Combination of seedling and adult plant resistance to leaf scald for stable resistance in barley

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Abstract

Rhynchosporium secalis can overcome a single resistance gene of barley in a relatively short period of time. Novel genes and quantitative trait locis (QTLs) are therefore vital to control scald in barley. A population of 220 double haploid lines was developed from a cross of Vlamingh and WABAR2147, where Vlamingh showed adult plant resistance (APR) and WABAR2147 showed seedling resistance to a group of isolates. The population was tested for APR to scald under natural infection in two consecutive seasons in addition to a seedling screen with three isolates. One single gene was mapped to chromosome 6H based on the seedling test, and two QTLs (*QSc.VlWa.4H* and *QSc.VlWa.6H*) were mapped to chromosomes 4H and 6H based on APR. Epistatic interaction was observed between the two QTLs, and environment/QTL interaction was only observed for *QSc.VlWa.6H* which co-segregated with the seedling resistance gene and contributed to basal resistance against scald during whole growth stages. *QSc.VlWa.4H* explained 42.5 and 57.8 % of the phenotypic variation in the two independent trials when the effect of *QSc.VlWa.6H* was excluded from the analysis. We developed a high-density consensus genetic map with 7,876 molecular makers and anchored 43 QTLs and 7 genes for scald resistance from different mapping populations. No known QTLs or genes were reported in a similar position to *QSc.VlWa.4H*, and it was the first major QTL for APR of scald on chromosome 4HS in barley. Combination of the two QTLs achieved better and stable scald resistance across four different environments.

Keywords

Leaf scald; *Rhynchosporium secalis;* Barley; Adult plant resistance; Durable resistance

Introduction

Scald [*Rhynchosporium secalis* (Oudem) J.J. Davis] of barley (*Hordeum vulgare* L.) is a serious leaf and stem disease worldwide, especially in temperate climate areas. It causes significant yield losses and reduces grain quality (Shipton et al. 1974). The disease is caused by *R. secalis*, a highly variable fungal pathogen (Zaffarano et al. 2006; Goodwin et al. 1993). Because of this variability for pathogenicity, the *R. secalis* population can change over a relatively short period of time and quickly overcome the single resistance gene in barley (Newton et al. 2001; Oxley 2003). Therefore, breeding

non-race-specific cultivars or pyramiding some related resistance genes into a single cultivar is an effective strategy for controlling scald (Cselenyi et al. 1998; Nelson 1978). Identification of novel genes or quantitative trait loci is of considerable importance.

Several resistance genes against *R. secalis* have been reported in cultivated barley and wild barley. *Rrs1* is a complex locus on the long arm of chromosome 3H with multiple alleles (Graner and Tekauz 1996; Bjornstad et al. 2002, 2004). Other genes include *Rrs13* on chromosome 6H (Abbott et al. 1995; Genger et al. 2003b), *Rrs2*, *Rrs12* and *Rrs15* on chromosome 7H (Abbott et al. 1992; Genger et al. 2003a, b, 2005; Schweizer et al. 1995), *Rrs14* on chromosome 1HS (Garvin et al. 1997, 2000) and *Rrs16* on chromosome 4HS (Pickering et al. 2006). In addition, a number of QTLs for scald resistance have been identified on all chromosomes except chromosome 5H (Backes et al. 1995; Li and Zhou 2011; Sayed et al. 2004; Spaner et al. 1998; Yun et al. 2005).

It is noteworthy that almost all the genes and quantitative trait locis (QTLs) against leaf scald in cultivated and wild barley are related to seedling resistance although some genetic characters are not expressed at the seedling stage (Cheong et al. 2006). This type of resistance can be readily overcome by changes in pathogen populations. In contrast, adult plant resistance (APR) involves partial levels of resistance which may provide more durable resistance. Understanding the genetics of resistance to scald at the adult plant stage will help to develop strategies for maintaining more durable resistance. We report a major QTL for APR against scald in a commercial malting barley variety Vlamingh and test resistance stability by a combination of the QTL and seedling resistance gene.

