

Genetics of Resistance of Barley to Quack Grass Crown Rust (*Puccinia coronata agropyrina*)

By

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**Thesis for Master of Sciences in Plant Sciences
Specialization Plant Breeding and Genetic resources**

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Summary

Crown rust is one of the fungal diseases of small grain cereals and grass species in the world. Recently, an isolate of crown rust, *Puccinia coronata agropyrina*, was found in Hungary on *Agropyron repens* (a grass species). In a preliminary barely seedling test, it appeared pathogenic to barley. The objectives of this study were five fold: first, to establish a preliminary host range of *Puccinia coronata agropyrina*; second, to determine the host status of barley: third, to map QTLs effective to *P. coronata agropyrina* in barley mapping populations Vada x SusPtrit (VxS) and Cebada Capa x SusPtrit (CCxS); fourth, to compare those QTLs with other QTLs mapped to other rusts in the same populations; and fifth, to determine the possible mechanism for the resistance involved.

A host range test on 36 grass and cereal species represented by 77 accessions showed that 23 of the species had a susceptible Infection Type (score 3 and 4) while 14 species had a resistant Infection Type (score 0-2). At genus level, *Aegilops*, *Agropyron*, *Bromus*, and *Hordeum* were susceptible, while *Avena*, *Dactylis*, *Lolium*, *Secale*, and *Triticum* were resistant. Among the susceptible species, percent of susceptible accessions ranged from 14.3 to 100.

A test at seedling stage on 108 barley accessions of diverse geographic origin showed that 83 % of them were fully susceptible (susceptibility score 4 and 5) and 14 % were moderately susceptible (score 3). Only 3% showed low susceptibility (score 2) and none were resistant (score lower than 2). A test on selected accessions at adult plant stage, however, showed that accessions with full or intermediate susceptibility at seedling stage appeared resistant.

In mapping experiments, 8 QTLs effective to *P. coronata agropyrina* were detected in VxS and CCxS mapping populations using Relative Latency Period and Relative Infection Frequency (four QTLs per mapping population). In both populations, transgressive segregation was observed for Relative Latency Period and Relative Infection Frequency indicating that both parents contributed the resistance/susceptibility alleles. The QTLs explained 60 % of the total phenotypic variation in both populations. Seven of the QTLs effective to *P. cor. agropyrina* co-localized with most of the QTLs effective to four heterologous rusts (*P. persistency*, *P. triticina*, *P. hor. secalini* and *P. hor. muruni*). Only a single QTL co-localized with a QTL for partial resistance (*Rphq4*).

The histology of the resistance, studied on 6 resistant and 3 susceptible lines, revealed nine classes of Infection Units under UV-microscopy. The lines varied significantly for four infection unit classes: Early aborted - host cell necrosis ($P=0.03$), Early aborted + host cell necrosis ($P<0.001$), Established medium sized + host cell necrosis ($P=0.04$) and Established large sized - host cell necrosis ($P<0.0001$). In a pair wise comparison of a resistant and susceptible line, higher proportions of the Early aborted $\pm$ host cell necrosis infection units were associated with most of the resistant lines while lower proportions were associated with all susceptible lines.

Chapter 1: Introduction

Types of Resistances in general

Resistance genes (both major and minor) are used as an option to protect crops from economically important diseases. In plant-pathogen system, two types of resistance (host and nonhosts) are known (Niks, 1987). Host resistance could be due to reduced level of basic compatibility between the pathogen and the plant genotype, or due to arresting further development of the pathogen in the presence of full basic compatibility (Niks, 1987). The nonhost type of resistance on the other hand is believed to be due to the absence of basic compatibility (Niks, 1982; 1987). Within the host resistance category, hypersensitive resistance and non-hypersensitive resistance (partial resistance) are recognized and known to be controlled by different sets of genes and they differ in durability. In barley for instance, hypersensitive resistance to leaf rust, also called race-specific resistance, is governed by major genes and is not durable (Parlevliet 1983). Partial resistance (PR) on the other hand is controlled by minor genes, has a polygenic inheritance and is not based on hypersensitivity (Parlevliet 1975).

Crown rust

Crown rust is one of the fungal diseases of small grain cereals and grass species in the world. It is caused by *Puccinia coronata* species complex in general. Buckthorn (*Rhamnus cathartica*) is the principal alternate host of crown rust (<http://www.ars.usda.gov/Maindocs>). The disease is commonly called 'Crown rust' due to the typical crown-like appendages on the apex of the teliospore (Szabo, 2006; Zambino and Szabo, 1993). Crown rust is most important where dews are frequent and temperatures are mild (15-25 C) during the oat growing season. (<http://www.ars.usda.gov/Main/docs.htm?docid=9919>). *Puccinia coronata* is a complex species that has a broad telial host range including more than 45 genera of grasses and a narrow aecial host range (Szabo, 2006).

Various varieties and forms are known to occur in cultivated small grain cereals and their wild types including other grass species. Some of them are *P. coronata* f. sp. *avenae*, *P. coronata* f. sp. *hordei*, *P. coronata* f. sp. *bromi*, *P. coronata* f. sp. *lolii* and *P. coronata* f. sp. *agropyri*, (<http://www.ars.usda.gov/Maindocs.htm?docid=9855>). *Puccinia coronata* f. sp. *avenae*

predominantly attacks oat (*Avena sativa*) causing the commonly known oat crown rust disease. It occurs worldwide wherever cultivated or wild oat species occur except in very arid areas. It is the most widespread and damaging disease of oat. During 1977-1980, Sebesta and Harder (1983) reported a wide spread infection of oats (*Avena sativa*) by *P. coronata* var. *avenae* over Europe with the most severe infections occurred in the southern part of the continent. *Puccinia coronata* f. sp. *hordei* infects barley (*Hordeum vulgare*) and rye (*Secale cereale*). The disease is named as crown rust of barley and was found first in a barley breeding nursery near Clay Center, Nebraska in 1992 (Jin and Stephenson 1999). According to Jin and Stephenson, the uredinial and telial states of this rust were found on many native and introduced gramineous species in Minnesota, North Dakota and South Dakota. *Puccinia coronata* f. sp. *bromi* is a recently described form in North America mainly prevalent on its host of origin *Bromus* sp. as described by Delgado et al. (2001) and Anikster et al. (2003). *P. coronata* f. sp. *loli* is a much known form of *P. coronata* complex and world wide problem on perennial rye grass (*Lolium perenne*). *P. coronata* f. sp. *agropri*, occurs on *Agropyron repense* (= *Elytrigia repens*) in North Dakota (Schwinghamer, 1955; Szabo, 2006). Quack grass (*Agropyron repens*) also called couch grass, or quick grass is a rapidly spreading grass of the family Poaceae. It is native to Europe and has been introduced into other north temperate areas for forage or erosion control. (<http://www.britannica.com/eb/article-9062130>).

Urban and Markova (1993) reviewed literature records on Crown rust mostly with respect to the geographical distribution, spore morphology and natural occurrence on various host plants in Europe. Their review showed that crown rust on *Agropyron repens* (L.) P. B has been observed in Russia, Ukraine, Finland, Sweden, Norway, Denmark, Lithuania, Poland, Czechoslovakia, E Germany, Rumania, Hungary, Switzerland, British Isles, Italy, and Madeira. They also indicated that there exist physiologic races alternating with *Frangula alnus* in some ecotypes like Sweden. In some other countries like Moravia Bohemia, however, *Rhamnus catharticus* is the alternate host.

Background of the study

In general, formae speciales of *P. coronata* rarely infect barley. Atienza et al. (2004) inoculated 109 barley accessions with *P. coronata* f. sp. *festucae*, *P. coronata* f. sp. *avenae*, *P. coronata* f. sp. *loli* and *P. coronata* f. sp. *holci* and reported a susceptibility score of 2 on 1%, 4%, 0% and 2% of the tested accessions respectively.

In Hungary, Niks has found an isolate of crown rust (*P. coronata agropyrina*) on *Agropyron repens*. A preliminary infection test at seedling stage showed that some barley lines like research line SusPtrit (Atienza et al. 2004) was extremely susceptible, while cultivars Cebada Capa and Vada were quantitatively resistant showing fewer pustules of susceptible infection type (Niks personal communication). Although this preliminary seedling test has shown that susceptibility in barley to *P. coronata agropyrina* may be common, it does not lead to comprehensive conclusion about the host status of barley to this rust. Therefore, extensive assessment for the host status quantification of barley using large number of accessions was necessary. Apart from that, no sufficient information is available about the host range of this pathogen, the genetics of the resistance and the associated possible mechanism involved (Niks personal communication).

Conducting a pathogenicity test on a collection of various grass and cereal species could help to quantify the host range of *P. coronata agropyrina*. On other hand, lines that are susceptible may still differ quantitatively in level of susceptibility and their difference can be captured by measuring the Latency period (LP) (Parlevliet, 1979). Moreover, parental pairs of mapping populations may differ in resistance/susceptibility (either complete resistance or difference in LP). Therefore, screening of mapping populations derived from such parental lines allows mapping of the loci for the resistance involved. Further more, histological investigation on resistant and susceptible lines could unravel the possible mechanism underlying the resistance.

Objectives of the study

- To establish a preliminary host range of *P. coronata agropyrina*
- To quantify the host status of barley
- To map QTLs effective to *P. coronata agropyrina* in Barley mapping populations Vada x SusPtrit (VxS) and Cebada Capa x SusPtrit (CCxS)
- To compare those QTLs with other QTLs mapped to other rusts in the same populations
- To determine the mechanism of resistance

In this study, four separate experiments were conducted to achieve the objectives stated above.

Chapter 2: Determination of Host range of *P. cor. agropyrina*

2.1 Materials and Methods

2.1.1 Plant material

The genera *Aegilops*, *Agropyron*, *Bromus*, *Hordeum*, *Avena*, *Dactylis*, *Lolium*, *Secale*, and *Triticum* were used to determine the hostrange of *Puccinia coronata agropyrina*. They encompassed, 36 grass and cereal species which in total represented by 77 accessions. Forty-six of the accessions were obtained from the collection of planting materials in the barley research unit of Wageningen University Department of Plant Breeding. Thirty accessions were kindly provided by USDA, ARS, WRPIS (Washington State University Regional Plant Introduction Station). More accessions of *Hordeum* and *Bromus* were used to compare the host range of this pathogen with hostrange of other forms of crown rust that specialize on *Hordeum* and *Bromus*. List of the species, accessions and their origin is given in Appendix 1.

2.1.2 Pathogen material and multiplication

Puccinia coronata agropyrina was used for the various infection experiments. It was collected in Hungary from *Agropyron repens* (Niks, personal communication). Fresh spores were multiplied on the host plant species *Agropyron repens* by dusting the inoculum (urediospores + Lycopodium powder) on the leaves using a brush. The inoculated plants were kept in an incubation cell under 100% relative humidity and 17 °C for 10 hours of darkness so that spores could germinate. After thirteen days of incubation in a greenhouse compartment, spores were ready and were collected using a cyclone spore collector. Before using them for inoculation, the spores were stored in desiccator for 2 - 7 days so that excess water could evaporate.

2.1.3 Inoculation and Incubation

The accessions of grass species and cereals mentioned above were subjected to an infection experiment. About 20 seeds of each grass and 10 seeds of the cereals were sown in 11 cm diameter plastic pot and seedlings were raised in a greenhouse compartment. The conditions for compartment were 20 °C & 16 °C during light and dark periods respectively; 16 hours of artificial light under 70% Relative Humidity throughout the experiment. After reliable seedling germination was found, one to four seedlings per accession were transplanted on rectangular planting boxes (37 cm wide, 39 cm long). As they took longer time to germinate, the grasses were transplanted two weeks after sowing, whereas the cereals were transplanted

one week after sowing. For the grasses inoculation was done about three weeks after sowing while for the cereals twelve days after sowing. In both cases, each seedling of the respective accessions was laid down in a horizontal position in such a way that the adaxial surface of the primary leaf face upwards. The inoculum was prepared by mixing 1 part of urediospores with 10 parts of lycopodium to achieve nearly a homogenous distribution of the spores. The inoculation was carried out in a settling tower (Hoogkamp et al., 1998) by applying 5 mg of urediospores per box. This is assumed to result in a deposition of 300 urediospores per cm² (Atienza et al., 2004). To trigger spore germination, the inoculated plants were incubated in a dew chamber under the same sets of conditions used for the multiplication of the pathogen. The next morning the boxes were transferred to another greenhouse compartment to stimulate development of uredia and sporulation and they stayed there until evaluation. The conditions of this compartment were the same as described for the seedling growing compartment.

2.1.4 Evaluation

Thirteen days after inoculation, seedlings were evaluated for reaction to the pathogen based on the 0 to 4 Infection Type (IT) scoring scale. As Niks (1987) reviewed, the values in this scale are given in the following description: 0: no symptom (immune), 0+: necrotic or chlorotic flecks (highly resistant), 1: minute pustules surrounded by necrosis or chlorosis (resistant) 2: small pustules surrounded by necrosis or chlorosis (moderately resistant), 3: medium sized pustules surrounded by some chlorosis (moderately susceptible), 4: large pustules, no chlorosis (susceptible). IT's of 0, 0+, 1, or 2 are considered as indicative of a resistant host response, whereas IT's of 3 and 4 are considered as a susceptible host response. An accession was considered susceptible when at least one plant showed susceptible IT (3 or 4). A species was considered susceptible (Host) when at least one accession was susceptible; otherwise Non-host. A genus was considered susceptible when at least one of its species is susceptible.

2. 2 Results

2.2.1 Pathogenicity of *P. cor. agropyrina* to the grass and cereal species

Of the grasses and cereal species, the genus *Aegilops*, *Agropyron*, *Bromus*, *Hordeum* contained susceptible species, while tested accessions of species in the genera *Avena*, *Dactylis*, *Lolium*, *Secale*, and *Triticum* were resistant (Table 1). Almost all the *Hordeum* and most of the *Bromus* species contained susceptible accessions.

Table 1. Host range of *Puccinia coronata agropyrina*: an isolate from quack grass (*Agropyron repens*)

Species	Number of plants	Number of accessions	% of Susceptible accessions.	IT ¹	Status of plant species
<i>Aegilops columnaris</i> Zhuk.	4	1	0	0;	Non-Host
<i>A. comosa</i> Sibth. et Sm.	9	3	67.7	4,0;	Host
<i>A. peregrine</i>	11	3	33.3	0;,3	Host
<i>A. speltoides</i>	14	7	14.3	0,1,0;,2,4	Non-Host
<i>Agropyron repens</i>	26	1	100	4,3	Host
<i>Avena sativa</i>	7	2	0	0	Non-Host
<i>Bromus carinatus</i>	7	2	0	1,2,0;	Non-Host
<i>B. catharticus</i> var. <i>catharticus</i>	4	1	0	0;	Non-Host
<i>B. danthoiae</i>	4	1	100	1,4	Host
<i>B. erectus</i>	8	2	100	0,3	Host
<i>B. inermis</i> subsp. <i>Inermis</i>	15	4	0	0,0;,2	Non-Host
<i>B. japonicus</i>	12	3	67.7	2,4,0;	Host
<i>B. mango</i>	4	1	0	2,1	Non-Host
<i>B. scoparius</i>	8	2	100	4	Host
<i>B. species</i>	8	2	100	4	Host
<i>B. tectorum</i>	10	3	100	4	Host
<i>Dactylis glomerata</i>	5	1	0	0	Non-Host
<i>Hordeum bogdanii</i>	1	1	100	4	Host
<i>H. brevisubulatum</i> subsp. <i>Violaceum</i>	2	1	100	4	Host
<i>H. bulbosum</i>	31	12	75	4,1,0;,2,3	Host
<i>H. chilense</i>	4	1	100	4	Host
<i>H. comosum</i>	1	1	100	4	Host
<i>H. jubatum</i>	8	2	100	4	Host
<i>H. lechleri</i>	4	1	100	4	Host
<i>H. marinum</i>	4	1	100	4	Host
<i>H. murinum</i>	4	1	100	4	Host
<i>H. parodii</i>	4	1	100	4	Host
<i>H. procerum</i>	4	1	100	4	Host
<i>H. pusillum</i>	4	1	100	4	Host
<i>H. secalinum</i>	8	2	0	0	Non-Host
<i>H. stenostachys</i>	6	2	100	4	Host
<i>H. vulgare</i> ²	300	108	95	4,3,1,2	Host
<i>Lolium perenne</i>	4	1	0	0	Non-Host
<i>Lolium westerwolds</i>	4	1	0	0	Non-Host
<i>Secale cereale</i>	4	1	0	0;	Non-Host
<i>Triticum aestivum</i>	15	6	0	0;	Non-Host
<i>T. boeoticum</i>	2	1	0	0;	Non-Host

¹ Infection type score: 0: no symptoms (immune), 0;: necrotic or chlorotic flecks (highly resistant), 1: minute pustules surrounded by necrosis or chlorosis (resistant), 2: small pustules surrounded by necrosis or chlorosis (moderately resistant), 3: medium sized pustules surrounded by some chlorosis (moderately susceptible), 4: large pustules, no chlorosis (susceptible); IT of 0-2 are considered resistant host reactions, while 3-4 is considered susceptible host reaction. Many infection type scores together indicate segregation occurred within or among accessions; values are given in decreasing order with the most frequent IT score put first.

² the data for *Hordeum vulgare* was included from the result of the host status experiment just to be complete

2.3 Discussion

Among the susceptible genera, the genus *Hordeum* was found to be the most susceptible genus where 14 out of the 15 species (93.3%) contained susceptible accessions. Only one species (*H. secalini*) showed a resistant infection type. Almost within all species of this the *Hordeum*, 100% of the accessions were susceptible with the most frequent infection type being 4. Only within two of the species segregation for susceptibility/resistance was observed. This shows that the genus could potentially be a predominant host of *P. cor. agropyrina* nearly as important as the host species *Agropyron repens*. Likewise, a great percentage (60%) of the species of the *Bromus* was found to be susceptible. However, only half of the susceptible species attained 4 as the most frequent IT score. Besides, more segregation occurred within/among susceptible accessions compared to *Hordeum*. This suggests that *P. cor. agropyrina* probably be more close to a form of crown rust that specializes on *Hordeum* than on *Bromus*. The *Aegilops* on the other hand was the least susceptible genus where both susceptible species involved segregation for infection type. Only one of the species attained 4 as the most frequent IT score while the other exhibited 0;. Among the resistant genera, *Avena sativa* and the *Lolium* sp. were characterized by immunity (IT=0) while *Secale cereale* and *Triticum* were found to be highly resistant (IT=0;) with no segregation for resistance with in /among their accessions. In general, of the 36 species tested in this study 64% were described as hosts while the remaining 36% were non-hosts of *P. cor. agropyrina*.

