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SSR allelic diversity in relation to morphological traits and resistance to grain mould in sorghum

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Abstract. Allelic variation at 46 simple sequence repeat (SSR) marker loci well distributed across the sorghum genome was used to assess genetic diversity among 92 sorghum lines, 74 resistant and 18 susceptible to grain mould. Of the 46 SSR markers, 44 were polymorphic, with the number of alleles ranging from 2 to 20 with an average of 7.55 alleles per locus. Genetic diversity among the sorghum lines was high as indicated by polymorphic information content (PIC) and gene diversity values. PIC values of polymorphic SSR markers ranged from 0.16 to 0.90, with an average of 0.54. Gene diversity among the sorghum lines varied from 0.16 to 0.91, with an average score of 0.58 per SSR marker. AMOVA indicated that 12% of the total variation observed among the sorghum lines was accounted for between grain mould resistant and susceptible types. Diversity based on six morphological traits and grain mould scores indicated major roles of panicle type and glumes coverage, followed by grain colour, in clustering of the lines. Seven grain mould resistant/susceptible pairs with dissimilarity indices >0.50, but with similar flowering time, plant height, and panicle type/inflorescence within each pair, were selected for use in developing recombinant inbred line mapping populations to identify genomic regions (and quantitative trait loci) associated with sorghum grain mould resistance.

Additional keywords: diversity, grain mould, sorghum, SSR.

Introduction

Availability of adequate genetic variation is one prerequisite for genetic improvement of crops. An accurate assessment of this variation in a gene pool of potential breeding material provides an objective basis to design efficient and cost-effective crop breeding strategies for sustainable long-term selection gains. Assessment of the degree and distribution of this variation provides a better understanding of evolutionary relationships and permits an objectively targeted utilisation of crop genetic resources for breeding and conservation. Advances in sorghum [*Sorghum bicolor* (L.) Moench] improvement have resulted in the development of high-yielding varieties for diverse agro-climatic conditions. Large-scale commercial cultivation of short-duration rainy season-adapted white-grained sorghum hybrids has contributed significantly to enhanced grain productivity in India. These short-duration cultivars were bred by manipulating maturity genes so that critical stages of crop growth (seedling establishment, flowering, and grain filling) coincide with periods of likely rainfall. However, this has significantly increased the risk of exposure of developing sorghum grain to conditions favouring infection by fungi. Although sorghum grain productivity during the rainy season has increased substantially following commercialisation of these short-duration rainy season-adapted hybrids, the area under sorghum cultivation in India has declined precipitously, in part due to grain mould susceptibility of these modern rainy season-adapted hybrids (Thakur *et al*. 2006).

Grain mould is one of the most important biotic constraints to production of grain sorghum worldwide (Williams and Rao 1981; Thakur *et al*. 2006). The term grain mould is used to describe the diseased appearance of sorghum grain resulting from infection by one or more fungi (Williams and Rao 1981). A complex of pathogenic and opportunistic fungi causes grain mould, and major fungi that are associated with early infection events are *Fusarium* spp., *Cochliobolus lunatus* (anamorph = *Curvularia lunata*), and *Alternaria alternata* (Thakur *et al*. 2003, 2006). Damage resulting from early infection includes reduced kernel development, discoloration of grains, colonisation and degradation of endosperm, decreased grain density, germination, and seedling vigour. Several species of *Fusarium* have been shown to produce mycotoxins, such as fumonisins and trichothecenes, which are harmful to human and animal health (Thakur *et al*. 2006). Developing grain mould resistant sorghum hybrids would provide the most economical disease management option for farmers. Despite long-term efforts made in breeding for resistance to grain mould in sorghum, the advances made in developing rainy season cultivars with adequate levels of resistance combined with farmers- and market-preferred grain traits have been limited. This is because host reaction to

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grain mould fungi is a complex trait and several morphological traits have been shown to be associated with resistance and/or escape (Audilakshmi *et al*. 1999, 2005).

Knowledge of genetic diversity in germplasm and breeding material is very important in planning appropriate strategies for crop improvement, especially for complex traits such as grain mould resistance. However, classification of germplasm accessions based on discrete morphological characters may not provide an accurate indication of their genetic divergence (Menkir *et al*. 1997). Molecular markers have been widely applied to characterise genetic diversity in sorghum germplasm collections and in breeding programs. Their direct application in applied breeding has been emphasised in reports on identification and characterisation of quantitative trait loci (QTL) associated with important traits, such as resistance to diseases and insects, and tolerance to aluminum and terminal drought stress (Oh *et al*. 1994, 1996; Klein *et al*. 2001; Agrama *et al*. 2002; Magalhaes *et al*. 2004; Mittal and Boora 2006; Harris *et al*. 2007; Nagy *et al*. 2007). Among DNA markers, microsatellites (also known as simple sequence repeats, SSRs) remain the markers of choice for practical breeding applications, in particular in developing countries. The SSRs are usually characterised by a high degree of length polymorphism, and are ideal single-locus, co-dominant markers for genetic studies. SSRs have been successfully used to assess genetic diversity in sorghum germplasm and to map QTLs associated with resistance to biotic and abiotic stresses (Agrama *et al*. 2002; Hash *et al*. 2003; Casa *et al*. 2005; Folkertsma *et al*. 2005; Geleta *et al*. 2006; Mittal and Boora 2006).

The objective of this study was to assess genetic diversity among selected grain mould resistant and susceptible sorghum lines, and identify parental pairs for developing new mapping populations for grain mould resistance, based on both genetic and morphological variation.

Materials and methods

Plant material

In total, 156 sorghum germplasm accessions and 50 advanced breeding lines, along with grain mould susceptible checks CSH 9, CSH 16, SPV 104, Bulk Y, and 296B, were evaluated in the sorghum grain mould nursery at ICRISAT-Patancheru during the 2005 and 2006 rainy seasons (June–September). Data were also recorded for various morphological traits: days to flowering (DTF, recorded as time from seedling emergence to complete panicle emergence of 50% of the plants), plant height (in cm, measured from base of the plant to the tip of the panicle at maturity), panicle type (recorded as density or panicle compactness at maturity), glume coverage (measured at maturity as percentage of the length of the grains covered by glumes), glume colour, and grain colour at the time of maturity. Ninety-two morphologically diverse lines having similar panicle grain mould ratings (PGMR) in 2005 and 2006 were selected for diversity analysis using SSR markers. Of the 92 selected lines, 74 were resistant (PGMR <3) and 18 susceptible (PGMR >7) to grain mould (Appendix 1).

