Mapping spot blotch resistance genes in four barley populations[1]

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Abstract *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) is the fungal pathogen responsible for spot blotch in barley (*Hordeum vulgare* L.) and occurs worldwide in warmer, humid growing conditions. Current Australian barley varieties are largely susceptible to this disease and attempts are being made to introduce sources of resistance from North America. In this study we have compared chromosomal locations of spot blotch resistance reactions in four North American two-rowed barley lines; the North Dakota lines ND11231-12 and ND11231-11 and the Canadian lines TR251 and WPG8412-9-2-1. Diversity Arrays Technology (DArT)-based PCR, expressed sequence tag (EST) and SSR markers have been mapped across four populations derived from crosses between susceptible parental lines and these four resistant parents to determine the location of resistance loci. Quantitative trait loci (QTL) conferring resistance to spot blotch in adult plants (APR) were detected on chromosomes 3HS and 7HS. In contrast, seedling resistance (SLR) was controlled solely by a locus on chromosome 7HS. The phenotypic variance explained by the APR QTL on 3HS was between 16 and 25% and the phenotypic variance explained by the 7HS APR QTL was between 8 and 42% across the four populations. The SLR QTL on 7HS explained between 52 to 64% of the phenotypic variance. An examination of the pedigrees of these resistance sources supports the

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common identity of resistance in these lines and indicates that only a limited number of major resistance loci are available in current two-rowed germplasm.

Keywords Barley · *Bipolaris sorokiniana* · *Cochliobolus sativus* · Spot blotch · Disease resistance · QTL · MAS

Introduction

Spot blotch of barley is caused by the fungus *Bipolaris sorokiniana* (Sacc. in Sorok) Shoem. (teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechsl. Ex Dastur). This major foliar disease occurs in the warmer and more humid growing regions of the world, including North and South America, Europe, Asia, Syria and Australia (Steffenson et al. 1996; Kumar et al. 2002; Joshi et al. 2004; Meldrum et al. 2004; Müller et al. 2005; Arabi and Jawhar 2007; Kuldeep et al. 2008). As the perfect state does not play an important role in the epidemiology of the disease in Australia, the imperfect state is used when referring to the pathogen throughout this paper.

In Australia, spot blotch of barley can lead to severe crop losses in sub-tropical areas of northern New South Wales and Queensland. Localized barley yield losses greater than 30% have been reported with an average annual loss of $1 million and potential annual losses of $2 million under highly favourable conditions (Murray and Brennan 2009). Spot blotch infection in seedlings starts as dark brown-to-black spots on leaf sheaths and progresses from lower to upper leaves during crop development. If infection occurs early in the crop cycle and conditions remain favourable for disease development, complete defoliation is possible, resulting in severe yield reductions (Kumar et al. 2002). On susceptible adult plants, lesions are generally oblong with chlorotic margins. These often coalesce to kill large portions of the leaf, with severely infected leaves senescing prematurely (Steffenson 1997).

Repeated fungicide applications can be used to control spot blotch, but the use of resistant cultivars offers the most economically and environmentally sound means of control (Fetch and Steffenson 1994). The development of resistant cultivars is, however, complicated by the occurrence of different pathotypes of the fungus. Knight et al. (2010) screened 35 Australian spot blotch isolates across 15 barley cultivars and identified 10 apparent pathotypes. In an earlier study a differential set consisting of 20 lines was screened and six pathotypes were identified among 34 Australian isolates of the fungus (Meldrum et al. 2004). Similarly, by using three barley differentials, three

pathotypes were identified from 58 isolates obtained from different regions of the world (Zhong and Steffenson 2001) and eight virulence groups were identified in Canada by evaluating 127 isolates on 12 barley genotypes (Ghazvini and Tekauz 2007). Recent studies have inferred that a simple gene-for-gene model does not fit all the data and that resistance may result from combinations of both qualitative major and quantitative minor genes (Ghazvini and Tekauz 2008; Knight et al. 2010)

In the United States spot blotch resistance obtained from the six-rowed line ND B112 and its derivatives has been effective for over 40 years (Steffenson et al. 1996). The six-rowed cultivar Morex, which includes ND B112 in its pedigree, has been used in a number of crosses to identify the chromosomal regions associated with resistance expression (Bilgic et al. 2005). Two-rowed breeding lines resistant to spot blotch have become available from various Canadian programs, including TR251 (Grewal et al. 2008) and WPG8412-9-2-1 (Platz et al. 2005).

The objective of this study was to examine the expression of seedling resistance (SLR) and adult plant resistance (APR) in four barley populations derived from North American resistance sources. Genetic maps have been constructed and markers for resistance loci identified in order to determine whether these different sources carry unique resistances that may provide a range of resistance genes for deployment in breeding programs.

