Australasian Plant Pathol. (2014) 43:197–203
DOI 10.1007/s13313-013-0264-5

Use of specific differential isolates of *Rhynchosporium commune* to detect minor gene resistance to leaf scald in barley seedlings

H. Wallwork · M. Grcic · C. D. Li · M. J. Hayden · K. Chalmers · D. E. Mather

Received: 22 May 2013 / Accepted: 18 December 2013 / Published online: 30 December 2013

Abstract Major gene resistance to leaf scald caused by *Rhynchosporium commune* is readily detected in barley seedlings. Screening of barley lines in this manner has been used to identify and map the presence of several such major genes. Similar detection systems have not been possible for minor genes, detection of which has come from field evaluation of plants at later growth stages. Resistance contributed by such minor genes has often therefore been termed adult plant resistance (APR) and most barley lines possess some degree of such resistance to scald. The presence and genetic control of minor gene resistance has been more difficult to study due to the requirement for field screening and the partial and apparent multigenic nature of this resistance. In this paper we report the identification of isolates of *R. commune* and methods which enable the presence of minor genes to be detected at the seedling stage in some key Australian varieties and breeding parents. A mapping population has been used to confirm that the QTL detected in seedlings and in the field are the same.

Keywords Adult plant resistance · Resistance QTL · Pathogen variation

H. Wallwork (✉) · M. Grcic
South Australian Research and Development Institute, Hartley Grove, Urrbrae, SA 5064, Australia
e-mail: hugh.wallwork@sa.gov.au

C. D. Li
Department of Agriculture and Food, 3 Baron Hay Court, South Perth, Western Australia 6150, Australia

H. Wallwork · K. Chalmers · D. E. Mather
School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Urrbrae, South Australia 5064, Australia

M. J. Hayden
Department of Environment and Primary Industry, AgriBioSciences Centre, La Trobe University, Bundoora, Victoria 3083, Australia

Introduction

Barley scald, caused by *Rhynchosporium commune* (formerly *R. secalis,* Zaffarano et al. 2011) is a fungal pathogen with a very high degree of variation in virulence on barley (Ali et al. 1976; Goodwin et al. 1992; Jackson and Webster 1976; Tekauz 1991; Wallwork and Grcic 2011; Williams et al. 2003; Zhang et al. 1992). Species of barley grass (*Hordeum glaucum* and *H. leporinum*) are alternative hosts of the fungus also with diverse genotypes varying in resistance to the fungus (Jarosz and Burdon 1996) and harbouring diverse pathotypes of the fungus (Ali 1981; Brown 1990). Control of scald in barley crops has been through host resistance combined with beneficial crop rotations and use of fungicides.

Resistance to scald can be detected at the seedling stage and/or in adult plants. Genetic analyses have attributed seedling-stage resistance to major genes that provide high levels of isolate-specific resistance at all growth stages (Summarised in Goodwin et al. 1990). 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 be under the control of multiple minor genes, each of which contributes a small effect. Consistent with this, several small-effect quantitative trait loci (QTL) have been reported for resistance of barley against scald (Jensen et al. 2002; Cheong et al. 2006; Zhan et al. 2008).

One of the benefits of resistance genes with minor effects is the possibility they provide for gene pyramiding to develop varieties with durable resistance. With visual selection of breeding lines with less severe symptoms than either parent, it should be possible to obtain transgressive segregant progeny with more minor-effect genes than either parent. With repeated cycles of similar selection, it may be possible to increase both the level and durability of resistance, especially if the genes confer resistance against different and/or multiple isolates of the pathogen.

Current methods for the evaluation of minor-gene resistance involve assessment of adult plants in inoculated field nurseries. Compared to methods used to assess seedling resistance, assessment of minor gene resistance takes longer (months compared to weeks), is more expensive and is more subject to variation in environmental conditions. Development of methods for rapid and reliable detection of minor gene resistance would be useful not only for selection of parents and progeny in barley breeding, but also for the genetic mapping of resistance loci which would permit the selection of parents with complementary resistance genotypes. This could lead to gene pyramiding via marker-assisted selection.

