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HOST STATUS AND GENETIC ANALYSIS OF QUANTITATIVE RESISTANCE OF BARLEY TO YELLOW STRIPE RUST CAUSED BY
Puccinia striiformis f.sp. *bromi*,
P. striiformis f.sp. *tritici* and
P. striiformis f.sp. *hordei*

By:

Skye van Heyzen

Thesis for Master of Sciences in Plant Sciences
Specialization: Plant Breeding and Genetic Resources

Supervisor:

Dr. Ir. Rients E. Niks

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Summary

Puccinia striiformis, the causal fungus of yellow stripe rust, is an economically important pathogen. Recently an isolate of stripe rust was collected from *Bromus carinatus* in Wageningen, The Netherlands. This has been temporarily classified as *P. striiformis* f.sp. *bromi* (*Psb*) and in preliminary studies showed a propensity to infect barley. Therefore it is of interest to test and compare this isolate with other stripe rust isolates that have been shown to infect barley; namely *P. striiformis* f.sp. *tritici* (*Pst*) and *P. striiformis* f.sp. *hordei* (*Psh*). The objectives of this study were four fold: Firstly to establish a preliminary host range of *Psb*, *Pst* and *Psh*; second to quantify the host status of barley to these three yellow stripe rust isolates; third to map those QTL's that are effective against *Psb*, *Pst* and *Psh* in barley mapping populations; and lastly to compare these QTL's in all three rust isolates to those QTL's that have been previously mapped to other heterologous rusts.

An indicative host range study on 46 grass and cereal species, consisting of a total of 162 accessions, showed *Psb* to be more versatile than *Pst* or *Psh*, in the sense that *Psb* was more successful on wild and cultivated accessions where both *Pst* and *Psh* were less successful. On a genus level, *Lolium* and *Secale* were resistant, *Avena* susceptible to only *Psb* and *Agropyron*, *Aegilops*, *Triticum*, *Hordeum* and *Bromus* were all susceptible to the three ff.spp..

A barley host-status seedling test was performed on 118 accessions, resulting in barley being classified a host for *Psh*, with 90% of the accessions showing a host-type response, and as a marginal host for *Psb* and *Pst*, with host-type responses in 47% and 11% of the accessions tested, respectively. At an adult plant stage, most accessions were resistant, except for those tested with *Psh*.

QTL mapping experiments revealed two QTLs conferring resistance against *Psb* and *Pst* and a third for resistance against *Psb* in the Vada × SusPtrit RIL population, using quantitative data from number of pustule and composite lesion length with Vada being the resistant parent and SusPtrit the susceptible parent. Mapping experiments in the L94 × Vada RIL population, also using quantitative data from number of pustule and composite lesion length but with L94 being the resistant parent and Vada the susceptible parent, mapped a major gene which has been attributed to be possibly *rpsGZ*.

Chapter 1: Introduction

1.1 Types of resistance

In plant-pathogen systems, there are many types of resistance that can be discerned, of which host and non-host resistance are two (Niks, 1987). A plant species is said to be a host when the majority of accessions of the species exhibit susceptibility, and thus a compatible reaction, to a particular pathogen (Jafary *et al.*, 2008; Niks, 1987, 1988). A plant species is said to be a non-host when the species displays immunity against all genotypes of a pathogen. Moreover it is said that non-host resistance mechanisms are dominated by the presence of a non-specific defence reaction which the pathogen cannot negate (Heath, 2000; Niks, 1987, 1988). However, the clear distinction between host and non-host status is not straightforward, as some plant species have a few accessions that exhibit susceptibility, or an intermediate susceptibility, to the pathogen or various genotypes of the pathogen. This then lends itself to be termed a near non-host or a marginal host (Jafary *et al.*, 2008; Niks, 1987).

Within the host resistance category two types of reactions are observed; namely a hypersensitive and non-hypersensitive reaction (Niks 1988). The hypersensitive reaction is typified by major gene resistance, which is based on a gene-for-gene relationship. This type of resistance has dominated breeding programs, owing to its simple inheritance pattern and large phenotypic effect. However, this type of resistance is easily broken down by pathogens; thus making this type of resistance non-durable (Niks 1982; Parlevliet 1977).

On the other hand, the presence of a non-hypersensitive resistance reaction has been said to have more durability and should thus be the preferred source of resistance. This has also been termed partial resistance and is controlled by many genes (Castro *et al.* 2002; Niks 1982). This type of resistance is quantitative and more difficult to score, and thus for use in breeding programs requires extensive field studies. Therefore making this type of resistance, although possibly more promising, more difficult to breed for (Castro *et al.* 2002).

1.2 Yellow stripe rust

Puccinia striiformis is the causal fungus of yellow stripe rust. This rust fungus has intermittently devastated cereal production worldwide through the years, and results in defoliation and shrivelled kernels on the affected plants. For example in the United States of

America, financial losses due to stripe rust on wheat in 2000, 2001, 2002 and 2003 were estimated at around $27 million, $119 million, $24 million and $267 million respectively (Chen 2005; Xi *et al.* 2007). In barley, also in the USA, a 72% grain yield loss was observed in the most susceptible cultivar to stripe rust. Moreover, it has been reported that this is a very economically important disease, not only in the USA but worldwide (Castro *et al.* 2002; Chen & Line 1992; Chen 2005; Xi *et al.* 2007).

Historically *P. striiformis* was first described by Gadd in 1777, and later Schmidt in 1827 described it under *Uredo glumarum*, and finally the rust was identified by Eriksson in 1894 (Line 2002; Stubbs 1985). It was, however, only changed to its accepted name of *Puccinia striiformis* in 1956 by Cummins and Stevenson.

There are different formae speciales (ff.spp.) of *P. striiformis* and these cause stripe rust disease on different grasses and cereals. They typically differ in their ability to attack the different species and are hence classified as different forms of *P. striiformis* (Xi *et al.* 2007). There are seven different ff.spp. classified which are characterized by the plant genera, or species, that they are able to successfully attack. *P. striiformis* f.sp. *hordei* (barley stripe rust), *P. striiformis* f.sp. *tritici* (wheat stripe rust), *P. striiformis* f.sp. *dactylis* (orchard grass stripe rust), *P. striiformis* f.sp. *poae* (blue grass stripe rust), *P. striiformis* f.sp. *elymi* (*Elymus* stripe rust), *P. striiformis* f.sp. *agropyri* (*Agropyron* stripe rust) and *P. striiformis* f.sp. *leymi* (*Leymus* stripe rust) (Chen 2005; Stubbs 1985).

It has been said that Europe has one of the longest histories of stripe rust, with epidemics occurring sporadically with varying frequencies in all wheat growing countries. In northwest and central Europe, for example, there was a serious epidemic occurring on wheat and barley in 1961. This was attributed to favourable weather conditions for both the overwintering and over-summering forms of the rust (Stubbs 1985). Apart from Europe, stripe rust has been detected in Northern America, Central America, Southern America, the Middle East, Central Asia, East Asia, South Asia, Africa, Australia and New Zealand (Chen *et al.* 1995; Chen 2005; Stubbs 1985).

Two of the most economically important ff.spp. are *P. striiformis* f.sp. *tritici* (*Pst*) and *P. striiformis* f.sp. *hordei* (*Psh*) (Xi *et al.* 2007). Of these two, *Pst* has been noted for a much

longer period of time than *Psh*. For example, *Pst* has been notably present in the USA since 1915, while *Psh* noticeably since only 1991 (Chen *et al.* 1995).

Although *Pst* and *Psh* have been classified as individual entities, they do have an overlapping host range, in that a few cultivars of wheat can be attacked by *Psh* and vice versa. However, in saying this typically there is hardly ever any damage caused by *Pst* on barley and *Psh* on wheat (Chen 2005; Pahalawatta & Chen 2005; Rodrigues *et al.* 2004).

Recently an isolate of *P. striiformis* was isolated from *Bromus carinatus* in Wageningen, in the Netherlands (Latitude 51° 58' 0" N; Longitude 5° 40' 0" E), and this is believed to be a different and new f.sp. of stripe rust (Niks, personal communication). Due to this being isolated from a *Bromus* grass it has been temporarily labelled *P. striiformis* f.sp. *bromi* (*Psb*). Furthermore, this is thought to be a more versatile f.sp. of stripe rust, as it was isolated from a wild Bromus grass and showed propensity to attack some barley lines (Niks, personal communication). Hence this rust would be interesting to study fundamentally as the other stripe rusts do not seem to have this versatility.

Typically stripe rust seems to thrive under, and show preference for, moister and cooler conditions, and the environment seems to play an important role in the stripe rusts ability to successfully attack the host plants. In particular it seems to play an important role in the resulting symptoms and effects of stripe rust; and, to our knowledge, even more so than with any other cereal rust (Chen 2005; Stubbs 1985; Zadoks 1961). In addition to these environmental factors, the effect of light intensity also seems to play a crucial role in the infectivity of stripe rust. Research performed on *P. striiformis* suggests that wheat seedlings exposed to high light intensities (>28.8 mol quanta m^{-2}) were more receptive than those grown in dark conditions to the subsequent inoculation (Vallavieille-Pope *et al.* 2002). In research done on other pathogens, however, infectivity seemed to be inversely proportional to pre-inoculation light exposure (Shafia *et al.* 2001; Zhang *et al.* 1995). In either case though, it can be said that light intensity as an environmental factor, and in the case of stripe rust – exposure to high intensities prior inoculation, directly effects successful infectivity and host receptivity and possibly host resistance response (Roberts & Paul 2006; Zadoks 1961).

Upon successful infection, the stripe rust lesion encompasses the entire width of the primary leaf, and is typically several centimetres in length. This is observed as a yellow fleck on

which several small pustules develop and sporulate abundantly. These pustules are closely packed together and arranged in lines on or between the veins of the leaf (Chen 2005; Zadoks 1961). In a greenhouse, when light is short, these pustules are more widely spaced. On mature plants narrow stripes, typically not wider than 1 to 2 mm, are observed. In comparison, the stripes on mature plants sporulate more abundantly than those on seedlings (Zadoks 1961).

1.3 Background and implications of this study

As the cereal rust diseases, including yellow stripe rust, have been long noted for their potential to devastate crops, and with the recent isolation of a potentially new form of yellow stripe rust, it is important to study the effects of rusts on crops and how to control these through acquired plant resistances (USDA 2008a).

The newly isolated *Psb* has shown propensity and ability to infect different barley lines (Niks, personal communication), and to our knowledge there have been no studies carried out on this rust. Therefore it is of interest to compare this rust to other yellow stripe rusts that can infect barley; these are notably *P. striiformis* f.sp. *hordei* (*Psh*) and, on a few barley accessions, *P. striiformis* f.sp. *tritici* (*Pst*) (Stubbs 1985; USDA 2008b). Furthermore, these tests also indicated that this rust may have a much broader host range than either *Psh* or *Pst*, as preliminary studies have shown that this rust has the propensity to infect several Poaceae genera, including cultivated barley, therefore indicating that this may be a more versatile pathogen (Niks, personal communication). Furthermore, as this was isolated from *Bromus carinatus* there is epidemiological relevance in studying this as the wild grasses may act as alternate hosts for the pathogen.

In addition to these initial host range and host status studies, if pathogenicity tests reveal lines of barley that differ in their resistance quantitatively, then this is also of interest as this indicates the presence of QTLs for resistance to the rust. Knowing if there are QTLs responsible for resistance in barley is of importance if we are to breed cultivated crops for non-host resistance; a more durable form of resistance. Furthermore, in the quest of nonhost breeding, it is important to note the QTLs that are responsible for resistance in one rust to other heterologous rusts, and to see if these QTLs overlap.

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1.4 Project objectives

There are four main objectives to this study:

- To establish a preliminary host range of *Puccinia striiformis* f.sp. *bromi*, *P. striiformis* f.sp. *tritici* and *P. striiformis* f.sp. *hordei*.

- To quantify the host status of barley to these three yellow stripe rust isolates.

- To map QTL's, and possibly major genes, that are effective against *P. striiformis* f.sp. *bromi*, *P. striiformis* f.sp. *tritici* and *P. striiformis* f.sp. *hordei* in barley mapping populations.

- To compare these QTL's in all three rust isolates to those QTL's that have been previously mapped to other heterologous rusts.

Chapter 2: Determination of host range of *Puccinia striiformis* f.sp. *bromi*, *P. striiformis* f.sp. *hordei* and *P. striiformis* f.sp. *tritici*.

2.1. Materials and methods

2.1.1 Plant material

The three rust ff.spp. isolates were tested on a range of *Bromus*, *Aegilops*, *Agropyron*, *Avena*, *Hordeum*, *Lolium*, *Secale* and *Triticum* accessions (Table 4; Appendix 1). All *Bromus* accessions were kindly provided by Dr. Tatjana Oja of the Institute of Botany and Ecology at the University of Tartu, Estonia. All other material was kindly provided by the Barley Research Unit in the Department of Plant Breeding, Wageningen University and Research Centre. These plants represent 46 grass and cereal species, and consist of a total of 162 accessions. These plants also represent diverse sources, in that some accessions are the same species but were obtained from different countries.

The accession list has been compiled using a previous host range study (Alemu 2008), with a few additional accessions being added, as these showed interesting results in literature (Chen & Line 1992; Chen *et al.* 1995; Moldenhauer *et al.* 2006; Pahalawatta & Chen 2005; Pathan *et al.* 2008; Pretorius *et al.* 2007; Rodrigues *et al.* 2004; Sandoval-Islas *et al.* 2002; Wellings 2007; Yan & Chen 2006).

2.1.2 Pathogen material and propagation

The rust isolate found and isolated from *Bromus carinatus* in Wageningen has been preliminarily classified as *Puccinia striiformis* f.sp. *bromi* (*Psb*) (Niks, personal communication). The isolates *Puccinia striiformis* f.sp. *tritici* (*Pst*) and *Puccinia striiformis* f.sp. *hordei* (*Psh*) were obtained from the collection of rust isolates at Plant Research International, Wageningen, The Netherlands (Niks, personal communication).

Two temperature settings in the humidity chamber were tested in the initial propagation experiment; as literature suggests that cold temperatures (10°C) are required for yellow stripe rust to establish itself successfully, and the current settings (16°C) of the humidity chamber are warmer than those described in literature (Chen & Line 1992; Pahalawatta & Chen 2005; Castro et al. 2003; Chen et al. 1995). The current settings are very successful for the

development of stem and leaf rusts, and for ease it was better to keep the temperature at the warmer settings. Moreover, despite these reports, it is also believed that stripe rust can successfully propagate under the current warmer conditions set in the humidity chamber (Niks, personal communication).

Psb and *Pst* were used in the first propagation experiment as there was a limited quantity of *Psh* inoculum. Known susceptible accessions were used for each rust; *Bromus tectorum*, *B. diandrus* and *Hordeum lechleri* used for *Psb* and Michigan Amber for *Pst.*

Each accession, represented by 4 plants in a single pot, were inoculated with 4 mg urediospores (of the appropriate rust) mixed with lycopodium powder (approximately 1:20 v/v) using a powder blower. A glass slide was placed with the accessions such that spore germination could be observed the following day. These were then placed in the humidity chamber at the settings to be tested and left overnight. The following morning germination of the spores could be noted by observing successful development of germ tubes by the spores on the glass slides.

After this experiment, *Psh* was tested on known susceptible barley accessions Braemar, RIFF and research line SusPtrit. Due to the results of the *Psb* and *Pst* experiment, the current warmer settings were used initially. However, due to first *Psh* spore germination results being poor, the cooler temperatures were also tried with this rust. Nonetheless after inoculation with freshly collected spores, and the corresponding results, it was decided that the current warmer settings would suffice. Therefore, for all remaining experiments the warmer temperature settings in the humidity chamber room were used.

Other research has indicated the importance of light intensity and quantity prior to inoculation of the plants. The research suggests that for successful infection the plants must be supplemented with light, at relatively high intensities (optimally 30.1 mol quanta m^{-2}), prior to inoculation for a 16 hour period (successful infection was calculated by the number of pustules or chloroses per leaf area divided by the number of deposited spores) (Vallavieille-Pope *et al.* 2002). Due to this concern, inoculations were carried out as late in the day as possible. Thus leaving the plants in the well illuminated greenhouse, where they were grown until young seedling stage, for as long as possible, before transferring them to the less-well

illuminated humidity chamber room, thereby giving the pathogen the best chance to successfully infect the plants.

Once sufficient sporulation had occurred, which varied between 12 and 16 days, spores were collected using a cyclone spore collector and placed in a desiccator to evaporate any excess water. In the case where more spores were produced than required, these were labelled and stored in liquid nitrogen.

2.1.3 Inoculation and incubation

The various grass and cereal species mentioned in Appendix 1 were subjected to an infection experiment. For the first inoculation experiment, for *Psb* testing, the seeds were planted and grown in 12 cm × 12 cm pots with 5 to 9 seeds sown per pot (depending on how many seeds were available for the trials). This was because it was expected that there would be variation in the seed germination and seedling development time. These plants were then raised in a greenhouse and once a suitable proportion of the seedlings had germinated to the appropriate development stage (after emergence of the second leaf), they were transplanted to rectangular planting boxes; which were either 36 cm × 44 cm long or 38 cm × 58 cm long (depending on availability of the inoculation boxes). Those seedlings that were not of the appropriate development stage (as mentioned above) were left to grow in the pots, and transplanted at a later stage and then subjected to a subsequent inoculation experiment.

For those boxes inoculated with either *Psb* or *Psh*, the research line SusPtrit was included as a reference line. For those boxes inoculated with *Pst* the wheat cultivar Michigan Amber was included as a reference line. These reference lines were selected as they were known to be susceptible to the associated pathogens (Niks, personal communication).

In subsequent inoculation experiments the seeds that were noted to have germinated at more or less the same time were planted in inoculation boxes together. Those boxes at the same development stage were inoculated together.

The inoculation was done using a powder blower in order to keep the treatment the same for all boxes as not all boxes could be inoculated using a settling tower as they were too large. The smaller boxes were inoculated with 4 mg of urediospores and the larger boxes with 5 mg

of urediospores. In both cases the spores were mixed with lycopodium powder (+/- 1:20 v/v) before application to promote a uniform spreading of the spores.

2.1.4 Evaluation

The various accessions were tested at seedling stage (after third or fourth leaf emergence) to evaluate their resistance level. In order to evaluate the resistance of the seedlings the 0-to-9 scale and descriptive scale as outlined by McNeal was used (McNeal *et al.* 1971).

Table 1 Numerical scale and descriptive scale for the evaluation of susceptibility to *Puccinia striiformis* f.sp. *bromi*, *P. striiformis* f.sp. *hordei* and *P. striiformis* f.sp. *tritici* (McNeal *et al.* 1971).

Score	Hypersensitivity	Sporulation	Description
0	None	None	Immune
1	Necrosis or chlorosis	None	Very resistant (VR)
2	Necrosis and chlorosis	None	Resistant (R)
3	Necrosis and/or chlorosis	Trace	Moderately resistant (MR)
4	Necrosis and/or chlorosis	Light	Low intermediate (LM)
5	Necrosis and/or chlorosis	Intermediate	Intermediate (M)
6	Necrosis and/or chlorosis	Moderate	High intermediate (HM)
7	Necrosis and/or chlorosis	Abundant	Moderately susceptible (MS)
8	Chlorosis behind sporulating area	Abundant	Susceptible (S)
9	None	Abundant	Very susceptible (VS)

A plant species was proposed to have a presumed host-status if one accession within the species showed a susceptible reaction otherwise it was proposed to have a presumed non-host status. This was pre-set at a score value of ≥ 4, as McNeal *et al.* (1971) prescribe scores of 1 to 3 to resistant plants, and anything value ≥ 4 is either intermediate or susceptible (Table 2). Thus for sake of ease for descriptive comparisons, this scale outlined was modified, by grouping scores together.

Table 2 Modified scores and descriptions for analysis for accessions tested against *Psb*, *Pst* and *Psh* (modified from McNeal *et al.* (1971))

Scores	Description
0	Immune (I)
1-3	Resistant (R)
4-6	Intermediate (M)
7-9	Susceptible (S)

Furthermore, for a more complete overview, for assessing the *Hordeum* genus, the data obtained from the host status experiments were included. However, the data needed to be transformed (Table 3). This was based on the scale outlined by McNeal *et al.* (1971), and the modified scores from table 2.

Table 3 Description and designation of associated pustule number for assessment of data from host status for inclusion in host range

Pustules	Description and designation
0	Immune (I)
0 (flecks) to 10	Resistant (R)
11 to 100	Intermediate (M)
≥ 101	Susceptible (S)

2.2 Results

2.2.1 Temperature settings

All three rust isolates had varying levels of successful spore germination at the cooler (10°C) and at the warmer (16°C) humidity chamber settings. However, the warmer settings provided a better spore germination rate than the cooler settings.

In terms of the genera and species tested during the host range, all of the results are summarised in table 4.

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2.2.2 *Lolium*, *Avena*, *Secale* and *Agropyron* accessions

All *Lolium*, *Avena* and *Secale* accessions were either immune or resistant to all three ff.spp., however, not many accessions were tested. The *Agropyron* accessions showed either an immune type or resistant response to *Pst* and *Psh*, while one of the accessions showed an intermediate response to *Psb*.

2.2.3 *Aegilops* and *Triticum* accessions

The *Aegilops* accessions had scores ≥4 to all three ff.spp. but were only fully susceptible to *Pst* with an intermediate response to *Psb* and *Psh*. Moreover, most accessions resistant against *Psb* are scores of 2, while most are scores of 3 against *Pst*, and about two thirds are scores of 2 against *Psh* (for details see appendix 1).