Materials and Methods

Plant material

Four populations were used in this study: VB9524/ND11231-12 (VB/ND-12), ND11231-11/WI2875-17 (ND-11/WI), TR251/Gairdner (TR/GA) and WPG8412-9-2-1/Lindwall (WP/LI). The doubled haploid (DH) population VB/ND-12 consists of 180 lines and was developed by Emebiri et al. (2005). ND11231-12, (ND7085/ND4994-15/ND7556), which shows a high level of resistance to spot blotch, is a two-rowed, narrow leafed sister line of the cultivar Logan (PI 592784, ND11231-11), which was released from the North Dakota Agricultural Experiment Station in 1995. The spot blotch susceptible line VB9524 (two-rowed) was developed by the Department of Primary Industries, Victoria, Australia, and is an advanced selection from the cross Arapiles/Franklin (Emebiri *et al.* 2005). The QTL identified for seedling and adult plant resistancein the DH population VB/ND-12 were validated using the population ND-11/WI which consisted of 85 DH lines. Spot blotch

resistance genes were also mapped in a TR/GA DH population consisting of 147 lines. This population was developed by the Department of Agriculture, Western Australia. TR251 (TR229//AC Oxbow/ND7556) is a two-rowed barley breeding line from Agriculture and Agri-Food Canada (AAFC), Brandon Research Centre, Canada (Grewal *et al.* 2008). Gairdner (Onslow/Tas83-587) is a two-rowed, high yielding malting barley developed for the higher rainfall areas of Western Australia (Paynter et al. 2001). Ninety-four randomly chosen lines of a WP//LI DH population were used to determine whether the major QTL present in the other three populations were also present in this population. WPG8412-9-2-1 (Bowman/TR473//Ellice/TR451) is a two-rowed Canadian line. Lindwall (Triumph/Grimmett) is a two-rowed barley developed by Queensland Primary Industries and Fisheries.

Fungal preparation and inoculation

Each population was screened with isolate SB61, which originated from a field epidemic in the barley variety Kaputar at Monto in Queensland in 1998. Trials were conducted at Redlands Research Station Cleveland, Queensland and at Grafton, New South Wales in 2007 only. The presence of significant extraneous inoculum was unlikely as both sites are distant from the main cropping areas. Spot blotch was not observed in any of the nurseries prior to inoculation of spreader rows with SB61. Inoculum was sub-cultured on starch nitrate agar and grown for 2 weeks at 25°C. Five mL aliquots of medium containing five drops of Tween 20 and 100 ml of milli-Q water were added to the fungal cultures and the conidia were gently dislodged with a camel hair brush. The suspension was filtered through a 400 micron mesh before the number of conidia per mL was determined using a Neubauer counter (haemocytometer). The average of 12 counts was calculated and the conidial suspension was diluted to a final concentration of 10,000 conidia per mL.

Seedling screens

Seedling experiments were carried out at the Hermitage Research Station, Warwick, Queensland in 2005 and 2006 for the VB/ND-12 population and in 2006, 2007 and 2003 for the ND-11/WI, TR/GA and WP/LI populations, respectively. A completely randomized design with two replicates was employed for all seedling trials. Between seven and ten seeds of each line were sown at three evenly spaced sites around the circumference of 10 cm sterile pots containing 1:1 scrub-loam soil and mushroom

compost. Seedlings were raised in the glasshouse and fertilized weekly. After approximately 15 days, when the second leaf was fully expanded, the seedlings were inoculated with a conidial suspension containing 10,000 conidia/mL at 2 mL/pot using a Paasche air brush (H series) at 220 KPa. The plants were placed immediately into a dew chamber for 24 hrs at 22°C (14 hrs dark/10 hrs light). The plants were then transferred to a growth room (25/15°C, 12 hrs light, 60% relative humidity). Seedlings were bottom watered until they were rated 13 days post-inoculation. A 1-9 scale based on lesion severity was used with 9 being the most severe/very susceptible) (Fetch and Steffenson 1999). The mean of the replicates was calculated and used as the infection response value in the QTL analysis.

Adult plant screens

Randomized field trials of the VB/ND-12 population were conducted in 2004 and 2006 at a warm, high humidity coastal site at Redlands Research Station, Queensland. Trials for the WP/LI and ND-11/WI populations were conducted at this same location in 2003 and 2006, respectively. The TR/GA population was screened in 2007 at Grafton Research Station in north eastern New South Wales. Two replicates of each line were sown as hill plots of 10-20 seeds 0.54 m apart within rows 0.75m apart. Spreader rows of the highly susceptible cultivar Tallon were sown between each pair of datum rows. When Tallon rows were at approximately Zadoks growth stage Z32 (stem elongation), they were artificially inoculated with a conidial suspension of isolate SB61 and the epidemic promoted by regular sprinkler irrigation. Assessment of infection response was determined on a 1-9 scale based on severity, with a score of 9 being the most severe. Plots were scored at least twice, with the first rating taken after anthesis (Z75), then at 2 week intervals during the grain fill period. The mean of the ratings was calculated to obtain a single rating for each replicate. The replicates in turn were averaged to obtain the infection response value used in the QTL analysis.

Seedling and adult plant trial data analysis

Frequency histograms of host infection responses and descriptive statistics of raw phenotypic data were generated in Microsoft Excel. Correlations between replicates of each trial were calculated with SAS Enterprise Guide 4.1.

Single sequence repeat (SSR) analysis

DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation). Primer sequences for SSR markers were obtained from GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) and a standard protocol was used to amplify the markers. The reaction mixture consisted of 20 ng DNA, 5 µM of each primer, 100 µM of each dNTP, 1.5 mM $MgCl_2$, 1x buffer (Bioline Pty Ltd., Australia) and 0.1 U Immolase™ DNA polymerase (Bioline Pty Ltd., Australia) in a total volume of 10 µl. The following polymerase chain reaction (PCR) cycle profile was used: 7 min at 95°C, followed by 35 cycles at 94°C for 30s, 50-60°C (depending on annealing temperature) for 30s and 72°C for 30s and one cycle at 72°C for 10 min. The amplified products were visualized using a Gel-Scan 2000™ (Corbett Life Sciences, Sydney, Australia).

