.1
BOPA1_ConsensusGBS0348-2	2H	112.33	AK369591.1
SCRI_RS_147203	2H	119.71	AK369872.1
SCRI_RS_227965	2H	119.71	AK365405.1
SCRI_RS_230508	2H	120.65	AK369188.1
SCRI_RS_179560	2H	121.05	AK373673.1
SCRI_RS_156045	2H	124.51	AK374410.1
SCRI_RS_16799	2H	125.22	AK373540.1
SCRI_RS_238606	2H	126.08	AK371708.1
SCRI_RS_149429	2H	128.13	AK353879.1

SCRI_RS_142593	2H	131.87	AK331385.1
SCRI_RS_192711	2H	134.23	AK372653.1
SCRI_RS_151129	2H	135.02	AK368018.1
BOPA1_13178-89	2H	135.02	AK374855.1
SCRI_RS_157929	2H	139.45	AK373001.1
SCRI_RS_157929	2H	139.45	AK363336.1
BOPA2_12_10579	2H	144.62	AK368583.1
SCRI_RS_118062	2H	145.74	AK364748.1
SCRI_RS_193100	2H	146.48	AK248742.1

2.1.5 Genotyping :

DNA extraction of SusPtrit, Steptoe, SusQ11 and the 20 homorecombinants was performed with the 'DNeasy Plant Mini Kit' protocol from QIAGEN following the manufacturer's instructions. Cuantification of the concentration of the DNA obtained was done with a Nanodrop and samples at a concentration of 7.5 ng/µl were prepared for PCR. Subsequently, PCRs were performed in SusPtrit, Steptoe, SusQ11 and a mix of the two parental lines with all the designed primer combinations to check if they produce any amplicon. These PCR reactions were run in 96-well plates in Bio-Rad PCR machines and they were performed as it is described in the table 2 and figure 2.4.4.. Next, the reactions were analysed in the LightScanner to see whether there is polymorphism between the parental lines. LightScanner is a methodology that can perform high throughput gene scanning and mutation detection. The analysis is based on the difference in the separation of DNA strands of a PCR template by temperature that are caused by the SNPs. This method has the great advantage of being very fast for genotyping and identifying polymorphism. PCR products were analysed in the LightScanner with settings as follows; start temperature of 77^0 C, hold temperature of 74^0 C and end temperature of 95^0C. After that, PCR samples were subjected to electrophoresis in 1.5% agarose gels (0.5X TBE as buffer) to check size, number and quality of the amplicons for every primer pair combination.

Table 2.4 The composition of master mix used for PCR reactions for LightScanner.

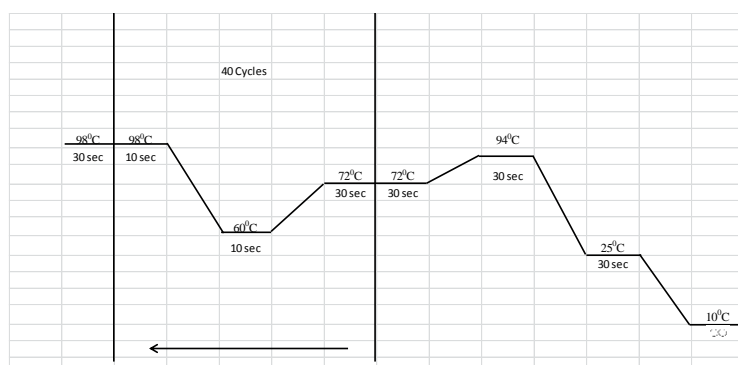| Component	Volume for 1 reaction (ul)
MQ Water	4.5
5X Phire enzyme	0.1
5X Buffer	2
dNTPs	0.4
LC-green	1
Forward Primer	0.25
Reverse Primer	0.25
DNA (7.5ng)	1
Mineral Oil	20

Figure 2.4 Illustration of the PCR program performed

The markers that produces clear amplicons were sequenced in order to identify SNP polymorphism between the parental lines. PCR fragments were purified using the 'QIAquick PCR Purification Kit' (QIAGEN) following the manufacturer's instructions and eluted in a final volume of 12 µl to ensure a high concentration of the PCR products. Sequencing was done using the GATC sequencing service (Germany). The DNA sequences were edited and aligned using MEGA4 program (BIBLIOGRAPHY). They were compared to the sequence of the array of the corresponding SNP loci in order to know if the polymorphisms found are the ones described in the array or new ones. Using this data, Cleaved Amplified Polymorphisms (CAP) candidates were identified using the programs designed for this purpose in 'Sol Genomics Network' (http://solgenomics.net/tools/caps_designer/caps_input.pl) and 'dCAPS finder' (http://helix.wustl.edu/dcaps/). At the end, all these information was put together and one marker was selected per locus every 5 cM and they were mapped in the 20 homorecombinants, SusQ11, SusPtrit, Steptoe and a sample formed by a mix the parental lines.

In table 2.5 the markers used finally for genotyping in the homorecombinants are listed along with the restriction enzymes, buffers and respective temperature used for the CAP genotyping when it was possible. Every restriction reaction was done adding 10µl of PCR product, 3µl of the corresponding buffer of the enzyme, 16µl of sterile MiliQ water and 1µl of the enzyme. Restriction was done overnight when the temperature was 37°C and for 3 hours 65 and 55°C. After that, the samples were electrophoresed during 1,5 hours in agarose gels at 2.5% (0.5x TBE).

Table2.5 List of markers for QTL *Rphq11* along with their position on Chromosome 2H. Restriction Enzymes (RE) used for the genotyping into recombinants.

Marker	SNP Loci	LG	CM2013	OWB	Other maps	N° SNP	CAPs candidates	Temperature	Buffer
53	SCRI_RS_135248	2H	105.72		94.90	3	HinfI	37	Red
57	BOPA2_12_30555	2H	110.03	122.26	106.46	2	MseI	37(DdeI & MseI)	Tango(DdeI), NEB nr 2(MseI)
61	BOPA1_ConsensusGBS0348-2	2H	112.33		108.61	1	No CAPs candidates		
1	SCRI_RS_147203	2H	119.71		64.10	1	DdeI	37	Tango
7	SCRI_RS_230508	2H	120.65			3	NlaIII	37	Green
25	SCRI_RS_179560	2H	121.05		96.92	3*	NlaIII	37	Green
11	SCRI_RS_156045	2H	124.51		106.44		DdeI	37	Tango
41	SCRI_RS_149429	2H	128.13		112.04	1	NlaIV	37	Tango
38	SCRI_RS_142593	2H	131.87		112.32	1	No CAPs candidates		
43	SCRI_RS_192711	2H	134.23		109.42	3	RsaI, TseI	37(RsaI),65(TseI)	Tango(RsaI),CutSmaart Buffer(TseI)
45	SCRI_RS_192711	2H	134.23		109.42	1	No CAPs candidates		
35	SCRI_RS_151129	2H	135.02		125.85	2*	DpnI	37	Tango
16	BOPA1_13178-89	2H	135.02	143.83	121.50	1	Hpy99I	37	CutSmart Buffer
47	SCRI_RS_157929	2H	139.45			1	DpnII	37	Tango
49	BOPA2_12_10579	2H	144.62		132.48	1	CviJI (CviKI-1)	37	CutSmart Buffer
21	SCRI_RS_118062	2H	145.74		126.77	1	TaqI	65	Unique
22	SCRI_RS_193100	2H	146.48		127.27	1	No CAPs candidates		

2.1.6 Statistical analysis:

Two sample t-test was carried out for each heterologous rust using the data of the each marker in the introgression area of *Rphq11*. This test was performed to check if there is any influence of QTL *Rphq11* over the resistance to heterologous rusts. The hypothesis was:

H_0: the mean of relative infection frequency (RIF) of heterologous rust with allele Steptoe is not less than allele SusPtrit
H_1: There is difference between the means of RIF for both alleles.

2.2 Fine mapping of Rnhq

Parental lines, Vada and L94, the experimental line SusPtrit and L94-Rnhq (the NIL developed in L94 background with an introgression from Vada) together with 20 homorecombinants (coming from the cross L94xL94-Rnhq) were used for the fine mapping of *Rnhq*. L94-Rnhq is a NIL which presents an introgression from Vada of around 37cM in 7H chromosome (from 63 to 99cM according to the data of the consensus map). This NIL has the QTL for nonhost resistance called *Rnhq* which has shown effective against Phm, Phs, Pt and Pgl. In this case the phenotyping of the recombinants the four heterologous rusts was done previously. Besides of the phenotyping, some markers were developed before from the SNP array before this thesis started to fine map the resistance. However, more markers are needed to have a better picture of the fine mapping of *Rnhq* and to allow teh selection of new homorecombinants in future. The DNA extraction, marker development and genotyping was done in the same way as it has been explained before for *Rphq11*. In the case of *Rnhq*, the

homorecombinants had been genotyped previously with 9K i-select Infinum array so we could compare the result of the genotyping with the array with the genotyping in our lab by lightscanner, CAPs or sequencing.

Table2.6 List of the Markers for QTL *Rnhq* along with their position on Chromosome 7H and Restriction Enzymes (RE) used for the genotyping into recombinants.

Marker	PCR product	LG	CM	OWB	i-select	N° SNP	Restriction Enzymes	Temperature	Buffer
66_AMS	SCRI_RS_186683	7H	62.96		50.85	2	NlaIII	37	GREEN
4_Abhay	SCRI_RS_146382	7H	63.32		50.71	2*	RsaI	37	Tango
5_AMS	SCRI_RS_146382	7H	63.32		50.71	1	HaeIII, DdeI	37 (HaeIII & DdeI)	Red (HaeIII)/Tango (DdeI)
1_abhay	BOPA1_12239-662	7H	63.32	61.49	56.81	1	ClaI	37	Tango
3_AMS	BOPA1_12239-662	7H	63.32	61.49	56.81	2*	ClaI	37	Tango
65_AMS	SCRI_RS_230478	7H	66.28		54.82	1	HpyCH4IV	37	Neb nr.1
26_AMS	SCRI_RS_236651	7H	71.29		62.18	1	TseI	65	CutSmart Buffer
9_Abhay	SCRI_RS_133026	7H	85.70		77.27	1	HaeIII	37	Red
9_AMS	BOPA1_1674-468	7H	86.00		86.44	1*	CviJI	37	CutSmart Buffer
44_AMS	SCRI_RS_206747	7H	87.31		77.27	3*	Sau96I, NlaIII	37 (Sau96I & NlaIII)	Tango (Sau96I)/ Green for NlaIII
12_AMS	BOPA1_11619-618	7H	87.31	98.97	87.97	1	TaqI	65	Unique
17_AMS	SCRI_RS_104566	7H	90.47		80.10	2	SatI	37	Green
20_AMS	BOPA2_12_21479	7H	94.75			1	Sau96I, SecI	37 (Sau96I)/55 (SecI)	Tango (Sau96I & SecI)
42_AMS	BOPA1_2444-437	7H	98.35		99.67	8	NlaIII, SphI	37 (NlaIII & SphI)	Green (NlaIII)/Blue (SphI)
24_Abhay	BOPA1_2444-437	7H	98.35		99.67	2	TaqI, NlaIII	65 (TaqI)/ 37 (NlaIII)	Unique (TaqI)/Green (NlaIII)
71_AMS	SCRI_RS_196885	7H	99.06		85.17	1	HpaII		
25_AMS	SCRI_RS_143884	7H	99.38		92.21	1*	MnlI, Sau96I	37(MnlI & Sau96I)	Green (MnlI), Tango(Sau96I)
25_Abhay	SCRI_RS_143884	7H	99.38		92.21	1	BslI	55	Tango
51_AMS	SCRI_RS_136590	7H			93.91	9	NlaIII, TspEI	37 (NlaIII)/ 65 (TspEI)	Green (NlaIII)/Blue (TspEI)
52_AMS	SCRI_RS_136586	7H			93.91	2	HaeIII, SecI	37(HaeIII)/55 (SecI)	Red (HaeIII)/ Tango for SecI
16_Abhay	SCRI_RS_136590	7H			93.91	1	TspEI	65	Blue
18_Abhay	BOPA1_1800-1101	7H		128.60	104.78	2	AluI, TaqI	37(AluI)/65(TaqI)	Tango(AluI)/Unique(TaqI)

