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Genomic regions associated with common root rot resistance in the barley variety Delta

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Common root rot (CRR) caused by *Bipolaris sorokiniana* is a serious disease constraint in the dry temperate cereal growing regions of the world. Currently little is known about the genetic control of resistance to CRR in cereals. In this study based on a Delta/Lindwall barley population we have undertaken a bulked segregant analysis (BSA) and whole genome mapping approach utilising Diversity Arrays Technology (DArT) to identified quantitative trait loci (QTL) associated with CRR expression. One QTL each was identified on chromosomes 4HL and 5HL explaining 12 and 11% of the phenotypic variance, respectively.

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Introduction

The fungal pathogen *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur), is the causal agent of the foliar disease spot blotch and the root disease common root rot (CRR) in winter cereals. Common root rot infected seedlings develop dark brown necrotic lesions on the roots, crown and lower leaf sheaths (Kumar *et al.* 2002). The disease impairs the functioning of roots and crown, resulting in fewer tillers and heads, which causes a reduction in grain yield and quality (Wildermuth *et al.* 1992). As high soil temperatures and low soil moisture appear to contribute to the severity of the disease, damage due to CRR can vary widely from year to year in a given location (Van Leur *et al.* 1997; Kumar *et al.* 2002; Mathre *et al.* 2003). This disease is a significant constraint in the dryer temperate cereal growing regions of the world, and has been particularly damaging in Syria, Canada, USA and Australia (Wildermuth 1986; Conner *et al.* 1996; Van Leur *et al.* 1997; Kumar *et al.* 2002; Tobias *et al.* 2009). In Australia, CRR has been reported in wheat and barley cropping fields in every mainland state, with estimated yield losses of up to 24% reported in Queensland (Wildermuth *et al.* 1992).

Integrated strategies for controlling *B. sorokiniana* on barley include soil and residue management, chemical control, crop rotation and resistance breeding (Mehta 1988; Steffenson 1997). Wildermuth and McNamara (1991) demonstrated that rotational strategies involving a range of other crops, in particular lucerne, resulted in the reduction of soil populations of *B. sorokiniana* and thus a reduction in the severity of CRR. However, after a

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single wheat crop, soil populations of *B. sorokiniana* were effectively restored to their previous levels, suggesting that crop rotations have a relatively short-term effect on the control of CRR.

Phenotypic screening for CRR resistance generally relies on assessment of visual discolouration along the sub-crown internode and on the roots (Tobias *et al.* 2009) of advanced plant materials sampled from the field. These methods are subjective, time consuming, expensive and prone to significant environmental interactions. Due to these limitations, molecular marker technologies combined with conventional breeding methods may provide a faster and more robust means of identifying resistant progeny, thus increasing the efficiency of selection in cereal breeding programs.

This study examined a population of recombinant inbred barley lines (RILs) developed from a cross between the moderately resistant parent variety Delta and the susceptible variety Lindwall, with the aim of identifying the genomic regions linked to the expression of CRR resistance.

Materials and methods

Plant materials

The moderately resistant variety Delta (Tyra/Claret) was released in England in 1983 and the susceptible variety Lindwall (Triumph/Grimmett) was released in Queensland in 1996. The Delta/Lindwall population was developed in 1998 by the barley breeding group at the Department of Employment, Economic Development and Innovation, Queensland Primary Industries and Fisheries (DEEDI-PI&F), Hermitage Research Station, Warwick, Queensland, in collaboration with Dr Graham Wildermuth, DEEDI-PI&F, Leslie Research Station, Toowoomba, Queensland.

Fungal Inoculum

The CRR field trials were set up in a four-way rotational block with two fallow years followed by a bulk planting of a CRR susceptible variety such as wheat GS50a in the season preceding the experimental trial. Soil populations of *B. sorokiniana* were determined as indicated in Wildermuth *et al.* (1992).