DNA extraction

For total genomic DNA extraction, 15–20 seeds of each selected accession were sown in a small plastic pot, watered, and allowed to grow for 14 days, until the seedlings were ~10 cm in height. Leaf tissues from all seedlings of a given accession were pooled and a sample of ~30 mg of leaf tissues was used for DNA isolation using a 3% CTAB mini-prep method (Mace *et al*. 2003) in a 96-well format. The quality and quantity of each DNA sample were determined based on agarose gel electrophoresis using uncut-λ DNA standards of known concentration and subsequently diluted to a working concentration of 2.5 ng/μL.

SSR amplification and capillary electrophoresis

A set of 46 sorghum SSR primer pairs, detecting single-copy SSR loci well distributed across the sorghum nuclear genome, was used for genotyping (Table 1). This included a set of 39 sorghum SSR primer pairs that was used for fingerprinting a composite germplasm collection of 3365 sorghum accessions in a Generation Challenge Program project (http://gcpcr.grinfo.net/index.php?app=datasets&inc=files_list). Primer pairs for the SSR markers used were previously defined by Brown *et al*. (1996) (*Xgap072*, *Xgap084*, and *Xgap121*); Taramino *et al*. (1997) (*XSbAGA01*); Kong *et al*. (2000) (*Xtxp012*, *Xtxp015*, *Xtxp021*, *Xtxp025*, and *Xtxp031*); Bhattramakki *et al*. (2000) (*Xtxp065*, *Xtxp088*, *Xtxp114*, *Xtxp136*(*Kaf3*), *Xtxp141*, *Xtxp265*, *Xtxp274*, *Xtxp278*, *Xtxp312*, *Xtxp320*(*phyB*), *Xtxp321*, *Xtxp348*, and *Xtxp354*); Schloss *et al*. (2002) (*Xcup06*, *Xcup11*, *Xcup14*, *Xcup28*, *Xcup53* and *Xcup61*); Ramu *et al*. (2009) (*Xisep0107*, *Xisep0310*); and CIRAD (2 *Xgpsb* and 12 *XmSbCIR* markers) (http://gcpcr.grinfo.net/index.php?app=datasets&inc=files_list). Forward primers were labelled with 6-carboxyflourescein (6-FAM), 4,7,2′,4′,5′,7′-hexachloro-6-carboxyflourescein (HEX), or 7′,8″-benzo,5′-fluoro-2′,4,7-trichloro-3-carboxyfluorescein (NED), allowing post-PCR pooling of the amplified products. PCR conditions, genotyping on an ABI 3700 Genetic analyzer (Applied Biosystems), and further analyses with associated software were done as described by Folkertsma *et al*. (2005). Repeatability of each PCR and capillary electrophoresis run was verified by including a control sample (BT×623) in every assay. In sorghum, many SSRs were originally isolated from BT×623 and this genotype has been used as a reference for sorghum molecular genotyping (Bhattramakki *et al*. 2000; Kong *et al*. 2000), BAC library development (Klein *et al*. 2000), and genome sequencing (Paterson *et al*. 2009).

Data analyses

All SSR markers showed high reproducibility, with high consistency in size of the amplified product of the control sample (BT×623) between PCR and ABI runs. Therefore, all 46 markers were included in the initial analysis. Raw allele sizes (in base pairs, bp) calculated to 2 decimal places by Genotyper software (Applied Biosystems) were assigned to their appropriate allele-size ‘bin’, based on the microsatellite repeat length using Allelobin v2.0. This software developed at ICRISAT (www.icrisat.org/gt-bt/Allelobin.htm) utilises the algorithm developed by Idury and Cardon (1997). Using the ‘binned’ dataset, PowerMarker v.3.25 (Liu and Muse 2005) was used to