3. Results:

3.1 Fine mapping of the resistance to the heterologous rusts in the *Rphq11* region

61 primer pairs were designed for the selected 21 SNP loci and genotyped in the parental lines (Steptoe and Susptrit), Vada genotype and a sample formed by a mix DNA 1:1 of Steptoe and SusPtrit and another one with a mix 1:1 of Vada and SusPtrit. The PCR reactions were analysed in LightScanner to detect differences in the melting curves between the parental lines and the mixes (which simulate a heterozygous sample). A total of 46 primer pairs produced clear amplicons and they were used in the next experiments. Table 3.1 shows all the 61 markers used in Rphq11 mapping and in the appendix the sequence of the primers is described. In other hand, figure 3.1 illustrate some the PCR amplifications performed.
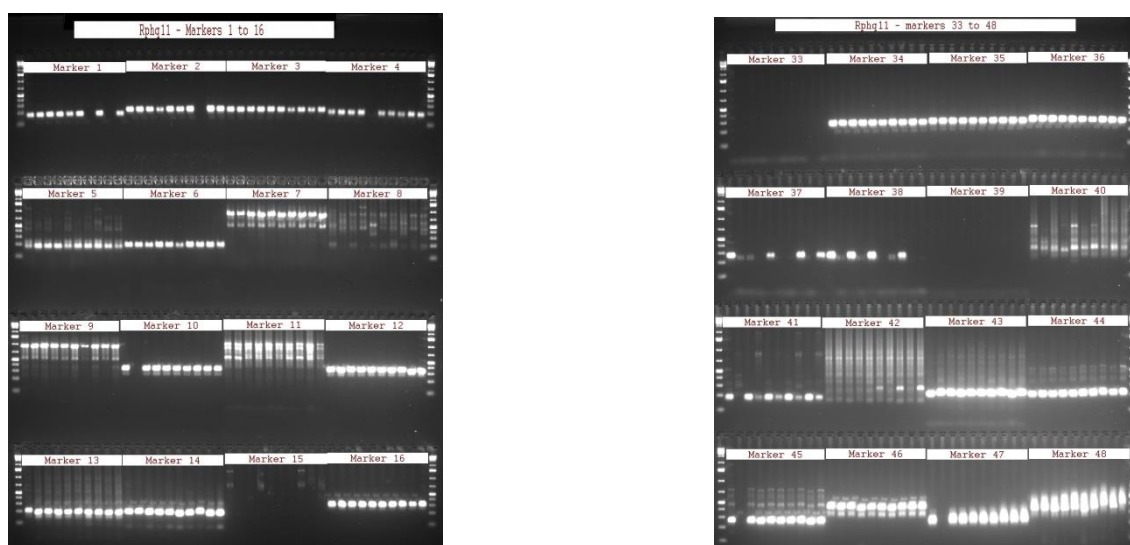
Figure 3.1 Gel showing the PCR products of some of the primer pairs designed to find SNPs between the parental lines. For every marker samples 1 and 2 correspond to Steptoe (St), 3 and 4 to SusPtrit (Sp), 5 and 6 to Vada (V), 7 and 8 to St+Sp and 9 and 10 to V+Sp.

During the LighScanner analysis, 32 primer pairs (out of 61) corrsponding to 16 SNP loci displayed difference in the melting curves of the parental lines. Consequently, the homozygous recombinants were genotyped with at least one of these polymorphic primer pairs per SNP loci but only 12 (11 SNP loci) of them produced interpretable results within the homo-recombinants.

Figure 3.2 LightScanner results for marker 45 (SCRI_RS_192711)in the parental lines (Steptoe, G1; SusPtrit, G2; Mix, G3; SusQ11, G4) and mapping population (From Rec 1, G5, to Rec 20, G12).

Table 3.1. Markers used in this study for the fine mapping of the resistance to heterologous rusts associated to *Rphq11*

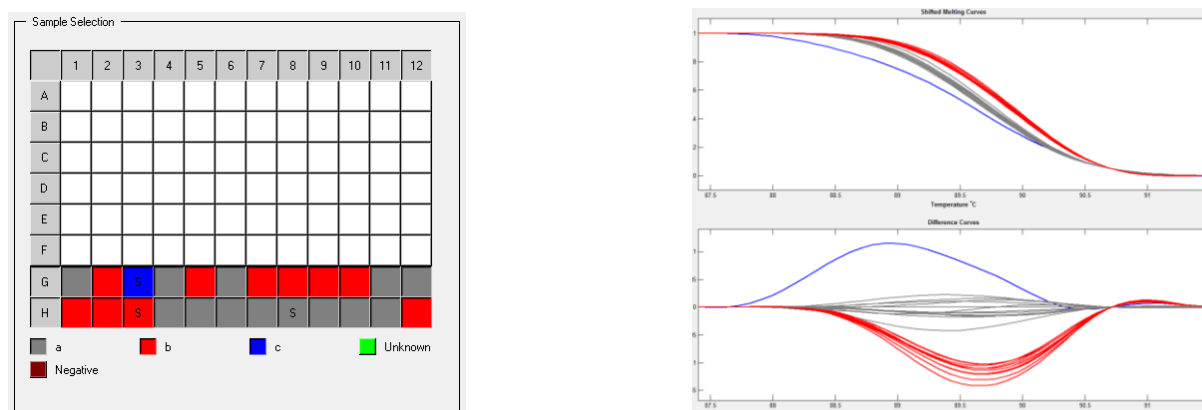| Marker Name	SNP loci	BLAST	Chromosome	Position CM	PCR amplification	Number SNPs	LightScanner	CAPs candidate	Mapped in Mapping pop.
M_52	SCRI_RS_135248		2H	105.72	Two bands				
M_53*	SCRI_RS_135248	AK367668.1	2H	105.72	Two bands	3	Polymorphic	HinfI	CAP
M_54	SCRI_RS_135248		2H	105.72	Two bands				
M_55	BOPA2_12_30555		2H	110.03	Two bands				
M_56	BOPA2_12_30555		2H	110.03	Two bands		Polymorphic		LightScanner
M_57*	BOPA2_12_30555	AK249620.1	2H	110.03	Two bands	2	Polymorphic	MseI	CAP
M_58	BOPA1_ABC13569-1-1-107		2H	111.18	Single band				
M_59	BOPA1_ABC13569-1-1-107		2H	111.18	Single band				
M_60	BOPA1_ConsensusGBS0348-2		2H	112.33	No Amplification				
M_61*	BOPA1_ConsensusGBS0348-2	AK369591.1	2H	112.33	Single band	1	Polymorphic		LightScanner
M_1*	SCRI_RS_147203:	AK369872.1	2H	119.71	Single band	1	Polymorphic	DdeI	LightScanner
M_2	SCRI_RS_147203:		2H	119.71	Single band				
M_3	SCRI_RS_147203:		2H	119.71	Single band				
M_4	SCRI_RS_227965		2H	119.71	Single band				
M_5	SCRI_RS_227965		2H	119.71	Single band				
M_6	SCRI_RS_227965		2H	119.71	Single band				
M_7*	SCRI_RS_230508		2H	120.65	Two bands	3		NlaIII	
M_8	SCRI_RS_230508		2H	120.65	Two bands	3	Polymorphic		LightScanner
M_9	SCRI_RS_230508		2H	120.65	Two bands				
M_25*	SCRI_RS_179560	AK373673.1	2H	121.05	Two bands	3*	Polymorphic	NlaIII	CAP
M_26	SCRI_RS_179560		2H	121.05	Two bands				
M_27	SCRI_RS_179560		2H	121.05	Two bands				
M_10*	SCRI_RS_156045	AK374410.1	2H	124.51	Two bands	0			
M_11*	SCRI_RS_156045	AK374410.1	2H	124.51	Three bands	0			
M_12	SCRI_RS_156045		2H	124.51	Two bands				
M_13*	SCRI_RS_16799	AK373540.1	2H	125.22	Single band	0			
M_14	SCRI_RS_16799		2H	125.22	Single band				
M_15	SCRI_RS_16799		2H	125.22	No Amplification				
M_28	SCRI_RS_238606		2H	126.08	No Amplification				
M_29	SCRI_RS_238606		2H	126.08	No Amplification				
M_30	SCRI_RS_238606		2H	126.08	No Amplification				
M_40	SCRI_RS_149429		2H	128.13	Two bands				
M_41*	SCRI_RS_149429	AK353879.1	2H	128.13	Single band	1	Polymorphic	NlaIV	CAP
M_42	SCRI_RS_149429		2H	128.13	No Amplification				
M_37	SCRI_RS_142593		2H	131.87	Single band				
M_38*	SCRI_RS_142593	AK331385.1	2H	131.87	Single band	1	Polymorphic		LightScanner
M_39	SCRI_RS_142593		2H	131.87	No Amplification				
M_43*	SCRI_RS_192711	AK372653.1	2H	134.23	Single band	3		RsaI, TseI	LightScanner
M_44	SCRI_RS_192711		2H	134.23	Single band				
M_45*	SCRI_RS_192711	AK372653.1	2H	134.23	Three bands	1	Polymorphic		LightScanner
M_16*	BOPA1_13178-89	AK374855.1	2H	135.02	Single band	1		Hpy99I	LightScanner
M_17	BOPA1_13178-89		2H	135.02	No Amplification				
M_18	BOPA1_13178-89		2H	135.02	No Amplification				
M_34	SCRI_RS_151129		2H	135.02	Single band				
M_35*	SCRI_RS_151129	AK368018.1	2H	135.02	Single band	2*		DpnI	Sequencing
M_36	SCRI_RS_151129		2H	135.02	Single band				
M_31	SCRI_RS_157929		2H	139.45	Three bands				
M_32	SCRI_RS_157929		2H	139.45	Two bands				
M_33	SCRI_RS_157929		2H	139.45	No Amplification				
M_46	SCRI_RS_157929		2H	139.45	Three bands				
M_47*	SCRI_RS_157929	AK363336.1	2H	139.45	Two bands	1		DpnII	Sequencing
M_48	SCRI_RS_157929		2H	139.45	Three bands				
M_49*	BOPA2_12_10579	AK368583.1	2H	144.62	Single band	1		CviJI	Sequencing
M_50	BOPA2_12_10579		2H	144.62	Single band				
M_51	BOPA2_12_10579		2H	144.62	Three bands				
M_19	SCRI_RS_118062		2H	145.74	Single band				
M_20	SCRI_RS_118062		2H	145.74	Single band				
M_21*	SCRI_RS_118062	AK364748.1	2H	145.74	Single band	1	Polymorphic	TaqI	CAP
M_22*	SCRI_RS_193100	AK248742.1	2H	146.48	Single band	1			LightScanner
M_23	SCRI_RS_193100	AK248742.1	2H	146.48	Single band		Polymorphic		LightScanner
M_24	SCRI_RS_193100		2H	146.48	Single band				

*: Sequenced markers in parental lines

Figure 3.3. CAP genotyping in the parental lines. For every marker sample 1 is Steptoe, 2 SusPtrit, 3 SusQ11 and 4 Steptoe + SusPtrit
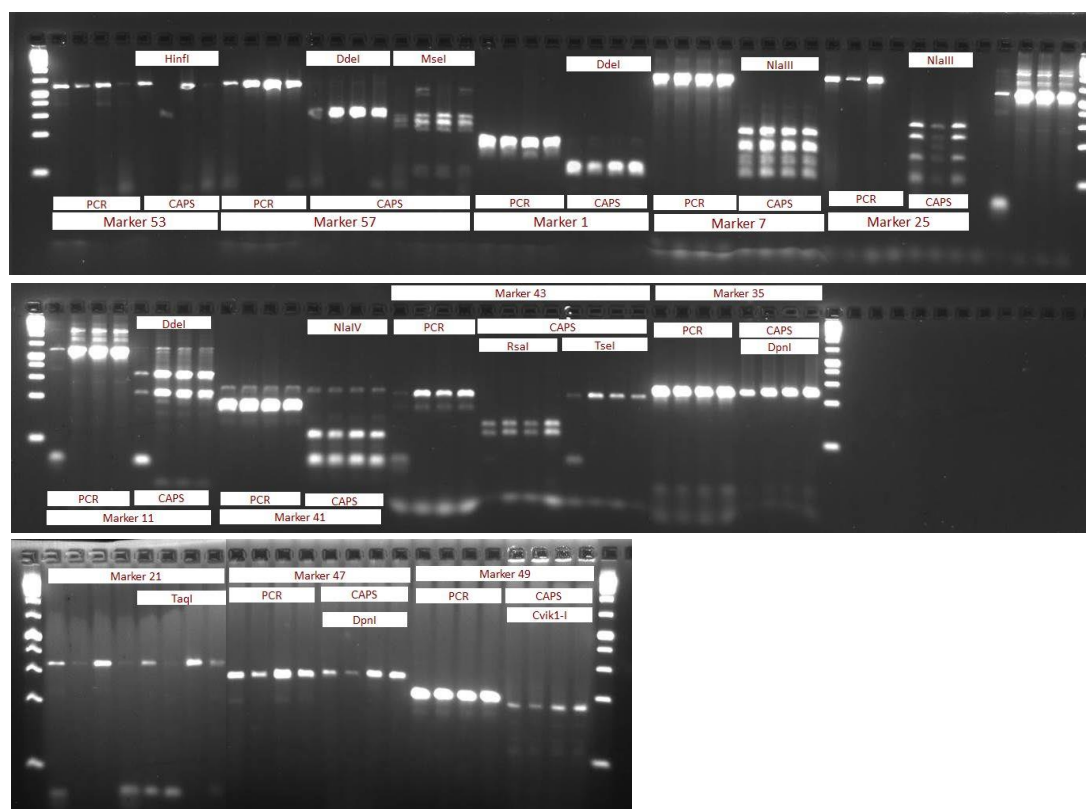
After genotyping with LightScanner, the PCR samples of 19 markers were sequenced in the parental lines (SusPtrit and Steptoe) and the mix (SusPtrit + Steptoe) in order to detect SNPs. After that, the sequences were used to find CAP candidates (with the "dCAPs finder" program) (see table 3.1).