Field trials

CRR field trials of this population were conducted at Wellcamp, Queensland in 2002, 2003, 2004 and 2005. Seed of individual barley lines were planted in 3 m single row plots (10 plots per row; 80 plants per plot) with a row spacing of 50 cm. Due to space constraints all lines of the population could not be included in each trial. The parents Delta and Lindwall, as well as the susceptible variety Timgalen and the RILs were replicated in each trial. The total number of lines grown in 2002, 2003, 2004 and 2005, were 41, 64, 67 and 85, respectively, with 92 lines scored at least twice. The method used to calculate disease severity was according to Wildermuth (1986). The CRR disease scores across years were standardised by converting them to disease severity scores as a percentage of the CRR susceptible line, Timgalen. After standardisation, the disease scores across the years 2002, 2004 and 2005 were averaged to one score per line. These scores were used in the quantitative trait loci (QTL) analysis. The 2003 data was excluded from the QTL analysis as it had a significantly lower ($P < 0.001$) average CRR severity score compared with the 2002, 2004 and 2005 data.

Bulked Segregant Analysis (BSA) and Diversity Arrays Technology (DArT) analysis

DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Sydney). Before investing in the construction of a whole genome linkage map for the Delta/Lindwall population, a bulked segregant analysis (BSA; Michelmore *et al.* 1991) was conducted, to determine whether the segregation of CRR resistance/susceptibility in the population was sufficient to detect

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QTL. CRR severity scores from the years 2002, 2003 and 2004 were used to determine the average CRR severity scores for BSA (the 2005 data were not available at this time). Equal quantities of DNA (20 ng) from eight lines with the highest and eight lines with the lowest average CCR severity score were pooled separately for BSA. DNA of the two bulks and each parent were sent to Triticarte Pty Ltd (http://www.triticarte.com.au/default.html) for Diversity Arrays Technology (DArT) analysis (Wenzl *et al*. 2004).

DArT data were analysed by examining the hybridisation intensity of parental and bulked DNA to individual DArT Markers. Markers were selected based on the contrast in hybridisation intensity of both the parents and the bulks using an arbitrary threshold of >1.0. Markers with the largest difference in hybridisation intensity between the bulks were considered to have the highest probability of association with CRR resistance.

As some putative markers showed association with CRR resistance in the BSA, a whole genome analysis of the population was pursued. DNA of all 147 RIL lines of the Delta/Lindwall population was sent to Triticarte Pty Ltd for DArT analysis.

Single Sequence Repeat (*SSR*) *analysis*

Primer sequences for SSR markers which mapped near putative QTL on a consensus map (Wenzl *et al*. 2006) were obtained from GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) and a standard protocol was used to amplify the markers (Bovill *et al*. 2010). The amplified products were visualised using a Gel-Scan 2000™ (Corbett Life Sciences, Sydney, Australia).

Linkage map construction and QTL analysis

A linkage map of the Delta/Lindwall population was constructed consisting of 468 DArT and 9 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 employed to order markers within linkage groups. The Kosambi function was applied to calculate map distances. Composite interval mapping was conducted with Windows QTL Cartographer ver. 2.5 (Wang *et al*. 2007). One thousand (1000) permutation tests at 5 cM intervals were conducted to calculate the minimum log-likelihood (LOD) score of 2.9 for a QTL to be significant. Map figures were produced with MapChart ver. 2.1 (Voorrips 2002).

Results

Descriptive statistics of the four growing seasons and the averaged dataset are given in Table 1. The frequency of the average adult infection responses to CRR for the years 2002, 2004 and 2005 displayed a continuous distribution, indicative of a quantitative trait (Fig. 1). The parental line, Delta, displayed partial resistance to the CRR infection with an average disease severity percentage score of 54.7% while the susceptible parental line, Lindwall, gave a mean score of 82.4%.

Using BSA and DArT analysis, 17 DArT markers were identified which showed a >1.0 difference in the hybridisation intensity both between the parents and between the resistant and susceptible bulks (Table 2). These were clustered in three genomic regions on chromosomes 2H (eight markers), 4H (seven markers) and 7H (two markers) according to the barley DArT consensus map (Wenzl *et al*. 2006). The region on 4H was identified as having a sub-group of seven closely linked DArT markers with a contrast in hybridisation intensity between the parents and the bulks ≥ 2.0, with the largest difference between the bulks (3.58) detected at marker bPb-3045. In comparison, the region on chromosome 2H only revealed one DArT marker (bPb-6052) with a difference in hybridisation intensity >2. The region on

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7H was considered minor, as the two DArT markers showed a contrast in the hybridisation intensity lying between 1.5 and 2.0.

