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ORIGINAL PAPER

Inheritance and molecular mapping of a gene conferring seedling resistance against *Puccinia hordei* in the barley cultivar Ricardo

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Abstract Genetic studies were undertaken to determine the inheritance and genomic location of uncharacterised seedling resistance to leaf rust, caused by *Puccinia hordei*, in the barley cultivar Ricardo. The resistance was shown to be conferred by a single dominant gene, which was tentatively designated *RphRic*. Bulk segregant analysis (BSA) and genetic mapping of an F_3 mapping population using multiplex-ready SSR genotyping and Illumina GoldenGate SNP assay located *RphRic* in chromosome 4H. Given that this is the first gene for leaf rust resistance mapped on chromosome 4H, it was designated *Rph21*. The presence of an additional gene, *Rph2*, in Ricardo, was confirmed by the test of allelism. The seedling gene *Rph21* has shown effectiveness against all Australian pathotypes of *P. hordei* tested since at least 1992 and hence represents a new and useful source of resistance to this pathogen.

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Introduction

Cultivated barley (*Hordeum vulgare* L. subsp. *vulgare*), an important cereal crop worldwide (Ulrich 2011), is affected by many diseases. Among these diseases, leaf rust can be one of the most devastating (Park 2003). It is caused by the fungus *Puccinia hordei* Otth. and affects barley production in many parts of the world (Clifford 1985). Barley leaf rust epidemics have caused significant yield losses in many countries (Arnst et al. 1979; Cotterill et al. 1992; Griffey et al. 1994; Melville et al. 1976), including Australia (Murray and Brennan 2010). The disease has been managed by developing and growing resistant cultivars, an approach that is regarded as one of the most cost effective and sustainable control measures. A total of 19 major seedling resistance genes (*Rph1* to *Rph19*; Weerasena et al. 2004) and a single adult plant resistance (APR) gene (*Rph20*; Hickey et al. 2011) conferring resistance to *P. hordei* have been characterised in barley. More recently, a major seedling resistance gene, temporarily designated *RphMBR1012*, conferring resistance to a highly virulent European isolate of *P. hordei*, was described (König et al. 2012). Most of the major genes have been overcome by new pathotypes of *P. hordei* (Park 2003). As only seedling resistance genes *Rph7*, *Rph11*, *Rph14*, *Rph15* and *Rph18* (Park 2003, 2010) and the APR gene *Rph20* remain effective in Australia (Park 2010, unpublished), there is an urgent need to discover and characterise new sources of resistance to *P. hordei*.

Genetic loci conferring seedling resistance to *P. hordei* have been characterised using trisomic analysis, isozyme markers, morphological markers, and molecular markers. Designated *Rph* genes are located on all barley chromosomes except 4H. Of the designated loci *Rph1* to *Rph19*, six are reported to involve alleles; *Rph5* and *Rph6* (Zhong

et al. 2003), *Rph9* and *Rph12* (Borovkova et al. 1998) and *Rph15* and *Rph16* (Weerasena et al. 2004). The seedling gene *Rph2* was mapped on chromosome 5HS (Borovkova et al. 1997; Franckowiak et al. 1997). According to Franckowiak et al. (1997), *Rph2* is a complex locus comprising many alleles. In addition to *Rph2*, the barley cultivar 'Reka 1' was reported to carry a second leaf rust resistance gene (Tan 1977), which was later characterised and designated as *Rph19* (Park and Karakousis 2002). Gene *Rph2* is reported to be allelic to *RphQ*, as no segregation was observed in F_2 populations derived from crosses between barley line Q21861 (*RphQ*) and sources of *Rph2* (Peruvian, PI531840 and PI531841) when inoculated with an *Rph2* avirulent *P. hordei* pathotype ND8702 (Borovkova et al. 1997). Pathotypes of *P. hordei* with different pathogenicities to *Rph* genes have been used to postulate new sources of resistance in barley germplasm (Cotterill et al. 1992; Golegaonkar et al. 2009; Park and Karakousis 2002; Tan 1977). In other studies, recombinant inbred lines (RILs) and DNA markers were used to locate loci conferring resistance to *P. hordei* to chromosomes in *H. vulgare*—for example *Rph2* (Borovkova et al. 1997; Franckowiak et al. 1997), *Rph5* (Mammadov et al. 2003), *Rph7* (Brunner et al. 2000; Graner et al. 2000) and *Rph19* (Park and Karakousis 2002).

