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Four QTLs determine crown rust (*Puccinia coronata* f. sp. *lolii*) resistance in a perennial ryegrass (*Lolium perenne*) population

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Crown rust resistance is an important selection criterion in ryegrass breeding. The disease, caused by the biotrophic fungus *Puccinia coronata*, causes yield losses and reduced quality. In this study, we used linkage mapping and QTL analysis to unravel the genomic organization of crown rust resistance in a *Lolium perenne* population. The progeny of a pair cross between a susceptible and a resistant plant were analysed for crown rust resistance. A linkage map, consisting of 227 loci (AFLP, SSR, RFLP and STS) and spanning 744 cM, was generated using the two-way pseudo-testcross approach from 252 individuals. QTL analysis revealed four genomic regions involved in crown rust resistance. Two QTLs were located on LG1 (*LpPc4* and *LpPc2*) and two on LG2 (*LpPc3* and *LpPc1)*. They explain 12.5, 24.9, 5.5 and 2.6% of phenotypic variance, respectively. An STS marker, showing homology to R genes, maps in the proximity of *LpPc2*. Further research is, however, necessary to check the presence of functional R genes in this region. Synteny at the QTL level between homologous groups of chromosomes within the *Gramineae* was observed. LG1 and LG2 show homology with group A and B chromosomes of oat on which crown rust-resistance genes have been identified, and with the group 1 chromosomes of the *Triticeae*, on which leaf rust-resistance genes have been mapped. These results are of major importance for understanding the molecular background of crown rust resistance in ryegrasses. The identified markers linked to crown rust resistance have the potential for use in marker-assisted breeding.
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Introduction

Perennial ryegrass (*Lolium perenne*) is an obligate outbreeding grass species used for either turf or forage purposes. In species grown for their vegetative organs, such as *L. perenne*, foliar diseases are especially important as they are often correlated with reductions in yield and quality. This is the case for crown rust infections caused by the biotrophic fungus *Puccinia coronata* (Potter, 1987; Plummer *et al*, 1990). The severity of crown rust infections can be reduced by good management practices, such as the use of resistant cultivars (Van Bockstaele and Baert, 2004). The use of resistant cultivars is especially needed due to the current tendency to minimize nitrogen input since, the less the nitrogen is applied, the more the crown rust invades ryegrass stands (Kimbeng, 1999). Given the economic impact of crown rust infections, improvement of genetic resistance to this disease is one of the major goals in ryegrass breeding programmes (Wilkins and Humphreys, 2003; Van Bockstaele and Baert, 2004). Currently, breeders identify genotypes carrying resistance genes for crown rust either after natural or artificial infection. A better understanding of the inheritance of the resistance and the identification of the genomic regions that influence resistance can help us to develop more efficient and effective breeding and selection schemes. For example, once identified, the different genomic regions involved in the determination of crown rust resistance can be combined and investigated in a single genotype of positive alleles at several of these loci.

To date, two studies have reported on genomic regions determining crown rust resistance in *Lolium*. Dumsday *et al* (2003) detected one major locus (designated *LpPc1* by the authors) on linkage group (LG) 2 of the *L. perenne* genetic map. Muylle *et al* (2005) detected two clusters of AFLP markers associated with crown rust resistance, explaining 35% of the variance observed in the segregating population investigated. One cluster mapped to LG 2; the map position of the other cluster was not determined. In both studies, bulked segregant analysis (BSA), followed by mapping of the markers displaying significant associations with the trait, was applied. Although this approach allows the identification of genomic regions that have major effects on the trait, a thorough QTL analysis is necessary to obtain a more general overview of the genetic factors determining resistance and to identify additional genomic regions with minor effects (Quarrie *et al*, 1999). An

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essential step for QTL analysis is the construction of a detailed linkage map of the population used for phenotypic analysis.

L. perenne displays gametophytic self-incompatibility, controlled by two loci designated *S* and *Z* (Thorogood *et al*, 2002). Linkage analysis in *L. perenne* is complicated by this self-incompatibility system and by severe levels of inbreeding depression. However, the two-way pseudo-testcross procedure, described by Grattapaglia and Sederoff (1994), can be applied for successful linkage mapping in this outcrossing species. In this procedure, the mapping family consists of an F_1 progeny derived from a pair-cross between two unrelated, highly heterozygous individuals. This mapping approach has been applied with success in several species (apple, Maliepaard *et al*, 1998; poplar, Wu *et al*, 2000), and also in *Lolium* (Faville *et al*, 2004; Warnke *et al*, 2004).

In this study, we first developed a linkage map based on AFLP, SSR, STS and RFLP markers of a *L. perenne* population segregating for crown rust resistance. Secondly, we used this map to perform QTL analysis. We identified the genomic regions involved in the determination of crown rust resistance in this crop and also studied the syntenic relationships at the genomic and QTL level between *Lolium* and other grass genomes.

Materials and methods

Plant materials and phenotypic evaluations

The mapping population (252 individuals) was derived from a pair cross between two heterozygous perennial ryegrass genotypes, SB2 and TC1, with contrasting phenotypes for crown rust resistance. SB2 was susceptible to crown rust, while TC1 was resistant. The mapping population was evaluated for crown rust resistance at the seedling stage using artificial inoculations, as described in Muylle *et al* (2005). Scores varied from 1 (=resistant) to 6 (=susceptible). The segregation of the trait indicated that resistance to crown rust in this population was determined by the action of two major loci of which the resistant alleles are dominant and do not interact. It was also demonstrated that additional genomic regions, with smaller individual effects, influence the expression of the trait in this population (Muylle *et al*, 2005).