Whole genome QTL analysis indicated that CRR resistance in the Delta/Lindwall population was conditioned by two QTL (Table 3). The QTL on chromosome 4HL, also detected by BSA, was located between markers bPb-2427 and bPb-8013 and explained 12% of the phenotypic variance (Fig. 2). The second QTL had not been detected by BSA and was located between markers bPb-9892 and bPb-4971 on chromosome 5HL and explained 11% of the phenotypic variance (Fig. 2). Both QTL were inherited from Delta, consistent with the absence of transgressive segregation towards resistance in the population. The putative QTL on 2H and 7H previously detected by BSA were not significantly linked to resistance in the mapping analysis.

Discussion

In this study BSA showed an association between DArT markers and CRR resistance. Once putative markers had been identified on chromosomes 2H, 4H and 7H, a whole genome mapping approach was undertaken to verify these markers and to determine whether there were other QTL which had not been detected using the BSA approach. Only the 4HL region, detected through BSA, was also detected through whole genome mapping and an additional region on 5HL was detected. Detection of the two putative regions on 2H and 7H by BSA suggests that using an arbitrary threshold of >1.0 for the difference in hybridisation intensity may have been too low. Furthermore, a region is more likely to be significant if multiple markers in the same region show a high difference in hybridisation intensity (≥ 2.0), as observed with the 4HL region. Difference observed between the BSA and whole genome QTL analysis results may also be due to the different datasets used in the two analyses, i.e. 2002, 2003 and 2004 data for BSA *v.* 2002, 2004 and 2005 data for whole genome QTL analysis. Some of the lines chosen for BSA would have been different had the 2005 data been included. Nevertheless, BSA was useful for determining whether a genetic effect for CRR resistance could be detected in the population without investing in a whole genome linkage map. However, it is necessary to perform whole genome or at least regional QTL mapping across the entire population to verify BSA results when dealing with a trait like CRR which is difficult to phenotype and shows large variations between years.

To our knowledge, only two other studies have identified QTL for CRR resistance. Random amplified polymorphic DNA (RAPD) markers were used together with BSA to identify markers linked to CRR resistance in Fr926–77/Deuce and Virden/Ellis crosses (Kutcher *et al*. 1996*b*). In both populations a RAPD marker, also associated with the two-rowed and six-rowed spike locus, was associated with CRR reaction. Genes associated with these spike morphologies are located on chromosomes 2HL and 4HS (Marquez-Cedillo *et al*. 2001). In a separate study, a RAPD marker allele associated with CRR resistance in the cultivar Bowman, was closely linked in repulsion to the morphological markers for glossy-sheath (*gs4*) and orange lemma (*o*) (Kutcher *et al*. 1996*a*). These independent and potential sources of resistance to CRR may provide opportunities for quantitative gene pyramiding with the Delta source to improve the level of resistance expression in derived lines. However, rigorous screening of alternative sources such as Bowman, Deuce and Virden for disease responses under a range of Australian conditions is required.

The CRR QTL identified in the present study were not located in regions previously associated with spot blotch resistance (Bovill *et al*. 2010), suggesting that genetic control of

CRR resistance and spot blotch resistance are independently inherited. This is in agreement with previous findings (Kutcher *et al*. 1996*a*). Furthermore, it has been shown recently that Australian isolates of *B. sorokiniana* collected from CRR infections were not able to induce susceptible spot blotch infection responses from host barley plants (Knight *et al*. 2010).

The CRR QTL on chromosome 4HL is in a similar location to a QTL identified for adult plant resistance to the net form of net blotch (*Pyrenophora teres* f. *teres*) in an Alexis/Sloop doubled haploid population (Lehmensiek *et al*. 2007), while Barr *et al*. (1998) have identified a cereal cyst nematode (*Heterodera avenae* Woll.) gene in the 5HL QTL region linked to CRR resistance in this study. Further studies are needed to determine whether these findings indicate the presence of a resistance gene cluster.

In view of the resource intensive nature of phenotypic screening for CRR, the QTL identified in this study may be useful in selecting targets for breeding programs incorporating this source of resistance. However, before that is possible, robust PCR-based markers for use in routine screening will need to be developed from sequence information for the relevant DArT probes (Bovill *et al*. 2010).

