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Homologues of the maize rust resistance gene *Rp1-D* are genetically associated with a major rust resistance QTL in sorghum

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Abstract As part of a comparative mapping study between sugarcane and sorghum, a sugarcane cDNA clone with homology to the maize *Rp1-D* rust resistance gene was mapped in sorghum. The cDNA probe hybridised to multiple loci, including one on sorghum linkage group (LG) E in a region where a major rust resistance QTL had been previously mapped. Partial sorghum *Rp1-D* homologues were isolated from genomic DNA of rust-resistant and -susceptible progeny selected from a sorghum mapping population. Sequencing of the *Rp1-D* homologues revealed five discrete sequence classes: three from resistant progeny and two from susceptible progeny. PCR primers specific to each sequence class were used to amplify products from the progeny and confirmed that the five sequence classes mapped to the same locus on LG E. Cluster analysis of these sorghum sequences and available sugarcane, maize and sorghum *Rp1-D* homologue sequences showed that the maize *Rp1-D* sequence and the partial sugarcane *Rp1-D* homologue were clustered with one of the sorghum resistant progeny sequence classes, while previously published sorghum *Rp1-D* homologue sequences clustered with the susceptible progeny sequence classes. Full-length sequence information was obtained for one member of a resistant progeny sequence class (*Rp1-SO*) and compared with the maize *Rp1-D* sequence and a previously identified sorghum *Rp1* homologue (*Rph1-2*). There was considerable similarity between the two sorghum sequences and less similarity between the sorghum and maize sequences. These results suggest a conservation of function and gene sequence homology at the *Rp1* loci of maize and sorghum and provide a basis for convenient PCR-based screening tools for putative rust resistance alleles in sorghum.

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Introduction

Sorghum rust (*Puccinia purpurea* Cooke) occurs widely in almost all of the sorghum growing areas of the world. The fungal disease itself has a minimal effect on sorghum yield; however, rust is important because it predisposes plants to other major diseases, such as the *Fusarium* stalk rots, charcoal rot and grain moulds (Frederiksen 1986). Although only two races of *P. purpurea* have been identified (Bergquist 1974), the genetics of rust resistance in sorghum appears complicated, with varying numbers of genes at dispersed loci having differing effects and possible modes of action reported (Miller and Cruzado 1969; Patil-Kulkarni et al. 1972; Rana et al. 1976).

In contrast, many different major race-specific resistance (R) genes conferring resistance to maize common

rust (*Puccinia sorghi*), a major pathogen of maize, have been identified (Wilkinson and Hooker 1968). Most of these genes map to the *rp1* locus on the short arm of maize chromosome 10 (Rhoades 1935; Saxena and Hooker 1968). The *rp1* locus is a complex locus, with the order and arrangement of the *Rp1* genes often ambiguous due to genetic re-assortment within the locus (Hulbert et al. 1993). The number of *Rp1* homologues at the locus can also vary considerably from possibly as few as one and as many as up to 20 in various haplotypes of *Rp1* (Collins et al. 1999; Sun et al. 2001; Ramakrishna et al. 2002; Webb et al. 2002). Such re-assortment can give rise to new rust resistance specificities (Richter et al. 1995; Sun et al. 2001).

Most R genes isolated to date encode a putative nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region, with conserved amino acid motifs in and around the putative NBS (Meyers et al. 1999). Using primers designed to the conserved motifs, Collins et al. (1998) amplified resistance gene analogues (RGAs) in maize and used them as probes to search for functional R genes. One of these RGAs identified a small gene family that co-segregated perfectly with the *rp1* locus (Collins et al. 1998). Further characterisation of the gene family, together with gene-tagging approaches, identified the *Rp1-D* gene (Collins et al. 1999), which is clustered with approximately eight other homologues at the *rp1* locus (Collins et al. 1999). *Rp1-D* encodes a protein of 1,292 amino acids containing a P-loop and kinase-2 NBS motifs and an LRR domain and is therefore classified as an NBS-LRR *R* gene.

Other grasses have also been shown to contain sequences homologous to genes at the *rp1* locus in maize (Ayliffe et al. 2000). In barley, nine members of a RGA family with homology to the maize rust R gene *Rp1-D* have been mapped to five different barley chromosomes (Rostoks et al. 2002), with five homologues clustered within approximately 400 kb on chromosome 1. These homologues are near, but do not co-segregate with, the barley stem rust resistance locus *Rpg1* (Rostoks et al. 2002). One of the homologues maps to barley chromosome 7 (Rostoks et al. 2002); this chromosome has been suggested to be syntenous with maize chromosome 10 (Moore et al. 1995). Examination of the GenBank database also reveals that three *Rp1-D* homologues are located on the same rice BAC clone (S. Hermann, unpublished observation). However, while these homologues have high homology to the maize rust R gene *Rp1-D*, they do not segregate with any known disease resistance and their function is unclear.