The use of molecular markers has fast-tracked breeding programmes by permitting marker-assisted selection (Langridge and Barr 2003). Microsatellites (SSRs) and single-nucleotide polymorphisms (SNPs) are the preferred types of molecular marker in cereal research due to their abundance throughout the genome, co-dominance, and ease of use (Close et al. 2009; Ganal et al. 2009; Gupta and Varshney 2000). An extensive SSR and SNP marker resource is now available for barley, as well as high-density SSR- and SNP-based genetic maps (Ramsay et al. 2000; Varshney et al. 2007; Muñoz-Amatriaín et al. 2011). When combined with bulk segregant analysis (BSA, Michelmore et al. 1991), SSR and SNP markers facilitate the rapid detection of markers linked to specific genes. For example, BSA has been used widely to identify molecular markers linked to stripe rust resistance in bread wheat (Bansal et al. 2010) and for leaf rust resistance in durum wheat (Singh et al. 2010). Based on BSA, a sequence tagged site (STS) marker *ITS1* (derived from *Rrn2*) was found to be closely linked (1.6 cM) to the *Rph2* allele *RphQ* (Borovkova et al. 1997).

Ricardo, a land race believed to have originated from Uruguay, carries *Rph2* (*Pa2*) (Henderson 1945; Moseman and Roan 1959; Zloten 1952) and an uncharacterised seedling gene (Park unpublished; Stöcker 1983; Wallwork et al. 1992; Yahyaoui et al. 1988). Ricardo was reported to be highly resistant to a pathotype of *P. hordei* with virulence for *Rph2* under field conditions and showed environmental sensitivity in the expression of seedling resistance to pathotypes with virulence for *Rph2* under greenhouse conditions (Golegaonkar 2007). In the present study, tests of allelism were conducted to confirm the presence of *Rph2* in Ricardo, and the inheritance and genomic location of the uncharacterised seedling resistance to *P. hordei* was investigated.

Materials and methods

Plant material

Seed of Ricardo, Gus (leaf rust susceptible), Peruvian (*Rph2*) and differential genotypes with known *Rph* genes was obtained from the germplasm collection held at the Plant Breeding Institute (PBI), University of Sydney. F_3 populations were developed from the crosses Ricardo/Gus (200 lines) and Ricardo/Peruvian (147 lines) at the PBI. An Australian series of differential genotypes described by Park (2003) was used as controls, with three additional lines carrying *Rph15*, *Rph17* and *Rph18*.

Pathogen material

Ten pathotypes of *P. hordei* were used in the studies, all of which are maintained in the PBI Cereal Rust Collection. The pathogenicities and passport information for these pathotypes are described in detail in Table 1.

Determining conditions for the optimal expression of seedling resistance in Ricardo

Previous studies showed that the low infection types (ITs) produced by the uncharacterised seedling resistance (hereafter referred to as *RphRic*) in Ricardo varied with environmental conditions and the *P. hordei* pathotype used (Park unpublished). Experiments were therefore conducted to determine the conditions leading to optimal expression of *RphRic*. Four sets of Ricardo, Peruvian and Gus, along with all differential genotypes, were sown in the greenhouse. Four clumps (parents) and five clumps (differentials) per pot (8–10 seeds per clump) were sown in 9-cm-diameter pots filled with a mixture of fine bark and coarse sand and fertilized using Aquasol® (100 gm per 10 l of water per 200 pots) prior to sowing. Following sowing, pots were kept in a rust-free growth room at 20 ± 2 °C for germination. Seven-day old seedlings were fertilized with granular urea (Incitec Pivot® *w/w* 46 % nitrogen; 50 g per 10 l of water per 200 pots). The experiment was replicated three times with four sets per replicate. Four pathotypes (viz. 5457P+, 5652P+, 4673P+ and 200P−) of *P. hordei* were used. Nine- to ten-day-old seedlings at the one and a half-leaf