Table 1. Characteristics of 44 polymorphic SSR loci screened across 92 genotypes

Marker name	Repeat motif	LG[A]	Bin[B]	No. of alleles	Range	Gene diversity[C]	Heterozygosity	PIC[D]
Xcup53	(TTTA)5	SBI-01	A (01.01)	03	187–199	0.29	0.05	0.26
Xtxp088	(AG)31	SBI-01	A (01.03)	20	109–167	0.91	0.05	0.90
XmSbCIR286	(AC)9	SBI-01	A (01.03)	06	104–126	0.68	0.03	0.64
Xtxp320(*phyB*)	(AAG)20	SBI-01	A (01.04) and G (10.04)	08	262–289	0.77	0.05	0.73
XmSbCIR306	(GT)7	SBI-01	A (01.05)	03	120–124	0.51	0.04	0.44
Xcup06	(CTGC)4	SBI-01	A (01.05)	02	202–206	0.47	0.00	0.36
XmSbCIR223	(AC)6	SBI-02	B (02.01)	03	106–114	0.50	0.01	0.43
Xtxp025	(CT)12	SBI-02	B (02.01)	08	116–158	0.70	0.00	0.66
XmSbCIR238	(AC)26	SBI-02	B (02.02)	12	063–103	0.81	0.06	0.78
Sb6-84 = Xgap084	(AG)14	SBI-02	B (02.03)	08	182–206	0.79	0.01	0.75
Xtxp348	(TAA)37	SBI-02	B (02.04)	16	282–336	0.89	0.09	0.88
Xcup14	(AG)10	SBI-03	C (03.01)	06	205–237	0.58	0.02	0.51
Xtxp114	(AGG)8	SBI-03	C (03.02)	03	230–239	0.36	0.00	0.30
Xtxp031	(CT)25	SBI-03	C (03.03)	19	203–269	0.80	0.08	0.78
XmSbCIR276	(AC)9	SBI-03	C (03.03)	04	225–233	0.52	0.02	0.41
Xisep0107	(TGG)4	SBI-03	C (03.04)	02	200–206	0.50	0.02	0.37
Xcup11	(GCTA)4	SBI-03	C (03.05)	02	163–171	0.44	0.00	0.34
Xcup61	(CAG)7	SBI-03	C (03.05)	02	196–199	0.43	0.04	0.34
Xgpsb050	(CT)10(CA)10	SBI-04	D (04.01)	09	208–256	0.55	0.05	0.53
Xgap121	(AC)14	SBI-04	D (04.01)	06	214–226	0.73	0.02	0.68
Xtxp012	(CT)22	SBI-04	D (04.03)	14	175–215	0.78	0.04	0.76
Xcup28	(TGAG)5	SBI-04	D (04.04)	03	152–164	0.47	0.07	0.37
Xtxp021	(AG)18	SBI-04	D (04.05)	08	169–195	0.63	0.05	0.58
Xtxp065	(ACC)4(CCA)3CG(CT)8	SBI-05	J (10.01)	06	122–136	0.70	0.04	0.65
Xtxp015	(TC)16	SBI-05	J (10.03)	08	198–224	0.64	0.05	0.58
Xtxp136(*Kaf3*)	(GCA)5	SBI-05	J (10.05)	03	237–243	0.45	0.07	0.36
Sb4-72=Xgap072	(AG)16	SBI-06	I (09.01)	04	182–192	0.23	0.01	0.22
Xtxp265	(GAA)19	SBI-06	I (09.03)	14	175–220	0.85	0.09	0.83
Xtxp274	(TTC)19	SBI-06	I (09.03)	14	230–347	0.84	0.07	0.83
Xgpsb127	TCG	SBI-06	I (09.03)	03	186–192	0.49	0.07	0.39
Xtxp057	(GT)21	SBI-06	I (09.05)	08	234–254	0.80	0.04	0.78
Xtxp312	(CAA)26	SBI-07	E (05.02)	11	086–224	0.61	0.04	0.59
Xtxp278	(TTG)12	SBI-07	E (05.03)	03	243–252	0.19	0.03	0.17
Xgpsb123	(CA)7+(GA)5	SBI-08	H (08.01)	05	287–295	0.60	0.03	0.52
Xtxp321	(GT)4(AT)6(CT)21	SBI-08	H (08.02)	16	187–227	0.87	0.06	0.85
Xtxp354	(GA)21(AAG)3	SBI-08	H (08.02)	09	121–169	0.74	0.07	0.71
Xgpsb067	(GT)10	SBI-08	H (08.03)	05	172–182	0.16	0.04	0.16
XmSbCIR240	(TG)9	SBI-08	H (08.04)	07	106–164	0.55	0.03	0.48
Xtxp273(*Pbbf*)	(TTG)20	SBI-08	H (08.05)	06	204–231	0.59	0.07	0.54
Xcup02	(GCA)6	SBI-09	F (06.02)	06	192–206	0.46	0.04	0.42
Xtxp289	(CTT)6(AGG)6	SBI-09	F (06.05)	12	264–330	0.59	0.05	0.57
XSbAGA01	(AG)33	SBI-10	G (07.01)	09	088–108	0.73	0.04	0.7
XmSbCIR283	(CT)8 (GT)8.5	SBI-10	G (07.03)	09	113–143	0.76	0.06	0.73
Xtxp141	(GA)23	SBI-10	G (07.05)	10	134–168	0.78	0.04	0.75
Mean				07.55		0.58	0.04	0.54
Maximum				20.00		0.91	0.09	0.90
Minimum				02.00		0.16	0.00	0.16

[A]Linkage group nomenclature as per Kim *et al*. (2005).
[B]Bin, marker positions as per Menz *et al*. (2002).
[C]Gene diversity as explained by Weir and Hill (2002).
[D]Polymorphic information content as per Botstein *et al*. (1980).

calculate the total numbers of alleles, the numbers of common alleles with frequencies of at least 5%, the observed allele size ranges (bp), the polymorphic information content (PIC) values (Botstein *et al*. 1980; Smith *et al*. 2000) and gene diversity. Analysis of molecular variance (AMOVA) was performed by using Arlequin v.3.1 (Excoffier *et al*. 2005). Classical *F*-statistics (Wright 1965) and population-specific *F*-statistics estimates (Weir and Hill 2002) were estimated using PowerMarker v.3.25. The locus-by-locus analysis, by pair-wise difference method, was performed using Arlequin v.3.1 to identify markers putatively associated with grain mould resistance/susceptibility. DARwin v.5.0 (Perrier *et al*. 2003; Perrier and

Jacquemoud-Collet 2006) was used to calculate pair-wise genetic dissimilarities of accessions using simple matching. The dissimilarity coefficients were used to perform principal coordinates analyses (PCoA) and construct weighted neighbour-joining trees (Saitou and Nei 1987) with a bootstrapping value of 10 000 using DARwin v.5.0.

The qualitative morphological traits, such as panicle type, glume colour, and grain colour, were assigned numerical ratings following the DUS (distinctiveness, uniformity and stability) ratings developed by the National Research Centre for Sorghum (Reddy *et al*. 2006) to facilitate statistical analysis. Pair-wise genetic dissimilarity values based on the Gower's distance (Gower 1985; Gower and Legendre 1986) were worked out using morphological data (SAS 9.1). The dissimilarity indices obtained were used to perform principal coordinate analyses using DARwin v.5.0 (Perrier and Jacquemoud-Collet 2006). The tree was plotted using hierarchical clustering following Ward's minimum variance method (Ward 1963) with a bootstrapping value of 10 000. Mantel's test (Mantel 1967) with 1000 permutations was performed to determine the significance of correlation between dissimilarity matrices derived from SSR data and from phenotypic traits associated with disease resistance using DARwin v.5.0.

Results

Allelic richness among resistant and susceptible lines

PCR amplifications were successful for all 46 SSRs across the genotypes (accessions/lines) screened. The observed allele sizes ranged from 63 bp (*XmsbCIR 238*) to 347 bp (*Xtxp274*). Of the 46 SSR markers considered, 44 were polymorphic and only 2 of the genic SSR loci (*Xcup63* and *Xisep0310*) were monomorphic in this diverse set of sorghum genotypes. Heterozygosity values of the 44 polymorphic SSR markers ranged from 0.00 to 0.09, with a mean of 0.04, suggesting that each detected a single genetic locus and that each of the sorghum accessions used was reasonably inbred and homogeneous. These 44 polymorphic SSRs revealed a total of 332 alleles with a range of 2 (EST-SSRs *Xcup06*, *Xisep0107*, *Xcup11*, and *Xcup61*) to 20 (genic SSR *Xtxp088*) alleles and an average of 7.55 alleles per primer pair (Table 1). However, excluding rare alleles (frequency of less than 5%), the average number of alleles per locus was reduced to 4.59, with a range of 1 (*Xgpsb067*) to 15 (*Xtxp088*). Of these 44 markers detecting polymorphism, all 44 were polymorphic among grain mould resistant accessions and 41 were polymorphic among susceptible accessions. On average, 6.8 alleles per locus were observed among the resistant genotypes, with a range of 2–17, whereas among the susceptible genotypes the average number of alleles per locus was 4.3, with a range of 2–11. The average gene diversities over loci for the resistant and susceptible groups were 0.53 and 0.54, respectively.