Sorghum and maize are both members of the Andropogonae tribe, and comparative mapping studies have revealed considerable synteny between the genomes of these two species (Whitkus et al. 1992; Grivet et al. 1994; Pereira et al. 1994; Moore et al. 1995; Gale and Devos 1998). These studies have shown some synteny between maize chromosome 10, the location of the *Rp1* locus and sorghum LG H (as defined by Peng et al. 1999) and LG E, as defined by Tao et al. (1998). They have also demonstrated that these two LGs are the same linkage group, hereafter referred to as LG E; both linkage groups contain markers TXS1128, RZ261, CDO344 and TXP18 (Peng et al. 1999; Tao et al. 2000). Tao et al. (1998) have previously identified a major QTL for rust resistance on LG E that explained approximately 40% of the variation in the population under study; three minor QTLs on other linkage groups were also identified.

The *Rp1*-orthologous region in sorghum has been recently identified and characterised (Ramakrishna et al. 2002). A 27-kb region on sorghum LG E was sequenced and found to contain five *Rp1* homologues, but only one appeared to encode a predicted full-length protein similar to that encoded by the maize *Rp1-D* gene (Peng et al. 1999). Two of the homologues were truncated, while the other two apparently full-length homologues contained a stop codon and a retrotransposon, both of which should result in a truncated protein.

Many genetic maps of sorghum have been produced (e.g. Whitkus et al. 1992; Chittenden et al. 1994; Dufour et al. 1997; Tao et al. 1998; Boivin et al. 1999; Peng et al. 1999; Bhattramakki et al. 2000; Kong et al. 2000; Tao et al. 2000; Menz et al. 2002). Almost all of the markers on these maps are anonymous markers, including RFLPs, SSRs and AFLPs. The selection of molecular markers for implementation in marker-assisted plant breeding depends on the value of the trait being tagged, the closeness of the marker to the trait of interest and its ease of use. Consequently, there is increasing interest in the use of PCR-based 'perfect' markers, where the marker is in the gene responsible for the phenotype (Ellis et al. 2002). In this study, we describe the molecular characterisation of *Rp1-D* homologues in resistant and susceptible sorghum progeny lines and the development of PCR-based 'perfect' markers for rust resistance.

Materials and methods

Mapping of the sugarcane *Rp1-D* homologue in sorghum

The sugarcane *Rp1-D* homologue was identified in a sugarcane EST collection (Casu et al. 2001; GenBank accession no. CF577018). The EST clone was used to screen DNA from four different sorghum inbred lines (QL39, QL41, 90562 and ICSV745) that are parents of current sorghum mapping populations (Tao et al. 2000, 2003). QL39 and QL41 are both moderately rust resistant, but QL41 is more rust resistant than QL39 (D.R. Jordan, unpublished data). The rust reaction of 90562 and ICSV 745 is unknown. The four sorghum lines were each digested with five different restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I, *Dra*I), using techniques described previously (Tao et al. 1998). After identifying a polymorphic probe–enzyme combination, the sugarcane *Rp1-D* homologue was used to screen 120 random recombinant inbred lines (RILs) developed from a cross between QL39 and QL41 by the Queensland Department of Primary Industries (Henzell 1992) and the data incorporated into the QL39 × QL41 map, using techniques described previously (Tao et al. 1998).

Table 1 Primers designed for use in amplification of specific *Rp1-D* homologous sequence classes

Primer name	Primer sequence
Rp1D-1	5′-CCATCGTCACACGCGAGAGATT-3′
Rp1D-2	5′-GGTATGTTCTAGCTTCATGCGCAAT-3′
Rp1D-3	5′-CCATCGTCACACAAGGGAGATT-3′
Rp1D-4	5′-GATATCAGGATGTGGGTCTGCAT-3′
Rp1D-5	5′-GCTTATTGTGCAAAAGTTGATCTCC-3′
Rp1D-6	5′-GAAATCACCTCTGCAGTATGGTCC-3′
Rp1D-7	5′-GATGTCAGGATATGGGTCTGCAT-3′
Rp1D-8	5′-GGGTAGAAGAAGGCTTTGATGTCA-3′
Rp1D-9	5′-CGAGCCTACTGCACAAAATCTG-3′
Rp1D-10	5′-CAAGAGGATAGAAGAAGGCTTTGATAT-3′
Rp1D-11	5′-CCAGCTTCGTGCGTAATACTTG-3′

Isolation of sorghum homologues of maize *Rp1-D*

Of the 120 RIL progeny from the QL39 × QL41 sorghum mapping population, six rust-resistant progeny with the QL41-derived resistance QTL allele on LG E and six rust-susceptible progeny with the QL39-derived susceptible QTL allele on LG E were identified (Tao et al. 1998). The six rust-resistant progeny had an average rust rating of 2.5 and ranged from 1.9 to 2.9 (using the industry 1–9 resistance rating, with 1 being most resistant and 9 being very susceptible), whereas the six rust-susceptible progeny had rust ratings from 6.9 to 7.9 and averaged 7.25. Equal quantities of DNA from the six rust-resistant progeny were combined to form a resistant progeny DNA bulk, while equal quantities of DNA from the six rust-susceptible progeny were combined to form a rust-susceptible progeny DNA bulk.