Molecular marker analysis

Before phenotypic evaluation, young leaf material was harvested, frozen in liquid nitrogen, lyophilized and stored under vacuum conditions until used for DNA extraction. Lyophilized material was ground using a mill (Retsch MM200). Genomic DNA was prepared from lyophilized material using a modified CTAB protocol (Weising *et al*, 1991).

AFLP markers

Fluorescent AFLP analysis was performed with *Eco*RI/*Mse*I and *Hind*III/*Mse*I enzyme combinations according to Roldán-Ruiz *et al* (2000). Eight primer combinations (PC) were used for linkage mapping (*Eco*RI-ACG-*Mse*I-CAA, *Eco*RI-AAC-*Mse*I-CAC, *Eco*RI-ACA-*Mse*I-CAT, *Eco*RI-AAG-*Mse*I-GCC, *Eco*RI-ACG-*Mse*I-GGC, *Hind*III-TGG-*Mse*I-CAT, *Hind*III-TAC-*Mse*I-GAT and *Hind*III-TGC-*Mse*I-GTT).

RFLP markers

A set of 51 RFLP probes, combined with three restriction enzymes (*Eco*RI, *Hind*III and *Dra*I), was used to screen for polymorphisms in the mapping population. The RFLP probes were derived from oat (CDO from Cornell University, USA), rice (RZ from Cornell University, USA; RGR, RGC and RGG from NIAR, Japan), barley (BCD from Cornell University, USA), maize (CSU from Californian State University, USA), wheat (PSR from JIC, UK) and fescue (IBF from AUN, Norway). RFLP analysis was performed using ^{32}P-labelled probes as described by Muylle (2003).

SSR markers

Four sources of SSRs were used: (1) 10 primer pairs from Kubik *et al* (1999, 2001), (2) 10 primer pairs from Jones *et al* (2001), (3) 11 primer pairs developed at DvP (Jensen *et al*, 2005) and (4) 100 unpublished primer pairs developed by Jones *et al* (La Trobe University, Australia) and licensed to Advanta Van der Have (Rilland, The Netherlands). PCR amplifications of SSR sets (1), (2) and (3) were performed using a Geneamp PCR reagent kit (Applied Biosystems). One primer of each pair was fluorescently labelled to allow fragment separation and detection on an ABI Prism 377 DNA sequencer (Perkin–Elmer). GS-500 Rox labelled size standard was loaded in each lane to enable automatic analysis of the data. Genescan Analysis Software 2.1 was used to translate the information collected by the ABI377 into fragment sizing information and Genotyper 2.5 software was used to score the fingerprints. The fourth set of SSRs was screened, tested and scored at Advanta Van der Have (Rilland, The Netherlands), using a LiCor Gene ReadIR 4200. The scoring of these SSRs was carried out using Gene ImagIR software (LiCor Inc.).

STS analysis

A total of 16 primer pairs developed by Lem and Lallemand (2003) were screened for polymorphisms. PCR amplifications were performed using a Geneamp PCR reagent kit (Applied Biosystems). STS fragments were separated on 8% polyacrylamide gels and visualized by UV illumination after staining with ethidium bromide. The STS fingerprints were scored manually.

We also mapped a marker derived from an RGA sequence, which we PCR-amplified in *Lolium* using the primer pair s1-NBS1 (Mago *et al*, 1999). In ryegrass, one fragment of approximately 540 bp displayed homology with known disease-resistance genes. Using the FASTA algorithm, homology was found with the *RPP13*, *RPP8*, *RPM1*-resistance genes isolated from *A. thaliana* and *I2*-resistance gene isolated from *Lycopersicon esculentum*. These genes all have NBS motifs. Sequence information of the amplified fragments from the parents of the mapping population (SB2 and TC1) were used to identify a restriction site polymorphism in order to transform this PCR fragment into a CAPS marker (denominated NBS-1).

Map construction and QTL analysis

RFLP, SSR and STS markers were scored codominantly if possible. Using this information, six marker classes were defined: (1) a-x--, (2) --xa-, (3) a-xa-, (4) abxab, (5) abxac and (6) abxcd, with the first two characters representing

the allelic composition of the resistant parent and the last two characters the allelic composition of the susceptible parent (a, b, c = detectable alleles; - = unknown allele). Class 3 markers were not included in the linkage analysis, as such markers contribute little information to the map and recombination frequency estimates obtained with such markers are typically inaccurate (Maliepaard *et al*, 1998). The χ^2 test integrated in JoinMap 3.0 software (Van Ooijen and Voorrips, 2001) was used to estimate departures from expected segregation ratios. A linkage map was constructed for each parent independently. Initially, distorted markers ($P < 0.001$) were omitted from the analysis. In a second round, distorted markers and markers unmapped during the first step were added to the map only if the map order was not drastically affected. Markers were grouped into linkage groups at LOD equal or higher than 4 using JoinMap 3.0 (Van Ooijen and Voorrips, 2001). Markers of classes (4), (5) and (6) were used as allelic bridges to integrate the parental linkage groups. Marker order was calculated at LOD = 1.00 and recombination threshold value (REC) of 0.40. Map distances were calculated using the Kosambi function.