Subsequently, the PCR samples of the 12 markers with CAP candidates were subjected to restiction with the corresponding enzymes. Unfortunately, not all the markers with CAP candidates produced a polymorphic pattern between the parental lines (see figure 3.3). All together, 14 markers were used for diggestion with different enzymes but it was found only 5 markers (of 5 SNP loci) which could be mapped in the homo-recombinans. Figure 3.3 and 3.4 shows two examples of CAP genotyping.

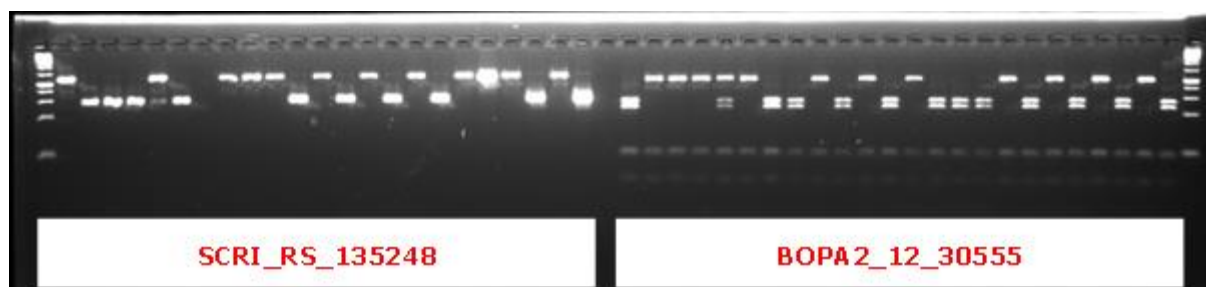Figure 3.4 CAPs genotyping showing the diggestion of the PCR products of the parental lines, mix and homo-recombinants with *HinfI* (SCRI_RS_135248) and *MseI* (BOPA2_12_30555) enzymes in an agarose gel at 2.5%.

Using LightScanner and CAPs genotyping it was possible to mapped at least one marker every 5 cM as it was defined at the begining except in the positions 124cM, 135cM, 139cM and 144cM. In order

to fill these gap and to map one marker in for every of those positions, the decision was to sequence markers 13, 35, 47, 49 in the homo-recombinants to determine whether their genotype were Steptoe or SusPtrit for these loci (Figure 3.5). There were problems with marker 13 because the SNP described in the array polymorphic between SusPtrit and Steptoe was not polymorphic in the sequences of parental lines and recombinants and it was not possible to find another one. To solve this, marker 10 was later sequenced but the problem was the same and all the sequences were identical. Before to this, marker 11 was sequenced in the parental lines but it did not produce a clear sequence, and a possible CAP candidate found did not work. Unfortunately, it was not possible to map a marker in position 124cM. At the end, with this information a marker of about every 5cM was mapped except for the gap between 121 and 128 and the result of this mapping in the recombinants is showed in table 3.2. A detailed description of the markers employed is done in table 3.3

Figure 3.5. Alignment of the sequences obtained for marker 49 (BOPA2_12_10579) in the parental lines, mix, SusQ11 and homo-recombinants, and also with the sequence of the array. In yellow it is indicated the bases inmediatly before to the SNP.

Table 3.2. Mapping in the recombinants the markers developed in this study

Position in CM	105.7	112-119	110.0	110.0	112.3	119.7	120.7	121.1	128.1	131.9	134.2	135.0	139.5	144.6	145.7	146.5
Marker Name	Marker 53	GBMS244	Marker 57	Marker 56	Marker 61	Marker 1	Marker 8	Marker 25	Marker 41	Marker 38	Marker 45	Marker 35	Marker 47	Marker 49	Marker 21	Marker 23
Sample/Genotyping	CAP	LightS	CAP	LightS	LightS	LightS	LightS	CAP	CAP	LightS	LightS	Seq	Seq	Seq	CAP	LightS
Rec_1_Q11	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Rec_2_Q11	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A
Rec_3_Q11	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
Rec_4_Q11	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B
Rec_5_Q11	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Rec_6_Q11	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B
Rec_7_Q11	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B
Rec_8_Q11	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	B
Rec_9_Q11	B	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B
Rec_10_Q11	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Rec_11_Q11	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Rec_12_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_13_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_13'_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_14_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_15_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_16_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_17_Q11	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B
Rec_18_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rec_19_Q11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
Rec_20_Q11	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B

Table 3.3. Markers used to map the resistance to the heterelogous rusts in *Rphq11* region

Marker name	SNP Loci	LG	CM_2013	OWB	i-select	N° SNP	Genotyping Method	CAPs genotyping
M_53	SCRI_RS_135248	2H	105.72		94.90	3	CAP	HinfI
M_57	BOPA2_12_30555	2H	110.03	122.26	106.46	2	CAP	MseI
M_56	BOPA2_12_30555	2H	110.03				LightScanner	
M_61	BOPA1_ConsensusGBS0348-2	2H	112.33		108.61	1	LightScanner	
M_1	SCRI_RS_147203	2H	119.71		64.10	1	LightScanner	
M_8	SCRI_RS_230508	2H	120.65			3	LightScanner	
M_25	SCRI_RS_179560	2H	121.05		96.92	3*	CAP	NlaIII
M_41	SCRI_RS_149429	2H	128.13		112.04	1	CAP	NlaIV
M_38	SCRI_RS_142593	2H	131.87		112.32	1	LightScanner	
M_45	SCRI_RS_192711	2H	134.23		109.42	1	LightScanner	
M_35	SCRI_RS_151129	2H	135.02		125.85	2*	Seq	
M_47	SCRI_RS_157929	2H	139.45			1	Seq	
M_49	BOPA2_12_10579	2H	144.62		132.48	1	Seq	
M_21	SCRI_RS_118062	2H	145.74		126.77	1	CAP	TaqI
M_23	SCRI_RS_193100	2H	146.48		127.27		LightScanner	

*: The polymorphisms are not the ones described in the array

Table 3.4 shows the results of the phenotyping experiments with the six heterologous rusts in terms of relative infection frecuency (RIF) compared to the susceptible control (SusPtrit). The data is presented considering the average value of the four inoculated plants. According to this data, inoculations performed with Phs, Phb ISR and Pp were considered quite reliable. In contrast, the inoculations with Phm, Pgl and Pt Swiss produced data not easy to interpretate. Considering the phenotypic value of every recombinant for every rust, it was assesed as resistant (letter A) or susceptible (B) to compare the resistance/susceptibility profile of the recombinants with the genotypings done in them. With this information it was possible to map the resistance to Phb ISR, Phs and Pp in the homorecombinants.

Putting together the phenotypic and genotypic data generated in this study (see table 3.5) it was observed that resistance to Phs and Phb, either Israel or Iran isolate, lies between 121 and 128cM (between marker 8 and 41). Resistance to Pp seems to be placed between 120 and 121 cM (between markers 8 and 25) but apparently it is not the same gene that the one for Phb and Phs (in the hypothetical situation that they would share) because the resistance/susceptibility profile in the recombinants is not the same.

Table 3.4. Data of the phenotyping experiments expressed in relative infection frecuency respect to SusPtrit. A: Steptoe and B: SusPtrit

Genotype	Phs		Phb ISR		Pp		Phm	Pgl	Pt SWISS
	Phs		Phb ISR		Pp		Phm	Pgl	Pt SWISS
Rec_1_Q11	75.7	B	33.1	B	185.1	B	45.2	117.3	53.2
Rec_2_Q11	12.2	A	6.1	A	17.9	A	35.7	87.3	31.0
Rec_3_Q11	131.8	B	65.8	B	117.9	B	84.2	150.5	89.8
Rec_4_Q11	46.4	A	10.2	A	46.4	A	19.8	36.5	33.2
Rec_5_Q11	112.6	B	58.1	B	63.0	B	44.7	224.8	90.6
Rec_6_Q11	34.5	B	26.1	B	315.6	B	41.8	48.5	26.6
Rec_7_Q11	92.7	B	38.1	B	97.2	B	74.7	135.1	59.6
Rec_8_Q11	100.2	B	45.7	B	66.2	B	56.6	70.0	76.9
Rec_9_Q11	70.3	B	40.8	B	240.0	B	55.7	78.4	66.2
Rec_10_Q11	48.7	B	49.0	B	152.3	B	52.3	62.0	49.4
Rec_11_Q11	65.1	B	76.1	B	153.5	B	102.6	83.7	69.6
Rec_12_Q11	36.7	A	15.2	A	10.1	A	55.0	55.9	30.6
Rec_13_Q11	10.1	A	23.5	A	20.2	A	37.8	24.7	44.9
Rec_13'_Q11	15.9	A	14.2	A	27.5	A	23.5	74.8	67.0
Rec_14_Q11	30.9	A	19.3	A	31.1	A	65.0	57.7	37.3
Rec_15_Q11	12.6	A	10.0	A	5.0	A	38.7	28.1	39.8
Rec_16_Q11	18.8	A	14.1	A	7.0	A	62.5	76.0	38.3
Rec_17_Q11	28.6	A	17.7	A	23.8	A	44.4	39.1	22.1
Rec_18_Q11	12.3	A	9.6	A	16.9	A	54.3	89.2	27.2
Rec_19_Q11	18.1	A	8.2	A	12.0	A	35.6	58.7	14.2
Rec_20_Q11	53.1	B	54.4	B	27.6	A	82.0	73.6	75.4
SusQ11	24.17582	A	7.4	A	25.0	A	37.3	38.9	43.1
SusQ11 Box 1	15.9292	A	7.7	A	25.0	A	39.3	66.0	19.9
SusQ11 Box 2	37.68116	A	7.1	A		A	35.6	24.7	57.9
Steptoe								19.7	
Steptoe Box 1								36.5	
Steptoe box 2								10.9	

3.1.1 Interaction between *Rphq11* and resistance to heterologous rusts

The data of the phenotyping experiment showed in table 3.4 was used to calculate the mean of relative infection frequency (RIF) of Steptoe and SusPtrit alleles for every heterologous rusts used in this study. Two sample t-test was carried out to check the significance level of the successful markers. In table 3.6 and table 3.7 the p-values of some markers were highlighted in green colour. These values are highly significant for the respective markers compared to other markers. According to the table 3.6 resistance to Phs lies in between position 119.71cM and 128.13cM, because markers in this position are with lowest p-value (<0.001) and it is highly significant. For Phb ISR, the resistance lies in between 119.71cM and 135.02cM . For Pp the highly significant marker is SCRI_RS_179560 and it is located at position 121.05cM. Substitution mapping (table 3.5) shows that resistance for those heterologous rusts (Phs, Phb ISR and Pp) is positioned at the same points mentioned above according to statistical analysis (table 3.6).

As previously, it was difficult to explain the location of the resistance for Pt, Phm and Pgl. The statistical analysis explains the hypothetical location of the resistance between 121.05cM and 131.87cM. Figure 3.6 display the graphs for each heterologous rusts showing the difference between means of RIF with Steptoe allele and SusPtrit allele calculated for every marker.