Of the *Triticum* accessions 29 out of 50 (58%) either showed a susceptible or intermediate response to *Pst* while 21 out of 50 (42%) were resistant and none were immune. For *Psb* only 3 of 52 (6%) accessions showed an intermediate response, while 37 of 52 (71%) were resistant and 12 of 52 (23%) accessions were immune, and none were fully susceptible. Of accessions tested against *Psh* 30 of 51 (59%) were resistant while 21 of 51 (41%) were immune.

2.2.4 *Hordeum* accessions

The majority of the *Hordeum* accessions, 75 of 127 (59%), were susceptible to *Psh*, with 33 of 127 (26%) showing an intermediate response, and 10 of 127 (8%) were resistant and 9 of 127 (7%) were immune. In addition, in terms of the cultivated barley tested, *Hordeum vulgare* accessions, 102 of 115 (89%) had scores of ≥4. The large majority of accessions were immune to *Pst*, 96 of 127 (76%), with 13 of 127 (10%) being resistant, and 14 of 127 (11%) having an intermediate response and 4 of 127 (3%) having a susceptible response. *Psb* was almost split down the middle, with 21 of 128 (16%) and 45 of 128 (35%) being either immune or resistant, while 42 of 128 (33%) and 20 of 128 (16%) were of intermediate type response or susceptible. However, of the wild *Hordeum* accessions tested, 10 had scores ≥4 when tested against *Psb*, while only 6 had scores ≥4 against *Psh*.

Table 4 Host range table showing the different species or sections tested and there response to *Psb*, *Pst* and *Psh*

Genus	Species/section	No. of acc [b]	*Psb* [a]				*Pst* [a]				*Psh* [a]			
			I	R	M	S	I	R	M	S	I	R	M	S
Lolium	*perenne*	1	1				1				1			
	multiflorum Lam.	1	1				1				1			
	Total *Lolium*	**2**	**2**				**2**				**2**			
Avena	*sativa*	2	1	1			1	1			2			
	Total *Avena*	**2**	**1**	**1**			**1**	**1**			**2**			
Secale	*cereale*	1		1			1				1			
	Total *Secale*	**1**		**1**			**1**				**1**			
Agropyron	*repens*	2	1		1		1	1			1	1		
	Total *Agropyron*	**2**	**1**		**1**		**1**	**1**			**1**	**1**		
Aegilops	*columnaris*	2		1	1		2				1		1	
	kotschyi	1			1			1			1			
	peregrina	4		4			4				2	2		
	speltoides	7	3	4			2	4		1	4	3		
	Total *Aegilops*	**14**	**3**	**9**	**2**		**6**	**6**	**1**	**1**	**7**	**6**	**1**	
Triticum	*aestivum* *	39	11	25	3			13	15	10	19	20		
	turgidum **	13	1	12				8	4		2	10		
	Total *Triticum*	**53**	**12**	**37**	**3**			**21**	**19**	**10**	**21**	**30**		
Hordeum	*bulbosum*	1			1			1			1			
	chilense	1			1			1			1			
	jubatum	3	1		2		2	1			2		1	
	lechleri	1			1		1					1		
	murinum	2		1	1		1		1		1			1
	parodii	1			1			1			1			
	procerum	1			1				1					1
	secalinum	1			1				1			1		
	stenostachys	1			1		1					1		
	vulgare [c]	118	20	44	32	20	93	10	8	4	7	6	29	73
	Total *Hordeum*	**130**	**21**	**45**	**42**	**20**	**96**	**13**	**14**	**4**	**9**	**10**	**33**	**75**
Bromus [d]	Bromopsis (3 species)	8	7	1			7	1			5	3		
	Bromus (14 species)	32	9	12	11		16	12	4		23	7	1	1
	Ceratochloa (1 species)	1				1	1						1	
	Genea (7 species)	32	3	14	15		5	21	6		5	17	9	1
	Total *Bromus* (25 species)	**73**	**19**	**27**	**26**	**1**	**28**	**35**	**10**		**33**	**27**	**11**	**2**

[a] *Psb* – *P. striiformis* f.sp. *bromi*, *Pst* – *P. striiformis* f.sp. *tritici*, *Psh* – *P. striiformis* f.sp. *hordei*. [b] acc is an abbreviation for accessions. [c] data from the host status experiment was included using transformed data. [d] sections defined as in Saarela *et al.* 2007 * one accession missing from *Pst*. ** one accession missing form *Psh*. ***I – immune; R – resistant; M – intermediate; S – susceptible.

2.2.5 *Bromus* accessions

In the *Bromus* sections 27 of 73 (37%) accessions had scores of ≥4 to *Psb*, while only 10 of 73 (14%) and 13 of 73 (18%) had scores of ≥4 to either *Pst* or *Psh* respectively. However, two accessions were fully susceptible to *Psh* whilst only one was fully susceptible to *Psb*.

2.3 Discussion

2.3.1 Temperature settings

Literature suggests that yellow stripe rust inoculations should be carried out at 10°C in order to achieve successful infection (Chen & Line, 1992; Pahalawatta & Chen, 2005; Castro *et al.* 2003; Chen *et al.* 1995). However, during this set of experiments it was observed that in fact the rusts germinated better at warmer conditions (16°C). Despite this, varying levels of spore germination were observed in the different experiments but the cause of this is unclear. It may be due to inoculations being carried out at different times, and because spores were left in a desiccator and were used anywhere from 1 day post collection until 25 days post collection, they may have become non-viable at the later inoculations.

2.3.2 *Lolium*, *Avena*, *Secale* and *Agropyron* accessions

As a genus only *Lolium* exhibited complete immunity to *Psb*, *Pst* and *Psh* and therefore is presumed to be a non-host to all three ff.spp. Zadoks (1961) also tested *Lolium multiflorum* and *L. perenne* and he tested different ff.spp. of yellow stripe rust and observed no response, and hence presumed immunity.

Although the *Secale* accession showed a resistant response to *Psb*, *Pst* and *Psh*, the resistances observed were all scores of 1 (Appendix 1). Therefore no trace sporulation was observed and thus *Secale* is also presumed to be a non-host to all three ff.spp. According to the USDA *Secale cereale* is not a host for *P. striiformis*, however, the American Phytopathology Society (APS) mention that *P. striiformis* is a disease of *S. cereale* but they do not mention which form of the rust is responsible for this response (APS, 1993; USDA, 2008b). Therefore, as further details are not available *Secale* is still presumed to be a non-host.

Although the *Avena* accessions are all either immune or resistant to *Psb*, *Pst* and *Psh*, the one accession that showed the resistant response to *Psb* did score a 3, and therefore trace

sporulation was observed. Based on previous definitions provided, *Avena* may be considered to be a potential marginal host of *Psb* (Jafary *et al.*, 2008; Niks, 1987). In addition both the USDA and APS mention that stripe rust does not occur on *Avena* (APS, 1993; USDA, 2008b). As *Psb* was only recently isolated, it is not surprising then that only *Psb* and not *Pst* or *Psh* were able to attack *Avena*.

Of the resistances observed in the *Agropyron* accessions, these were all scores of 3. Therefore, *Agropyron* is scored descriptively as resistant, however, trace sporulation was observed to all three ff.spp., therefore lending itself to be classified as a potential marginal host to the three ff.spp.. Zadoks (1961) noted that there were a few exceptions in the *Agropyron* genus that were susceptible to *Pst*, however, he did find that none of the isolates of *Psh* that he tested were successful at attacking *Agropyron repens*. Therefore this study agrees with his *Pst* findings, and promotes the idea that the *Psh* isolate tested in this study is different to the one used by Zadoks (1961), and thus with a new isolate being used *Agropyron* may now potentially be a marginal host.

2.3.3 *Aegilops* and *Triticum* accessions

In the *Aegilops* genus there were scores of ≥ 4 observed for *Psb*, *Pst* and *Psh*, however immunity, resistant and intermediate responses were observed. Therefore, *Aegilops* appears to be a marginal host for all three ff.spp. Moreover, *Aegilops* seems to be a better and more frequent host of *Pst* than either *Psb* or *Psh*. This is interesting as authors have described *Aegilops* and *Triticum* to be phylogenetically related, thus consequently it seems intuitive that *Pst* would be more successful than either *Psb* or *Psh* as is the case in this study (Sasanuma *et al.*, 1996; Tsunewaki *et al.*, 1976). In addition, *Psb* is more successful than *Psh*, and this is not surprising either as *Psb* was isolated off wild grass, and *Aegilops* is wild-goat grass, thus it is expected that *Psb* is more adapted to attacking wild grasses than *Psh* (California Department of Food and Agriculture, 2009; TheFreeDictionary.com, 2009).

Only two species of *Triticum* were tested; *T. aestivum* (bread wheat - hexaploid) and *T. turgidum* (durum wheat - tetraploid). All of the *Triticum* accessions showed either an immune or resistant type response to *Psh*. However, five of the accessions (four bread and one durum wheat – for details refer to appendix 1) did score a 3, and therefore *Triticum* may be a potential marginal host for *Psh*, as a score of 3 although classed as descriptively resistant does

have trace sporulation. This is in accordance with what has been reported in literature, as it has been observed that some *Triticum* accessions can be successfully attacked by *Psh* (Chen *et al.*, 1995; Pahalawatta & Chen, 2005). Of the specific cultivars mentioned by Chen *et al.* (1995), Chinese-166 gave an intermediate to susceptible response, whist this study only observed trace sporulation. The other cultivar, Morocco, was reported to have an intermediate or very susceptible response, and in this study Morocco was immune to the *Psh* isolate tested. This may indicate that the isolate used by Chen *et al.* (1995) was different to the isolate used in this study. Of the accessions inoculated with *Psb* that show a resistant response, seven bread and two durum wheats scored 3 (for details refer to appendix 1). Therefore, the majority of the wheats show an immune or resistant response, however, as accessions showed an intermediate response *Triticum* is presumed to be a marginal host for *Psb*. Of those accessions described as resistant to *Pst*, only one bread and three durum wheats scored lower than 3 (for details refer to appendix 1). Therefore, *Triticum* is not only a host for *Pst* but also *Pst* is more successful in attacking the genus *Triticum* than either *Psb* or *Psh*. This is what is expected as *Triticum* is a known host for *Pst*.

In addition to this, *Triticum* and *Aegilops* are phylogenetically related as mentioned above, and the results almost match. The only considerable difference is that there is immunity observed in several *Aegilops* accessions when inoculated with *Pst*, whereas there is no immunity observed in the *Triticum* accessions. It could be hypothesized that the isolate of *Pst* used in this study, through ages of selective pressure, may have become more adapted and better suited to attack cultivated wheat than *Aegilops*.

2.3.4 *Hordeum* accessions

As 86% of the *Hordeum* accessions exhibited scores of ≤3 while 14% scores of ≥4, when inoculated with *Pst*, *Hordeum* is presumed to be a marginal host for *Pst*. Much like the results for *Triticum* accessions inoculated with *Psh*, this is not a surprising result, as some *Hordeum* accessions have been noted in literature to be susceptible to *Pst* (Pahalawatta & Chen, 2005); these specific accessions will be explained in further detail in chapter 3. When inoculated with *Psb* 52% of the *Hordeum* accessions had scores of ≥4, and therefore *Hordeum* is proposed to be a marginal host for *Psb*. When inoculated with *Psh* 91% of the *Hordeum* accessions had scores of ≥4. This is expected as barley is a host for *Psh*. However, what is apparent and interesting is that of the wild *Hordeum* accessions tested, 83% show a host-type response to *Psb*, whilst only 58% show this same response to *Pst* and an even fewer, 50%, of

the wild *Hordeum* accessions tested showed a host-type response to *Psh*. The reason for this may again be hypothesized that, as *Pst* was proposed to be more adapted to wheat cultivars, the *Psh* isolate used may have become adapted to attacking barley cultivars, and in essence may have lost the ability to successfully attack wild accessions of the same genus.

2.3.5 *Bromus* accessions

The *Bromus* genus has been analysed according to the taxonomic sections tested. As *Bromus* taxonomic classification is still a debated subject today, sections are as defined by Saarela *et al.* (2007). It was initially expected that the *Bromus* accessions would be more susceptible to *Psb* than to either *Pst* or *Psh*, and this was the case. *Bromus* had 59% of the accessions with a score ≥4, and is presumed to be a marginal host for *Psb*. While none of the accessions showed full susceptibility to *Pst* or *Psh*, there are still intermediate responses and therefore *Bromus* is presumed to be a marginal host for *Pst* and *Psh* as well.

2.4 Conclusion

The importance of inoculating plants at low temperatures seems to be not as important as outlined in literature.

Lolium and *Secale* are presumed to be non-host to all three ff.spp.. *Avena* is presumed to be a marginal host for only *Psb* and nonhost for *Pst* and *Psh*. *Agropyron* and *Aegilops* are marginal hosts to all three ff.spp.. *Triticum* is a host for *Pst* and a marginal host for *Psb* and *Psh*, while *Hordeum* is a host for *Psh* and a marginal host for *Psb* and *Pst,* and *Bromus* is a host for *Psb* and a marginal host for *Pst* and *Psh*. In addition, the sections of Bromus differ in their response to the different ff.spp. with the most susceptible being Genea, then Bromus, then Ceratochloa and then Bromopsis. Also *Psb* seems to be more efficient at infecting the wild accessions of all genera tested, whilst *Pst* and *Psh* are more efficient at infecting their respective cultivated species. Therefore, it seems that *Psb* is more versatile than either *Pst* or *Psh*, as it is more successful on wild accessions and is also successful on cultivated accessions. The versatility of *Psb* may be important in an epidemiological way, as then there are a variety of genera that can be alternate hosts for this rust, and if this f.sp. can cause epidemics on

cultivars of the *Hordeum* and *Triticum* genera, then this rust may be seen as an economically important pathogen.

However it is important to note that due to the limited number of accessions tested in this host range study, no one species, or one genus, can be conclusively determined as a host or a non-host. Therefore these tests merely aid in indicating a potential host range, but more importantly these tests help in indicating the versatility of the pathogens in relation to one another.

Chapter 3: Determination of the host status of barley accessions to *P. striiformis* f.sp. *bromi*, *P. striiformis* f.sp. *tritici* and *P. striiformis* f.sp. *hordei*.

3.1 Materials and Methods

3.1.1 Seedling stage testing

a. Plant material

The various barley accessions mentioned in Appendix 2 were subjected to an infection experiment. In this experiment 118 barley accessions were tested to determine their susceptibility or resistance level to the three yellow stripe rust isolates, in order to determine the host status of barley. The accessions were selected using a previous study (Alemu, 2008; Atienza *et al.*, 2004) as well as some others from literature and other thesis work that were determined as interesting; namely Calicuchima (Castro *et al.*, 2003; Sandoval-Islas *et al.*, 2002), Mazurka (Chen & Line, 2002; Senden, 1993), Berac, Delibes and Golden Promise (Rodrigues *et al.*, 2004). Moreover, included in these 118 accessions were the various parents of the mapping populations. This was to not only aid in determining the host status but also in selecting the populations to be used for the later QTL mapping experiments.

Accessions were planted in 38 cm × 58 cm planting boxes, except for the parents of the mapping populations which were planted in the smaller 36 cm × 44 cm planting boxes. Again susceptible reference lines were included in all boxes; the experimental line SusPtrit for those to be inoculated with either *Psb* and *Psh*, and wheat cultivar Michigan Amber for those to be inoculated with *Pst*.

b. Inoculation and incubation

Inoculation was performed using a midpoint inoculation method. For this method the leaves of the seedlings, in the first leaf stage, were laid flat on the soil surface with the adaxial surface facing upwards. U-shaped pins were used to hold the leaves in this position. A black permanent marker pen was then used to place a single dot on the leaf surface more-or-less two thirds of the way up the leaf measured from the bottom to the tip of the leaf. Urediospores were applied as a stripe across the width of the leaf, where the dot had been placed, using a fine paint brush and a mixture of 5 mg of urediospores mixed with lycopodium powder (+/- 1:20 v/v).

The purpose behind using this method is due to the nature of the rust, in the sense that one spore of this rust can produce multiple pustules, not to mention that on occasion there can be pustule fusion between two or more pustules. Therefore, this makes direct calculation of the latency period (LP – time at which 50% of mature pustules have developed) and infection frequency (IF – number of pustules per square centimetre), as done for other heterologous rusts, almost impossible. However, with the employment of this method, it was assumed that one can indicate the LP and IF by comparison to the reference lines within the same inoculated box. However, the definition of IF would be altered to the number of pustules per composite lesion length as the whole leaf was measured and not just a set area.

Incubation was performed as in the previous study on the host range.

c. Evaluation

Evaluation was performed differently from before as the use of the scale outlined by McNeal *et al.* (1971) is not suitable for this inoculation method. Here the number of pustules, the length of the composite lesion (measured in millimetres) and the presence/absence of chlorosis and/or necrosis were counted, measured and/or observed, respectively. For simplicity a 0-5 scale was created to see differences. Those accessions showing either a 0 or 1 score can be seen as resistant, and those with a 2 to 5 score as susceptible (Table 5).

Table 5 Numerical 0-to-5 and descriptive scale for the assessment of resistances and susceptibilities of accessions tested in the host status

Score	Pustules	Description
0	0	Resistant
1	0 with flecks	Resistant
2	3 to 20	Susceptible
3	21 to 100	Susceptible
4	101 to 200	Susceptible
5	≥ 201	Susceptible

3.1.2 Adult plant testing

This test was performed as some plants that are susceptible at the seedling stage may present resistance in the adult plant stage; known as adult plant resistance.

a. Plant material

Based on the seedling test results in 4.1, those accessions that showed the most susceptible reaction were selected and tested for the level of resistance or susceptibility in the adult plant phase (Appendix 3).

Plants were transplanted from there boxes into pots. Plants were cropped just below the first leaf, and allowed to grow until the flag leaf emerged.

b. Inoculations, incubations and evaluation

Once the flag leaf emerged, inoculations were carried out. Inoculations were done using a powder blower; incubations were carried out as previously described in the seedling test. Evaluation was based on amount of pustules present and the composite lesion length. Plants were then classified as showing a susceptible response or a resistant response as per prior definition given in the introduction.

3.2 Results

3.2.1 Susceptibility across all barley accessions at seedling stage

Susceptibilities were seen across accessions for *Psb*, *Pst* and *Psh* (Table 6). Only 12% (additive percentages of scores 2 to 5 – table 6) of the cultivars were susceptible to *Pst*, 47% to *Psb* and a much larger 91% to *Psh*. Of all the accessions only 2% showed the most susceptible reaction to *Pst*, only 5% this same reaction to *Psb* and 25% to *Psh*. For *Pst* the exceptional lines showing full susceptibility Dom and SusPtrit (Appendix 2). For *Psb* the exceptional lines showing full susceptibility Freya Jerusalem, Jerusalem II, Dom, Morex, Steptoe and SusPtrit (Appendix 2). There is also a significant proportion of accessions that have an intermediate type response to *Psb* and *Psh* and a lower proportion to *Pst*; 42%, 65% and 9% respectively. The highest proportion of immunity is seen in those accessions inoculated with *Pst* (Figure 1). What is noteworthy is that the reference lines did not always show the most susceptible reaction.

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Only the research line SusPtrit showed the most susceptible response to all three ff.spp. Nevertheless, there were some exceptional susceptibilities seen in some accessions as seen in Figure 2.

Table 6 Susceptibility of all barley accessions using a 0-5 scale in relation to respective rusts *Psb*, *Pst* and *Psh* and their respective reference lines; SusPtrit (*Psb* and *Psh*) and Michigan Amber (*Pst*). Values are given in percentages.

Lines	*Psb*					
	0	**1**	**2**	**3**	**4**	**5**
Barley accessions	17	36	11	19	12	5
SusPtrit	0	0	0	0	0	100
	Pst					
Barley accessions	79	9	2	6	2	2
Michigan Amber	0	0	0	18	27	55
	Psh					
Barley accessions	6	3	5	24	37	25
SusPtrit	0	0	0	0	20	80

Percentages are calculated as the number of accessions exhibiting the particular score value, divided by the number of barley accessions (n=118), multiplied by 100.

Of the lines that were included from literature, Mazurka showed an immune response to *Pst*, where literature has shown this line to be either susceptible or resistant (Table 7). Furthermore, Mazurka's response to *Psh* was an intermediate susceptibility, where literature describes either resistance or very susceptible responses to this f.sp..Berac, Golden Promise and Delibes all showed the same responses as outlined in literature, namely very susceptible, very susceptible and resistant respectively (Table 7). Calicuchima has been described as resistant to *Psh* in literature; however, in this study Calicuchima exhibited a susceptible response (Table 7).

