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Identification and validation of molecular markers associated with Pachymetra root rot and brown rust resistance in sugarcane using map- and association-based approaches

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Abstract

Marker-assisted selection for traits that are difficult to screen for, such as resistance to many sugarcane diseases, has the potential to facilitate the development of improved cultivars in sugarcane. Pachymetra root rot (PRR) and brown rust resistance ratings were obtained over two years for 192 I1 progeny (progeny produced by two heterozygous, non-inbred parental lines) of a sugarcane (*Saccharum* spp. hybrid) cross between two elite sugarcane clones, Q117 and 74C42. Approximately 1000 single-dose markers, including microsatellite (SSR), amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers, were scored across the population and maps containing approximately 400 markers were constructed for each parent. At $p \leq 0.01$, two genomic regions, one from the female Q117 map and a different region from the 74C42 male map, plus an unlinked bi-parental simplex marker (single-dose marker present in both parents) were identified as associated with PRR over both years of data collection. These regions explained between 6 and 16% of the phenotypic variation. An additional region was identified in the female map as associated with PRR at $p \leq 0.01$ in one year and $p \leq 0.05$ in the second year. This region explained between 4 and 8% of the phenotypic variation. For brown rust, two genomic regions, one from the female map and one from the male map, plus an unlinked marker from both maps, were identified as associated with brown rust resistance at $p \leq 0.01$ over two years of phenotypic data. Each region explained between 7 and 18% of the phenotypic variation. Several additional regions were identified in both maps as associated with brown rust at $p \leq 0.01$ in one year and $p \leq 0.05$ in the second year. These regions also explained between 5 and 11% of the phenotypic variation. To validate these markers and determine whether they would be useful in alternative germplasm, markers from each genomic region associated with PRR or brown rust were screened across a set of 154 elite sugarcane clones; PRR and brown rust ratings were available for 131 and 72 of the clones, respectively. For PRR, three of the 6 markers tested remained significantly associated ($p \leq 0.01$) with resistance ratings in the elite clone set. For brown rust, only one of the seven markers tested remained significantly associated ($p \leq 0.01$) with resistance in the elite clone set, with one other marker associated at $p \leq 0.05$. These results suggest that

these markers could be broadly effective in selecting for PRR and/or brown rust resistance in sugarcane breeding programs.

Introduction

Resistance to pests and diseases is a major objective of all crop breeding programs, including sugarcane. In the Australian sugarcane industry, the cost of control and loss of production due to major sugarcane pests and diseases was estimated to be 10% of the total value of the sugarcane crop in 1996 (McLeod et al. 1999). Of the major pathogens considered, soil-borne pathogens were the most serious, causing an estimated 75% of the losses, followed by canegrubs (caused by larvae of species of Scarabaeidae) (10%), ratoon stunting disease (caused by *Clavibacter xyli* subsp. *xyli*) (6%) and brown rust (caused by *Puccinia melanocephala* H and P Sydow) (3%).

PRR, caused by *Pachymetra chaunorhiza* Croft and Dick (Dick et al. 1989), is one of the major soil-borne diseases affecting sugarcane in Australia. It is estimated that industry losses to PRR alone are commonly between 15–35%, depending on the level of the disease and resistance of the commercial cultivars grown (Magarey et al. 2002), with reports of losses up to 40% in individual cultivars (Magarey 1994). In northern Queensland, surveys have suggested that almost every field is infected with the disease (Magarey et al. 1987; Magarey 1996). PRR is a disease unique to Queensland sugarcane fields and has never been recorded outside Queensland or outside a commercial sugarcane field (Magarey 1996). Little is known about the genetic basis of resistance to PRR but estimates of heritability range from 0.57–0.64 (B.J. Croft and N. Berding unpublished). However, because of the importance of this disease, all elite material being considered for commercial release in northern Queensland, as well as selected entries from other regions, are screened for resistance to PRR (Magarey and Bull 2003).

By contrast, brown rust, caused by *Puccinia melanocephala* H and P Sydow, occurs worldwide and tends to only influence yield in very susceptible cultivars. Rust resistance is generally considered to be a quantitatively inherited trait with high heritability (Tai et al. 1981; Hogarth et al. 1983, 1993). However, a major rust resistance gene has been identified in a French cultivar, R570 (Daugrois et al. 1996); this is the only confirmed report of a monogenic trait in sugarcane. As rust resistance in Australia appears to be quantitatively inherited, and as highly susceptible clones are eliminated by selection in the breeding program, there are no specific trials to rate clones for resistance to brown rust.