QTL analysis was carried out with MapQTL 4.0 (Van Ooijen *et al*, 2002) using automatic cofactor selection and MQM mapping. This analysis was based on the parental linkage maps produced in round 2 in the JoinMap3.0 software, in which markers are not forced to map onto the linkage group.

Comparative analysis

Comparative mapping was performed as described by Jones *et al* (2002b). The comparative location of probes in the *Triticeae* and rice genomes were as reported by Jones *et al* (2002b) and/or ascertained using the Graingenes (http://grain.jouy.inra.fr/ggpages/) and Gramene (http://www.gramene.org/) databases.

Results and discussion

Segregation of DNA markers

The eight AFLP primer combinations revealed a total of 164 polymorphic markers and included the markers selected in a BSA analysis on the same segregating population that has been reported elsewhere (Muylle *et al*, 2005). The 29 heterologous RFLP probes polymorphic in the mapping population included both cDNA and genomic DNA probes with known map positions in rice. These markers were included in the *L. perenne* map to enable the study of syntenic relationships between *Lolium* and other *Gramineae* (see below). We were able to score the RFLP markers codominantly in 19 cases out of 29, showing once more the value of RFLP as a codominant marker system. These RFLP probes detected a total of 47 polymorphic loci. Of the 131 SSR primer pairs, 31 (Kubik *et al*, 1999, 2001; Jones *et al*, 2002a; Jensen *et al*, 2005) revealed useful polymorphisms in the mapping population and amplified a total of 54 polymorphic loci. Five STS markers developed by Lem and Lallemand (2003), and the RGA marker developed in this study (NBS-1) were polymorphic in the mapping population and resulted in 10 polymorphic loci useful for linkage analysis.

Overall, 15% of the markers showed distorted segregation ($P < 0.001$). This figure is in the same order of magnitude as that previously reported in other *Lolium* studies (Armstead *et al*, 2002; Jones *et al*, 2002a, b; Thorogood *et al*, 2002; Faville *et al*, 2004; Inoue *et al*, 2004; Warnke *et al*, 2004). Apparent segregation distortion can be due to statistical error, genotyping and scoring errors or can be a real biological phenomenon. Genotyping and scoring errors might explain the distorted patterns in our study. In all, 26 of the 275 markers exhibited bias towards the 'presence' allele. For AFLP markers, this can be due to the superimposition on the gels of nonallelic PCR products, corresponding to bands from different loci (ie fragment homoplasy, as has been demonstrated by Vekemans *et al* (2002) for AFLP markers). A total of 20 AFLP markers deviated towards excess of the 'absence' allele, however, in most of these cases the fragments displayed faint amplification. Possible biological reasons for segregation distortion include the result of pollen tube competition, pollen lethals, preferential fertilization and selective elimination of zygotes (Lu *et al*, 2002).

Linkage map construction

Map construction was carried out according to the two-way pseudo-testcross procedure. A total of 275 markers were included in the linkage analysis: 39% of the markers were derived from the susceptible parent, 42% of the markers were from the resistant parent and 19% of the markers were heterozygous in both parents. An integrated map was obtained by aligning the two parental maps on the basis of allelic bridges (Figure 1). This *L. perenne* genetic map was generated from 252 individuals, spans 744 cM, consists of 227 loci grouped into seven linkage groups corresponding to the haploid chromosome number of *Lolium*. The mean distance between two consecutive loci is 3.28 cM. The length of the linkage groups (LGs) vary from 83 till 133 cM. The number of markers per linkage group ranges from 17 (LG6) to 48 markers (LG1), with an average of 32 markers per LG. Marker clusters (defined here as 10 cM intervals with more than nine markers) were found in all LG except LG4 and LG6. Major gaps (0 markers/10 cM) were present in all LG, except LG3; gaps between two adjacent markers exceeding 20 cM were found on LG2, LG6 and LG7.

Using the observed mean chiasma frequency per chromosome of 1.7 obtained from an interspecific cross of *L. perenne* × *L. multiflorum* (Naylor, 1960), the theoretical genome length of *Lolium* was estimated to be 1190 cM. The map obtained here spans 744 cM and thus covers 63% of the whole genome. Partial genome coverage is also indicated by the substantial number of markers (48 out of 275) that were not mapped into a linkage group. Additional markers, preferrably codominant loci such as SSRs or markers derived from expressed sequences, are needed to integrate these ungrouped markers into the current linkage map.