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References

<jrn>Barr AR, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Lance RCM, Lewis J, Jeffries SP, Langridge P (1998) RFLP mapping of a new cereal cyst nematode resistance locus in barley. *Plant Breeding* **117**, 185–187. doi:10.1111/j.1439-0523.1998.tb01477.x</jrn>

<jrn>Bovill J, Lehmensiek A, Platz GJ, Usher T, Franckowiak J, Mace E, Sutherland MW (2010) Mapping *Bipolaris sorokiniana* resistance genes in four barley populations. *Molecular Breeding*, doi: 10.1007/s11032-010-9401-9

<jrn>Conner RL, Duczek LJ, Kozub GC, Kuzyk AD (1996) Influence of crop rotation on common root rot of wheat and barley. *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* **18**, 247–254.</jrn>

<jrn>Knight N, Platz G, Lehmensiek A, Sutherland MW (2010) An investigation of genetic variation among Australian isolates of *Bipolaris sorokiniana* from different cereal tissues and comparison of their abilities to cause spot blotch on barley. *Australasian Plant Pathology*, (In press)</jrn>

<jrn>Kumar J, Schafer P, Huckelhoven R, Langen G, Baltruschat H, Stein E, Nagarajan S, Kogel KH (2002) *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Molecular Plant Pathology* **3**, 185–195. doi:10.1046/j.1364-3703.2002.00120.x</jrn>

<jrn>Kutcher HR, Bailey KL, Rossnagel BG, Franckowiak JD (1996*a*) Linked morphological and molecular markers associated with common root rot reaction in barley. *Canadian Journal of Plant Science* **76**, 879–883.</jrn>

<jrn>Kutcher HR, Bailey KL, Rossnagel BG, Legge WG (1996*b*) Identification of RAPD markers for common root rot and spot blotch (*Cochliobolus sativus*) resistance in barley. *Genome* **39**, 206–215. doi:10.1139/g96-027</jrn>

<jrn>Lehmensiek A, Platz J, Mace E, Poulsen D, Sutherland MW (2007) Mapping of adult plant resistance to net form of net blotch in three Australian barley populations. *Australian Journal of Agricultural Research* **58**, 1191–1197 doi:10.1071/AR07141</jrn>

<jrn>Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* **12**, 930–932. doi:10.1007/s00335-001-1016-3</jrn>

<jrn>Marquez-Cedillo LA, Hayes PM, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2001) QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. *Theoretical and Applied Genetics* **103**, 625–637. doi:10.1007/PL00002919</jrn>

<jrn>Mathre DE, Johnston RH, Grey WE (2003) Diagnosis of common root rot of wheat and barley. *Plant Health Progress* **August issue**, 1–6. doi:10.1094/PHP-2003-0819-01-DG</jrn>

<edb>Mehta YR (1988) Constraints on the integrated management of spot blotch of wheat. In ‘Helminthosporium blights of wheat: spot blotch and tan spot’. (Eds E Duveiller, HJ Dubin, J, Reeves, A McNab) pp. 18–27. (CIMMYT: Mexico)</edb>

<jrn>Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 9828–9832. doi:10.1073/pnas.88.21.9828</jrn>

<edb>Steffenson BJ (1997) Spot blotch. In ‘Compendium of barley diseases’. (Ed. DE Mathre) pp. 35–36. (American Phytopathological Society: St. Paul)</edb>

<jrn>Tobias DJ, Stack RW, Puri KD, Riveland N, Zhong SB (2009) Reactions of hard red spring wheat to common root rot under field conditions of Northern United States of America. *Euphytica* **167**, 165–172. doi:10.1007/s10681-008-9853-8</jrn>

<jrn>Van Leur JAG, Alamdar MZ, Khawatmi S (1997) Effect of common root rot (*Cochliobolus sativus*) on yields of barley under experimental conditions in northern Syria. *Australian Journal of Agricultural Research* **48**, 351–357. doi:10.1071/A93126</jrn>

<jrn>Van Os H, Stam P, Visser RGF, Van Eck H (2005) RECORD: a novel method for ordering loci on a genetic linkage map. *Theoretical and Applied Genetics* **112**, 30–40. doi:10.1007/s00122-005-0097-x</jrn>