This paper presents the results of an investigation aimed at determining whether differences in minor gene resistance levels can be detected under controlled-environment conditions after inoculation at the seedling stage with less virulent isolates. This work was prompted partly by the observation (Wallwork and Grcic 2011) of small but consistent differences in the level of seedling resistance between the barley varieties Gairdner (Franklin-sib/Onslow) and Onslow (Forrest/Aapo), both of which are thought to have inherited the seedling resistance gene *Rrs2* from Forrest. Despite this apparent common determinant of resistance, Gairdner seemed more resistant than Onslow against many isolates. This led to the suggestion (Wallwork and Grcic 2011) that Gairdner inherited minor-gene resistance from its Franklin-sib parent, and that this resistance is detectable at the seedling stage. Franklin itself expresses no resistance in most seedling tests but has shown good resistance in many field trials in South Australia (Wallwork personal observation).

Materials and methods

Germplasm

Seed of 312 doubled haploid (DH) lines were obtained from a cross between the barley varieties Vlamingh and Buloke. Vlamingh is a malting quality variety grown in Western Australia and bred by the Department of Agriculture and Food, Western Australia (DAFWA). It came from a cross WABAR570/TR118 where WABAR570 is a DAFWA breeding line and TR118 is a breeding line from the University of Saskatchewan. Buloke is a widely grown malting variety in South Australia, Victoria and Western Australia that was bred by the Department of Primary Industries, Victoria, Australia (DPI-Victoria). It came from a cross Franklin/VB9104// VB9104 where VB9104 is a breeding line from DPI-Victoria. This set of DH lines is one of two such sets that have been derived from the Vlamingh/Buloke parental combination, the other one having been reported on by Emebiri (2013). Other barley varieties and lines listed in Table 1 were obtained from various sources, with each being selected as true to type and multiplied in a glasshouse after covering the heads to prevent outcrossing. Turk and Atlas are internationally used differential varieties with seedling resistance described in Wallwork and Grcic (2011). Franklin and Keel were widely grown malting and feed varieties in Southern Australia that express partial resistance and which are important parents in many crosses. Schooner is an older malting variety that was widely grown across Southern Australia. It has a low level of partial resistance that has proven durable. O'Connor and Tilga are feed varieties with partial resistance. Sloop, a widely grown malting-quality variety from Southern Australia, was included as a susceptible control.

Collection and storage of isolates

Isolate 332a was collected in 2000 from a barley research plot of breeding line WI3152 at Kingsford in South Australia. Isolate 1,286 was collected in 2007 from roadside barley grass in McLaren Vale, South Australia. Isolate 1,302 was collected in 2005 from barley grass growing close to barley plots at Horsham Research Station, Victoria. Isolate 341b was collected in 2000 from barley grass at Cherry Gardens in the Adelaide Hills in South Australia. The other isolates listed in Table 1 were collected from commercial barley crops and barley trial plots in

Table 1 Reactions of a selection of barley varieties and breeding lines to some differential isolates of *R. commune*

Lines	6	8	29	102f	332a	341b	385	1001	1002	1005	1297	1286	1302
Turk (Rrs1)	R	S	R	R	S	S	R	R	R	R	R	R	MS
Atlas (Rrs2)	R	MR	MR	S	S	MR	S	MS	S	MR	MS	R	R
Franklin	S	MS	MR	S	S	S	S	S	S	MR-MS	S	MR	MR
Tilga	MR	MR	MS	MS	S	S	S	S	S	MS	S	MR	R
Keel	MR-MS	MR	MR	-	S	S	S	S	S	MS	S	R	MR
VB9104	S	MR-MS	S	S	S	S	S	S	S	S	S	MR	MR
O'Connor	MR	MS	MS	MS	S	S	S	S	S	MS	S	MR	MR
Schooner	S	S	S	S	S	S	S	S	S	S	S	MS	MS-S
Sloop – S check	S	S	S	S	S	S	S	S	S	S	S	S	MS-S

South Australia (isolates 29, 102f), Victoria (isolates 385, 1001, 1002 1005 and 1297) and Western Australia (isolate 6).

