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Mapping of a novel QTL for resistance to cereal cyst nematode in wheat

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Abstract Cereal cyst nematode (CCN; *Heterodera avenae* Woll.) is a root pathogen of cereals that can cause severe yield losses in intolerant wheat cultivars. Loci for resistance to CCN, measured by a seedling bioassay, were identified by creating a genetic map based on a Trident/Molineux doubled haploid population of 182 lines. A novel locus accounting for up to 14% of the resistance to CCN was mapped to chromosome 1B of Molineux by association with microsatellite marker loci *Xwmc719* and *Xgwm140*. This locus acts additively with the previously identified CCN resistance loci identified on chromosomes 6B (*Cre8*) and 2A (*Cre5* on the VPM1 segment) in this population to explain 44% of the genetic variance for this major wheat pathogen.

Introduction

Cereal cyst nematode (CCN; *Heterodera avenae* Woll.) is a root pathogen of cereals found in more than 31 countries, spanning the world's wheat growing areas (Meagher 1977; Eastwood et al. 1994). Yield losses in an intolerant wheat cultivar may be up to 30% (Fisher 1982), and damage is often more severe in rainfed soils with poor nutrient status. Crop rotation, host resistance (O'Brien and Fisher 1974) and host tolerance (Fisher et al. 1981) are the only economically and environmentally sustainable methods of controlling damage from this nematode. Seven genes for resistance to CCN have been identified in hexaploid wheat and its relatives: *Cre1* (Cereal root eelworm—locus 1) (*Triticum aestivum* 2B) (Williams et al. 1994), *Cre2* (transferred to wheat from *Aegilops ventricosa*) (Delibes et al. 1993), *Cre3* (2D, transferred from *Aegilops tauschii*) (Eastwood et al. 1994), *Cre4* (*A. tauschii*) (Eastwood et al. 1991), *Cre5* (2A, VPM1 segment from *A. ventricosa*) (Jahier et al. 2001), *Cre6* (*A. ventricosa* $5N^V$) (Ogbonnaya et al. 2001), *Cre7* (*Aegilops truincialis)* (Romero et al. 1998) and *Cre8* (*T. aestivum* 6B) (Williams et al. 2003). CCN resistance genes have also been mapped in rye (6R, Taylor et al. 1998) and barley (2H, Kretschmer et al. 1997; 5H, Barr et al. 1998). A linkage disequilibrium study (Paull et al. 1998) found an RFLP locus, *Xcdo347*, that was associated with the Festiguay-derived CCN resistance of the wheat cultivars Molineux, Frame and Barunga. Williams et al. (2003) used this RFLP as a starting point to genetically locate, with RFLP markers, the gene *Cre8* which provides CCN resistance (and tolerance) in the cultivar Molineux. However, wheat breeders using Molineux as a source of CCN resistance in new crosses have reported that marker-assisted selection for *Cre8* has not produced progeny with high-level resistance to CCN, possibly indicating the existence of further genes in this variety. This paper reports the identification, by genetic mapping, of a second locus contributing to CCN resistance in the wheat cultivar Molineux, its genetic relationship with other CCN resistance and tolerance genes in the Trident/Molineux population, and the identification of closely-linked, PCR-based molecular markers available for marker-assisted selection of these loci.

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Materials and methods

Plant materials

A doubled haploid population consisting of 182 individuals produced from a cross between 'Trident' and

'Molineux' was used as the basis for this study (Ranjbar 1997). Pedigrees and CCN resistance reactions of population parents, related cultivars and a susceptible check variety are given in Table 1.

Cereal cyst nematode tolerance and resistance screening

Lines were screened for resistance using the bioassay method described by Fisher (1982) except that seedlings were inoculated with 100 juveniles rather than 75. The population was screened in two consecutive years, with five seedlings assayed per experiment.

Genotyping

Simple sequence repeat (SSR)

A total of 678 SSR markers were screened for polymorphism using standard PCR conditions and electrophoresis of amplified fragments as described by Karakousis et al. (2003). Subsequent genotyping of each DH line was achieved by visually scoring each marker in accordance with the corresponding parental alleles.