Table 7 Barley lines of interest taken from literature; indicating descriptions outlined in literature as well as the noted response in this study

Barley line	Isolate	Literature description*	Author/s	Response*
Mazurka	*Pst*	S and R	Senden (1993)	I
	Psh	R (4 races); VS (1 race)	Chen & Line (2002)	M
Berac	*Psh*	VS	Rodrigues *et al.* (2004)	VS
Golden Promise	*Psh*	VS	Rodrigues *et al.* (2004)	VS
Delibes	*Psh*	R	Rodrigues *et al.* (2004)	R
Calicuchima	*Psh*	R	Castro *et al.* (2003)	S

*R – resistant; M – intermediate; S – susceptible; VS – very susceptible
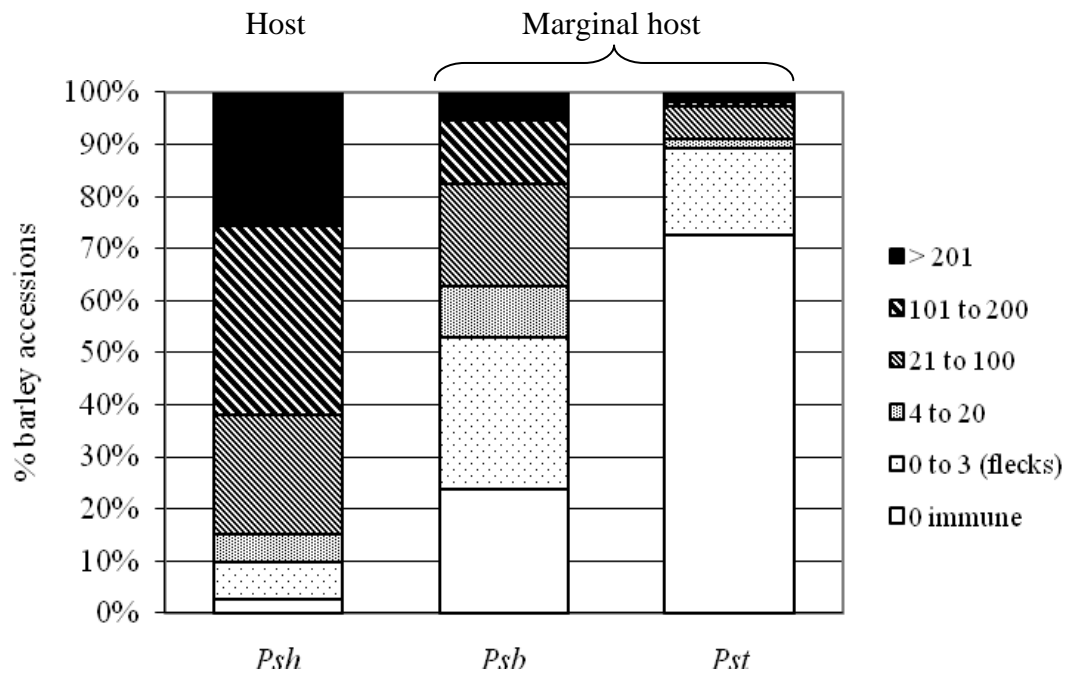
Figure 1 Host status of barley to *Psh*, *Psb* and *Pst*. Percentage of accessions (n=113) showing specific amount of pustules.
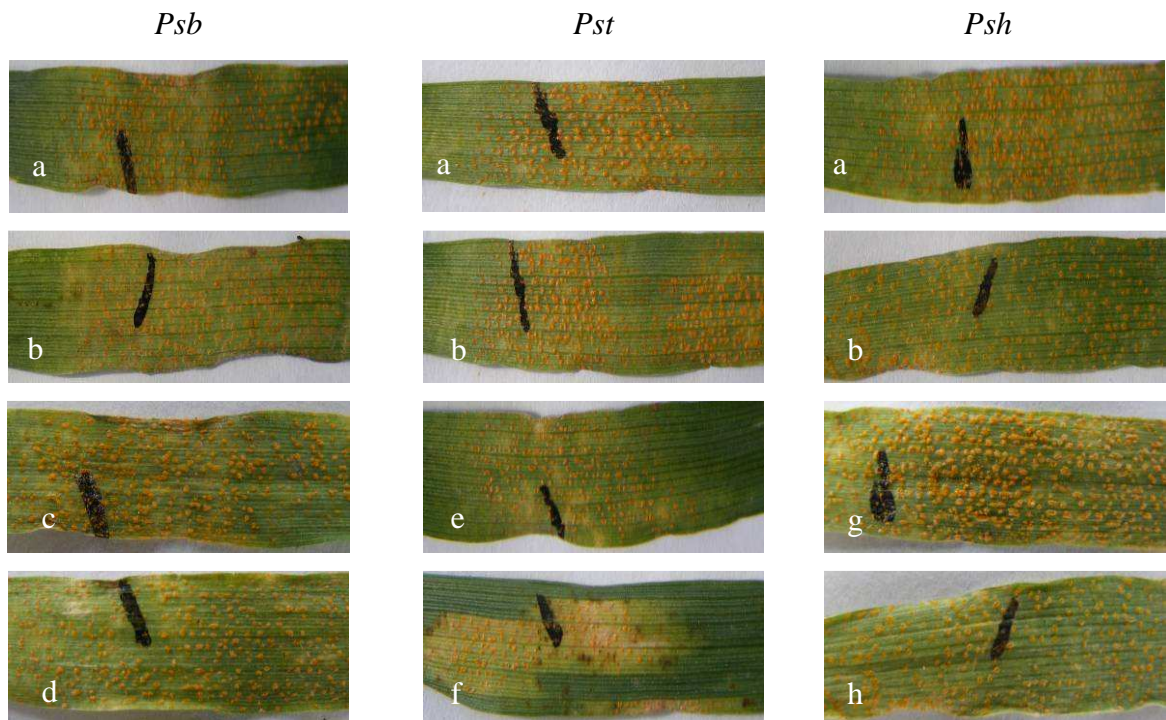
Figure 2 Barley cultivar lines of exceptional susceptibility to *Psb*, *Pst* and *Psh*. a – SusPtrit; b – Dom; c – Freya Jerusalem; d – Jerusalem II; e – Calicuchima (RphX); f – Speciale; g – C118; h – Egypt IV.

3.2.2 Susceptibility based level of agronomic application

For all accessions tested the landraces showed the most resistance to *Psb* and *Psh* (table 8), while the research lines were the most susceptible to *Pst* (table 8). The wild species, *H. spontaneum*, always had susceptible accessions and were the most susceptible to *Psh*, in comparison to *Psb* and *Pst* (table 8). The cultivars ranged from immune type responses through to the most susceptible reaction noted (table 8). There is no overlap in resistances or susceptibilities seen in the level of agronomic application when comparing all three ff.spp..

Table 8 Susceptibility of accessions using a 0-5 scale[a] to *Psb*, *Pst* and *Psh* in relation to the level of agronomic application; numbers are in percentages.

Level of agronomic application	No. of acc.[b]	*Psb* 0	1	2	3	4	5
Wild species (*H. spontaneum*)	4	0	0	0	75	25	0
Research lines	8	0	13	13	50	0	25
Cultivars	81	16	38	12	19	10	5
Landraces	24	29	38	8	4	21	0
Unknown	1	0	100	0	0	0	0
		Pst					
Wild species (*H. spontaneum*)	4	50	25	0	25	0	0
Research lines	8	50	13	0	13	0	25
Cultivars	80	86	6	4	4	0	0
Landraces	25	72	12	0	8	8	0
Unknown	0	0	0	0	0	0	0
		Psh					
Wild species (*H. spontaneum*)	3	0	0	0	0	67	33
Research lines	8	0	13	0	0	25	63
Cultivars	80	8	0	6	24	38	25
Landraces	26	4	12	4	35	35	12
Unknown	0	0	0	0	0	0	0

[a] 0-5 scale: 0 – Immune; 1 – 0 pustules with flecks to 3 pustules; 2 – 4 to 20 pustules; 3 – 21 to 100 pustules; 4 – 101 to 200 pustules; 5 – >200 pustules. [b] acc. is an abbreviation for accession.

3.2.3 Susceptibility based on origin of accessions

For all three ff.spp., the North American accessions are the most susceptible and then the Asian, then South American, then European and then the most resistant are the African accessions (table 9). For *Psb* and *Pst* all African accessions were resistant, whilst the other

accessions from other origins showed a range from an immune type response to the most susceptible response. Although the African accessions showed the highest amount of resistance, in comparison to other origins, to *Psh*, there is still some susceptibility observed, and there is also no resistance seen in the North American and Asian accessions.

Table 9 Susceptibility of accessions using a 0-5 scale[a] to *Psb*, *Pst* and *Psh* in relation to the origin of the accessions tested; numbers are in percentages.

Origin [b]	No. of acc. [c]	*Psb*					
		0	1	2	3	4	5
North America	10	10	10	0	20	30	30
Asia	9	11	33	0	11	44	0
South America	14	29	29	14	7	21	0
Europe	66	17	41	14	20	5	5
Africa	9	33	67	0	0	0	0
Unknown	6	0	17	33	50	17	0
		Pst					
North America	9	33	22	0	22	11	11
Asia	10	70	10	0	10	10	0
South America	14	79	7	0	14	0	0
Europe	66	88	6	3	2	0	2
Africa	9	89	11	0	0	0	0
Unknown	6	67	0	17	17	0	0
		Psh					
North America	9	0	0	11	22	33	33
Asia	11	0	0	9	45	36	9
South America	14	7	0	7	21	57	7
Europe	66	8	2	5	23	33	30
Africa	9	11	33	0	33	22	0
Unknown	5	0	0	0	0	20	80

[a] 0-5 scale: 0 – Immune; 1 – 0 pustules with flecks to 3 pustules; 2 – 4 to 20 pustules; 3 – 21 to 100 pustules; 4 – 101 to 200 pustules; 5 – >200 pustules. [b] origins are ordered from most susceptible to most resistant for each f.sp., and wild species accessions (*H. spontaneum*) are excluded as this trait does not apply to them. [c] acc. is an abbreviation for accession.

3.2.4 Susceptibility based on morphological traits

Table 10 Susceptibility of accessions using a 0-5 scale [a] to *Psb*, *Pst* and *Psh* in relation to observed morphological traits; numbers are in percentages.

Morphological trait	No. of acc. [b]	*Psb*					
		0	**1**	**2**	**3**	**4**	**5**
Awned	111	17	35	11	20	13	5
Awnless	3	33	67	0	0	0	0
Unknown	4	0	25	25	25	0	25
Six Rowed	33	21	21	0	21	27	9
Two Rowed	81	16	42	15	19	6	2
Unknown	4	0	25	25	25	0	25
Covered seed	109	18	35	12	19	11	5
Naked seed	9	0	44	0	22	22	11
Black seed colour [c]	9	0	56	0	22	11	11
White seed colour [c]	105	18	34	12	17	11	5
		Pst					
Awned	110	80	9	3	5	2	1
Awnless	3	100	0	0	0	0	0
Unknown	4	50	0	0	25	0	25
Six Rowed	34	65	12	6	9	6	3
Two Rowed	79	87	8	1	4	0	0
Unknown	4	50	0	0	25	0	25
Covered seed	108	81	9	3	6	1	1
Naked seed	9	67	0	0	11	11	11
Black seed colour [c]	8	50	38	0	0	0	13
White seed colour [c]	105	80	6	2	6	2	1
		Psh					
Awned	110	6	3	5	24	37	25
Awnless	3	0	33	0	33	33	0
Unknown	4	0	0	0	25	25	50
Six Rowed	35	0	3	6	37	34	20
Two Rowed	78	9	4	5	19	38	26
Unknown	4	0	0	0	25	25	50
Covered seed	107	6	3	6	23	38	24
Naked seed	10	10	10	0	30	20	30
Black seed colour [c]	8	13	25	0	13	25	25
White seed colour [c]	106	6	2	6	25	36	24

[a] 0-5 scale: 0 – Immune; 1 – 0 pustules with flecks to 3 pustules; 2 – 4 to 20 pustules; 3 – 21 to 100 pustules; 4 – 101 to 200 pustules; 5 – >200 pustules. [b] acc. is an abbreviation for accession. [c] wild species accessions (*H. spontaneum*) are excluded as this trait does not apply to them.

The results of susceptibilities and resistances in terms of morphological traits merely suggest differences that awned accessions are more susceptible than awnless accessions; six rowed accessions are more susceptible than two rowed accessions; accessions with naked seed seem to be more susceptible than those with covered seed except when inoculated with *Psh*, and that accessions with white seed tend to be more susceptible than those with black seed (table 10). Therefore, if there were to be clear morphological traits associated with susceptibility for example, the most susceptible accessions to *Psb* and *Pst* would be accessions that are awned, six rowed and have naked white seed. However, there are large differences observed when assessing individual accessions. Such an example is Kobakintagi that has an average pustule number of 111 when inoculated with *Psb*, but shows an immune type response to *Pst*, whilst SusPtrit has average pustule numbers of 247 and 203 when inoculated with *Psb* or *Pst* respectively (Appendix 2), and both fit the previous description. In addition, the most susceptible accession to *Psh* should be awned, six rowed and have covered white seed, if there was an association. However, yet again a large variation is observed, for example accession Calicuchima (RphX) has an average pustule number of 184, whilst L98 has an average of 0 pustules; and both these accessions fit the above description.

3.2.5 Susceptibility comparison with all categories: Level of agronomic application, origin and morphological traits

Asian landraces that are six-rowed, awned and have white covered seed showed both the most extreme resistance (0(I)-to-5 pustules) and the most susceptible response (≥200 pustules) when inoculated with *Psb* (table 11). In addition, this is also seen when accessions with the same description are inoculated with *Pst*, however, in these Asian landraces there is no shared seed type characteristic. Two-rowed awned European accessions exhibit resistance to *Psb* and *Psh*, however, there are some two-rowed awned European accessions that exhibit a susceptible response the *Psb* and *Psh*. The North American six-rowed cultivars showed a range of resistant and susceptible responses to all three ff.spp. These cultivars are typically susceptible to *Psh* and *Psb*, while resistant and susceptible to *Pst*. The African landraces were resistant to *Psh*, and did not show extreme susceptibilities or resistances to either *Psb* or *Pst*. Although there are extreme resistances and susceptibilities observed in South American accessions, the only characteristic that they share is awn-type and seed colour.

Table 11 Resistance and susceptibility responses of accessions based on shared characteristics: Origin, Type, Awn type, Seed colour and Seed type; only accessions with extreme responses are considered for analysis.

Isolate	Response	Origin	Type	Spike row	Awn type	Seed colour	Seed type
Psb	R	Asia	Landrace	Six-row	Awned	White	Covered
	R	Europe	-	Two-row	Awned	-	Covered
	R	South America	-	-	Awned	White	Covered
	S	Asia*	Landrace	Six-row	Awned	White	Covered
	S	Europe	-	-	Awned	White	-
	S	North America	-	Six-row	Awned	White	Covered
Pst	R	Asia	Landrace	Six-row	Awned	White	-
	R	Europe	-	-	Awned	-	Covered
	R	North America	Cultivar	Six-row	Awned	White	Covered
	R	South America	-	-	Awned	White	Covered
	S	Asia	Landrace	Six-row	Awned	White	Naked
	S	Europe	Res. Line	Six-row	Awned	White	Naked
	S	North America	-	Six-row	Awned	-	-
	S	South America	Cultivar	-	Awned	White	Covered
Psh	R	Africa	Landrace	-	-	-	-
	R	Europe	-	Two-row	Awned	-	Covered
	R	South America*	Cultivar	Two-row	Awned	White	Covered
	S	Asia*	Landrace	Six-row	Awned	White	Naked
	S	Europe	-	-	Awned	-	-
	S	South America*	Landrace	Six-row	Awned	White	Covered
	S	North America	-	Six-row	Awned	White	-

*Data represented by one accession only. **(-): indicates no shared characteristic. *** R: ≥200 pustules for *Psb*; ≥30 pustules for *Pst*, ≥200 pustules for *Psh*. S: 0 (I)-5 pustules for *Psb*, *Pst* and *Psh*.

3.2.6 Comparison of seedling stage and adult stage susceptibility

Of those accessions that showed an exceptional level of susceptibility in the seedling stage and were then selected for adult plant testing (appendix 3), most attained a considerable level of resistance in the adult plant stage. However, there are some accessions that remained susceptible (table 12). The accessions inoculated with *Psb* remained more-or-less consistent in the average amount of pustules whether in seedling or adult stage, whereas Speciale and Dom had a reduced number of pustules. Dom was the only accession to remain susceptible to *Pst* in adult plant stage, but with a much reduced average number of pustules. The accessions inoculated with *Psh* all showed a reduced average number of pustules in the adult plant stage, except for accession Trigo Biasa which showed an increase in susceptibility.

Table 12 Comparison of the susceptibility of accessions in seedling and adult stages that remained susceptible to *Psb*, *Pst* and *Psh*; lines used as seedling test reference lines are also included.

Accession	*Psb* (seedling)		*Psb* (adult)	
	avg pust.[a]	Status[c]	avg pust.[a]	Status[c]
SusPtrit (Ref)[b]	203	S	0	R
Magnif 102	153	S	150	S
Speciale	180	S	50	S
Dom	220	S	50	S

Accession	*Pst* (seedling)		*Pst* (adult)	
	avg pust.[a]	Status	avg pust.[a]	Status
Michigan Amber (Ref)[b]	500	S	300	S
Dom	253	S	50	S

Accession	*Psh* (seedling)		*Psh* (adult)	
	avg pust.[a]	Status	avg pust.[a]	Status
SusPtrit (Ref)[b]	203	S	150	S
Georgie	240	S	20	S
Union	257	S	10	S
Brage	310	S	20	S
C118	400	S	60	S
Stander	240	S	60	S
Trigo Biasa	260	S	1000	S
Vada	260	S	10	S
Steptoe	270	S	100	S

[a] avg. pust. is an abbreviation for average pustule, calculated as the mean pustule number of all plants tested. [b] (Ref) is an abbreviation for reference line. [c] R – resistant; S – susceptible.

3.3 Discussion

3.3.1 Susceptibility across all barley accessions at seedling stage

Susceptibility was found in some accessions for each of the ff.spp.. Moreover, for each of the ff.spp., the most susceptible response ($\geq$200 pustules) was observed. However, only the research line SusPtrit showed this level of susceptibility to all of the ff.spp.. From the results the highest numbers of accessions were susceptible to *Psh* followed by *Psb* with the least susceptibility to *Pst*. Based on definitions provided in literature (Atienza *et al.*, 2004; Niks & Marcel, 2009), barley is therefore a host for *Psh* and marginal host for *Psb* and *Pst* (Figure 1). This is what was expected as barley is a known host for *Psh* and some barley accessions have been noted to be susceptible to *Pst* (Pahalawatta & Chen, 2005), therefore indicating that barley should be classified as marginal host for *Pst*. Moreover, from what was observed in the host range experiments, *Psb* seemed to be more versatile than *Pst* in its ability to successfully

attack different genera, and was shown to be more successful on *Hordeum* accessions (refer to previous chapter). Therefore it was expected that *Psb* would infect more barley cultivars than *Pst*, and accordingly it was thus anticipated that barley would be a marginal host.

It is important to note, though, that there were differences observed in the response of the reference lines, as they did not always exhibit the most susceptible response. This could be looked at as a form of non-genetic variation, as observations within boxes were reasonably uniform (phenotypic observation data not included). Therefore, the differences observed are noted between boxes and could be attributed to, and explained by, different effects. A possible explanation could be that reference lines may have "escaped" spore deposition. That is, there is a possibility that the amount of spores (that came into contact with the leaf surface) was different on the separate leaves. This may be due to the midpoint inoculation technique, as with this technique one cannot ensure the quantity of spores applied will be identical on each leaf. However, neither can the other techniques, but, these other techniques do provided more of an even distribution of the spores over the box, and thereby applying a reasonably more even distribution of the amount of spores per leaf.

As mentioned in the materials and methods and results, there were certain interesting accessions that were found in the literature and these were included in this study. Literature showed barley cultivar Mazurka to have a susceptible and resistant response to a *Pst* isolate (Senden, 1993), where this study showed an immune type response. Chen & Line (2002) tested several *Psh* races and found Mazurka to be resistant to four races and quite susceptible to one of the races (they used a 0-9 scale similar to the scale outlined by McNeal (1971)). This study found Mazurka to have an intermediate response to *Psh*. A possible explanation for this observation could lie in the use of different isolates. In the sense that the isolates used by Senden and Chen & Line may have been different, as the origins of the isolates in these studies are not known.

The descriptions of Rodrigues *et al.* (2004) in terms of responses of lines Berac, Delibes and Golden Promise (tested against *Psh*), concur with the observations in this study. This may infer that these particular lines respond similarly to different races of *Psh*.

Castro *et al.* (2003) tested the Ecuadorian barley line Calicuchima against *Psh*, and inferred that at seedling stage Calicuchima is resistant by tracing two QTLs for resistance back to the

Calicuchima-sib in their Orca mapping population (a mapping population developed by crossing Calicuchima and Bowman cultivars for the assessment of *Psh* resistance). Moreover, Sandoval-Islas *et al.* (2002), note Calicuchima to have a low infection severity (average between 3 to 15% at different temperatures) at an adult plant stage, and Castro *et al.* (2003) also note adult stage resistance in Calicuchima. What is interesting is that Calicuchima seedlings tested in this study were highly susceptible to *Psh*, with an average pustule number of 184. The only possible explanation for the differences observed could be that the isolates used are different, and that Calicuchima is resistant to the North American isolates used by Castro *et al.* (2003), but not the European isolate used in this study.

3.3.2 Susceptibility based on level of agronomic application

When looking at the different agricultural application categories, the wild species (*H. spontaneum*) tend to be susceptible to all three ff.spp. despite their being resistance observed when inoculated with *Pst*. It is not surprising to note that *Psb* was more successful on the wild accessions than *Pst*, as this result was expected following the results of the host range.

The landraces tended to be the most resistant accessions to the three ff.spp., except for *Pst* where the cultivars were the most resistant. However, there is still resistance observed for landraces inoculated with *Pst*. Therefore, the landraces may provide a potential source of resistance to all three ff.spp.. Yet as a range of susceptibilities and resistances were observed within research lines, cultivars and landraces, this does potentially infer that there is large genetic diversity present in these accessions, and this variation provides breeders with resources to breed for resistance.

