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Genetic divergence and molecular characterization of sorghum hybrids and their parents for reaction to *Atherigona soccata* (Rondani)

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Summary

Simple sequence repeat (SSR) markers linked to quantitative trait loci (QTL) associated with resistance to sorghum shoot fly, *Atherigona soccata* were used to characterize the genetic and phenotypic diversity of 12 cytoplasmic male-sterile (CMS) and maintainers, 12 restorer lines, and 144 F_1 hybrids. The genetic diversity was quite high among the shoot fly-susceptible parents and the hybrids based on them, as indicated by high polymorphic information content (PIC) values, while limited genetic diversity was observed among shoot fly-resistant lines. The phenotypic and genotypic dissimilarity analysis indicated that the shoot fly-resistant and -susceptible parents were 73.2 and 38.5% distinct from each other, and the morphological and genetic distances of certain resistant and susceptible cross combinations was more than their resistant or susceptible parents. Genetic variability among the groups was low (10.8%), but high within groups (89.2%). The genetic and morphological distances suggested that the F_1 hybrids were closer to CMS (5 to 12% dissimilar) than the restorer (11 to 87% dissimilar), suggesting that CMS influences the expression of resistance to sorghum shoot fly. The SSR markers can be used to characterize the homologous traits in sorghum germplasm.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops in Africa, Asia, USA, Australia, and Latin America. Several biotic and abiotic constraints limit the production and productivity of this crop. Amongst the biotic constraints, insect-pests are predominant, causing nearly US$1 billion loss to the actual produce in semi-arid tropics (ICRISAT, 1992). More than 150 species of insects have been recorded as pests of sorghum, of which sorghum shoot fly, *Atherigona soccata* (Rondani) (Muscidae: Diptera) is an important pest in Asia, Africa, and the Mediterranean Europe (Jotwani et al., 1980; Sharma, 1993). Host plant resistance is one of the most effective means of keeping shoot fly populations below economic threshold levels. A number of genotypes with resistance to shoot fly have been identified, but the levels of resistance are low to moderate (Sharma et al., 2003). Since, most of the area under improved cultivars is planted to high-yielding hybrids, the future breeding efforts should focus on developing high yielding shoot fly-resistant hybrids. The discovery of usable sources of cytoplasmic male-sterility (CMS) (Stephens & Holland, 1954) has made it easier to incorporate the desired traits into hybrid parents (House, 1985). However, large-scale cultivation of hybrids based on a single source of CMS may be a potential danger to the stability of crop production due to their vulnerability to insect pests and diseases (Yang et al., 1989; Sharma et al., 2004). Therefore, it is important to identify shoot fly-resistant cytoplasmic male-sterile (A-lines), maintainer (B-lines), and restorer (R-lines) lines with different mechanisms of resistance, understand the nature of gene action, and transfer genes conferring resistance to shoot fly into high yielding genetic cultivars (Dhillon, 2004).

Knowledge of germplasm diversity and genetic relationships in the breeding material is an invaluable aid in planning appropriate strategies for crop improvement. Classification of germplasm accessions solely based on discrete morphological characters may not provide an accurate indication of their genetic divergence (Menkir et al., 1997), and phenotypic selection based on traits that are conditioned by additive allelic effects can produce dramatic and economically important changes in breeding populations. Molecular markers have been used for rapid, detailed, and directed genetic manipulation of crop plants, and to identify and characterize quantitative trait loci (QTLs) associated with plant height and days to maturity (Pereira & Lee, 1995), plant domestication (Paterson et al., 1995), resistance to diseases (Gowda et al., 1995) and insects (Agrama et al., 2002), and tolerance to drought (Tuinstra et al., 1998). The SSR markers are co-dominant, flanking, uniformly distributed, and highly polymorphic in plants, and even among the closely related accessions (Morgante & Olivieri, 1993; Wang et al., 1994; Rongwen et al., 1995; Yang et al., 1995; Brown et al., 1996). Although the development of SSR markers is more expansive than the specific sequence information based markers (such as RFLPs, AFLPs and RAPDs), but once developed, the analysis of SSR polymorphism among genotypes is straightforward and inexpensive.

The advent of PCR based molecular marker techniques has facilitated the analysis of sorghum genome (Williams et al., 1990). A high level of genetic variation has been detected among the sorghum accessions, which was high for *bicolor*, and *guinea* races and low for *kafir* race (Ejeta et al., 2000). The sorghum genotype BTx 623 has been used as a reference for molecular genotyping of sorghum. It has been used as source of DNA to construct enriched libraries and the two sorghum bacterial artificial chromosome (BAC) libraries that are currently available (Bhattramakki et al., 2000). The probability of alleles at a locus depends upon the working group to which the accessions belong. Kong et al. (2000) reported 0.88 to 0.67 alleles per locus, while Bhattramakki et al. (2000) reported 3.88 alleles per locus in their respective working materials. In addition, the number of alleles per locus is positively correlated with the number of repeated units at the loci in BTx 623, the strain from which the SSRs were originally isolated, confirming the usefulness of SSR loci in marker-assisted selection in sorghum (Kong et al., 2000). The SSR primers and linkage map locations have been published for sorghum (Taramino et al., 1997; Tao et al., 1998, 2000; Kong et al., 2000), but their number is too small to develop a fine map and utilize favorable genes for resistance to insects for crop improvement.