Sequence tagged site (STS) markers

Vernalisation (Vrn)

Primers VRN1AF and VRN1AR were amplified according to Yan et al. (2004), producing 750 and 500 bp products from Trident and Molineux VrnA1 alleles, respectively. Amplified products from DH population were separated on 1.5% agarose gels and visualised on a UV Transilluminator after ethidium bromide staining.

Reduced height (Rht1 and Rht2)

PCR and electrophoresis conditions were used as described by Ellis et al. (2002).

RFLP analysis

DNA extraction was achieved using a DNA mini-prep method adapted from Rogowsky et al. (1991). Variations to the method were as described below. For the initial extraction, 600 µl of extraction buffer and phenol-chloroform-isoamyl alcohol (25:24:1) were used. The extraction buffer was 0.1 M Tris–HCl, pH 8, 10 mM EDTA, 0.1 M NaCl, 1% sarkosyl. DNA was precipitated by the addition of 0.1 vol. of 3 M sodium acetate (pH 4.8) and one volume of propan-2-ol. For restriction fragment length polymorphism (RFLP) marker analysis restriction endonuclease digestion and Southern hybridisation followed the methods described by Guidet et al. (1991).

Map construction and quantitative trait loci (QTL) analysis

The map was constructed using Map Manager QTX [version QTXb20, Manly et al. (2001)] using Kosambi mapping function with a threshold value of $P = 0.01$. Genotypic data from the doubled haploid population was initially arranged into groups via the "Make Linkage Groups" function. New markers were integrated into these chromosomes using the "Links report" function, then in conjunction with the "ripple" function and published maps, an order of markers was established, with the aim of minimising double recombinants and chromosome length. Raw data was rechecked if anomalies were observed. Quantitative trait loci for CCN resistance was characterised by simple interval mapping using Map Manager QTXb20 (Manly et al. 2001) to generate a likelihood ratio statistic (LRS) (Haley and Knott 1992) and the amount of the total trait variance which would be explained by a QTL at this locus, as a percent. The effect of the three QTL on CCN resistance was determined by multiple linear regression using Genstat sixth edition (Payne et al. 2002). The genotype for each of the DH lines at the three loci was determined using the closest linked marker(s). Where a recombination had occurred a missing value was assigned to the locus.

Results

Segregation of progeny for CCN resistance

The CCN resistance phenotypes of the parents of this cross are given in Table 1. Segregation of DH progeny for CCN resistance from the Trident/Molineux cross was skewed towards resistance and considerable transgressive segregation was observed (Fig. 1). Broad sense heritability for CCN resistance was 0.88 in 2005 and 0.93 in 2004.

Table 1 Pedigree and phenotypes of cultivars

Parent	Pedigree	CCN cysts (2005)
Molineux	Pitic62/Festiguay//2*Warigal	17 (Resistant)
Trident	VPM1/5*Cook//4*Spear	52 (Moderately susceptible)
Spear	Sabre/MEC3//Insignia	71 Susceptible
Frame	Molineux/3*Dagger	18 Resistant
Egret	Heron/2*WW15	67 Susceptible

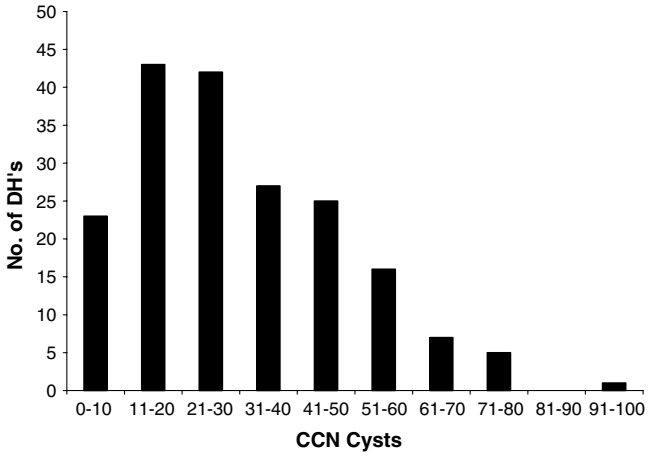

Fig. 1 Cereal cyst nematode cyst counts of Trident/Molineux doubled haploids in 2005 experiment (Trident = 52, Molineux = 17)

Map construction

The map consisted of a total of 251 markers, spread over 21 chromosomes with a total length of 3,061 cM and an average chromosome length of 146 cM (Fig. 2). Over 90% of markers were linked together with LOD scores >3. In total, 240 SSR markers, seven gene-based markers and four RFLP (CDO347, BCD175, BCD1, AWBMA20) markers were used to create the map. Twelve markers showed significant segregation distortion.