3.3.3 Susceptibility based on origin of accessions

The modern North American accessions were always the most susceptible and the African accessions the most resistant, with a range of responses in accessions from Asia, South America and Europe. This is not completely unexpected as the African accessions (barring one – Ribari) are all landraces. However, a study performed on heterologous rusts states that African landraces are more susceptible to heterologous rust fungi than modern European accessions (Atienza *et al.*, 2004). For example Atienza *et al.* (2004) describe L94 to have a relatively high level of susceptibility. However, in this study L94 is one of the most resistant

accessions to all three ff.spp. with average pustule numbers of 0 to *Psb*, 0 to *Pst* and 3 to *Psh* (for details see Appendix 2). Thus this indicates that the mechanisms conferring resistance to heterologous rust fungi are not the same as to those required for resistance to yellow stripe rust. Although it should also be mentioned that only one race of each isolate was tested in this study, and other races may be more successful and effective at attacking barley.

Moreover, when comparing accessions within origins a range of susceptibilities and resistances were observed (except in African accessions inoculated with either *Psb* or *Pst*), thus indicating that within origins there is a large genetic diversity present. Therefore there are different potential sources of resistance, for utilization by breeders, of which the African accessions are the most promising if breeders are to breed for resistance in barley to only yellow stripe rust. However, as the other study indicated that African landraces show susceptibility to heterologous rust fungi (Atienza *et al.*, 2004), breeders would need to consider only the introgression of the regions of interest from the African landraces into possibly modern European cultivars that harbour more resistance to heterologous rust fungi, as clearly the African landraces alone cannot provide adequate resistance to heterologous rust fungi.

3.3.4 Susceptibility based on morphological traits

There are no large discernable differences when analysing accessions in relation to morphological traits, in that it is not possible to group specific traits and conclude a possible association with either resistance or susceptibility. Therefore the results only suggest, or rather imply, that some morphological traits may be somehow associated to the observed susceptibilities or resistances. It is important to note that, in some cases the amount of accessions tested for a specific morphological trait, such as awned versus awnless, is so low that this adds to the difficulty of conclusively distinguishing whether or not specific traits may be linked to specific susceptibilities observed.

As shown and explained in results it is not always the case that the morphological traits are associated with susceptibility, with given examples of Kobakintagi and SusPtrit for *Psb* and *Pst*, as well as Calicuchima and L98 for *Psh*. These examples support the idea that the morphological traits do not provide any conclusive evidence that they may be associated with observed susceptibility. Furthermore, all morphological traits (except for the awnless trait)

exhibit the whole range of susceptibilities/resistances observed. This indicates that there is a large genetic diversity in the accessions within morphological traits.

3.3.5 Susceptibility comparison with all categories: Level of agronomic application, origin and morphological traits

Although this analysis was based on extreme resistances (0-to-5 pustules for *Psb*, *Pst* and *Psh*) and susceptibilities (≥200 pustules for *Psb*; ≥30 pustules for *Pst*, ≥200 pustules for *Psh*) of accessions, it does still provide some insight into associations of characteristics of accessions within origins in terms of their response.

In saying this there is grouping of characteristics between accessions within origins. As in the North American six-rowed awned accessions exhibit susceptibilities to *Psb* and *Psh*, which may be explained by the relatively new presence of *Psh* (Chen *et al.*, 1995) and the very new presence of *Psb*, to which the North American accessions have never been exposed to or bred for resistance to. However, there are North American six-rowed awned accessions that are resistant and others that are susceptible to *Pst*. Therefore, the potential association of specific characteristics within origins to susceptibilities seems to be present for *Psb* and *Psh*, but not for *Pst*. It is unclear as to why this is the case, however, potential reasoning could be that the ancestors of the North American accessions were never selected for resistance to these ff.spp., and thereby promoting the differences observed.

There are some observations that suggest that what is observed in those specific characteristics, which may be associated with resistance or susceptibility, are not always steadfast or exempt from one another. Such an example is the two-row European accessions that exhibit resistance to *Psb* and *Psh*, however, awned European accessions exhibit susceptibility to *Psb* and *Psh*. The reason that this is important to note is that both two-row and six-row accessions were found to be susceptible to *Psb* and *Psh*, hence this characteristic is not shared (Table 11). Therefore, no association of two-row awned European accessions with resistance to *Psb* and *Psh* can be made. The same goes for six-row awned Asian landraces tested against *Psb* and *Pst*, in that for both rusts these type of landraces exhibit both resistance and susceptibility to the ff.spp..

Another interesting observation is upon observing seed colour by itself (see 3.2.4 – Table 10), black seed is shown to be more of a characteristic of resistant accessions and white more of susceptible accessions, but in accessions that exhibit the most extreme resistance (immune to 5 pustules), they are predominantly white seeded (Table 11). This further promotes the idea that specific characteristics of accessions within origins is not steadfast, and should not be looked at as conclusive, at least not in terms of yellow stripe rust.

3.3.6 Comparison of seedling stage and adult stage susceptibility

Adult plant testing revealed that typically susceptibility in barley is growth stage dependent, however, for each of the ff.spp. there was always at least one accession that was susceptible, albeit at a reduced level, to the individual f.sp. being tested. Atienza *et al.* (2004) reported a similar phenomenon between barley and several heterologous rusts, by noting full resistance in most accessions tested but also some accessions with a fairly susceptible response. This could be attributed to the genes effective for resistance in the seedling stage, are not the same genes that are effective for resistance in the adult plant stage.

It is interesting to note that accession Trigo Biasa showed a more susceptible response in the adult plant stage. This could be an artefact of the different inoculation techniques used; as the midpoint inoculation technique used in the seedling stage does not deliver the same amount of spores as with the use of the powder blower. In any case though Trigo Biasa did exhibit a more susceptible response than the reference line SusPtrit and Trigo Biasa was one of the susceptible parents used in the development of SusPtrit (Atienza *et al.*, 2004). This then brings in to question whether or not there are multiple genes responsible for the difference in the level of susceptibility/resistance observed, and then whether or not Trigo Biasa has all the genes required for susceptibility (or rather lack of any resistance genes), while SusPtrit has accumulated genes from other accessions that have provided it with some resistance.

3.4 Conclusion

Barley is a host for *Psh* and a marginal host for *Psb* and *Pst* at the seedling stage. As the majority of accessions are susceptible to *Psh*, whilst only a varying proportion of accessions are susceptible to *Psb* and *Pst*.

It appears that landraces exhibit the most resistance to all of the ff.spp.. There also seems to be a large genetic diversity present, and therefore it becomes difficult to conclusively associate an agronomic application or origin to susceptibilities/resistances observed. In saying that though there is complete resistance observed in African accessions to *Psb* and *Pst*. In addition, no resistance is observed in Asian and North American accessions to *Psh*. This is more than likely due to the absence of the isolates used in these areas of the world. Furthermore, there appears to be a lack of association of specific characteristics, even within origins, in terms of resistances and susceptibilities observed.

It is apparent that resistance observed in barley is stage dependent, as most accessions in the adult plant stage appear to be resistant. Whether or not this is attributable to resistance genes being present is unclear, as histology of the ff.spp. tested was neither observed nor evaluated.

Chapter 4: Mapping of QTL's effective to *P. striiformis* f.sp. *bromi*, *P. striiformis* f.sp. *tritici* and major gene effective to *P. striiformis* f.sp. *hordei*, and comparison to known QTL's/genes mapped for other rusts.

4.1 Materials and Methods

4.1.1 Testing parental lines

The parental lines of the mapping population were grown, as described previously, at the same time as the barley host status test, and tested against all three ff.spp. using the midpoint inoculation technique as outlined for the seedling tests. Mapping populations were then selected based on the results of the parental lines, for which the parents were contrasting in their level of resistance. The criteria for selection were parents that exhibited a high level of resistance and those that exhibited a high level of susceptibility.

4.1.2 Phenotyping mapping populations

The Vada × SusPtrit recombinant inbred line (RIL) mapping population was selected for *Psb* and *Pst*, as Vada exhibited resistance and SusPtrit susceptibility to both ff.spp.. This mapping population has also been used to study the inheritance of resistance to heterologous rust species, and is hence of interest (Jafary *et al.*, 2006; 2008). The RILs were planted in boxes and three replications per mapping population were carried out for each ff.spp.. For each replicate, three plants represented each RIL in each box. For *Psh* the L94 × Vada RIL mapping population was selected. This selection was based on L94 exhibiting an immune response whilst Vada a susceptible response. Only one replicate was carried out, as it was believed to be all that was necessary given the time frame and confidence in the result. The growing of the seedlings was conducted as described previously, also with three plants representing each RIL in each box. These mapping populations are F_8-derived RIL populations, of which each RIL was derived after seven generations of single seed decent from 200 F_2 plants from the crosses Vada × SusPtrit and L94 × Vada, respectively. The populations were developed at the barley research unit of Wageningen University and Research Centre, the Netherlands, in the Department of Plant Breeding.

Due to the nature in which stripe rust infects plants systemically, assessments could not be carried out as described for heterologous rusts by previous studies. Owing to this the midpoint

inoculation technique was favoured. However, due to problems experienced, after the first two replicates for *Psb* and *Pst*, a second inoculation method needed to be used; namely the inoculation tower method. The primary reason for this was that there were observations of inconsistent results (large variation in number of pustules) between plants within RIL within box. It was thus thought that the inoculation tower could solve this problem.

a. Midpoint inoculation

In replicates one and two, seeds were planted and grown using the larger boxes (38 cm × 58 cm) and the method was performed by applying spores directly to a predetermined point on the leaves. This is the same inoculation and incubation procedure as described previously for the barley host status seedling tests.

b. Inoculation tower

For the third replicates of *Psb* and *Pst*, seeds were planted and grown in the smaller boxes (36 cm × 44 cm). At the first leaf stage the leaves were pinned down, with the adaxial surface facing upwards, using U-shaped pins as per the midpoint inoculation technique. Boxes were placed inside the inoculation tower and 6 mg of urediospores mixed with lycopodium powder (+/- 1:20 v/v) was applied. After application of spores, the boxes were left in tower for approximately 4 minutes to let the spores settle. Glass slides were included in the boxes such that spore germination could be checked the following day. Incubation was performed as outlined by the previous host range and host status studies.

c. Assessment

In replicates one (for *Psb*, *Pst* and *Psh*) and two (for *Psb* and *Pst*) the midpoint inoculation assessment was carried out at the time that the reference lines SusPtrit (for *Psb* and *Psh*) or Michigan Amber (for *Pst*) showed between 50 and 100 mature pustules. The RILs were assessed for the number of pustules as well as the composite lesion length (measured in millimetres as the addition of the lengths of all lesions present on a single leaf blade). In addition, the plants were accessed for the level of hypersensitivity; in the presence or absence of chlorosis and/or necrosis. The correlation coefficients, and associated significance tests, were calculated in order to determine whether or not replicates one and two, or phenotypes measured in these replicates, were significantly different from one another.

The assessment for the RILs inoculated using the inoculation tower was performed differently. As soon as a seedling of a RIL begun producing pustules, counting of the pustules of that seedling began. Counting was continued until the rust had produced 50 or more pustules on the seedling, unless pustule formation had been inhibited. Pustule formation was considered to have been stopped after three consecutive days of counting the same number of pustules on the specific seedling. This was done for every RIL up until and including 18 days post inoculation for *Psb* and 21 days post inoculation for *Pst*, as this was the point at which leaves that had shown early pustule formation begun senescing. Evaluation was performed using the rate of development of the rust (pustules per hour), the latency period (LP – days) in that the time until the first pustule was observed, LP low missing (LPLM – in using the lowest LP observed between plants within RIL and using missing data (*) for those RILs that did not develop any pustules), and a 0-9 scale (outlined in appendix 4).

4.1.3 QTL mapping and analysis

MapQTL 5.0 mapping software was used to map QTLs that showed as effective against *Psb*, *Pst* and *Psh* using the quantitative data obtained from the replicates. For all replicates carried out using the midpoint inoculation method, the quantitative data used was the number of pustules and composite lesion length from the individual replicates as well as the averages of these replicates.

For the inoculation tower a modification was necessary. It has been hypothesized that there may be different biological mechanisms responsible for the differences observed (Maliepaard, personal communication). As in pre- and post-successful infection mechanisms that control the resistance and susceptibility levels observed. Due to this, the quantitative data used to map QTLs for the third replicates of *Psb* and *Pst* was the LP (which I have defined as the number of hours before pustulation was first observed), as well as the rate of development (measured as the number of hours it took for a RIL to develop pustules from the time that pustulation began until counting was stopped for that RIL; each RIL value was an average of the seedlings per RIL), and the LPLM (described in 4.1.2).

The locus and map files were obtained from the Barley research unit of Wageningen University, Department of Plant Breeding. Interval mapping was performed, setting the

threshold LOD value at 3. Automatic cofactor selection was performed to give a suggestion on the most likely marker to be found in the region of peaks observed, such that these were then used as cofactors for Multiple QTL Mapping (MQM). In order to determine consistency of peaks, a trial and error method was used with the suggested cofactors. After which Restricted Multiple QTL Mapping (rMQM) was performed using those cofactors that showed as consistent peaks above or near the threshold value. These QTLs were then incorporated into the genetic maps of the populations.

A correlations test between replicates 1 and 2 (for both *Psb* and *Pst*) was performed in order to check the reliability of data obtained from the replicates. The purpose of this is twofold; firstly it is used to determine that the conclusions of the replicates are reliable in the sense that replicates can be compared, and secondly if the replicates correlated well then the average across the replicates could be calculated and an additional QTL mapping procedure could be performed. The correlations test was performed treating the number of pustules and the composite lesion length (mm) as separate entities, and then also checking the correlation between the number of pustules and composite lesion length.

Moreover, a correlations test was required for the third replicate of *Psb*. As many seedlings varied in leaf size within RIL and between RILs. The smaller leaves cannot accommodate as much inoculum, number of pustules or composite lesion length, in comparison to the larger leaves; therefore it is important to calculate and assess if the size of the leaves influences the susceptibility response.

The QTLs found were compared to other QTLs that have been found to be effective to heterologous rusts. This was achieved by incorporating the QTLs into the high density consensus barley linkage map (Jafary *et al.* 2008; Marcel *et al.* 2007).

4.2 Results

4.2.1 Parental line analysis

There was a large amount of segregation observed in the parental lines (Table 13). The most susceptible accessions to *Psb* were SusPtrit, Steptoe, Morex, Dom and Henni, while the most resistant to were Gei, Cebada capa, L94, Vada and Tremois. There were only three parents

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susceptible to *Pst* with the most susceptible being Dom, then SusPtrit and then Rec. Whilst the rest were either immune or showed a low level of hypersensitivity. None of the parents on average were immune to *Psh*. However, several individual plants of accession L94 did show an immune type response, and of the plants that developed pustules a necrosis surrounding the pustules was observed (data not presented). L94 was the most resistant to *Psh*, while Henni, Steptoe, Vada, Nure, SusPtrit, Gunhild and C123 were the most susceptible (table 13).

Table 13 Response of the parental lines of the mapping populations tested against *Psb*, *Pst* and *Psh* in relation to the average number of pustules (NPUS)[a]

MP [b] parents	*Psb* (NPUS)	MP [b] parents	*Pst* (NPUS)	MP [b] parents	*Psh* (NPUS)
SusPtrit	**247**	Dom	253	Henni	310
Steptoe	223	**SusPtrit**	**203**	Steptoe	270
Morex	220	Rec	73	**Vada**	**260**
Dom	220	L94	0	Nure	247
Henni	200	Steptoe	0	SusPtrit	238
Gunhild	99	116-5	0 (I)	Gunhild	225
C123	97	C123	0 (I)	C123	217
Rec	71	Cebada capa	0 (I)	Rec	207
116-5	64	Gei	0 (I)	Gei	200
Nure	42	Gunhild	0 (I)	Morex	195
Meltan	4	Henni	0 (I)	116-5	172
Tremois	1	Meltan	0 (I)	Dom	168
Vada	**1**	Morex	0 (I)	Cebada capa	77
L94	0	Nure	0 (I)	Meltan	65
Cebada capa	0 (I)	Tremois	0 (I)	Tremois	31
Gei	0 (I)	**Vada**	**0 (I)**	**L94**	**3**

[a] NPUS is the average number of pustules calculated as the mean pustule count of the plants counted that represented the accession, where 0(I) indicates an immune type response. [b] MP is an abbreviation for mapping population. *Accessions highlighted in bold were the parents responsible for the selection of the mapping populations.

4.2.2 Segregation of the mapping populations

The Vada × SusPtrit mapping population was selected, as previously mentioned, to test against *Psb* and *Pst*. Vada exhibited a very low number of pustules, while SusPtrit a very high number of pustules, and there was no transgressive segregation observed in the mapping populations (figure 3). Similar histograms, as in figure 3, were constructed for the third replicates of *Psb* and *Pst*, however the segregation pattern was extremely similar; therefore data were not included. There was, however, segregation observed with many plants exhibiting intermediate responses as well as the parental line response.
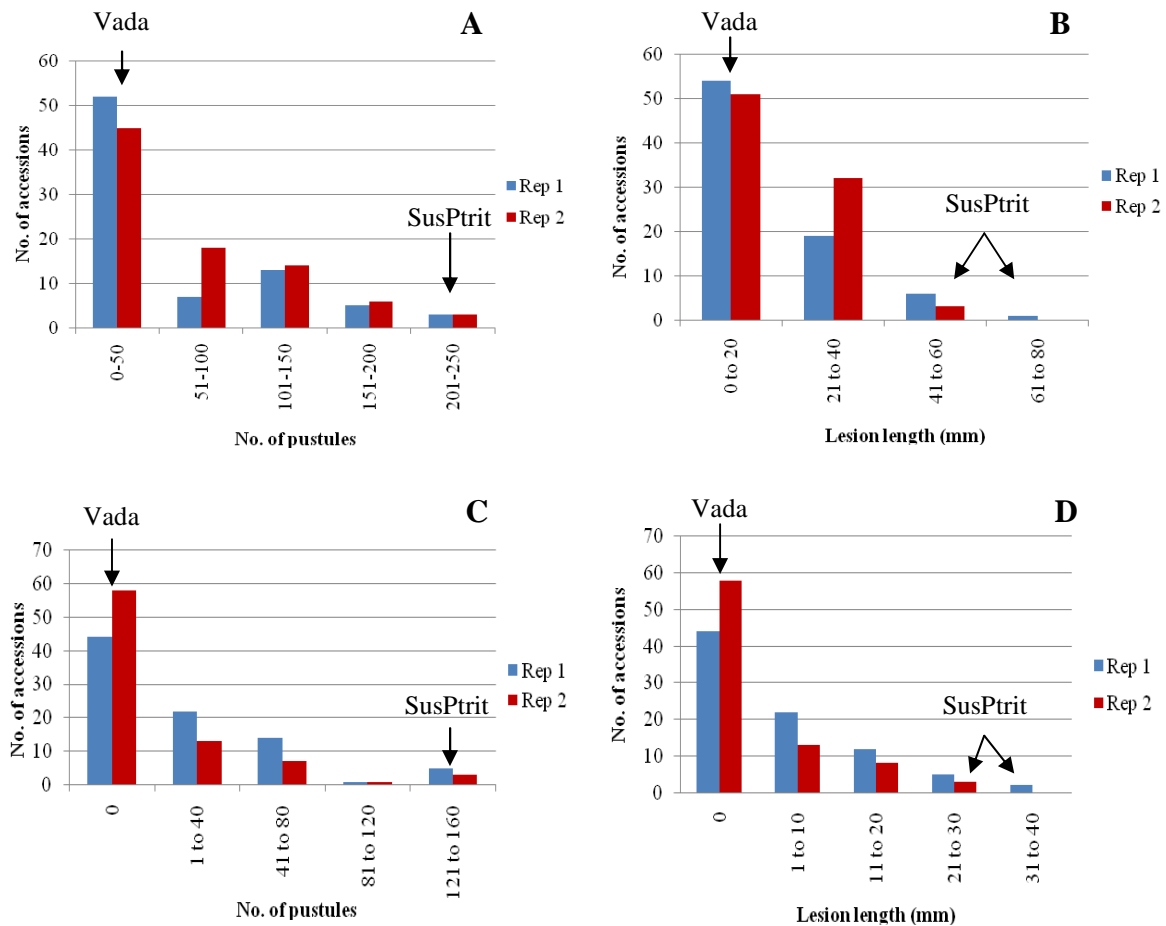
Figure 3 Frequency distribution of phenotypes (No. of pustules; Lesion length - mm) for resistance to *Psb* (A & B) and *Pst* (C & D) in barley mapping population Vada × SusPtrit for replicates 1 & 2; arrows indicate parental line values.

