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Association analysis of charcoal rot disease component traits in sorghum minicore germplasm with EST-SSR markers

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Abstract

Sorghum is an important crop of semi arid regions where it suffers from several biotic stresses. Among biotic stresses, charcoal rot/stalk rot is a major constraint for *rabi* (post rainy) sorghum production. Identification of stable resistant sources and incorporation of the genes or quantitative trait loci (QTL1) governing resistance are the prerequisite to make progress in breeding for charcoal rot resistance. A set of 242 sorghum minicore collection were phenotyped in sick plot of charcoal rot disease. A total of 31 polymorphic EST-SSR markers were developed and mapped through association analysis. The population structure analysis indicated the partitioning of the genetic population structure into four clusters. In the current study six new markers were identified for charcoal rot disease resistance (*Xiabt 210, Xiabt 527, Xiabt 301, Xiabt 37, Xiabt 77, Xiabt 81*) and was validated previously identified flanking markers for charcoal rot disease QTLs (locus *Xiabt 275*) at RARS Vijayapura location. Six marker trait associations were stable at two locations and these significant associations are useful and suitable for marker assisted selection in charcoal rot disease resistance breeding programs.

Key words: Sorghum, charcoal rot disease, QTLs, association analysis

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth major cereal crop of the world grown in arid and semi arid tropics, including India. It is well adapted to low input and low moisture situations and thereby meet food, nutrition of humans and fodder requirements of cattle. In India sorghum is grown both during south west monsoon (*kharif*) and post monsoon season (*rabi*). Even though *rabi* sorghum is highly valued because of its excellent grain and fodder quality, the grain productivity in India is lower (819 kg/ha) than *kharif* sorghum (1100 kg/ha) (Usha and Chithra, 2015). Low levels of productivity in *rabi* sorghum has been due to diseases caused by fungal pathogens and are greatest concern next only to moisture stress. Among fungal diseases charcoal rot disease has a potential to cause total crop lodging especially when there is a dry spell during grain filling and maturation stage. In India, *rabi* sorghum generally experiences a receding moisture situation during maturation, which indeed is congenial for charcoal rot. Charcoal rot is caused by soil born fungi *Macrophomina phaseolina* Tassi (Goid) and first indication of the disease is lodging of plants at basal node as they approach maturity. The fungus generally disintegrates the pith and characteristic black patchy charcoal like appearance occurs on the vascular tissue. Generally the plants show senescence and lodge due to stem breaking at or just above ground level (Reed et al. 1980).

Earlier studies have reported significant genetic variability for reaction to charcoal rot or stalk rot pathogens, identified sources of resistance and determined the mode of inheritance of the traits for charcoal rot (Sahib et al. 1990; Pecina-Quintero et al. 1999; Tesso et al. 2005). Many of the resistance sources were identified in stay-green backgrounds, because previous studies showed strong association between charcoal rot resistance and stay-green mediated post flowering drought tolerance. Therefore, breeding efforts to improve the traits have mainly focused on indirect selection for the stay-green trait. Although this approach has worked well, it obviously leaves out potential resistance sources that may be available in non stay-green backgrounds. Studies on

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the reaction of sorghum to charcoal rot suggested that both dominant and recessive genes were involved in active resistance (Bramel-cox and Clafin, 1989). Therefore, resistance for complex disease like charcoal rot can be achieved if we opt for the quantitative resistance through breeding. The application of molecular markers allows us to understand and identify quantitative trait loci involved in disease resistance. In sorghum, studies have used specifically designed populations to analyse and find QTLs associated with traits of economic significance in sorghum including plant height and maturity (Pereira and Lee, 1995), characters concerned with plant domestication (Patterson et al. 1995), disease resistance (Gowda et al. 1995) and drought tolerance (Tuinstra et al. 1998). Similarly at UAS Dharwad, 93 RILs derived from IS22380 × E36-1 were evaluated at different locations and stable QTLs were identified for charcoal rot disease resistance with a variety of molecular markers over time and space (Reddy et al. 2008; Patil et al. 2012).