Location of Molineux CCN tolerance loci

Simple interval analysis revealed three QTLs that contributed to CCN resistance in this population, on chromosomes 1B, 2A and 6B (Table 2, Fig. 2). The QTLs on chromosomes 1B and 6B were contributed by Molineux, and explained up to 8 and 24% of the mean trait variation, respectively (Table 2). The QTL on chromosome 2A was derived from Trident, and explained up to 12% of the mean variation for CCN resistance. Composite interval mapping using the CCN mean data confirmed the simple interval analysis, showing that the three QTLs on chromosomes 1B, 2A and 6B explained up to 8, 11 and 20%, respectively, or a total of 40% of the variation for CCN resistance. Multiple linear regression was also used to show that markers linked to the 1B, 2A and 6B QTLs accounted for 44.7% of the variation for CCN resistance. Based on the markers linked to the chromosome 2A and 6B QTLs, it is likely that these are allelic to the previously mapped *Cre5* and *Cre8* genes, respectively. The QTL for CCN resistance on chromosome 1B is designated *QCre.srd-1B* according to the rules in the Catalogue of Gene Symbols for Wheat—1998 edition. As the markers defining the *QCre.srd-1B* QTL are located distally on the long arm of chromosome 1B, their derivation from this chromosome was confirmed by nullisomic analysis. According to nulli-tetra analysis, the SSR locus Xgwm140 linked to *QCre.srd-1B* is indeed derived from chromosome 1B (Fig. 3). The contribution of the two Molineux loci on resistance of the DHs can be determined by taking a mean of cyst counts from DHs with homozygous markers flanking the resistance loci on chromosomes 1B, 2A and 6B. The chromosome 6B locus has a greater effect on suppression of CCN cyst multiplication than the locus on 1B, and when both are present, the mean CCN resistance level is approximately that of the resistant parent, Molineux, while progeny carrying all three loci have much greater resistance than either parent (Table 3). The *QCre.srd-1B* QTL for CCN resistance did not contribute to tolerance to CCN (data not shown).

Discussion

The aim of this study was to complete the dissection of the genetics of CCN tolerance in Molineux by genetic mapping, and to identify new, PCR-based, molecular markers linked to CCN resistance loci.

Identification of the first CCN tolerance/resistance locus *Cre8* in Molineux (Williams et al. 2003) was achieved by the use of candidate markers identified in one of the first linkage disequilibrium studies of wheat by Paull et al. (1998). In the *Cre8* mapping study, determination of the genetics of Molineux CCN resistance in the Trident/Molineux population was initially confounded by the presence of a weak gene(s) for CCN resistance on the VPM1 segment of chromosome 2A carried by Trident (Bonhomme et al. 1995). An RFLP diagnostic for VPM1 was consequently deployed to exclude lines carrying this segment from the analysis.

A recent study concluded that although some lines with the VPM1 segment on chromosome 2AS have CCN resistance to European (Rivoal et al. 1986) and Australian (Ogbonnaya et al. 2001) pathotypes conferred by the locus *Cre5*, Trident did not, possibly losing it through recombination or suppression (Jahier et al. 2001). However, in this study we show by QTL analysis that a small contribution to CCN resistance is provided by the VPM1 segment of Trident. This accords with the observation that Spear, to which the VPM1 segment was introgressed to create Trident, is a more CCN susceptible cultivar (Table 1).