Comparison of the present result with host range studies of some crown rust forms/isolates gives information about the identity of *P. cor. agropyrina* (Table 2). Anikster et al. (2003) tested pathogenicity of *P. coronata* f. sp. *bromi*, *P. coronata* var. *hordei*, and *P. coronata* var. *avenae* on cereals and grass species of which 12 are included in the present study. The isolates in their study were mainly from USA and some from Israel. They reported that the host range of *P. coronata* f. sp. *bromi* was restricted to *Bromus inermis*, *B. japonicus*, *B. tectorum*, and *B. scoparius*. All the *Hordeum* spp. were resistant besides *Avena*, *Secale cereale*, *Triticum* and *Agropyron repens* (= *Elytrigia repens*). In the present study, three of these *Bromus* spp. and all the *Hordeum* spp. appeared to be susceptible to *P. cor. agropyrina*.

Compared to the data (Anikster et al., 2003) for *P. cor. var. hordei*, Almost all (seven out of eight) the susceptible species to *P. cor. var. hordei* were also appeared susceptible to *P. cor. agropyrina*. Another report by Jin and Steffenson (1992) indicates some host range difference

Table 2. Host range of *Puccinia coronata agropyrina* compared to *P. cor. f. sp. bromi*, *P. cor. var. hordei* and *P. cor. var. avenae*.

Species	Infection Type (IT) ¹				
	<i>P. cor. agropyrina</i>	<i>P. cor. f. sp. bromi</i> ²	<i>P. cor. var. hordei</i> ²	<i>P. cor. var. avenae</i> ²	<i>P.c. var. hordei</i> ³
<i>Aegilops columnaris</i> Zhuk.	0;				
<i>A. comosa</i> Sibth. et Sm.	4,0;				
<i>A. peregrina</i>	0;;3				
<i>A. speltoides</i>	0,1,0;;2,4				
<i>Agropyron repens</i> ⁴	4,3	0	3	0;	43
<i>Avena sativa</i>	0	0	0;	3+	00;/3
<i>Bromus carinatus</i>	1,2,0;				
<i>B. catharticus</i> var. <i>catharticus</i>	0;				
<i>B. danthoiae</i>	1,4				
<i>B. erectus</i>	0,3				
<i>B. inermis</i> subsp. <i>Inermis</i>	0,0;;2	3	0	0	
<i>B. japonicus</i>	2,4,0;	3	3	0	34/23
<i>B. mango</i>	2,1				
<i>B. scoparius</i>	4	3	3	0	
<i>B. species</i>	4				
<i>B. tectorum</i>	4	3,3+	1,2	;N	34/21
<i>Dactylis glomerata</i>	0				
<i>Hordeum bogdanii</i>	4				4
<i>H. brevisubulatum</i> subsp. <i>violaceum</i>	4				
<i>H. bulbosum</i>	4,1,0;;2,3	;N	3	0; ,1	43/0;
<i>H. chilense</i>	4				43
<i>H. comosum</i>	4				4
<i>H. jubatum</i>	4				34
<i>H. lechleri</i>	4				43
<i>H. marinum</i>	4	;N-	3	0;	43
<i>H. murinum</i>	4				43
<i>H. parodii</i>	4				43
<i>H. procerum</i>	4				43
<i>H. pusillum</i>	4	0	3	0;	
<i>H. secalinum</i>	0				
<i>H. stenostachys</i>	4				
<i>H. vulgare</i>	4, 3, 1, 2	;N, 0;;N	3	;N, 0;C, ;N	34/0;
<i>Lolium perenne</i>	0				0/12
<i>Lolium westerwolds</i>	0				
<i>Secale cereale</i>	0;	0;	3	0;	23/0;
<i>Triticum aestivum</i>	0;	0;	0;	0;	0;/32
<i>T. boeoticum</i>	0;				

¹ Infection Type is as described for Table 1. But with slight difference in description among the Authors of the results presented for the other rusts: 0; C = large chlorotic flecks, N= necrotic flecks, ";" = necrotic flecking without sporulation.. "/" segregation occurred within or among accessions with the most prevalent IT presented first.

^{2,3} Data extracted from; Anikster, et al. (2003) and Jin & Steffenson (1992) respectively.

⁴ *Agropyron repens* is synonymous to *Elytrigia repens*; hence, data corresponding to *Elytrigia repens* was adopted from the respective sources.

between our isolate and *P. cor. var. hordei*. Jin and Steffenson (1992) evaluated the disease reactions of 87 gramineous species to *P. cor. var. hordei* of which 18 (Table 3) were included in the present study. Similar to their report for these 18 species (Table 3), all the *Hordeum* and some *Bromus spp.* which were susceptible to *P. cor. var. hordei* were also susceptible to *P. cor. agropyrina* in the present study. However, *Secale cereale*, *Avena sativa* and *Triticum aestivum* were resistant to *P. cor. agropyrina* despite their susceptibility to *P. cor. var. hordei*. Unlike the report of Anikster et al. (2003), the later two genera were susceptible to *P. cor. var. hordei* indicating that it has relatively wider host range than *P. cor. agropyrina*.

The fact that *Secale cereale* and some *Triticum spp.* are susceptible to a form/an isolate of crown rust is also supported by reports of other studies in USA/Canada. Schwinghamer (1955) described a crown rust pathogen observed on *Agropyron spp.* in eastern North Dakota and in western Minnesota. He reported that *Secale cereale* was also susceptible. Similarly, Sampson and Watson (1985) determined the host range of *Agropyron* (Quack grass) isolates of *P. coronata*, and reported that *Secale cereale* and *Triticale* were susceptible. This implies that forms of crown rust in USA and Canada could have wider host range compared to European crown rust that occur on *Agropyron*. On the other hand, the data presented (Anikster et al., 2003) for *P. cor. var. avenae* clearly shows that all the species susceptible to *P. cor. agropyrina* are hardly infected by *P. cor. var. avenae*. Rather, *Secale cereale* and *Triticum aestivum* occur as resistant species for both of them. Therefore, *P. cor. agropyrina* is undoubtedly different from *P. cor. var. avenae* at least in host range.

2.4 Conclusion

Based on the comparison on the limited host range data, *Puccinia coronata agropyrina* has narrow host range compared to *P. cor. var. hordei*, but wider host range compared to *P. cor. f. sp. bromi* and *P. cor. var. avenae*.

Due to the limited literature based comparison we made, the conclusion on the degree of similarity between the European and the American isolates would be rather weak. Therefore, it is difficult to conclude that *P. cor. agropyrina* is more similar in host range to either *P. cor. f. sp. bromi* or *P. cor. var. hordei*. So, we would rather say that, it could be a different form of *P. coronata species* complex which combines some features of *P. cor. f. sp. bromi* and *P. cor. var. hordei*.

Chapter 3: Determination of Host status of barley accessions to *P. cor. agropyrina*

3.1 Materials and Methods

3.1.1 Seedling stage testing

Plant material

A collection of 108 barley accessions of different geographical origin and type were used for quantification of the host status of barley. The accessions were almost the same set of lines as used by Atienza et al., (2004) to determine the host status of barley to several heterologous rusts. A list of the accessions including their description is given in Appendix 2.

Inoculation and Incubation

Seedlings of 10 accessions were directly grown in a box of 37x39 cm. In each box, the grass *Agropyron repens* (the host of the pathogen) and the experimental line SusPtrit (Atienza et al., 2004) were grown as a reference. Per accession, 5 seedlings were grown and later were cut in to 3 seedlings. Inoculation and incubation were done as described before.

Evaluation and data analysis

Thirteen days after inoculation, number of pustules and flecks without pustules per infected leaf were estimated instead of direct counting which was quite laborious and time consuming. In some cases, where the number of pustules was few, actual count was taken. The count data obtained was converted to the 0 to 5 scale of Atienza et al. (2004). The number of pustules per leaf together with the extent of flecks, averaged over three seedlings per accessions, was considered to reflect the level of susceptibility of each accession. The 0 - 5 susceptibility score was used as in the following description: 0: immune or near immune (less than 3 pustules and no or few flecks); 1: less than 3 pustules and medium or many flecks, 2: 3–10 pustules; 3:10–100 pustules; 4: 100 -500 pustules, 5 more than 500 pustules. Score values of 2 or higher were considered a (somewhat) susceptible reaction (Atienza et al., 2004). Where accessions showed a hypersensitive reaction, the response was evaluated according to the 0 to 4 Infection Type (IT) score as described in chapter 1.

The level of susceptibility of the tested barley accessions was presented by calculating the proportion of accessions per each susceptibility score. For better understanding of the result, data of the susceptible reference lines were also presented together. The level of susceptibility reported in the present study was also compared with susceptibility to heterologous rusts reported in other studies.

3.1.2 Adult plant testing

Plant material

Based on the result obtained at seedling stage, 6 accessions were selected and tested to see the level of susceptibility at adult plant stage. Three of the selected accessions (SusPtrit, SusPmur, and Trigo Biosa) were from the highly susceptible (score 4 & 5) and the other three (Vada, C118 and Bavaria) were from the moderately (score of 3) susceptible classes.

Inoculation and Incubation

Per accession, 5 plants were grown in pots of 11 cm diameter which later were reduced in to 2 plants. Each accession was replicated 10 times. About one and half month after sowing, inoculation was done by spraying the inoculum on a marked spot of the first leaf from the top of the plant. Incubation was carried out as described before.

Evaluation and data analysis

Also for adult plants, thirteen days after inoculation, the accessions were evaluated for their reaction using the 0-4 Infection Type scoring scale as described in chapter 1. Proportions of plants showing susceptible/resistant reaction for seedling stage were compared with that of the adult stage to find out the changes occurred in the level of susceptibility.

3.2 Results

3.2.1 Susceptibility across accessions

All the tested accessions were susceptible to *P. cor. agropyrina* as per the 0-5 susceptibility score. From the total accession, 83 % showed susceptibility score of 4 and 5 (full susceptibility) while 14 % and 3% showed 2 and 3 (low and intermediate susceptibility) respectively. None of the accessions showed susceptibility score of 0 and 1 (Table 3). The

value 1% for the accessions is attributed to SusPmur which showed extreme susceptibility (Score of 5 i.e. >500 pustules per leaf) in all the plants tested.

Table 3. Percentage of barley accessions and susceptible references per susceptibility score¹ for *Puccinia coronata agropyrina*.

Tested lines	Percentage of accessions/plants					
	0	1	2	3	4	5
Barley accessions	0	0	3	14	82	1
SusPtrit ²	0	0	0	0	69	31
<i>Agropyron repens</i> ³	0	0	0	10	87	3

¹Susceptibility score per leaf:

0: Immune or near immune (less than 3 pustules and few flecks), 1: less than 3 pustules and medium or many flecks, 2: 3-10 pustules, 3: 10-100 pustules, 4: 100-500 pustules, 5: More than 500 pustules. Score values of 2 or higher were considered a susceptible reaction (Atienza et al., 2004). Susceptibility score values are highlighted in bold

²a susceptible experimental line (SusPtrit) used as reference for susceptibility of barley to *P. cor. agropyrina*.

³susceptible host species (*Agropyron repens*)

Three accessions, Albert, Archer and Decortdatum showed exceptionally low level of susceptibility (susceptibility score of 2) which covered the 3% of the total (Table 3). The research line SusPtrit, showed a higher susceptibility (more plants with score of 5) than the host species *Agropyron repens* (mainly score of 4 but a single plant had score of 5).

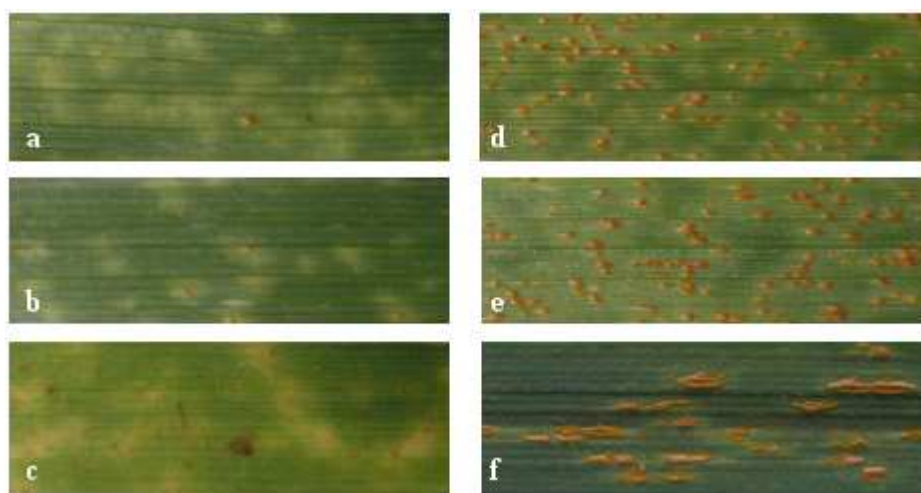


Figure 1. Barley lines of exceptional susceptibility to *Puccinia coronata agropyrina*: cultivars Albert (a), Archer (b); research lines Decortdatum (c) and SusPmur (d) as compared to a susceptible line SusPtrit (e) and a host species *Agropyron repens* (f).

3.2.2 Susceptibility by different categories of accessions: level of agronomic application, origin and morphological traits

Accessions from land races and research lines showed most of the susceptibility scores (2, 3, 4 & 5) while cultivars released before and in/after than 1945 showed susceptibility score of 2, 3 & 4. The wild species (*H. spontaneum*) exclusively showed a susceptibility score of 4. Accessions of African, Asian and South American origin showed nearly similar susceptibility score except that a single accession of African origin attained a score of 2.

Table 4. Level of susceptibility¹ (%) of barley accessions of various categories per susceptibility* score for *Puccinia coronata agropyria*.

Categories	Number of accessions	Percentage of accessions					
		0	1	2	3	4	5
Level of agronomic application							
Wild species (<i>H. spontaneum</i>)	5	0	0	0	0	100	0
Line from landrace and research lines	31	0	0	3	23	71	3
Cultivars	59	0	0	3	9	88	0
Unknown	13	0	0	0	23	77	0
Origin²							
Africa	10	0	0	10	30	60	0
Asia	12	0	0	0	8	92	0
Europe	56	0	0	4	12	82	1
North America	6	0	0	0	0	100	0
South America	13	0	0	0	23	77	0
Unknown	7	0	0	0	14	86	0
Morphological traits							
Awed	99	0	0	3	11	86	0
Awnless	3	0	0	0	100	0	0
Unknown	6	0	0	0	17	67	17
Six rowed	32	0	0	3	13	84	0
Two rowed	70	0	0	3	14	83	0
Unknown	6	0	0	0	17	67	17
Naked	10	0	0	10	10	80	0
Non naked	96	0	0	2	15	83	0
Unknown	2	0	0	0	0	50	50
Black ²	7	0	0	29	0	71	0
White ²	95	0	0	1	16	83	0
Unknown ²	2	0	0	0	0	50	50
All accessions	108	0	0	3	14	82	1

¹ Susceptibility refers to at least 3 pustules per leaf; Susceptibility score values are highlighted in bold

² Accessions of *Hordeum spontaneum* are not included for the corresponding trait does not apply to them

Modern cultivars of North American origin showed solely higher level of susceptibility (score of 4). On the other hand, The European origin cultivars and research lines covered susceptibility score of 2-5. From morphological trait perspective, susceptibility appeared to be common among the accessions of different subcategories. Except for number of spike rows, for each pair of the other traits the accessions considerably differed in susceptibility and were described by score of 3 and 4.

3.2.3 Hypersensitivity

A large proportion of the tested accessions showed susceptible infection type despite limited hypersensitivity exhibited by very few of them. Only 5 % of the total showed resistant infection type; while the remaining 95% were susceptible. None of them appeared to be Immune. Depending on the degree of chlorotic and necrotic flecks combined with the size of the pustules, cultivars Albert, Archer, L92, L98, and the research line Decortdatum showed a resistant infection type.

Table 5. Percentage of barley accessions and susceptible references per classes of infection type for *Puccinia coronata agropyrina*.

Tested lines	Infection Type score ¹					
	0	0;	1	2	3	4
Barley accessions	0	0	4	1	11	84
SusPtrit ²	0	0	0	0	0	100
<i>Agropyron repens</i> ³	0	0	0	0	0	100

¹ Infection type score per leaf:

0: no symptoms (Immune), 0; : necrotic or chlorotic flecks (highly resistant), 1: minute pustules surrounded by necrosis or chlorosis (resistant), 2: small pustules surrounded by necrosis or chlorosis (moderately resistant), 3: medium sized pustules surrounded by some chlorosis (moderately susceptible), 4: large pustules, no chlorosis (susceptible). Score values of 0, 0;, 1, and 2 are considered as indicative of a resistant host response, whereas IT's of 3 and 4 are considered as a susceptible host response. IT score values are highlighted in bold.

^{2&3} Reference lines as described in Table 3

3.2.4 Susceptibility at Seedling stage compared to Adult plant stage

All the tested accessions were susceptible during the seedling stage showing Infection Type score of 3 and 4 while attaining considerable level of resistance in the adult stage (Table 6; Figure 2). Percentage of susceptible plants per selected accession at adult stage ranged from 0% (Bavaria) to 65 % (SusPtrit). In other words, the percentage of plants showing resistant infection type (IT scores 0-2) rose from 35% (SusPtrit) to 100% (Bavaria).

Table 6. Percentage of plants showed susceptible and resistant Infection type* for *Puccinia coronata agropyrina* per selected accessions tested at seedling and adult plant stages.