Materials and methods

Plant material

Vlamingh is a major malting barley variety released by the Department of Agriculture and Food Western Australia in 2006. The variety is susceptible to scald at the seedling stage, but expresses moderate-to-high levels of resistance at the adult plant stage. WABAR2147 is an advanced breeding line with good seedling resistance to scald and high malting quality. A population of 220 double haploid (DH) lines was developed from the Vlamingh × WABAR2147 cross using anther culture.

Rhynchosporium secalis assay

Seedling resistance test using isolates

The detailed method for seedling resistance has been reported (Wallwork and Grcic 2011). Barley seeds from different lines were sown in 80-mm^2 pots which were placed in open-bottomed plastic trays (12 pots per tray). Each half pot had two or three seeds sown of the differential DH lines. Plants were grown in a controlled environment room (CER) with a 10/14-h photoperiod set at 15/13 °C until the three-leaf stage at which time they were ready for inoculation. Testing of each line by isolate combination involved two or three seedlings per half pot, with three replicates per test using three isolates. Seedlings were rated using a simple four-point scale (R, MR, MS and S): R = no symptoms, MR = minor lesions, mostly on leaf sheaths or leaf margins, MS = few and/or late-appearing susceptible leaf lesions, S = many large lesions or death of seedling leaves. Three isolates with different virulent to the known seedling resistance genes were used to inoculate the DH population. The isolates were selected from the 12 isolates in Wallwork and Grcic (2011), which represent majority of the known *Rhynchosporium* isolates in Australia. The DH lines and parents were randomly arranged in the CER, and the consensus rating of the three replicates was recorded as the resistance scale.

Field screening

The DH population was sown at the Department of Agriculture and Food Research Stations in Wongan Hills, Western Australia (Lat 30.54 South and Long 116.43 East), in two consecutive years (2004 and 2005). All DH lines were sown in a full randomized block design with two replicates. Each plot comprised 10–15 plants in one meter rows. Barley straw infested with scald was applied at 50 g/m^2 at the seedling stage (4–5 leaf). Plants in each plot were assessed at anthesis according to the CIMMYT scale (0–9) (Saari and Prescott 1975). Half of the population was randomly selected for further testing APR at Shenton Park (Lat 31.96 South and Long 115.81 East) and Medina (Lat 32.24 South and Long 115.81 East), Western Australia, for validation in the following year.

Statistical analysis and genetic mapping

A genetic map of the Vlamingh × WABAR2147 DH population was constructed with JoinMap 4.1 (Van Ooijen 2011). The linkage map of this population includes 435 diversity arrays technology (DArT) and 33 microsatellite markers. The DArT markers were conducted by commercial service (http://www.diversityarrays.com), and the microsatellite markers were selected from Graingenes (http://wheat.pw.usda.gov/GG2/index.shtml).

All statistical analyses were carried out with SPSS, version 20. Correlation of scald severity for the DH lines between years/sites was calculated. A significance analysis was used to assess the variance of phenotype at the two growth stages.

QTL analysis

QTL analysis of *Rhynchosporium* resistance was performed using MapQTL software, version 5 (Van Ooijen 2004). First, an interval mapping (IM) analysis was performed to identify the major QTL. Automatic cofactor selection was used to detect significantly associated markers as possible cofactors for multiple-QTL mapping (MQM) analysis. In order to determine the significant threshold of the LOD score, a permutation test was performed to calculate the LOD threshold corresponding to 10,000 iterations and a significance level $\alpha = 0.05$. A threshold LOD >3 was used to declare the presence of a QTL in the IM and MQM analyses. QTL positions were determined on the individual map and then extrapolated to the barley consensus map.