Primers were designed to regions conserved between the maize *Rp1-D* and sugarcane *Rp1-D* homologue sequences (MCS2F: 5′-GCTTGTCTTGGATGATGTTTGG-3′, MCS2R: 5′-GGATAT-CATGCATGACATAG-3′). These primers were used to amplify sorghum *Rp1-D* homologues from the two bulked DNA samples. Reaction mixes contained 10 mM dNTPs, 10× buffer, 5 mM $MgCl_2$, 20 pmol each primer, 1 U Expand HiFi *Taq* polymerase (Roche) and 25 ng DNA in a reaction volume of 25 µl. Cycling parameters were: initial denaturation for 5 min at 94°C, followed by 39 cycles of 1 min at 94°C (with ramping at 0.3°C/s to 55°C), 30 s at 55°C and 2 min at 72°C and a final incubation at 72°C for 7 min.

The amplification products from the two bulks were cloned into a plasmid vector and approximately 60 clones from each bulk sequenced using standard techniques (Sambrook et al. 1989). The sequences were aligned using Clustal W, as implemented with the software MacVector, version 7.0 (Accelrys), with the following multiple alignment parameters: open gap penalty = 10.0, extend gap penalty = 0.1, delay divergent = 40%, gap distance = 8, similarity matrix = blosum.

Primers were designed to each sequence class such that the most 3′ base of the primer was the polymorphic nucleotide (Table 1). Annealing temperatures were varied to optimise the ability of primer pairs to only amplify the single-sequence class from which they were derived (Table 2). Each primer pair was used to amplify DNA from individual RILs from the sorghum mapping population, using the reaction mixes and cycling parameters described above.

Isolation of a genomic sorghum *Rp1-D* homologue

A sorghum cosmid library was constructed from a rust-resistant line, QL41, using the EpiFOS Fosmid Library production kit (Epicentre). This line was one of the parents of the sorghum mapping population used to map the rust resistance QTLs (Tao et al. 1998). The library was screened for maize *Rp1-D* homologues, using the sugarcane *Rp1-D* homologue as a probe. Positive cosmid clones were restriction digested with *Dra*I, Southern blotted onto a nylon membrane and re-hybridised with the probe. Hybridising cosmid clone fragments were subsequently sub-cloned into a plasmid vector pSMART (Lucigen) and sequenced using standard techniques (Sambrook et al. 1989). Sequence analysis and alignment of all putative *Rp1-D* homologues was undertaken using the GAP sequence alignment program (Genetic Computer Group, University of Wisconsin). Cluster analysis was undertaken after estimating the rates of nucleotide substitution, using the distance measures of Nei and Gojobori (1986) and the Jukes–Cantor correction as implemented in the MEGA2 (molecular evolutionary genetic analysis) package.

Results and discussion

A major QTL for rust resistance in sorghum co-locates with homologues of *Rp1-D*, a maize rust R gene

In an independent study undertaking comparative mapping of sugarcane and sorghum, a sugarcane cDNA clone with homology to the maize *Rp1-D* gene was identified in a sugarcane EST collection (Casu et al. 2001). This cDNA clone was approximately 1.6 kb in length, and translation of its sequence demonstrated that it shared an overall 83.6% amino acid identity with the maize *Rp1-D* protein, with 87.1% amino acid identity in the NBS region (GenBank accession no. AAD47197; Fig. 1). As sugarcane and sorghum genes are usually highly homologous (R.E. Casu, unpublished data), the cDNA homologue to *Rp1-D* was used to analyse loci with potential R gene functions in sorghum. The sugarcane *Rp1-D* homologue (*rp1-su*) was hybridised to genomic digests of four different sorghum inbred lines (Fig. 2), which are parents of two sorghum mapping populations (Tao et al. 2000, 2003). The hybridising restriction fragments suggested a small multigene family (Fig. 2), as estimated by the number and intensity of bands. To genetically map polymorphic fragments hybridising to the *Rp1-D* homologue probe, the sugarcane *Rp1-D* homologue was used to screen 120 RILs of the QL39 × QL41 RIL population that

Table 2 Primer combinations and annealing temperatures for amplification of each major *Rp1-D* homology sequence class

Sequence class	Forward primer	Reverse primer	Optimum annealing temperature (°C)
RC1	Rp1D-1	Rp1D-2	71.5
RC2	Rp1D-8	Rp1D-9	69.5
RC3	Rp1D-10	Rp1D-9	70
SC1	Rp1D-4	Rp1D-5	69
SC2	Rp1D-7	Rp1D-6	68