Genetic diversity in grain mould resistant and susceptible sorghum lines

Genetic diversity among the grain mould resistant and susceptible sorghum lines was quite high as indicated by polymorphic information content (PIC) and gene diversity values of the SSR markers. PIC values of the 44 polymorphic SSR markers ranged from 0.16 (*Xgpsb067*) to 0.90 (*Xtxp088*), with an average of 0.54. Similarly, gene diversity among the sorghum lines also varied from 0.16 (*Xgpsb067*) to 0.91 (*Xtxp088*), with an average of 0.58 (Table 1). The observed heterozygosity among resistant and susceptible accessions was quite low (0.037 and 0.058, respectively) compared with average expected heterozygosity of 0.555 and 0.566, respectively, for resistant and susceptible accessions indicating that the loci were not in Hardy-Weinberg equilibrium. The locus-wise and population-wise *F*-statistics (frequencies) revealed that there were 139 and 25 alleles associated with resistant and susceptible groups of genotypes, respectively.

The weighted neighbour-joining clustering-based dendrogram generated using simple matching dissimilarity indices clustered the sorghum accessions into 4 major groups (Fig. 1). Group III included 50% of the resistant accessions (37 out of 74) used in the study along with an exceptional susceptible line (ICSB 370-2-9) that originated as a single-plant selection from one of the resistant lines (ICSB 370-2). Similarly, 50% of susceptible accessions (9 out of 18) were clustered into group IV. Groups I and II contained both resistant and susceptible accessions. Genetic diversity among resistant and susceptible accessions was also confirmed by scatter plots derived through principal coordinate analysis (PCoA) (not shown). Most of the resistant accessions were clustered in the right portions of the plot, and susceptible accessions were mixed with resistant accessions in the left portion of the plot (axes1/2).

Analysis of molecular variance (AMOVA) was performed on the dataset to partition the total genetic variation within and between the populations of resistant and susceptible accessions. AMOVA indicated that 12% of the total variation among the sorghum accessions used in this study was due to differences between the resistant and susceptible groups, 81% was due to differences within these groups, and the remaining 7% due to allelic variation within genotypes.

Putative association of SSR loci with grain mould resistance

A locus-by-locus AMOVA was performed to calculate the contribution of each locus to the differentiation of resistant and susceptible groups among the accessions tested. Allelic variation at 5 SSR loci distributed across 3 sorghum linkage groups, out of 44 tested across the 10 sorghum linkage groups, exhibited significant association with grain mould reaction (Table 2). Furthermore, markers *Xcup11* (50%) and *XmSbCIR276* (38%) (the latter genetically linked to the *R* locus, which is involved in epistatic control of pericarp colour) contributed significantly to total genetic differences between the 2 disease-response groups.

Diversity based on morphological traits

The morphological data for 6 traits and grain mould scores were used for dissecting the diversity in the selected grain mould resistant and susceptible accessions. A dissimilarity matrix based on Gower's genetic distance was used for plotting the tree and PCoA. The tree based on hierarchical clustering grouped