Association analysis a novel tool for QTL detection involves search for a marker significantly co-segregates with any trait in a diverse collection of germplasm or breeding materials (Myles et al. 2009). This approach exploits the historical recombination events that have occurred in natural populations, thereby reducing the cost and time taken to develop segregating populations such as F_2, double haploid or back-cross population and examination of a higher proportion of polymorphic molecular markers which provides better genome coverage than bi-parental population which depends on restricted allelic variation with a small number of recombination events (Flint-Garcia et al. 2005; Jannink et al. 2001). The major concern is false discovery attributed to spurious associations caused by population stratification and unequal relatedness among individuals in a given population (Abiola et al. 2003). Population stratification was initially addressed using general linear model (GLM) based methods such as structured association (Pritchard et al. 2000), genomic control (Devlin and Roeder 1999) and family-based tests of association (Abecasis et al. 2000). Markers that have statistically significant association with any trait facilitate and speed up selection of disease resistant and high yielding varieties through marker assisted selection (MAS).

Previously minicore collection of sorghum germplasm have been screened with SSR markers flanking through three charcoal rot resistance QTLs (*qCr1,qCr2,qCr3*), which displayed variation in combination of QTLs for charcoal rot disease resistance. Some of the sorghum accessions (IS 30533, IS 12735, IS 29950) showed highly resistant response even in the absence of one or two of these QTLs. There may be still unknown genomic regions responsible for charcoal rot resistance in sorghum. Therefore, in the present study an attempt was made to identify marker trait associations for component traits of charcoal rot resistance.

Materials and methods

Seed material

For phenotypic evaluation, a collection of 242 accessions of the sorghum minicore was obtained from the Genetic Resources Division, ICRISAT, Patancheru, India. The minicore included all five basic races (number is given in parenthesis) [*bicolor* (20), *caudatum* (39), *durra* (30), *guinea* (29) and *kafir* (21)] and 10 intermediate races [*caudatum-bicolor* (30), *durra-bicolor* (7), *durra-caudatum* (19), *guinea-bicolor* (2), *guinea-caudatum* (27), *guinea-durra* (2), *guinea-kafir* (3), *kafir-bicolor* (2), *kafir-caudatum* (7) and *kafir-durra* (4)]. Two check varieties for evaluation of charcoal rot incidence *i.e.,* SPV 86 (a high yielding variety which is highly susceptible to charcoal rot) and E36-1 (resistant to charcoal rot and a stay green source adapted to tropics) were used along with above mentioned minicore accessions for the field evaluation.

Phenotyping for charcoal rot disease component traits

The above listed materials were evaluated at Regional Agricultural Research Station (RARS), Vijayapura during post-rainy season of 2014-15 for charcoal rot disease component traits. Randomized complete block design (RCBD) was adapted on a total area of 32 x 16 m^2 with two replications. A replication consisted of 4 blocks, each with 61 lines, planted randomly. Each accession was planted in single row with 15 plants with a spacing of 45 x 15 cm in the month of October. Evaluation was under natural sick plot and typical receding moisture situation i.e., no rains from pre-flowering stage of the crop till the end which is congenial for the stalk rot manifestation. Observations were made 20 days after complete grain filling (complete maturity) on three parameters, *viz*., per cent lodging, number of internodes crossed by the fungi and length of infection on individual plant basis. The number of lodged plants at the time of observation was counted. The stems of all plants were slit open to measure the length of infection (cm) and the number of internodes crossed by the rot. Phenotypic data

analyses like analysis of variance (ANOVA), estimation of phenotypic and genotypic coefficients of variation (GCV and PCV), heritability (h^2), phenotypic correlation and genetic advance as percent of mean (GAM) were carried out for all the traits using Windostat version 8.