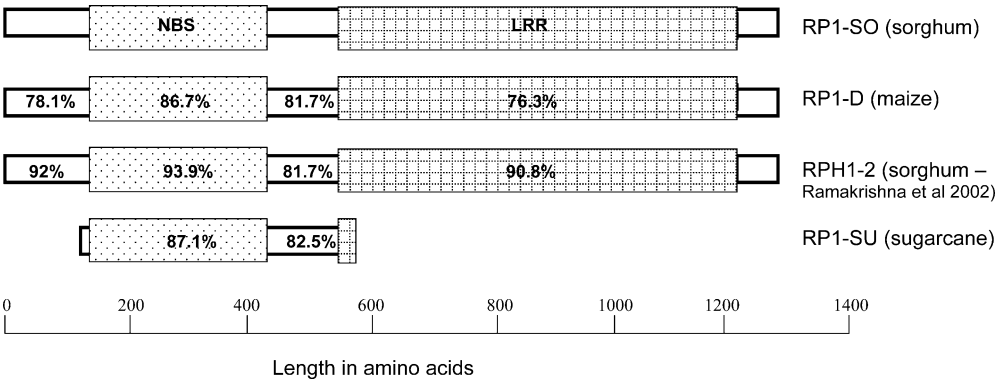

Fig. 1 Comparison of the predicted amino acid sequence of the sorghum *Rp1-D* homologue (*RP1-SO*) with the sugarcane (*RP1-SU*) and sorghum *Rp1-D* homologues (*RPH1-2*, Ramakrishna et al. 2002) and the maize *Rp1-D* gene (*RP1-D*). Each protein has been drawn to scale, and the *bar at the bottom of the figure* indicates length in amino acids. Identified protein domains and motifs are shown within boxes. The percentage values between the proteins indicate the amino acid sequence identity between sugarcane and maize and between sorghum and maize, as determined by the GAP sequence alignment program (Genetic Computer Group, University of Wisconsin). *LRR* Leucine-rich repeat domain, *NBS* nucleotide-binding site

had been digested with *Dra*I. Of the approximately four to six hybridising fragments in each parent, only one of the *Dra*I fragments was found to segregate in the RILs (a 3-kb fragment from QL41 and a 4-kb fragment from QL39). Linkage analysis was undertaken using the MapQTL software to incorporate the hybridisation information into the existing map and QTL datasets for this population (Tao et al. 1998, 2000). The sugarcane *Rp1-D* probe was found to map to sorghum LG E and to the centre of a major QTL for rust resistance (Fig. 3) previously identified by Tao et al. (1998). This QTL had been shown to explain between 32% and 43% of the phenotypic variation in rust resistance in this RIL population (Tao et al. 1998). The 3-kb fragment from QL41 was associated with increased rust resistance, and the 4-kb fragment was associated with increased rust susceptibility.

This result is consistent with the synteny between maize chromosome 10, the location of the *rp1* locus, and sorghum LG H (as defined by Peng et al. 1999) and LG E (as defined by Tao et al. 2000), previously suggested by Gale and Devos (1998). It also provides evidence of the co-location of putative rust R genes and a major QTL for rust resistance in sorghum and suggests a possible conservation of gene function at this locus for rust resistance between maize and sorghum.

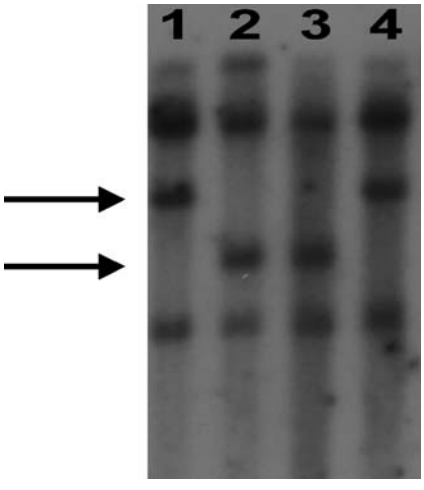

Fig. 2 Sorghum genotypes digested with different restriction enzymes and probed with the sugarcane homologue of *Rp1-D* reveal a small multigene family. *Lanes 1–4* Sorghum inbred lines QL39, QL41, 90562 and ICSV 745 digested with *Dra*I. *Arrows* illustrate the 4-kb (*top arrow*) and 3-kb (*bottom arrow*) polymorphism mapped and incorporated into the sorghum maps of Tao et al. (1998, 2000)

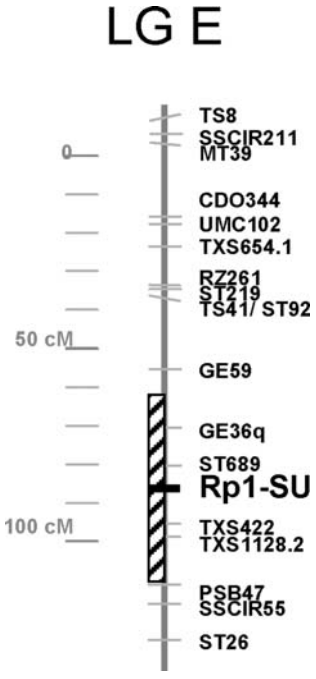

Fig. 3 Sorghum LG E (Tao et al. 1998, 2000; the same as LG H as defined by Peng et al. 1999) showing the map position of the sugarcane homologue of *Rp1-D* (*Rp1-SU*)

Distinct *Rp1-D* homologues co-segregate with resistance and susceptibility at the LG E rust resistance locus