Leaf pieces of 4 mm^2 excised from scald lesions were surface sterilised by dipping in 70 % ethanol, then into 1 % sodium hypochlorite for 60 s, before being rinsed in sterile reverse osmosis (RO) water and dried on sterile filter paper. Pieces were then placed on 3.9 % potato dextrose agar (Difco) amended with 0.01 g/l streptomycin sulphate and incubated at 16 °C with a 12-h photoperiod. After 2 to 10 d, conidia that had been exuded in small clear, white-to-pink droplets were streaked with a sterile needle on to lima bean agar (LBA) amended with 0.01 g/l streptomycin sulphate (amended LBA). After 24 h at 16 °C with a 12-h photoperiod, a germinated spore observed under a stereomicroscope at 100× magnification was excised from the agar using a flattened end of a platinum wire and transferred to amended LBA. The agar plate was incubated as described above and after a colony had formed it was spread over the plate with a sterile scalpel to increase growth. After 7 d incubation the plate was flooded with sterile RO water and the spores scraped into solution. For long term storage, 500 μl of the concentrated spore solution were pipetted into 1-ml Nunc cryotubes which were then filled with a mixture of sterilised silica (100 to 200 mesh) and self-indicating silica gel, ensuring that there were at least a few indicator beads per tube. These cryotubes were stored at −80 °C.

All isolates were initially tested against a small set of barley varieties to identify those with good growth, sporulation and pathogenicity characteristics. Those with novel or useful virulence features were then used in later tests for more detailed studies.

Seedling inoculation

Cultures were grown on amended LBA at 16 °C with a 12-h photoperiod for 7 d to produce plates covered in spores. A spore solution was produced as described above and diluted to 10^6 spores/ml in sterile RO water by use of a haemocytometer. One droplet of Tween 20 was added to 100 ml of spore solution which was then sprayed onto two seedlings at the three-leaf stage that had been grown in 8-cm pots. Each variety × isolate combination had three replicates. Following inoculation, the seedlings were placed in the dark with 100 % humidity in a controlled-atmosphere growth room at 13 ° C for 24 h and then transferred to a 10/14 h photoperiod with similar humidity and temperature. The first signs of infection were observed 7 to 10 d later and assessments made 14 d after inoculation.

Individual seedlings were rated using a simple 5-category scale (*R* = no symptoms, LS = symptoms only on leaf sheaths, MR = very minor lesions on leaves, often only on leaf margins, MS = a few leaf blotches, often only on the margins of leaves and *S* = many large lesions on leaves or death of whole leaves) since symptoms are ill-defined and preclude more precise ratings. Final ratings of lines were based on the scores of four seedlings. For the Vlamingh/Buloke DH lines, these ratings were converted to a numerical scale in which *R*=1, LS=2, MR=3, MS=5 and *S*=7, and mean scores were computed across 5 seedlings. This numerical scale was designed to align with a 1–9 numbering system used in National Variety Trial disease ratings for cereals in Australia.

Field screening

Field screening of lines was carried out in a disease nursery at Turretfield Research Station near Gawler in South Australia. For each of 300 lines of the Vlamingh/Buloke DH population, 9 g of seed was sown in one pair of 4-m-long rows in each of two randomised blocks. All plots were inoculated with spore suspensions of isolate 332a on several damp evenings, starting at the mid-tillering stage. Inoculation was carried out with a HARDI Model-C5 knapsack sprayer in which the conidia were diluted in water. Rating of lines was based on a visual comparative assessment of total leaf area infected on 8 October 2009 using a 1–9 scale (1 = Resistant, 3 = Moderately Resistant, 5 = Moderately Susceptible, 7 = Susceptible and 9 = Very Susceptible). Variation in growth stage between the lines at assessment date was not thought significant enough to warrant recording.

Mapping of Vlamingh/Buloke

DNA samples of the parents and DH lines were assayed on a both a DArT (Diversity Arrays Technology) array (Wenzl et al. 2004) and an Illumina GoldenGate Oligo Pool Array. The latter array was designed to include 384 single-nucleotide polymorphisms (SNPs) selected from among the 1536 SNPs of the Barley Oligo Pool Array 1 (BOPA1) described by Close et al. (2009). In addition, Buloke and its parents VB9014 and Franklin were genotyped using the full 1536-SNP BOPA1 array. A linkage map was constructed using MultiPoint software (Mester et al. 2003). Quantitative trait locus (QTL) analysis was carried out using simple interval mapping (Lander and Botstein 1989) as implemented in the software MapManager QTX 2.0 (Manly et al. 2001) with a genome-wide significance level of 0.05 determined by 1,000 permutations. Analysis of variance was used to test the statistical significance of QTL × QTL interactions. Genetic maps were drawn using the software MapChart (Voorrips 2002).