Modern sugarcane cultivars are derived from a limited number of inter-specific hybridisation events between *Saccharum officinarum* ($2n = 80$) and *S. spontaneum* ($2n = 48$–124). Chromosome numbers in sugarcane cultivars typically range from 100–120 with approximately 80% of the genome contributed by *S. officinarum*, 10–15% by *S. spontaneum* and 5–10% from recombination between the two species (D'Hont et al. 1996; Piperidis and D'Hont 2001). *S. officinarum* and *S. spontaneum* have also been shown to have different basic chromosome numbers of $x = 10$ and $x = 8$, respectively (D'Hont et al. 1995). Despite this structural complexity, genetic maps have been constructed in sugarcane cultivars (Hoarau et al. 2001; Rossi et al. 2003; Aitken et al. 2005) as well as in ancestral *Saccharum* species (Al-Janabi et al. 1993; Da Silva et al. 1995; Mudge et al. 1996; Ming et al. 2002). As sugarcane is highly heterozygous, these maps have been constructed in either populations consisting of selfed progeny, or the I1 progeny (progeny from two highly heterozygous parents) from a biparental cross; in the latter case, maps are constructed for both parents (Grattapaglia and Sederoff 1994).

The largest maps in sugarcane have been constructed in two sugarcane cultivars, R570 from Reunion Island and Q165 from Australia. Each map contains more than 1000 markers, mainly AFLPs and SSRs, distributed on approximately 100 linkage groups (LGs) (Rossi et al. 2003; Aitken et al. 2005). Using SSRs, these LGs have been grouped into homology groups (HGs), each containing multiple homologous/homoeologous LGs. In R570, 66 of the 128 LGs could be grouped into 7 HGs on the basis of 2 common SSRs (Rossi et al. 2003). In Q165, 126 of the 134 LGs could be assigned to 8 HGs (Aitken et al. 2005), the expected number based on the basic chromosome number of *S. spontaneum* (D'Hont et al. 1995).

The varietal and ancestral species maps, have been used for QTL analyses. Most of these analyses have involved sugar-related traits and each QTL typically accounts for less than 15% of the variation (e.g. Hoarau et al. 2002; Ming et al. 2002; Reffay et al. 2005). Rust is the only disease trait and only single gene trait mapped in sugarcane to date (Daugrois et al. 1996) although there has been a report of a single gene for eyespot resistance (caused by *Helminthosporium sacchari* Van Breda de Haan) mapped in *S. officinarum* (Mudge et al. 1996).

This study was undertaken to identify molecular markers associated with two traits in sugarcane, PRR and brown rust resistance, for application in marker-assisted selection. Additional objectives of the study were to investigate the genetic basis of PRR resistance in Australian sugarcane and to compare the genetic complexity of brown rust resistance in Australian sugarcane with that published for a French sugarcane cultivar.

Materials and methods

Plant material

A biparental cross between the cultivar Q117 and a high-performing elite clone 74C42 was made by Dr. Nils Berding, BSES Ltd, Meringa, Queensland, Australia and generated 192 I1 progeny. The population was designated the Q1 population.

One hundred and fifty-four diverse cultivars and elites sugarcane lines were selected from within Australian sugarcane germplasm and termed the Elite Clone Set (ECS). Based on pedigree information, the lines were selected as representative of the diversity within the Australian sugarcane breeding program (PA Jackson, pers. comm.) and are listed in Supplementary Data Table 1.

PRR and brown rust resistance ratings in the Q1 population and elite clone set (ECS)

Due to space limitations and to enable accurate phenotypic measurement of the Q1 progeny, the 192 Q1 progeny were divided into two sets of approximately equal numbers. PRR ratings were obtained with the method used to evaluate all near release commercial sugarcane varieties (Croft 1989). The first set of Q1 progeny was screened for PRR resistance in a glasshouse at the BSES Tully Sugar Experiment Station, Queensland, Australia, in 2002 and the second set of progeny in 2003. Briefly, oospores of *P. chaunorhiza* were prepared from a mix of isolates grown in pure culture. The clones were planted into potting mix infested with 20 oospores/g of *P. chaunorhiza*, grown for 12 weeks, and the percentage of rotted roots (from the number of rotted roots and the total number of roots) calculated. A set of standard cultivars of known PRR resistance were included in each trial. The % rotted roots of the test clones was regressed against the long-term standard ratings and the test clone ratings converted to PRR ratings on a 1–9 scale where 1 is no disease and 9 is severe root rot. Each trial had five replicates.