Accessions	Susceptible(IT= 3-4)		Resistant (IT= 0-2)	
	seedling	adult	seedling	adult
SusPtrit	100	65	0	35
SusPmur	100	40	0	60
Trigo Biasa	100	25	0	75
Vada	100	5	0	95
C 118	100	5	0	95
Bavaria	100	0	0	100

* Infection Type score: as described in Table 5

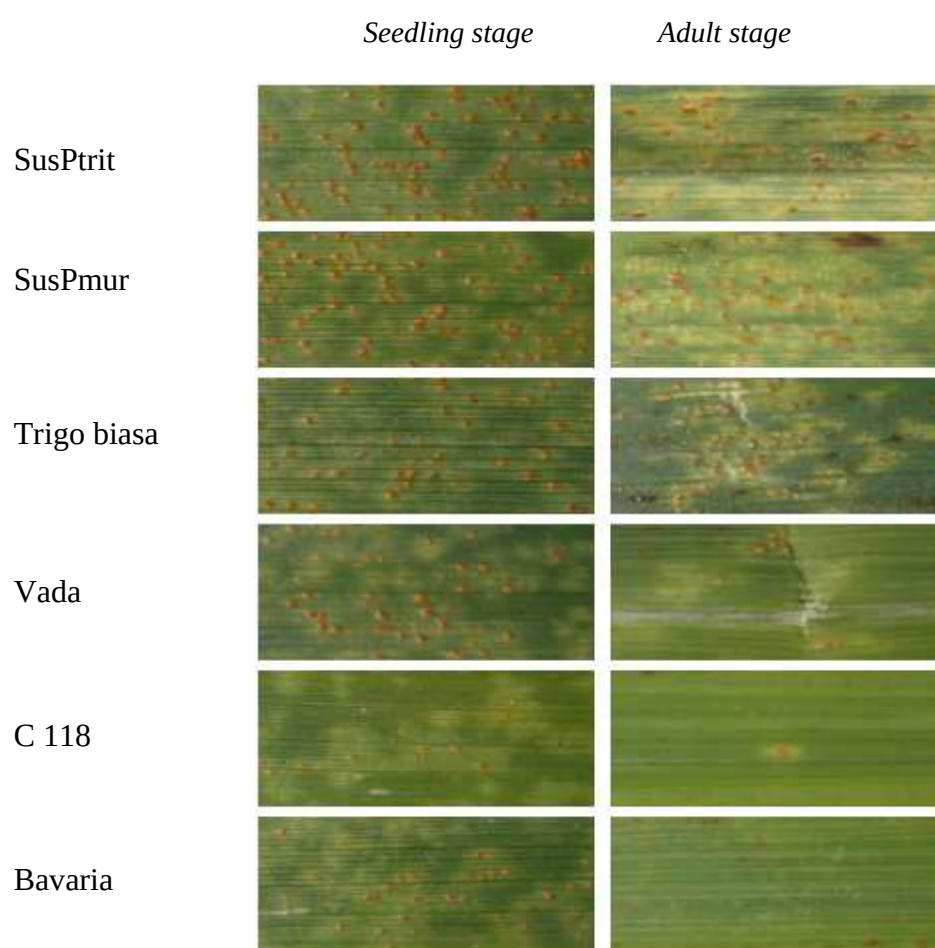


Figure 2 Uredia of *P. cor. agropyrina* on selected barley accessions at seedling and adult plant stage: the pictures with visible urediospores for the adult stage represent the few plants showing the susceptible IT out of 20 plants tested per accession.

Table 7. Susceptibility of barley accessions to *P. coronata agropyrina* compared with susceptibility to other ¹ heterologous rust species and *P. hordei* at seedling stage.

Rust species	Susceptibility score ²					
	0	1	2	3	4	5
<i>P. agropyrina</i>	39	7	9	37	8	0
<i>P. triticina</i> Flamingo	55	10	19	13	3	0
<i>P. triticina</i> French	54	10	17	19	0	0
<i>P. hordei.-secalini</i>	30	37	9	16	7	1
<i>P. graminis</i> f. sp. <i>lolii</i>	72	0	14	11	3	0
<i>P. hordei.-murini</i> Rhenen	59	27	3	3	8	0
<i>P. hordei.-murini</i> Cordoba	31	11	44	8	6	0
<i>P. coronata</i> f.sp. <i>festucae</i>	67	32	1	0	0	0
<i>P. coronata</i> f. sp. <i>Avenae</i>	67	29	4	0	0	0
<i>P. coronata</i> f. sp. <i>lolii</i>	67	33	0	0	0	0
<i>P. coronata</i> f.sp. <i>holci</i>	67	31	2	0	0	0
<i>P. holcina</i>	97	3	0	0	0	0
<i>P. bromina</i>	98	2	0	0	0	0
<i>P. recondita</i> f.sp. <i>recondita</i>	100	0	0	0	0	0
<i>P. hordei</i> (1.2.1)	3	0	0	5	66	26
<i>P. coronata agropyrina</i>	0	0	3	14	82	1

¹ Data for susceptibility to other rusts were adopted from Atienza et al., (2004)

² Susceptibility score per leaf: as described in Table 3

3.3 Discussion

The full susceptibility exhibited by a great percentage of the accessions leads us to consider barley as a host of *P. cor. agropyrina*. Nearly, a susceptibility level (83%) as high as the host species *Agropyron repens* (90%) was observed among the accessions. Compared to SusPtrit (the susceptible barley line used as reference), still larger percentage of the accessions were fully susceptible. Nevertheless, there were more with susceptibility score 5 in SusPtrit than in the accessions. In any case, the lower percentage of low / intermediate susceptible classes (17%) and the higher percentage of the fully susceptible classes (83%) clearly suggest that barley could be regarded as a true host of *P. coronata agropyrina*.

Exceptionally, SusPtrit and SusPmur have displayed an extreme susceptibility higher than any of the accessions including the host species (*Agropyron repens*). This may be attributed to difference in the pattern of uredia (forms some how individual uredia on barley but rows on *Agropyron*) on the leaves which may lead to underestimation of number of pustules. Besides, the narrow leaf area of *Agropyron* might have contributed to the lower number of pustules per leaf and hence to the observed difference. On the other hand, it may be true that SusPtrit and SusPmure are more susceptible than the host species. These lines were developed to study the mechanism and inheritance of nonhost instance in barley to heterologous rusts (Atienza et al.,

2004). They were meant for accumulation of susceptibility for target pathogens *P. triticina* and *P. hordei-muruni* respectively. However, in the end, the authors reported that the lines were also susceptible to other non-target heterologous rust pathogens. So, this accumulated gene dose for susceptibility may also be the cause for their extreme susceptibility to *P. coronata agropyrina* as well. On the other extreme, cultivars Albert, Archer and the research line Decortdatum appeared as the lines with the lowest level of susceptibility compared to any of the accessions tested (figure 1). Albert and Archer reacted in such a way that there would be very little number of small sized sporulating pustules accompanied by many flecks. Decortdatum however, restricted the pustule development to a very minute size and was accompanied by many chlorotic and necrotic flecks. Sporulation in this case was relatively rare.

Evaluation on the level of hypersensitivity among the tested accessions had distinguished as to which type of the host resistance was prevalent. Obviously, hypersensitivity was limited to two modern cultivars (Albert and Archer respectively) and two landraces and a research line (L92, L98, and Decortdatum). This hints that in barley, complete resistance to *P. cor. agropyrina* is less likely than Partial resistance. The variation among the accessions seems to be mainly due to quantitative difference rather than qualitative. The fact that the hypersensitivity is limited to modern cultivars and landraces also suggests that they may be related in pedigree.

The level of agronomic application of the accessions seemed to be associated with level of genetic diversity for resistance/susceptibility to the pathogen under study. Accordingly, with in the category of lines from land races and research lines, susceptibility score of 2-5 were represented although the accessions cover only 31% of the total. However, with in the cultivars, covering 59% of the total accessions, score of 2-3 were represented of which 80 % is score value of 4. This indicates that there is a wider genetic diversity in resistance/susceptibility to *P. cor. agropyrina* among landraces and research lines compared to among modern cultivars. This is expected because modern cultivars are improved lines in which the resistant aspect is largely exploited from any possible source through recombination and put together in to one line. That creates a narrow variation among them compared to among the land races.

To some extent, susceptibility seemed to be more allied to accessions of Asian, European, North and South American origins than to accessions of African origin. This difference may not sound that significant. However, it may suggest that an isolate similar to the European one may also present in the other regions for which the accessions of the respective regions differ in adaptation. In relation to morphological traits, susceptibility appeared to be associated with awned and white seed accessions. For both traits, at least 80% of the accessions were fully susceptible (Table 4). This could be an indication for linkage between gene for susceptibility and the genes for awned seed or white seed traits.

Adult plant testing showed that susceptibility in barley tends to be growth stage dependent (Table 6; figure 2). Even the highly susceptible lines SusPtrit and SusPmur have shown a limited level of susceptibility and they seemed as if gaining a considerable level of resistance at the adult stage. Like wise, accessions of intermediate susceptibility (Vada C118, and Bavaria) also turned to nearly completely resistant. The same phenomenon was reported by Atienza et al., (2004) for the interaction between barley and heterologous rust species. This may indicate that genes involved in resistance at seedling stage may be different from those involved at the adult stage. May be, difference in the leaf morphology between the seedling and adult plant stage could be another reason for the observed difference in resistance. For instance, leaves may turn to harder and hairy during adult stage than they were at seedling stage (physiological maturity). This may hamper success of intimate contact between the pathogen and the plant leading to consideration of the plants as resistant while they are susceptible.

Compared to previous results reported by Atienza et al., (2004) on the non-host status of 109 barley accessions (similar sets like we used), our result confirms that barley is a host rather than nonhost of *P. coronata agropyrina*. Atienza et al., (2004) quantified the nonhost status of barley to heterologous rust species and a heterologous powdery mildew in reference to *P. hordei*. They reported that less than 10% of the accessions showed full susceptibility for any of the heterologous rusts. Specifically, for a mixture of four forma speciales of *P. coronata* Corda, they reported a marginal infection just on 3% of the accessions. On the other hand, they reported 92 % of the accessions were fully infected by *P. hordei*. And they concluded that barley could be considered a true nonhost for many of the heterologous rusts and a near-nonhost to some of them. In the present study, we found full susceptibility to *P. coronata agropyrina* on 83 % of the accessions. This is more similar to the pathogenicity of *P. hordei*

than the pathogenicity of any of the heterologous rusts on the tested accessions. Therefore, our result confirms that barley can be considered as a true host of *P. coronata agropyrina*. Moreover, the higher pathogenicity of *P. coronata agropyrina* hints that it could be a different form of *P. coronata species* different from the marginally infective forms used by Atienza et al., (2004).

3.4 Conclusion

In general, in seedling stage, barley has shown some how full susceptibility to *P. cor. agropyrina* as high as the host species *Agropyron repens* and hence can be regarded as a true host of *P. cor. agropyrina*.

Association of susceptibility with awned and white seeded accessions entails presence of linkage between the traits and genes for susceptibility to *P. coronata agropyrina*.

Though seedling test showed full susceptibility, build up of resistance at adult plant stage suggest that susceptibility in barley to *P. coronata agropyrina* may be growth stage dependent. However, difference in leaf morphology between seedling and adult plant stage due to physiological maturity may also lead to resistance based on avoidance.

Chapter 4: Mapping QTLs Effective to *P. cor. agropyrina* and comparing them with other QTLs mapped to other rusts

4.1 Materials and Methods

4.1.1 Testing Parental lines

Plant material, Inoculation and Incubation

In this small experiment the six Barley parental lines were subjected to infection experiment to confirm the level of contrast in resistance/susceptibility observed between the parental lines in a preliminary seedling test (Niks personal communication). The parental lines were Vada, Cebada Capa, SusPtrit, L94, Steptoe and Morex. Our focus was on the lines Cebada Capa, Vada and SusPtrit as they were the parents of the mapping populations selected for mapping QTLs. The experiment was conducted in three replications. All the lines were grown in a box (as described above) and represented by eight seedlings which later were cut in to five plants. Eleven days after sowing, Inoculation and incubation were carried out as mentioned before.

Evaluation and calculations

Latency period (LP) and Infection Frequency (IF) were evaluated per each line to elucidate the existing difference among the lines. About seven days after inoculation, a small region of infected leaves was marked for subsequent monitoring of the pustule development. From the eighth day onwards, the number of mature pustules per marked region of the infected leaf was counted each day approximately 24 hours after the previous counting. This way, the counting was continued until the time when no further increase in the number of pustules was observed. During each counting, the starting and ending time required to accomplish counting per box were recorded. When no significant increase in pustule numbers was observed the counting was stopped for two days and the final counting was done. Half of this final count (the value 50% of final matured pustules) was then used as a basis for the calculation of the LP for each RIL. At the day of the final counting, IF was determined by counting the number of the urediospores (pustules) per cm² of infected leaf.

The LP, in terms of hours after inoculation (hai) corresponding to the 50% final matured pustules was computed by applying linear interpolations: i.e. $LP = \text{Time left} + [(50\% \text{ of Final count} - \text{Count Left}) / (\text{Count right} - \text{Count left})] * (\text{Time right} - \text{Time left})$. Where: Time left and Time right are the corresponding hours recorded for the counts bordering the 50% value

of the final pustule count; in this case Count Left and Count right respectively. Relative Latency Period (RLP) and Relative Infection Frequency (RIF) were calculated by setting the LP and IF of SusPtrit to 100. Analysis of variance for both RLP and RIF was carried out using Genstat statistical software (10th edition version 10.2. 0.175) to test differences among the parental lines and to analyze the correlation between the RLP and RIF.

4.1.2 Phenotyping mapping populations

Two mapping populations of barley were used in this experiment. They were F8-derived recombinant inbred lines (RILs) each of which was derived after seven generations of single-seed decent from 200 F2 plants of the crosses Vada x SusPtrit and Cebada Capa x SusPtrit. They were developed in the barley research unit of Wageningen University department of Plant Breeding.

The experiment was conducted in three replications per mapping population. In Vada x SusPtrit, 140 RILs and in Cebada Capa x SusPtrit 110 RILs were used. In both populations a RIL was represented by a single plant and per box, 32 lines were grown together with the respective parental lines. Raising of seedlings, inoculation and incubation procedures were done as described for the parental line testing. However, in the third replication of Vada x SusPtrit and all replication of Cebada Capa x SusPtrit, 3mg of urediospores were used per box. This was done because, higher density of pustules were observed when 5mg of spores was used making counting of individual pustules difficult. Besides, it was believed that differences between lines may be obscured due to higher density of pustules.

Evaluation, calculations and analysis of phenotypic data

The same methodology as used in the parental line testing was followed for evaluation of LP and IF. However, RLP and RIF were calculated by setting the average of the RILs in a box to 100 to achieve some how a random distribution of the error among the lines within a box. The reason for this was the assumption that calculation of the RLP and RIF on LP and IF values of a single susceptible plant (in this case SusPtrit) may cause underestimation /overestimation of actual values that may lead to higher experimental error. For both RLP and RIF, the average of the three replications was considered to represent the level of resistance of each RIL and parental lines. The segregation pattern of the mapping populations for both RLP and RIF was inferred from their Frequency distributions. Also, the correlation between the RLP and RIF was analyzed using Genstat, 10th edition version 10.2. 0.175.

4.1.3 QTL mapping and analysis

The software Map QTL version 5.0; (Ooijen, 2004) was used to map QTLs effective to *P. cor. agropyrina*, using the quantitative data RLP and RIF. For both mapping populations, the quantitative data were the averages of the three replications and they were converted in to a text file according to the format of the software. The respective locus file(marker data) and map files were obtained from the Barely research unit of Wageningen University Department of Plant Breeding. In the marker data of both populations, Alleles from SusPtrit were designated as “a” where as alleles from Vada and Cebada Capa were designated as “b”.

Interval Mapping (IM) was carried out by setting the threshold LOD value at 3. Automatic cofactor selection (ACS) was also done to find suggestion on most likely marker in the region of a peak that could be used as a cofactor in Multiple QTL Mapping (MQM). A trial and error procedure was followed with the suggested cofactor markers in MQM to see the consistency of peaks. Finally, Restricted Multiple QTL Mapping was carried out using the cofactor markers that gave consistent peak above and including the threshold in MQM. The Detected QTLs were incorporated in the genetic maps of the populations using MapChart 2.2 (Voorrips, 2002) to show their positions.

Apart from that, comparison was made between the QTLs detected in the present study and those mapped for other rusts in the same populations. For easy understanding of the comparisons, the QTLs were incorporated in the high density consensus barley linkage map (Marcel et al., 2007) where QTLs for partial resistance to leaf rust (*P. hordei*) and nonhost resistance (Jafary et al., 2008) are shown. The LOD-1 and LOD-2 values of the QTLs were converted according to the position of the Peak markers in the same consensus map (Marcel et al., 2007).

4.2 Results

4.2.1 Variation in RLP and RIF among parental line

The parental lines were significantly varied for Relative latency period ($P < 0.001$). According to Duncan multiple range tests, they fall in to four groups. The list significant difference was found to be 7.2 relative latency period at 5% probability. Cebada Capa had the longest latency period while SusPtrit had the shortest. Relative infection frequency was also significant

among the parental lines ($P=0.03$). Three groups were discerned with Duncan multiple range tests at 5% probability. The least significant difference was 49.42 relative infection frequencies. Cebada Capa had the lowest Infection frequency while SusPtrit had the highest infection frequency.

Table 8. Summary of Relative Latency period and Relative Infection Frequency of six barley parental lines as resulted from one way analysis of variance: values are means of three replications

Cultivar	Relative Latency Period *	Relative Infection Frequency*
Cebada Capa	122.5 ^a	15.7 ^a
Morex	116.7 ^{ab}	44.5 ^{ab}
Steptoe	109.6 ^{bc}	36.0 ^{ab}
Vada	108.7 ^c	44.5 ^{ab}
L94	104.9 ^{cd}	79.9 ^{bc}
Susptritt	100.0 ^d	100.0 ^c
Grand mean	110.4	53.4
CV %	3.7	52.0

* Means with the same letter are not significantly different while means with different letter are significantly different at 5% probability according to Duncan multiple range test.

