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ORIGINAL PAPER

QTL analysis of ergot resistance in sorghum

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Abstract Sorghum ergot, caused predominantly by *Claviceps africana* Frederickson, Mantle, de Milliano, is a significant threat to the sorghum industry worldwide. The objectives of this study were firstly, to identify molecular markers linked to ergot resistance and to two pollen traits, pollen quantity (PQ) and pollen viability (PV), and secondly, to assess the relationship between the two pollen traits and ergot resistance in sorghum. A genetic linkage map of sorghum RIL population R931945-2-2 × IS 8525 (resistance source) was constructed using 303 markers including 36 SSR, 117 AFLP™, 148 DArT™ and two morphological trait loci. Composite interval mapping identified nine, five, and four QTL linked to molecular markers for percentage ergot infection (PCERGOT), PQ and PV, respectively, at a LOD >2.0. Co-location/linkage of QTL were identified on four chromosomes while other QTL for the three traits mapped independently, indicating that both pollen and non pollen-based mechanisms of ergot resistance were operating in this sorghum population. Of the nine QTL identified for PCERGOT, five were identified using the overall data set while four were specific to the group data sets defined by temperature and humidity. QTL identified on SBI-02 and SBI-06 were further validated in additional populations. This is the first report of QTL associated with ergot resistance in sorghum. The markers reported herein could be used for marker-assisted selection for this important disease of sorghum.

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Introduction

Ergot is a fungal disease of the sorghum inflorescence and is caused predominantly by *Claviceps africana* Frederickson, Mantle, de Milliano (Bandyopadhyay et al. 1998; Pazoutova 2000). The disease poses a significant threat to the sorghum industry worldwide and in Australia since its first outbreak in Australia in the mid 1990s. The pathogen mainly infects unfertilised ovaries and, as a result, hybrid seed production that utilises male sterile seed parents is most vulnerable. In India and in Zimbabwe, regular yield losses and occasionally total yield losses in hybrid seed production plots have been reported due to the disease (Frederickson et al. 1994; Bandyopadhyay et al. 1998). In addition, sorghum grain contaminated with sclerotes

(vegetative fungal propagules) can cause toxicity when fed to livestock (Blaney et al. 2000).

To date, two physiological mechanisms of ergot resistance have been reported: (1) pollen-based; in which resistance is related to the ability of plants to pollinate and fertilise rapidly before infection can occur (Bandyopadhyay et al. 1998), and (2) non pollen-based; in which a combination of floral characteristics, e.g. least exposure time of stigma to inoculum before pollination, rapid stigma drying after pollination and small stigma are associated with resistance (Dahlberg et al. 2001). While pollen-based mechanisms of resistance have value in some environments they are limited by the overriding effect of the environment (e.g. ergot favourable conditions) on pollen activity (Frederickson et al. 1994; Bandyopadhyay et al. 1998). Non pollen-based mechanisms of ergot resistance were first reported in the sorghum line IS 8525 (Dahlberg et al. 2001). The corresponding A3 male sterile line also showed moderate resistance but resistance was reported to be unstable across environments (Reed et al. 2002).

Genetic resistance to plant diseases is a major objective of most plant breeding programs. In a recent study, Parh et al. (2006) reported that ergot resistance in sorghum is controlled by many genes and that the pollen traits, PQ and PV have moderate genetic correlation with PCERGOT. Determining the number and location of genes controlling resistance as well as studying the association of pollen traits with ergot resistance can assist in the rapid and efficient development of new ergot-resistant varieties. Moreover, markers tightly linked to major genes or quantitative trait loci (QTL) in one population can be used for marker-assisted selection (MAS) screens if they are polymorphic in another population. The development of varieties resistant to sorghum ergot has not been successful to date. We have utilised sorghum line IS 8525, one of the recently reported non pollen-based sources of ergot resistance, in the development of a recombinant inbred line (RIL) population to reveal the genetic complexity of ergot resistance. Our objectives were to construct a genetic linkage map of the RIL population R931945-2-2 (a commercially accepted restorer line in Australia) × IS 8525 (resistance source), to determine the number and map location of QTL underlying ergot resistance, to assess the relationship of two pollen traits, pollen quantity (PQ) and pollen viability (PV) with ergot resistance and to validate the identified QTL in other populations to examine their potential for MAS in sorghum.

Materials and methods

Mapping population and data analyses

A subset of 146 individuals of sorghum RIL population R931945-2-2 × IS 8525 (alias 31945-2-2 × IS 8525 as described previously by Parh et al. 2006) was used for map construction and subsequent QTL analyses. Data for PCERGOT and the two pollen traits, PQ and PV, were collected from the two field trials conducted at Hermitage Research Station, Warwick, QLD, Australia during the 2001 and 2002 growing seasons. In each year there were two planting dates and a number of sampling dates for recording data on PCERGOT and PQ and PV as described previously by Parh et al. (2006). PCERGOT was measured as the percentage of florets infected per panicle. PQ was measured visually on a 1–10 scale (1 for no visible pollen and 10 for copious quantity of visible pollen) by observing the resultant clouds of pollen flicked from flowering sorghum heads. PV was measured under a compound microscope from the pollen collected on a water-agar plate based on percentage of viable (those which took dark brown to black iodine stain) and non-viable (those which were empty, pale and disrupted) pollen. Field data was analysed using a linear mixed model. A two-factor analytical model was used to model the genotype × sampling date interaction and clustering of sampling dates into groups (Parh et al. 2006). The predicted mean of each RIL from the overall and group data for PCERGOT, PQ and PV was then used for QTL analysis.

Validation populations

The Department of Primary Industries and Fisheries, QLD (DPI&F) developed a number of populations using IS 8525 as a source of resistance to ergot. These populations were used for validation of some of the QTL regions reported in this study. The pedigree, number of individuals tested and the expected segregation ratio of marker alleles in each population are shown in Table 1. In each of these populations, selections were made for ergot resistance as well as other desirable crop characteristics. DNA was extracted from the selected individuals and PCR was performed following the protocols as described below. A *t*-test was used to examine the difference between mean PCERGOT of the linked loci carrying either the IS 8525 (AA) or the R931945-2-2/other parent (BB) allele.