Genotyping

For genotyping 40 EST-SSR markers were selected from a set of 63 EST-SSRs developed by mining publicly available sorghum ESTs at NCBI (Arun 2006) and mapped at IABT UAS-Dharwad (Patil et al. 2012). These markers had reasonable coverage on sorghum genome representing all ten linkage groups of sorghum. Genomic DNA was prepared from leaf samples which were collected from fifteen to twenty day old seedlings of individual plants following the method of Krishna and Jawali (1997). PCR was set up with 20 μl reaction mixture comprising 100 ng of template DNA, 10 Pm each of forward and reverse primers, 2.5 mM dNTPs, 10X PCR buffer and 3U of *Taq* polymerase (New England Biolabs). PCR reaction was carried out using Master Cycler gradient 5331(Eppendorf, Germany) with initial denaturation at 94°C for 3 min, annealing temperature for 30 sec, primer extension at 72°C for 30 sec repeated for 40 cycles. Separation and visualization of PCR products was done on agarose (3%) gel followed by Ethidium bromide staining (Etbr). Only clear and unambiguous bands of EST-SSR markers were scored. Markers were scored for the presence and absence of the corresponding band among the accessions. Polymorphism information content (PIC) was calculated according to Nei (1973). Population structure was estimated by the Structure v. 2.2 Program (Pritchard et al. 2000) using the allelic frequencies of polymorphic markers. The hypothetical number of expected populations (K) was set to range from 1 to 10. Data was processed by an admixture model using a burn-in Main Agricultural Research Station period of 5000 and iterations of 10. The presented clusters were corrected according to Evanno et al. (2005). For marker and trait association analysis, phenotypic data obtained from two locations, *viz.,* MARS, Dharwad and RARS, Vijayapura were considered. Marker and trait association analysis was tested using the General Linear Model (GLM) in the Tassel v. 2.0.1 software program (Bradbury et al. 2007). Values of the Q matrix obtained in Structure were presented as covariates. The *P* value determines whether a trait is associated with the marker and R^2 for a marker evaluates the magnitude of trait effects.

Results

Phenotypic analysis

Analysis of variance revealed highly significant differences among 242 sorghum minicore collection for component traits of charcoal rot disease, *viz.,* per cent lodging, number of internodes crossed, length of infection of fungus and plant height. For plant height significant variation ranged from 113.4 to 360.8 cm. High variation with a minimum of 0 and maximum value of 93.53 per cent was recorded for per cent lodging. The length of fungal infection varied from 1.35 to 50.8 cm and number of internodes crossed varied with a minimum of 3.58 and maximum value of 1.15, respectively. High PCV, GCV (>20%) with high heritability (>60%) was observed for plant height and percent lodging whereas length of infection and number of internode crossed were observed with high PCV, GCV with moderate heritability (Table 1). The charcoal rot component traits showed significant positive correlation among them. But percent lodging and length of infection was positively correlated with plant height whereas number of internode crossed was negatively correlated with plant height (Table 2).

Table 1. Genetic variability parameters of charcoal rot disease component traits in sorghum minicore collection

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
	Minimum	Maximum					
PL	113.4	360.80	186.52	23.43	21.54	84.55	40.81
LD%	0.0	93.5	22.57	32.35	31.50	94.3	63.23
SP	1.35	50.8	18.17	31.41	30.87	48.3	22.72
NIC	1.15	3.58	2.07	27.39	20.96	58.54	33.03

PL=Plant height (cm), LD%=Percent lodging, SP=Length of infection (cm), NIC=Number of internodes crossed, PCV= Phenotypic coefficient of variation, GCV= Genotypic coefficient of variation

Table 2. Phenotypic correlation coefficients among charcoal rot component traits in sorghum minicore collection

Trait	PL	LD%	SP	NIC
PL	1	0.040	0.002	–0.010
LD%		1	0.855**	0.263**
SP			1	0.364**
NIC				1

**Significant at 1% probability level; PL=Plant height (cm), LD%=Percent lodging, SP=Length of infection (cm), NIC=Number of internodes crossed

Genotypic analysis

Among the 40 selected EST-SSR markers, 31 were polymorphic among the sorghum minicore. These 31 markers were used to evaluate allele diversity. The size of the amplicons ranged from 50bp to 500bp. A total of 129 alleles were observed among minicore accessions with a minimum of two alleles with the marker *Xiabt243* and a maximum of eight alleles were amplified by the marker *Xiabt224* with an average of four alleles per locus. The polymorphism information content for the SSR loci in this study ranged from 0.1945 (*Xiabt405*) to 0.6177 (*Xiabt389*) with a mean of 0.475. In the present study, 14 markers were identified to have PIC value of 0.5 which are considered to have genotype discriminating power (Table 3).