4.2.2 Correlation between RLP and RIF for parental lines

Values for correlation coefficients between and among latency period and infection frequency are presented below (Table 9). Slightly higher correlation was observed between Latency period and Infection Frequency ($r = -0.91$) than between the relative values ($r = -0.89$). Both correlation coefficients were significant at 5% probability with non-directional t-test ($P=0.01$ and 0.02 respectively).

Table 9. Correlation coefficients (r) * of Latency period and infection frequency (absolute and relative values).

Compared Traits	LP	IF	RLP	RIF
LP	1.00			
IF	-0.91	1.00		
RLP	1.00	-0.91	1.00	
RIF	-0.89	1.00	-0.89	1.00

* r values are on the basis of 6 observations (each parental line was represented by the average of 5 plants)
LP= Latency period; **IF**= Infection Frequency; **RLP**= Relative latency period and **RIF**= Relative infection frequency

4.2.3. Transgressive segregation of the mapping populations

In Vada x SusPtrit population, the RILs showed a transgressive segregation for resistance/susceptibility to *P. coronata agropyrina* (Figure 3). For RLP, 45% of the RILs showed shorter latency period than Vada, and 12% longer latency period than SusPtrit. The remaining 43% showed a value between and including the parents. For RIF, 36% of the RILs attained higher than Vada; 41% showed lower than SusPtrit and the rest 23% showed a value between and including the parents.

Also in Cebada Capa x SusPtrit population, the RILs segregated transgressively for resistance /susceptibility to *P. coronata agropyrina* (Figure 3). Here, for RLP, 13 % of the RILs attained a longer latency period than Cebada Capa while 7 % showed a shorter latency period than SusPtrit. The remaining 80% showed a value between and including the parents. For RIF, 1 % of the RILs showed a lower infection frequency than Cebada Capa, 8% showed higher infection frequency than SusPtrit while 91 % showed a value between and including the parents.

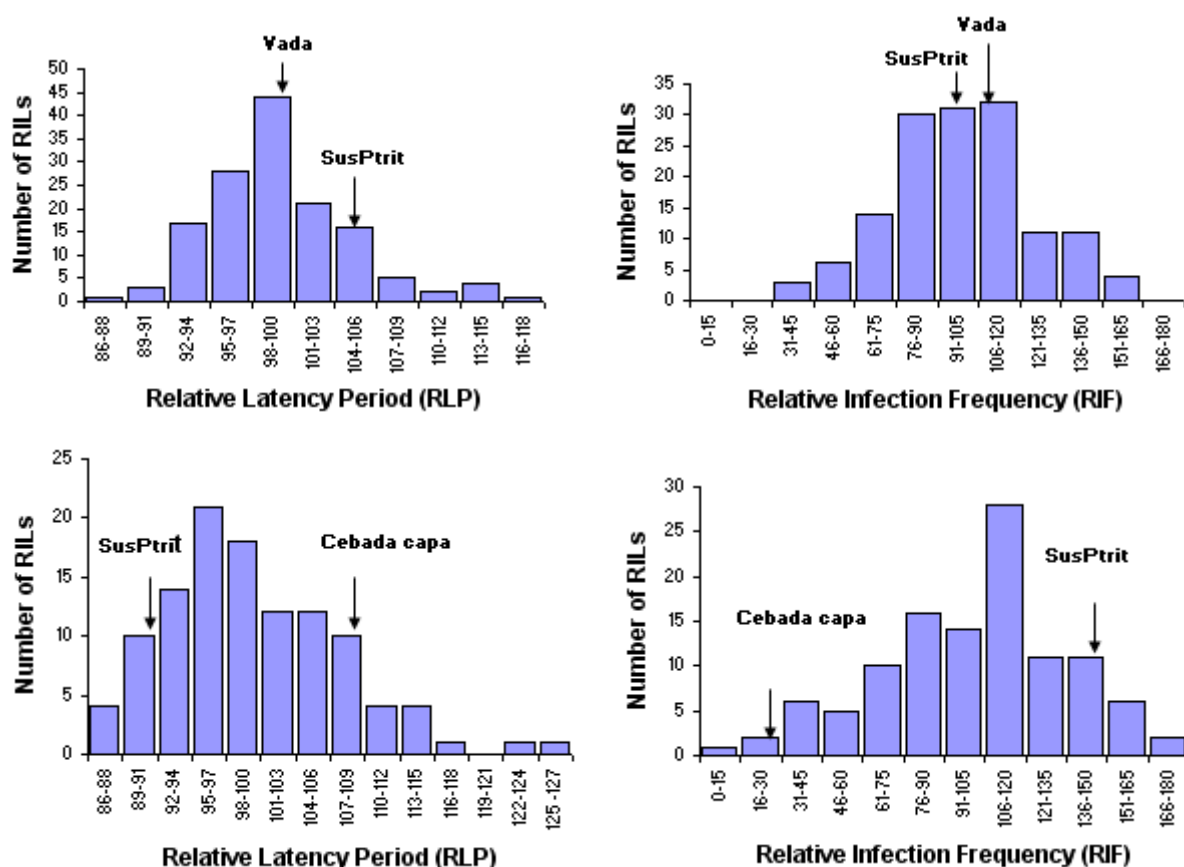


Figure 3. Frequency distribution of phenotypes (RLP & RIF) for resistance to *Puccinia coronata agropyrina* in barley mapping populations Vada x SusPtrit and Cebada Capa x SusPtrit. Arrows indicate values of the two parental lines.

4.2.4 Correlation between RLP and RIF within mapping populations

A very highly significant ($P = 0.0000$) correlation was observed between RLP and RIF for both populations based on the non-directional two sided t-test at 5% probability. However, the correlation coefficient is relatively low (Table 10).

Table 10. Correlation coefficients (r)* between average values of relative and absolute Latency period and infection frequency for mapping populations Vada x SusPtrit (A) and Cebada Capa x SusPtrit.

A. Vada x SusPtrit				
variables	LP	IF	RLP	RIF
LP				
IF	-0.55			
RLP	0.90	-0.52		
RIF	-0.47	0.91	-0.55	
B. Cebada Capa x SusPtrit				
LP				
IF	-0.50			
RLP	0.98	-0.54		
RIF	-0.53	0.98	-0.55	

*Correlations coefficient values are based on 142 observations including parental lines

LP= Latency period; IF= Infection Frequency; RLP= Relative latency period and RIF= Relative infection frequency

4.2.5 QTLs detected

In total, 8 QTLs conferring resistance to *P. coronata agropyrina* were identified (Table 11; Figure 4; Figure 5). In each population, 3 QTLs were mapped with RLP and 2 QTLs with RIF. Except in chromosomes 4(4H) and 5(1H), at least one QTL was mapped in all the other chromosomes. In Vada x SusPtrit, *Rpcq3* (on chromosome 1(7H)) was the most effective QTL while the rest were nearly equally effective. In Cebada Capa x SusPtrit, *Rpcq5* was the one with the greatest effect while *Rpcq7* (for RLP) was with the lowest effect. The other QTLs had some how an intermediate effect.

For three of the QTLs identified in Vada x SusPtrit, the resistance allele came from Vada and for the two QTLs it came from SusPtrit. For Cebada Capa x SusPtrit however, the situation was a bit different. For all the QTLs mapped with RLP and a QTL mapped with RIF, alleles for resistance came from Cebada Capa. SusPtrit contributed for the resistance allele of just one QTL. In both populations, a QTL mapped with RLP overlapped with a QTL mapped with RIF.

Table 11. Summary of QTLs conferring resistance to crown rust isolate *Puccinia coronata agropyrina* at seedling stage in two barley mapping populations Vada x SusPtrit and Cebada Capa x SusPtrit.

Population	Trait	QTL	Chr.	cM	LOD ^b	LOD-2 ^c	% Expl.	Additive	Donor ^d
Vada x SusPtrit	RLP	<i>Rpcq1</i>	2(2H)	103.3	5.38	101-110	10.9	-1.75	Vada
		<i>Rpcq2</i>	7(5H)	6.8	4.78	0-20	9.9	1.70	SusPtrit
		<i>Rpcq3</i>	1(7H)	109.9	5.31	87-113	10.5	-1.74	Vada
		Total					31.3	-1.79	
	RIF	<i>Rpcq3</i>	1(7H)	95.5	7.28	82-117	18.1	11.16	Vada
		<i>Rpcq4</i>	7(5H)	54.8	4.44	12-78	10.6	-8.97	SusPtrit
		Total					28.7	2.19	
C. Capa x SusPtrit	RLP	<i>Rpcq5</i>	2(2H)	137.3	7.21	132-153	20.7	-3.37	C. Capa
		<i>Rpcq6</i>	3(3H)	132.3	3.55	127-145	8.6	-2.25	C. Capa
		<i>Rpcq7</i>	6(6H)	147.2	3.1	131-156	8.2	-2.19	C. Capa
		Total					37.5	-7.82	
	RIF	<i>Rpcq7</i>	6(6H)	153.8	3.03	115-156	9.5	10.25	C. Capa
		<i>Rpcq8</i>	1(7H)	0	3.96	0-16	12.8	-15.36	SusPtrit
		Total					22.3	-5.11	

^a Position of the peak marker on the individual linkage maps

^b LOD values 3.00 and above were considered QTL

^c Two LOD support interval of the QTLs from peak marker based on the result of Restricted MQM.

^d C. Capa is an abbreviation for Cebada Capa

QTLs with identical designation are considered the same QTL due to their overlapping in the same chromosomal region.

4.2.6 Comparison of QTLs with QTLs mapped to other rusts in the same mapping populations

Of the five QTLs mapped in Vada x SusPtrit, *Rpcq1* and *Rpcq3* overlapped with at least one QTL effective to any of the three heterologous rusts (*P. persistency*, *P. tritcina* and *P. hor.-secalini*). No overlapping was found for *Rpcq4* with any of the QTLs effective to the heterologous rusts (Table 12 and Figure 6). *Rpcq2* on the other hand, overlapped with a QTL (*Rphq4*) for partial resistance. Marcel et al (2007) quoting Qi et al (1998b, 1999) described that this QTL was mapped in Vada x L94 population. Interestingly, it has identical peak marker with our QTL *Rpcq2* indicating that most likely they are the same QTL. This identity of the QTLs suggests a possible resemblance between the type of resistance to *P. cor. agropyrina* and to *P. hordei*; presumably, partial resistance.

Table 12. Summary of QTLs conferring resistance to crown rust *Puccinia coronata agropyrina* compared with QTLs for nonhost and host resistance mapped to heterologous rusts and *P. hordei* in the same barley mapping populations Vada x SusPtrit and Cebada Capa x SusPtrit at seedling stage.

Populations	Crown rust (<i>P. cor. agropyrina</i>)				Other rusts ¹		
	QTL ²	Chr.	LOD	LOD – 2 ³	LOD	LOD – 2	rust species
Vada X SusPtrit	<i>Rpcq1</i>	2(2H)	5.38	121-131	4.7	123.2-145.4	<i>P. persistency</i>
	<i>Rpcq2</i> ⁴	7(5H)	4.78	6-26	-	9.1-15.3	<i>P. hordei</i>
	<i>Rpcq3</i>	1(7H)	5.31	78-105	5.3	83.1-94.4	<i>P. persistency</i>
					2.8	29.9-101.9	<i>P. persistency</i>
					7.8	99.7-104.2	<i>P. persistency</i>
					11.3	86.3-91.4	<i>P. triticina</i>
					8.6	101.7-120.6	<i>P. triticina</i>
					3.1	51.2-91.4	<i>P. hor. secalini</i>
	<i>Rpcq3</i>	1(7H)	7.28	75-111	5.3	83.1-94.4	<i>P. persistency</i>
					2.8	29.9-101.9	<i>P. persistency</i>
					7.8	99.7-104.2	<i>P. persistency</i>
					11.3	86.3-91.4	<i>P. triticina</i>
					8.6	101.7-120.6	<i>P. triticina</i>
					3.1	51.2-91.4	<i>P. hor. secalini</i>
	<i>Rpcq4</i>	7(5H)	4.44	24-89	-	-	-
C. Capa X SusPtrit	<i>Rpcq5</i>	2(2H)	7.21	101-123	-	-	-
	<i>Rpcq6</i>	3(3H)	3.55	99-117	7.3	100.4-124.2	<i>P. persistency</i>
					8.3	98.4-133.3	<i>P. triticina</i>
					6.1	92.8-125.4	<i>P. hor. muruni</i>
					6.5	94.5-125.8	<i>P. hor. secalini</i>
	<i>Rpcq7</i>	6(6H)	3.1	106-130	5.7	38.1-127.7	<i>P. hor. muruni</i>
	<i>Rpcq7</i>	6(6H)	3.03	79-120	5.7	38.1-127.7	<i>P. hor. muruni</i>
					3.8	53.5-82.7	<i>P. triticina</i>
	<i>Rpcq8</i>	1(7H)	3.96	38-54	2.8	29.9-101.9	<i>P. persistency</i>
					2.9	25.3-66.5	<i>P. hor. secalini</i>

¹Data for QTLs of other rusts is extracted from (Jafary et al., 2008) and partial resistance (Marcel et al., 2007)

²Designation for the name of the QTLs applies only for *P. cor. agropyrina* in the present study

³Two LOD support interval of the QTLs (from peak marker) based on the result of rMQM; the values were calculated from the corresponding positions of the peak markers on the consensus map of Marcel et al. (2007).

⁴Peak marker of this QTL is a bin marker located in BIN 5H_02.2 on the consensus map as described by Marcel et al. (2007)

QTLs with identical designation are considered the same QTL due to their overlapping in the same chromosomal region.

Similarly, in Cebada Capa x SusPtrit, *Rpcq6*, *Rpcq7*, and *Rpcq8* overlapped with at least one QTL effective to the heterologous rusts. No overlapping QTL was found for *Rpcq5*. Typical in this population is that *Rpcq6* overlapped with four QTLs each of them are effective to one of the four heterologous rusts (*P. persistency*, *P. triticina*, *P. hor.-muruni* and *P. hor.-secalini*). This may indicate that QTLs in Cebada Capa x SusPtrit are some how more diverse in their effectiveness to heterologous rust than QTLs in Vada x SusPtrit. As described above,

Rpcq4 and *Rpcq5* did not co-localized with any of the QTLs used in the comparison. This indicates that probably they can be specific to *P. cor. agropyrina*. However, in the present study, only a single isolate was used. Therefore, the scope of this study limits further discussion on the specificity of these QTLs.

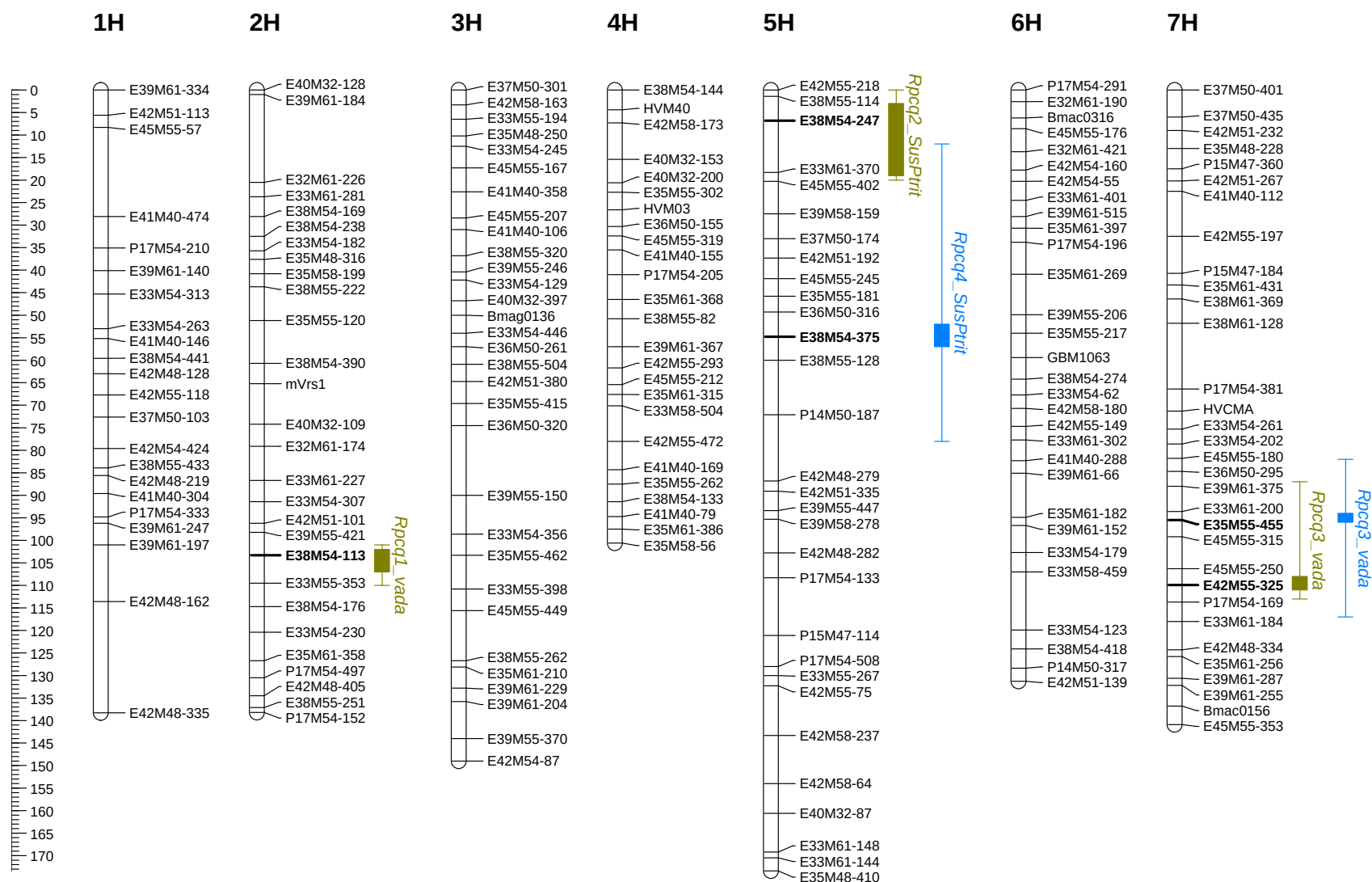


Figure 4. Positions of QTLs mapped for resistance to *P. coronata agropyrina* in barley mapping population Vada x SusPrit at seedling stage. Peak markers of the highest LOD are highlighted in bold. QTL bars (boxes and Lines) represent approximately one and two LOD support intervals respectively from peak marker; the ruler in the left side indicates distances in centiMorgans. ■ QTLs mapped with Relative Latency period (RLP); ■ QTLs mapped with Relative Infection Frequency (RIF)