Diversity Arrays Technology (DArT) and conversion of DArT markers to PCR-based markers

DNA of 94 DH lines of the TR/GA population was sent to Triticarte Pty Ltd (http://www.triticarte.com.au/default.html) for Diversity Arrays Technology (DArT) analysis (Wenzl et al. 2004).

Three DArT markers, located in QTL regions, were converted to PCR-based markers. Sequences for the DArT markers were obtained from Triticarte Pty Ltd. Primer3 version 0.4.0 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used to design primers for the DArT markers. The PCR-based DArT markers were amplified using a standard PCR reaction as described under SSR analysis.

Expressed sequence tag (EST) amplification

Primers were designed for 4 ESTs (BF256735, BF261183, BF065489, BF474338) mapping close to the *Rcs5* gene on chromosome 7H (Drader et al. 2009). Sequences of ESTs were obtained from the NCBI website (http://www.ncbi.nlm.nih.gov) and Primer3 version 0.4.0 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used to design primers. The PCR-based EST markers were amplified using a standard PCR reaction as described under SSR analysis above.

Linkage map construction and QTL analysis

A linkage map had previously been constructed for the VB/ND-12 population consisting of 270 markers (Emebiri *et al.* 2005) and was further curated according to Lehmensiek *et al.* (2005). For the TR/GA population a genetic linkage map was produced consisting of 492 DArT and 50 SSR markers. MapManager QTXb20 (Manly *et al.* 2001) was used to partition DArT and SSR markers into linkage groups and RECORD (Van Os *et al.* 2005) was used to order markers within linkage groups. The Kosambi function was used to calculate map distances.

Composite interval mapping was conducted with Windows QTL Cartographer version 2.5 (Wang *et al.* 2007) employing model 6 with a 10 cM window and 5 cM walk speed. One thousand permutation tests at 5 cM intervals were conducted to determine significance thresholds for QTL detection.

For the ND-11/WI and WP/LI populations no whole genome genetic map was produced. Rather, individual markers located in QTL regions identified in other populations were amplified across the populations and the single marker regression analysis function in MapManager QTXb20 was used to identify any significant marker trait associations. Map figures were produced with MapChart version 2.1 (Voorrips 2002).

Results

Phenotypic data

The infection responses (IRs) of parents and progeny were consistent between the replicates within the experimental trials as indicated by the correlation co-efficient (Table 1), and a mean of the replicates was used in the analysis of the data. For all four populations the mean IR was lower at the seedling stage than at the adult plant stage and the seedling data displayed a greater range of IRs to spot blotch when compared with the range of IRs observed under field conditions (Table1; Fig. 1). Progeny responses in the field trials of the VB/ND-12 and TR/GA populations were skewed towards susceptibility (Table 1). In the seedling trials, the resistant parents, ND11231-12, ND11231-11, TR251 and WPG8412-9-2-1 exhibited a low (1-3) IR whereas the susceptible parents displayed a high (7-8) IR to the spot blotch disease (Fig. 1). In comparison, the six-rowed barley line, ND B112 had seedling IR scores equal to or lower than the ND11231-12 and ND11231-11 lines but higher than the TR251 and WPG8412-9-2-1 lines (Table 1). In the field trials, the resistant parents exhibited an intermediate (4-5) IR whereas the susceptible parents again displayed a

high (7-8) IR (Fig. 1) and ND B112 had IR scores equal to or lower than the parents of the populations (Table 1). A number of transgressive segregants having lower IR scores than the resistant parent were observed in the VB/ND-12 population at both the seedling and adult stages and in the TR/GA population at the adult stage.

QTL identification and analysis

Whole genome genetic linkage maps were available for the VB/ND-12 and TR/GA populations and were used in the QTL analysis. Permutation tests indicated that 2.8 was the critical log-likelihood (LOD) score for a QTL to be significant. QTL analysis of the seedling trials revealed that SLR to spot blotch is conditioned by a major QTL on the same region of the short arm of chromosome 7H in both populations (Table 2, Fig. 2), which explained 64% and 58% of the phenotypic variation in the VB/ND-12 and TR/GA population, respectively. The marker Est3 1>2 was closest to the spot blotch resistance gene in the VB/ND-12 population, whereas DArT marker bpb-8660 was closest in the TR/GA population. A minor QTL for SLR, contributed by the susceptible parent VB9524 and explaining 3% of the phenotypic variance was detected on chromosome 5HL (Table 2). This QTL was, however, not consistent across both trials.

Composite interval mapping of the adult plant data identified several QTL conferring APR to spot blotch (Table 3). Two significant QTL contributed by the resistant parents were identified on chromosomes 3HS and 7HS in both the VB/ND-12 and TR/GA populations (Fig. 2). The amplified fragment length polymorphism (AFLP) marker, P13M61-168 was at the peak of the 3HS QTL in the VB/ND-12 population, whereas the SSR marker Bmag919 was at the QTL peak in the TR/GA population. The QTL in this region explained from 13% (2006) to 17% (2004) and 25% (2007) of the phenotypic variance in the VB/ND-12 and TR/GA population, respectively. The 7H APR QTL mapped to the same region as the SLR QTL on 7H and explained 42% (both years) and 19% of the phenotypic variance in the VB/ND-12 and TR/GA population, respectively. Minor QTL (4% phenotypic variation each detected only in the 2006 trial) contributed by the susceptible parent were identified on chromosomes 2HS and 5HS in the VB/ND-12 population (Table 3). As the QTL on chromosome 3HS in the VB/ND-12 population was in a region consisting only of AFLP markers (Fig. 2), it could not be determined whether this region was in a similar location to the region of the QTL identified in the TR/GA population. For comparison, the same