Rp1 homologues have been shown to cluster in several species, including maize (Ayliffe et al. 2000), barley (Rostoks et al. 2002), rice (S. Hermann, unpublished data) and sorghum (Ramakrishna et al. 2002; this paper). However, not all homologues are functional R genes. In maize, the *Rp1-D* haplotype contains nine homologous genes, including *Rp1-D* (Collins et al. 1999), while in sorghum, only one of the five homologues at this syntenous region appears to encode a full-length product (Ramakrishna et al. 2002). To identify potential R genes within the cluster on sorghum LG E, primers were designed to regions conserved between the maize and sugarcane *Rp1-D* homologues and used to amplify an approximately 400-bp fragment from rust-resistant sorghum progeny and rust-susceptible sorghum progeny DNA bulks. The resistant progeny bulk contained six lines with a rust rating of between 1.9 and 2.9, each with the QL41-derived resistance QTL allele on LG E. Conversely, the susceptible progeny bulk contained six lines with a rust rating of between 6.9 and 7.9, each with the QL39-derived susceptible QTL allele on LG E. The amplification products from the two bulks were cloned, and approximately 60 clones from each bulk sequenced by a single sequencing pass. With a stringency of 99%, alignment of the approximately 120 sequences revealed eight different sequence classes, five major sequence classes with multiple representations and three singleton classes (Fig. 4). The presence of five major sequence classes within the 200 sequences suggests five distinct *Rp1-D* gene variants. Three of the sequence classes and one singleton class originated from the resistant progeny DNA bulk (RC1, RC2, RC3, RS1), while the remaining two sequence classes and two singleton classes originated from the susceptible progeny DNA bulk (SC1, SC2, SS1, SS2). None of the sequences derived from the resistant progeny DNA bulk grouped with sequences derived from the susceptible progeny DNA bulk and vice versa. The three resistance sequence classes contained 27, 15 and eight sequences, respectively, while the two susceptible sequence classes contained 42 and nine sequences each.

The sequences and sequence classes discussed above were identified from sorghum progeny assigned to bulks solely on the basis of the presence of the QL39 (more susceptible) or QL41 (more resistant) version of the LG E

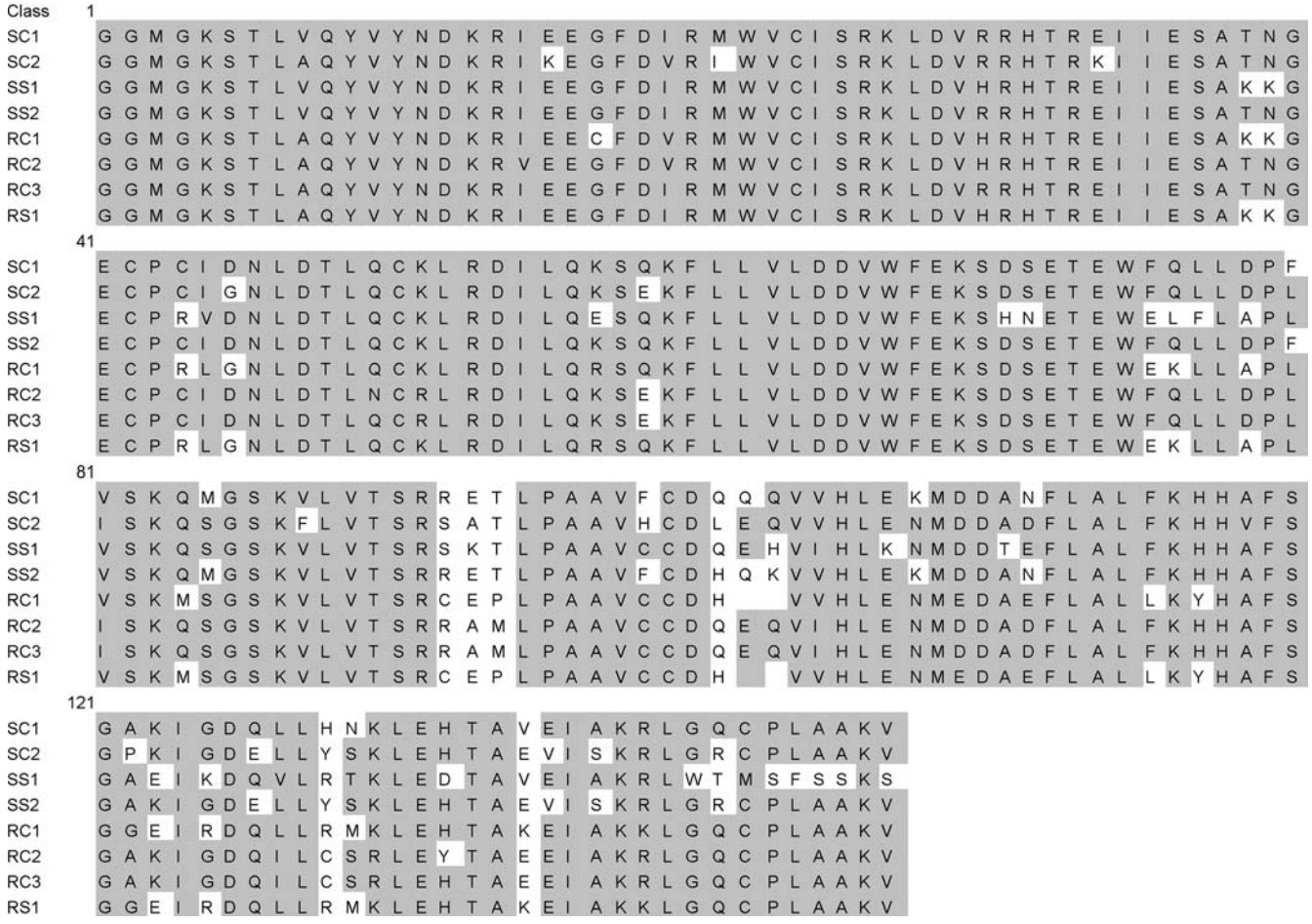

Fig. 4 Amino acid consensus sequence for each sequence class. *SC1* Susceptible sequence class 1, *SC2* susceptible sequence class 2, *SS1* susceptible singleton sequence 1, *SS2* susceptible singleton sequence 2, *RC1* resistance sequence class 1, *RC2* resistance sequence class 2, *RC3* resistance sequence class 3, *RS1* resistance singleton sequence 1