Construction of the barley integrated map

Seventeen genetic populations were used to generate a high-density integrated map of barley (Electronic Supplementary Material Table S1). The populations consisted of 11 DH populations: Barque-73/CPI71284-48, Clipper/Sahara, Dayton/Zhepi2, Igri/Atlas68, Steptoe/Morex, TX9425/Franklin, Yerong/Franklin, OWBRec/OWBDom, Morex/Barke, Igri/Franka and Lina/Canada Park; and 6 recombinant inbred line (RIL) populations: Frederickson/Stander, Patty/Tallon, Foster/CI4196, SusPtrit/Vada, L94/Vada and Haruna Nijo/Akashinriki (Electronic Supplementary Material Table S1).

Segregating makers from the consensus map consisted of AFLP, RFLP, RARD, SSR, DArT and SNP markers. Segregating data sets were obtained from Varshney et al. (2007) and Hearnden et al. (2007) for SSR; and Wenzl et al. (2006) for DArT; SNP maker data sets from Close et al. (2009), Sato et al. (2011) and Szucs et al. (2009).

The integrated map was constructed using MergeMap software developed from the University of California, USA (Wu et al. 2007, 2008a, b, 2011). The software compares individual maps and then combines them into a single, directed graph on the basis of shared vertices. Inconsistencies among individual maps generates cycles in the combined graph that are resolved by deleting the smallest set of marker occurrences (Jackson et al. 2005, 2008; Yap et al. 2003). The value of weight was assigned to all individual maps with high value of weight associated with high confidence. The data sets from

Hearnden et al. (2007) and Szucs et al. (2009), which connected other maps, were given a higher value of weight.

The implementation of Mergemap will inflate the genetic distance in the consensus map. Therefore, the genetic length of the consensus map was normalized to the mean centimorgans (cM) distance for each linkage group from the different individual maps (Close et al. 2009).

Results

Phenotypic variation of resistance against *Rhynchosporium*

Field test for scald resistance

Scald resistance was scored from 0 (non-infected) to 9 (highly susceptible) in the field trials. Both parents showed moderate tolerance to scald at the adult plant stage. Vlamingh (score 2.2–3.3) exhibited slightly better tolerance than WABAR2147 (score 2.2–4.2) in the two trials (Table 1). Transgressive segregation was observed in both years among the DH population with disease scores varying from 1 (high resistance) to 9 (high susceptibility) (Table 1; Fig. 1a). Correlation between the values for DH lines in two trials was highly significant ($R^2 = 0.7646$, $P < 0.001$), which indicated that APR to scald was predominantly controlled by genetic factors.

Seedling test for scald resistance

In contrast to the APR in field trials, the two parental lines significantly differed for scald response when inoculated at the seedling stage using race-specific isolates in the glasshouse. Vlamingh was highly susceptible, while WABAR2147 was highly resistant to scald. The DH population was segregated in a 1:1 ratio for resistance and susceptibility (Electronic Supplementary Material Fig. S1), which suggested that one major gene controlled seedling resistance. The seedling resistance gene was mapped to chromosome 6H and flanked by markers bPb-6331 and bPb-5252 (Fig. 2). The segregation of seedling resistance was further considered as a genetic maker (scald-seedling) when conducting QTL analysis for APR.

According to the seedling resistance results, we divided the whole population into seedling resistant and seedling susceptible groups. The frequency distribution of scald severity distorted towards susceptibility in the seedling susceptible group (Fig. 1b), when comparing the whole population (Fig. 1a), which indicated that the seedling resistance gene interacted with APR in the field. Therefore, we conducted QTL analysis for APR in the full population and the seedling susceptible subpopulation.

QTL adult plant resistance

Two QTLs for APR to scald were identified in the population of Vlamingh and WABAR2147 in both trials (Table 2). The resistance from Vlamingh was mapped to chromosome 4H, designated as *QSc.VlWa.4H*. The resistance from WABAR2147 was mapped to chromosome 6H, designated as *QSc.VlWa.6H*.*QSc.VlWa.6H* co-segregated with the seedling resistance gene on chromosome 6H, explaining 30.7 and 27.1 % of phenotypic variation with LOD scores of 12.9 and 11.4 in the two trials, respectively.