There was also no transgressive segregation observed in the L94 × Vada mapping population when inoculated with *Psh*. However, there is an almost bimodal distribution seen, therefore segregation, and this could possibly indicate the presence of a major gene for resistance (figure 4).
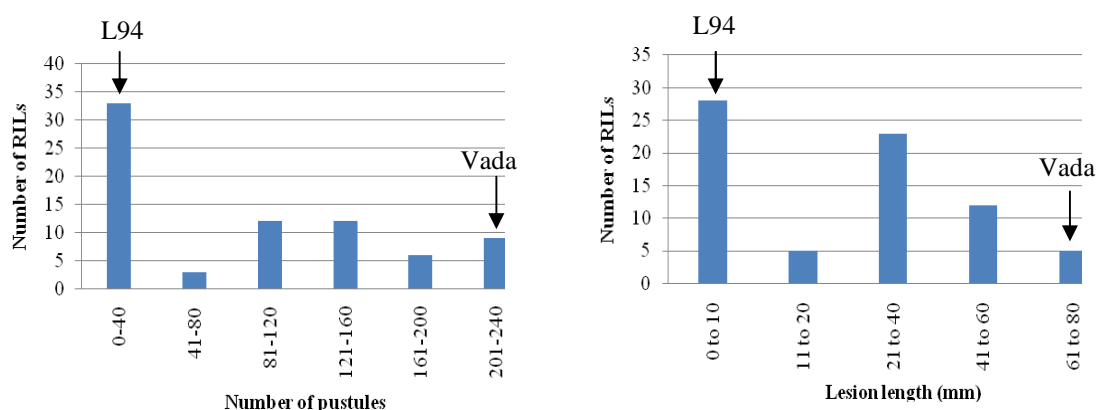
Figure 4 Frequency distribution of phenotypes (number of pustules; lesion length – mm) for resistance to *Psh* in barley mapping population L94 × Vada; arrows indicate parental line values.

4.2.3 Correlations

When running the correlations test between replicates for number of pustules and composite lesion length, that is comparing data from replicate 1 and 2 for the different phenotypes measured during the mapping population testing, strong correlations of 0.89 and 0.80 were observed for *Psb* replicates, however, weaker correlations of 0.65 and 0.61 were observed for *Pst* replicates (table 14). Moreover, when performing a correlation test between phenotypes (number of pustules and composite lesion length), strong correlations were observed for *Psb*, *Pst* and *Psh* for all replicates (table 14). All correlations tested at α=0.01 level of significance using a one tailed test have p<0.01, therefore these values are statistically significant.

Table 14 Correlation coefficients (r) for number of pustules (NPUS) and composite lesion length (MML) calculated between replicates and within replicates for *Psb* and *Pst*, and within replicate for *Psh*.

Rep	Corr. Co. (r) (*Psb*)		Corr. Co. (r) (*Pst*)		Corr. Co. (r) (*Psh*)	
	NPUS	**MML**	**NPUS**	**MML**	**NPUS**	**MML**
1 & 2	0.89	0.80	0.65	0.61		
1	0.90		0.93		0.93	
2	0.91		0.94			

Weaker correlations were observed between all phenotypes measured and leaf length, taken during the third replicate of *Psb* (table 15). All correlations tested at α=0.01 level of significance using a one tailed test have p<0.01, therefore these values are statistically significant.

Table 15 Correlation coefficients (r) for pustule difference, time to development, rate of development and latency period, to leaf length.

Trait measured	r
Pustule difference [a]	0.30
Time to development [b]	-0.01
Rate of development [c]	0.31
Latency Period [d]	-0.24

[a] pustule difference – calculated as the difference between the last count and first count of pustules on the RILs (this is a mean value). [b] Time to development – calculated as the amount of hours it took to develop the pustules from the pustule difference. [c] Rate of development – calculated by taking the pustule difference and dividing this by the time to development (pustules.hr^{-1}). [d] – Latency period – amount of days till first pustule/s is observed.

4.2.4 Detected QTLs and major gene

Three QTLs in total were detected that conferred resistance to *Psb* in barley mapping population Vada × SusPtrit (table 16; figure 5; figure 7). One of the QTLs, located on chromosome 5 (1H), was mapped using number of pustules (NPUS) (replicates 1 and 2), composite lesion length (MML) (replicates 1 and 2) and a scale (third replicate). Another QTL was mapped using the data averaged over replicates 1 and 2 for MML, and despite this marker only reaching above LOD score 3 when using the averaged data, it was a consistent peak marker in other mapping experiments (appendix 5). The third QTL was mapped using a scale by Rients Niks (appendix 4) as well as the LPLM data (as outlined in the assessment part of materials and methods) from the third replicate.

Table 16 Summary of QTLs conferring resistance to *Psb* at seedling stage in Vada × SusPtrit barley mapping population.

Trait	Rep.[a]	Chr.	cM	Locus	LOD[b]	LOD-2[c]	% Ex.	Add.	Donor
NPUS	1	5 (1H)	28.12	E41M40-474	3.37	10.8-33.5	16.5	24.9	Vada
	2	5 (1H)	28.12	E41M40-474	6.55	20.3-32.9	25.8	32.5	Vada
	1 &2	5 (1H)	28.12	E41M40-474	5.36	19.9-32.7	25.1	32.0	Vada
MML	1	5 (1H)	28.12	E41M40-474	3.69	13.2-33.3	18.2	6.4	Vada
	2	5 (1H)	28.12	E41M40-474	4.27	15.9-34.1	17.6	5.4	Vada
	1 & 2	5 (1H)	28.12	E41M40-474	3.96	16.8-33.9	21.3	6.5	Vada
	1 & 2	2 (2H)	65.18	mVrs1	3.46	62-70.9	18.2	6.1	Vada
Scale	4	1 (7H)	132.2	E39M61-255	3	126.6-140.9	10.9	0.9	Vada
	3	5 (1H)	28.12	E41M40-474	3.67	14.9-32.9	12.1	12.5	Vada
	3	1 (7H)	125.8	E35M61-256	5.42	122.6-135.7	18.6	16.3	Vada
LPLM	3	1 (7H)	130.6	E39M61-287	3.12	122.4-139.1	12.8	-0.8	Vada

[a] 1 & 2 indicate QTLs mapped using combined data. [b] LOD values of 3.00 and above were considered to be QTLs. [c] Two LOD support interval calculated from peak marker based on rMQM results.

Two QTLs were mapped and detected that conferred resistance to *Pst* in barley mapping population Vada × SusPtrit (table 17; figure 6; figure 7). One of the QTLs was located on chromosome 1 (7H) and was mapped using NPUS and MML data from replicate 2 and averaged replicate data. The other QTL was mapped on chromosome 5 (1H) using NPUS (replicate 1), MML (replicates 1 and 2), and the rate of development (replicate 3) data.

Table 17 Summary of QTLs conferring resistance to *Pst* at seedling stage in Vada × SusPtrit barley mapping population.

Trait	Rep.[a]	Chr.	cM	Locus	LOD[b]	LOD-2[c]	% Ex.	Add.	Donor
NPUS	1	5 (1H)	28.12	E41M40-474	3.24	14.3-34.4	11.2	13.2	Vada
	2	1 (7H)	130.6	E39M61-287	3.2	113.2-136	13.3	11.7	Vada
	1 &2	1 (7H)	113.7	P17M54-169	4.38	109.9-117.3	14.8	14.9	Vada
MML	1	5 (1H)	28.12	E41M40-474	3.86	16.6-35.9	15	3.7	Vada
	2	5 (1H)	28.12	E41M40-474	3.1	10.4-38.4	10.5	2.1	Vada
	2	1 (7H)	130.6	E39M61-287	3.81	126.8-134.1	13.2	2.3	Vada
	1&2	1 (7H)	113.7	P17M54-169	3.39	110.2-117.6	11.2	2.7	Vada
Rate dev.	3	5 (1H)	28.12	E41M40-474	3.28	14.3-40.1	12.3	0.1	Vada

[a] 1 & 2 indicate QTLs mapped using combined data. [b] LOD values of 3.00 and above were considered to be QTLs. [c] Two LOD support interval calculated from peak marker based on rMQM results.

A major gene for resistance was mapped and located on chromosome 4 (4H) in barley mapping population L94 × Vada, conferring resistance to *Psh* (table 18; figure 8). This gene was mapped using NPUS and MML data from the single replicate carried out, and had a LOD score of >15. Examples of the LOD profiles for *Psb*, *Pst* and *Psh* can be seen in appendices 5, 6 and 7 respectively.

Table 18 Summary of major gene conferring resistance to *Psh* at seedling stage in L94 × Vada barley mapping population.

Trait	Rep.	Chr.	cM	Locus	LOD[a]	LOD-2[b]	% Ex.	Add.	Donor
NPUS	1	4 (4H)	72.7	EBmac0701	15.15	71.1-74.4	46	-55.2	L94
MML	1	4 (4H)	75.1	E40M32-660	17.25	73.3-76.2	49.1	-15.5	L94

[a] LOD values of 3.00 and above were considered to be QTLs. [b] Two LOD support interval calculated from peak marker based on rMQM results.

Interestingly, the QTLs mapped on chromosomes 1 (7H) and 5 (1H) were mapped as effective to both *Psb* and *Pst*, with the respective regions on each chromosome overlapping. Furthermore, of the QTLs mapped for resistance to either *Psb* or *Pst*, only those mapped on chromosome 1 (7H) co-localised with QTLs mapped for resistance to other heterologous rusts *P. hordei* (24), *P. hordei murini* and *P. triticina* (figure 9). There were other QTLs that were reasonably close, however, did not overlap and therefore were not considered to co-localise. The major gene that was mapped for resistance to *Psh* interestingly co-localised with QTLs mapped for resistance to five heterologous rusts; namely *P. hordei* 1.2.1, *P. hordei murini*, *P. hordei secalini*, *P. persistens* and *P. triticina* (figure 9).
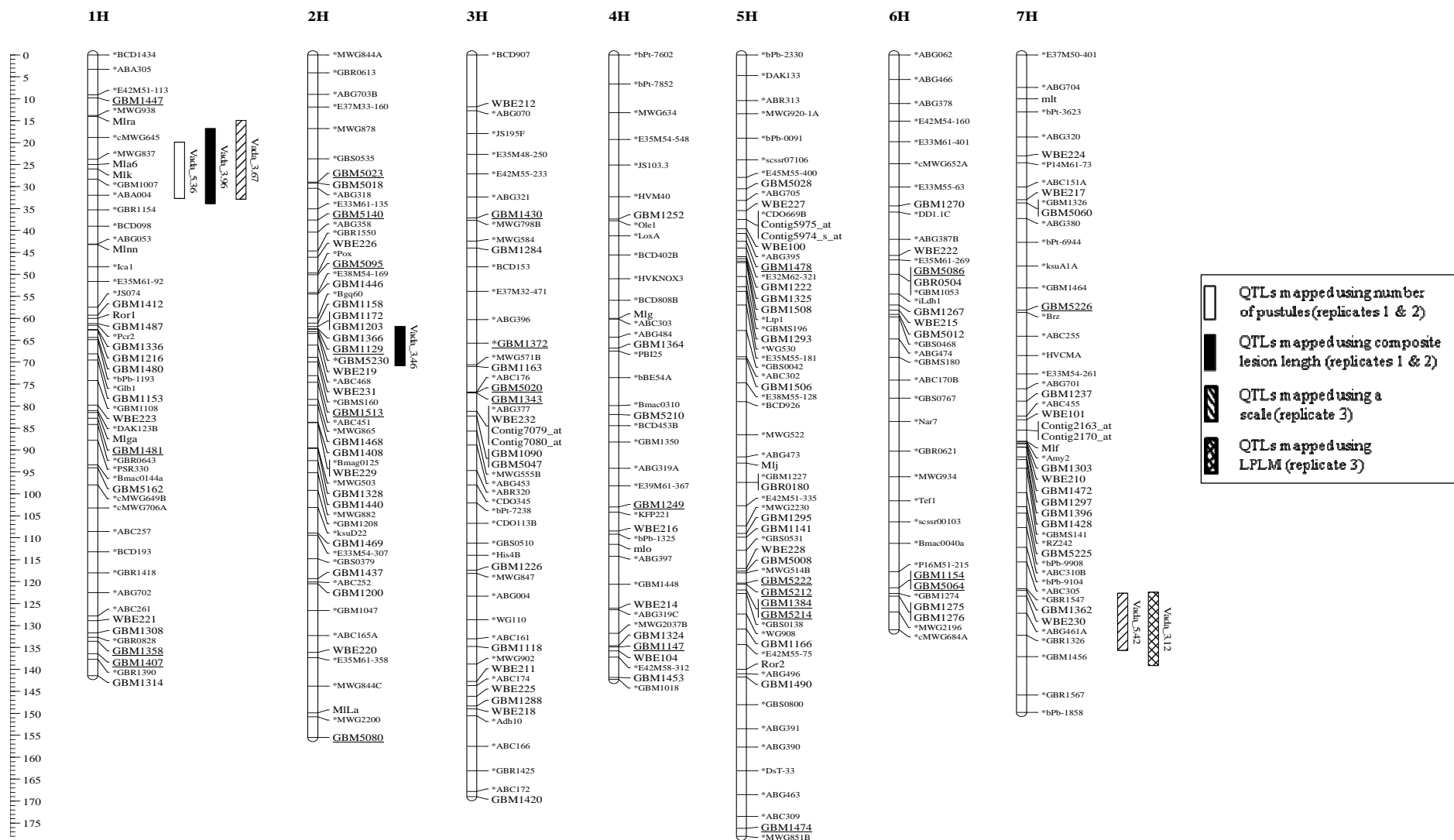
Figure 5 Position of QTLs mapped for seedling stage resistance to *Psb* in barley mapping population Vada × SusPtrit using an integrated map of the barley genome. Bars represent a two LOD support interval. The ruler indicates distances in centiMorgans (cM).

Figure 6 Position of QTLs mapped for seedling stage resistance to *Pst* in barley mapping population Vada × SusPtrit using an integrated map of the barley genome. Bars represent a two LOD support interval. The ruler indicates distances in centiMorgans (cM).
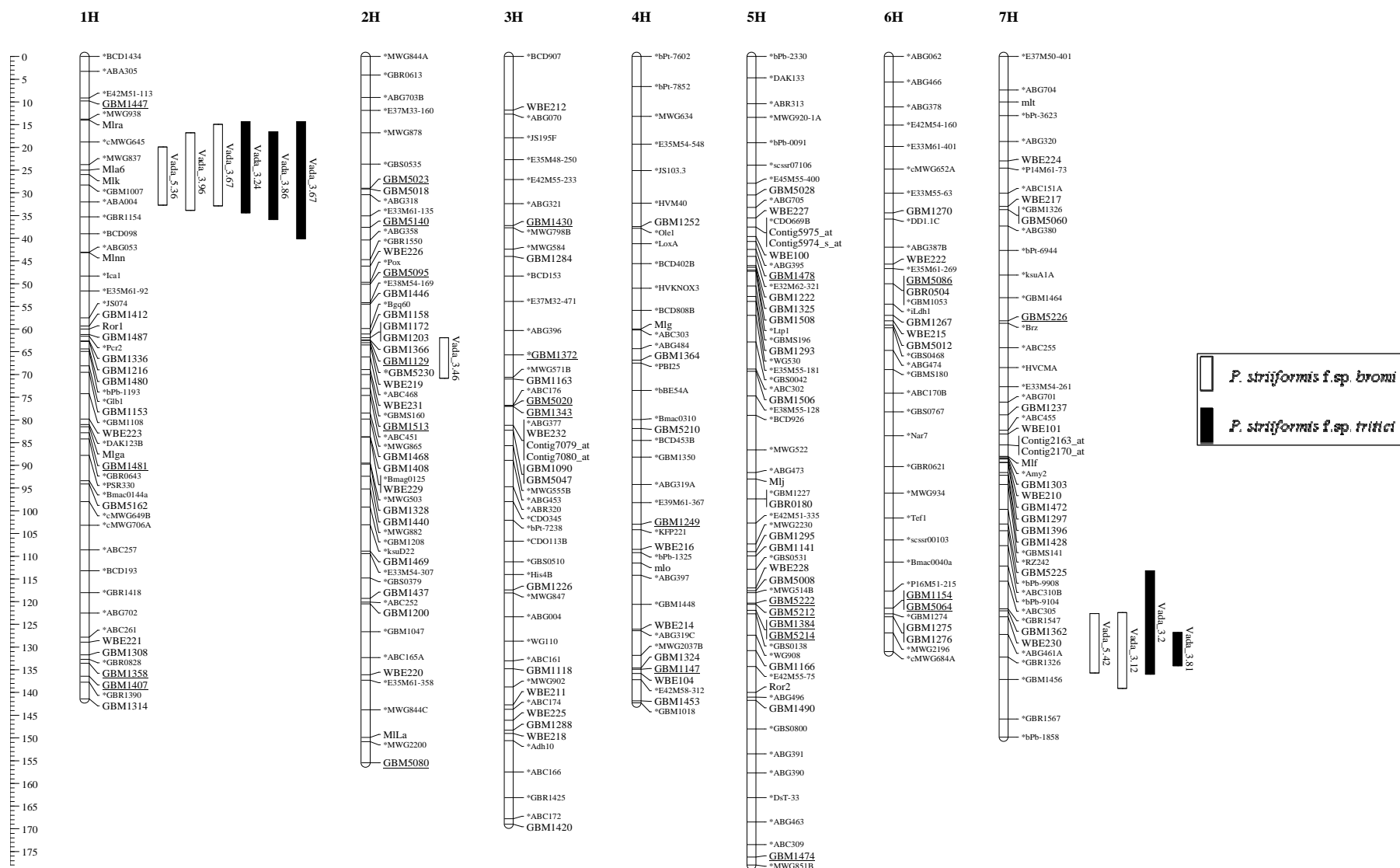
Figure 7 Position and comparison of QTLs mapped for seedling stage resistance to *Psb* and *Pst* in barley mapping population Vada × SusPtrit using an integrated map of the barley genome. Bars represent a two LOD support interval. The ruler indicates distances in centiMorgans (cM).

1H **2H** **3H** **4H** **5H** **6H** **7H**
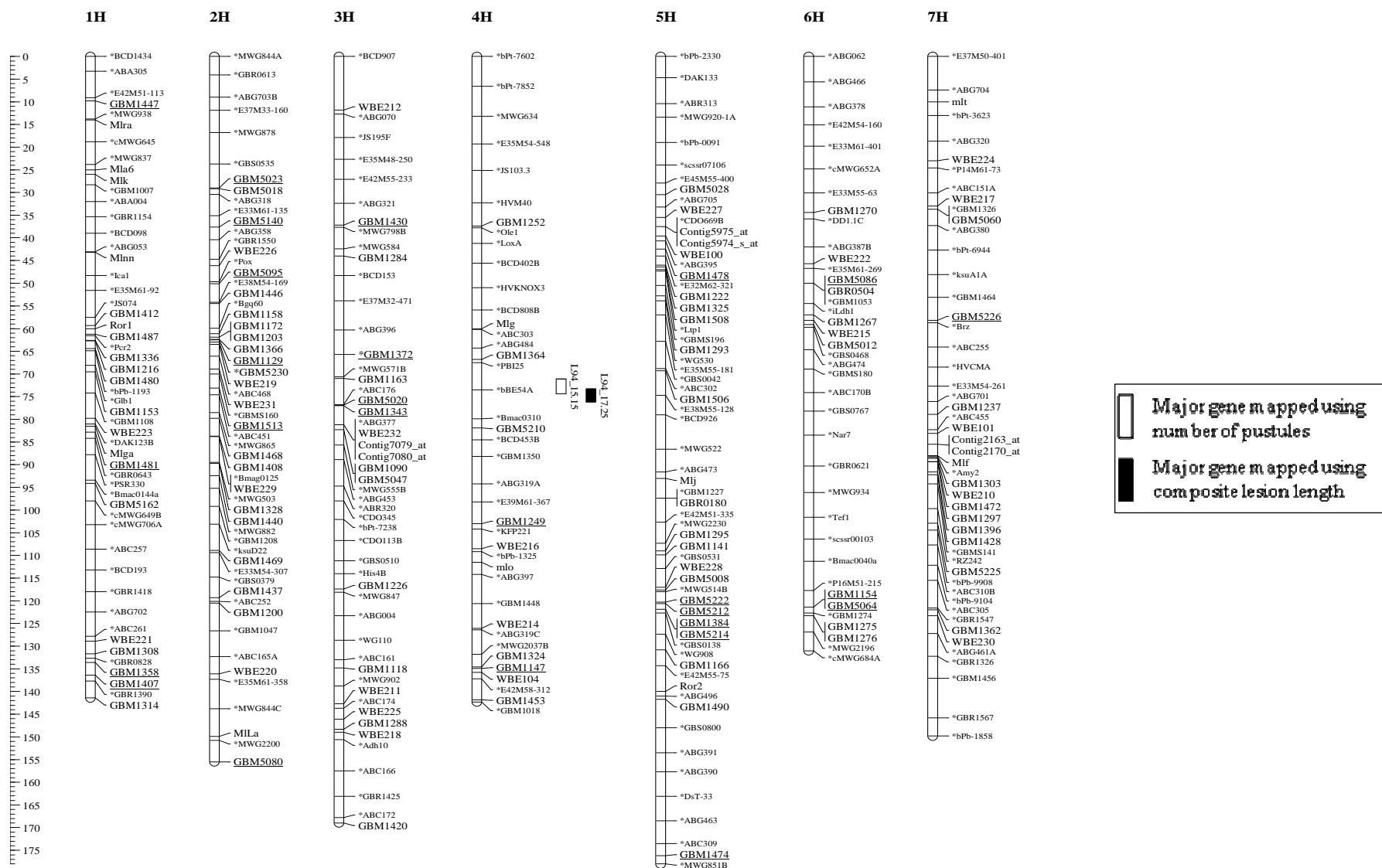
Figure 8 Position of major gene mapped for seedling stage resistance to *Psh* in barley mapping population L94 × Vada using an integrated map of the barley genome. Bars represent a two LOD support interval. The ruler indicates distances in centiMorgans (cM).
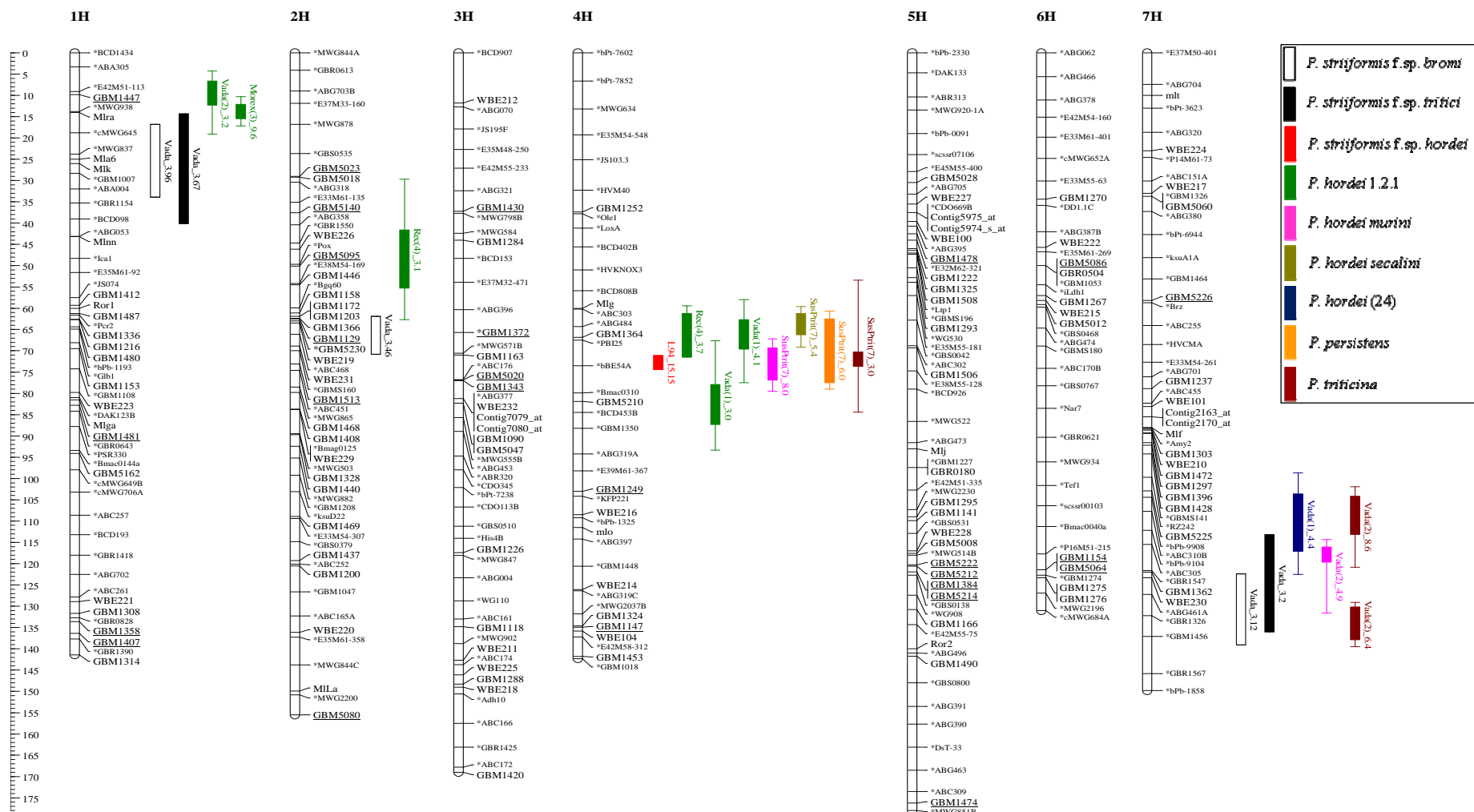
Figure 9 Position and comparison of QTLs and major gene mapped for seedling stage resistance to *Psb*, *Pst* and *Psh* to those QTLs (in a similar region) that have been previously mapped for nonhost resistance (Jafary *et al.* 2008) and partial resistance (Marcel *et al.* 2007). Bars (inner and outer) represent one and two LOD support intervals respectively. Integrated map and data for QTLs to heterologous rusts was kindly provided by Dr. Reza Aghnoum. The ruler indicates distances in centiMorgans (cM).