markers needed to be positioned in maps of both populations. Initial screens for polymorphisms indicated that the 3HS region of the VB/ND-12 population showed low polymorphism with only one SSR marker (scssr10559) out of twenty tested being polymorphic and the only other polymorphic markers being AFLPs. This rendered comparisons of QTL positions between populations very difficult. However successful conversion to a PCR-based marker of the DArT marker bpb-3865, which flanked the resistance locus in the TR/GA population, enabled this comparison to be made. The converted marker was dominant in the VB/ND-12 population producing a 240 base pair (bp) band with the ND11231-12 parent only (Table 4). This marker was mapped 0.6 cM distal to AFLP marker P13M61-168 (Fig. 3). Re-analysis of the QTL indicated that bpb-3865 was the closest marker characterised to the resistance locus on chromosome 3H.

Genetic linkage maps were not available for the ND-11/WI and WP/LI populations, therefore a single marker regression approach was undertaken to determine whether the major resistance genes on chromosome 3H and 7H identified in the VB/ND-12 and TR/GA population could also be identified in these populations. As the DArT markers bpb-8660 and bpb-5172 were closest to the 7HS QTL in the TR/GA population (Fig. 2), these DArT markers were also converted to PCR-based markers (Table 4). The converted bpb-8660 marker was only polymorphic in the TR/GA and WP/LI populations and amplified a dominant 220 bp band on DNA from Gairdner and Lindwall. The converted DArT marker bpb-5172 was not polymorphic in either of the ND-11/WI and WP/LI populations. ESTs identified by Drader et al. (2009) to be close to the spot blotch resistance gene on 7HS were also converted to PCR-based markers and screened across the parents of the four populations (Table 4). Of the 4 converted EST markers only BF256735 was polymorphic in all four populations.

One marker (bpb-3865) in the 3HS QTL region and four markers (EBmag794, HVM4, BF256735 and EBmac603) in the 7HS QTL region were polymorphic in the ND-11/WI population and were amplified across all lines of this population. Single

marker regression analysis confirmed the SLR QTL on chromosome 7HS with a phenotypic variance explained of 62% (Table 2) and the APR QTL on 3H with a phenotypic variance explained of 21% (Table 3). The 7HS markers were suggestive of an association with APR (LOD = 1.8; Table 3).

Six markers in the 3HS QTL region (bpb-3865, Bmag919, GBM1159, HVGLTP4X3D0001, HvLTTP and EBmac705) and four markers (HVM4, BF256735, bpb-8660 and EBmac603) in the 7HS QTL region were polymorphic in the WP/LI population and were used in the single marker regression analysis. The analysis indicated that the 7HS QTL was also highly significant in this population, with the closest marker, EBmac603, explaining 52% of the phenotypic variance for SLR resistance (Table 2; Fig. 3). A significant association between the markers on 3HS and APR was observed, with marker GBM1159 explaining 15% of the phenotypic variance (Table 3; Fig. 3). A suggestive association (LOD 2.2) was observed with the markers on 7HS and APR, explaining 10% of the phenotypic variance (Table 3; Fig. 3).

Discussion

This study has identified QTL that condition resistance to spot blotch at both the seedling and adult-plant stages of barley development in four independent mapping populations. While some QTL are only effective in field tests of adult plants, other QTL appear effective throughout the life of the plant. This situation mirrors that observed in resistance of barley to the net blotch (net form of *Pyrenophora teres*) pathogen (Lehmensiek et al. 2007). QTL analysis identified a highly significant SLR QTL on chromosome 7HS in the VB/ND-12, ND-11/WI, TR/GA and WP/LI populations. Based on close linkage to EST marker BF256735, located on a 2.8 cM

segment containing the *Rcs5* gene (Drader et al. 2009), this 7HS QTL is also closely co-incident with the *Rcs5* gene. In North Dakota, the *Rcs5* gene was significant in the expression of seedling resistance to *B. sorokiniana* (*C. sativus*) in three DH populations derived from the resistant source Morex and in a TR306/Harrington population with TR306 as the resistant source (Fig. 4)(Bilgic et al. 2005).

A significant SLR QTL, contributed by VB9524, was detected on chromosome 5HL in the VB/ND-12 population. The detection of a significant resistant QTL from the susceptible parent is consistent with the observation of transgressive segregants in this population (Fig. 1). Even though this QTL only explained 3% of the phenotypic variance and was detected in only one seedling trial, it is in a region where a minor APR QTL has previously been identified by Bilgic et al. (2005) (Fig. 4). Significantly, Knight et al. (2010) observed that VB9524 showed intermediate seedling reactions to the majority of *B. sorokiniana* isolates screened across it, suggesting the presence of at least one minor gene for resistance.

A region on the short arm of chromosome 3H was a major contributor to the expression of adult plant resistance, accounting for 13 to 25% of the phenotypic variance. This region on 3HS has previously been reported for spot blotch resistance in the cultivar Bowman and a minor QTL in this region was identified in Steptoe (Fig. 4) (Bilgic et al. 2005; Bilgic et al. 2006). A major spot blotch resistance gene was also detected on 3HS in the Dicktoo/Morex population (Bilgic *et al.* 2005). The placement of the QTL on the consensus map however, suggests that this QTL may be in a different region (Fig. 4) of 3HS. Amplification of polymorphic markers in these regions common to both populations is required to decide this issue.