Analysis of population structure

Population structure was tested by checking the number of sub-populations (K) from one to ten (K = 1 to K =10) by following a modified method proposed by Evanno *et al.* (2005). In this case DK (delta K) was plotted against the number of subpopulations (K). The analysis showed the presence of the highest DK value at K=4, indicating a deep partitioning of the genetic population structure into four clusters. The population structure obtained at K=4 is depicted in Fig. 1. The four subpopulations were classified on major race group such as Q1-race *guinea*; Q2-race *kafir*; Q3-race *durra* and Q4-race *caudatum*. From the total number of accessions grouped as subpopulation Q1, 20.7% belonged to the race *guinea*, followed by *durra, durra-caudatum, durra- bicolor, caudatum, caudatum- bicolor, bicolor and kafir*. 16.6% of the accessions grouped as subpopulation Q2 corresponded to the race *kafir* followed by *kafir-bicolor, kafir-caudatum, kafir-durra*, *guinea, guinea - caudatum, guinea-kafir, durra, durra-caudatum, durra-bicolor, caudatum, caudatum-bicolor and bicolor.* From the total number of accessions grouped that corresponded to subpopulation Q3, 16.6% belonged to the race *durra* and followed by *durra-caudatum, durra-bicolor, caudatum, caudatum- bicolor, bicolour, guinea, guinea - bicolor, guinea - caudatum,*

Table 3. Polymorphism information content (PIC) for EST-SSR markers screened across the sorghum minicore collection

S.No.	Marker	Amplicon size in (bp)	No. of alleles	PIC
1	*Xiabt 30*	100-130	3	0.3234
2	*Xiabt 47*	250-300	3	0.3666
3	*Xiabt465*	150-230	3	0.3623
4	*Xiabt457*	200-250	4	0.5349
5	*Xiabt445*	180-250	4	0.4899
6	*Xiabt420*	200-300	3	0.3791
7	*Xiabt405*	150-200	3	0.1945
8	*Xiabt527*	290-300	4	0.4127
9	*Xiabt81*	130-150	4	0.4705
10	*Xiabt37*	170-185	4	0.5153
11	*Xiabt389*	200-500	5	0.6177
12	*Xiabt380*	200-300	4	0.5035
13	*Xiabt378*	150-200	5	0.5955
14	*Xiabt374*	190-230	4	0.5688
15	*Xiabt72*	150-200	5	0.5389
16	*Xiabt349*	180-200	3	0.3837
17	*Xiabt322*	170-190	5	0.4970
18	*Xiabt397*	170-230	4	0.5130
19	*Xiabt301*	250-380	6	0.5996
20	*Xiabt77*	200-300	5	0.5798
21	*Xiabt80*	190-220	3	0.3850
22	*Xiabt82*	180-200	4	0.5356
23	*Xiabt65*	50-100	4	0.3997
24	*Xiabt85*	200-300	5	0.5812
25	*Xiabt62*	150-180	5	0.5388
26	*Xiabt87*	200-250	3	0.3621
27	*Xiabt275*	150-250	6	0.5465
28	*Xiabt243*	100-130	2	0.3697
29	*Xiabt241*	200-250	4	0.4880
30	*Xiabt210*	200-150	4	0.4637
31	*Xiabt224*	350-100	8	0.6164
Total number of alleles			129	
Maximum value			8	0.6177
Minimum value			2	0.1945

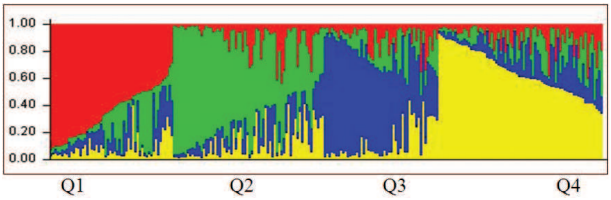

Fig. 1. Population structure plot of 242 sorghum minicore accessions based on 31 EST-SSR markers under the assumption of subpopulation *K* = 4. Minicore accessions are represented by a bar which is divided into several parts with different colors according to the accessions estimated fractions of the four clusters. The *x*-axis indicates the accession and numbers on the *y*-axis shows the group membership in percent. Q1=First subpopulation, Q2=Second subpopulation, Q3=Third subpopulation and Q4=Fourth subpopulation

guinea – durra, kafir and *kafir-bicolor*. 33.3% of the accessions grouped as subpopulation Q4 corresponded to the race *caudatum* followed by *caudatum- bicolor, durra, durra- caudatum, durra-bicolor, guinea*, *guinea - caudatum, guinea-bicolor, kafir, kafir-bicolor, kafir-caudatum.*