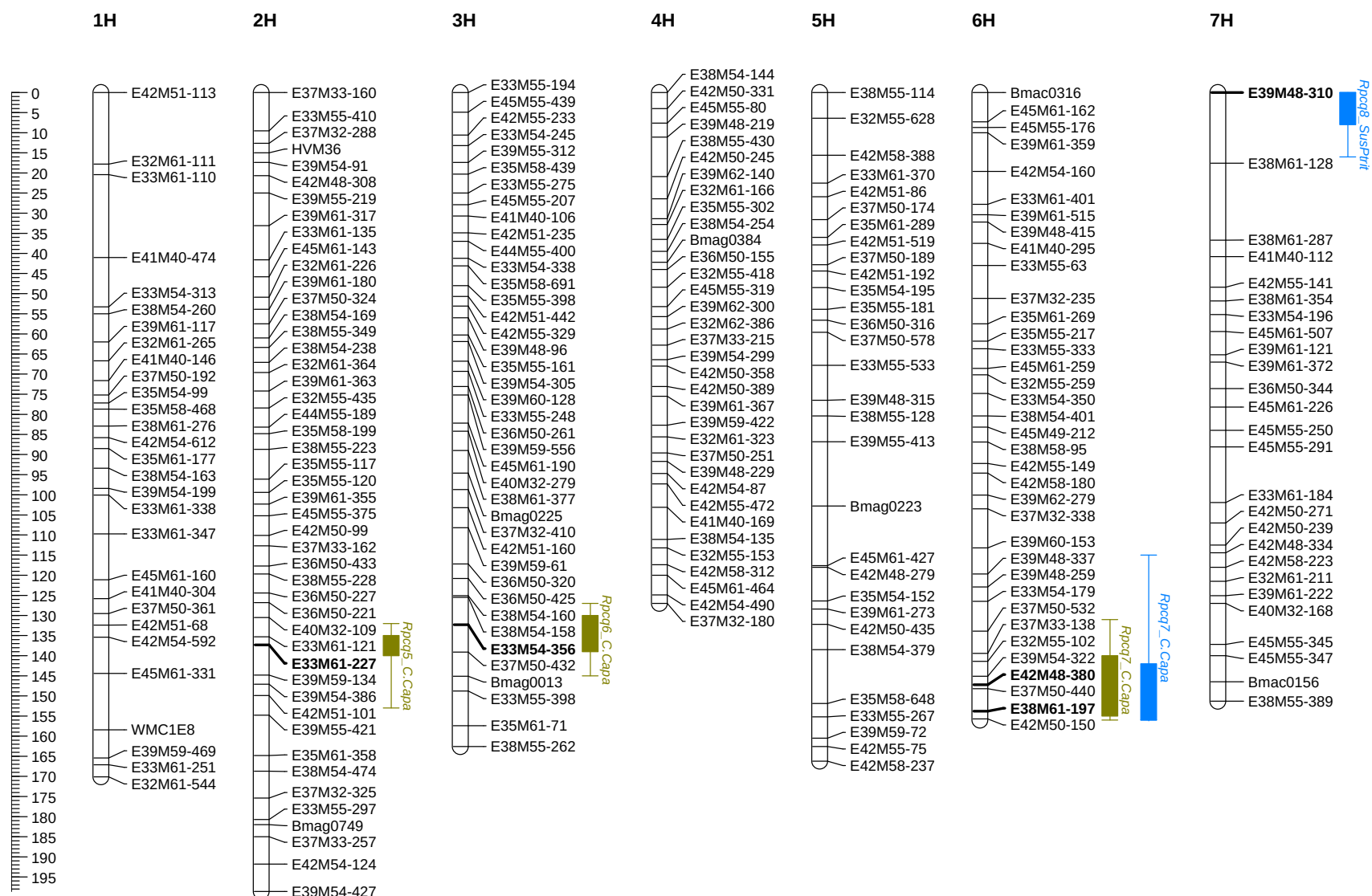


Figure 5. Positions of QTLs mapped for resistance to *P. coronata agropyrina* in barley mapping population Cebada Capa x SusPtrit at seedling stage. Peak markers of the highest LOD are highlighted in bold. QTL bars (boxes and Lines) represent approximately one and two LOD support intervals respectively from peak marker; the ruler in the left side indicates distances in centiMorgans. ■ QTLs mapped with Relative Latency period (RLP); ■ QTLs mapped with Relative Infection Frequency (RIF)

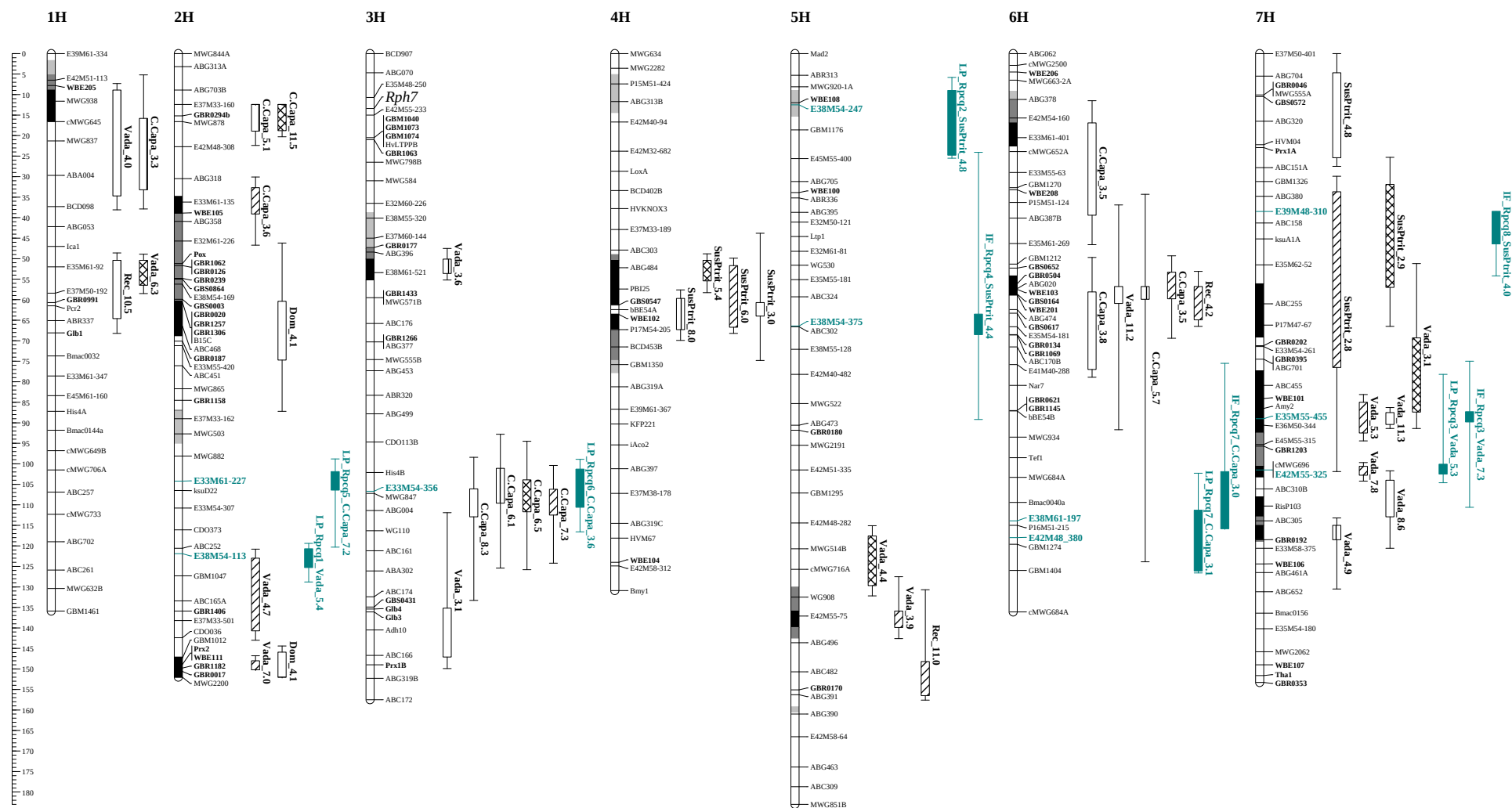


Figure 6. Location and comparison of detected QTLs effective to *P. cor. agropyrina* with position of QTLs for nonhost resistance (Jafary et al., 2007) and of partial resistance (Marcel et al., 2007). QTL bars (inner and outer) represent approximately One and Two LOD support intervals respectively based on the rMQM analysis. The data and the Map chart file for QTLs of the other rusts was kindly provided by Dr. Thierry C. Marcel.

4.3 Discussion

Parental lines

There was a significant difference among the six parental lines both in Relative latency period and Relative infection frequency. Cebada Capa is the most resistant parental line and SusPtrit is the most susceptible line. The other lines lied between these two extremes. Statistically speaking, it appears that Relative latency period has explained the associated genetic variation in resistance to *P. coronata agropyrina* better than Relative Infection Frequency. This is evident from the observed level of significance: $P < 0.001$ for RLP and $P = 0.03$ for RIF. Apart from that very low coefficient of variation (3.7%) has been observed in the case of Latency period as opposed to Infection frequency (52%). This shows that there was a negligible experimental error in evaluation of Latency period as compared to the huge experimental error associated with Infection frequency. Therefore, the observed difference among the parental lines is more reliable in the case of RLP than the one resulted from RIF. Of the components of partial resistance of barley to leaf rust for instance, Parlevliet (1979) stated that LP is the reproducible and easy to measure.

The correlation between RLP and RIF has appeared to be slightly lower than in the case of LP and IF. Perhaps, this is attributed to conversion of the data in to relative values. May be, relative values lead to a certain level of precision by correcting for unwanted variation among data points. However, it is possible that the precision is achieved at the expense of losing a certain level of the real relationship between absolute values in this case LP and IF.

The negative correlation between Latency period and Infection frequency (for both relative and absolute values) is expected. By logical reasoning, longer latency period would mean lower infection frequency and vice versa. In our result however, this relation seems to be violated by Morex and Steptoe. For Relative Latency period, Morex had a higher value than Steptoe; accordingly it should have shown a lower Relative Infection frequency than Steptoe. Difficulty in distinguishing between primary pustules and secondary pustules is believed to be a reason for the observed discrepancy. During observation, evaluation for Infection frequency was complicated to some extent by secondary pustules. These pustules were believed to have developed from the early sporulating uredia established by inoculation in the settling tower. At the time of counting, some of them have already attained a size big enough to confuse

identification of the real primary pustules. Consequently, it was likely that they led to overestimation of infection frequency on the specific plant under evaluation.

Phenotyping mapping populations

As opposed to what we found in the parental line testing, Vada appeared as more susceptible than SusPtrit. This inconsistency partly could be due to the inaccuracy associated with evaluation of infection frequency as explained before. In addition to that; difference in the patterns of uredia on the various RILs could have contributed as well. From our observation for instance, on SusPtrit, uredia were larger and to some extent fused together. Where as, on Vada and some other RILs, they were more like scattered and individual uredia. There fore, during making the last few and the final counts especially on SusPtrit, it was very tempting to judge such a pustule was just a single pustule or more. So, this might have caused under-estimation of infection frequency (over-estimation of latency period) on SusPtrit. On the other hand, it could be true that in nature, just Vada is more susceptible than SusPtrit. In Cebada Capa x SusPtrit however, the situation seems to be consistent. Evidently, Cebada Capa appeared as the resistant parent while SusPtrit appeared as the susceptible parent as it was quite clear from the histograms of RLP and RIF.

In both populations, the frequency distributions have shown a transgressive segregation for resistance/susceptibility to *P. coronata agropyrina*. Obviously, in Vada x SusPtrit, a large percentage of the RILs had the higher values of RLP and RIF than the parents. In Cebada Capa x SusPtrit however, quite a smaller percentage of the RILs attained extreme phenotypic values. This indicates that Vada and SusPtrit are very closer in phenotypic value than Cebada Capa and SusPtrit are. In general, as the parental lines become closer in terms of the phenotype of the trait, there would be a greater chance for larger proportion of the population to be extremely resistant or susceptible segregants.

Highly significant correlation has been found between RLP and RIF for both populations. However, the correlation coefficients ($r = -0.55$ for both populations) are much lower than what we found for the parental lines testing with only 6 observations (Table 9). It is important to notice that when larger number of observations used, correlation coefficients of weak relationships could yield significant p values. Therefore, the observed level of significance could be mainly because of the larger number of observation (142 and 112 respectively) used in the populations rather than the magnitude of the correlation coefficient (r) alone

Mapping QTLs

The fact that one QTL for RLP overlapped with a QTL mapped with RIF in both mapping populations reduces the total number of the identified QTLs to eight. Obviously, the extent of overlap was stronger in Cebada Capa x SusPtrit than it is in Vada x SusPtrit. The former involves an overlap at one and two LOD support intervals while the latter displayed an overlap only at two LOD support interval. Besides, the distance between the peak markers for the overlapped QTLs is 6.6 and 14.4 centiMorgans respectively (Table 11). Despite these differences, the resistance alleles of these QTLs have come only from one of the parent, and the susceptibility allele from the other parent. Moreover they are located on nearly similar and closer regions of the genome. Therefore, they can still be considered as the same QTL instead of two.

As expected from transgressive segregation, both parents have contributed for the resistance and susceptibility alleles. In Cebada Capa x SusPtrit, Cebada Capa has contributed the resistance allele's for 75% of the QTLs, while SusPtrit contribute just only one QTL (25%). In Vada and SusPtrit however, each of the parents has contributed equally for the resistance and susceptible allele. The comparison is based on the consideration that the overlapped QTLs are counted as a single QTL. It seems that the wider the difference in the phenotypic value of the parents, the more likely that the resistance alleles have come mainly from one of the parents.

There was a situation where it was difficult to decide whether a LOD profile of two peaks represent two QTLs or just a QTL (Figure 7). This was true for QTLs *Rpcq3*, *Rpcq4* and *Rpcq5*. The situation was that one marker appears as a peak marker with the highest LOD score while another one appears with smaller LOD score but still above the threshold value (3 in this case). Hence we preferred to consider them as one QTL by assuming the lower peaks as a decreasing effect of the QTL with the highest peak. In that case, the LOD-1 interval of the QTL was calculated on the basis of the peak marker with the highest LOD score and the LOD-2 interval on the basis of the peak with the lower LOD score. This was done because if further investigation like map-based cloning is needed, we think that it is more likely to find a gene in a region of QTL defined by wider interval than the narrow one.

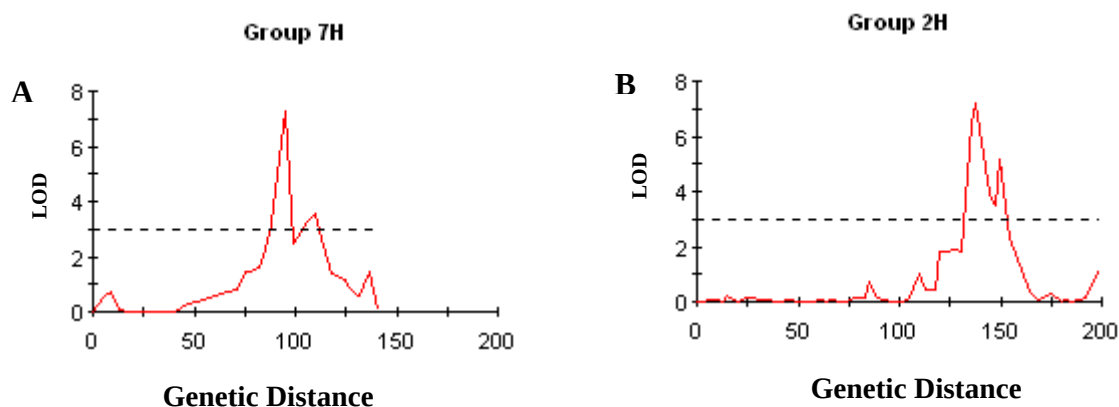


Figure 7. LOD profiles of QTLs *Rpcq3* (on Group 7H) and *Rpcq5* (on Group 2H) representing examples of the situation where the two peaks are considered as if standing for one QTL. A: is for RIF in Vada x SusPtrit; B: is for RLP in Cebada Capa x SusPtrit

4.4 Conclusion

There was sufficient variation among the parental lines which allowed further investigation by mapping QTLs effective to *P. cor. agropyrina*.

Considering the overlapping QTL pairs as one, eight QTLs effective to *P. cor. agropyrina* were identified. In both populations, one QTL for RLP overlapped with a QTL for RIF indicating that a gene involved in controlling RLP can also be involved in controlling RIF.

Compared to QTLs mapped to other rust, 63% of the QTLs effective to *P. cor. agropyrina* have shown co-location with the QTLs of the heterologous rusts and can be considered QTLs of multiple effects. This suggests that either similar or the same set of genes are involved in resistance to *P. cor. agropyrina* and to heterologous rusts.

The limited collocation with a QTL of partial resistance indicates that to some extent, genes involved in partial resistance can also take part in resistance to *P. cor. agropyrina*.

However, the absence of co-location between two of the newly mapped QTLs and any of the other QTLs indicates that resistance to *P. coronata agropyrina* may be dictated by different sets of genes as well.

Chapter 5: Histology of resistance to *P. cor. agropyrina*

5.1 Material and Methods

5.1.1 Raising planting material

Nine barley lines of which 6 are most resistant and three are most susceptible were selected from the accessions and the mapping populations. Namely, the lines were Albert Decorticum, Vada, Cebada Capa, SusPtrit, VxS 70, VxS 85, CCxS 46 and CCxS108. Except for VxS 70 and Decorticum, Each line was represented by 6 seedlings. Three of these were used for microscopic investigation and the remaining three were used for evaluation of LP, IF and IT. Raising of seedlings, inoculation and evaluation of LP and IF were done as described before.