Results and discussion

Included amongst barley lines that have been tested against a wide collection of scald isolates are six varieties and breeding lines (Franklin, Keel, O'Connor, Schooner, Tilga and VB9104) that show useful levels of resistance in the field

Table 2 Reaction of the parents of the mapping population Vlamingh/Buloke to isolate 332a of *R. commune* in the field and to isolates 1,286 and 1,302 in seedling tests

Lines	332a field	332a seedling	1286 seedling	1302 seedling
Vlamingh	R	MR	MR	R-MR
Buloke	MS	MS-S	MS	MR

('field resistance') but susceptible or moderately susceptible reactions against most isolates in seedling tests. Among 13 isolates for which results are presented in Table 1, eight (102f, 332a, 341b, 385, 1001, 1002, 1005 and 1297) were virulent (resistance rating MS or S) on the six lines that exhibit field resistance. These isolates were also virulent on controls carrying *Rrs1* (Turk, isolates 332a and 341b) or *Rrs2* (Atlas, isolates 102f, 332a, 385, 1001, 1002, 1297). Isolates 6, 8 and 29 which are used as differentials in seedling screening (Wallwork and Grcic 2011), were virulent on some of the lines with field resistance, but not on others. Two isolates, 1286 and 1302, however, were avirulent on each of the lines that displayed good field resistance (Franklin, Keel, O'Connor, Tilga and VB9104) and partially avirulent on Schooner. These two isolates were collected from barley grass in South Australia and Victoria respectively. Isolate 1,302 differs from 1,286 principally in that it has virulence on Turk and Halcyon, both of which carry the major gene *Rrs1*. Both of these isolates elicited a susceptible reaction on the check variety Sloop; this demonstrates that neither isolate lacks pathogenicity or aggressiveness.

The variety Schooner was selected for this research because it has shown a low but durable level of resistance to scald despite being grown over a very large area of South Australia (up to 42 % of the barley area in 1998 and still around 10 % by 2009) and Victoria (50 % in 1998) since it was first released in 1983. In field nurseries over many years, Schooner has consistently been rated as moderately susceptible (MS or MS-S) to scald whilst some other varieties and breeding lines have been rated susceptible or very susceptible (S or VS). Screening of 31 different isolates of *R. commune* on Schooner seedlings has shown that 29 induce a fully susceptible (S) reaction, whilst just two, 1,286 and 1,302, induce a moderately susceptible (MS and MS-S) reaction (data not shown).

Detection of minor gene loci

While the results presented in Table 1 seem to indicate that differences in field resistance can be detected by inoculating seedlings with the pathogenic but quite avirulent isolates 1,286 and 1,302, this does not necessarily mean that the same genes are responsible for differences detected in these seedling tests and on adult plants in field tests. To investigate the genetic basis of these differences, 312 doubled haploid lines of a Vlamingh/Buloke population were assessed using isolate 332a in the field and isolates 332a, 1,286 and 1,302 in seedling tests. Seedling tests had shown that Vlamingh has seedling resistance to all three isolates, 332a, 1,286 and 1,302, whereas Buloke is susceptible to 332a in seedling tests but partially resistant against this isolate in the field (Table 2).