Table3.6 Statistical analysis of Phs, Phb ISRand Pp resistance showing RIF for Alleles of Steptoe and SusPtrit

MARKER NAME	SNP Loci	POSITION (cM)	Phs			Phb ISR			Pp		
			Steptoe Allele	SusPtrit Allele	P-value	Steptoe Allele	SusPtrit Allele	P-value	Steptoe Allele	SusPtrit Allele	P-value
WBE 144	WBE 144	91.28	52.22	42.31	0.715	27.11	36.54	0.173	72.15	89.44	0.338
GBM 1062	GBM 1062	100.26	35.05	64.16	0.033	20.78	40.67	0.013	42.05	117.37	0.021
M_53	SCRI_RS_135248	105.72	25.97	69.78	0.001	16.48	42.77	0.001	33.87	117.95	0.011
M_57	BOPA2_12_30555	110.03	25.77	74.37	0.001	17.85	43.89	0.001	20.69	140.86	0.001
M_56	BOPA2_12_30555	110.03	29.48	74.82	0.004	19.77	44.24	0.002	38.96	129.85	0.006
M_61	3OPA1_ConsensusGBS0348-2	112.33	29.87	79.87	0.003	20.26	46.5	0.001	60.24	106.63	0.121
M_1	SCRI_RS_147203	119.71	28.15	82.65	< 0.001	18.72	49	< 0.001	37.34	143.85	0.002
M_8	SCRI_RS_230508	120.65	28.15	82.65	< 0.001	18.72	49	< 0.001	37.34	143.85	0.002
M_25	SCRI_RS_179560	121.05	22.05	78.47	< 0.001	13.46	48.72	< 0.001	19.8	141.84	< 0.001
M_41	SCRI_RS_149429	128.13	27.94	76.89	< 0.001	15.52	49.9	< 0.001	26.25	146.8	0.002
M_38	SCRI_RS_142593	131.87	33.49	73.97	0.005	17.84	50.43	< 0.001	29.32	156.88	0.003
M_45	SCRI_RS_192711	134.23	32.41	70.92	0.006	18.48	45.96	< 0.001	27.9	144.6	0.003
M_35	SCRI_RS_151129	135.02	32.41	70.92	0.006	18.48	45.96	< 0.001	27.9	144.6	0.003
M_47	SCRI_RS_157929	139.45	32.76	66.69	0.014	18.55	43.13	0.002	28.27	132.52	0.004
M_49	BOPA2_12_10579	144.62	32.76	66.69	0.014	18.55	43.13	0.002	28.27	132.52	0.004
M_21	SCRI_RS_118062	145.74	32.76	59.45	0.03	18.55	43.13	0.002	28.27	132.52	0.004
M_23	SCRI_RS_193100	146.48	31.25	62.16	0.026	19.76	38.12	0.023	28.17	115.22	0.006

Table3.7 Statistical analysis of heterologous rusts Pt, Phm and Pgl showing means of RIF for Alleles of Steptoe and SusPtrit

| MARKER NAME	SNP Loci	POSITION (cM)	Pt			Phm			Pgl		
			Steptoe Allele	SusPtrit Allele	P-value	Steptoe Allele	SusPtrit Allele	P-value	Steptoe Allele	SusPtrit Allele	P-value
WBE 144	WBE 144	91.28	51.33	46.3	0.679	48.93	60.98	0.105	84.8	69.27	0.825
GBM 1062	GBM 1062	100.26	43.74	56.16	0.108	49.66	56.57	0.227	72.79	87.14	0.246
M_53	SCRI_RS_135248	105.72	37.45	60.75	0.007	46.2	59.08	0.077	62.16	95.5	0.05
M_57	BOPA2_12_30555	110.03	39.08	61.28	0.01	47.14	59.33	0.089	55.86	105.77	0.008
M_56	BOPA2_12_30555	110.03	41.35	60.73	0.024	47.85	59.74	0.097	57.74	108.81	0.013
M_61	3OPA1_ConsensusGBS0348-2	112.33	40.21	65	0.005	47.39	61.98	0.057	57.03	116.34	0.008
M_1	SCRI_RS_147203	119.71	40.55	64.45	0.007	46.92	62.74	0.042	60.01	111.49	0.021
M_8	SCRI_RS_230508	120.65	40.55	64.45	0.007	46.92	62.74	0.042	60.01	111.49	0.021
M_25	SCRI_RS_179560	121.05	35.05	65.72	< 0.001	42.94	63.96	0.007	57.1	104.4	0.012
M_41	SCRI_RS_149429	128.13	37.09	66.4	< 0.001	45.58	62.76	0.027	63.6	100.98	0.033
M_38	SCRI_RS_142593	131.87	40.15	65.09	0.005	46.43	63.54	0.03	64.1	104.86	0.049
M_45	SCRI_RS_192711	134.23	40.73	61.55	0.016	48.65	58.68	0.138	66.4	97.26	0.09
M_35	SCRI_RS_151129	135.02	40.73	61.55	0.016	48.65	58.68	0.138	66.4	97.26	0.09
M_47	SCRI_RS_157929	139.45	42.43	57.6	0.064	49.04	57.25	0.185	68.88	91.44	0.138
M_49	BOPA2_12_10579	144.62	42.43	57.6	0.064	49.04	57.25	0.185	68.88	91.44	0.138
M_21	SCRI_RS_118062	145.74	42.43	57.6	0.064	49.04	57.25	0.185	68.88	91.44	0.138
M_23	SCRI_RS_193100	146.48	45.1	53.07	0.219	50.74	54.6	0.34	71.59	85.65	0.252
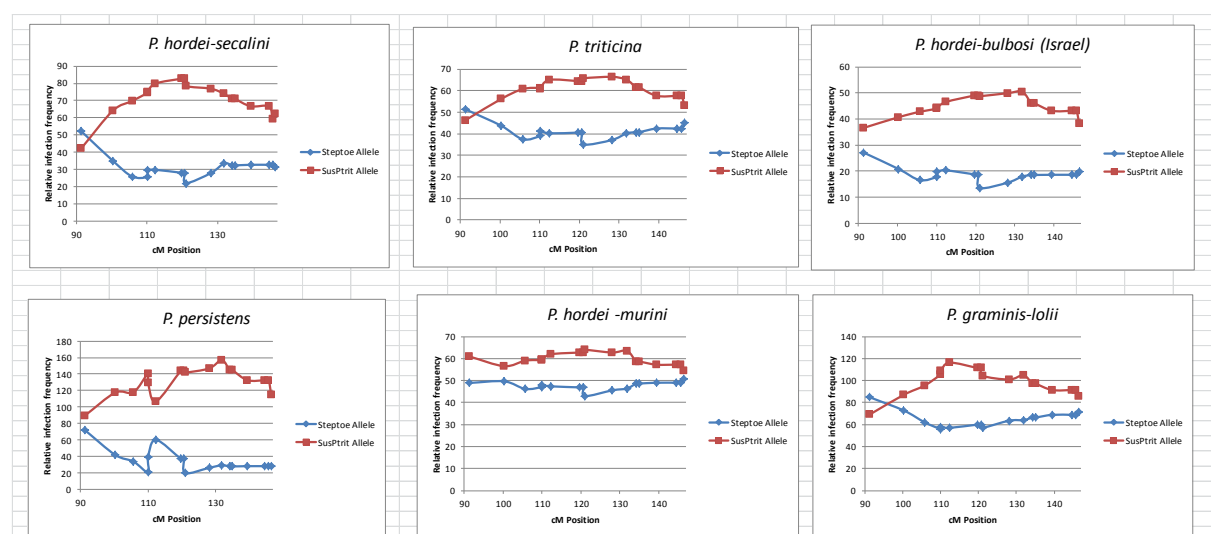
Figure 3.6 graphs of every heterologous rusts showing the difference between means of RIF with Allele Steptoe and Allele SusPtrit for every marker.

Table 3.5. Substitution mapping for the resistance to heterologous rusts (Phb, Phs and Pp) in *Rphq11* region (A: Steptoe and B: SusPtrit). For every marker it is indicated the position in 2H according to the consensus map (when it is known) and the genotyping methodology used.

3.2 Fine mapping of *Rnhq*

Fourteen SNP markers were selected along the introgression from Vada containing *Rnhq* in L94 background. The idea was to develop more markers for *Rnhq* region and put them together with the ones developed before at Niks' group in order to improve the fine mapping of the sub-QTLs for Phm/Phs, Pt and Pgl. A total of twenty six primer pairs were developed for 13 SNP loci from the i-select array (Table 3.2.1). The PCR reactions were performed using these primer pairs in the parental lines (Vada and L94) ,SusPtrit, one mix of parental lines (Vada+L94) and one mix of Vada+SusPtrit (Figure 3.2.1). The way of working was the same that it was explained before for *Rphq11* study. 17 primer pairs produced clear amplicons (Single band) (see Figure 3.2.1) and thirteen primer pairs of them showed polymorphism between the parental lines when analysed in the LightScanner. 12 of these markers were sequenced in the parental lines to find out the number of SNP polymorphic between them. After that they were genotyped in the homo-recombinants following the LightScanner methodology but it was only possible to have reliable results on 6 markers (from 5 SNP loci).These are markers 1, 4,9, 24, 25 and 26. A summary of this information is shown in Table 3.2.1. Previous data generated in the group about 71 primer pairs of 39 SNP loci in *Rnhq* region pointed out that nine markers produced good data for LightScanner genotyping in the homorecombinants (Markers 5, 0, 11, 44, 50, 59 and 65 from AMS primer design). Thus, they were also genotyped in the homo-recombinants and the data was used later for the mapping.

Table 3.2.1 List of the primers designed in this study to contribute to the fine mapping .

| Primer number	SNP loci	BLAST	LG	Marker position CM_2013	OWB	i-select	PCR	Sequenced Parental lines	Number SNPs	Lightscanner genotyping	CAPs genotyping	Genotyped Map. Pop
1	BOPA1_12239-662	AK355306.1	7H	63.32	61.49	56.81	single band	Yes	1	Polymorphic		LightScanner
2	BOPA1_12239-662	AK355306.2	7H	63.32	61.49	56.81	single band			Polymorphic		
3	BOPA1_12239-662	AK355306.3	7H	63.32	61.49	56.81	single band					
4	SCRI_RS_146382	Barley1_20068	7H	63.32		50.71	single band	Yes	2*	Polymorphic	*RsaI*	CAP
5	SCRI_RS_136556	CD863131	7H	62.96		47.30	two bands					
6	SCRI_RS_136556	CD863131	7H	62.96		47.30	two bands					
7	SCRI_RS_150062	AK356490.1	7H	84.82		76.56	single band	Yes	1*	Polymorphic		
8	SCRI_RS_150062	AK356490.2	7H	84.82		76.56	some bands					
9	SCRI_RS_133026	AK363024.1	7H	85.70		77.27	single band	Yes	1		*HaeIII*	CAP
10	BOPA1_4589-131	AK377085.1	7H	86.43	98.97	87.21						
11	BOPA1_4589-131	AK377085.1	7H	86.43	98.97	87.21	single band			Polymorphic		
12	SCRI_RS_136586	AK371770.1	7H			93.91	some bands					
13	SCRI_RS_136586	AK371770.2	7H			93.91	single band					
14	BOPA2_12_21479	AK365803.1	7H	94.75			single band	Yes	0			
15	BOPA2_12_21479	AK365803.2	7H	94.75			single band	Yes	1	Polymorphic		
16	SCRI_RS_136590	Barley1_11960	7H			93.91	single band	Yes	1	Polymorphic		
17	SCRI_RS_136590	Barley1_11961	7H			93.91	single band			Polymorphic		
18	BOPA1_1800-1101	AK367663.1	7H		128.60	104.78	single band	Yes	2	Polymorphic		Seq??
19	BOPA1_1800-1101	AK367663.2	7H		128.60	104.78	single band	Yes	0	Polymorphic		
20	BOPA2_12_21464	AK364970.1	7H		128.60	104.78	some bands			Polymorphic		
21	BOPA2_12_21464	AK364970.2	7H		128.60	104.78						
22	BOPA1_12027-128	AK250887.1	7H	99.63	124.86	102.85	some bands					
23	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	some bands					
24	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	single band	Yes	2	Polymorphic	*TaqI*	CAP
25	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	single band	Yes	1	Polymorphic		LightScanner
26	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	single band	Yes	1	Polymorphic		LightScanner
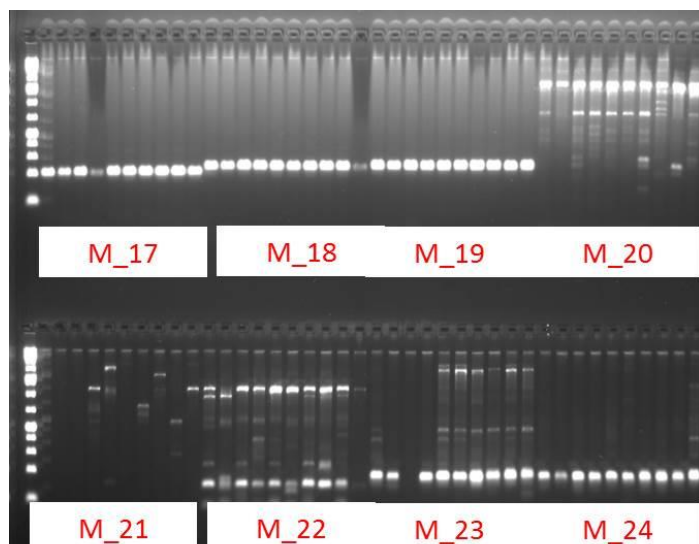
Figure 3.2.1 Gel showing the PCR products with some of the primer pairs designed for the *Rnhq* region.