DNA isolation

DNA was extracted from 3 to 4-week-old seedlings using a modified version of the CTAB method as described by Saghai Maroof et al. (1984). About 1–2 g of freeze-dried leaf sample was ground to a fine powder, mixed with 4.0–8.0 ml freshly made pre-warmed CTAB buffer (100 mM Tris–HCl, pH 8.0; 20 mM EDTA, 2.8 M NaCl, 2% CTAB) and incubated in a water-bath at 65°C for about an hour with occasional agitation. The contents were then extracted with a half volume of chloroform/isoamyl alcohol (24:1). The

Table 1 Description of populations used in the validation test for the QTL regions identified in the sorghum RIL population R931945-2-2 × IS 8525

Population	Generation	No. of lines tested	Expected allele segregation[c]
B972422/IS 8525[a]	F6	20	1 (AA):1(BB)
B971990/IS 8525	F7	24	1 (AA):1(BB)
ms3*3_R931945//R9R931945-2-2/IS 8525[b]	BC1F5	38	1 (AA):1(BB):2 (CC)[d]
IS 8525/R960535//R960535	BC1F6	17	1 (AA):3(BB)
ms3*3_R931945//R960535/IS 8525	F6	34	1 (AA):1(BB):2 (CC)

[a] Prefix B indicates B-line

[b] ms3 indicates male sterile line

[c] *AA* IS 8525, *BB*, *CC* other parents of each cross

[d] *BB*, *CC* alleles were counted together and written as BB allele in the validation test in Table 7

aqueous phase was removed and the DNA was precipitated with a 1/10 volume of 7.5 M ammonium acetate and one volume of cold absolute ethanol. The precipitated DNA was washed with 70% ethanol, dried and dissolved in 0.1× TE buffer. RNA was digested by adding RNase (10 mg/ml). DNA concentration and quality were determined using fluorometric methods and running samples on agarose gels, respectively.

Genotyping procedures

Genotyping of the individual RIL was performed using SSR, AFLP™, and DArT™ technologies as described below:

SSR markers

PCR amplification of SSRs was performed in a 20 µl reaction volume containing 25 ng of genomic DNA, 1× PCR buffer (Promega), 4 mM $MgCl_2$, 0.125 mM each of dTTP, dCTP, dGTP and 0.0125 mM (1/10) of dATP, 25 ng of each forward and reverse SSR primer, 0.5 U of *Taq* DNA polymerase and 1 µCi of [α ^{33}P] dATP in either MJ research (PTC 100) or Perkin-Elmer (GeneAmp 9700 or 2700) Thermal Cycler. After an initial denaturation at 94°C for 4 min, the reaction mixture was subjected to ten cycles of 94°C (30 s), 60–1°C/cycle (30 s), 72°C (1 min) followed by 30 cycles of 94°C (15 s), 50°C (30 s), 72°C (1 min) and a final extension of 72°C for 8 min. The amplified products were separated on 4% denaturing polyacrylamide gels in 1× TBE at a constant voltage of 80 w for about 2 h. Following electrophoresis, the gel was dried and exposed onto Kodak Biomax MR or Diagnostic XK-1 film for autoradiography. The film was developed using Kodak developer and fixer. SSR markers used in this study were reported previously by Kong et al. (2000), Bhattramakki et al. (2000) and Brown et al. (1996). The "TxP" and "Sb" markers were designated as Xtxp and Xgap, respectively, in the map of Bhattramakki et al. (2000).

AFLP™ markers

The AFLP™ method of Vos et al. (1995) was modified as follows. Two restriction enzymes, *Eco*RI and *Mse*I, were used to digest the genomic DNA (300 ng/µl). The digestion buffer included 6 U of *Eco*RI and 4 U of *Mse*I enzymes, 1× NEBS buffer, 0.1 mM BSA, 5 mM Spermidine and 1 mM DTT in a 40 µl of reaction volume. The reaction mixture was incubated overnight at 37°C followed by inactivation of enzymes at 70°C for 10 min. Ten µl of a solution containing 5 pmol of *Eco*RI-adapters, 50 pmol of *Mse*I-adapters, 0.5 µl of 0.1 M ATP, 1× T4 DNA ligation buffer and 1.5 U of T4-DNA ligase was added to each digest and incubated for 1 h at 37°C. The ligated mixture was diluted to 500 µl with 0.1× TE, pH 8.0 and stored at −20°C.

DNA was selectively pre-amplified in a 20 µl reaction mix containing 5 µl of diluted ligation mix, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.160 mM dNTPs (Promega), a single base extension of *Eco*RI (5′-GTAGACTGCGTACCAAT TC + A-3′) and *Mse*I (5′-ATGAGTCCTGAGTAA + C-3′) (50 ng/µl each) primers and 0.44 U of *Taq* DNA polymerase (Promega). The thermal profile for pre-amplification was a 30 s denaturing step at 94°C, a 30 s step-down annealing step, and a 1 min extension step at 72°C. The annealing temperature was 65°C in the first cycle, subsequently reduced by 0.7°C per cycle for the next 12 cycles, and finally stabilizing at 56°C for the remaining 23 cycles.

Selective amplification was carried out with two oligonucleotide primers, one corresponding to the *Eco*RI-end and the other corresponding to the *Mse*I-end. A total of 40 primer combinations (32 primer combinations each contained 3 selective nucleotides while 8 primer combinations contained *Eco*RI primers with 2 and *Mse*I primers with 3 selective nucleotides) were used to amplify AFLP bands.

For selective amplification, the *Eco*RI primer was end-labelled using [γ-^{33}P] dATP and T4-polynucleotide kinase. The reaction mixture and the thermal profile used for the selective amplification were the same as used for pre-amplification except that one of the primers (*Eco*RI) was end-labelled and the template DNA was the diluted (20-fold in 0.1× TE) pre-amplification product.

The procedure for gel electrophoresis and detection of AFLP™ bands was as described before for SSR analysis except that the gel was exposed onto more sensitive Kodak Biomax MR film. The polymorphic bands were named after the primer combination used in selective amplification and numbered serially in decreasing order of molecular weight.

DArT™ markers

The genotyping technique using diversity arrays technology (DArT™) has recently been applied to sorghum. The protocol has been described by Mace et al. (2008) which is a modification of the technique developed originally for rice (Jaccoud et al. 2001) and subsequently applied to many other plant species, including barley (Wenzl et al. 2006), cassava (Xia et al. 2005), *Arabidopsis* (Wittenberg et al. 2005), pigeonpea (Yang et al. 2006) and wheat (Akbari et al. 2006).

Data for morphological characters

Data for PCERGOT, PQ and PV were scored in the population as described previously by Parh et al. (2006). In addition, two morphological traits, coleoptile colour and red leaf were scored. Coleoptile colour, purple (IS 8525) versus green (R931945-2-2), was scored 3–4 weeks after emergence from both years' trials and red leaf, present (IS 8525) versus absent (R931945-2-2) was scored only once on planting date 2 of 2001, as the expression of this trait is environmentally dependent and requires the plant to be infected with the Johnson grass strain of sugarcane mosaic virus (Persley et al. 1977).