The APR QTL on 7HS was located in the same region as the major seedling resistance QTL and the *Rcs5* gene. Even though this QTL was present in all

populations, it was only significant in the VB/ND-12 and TR/GA populations, while it was rated as statistically suggestive in the other two populations. The *Rcs5* gene region has been reported for the field expression of spot blotch resistance in diverse DH populations in North Dakota and it was also observed that it explained varied levels of the phenotypic response observed in the different populations (Fig. 4) (Steffenson et al. 1996; Bilgic et al. 2005). Hence while the *Rcs5* region is the principal (and often sole) contributor to seedling resistance in glasshouse tests, it is perhaps prone to environmental interactions and/or influenced by genetic background in adult plants under field conditions.

Two minor QTL for adult plant resistance were identified in the VB/ND-12 population on chromosomes 2HS and 5HS, which were contributed by the susceptible parent VB9524. The 2HS QTL is in a similar region to a minor QTL identified for adult plant resistance in the Steptoe/Morex population (Fig. 4) (Bilgic *et al.* 2005). Neither the 2HS nor 5HS QTL were consistently detected in both trial years and require further investigation in a range of environments and backgrounds to determine the significance of their contribution.

This study has demonstrated the usefulness of sequenced DArT makers as a resource for rapid development of PCR-based markers for routine screening of specific regions. Due to low levels of polymorphism observed in the 3HS region in the VB/ND-12 population, only one SSR marker was available for marker-assisted selection (MAS). As a result, a DArT marker associated with spot blotch resistance in the same region in the TR/GA population was successfully converted to a PCR-based marker and mapped in the VB/ND-12 population. Two DArT markers on 7HS were also converted to PCR-based markers, one of which amplified a fragment in the susceptible parents of populations TR/GA and WP/LI.

Molecular markers for use in MAS are most effective for breeding programs if they can consistently identify trait-linked loci within diverse populations. Therefore it is important to validate QTL within several genetic backgrounds. Here we have illustrated that the major QTL on 3HS and 7HS are consistently associated with spot blotch resistance in four independent but related populations. The markers associated with these loci might prove very useful for selection of spot blotch resistance within Australian and international barley breeding programs. At the same time, this study indicates that several North American two-rowed barley lines introduced into Australia carry the same two resistance loci, located on 3HS and 7HS (*Rcs5* gene region). Indeed a review of the international literature, as summarized in Fig. 4, indicates that the only other loci identified to date which confer major effects on phenotypic variation for resistance are located on 1HS and on 2HS. Therefore, current efforts (Steffenson et al. 2008) to source new resistances from wild cereal species should be encouraged.

The resistance to spot blotch infection in ND11231-12 (ND7085/ND4994-15/ND7556) can be traced back through the two-rowed line ND7556 [PI 643244, Norbert//ND4856/M37] to the six-rowed breeding line M37 (Manker/Karl//M18) and through the two-rowed line ND4856 [PI 643227, Klages//H316/ND B130] to ND B112, which carries a high level of spot blotch resistance. A second parent ND4994-15 [PI 643230, Klages//Fergus/Nordic/3/ND1156/4/Hector], a sister line of Bowman (PI 483237), also traces back to ND B112 via six-rowed lines, Nordic and ND1156. TR251 (TR229//AC Oxbow/ND7556) is related to these ND lines since ND11231-12, ND11231-11 and TR251 are thought to have inherited their spot blotch resistance genes from ND7556. WPG8412-9-2-1 (Bowman/TR473/Ellice/TR451) probably derived its resistance genes from Bowman. Thus the resistant lines used in this study

and currently being introgressed into Australian germplasm have related pedigrees and may share a common spot blotch resistance source, as indicated by QTL analysis. In the future, enhanced spot blotch disease pressure is expected in some barley growing regions in Australia as a result of changing climate and rainfall patterns. Additional and independent sources of resistance, such as the 1HS and 2HS genes detected in Calicuchima-sib (Biligic et al. 2006), should also be recruited into local germplasm, to enhance the effectiveness and durability of resistance in the next generation of barley cultivars.

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Table 1 Descriptive statistics of the four populations, VB/ND*12, ND*11/WI, TR/GA and WP/LI

Table 2 Summary of QTL associated with spot blotch seedling resistance in four DH populations VB/ND*12, TR/GA, ND*11/WI and WP/LI

Table 3 Summary of QTL associated with spot blotch adult plant resistance in four DH populations

Table 4 Primer sequences of PCR-based DArT and EST markers

Fig. 1 Frequency of infection responses to spot blotch disease at the seedling (A) and adult plant (B) stage for the VB/ND-12, ND-11/WI, TR/GA, WP/LI populations. Mean parental scores are indicated by arrows

Fig. 2 Genetic linkage map of chromosomes 3H and 7H of the VB/ND-12 and TR/GA populations indicating locations of seedling resistance (SLR) and adult plant resistance (APR) QTL. Distances (cM) are indicated on the far left side. Markers are given on the left and QTL intervals are indicated by bars on the right. The marker closest to the QTL peak is indicated by the perpendicular mark on the QTL interval.