Of the 12 sequenced markers, SNPs were identified for ten of them. After analysing the sequences, it was detected CAPs candidates and they were checked in the parental lines. Markers 4, 9 and 24 (Abhay's design) showed clear polymorphisms between the parental lines. Moreover, from the other group of 71 primer pairs (corresponding to 39 SNPs) designed before to the present study, 34 markers had been sequenced in the parental lines and they were also evaluated for CAPs candidates finding 7 markers polymorphic. All these 10 markers were genotyped in the homorecombinants by the digestion with the corresponding restriction enzymes. Figure 3.2.2 display the part of the results of the CAPS genotyping performed in the parental lines.
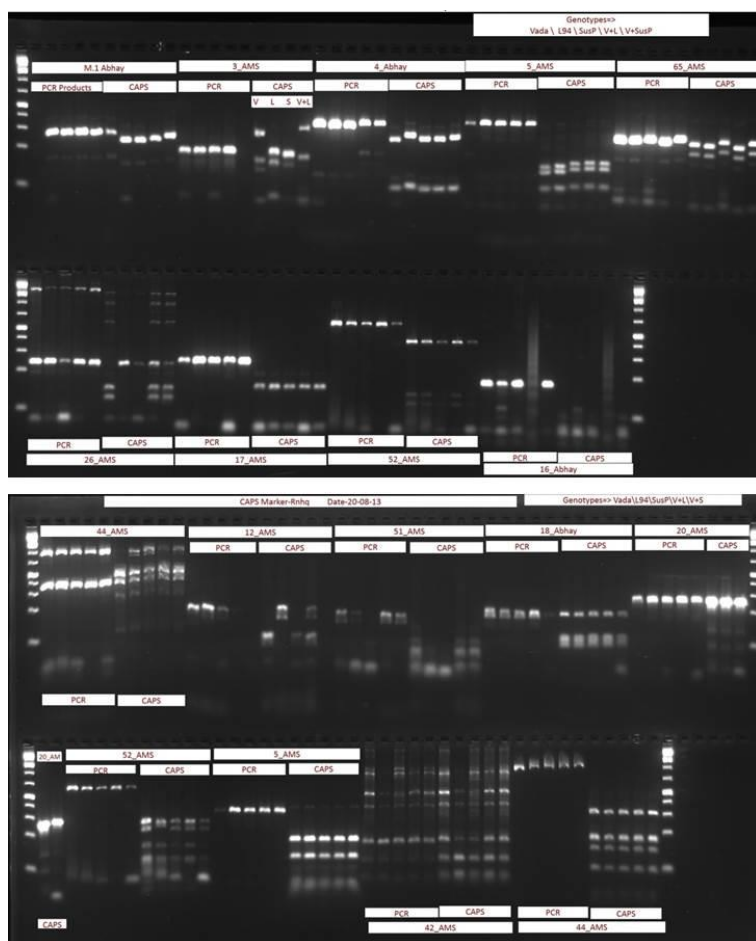
Figure 3.2.2 Pictures of the CAPS genotyping in the parental lines(Vada and L94), SusPtrit and Mixture of DNA of Vada+L94

Table 3.2.2 shows the markers that were sequenced in this project (Abhay's design) but also the ones that were developed and sequenced before. There is a total of 46 markers corresponding to 31 SNPs. A total of 30 of these SNPs can be used for the fine mapping but in this project we only used 15 because they are the ones for which is possible to do the genotyping by CAPs or LightScanner. Thus, these 15 markers were genotyped in the homo-recombinants with these methodologies. These results are presented in the figure 3.2.3. In this figure it is indicated the relation of the marker with the QTL (flanking or cosegregating) observing that there are flanking and cosegregating markers for the three QTLs with the exception of Rnhq-Phm/Phs. In this case it was not possible to find a flanking marker upstream of the QTL. According to this data, Rnhq-Phm/Phs is mapped in a region of 3.4 cM (between cM), Rnhq-Pt in a 1.5 cM interval (between cM) and Rnhq-Pgl in 5.2 cM (between cM). Figure 3.2.4 is included to have a better picture of the position of the QTLs, and the peak and flanking markers. The genotyping performed with the 15 markers allow us to compare the results with the ones obtained in the 9K i-select array (see figure 3.2.3). In general the results of the different genotypings carried out are quite coincident but there some discrepancies that they will be explained later in the discussion.

Figure 3.2.3. Genotyping in the homorecombinants of the markers used in this study

Table 3.2.2. Complete list of markers sequenced for the fine mapping of *Rnhq*

Marker	Primer design	SNP Loci	LG	CM	OWB	i-select	Number SNP	Lightscanner Genotyping	CAPs Genotyping	Genotyped MP	QTL
1	Abhay	BOPA1_12239-662	7H	63.32	61.49	56.81	1	Good		LighScanner	Phs/Phm (Co)
4	Abhay	SCRI_RS_146382	7H	63.32		50.71	2*	Very good	RsaI	CAP	Phs/Phm (Co)
7	Abhay	SCRI_RS_150062	7H	84.82		76.56	1*	Bad data			
9	Abhay	SCRI_RS_133026	7H	85.70		77.27	1	Very good	HaeIII	CAP	Pt (Fl)
14	Abhay	BOPA2_12_21479	7H	94.75			0	No polymorphism			
15	Abhay	BOPA2_12_21479	7H	94.75			1	Bad data			
16	Abhay	SCRI_RS_136590	7H			93.91	1	Bad data			
18	Abhay	BOPA1_1800-1101	7H		128.60	104.78	2	Bad data		Seq	
19	Abhay	BOPA1_1800-1101	7H		128.60	104.78	0	No polymorphism			
24	Abhay	BOPA1_2444-437	7H	98.35		99.67	2	No polymorphism	TaqI	CAP	Pgl (Co)
25	Abhay	SCRI_RS_143884	7H	99.38		92.21	1	Good		LighScanner	Pgl (Fl)
26	Abhay	SCRI_RS_143884	7H	99.38		92.21	1	Good		LighScanner	Pgl (Fl)
3	AMS	BOPA1_12239-662	7H	63.32	61.49	56.81	2*	Good	ClaI	CAP	Phs/Phm (Co)
5	AMS	SCRI_RS_146382	7H	63.32		50.71	1	Very good		LighScanner	Phs/Phm (Co)
8	AMS	SCRI_RS_150062	7H	84.82		76.56	0	No polymorphism			
9	AMS	BOPA1_1674-468	7H	86.00		86.44	1*	Very good		LighScanner	Pt (Co)
11	AMS	SCRI_RS_133026	7H	85.70		77.27	1	Good		LighScanner	Pt (Fl)
12	AMS	BOPA1_11619-618	7H	87.31	98.97	87.97	1	Bad data	TaqI	CAP	Pt (Fl)
13	AMS	BOPA1_11619-618	7H	87.31	98.97	87.97	0	No polymorphism			
14	AMS	BOPA1_1676-557	7H	88.18	98.97	87.97	0	No polymorphism			
15	AMS	SCRI_RS_194291	7H	88.17		77.41	2	Bad data		Seq	
16	AMS	SCRI_RS_194291	7H	88.17		77.41	0	No polymorphism			
17	AMS	SCRI_RS_104566	7H	90.47		80.10	2	Bad data		Seq	
20	AMS	BOPA2_12_21479	7H	94.75			1	Bad data		Seq	
25	AMS	SCRI_RS_143884	7H	99.38		92.21	1 (SxV)	Good			
26	AMS	SCRI_RS_236651	7H	71.29		62.18	1	Bad data	TseI (Not done yet)	CAP ???	Phs/Phm (Fl)
27	AMS	SCRI_RS_236651	7H	71.29		62.18	1	Bad data			
28	AMS	BOPA1_4054-1326	7H	72.38		68.46	1*	Bad data			
33	AMS	BOPA2_12_10657	7H	67.49	71.46	68.46	1	Bad data			
40	AMS	SCRI_RS_2914	7H	84.03		70.96	2	Bad data			
42	AMS	BOPA1_2444-437	7H	98.35		99.67	8		SphI	CAP	Pgl (Co)
44	AMS	SCRI_RS_206747	7H	87.31		77.27	3*	Very good		LighScanner	Pt (Fl)
45	AMS	SCRI_RS_124478	7H	87.31		77.27	2	Good			
47	AMS	SCRI_RS_171080	7H	87.75		77.41	1	No polymorphism			
50	AMS	SCRI_RS_122512	7H			76.70	1	Very good		LighScanner	Pt (Fl)
51	AMS	SCRI_RS_136590	7H			93.91	9	Good	NlaIII	CAP	Pgl (Co)
52	AMS	SCRI_RS_136586	7H			93.91	2	Bad data	Sau96I	CAP	Pgl (Fl)
55	AMS	SCRI_RS_208890	7H	106.61		97.24	1*(VxS)	Not tested			
56	AMS	SCRI_RS_208890	7H	106.61		97.24	1*	Not tested	ScrFI (Not done yet)	CAP ???	
58	AMS	SCRI_RS_15864	7H	69.44		58.14	0	Not tested			
59	AMS	SCRI_RS_161111	7H	63.35		52.27	1	Very good		LighScanner	Phs/Phm (Co)
65	AMS	SCRI_RS_230478	7H	66.28		54.82	1	Good		LighScanner	Phs/Phm (Fl)
66	AMS	SCRI_RS_186683	7H	62.96		50.85	2	Not tested	NlaIII	CAP	Phs/Phm (Co)
69	AMS	SCRI_RS_219581	7H	86.39		77.27	1	Not tested			
70	AMS	SCRI_RS_168994	7H	98.42		89.52	1	Bad data		Seq	
71	AMS	SCRI_RS_196885	7H	99.06		85.17	1	Not tested	HpaII	CAP	Pgl (Fl)

*: the SNPs are not the ones described in the array

Co= Cosegregating
Fl= Flanking

Table 3.2.4. Representation of the introgressed area of Vada with Rnhq sub-QTLs indication the ditances (in cM) and marker developed for the fine mapping.

Key: **A: L94** (susceptible parental line) — **B: Vada** (Resistant parental line) — **U: Heterozygous**

Column headers — Flanking markers / Peak markers / No SNP. Individual IDs (top row P#, bottom row Rec#):

QTL	Dev	Developed Marker	LG	CM_Martin-Sanz	OWB	i-select	P7 / Rec17	P12 / Rec22	P6 / Rec16	P19 / Rec30	P17 / Rec29	P14 / Rec25	P3 / Rec11	P1 / Rec1	P20 / Rec4	P15 / Rec26	P8 / Rec18	P13 / Rec23	P22 / Rec9	P18 / Rec3	P4 / Rec14	P16 / Rec28	P2 / Rec10	P11 / Rec20	P5 / Rec15	P21 / Rec5	P10 / Rec2
		SCRI_RS_136918	7H	59.54		45.14	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
		SCRI_RS_171008	7H	59.54		49.86	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
		SCRI_RS_134872	7H	60.12		49.72	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
QTL_1_Phs	x	SCRI_RS_186683	7H	62.96		50.85	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs		SCRI_RS_136556	7H	62.96		47.30	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs		SCRI_RS_209511	7H	63.16		52.27	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs		BOPA1_5028-1261	7H	63.32		56.81	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs	x	BOPA1_12239-662	7H	63.32	61.49	56.81	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs	x	SCRI_RS_146382	7H	63.32		50.71	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs		SCRI_RS_137626	7H	63.35		52.27	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
QTL_1_Phs	x	SCRI_RS_161111	7H	63.35		52.27	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A
		BOPA2_12_31357	7H	66.20		54.82	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A
	x	SCRI_RS_230478	7H	66.28		54.82	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A
		BOPA1_2669-1012	7H	66.52		55.63	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A
		SCRI_RS_15864	7H	69.44		58.14	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	A	A
		BOPA1_4475-478	7H	67.49	71.46	68.46	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		BOPA2_12_10657	7H	67.49	71.46	68.46	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		BOPA1_5695-922	7H	70.74		63.66	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		BOPA1_7810-113	7H	70.88	71.46	68.46	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		SCRI_RS_11068	7H	71.20		62.11	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		SCRI_RS_236651	7H	71.29		62.18	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		SCRI_RS_132425	7H	71.44		62.39	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		BOPA2_12_30149	7H	71.49		63.66	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	A
		WBE101	7H				A	A	A	A	A	U	A	A	A	B	B	B	U	B	B	A	A	A	B	B	B
		BOPA1_4054-1326	7H	72.38		68.46	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	B
		SCRI_RS_139962	7H	72.38		64.80	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	B
		BOPA1_1735-1424	7H	72.38		73.75	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	B
		SCRI_RS_182	7H	72.65		63.95	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	B
		BOPA1_3186-1560	7H	72.80		71.10	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	B
		BOPA2_12_30496	7H	74.50		73.75	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	B	B	B
		GBM1359	7H				A	A	A	A	A	U	A	B	B	A	A	B	U	B	B	A	A	U	A	A	B
		SCRI_RS_194085	7H			77.41	A	A	A	A	A	A	A	A	B	B	A	B	B	B	B	A	A	A	B	B	B
		SCRI_RS_2914	7H	84.03		70.96	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	A	A	A	B	B	B
		SCRI_RS_150062	7H	84.82		76.56	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	A	A	A	A	A	B
		SCRI_RS_204771	7H	84.82		76.56	A	A	A	A	B	B	B	B	B	B	U	B	B	B	B	A	A	A	A	A	B
		SCRI_RS_230083	7H	84.82		76.42	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	A	A	A	A	A	B
	x	SCRI_RS_122512	7H			76.70	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	A	A	A	A	A	B
		BOPA2_12_30199	7H		97.74	86.44	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	A	A	A	A	A	B
QTL_2_Pl	x	BOPA1_1674-468	7H	86.00		86.44	A	A	A	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	A	A	A
QTL_2_Pl		SKT1	7H				A	A	A	B	B	U	B	B	B	B	A	A	B	U	B	B	B	B	A	A	A
	x	SCRI_RS_133026	7H	85.70		77.27	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
		SCRI_RS_207238	7H	86.39		77.27	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
		SCRI_RS_219581	7H	86.39		77.27	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
		BOPA1_2462-971	7H	86.39		87.97	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
		BOPA2_12_30999	7H	86.39		77.27	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
		BOPA1_4589-131	7H	86.43	98.97	87.21	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
	x	SCRI_RS_206747	7H	87.31		77.27	A	A	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A
		P14M61_275	7H				A	A	B	B	B	U	B	B	B	U	A	A	A	U	B	B	B	B	A	A	A
	x	BOPA1_11619-618	7H	87.31	98.97	87.97	A	B	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A
		SCRI_RS_124478	7H	87.31		77.27	A	B	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A
		SCRI_RS_171080	7H	87.75		77.41	A	B	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A
		E33M61_85	7H				A	B	B	B	B	U	B	B	B	B	A	A	A	U	B	B	B	B	A	A	A
		MWG2031	7H				A	B	B	B	B	U	B	B	B	B	A	A	A	U	B	B	B	B	A	A	A
		BOPA1_1676-557	7H	88.18	98.97	87.97	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	B	A	A	A	A	A
		SCRI_RS_194291	7H	88.17		77.41	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	B	A	A	A	A	A
		SKT7	7H				B	B	B	B	B	U	B	B	B	B	A	A	A	U	A	B	A	A	A	A	A
		MN	7H				B	B	B	B	B	U	B	B	B	B	A	A	A	U	A	B	A	A	A	A	A
		GBM1303	7H				B	B	B	B	B	U	B	B	B	B	A	A	A	U	A	B	A	A	A	A	A
		SCRI_RS_104566	7H	90.47		80.10	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	A	A	A
		SCRI_RS_194841	7H	91.62		81.52	B	B	B	B	B	B	B	B	B	B	U	A	A	A	A	A	A	B	A	U	A
	x	SCRI_RS_136586	7H			93.91	B	B	B	B	B	B	B	B	B	B	U	A	A	A	A	A	B	B	U	A	A
QTL_3_Pgl	x	SCRI_RS_136590	7H			93.91	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl		BOPA2_12_21479	7H	94.75			B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl	x	BOPA1_2444-437	7H	98.35		99.67	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl		SCRI_RS_168994	7H	98.42		89.52	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl	x	SCRI_RS_196885	7H	99.06		85.17	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl	x	SCRI_RS_143884	7H	99.38		92.21	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl		BOPA1_12027-128	7H	99.63	124.86	102.85	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl		BOPA2_12_21464	7H		128.60	104.78	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
QTL_3_Pgl		BOPA1_1800-1101	7H		128.60	104.78	B	B	B	B	B	B	B	B	B	B	U	A	A	U	A	A	B	B	U	A	A
		SCRI_RS_182503	7H	109.61		100.00	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
		BOPA1_6541-1329	7H	109.61		110.99	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