In total, 23 of the mapped loci deviated significantly from the expected Mendelian segregation ratios. In the present study, a high number of distorted markers were found in LG1 (eight markers) and LG2 (six markers), while the highest percentage of distorted markers was found in LG6, where four out of 18 markers were

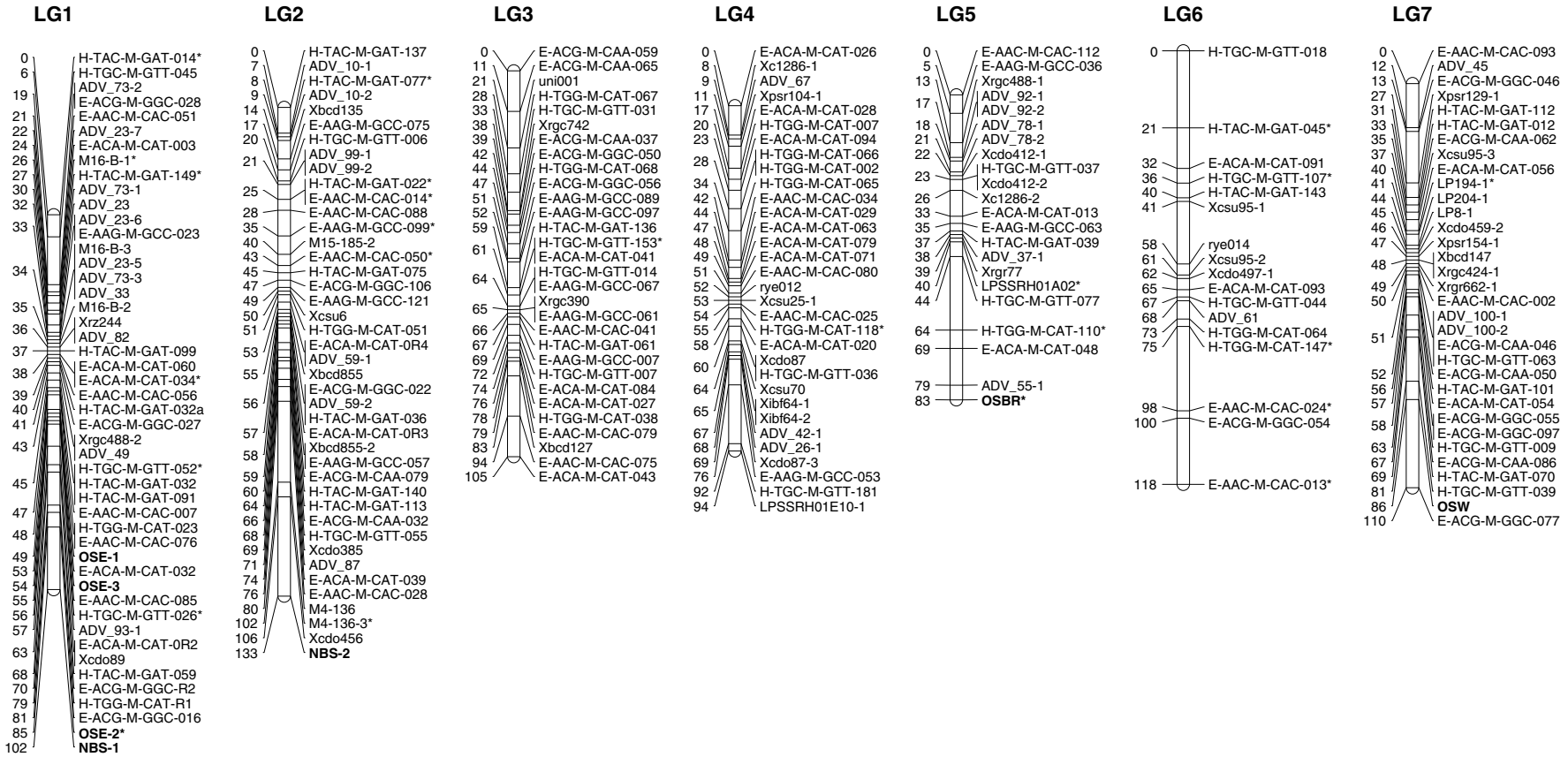

Figure 1 Genetic linkage map for the *L. perenne* cross based on SSR, RFLP, STS and AFLP markers. RFLPs are shown with prefix X; ESTs are indicated in bold; and SSRs are shown with prefix adv, lp, M, rye or uni; AFLP markers selected during the BSA analysis as being significantly associated with crown rust resistance end with −Rx (with x = number). Distorted markers ($P < 0.001$) are marked with *. Linkage groups are numbered according to the ILGI linkage map.

distorted. LG1 and LG2 represent the chromosomes where the self-incompatibility (SI) loci *S* and *Z* have been mapped by Thorogood *et al* (2002). In LG2, all distorted loci (except rye024_b) mapped in the proximity of the *Z* locus, which is located in the neighbourhood of BCD135 (http://ukcrop.net/perl/ace/search/FoggDB). Therefore, the presence of a high number of distorted loci in this region can be explained by the SI system present in *L. perenne*. This phenomenon of distorted segregation ratios of molecular markers due to self-incompatibility has been reported in other species including *Fragaria* (Sargent *et al*, 2004), citrus (Ruiz and Asins, 2003), and *Phalaris coerulescens* (Bian *et al*, 2004). However, on LG1, just three out of the eight distorted loci mapped close to CDO89 and OSE, markers known to map in the proximity of *S* (http://ukcrop.net/perl/ace/search/FoggDB). In LG1 and LG6, distortion can be attributed to the presence of genes related to viability, as also suggested by Thorogood *et al* (2002) for markers on LG3 in their mapping population. In the *Lolium* maps published to date, segregation distortion appears in different LGs, indicating that genes that have influence on the generation and survival of the progeny are located at different regions of the genome.