For brown rust ratings, the first set of progeny was planted in the field at the BSES Woodford Sugar Experiment Station, Queensland, Australia, in 1999 and the second set in 2001. Brown rust ratings were obtained for both progeny sets as described in McIntyre et al. (2004). The percent leaf area affected by brown rust was estimated visually on the fifth fully expanded leaf and the ratings were assigned on a 1–9 scale relative to a set of eight standard cultivars of known reaction.

PRR and brown rust ratings for the ECS were obtained from the BSES database and are listed in Supplementary Data Table 1. The PRR and brown rust reactions of the ECS were obtained from past resistance trials conducted by BSES using methods similar to those described above.

Map construction and QTL analysis in the Q1 population

Leaf material from the 192 I1 progeny was obtained from field-grown plants, freeze-dried and stored at $-20\,^{\circ}C$. DNA was isolated using the method of Hoisington (1992) and diluted appropriately for RFLP, SSR and AFLP analysis. RFLP, SSR and AFLP markers were scored as described in McIntyre et al. (2004). Briefly, seven RFLP and 31 sugarcane Resistance Gene Analogue (RGA) clones used as RFLP probes, were screened over the Q1 population using techniques described in Hoisington (1992). Thirty polymorphic SSR primers were obtained from the Sugarcane Microsatellite Consortium collection (Cordeiro et al. 2000) and amplified in the Q1 progeny. The Q1 progeny were also screened with

approximately 30 AFLP primer pairs. All markers were scored as dominant markers as the alternative allele for each marker was unknown. Consequently, maps were produced for each parent. All segregating bands that were clear and unambiguous were scored as 1 for present and 0 for absent for all 3 marker methods. Each marker was tested against the expected segregation ratios using a χ^2 test for single-dose markers (SD – markers present only once in the genome) (1:1, markers present once in one parental genome or 3:1, marker present in both parents segregating as single-dose markers). The parental origin of each marker was noted. All progeny were checked to see that they contained markers from the male parent, 74C42, to confirm that they were hybrids and not selfed progeny of the female parent, Q117. AFLP markers were labelled using the 3 selective nucleotides in the *Eco*RI primer followed by the 3 selective nucleotides in the *Mse*I primer followed by numbers in descending molecular-weight order. SSR markers were labeled M then the identity number from the Sugarcane Microsatellite Consortium collection (Cordeiro et al. 2000) and a letter denoting the allele by descending molecular weight. RGA markers were labeled RGA then a number specific to that RGA (McIntyre et al. 2004), followed by a letter denoting the allele generated by the probe in descending molecular weight.

Linkage analysis was undertaken for each parental map as described in McIntyre et al. (2004). Linkage groups (LGs) were assembled into homology groups (HGs) on the basis of common RFLPs and SSRs, using the maps of R570 (Rossi et al. 2003) and Q165 (Aitken et al. 2005) as references. Common RFLP and SSR markers also enabled partial alignment of the male and female LGs and HGs. QTL analysis for both traits was carried out with single-dose (SD) markers (markers from one parent that segregate 1:1 in the progeny), or bi-parental simplex markers (single-dose markers present in both parents that segregate 3:1 in the progeny, as described by da Silva et al. 1995). A single factor analysis was conducted using MapManager QTxB v.17 (Meer et al. 2002) to determine associations at $p \leq 0.01$ and $p \leq 0.05$.

Validation of markers in the Elite Clone Set (ECS)

Leaf material from the ECS was also obtained from field-grown plants, stored and DNA extracted as described above. SSR and AFLP primer combinations that identified potential markers in the QTL analyses were run across the 154 samples of the ECS. Markers of interest from the Q1 population QTL analysis were scored as present or absent for each of the 154 samples in the ECS. For each marker, ECS clones were grouped into two classes: those with the marker and those without. The phenotypic mean of the two classes was then statistically compared.

Results

PRR and brown rust resistance ratings in the Q1 population and ECS

Q117, the female parent of the Q1 population, is rated 5 for PRR and 5.5 for brown rust while 74C42, the male parent, is rated 5.5 and 2.5 for PRR and brown rust, respectively. The resistance rating distribution of both progeny sets (Figure 1) suggests that PRR resistance is a multigene trait; this suggestion is further supported by the observed transgressive segregation in the Q1 progeny. The PRR resistance ratings in the progeny ranged from 1–9 and averaged 4.8, with an average of 4.2 and 5.8 for the two progeny sets. The correlation between the PRR resistance ratings for the standards in the two trials, including the two parents, was $r^2 = 0.748$ which is highly significant and suggests that the test clone results are comparable between the two trials.