When the *QSc.VlWa.6H* effect was fixed, only *QSc.VlWa.4H* was identified for APR which was detected in both trials with the same confidence interval on chromosome 4H near SSR maker GBM1501. The total phenotypic variation explained by *QSc.VlWa.4H* was 42.5 and 57.8 % in the two trials, respectively.

Genetic effect of the two QTL combinations

The DH population was further divided into 4 groups based on the two QTLs on chromosomes 4H and 6H (Table 3). The combination of the 6H QTL from WABAR2147 and the 4H QTL from Vlamingh significantly improved scald resistance (average score 2.1) over the single gene on 6H (average score 3.0) or the single QTL on 4H (average score 3.9). More importantly, the combination of the two QTLs/genes improved resistance stability in the different environments (Table 3). This was more significant at the Shenton Park and Medina sites where the diversity of the pathogen was higher.

The new barley integrated map

In order to compare the QTL mapping results with the known scald-resistant genes/QTLs, 17 mapping populations were used to construct the consensus map. Of these populations, segregating data sets of Barque-73/CPI71284-48 and OWBRec/OWBDom formed the framework map which contributed more than 40 % of the markers to the final consensus map. Among these mapping populations, 518 SNPs, 257 DArTs and 24 SSR markers were shared in at least two populations. In order to inspect the reliability of this consensus map, we compared it to the consensus map developed by Aghnoum et al. (2010) and the SNP consensus map developed by Munoz-Amatriain et al. (2011) (http://wheat.pw.usda.gov). The marker orders between all maps were highly consistent with only a few inversions in some regions.

A total of 7,876 molecular makers were integrated into the barley consensus map covering a total genetic distance of 1,363 cM with an average distance between two adjacent makers of 0.17 cM (Electronic Supplementary Material Table S2). This consensus map contained 40 % DArTs, 37.5 % SNPs, 11.7 % SSRs, 6.1 % RFLPs and 0.6 % AFLPs. More than 90 % of the markers on this consensus map were designed on the basis of the gene sequence or expressed sequence tags and can be used to anchor related candidate genes to the fingerprinted contigs of barley. We used this consensus map to compare all known QTLs and genes for scald resistance.

Discussion

Diversity of QTLs and genes for *Rhynchosporium* resistance in barley

In total, 43QTLs and seven genes conferring quantitative and qualitative resistance to *R. secalis* have been located on individual maps (Abbott et al. 1992, 1995; Backes et al. 1995; Bjornstad et al. 2002, 2004; Cheong et al. 2006; Garvin et al. 1997, 2000; Genger et al. 2003a, b, 2005; Gronnerod et al. 2002; Jensen et al. 2002; Li and Zhou 2011; Patil et al. 2003; Pickering et al. 2006; Sayed et al. 2004; Schweizer et al. 1995, 2004; Shtaya et al. 2006; Spaner et al. 1998; von Korff et al. 2005; Wagner et al. 2008; Yun et al. 2005). Here, we integrated all these QTLs and genes into a consensus map to assess the genetic variation of scald resistance (Fig. 2). The QTLs and genes for scald resistance were distributed on six chromosomes except chromosome 5H. In this study, two QTLs were identified in the Vlamingh × WABAR2147 population. *QSc.VlWa.6H* was located on the short arm of chromosome 6H. Previous reports identified other QTLs for scald resistance in a similar region (Cheong et al. 2006; Gronnerod et al. 2002; Jensen et al. 2002; Shtaya et al. 2006; Spaner et al. 1998). This suggests that *QSc.VlWa.6H* might be the same as QTLs reported in other genetic materials. *QSc.VlWa.4H*was the first major QTL for APR identified on chromosome 4HS. There were no other QTLs for scald resistance of barley reported in a similar position to *QSc.VlWa.4H*.