Linkage map construction

Linkage analysis was conducted using MultiPoint software (http://www.multiqtl.com). In preliminary analysis of the data, markers were deleted which had high segregation distortion ($P \leq 0.001$). The RIL selfing population setting was selected and a maximum threshold recombination fraction (rf_s) value of 0.35 was used to initially group the markers into clusters. The Kosambi (Kosambi 1944) mapping function was used to calculate map distances (cM) from rf_s. Multipoint linkage analysis of loci within each cluster was then performed and marker order was further verified through re-sampling for quality control via jack-knifing (Mester et al. 2003). Markers that could be ordered with a jack-knife value of 90% or greater were included as "framework" markers, with any remaining markers causing unstable neighbourhoods being initially excluded from the map. Following a repeated multipoint linkage analysis with the reduced set of markers for each cluster to achieve a stabilised neighbourhood, the previously excluded markers were attached by assigning them to the best intervals on the framework map. Within these ordered individual clusters, the SSR locations were compared to previously published sorghum maps (Bhattramakki et al. 2000; Menz et al. 2002) in order to allow the clusters to be assigned to sorghum chromosomes, SBI-01 to SBI-10 according to recent nomenclature (Kim et al. 2005).

QTL detection

The most likely location of QTL and their genetic effects were initially detected by composite interval mapping (CIM) (Zeng 1993, 1994) using QTL Cartographer version 2.5 software (Basten et al. 2002). Model 6 (standard model), forward step-wise regression with five markers as cofactors to control genetic background and a 10 cM genome-wide scan window, were used for the detection of QTL. Further, single marker analysis (SMA) and QTL Network-2.0 (Yang et al. 2005) were used to confirm the results generated by CIM. A permutation (1,000 permutations) based LOD threshold of 3.0 was used to declare putative QTL (Churchill and Doerge 1994) for PCERGOT obtained from the overall data set. However, QTL exceeding a LOD threshold of >2.0 were also reported to indicate suggestive QTL in the region with group data of PCERGOT and the overall and group data of PQ and PV. When separated by a minimum distance of 20 cM (Ungerer et al. 2002) or 1-LOD support interval (Lander and Botstein 1989), two peaks on one chromosome were considered as two different QTL. Otherwise, the higher peak was chosen to more closely approximate the position of the QTL.

Results

Phenotypic data for PCERGOT and pollen traits

The means and ranges of the predicted means of 146 individuals of the mapping population for PCERGOT and the two pollen traits, PQ and PV are shown in Table 2. The distribution of the square-root transformed predicted means followed an assumption of normality for the overall and group data of PCERGOT as reported previously by Parh et al. (2006) but skewness and kurtosis were observed in the histograms for PQ and PV with the reduced set of data. For the ease of discussion, the predicted means of the RILs were subsequently used for QTL analysis throughout the paper.

Table 2 Predicted means and ranges of the group and overall data for percentage ergot infection (PCERGOT), pollen quantity (PQ) and pollen viability (PV) in the sorghum RIL population R931945-2-2 × IS 8525 (n = 146)

Group[a]	Genotypes	PCERGOT Range	Mean	PQ Range	Mean	PV[b] Range	Mean
G1	RILs	0.64–49.62	19.40	2.85–6.72	4.82	–	–
	R931945-2-2	–	30.84	–	4.49	–	–
	IS 8525	–	2.95	–	5.25	–	–
G2	RILs	2.82–40.65	12.94	1.25–6.78	4.66	–	–
	R931945-2-2	–	28.34	–	2.77	–	–
	IS 8525	–	3.19	–	4.46	–	–
G3	RILs	1.44–47.48	12.32	–	–	63.73–95.89	85.96
	R931945-2-2	–	34.79	–	–	–	83.47
	IS 8525	–	1.97	–	–	–	91.95
Overall	RILs	3.60–44.29	15.20	2.64–6.39	4.69	72.40–94.75	86.98
	R931945-2-2	–	30.18	–	3.09	–	79.51
	IS 8525	–	4.08	–	4.76	–	92.63

[a] The groups are specific to each trait and are not comparable across traits. For PCERGOT group1 (G1) to group3 (G3) represent 4, 6 and 5 sampling dates; for PQ, group1 (G1) and group2 (G2) represent 4 and 7 sampling dates and for PV, group3 (G3) represent 3 sampling dates, respectively

[b] For PV, only G3 is considered. The RILs of the other groups contain many missing values for PV and are not useful for subsequent analysis

Linkage map of the R931945-2-2 × IS 8525 RIL population and its comparison with other *Sorghum bicolor* maps

The framework map of the *S. bicolor* R931945-2-2 × IS 8525 RIL population consists of 303 marker loci, including 36 SSR, 117 AFLP™ and 148 DArT™ markers and two morphological trait loci, coleoptile colour and red leaf (Fig. 1). The ten linkage groups of the map spanned a total length of 1,625.2 cM with an average distance of 5.36 cM between loci along the chromosome. In general, the 36 SSR markers were distributed evenly throughout the chromosomes and in chromosomes where two or more SSRs were mapped, the marker order and positions were in good agreement with the published map of Bhattramakki et al. (2000) and the high density map of Menz et al. (2002). With the exception of four gaps greater than 30 cM on SBI-01, SBI-08, SBI-09 and SBI-10, and a slight clustering of markers in a few regions, the markers were distributed evenly in the map. The map position of the locus controlling coleoptile colour (Rs* on SBI-06) is very similar to that reported for the plant colour gene by Klein et al. (2001) while the map position for red leaf (rlf on SBI-10) is reported for the first time.

QTL for PCERGOT

The CIM analysis identified five QTL for PCERGOT on SBI-01, SBI-02, SBI-06, SBI-07 and SBI-08 using the overall data set (Table 3). In addition, four QTL were identified that were specific to the group data sets only. With the exception of the QTL on SBI-08, the QTL identified with the overall data set were also identified with 1–3 group data sets. All of these QTL, including the QTL on SBI-08, were identified by SMA (data not shown). QTL Network-2.0 identified all but the QTL on SBI-02 with the overall data set, however, with less statistical significance (P = >0.0001). The statistical significance increased substantially (P = <0.0001) when analysis was performed across all of the group data sets; the number, location and additive effects of QTL were approximately similar to those as identified by CIM analysis (data not shown).