With isolate 332a, QTL were detected on the short arms of chromosome 4H and 7H (Table 3, Fig. 1), with the resistance alleles coming from Vlamingh and Buloke, respectively. The QTL on 4H had very large effects, explaining 62 % of the variation observed at the adult stage in the field and 50 % of the variation observed in the seedling test. In contrast, the QTL on 7H explained only 8 % of the adult-stage variation and 5 % of the seedling variation. Both of these QTL were also detected when the avirulent isolates 1,286 and 1,302 were used in seedling tests. With these isolates, the QTL on 4H still had the larger effect, but the effect of the QTL on 7H was much more substantial than that detected with isolate 332a. Thus, it seems that the use of relatively avirulent isolates in a seedling test conducted under controlled environment conditions has facilitated the detection and mapping of a minor gene that was difficult to detect under the more variable conditions in the field, while still permitting the detection of a major gene that exerts strong effects on both seedlings and adult plants. From an analysis of variance of the QTL effects in each of the

Table 3 Map positions, peak test statistic values (LRS), percentage of phenotypic variation explained (R^2) and estimated additive effects (*a)* for QTL that affected scald resistance in a Vlamingh/Buloke mapping population evaluated in seedling tests and in a field trial

Experiment	Isolate	QTL detected on 4HS					QTL detected on 7HS					4HS × 7HS interaction
		Position	Nearest markers	LRS[a]	R^2	d^b	Position	Nearest markers	LRS	R^2	*a*	*p*
Seedling test	1286	1 cM	bPb-9304	116.8	33 %	1.34	7 cM	2_0710	51.6	16 %	−0.92	<0.001
Seedling test	1302	2 cM	bPb-14836	178.2	45 %	1.17	1 cM	bPb-25565	81.4	24 %	−0.85	<0.001
Seedling test	332a	1 cM	bPb-9304	202.8	50 %	1.82	1 cM	bPb-25565	15.3	5 %	−0.58	0.046
Field trial	332a	2 cM	bPb-14836	288.3	62 %	1.71	10 cM	2_0710	25.6	8 %	−0.65	0.163

[a] LRS values greater than 13.3 for the seedling tests or 13.0 for the field trial are statistically significant ($P<0.05$, genome-wide)

[b] Positive additive effects indicate resistance effects from Vlamingh allele and negative additive effects indicate resistance effects from the Buloke allele