Association analysis

Since the same minicore collection was evaluated earlier in sick plot at MARS, Dharwad (Borphukan, 2014). The phenotypic data obtained in this location was also considered for analysis. The markers which showed association with a phenotype at both the location (MARS, Dharwad and RARS, Vijayapura) were considered as stable (Table 4). The genotypic data of 31 EST-SSR markers used in the present study amplified a total of 129 alleles, however after filtering the minor alleles (with allele frequency less than 1%) only 78 alleles could be finally used for association studies. Of these, 6 markers *Xiabt 210, Xiabt 527, Xiabt 301, Xiabt 37, Xiabt 77 and Xiabt 81*) showed associations with traits at both locations. *Xiabt 527* showed significant association with all the three component traits of charcoal rot resistance at both the locations. R^2 value for the component traits of charcoal rot resistance ranged between 2-3.5%. Charcoal rot component traits such as per cent lodging and length of infection were significantly associated with *Xiabt 210* and *Xiabt 77.* The maximum R^2 values for per cent lodging ranged from 2.4-6.5% in *Xiabt 210* and it was 2.8-3.1% in *Xiabt 77*. In addition, three markers showed association with only one component traits of charcoal rot *viz., Xiabt37* showed association with per cent lodging with R^2 values ranging from 2.1-2.7%, *Xiabt81* with length of infection with R^2 values ranging from 2.8-3.7% and *Xiabt 301* with number of internode crossed with R^2 values ranging from 2.6-4.3%.

Discussion

Genetic improvement of *rabi* sorghum is hindered by lack of phenotypic variability as most of the *rabi* sorghum varieties are of *durra* type where as *kharif* cultivars belong to *caudatum* and *kafir* races. Previous efforts for breeding *rabi* sorghum with drought and disease resistance utilised mainly elite breeding lines with a narrow genetic base. Utilization of available germplasm can enhance the level of resistance to charcoal rot as it covers all the allelic combinations. Evaluation, characterization and utilization of exotic germplasm resources in breeding programs to enhance the diversity of cultivars, may nullify the bottlenecks in crop breeding program. Studies on reaction of different sorghum genotypes to charcoal rot suggested the involvement of both dominant and susceptible genes (Tenkouano et al. 1993) and involvement of many genes each with little effect and highly influenced by the environment. Phenotypic selection for such traits will be generally difficult. Selection based on markers could theoretically facilitate the manipulation of such trait without affecting other important agronomic traits. Further it is advantageous because selection can be excised even in the absence of ephiphytotic condition.

High values of GCV and PCV were found for plant height, length of infection and per cent lodging which indicated variation for these characters and their contribution towards total genetic variability. For disease related trait, *viz.,* number of internodes crossed, PCV was higher than the GCV showing moderate variation among the minicore accessions. Earlier reports (Reddy et al. 2008) indicated moderate variation for number of internode crossed in sorghum minicore collection, evaluated at MARS Dharwad. High estimates of broad sense heritability noticed for per cent lodging and plant height indicates that these traits were less subjected to environmental influences. Moderate heritability was observed for length of infection and number of internode crossed by *M. phaseolina.* High heritability coupled with high genetic advance over mean was observed for the traits, *viz.,* plant height and percent lodging indicates that additive gene effects are operating and selection for superior genotype is possible. However, moderate heritability

Table 4. Marker loci and their alleles significantly associated with charcoal rot traits in sorghum minicore collection