All of the additive effects identified using the overall data set were negative indicating that the QTL alleles associated with decreased PCERGOT were derived from the resistant parent IS 8525 of the cross. The phenotypic variation explained by the individual QTL varied from 7.0 to 14.1 and collectively the five QTL identified by CIM analysis using the overall data accounted for 53.7% of the total phenotypic variation for PCERGOT. In addition, QTL specific to the group data sets explained a substantial portion of phenotypic variation for PCERGOT. These QTL alleles had both positive and negative additive effects, indicating that alleles associated with increased and decreased PCERGOT were derived from both parents (Table 3).

QTL for PQ

Using the overall data set three QTL were identified for PQ on SBI-04, SBI-06 and SBI-07 (Table 4). The QTL on SBI-04 and SBI-06 were also identified by the G1 and G2 data sets of PQ and that of SBI-07 by the G1 data set only. In addition, two independent regions, one on SBI-01 and another on SBI-10 were identified by the G1 and G2 data sets, respectively (Table 4). SMA identified all but the QTL on SBI-07 and QTL Network-2 identified only the QTL on SBI-04, SBI-06 and SBI-10 with less statistical significance

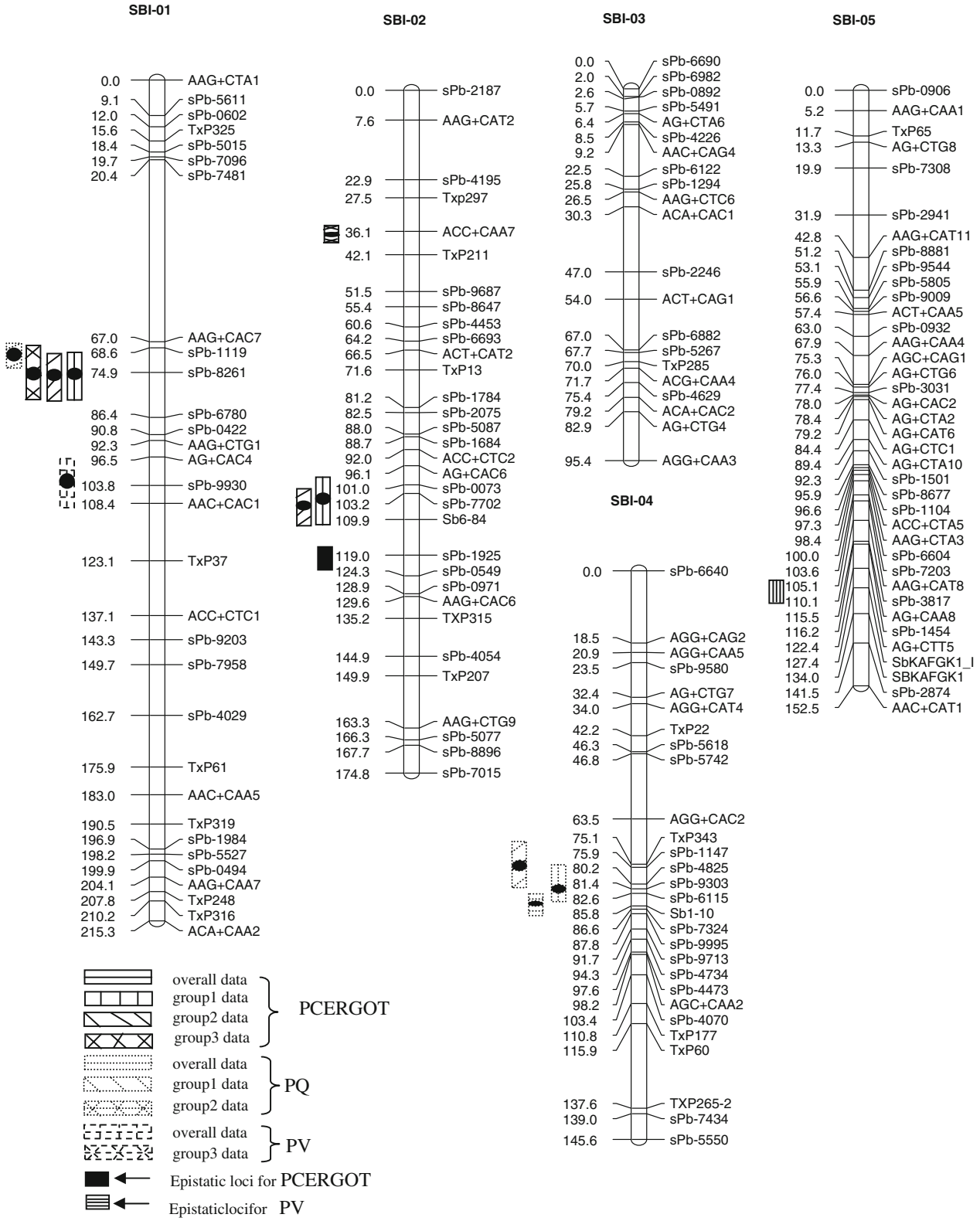

Fig. 1 *S. bicolor* chromosomes showing QTL locations of percentage ergot infection (PCERGOT) and pollen traits (PQ and PV) identified by overall and group data. The locations of two morphological trait loci, coleoptile colour (Rs*) and red leaf (*rlf*), are shown on SBI-06 and SBI-10, respectively. Rs* is similar to plant colour gene reported earlier by Klein et al. (2001). Locations of QTLs are indicated by *solid*, *dotted* and *broken bars* for PCERGOT, PQ and PV, respectively. Epistatic loci for PCERGOT and PV are shown using *black* and *lined bars*, respectively. QTL bars represent one-LOD support interval surrounding the peak score (indicated by *black dot* inside the *bar*). Distances between markers are shown in centiMorgan using Kosambi mapping function