5.1.2 Staining leaf samples for Fluorescence Microscopy

Six days after inoculation, i.e. when the flecks started to be visible, three leaf segments about 2 to 3 cm² were collected from the middle part of the leaf per each line and put in six separate test tubes. Labels for each line were written on strip of paper by pencil to avoid possible washing away by water and alcohol. The leaf segments were immediately fixed and bleached by boiling for 1.5 minutes in a water bath in lactophenol-ethanol (1:2 v/v). To prevent sudden eruptions of the contents of the tubes, some boiling stones were added to the water bath and one stone to each of the test tubes. After the leaves were bleached, the lactophenol-ethanol was poured off and they were washed 1x 30 minutes in ethanol (50%) and in 0.05N NaOH (2g/l) respectively one after the other. The washed leaf segments were rinsed 3 x in water and soaked for 30 minutes in 0.1 M Tris/HCl buffer (pH 8.3). After 5 minutes of staining in a solution of 0.1% Uvitex in the same buffer, they were rinsed thoroughly 4 x in water and then washed for 30 minutes in a solution of 25% glycerol. Finally, the leaves samples were embedded in glycerol on an object slide with the adaxial side facing up.

5.1.3 Examination of Infection units under UV-Microscope

For ease of investigation, different classes of Infection Units (IU) (Table 13) were set based on status of infection unit where an infection unit is described as Non-penetrating, Early Aborted and Established. The Early Aborted and Established classes were further classified in to two based on association with host cell necrosis. A third level classification was done within the Established colonies based on colony size in which case small, medium and large colonies were recognized depending on the number of extensive hyphae branches per IU.

Direct measurement of colony diameter was not possible due to the extensive nature of the hyphae branches over a longer distance

The preparations were then observed under UV-Microscope with a 10x10x1.6 magnification. The infected leaf preparations were screened in a zigzag manner starting from one of the corners and moving horizontally along longitudinal axis of the leaves. The outmost stomatal rows were excluded from observation to avoid possible border effects. In this way, 50 infection units were examined per a single leaf segment of a line (150 IU per line except for two lines). Each of IU was categorized in the predetermined classes depending on the feature by which they were distinguished.

Table 13. Classess of Infection Units on the basis of which microscopic investigation was done on infected leaf segments stained with UVITEX.

Designation of type of infection unit	Descriptions
Non-Penetrating (NP)¹	Germ tubes present; only appresorium on the stomatal openings & no penetration peg.
Early Aborted (EA)	Hyphae tips (contact points) ≤ 6
Early Aborted without Necrosis (EA-N)	appresorium , penetration peg , and SSV² no host cell necrosis
Early Aborted & associated with Necrosis (EA+ N)	appresorium , penetration peg , SSV and host cell necrosis present
Established (Es)	Hyphae tips (contact points) > 6 ,
Established , Small & associated with Necrosis (EsS+N)	extended hyphae branches = 0 ; presence of host cell necrosis
Established , medium & associated with Necrosis (EsM+N)	extended hyphae branches ≤ 5; presence of host cell necrosis
Established , Large & associated with necrosis (EsL+N)	extended hyphae branches > 5; presence of host cell necrosis
Established , small & without necrosis (EsS –N)	extended hyphae branches = 0; absence of host cell necrosis
Established ,medium & without necrosis (EsM-N)	extended hyphae branches ≤ 5; absence of host cell necrosis
Established , large & without necrosis (EsL-N)	extended hyphae branches > 5; absence of host cell necrosis

¹ classification by size and associated host cell necrosis were not relevant for the Non-Penetrating Infection units. Also size was not relevant for the Early Aborted ones.

² SSV : Sub-Stomatal Vessicle

5.1.4 Analysis

Proportions of the infection units in the various classes were subjected to statistical analysis (One way ANOVA) to see differences among the lines. Further analysis within a resistant and susceptible line pairs was done based on the Infection Unit classes which gave significant difference among the lines.

5.2 Results

5.2.1 Variation among lines for the different classes of Infection Units

The lines differed significantly for some of the infection unit classes with in three of the classification categories (Table 14). Within the status of infection units category, proportion of EA and Es infection units were highly significant among the lines. With in colony size classification category, EsL colonies displayed a highly significant difference among the lines. no significant variation was found for the category host cell necrosis association. Further division of the EA, Es and EsL classes in combination with host cell necrosis gave a better picture on the relation between the resistance and the these infection unit classes (Table 15; Table 16).

Table 14. IT, RLP, RIF and Mean proportions of infection units of tested lines per classes of infection units: Probability of significance for difference (F-test) among the means of Infection units within each class is presented as resulted from one-way analysis of variance.

Line	IT	RLP	RIF	Status of Infection Unit			Size of colony			Host cell necrosis	
				NP	EA	Es	Es S	Es M	Es L	associated	Not-associated
Vada	4	101.6	67.9	0.09	0.11	0.80	0.07	0.23	0.51	0.41	0.51
VxS 85	3	134.7	38.6	0.16	0.33	0.51	0.05	0.24	0.21	0.37	0.47
VxS 70	4	95.9	81.7	0.06	0.06	0.88	0.06	0.24	0.58	0.24	0.70
Cebada Capa	2	120.9	9.8	0.11	0.61	0.28	0.04	0.10	0.14	0.20	0.69
CCxS 46	2	120.3	16.7	0.14	0.47	0.39	0.07	0.13	0.19	0.43	0.43
CCxS 108	4	98.2	86.6	0.07	0.11	0.81	0.09	0.29	0.43	0.31	0.62
SusPtrit	4	100.0	100.0	0.09	0.15	0.75	0.03	0.27	0.45	0.22	0.69
Albert	2	126.1	49.6	0.16	0.34	0.50	0.06	0.26	0.18	0.40	0.44
Decorticum	1	135.7	1.6	0.17	0.15	0.68	0.13	0.35	0.20	0.59	0.24
F-test*				0.119	<.001	<.001	0.274	0.114	0.001	0.084	0.024

* Probabilities showing significance difference among the lines are highlighted in bold.

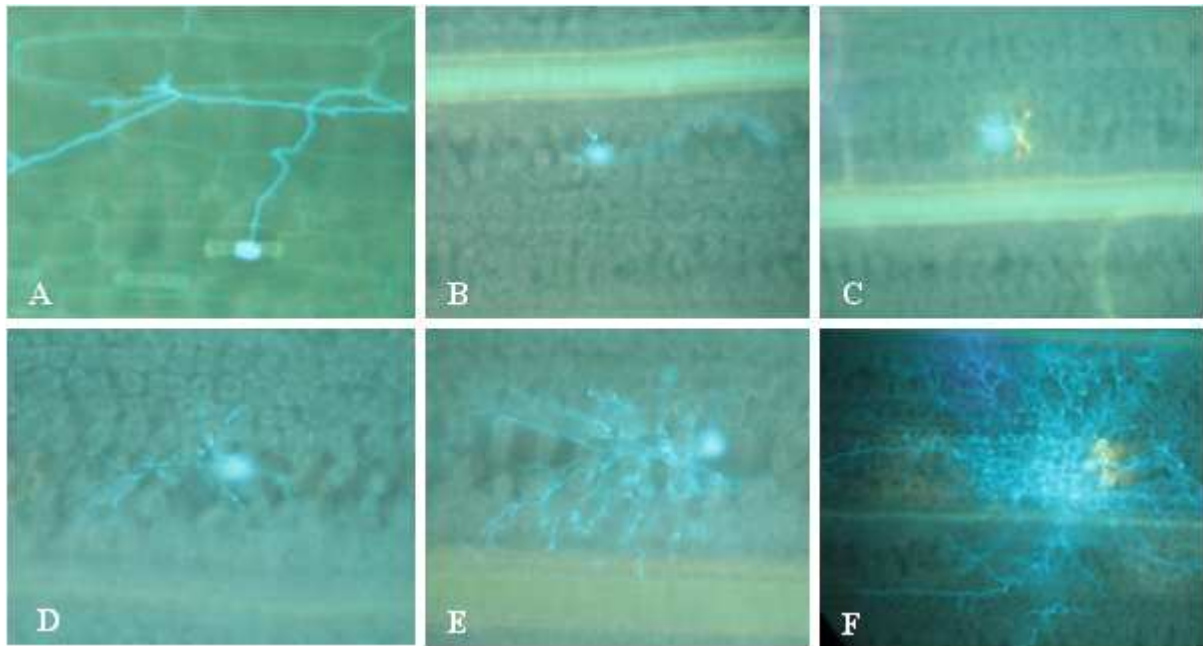


Figure 8. Some representative Infection Units of *P. coronata agropyrina* on barley. **A:** Non-penetrating; **B:** Early aborted; **C:** Early aborted associated with host cell necrosis; **D:** Established small; **E:** Established medium; **F:** Established large associated with host cell necrosis. Magnification =x160 for all pictures. Direct measurement of colony diameter was not possible due to the extensive nature of the hyphae branches over a longer distance almost in all directions like in F.

Further dissection of the EA class in to EA+N and EA-N classes and analysis revealed that the difference is largely attributed to EA component with out host cell necrosis. Although not so big, the EA+N infection unit also showed significant difference and seemed to play a role in the observed variation (Table 15). Similarly, a better explanation of the variation among the lines was achieved by EsL–N compared to the other classes of the established infection unit. To some extent, the lines also appeared to vary significantly for the EsM+N infection units (Table 15).

Nearly a similar result was found by pair-wise comparison of the relatively resistant and susceptible lines (Table 16). In four of the six pair-wise comparisons, the lines varied significantly either for EA ± N or EsL-N and EsM-N infection unit classes. Exceptionally, the comparisons CCxS 46 vs. CCxS 108 and Decortatum vs. SusPtrit varied significantly for EsM-S and EsM+N infection unit classes respectively. No significant variation was observed between Vada and SusPtrit.

Table 15. Mean proportions of infection units of tested lines per classes of infection units and their probabilities of significance for difference (F-test) as resulted from one-way analysis of variance.

Lines	NP	EA+N	EA-N	EsS+N	EsS -N	EsM+N	EsM-N	EsL+N	EsL-N
Vada	0.09	0.05	0.06	0.05	0.01	0.09	0.13	0.21	0.30
VxS 85	0.16	0.12	0.21	0.01	0.05	0.11	0.13	0.13	0.09
VxS 70	0.06	0.02	0.04	0.04	0.02	0.06	0.18	0.12	0.46
Cebada Capa	0.11	0.10	0.51	0.01	0.03	0.03	0.07	0.06	0.08
CCxS 46	0.14	0.14	0.33	0.04	0.03	0.08	0.05	0.17	0.02
CCxS 108	0.07	0.06	0.05	0.03	0.05	0.09	0.20	0.12	0.31
SusPtrit	0.09	0.03	0.12	0.01	0.03	0.05	0.23	0.13	0.31
Albert	0.16	0.14	0.20	0.04	0.02	0.10	0.16	0.12	0.06
Decortcatum	0.17	0.04	0.11	0.04	0.09	0.31	0.04	0.20	0.00
F-test*	0.12	0.03	<.001	0.41	0.11	0.04	0.11	0.13	0.00

* Probabilities showing significance difference among the lines are highlighted in bold.

Table 16. Pair wise comparison of mean proportion of infection units between resistant and susceptible lines per classes of infection units.

Compared line pairs	NP	EA+N	EA-N	EsS+N	EsS -N	EsM+N	EsM-N	EsL+N	EsL-N
Vada	0.09	0.05	0.06	0.05	0.01	0.09	0.13	0.21	0.30
SusPtrit	0.09	0.03	0.12	0.01	0.03	0.05	0.23	0.13	0.31
F-test*	0.88	0.25	0.22	0.24	0.23	0.28	0.41	0.12	0.91
VxS 85	0.16	0.12	0.21	0.01	0.05	0.11	0.13	0.13	0.09
VxS 70	0.06	0.02	0.04	0.04	0.02	0.06	0.18	0.12	0.46
F-test*	0.13	0.16	0.21	0.13	0.18	0.38	0.27	0.93	0.02
Cebada Capa	0.11	0.10	0.51	0.01	0.03	0.03	0.07	0.06	0.08
SusPtrit	0.09	0.03	0.12	0.01	0.03	0.05	0.23	0.13	0.31
F-test*	0.75	<.001	0.02	1.00	0.52	0.52	0.17	0.11	0.08
CCxS 46	0.14	0.14	0.33	0.04	0.03	0.08	0.05	0.17	0.02
CCxS 108	0.07	0.06	0.05	0.03	0.05	0.09	0.20	0.12	0.31
F-test*	0.24	0.20	0.04	0.80	0.35	0.88	0.01	0.36	0.02
Albert	0.16	0.14	0.20	0.04	0.02	0.10	0.16	0.12	0.06
SusPtrit	0.09	0.03	0.12	0.01	0.03	0.05	0.23	0.13	0.31
F-test*	0.16	0.03	0.27	0.07	0.64	0.07	0.56	0.69	0.05
Decortcatum	0.17	0.04	0.11	0.04	0.09	0.31	0.04	0.20	0.00
SusPtrit	0.09	0.03	0.12	0.01	0.03	0.05	0.23	0.13	0.31
F-test*	0.24	0.72	0.87	0.15	0.20	0.05	0.22	0.41	0.07

* Probabilities showing significance difference between the lines in a pair are highlighted in bold.

5.3 Discussion

Of the nine infection unit classes, it seems that EA and EsL infection units appeared as the best parameters to elucidate the existing variation among the resistant and susceptible lines. Interestingly, higher proportions of the EA $\pm$ N and EsM+N were associated with most of the resistant lines while lower proportions were associated with all susceptible lines. Conversely, lower proportions of the EsL-N and EsM-N infection units were associated with resistant lines while higher proportions were associated with susceptible lines (Table 16). In all cases of the analysis, Non-penetration was not important showing that the pathogen was so successful at least in finding its way towards the stomata. Apparently, in none of the lines the proportion of NP infection units exceeded 0.17 (17%) indicating that the mechanism underlying the resistance is most likely of post-penetration type.

Considerable occurrence of early abortion, combined with limited level of host cell necrosis has been described as a typical feature of nonhost reaction (Heath 1977 in Niks 1982). On non-host plant, infection units are arrested between formation of haustorial mother cell and first haustoria often accompanied by negligible host cell collapse (Niks 1982 quoted Heath 1977). Studding Early abortion of colonies of leaf rust (*P. hordei*) in partially resistant barley seedlings, Niks (1982) stated that the early abortion in partially resistant barely seedlings resembles the non-host reaction. The present result some how agrees with these facts. However, as the UVITEX staining technique did not allow to see the haustorium and post-haustorial events, it is not clear whether the abortion occurred before or after the formation of the first haustorium. Significantly, larger sized colonies with no associated host cell necrosis have appeared nearly as peculiarity of susceptible lines (Table 14; 15; 16 and Figure 8). It seems that the infection units looked as if doing successive host cell invasion and hardly arrested by the susceptible lines. In the present study, the biological reason behind this phenomenon is not clear.

On the other hand, some what a different way of reaction is exhibited by the line Decorticatum. For this line, no EsL-N infection units and low proportion of EA $\pm$ N infection units were observed. In contrast, a large proportion of the infection units were of the type EsM+N for which it differed significantly from the susceptible line. Apparently, the line was the most resistant one among the tested lines as it attained the Lowest IT score (1), longest RLP and the lowest RIF (Table 14). This may suggest that the colonies were arrested in the

post-establishment phase of infection units. Most probably, late abortion could be the possible cause for the observed reaction of this line. Besides, the considerable level of associated host cell necrosis might have played a major role in the resistance involved. Possibly, this type of resistance could be due to a reduced expression of a gene for hypersensitivity as stated by Niks (1982).

5.4 Conclusion

In general, the observed results suggest that the underlying mechanism of resistance could be mainly based on early abortion of colonies and to some extent combined with a reduced level of host cell necrosis.

6. Future direction

For the host range study, more comprehensive conclusions could be reached if further investigations are made on uredial and or telial morphology combined with host range. Besides, many European isolates should be used to attest the possible existence of host-pathogen specificities for *P. cor. agropyrina*. Comparison at molecular level based on nuclear ribosomal ITS (Internal Transcribed Spacer) sequences of the pathogens could be a better dissection tool to investigate the identity of the European crown rusts with those that occur in USA/Canada.

In the present study, it has been shown that resistance in barley to *P. cor. agropyrina* seems to depend on plant growth stage. However, lack of intimate contact between the pathogen and the host plant cell due to physiological maturity of leaves may confuse whether the resistance is based on post-penetration host response or avoidance. Comparative histology of the infection process at seedling and adult stage may help to confirm whether the resistance (lose in susceptibility) is based on avoidance or not .

The limited histological investigation has indicated possible resemblance or overlap between the mechanisms underlying host/nonhost resistance and resistance to *P. cor. agropyrina*. Investigation on post-haustorial events with a focus on Early aborted and Large Established colonies with other staining techniques may help to unravel the actual mechanism.

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Appendices

Appendix 1. List of species and accessions tested for host range determination experiment.