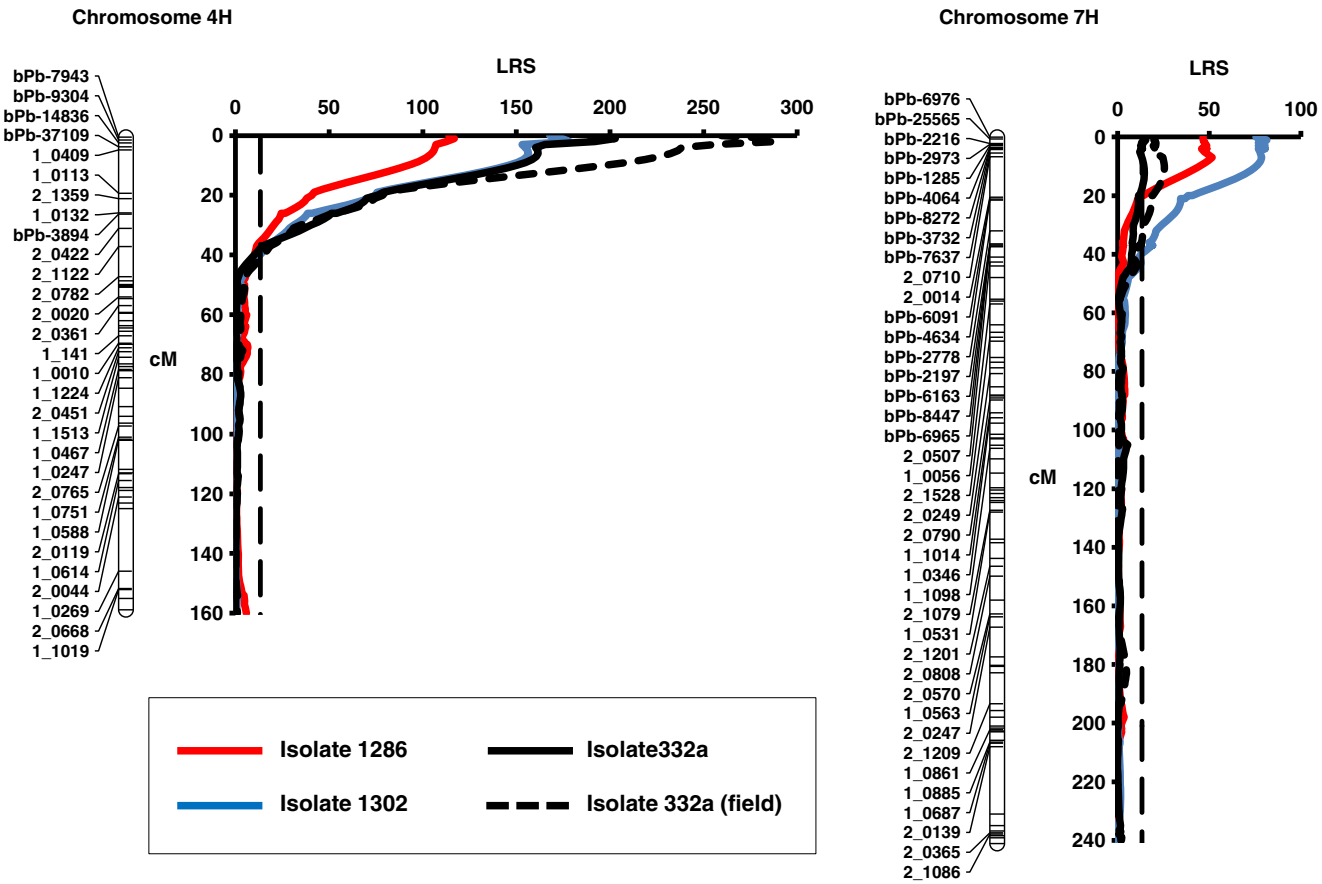

Fig. 1 LRS values for QTL detected on chromosomes 4H and 7H with isolates 1,286, 1,302 and 332a in seedling tests and isolate 332a in a field experiment. The *dashed line* parallel to the axis indicates the approximate threshold for a genome-wide significance level of 0.05. On the first 40 cM of each linkage map, all marker names are shown (for both DArT and SNP markers). Beyond 40 cM, marker names are shown for SNP markers only

tests it was evident that in the seedling tests significant interactions between the QTL could be detected whilst no significant interaction could be detected from the field data (Table 3).

The minor-effect QTL detected on chromosome 7H is in the same genomic region as the major-effect gene *Rrs2* (Schweizer et al. 1995; Schmidt et al. 2001; Hanemann et al. 2009). Considering that there are other minor-effect QTLs detected from field trials collocating with major-effect genes detected at the seeding stage (Newton et al. 2004; Cheong et al. 2006), it is possible that Buloke carries a minor-effect allele of *Rrs2*. Based on its phenotype, Buloke (pedigree Franklin/VB9104//VB9104) could have inherited this resistance from either of its parents since both have been shown to have minor gene resistance as illustrated in Table 1. Based on the SNP haplotypes of Buloke (pedigree Franklin/VB9104//VB9104), Franklin and VB9104 when genotyped on the BOPA1 array, it seems that Buloke inherited the distal 17 cM of its 7HS chromosome arm (and thus the resistance allele on 7HS) from VB9104 (Fig. 2).

Conclusions

These data indicate that seedling tests using avirulent isolates of *R. commune* can be used to detect minor gene resistance. With the use of a Vlamingh/Buloke mapping population, it was confirmed that the Buloke-derived allele at a QTL on 7H contributes to partial resistance in both seedling and field tests. Further, that QTL was most readily detected (highest LRS values) in seedling tests using avirulent isolates and was detectable even though a large-effect seedling resistance gene on chromosome 4H was also segregating in the population. Further, it was observed that QTL interactions between the 4H and 7H QTL could be detected in the seedling tests but not in the field trial indicating the greater precision possible with the seedling tests as against the field trial.