For brown rust, the distribution of resistance ratings for both sets of progeny are illustrated in Figure 2; it would appear that the results from Trial 2 are distributed more towards resistance. The brown rust ratings of the progeny also ranged from 1–9 with a mean rating of 3.5, and mean rating of 4.1 and 2.9 in the two trials. The correlation between the brown rust resistance ratings for the standards in the two trials, including the two parents, was $r^2 = 0.916$, which is very high.

PRR and brown rust ratings for the ECS are illustrated in Figure 3 and also range from 1–9. The PRR ratings were distributed evenly around the rating of 5, whereas the brown rust ratings were skewed towards resistance.

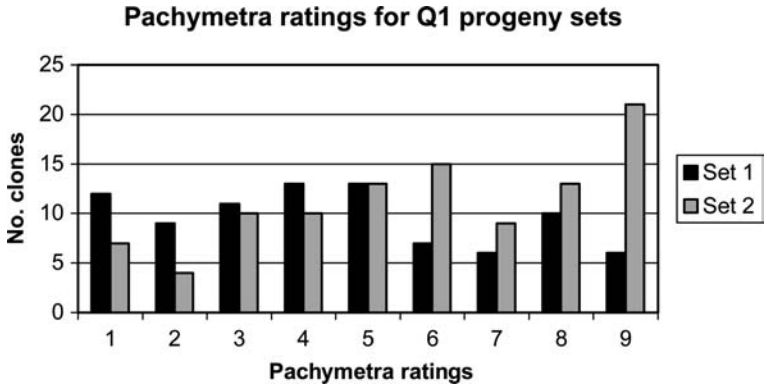

Figure 1. Frequency distribution illustrating the PRR ratings for the two sets of Q1 progeny. Ratings are on a 1–9 scale where 1 is no disease and 9 is severe root rot.

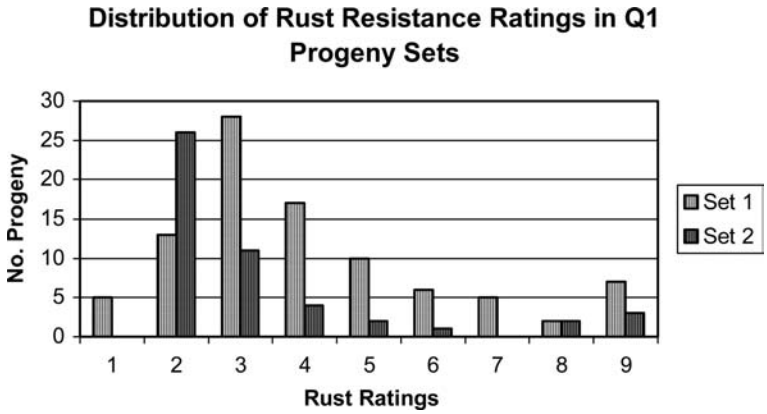

Figure 2. Frequency distribution illustrating the brown rust ratings for the two sets of Q1 progeny. Ratings are on a 1–9 scale where 1 is no disease and 9 is severe rust.

The Q117 and 74C42 maps

More than 1000 RFLP, SSR and AFLP markers were scored in the Q1 population. A total of 405 markers were incorporated into the female Q117 map, comprising 22 RGA (14 different RGAs), 66 SSR (35 primer pairs), 313 AFLP and 4 other RFLP (3 RFLP probes) markers. The map contains 75 LGs (Supplementary Figure 1), of which 53 contain more than 2 markers; there are also 46 unlinked markers. Using the RFLP, RGA and SSR markers, 42 of the 75 LGs could be assigned to 6 of the 8 HGs (as defined by Aitken et al. 2005). The number of LGs per HG varied from 2 in HG V to 11 in HG II (Supplementary Figure 1).

The male map (74C42) contains 445 single-dose markers comprising 67 SSR (36 SSR primers), 350 AFLP, 21 RGA (14 RGA probes) and 7 RFLP (4 RFLP probes) markers. The 74C42 map has 85 linkage groups (Supplementary Figure 2), with 55 containing more than 2 markers and there are 58 unlinked markers. Of the 85 LGs, 48 could be incorporated into 6 of the 8 HGs. The number of LGs per HG varied from 3 in HG VI to 16 in HG III.