The different marker types used to construct the map were distributed over the different linkage groups. A total of 137 AFLP markers were incorporated to the map. No significant clustering was observed for *Hind*III-generated or *Eco*RI-generated AFLPs. Although AFLP markers are not very informative in a two-way pseudo-testcross, they fulfilled an important role in expanding the genome coverage of the linkage groups (eg in LG6 and LG7). AFLP markers also filled in large gaps between codominant markers (eg LG4).

The AFLP markers identified in the BSA analysis by Muylle *et al* (2005), with putative associations with crown rust resistance (E-ACA-M-CAT-R3, E-ACA-M-CAT-R4, E-ACG-M-GGC-R2 and H-TGG-M-CAT-R1; Muylle *et al*, 2005), were also mapped in this study. E-ACA-M-CAT-R3 and E-ACA-M-CAT-R4 mapped in close proximity on LG2; E-ACG-M-GGC-R2 and H-TGG-M-CAT-R1 mapped to LG1. The marker cluster explaining the highest percentage of variation in the crown rust data (E-ACG-M-GGC-R2 and H-TGG-M-CAT-R1) mapped close to the resistance gene analogue, NBS-1.

Alignment with other linkage maps

The linkage map was aligned with the ILGI (International *Lolium* Genome Initiative) map (Jones *et al*, 2002a, b). Alignment was based on 32 SSRs, 10 RFLPs and five STSs in common on both linkage maps. Four inconsistencies (three SSRs and one RFLP) with the ILGI map were observed. These inconsistencies may be due to the detection of multiple loci by the respective SSR primer pairs or RFLP probes. This could certainly be the case for markers ADV87, ADV26 and Xcdo459 as each of these markers detected an extra monomorphic fragment in our mapping population, in addition to the polymorphic mapped fragments.

Although our map was constructed using the two-way pseudo-testcross approach, we could align it with the ILGI map, which was constructed using the one-way pseudo-testcross approach. Marker order was highly conserved and the total map length was in the same order of magnitude. This demonstrates the effectiveness of the two-way pseudo-testcross approach for linkage map construction in outcrossing species. The main advantage of this approach is that the mapping population can be constructed using plants from the breeding pool that show extreme phenotypes in the traits of interest. In this way, the segregation of the traits of interest is ensured in the progeny studied.

QTL analysis

Phenotypic analysis (Figure 2) of the mapping population revealed two major crown rust-resistance genes (see Muylle *et al* (2005) for a detailed discussion). To identify the position of genomic regions involved in crown rust resistance, QTL analysis was performed on each parental linkage map (TC1 and SB2) and on the integrated map. Using interval mapping, markers with an LOD score >3 associated with crown rust resistance were found on the TC1 (resistant parent) map only and not on the SB2 map. Automatic cofactor selection and MQM analysis on the TC1 map and on the integrated map identified four genomic regions with an LOD score >5, on LG 1 and LG2 associated with crown rust resistance (Figure 3). Two QTL regions (QTL1 and QTL2) were the two genomic regions associated with crown rust resistance previously identified by BSA (Muylle *et al*, 2005). QTL3 and QTL4 represent two novel genomic locations determining crown rust resistance in *L. perenne*. The RGA marker (NBS-1) mapped in the proximity of QTL2. Given the abundance of RGA sequences in plant genomes, many of which are not functional (Leister, 2004; Monosi *et al*, 2004), the colocation of this RGA marker with QTL2 is not sufficient to conclude that the identified RGA is involved in crown rust resistance. A more detailed study of the identified RGA sequence is necessary before it can be determined whether it is part of a functional gene and whether this gene is involved in crown rust resistance.

Simulations showed that 1-LOD and 1.5-LOD support intervals provide a QTL coverage probability of approximately 90 and 95%, respectively (Dupuis and Siegmund, 1999). The 1.5-LOD support intervals of the QTLs detected in the integrated map using MQM are given in Table 1.

The proportion of variance explained by the four QTLs detected was estimated using MQM (Table 1). QTL1, QTL2, QTL3 and QTL4 explained 2.6, 24.9, 5.5 and 12.5% of phenotypic variance, respectively (Table 1). The QTLs

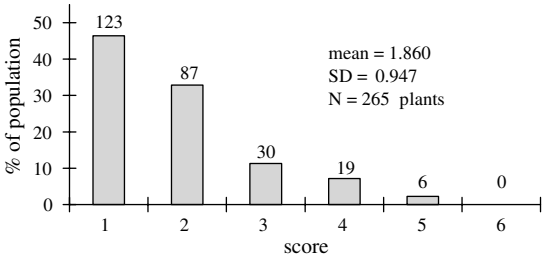

Figure 2 Frequency distribution of mean crown rust scores of the F_1 population. Crown rust scores vary from 1 = resistant to 6 = susceptible. The number of plants in each category are noted on top of each bar. The population mean, the standard deviation and the total number of plants are also given.