Table 1 *Puccinia hordei* pathotypes used in the present study

Pathotype	Culture no.	Virulence
243P−	487	*Rph1*, *Rph2*, *Rph6*, *Rph8*
253P−	490	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*
200P−	518	*Rph8*
5610P+	520	*Rph4*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph19*
5653P++*Rph13*	542	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph13*, *Rph19*
5453P−	560	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12*
5652P+	561	*Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph19*
4673P+	562	*Rph1*, *Rph2*, *Rph4*, *Rph5*, *Rph6*, *Rph8*, *Rph9*, *Rph12*, *Rph19*
5653P+	584	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph19*
5457P+	612	*Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12*, *Rph19*

growth stage were inoculated in the greenhouse. The seedlings were moved to an enclosed chamber and urediniospores (10–12 mg/10 ml/200 pots) were suspended in a light mineral oil (Shellsol®, mobil oil) and atomised over seedlings using an aerosol hydrocarbon propellant pressure pack. The chamber door was kept closed for 5 min to allow urediniospores to settle on the leaves completely. Leaf rust-inoculated seedlings were incubated for 24 h at room temperature in a dark chamber where continuous mist was created by an ultrasonic humidifier. After incubation, seedlings were moved to naturally lit microclimate rooms maintained at 17 ± 2, 23 ± 2 and 27 ± 2 °C. Infection type responses were scored 10–12 days after inoculation according to the 0–4 scale used by Park and Karakousis (2002).

Multipathotype testing

Parents Ricardo, Peruvian and Gus, along with all differential genotypes, were tested in the greenhouse against ten pathotypes of *P. hordei* (Table 1) according to the method described above, at post incubation temperatures of 23 ± 2 °C.

Inheritance of *RphRic* in Ricardo and *Rph2* allelism test

A total of 200 F_3 lines (Ricardo/Gus) and two sets of populations comprising of 79 and 68 F_3 lines each (Ricardo/Peruvian), parents Ricardo, Gus and Peruvian, and all differential genotypes, were sown in the greenhouse using 30–35 seeds per F_3 line, four clumps (parents) and five clumps (differentials) per 9-cm diameter pot according to the method described above. Seedlings were tested with *P. hordei* pathotypes 5457P+ or 200P− at 23 ± 2 °C as described above.

DNA extraction

Genomic DNA was extracted according to Bansal et al. (2010) from leaf tissue of seedlings of Ricardo, Gus and all lines of the Ricardo/Gus F_3 population.

Molecular analyses and mapping of *RphRic*

The multiplex-ready SSR technique developed by Hayden et al. (2008) was used to perform BSA (Michelmore et al. 1991) and genetic mapping of the uncharacterised locus conferring seedling resistance in Ricardo. Equal amounts of genomic DNA were pooled from ten non-segregating resistant and ten non-segregating susceptible F_3 lines to constitute the resistant and susceptible bulks, respectively. A total of 488 SSRs selected for genome-wide coverage and high information content (barley whole genome scan kits 1 and 2; http://www.genica.net.au) were used to identify marker-trait associations. SSRs revealing putative linkage between the bulks and parents were genotyped in the entire Ricardo/Gus F_3 population. Multiplex-ready PCR products generated for bulk segregant analysis were separated on an ABI3730 DNA fragment analyser (Applied Biosystems), while those produced for genetic mapping were separated on a GeneScan2000 (Corbett Research) using a 6 % (19:1 acylamide:bisacrylamide) gel, according to the manufacture's instructions. Reported SSR allele sizes were calculated from the ABI3730 analysis using GeneMapper v.3.7 (Applied Biosystems). Primer sequences for SSRs mapped in this study are available from GrainGenes (http://wheat.pw.usda.gov/cgi-bin/graingenes).

A custom oligo pool assay (OPA) comprising 384 SNPs derived from Barley POPA (Close et al. 2009) was used to enhance genetic mapping of the seedling resistance locus in Ricardo. The custom OPA consisted of highly informative (PIC > 0.4) SNPs selected for genome-wide coverage (average 5 cM marker spacing based on BOPA1 genetic map; Close et al. 2009). The Illumina BeadXpress was used to genotype each Ricardo/Gus F_3 line utilising the GoldenGate assay, as described by Fan et al. (2006). SNP allele calls were performed with the clustering algorithm GenTrain available in GenomeStudio v2011.1 (Illumia Inc., http://www.illumina.com). Each SNP was checked manually in GenomeStudio for genotype calling accuracy. Information for mapped SNPs is available at http://thehordeumtoolbox.org.

Linkage analyses and construction of consensus map

Map Manager QTXb20 version 0.30 (Manly et al. 2001) was used to perform linkage analysis between the

resistance gene and markers. Recombination fraction percentages were converted to cM using the Kosambi (1944) mapping function. Map Chart 2.2 (Voorrips 2002) was used to draw the linkage map.