rust resistance QTL. To test further whether all five *Rp1-D* gene variants mapped to this locus and to identify PCR-based markers for the QL41-derived resistance QTL, primers were designed to regions of polymorphism for the five major sequence classes (Table 1) and used to screen the 120 sorghum RILs (Fig. 5). For all 120 RILs, the three primer pairs designed to the resistance sequence classes always amplified together (data not shown), suggesting that these three sequence classes represent genes located very near each other that are segregating as a single unit in this small population. They co-segregated perfectly with the presence of the 3-kb *Dra*I RFLP from QL41 in Fig. 2 that mapped to the region which had previously been shown to be associated with rust resistance (Tao et al. 1998) and thus can be used as markers for this QTL. These three sequence classes thus appear to be derived from QL41. The two primer pairs designed to the susceptible sequence classes also co-segregated with each other and with the 4-kb *Dra*I RFLP from QL39 (Fig. 2) that mapped to the region which had previously been shown to be associated with rust resistance (Tao et al. 1998), suggesting that these two *Rp1-D* gene variants are located very near each other and are therefore segregating as a single unit. These two sequence classes appear to be derived from QL39.

These results suggest that the sorghum locus is as complex as it is in maize, with five sorghum *Rp1-D* gene variants identified in this sorghum population—three from the resistant progeny bulk and two from the susceptible progeny bulk—that are potentially rust resistance alleles.

Ramakrishna et al. (2002) identified five *Rp1* homologues from sorghum clustering in the *Rp1*-orthologous region. Of the five homologues, however, only three are apparently full-length, while the other two homologues are truncated and, of the three apparently full-length homologues, only one appears to encode a predicted full-length protein like that encoded by the maize *Rp1*-D gene. A second homologue contains a stop codon, while the third homologue contains a retrotransposon insertion, both of which should result in a truncated product. The observed truncation of two of the five *Rp1* homologues may explain why only three sequence classes were isolated from the rust-resistant progeny in this study, as the truncation would cover the region amplified. The three *Rp1-D* sequence variants that were derived from the resistant progeny bulk appear to be segregating as a single unit and are potential candidates for rust resistance alleles. It is possible that these three variants may be required to provide the resistance affected by the QTL or, alternatively, a sub-set may be sufficient. Only two sequence classes were identified from the rust-susceptible progeny bulk. If the two susceptible sequence classes are allelic to the resistance sequence classes, it may be that the third susceptible sequence class was not isolated due to the small number of clones sequenced from each progeny bulk. Sequencing of the bulk was not exhaustive, as evidenced by the varying numbers of members in each of the five sequence classes and the presence of singletons. Alternatively, it could also be due to variation in the number of *Rp1* genes per genotype at this locus, as observed in maize (Collins et al. 1999; Sun et al. 2001; Ramakrishna et al. 2002; Webb et al. 2002). Another reason for the difference in number of sequence classes could be that the resistant and susceptible sequence classes represent distinct loci at this region of LG E and could not be distinguished in our small mapping population.

Isolation of a full-length sorghum *Rp1-D* homologue co-segregating with resistance at the LG E rust resistance locus

The three *Rp1-D* gene variants identified from the resistance progeny bulks above were partial sequences only. As truncated *Rp1-D* homologues have been reported in several species, including sorghum (Ramakrishna et al.