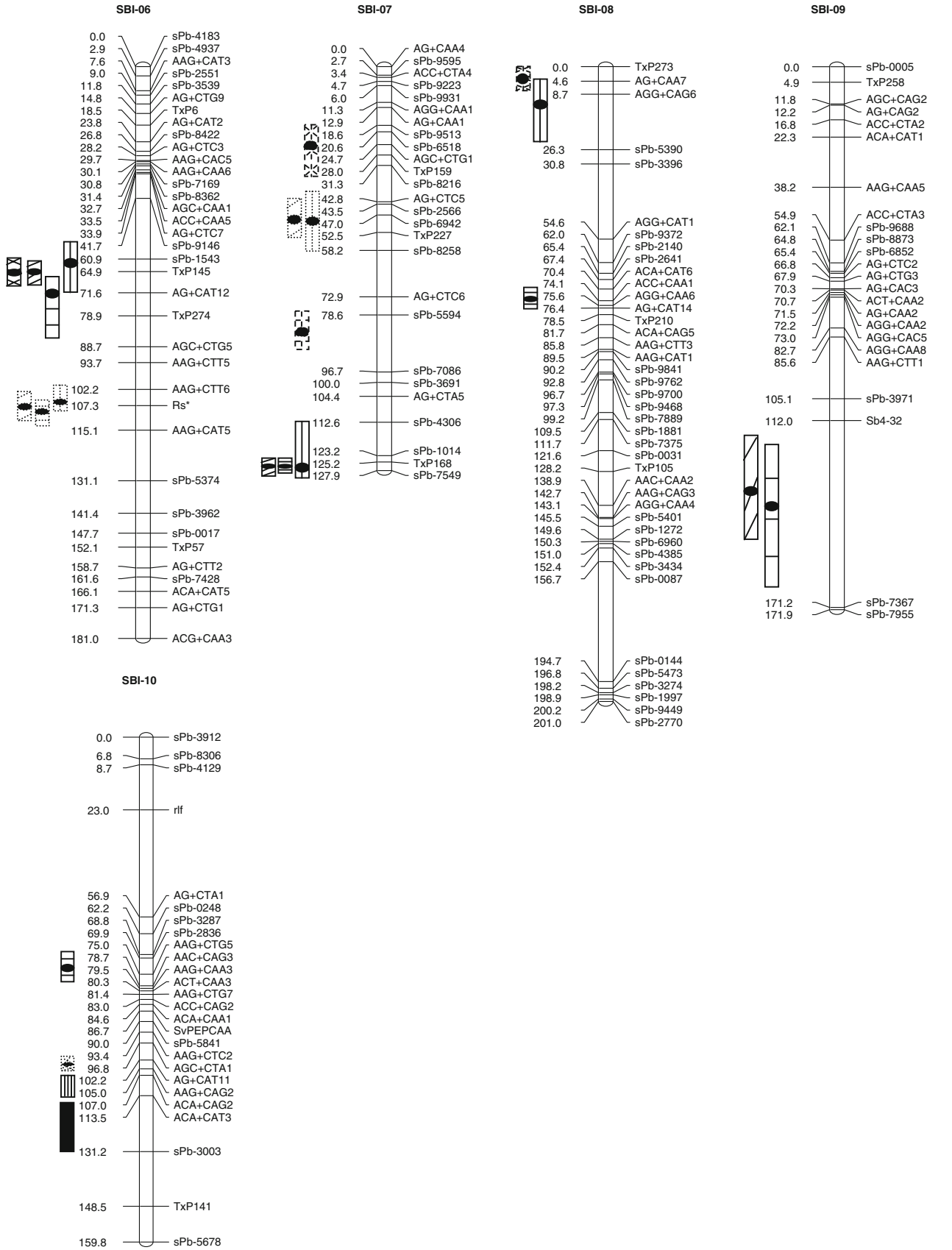

Fig. 1 continued

Table 3 QTL and their associated effects for percentage ergot infection (PCERGOT) as identified by composite interval mapping using overall (LOD > 3.0) and group data sets (LOD > 2.0) in sorghum RIL population R931945-2-2 × IS 8525

Chromosome	Identified by	Marker[a]	Position	RecombiL[b]	RecombiR[c]	LOD[d]	Additive[e]	R^{2f}
SBI-01	Overall, G2, G3	sPb-8261	74.9	0.0001	0.1131	4.78	−2.25	0.118
SBI-02	Overall, G3	sPb-7702	103.0	0.0201	0.0017	3.36	−1.79	0.070
SBI-02	G3	ACC + CAA7	36.2	0.0001	0.0587	3.53	−1.81	0.071
SBI-06	Overall, G1–G3	sPb-1543	62.9	0.0201	0.0203	6.23	−2.40	0.141
SBI-07	Overall, G1, G2	TxP168	127.2	0.0201	0.0069	4.48	−1.98	0.098
SBI-08	Overall	AGG + CAG6	12.7	0.0400	0.1331	3.71	−2.10	0.110
SBI-08	G1	AGG + CAA6	75.7	0.0001	0.0077	2.52	2.20	0.053
SBI-09	G1, G2	Sb4-32	142.0	0.2686	0.2634	3.19	−4.48	0.195
SBI-10	G1	AAG + CTG5	75.0	0.0001	0.0368	2.59	2.32	0.059

[a] The marker with the highest LOD score is shown

[b] Recombination of the identified markers with left flanking marker

[c] Recombination of the identified marker with right flanking marker

[d] Log_{10}-likelihood value

[e] Effect of substitution of AA (IS 8525) allele by BB (R931945-2-2) allele. Negative and positive signs of the estimate indicate allele contributed by IS 8525 and R931945-2-2, respectively

[f] Percentage total phenotypic variation explained

Table 4 QTL and their associated effects for pollen quantity (PQ) as identified by composite interval mapping (LOD > 2.0) using overall and group data sets in the sorghum RIL population R931945-2-2 × IS 8525

Chromosome	Identified by	Marker[a]	Position	RecombiL[b]	RecombiR[c]	LOD[d]	Additive[e]	R^{2f}
SBI-04	Overall, G1, G2	sPb-1147	79.88	0.0401	0.0028	2.56	0.21	0.066
SBI-06	Overall, G1, G2	AAG + CTT6	106.16	0.0400	0.0110	6.65	0.42	0.199
SBI-07	Overall, G1	sPb-6942	49.02	0.0201	0.0347	2.71	0.23	0.073
SBI-01	G1	AAG + CAC7	67.03	0.0001	0.0156	2.42	−0.20	0.060
SBI-10	G2	AGC + CTA1	100.85	0.0400	0.0132	2.24	−0.30	0.063

[a] The marker with the highest LOD score is shown

[b] Recombination of the identified markers with left flanking marker

[c] Recombination of the identified marker with right flanking marker

[d] Log_{10}-likelihood value

[e] Effect of substitution of AA (IS 8525) allele by BB (R931945-2-2) allele. Positive and negative signs of the estimate indicate allele contributed by IS 8525 and R931945-2-2, respectively

[f] Percentage total phenotypic variation explained

when analysis was performed across two group data sets of PQ (data not shown). Collectively, the five QTL explained 46.2% of the phenotypic variation for PQ. All of the QTL identified using the overall data set had a positive additive effect, indicating that the alleles associated with increased PQ were derived from IS 8525. However, the two QTL identified in either the G1 or G2 data sets had a negative additive effect, indicating that the alleles associated with decreased PQ were derived from R931945-2-2.