Trait	Marker	Allele (bp)	PARS Vijayapura		MARS Dahrwad	
			P value (0.01)	R^2 (%)*	P value (0.01)	R^2 (%)*
Per cent lodging	*Xiabt 210*	200	0.000053	6.5	0.0078	2.9
	Xiabt 210	180	0.00783	4.2	0.0141	2.4
	Xiabt 210	150	0.01412	2.9	0.0039	3.4
	Xiabt 527	300	0.002486	2.0	0.0142	2.2
	Xaibt 527	250	0.000264	5.4	0.0014	3.8
	Xiabt 81	150	0.00438	3.3	-	-
	Xiabt 81	100	0.0057	3.1	-	-
	Xiabt 65	100	0.0129	2.5	-	-
	Xiabt 65	50	0.0077	2.9	-	-
	Xiabt 37	175	0.002322	2.1	0.0097	2.7
	Xiabt 275	175	0.00048	4.9	-	-
	Xiabt 77	200	0.00832	2.8	0.0087	3.1
	Xiabt 301	300	0.01449	2.4	-	-
	Xiabt 380	200	-	-	0.0151	2.4
Length of infection	*Xiabt 210*	200	0.000365	5.1	0.0068	3.0
	Xiabt 210	150	0.00109	4.3	0.00042	5.1
	Xiabt 527	250	0.00173	4.0	0.0102	2.4
	Xiabt 527	280	0.00256	3.7	0.0025	3.7
	Xiabt 37	200	0.00785	2.9	-	-
	Xiabt 37	175	0.002206	2.1	-	-
	Xiabt 301	300	0.01349	2.5	-	-
	Xiabt 389	250	0.01377	2.5	-	-
	Xiabt 77	200	0.01446	2.4	0.0097	2.7
	Xiabt 275	200	0.002512	2.0	-	-
	Xiabt 275	175	0.00083	4.5	-	-
	Xiabt 81	100	0.00242	3.7	0.0024	3.7
	Xiabt 81	150	0.00244	3.7	0.0086	2.8
	Xiabt 380	200	-	-	0.0190	2.0
No. of internode crossed	*Xiabt 210*	200	0.00175	4.0	-	-
	Xaibt 210	150	0.00985	2.7	-	-
	Xiabt 81	175	0.00399	3.4	-	-
	Xiabt 37	200	0.00615	3.0	-	-
	Xiabt 37	175	0.00947	2.7	-	-
	Xiabt 301	380	0.00869	2.8	0.0113	2.6
	Xiabt 301	300	0.01103	2.6	0.0008	4.3
	Xiabt 457	250	0.01844	2.2	-	-
	Xiabt 457	200	0.002167	2.1	-	-
	Xiabt 527	250	0.00309	3.5	0.0115	2.5
	Xiabt 77	200	-	-	0.0119	2.5

GLM- General linear model (based on Q-matrix), Markers used for analysis are EST-SSR and are named as *Xiabt* and markers associated with the trait at significant *P value <0.01 were selected from GLM, R^2 represents the phenotypic variation explained in per cent

coupled with high genetic advance for length of infection and number of internode crossed was observed. All the charcoal rot component traits are found to be significantly and positively correlated with each other. However, per cent lodging has been observed with positive correlation with plant height. These results of positive association between characters indicate that these traits could be utilized for the crop improvement which could enhance the genetic constituent for the upliftment of traits of interest to express better phenotype.

The PIC values of markers can provide an estimate of the discrimination power in a set of accessions by taking not only the number of alleles, but also the relative frequencies of each allele (Smith et al. 2000). All except one EST-SSR locus had PIC values greater than or equal to 0.5 with a mean of 0.475. The average PIC value of EST-SSR markers is similar with previous studies using EST-SSR markers for genetic diversity analysis in other crops, e.g., 0.443 in bread wheat (Gupta et al. 2003) and 0.45 in barley (Thiel et al. 2003). However, the average PIC value was lower compared to the PIC values (0.62) of genomic SSR markers in sorghum reported earlier by Mbeyagala et al. (2012) and Adugna (2014). Generally it is shown that high PIC values and large number of alleles per markers for genomic-derived SSRs were significantly higher than EST-SSRs, as indicated by the reports on flax wheat (Eujayl et al. 2002), sunflower (Pashley et al. 2006) and sugar beet (Laurent et al. 2007), levant cotton (Jena et al. 2012). Furthermore, EST-SSR markers originated from highly conserved genomic regions, which may present lower degree of polymorphism compared to microsatellites originating from genomic libraries (Varsheny et al. 2005).