Species tested	Origin/seed source	Accession No./ English name/variety*	No. of plants tested	IT score**
<i>Aegilops columnaris</i> Zhuk.	Wageningen University	AE 111/78 (96443)	4	0;
<i>A. comosa</i> Sibth. et Sm.	Wageningen University	AE 115/78 (96446)	4	0;
<i>A. comosa</i> Sibth. et Sm.	Wageningen University	AE 116/78 (96447)	1	4
<i>A. comosa</i> Sibth. et Sm.	Wageningen University	AE 873/85 (96450)	4	4
<i>A. peregrina</i>	Wageningen University	AE 547/78 (96403)	4	3
<i>A. peregrina</i> (Hackel) Maire et Weiller	Wageningen University	AE 381/83 (96401)	3	0;
<i>A. peregrina</i> (Hackel) Maire et Weiller	Wageningen University	AE 548/78 (96402)	4	0;
<i>A. speltoides</i>	Wageningen University	842105 (96458)	1	0
<i>A. speltoides</i>	Wageningen University	842110 (96465)	1	0
<i>A. speltoides</i>	Wageningen University	842112 (96464)	1	4
<i>A. speltoides</i>	Wageningen University	842106 (96459)	3	0,1,2
<i>A. speltoides</i>	Wageningen University	842107 (96460)	4	0,0,1,1
<i>A. speltoides</i>	Wageningen University	842104 (96461)	3	0,0; 0;
<i>A. speltoides</i>	Wageningen University	842108 (96462)	1	1
<i>Agropyron repens</i>	Wageningen University		26	3,4(25)
<i>Avena sativa</i>	Wageningen University	Cebeco (2001012)	3	0
<i>A. sativa</i>	Wageningen University	Alfred (200518)	4	0
<i>Bromus carinatus</i>	United States, Iowa	PI 278697	3	0,1,1
<i>B. carinatus</i>	Wageningen University	2007338	4	1,1,2,2
<i>B. catharticus</i> var. <i>catharticus</i>	Australia, Austr. Capital	PI 168556	4	0;
<i>B. danthoiae</i>	Turkey	PI 206416	4	1,1,4,4
<i>B. erectus</i>	Romania, Cluj	PI 111279	4	0,0,0,3
<i>B. erectus</i>	Turkey	PI 172397	4	0
<i>B. inermis</i> subsp. <i>Inermis</i>	Turkey	PI 172395	4	0
<i>B. inermis</i> subsp. <i>Inermis</i>	Poland, Poznan	PI 255870	3	0
<i>B. inermis</i> subsp. <i>Inermis</i>	Former Soviet Union	PI 262456	4	0,0,0,2
<i>B. inermis</i> subsp. <i>Inermis</i>	Former Soviet Union	PI 370660	4	0,0,0,0;
<i>B. japonicus</i>	Turkey	PI 204399	4	4
<i>B. japonicus</i>	Pakistan	PI 219726	4	4
<i>B. japonicus</i>	Iran	PI 239720	4	0,2,2,2
<i>B. mango</i>	Argentina	PI 598721	4	1,2,2,2
<i>B. scoparius</i>	Afghanistan	PI 220514	4	4
<i>B. scoparius</i>	Former Soviet Union	PI 314229	4	4
<i>B. species</i>	Barcelona	20031752	4	4
<i>B. species</i>	Spain	2004101	4	4
<i>B. tectorum</i>	Afghanistan	PI 219992	4	4
<i>B. tectorum</i>	Afghanistan	PI 220575	4	4
<i>B. tectorum</i>	Iran	PI 228397	2	4
<i>Dactylis glomerata</i>	Wageningen University	Cock's-foot (2003099)	5	0
<i>Hordeum bogdanii</i>	Afghanistan, Parwan	PI 269406	1	4
<i>H. brevisubulatum</i> subsp. <i>Violaceum</i>	Iran	PI243220	2	4
<i>H. bulbosum</i>	Wageningen University	82 (2005655)	1	0;
<i>H. bulbosum</i>	Wageningen University	Gra.24/61 (2005657)	1	1
<i>H. bulbosum</i>	Wageningen University	CPI 23529 (2005660)	1	4
<i>H. bulbosum</i>	Wageningen University	CPI 15012 (2005661)	1	4
<i>H. bulbosum</i>	Wageningen University	Gra.60/61 (2005662)	3	4
<i>H. bulbosum</i>	Wageningen University	CPI 18973 (2005663)	4	2,1,1,1
<i>H. bulbosum</i>	Wageningen University	CGN 13032 (2006115)	4	4
<i>H. bulbosum</i>	Wageningen University	CGN 13033 (2006116)	4	3,2,1,0;
<i>H. bulbosum</i>	Wageningen University	CGN 13035 (2006117)	2	4
<i>H. bulbosum</i>	Wageningen University	CGN 13043 (2006119)	3	0,4,4
<i>H. bulbosum</i>	Wageningen University	CGN 13068 (2006120)	3	4
<i>H. bulbosum</i>	Wageningen University	—	4	0,2,3,3
<i>H. chilense</i>	Argentina, Rio Negro	PI 531781	4	4
<i>H. cmosum</i>	Argentina	PI269648	1	4
<i>H. jubatum</i>	Canada, British Columbia	PI 234683	4	4
<i>H. jubatum</i>	United States, Colorado	W6 27314	4	4
<i>H. lechleri</i>	Argentinian, Chubut	PI 531784	4	4

Appendix 1. Continued

<i>H. marinum</i>	952194	PI204582	4	4
<i>H. murinum</i>	Wageningen University	—	4	4
<i>H. Parodii</i>	Argentinian, Buenos Aires	PI 531786	4	4
<i>H. procerum</i>	Argentina, La Pampa	PI 531787	4	4
<i>H. pusillum</i>	United States, Kentucky	Ciho 15663	4	4
<i>H. secalinum</i>	Wageningen University	—	4	0
<i>H. secalinum</i>	Wageningen University	2000617	4	0
<i>H. stenostachys</i>	Argentina, La Pampa	PI 266195	4	4
<i>H. stenostachys</i>	Argentina, Cordoba	PI 531791	2	4
<i>Lolium perenne</i>	Wageningen University	2007402	4	0
<i>L. westerwolds</i>	Wageningen University	2007401	4	0
<i>Secale cereale</i>	Wageningen University	Rye (200517)	4	0;
<i>Triticum aestivum</i>	Wageningen University	Morocco (96306)	1	0;
<i>T. aestivum</i>	Wageningen University	BH1146 (99046)	4	0;
<i>T. aestivum</i>	Wageningen University	Chinese Spring (96531)	2	0;
<i>T. aestivum</i>	Wageningen University	Vivant (2003004)	1	0;
<i>T. aestivum</i>	Wageningen University	Morocco (2003010)	3	0;
<i>T. aestivum</i>	Wageningen University	Thatcher (200504)	4	0;
<i>T. boeoticum</i>	Wageningen University	1-1082?(96514)	2	0;

*the numbers in bracket “()” indicate the green number code according to the list of plant materials with in the research group Breeding for Resistance to Biotic stress, barley research unit of Wageningen University and Research Center.

** Presence of only one score value means all the tested plants had the same IT score. For Agropyron, 4(25) means , 25 plants had IT score of 4

Appendix 2. List of Barley accessions used for the host status determination experiment

No	Accession Name	Seed type	Origin	Type	Year of Release	Spike Row	Awn Type	Color of seed	NPPL*	0-5 score**
1	116-5	Naked		Res. line				white	180	4
2	Ab 14 Köln	Non naked	Ethiopia	Landrace	<1945	Six Rowed	Awmed	white	102	4
3	Akka	Non naked	Sweden	Cultivar	1969	Two Rowed	Awmed	white	26	3
4	Albert	Non naked	France	Cultivar	<1949	Six Rowed	Awmed	Black	3	2
5	Alfa	Non naked	Denmark	Cultivar	<1947	Two Rowed	Awmed	white	228	4
6	Allegro	Non naked	Netherlands	Cultivar	1978	Two Rowed	Awmed	white	185	4
7	Aramir	Non naked	Netherlands	Cultivar	1972	Two Rowed	Awmed	white	228	4
8	Archer	Non naked	United Kingdom	Cultivar	<1931	Two Rowed	Awmed	white	6	2
9	Ark Royal	Non naked	United Kingdom	Cultivar	1976	Two Rowed	Awmed	white	308	4
10	Armella	Non naked	France	Cultivar	<1974	Two Rowed	Awmed	white	115	4
11	Aura	Non naked	Germany	Cultivar	<1975	Two Rowed	Awmed	white	257	4
12	Berg	Non naked	Western Eur.	Cultivar	<1938	Six Rowed	Awmed	white	110	4
13	Bolivia	Non naked	Bolivia	Landrace	<1913	Six Rowed	Awmed	white	153	4
14	Brage	Non naked	Sweden	Cultivar	1925	Two Rowed	Awmed	white	150	4
15	Burton Malt	Non naked	United King.	Cultivar	<1920	Two Rowed	Awmed	white	333	4
16	C118	Non naked		Res. line				white	295	4
17	Cebada Capa	Non naked	Argentina	Cultivar	<1936	Six Rowed	Awmed	white	28	3
18	Dabat	Non naked	Ethiopia	Landrace		Six Rowed	Awnless	white	50	3
19	Decorticatorum	Naked	Ethiopia	Res. line	1920	Two Rowed	Awmed	Black	9	2
20	Drossel	Non naked	Germany	Cultivar	1971	Two Rowed	Awmed	white	113	4
21	Effendi	Non naked	Netherlands	Cultivar	<1972	Two Rowed	Awmed	white	400	4
22	Egypt IV	Non naked	Germany	Cultivar	<1938	Six Rowed	Awmed	white	190	4
23	Emir	Non naked	Netherlands	Cultivar	1962	Two Rowed	Awmed	white	110	4
24	Firibach III	Non naked	Germany	Cultivar	1948	Two Rowed	Awmed	white	298	4
25	Frankengold	Non naked	Germany? (Brei	Cultivar	1975	Two rowed	Awmed	white	Missing	Missing
26	Freegold	Non naked	United King.	Cultivar	1971	Two Rowed	Awmed	white	90	3
27	Freya Y	Non naked	Sweden?	Cultivar	1942?	Two Rowed	Awmed	white	500	4
28	Georgie	Non naked	United King.	Cultivar	1975	Two Rowed	Awmed	white	442	4
29	Gold	Non naked	Sweden	Cultivar	<1913	Two Rowed	Awmed	white	90	3
30	Gospick	Non naked	Yugoslavia	landrace	<1949	Two Rowed	Awmed	white	66	3
31	Hassan	Non naked	Netherlands	Cultivar	1971	Two Rowed	Awmed	white	242	4
32	Japan 1	Non naked	Japan	Landrace	<1963	Six Rowed	Awmed	white	290	4
33	Japan 15	Non naked	Japan	landrace		Six Rowed	Awmed	white	156	4
34	Japan 18	Non naked	Japan	landrace		six rowed	Awmed	white	283	4
35	Japan 20	Non naked	Japan	Landrace		Six Rowed	Awmed	white	60	3
36	Japan 6	Naked	Japan	Landrace		Six Rowed	Awmed	white	373	4
37	Japan 8	Naked	Japan	Landrace		Six Rowed	Awmed	white	260	4
38	Jeruzalem II	Non naked	Israel	Cultivar	<1990	----	----	white	278	4
39	Kobakintagi	Naked	Japan	Landrace	<1950	Six Rowed	Awmed	white	162	4
40	Kuckuck	Non naked	Western Eu.	Landrace	1961	Two Rowed	Awmed	white	44	3
41	Kwan	Non naked	United States	Cultivar	<1968	Six Rowed	Awmed	white	129	4
42	Goudgerst	Non naked	Sweden	Cultivar	<1913	Two Rowed	Awmed	white	278	4
43	L92	Naked	Ethiopia	Landrace	<1963	Two Rowed	Awnless	white	31	3
44	L98	Non naked	Ethiopia	Landrace	<1963	Six Rowed	Awmed	white	123	4
45	La Estanzuela	Non naked						white	53	3
46	Lago	Non naked	Western Eur.	Cultivar	----	Two Rowed	Awmed	white	408	4
47	Lechtaler	Non naked	Portugal	Landrace	<1938	Two Rowed	Awmed	white	197	4
48	Lofa Abed	Non naked	Denmark	Cultivar	1970	Two Rowed	Awmed	white	185	4
49	Magnif 102	Non naked	Argentina	Cultivar	<1968	Two Rowed	Awmed	white	287	4
50	Magnif 104	Non naked	Argentina	Cultivar	<1968	Two Rowed	Awmed	white	220	4
51	Bavaria	Non naked	Germany	Cultivar	<1903	Two Rowed	Awmed	white	365	4
52	Meta	Non naked	Netherlands	Cultivar	1981	Two Rowed	Awmed	white	103	4
53	Midas	Non naked	United King.	Cultivar	1970	Two Rowed	Awmed	white	104	4
54	Morgenrot	Non naked	Germany	Cultivar	<1944	Six Rowed	Awmed	white	300	4
55	Mosane	Non naked	Belgium	Cultivar	1961	Two Rowed	Awmed	white	272	4
56	Multan	Non naked	Pakistan	Landrace	<1923	Six Rowed	Awmed	white	292	4
57	Nadrine	Naked	----			Two Rowed	Awmed	Black	270	4
58	Harrington	Non naked	Canada	Cultivar	1981	Two Rowed	Awmed	white	Missing	Missing
59	Isaria	Non naked	Germany	Cultivar	1924	Two Rowed	Awmed	white	447	4
60	Opal	Non naked	Denmark	Cultivar	<1924	Two Rowed	Awmed	white	210	4
61	Peruvian	Non naked	Peru	Landrace	<1917	Six Rowed	Awmed	white	228	4
62	Porthos	Non naked	France	Cultivar	1975	Two Rowed	Awmed	white	130	4
63	Printa	Non naked	Netherlands	Cultivar	> 1942	Two Rowed	Awmed	white	408	4
64	Probst	Non naked	Austria	Cultivar	<1949	Two Rowed	Awmed	white	359	4
65	Ramona	Non naked	Netherlands	Cultivar	<1974	Two Rowed	Awmed	white	126	4
66	Ribari	Non naked	Egypt	Cultivar	<1960	Six Rowed	Awmed	white	272	4
67	Ruby	Non naked	U K	Cultivar	1966	Two Rowed	Awmed	white	309	4
68	Speciale	Non naked	USA	Landrace	<1947	Six Rowed	Awmed	white	172	4

Appendix 2. Continued

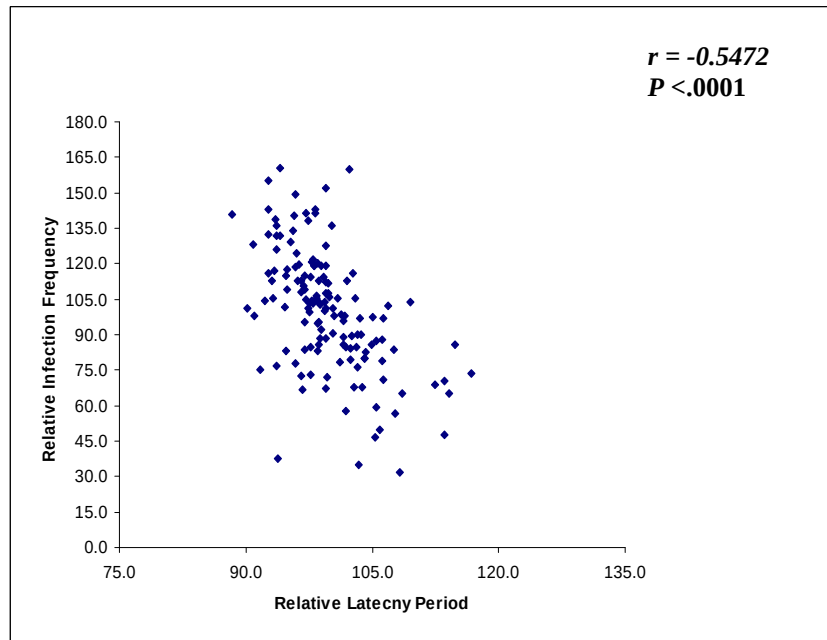
69	Spiti	Non naked China	Landrace	<1926	Six Rowed	Awmed	white	228	4
70	Spratt Archer	Non naked United King.	Cultivar	<1929	Two Rowed	Awmed	white	218	4
71	Sudan	Non naked Sudan	Landrace	<1938	Six Rowed	Awmed	white	16	3
72	Sultan	Non naked Netherlands	Cultivar	1966	Two Rowed	Awmed	white	328	4
73	Topper	Non naked Germany	Cultivar	<1959	Six Rowed	Awmed	white	327	4
74	Tresor de V	Non naked France	Cultivar	1940	Two Rowed	Awmed	white	261	4
75	Lacey	Non naked USA	Cultivar	2000	Six Rowed	Awmed	white	244	4
76	Union	Non naked Germany	Cultivar	1955	Two Rowed	Awmed	white	256	4
77	Valeta	Non naked Netherlands	Cultivar	<1972	Two Rowed	Awmed	white	354	4
78	Fong Tien	Non naked China	Landrace	1926	Six Rowed	Awmed	white	321	4
79	Vada	Non naked Netherlands	Cultivar	<1956	Two Rowed	Awmed	white	256	4
80	Gei	Non naked Netherlands	Cultivar		Two Rowed	Awmed	white	126	4
81	Gunhild	Non naked Denmark	Cultivar	<1980	Two Rowed	Awmed	white	270	4
82	Menelik	Non naked Ukraine	Landrace	<1930	Two Rowed	Awmed	white	43	3
83	L100	Naked Ethiopia	Landrace		Six Rowed	Awmed	Black	446	4
84	C123	Non naked	Res. line	<1976	Six Rowed	Awmed	Black	142	4
85	Nigrimiden	Naked Ethiopia	Landrace	<1962	Two Rowed	Awmed	Black	223	4
86	nhQTL-L94	Unknown						355	4
87	Trigo Biasa	Naked Indonesia	Landrace	<1993	Six Rowed	Awmed	white	298	4
88	Morex	Non naked United States	Cultivar	1978	Six Rowed	Awmed	white	209	4
89	L94	Non naked Ethiopia	Landrace		Two Rowed	Awmed	Black	343	4
90	PI391136	Non naked	Wild barley		Two Rowed	Awmed	white	164	4
91	Suspmur	Unknown						588	5
92	Robust	Non naked United States	Cultivar	1983	Six Rowed	Awmed	white	286	4
93	Stander	Non naked United States	Cultivar	1993	Six Rowed	Awmed	white	294	4
94	H. spon. Ashkelon	Non naked	Wild barley		Two Rowed	Awmed	white	213	4
95	H. spon. Mehola	Non naked	wild barley		Two Rowed	Awmed	white	228	4
96	H. spon. Maalot	Non naked	Wild barley		Two Rowed	Awmed	white	143	4
97	H. spon. Mount Mer	Non naked	Wild barley		Two Rowed	Awmed	white	176	4
98	ANA	Non naked Argentina	Cultivar		Two Rowed	Awmed	white	266	4
99	FNC 1	Non naked Uruguay	Cultivar		Two Rowed	Awmed	white	112	4
100	FNC 6-1	Non naked Uruguay	Cultivar		Two Rowed	Awmed	white	273	4
101	CLE 182	Non naked Uruguay	Cultivar		Two Rowed	Awmed	white	209	4
102	CLE 187	Non naked Uruguay	Cultivar		Two Rowed	Awmed	white	37	3
103	CLE 194	Non naked CIMMYT	Cultivar		Two Rowed	Awmed	white	23	3
104	CLE 152	Non naked Uruguay	Cultivar		Two Rowed	Awmed	white	179	4
105	CLE 157	Non naked Uruguay	Cultivar		Two Rowed	Awmed	white	227	4
106	Varunda	Non naked Netherlands	Cultivar	1969	Two Rowed	Awmed	white	92	3
107	Volla	Non naked Germany	Cultivar	1957	Two Rowed	Awmed	white	305	4
108	Apex	Non naked Netherlands	Cultivar	<1982	Two rowed	Awmed	white	244	4
109	Haisa	Non naked Germany	Cultivar	1939	Two Rowed	Awmed	white	213	4
110	Prisma	Non naked Netherlands	Cultivar	<1980	Two Rowed	Awmed	white	197	4
111	Susptrit	Naked Netherlands	research line					543	5
112	Agropyron							261	4

* NPPL : Number of Pustules Per Leaf represents average value of three plants per accession.