Identification of markers associated with PRR and brown rust resistance

For both PRR and brown rust, approximately 30 markers were identified as associated with each trait at $p \leq 0.01$ in one or both year's data. Of these 30 markers, approximately one-half were associated at $p \leq 0.01$ in both years or $p \leq 0.01$ in one year and $p \leq 0.05$ in the other year.

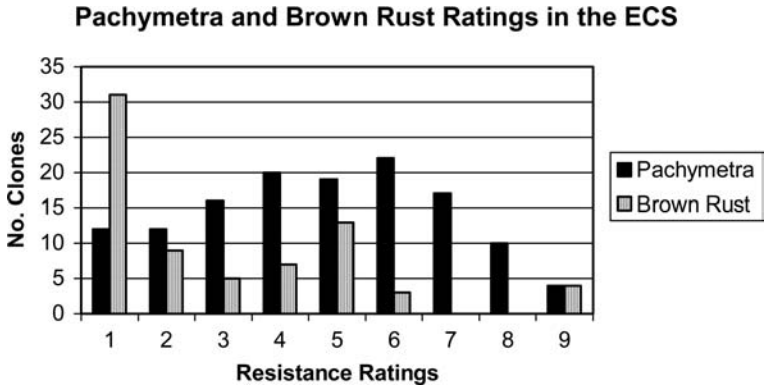

Figure 3. Frequency distribution illustrating the range of PRR and brown rust ratings for clones within the ECS. Ratings are on a 1–9 scale where 1 is no disease and 9 is severe disease.

Seven markers (1 SSR marker and 6 AFLP markers) were identified as associated with PRR at $p \leq 0.01$ over both years of testing (Table 1). These seven markers were distributed onto 1 LG in the female Q117 map (LG 22F in HG II) and one LG in the male 74C42 map (LG 5M in HG III) (Supplementary Figures 1 and 2) with one marker, a bi-parental simplex marker, remaining unlinked. A further six AFLP markers were identified as associated at $p \leq 0.01$ in one year and $p \leq 0.05$ in the second year (Table 1). Four of the six additional markers were linked on LG 2F (HG IV) in the female map, while the other two AFLP markers were on LG 5M (HG III) of the male map. Both female LGs and one of the male LGs contained multiple linked significantly associated markers (Table 1). Individual markers explained between 4 and 16% of the phenotypic variation in PRR resistance, with the 5 linked markers on LG22F explaining the most variation (Table 1). These 3 LGs (LG22F, LG 5M and LG 15M) were associated with increased susceptibility to PRR while the QTL on LG 2F was associated with resistance (Table 1).

Four markers (1 SSR and 3 AFLP markers) were identified as associated with brown rust at $p \leq 0.01$ over both years of testing (Table 2).

Table 1. Markers associated with PRR resistance at $p < 0.01$ in at least one year of phenotypic data.

Map	Marker[a]	LG	HG	2001		2002		
				p value	% Var'n[b]	*p* value	% Var'n[b]	Marker Effect[c]
Q117	Aacctg.29	2	IV	0.010	8	0.022	4	−1.4
	Acgcta.10	2		0.010	8	0.023	5	−1.5
	Acccta.11	2		0.010	8	0.043	4	−1.5
	Acccta.21	2		0.005	9	0.036	4	−1.5
	Acacta.9	22	II	0.005	10	0.001	6	+1.6
	M44.5	22		< 0.001	16	0.002	9	+2.0
	Acgcag.3	22		< 0.001	14	0.001	9	+1.9
	Acccag.11	22		0.006	9	0.003	9	+1.5
	Acccag.4	22		0.002	13	0.003	9	+1.8
74C42	Acgctg.2	5	III	0.006	9	0.026	5	+1.5
	Acactt.10	5		0.002	11	0.021	5	+1.7
	Aggcac.3	5		0.008	8	0.010	6	+1.5
3:1[d]	**Acgctg.5**			0.007	9	0.005	8	+1.5

[a] markers in bold were significantly associated at $p \leq 0.01$ in both years of phenotypic screening.
[b] % phenotypic variation explained.
[c] average additive marker effect over both years.
[d] bi-parental simplex marker = single dose marker present in both parents and segregating 3:1 in the progeny.

Table 2. Markers associated with brown rust resistance at $p < 0.01$ in at least one year of phenotypic data.