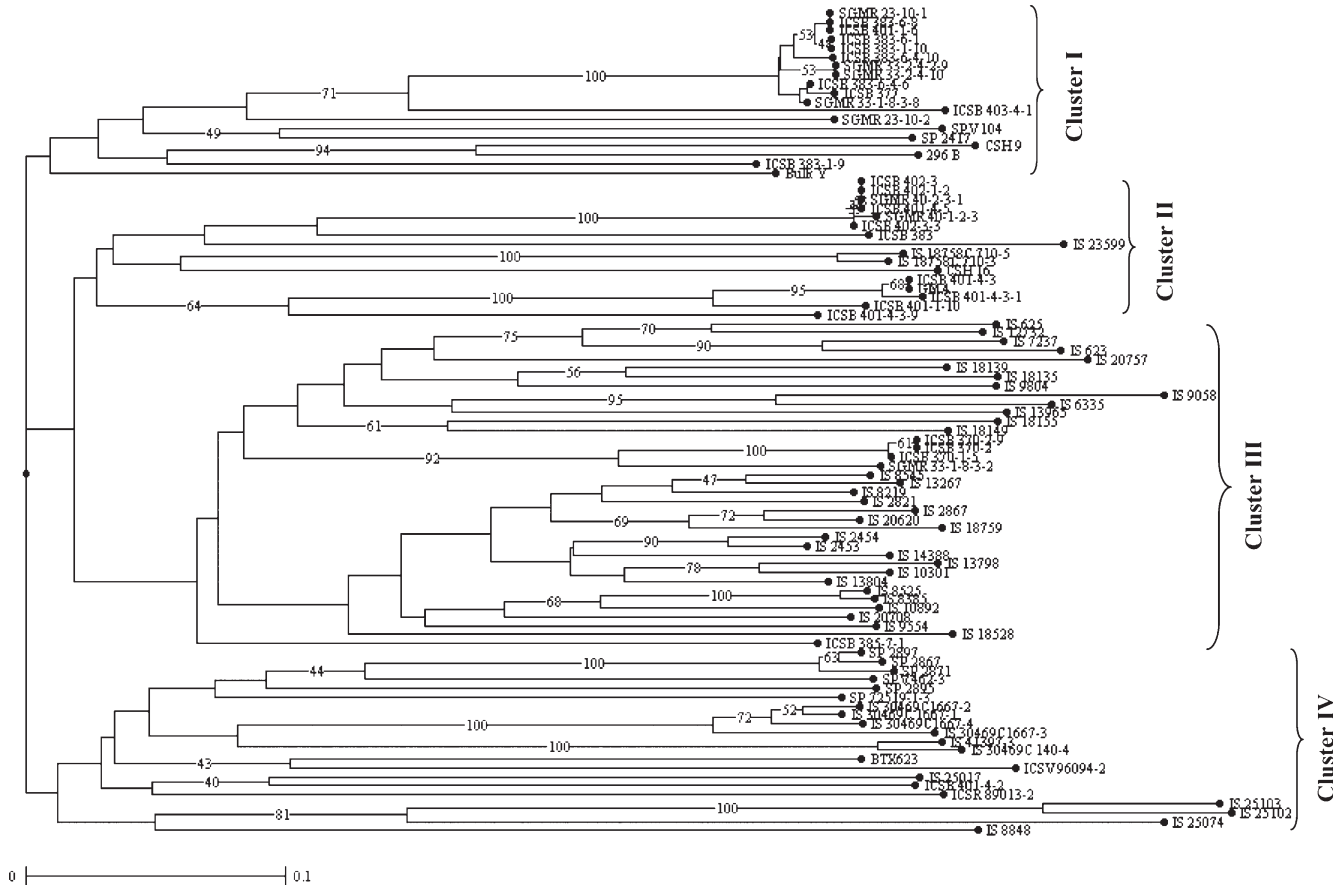

Fig. 1. Tree constructed based on 44 polymorphic sorghum SSR markers using the simple matching dissimilarity index and weighted neighbour-joining clustering for the 92 sorghum accessions expressing resistant and susceptible reaction to grain mould. Cluster I: 296B, Bulk Y, CSH 9, ICSB 377, -383-1-10, -383-1-9, -383-6-1, -83-6-4-10, -383-6-4-6, -383-6-8, -401-1-6, -403-4-1, SGMR 23-10-1, -23-10-2, -33-1-8-3-8, -33-2-4-10, -33-2-4-2-9, SP 2417, SPV 104. Cluster II: CSH 16, GM 4, ICSB 383, -401-1-10, -401-4-3, -401-4-3-1, -401-4-3-9, -401-4-5, -402-1-2, -402-3, -402-3-3, IS 18758C 710-3, -18758C 710-5, -23599, SGMR 40-1-2-3, -40-2-3-1. Cluster III: ICSB 370-1-5, -370-2, -370-2-9, -385-7-1, IS 10301, -10892, -12732, -13267, -13798, -13804, -13965, -14388, -18135, -18139, -18149, -18155, -18528, -18759, -20620, -20708, -20757, -2453, -2454, -2821, -2867, -623, -625, -6335, -7237, -8219, -8385, -8525, -8545, -9058, -9554, -9804, SGMR 33-1-8-3-2. Cluster IV: ICSB 401-4-2, ICSR 89013-2, ICSV 96094-2, IS 25017, -25074, -25102, -25103, -30469C 140-4, -30469C 1667-1, -30469C 1667-2, -30469C 1667-3, -30469C 1667-4, -41397-3, -8848, SP 2867, -2871, -2895, -2897, -72519-1-3, SPV 462-3.

Table 2. List of loci putatively linked with grain mould resistance as determined through locus-by-locus AMOVA

Locus	Linkage group	SSD[A]	Va[B]	Contribution value (%) to differentiation between resistant and susceptible groups
Xcup14	SBI-03	6.12	0.092	26.14
XmSbCIR276	SBI-03	8.64	0.136	38.39
Xcup11	SBI-03	10.41	0.167	50.08
Xgpsb127	SBI-06	4.67	0.071	23.83
Xtxp273(*Pbbf*)	SBI-08	6.38	0.104	28.19

[A]Sum of squares for variation.
[B]Variance component for differentiation among the populations.

the accessions into 4 clusters, with 2 clusters for resistant (Cluster I and IV) and one for susceptible (Cluster II) accessions (Fig. 2). The PCoA (not shown) revealed clear distinction of grain mould resistant and susceptible accessions, with susceptible accessions grouped in 2, and resistant accessions in several clusters. The Gower's dissimilarity distance values for this dataset ranged from 0.03 to 0.86. Further to this, the scores for morphological traits, *viz*. panicle type, glume colour, grain colour, and grain mould, were associated with the SSR-based tree constructed using DARWin v.5.0. When this morphological information was overlaid on the SSR-based tree, the relative importance of these traits was assessed by comparing the SSR-based grouping with the distribution of these traits in each group. A major role of grain colour for clustering was observed, with the racial differentiation and shared pedigrees defining the within-cluster differentiation. The Mantel's test further revealed a significant association (standard normal variate, $g = 38.58$; Mantel's coefficient, $Z = 2605.46$; and correlation coefficient, $r = 0.80$) between the SSR- and morphological-based dissimilarity matrices at $P = 0.05$ for the critical value of $g = 2.575$.