It is of interest that both QTLs were located at or near the gene-specific seedling resistance genes. The *Rrs13* gene, originating from *H. vulgare* ssp. *spontaneum*, was close to the location of *QSc.VlWa.6H* (Abbott et al. 1995; Genger et al. 2003b), which may partially explain that WABAR2147 showed resistance to various isolates. *QSc.VlWa.4H* was in a similar position to

the *Rrs16* gene, which was identified in *Hordeum bulbosum*. In contrast to *QSc.VlWa.6H*, no seedling resistant effect was observed for *QSc.VlWa.4H.* These QTLs and resistance genes were identified from different genetic backgrounds and field trials, indicating that more evidence is needed to determine whether *QSc.VlWa.6H* and *QSc.VlWa.4H* are alleles of major resistance genes.

Growth-stage specificity of QTL for scald resistance

Several studies have revealed that most QTLs for leaf scald resistance in barley are highly growth-stage specific and that a high level of genetic diversity is involved in consistent resistance to scald (Cheong et al. 2006). We found that *QSc.VlWa.6H* was detected at both growth stages and contributed to basal resistance against scald during whole growth stages. It suggests that *QSc.VlWa.6H* for leaf scald resistance is growth stage independent. This also agrees with the previous reports, where several genes and QTLs for different plant stage resistance were mapped in a similar position to *QSc.VlWa.6H* (Abbott et al. 1995; Cheong et al. 2006; Jensen et al. 2002; Spaner et al. 1998). On the other hand, *QSc.VlWa.4H* was identified for APR and the high effect of *QSc.VlWa.4H* confirmed that it was responsible for scald APR in Vlamingh. As no seedling resistance was detected in Vlamingh, *QSc.VlWa.4H*should be a real APR gene.

Development of barley variety with more durable resistance

The scald pathogen can rapidly change pathotype composition and frequency. This makes it difficult to develop durable barley scald resistance. Various strategies have been proposed to develop barley varieties with more durable resistance, including the identification of novel genes, QTLs and gene pyramids (Cheong et al. 2006; Wallwork and Grcic 2011). Another difficulty is the negative association of durable scald resistance with yield and malting quality, which makes it more challenging when breeding malting barley varieties with good resistance for scald and reasonable yield (Juskiw et al. 2008). In the present study, one major QTL for APR and one gene-specific resistance gene were identified with good malting quality. Pyramiding the two QTLs/genes not only enhanced scald resistance but also improved resistance stability across different environments (Table 3). Hopefully, the QTL/gene combination may provide more durable resistance. As the QTL/gene combination was pyramided in the malting quality background, it should provide valuable genetic materials and molecular markers for developing malting barley varieties with durable scald resistance.

Acknowledgments

This project is supported by the Australian Grain Research and Development Corporation.

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Fig. 1 Frequency distribution of phenotypes for scald derived from a population of Vlamingh × WABAR2147 (**a**) and the seedling susceptible group (**b**) at the adult growth stage in 2004 and 2005