Map	Marker[a]	LG	HG	2001		2002		
				p value	% Var'n[b]	*p* value	% Var'n[b]	Marker Effect[a]
Q117	**Acactg.16**	U	–	0.011	7	0.005	8	+1.1
	Actcat.14	13	U	0.010	7	0.028	5	−1.1
	Accctc.18	13		0.010	7	0.037	5	−1.1
	RGA-Q18	13		0.010	7	0.022	6	−1.1
	Aacctc.12	18	U	0.050	4	0.005	8	−0.9
	Aggctc.20	60	U	0.010	7	0.006	8	−1.2
	Aggctc.12	67	U	0.010	7	0.034	6	−1.1
74C42	**Uaccctc.17**	U	–	0.008	8	<0.001	11	+1.2
	Accctc.20	5	III	0.010	7	0.032	5	+1.1
	Agccag.4	10	III	0.01	7	0.023	6	−1.1
	Aagctc.6	10		0.006	8	0.013	7	−1.2
	Acacta.29	10		0.009	7	0.027	5	−1.1
	Aagcac.20	23	U	0.013	11	0.004	9	−1.4
	Actcac.15	23		0.007	8	0.032	5	−1.2
	M39.5	30	V	<0.001	16	<0.001	18	−1.7
	M51.1	56	III	0.021	6	0.003	9	+1.0

[a] markers in bold were significantly associated at $p \leq 0.01$ in both years of phenotypic screening.
[b] % phenotypic variation explained.
[c] average additive marker effect over both years.

Two of the 4 markers were mapped to LGs, one female and one male LG, but the other two markers were unlinked. A further 12 markers (1 SSR, 1 RGA and 9 AFLP markers) were identified as associated at $p \leq 0.01$ in one year and $p \leq 0.05$ in the second year. Eight of the twelve markers were linked on one female (LG 13, unassigned to a HG) and two male LGs (LG 10M and 23M in HG III and unassigned, respectively) but the remaining four markers were distributed onto different male and female LGs (Supplementary Figures 1 and 2). However, two of these LGs were also part of HG III (Table 2). Individual markers explained between 4 and 18% of the phenotypic variation in brown rust resistance (Table 2). Most of the markers identified were associated with resistance to brown rust; only four of the 16 markers were associated with susceptibility (Table 2).

LG 5M was the only LG in either map associated with both diseases. This LG contains 16 markers (Supplementary Figure 2) and spans approximately 160 cM. Three AFLP markers at one end of the LG were associated with susceptibility to PRR. One AFLP marker was associated with increased susceptibility to brown rust and was located towards the other end of the LG, approximately 90 cM distant from the 3 PRR-associated markers.

Validation of markers in alternative germplasm

Markers representing the different genomic regions identified in the Q1 population as associated with PRR and brown rust (Tables 1 and 2) were screened over the ECS to determine if the association between marker and trait was maintained over a broader range of germplasm. Six of the 13 markers identified in the Q1 population as associated with PRR and seven of the 16 markers identified as associated with brown rust were screened over the ECS and the results are given in Table 3.

For PRR, all six markers were present in at least 20% of the 154 lines within the ECS and were assessed for their association with PRR resistance (Table 3). Three of the six markers remained significantly associated with PRR in the ECS and at higher levels of significance than in the Q1 population. Two of the three markers were associated with resistance to PRR and one was associated with susceptibility (Table 2). In all 3 cases, the direction of the association was consistent with the direction of the marker association in the Q1 population. Two of the markers were linked in the one Q117 LG, LG 2F. The origin of the third marker, acgctg.5, is unknown as it was present in both parents (it is a bi-parental simplex marker).

Table 3. Validation of PRR and brown rust resistance markers, identified in the Q1 population, in the Elite Clone Set.

Trait	Map	Q1 Marker	LG	HG	Elite Clone Set Results		
					No. Clones with Marker	*t*-test *p* value	Marker Effect[a]
PRR	Q117	Acgcta.10	2	IV	33	0.009	−1.1
		Acccta.11	2		65	< 0.001	−1.37
		Acccta.21	2		25	0.15	ND[b]
		M44.5	22	II	73	0.688	ND
	74C42	Acgctg.2	5	III	85	0.16	ND
		Xacgctg.5	15 3:1	I	52	< 0.001	+ 1.39
Brown Rust	Q117	Acactg.16	U	–	29	0.244	ND
		Accctc.18	13	U	3	DNC[c]	
		Aacctc.12	18	U	38	0.63	ND
		Aggctc.20	60	U	31	0.009	−1.37
	74C42	Accctc.20	5	III	46	0.68	ND
		Actcac.15	23	U	32	0.055	−1.0
		M39.5	30	V	50	0.080	−1.0

[a] additive marker effect.
[b] ND = no difference.
[c] DNC = did not calculate as too few clones with markers.