4. Discussion:

The aim of this study for *Rphq11* QTL was to develop molecular markers at every 5 cM interval around *Rphq11* region in order to map the resistance to some non-adapted rust: Pp, Phb Iran, Phb Israel, Phm, Phs, Pt Swiss and Pgl. Later the association between host and nohst resistance was investigated. Secondly, for Rnhq QTLs the objective was to develop some markers to go further in the fine mapping of the resistance to Phm, Phs, Pgl and Pt Swiss.

For the molecular analysis, various genotyping methods were used in this study. Firstly, LighScanner genotyping was performed. This methodology was found very useful to identify polymorphism between the parental lines but then when the complete mapping population was genotyped it was not easy to find markers with clear interpretations. Even so XX markers for *Rphq11* and XX *Rnhq* could be mapped with this methodology. Some of these markers were dominant but some co-dominants (see figures of LightScanner results before). The experience show in this thesis is that markers with no difference in the melting curves in the LightScanner for the parental lines had no SNPs so it is not worth to sequence them.

The second genotyping methodology was CAP genotyping. The problem of this technic is that you need to have an enzyme for the target SNP and this is not easy. For example, it can be easily observed in table 3.2.2 that only 12 out of 46 markers could be genotyped with CAPs. The good thing is that this genotyping it is easy and very powerful. You can establish easily the alleles of the samples while with LightScanner sometimes it is not easy and you need to do several repetitions. One problem found in this thesis is that when the sequences were evaluated for CAPs candidates, many were found but the real situation is that when the restriction was done in the parental lines no polymorphism was observed and I cannot explain the reason of failure. Some markers could be genotyped at the same time with LightScanner and CAPs and the results were in general very similar.

The third methodology for genotyping used in this study was sequencing. Because you are going directly to the sequence it was the most trustful method but also the most expensive. However, considering that nowadays the price of the sequencing is decreasing, it is very advisable to use it when you do not have other clear way of genotyping.

Sequences of all the generated markers are available and they could be used in future for other genotyping methodologies like "KASP method". This is especially useful when it is required to genotype a lot of plants with a few markers and this could be the situation in a short future.

The phenotyping performed with six rust in the *Rphq11* recombinants showed data of easy interpretation for Phs, Phb and Pp. In other hand, it was not easy to decide if the recombinants were resistant or susceptible to Pt Swiss, Pgl and Phm. Inoculations were done in 4 plants but in only one repetition because there were no more seeds available. During the development of this thesis more seeds were produced so it is possible to continue with the subsequent phenotypings. For future inoculations of these recombinants it is highly recommended to include less plants per repetition/genotype but with all the recombinants in only one box to avoid high variations in the quantity of spores apply. Actually, this is the most likely explanation for the differences observed for some rusts in SusQ11 phenotype. It is expected that the future phenotyping experiments establish the exact location for the resistance genes to the different rusts.

When the phenotypic data of the heterologous rusts and *P. hordei* (homologous) are compared we observed a clear interaction of Rphq11 with the resistance to Phb Iran as it is shown in table 4.1. It is also possible to observe an interaction with the resistance to Phb ISR, Pp and Phm but more experiments are needed to confirm it.

Table 4.1. Phenotypic data in RIF comparing the different allelic configurations for Rphq11 and the resistance to other rusts. A: Steptoe (resistance); B: SusPtrit (Susceptible)

Rphq11	Other rusts	Phs	Pt	Phb ISR	Phb IRAN	Pp	Phm	Pgl
A	A	19.6	37.2	14.1	4.5	17.0	35.9	34.4
B	A	19.7	36.5	11.8	30.7	20.1	40.0	39.1
A	B	84.8	76.8	44.5	59.7	137.6	79.4	117.0
B	B	59.3	70.8	55.1	101.4	181.9	92.3	81.2

Table 4.2. Resistance QTLs mapped previously to this study in other mapping populations

Pathogen	SNP Loci	LG	CM 2013	Population	Trait	LOD	% exp	Add	Donor	QTL Name	Reference
Phb ISR/Pp	BOPA1_8523-316	2H	108.178	VxS/SxGP	IU-N/RIF	3.05/3.36	8/8.3	4.92/10.9	Vada/GP		Jafary, unpublished (updated)/Yeo et al., 2014
Pt	SCRI_RS_128484	2H	121.029	SxGP	RIF	4.61	11.5	11.98	GP		Yeo et al., 2013
Phb ISR/Pp	BOPA1_868-675	2H	123.338	VxS/L94xS	EA/IF	2.88/-	7.9/-	'-3.41752/-	SusPtrit/L94		Jafary, unpublished (updated)/Chisenga (unpublished)
Pca	E33M61-227	2H	123.821	CCxS	RLP50S	7.21	20.7	-3.37	Cebada Capa	Rpcq5	Alemu, unpublished
Phs	SCRI_RS_156045	2H	124.508	SxGP	RIF	4.16	10.4	22.33	GP		Yeo et al., 2013
Phb ISR/Pp	BOPA1_1381-547	2H	132.302	L94xS	IF				L94		Chisenga, unpublished
Pca	E38M54-113	2H	141.394	VxS	RLP50S	5.38	10.9	-1.75	Vada	Rpcq1	Alemu, unpublished

Finally, it has been checked the consensus mapped 2013 developed at Niks' group to see which resistance QTLs had been mapped before in other mapping populations and this data is presented in table 4.2. Three QTLs for resistance to Phb ISR and Pp, one for Phs, another one for Pt and two for Pca were mapped previously in other material in the interval of the introgression of Steptoe in SusPtrit. It is very interesting to notice that in the area between 121 and 124cM which is the candidate region for Phb ISR, Phs and Pp in this thesis, QTLs for resistance to Pt, Phb ISR and Pp are located so it is possible that they are the same QTLs found in the present thesis. Resistance to the other pathogen of the table, Pca, it is observed that the QTLs are in positions 123 and 141cM. Considering this date, it would advisable for future studies to inoculate SusPtrit, Steptoe and SusQ11 with this rust to see if SusQ11 is resistant and if it is to continue with the inoculation of the homo-recombinants.

In the case of the other QTL(s), *Rnhq*, the contribution of this study is very important to continue with the fine mapping of this QTLs because 10 new markers corresponding to 9 SNPs have been successfully developed. The 15 SNPs genotyped in the homo-recombinants by LightScanner and CAP methods were compared with the results obtained by 9K i-select array. In general the results of the genotyping were very similar but there are some unexpected results. For instance it is evident that the samples used in this project as Rec28 are not the same than the one genotyped in the array. It is needed to collect new samples of Rec28 to check what are the wrong results, if the ones of this thesis or the ones in i-select. For Rnhq-Phm/Phs the only discrepancy is found in Rec11 but this could be due to mistakes in the LightScanner analysis. For Rnhq-Pt the only clear different results is in Rec 9 for marker 12_AMS. In the case of Rnhq-Pgl there are more discrepancies as it is possible to see for markers 71_AMS, 25_Abhay and 26 Abhay for Rec26 and marker 52_AMS in Rec15 and Rec26. It is of a great importance to repeat this results with new DNA samples to confirm this data before of continuing with new experiments. If it is confirmed, the area of Rnhq-Pgl would be smaller than what was expected according to the data got from i-select.

5. Conclusions

- In *Rphq11* region, resistance to Pp has been mapped in an area between 120 and 121cM while the resistance to Phs and Phb (Iran and Israel isolates) has been located in an interval between 121 and 128 cM.

- Association between host and nonhost resistance in *Rphq11* region have been observed for the resistance to Phb Iran and possibly for Phb ISR and Pp.

- Co-segregating and flanking markers to *Rnhq* sub-QTLs (Phm/Phs, Pt and Pgl) have been developed and studied in the recombinants to continue with the fine mapping towards their cloning in future