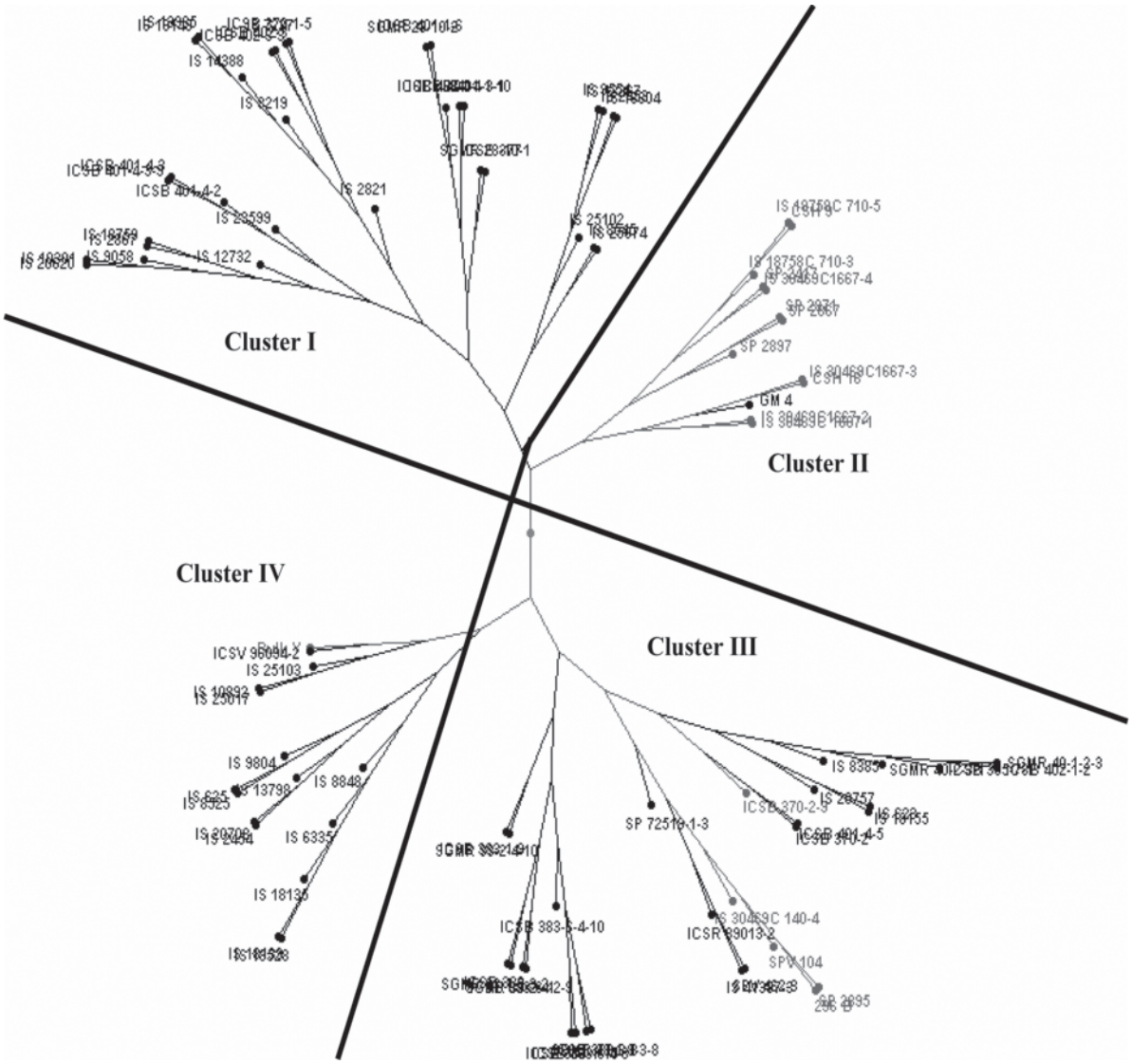

Fig. 2. Tree constructed based on morphological traits using the Gower's dissimilarity indices and Ward's minimum variance method of clustering for the 92 sorghum accessions expressing resistant (dark nodes) and susceptible (light nodes) reaction to grain mould. Cluster I: ICSB370-1-5,-377,-401-1-10,-401-1-6,-401-4-2,-401-4-3,-401-4-3-1,-401-4-3-9,-402-3,-402-3-3,-403-4-1,IS10301,-12732,-13267,-13804, -3965, -14388, -18149, -18759, -20620, -23599, -2453, -25074, -25102, -2821, -2867, -7237, -8219, -8545, -9058, -9554, SGMR 23-10-1, -23-10-2. Cluster II: CSH 16, -9, GM 4, IS 18758C 710-3, -18758C 710-5, -30469C 1667-1, -30469C 1667-2, -30469C 1667-3, -30469C 1667-4, SP 2417, -2867, -2871, -2897. Cluster III: 296B, ICSB 370-2, -370-2-9, -383, -383-1-10, -383-1-9, -383-6-1, -383-6-4-10, -383-6-4-6, -383-6-8, -385-7-1, -401-4-5, -402-1-2, ICSR 89013-2, IS 18155, -20757, -30469C 140-4, -41397-3, -623, -8385, SGMR 33-1-8-3-2, -33-1-8-3-8, -33-2-4-10, -33-2-4-2-9, -40-1-2-3, -40-2-3-1, SP 2895, -72519-1-3, SPV 104, -462-3. Cluster IV: Bulk Y, ICSV 96094-2, IS 10892, -13798, -18135, -18139, -18528, -20708, -2454, -25017, -25103, -625, -6335, -8525, -8848, -9804.

Discussion

The genetic diversity among the grain mould resistant and susceptible sorghum accessions used in this study was high as indicated by polymorphic information content (PIC) and gene diversity values. The PIC of a SSR marker gives an idea about the discriminatory power of that marker by taking into account the number of alleles detected and their relative frequencies (Smith *et al*. 2000). Most of the SSR markers used in this study showed a moderate discriminatory power, hence the number of markers used in this study should be sufficient to assess genetic variation among these grain mould resistant/susceptible sorghum accessions.

Sorghum is primarily an inbreeding species resulting in a low level of observed heterozygosity, but the gene pool as a whole maintains a high level of allelic variation. The high level of allelic variability but low level of heterozygosity observed in this study agrees with a previous study on diversity analysis of Eritrean sorghum landraces with SSR markers (Ghebru *et al*. 2002) and fits well with the expected inbreeding behaviour of sorghum. A large proportion (38%) of rare alleles (frequency

<5%) was observed in this study. The large proportion of rare alleles and overall divergence observed between grain mould resistant and susceptible sorghum accessions indicate the opportunity to select phenotypically and genotypically divergent parental lines for generating biparental populations for mapping of genes/QTLs associated with grain mould resistance and for marker-assisted introgression of favourable alleles into elite breeding lines.

Diversity analysis based on sorghum SSR marker allelic variation revealed clear patterns of genetic divergence between and among grain mould resistant and susceptible accessions, which grouped in several distinct clusters. The SSR-based groupings were then associated with the morphological descriptors for each trait using DARwin v.5.0. This clearly indicated the role of grain colour (controlled by pericarp and testa pigmentation) in the clustering. The link among the high tannin content (Harris and Burns 1973), presence of a pigmented testa (Esele *et al*. 1993), and grain mould resistance in sorghum is well documented. There were two main clusters, one with red, brown, and brown-red (predominantly grain mould resistant), and another mostly of white grain accessions (predominantly grain mould susceptible). White-grained sorghums have been found to be more susceptible to grain mould than red- and brown-grained sorghums (Audilakshmi *et al*. 1999). Clustering within the resistant and susceptible groups was driven by racial differentiation and shared pedigrees of the accessions studied. A similar trend was observed in SSR-based characterisation of a large sorghum germplasm composite collection, where sorghum accessions were grouped primarily on the basis of origin, and clustering within groups was driven by racial classification (Hash *et al*. 2007).