4.3 Discussion

4.3.1 Parental line analysis and mapping population segregation

There was a large variation observed in parental line testing, which was anticipated, and subsequently the Vada × SusPtrit mapping population was selected for testing *Psb* and *Pst* as this population has been studied for the inheritance of resistance to several heterologous rusts (Jafary *et al.*, 2006; 2008). However, this population could not be selected for testing *Psh* as both Vada and SusPtrit showed susceptible responses. Instead the L94 × Vada mapping population was selected as it was hypothesized that there may be a major conferring resistance in L94. This was based on observations where, despite having an average pustule number of 3, more often than not L94 exhibited an immune type response, and when pustules were observed they were surrounded by necrotic tissue.

The mapping population Vada × SusPtrit did not show transgressive segregation in any phenotype measured. This is not particularly surprising as Vada always had a low average pustule number (for *Psb* and *Pst*) whilst SusPtrit on average tended to have the highest or one of the highest pustule numbers. Therefore the resistances were expected to be derived from Vada and not SusPtrit, and upon further analysis this was the case (further details provided in 4.3.2). The L94 × Vada mapping population also did not show transgressive segregation, however, an almost bimodal distribution was seen, and under the assumption that a major gene may confer the resistance observed in L94, this was expected and in essence promotes the idea that a major gene might be present and derived from L94.

4.3.2 QTL analysis

Three QTLs were mapped for resistance to *Psb*, with Vada being the contributing parent for all three. Of these one found on chromosome 5(1H) was mapped in all replicates, strongly indicating its presence and effectively at contributing to resistance against *Psb*. In addition, there was a strong correlation observed between replicates using either NPUS or MML data, providing an opportunity to average the data from the two replicates, and with this averaged data the same QTL was mapped again. It is important to note that when tested for significance, all correlations that were calculated from replicate 1 and 2 data were statistically significant and therefore strong conclusions can be drawn and relied upon.

It was also initially suspected that the growth of the lesions, and therefore resultant MML, may be controlled by a separate mechanism to pustule development, and the hence the resultant NPUS. However, there was a strong correlation between NPUS and MML data calculated in all replicates. Thus this indicates that when NPUS is high MML will be large as well and vice versa; indicating that more than likely NPUS and MML are controlled by the same or similar mechanism, or at least are inhibited by the same or similar mechanism.

The other two QTLs mapped for resistance to *Psb*, were mapped only with averaged data (chromosome 2(2H)) or from data obtained from the third replicate as well as a replicate run by Dr. Ir. Rients Niks (chromosome 1(7H)). Although the QTL mapped on chromosome 1(7H) was only mapped using data from the third replicate and data obtained from Dr. Ir. Rients Niks, there was always a consistent peak in this region for the first two replicates (appendix 5 – graph b). Interestingly, this QTL on chromosome 1 also co-localises with other heterologous rusts *P. hordei* (24), *P. hordei murini* and *P. triticina* (figure 9). This may then be of interest, as this region has then been mapped for conferring resistance to both host and non-host pathogens and could therefore play role in breeding for an increased quantitative basal defence resistance; where a plant species may reduce the spread of the disease after successful infection (Niks & Marcel, 2009).

Two QTLs were mapped conferring resistance to *Pst* with Vada being the contributing parent for both. These were mapped using quantitative data of NPUS and MML and were found on chromosomes 1(7H) and 5(1H). What is interesting and apparent is that these QTLs overlap with the QTLs mapped conferring resistance to *Psb*, and could therefore be considered as the same QTLs. When averaging replication data for *Pst*, the QTL mapped on chromosome 5(1H) is "lost" (appendix 6). This can be explained by the poor correlation between replicates one and two. Moreover, because of this poor correlation it was of interest to perform another replication. However, during the third replication problems arose, in the sense that the entire population needed to be sprayed against aphids. Although, to our knowledge, no tests have been performed to see whether or not the chemicals used affects pustule development in yellow stripe rust, it has been observed that spraying with chemicals against aphids does result in inhibition of pustule formation (Niks, personal communication). Therefore, due to this concern there may have been QTLs that were masked during the third replicate and the results obtained thus cannot be seen as conclusive or complete until another replicate is performed. Despite this the same QTL on chromosome 5(1H) was mapped using data from the third replicate.

In addition, the correlations to the leaf lengths (table 15) were calculated from data collected during the third *Psb* replicate. These correlations are all statistically significant, suggesting that the size of the leaf does not have an influence on the success or development of the infection. However, it may be important to note that as data was not collected from the *Pst* replicate, therefore the correlations may be different in this replicate, although it this seems unlikely.

4.3.3 Major gene analysis

A major gene for conferring resistance to *Psh* was mapped in the L94 × Vada mapping population with L94 contributing the resistance gene. When using different data to map the gene it is associated with different markers, however, these regions do overlap. The peak markers found to be linked to this gene are *EBmac0701* and *E40M32-660* for NPUS and MML data respectively.

Yan and Chen (2006) mapped the *rpsGZ* gene which is a recessive gene that is said to confer complete resistance to all races of *Psh* found thus far in the USA. They developed F_8 recombinant inbred lines (RILs) from the cross Steptoe × Grannelose Zweizeilige (GZ) through single seed decent, and then evaluated the parents and RILs at seedling stage for resistance to *Psh*. They subsequently mapped the gene closest to the SSR marker *EBmac0679*, and noted markers *EBmac0701*, *WMS6* and *Bmag0138* to be linked to the resistance locus with 9.9, 17.4 and 23.3 cM genetic distances from the gene, respectively.

Therefore, this can be considered to be the same gene as what was mapped in this study, due to the common association with marker *EBmac0701*. To further support this finding both of the contributing parents L94 and GZ are Ethiopian landraces. The detailed history of these landraces has been unobtainable thus far and therefore to determine whether or not L94 was used in a cross to develop GZ, or vice versa, is not possible. There are different designations, or names, found for the accessions, such as Abyssinian 1102, HOR3036 and BBA 1465 for L94, and HOR3028 and BBA1437 for GZ (Jorgensen, 1992); however, further detail could not be obtained. Other researchers such as Collins *et al.* (2001) simply refer to GZ as an Ethiopian landrace. Despite this, due to the marker association and Ethiopian landrace heritage this gene can be proposed to be *rpsGZ* mapped by Yan and Chen (2006).

What is also quite interesting is that this gene has been mapped in a region where several QTLs noted for conferring resistance to heterologous rusts *P. hordei* 1.2.1, *P. hordei murini*, *P. hordei secalini*, *P. persistens* and *P. triticina* (figure 9) have been previously mapped (Jafary *et al.*, 2008;

Marcel *et al.*, 2007). However, these genes were mapped in different populations and the donors for the QTLs are different. Therefore it may be considered that this segment of the genome, or at least an intricate part of it, might be conserved and that when present is able to confer resistance to multiple rusts.

4.4 Conclusion

There was satisfactory variation observed in parental line testing, therefore mapping experiments could be carried out. The Vada × SusPtrit mapping population was selected for mapping QTLs conferring resistance to *Psb* and *Pst*. It was believed that a major gene was conferring resistance in L94 to *Psh*, thus the L94 × Vada mapping population was selected.

There was no transgressive segregation of any phenotypes measured in any of the populations, however, there was an almost bimodal distribution observed in the L94 × Vada population supporting the idea of the presence of a major gene. In addition, strong correlations were calculated between and within replicates 1 and 2 for each of the ff.spp.; all were statistically significant. Leaf length was thought to possibly affect pustule development and lesion length measurements in the third replicate for *Psb* and *Pst*, however, weak correlations were observed between all phenotypes, all of which were statistically significant.

Three QTLs were mapped for resistance to *Psb* on chromosomes 1(7H), 2(2H) and 5(1H). Two QTLs were mapped for resistance to *Pst* on chromosomes 1(7H) and 5(H). The QTLs mapped for both *Psb* and *Pst* overlapped, and can therefore be considered as the same, thus there was a net two QTLs mapped conferring resistance to both *Psb* and *Pst*. A major gene was mapped conferring resistance to *Psh* on chromosome 4(4H). Yan & Chen (2006) mapped the recessive resistance gene *rpsGZ* which is associated with marker *EBmac0701* which is also associated to the gene mapped in this study. Both donors L94 and GZ are Ethiopian landraces, and despite the lack of detailed history it is more than likely that these two donors are related and therefore share the *rpsGZ* gene.

Chapter 5: Conclusion

The indicative host range study revealed that *Psb* appears to be more versatile than either *Pst* or *Psh*. This versatility may enable *Psb* to have a variety of hosts, and if epidemics on *Hordeum* or *Triticum* genera were a potential then this rust may be seen as an economically important pathogen. Interestingly *Psb* was more successful than *Psh* at attacking wild *Hordeum* accessions, although this may be contributed to ages of selective pressure on *Psh* on the presence of cultivated *Hordeum* and not wild *Hordeum* accessions, thereby *Psh* becoming adapted more too cultivated *Hordeum*, than to wild species. Moreover, it was also apparent that *Psb* was more successful than *Pst* at attacking *Aegilops* accessions (a wild goat grass phylogenetically related to *Triticum*). This could also be attributed to ages of selective pressure but on the *Pst* isolate on cultivated *Triticum*, thereby *Pst* becoming adapted more too cultivated *Triticum*, than to wild related species.

The host status tests not only further supported the findings of the host range (in terms of the versatilities of the ff.spp.), but also determined that barley is a host for *Psh* and a marginal host for both *Psb* and *Pst*. This is not surprising as *Psh* is known to be a host, and *Pst* is known to be able to attack a few *Hordeum* accessions. In addition there were no specific characteristics, when considered either by themselves or within origins of the accessions, that could be associated to resistances or susceptibilities that were observed.

The presence of two QTLs conferring resistance to both *Psb* and *Pst* were mapped in the Vada × SusPtrit mapping population. These were mapped on chromosomes 1 (7H) and 5 (1H), and the donating parent was Vada. The QTL on chromosome 1 (7H) co-localises with QTLs previously mapped for resistance to *P. hordei* (24), *P. hordei murini* and *P. triticina*. Although these were mapped in separate populations, the comparison has been made with the use of integrated maps. Therefore, this region is of further interest as it has been mapped conferring resistance to host and non-host pathogens. There was also a major gene for resistance mapped in the L94 × Vada mapping population that conferred resistance to *Psh* with L94 being the donor parent. Interestingly this major gene co-localises with QTLs mapped for resistance to *P. hordei* 1.2.1, *P. hordei murini*, *P. hordei secalini*, *P. persistens* and *P. triticina*. These too were mapped in different populations; however, integrated maps were used for comparative purposes. Therefore, this region is also of further interest as it has been a region mapped conferring resistance to host and non-host pathogens.

Chapter 6: Future direction and suggestions

A more comprehensive host range study should be performed, if a true host range is to be established. Furthermore, different isolates should be obtained and tested to try to reveal true differences. In addition the isolates used in this study, as well as possibly more isolates, should be tested at a molecular level, as well as in disease trials. Such experiments may incorporate the use of expressed sequence tags as used by Chen *et al.* (2009) to try to identify differences between isolates.

As not much is known about the pathogenicity and infectivity mechanisms of this rust, its histology should to be studied. With the knowledge gained from histological investigations, researchers may gain insight not only into how this rust infects plants and establishes itself, but also into identifying the underlying mechanisms responsible for the resistances observed. These studies may also be considered for testing selected accessions in the host range study, for the same reasons. These studies should include a range of accessions differing in their response to the fungi (notably immune, resistant, intermediate and susceptible), such that the mechanisms of action may be discerned.

More adult plant testing needs to be performed, as replicates will provide valuable knowledge. Moreover, an accessions list needs to be derived such that all three ff.spp. are tested against the same adult plants. This would mean more conclusive comparisons can be made for resistances at an adult plant stage.

Further QTL mapping replications for all three ff.spp. should be performed, especially for *Pst* as there were poor correlations calculated between the replicates performed, and with the spraying against aphids the results may be false or masked. The use of the inoculation tower would be the suggested method, rather than the midpoint inoculation, for the replications. Furthermore, the use of freshly collected spores is preferable over those spores that have been left in the desiccator for several days. As then it can be more firmly guaranteed that all plants will be exposed to viable spores, and spore germination did decrease in spores that had been left in the desiccator.

Although this study had its flaws and more work is needed to identify a true host range for *Psb*, an indication of the versatility of this rust in comparison to *Pst* and *Psh* was provided. Furthermore, with the identification of this versatility and results of the host status experiments, *Psb* may hold a potential economic importance. The QTL studies give a good preliminary result and indication of

the potential to find genes conferring resistance not only to *Psb* but also to *Pst*. Finally although it seems that the major gene for resistance to Psh has been previously mapped, this discovery does open questions of the history of the genes and accessions involved.

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Appendix 1: Host range accessions

Species/Variety tested	Origin/Source of seed	Accession No.	Psb		Pst		Psh		Presumed host status
			Score	Level	Score	Level	Score	Level	
Aegilops columnaris	?	CGN 96443	2	HM (6)	1	MR (3)	0	LM (4)	B; T; H
Triticum columnare	?	CGN 06607	6		3		4		
T. kotschyi	Israel	CGN 06606	6	HM (6)	5	M (5)	2	R (2)	B; T
A. peregrina	?	96403	1		0		0		
A. peregrina	?	CGN 96402	2	R (2)	0	I (0)	2	R (2)	N
A. peregrina	?	CGN 96403	2		0		0		
T. peregrinum	?	CGN 16017	2		0		2		
A. speltoides	?	96459	0		0		0		
T. speltoides	?	CGN 10689	2		1		0		
T. speltoides	Israel	CGN 10692	3		3		3		
T. speltoides	Turkey	CGN 10693	2	MR (3)	3	MS (7)	0	MR (3)	B; T; H
T. speltoides	Turkey	CGN 10695	3		7		3		
T. speltoides	Israel	CGN 13123	0		1		2		
T. speltoides	?	CGN 16011	0		0		0		
Avena sativa (Alfred - haver)	Wageningen	200518	0	I (0)	0	I (0)	0	I (0)	N
A. sativa (Haver Cebeco)	Wageningen	2001012	3	MR (3)	1	VR (1)	0	I (0)	B
Agropyron repens	Wageningen	from root	0	LM (4)	0	MR (3)	0	MR (3)	B; T; H
Agropyron repens (GRA 845/83)	Wageningen	96385	4		3		3		
Bromus alopecuros	Germany	2008994	3	MR (3)	1	VR (1)	0	I (0)	B
B. alopecuros	Israel	2008995	1		0		0		
B. arvensis	Bulgaria	2008996	3	MR (3)	0	I (0)	0	I (0)	B
B. briziformis	Soviet Union	2008998	0	I (0)	0	I (0)	0	I (0)	N
B. chrysopogon	France	20081000	4	LM (4)	3	MR (3)	2	R (2)	B; T
B. commutafus	Germany	20081001	0	I (0)	0	I (0)	0	I (0)	N
B. danthoniae	Turkey	206416	6	HM (6)	4	LM (4)	3	MR (3)	B; T; H
B. diandrus	Greece	20081002	0		3		0		
B. diandrus	Spain	20081003	6		0		4		
B. diandrus	France	20081004	3	HM (6)	2	LM (4)	2	LM (4)	B; T; H
B. diandrus	Spain	O31752	4		4		3		
B. diandrus	Turkey	20081026	0		1		1		

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Species	Country	Number							
B. erectus	Romania	111279	0	MR (3)	0	I (0)	0	I (0)	B
B. erectus	Turkey	172397	3		0		0		
B. fasciculatus	Israel	20081006	3	MR (3)	2	R (2)	3	MR (3)	B; H
B. hordeaceus	Ukraine	20081007	0		0		0		
B. hordeaceus	Spain	20081009	0	I (0)	0	VR (1)	0	R (2)	N
B. hordeaceus	France	20081011	0		1		2		
B. inermis subsp inermis	Turkey	172395	0		0		0		
B. inermis subsp inermis	Poland	255870	0	I (0)	0	I (0)	1	VR (1)	N
B. inermis subsp inermis	Former Soviet Union	262456	0		0		0		
B. inermis subsp inermis	Former Soviet Union	370660	0		0		0		
B. japonicus	Turkey	204399	5		4		0		
B. japonicus	Pakistan	219726	5		3		4		
B. japonicus	Iran	239720	1		0		0		
B. japonicus	Bulgaria	20081013	0	HM (6)	0	LM (4)	0	LM (4)	B; T; H
B. japonicus	Central Russia	20081014	6		4		0		
B. japonicus	China	20081015	5		1		2		
B. japonicus	Pakistan	20081016	2		1		0		
B. lanceolatus	Czech Republic	20081017	3		3		0		
B. lanceolatus	France	20081018	4	LM (4)	1	MR (3)	0	I (0)	B; T
B. lanceolatus	Turkey	20081019	3		0		0		
B. lanceolatus	Iran	20081020	3		0		0		
B. madritensis	France	20081021	2		2		2		
B. madritensis	USA	20081022	5		3		4		
B. madritensis	Greece	20081023	4	M (5)	3	LM (4)	4	VS (9)	B; T; H
B. madritensis	Ukraine	20081024	3		1		4		
B. madritensis	Iraq	20081025	5		4		9		
B. mango	Argentina	598721	7	MS (7)	2	R (2)	4	LM (4)	B; H
B. pectnatus	Afghanistan	20081027	5	M (5)	1	LM (4)	9	VS (9)	B; T; H
B. pectnatus	Belgium	20081028	3		4		0		
B. pseudodantonae	Turkey	20081029	0	I (0)	0	I (0)	3	MR (3)	H
B. rigidus	USA	20081031	2	R (2)	0	I (0)	1	VR (1)	N
B. rubens	France	20081032	4		5		2		
B. rubens	Spain	20081034	3	M (5)	4	M (5)	0	LM (4)	B; T; H
B. rubens	France	20081035	5		4		3		