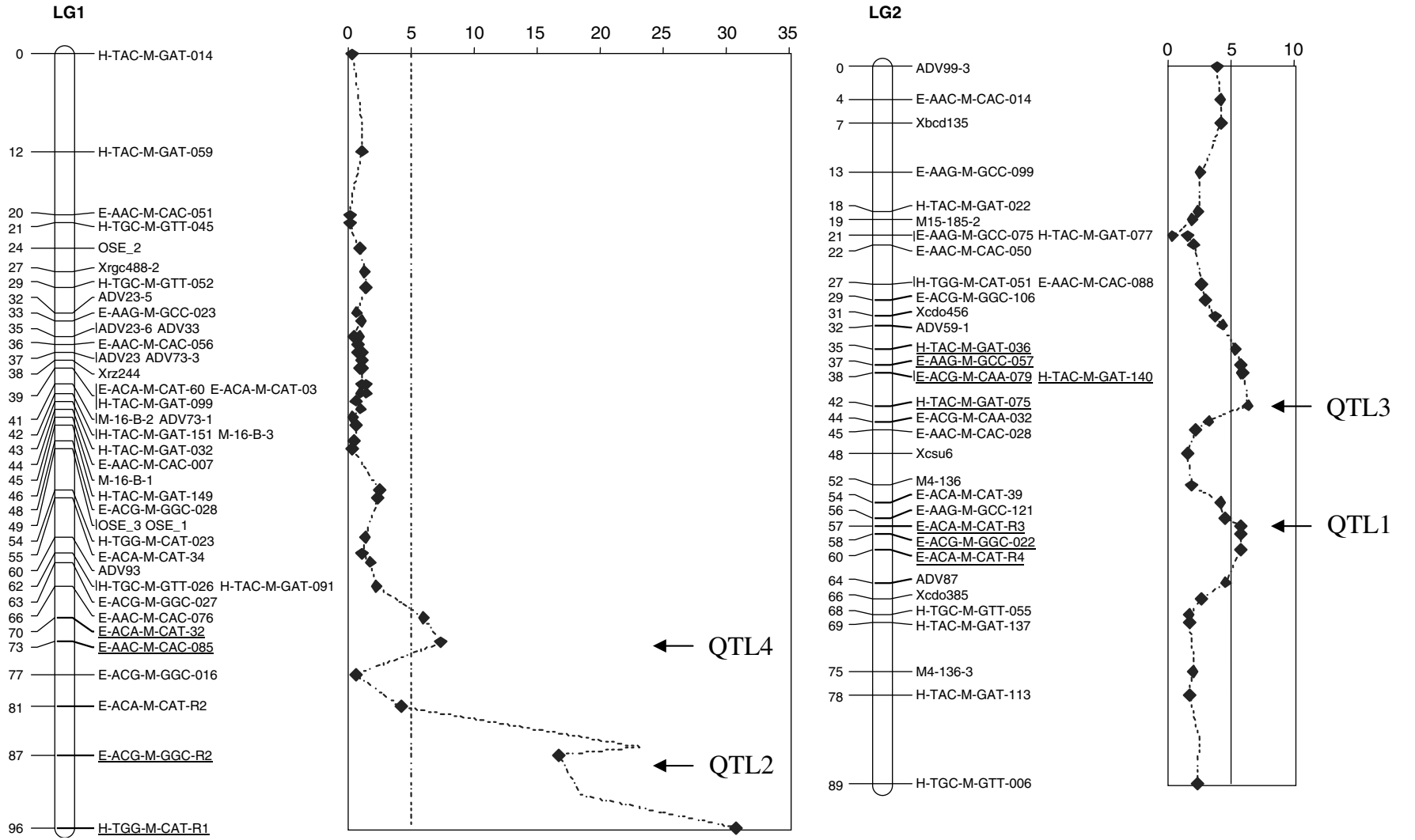

Figure 3 MQM results obtained using the integrated map. The 'map 2' version in the JoinMap 3.0 software procedure was used for QTL analysis. Markers with LOD score higher than five were retained for MQM analysis; markers that were selected after MQM are underlined.

Table 1 1.5 LOD support intervals giving a 95% probability of QTL location in the integrated map using MQM and the variance explained by each QTL calculated using three different methods: simple linear regression (LR), Interval mapping (IM) and multiple QTL mapping (MQM)

	MARKER	*LG*	*Position (cM)*	*LOD score*	*Left border (cM)*	*Right border (cM)*	*Size of 1.5 LOD-interval (cM)*	*Variance explained*		
								LR (%)	*IM (%)*	*MQM (%)*
QTL1	E-ACG-M-GGC-22	2	58.4	5.81	55.4	65.6	10.2	3.7	6.4	2.6
QTL2	H-TGG-M-CAT-R1	1	96.1	30.76	95.2	—	0.9[a]	20.7	23.7	24.9
QTL3	H-TAC-M-GAT-075	2	42.2	6.35	24.6	43.0	18.4	9.0	10.6	5.5
QTL4	E-AAC-M-CAC-085	1	72.5	7.37	70.2	73.4	3.2	7.4	13.4	12.5

[a]Only the left border was calculated as H-TGG-M-CAT-R1 mapped to the distal end of LG1.

explaining over 20% of the phenotypic variance, such as QTL2, are strong QTLs. The traits controlled by such QTLs can be considered almost Mendelian and are of extreme interest to breeders for use in selection programmes (Manly and Olson, 1999). QTL pairs were tested for epistatic interactions using GLM analysis (Kover and Caicedo, 2001) but no epistatic effects were identified.