The creation of a complete genetic map of the Trident/Molineux population has facilitated the identification of major and minor loci for CCN resistance. The impetus for searching for extra loci in Molineux after the *Cre8* gene had been identified was provided by plant breeders in Australia and internationally, who are using this cultivar as a source of resistance to this damaging nematode, but had not recovered high levels of CCN resistance in progeny selected by MAS. The ability to select for both Molineux CCN resistance loci with high-throughput markers should lead to more rapid breeding

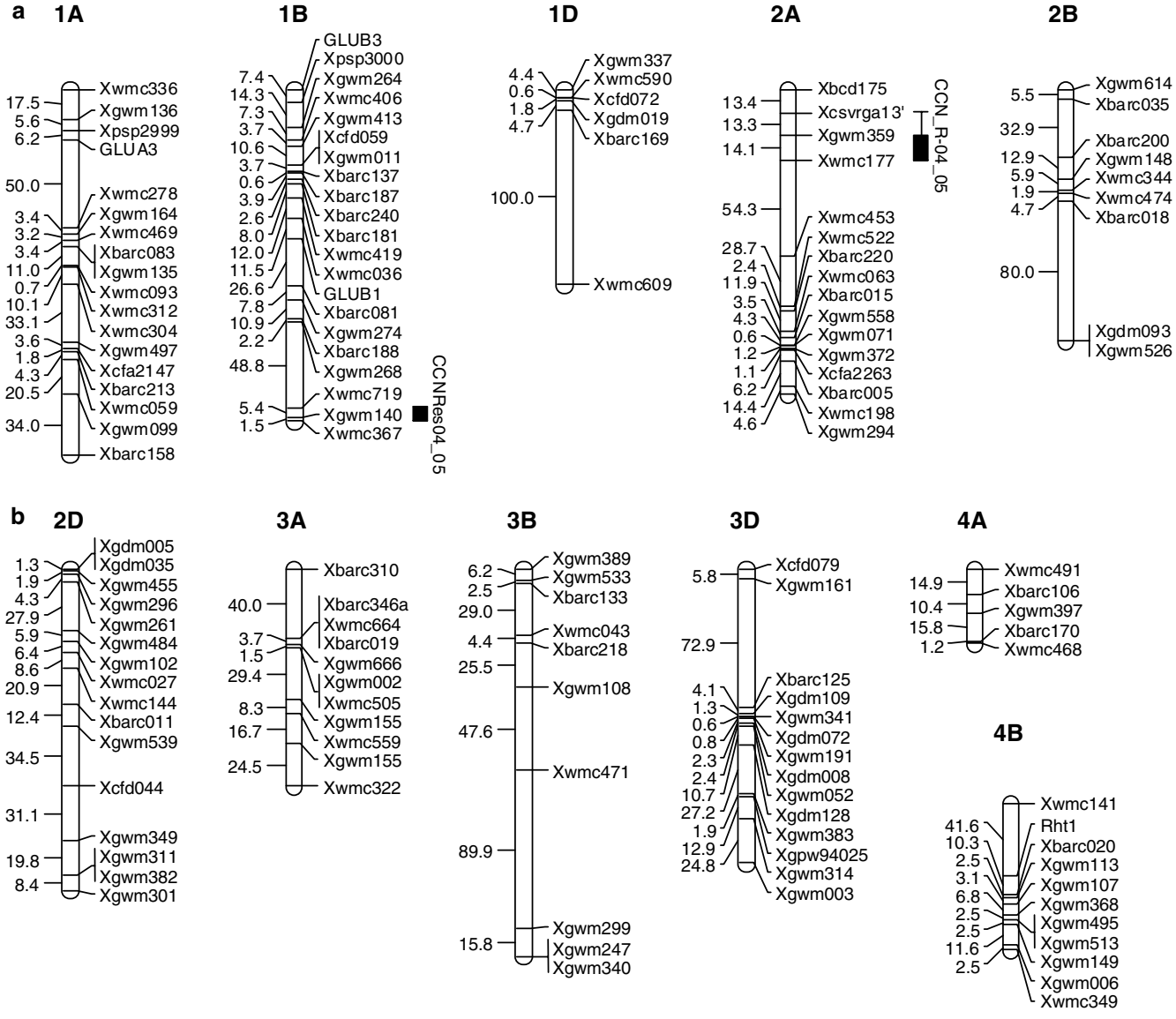

Fig. 2 Molecular linkage map of wheat based on the cross Trident × Molineux. Map distances are given in centi-Morgans (Kosambi function). Putative QTLs are shown on the right, with *solid black bars* and *black lines* indicating significance ($P < 0.001$ and $P < 0.05$, respectively)

of resistant lines, as simulation has shown that MAS can increase genetic gain and reduce costs of wheat breeding (Kuchel et al. 2005).