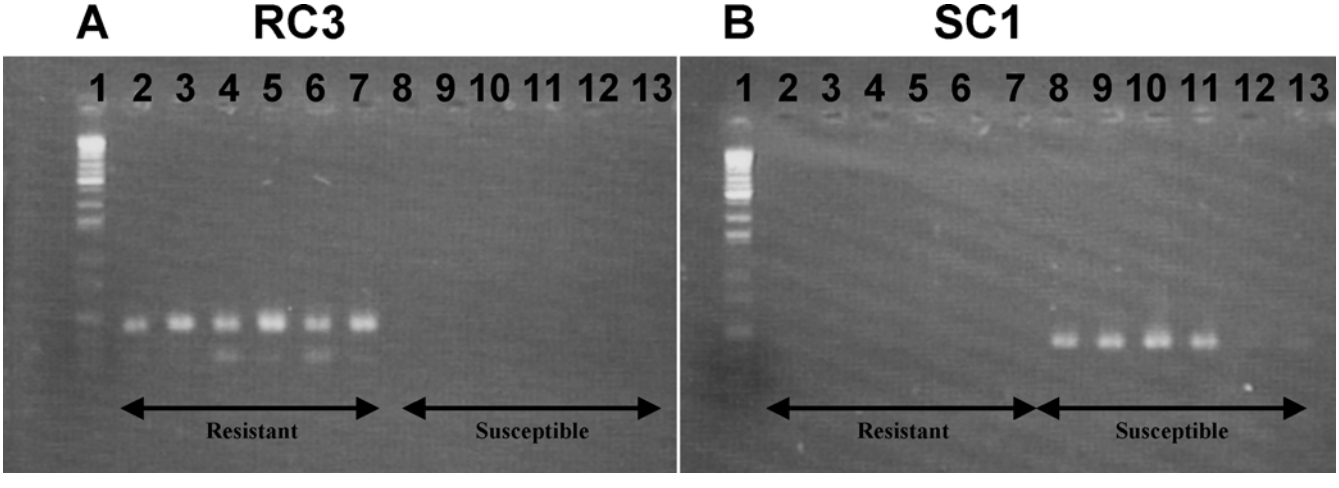

Fig. 5a, b Amplification using sequence class-specific primers. **a** Primers designed to resistance sequence class 3 (*RC3*). **b** Primers designed to susceptible sequence class 1 (*SC1*). *Lane 1* Size marker, *lanes 2–7* six rust-resistant sorghum recombinant inbred line (RIL) progeny from the QL39 × QL41 population and used in the resistance bulk, *lanes 8–13* six rust-susceptible sorghum RIL progeny from the QL39 × QL41 population and used in the susceptible bulk

2002), it was therefore important to ascertain whether a full-length *Rp1-D* homologue was located at this locus on LG E in this population. The sequence of this full-length clone could then be compared with the full-length sequence obtained by Ramakrishna et al. (2002).

A cosmid library of approximately 35,000 clones was generated from QL41, the source of the 'resistant' QTL allele on LG E, and screened with the sugarcane *Rp1-D* homologue. A Southern blot was performed on a *Dra*I digestion of the cosmids, using the sugarcane *Rp1-D* homologue as a probe (data not shown) to identify cosmids that contained a 3-kb *Dra*I fragment, the same size as the *Dra*I RFLP fragment from the rust-resistant parent QL41 that was originally mapped to the major rust resistance QTL on LG E in sorghum (Tao et al. 1998). One cosmid containing a 40-kb insert was identified as containing the correctly sized *Dra*I RFLP fragment. Following digestion with several different restriction enzymes and Southern hybridisation, using the sugarcane *Rp1-D* homologue as a probe, it was concluded that the cosmid contained a single *Rp1* -D homologue (data not shown). The cosmid was digested with *Dra*I, sub-cloned and a colony blot performed using the maize *Rp1-D* gene as a probe. Hybridising sub-clones were identified and sequenced.

The sorghum *Rp1-D* homologue (*Rp1-SO*) contained a large open reading frame of 3,871 bp potentially encoding a protein of 1,296 amino acids (accession no. AY369028). Comparison of the entire putative amino acid sequence with the maize RP1-D protein and a previously isolated sorghum full-length *Rp1* homologue (*Rph1-2*, Ramakrishna et al. 2002) showed a higher level of amino acid identity between the two sorghum clones (Fig. 1). Further investigation of the amino acid identity of the NBS and LRR domains indicated that the NBS region shared a higher identity than other regions (Fig. 1). The LRR domain could be arranged to resemble the consensus proposed by Jones and Jones (1997, data not shown) and was similar to that reported for the maize RP1-D protein (Collins et al. 1999).

Further comparison between the two sorghum *Rp1* homologues (*Rp1-SO* and *Rph1-2*) revealed scattered differences and two small regions of amino acid variation in the region between the NBS and LRR. *Rp1-SO* has the amino acids ISGRR between positions 483 and 487 and QRYFVP between positions 511 and 516, whereas *Rph1-2* (and *Rph1 4*) have NLSSM and ETEYYS at these respective positions. The maize *Rp1-D* gene has NLSRR and QMYCDS in these regions. The sequence of *Rp1-SO* suggested that this gene was a member of R gene class 3