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Appendix

Table A.1. Primers used for the genotyping of the *Rphq11* region

Marker Name	SNP loci	BLAST	LG	Map Position CM	OWB	i-select	Primer Forward	Primer Reverse
M_52	SCRI_RS_135248	AK367668.1	2H	105.72		94.90	GATCCACAATCACCGAATCA	GGAGAGACTGGGGCTGAATA
M_53*	SCRI_RS_135248	AK367668.1	2H	105.72		94.90	TCCATCCACTCCGAAGTTCT	TGTTCCAAAAATCTCCTCTGC
M_54	SCRI_RS_135248	AK367668.1	2H	105.72		94.90	CCTCTTCAAATACTTCCAGCAGA	GACTTGATCCATTCCATGATGA
M_55	BOPA2_12_30555	AK249620.1	2H	110.03	122.26	106.46	GACTTTCAGTCTGGGCTTCG	TCAACACGCTCTCATTCTTTTG
M_56	BOPA2_12_30555	AK249620.1	2H	110.03	122.26	106.46	TACCTGGGGATTACGCACA	CACGCTCTCATTCTTTTGAACA
M_57*	BOPA2_12_30555	AK249620.1	2H	110.03	122.26	106.46	GGTTTACCTGGGGATTACGC	CGCTCTCATTCTTTTGAACAAC
M_58	BOPA1_ABC13569-1-1-107	AK252242.1	2H	111.18	122.26	106.46	GCTTAAATCAGCCTTGGTGAC	CAGTAGCAGAGATTGGGGATG
M_59	BOPA1_ABC13569-1-1-107	AK252242.1	2H	111.18	122.26	106.46	CCACAAGGATGACTGCAAGA	CAGTAGCAGAGATTGGGGATG
M_60	BOPA1_ConsensusGBS0348-2	AK369591.1	2H	112.33		108.61	ATACCCTCGCCGTCTCTCTC	TTGTCCTGGGCCTCATACTC
M_61*	BOPA1_ConsensusGBS0348-2	AK369591.1	2H	112.33		108.61	TGTTGGTGTTTCTATCCGATG	AAACAGCAGCTTTGCCTCAG
M_1*	SCRI_RS_147203	AK369872.1	2H	119.71		64.10	GGCAAGTAAACAGGCGAAAC	TCAAGACAAGGGTACCACACA
M_2	SCRI_RS_147203	AK369872.1	2H	119.71		64.10	GCGTTTATCATTGGGATCGT	TCAAGACAAGGGTACCACACA
M_3	SCRI_RS_147203	AK369872.1	2H	119.71		64.10	AGGCAAGTAAACAGGCGAAA	GCAAGAAAAGTACAACGGCATA
M_4	SCRI_RS_227965	AK365405.1	2H	119.71			TACGCATCCGACATCCATTA	TCCAAATTAACGGGACGAAC
M_5	SCRI_RS_227965	AK365405.1	2H	119.71			ACTACGCATCCGACATCCAT	AATTAACGGGACGAACATCG
M_6	SCRI_RS_227965	AK365405.1	2H	119.71			TACGCATCCGACATCCATTA	ACGCAACAATCCATCCAAAT
M_7*	SCRI_RS_230508	AK369188.1	2H	120.65			AGTGCATCAGGTGGAGGAAG	GCTCAGCAGCTTATCGGAAG
M_8	SCRI_RS_230508	AK369188.1	2H	120.65			CTGTAGAAGGAGGCGACGAC	AACTTCAGCATCAGGGAAGG
M_9	SCRI_RS_230508	AK369188.1	2H	120.65			GATGCATCTGTTCCCTCCTC	TGCTCCAGTTTCTCCACCTT
M_25*	SCRI_RS_179560	AK373673.1	2H	121.05		96.92	GAATTGTGCTCTGCCTCTCC	AACCACCCAAAACTGAATGC
M_26	SCRI_RS_179560	AK373673.1	2H	121.05		96.92	GCTACAGTATCTGGCGTTCG	CAGGAAAAACCACCCAAAAC
M_27	SCRI_RS_179560	AK373673.1	2H	121.05		96.92	GAATTGTGCTCTGCCTCTCC	AACCACCCAAAACTGAATGC
M_10*	SCRI_RS_156045	AK374410.1	2H	124.51		106.44	GAAGTGGGAAAGGGGAAGAG	GAGCCACGTTGTAAACACCA
M_11*	SCRI_RS_156045	AK374410.1	2H	124.51		106.44	CTCGCCTCTCTAGCATCCAG	GGTGTTGCTTCTTCCCACTC
M_12	SCRI_RS_156045	AK374410.1	2H	124.51		106.44	GTGGGAAAGGGGAAGAGTTC	GCCACGTTGTAAACACCAAA
M_13*	SCRI_RS_16799	AK373540.1	2H	125.22		92.58	AGTTCAAACCACCCATCACC	GATCTTGGCCTTGACGTTGT
M_14	SCRI_RS_16799	AK373540.1	2H	125.22		92.58	GCCTCCAGAGTTCAAACCAC	ATCTTGGCCTTGACGTTGTC
M_15	SCRI_RS_16799	AK373540.1	2H	125.22		92.58	GCCTCCAGAGTTCAAACCAC	CTTGACGTTGTCGATGGTGT
M_28	SCRI_RS_238606	AK371708.1	2H	126.08		109.28	TCCCTCCCTACCATCTCCTC	ATCTCTCAGCACCACCGATT
M_29	SCRI_RS_238606	AK371708.1	2H	126.08		109.28	GTCCCTCCCTACCATCTCCT	ATCTCTCAGCACCACCGATT
M_30	SCRI_RS_238606	AK371708.1	2H	126.08		109.28	TCCCTCCCTACCATCTCCTC	ATGCCGCGTCAACTATCAAT
M_40	SCRI_RS_149429	AK353879.1	2H	128.13		112.04	ACCATGTCCGCAATTCCA	ATCTCCTCCCCCTTCTCCTC
M_41*	SCRI_RS_149429	AK353879.1	2H	128.13		112.04	GTTCCGCAATGTCCTCTGAC	CCTTCTCCTCTCCCTCGATT
M_42	SCRI_RS_149429	AK353879.1	2H	128.13		112.04	CCACCATGTCCGCAATTC	ATCTCCTCCCCCTTCTCCTC
M_37	SCRI_RS_142593	AK331385.1	2H	131.87		112.32	CAGTCATGGCAACTGGGAAC	TAGGCAAAACTGCGAGTCCT
M_38*	SCRI_RS_142593	AK331385.1	2H	131.87		112.32	CAGTCATGGCAACTGGGAAC	GCAAAACTGCGAGTCCTCTT
M_39	SCRI_RS_142593	AK331385.1	2H	131.87		112.32	TGCGTGGAACACCAGTTATG	CGGACAATGACCAGCAACTA
M_43*	SCRI_RS_192711	AK372653.1	2H	134.23		109.42	TCTTCCTTTGCTGATGACGAT	ACAAACAGAGGACGGCAGAC
M_44	SCRI_RS_192711	AK372653.1	2H	134.23		109.42	TCTTCCTTTGCTGATGACGAT	GGCAGACCTACCACACATGA
M_45*	SCRI_RS_192711	AK372653.1	2H	134.23		109.42	GGCTGGCTGCTACCCTATTA	CAATACCATGCTTGCACGAC
M_16*	BOPA1_13178-89	AK374855.1	2H	135.02	143.83	121.50	GGCAAGAAGAACAAGACGAGA	GCTGGGTGTAGGATGGACTT
M_17	BOPA1_13178-89	AK374855.1	2H	135.02	143.83	121.50	GGCAAGAAGAACAAGACGAGA	CATGGCTGGGTGTAGGATG
M_18	BOPA1_13178-89	AK374855.1	2H	135.02	143.83	121.50	GGCAAGAAGAACAAGACGAGA	AGACCTTCTCTTCCCTGATGC
M_34	SCRI_RS_151129	AK368018.1	2H	135.02		125.85	CTTCTGAACTCGAAGCAGCA	TGAGATTCTGTGCAATGTCCA
M_35*	SCRI_RS_151129	AK368018.1	2H	135.02		125.85	GGAAGACGCTTCTGAACTCG	TGAGATTCTGTGCAATGTCCA
M_36	SCRI_RS_151129	AK368018.1	2H	135.02		125.85	TGTGATGGAGAGCTTGAGGA	TGAGATTCTGTGCAATGTCCA
M_31	SCRI_RS_157929	AK373001.1	2H	139.45			CGAGAGGATGAAGGTCAAGG	GAAGGTGTCAGATCGCTGAA
M_32	SCRI_RS_157929	AK373001.1	2H	139.45			ACGCTTGTCGTCATCTCAG	GAAGGTGTCAGATCGCTGAA
M_33	SCRI_RS_157929	AK373001.1	2H	139.45			CGAGAGGATGAAGGTCAAGG	TCCTGCCAACGAATCAAGTA
M_46	SCRI_RS_157929	AK373001.1	2H	139.45			AGGCTTTATGTCACCGAAGG	ATCCTGCCAACGAATCAAGT
M_47*	SCRI_RS_157929	AK373001.1	2H	139.45			CGAGAGGATGAAGGTCAAGG	TCCTGCCAACGAATCAAGTA
M_48	SCRI_RS_157929	AK373001.1	2H	139.45			CGAGAGGATGAAGGTCAAGG	TGCAGATCACCAGAGCTGTC
M_49*	BOPA2_12_10579	AK368583.1	2H	144.62		132.48	TATGACCACTGCCGACTTCA	AACAATTCCCGCATCAAGAG
M_50	BOPA2_12_10579	AK368583.1	2H	144.62		132.48	GACCACTGCCGACTTCATCT	AACAATTCCCGCATCAAGAG
M_51	BOPA2_12_10579	AK368583.1	2H	144.62		132.48	TATGACCACTGCCGACTTCA	CCTTCCTGTGCTTCCACTGT
M_19	SCRI_RS_118062	AK364748.1	2H	145.74		126.77	TAGCAACCTTGTCCCTGGTC	CAAAATTCTCCCGTCCAATG
M_20	SCRI_RS_118062	AK364748.1	2H	145.74		126.77	GAGAAGCTGCTGCCTCTGAT	CAAAATTCTCCCGTCCAATG
M_21*	SCRI_RS_118062	AK364748.1	2H	145.74		126.77	CATGTTTGAAGGGGACAACG	TGGGCACAAAGAACTCACAC
M_22*	SCRI_RS_193100	AK248742.1	2H	146.48		127.27	CAGGTTCTATCAGGCATCCA	GATTCCTCACATCCTCTCTACCA
M_23	SCRI_RS_193100	AK248742.1	2H	146.48		127.27	TTCGGGCAAGAACTACAACC	TTCCTCACATCCTCTCTACCA
M_24	SCRI_RS_193100	AK248742.1	2H	146.48		127.27	TTCGGGCAAGAACTACAACC	GATTCCTCACATCCTCTCTACCA