In this population, selection for both Molineux CCN resistance loci with molecular markers would identify progeny with an average of 41 or 19 cysts in 2004 or

Table 2 Genetic variation explained by markers linked to CCN resistance

Locus	Donor	Marker	CCN mean		CCN (2005)		CCN (2004)	
			LRS[a]	R^{2b}	LRS	R^2	LRS	R^2
Cre8	Molineux	*Xgdm147*	50.8	24.0	40.2	22.7	40.0	20.4
		Xcdo347	24.0	12.0	14.5	9.1	22.9	12.9
Cre5	Trident	*Xwmc177*	16.0	8.0	23.8	13.6	12.3	7.5
		Xgwm359	13.9	7.0	15.9	9.1	9.5	5.3
QCre.srd-1B	Molineux	*Xwmc719*	23.8	12.0	10.3	6.8	24.0	12.9
		Xgwm140	22.9	12.0	16.3	10.2	19.2	10.7

[a]Likelihood ratio statistic for the association of the trait with this locus
[b]The amount of the genetic variance which would be explained by a QTL at this locus, as a percent

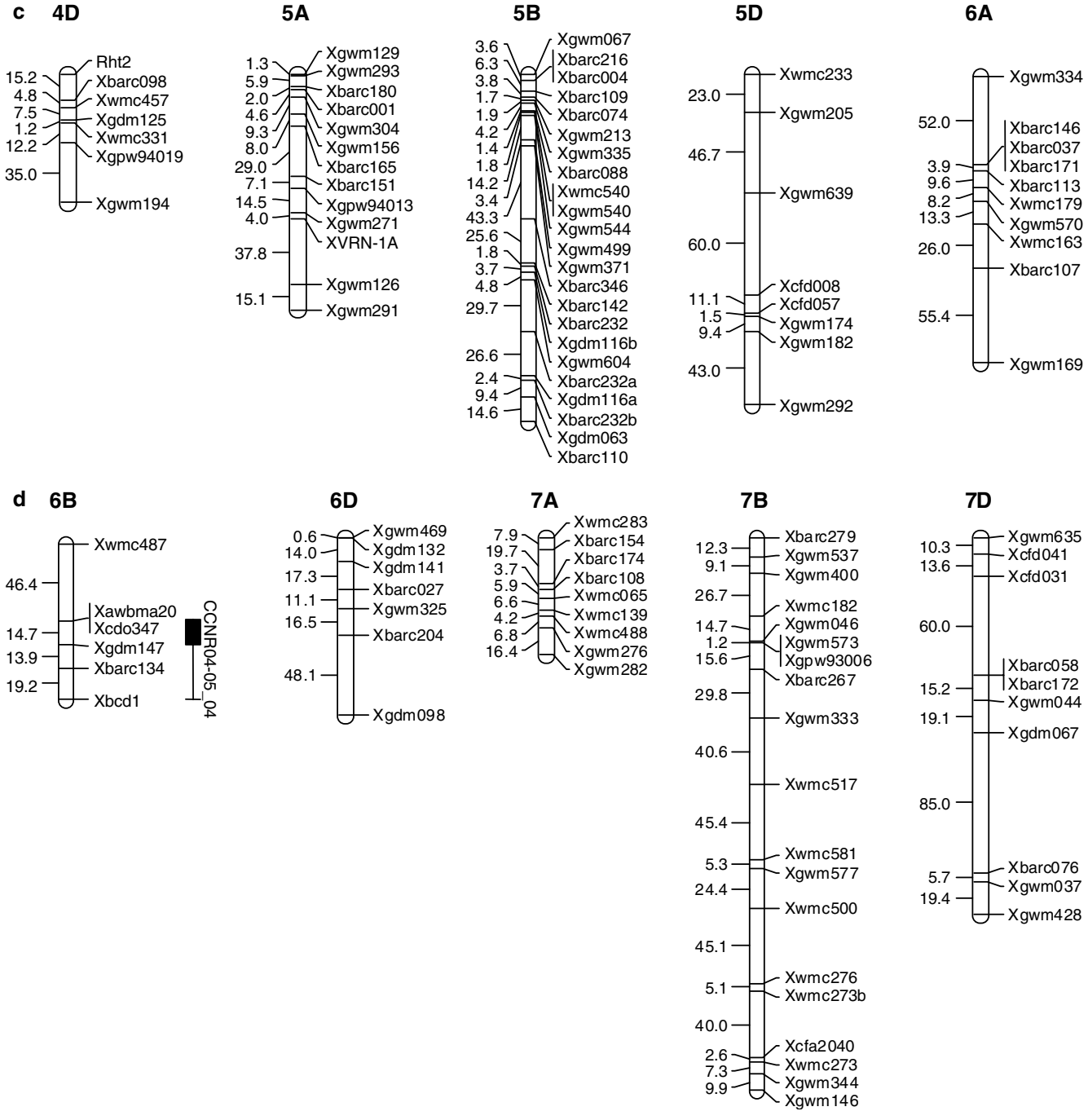

Fig. 2 (Contd.)

2005, a similar level of resistance to the Molineux parent (65 or 17 cysts in 2004 or 2005). Prior to this study, selection of the *Cre8* gene alone, would have resulted in a higher mean number of 58 or 37 cysts in 2004 or 2005, and the previously identified RFLP marker was technically unsuited to the high-throughput required by current wheat breeding programs. Also, through the additional selection of the VPM1 segment carried by Trident, a level of CCN resistance greater than that of Molineux may be recovered in progeny carrying all three loci (18 or 12 cysts in 2004 or 2005).