Chi squared analyses

Goodness-of-fit of observed segregation ratios with the expected genetic ratios of phenotypic data from the F_3 populations was tested using Chi-squared (χ^2) analysis.

Results

Expression of seedling resistance in Ricardo

To determine the optimal temperature for expression of *RphRic*, Ricardo was inoculated with four pathotypes of *P. hordei*, all of which, except one (200P−), were virulent for *Rph2*. The plants were then incubated at three post-inoculation temperatures. Ricardo expressed low ITs ("11++C++" to "1++2C") against pathotype 5457P+, and slightly higher ITs against pathotypes 4673P+ ("1++2++" to "2++3C") and 5652P+ ("12++C" to "2++3-C") over a range of temperatures (17 ± 2, 23 ± 2 and 27 ± 2 °C) under greenhouse conditions (Table 2). The lowest ITs of "11++C++" were noted against pathotype 5457P+ compared with "2++3C" against 4673P+ and "2++3-C" against pathotype 5652P+ at 23 ± 2 °C, where Ricardo produced a higher level of chlorosis at 23 ± 2 °C in comparison with 17 ± 2 and 27 ± 2 °C when inoculated with pathotype 5457P+ (Fig. 1). Peruvian showed the expected susceptibility against all three *Rph2*-virulent pathotypes and resistance against the *Rph2*-avirulent pathotype 200P−, while Gus was susceptible to all the pathotypes used (Table 2).

Table 2 Infection types produced by Ricardo, Gus and Peruvian with different pathotypes of *Puccinia hordei* at three post-inoculation temperatures in the greenhouse under natural lighting

Pathotype	Temperature (°C)	Ricardo	Peruvian	Gus
5457P+	17 ± 2	1++2C	3+	3+
	23 ± 2	11++C++	3+	3+
	27 ± 2	1++2C	3+	3+
5652P+	17 ± 2	12++C	3+	3+
	23 ± 2	2++3-C	3+	3+
	27 ± 2	2++C	33+	3+
4673P+	17 ± 2	1++2 ++	33+	3+
	23 ± 2	2++3C	3+	3+
	27 ± 2	2++3C	3+	3+
200P−	17 ± 2	;N	;N	3+
	23 ± 2	;1 = CN	;1 = CN+	3+
	27 ± 2	;C	;CN	3+

Pathotypes 5457P+, 5652P+ and 4673P+, while virulent on *Rph2* (Peruvian), were avirulent on *RphRic* present in Ricardo; and pathotype 200P− was avirulent on *Rph2* and *RphRic*

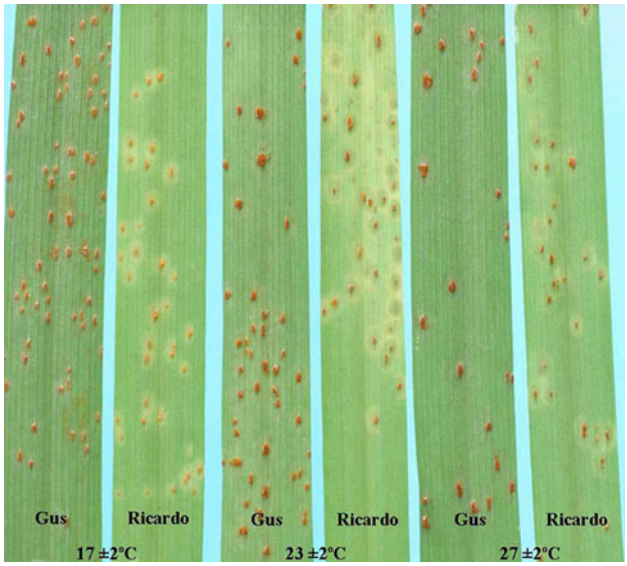

Fig. 1 Greenhouse infection types of Gus and Ricardo (in three sets) at three different post-inoculation temperatures against *Puccinia hordei* pathotype 5457P+

Multipathotype testing for seedling resistance

To postulate and confirm the seedling resistance genes present in the parents Ricardo, Peruvian and Gus, each was tested against ten different pathotypes of *P. hordei* at 23 ± 2 °C in the greenhouse. Ricardo was resistant and Gus was susceptible to all ten pathotypes. Peruvian was susceptible to all pathotypes except 200P− and 5610P+ (both avirulent for *Rph2*). The results obtained were consistent with the presence of seedling resistance genes *RphRic* and *Rph2* in Ricardo and *Rph2* in Peruvian, where all differential lines produced the expected ITs against all used pathotypes (Table 3).