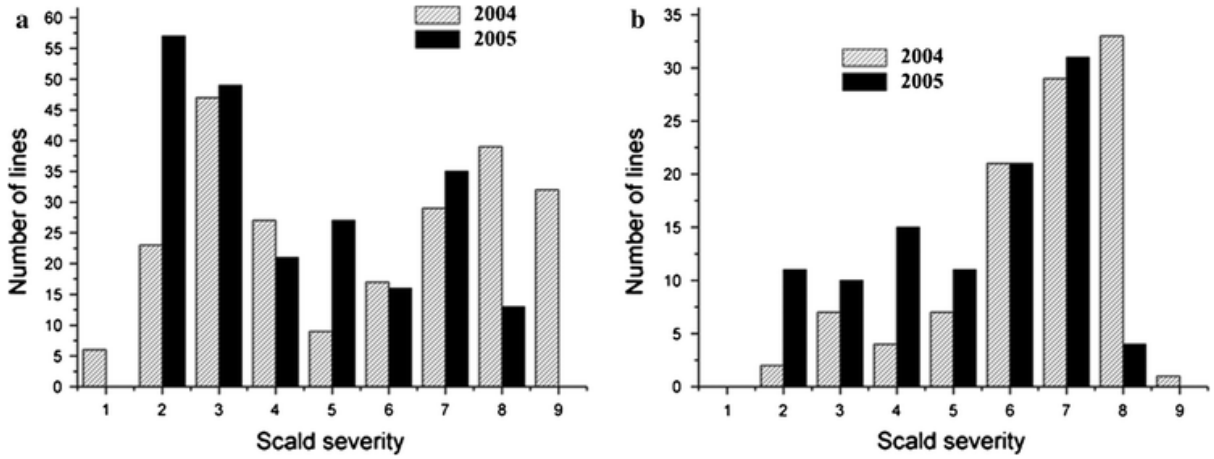

Fig. 2 Locations of quantitative trait loci and major genes for resistance to *R. secalis* on the new barley consensus map