QTL for PV

For PV, four independent QTL, one each on SBI-01 and SBI-08 and two on SBI-07 were identified using the overall and G3 data sets (Table 5). SMA identified all of the above QTL but QTL Network-2.0 identified only the QTL on SBI-01 and SBI-07 in a similar position but with less statistical significance (data not shown). The four QTL explained 37.7% of the total phenotypic variation for PV. The additive effect for all QTL was positive, indicating that IS 8525 contributed the beneficial alleles associated with increased PV.

Pleiotropic effects versus linkage

CIM analysis identified 15 chromosomal regions which harbour QTL underlying traits controlling PCERGOT and/or PQ and PV (Fig. 1). These QTL were dispersed on eight of the ten sorghum chromosomes; only chromosomes SBI-03 and SBI-05 were without QTL for these traits. Congruency

Table 5 QTL and their associated effects for pollen viability (PV) as identified by composite interval mapping (LOD > 2.0) using overall and group data sets in the sorghum RIL population R931945-2-2 × IS 8525

Chromosome	Identified by	Marker[a]	Position	RecombiL[b]	RecombiR[c]	LOD[d]	Additive[e]	R^{2f}
SBI-01	Overall	AG + CAC4	102.51	0.0598	0.0130	3.18	1.14	0.092
SBI-07	Overall	sPb-5594	82.65	0.0400	0.1365	3.09	1.33	0.125
SBI-07	G3	AAC + CAG3	24.75	0.0001	0.0327	2.39	1.64	0.067
SBI-08	G3	TxP273	4.01	0.0400	0.0059	3.26	1.89	0.095

[a] The marker with the highest LOD score is shown

[b] Recombination of the identified markers with left flanking marker

[c] Recombination of the identified marker with right flanking marker

[d] Log_{10}-likelihood value

[e] Effect of substitution of AA (IS 8525) allele by BB (R931945-2-2) allele. Positive sign of the estimate indicates allele contributed by IS 8525

[f] Percentage total phenotypic variation explained

of QTL for PCERGOT and/or PQ and PV were found only in two regions, one on SBI-01 where QTL for PCERGOT and PQ were mapped in approximately similar regions and on SBI-08, one QTL each for PCERGOT and PV were mapped within a 1-LOD support interval (Fig. 1). Similarly, linkage of QTL for PCERGOT and PQ/or PV were observed in two regions: on SBI-01, QTL for PCERGOT and PV were mapped approximately 25 cM apart and on SBI-10, QTL for PCERGOT and PQ were mapped approximately 25 cM apart (Fig. 1). On SBI-07, four different regions were associated with QTL for PCERGOT, PQ and PV. In this chromosome, a significant region for PCERGOT was mapped at the distal end (at position 127 cM), two QTL for PV were mapped at positions 25 and 83 cM and a QTL for PQ was mapped in between the QTL of PV at position 49 cM.

Epistasis between PCERGOT, PQ and PV

No significant epistatic interaction was identified among the main effect QTL for PCERGOT, PQ and PV using CIM analysis. This was confirmed by QTL Network-2.0 (data not shown). However, QTL Network-2.0 identified one significant epistatic interaction between two marker alleles, sPb-1925 and ACA + CAT3 on SBI-02 and SBI-10, respectively, which were not involved in main effect QTL (Table 6). Similarly for PV, one significant epistatic interaction involving two marker alleles, one on SBI-10 and another on SBI-05 was found (Table 6). These epistatic QTL explained about 3.9 and 13.7% of the phenotypic variance for PCERGOT and PV, respectively, as indicated by the heritability estimate of the epistatic allele by QTL Network-2.0 (Table 6).

QTL by environment interaction

The sign of the additive by environment interaction was inconsistent and not significant as observed for the QTL identified by the group data sets using QTL Network-2.0 (data not shown). However, CIM analysis identified four QTL for PCERGOT in the group data sets only which were not detected using the overall data; one region on SBI-09 identified by both G1 and G2 data sets, and the others on SBI-08 and SBI-10 by G1 and on SBI-02 by G3 data sets. Two of these QTL regions (SBI-09 and SBI-02) had LOD > 3.0 and were also identified by the QTL Network-2.0 when analysis was performed across all of the group and overall data for PCERGOT (data not shown). The additive effect of the two QTL on SBI-08 and SBI-10 was positive indicating that the QTL alleles associated with increased PCERGOT were derived from the susceptible parent R931945-2-2 of the cross. The other two QTL on SBI-02 and SBI-09 were derived from the resistant parent IS 8525. The QTL on SBI-09 linked to SSR marker Sb4-32 explained the highest amount of phenotypic variance for PCERGOT (19.5% on average).

Table 6 Epistatic loci associated with percentage ergot infection (PCERGOT) and pollen viability (PV) as indicated by QTL Network-2.0 in the sorghum RIL population R931945-2-2 × IS 8525

Trait	Chromoso-me	Marker interval	Chromoso-me	Marker interval	AAij[a]	*P*-value	h^{2b}
PCERGOT	SBI-02	sPb-1925/sPb-0549	SBI-10	ACA + CAT3/sPb-3003	−1.57	0.000009	0.039
PV	SBI-05	AAG + CAT8/sPb-3817	SBI-10	AG + CAT11/AAG + CAG2	−2.35	0.000006	0.137

[a] Additive × additive epistatic effect

[b] Heritability of the epistatic allele

Validation of QTL for PCERGOT

The polymorphic SSR markers that mapped nearby to the reported QTL regions of PCERGOT in this study were used in the validation test. We were able to validate the marker-QTL associations only for two regions: one on SBI-02 where SSR marker Sb6-84 mapped approximately 6.7 cM away and another on SBI-06 where SSR markers TxP145 and TxP274 mapped approximately 4 and 18 cM away from the reported markers sPb-7702 and sPb-1543 linked to the QTL in these regions, respectively. These SSR markers were found to be polymorphic in three, four and five (all) validation populations, respectively. The segregation of these markers, together with the segregation of SSR marker TxP168 which was found to be polymorphic in one validation population and representing one significant QTL region on SBI-07, is shown in Table 7. Across all segregating populations, the mean difference of PCERGOT for the progeny carrying the AA (IS 8525) and the BB (other parents) allele were found to be highly significant ($P > 0.01$) for the QTL region on SBI-06 and that on SBI-02 was significant at the $P > 0.05$ level. Individually, either a positive trend or a significant marker-QTL association was observed in all but the population B971990/IS 8525 and B972422/IS 8525 where SSR markers TxP274 and Sb6-84 used in validation were found to be not associated with the QTL of PCERGOT.