Fig. 3 Enlarged maps of chromosomes 3H and 7H of the VB/ND-12, TR/GA, ND-11/WI, WP/LI populations. Markers in common are connected by lines. Markers showing the highest association with spot blotch resistance are underlined

Fig. 4 Locations of spot blotch resistance QTL identified to date based on consensus map by Wenzl et al (2006). Some markers on the original map have been removed for display purposes. Markers are listed on the left and QTL are indicated on the right. SLR QTL intervals are indicated by white bars, APR QTL intervals are indicated by black bars. QTL names denoting: reference for QTL.the population(^ = resistance donor).the percentage phenotypic variance explained [CA=Calcuchima-sib; BO=Bowman; ST=Steptoe; MO=Morex, DI=Dicktoo; HA=Harrington; TR=TR306; Bovill10 refers to this publication]. Map distance in cM is given by the ruler on the left side of the figure

Table 1

Trial	*N	#Min	#Max	#Mean	#Std. Dev.	#Skewness	^r	€NDB112 IR
VB/ND-12 SLR05	180	2.0	9.0	6.07	1.74	-0.276	0.8	4.0
VB/ND-12 SLR06	149	2.0	9.0	5.85	2.14	-0.305	0.8	3.5
VB/ND-12 APR04	180	3.5	8.5	6.62	1.08	-0.580	0.9	3.0
VB/ND-12 APR06	180	3.8	8.3	6.46	1.01	-0.766	0.8	2.0
ND-11/WI SLR06	83	2.0	9.0	6.11	2.17	-0.276	0.9	3.0
ND-11/WI APR06	84	5.3	8.0	6.67	0.72	-0.213	0.6	4.0
TR/GA SLR07	134	2.0	9.0	5.16	1.78	-0.121	0.8	2.5
TR/GA APR07	133	3.3	8.3	6.27	1.09	-0.553	0.7	5.5
WP/LI SLR03	94	2.0	8.5	5.54	1.73	-0.226	0.9	3.0
WP/LI APR03	94	4.0	8.0	6.09	0.87	-0.242	0.5	3.5

*Number of lines evaluated
Minimum, maximum, mean, standard deviation and skewness for spot blotch infection response scores evaluated using a 1 to 9 scale at the seedling (SLR) and adult plant stage (APR), with a score of 9 being the most severe
^r Correlation co-efficient ($P<0.0001$) indicating correlation between replicates of trials
€Infection response scores for ND B112

Table 2

Population	Chromo-some	LOD score (trial)	Phenotypic variation (%)	Donor	Closest marker
VB/ND-12	7HS	32.9 (2005)	64	ND	EST31>2
		43.1 (2006)	64	ND	
	5HL	3.6 (2006)	3	VB	P22M50-304
TR/GA	7HS	27.5	58	TR	bpb-8660
ND-11/WI	7HS	15.4	62	ND	EBmac603
WP/LI	7HS	18.4	52	WP	EBmac603

Table 3

Population	Chromo-some	LOD score (trial)	Phenotypic variation (%)	Donor	Closest marker
VB/ND-12	2HS	3.3 (2006)	4	VB	MWG64
	3HS	17.0 (2004)	17	ND	P13M61-168/bpb-3865
		11.3 (2006)	13	ND	P13M61-168/bpb-3865
	5HS	3.8 (2006)	4	VB	P11M47-122
	7HS	33.4 (2004)	42	ND	Est31>2
		24.8 (2006)	42	ND	Est31>2
TR/GA	3HS	15.3	25	TR	Bmag919
	7HS	12.2	19	TR	bpb-8660
ND-11/WI	3HS	4.3	21	ND	bpb-3865
	7HS	1.3	8	ND	EBmag794
WP/LI	3HS	3.4	16	WP	GBM1159
	7HS	2.2	10	WP	bpb-8660/BF256735

Table 4

Chromo-some	Primer name	Sequence	Size (base pairs)
3H	bpb-3865 forward	5’-CTGACAATCACGCAGGAAGA	240
	bpb-3865 reverse	5’-TGGCAGGGTTGACCTTAAAC	
7H	bpb-8660 forward	5’-GCAATATTTTGCGGAGCTGT	210
	bpb-8660 reverse	5’-AGCCTGAGGCAGAGGTAGTG	
7H	bpb-5172 forward	5’-AATACGGCGGGATATGATGA	174
	bpb-5172 reverse	5’-GCTTTCGGTCTCACTCGAAC	
7H	BF256735 forward	5’- CAAAACAGAGCGTCTGGTCA	240
	BF256735 reverse	5’- TACCGCAAATACATGCCAAA	
7H	BF261183 forward	5’-TCATGTTTGGAATGGAGCTG	210
	BF261183 reverse	5’-GATGCATGAGCAACCAAAGA	
7H	BF065489 forward	5’- GGTTTGTCAAGGATGGTGCT	224
	BF065489 reverse	5’- GGAACGGAGGGAGTACATGA	
7H	BF474338 forward	5’- AGAATACCGCTTCGACGAGA	174
	BF474338 reverse	5’- GCCCACTGCCTACGATACAC	