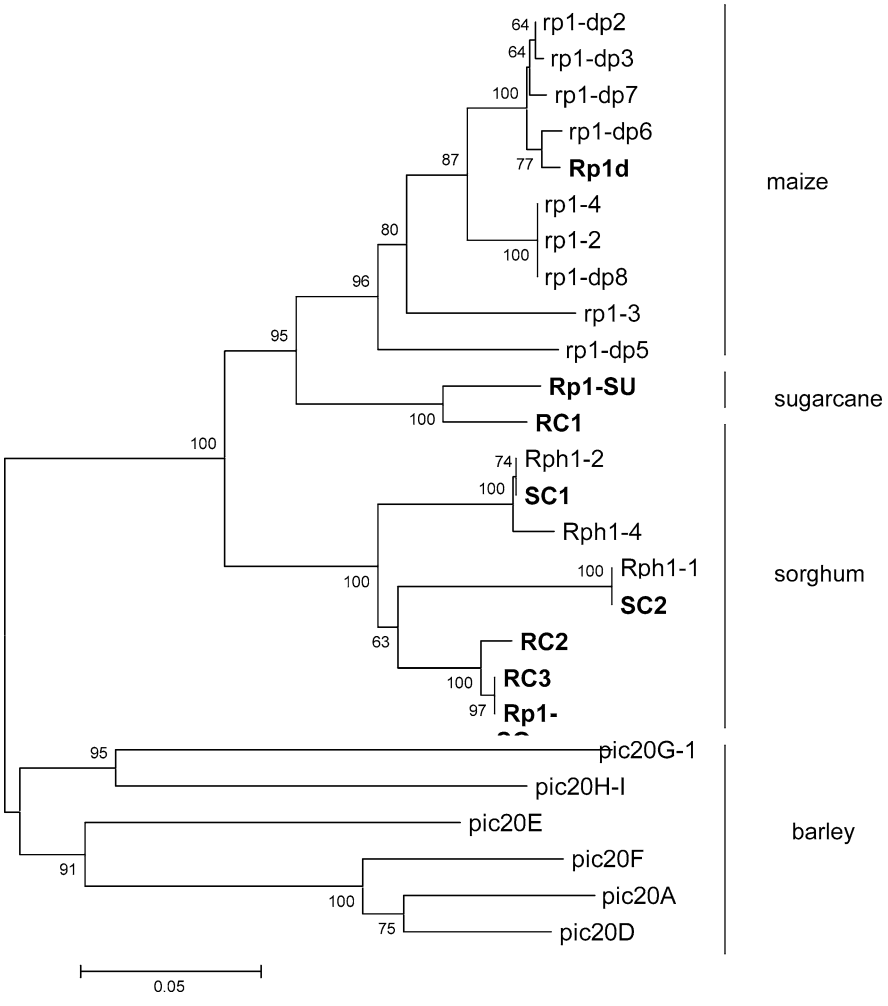

Fig. 6 A neighbour-joining tree of sorghum, sugarcane, maize and barley *Rp1* homologues. *Rp1-SU* sugarcane *Rp1-D* homologue, *RC1* sorghum resistance progeny class 1, *RC2* sorghum resistance progeny class 2, *RC3* sorghum resistance progeny class 3, *SC1* sorghum susceptible progeny class 1, *SC2* sorghum susceptible progeny class 2, *Rp1-D* maize *Rp1-D* gene, *Rph1-1*, *Rph1-2* and *Rph1-4* sorghum *Rp1-D* homologues (Ramakrishna et al. 2002). Other maize and barley sequences were obtained from the GenBank database. Bootstrap values based on 1,000 replicates are indicated at the nodes

(RC3). It is not known whether full-length members of R gene classes 1 and 2 also exist in QL41 and whether they encode apparently full-length proteins or, as reported by Ramakrishna et al. (2002), the other two homologues contain stop codons or insertions/deletions that result in truncated products.

Relationship amongst sorghum *Rp1* homologues

To investigate the relationship amongst the partial sequences of the five *Rp1-D* homologues identified in this study, the three full-length *Rp1-D* homologues identified by Ramakrishna et al. (2002) (*Rph1-1*, *Rph1-2* and *Rph1-4*), the partial *Rp1* homologue identified from sugarcane and the maize *Rp1-D* gene, a neighbour-joining tree was constructed based on synonymous substitutions in the N-terminal region (Fig. 6). Of the five *Rp1-D* homologues identified in this study, RC2 and RC3 are very similar, while RC1 is quite distinct. SC1 and SC2 are also very similar to each other. The three *Rp1-D* homologues isolated by Ramakrishna et al. (2002) clustered with SC1 and SC2. Overall, the tree suggests that there is as much diversity within the sorghum locus as there is between maize, sugarcane and sorghum at this locus, as firstly, the sorghum RC1 *Rp1-D* homologue clusters with the sugarcane *Rp1-D* homologue (*rp1-su*) and not with the other sorghum *Rp1-D* homologues, and secondly, this sorghum-sugarcane cluster groups with the maize *Rp1* homologues. This suggests that following the original gene divergence and speciation, there has been ongoing diversification at this locus within sorghum. The result differs a little from that reported by Ramakrishna et al. (2002), where the barley, sorghum and maize *Rp1* homologues clustered in separate branches. Their result suggested that either multiple gene duplication events of a single *Rp1* gene resulted in the multiple *Rp1* homologues within each species after the initial species divergence, or alternatively, conversion processes have resulted in a homogenisation of the *Rp1* gene family within each species (Ramakrishna et al. 2002). However, in the present study, a comparison has been made between sorghum *Rp1-D* homologues isolated from three sorghum lines, BTx623 (Ramakrishna et al. 2002), QL39 and QL41 (this study), and one sugarcane line (Q117), whereas Ramakrishna et al. (2002) analysed several *Rp1* homologues from single sorghum (BTx623), maize and barley lines.

There are many studies currently underway to isolate and characterise RGAs in crop species. In these studies, the information is being used to provide molecular markers for marker-assisted selection, as a pool of potential R genes and to improve our understanding of the mechanisms of disease resistance. In our study, we have mapped a sugarcane homologue of the maize rust R gene *Rp1-D* to a major rust resistance QTL in sorghum. We have then used primers to amplify sorghum homologues of the maize *Rp1-D* gene and identified five gene variants, three of which were only found in rust-resistant sorghum progeny bulks and two of which were only found in rust-susceptible sorghum progeny bulks. We have cloned and sequenced one of the R genes and shown it to be highly homologous to the maize *Rp1-D* gene. Primers specific to the five genes have been designed and are available for marker-assisted selection for this rust resistance QTL in sorghum.

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