Genetic diversity among resistant and susceptible accessions was confirmed using scatter plots derived through principal coordinate analysis (PCoA) based on the marker-derived dissimilarity indices. This PCoA revealed a similar trend of clustering, with resistant and susceptible accessions tending to group separately, and subclusters being formed on the basis of racial differences and shared pedigrees. Analysis of molecular variance (AMOVA) could partition the total genetic variation within and between the populations of resistant and susceptible accessions. It revealed that 12% of the total SSR allelic variation among the sorghum accessions used in the study was accounted for by accession reaction type (susceptible or resistant) to grain mould fungi. It was evident that the variance component for the susceptible group was less than that of the resistant group because of smaller numbers of susceptible accessions in the set. Nonetheless, the within-subpopulation *F*-statistic for the susceptible group (0.904) was as good as that of the resistant group (0.944). This was also evident from the average gene diversity values over loci for resistant (0.53) and susceptible (0.54) groups. The F_{ST} value of 0.116 revealed that the level of genetic differentiation was moderate. This indicates that both the resistant and susceptible groups have enough genetic diversity for further utilisation in identifying diverse genotypes for the development of mapping populations segregating for grain mould resistance.

For the morphological traits-based dendrogram (Fig. 2), scores for panicle type, plant height, and glume coverage were the main factors driving the clustering of the accessions into different groups, followed by PGMR and grain colour scores. Grain mould resistance is not only reported to be correlated with open panicles and long glumes (Glueck *et al*. 1977), but also with greater glume coverage (%), glume length, and glume area (Mansuetus *et al*. 1990). The grouping of resistant accession GM 4 in a susceptible cluster and of susceptible accession Bulk Y in a resistant cluster indicated that the clustering was based on morphological traits. Similar trends were also observed in the PCoA based on morphological traits. The results obtained from the SSR-based and morphological trait-based diversity assessment were considerably similar as indicated by significant correlation between the SSR- and morphological traits-based diversity matrices ($r = 0.80$).

A previous study attempting to map QTLs for grain mould resistance (Klein *et al*. 2001) largely resulted in mapping of major flowering and plant height genes (most 'grain mould resistance' QTLs were found in genomic regions harbouring these genes). The roles of flowering time, panicle compactness, glume coverage, and grain colour (due to pericarp and testa pigmentation) in sorghum grain mould resistance are well documented (Glueck *et al*. 1977; Audilakshmi *et al*. 1999; Thakur *et al*. 2006).

We selected 5 SSR marker loci contributing most to differentiation of the grain mould resistant and susceptible reaction classes based on locus-by-locus AMOVA (Table 2). SSR marker locus *Xcup11* maps near to a flowering QTL on sorghum chromosome SBI-03 (M. T. Vinayan, pers. comm.). When *Xcup11* was checked for its physical position on the sorghum genome sequence on chromosome SBI-03, it was found to be located (at 1.91 Mb) between *ZCN14* (1.51 Mb) and *ZCN23* (2.27 Mb) (Ramu *et al*. 2010). The *ZCN* gene-family is well characterised for its role in flowering and developmental processes in plant growth. Similarly, locus *Xgpsb127* may be linked with Ma_1/ma_1, a major gene on SBI-06 controlling photoperiod-temperature response of flowering time (Feltus *et al*. 2006; Mace *et al*. 2009). Finally, locus *XmSbCIR276* was mapped *in-silico* near the red pericarp (*R*/*r*) gene on chromosome SBI-03 (Ramu *et al*. 2010). The red-grained sorghum lines have been reported to be mostly resistant to grain mould (Audilakshmi *et al*. 1999). These findings provide initial clues for future QTL mapping and marker-assisted backcrossing programs.

The main aim of this research was to assess genetic diversity among selected grain mould resistant and susceptible sorghum accessions in order to identify parental pairs for the development of mapping populations for grain mould resistance. After considering the SSR-based genetic diversity and morphological diversity for these lines, we chose parental pairs, based on maximum SSR-based genetic distance and minimum variation in flowering time, plant height, panicle type/inflorescence, and contrasting grain tannin contents. On the basis of these criteria, 7 pairs of genetically diverse susceptible and resistant parent lines (Bulk Y/ICSB 377, IS 30469C 1667-2/SGMR 40-1-2-3, SP 2417/IS 41397-3, SPV 104/ICSV 96094-2, IS 18758C 710-3/IS 25103, ICSB 370-2-9/IS 8385, and ICSB 370-2-9/IS 8219) were chosen with dissimilarity indices >0.50 to develop mapping populations (recombinant inbred line sets) for mapping genomic regions contributing to sorghum grain mould resistance and marker-

assisted introgression of QTLs for mould resistance into elite breeding lines.

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Appendix 1. Morphological characterisation of sorghum lines involved in diversity study