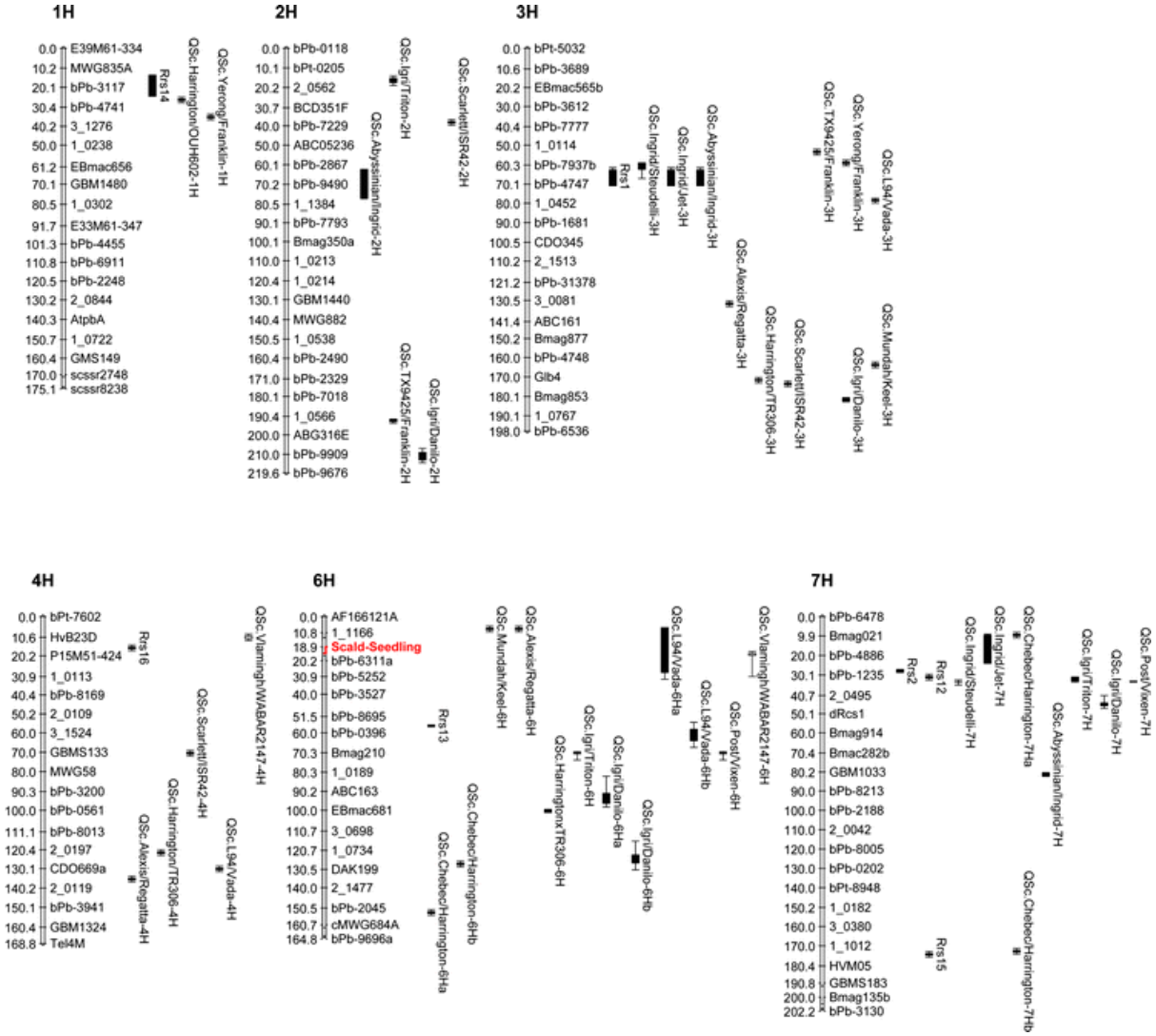

Table 1

Descriptive statistics for scald severity (mean ± SE) in populations of Vlamingh × WABAR2147 measured in different trials

Year	**Means for parents**		**DH lines**		
	WABAR2147	**Vlamingh**	**Minimum**	**Maximum**	**Mean**
Year 1 (adult stage)	2.2 ± 0.50	2.2 ± 0.24	1.0	9.0	3.3 ± 0.15
Year 2 (adult stage)	4.2 ± 0.51	3.3 ± 0.33	1.0	9.0	4.1 ± 0.16
Seedling stage	Resistance	Susceptible			

Table 2

Summary of QTLs for disease severity of scald (*R. secalis*) identified in the population of Vlamingh × WABAR2147 and the susceptible group

Population	QTL	Position (cM)	Nearest marker	LOD	Exp%	Additive
Year 1						
Whole population	QSc.VlWa.4H	11.42	GBM1501	6.3	10.3	−0.64497
	QSc.VlWa.6H	18.9	Scald-seedling	12.89	30.7	1.1034
	Total				41	0.458435
Susceptible group	QSc.VlWa.4H	9.9	bPb-9304	9.12	42.5	−1.68606
Year 2						
Whole population	QSc.VlWa.4H	11.42	GBM1501	4.17	7.2	−0.63499
	QSc.VlWa.6H	18.9	Scald-seedling	11.35	27.1	1.24815
	Total				34.3	0.613161
Susceptible group	QSc.VlWa.4H	11.42	GBM1501	16.98	57.8	−1.37645

Table 3

Adult plant resistance of the four genotypes at four trial sites (mean ± SE) based on the seedling resistance gene on chromosome 6H from WABAR2147 (W6H) and the adult plant resistance QTL on chromosome 4H from Vlamingh (V4H)

Genotypes	Shenton Park	Medina	Wongan Hills	Wongan Hills2
W6H/V4H	2.0 ± 0.18a	2.4 ± 0.10a	2.4 ± 0.12a	1.7 ± 0.10a
W6H/W4H	4.1 ± 0.35c	3.4 ± 0.10b	2.5 ± 0.28a	1.9 ± 0.09a
V6H/V4H	2.4 ± 0.21b	5.9 ± 0.28c	3.7 ± 0.32b	3.4 ± 0.22c
V6H/W4H	6.0 ± 0.30d	7.4 ± 0.13d	6.6 ± 0.16c	5.7 ± 0.13d

The different letter after the data represented statistically significance ($P < 0.05$). The disease scale is scored 1–9 where 9 is the most susceptible