Inheritance of seedling resistance in Ricardo

F_1 and F_3 lines derived from intercrossing Ricardo and Gus were tested with pathotype 5457P+ in the greenhouse. All F_1 plants produced low ITs ("11++C++"), indicating dominant inheritance of *RphRic*. Of the 200 F_3 lines, 13 showed poor germination and were excluded. The remaining 187 F_3 lines were scored as 37 non-segregating resistant, 104 segregating, and 46 non-segregating susceptible when tested with pathotype 5457P+ in the greenhouse. Chi-squared analyses confirmed the goodness-of-fit to a 1:2:1 ratio ($\chi^2 = 3.22$, $p = 0.19$), expected for monogenic inheritance of *RphRic* (Table 4).

Table 3 Responses of Ricardo, Peruvian, Gus and differential genotypes in the greenhouse tests with 10 pathotypes of *Puccinia hordei*

Genotypes	243P−	253P−	200P−	5610P+	5653P++*Rph13*	5453P−	5652P+	4673P+	5653P+	5457P+	Postulated *Rph* gene
Ricardo	2+3−C	2+3−C	;1CN	12+CN	22+C	3−C	2++3−C	2++3C	122+C	11++C++	*Rph2* + *RphRic*
Peruvian	33+	33 + C	;1CN	;1CN	33+	33+	3+	33+	33+	3+	*Rph2*
Gus/susceptible	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	Nil
Sudan/*Rph1*	3+	3+	0;	0;–	3+	3+	;N	3+	3+	3+	*Rh1*
Peruvian/*Rph2*	3+	33+	;1CN	;1CN	33+	33+	3+	33+	33+	3+	*Rph2*
Estate/*Rph3*	0;	0;–	0;=	0;−C	0;=	0;1 = C	;−C	;–	;C	3+	*Rph3*
Gold/*Rph4*	;1+	3+	;11−	3+	3+	3+	3+	3+	33+	3+	*Rph4*
Magnif 104/*Rph5*	0;N	;-N	0;=	0;–	;	0;	;N	3+	0;–	0;C	*Rph5*
Bolivia/*Rph2* + *Rph6*	3+	33+	;C	;CN	3+	3+	33+	33+	3+	3+	*Rph2* + *Rph6*
Cebada capa/*Rph7*	0;	0;N	0;=	0;=	;CN+	;N	0;N	0;N	0;=	;1 = CN	*Rph7*
Egypt 4/*Rph8*	3+	3+	3+	3+	3+	;1CN	3+	33+	3+	12++C	*Rph8*
Abyssinian/*Rph9*	233+	233+	;CN	3+	33+	3+	3+	3+	3+	3+	*Rph9*
Clipper BC8/*Rph10*	;12+	;12+	;1 = C	33+	33+	3+	3+	3	3+	3+	*Rph10*
Clipper BC67/*Rph11*	;12C	;1+2+C	;11+C	1++C	;1C	12+C	12+C	12+C	2++3	2++3C	*Rph11*
Triumph/*Rph12*	22+	12+C	0;CN	3+	33+	3+	3+	3+	3+	3+	*Rph12*
Berg/*Rph1*	3+	3+	0;C	0;–	3+	3+	;N	3+	3	3+	*Rph1*
Reka 1/*Rph2* + *RphP*	;1+	;1+N	;1 = C	;C	3+	3+	3+	3+	;C	3+	*Rph2* + *RphP*
Ricardo/*Rph2* + ?	2 + 3−C	2 + 3−C	;1CN	12+C	22++C	3 = C	2++3−C	12++3C	122+C	11++C++	*Rph2* + ?
Quinn/*Rph2* + *Rph5*	;N	;N	0;C	0;–	0;–	0;=	;−N	3+	0;–	0;C	*Rph2* + *Rph5*
PI 531849/*Rph13*	0;N	0;-	0;=	0;=	3+	;1 = C	;-N	;1N	;CN	;CN	*Rph13*
PI 584760/*Rph14*	;12−	;12+C	11++2+C	3	;CN+	;1−N	;12−N	;12N	;CN	;1−CN	*Rph14*
Prior/*Rph19*	;1−N	;1−N	0;=	3+	3+	11−C	3+	3+	3+	3+	*Rph19*
Q21861/*RphQ*	33+	3+	0;	;C	3+	3+	3+	3+	33+	3+	*RphQ*
Cantala/*RphC*	12−C	;1−C	3+	3+	33+	3+	3+	3+	3+	3+	*RphC*
PI366444/*RphB37*	3	2 ++3C	3+	3+	3+	3+	3+	3+	3+	3+	*RphB37*
Gatam/*RphGat*	33+	33+	0;C	0;–	3+	;11++C	33+	3+	33+	3+	*RphGat*
Bowman + *Rph15*/*Rph15*	;N	;N	0;C	;CN	;CN+	;CN	;1−N	;N	;CN	;CN	*Rph15*
81882/BS1/*Rph17*	;1 N	;1 N	;C	;11+C	;1−CN	0;C	;N	;1+N	;C	0;C	*Rph17*
38P18/8/1/10/*Rph18*	0;N	0;N	0;=	0;=	0;=	0;=	0;N	0;N	0;-	0;=	*Rph18*