Association analysis is strongly affected by factors like selection, population structure and family relationships which may lead to incorrect marker and trait association (Buckler and Thornsberry 2002). Therefore in the present study an attempt was made for marker and trait association analysis adopting GLM along with population structure to avoid false positive association. The number of

sub-populations was obtained based on the delta K value derived from Evanno's method (Evanno et al. 2005). At K=4, delta K value was found to be maximum and there was deep partioning of population into four sub-populations. The identified four subpopulations were characterised based on major race group such as Q1, which consists of the race *guinea*, Q2 consists of the race *kafir*, Q3 consists of the race *durra*, and Q4 consists of the race *caudatum*. But the race bicolour did not form a major group instead it was found distributed in all the subpopulation.

Brown et al. (2011) reported the population structure of 216 sorghum lines using 434 markers (SNPs and SSRs). In this study, 4 subpopulations were clearly identified corresponding to the four races *guinea, kafir, durra* and *caudatum*, while the *bicolor* race was not identified. Four subpopulations were observed with major race as *guinea, kafir, durra and caudatum* in an association panel consisting of 300 genotypes (Adeyanju et al. 2015). Results suggest that the *bicolor* race is more heterogeneous than the other four sorghum races. Genetic admixture is observed since certain individuals were not clearly identified as members of one of the subpopulations and could belong to another group as well. This phenomenon has also been reported in other studies in sorghum (Casa et al. 2008; Morris et al. 2013).

Identification of QTLs that is present in most of the environments would be important for practical breeding applications. Hence, stability of the QTLs is important for MAS. In the present investigation, the marker trait associations commonly detected at two locations were considered as stable. Some of the markers used in the present study were also included in the previous QTL mapping studies for charcoal rot disease and yield related traits in 98 RILs derived from the cross IS 22380 × E 36-1which were contrasting parents for charcoal rot disease in sorghum. Therefore an effort was made to identify the position of the associated markers in the linkage map constructed earlier.

A marker, *Xiabt527* is associated with all the three charcoal rot disease component traits in minicore collection. It is 89.5 cM away from number of internode crossed QTL (36.8-42.1c M) in linkage group E identified earlier. Similarly *Xiabt 210* showed significant association with per cent lodging and length of infection with relatively high phenotypic variation. The locus *Xiabt 210* is 192.5 cM away from length of infection QTL (96.1-110.1 cM) in linkage group A. *Xiabt 77* was significantly associated with per cent lodging and length of infection in minicore collection and is 6.6 cM away from per cent lodging QTL (1.8-3.9 cM) in linkage group D. Similarly *Xiabt301* was significantly associated with number of internode crossed in minicore collection. It is 235.4 cM away from per cent lodging QTL (1.8-3.9 cM) in linkage group D. *Xiabt37* which is significantly associated with per cent lodging is 84.3 cM away from length of infection QTL (96.1-110.1 cM) in linkage group A.

Two major QTLs for number of internode crossed in 93 recombinant inbred lines (RILs) derived from IS22380 × E36-1 on linkage group B and D were reported by Reddy et al. (2008). The QTL on linkage group B (147.3-164.1 cM) was flanked by the markers *xtxp*297-*xtxp*298 and accounted for 19 % phenotypic variation. Another QTL on linkage group D (33-49.1 cM) was flanked by *xtxp*213-*iabtgs*1 and accounted for 12.5 % phenotypic variation. Likewise one major QTL was detected for length of infection on linkage group A (45.3-55.9 cM) flanked by the markers AC13-AT5 and accounted for 10.7% phenotypic variation. Three QTLs on linkage groups B, D and F and seven QTLs on linkage groups A, B, D and F respectively for length of infection in sorghum have been identified earlier. Reddy et al. (2008) reported one major and one minor QTLs for per cent lodging on linkage groups D and I. They explained a total 18.89 per cent total phenotypic variation. The QTL on linkage group D was located at 0.01 cM from *xtxp*343, and the QTL on linkage group I (25.8-34.5 cM) flanked by *xtxp*176-*xtxp*312. Significant associations found in the present investigation may be useful and suitable for marker assisted selection in charcoal disease resistance breeding.

Authors' contribution

Conceptualization of research (BF, SB); Designing of the experiments (SB, BF, BNHK); Contribution of experimental materials (SB); Execution of field/lab experiments and data collection (BNHK, SB); Analysis of data and interpretation (BNHK, SB); Preparation of manuscript (BNHK, SB).

Declaration

The authors declare no conflict of interest.

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