Dumsday *et al* (2003) detected a major QTL for crown rust resistance on LG2. In our study, QTL1 is also located on LG2, in the proximity of Xcdo385 (Figure 3). According to Jones *et al* (2002b), Xcdo385 maps to LG2 in the ILGI mapping population. It is in this genomic region that Dumsday *et al* (2003) also located SSR markers associated with crown rust resistance. This indicates that QTL1 in our study corresponds to the same region as *LpPc1* in the study of Dumsday *et al* (2003). However, QTL1 does not explain a high proportion of variance (2.6%), in contrast to the high proportion of variance explained in the study of Dumsday *et al* (2003). In our study, QTL2 is of major importance (>20% variance explained). Following the nomenclature introduced by Dumsday *et al* (2003) to designate genomic regions involved in crown rust resistance in *L. perenne*, it is proposed that this locus is designated *LpPc2*. Likewise, QTL 1, QTL 3 and QTL 4 can be designated *LpPc1*, *LpPc3* and *LpPc4*, respectively.

Synteny at the marker and QTL level

We mapped 31 loci revealed by 29 heterologous RFLP probes. These probes had been previously mapped in rice by Stephenson (1999) and therefore were suitable for comparative mapping. An overview of the location of these probes on the rice map (Stephenson, 1999; gramene database), oat map (Van Deynze *et al*, 1995a; gramene database), wheat and barley map (Gale *et al*, 1995; Nelson *et al*, 1995a, b; Van Deynze *et al*, 1995b; Marino *et al*, 1996; gramene database) and the ILGI reference map (Jones *et al*, 2002b) is given in Table 2.

Jones *et al* (2002b) carried out a thorough comparative study between the ILGI map and three other *Poaceae* maps (*Triticeae*, rice and oat) on the basis of 109 heterologous probes. Our data provides new additional information for comparative studies. For LG1 of *Lolium*, we can confirm synteny with LG1 of *Triticeae*, LG5 of rice, and LGA of oat. For LG2 of *Lolium*, we have mapped an additional probe (BCD855), which confirms synteny with LG2 of *Triticeae*, and two probes, which confirm synteny with LG4 and LG7 of rice (CSU6 and BCD855, respectively). In LG3 of *Lolium*, we mapped two additional probes (RGC742 and BCD127), which identified synteny with rice LG1. In LG4 of *Lolium*, we mapped two probes (PSR104 and CDO87), which confirm synteny with LG3 of rice. However, the other three probes mapped in LG4 of *Lolium* did not show synteny with LG3. Synteny with LGE of oat and LG4 of the *Triticeae* was identified, but one probe (CDO87) mapped in barley on LG3H. In LG7 of *Lolium*, we found three additional probes showing synteny with rice LG6 and LG8. One of

Table 2 Overview of map positions of heterologous RFLP probes on the rice map (Stephenson, 1999; gramene database), oat map (Van Deynze *et al*, 1995a), wheat map, barley map (gramene database; Nelson *et al*, 1995a, b; Van Deynze *et al*, 1995a; Gale *et al*, 1995; Marino *et al*, 1996) and the ILGI map (Jones *et al*, 2002a)

LG in this study	*Probe*	*LG*				
		Rice	*Oat*	*Barley*	*Wheat*	*ILGI*
1	RZ244	1/5	A/C	—	1A	—
	RGC488	10	—	—	—	—
	CDO89	5	D	—	1A/1D	—
2	BCD135	4	B	—	2B/4A-	2
	CSU6	2/4	—	—	—	—
	BCD855	7	—	—	2A	—
	CDO385	7	B	—	—	2
	CDO456	4	B	—	2A/2B/2D	2
3	RGC742	1	—	—	—	—
	RGC390	8	—	—	—	3
	BCD127	1	—	—	—	—
4	C1286	10	—	3H	—	—
	PSR104	3	—	4H	—	—
	CSU25	11	E	—	4B	—
	CDO87	3	E	—		—
	CSU70	1/12	—	—	—	—
5	RGC488	6/10	—	—	—	—
	CDO412	9	E	—	5A/5B/5D-	5
	RGR77	3/11/12	—	—	—	—
	C1286	10	—	3H	—	—
6	CSU95	2/6	—	—	—	—
	RGC424	2/7/8	G	—	7	6
7	CSU95	6	—	—	—	—
	RGC424	2/7/8	—	—	—	—
	PSR154	8	—	6H	—	7
	BCD147	8	D	—	3B	7
	CDO459	12	F	—	—	5
	RGR662	8	—	—	—	—
	PSR129	6	—	7H	7B	—

these probes on LG7 of *Lolium* confirmed synteny with LG7 of the *Triticeae*.

Although our data set was very small for comparative mapping, it provided additional information to the study made on the basis of the ILGI map. The information obtained from our study includes some confirmation but also some inconsistencies to the results obtained by Jones *et al* (2002b). Further integration of all available *Lolium* maps will improve the knowledge about the existence of syntenic relationships between *Lolium* and other *Gramineae*.