Table 4 Observed segregation frequencies in F_3 populations derived from the crosses Ricardo/Gus and Ricardo/Peruvian (*Rph2*), when inoculated with the *Rph2*-virulent *P. hordei* pathotype 5457P+ and *Rph2*-avirulent *P. hordei* pathotype 200P− respectively, at seedling stage in the greenhouse

Cross	Pathotype	Number of F_3 lines				Predicted ratio	χ^2	*p*	Number of genes
		Non-segregating resistant	Segregating	Non-segregating susceptible	Total				
Ricardo/Gus	5457P+	37	104	46	187	1:2:1	3.22	0.19	1
Ricardo/Peruvian	200P−	79	0	0	79	No Seg.			*Rph2*
Ricardo/Peruvian	200P−	68	0	0	68	No Seg.			*Rph2*

20–25 plants assessed per F_3 line, χ^2 table value at $p = 0.05$ is 5.99 (2 d.f.) and at $p = 0.01$ is 9.21 (2 d.f.)

Seg. segregation

Molecular mapping of *RphRic*

A total of 488 SSR were used to identify markers linked to the uncharacterised locus responsible for seedling resistance to *P. hordei* in cultivar Ricardo. Twelve markers (*EBmac0635*, *EBmac0701*, *HvBTAI0003*, *HvHVO0003*, *HVMLOE*, *HvPEPD1PR*, *GBM1220*, *GBM1003*, *GBM 1015*, *GBM1028*, *GBM1044* and *Bmy1_INDEL6*) were polymorphic between the parents and showed linkage with the resistant and susceptible bulks. The allele sizes for each of these linked SSRs are given in Table 5. All markers were mapped previously on chromosome 4H, providing strong evidence for the presence of *RphRic* in this chromosome.

The 12 linked SSRs were used to genotype the 187 Ricardo/Gus F_3 lines. Linkage analyses grouped seven markers (*HvHVO0003*, *HVMLOE*, *HvPEPD1PR*, *GBM 1220*, *GBM1003*, *GBM1015*, and *GBM1044*) into a single linkage group of 85.1 cM, with the *RphRic* locus flanked by *GBM1220* on the proximal end and by *GBM1003* on the distal end, at distances of 17.4 and 20.4 cM, respectively. To further enhance genetic mapping of the *RphRic* locus, 384 SNPs were also genotyped on the Ricardo/Gus F_3 lines. Linkage analysis of both marker sets resulted in a linkage group of 173.5 cM, comprised of 8 SSR and 15 SNP loci, with the *RphRic* locus flanked by markers *2_0765* and *GBM1044* on the proximal end by 14.1 and 18.1 cM, respectively, and by *2_0119* and *GMB1220* on the distal end, at distances 16.4 and 16.9 cM, respectively (Fig. 2). The alleles for each linked SNP are given in Table 5.