Loci with minor effects have been reported widely in other cereal pathogen systems, particularly the wheat rust diseases, and are frequently described as providing adult plant resistance (APR) since the plants carrying the resistances are often described as being susceptible at the seedling stage. The barley varieties and lines described here as carrying minor-

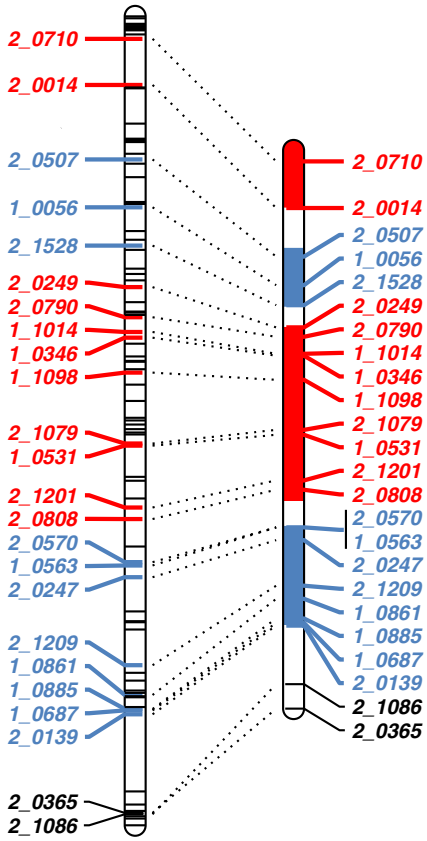

Fig. 2 A comparison of the positions of the SNP markers mapped on chromosome 7H in the Vlamingh/Buloke population used here (*left*) with the positions of the same markers on the consensus SNP map of chromosome 7H of Close et al. (2009). Shading (*red, blue* or *none*) on the consensus map provides a graphical genotype of Buloke constructed based on examination of the SNP haplotypes of Buloke and its parents VB9104 and Franklin. Shading indicates inheritance from VB9104 (*red*) or Franklin (*blue*). Non-shaded regions are those for which SNP polymorphism between VB9104 and Franklin was not sufficient to determine the origin of the Buloke segment

effect genes could be described as carrying APR based on susceptible reactions in seedling tests with common isolates virulent of *R. commune*. However, the term APR may not be appropriate for the results reported here, given that differences in resistance were detectable at the seedling stage when appropriate avirulent isolates were used.

In many host pathogen systems, APR and partial resistance have frequently been referred to as being race-non specific. This is demonstrated not to be the case with the six partial resistances detected here, as race-specificity has been demonstrated with isolates that are more virulent than isolates 1,286 and 1,302.

Genes of minor effect against scald provide a basis for an alternative to using and pyramiding major genes for seedling resistance. Partial resistance is widespread in barley varieties and germplasm collections. The genetic basis of partial resistance has not however received as much attention as the more complete resistance conferred by major-effect genes, probably because the latter has been easier to study at the seedling stage. Those studies that have been conducted show that QTL with minor effects in field trials are often located at or very close to loci that confer major effects at the seedling stage (Newton et al. 2004; Cheong et al. 2006). The results shown here demonstrate a rapid and reliable method for detecting the effects of such loci based on evaluations conducted with seedlings. This method is useful not only for screening and selection in barley breeding programs, but also for the mapping of genetic loci responsible for both major-gene and minor-gene resistance. The test is currently being used by SARDI as a service to barley breeding programs in Australia. The use of isolate 1,302 is particularly useful for tests on breeding lines derived from crosses with cv Hindmarsh which carries the gene Rrs1 but which is otherwise very susceptible to scald.

Although the current work has demonstrated collocation of a seedling-detected locus with an adult-stage QTL detected in the field, this cannot be assumed to hold for all minor-effect genes for scald resistance. For this reason validation needs to occur separately for each resistance source.

Acknowledgments The Grains Research and Development Corporation for providing the research grant that funded this work. Grant Hollaway and Mark Mclean (Department of Primary Industries, Victoria) and Celeste Linde (Australian National University) for providing some isolates of *R. commune*. Elysia Vassos, Rebecca Fox and Greg Lott for technical assistance.

References

Ali SM (1981) Barley grass as a source of pathogenic variation in *Rhynchosporium secalis*. Aust J Agric Res 32:21–25

Ali SM, Mayfield AH, Clare BG (1976) Pathogenicity of 203 isolates of *Rhynchosporium secalis* on 21 barley cultivars. Physiol Plant Pathol 9:135–143

Brown JS (1990) Pathogenic variation among isolates of *Rhynchosporium secalis* from barley grass growing in South Eastern Australia. Euphytica 50:81–89

Cheong J, Williams K, Wallwork H (2006) The identification of QTLs for adult plant resistance to leaf scald in barley. Aust J Agric Res 57: 961–965