*: Sequenced in the parental lines

Table A.2. Primers used for genotyping of the *Rnhq* region

Marker Name	SNP loci	BLAST	LG	Map position			Primer Forward	Primer Reverse
				CM_2013	OWB	i-select		
1_Abhay	BOPA1_12239-662	AK355306.1	7H	63.32	61.49	56.81	ATGGCTCAAAGCTCACGTCT	TACACACCACCCCCACAAC
2_Abhay	BOPA1_12239-662	AK355306.2	7H	63.32	61.49	56.81	ACGTCTCCTGTGTGGCAAT	CCACCCAACTCACCAAAATAA
3_Abhay	BOPA1_12239-662	AK355306.3	7H	63.32	61.49	56.81	TACAACAACGATGCCAACCA	ATGGCTCAAAGCTCACGTCT
4_Abhay	SCRI_RS_146382	Barley1_20068	7H	63.32		50.71	tcttacaaatccgacgcaca	aatttcccagcactccattg
5_Abhay	SCRI_RS_136556	CD863131	7H	62.96		47.30	gatcgctcgctaatggagtc	ttcctagaacttgcccgaaa
6_Abhay	SCRI_RS_136556	CD863131	7H	62.96		47.30	aaccagatcgctcgctaatg	ttcctagaacttgcccgaaa
7_Abhay	SCRI_RS_150062	AK356490.1	7H	84.82		76.56	TCCCTCTCCTCCTACTGCTC	CGTGAGGTCCAGAGAGAAGC
8_Abhay	SCRI_RS_150062	AK356490.2	7H	84.82		76.56	CATCCTCCTCTACCCGTCCT	GGATGGAGAAGCTGTTGGTC
9_Abhay	SCRI_RS_133026	AK363024.1	7H	85.70		77.27	TGCCTCGCTCTCATCACA	CAAGAAATAGCTACAATCACCGAGT
10_Abhay	BOPA1_4589-131	AK377085.1	7H	86.43	98.97	87.21	AGCTCAGATCCGACGAGATG	CCCAGGAACACAGAAGCAAT
11_Abhay	BOPA1_4589-131	AK377085.1	7H	86.43	98.97	87.21	AGCTCAGATCCGACGAGATG	CAGACATAAACACCGCTTGC
12_Abhay	SCRI_RS_136586	AK371770.1	7H			93.91	CTGCTTCACCCACTCTGCTT	GGATATGGGAGATGGCAGTG
13_Abhay	SCRI_RS_136586	AK371770.2	7H			93.91	CTGCTTCACCCACTCTGCTT	ATCCTCCACGAGCTGATTTG
14_Abhay	BOPA2_12_21479	AK365803.1	7H	94.75			TTCTCATAGAAGCCTCGTGGA	GTCTCGTTTCTTCTTCTATTGCTG
15_Abhay	BOPA2_12_21479	AK365803.2	7H	94.75			TCTCATAGAAGCCTCGTGGAA	AGTCTCGTTTCTTCTTCTATTGCTG
16_Abhay	SCRI_RS_136590	Barley1_11960	7H			93.91	cccccttttgcttttctttt	gctacaatggagggcatgta
17_Abhay	SCRI_RS_136590	Barley1_11961	7H			93.91	tggcaatttcttccctgttc	gctacaatggagggcatgta
18_Abhay	BOPA1_1800-1101	AK367663.1	7H		128.60	104.78	AAGCTCCGCTGATGAGAATG	CCGCGTAACAACAGACACAA
19_Abhay	BOPA1_1800-1101	AK367663.2	7H		128.60	104.78	AAGCTCCGCTGATGAGAATG	GATCCCGCGTAACAACAGAC
20_Abhay	BOPA2_12_21464	AK364970.1	7H		128.60	104.78	AACCCCACACACATCCTGTT	ACGTGTCCGTGCAGTAGTTG
21_Abhay	BOPA2_12_21464	AK364970.2	7H		128.60	104.78	GAACCCCACACACATCCTGT	GTCCAGCTCCTGGTACATCC
22_Abhay	BOPA1_12027-128	AK250887.1	7H	99.63	124.86	102.85	ATCCCTCTCCGTTCCTCCT	ACCGTCACGTAGGATTCTGG
23_Abhay	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	TCAAACTAGGCATGGCATCA	CAAGGCTGAGGAGAAGAAGG
24_Abhay	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	CAAGGCTGAGGAGAAGAAGG	TCAAACTAGGCATGGCATCA
25_Abhay	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	GAAGAAGGCGTTGAAGGACA	AGTTTAGCCAGCCAGTCAGC
26_Abhay	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	AAGGCGTTGAAGGACATAGC	AGTTTAGCCAGCCAGTCAGC
1_AMS_Tereza	SCRI_RS_161111	AK354560.1	7H	63.35		52.27	TGGCCTGTGCACTAAGACAG	CCGAGAATGGTCGAAAGGTA
2_AMS_Tereza	SCRI_RS_161111	AK354560.1	7H	63.35		52.27	ACGATTCAGGAAACGGGCTT	TCCCTGCAGCTGAAGAACAG
3_AMS_Tereza	BOPA1_12239-662	AK355306.1	7H	63.32	61.49	56.81	CGTAAAATTGGGCATGTGTG	AGTCCAAGCTTGCTCGTGAT
4_AMS_Tereza	BOPA1_12239-662	AK355306.1	7H	63.32	61.49	56.81	AAGCTCACGTCTCCTGTGTG	GCCACTGGCCTATATGTCCC
5_AMS_Tereza	SCRI_RS_146382	Barley1_20068	7H	63.32		50.71	AATTTCCCAGCACTCCATTG	TCTTACAAATCCGACGCACA
6_AMS_Tereza	SCRI_RS_136556	AK358254.1	7H	62.96		47.30	GAGGTCCCCGTACGTAGCTC	GTGAGGAGGGTCATGGAGTG
7_AMS_Tereza	SCRI_RS_230478	XM_002443886.1	7H	66.28		54.82	ACCCTGTTCTGCTTCACACC	CCGCACTTTCATCTTTCCAT
8_AMS_Tereza	SCRI_RS_150062	AK356490.1	7H	84.82		76.56	CTTCCTCCTCTCGGCCTACT	CAGGATGGAGAAGCTGTTGG
9_AMS_Tereza	BOPA1_1674-468	JN107540.1	7H	86.00		86.44	CGAGGTCCTGAAAACTCCTG	AGGAAGACCAGCAGCAGAAA
10_AMS_Tereza	BOPA1_1674-468	JN107540.1	7H	86.00		86.44	AAGGCTGTTGTGCAGGTCTT	TATCGGAGGGCCATTATCAA
11_AMS_Tereza	SCRI_RS_133026	AK363024.1	7H	85.70		77.27	TCACCTCAAATCTGCAGTCG	TCACTCGTCTATCATCCAGACA
12_AMS_Tereza	BOPA1_11619-618	AK367043.1	7H	87.31	98.97	87.97	TTTGCGATAACAGCTTTGGA	ATGAGTCACAAAACGCGATG
13_AMS_Tereza	BOPA1_11619-618	AK367043.1	7H	87.31	98.97	87.97	ATGTTTCGGGAGAAAATGCT	ATTGACCATGCGACAAACTG
14_AMS_Tereza	BOPA1_1676-557	AK375073.1	7H	88.18	98.97	87.97	GAAGTCACGCAAGCAGATCA	TCACCCTTGGACACGACATA
15_AMS_Tereza	SCRI_RS_194291	AK248533.1	7H	88.17		77.41	ATTGTGTCCCTGTTGGTCGT	ATTTCTCGCGCAATTGTGAT
16_AMS_Tereza	SCRI_RS_194291	AK248533.1	7H	88.17		77.41	TTGTGTCCCTGTTGGTCGT	ATTTCTCGCGCAATTGTGAT
17_AMS_Tereza	SCRI_RS_104566	AK332506.1	7H	90.47		80.10	CTGCTGGCGTACCTCAAATC	GACATCTCCATCCCCTTCAA
18_AMS_Tereza	SCRI_RS_194841		7H	91.62		81.52	CAGAGGGAGGGAGGGAAGAA	ATGATGACGACGACCTTGGG
19_AMS_Tereza	BOPA2_12_21479	AK365803.1	7H	94.75			AGTCAGGAACGTCAGCCAAGG	GGAACCTGTGCATGAGACCA
20_AMS_Tereza	BOPA2_12_21479	AK365803.1	7H	94.75			GCTTCTTTCAGTGGGTGGAA	GAAAAGGGTCTAGGGGAGGA
21_AMS_Tereza	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	TCCTCCTTCACCATGGTCTC	TCATTTCTGGCGTGCAATAG
22_AMS_Tereza	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	GCCATCTTCCAGGTGGTCAT	CCATGTCATTTCTGGCGTGC
23_AMS_Tereza	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	GGAAATTCCTGACCCTGCTT	GTACGCTGCCTTCCCTGATA
24_AMS_Tereza	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	CTATCAACCGCGTCCTCTCC	CCTCCCCTAGGCCTCTTTCT
25_AMS_Tereza	SCRI_RS_143884	AK366098.1	7H	99.38		92.21	AGCACCAGAACTCATTCCCG	TCCTCCCCTAGGCCTCTTTC
26_AMS_Tereza	SCRI_RS_236651	AK367938.1	7H	71.29		62.18	ACAAGACGGACCTACGGATG	ATCAAAAGCCTCACCACAGG
27_AMS_Tereza	SCRI_RS_236651	AK367938.1	7H	71.29		62.18	CAATGGGCTTGTGGCTAGGA	ATCGTACTCACTCACGGGGA
28_AMS_Tereza	BOPA1_4054-1326	AB447484.1	7H	72.38		68.46	AGGTGATATCGGAGCTGGTG	ACCTGAATCCAGGGGAAATG
29_AMS_Tereza	BOPA1_4054-1326	AB447484.1	7H	72.38		68.46	GTGATATCGGAGCTGGTGG	CGGAACCAACTGCTAACCCT
30_AMS_Tereza	SCRI_RS_2914	AK366162.1	7H	84.03		70.96	CGGTGAAGAAGCTCTGGAAG	CCTGCAGGTTACCATTAGGG
31_AMS_Tereza	SCRI_RS_2914	AK366162.1	7H	84.03		70.96	GTCAAACTCCTACACCGGCA	GCTCGTAGCTCCCCATCTTC
32_AMS_Tereza	SCRI_RS_194085	AK251866.1	7H			77.41	ATCCATTCGCTTCAAGTTCC	AGAATAGTCCCGTTGGCTCA
33_AMS_Tereza	BOPA2_12_10657	AK366264.1	7H	67.49	71.46	68.46	CGGAAGGATCTTTCTTGCTAA	TGCCCGTGCATATACATACC
34_AMS_Tereza	BOPA2_12_10657	AK366264.1	7H	67.49	71.46	68.46	CCGACGCGTATGCTGATCTT	AAGGTGTTGCGGTCGTACTT
35_AMS_Tereza	SCRI_RS_161111	AK354560.1	7H	63.35		52.27	cgggtggttctggaatatct	aatggcaactgctgtcacac
36_AMS_Tereza	SCRI_RS_136556	AK358254.1	7H	62.96		47.30	ccagctccttcctcagcttat	caagaacttgcccgaaatgt
37_AMS_Tereza	SCRI_RS_230478	XM_002443886.1	7H	66.28		54.82	AGCAAAGTGCGTCGTCTTTT	TTAATTGCCCGGATGATTG
38_AMS_Tereza	SCRI_RS_230478	XM_002443886.1	7H	66.28		54.82	CCTCCTTTGCACGAATTCTC	TTAATTGCCCGGATGATTG
39_AMS_Tereza	SCRI_RS_230478	XM_002443886.1	7H	66.28		54.82	agcctccgcactttcatct	gctcccacaacaggaggata
40_AMS_Tereza	SCRI_RS_2914	AK366162.1	7H	84.03		70.96	aagctctggaagcagaccaa	ttgtaccgtgtgtcccagtc
41_AMS_Tereza	BOPA2_12_21479	AK365803.1	7H	94.75			tctacttgagtctcgtttctttc	tctcatagaagcctcgtggaa
42_AMS_Tereza	BOPA1_2444-437	AK358239.1	7H	98.35		99.67	ccaaggctgaggagaagaag	tcaaactaggcatggcatca
43_AMS_Tereza	BOPA1_4589-131	AK377085.1	7H	86.43	98.97	87.21	CTCAGATCCGACGAGATGGC	AGAAGCAATGGACGCTGTGA
44_AMS_Tereza	SCRI_RS_206747	AK368017.1	7H	87.31		77.27	GAACGCATCAAGCACAAAGA	ATTCCAAGGGCCTCCAATAG
45_AMS_Tereza	SCRI_RS_124478	AK370797.1	7H	87.31		77.27	AGCGGTAAACCACCTGCTTA	TTTGCTTCCAAGAGCTTCAA
46_AMS_Tereza	BOPA1_11619-618	AK367043.1	7H	87.31	98.97	87.97	CTTCCGCGTTGAAGATGAGT	TCCCTGACCTTCTAAGCCCTA
47_AMS_Tereza	SCRI_RS_171080	AK373441.1	7H	87.75		77.41	CACCACCACCACTTCTCCTT	GGTCCTGGCCTCTCTCTTTC
48_AMS_Tereza	BOPA1_1676-557	AK375073.1	7H	88.18	98.97	87.97	AGGGTACACCACTTGGGTTG	GACCGCGAGTTTGTCTTCAC
49_AMS_Tereza	BOPA1_12027-128	Array sequence	7H	99.63	124.86	102.85	ATCAATCCCTCTCCGTTCCT	ATCGACACCGTCACGTAGG
50_AMS_Tereza	SCRI_RS_122512	AK357827.1	7H			76.70	GAGTTGCCGACCACATTCTT	CCATCCACATCCCAACATCAA
51_AMS_Tereza	SCRI_RS_136590	Barley1_11960	7H			93.91	tgctacaatggagggcatgt	tggcaatttcttccctgttc
52_AMS_Tereza	SCRI_RS_136586	AK371770.1	7H			93.91	CTGCTTCACCCACTCTGCTT	GGCAAATGACCAAATCTTCC
53_AMS_Tereza	BOPA1_1800-1101	AK367663.1	7H		128.60	104.78	TCTTCCAGGACTCGGAGATG	CTGCGACGACAGGTAGAAGG
54_AMS_Tereza	BOPA2_12_21464	AK364970.1	7H		128.60	104.78	GACGGGGTTTCCCTACCT	GTCCAGCTCCTGGTACATCC
55_AMS_Tereza	SCRI_RS_208890		7H	106.61		97.24	CTCGTCGATCCGTCTCTAGG	TGTAGATGCCGTGCTTTCAC
56_AMS_Tereza	SCRI_RS_208890		7H	106.61		97.24	AGCTCGTCGATCCGTCTCTA	TGTAGATGCCGTGCTTTCAC
57_AMS_Tereza	SCRI_RS_15864		7H	69.44		58.14	CATCAGCGAAAGATCGGTTT	TGCTTTTGCACAAATGAAGC
58_AMS_Tereza	SCRI_RS_15864		7H	69.44		58.14	GGCGTACATCAGCGAAAGAT	TGCTTTTGCACAAATGAAGC
59_AMS_Tereza	SCRI_RS_161111	AK354560.1	7H	63.35		52.27	TCTTCTGCAGGAAAGGTTG	TTGCAGCTTAAATGGCTCCT
60_AMS_Tereza	SCRI_RS_161111	AK354560.1	7H	63.35		52.27	ACGATTCAGGAAACGGGCTT	TCCCTGCAGCTGAAGAACAG
61_AMS_Tereza	SCRI_RS_136556		7H	62.96		47.30	GAGGTCCCCGTACGTAGCTC	GAGGAGGGTCATGGAGTGAA
62_AMS_Tereza	SCRI_RS_136556		7H	62.96		47.30	ccagctccttcctcagcttat	caagaacttgcccgaaatgt
63_AMS_Tereza	SCRI_RS_136556		7H	62.96		47.30	gagttcccgtacgtagctc	gaggagggtcatggagtgaa
64_AMS_Tereza	SCRI_RS_136556		7H	62.96		47.30	gaacccagctccttcctca	caagaacttgcccgaaatgt
65_AMS_Tereza	SCRI_RS_230478	XM_002443886.1	7H	66.28		54.82	gcttccaggcaaaggtatca	gttgacaggggtttgatgct
66_AMS_Cynara	SCRI_RS_186683	AK374195.1	7H	62.96		50.85	TCGTATGGTTGTCGTCTGAAA	AATCCCGTGTCGGTGAAAG
67_AMS_Cynara	SCRI_RS_209511		7H	63.16		52.27	CGTGCTTATGCGTGGTGATA	GGTCCTCCTTGATGAACAGC
68_AMS_Cynara	BOPA2_12_30199		7H		97.74	86.44	CAAATGGAGCTACAAATATAAGAGG	AAGAATCCTGCATTTTGACAAG
69_AMS_Cynara	SCRI_RS_219581	AK365617.1	7H	86.39		77.27	AAACAGAATTGGGGTTGTCG	AAGGGGGTCCAAATTATTGC
70_AMS_Cynara	SCRI_RS_168994	AK358967.1	7H	98.42		89.52	GGACAGCAACCTCCTGAAGA	GCTCTGGGTAACAATTTGACG
71_AMS_Cynara	SCRI_RS_196885	AK356549.1	7H	99.06		85.17	TGAGGCAGAAACCTACACCA	CGTCGGCTCTTATTGTTCCT