For brown rust, six of the seven markers screened over the ECS were present in at least 20% of the 154 lines within the ECS and were assessed for their association with brown rust resistance (Table 3). Of the six markers, only one remained significantly associated with brown rust at a similar level of significance and it was associated with resistance, as in the Q1 population. This marker originated also from Q117 and was located on LG 60F. Two of the six markers were associated with resistance in the ECS at weaker significance levels. Actcac.15, a marker originating from 74C42 and located on LG 23M (Table 3) was associated with brown rust resistance at $p \leq 0.05$, while M39.5, also from 74C42 and on LG 30M (Table 3), was associated with brown rust resistance at $p < 0.1$.

Discussion

The female (Q117) and male (74C42) maps constructed in this population each contain more than 400 markers distributed onto 75 and 85 LGs, respectively. This number of markers is far fewer than the approximately 1000 markers mapped in the R570 (Rossi et al. 2003) and Q165 (Aitken et al. 2005) maps, which cover an estimated 33–50% of the sugarcane genome. Nevertheless, using common SSRs and RFLPs, it has been possible to assign many of the LGs in each map to previously described HGs (Rossi et al. 2003; Aitken et al. 2005) and numerous markers have been identified as associated with resistance to PRR or brown rust.

For PRR, the parents of the population were rated 5 and 5.5, and the progeny ratings were relatively evenly spread between 1 and 9. This suggests that inheritance of resistance to PRR is complex. Four genetic regions were identified as associated with PRR in both years of resistance screening data with 3 regions associated with resistance and one region associated with susceptibility. Individual markers were associated with relatively small effects, explaining from 4–16% of the phenotypic variation, which is consistent in size with those reported for QTLs for other sugarcane traits (Hoarau et al. 2002; Ming et al. 2002; Jordan et al. 2004). The four genetic regions, two from Q117 and two from 74C42, were assigned to different homology groups, suggesting that they are different loci. However, as many of the LGs were assigned to HGs on the basis of the presence of only one SSR, some of which map to more than one HG, further work is required to confirm that the four genomic regions represent separate loci.

The distribution of brown rust ratings in the Q1 population was quite different to that previously observed in the R570 population (Daugrois et al. 1996). It is important to note, however, that the R570 population was generated by selfing R570, a

rust resistant cultivar. The Q1 population was generated by crossing a moderately susceptible cultivar with a resistant elite line. Whereas the distribution of rust resistance ratings in the R570 population was ''L-shaped'' (highly skewed towards resistance) (Daugrois et al. 1996), indicative of a single major gene, the normal distribution of resistance ratings in the Q1 population and the observed transgressive segregation of some progeny is consistent with the involvement of many genes. The QTL analysis also supports this suggestion. In the Q1 population, individual markers accounted for 4–18% of the phenotypic variation in the trait, and most were associated with 8% or less. The results obtained in the present study suggest that the single major gene identified in R570 is not present in either of the two parental lines, and, given the degree of relatedness amongst Australian sugarcane cultivars (PA Jackson, unpubl. obs.), may not be present in Australian sugarcane germplasm.

In R570, the rust locus maps to R570 Homology Group VII (Rossi et al. 2003) (= HG I in Aitken et al. 2005). Many different microsatellites have been mapped to LGs within this HG (Rossi et al. 2003; Aitken et al. 2005), including mSSCIR36 and mSSCIR21. Alleles of both of these microsatellites mapped to several LGs in HG I in the both the female and male maps in the present study. No markers were identified as associated with brown rust resistance on any of these LGs in HG I at $p \leq 0.01$ in either map. However, an AFLP marker on LG 45F in HG I was associated with rust resistance in Q117 at $p \leq 0.05$ in both years of field screening (data not shown) which may indicate that there is a minor locus for rust resistance in Q117 in this HG; further studies would be required to determine whether it is the same locus as the major rust resistance locus in R570.