Many instances of orthology between grasses are reported (reviewed by Devos, 2005). Armstead *et al* (2004) report on synteny between a major heading-date QTL in perennial ryegrass and the Hd3 heading-date locus in rice. In addition, we made a comparison between R genes identified and mapped in other monocots. Several agreements with previous QTL-mapping studies were found. Leaf rust (*Puccinia triticina*)-resistance genes are located on the group 1 chromosomes of the *Triticeae* species (Van Deynze *et al*, 1995a). The homologous chromosome in oat, the group A chromosomes, contain resistance genes against *Puccinia coronata* Cda f. sp. *avenae*. Yu *et al* (1996) identified homologous regions for resistance to obligate biotrophs in *Avena*, *Hordeum* and *Zea mays* on the homologous group 1. The group 1 chromosomes of the *Triticeae* and the group A chromosomes of oat are homologous to the LG1 of *Lolium*; on this LG, we also found two QTLs (*LpPc2* and *LpPc4*) linked with crown rust resistance.

Yu and Wise (2000) mapped a cluster of crown rust-resistance loci (*Pca* cluster) to LG B of diploid oat. This LG is homologous to LG2 of the *Triticeae* and LG2 of *Lolium*, on which we identified two QTLs (*LpPc1* and *LpPc3*) for crown rust resistance.

These findings indicate the presence of homologous regions for resistance genes in the *Gramineae*. If group 1 and 2 chromosomes of the *Triticeae* descended from the same chromosomes of a common ancestor, the disease-resistance loci on them may be orthologous. However, we have very fragmentary data on the synteny between LG1 and LG2 of *Lolium* and other monocots to make a more detailed synteny analysis for QTLs linked with resistance.

Potential applications

Although the confidence intervals of the four identified QTLs are large, plant breeders may not need to know the QTL locations with great accuracy if they intend to introgress these regions by marker-assisted selection. Plant breeders are mainly interested in those QTLs, which have a large effect, to incorporate them in elite plants. Marker information can be used to increase the frequency of positive QTLs (and to decrease the frequency of negative QTLs) in these plants. Probably the greatest value of markers in this context is the reduction of linkage drag during introduction of QTLs. Marker information can help to break the unfavourable correlations between quantitative characters of interest. Furthermore, marker information around and within a QTL can be used to develop selection indexes (Kearsey and Farquhar, 1998).

The dissection of a complex trait was carried out using parents with high breeding values and a first products of this study for the breeding programme are resistant genotypes with specific (favourable) QTL configurations. Some questions remain to be answered before the broad-scale exploitation of those markers identified as being linked to QTLs with major effects. It is likely that the effect and action of a QTL may vary across environments, across different genetic backgrounds and in response to infection with different spore mixtures. It is, therefore, necessary to test the QTLs identified in this study in different environments with different spore mixtures. An assessment of the diversity of QTL alleles and their action present in a broad genetic population should also be made (Marques *et al*, 1999; King *et al*, 2000; Miflin, 2000).

However, taking into account our discovery of synteny, at the QTL level, with other grass species, it is possible that at least some of the QTLs identified in this study represent genomic regions involved in general resistance or genomic regions in which R genes are clustered (gene-for-gene resistance). Evidence for gene-for-gene resistance towards crown rust in *Lolium* has been reported by several authors (Dumsday *et al*, 2003; Roderick *et al*, 2003; Aldaoud *et al*, 2004). In the case of QTL2 (*LpPc2*), it is likely that we detect a genomic region coding for gene-for-gene resistance as the mapped RGA marker (NBS-1) coincides with *LpPc2*. It would therefore be interesting to analyse this population for resistance to other diseases affecting *Lolium*, including bacterial wilt and stem rust and to identify the QTLs which determine resistance to these diseases.

Conclusions

A linkage map of *L. perenne*, based on the two-way pseudo-testcross approach, has been constructed for a population derived from two parents with extreme contrasting phenotypes for crown rust resistance. The genetic map spans 744 cM and consists of 227 loci (RFLP, STS, AFLP and SSR), grouped into seven linkage groups. Markers generated with different marker techniques are evenly distributed on the genetic map. The map size (744 cM) is in the range of other published linkage maps of *Lolium*. The estimated genome coverage is 63% of the theoretically expected linkage map length for *Lolium*. The coverage of this map is far from complete as demonstrated by the number of markers that could not be mapped to the linkage groups. Additional markers, particularly codominant and gene-specific markers will help to expand the coverage of the genome in future research.

In this study, four QTLs involved in crown rust resistance have been identified and localized. Two QTLs are located on LG1 (*LpPc4* and *LpPc2*) and two on LG2 (*LpPc3* and *LpPc1)*. They explain 12.5, 24.9, 5.5 and 2.6% of phenotypic variance, respectively. No epistatic interactions were found between these four QTLs.

As reported in previous studies, a high level of synteny between *Lolium* and the *Triticeae* was found. Some additional information to the comparative mapping study between *Lolium* and related species made by Jones *et al* (2002b) was obtained. Expanding the knowledge of synteny between these species will allow the transfer of information from model species such as rice to less-studied species with larger genomes, like *Lolium*.

Mapping of genes or loci with known function are of special interest.

The presence of synteny at the QTL level between homologous groups of chromosomes within the *Gramineae* was identified. LG1 and LG2 of *Lolium* show homology with group A and B chromosomes of oat, on which crown rust-resistance genes have been identified. LG1 and LG2 also show homology with the group 1 chromosomes of the *Triticeae*, on which leaf rust-resistance genes have been identified.

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