Close TJ, Bhat PR, Lonardi S, Wu Y, Rostocks N, Ramsay L, Druka A, Stein N, Svensson JT, Wanamaker S, Bozdag S, Roose ML, Moscou MJ, Chao S, Varshney RK, Szűcs P, Sato K, Hayes PM, Matthews DE, Kleinhofs A, Muehbauer GJ, DeYoung J, Marshall DF, Madishetty K, Fendon RD, Condamine P, Graner A, Waugh R (2009) Development and implementation of high-throughput SNP genotyping in barley. BMC Genomics 10:582

Emebiri LC (2013) QTL dissection of the loss of green colour during post-anthesis grain maturation in two-rowed barley. Theor Appl Genet 126:1873–1884

Goodwin SB, Allard RW, Webster RK (1990) A nomenclature for *Rhynchosporium secalis* pathotypes. Phytopathology 80:1330–1336

Goodwin SB, Allard RW, Hardy SA, Webster RK (1992) Hierarchical structure of pathogenic variation among *Rhynchosporium secalis* populations in Idaho and Oregon. Can J Botany 70:810–817

Hanemann SGF, Cossu R, Wicker T, Röder MS (2009) Fine mapping, physical mapping and development of diagnostic markers for the *Rrs2* scald resistance gene in barley. Theor Appl Genet 119:1507–1522

Jackson LF, Webster RK (1976) Race differentiation, distribution and frequency of *Rhynchosporium secalis* in California. Phytopathology 66:719–725

Jarosz AM, Burdon JJ (1996) Resistance to barley scald (*Rhynchosporium secalis*) in wild barley grass (*Hordeum glaucum* and *Hordeum leporinum*) populations in south-eastern Australia. Aust J Agric Res 47:413–425

Jensen J, Backes G, Skinnes H, Giese H (2002) Quantitative trait loci for scald resistance in barley localized by a non-interval mapping procedure. Plant Breed 121:124–128

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Manly KF, Cudmore RH, Meer JM (2001) Map Manager QTL, cross-platform software for genetic mapping. Mamm Genome 12:930–932

Mester D, Ronin Y, Hu Y, Peng I, Nevo E, Korol AB (2003) Efficient multipoint mapping: making use of dominant repulsion-phase markers. Theor Appl Genet 107:1102–1112

Newton AC, Meyer RC, Young GR et al. (2004) Disease resistance mapping in spring barley. In Proceedings of the 9th international barley genetics symposium, Brno, Czech Republic, 20–26 June 2004: 720–725.

Schmidt D, Röder MS, Dargaz H, Wolf N, Schweizer GF, Tekauz A, Ganal MW (2001) Construction of a YAC library from barley cultivar Franka and identification of YAC-derived markers linked to the *Rh2* gene conferring resistance to scald (*Rhynchosporium secalis*). Genome 44:1031–1040

Schweizer G, Baumer M, Daniel G, Rugel H, Röder M (1995) RFLP markers linked to scald (*Rhyncosporium secalis*) resistance gene *Rh2* in barley. Theor Appl Genet 90:920–924

Tekauz A (1991) Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. Can J Plant Pathol 13:298–304

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wallwork H, Grcic M (2011) The use of differential isolates of *Rhynchosporium secalis* to identify resistance to leaf scald in barley. Australas Plant Pathol 40:490–496

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Killian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. PNAS 101:9915–9920

Williams K, Donellan S, Smyl C, Scott L, Wallwork H (2003) Molecular variation in *Rhynchosporium secalis* isolates obtained from hotspots. Australas Plant Pathol 32:257–262

Zaffarano PL, McDonald BA, Linde CC (2011) Two new species of *Rhynchosporium*. Mycologia 103:195–202

Zhan J, Fitt BDL, Pinnschmidt HO, Oxley SJP, Newton AC (2008) Resistance, epidemiology and sustainable management of *Rhynchosporium secalis* populations on barley. Plant Pathol 57:1–14

Zhang QF, Webster RK, Crandall BA, Jackson LF, Maroof MAS (1992) Race composition and pathogenicity associations of *Rhynchosporium secalis* in California. Phytopathology 82:798–803