Comparative mapping studies have indicated that the major rust locus in R570 (Rossi et al. 2003) on R570 HG VII is not syntenic with the major rust resistance locus in maize, *Rp1* (Rossi et al. 2003). However, the sorghum region orthologous to the maize *Rp1* has been recently identified and has been shown to contain homologues of *Rp1-D* (Ramakrishna et al. 2002; McIntyre et al. 2004). This sorghum region (sorghum LG E) has been also shown to contain a major rust resistance QTL (Tao et al. 1998). We have recently mapped a suite of RGAs in sorghum and several of these RGAs mapped to this region on LG E (McIntyre et al. 2004). One of the RGAs, RGA-Q18, mapped to LG13F in the Q117 map and was also associated with rust resistance. This observation suggests that this LG in sugarcane may be syntenic with LG E of sorghum and chromosome 10S of maize and may again suggest conservation of resistance gene function across all three species. Unfortunately, LG 13 F has not been assigned to a HG.

Three LGs assigned to HG III in the male map were also shown to be associated with brown rust. However, due to a lack of common markers, it was not possible to determine if the regions identified in these LGs were the same. Other LGs in this HG contain RGA markers and a sugarcane SSR that have been shown to map to sorghum LG E (McIntyre et al. 2004). More markers common to sorghum and sugarcane maps are required to determine whether sugarcane HG III is syntenic with sorghum LG E and maize 10S.

Markers identified using map-based approaches are polymorphic between the parents of the population and detect genes segregating in the experimental population. For a marker to be effective in a breeding program, it needs to be effective in a range of germplasm. The ECS was selected to contain a diverse range of Australian sugarcane germplasm, including current cultivars, current parental lines, and important ancestral parental lines. A subset of markers identified as associated with PRR or brown rust resistance using the map-based QTL analysis were screened over the ECS. Three PRR markers remained significantly associated in the ECS and may be useful in selecting for increased resistance to PRR in sugarcane germplasm. For brown rust, only 1 of the assessable markers remained significantly associated with resistance with two other markers remaining weakly associated. All three markers could be used for selection for increased resistance to brown rust in sugarcane germplasm.

Curiously, two of the PRR markers (Acgcta.10 and Acccta.11), despite being tightly linked on LG 2F in the Q117 map and remaining associated with resistance in the ECS, appeared to be in linkage equilibrium in the ECS; the number of ECS clones containing each marker varied greatly. This is surprising as the two markers are tightly linked at a distance of only 0.5 cM on LG 2F in Q117. Possible explanations for this unexpected result include: the distance between these two markers is

greater in most sugarcane cultivars than in Q117, allowing a greater possibility of recombination in other sugarcane clones to break the linkage between the two markers; chromosomes with a recombination event(s) between the two markers are widespread in sugarcane clones within the ECS; and, the ECS contain unrelated DNA fragments of identical size that co-migrates with the two PRR markers. Further research is required to determine the basis of this unexpected result.

The association between marker and trait in the ECS was lost for three of six PRR markers and five of six brown rust markers. There are many possible reasons for the loss of association between marker and trait. The ECS clones may contain different resistance genes for the two traits at different locations compared to the resistance genes tagged in the Q1 population. The markers identified in the Q1 population may be too loosely linked to the resistance genes for the two traits and linkage between the marker and the resistance gene has been broken in the ECS clones. Both suggestions are possible given the limited genome coverage of the current sugarcane maps preventing detection of all genomic regions associated with the targeted traits, the large number of homo(eo)logous chromosomes potentially containing resistance loci that are different to the ones tagged in this population and the observation by Jannoo et al. (1999) that linkage disequilibrium in sugarcane is maintained only over approximately 10 cM.

Two other possibilities are that the marker scored in the Q1 population and in the ECS are identical in size but are not tagging the same genomic region or that the initial association between the marker and the trait identified in the Q1 population was spurious. The use of linkage disequilibrium-based approaches for validation of mapped markers or identification of markers *per se* has been widely used in human genetics, but have only recently been used in plants. This approach has been used successfully in potatoes to validate mapped markers associated with *Verticillium* wilt resistance, caused by *Verticillium dahliae* (Simko et al. 2004), and late blight, caused by *Phytophthora infestans* (Gebhardt et al. 2004). In the latter study, 4 of the 5 markers identified as associated with late blight resistance using map-based approaches remained associated with resistance to the disease in the association mapping study. The one marker that failed in the association test was suggested to be too distant (0.6–0.9 cM) to the genes causal to the QTL and hence was in linkage equilibrium (Gebhardt et al. 2004).

This study has identified markers associated with two diseases of sugarcane. Of particular interest are the markers associated with PRR, a major disease of sugarcane in Australia. We have used both map-based and association-based approaches to identify several markers that appear to be broadly associated with resistance; such markers should have application for marker-assisted selection.

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