Fig. 1

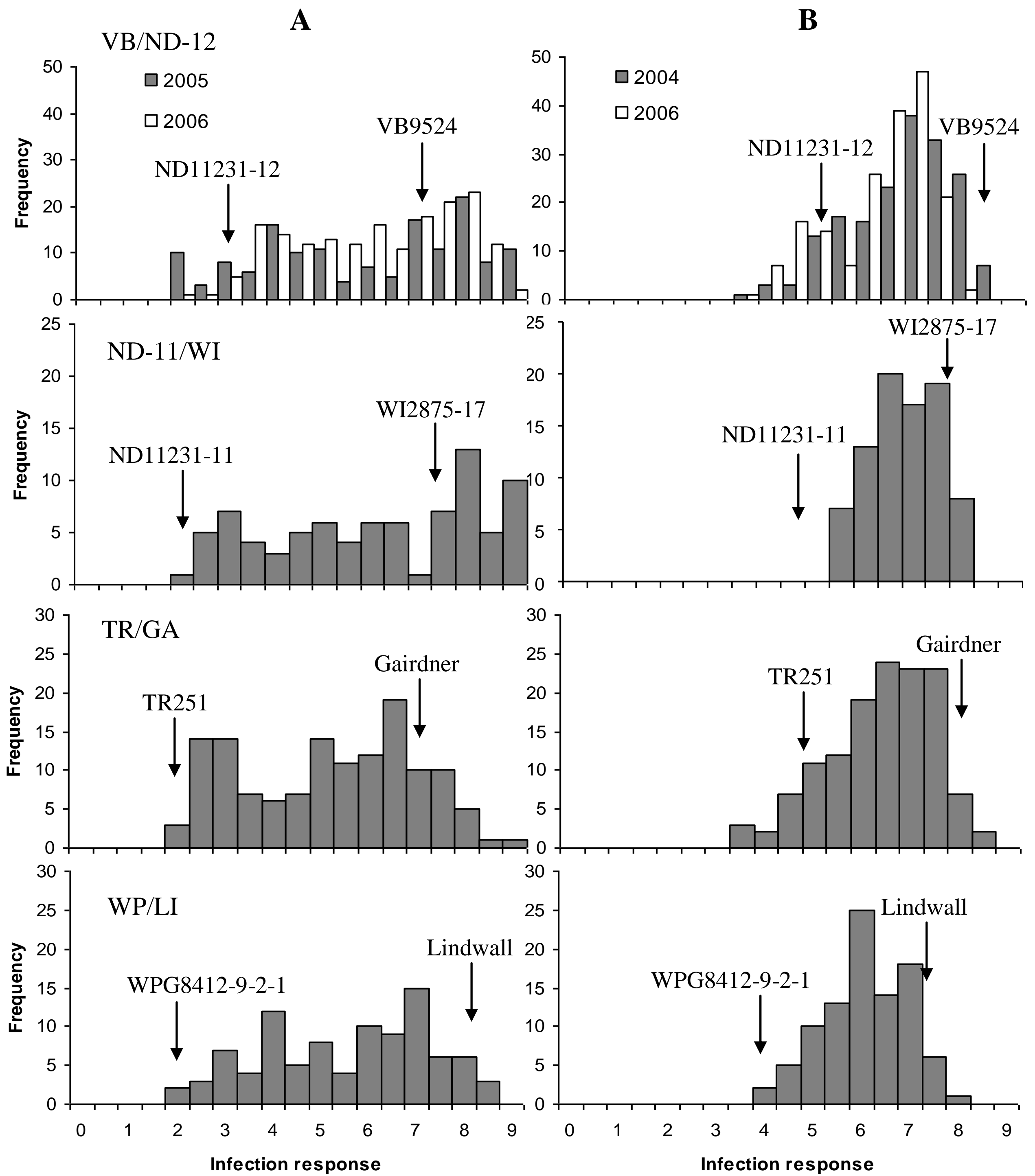


Fig. 2

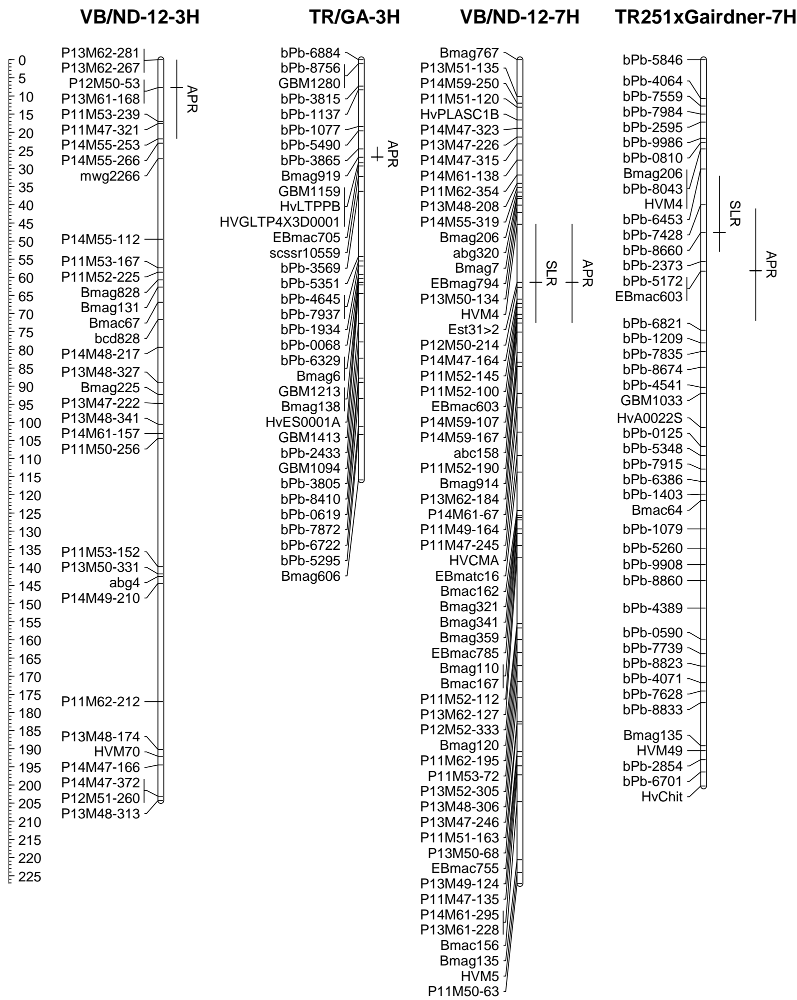
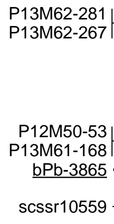
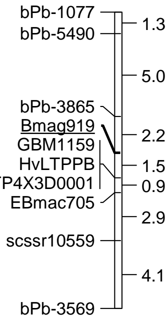