Discussion

We reported recently the genetics of sorghum ergot resistance in an F_5 recombinant inbred line population

Table 7 Mean percentage ergot infection (PCERGOT) for the individuals in the validation populations carrying AA (IS 8525) allele and BB (other parents of the cross) allele (number of individuals in each and overall populations for the two alleles is shown in parenthesis) at different SSR loci near to the linked marker underlying ergot resistance in sorghum

Population	Marker	Allele (no.)	Mean PCERGOT	*t*-test (*P*-value)
IS 8525/R960535//R960535	Sb6-84	AA (10)	3.77	0.101
		BB (7)	6.50	
	TxP145	AA (6)	2.65	0.036
		BB (11)	6.36	
	TxP274	AA (7)	2.27	0.018
		BB (11)	6.36	
ms3*3_ R931945-2-2//R960535/IS 8525	Sb6-84	AA (11)	7.34	0.048
		BB (26)	13.40	
	TxP274	AA (8)	9.35	0.227
		BB (25)	12.63	
ms3*3_ R931945-2-2//R931945-2-2/IS 8525	TxP274	AA (7)	8.31	0.064
		BB (29)	12.57	
	TxP145	AA (6)	8.101	0.065
		BB (30)	13.940	
	TxP168	AA (10)	10.809	0.238
		BB (26)	13.164	
B971990/IS 8525	TxP145	AA (12)	4.96	0.048
		BB (8)	10.77	
	TxP274	AA (13)	6.98	0.41
		BB (7)	7.85	
B972422/IS 8525	Sb6-84	AA (10)	6.40	0.40
		BB (14)	7.40	
	TxP145	AA (9)	4.40	0.10
		BB (9)	11.19	
	TxP274	AA (11)	5.56	0.16
		BB (10)	10.38	
Overall (3 populations)	Sb6-84	AA (31)	5.89	0.0340
		BB (47)	10.59	
Overall (4 populations)	TxP145	AA (33)	4.96	0.0002
		BB (59)	11.64	
Overall (5 populations)	TxP274	AA (46)	6.44	0.0041
		BB (81)	10.19	

R931945-2-2 × IS 8525 (Parh et al. 2006). In the current study, in a subset of the same population, we were able to map a number of QTL associated with PCERGOT and its two component pollen traits, PQ and PV, which have been previously reported to be implicated with ergot resistance. QTL were detected by CIM using QTL Cartographer. In addition, two other approaches, SMA and QTL Network-2.0 software, were used to verify the QTL-marker association and the chromosomal location of putative QTL identified in the study. For PCERGOT, five genomic regions with significant marker-QTL association were detected based on a permutation-based LOD score of >3.0 using the overall data set. Four of these QTL were also identified with the data sets for groups 1–3, but some of these QTL were detected with a lower threshold level (LOD >2.0). SMA identified the same QTL as QTL Cartographer CIM analysis whereas QTL Network-2.0 identified all but the QTL on SBI-02. QTL Network-2.0, however, identified one epistatic allele in this region of SBI-02. The genomic locations of the identified QTL in both approaches (CIM and QTL Network-2.0) were very similar.

In addition to ergot resistance, five genomic regions for PQ and four for PV were identified using the overall and group data sets by QTL Cartographer's CIM analysis. With only one exception, SMA identified all of these QTL, and QTL Network-2.0 identified some of these QTL for PQ and PV, however, with less statistical significance. This is not unlikely, given that the statistical methodology and the power of the test statistic used for the QTL analysis in the three different approaches are different.

The QTL-marker associations identified for ergot resistance in this study were further validated in a number of populations developed by DPI&F. The SSR markers that mapped closest to the reported QTL regions for PCERGOT were used in validation. The overall mean difference of PCERGOT carrying two alternate marker alleles for the two QTL regions on SBI-06 and SBI-02 was found to be significant at $P > 0.01$ and >0.05 levels, respectively. In individual population either a significant or a positive trend of the QTL-marker association was maintained suggesting that the QTL-marker associations reported for the two genomic regions in the mapping population are real. One exception, however, were markers TxP274 and Sb6-84, which were found to not be associated with ergot resistance in the population B971990/IS 8525 and B972422/IS 8525, respectively. Possible reasons for the non-association in these two populations include linkage disequilibrium not being maintained either due to recombination between the QTL and the marker used for validation or the introgressed QTL being linked to unfavourable effects for major agronomic traits. The consistency of QTL in different genetic backgrounds was less obvious particularly when there are several recipient parents as reported by Sebolt et al. (2000) and Yousef and Juvik (2002). Furthermore, the QTL on SBI-02 linked to the marker sPb-7702 and validated by TxP274 was not identified by QTL Network 2.0 but QTL Network 2.0 did detect a pair of epistatic alleles, one of which, sPb-1925, maps near to the QTL allele as identified by the CIM analysis on SBI-02. QTL allele and epistatic loci are interchangeable depending on the genetic background as reported by Liao et al. (2001) but our results suggest that it could happen due to method of analysis too. Most of the other SSR markers near to the QTL regions were not found to be polymorphic in the validation populations except TxP168 on SBI-07 which was found polymorphic only in the population ms3*3_R931945-2-2// R960535/IS 8525. However, this marker only showed a positive trend for QTL-marker association in this validation population. Further experimentation is necessary to validate the other QTL regions reported in this study.

QTL for PCERGOT and pollen traits in this study were determined by a number of data sets. Each data set in this study is representative of a different environment being influenced by variable temperature and humidity as reported earlier by Parh et al. (2006). Both PCERGOT and the pollen traits were reported previously to be influenced by G × E interactions (McLaren 1992; McLaren and Flett 1998; Wang et al. 2000). Therefore, it is not surprising that some QTL for these traits were detected only in one or a few environments. However, genomic regions with the fewest QTL × environment interactions and high genotypic main effects would be expected to express in the same way across different environments (Hittalmani et al. 2003). QTL for PCERGOT on SBI-01, SBI-02 and SBI-06 and SBI-07 were identified by both overall and 1–3 group data sets indicating that the expression of QTL in these genomic regions was less influenced by environmental factors. A non-significant additive by environment interaction was observed for the QTL on SBI-01, SBI-06 and SBI-07 plus the QTL on SBI-08 when analysis was performed across group data sets using QTL Network-2.0 which also indicated that these genomic regions were less influenced by environmental factors (data not shown). The number of QTL expressed was the highest with G1 data set in which three additional QTL were identified in this environment compared to one additional QTL with the G2 and G3 data sets. The mean PCERGOT of RILs was the highest with the G1 data set and was influenced by the lowest mean maximum and minimum temperatures and highest relative humidity as reported earlier by Parh et al. (2006). Our data therefore suggests that a number of additional QTL may need to be exploited by breeding programs to develop ergot resistance varieties for ergot favourable environments being characterised by low temperature and high humidity.