<jrn>Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity* **93**, 77–78. doi:10.1093/jhered/93.1.77</jrn>

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DOI: 10.1071/AP09089; TOC Head:

<eref>Wang S, Basten CJ, Zeng Z-B (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)</eref>

<jrn>Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 9915–9920. doi:10.1073/pnas.0401076101</jrn>

<jrn>Wenzl P, Li HB, Carling J, Zhou MX, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna J, Cakir M, Poulsen D, Wang JP, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* **7**, 206–227. doi:10.1186/1471-2164-7-206</jrn>

<jrn>Wildermuth GB (1986) Geographic distribution of common root rot and *Bipolaris sorokiniana* in Queensland wheat soils. *Australian Journal of Experimental Agriculture* **26**, 601–606. doi:10.1071/EA9860601</jrn>

<jrn>Wildermuth GB, McNamara RB (1991) Effect of cropping history on soil populations of *Biploaris sorokiniana* and common root rot of wheat. *Australian Journal of Agricultural Research* **42**, 779–790. doi:10.1071/AR9910779</jrn>

<jrn>Wildermuth GB, Tinline RD, McNamara RB (1992) Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. *Australian Journal of Agricultural Research* **43**, 43–58. doi:10.1071/AR9920043</jrn>

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Fig. 1. Frequency of average adult infection responses to CRR. Disease severity is expressed as a percentage of the susceptible cultivar Timgalen. Arrows indicate average parental scores

Fig. 2. Map of chromosomes 4H and 5H indicating QTL intervals (bars on the right side) for CRR resistance in the Delta/Lindwall population. Distances are indicated in centimorgans on the right. Markers on chromosome 4H which were significant in the BSA are underlined

Table 1. Descriptive statistics of the Common Root Rot field trials of the Delta/Lindwall barley population

Trial	[A]N	[B]Minimum	Maximum	Mean	[C]S.d.	Delta	Lindwall
2002	41	52.5	123.0	82.9	19.1	41.0	98.9
2003	64	31.2	85.1	54.7	12.5	33.8	60.5
2004	67	50.2	103.2	77.5	12.0	46.2	49.9
2005	85	50.9	108.8	89.1	13.1	76.8	98.5
[D]Avg	146	50.9	120.6	83.6	13.8	54.7	82.4

[A]Number of lines scored.

[B]Minimum, maximum and mean disease scores.

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[C]Standard deviation.

[D] Average of the 2002, 2004 and 2005 trial, i.e. data used in QTL analysis.

Table 2. Hybridisation intensity between parents and resistant and susceptible bulked DNA using DArT analysis

Marker	Chromosome	[A]Position (cM)	Hybridisation intensity differences	
			Parents	Bulks
bPb-3608	2HS	3.6	1.43	1.15
bPb-4148	2HS	3.6	1.73	1.46
bPb-6052	2HS	5.0	1.30	3.76
bPb-0485	2HS	5.0	1.58	1.40
bPb-8750	2HS	33.0	2.17	1.13
bPb-2501	2HS	47.4	3.22	1.72
bPb-3190	2HS	47.4	2.99	1.57
bPb-7906	2HS	65.3	3.14	1.65
bPb-0130	4HS	65.3	3.62	3.16
bPb-0365	4HS	65.3	3.56	3.07
bPb-0513	4HS	65.3	3.61	3.28
bPb-2427	4HS	65.3	2.73	2.31
bPb-3045	4HS	65.3	3.60	3.58
bPb-4333	4HS	65.3	2.92	3.07
bPb-9504	4HS	67.9	2.83	1.99
bPb-9202	7HS	17.0	1.73	1.90
bPb-4674	7HS	27.1	1.08	1.50

[A]Cumulative distance in centiMorgans indicating position of DArT marker according to DArT consensus map (Wenzl *et al*. 2006).

Table 3. Summary of quantitative trait loci (QTL) associated with common root rot in the Delta/Lindwall barley population

Chromosome	LOD[A] score	Phenotypic Variation (%)	Donor	Flanking Markers	Closest marker
4HL	4.3	11.6	Delta	bpb-2427 bpb-8013	bpb-0561
5HL	4.0	10.8	Delta	bpb-9892 bpb-4971	bpb-1719

[A]Minimum log-likelihood