Fig. 3

3H

VB/ND-12 TR/GA ND-11/WI WP/LI





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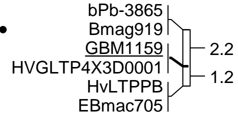
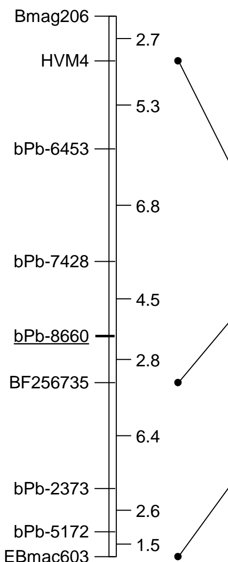
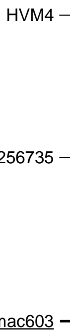


7H

VB/ND-12 TR/GA ND-11/WI WP/LI





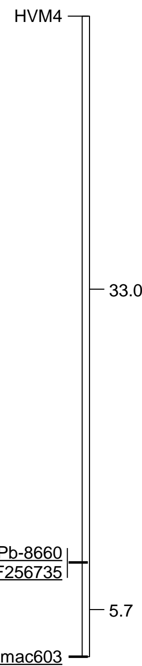


Fig. 4

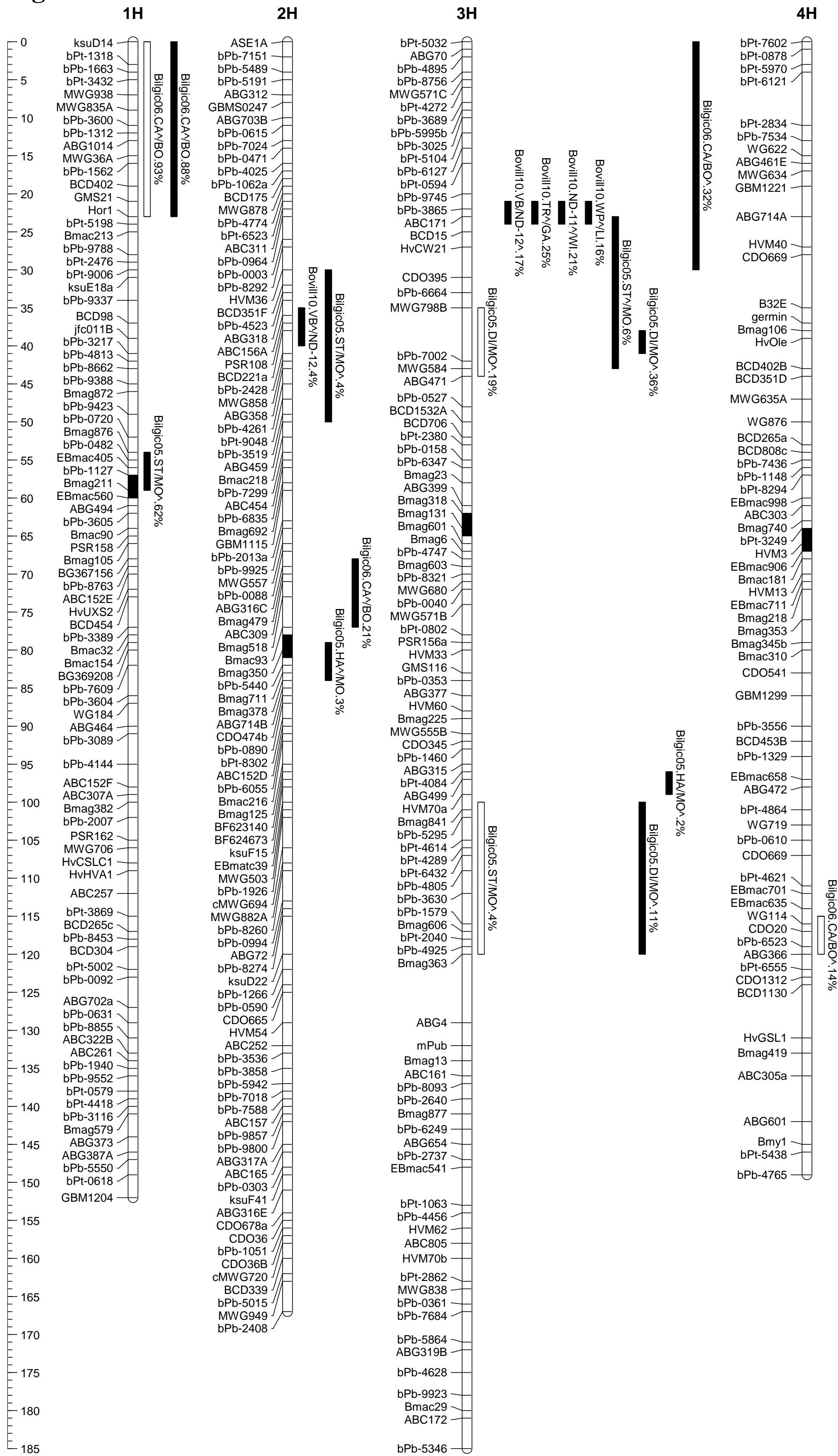


Fig. 4 continued