B. rubens	USA	20081036	4		3		4		
B. scoparius	Afghanistan	220514	5	M (5)	3	MR (3)	0	I (0)	B; T
B. scoparius	Former Soviet Union	314229	3		3		0		
B. secalinus	France	20081037	3		0		3		
B. secalinus	France	20081038	0	MR (3)	0	I (0)	0	MR (3)	B; H
B. secalinus	Germany	20081039	0		0		0		
B. squarossus	Ukraine	20081046	3		0		0		
B. squarossus	Bulgaria	20081047	4	LM (4)	3	MR (3)	0	MR (3)	B; T; H
B. squarossus	Iran	20081048	4		0		3		
B. sterilis	Ukraine	20081040	3		2		2		
B. sterilis	Ukraine	20081041	4		1		1		
B. sterilis	France	20081042	1	LM (4)	2	VR (1.17)	2	R (2)	B
B. sterilis	Bulgaria	20081043	1		1		2		
B. sterilis	Italy	20081044	0		1		0		
B. sterilis	Spain	20081045	2		0		2		
B. tectorum	Afghanistan	219992	2		1		9		
B. tectorum	Afghanistan	220575	4		4		5		
B. tectorum	Iran	20081049	5		3		5		
B. tectorum	Ukraine	20081050	2		0		1		
B. tectorum	Spain	20081051	1	M (5)	3	LM (4)	0	VS (9)	B; T; H
B. tectorum	Spain	20081052	2		3		1		
B. tectorum	Iran	20081053	4		3		5		
B. tectorum	USA	20081054	5		0		3		
B. tectorum	Estonia	20081055	4		2		0		
B. tectorum	Bulgaria	20081056	5		2		3		
B. tomentellus	Ukraine	20081057	0	I (0)	0	I (0)	1	VR (1)	N
Hordeum bulbosum	Wageningen	from pot	3	MR (3)	0	I (0)	0	I (0)	B
H. chilense	Argentina	531781	4	LM (4)	4	LM (4)	2	R (2)	B; T
H. jubatum	Wageningen	27314	4		3		3		
H. jubatum	Wageningen	27314	4	LM (4)	0	MR (3)	0	MR (3)	B; T; H
H. jubatum	Canada	234683	0		0		0		
H. lechleri	Argentina	531784	5	M (5)	3	MR (3)	5	M (5)	B; T; H
H. murinum	Wageningen	952194	1	HM (6)	0	LM (4)	1	VS (9)	B; T; H
H. murinum	Wageningen	from pot	6		4		9		

H. parodii	Argentina	531786	4	LM (4)	3	MR (3)	2	R (2)	B; T
H. procerum	Argentina	531787	4	LM (4)	5	M (5)	9	VS (9)	B; T; H
H. secalinum	Wageningen	from pot	4	LM (4)	4	LM (4)	4	LM (4)	B; T; H
H. stenostachys	Argentina	266195	4	LM (4)	2	R (2)	4	LM (4)	B; H
H. vulgare (Braemer)	Wageningen		3		1		8		
H. vulgare (RIFF)	Wageningen		3	MR (3)	1	R (2)	8	VS (9)	B; H
H. vulgare (Topper)	Wageningen	2005344	3		2		9		
Lolium perenne	Wageningen	2007402	0	I (0)	0	I (0)	0	I (0)	N
L. multiflorum Lam. (Westerwolds ryegrass)	Wageningen	2007401	0	I (0)	0	I (0)	0	I (0)	N
Secale cereale (Rogo)	Wageningen	200517	1	VR (1)	0	I (0)	0	I (0)	N
T. aestivum (Canimbla)	Australia	CGN12794	0	I (0)	3	MR (3)	0	I (0)	T
T. aestivum (Ching Hung nr.3)	China	CGN12654	1	VR (1)	4	LM (4)	0	I (0)	T
T. aestivum (Duiker)	South Africa	CGN12603	1	VR (1)	4	LM (4)	1	VR (1)	T
T. aestivum (Ford)	Australia	CGN12796	1	VR (1)	4	LM (4)	1	VR (1)	T
T. aestivum (Ghirka Krasnaia)	Eastern Europe	CGN12572	3	MR (3)	5	M (5)	1	VR (1)	B; T
T. aestivum (Klein Lucero)	Argentina	CGN12765	3	MR (3)	6	HM (6)	2	R (2)	B; T
T. aestivum (Koala)	Australia	CGN08512	3	MR (3)	6	HM (6)	0	I (0)	B; T
T. aestivum (Kung Chiao 288)	China	CGN12643	2	R (2)	6	HM (6)	1	VR (1)	T
T. aestivum (Simonsberg)	South Africa	CGN12607	0	I (0)	4	LM (4)	1	VR (1)	T
T. aestivum (Snabbe)	Sweden	CGN12397	4	LM (4)	5	M (5)	1	VR (1)	B; T
T. aestivum (Lal bahadur Lr 46 - type b)	Wageningen	200511	0	I (0)	8	S (8)	0	I (0)	T
T. aestivum (Michigan Amber)	Wageningen		1	VR (1)	7	MS (7)	1	VR (1)	T
T. aestivum (Morocco)	Wageningen	T2003010	0	I (0)	8	S (8)	0	I (0)	T
T. aestivum (Scalavatis 56 - gebaard)	Wageningen	200513	0	I (0)	7	MS (7)	0	I (0)	T
T. aestivum (Thatcher)	Wageningen	200504	0	I (0)	3	MR (3)	1	VR (1)	T
T. aestivum grp Aestivum (Atai)	Iran	CGN04063	1	VR (1)	7	MS (7)	2	R (2)	T
T. aestivum grp Aestivum (Bahatane)	Algeria	CGN06035	4	LM (4)	3	MR (3)	3	MR (3)	B; T; H
T. aestivum grp Aestivum (Beladi)	Egypt	CGN06092	0	I (0)	4	LM (4)	0	I (0)	T
T. aestivum grp Aestivum (Ble de Oi Liging)	China	CGN12113	2	R (2)	3	MR (3)	0	I (0)	T
T. aestivum grp Aestivum (Boxer)	United Kingdom	CGN16114	0	I (0)	2	R (2)	0	I (0)	N
T. aestivum grp Aestivum (Ch 34 Shin Pin 83)	China	CGN09152	0	I (0)	3	MR (3)	0	I (0)	T
T. aestivum grp Aestivum (Chinese 166)	China	CGN 04314	2	R (2)	7	MS (7)	2	MR (3)	T; H
T. aestivum grp Aestivum (Chinese 166)	China	CGN 09095	1		7		3		
T. aestivum grp Aestivum (Chinese spring)	China	CGN 04086	1	VR (1)	4	M (5)	0	I (0)	T

			Psb		Pst		Psh		
T. aestivum grp Aestivum (Chinese spring)	China	CGN 12743	1		5		0		
T. aestivum grp Aestivum (Fenman)	United Kingdom	CGN05450	1	VR (1)	3	MR (3)	1	VR (1)	T
T. aestivum grp Aestivum (Jaerae Chong)	Rep. of Korea	CGN05503	5	M (5)	5	M (5)	2	R (2)	B; T
T. aestivum grp Aestivum (K'amadi Sinde)	Ethiopia	CGN07999	2	R (2)	3	MR (3)	0	I (0)	T
T. aestivum grp Aestivum (Laria)	Eastern Europe	CGN19272	0	I (0)	7	MS (7)	0	I (0)	T
T. aestivum grp Aestivum (Little Joss)	United Kingdom	CGN08769	1	VR (1)	4	LM (4)	0	I (0)	T
T. aestivum grp Aestivum (Maris Huntsman)	United Kingdom	CGN08782	1	VR (1)	3	MR (3)	1	VR (1)	T
T. aestivum grp Aestivum (Mokhtar)	Egypt	CGN04163	1	VR (1)	4	LM (4)	2	R (2)	T
T. aestivum grp Aestivum (Nakhichevan)	Eastern Europe	CGN11900	1	VR (1)	3	MR (3)	0	I (0)	T
T. aestivum grp Aestivum (Nech Sinde)	Ethiopia	CGN08039	3	MR (3)	3	MR (3)	3	MR (3)	B; T; H
T. aestivum grp Aestivum (Nepal 66)	Nepal	CGN13675	1	VR (1)	7	MS (7)	0	I (0)	T
T. aestivum grp Aestivum (Seu Seun)	Rep. of Korea	CGN09132	1	VR (1)	missing		0	I (0)	N *
T. aestivum grp Aestivum (Suwon 92)	Rep. of Korea	CGN09133	2	R (2)	3	MR (3)	0	I (0)	T
T. aestivum grp Aestivum Spring (Gandumi Saman)	Iran	CGN06575	2	R (2)	7	MS (7)	3	MR (3)	T; H
T. aestivum grp Compactum (El Kreloff)	Algeria	CGN06534	0	I (0)	1	VR (1)	1	VR (1)	N
T. durum (Meridiano)	Wageningen	200501	0	I (0)	6	HM (6)	1	VR (1)	T
T. turgidum grp Dicoccon (Abessinischer Emmer)	Ethiopia	CGN07975	1	VR (1)	1	VR (1)	0	I (0)	N
T. turgidum grp Durum (Azzaidi)	Italy	CGN08151	1	VR (1)	3	MR (3)	1	VR (1)	T
T. turgidum grp Durum (Capelli)	Italy	CGN08238	1	VR (1)	1	VR (1)	1	VR (1)	N
T. turgidum grp Durum (Ekdani)	India	CGN08216	1	VR (1)	3	MR (3)	missing		T *
T. turgidum grp Durum (Francesa)	Italy	CGN08204	3	MR (3)	4	LM (4)	1	VR (1)	B; T
T. turgidum grp Durum (Gubieha Auttma)	Jordan	CGN06589	1	VR (1)	3	MR (3)	1	VR (1)	T
T. turgidum grp Durum (Hansia Broach)	India	CGN06567	1	VR (1)	5	M (5)	2	R (2)	T
T. turgidum grp Durum (Psathas)	Cyprus	CGN08232	1	VR (1)	4	LM (4)	1	VR (1)	T
T. turgidum grp Durum (Sonora)	Mexico	CGN12023	1	VR (1)	6	HM (6)	1	VR (1)	T
T. turgidum grp Durum (Tunisi)	Italy	CGN16061	1	VR (1)	3	MR (3)	1	VR (1)	T
T. turgidum grp Durum (Westphal 46)	Ethiopia	CGN07981	3	MR (3)	1	VR (1)	3	MR (3)	B; H
T. turgidum grp Durum (Westphal 96)	Ethiopia	CGN13141	missing		missing		missing		*
T. turgidum grp Turgidum (Baragon Bajio)	Mexico	CGN12285	1	VR (1)	3	MR (3)	0	I (0)	T

* Groups separated based on genera ** Score and level: based on 0-to9 and descriptive scale as outlined by McNeal et al. 1971 *** Number in brackets next to level indicates the most susceptible score observed for all accessions of that species tested **** Accessions with score of 3 or greater are presumed to be hosts; where: B - Psb; T - Pst; H - Psh; N - non-host

Appendix 2: Barley host status (seedling test) accessions

No.	Accession Name	Seed type	Origin	Type	Release year	Spike Row	Awn Type	Seed colour	Psb NPUS	Pres. status	Pst NPUS	Pres. status	Psh NPUS	Pres. status
1	Ab 14 Köln	Covered	Ethiopia	Landrace	<1945	Six Rowed	Awned	white	0 (I)	R	0 (I)	R	115	S
2	Akka	Covered	Sweden	Cultivar	1969	Two Rowed	Awned	white	20.67	S	0 (I)	R	252.67	S
3	Albert	Covered	France	Cultivar	<1949	Six Rowed	Awned	Black	122	S	0	R	134.67	S
4	Alfa	Covered	Denmark	Cultivar	<1947	Two Rowed	Awned	white	46	S	0 (I)	R	0 (I)	R
5	Allegro	Covered	Netherlands	Cultivar	1978	Two Rowed	Awned	white	0	R	0	R	184.67	S
6	ANA	Covered	Argentina	Cultivar		Two Rowed	Awned	white	0	R	0	R	190.67	S
7	Apex	Covered	Netherlands	Cultivar	<1982	Two rowed	Awned	white	0	R	0 (I)	R	14	S
8	Aramir	Covered	Netherlands	Cultivar	1972	Two Rowed	Awned	white	0	R	0 (I)	R	226	S
9	Archer	Covered	United Kingdom	Cultivar	<1931	Two Rowed	Awned	white	0	R	0 (I)	R	118	S
10	Ark Royal	Covered	United Kingdom	Cultivar	1976	Two Rowed	Awned	white	0	R	0 (I)	R	128	S
11	Armella	Covered	France	Cultivar	<1974	Two Rowed	Awned	white	0	R	0 (I)	R	172.67	S
12	Aura	Covered	Germany	Cultivar	<1975	Two Rowed	Awned	white	82.67	S	0 (I)	R	101	S
13	Bavaria	Covered	Germany	Cultivar	<1903	Two Rowed	Awned	white	0	R	0 (I)	R	290	S
14	Berac	Covered	Netherlands	Cultivar	1970	Two Rowed	Awned	white	79.67	S	0 (I)	R	300	S
15	Berg	Covered	Western Europe	Cultivar	<1938	Six Rowed	Awned	white	36.5	S	0 (I)	R	82.5	S
16	Brage	Covered	Sweden	Cultivar	1925	Two Rowed	Awned	white	16.5	S	0 (I)	R	310	S
17	Braemar	Covered	*	Cultivar		Two Rowed	Awned	white	7	S	4	S	200	S
18	Burton Malt	Covered	United Kingdom	Cultivar	<1920	Two Rowed	Awned	white	0	R	0 (I)	R	130.5	S
19	C118	Covered	*	Res. line		*	*	white	4	S	0 (I)	R	400	S
20	Calicuchima (RphX)	Covered	Ecuador	Cultivar	1992	Six Rowed	Awned	white	0 (I)	R	63.67	S	184.33	S
21	CLE 152	Covered	Uruguay	Cultivar		Two Rowed	Awned	white	11.67	S	0 (I)	R	48	S
22	CLE 157	Covered	Uruguay	Cultivar		Two Rowed	Awned	white	0	R	0 (I)	R	144.67	S
23	CLE 182	Covered	Uruguay	Cultivar		Two Rowed	Awned	white	136	S	46.5	S	159.33	S
24	CLE 187	Covered	Uruguay	Cultivar		Two Rowed	Awned	white	0 (I)	R	0 (I)	R	20.67	S
25	CLE 194	Covered	CIMMYT	Cultivar		Two Rowed	Awned	white	0	R	0 (I)	R	0 (I)	R
26	Dabat	Covered	Ethiopia	Landrace		Six Rowed	Awnless	white	0 (I)	R	0 (I)	R	45.5	S

27	Delibes	Covered	United Kingdom	Cultivar		Two Rowed	Awned	white	0	R	0 (I)	R	7	S
28	Drossel	Covered	Germany	Cultivar	1971	Two Rowed	Awned	white	2.67	R	0 (I)	R	100	S
29	Effendi	Covered	Netherlands	Cultivar	<1972	Two Rowed	Awned	white	1.67	R	0 (I)	R	201.67	S
30	Egypt IV	Covered	Germany	Cultivar	<1938	Six Rowed	Awned	white	178	S	10.33	S	300	S
31	Emir	Covered	Netherlands	Cultivar	1962	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	85.33	S
32	Firlbach III	Covered	Germany	Cultivar	1948	Two Rowed	Awned	white	87.33	S	0 (I)	R	139.33	S
33	FNC 1	Covered	Uruguay	Cultivar		Two Rowed	Awned	white	32.5	S	0 (I)	R	101	S
34	FNC 6-1	Covered	Uruguay	Cultivar		Two Rowed	Awned	white	16.33	S	0 (I)	R	140	S
35	Fong Tien	Covered	China	Landrace	1926	Six Rowed	Awned	white	168.33	S	22.33	S	128	S
36	Freya Jerusalem	Covered	Sweden?	Cultivar	1942?	Two Rowed	Awned	white	222.5	S	0 (I)	R	85.5	S
37	Georgie	Covered	United Kingdom	Cultivar	1975	Two Rowed	Awned	white	2	R	0 (I)	R	240	S
38	Gold	Covered	Sweden	Cultivar	<1913	Two Rowed	Awned	white	3.67	S	0 (I)	R	229.33	S
39	Golden promise	Covered	United Kingdom	Cultivar		Two Rowed	Awned	white	0 (I)	R	0 (I)	R	120.33	S
40	Gospick	Covered	Yugoslavia	landrace	<1949	Two Rowed	Awned	white	13.33	S	0 (I)	R	203	S
41	Goudgerst	Covered	Sweden	Cultivar	<1913	Two Rowed	Awned	white	1.67	R	0 (I)	R	226.67	S
42	H. spon. (PI391136)	Covered	*	Wild barley		Two Rowed	Awned	white	122.5	S	0 (I)	R	105	S
43	H. spon. Ashkelon	Covered	*	Wild barley		Two Rowed	Awned	white	82	S	0 (I)	R	*	*
44	H. spon. Maalot	Covered	*	Wild barley		Two Rowed	Awned	white	23	S	38.33	S	225	S
45	H. spon. Mehola	Covered	*	wild barley		Two Rowed	Awned	white	86.67	S	0	R	140	S
46	Haisa	Covered	Germany	Cultivar	1939	Two Rowed	Awned	white	0	R	0 (I)	R	112	S
47	Harrington	Covered	Canada	Cultivar	1981	Two Rowed	Awned	white	0 (I)	R	*	*	*	*
48	Hassan	Covered	Netherlands	Cultivar	1971	Two Rowed	Awned	white	0	R	0 (I)	R	73.33	S
49	Isaria	Covered	Germany	Cultivar	1924	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	50.67	S
50	Japan 1	Covered	Japan	Landrace	<1963	Six Rowed	Awned	white	0	R	0 (I)	R	78.67	S
51	Japan 15	Covered	Japan	landrace		Six Rowed	Awned	white	0 (I)	R	0 (I)	R	48.67	S
52	Japan 18	Covered	Japan	landrace		six rowed	Awned	white	200	S	0	R	42.33	S
53	Japan 20	Covered	Japan	Landrace		Six Rowed	Awned	white	0	R	0 (I)	R	18	S
54	Japan 6	Naked	Japan	Landrace		Six Rowed	Awned	white	*	*	*	*	96	S
55	Japan 8	Naked	Japan	Landrace		Six Rowed	Awned	white	*	*	114	S	45	S
56	Jerusalem II	Covered	Israel	Cultivar	<1990	Two Rowed	Awned	white	280	S	0 (I)	R	0 (I)	R

No	Name	Hull	Origin	Type	Year	Row	Awn	Color	V1	R1	V2	R2	V3	R3
57	Kobakintagi	Naked	Japan	Landrace	<1950	Six Rowed	Awned	white	111.33	S	0 (I)	R	142	S
58	Kuckuck	Covered	Western Europe	Landrace	1961	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	157	S
59	Kwan	Covered	United States	Cultivar	<1968	Six Rowed	Awned	white	0	R	0 (I)	R	15	S
60	L100	Naked	Ethiopia	Landrace		Six Rowed	Awned	Black	0	R	0 (I)	R	52	S
61	L92	Naked	Ethiopia	Landrace	<1963	Two Rowed	Awnless	white	0	R	0 (I)	R	0	R
62	L98	Covered	Ethiopia	Landrace	<1963	Six Rowed	Awned	white	0 (I)	R	0 (I)	R	0	R
63	La Estanzuela	Covered	Uruguay	Cultivar		*	*	white	0	R	0 (I)	R	68	S
64	Lacey	Covered	USA	Cultivar	2000	Six Rowed	Awned	white	40.33	S	1	R	127.33	S
65	Lago	Covered	Western Europe	Cultivar		Two Rowed	Awned	white	0	R	0 (I)	R	134.5	S
66	Lechtaler	Covered	Portugal	Landrace	<1938	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	60.33	S
67	Lofa Abed	Covered	Denmark	Cultivar	1970	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	91.67	S
68	Magnif 102	Covered	Argentina	Cultivar	<1968	Two Rowed	Awned	white	152.5	S	0 (I)	R	165	S
69	Magnif 104	Covered	Argentina	Cultivar	<1968	Two Rowed	Awned	white	125	S	0 (I)	R	124	S
70	Mazurka	Covered	Netherlands	Cultivar	1975	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	6	S
71	Menelik	Covered	Ukraine	Landrace	<1930	Two Rowed	Awned	white	0	R	22.33	S	154	S
72	Meta	Covered	Netherlands	Cultivar	1981	Two Rowed	Awned	white	15	S	0 (I)	R	137	S
73	Midas	Covered	United Kingdom	Cultivar	1970	Two Rowed	Awned	white	80.33	S	0 (I)	R	47.33	S
74	Morgenrot	Covered	Germany	Cultivar	<1944	Six Rowed	Awned	white	24.5	S	0 (I)	R	89	S
75	Mosane	Covered	Belgium	Cultivar	1961	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	253.33	S
76	Multan	Covered	Pakistan	Landrace	<1923	Six Rowed	Awned	white	87.5	S	0 (I)	R	103.33	S
77	Nadrine	Naked	*			Two Rowed	Awned	Black	0	R	*	*	*	*
78	nhQTL-L94	Covered	Netherlands	Res. line		Two Rowed	Awned	Black	0	R	0	R	0	R
79	Nigrimiden	Naked	Ethiopia	Landrace	<1962	Two Rowed	Awned	Black	0	R	0 (I)	R	0 (I)	R
80	Opal	Covered	Denmark	Cultivar	<1924	Two Rowed	Awned	white	21.33	S	0 (I)	R	270	S
81	Peruvian	Covered	Peru	Landrace	<1917	Six Rowed	Awned	white	0 (I)	R	0 (I)	R	220	S
82	Porthos	Covered	France	Cultivar	1975	Two Rowed	Awned	white	0	R	0 (I)	R	73.67	S
83	Printa	Covered	Netherlands	Cultivar	> 1942	Two Rowed	Awned	white	17.33	S	0 (I)	R	206.67	S
84	Prisma	Covered	Netherlands	Cultivar	<1980	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	0 (I)	R
85	Probst	Covered	Austria	Cultivar	<1949	Two Rowed	Awned	white	12	S	0 (I)	R	107	S
86	Ramona	Covered	Netherlands	Cultivar	<1974	Two Rowed	Awned	white	0	R	0 (I)	R	196	S