Detection of *Rph2* in Ricardo

Two independent populations derived from the cross Ricardo/Peruvian, consisting of 79 and 68 F_3 lines, were all resistant and showed no segregation in ITs when tested with the *Rph2*-avirulent pathotype 200P−, consistent with

Table 5 Details of polymorphic SSRs and SNPs published for chromosome 4H of barley used to genotype 187 F_3 lines derived from the cross Ricardo/Gus

Marker	Size (bp) or SNP allele			
	Resistant bulk	Ricardo	Gus	Susceptible bulk
HvHVO0003	269	269	266	266
HVMLOE	269	269	266	266
GBM1220	197	197	195	195
GBM1003	229;235	235	229	229
GBM1044	265	265	262	262
HvPEPD1PR	211;218	211	218	218
GBM1015	283;295	283	295	283;295
EBmac0635	141	141	Null	Null
EBmac0701	175	175	Null	Null
HvBTAI0003	263	263	Null	Null
GBM1028	314	314	316	314;316
Bmy1_INDEL6	262	262	262;272	262;272
2_1359	NA	T	G	NA
1_0031	NA	C	G	NA
1_0793	NA	C	G	NA
1_0010	NA	T	C	NA
1_1224	NA	A	G	NA
1_1513	NA	T	C	NA
1_1004	NA	C	T	NA
1_0247	NA	G	C	NA
1_0724	NA	C	G	NA
2_0765	NA	A	C	NA
2_0119	NA	A	C	NA
2_0974	NA	A	C	NA
1_1066	NA	G	C	NA
2_0668	NA	A	G	NA
1_0387	NA	T	G	NA

Values separated by ; indicates recombination shown by marker

Null null allele, *NA* not applicable

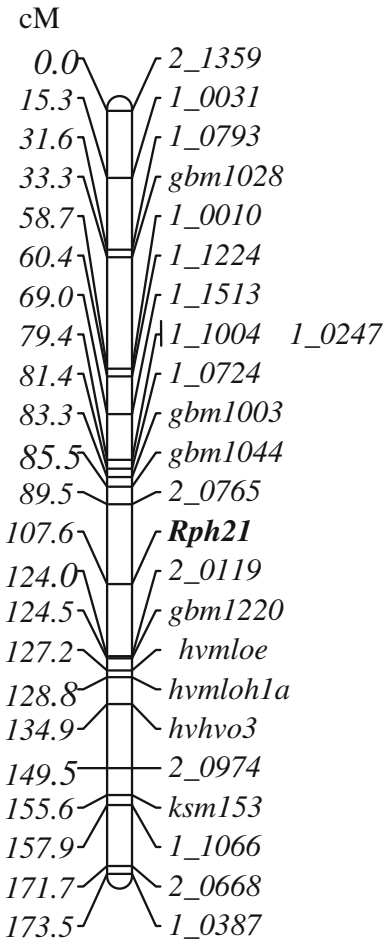

Fig. 2 Genetic map for *Rph21* (*RphRic*) in chromosome 4H in Ricardo/Gus F_3 population

the presence of *Rph2* in both Ricardo and Peruvian (Table 4). To ensure that the Ricardo/Peruvian crosses did not involve selfing, 15 F_3 lines from both populations were selected randomly and tested with pathotype 5457P+ (virulent to *Rph2* and avirulent to Ricardo). Segregation was observed in both tests [non-segregating resistant: segregating: non-segregating susceptible (4:7:4 and 3:5:7)], indicating that selfing was not involved, and the observed segregation pattern conformed to that expected from a single gene ($\chi^2 = 0.02$, 3.75 and $p = 0.99$, 0.15, respectively).

Discussion

The present study aimed to characterise a new source of resistance to *P. hordei* to help diversify the resistance sources currently available to control this disease. Cultivar Ricardo was reported to carry the leaf rust resistance gene *Rph2* (*Pa2*) (Henderson 1945; Moseman and Roan 1959; Zloten 1952) plus additional uncharacterised seedling resistance (Park unpublished; Stöcker 1983; Wallwork et al. 1992; Yahyaoui et al. 1988), referred to here as *RphRic*. Studies were therefore conducted to characterise *RphRic* and to prove the presence of *Rph2* in Ricardo. To ensure accurate phenotyping of *RphRic*, studies were first conducted to identify the most congenial environment for optimal expression of this gene.