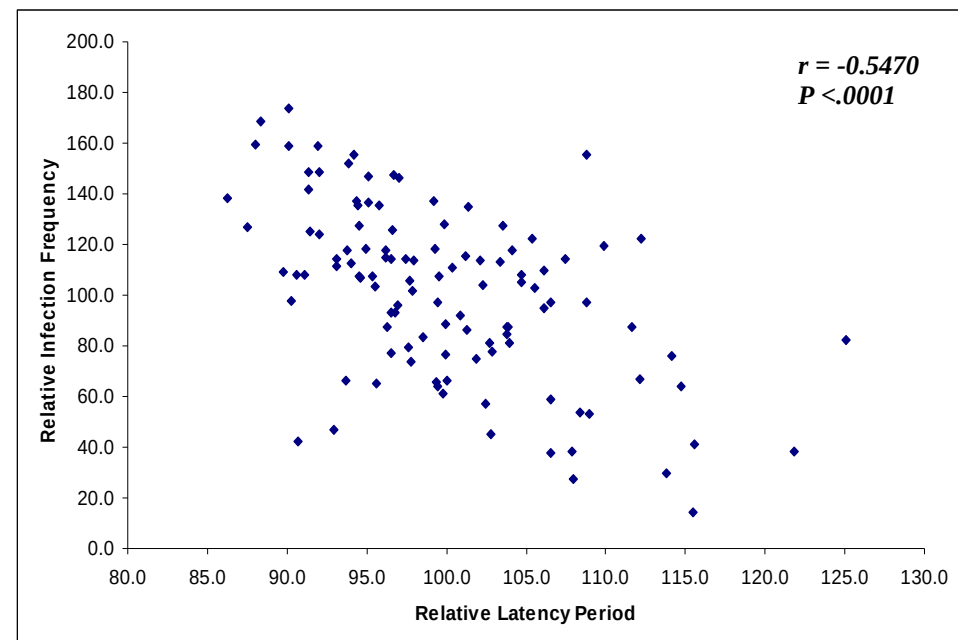
**the 0-5 score was given based on the average number of pustules per leaf as indicated in " * "

Appendix 3. Scatter plots of correlations between Average Relative Latency Period and Average Relative Infection Frequency in Vada x SusPtrit and Cebada Capa x SusPtrit mapping Populations

(a) Vada x SusPtrit

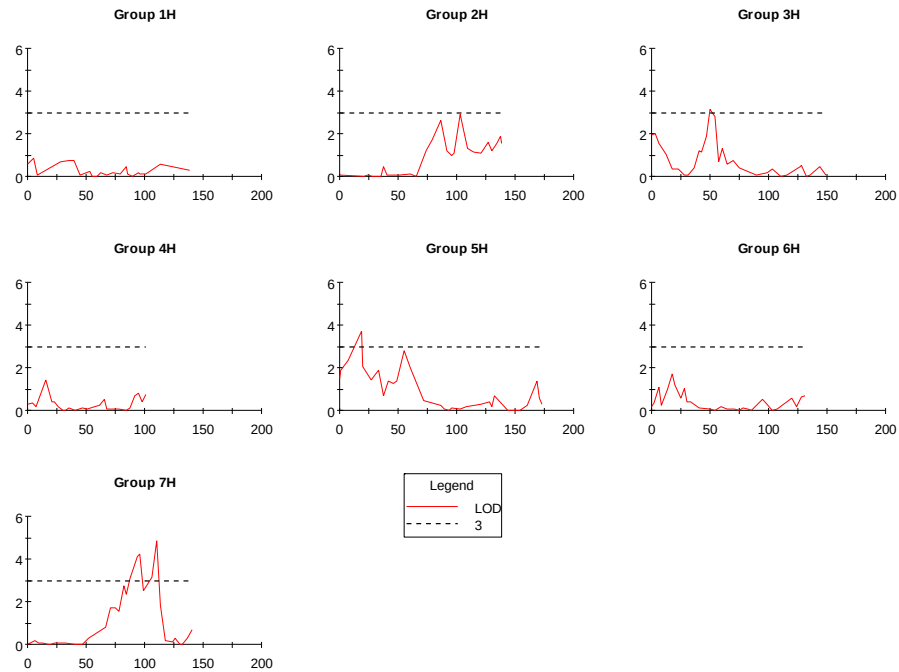


(b) Cebada Capa x SusPtrit

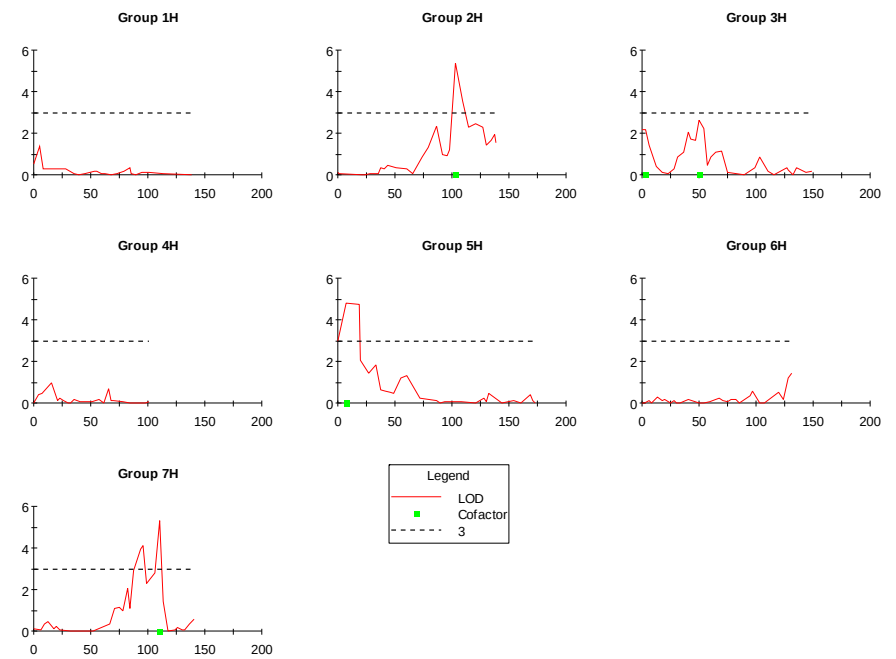


Appendix 4. LOD Profiles from Interval Mapping and rMQM mapping for RLP and RIF in Vada x SusPtrit mapping Population

(a) Interval Mapping: RLP

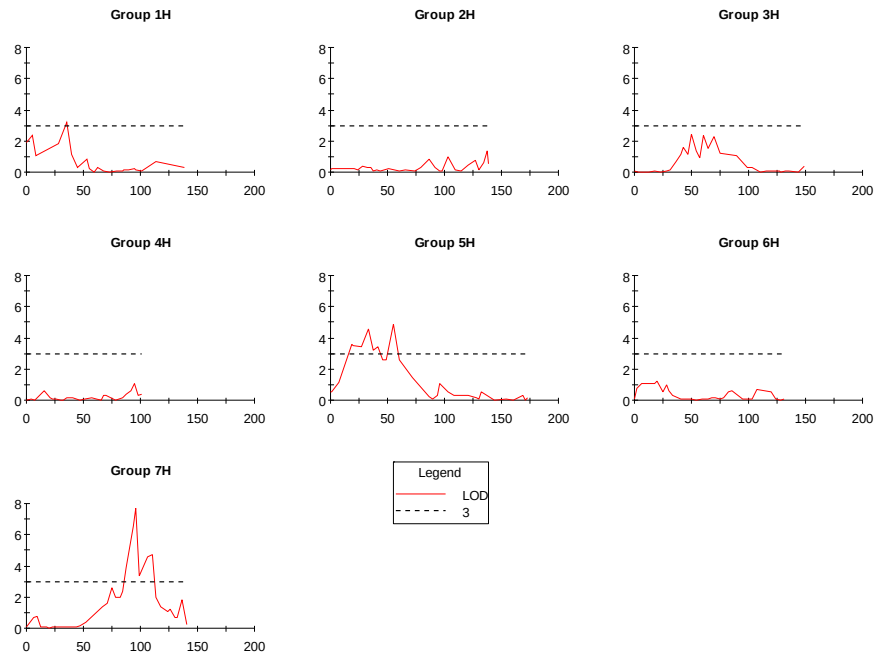


(b) Restricted MQM Mapping: RLP

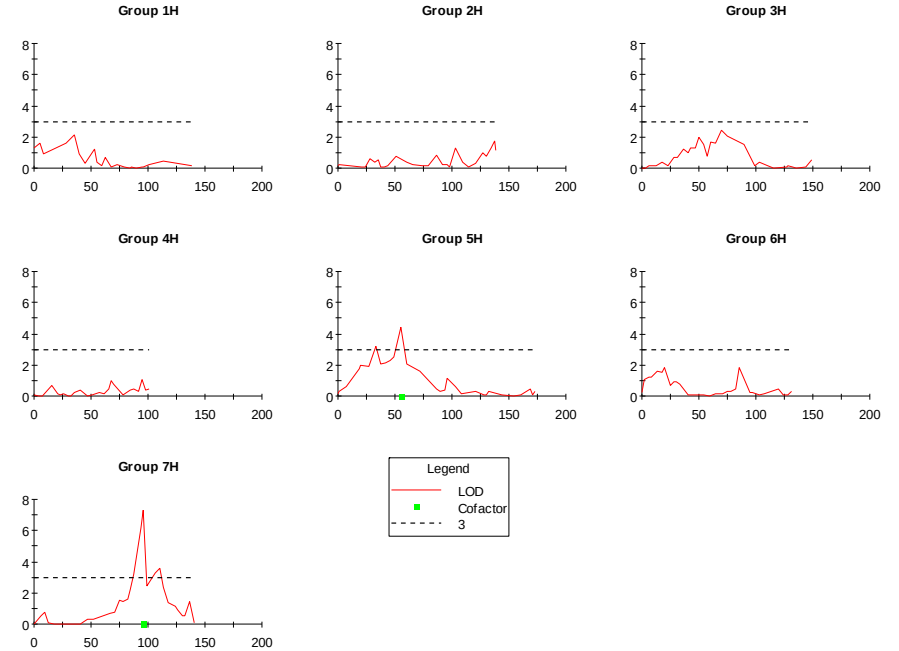


Appendix 4. Continued

(c) Interval Mapping: RIF

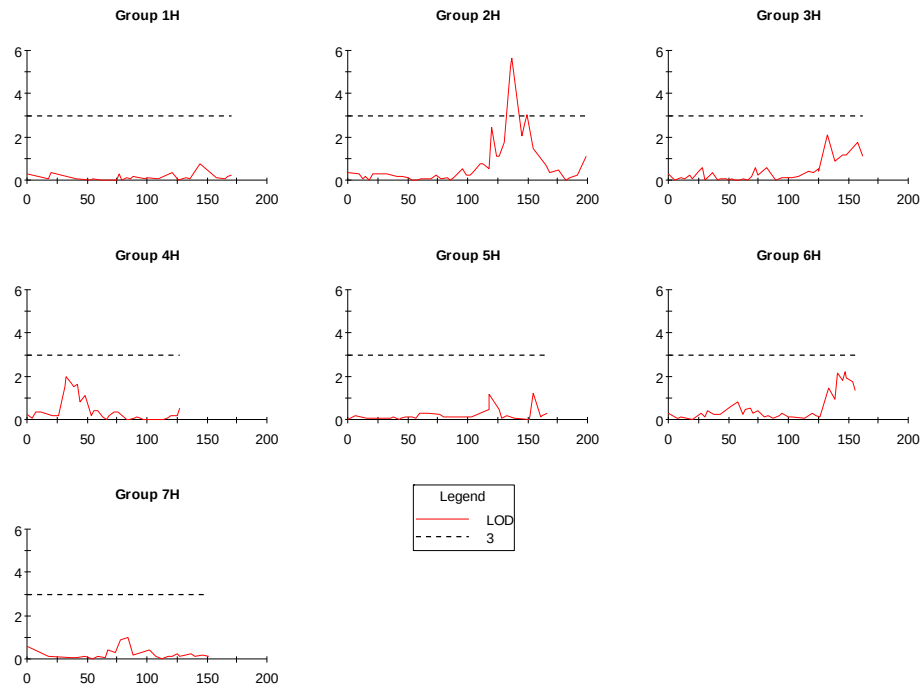


(d) Restricted MQM Mapping: RIF

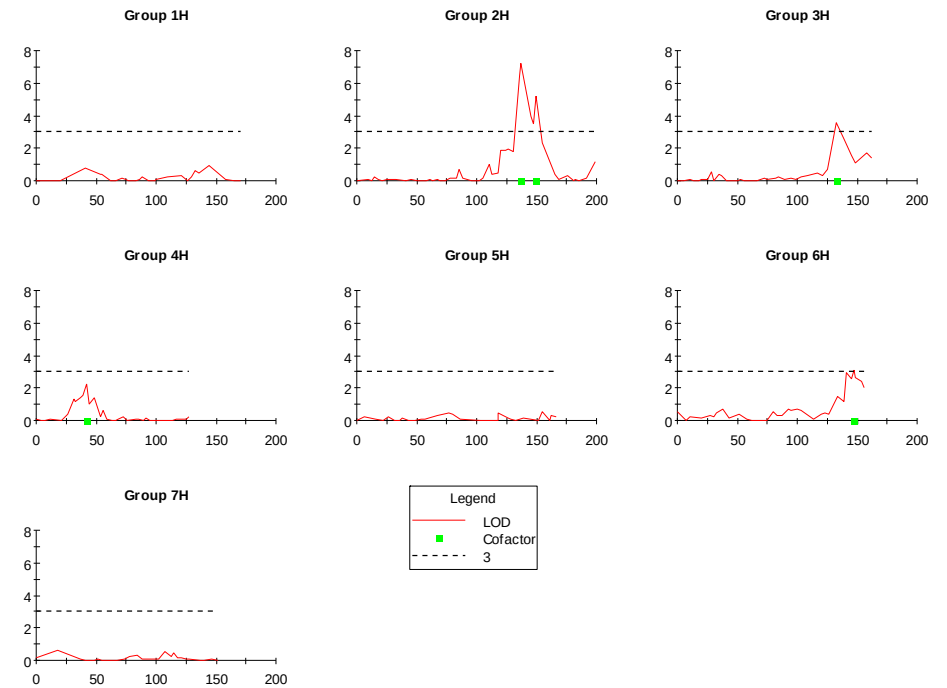


Appendix 5. LOD Profiles from Interval Mapping and rMQM mapping for RLP and RIF in Cebada Capa x SusPtrit mapping Population

(a) Interval Mapping: RLP

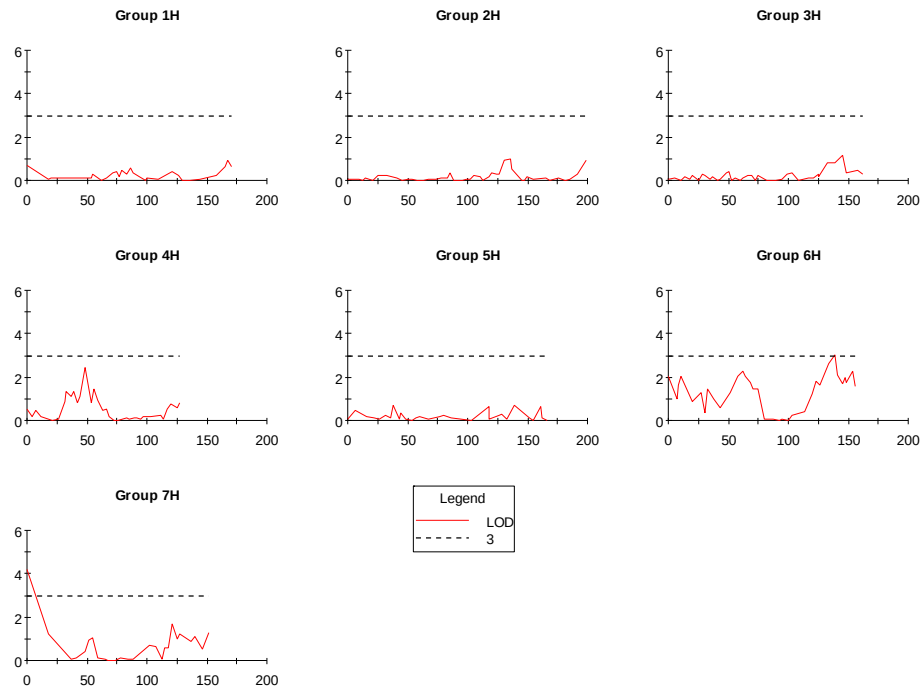


(b) Restricted MQM Mapping: RLP



Appendix 5. Continued

(c) Interval Mapping: RIF



(d) Restricted MQM Mapping: RIF