87	Ribari	Covered	Egypt	Cultivar	<1960	Six Rowed	Awned	white	0	R	0 (I)	R	100	S
88	Riff	Covered	Netherlands	Landrace		Two Rowed	Awned	white	8	S	3	S	200	S
89	Robust	Covered	United States	Cultivar	1983	Six Rowed	Awned	white	137	S	36.33	S	100.33	S
90	Ruby	Covered	United Kingdom	Cultivar	1966	Two Rowed	Awned	white	1	R	0 (I)	R	73.33	S
91	Speciale	Covered	USA	Landrace	<1947	Six Rowed	Awned	white	179.67	S	125.67	S	82	S
92	Spiti	Covered	China	Landrace	<1926	Six Rowed	Awned	white	0	R	0 (I)	R	137	S
93	Spratt Archer	Covered	United Kingdom	Cultivar	<1929	Two Rowed	Awned	white	11.67	S	0 (I)	R	0 (I)	R
94	Stander	Covered	United States	Cultivar	1993	Six Rowed	Awned	white	151	S	0 (I)	R	240	S
95	Sudan	Covered	Sudan	Landrace	<1938	Six Rowed	Awnless	white	0	R	0 (I)	R	108.5	S
96	Sultan	Covered	Netherlands	Cultivar	1966	Two Rowed	Awned	white	2.67	R	0 (I)	R	171	S
97	Suspmur	Covered	Netherlands	Res. line		Two Rowed	Awned	Black	63	S	0 (I)	R	228	S
98	Topper	Covered	Germany	Cultivar	<1959	Six Rowed	Awned	white	52.33	S	9	S	129.5	S
99	Tresor de V	Covered	France	Cultivar	1940	Two Rowed	Awned	white	42	S	0 (I)	R	87.33	S
100	Trigo Biasa	Naked	Indonesia	Landrace	<1993	Six Rowed	Awned	white	165	S	0 (I)	R	260	S
101	Union	Covered	Germany	Cultivar	1955	Two Rowed	Awned	white	0	R	0 (I)	R	256.67	S
102	Valeta	Covered	Netherlands	Cultivar	<1972	Two Rowed	Awned	white	0 (I)	R	0 (I)	R	171	S
103	Varunda	Covered	Netherlands	Cultivar	1969	Two Rowed	Awned	white	0	R	0 (I)	R	0 (I)	R
104	Volla	Covered	Germany	Cultivar	1957	Two Rowed	Awned	white	30	S	0 (I)	R	142.5	S
105	116-5	Naked	*	Res. line		Six Rowed	Awned	white	63.67	S	0 (I)	R	172	S
106	C123	Covered	*	Res. line	<1976	Six Rowed	Awned	Black	96.67	S	0 (I)	R	216.67	S
107	Cebada Capa	Covered	Argentina	Cultivar	<1936	Six Rowed	Awned	white	0 (I)	R	0 (I)	R	77	S
108	Dom	Covered	North America	Res. line		*	*	Black	220	S	253.33	S	167.5	S
109	Gei	Covered	Netherlands	Cultivar		Two Rowed	Awned	white	0 (I)	R	0 (I)	R	200	S
110	Gunhild	Covered	Denmark	Cultivar	<1980	Two Rowed	Awned	white	99	S	0 (I)	R	225	S
111	Henni	Covered	Germany	Cultivar		Two Rowed	Awned	white	200	S	0 (I)	R	310	S
112	L94	Covered	Ethiopia	Landrace		Two Rowed	Awned	Black	0	R	0	R	3.33	S
113	Meltan	Covered	Sweden	Cultivar		Two Rowed	Awned	white	4	S	0 (I)	R	64.67	S
114	Morex	Covered	United States	Cultivar	1978	Six Rowed	Awned	white	220	S	0 (I)	R	195	S
115	Nure	Covered	*	Cultivar		Two Rowed	Awned	white	42.33	S	0 (I)	R	246.67	S
116	Rec	Naked	North America	Res. line		*	*	white	71	S	72.67	S	206.67	S

117	Steptoe	Covered	United States	Cultivar	1971	Six Rowed	Awned	white	223.33	S	0	R	270	S
118	SusPtrit	Naked	Netherlands	Res. line		Six Rowed	Awned	white	246.7	S	203.33	S	238.44	S
119	Tremois	Covered	France	Cultivar		Two Rowed	Awned	white	1.33	R	0 (I)	R	31	S
120	Vada	Covered	Netherlands	Cultivar	<1956	Two Rowed	Awned	white	1	R	0 (I)	R	260	S

* missing data or unknown data is represented by (*) ** in some cases averages are indicated by the presence of only 1 plant, therefore may not be an accurate representation of the true status of the accession *** Pres. status indicates: the presumed status **** threshold for determining status: any accession showing an avg pustule count of 3 or greater has been given the presumed status of host **** NPUS - indicates the average number of pustules.

Appendix 3: Barley host status (adult plant testing) accessions

Accession no.		Accession	*Psb* (seedling)		*Psb* (adult)	
			avg pust.	Status	avg pust.	Status
	from pot	SusPtrit	203.33	S	0	R
G	20031662	Japan 18	200	S	0	R
G	20082260	Magnif 102	152.5	S	150	S
G	20082233	Robust	137	S	0	R
G	20082274	Speciale	179.67	S	50	S
G	20082251	Jerusalem II	280	S	0	R
G	20082240	Egypt IV	178	S	0	R
G	20082283	Fong Tien	168.33	S	0	R
G	20081268	Dom	220	S	50	S
G	20081269	Steptoe	223.33	S	0	R

Accession no.		Accession	*Pst* (seedling)		*Pst* (adult)	
			avg pust.	Status	avg pust.	Status
		Michigan Amber	500	S	300	S
G	20082240	Egypt IV	10.33	S	0	R
G	20082299	CLE 182	46.5	S	0	R
G	20031665	H. spon. Maalot	38.33	S	0	R
G	20082287	Menelik	22.33	S	0	R
G	20061106	Calicuchima (RphX)	63.67	S	0	R
G	20082233	Robust	36.33	S	0	R
G	20082274	Speciale	125.67	S	0	R
G	20082250	Japan 8	114	S	0	R
G	2007445	Rec	72.67	S	0	R
G	20081268	Dom	253.33	S	50	S
G	20081515	SusPtrit	203.33	S	0	R

| Accession no.		Accession	*Psh* (seedling)		*Psh* (adult)	
			avg pust.	Status	avg pust.	Status
		SusPtrit	203.33	S	150	S
G	20082239	Effendi	201.67	S	0	R
G	20082244	Georgie	240	S	20	S
G	20082281	Union	256.67	S	10	S
G	20082230	Brage	310	S	20	S
G	20082252	Kuckuck	157	S	0	R
G	20082268	Opal	270	S	0	R
G	20051029	C118	400	S	60	S
G	20082293	Stander	240	S	60	S
G	20082290	Trigo Biasa	260	S	1000	S
G	20082284	Vada	260	S	10	S
G	20081269	Steptoe	270	S	100	S

* Psb (seedling), Pst (seedling), Psh (seedling) refers to the results from the seedling tests ** Psb (adult), Pst (adult), Psh (adult) refers to the results from the adult tests *** avg pust. refers to the average number of pustules calculated as the mean of observed values.

Appendix 4 0-9 scale derived for 3rd replication analysis of RIL populations

| Score	Description
0	Immune
1	Hypersensitivity
2	<10 pustules; long LP
3	<10 pustules; short LP
4	11-50 pustules; long LP
5	11-50 pustules; short LP
6	>51 pustules; slow development; long LP
7	>51 pustules; fast development; long LP
8	>51 pustules; slow development; short LP
9	>51 pustules; fast development; short LP

*LP refers to latency period defined as the time (hours) it took for the first pustule to be observed.** Slow / fast development refers to the rate of development (pustules.hr^{-1})

Appendix 5: LOD profiles of restricted MQM mapping for traits analysed in Vada × SusPtrit mapping population for *Psb*
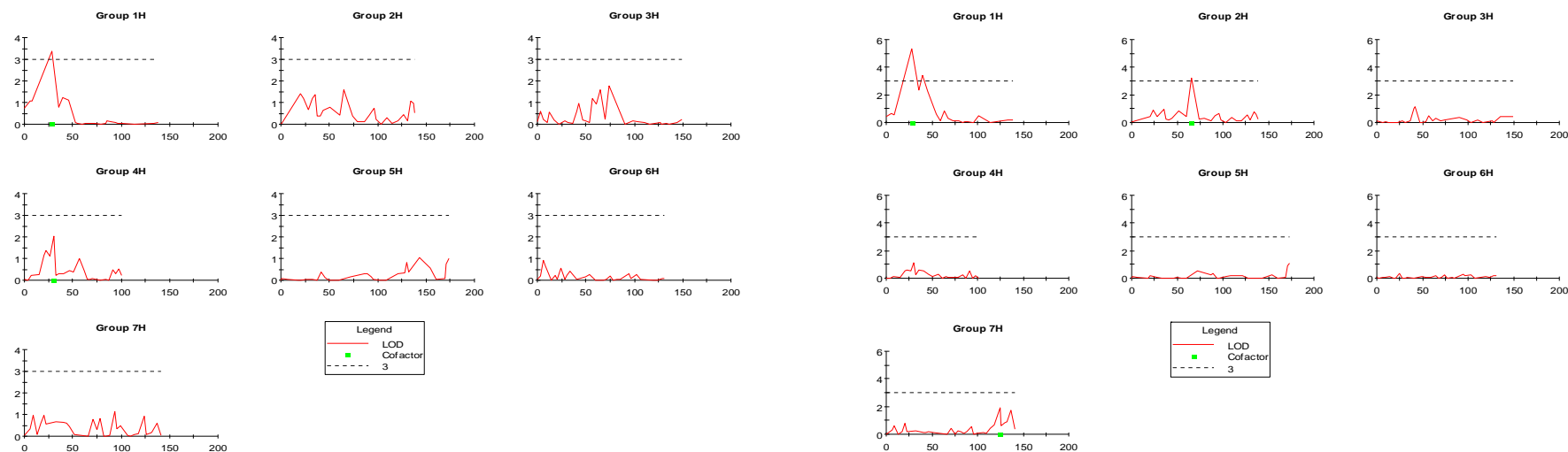
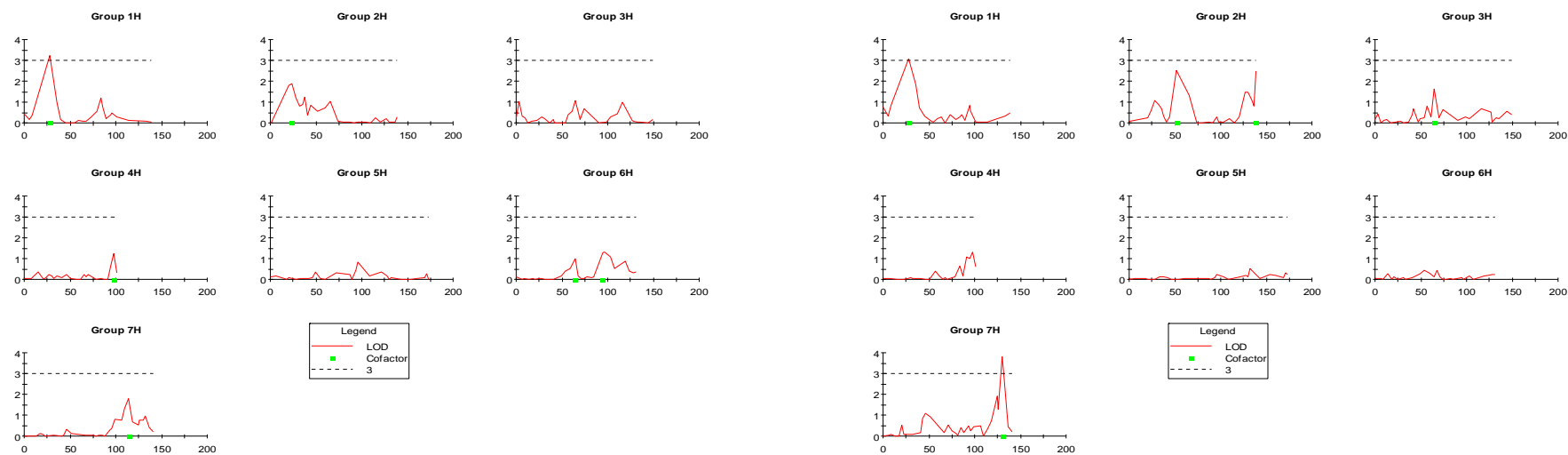a. Restricted MQM mapping using number of pustules (replicate 1).

b. Restricted MQM mapping using number of pustules (replicate combo).
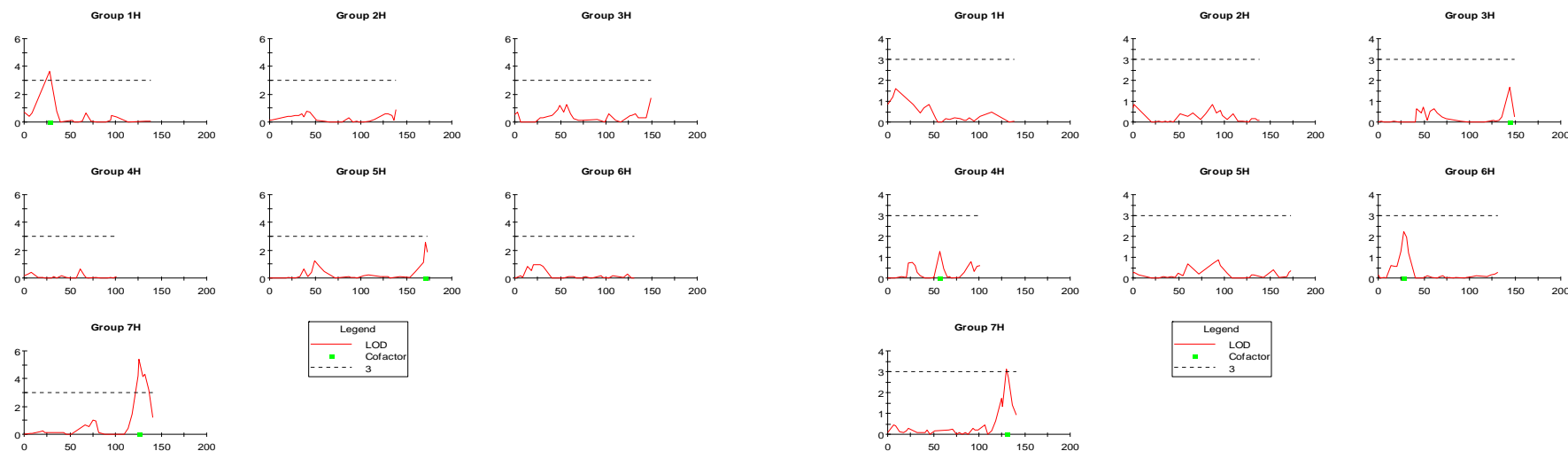
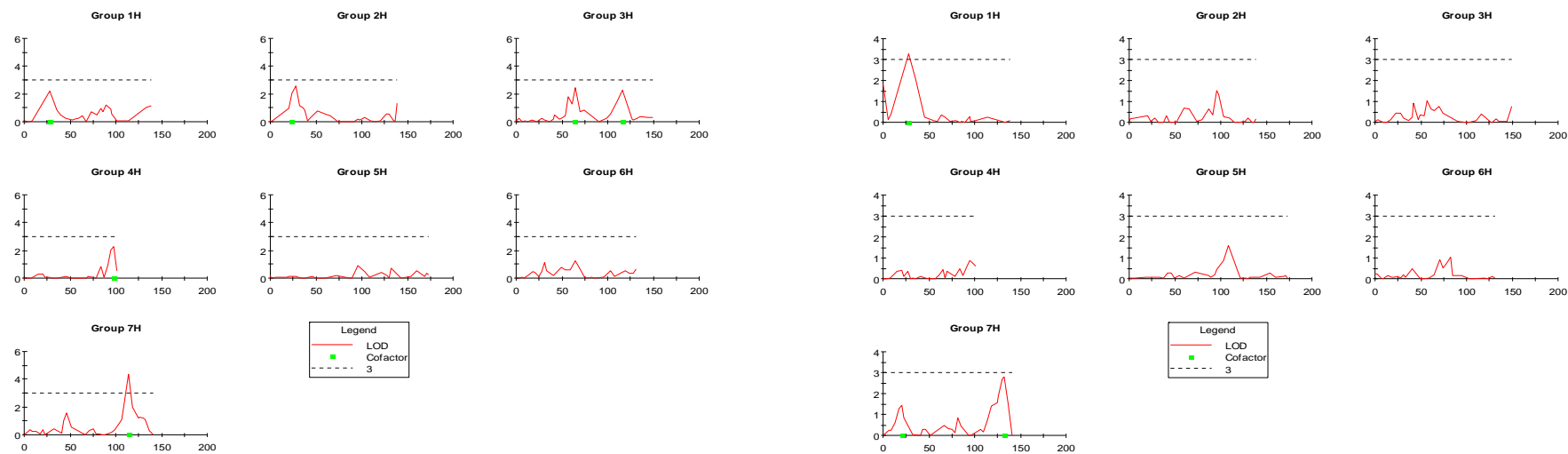c. Restricted MQM mapping using scale (Rients Niks replicate).

d. Restricted MQM mapping using LP low with missing data (replicate 3).

Appendix 6: LOD profiles of restricted MQM mapping for traits analysed in Vada × SusPtrit mapping population for *Pst*

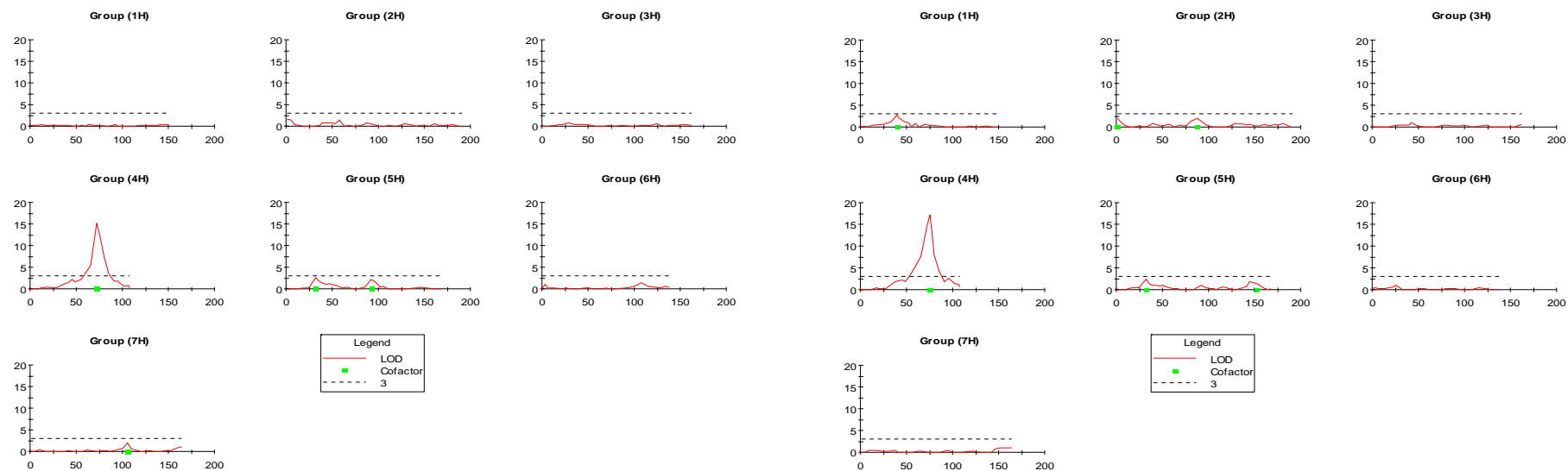a. Restricted MQM mapping using number of pustules (replicate 1).

b. Restricted MQM mapping using composite lesion length (replicate 2).

c. Restricted MQM mapping using number of pustules (replicate combo).

d. Restricted MQM mapping using rate of development (replicate 3).

Appendix 7: LOD profiles of restricted MQM mapping for traits analysed in L94 × Vada mapping population for *Psh*

a. Restricted MQM mapping using number of pustules.

b. Restricted MQM mapping using composite lesion length (mm).