The expression of rust resistance genes can differ with environmental conditions. In previous greenhouse studies, Ricardo produced ITs ranging from "1+"–"3" against different *P. hordei* pathotypes (Park unpublished; Golegaonkar 2007). Ricardo, Gus and Peruvian seedlings were therefore inoculated with four different pathotypes of *P. hordei*, three of which were virulent for *Rph2*, and one of which was avirulent (200P−), and incubated at a range of post-inoculation temperatures. The expression of the uncharacterised resistance in Ricardo varied with post-inoculation temperature and was found to be most strongly expressed at 23 ± 2 °C. Temperature sensitivity of rust resistance genes has been reported previously in both barley and other cereals. For example, seedlings of barley genotypes carrying the stem rust resistance gene *rpg4* produced different ITs against pathotype QCCJ of *P. graminis* f. sp. *tritici* when incubated at 18–19 and at 27–28 °C (Sun and Steffenson 1997). Similarly, wheat seedlings carrying *Yr17* expressed higher levels of resistance at 15–20 °C, and were susceptible at 12–15 °C to *P. striiformis* f. sp *tritici* (Qamar et al. 2008). At 18 °C and below, low ITs were produced by wheat seedlings carrying *Sr15*, while high ITs were produced at 26 °C and above when inoculated with *P. graminis* f. sp. *tritici* (Gousseau et al. 1985).

To confirm the presence of *RphRic* in Ricardo (Henderson 1945; Moseman and Roan 1959; Zloten 1952), multipathotype tests were carried out in the greenhouse. In addition to *Rph2*, the detection of *RphRic* in Ricardo (Table 3) was in accordance with earlier studies (Park unpublished; Stöcker 1983; Wallwork et al. 1992; Yahyaoui et al. 1988). Inheritance studies and Chi-squared analyses of F_3 Ricardo/Gus families confirmed dominant monogenic inheritance of *RphRic*. As pathotype 5457P+ was virulent on *Rph2*, the observed segregation was for gene *RphRic* only. Genetic mapping in the Ricardo/Gus population located *RphRic* on chromosome 4H, flanked proximally and distally by SSR markers *gbm1044* and *gbm1220,* and SNP markers *2_0765* and *2_0119*, respectively (Fig. 2). As no catalogued seedling gene conferring resistance to *P. hordei* has been located in chromosome 4H, the locus symbol *Rph21* is designated for *RphRic*. As virulence for *RphRic* has not yet been detected in Australia (Park, unpublished), it is potentially a useful new source of resistance to *P. hordei*.

Many genotypes of barley are reported to carry the seedling gene *Rph2* alone or in combination with other leaf

rust resistance genes. For example, the barley cultivar Peruvian carries *Rph2* (Levine and Cherewick 1952; Starling 1956; Steffenson and Jin 1997), Reka 1 carries *Rph2* and *Rph19* (Park and Karakousis 2002), Quinn carries *Rph2* and *Rph5* (Roane and Starling 1967; Starling 1956), and Bolivia carries *Rph2* and *Rph6* (Henderson 1945; Roane and Starling 1967; Starling 1956). Previous reports of the presence of *Rph2* in Ricardo are based solely on gene postulation. In the present study, two sets of F_3 populations, each derived from a separate F_1 seed from the cross Ricardo/Peruvian (*Rph2*), were tested with the *Rph2*-avirulent pathotype 200P− in the greenhouse. No segregation was observed in either F_3 population, providing genetic evidence that Ricardo carries *Rph2*. Considering this, it was necessary to use an *Rph2*-virulent pathotype in studies that led to the characterisation of *Rph21*. When 15 randomly selected F_3 lines from each population derived from the cross Ricardo/Peruvian were tested with the *Rph2*-virulent pathotype 5457P+, single gene segregation at the *RphRic* locus was observed, further supporting the presence of *Rph2* in both parents. In similar studies, Borovkova et al. (1997) reported no segregation in F_2 populations derived from an intercross between *RphQ* (Q21861) and *Rph2* (Peruvian, PI531840 and PI531841), when inoculated with a pathotype of *P. hordei* avirulent on *Rph2*, indicating the presence of the same resistance locus in both parents.

The present study established the presence of *Rph2* and *Rph21* in Ricardo. Seedling gene *Rph21* represents a new and useful source of resistance to *P. hordei* for the breeding of barley varieties with resistance to leaf rust. Given that the present study sought only to characterise and map *Rph21*, further work in finding more closely linked markers for efficient screening of this novel gene in breeding programmes would now be desirable. The gene-based SNP markers identified as flanking *Rph21* in this study provide a basis for the development of more closely linked markers by comparative mapping approach (Perovic et al. 2004) or with the use of newly developed genomic resources of barley (Mayer et al. 2011).

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