S. No.	Designation	Race type	PGMR[A]	DTF[B]	Height (cm)	Panicle type[C]	Glumes (%)	Glumes colour[D]	Grain colour[E]
1	ICSB 377	–[F]	1.3	57	153	SL	25	R	R
2	ICSB 383	–	2	69	228	SC	25	R	R
3	GM 4	–	2	81	193	SL	25	R	W
4	SGMR 23-10-1	–	2	72	217	SL	33	R	R
5	ICSB 383-1-9	–	2	72	237	SC	25	R	B
6	ICSB 401-4-2	–	2	69	203	SL	25	B	B
7	ICSB 403-4-1	–	2	66	165	SL	25	R	B
8	SGMR 33-1-8-3-2	–	2	70	227	SC	25	R	R
9	SGMR 23-10-2	–	2	76	218	SL	25	R	B
10	ICSB 383-1-10	–	2	72	240	SC	38	R	R
11	ICSB 383-6-1	–	2	72	240	SC	25	R	R
12	ICSB 383-6-8	–	2	72	228	SC	38	R	R
13	ICSB 401-1-6	–	2	76	203	SL	25	R	B
14	ICSB 401-1-10	–	2	68	220	SL	38	R	B
15	ICSB 401-4-3	–	2	70	210	SL	38	B	B
16	ICSB 402-3-3	–	2	60	150	SL	25	B	B
17	ICSB 401-4-5	–	2	59	148	SC	25	B	R
18	SGMR 33-1-8-3-8	–	2	72	220	SC	38	R	R
19	SGMR 33-2-4-2-9	–	2	72	225	SC	25	R	R
20	SGMR 33-2-4-10	–	2	71	228	SC	25	R	B
21	ICSB 401-4-3-1	–	2	70	203	SL	38	R	B
22	ICSB 401-4-3-9	–	2	68	210	SL	38	B	B
23	ICSB 383-6-4-6	–	2	72	238	SC	38	R	R
24	ICSB 383-6-4-10	–	2	71	245	SC	50	R	R
25	IS 6335	*caudatum*	1.1	53	230	L	75	B	R
26	IS 20708	*bicolor*	2	49	195	L	25	B	B
27	IS 8545	*caudatum*	2	50	168	SC	25	LR	LB
28	IS 8525	*caudatum*	2	50	185	L	38	B	B
29	IS 18759	*caudatum*	2	50	190	SL	38	B	B
30	IS 2821	*caudatum*	2	51	165	SL	25	B	LB
31	IS 8385	*caudatum-bicolor*	2	53	155	SC	25	B	B
32	IS 625	*caudatum*	2	53	193	L	38	B	B
33	IS 8219	*caudatum*	2	54	168	SL	25	B	B
34	IS 13965	*caudatum-bicolor*	2	54	193	SL	25	B	B
35	IS 13267	*durra-bicolor*	1.8	55	168	SL	25	LR	LB
36	IS 9804	*caudatum-bicolor*	2	57	180	L	38	B	B
37	IS 7237	*caudatum*	2	60	190	SL	25	B	B
38	IS 25102	*caudatum*	1.5	76	200	SL	38	BL	LB
39	IS 25103	*guinea-caudatum*	2.2	72	235	L	38	LR	W
40	IS 25074	*guinea-caudatum*	1.5	67	240	SC	25	LR	LB
41	IS 18155	*caudatum*	2	66	245	SC	25	B	B
42	IS 10892	*caudatum-bicolor*	1.5	65	245	L	25	R	LB
43	IS 9554	*caudatum*	1.7	64	250	SL	25	LR	LB
44	IS 623	*caudatum*	2	59	218	SC	25	B	B
45	IS 12732	*durra*	1.7	59	235	SL	38	B	B
46	IS 18135	*bicolor*	2	59	245	L	75	B	B
47	IS 2453	*caudatum*	2	58	203	SL	38	LR	LB
48	IS 18528	*bicolor*	2	58	220	L	63	B	B
49	IS 13798	*kafir*	1.9	58	225	L	25	B	B
50	IS 13804	*caudatum-bicolor*	1.6	57	220	SL	50	LR	LB
51	IS 14388	*durra-caudatum*	2	56	205	SL	25	B	B
52	IS 20757	*caudatum*	2	55	200	SC	38	B	B
53	IS 9058	*caudatum*	2	55	215	SL	38	B	B
54	IS 2454	*caudatum*	1.7	54	205	L	25	B	B
55	IS 8848	*caudatum-bicolor*	1	54	210	L	75	BL	B
56	IS 18149	*durra-caudatum*	2	54	215	SL	25	B	B
57	IS 20620	*caudatum*	1.9	53	205	SL	38	B	B
58	IS 10301	*kafir*	2	52	203	SL	38	B	B
59	IS 18139	*caudatum-bicolor*	1.9	51	230	L	63	B	B

(*Continued next page*)

Appendix 1. (*continued*)

S. No.	Designation	Race type	PGMR[A]	DTF[B]	Height (cm)	Panicle type[C]	Glumes (%)	Glumes colour[D]	Grain colour[E]
60	IS 2867	*caudatum*	2	50	215	SL	38	B	B
61	ICSB 402-3	–	2	62	160	SL	25	B	B
62	ICSB 370-1-5	–	2	59	175	SL	25	B	B
63	ICSV 96094-2	–	2	62	180	L	50	B	W
64	ICSR 89013-2	–	3	62	145	SC	25	B	W
65	ICSB 370-2	–	2	61	180	SC	25	B	R
66	ICSB 402-1-2	–	2	62	175	SC	25	B	B
67	SGMR 40-1-2-3	–	2	61	155	SC	25	B	B
68	SGMR 40-2-3-1	–	1	62	165	SC	25	B	B
69	ICSB 385-7-1	–	3	61	160	SC	25	B	B
70	IS 41397-3	–	2	63	165	SC	25	W	W
71	SPV 462-3	–	2	66	255	SC	25	W	W
72	SP 72519-1-3	–	4	62	150	C	25	W	W
73	IS 23599	*guinea-caudatum*	2	76	340	SL	25	B	B
74	IS 25017	*caudatum*	2	81	345	L	25	R	W
75	ICSB 370-2-9	–	7	54	175	SC	25	B	R
76	CSH 9	–	8.9	66	195	SL	25	W	W
77	CSH 16	–	9	69	168	SL	38	R	W
78	SPV 104	–	9	64	180	SC	25	W	W
79	Bulk Y	–	8.8	57	148	L	25	B	W
80	IS 18758C 710-3	*guinea-caudatum*	8.6	63	230	SL	25	W	W
81	IS 18758C 710-5	*guinea-caudatum*	8.5	65	210	SL	25	W	W
82	IS 30469C 140-4	*guinea-caudatum*	9	66	153	SC	25	R	W
83	IS 30469C 1667-1	*guinea-caudatum*	9	65	183	SL	25	LR	W
84	IS 30469C 1667-2	*guinea-caudatum*	9	63	168	SL	25	LR	W
85	IS 30469C 1667-3	*guinea-caudatum*	9	63	178	SL	25	R	W
86	IS 30469C 1667-4	*guinea-caudatum*	9	63	175	SL	25	W	W
87	SP 2417	–	8.6	63	163	SL	33	W	W
88	SP 2895	–	8.2	69	148	SC	25	W	W
89	SP 2871	–	8.2	68	142	SL	25	W	W
90	SP 2897	–	8.5	69	147	SL	42	W	W
91	SP 2867	–	8.3	68	148	SL	25	W	W
92	296B	–	8	69	143	SC	25	W	W

[A]The visual panicle grain mould rating using a progressive 1–9 scale: 1, no mould infection; 2, 1–5%; 3, 6–10%; 4, 11–20%; 5, 21–30%; 6, 31–40%; 7, 41–50%; 8, 51–75%; and 9, 76–100% moulded grains on a panicle.

[B]Days to 50% flowering.

[C]Panicle type: L, loose; SL, semi-loose; SC, semi-compact; C, compact.

[D]Glume colour: R, red; B, brown; LR, light red; BL, black; W, white.

[E]Grain colour: R, red; B, brown; W, white; LB, light brown; CW, chalky white.

[F]Race type for designated B/R-lines and cultivars is not available.