The expression of QTL for PQ was consistent across environments. Three QTL on SBI-04, SBI-06 and SBI-07

were identified using the overall data set as well as 1–2 group data sets indicating the stability of expression of QTL across environments. Two other QTL for PQ identified on SBI-01 and SBI-10 were specific to the G1 and G2 environments, respectively. By contrast, QTL for PV exhibited considerable variability in their expression. The QTL identified by the overall and G3 data were mapped either on a different chromosome or on the same chromosome but some distance away. The estimated heritability value of G3 data (0.339) for PV as reported earlier by Parh et al. (2006) also suggests that the expression of this trait is environmentally prone.

One of the important objectives of this study was to elucidate the genetic basis of ergot resistance and to explore the relationship of two pollen traits, PQ and PV. The genetic correlation found between PCERGOT and the two pollen traits, as reported previously by Parh et al. (2006), was moderate. These genetic correlations could be explained by the co-localisation of some of the QTL for PCERGOT and/or PQ and PV, either due to pleiotropy or linkages of various degrees. Most of the other QTL for PCERGOT mapped independently of the QTL for the two pollen traits, PQ and PV, at a distance of more than 40 cM. These observations indicate that a certain part of the variation for ergot resistance in this population may be explained by pollen traits but that the majority of variation is non pollen-based, as reported previously by Parh et al. (2006) and Dahlberg et al. (2001). However, a non pollen-based mechanism of ergot resistance (Dahlberg et al. 2001), reported during the course of the current study, was not examined in this investigation and awaits further investigation.

When multiple QTL control a trait, alleles of positive and negative effect (increasing or decreasing trait value) tend to be dispersed between parents, each with positive alleles at one or some loci and negative alleles at other loci. These dispersed alleles can be cryptic transgressive, and can be found even in parents with similar phenotype (Ungerer et al. 2002; Xu 2002). It is such extreme transgressive individuals that are often of greatest value to plant breeding. Plant breeding is directed towards accumulating such alleles (favourable alleles) for a trait by exerting selection. In the mapping population, most of the favourable QTL alleles underlying ergot resistance were contributed by the resistant parent, IS 8525, as expected. Of the nine QTL identified for PCERGOT, only two QTL specific to the group data and linked to the AFLP loci, AGG + CAA6 and AAG + CTG5 came from the susceptible parent, R931945-2-2. With the exception of one QTL identified by the group data on SBI-09, the additive effect of the QTL contributed by the resistant parent ranged from 1.79 to 2.40 indicating that none of the QTL identified has a large individual effect on ergot resistance and the effect is quantitative in nature. This is supported by the quantitative genetic analysis where a normal distribution was observed for the phenotypic data of PCERGOT (Parh et al. 2006). The additive effect of the QTL identified on SBI-09 differed substantially for the two group data, 4.48 (G1) versus 2.55 (G2), which could be computationally biased as there is a large gap in this genomic region of SBI-09.

Epistasis has been reported to be an important component of the genetic basis of complex traits (Li et al. 1997; Yu et al. 1997; Xing et al. 2002). However, our study suggests that epistasis is relatively unimportant compared to the additive effects controlling ergot resistance in this population of sorghum. This is consistent with our previous observations based on phenotypic data (Parh et al. 2006). We would not, however, rule out the effect of the small population size used in this study which might restrict the detection of epistatic alleles when compared to using a larger population. A genome wide scan for epistasis indicated that only a single pair of marker loci was involved in digenic interactions controlling PCERGOT. However, this interaction did not appear to have a "main" effect on PCERGOT at the single locus level; a main effect QTL was found to be located nearby on SBI-02 as identified by QTL Cartographer CIM analysis. A direct implication of epistasis, especially the involvement of QTL in the epistatic interactions, is that the effects of the single-locus QTL are mostly dependent on the genotypes of the other loci. However, in this analysis, the epistatic allele contributed only a small part of the total phenotypic variance of ergot resistance as indicated from the heritability estimate (3.9%) by QTL Network 2.0. Thus the proportion of phenotypic variation negated by the genotypes of the second locus would be less compared to the main effect of the QTL allele, assuming that the QTL identified by QTL Cartographer CIM is the same as that of the epistatic loci identified by QTL Network-2.0.

Clustering of resistance genes has been reported in a number of studies (George et al. 2003; Klein et al. 2001; McMullen and Simcox 1995). It is interesting to note that some of the QTL for ergot resistance identified in this study mapped in regions containing QTL for resistance to other diseases in sorghum. For instance, previous research has shown that QTL for a number of sorghum diseases including grain mould, anthracnose, zonate leaf spot, and bacterial leaf spot are located on SBI-06 (Klein et al. 2001), near to the region which we have shown contains a significant QTL for ergot resistance (PCERGOT). Three other regions on SBI-07 and SBI-10 and SBI-08 that are known to contain QTL for grain mould and rust (Klein et al. 2001; Tao et al. 1998) also appear to contain a QTL for ergot resistance. The question of whether these resistance genes are functionally related or not is unclear as the biochemical and physiological bases of resistance to these pathogens have yet to be elucidated in sorghum.

Ergot continues to be a threat to the sorghum industry in Australia and worldwide. The incidence and severity of the disease is highly environmentally dependent and screening for resistance is a cumbersome business. The five QTL identified for ergot resistance in this study on SBI-01, SBI-02, SBI-06, SBI-07 and SBI-08 linked to the markers sPb-8261, sPb-7702, sPb-1543, TxP168 and AGG + CAG6 were identified by the overall data set with high LOD score (>3.0). Except for the QTL on SBI-02, the other QTL showed non-significant additive by environment interaction indicating their stability across environments. We were also able to validate two QTL regions on SBI-02 and SBI-06, though some variation with the genetic background exists. The QTL on SBI-02 was not identified by QTL Network 2.0 but an epistatic allele was identified nearby. So caution should be undertaken when utilising the QTL on SBI-02 (assuming epistatic allele is the QTL allele identified by CIM) in a breeding program as to its desired effect will also depend on the interacting epistatic allele. The QTL on SBI-01, SBI-06 and SBI-07 were also identified by 2–3 group data sets indicating that these are the major QTL regions underlying ergot resistance in sorghum. These five QTL collectively explain about 53.7% of the total phenotypic variation for PCERGOT. Additionally, QTL specific to the group data representing different environments explain a substantial portion of phenotypic variance for PCERGOT. The study also revealed that two of the QTL for PQ and/or PV are located on SBI-01 and SBI-06. Therefore, identification and analysis of candidate genes to deduce information about the nature and function of the detected loci for these traits will be helpful in determining the genetic architecture of resistance to sorghum ergot.

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