Many of the previously identified CCN resistance genes have been mapped to syntenous locations in related grasses, possibly indicating the existence of a limited number of resistance genes that evolved in response to this obligate biotroph. However, this is the first report of a CCN resistance gene mapping on a group one chromosome of any grass species.

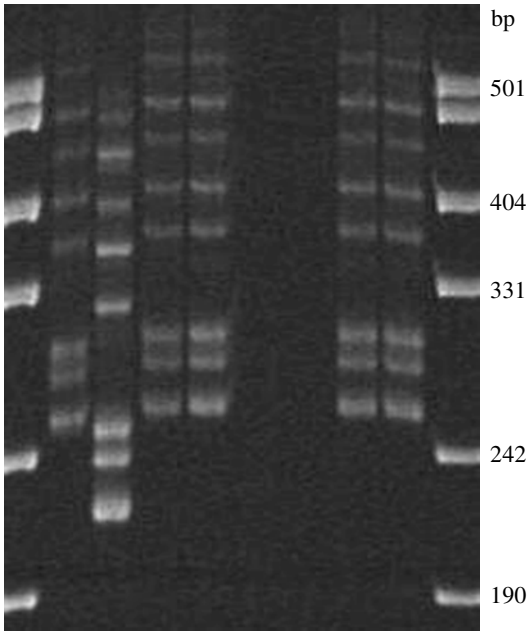

Fig. 3 Chromosomal location of QCre.srd-1B linked SSR marker *Xgwm140* by nullisomic analysis. Lanes are: *1*, *10* Size ladder; *2* Trident; *3* Molineux; *4* Nulli 1A-Tetra 1B; *5* Nulli 1A-Tetra 1D; *6* Nulli 1B-Tetra 1A; *7* Nulli 1B-Tetra 1D; *8* Nulli 1D-Tetra 1A; *9* Nulli 1D-Tetra 1B

Table 3 Effect of Molineux CCN resistance loci on CCN cysts

Locus	Mean CCN cysts (2004)	Mean CCN cysts (2005)
Cre8 (6B)	58	37
QCre.srd-1B	98	39
Cre8 and *QCre.srd-1B*	41	19
Cre8 and *QCre.srd-1B* and *Cre5* (2A)	18	12
Trident	108	52
Molineux	65	17

In summary, dissection of the genetics of resistance to CCN has been completed by the creation of a genetic map based on a large population of homozygous lines, followed by QTL analysis of two year's nematode bioassay data. A novel locus was identified and was found to provide more effective resistance when selected in conjunction with the previously mapped *Cre8* gene. PCR-based markers linked to both genes were identified. In addition, a genetic map is now available for mapping end use quality and agronomic traits in this population.

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