The objectives of our research were to analyze the genetic divergence in a set of 36 shoot fly-resistant and -susceptible inbred lines and their hybrids using SSR markers linked to QTL associated with resistance to sorghum shoot fly, and comparing the genotypic and phenotypic relatedness of the hybrids with their parents to understand the effect(s) of cytoplasmic male-sterility on the expression of resistance to sorghum shoot fly, *A. soccata*.

Materials and methods

Plant material

The test material consisting of 12 restorer (5 shoot fly-susceptible and 7 shoot fly-resistant), 12 cytoplasmic male-sterile (CMS) and their maintainer lines (5 shoot fly-susceptible and 7 shoot fly-resistant) (Table 1) and their 144 F_1 hybrids, were phenotyped at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (17° 32′ N, 78° 16′ E), Andhra Pradesh, India for three seasons [2003 rainy (July–November), early post-rainy (September–January), and late post-rainy (October–March)] along with shoot fly-resistant (IS 18551) and -susceptible (Swarna) checks under field conditions using the interlard fish-meal technique (Sharma et al., 1992). Each entry was sown on ridges in 2-row plots of 2-meter length, and 75 cm apart. There were three replications in a balanced alpha-design. The seedlings were thinned at 10 days after seedling emergence (DAE) to a plant-plant spacing of 10 cm. Data were recorded on deadheart (%) formation at 14 DAE, leaf glossiness at 5 DAE on a scale of 1 to 5 [1 = highly glossy (light green, shining, narrow and erect leaves), and 5 = non-glossy (dark green, dull, broad and drooping leaves)], and trichome density on central-portion of the 5th leaf at 12 DAE (both on the abaxial and adaxial surfaces of the leaf) (Sharma & Nwanze, 1997).

DNA extraction

For DNA extraction, the test material was planted in the greenhouse in small pots (10 cm dia.) using a potting

Table 1. Pedigrees of cytoplasmic male-sterile (A), maintainer (B), and restorer (R) lines of sorghum

Genotypes	Pedigree	Development year
A/B lines		
SPSFR 94011A/B	[(((BTx 623 × ((SC108-3 × GPR 148)-18-4-1)) × B lines bulk) -5-1-2-5) × ((PS 21194 × SPV 351)-3-1-2-3-3)]-13-3-1-1	1998
SPSFR 94012A/B	[(((BTx 623 × ((SC 108-3 × GPR 148)-18-4-1)) × B lines bulk) -5-1-2-5) × ((PS 21194 × SPV 351)-3-1-2-3-3)]-13-3-1-4	1998
SPSFR 94006A/B	[(((BTx 623 × ((SC 108-3 × GPR 148)-18-4-1)) × B lines bulk) -5-1-2-5) × ((PS 21194 × SPV 351)-3-1-2-3-3)]-13-2-2-1-1	1998
SPSFR 94007A/B	[(((BTx 623 × ((SC 108-3 × GPR 148)-18-4-1)) × B lines bulk) -5-1-2-5) × ((PS 21194 × SPV 351)-3-1-2-3-3)]-13-3-2-2-1	1998
SPSFR 94010A/B	[((((BTx 623 × ((SC 108-3 × GPR 148)-18-4-1)) × B lines bulk) -5-1-2-5) × PS 30715-1) × PS 19349B]-2-4-1	1998
SPSFR 94034A/B	[((Indian Synthetic 89-2 × Rs/R 20-682)-5-4-2) × ((PS 21194 × SPV 391)-3-1-2-3-1-1)]-4-2-1-1	1998
SP 55299A/B	(PS 21303 × SPV 386)-3-2-2-2-1	1998
SP 55301A/B	[((((BTx 623 × ((SC108-3 × GPR 148)-18-4-1)) × B lines bulk) -5-1-2-5) × ((PS 21194 × SPV 351)-3-1-2-3-3) × PS 19349B]-10	1998
296A/B	IS 3922 × Karad local	–
Tx 623A/B	IS 40583 (kafir) × IS 21807 (caudatum)	–
CK 60A/B	*Black hull kafir* × *Day milo*	–
ICSA 42A/B	[(BTx 623 × ((SC 108-3 × GPR 148)-18-4-1)) × B lines bulk]-5-3-6-3	1984
Restorer (R) lines		
ICSV 705	((RS/R × EN 3257-4)-1-5-6-1-1-1 × (SC 108-3 × CS 3541)-19-1) -3-1-2-3-3	–
ICSV 700	(IS 1082 × SC 108-3)-1-1-1-1-1	–
ICSV 708	((IS 5622 × CS 3541)-20-1-1-1-1-1-1 × (UCh V2 × Bulk Y-55) -1-5-1)-5-2-5-1-1	–
PS 30710	[(IS 5622 × CS 3541)-20-1-1-1-1-1-1 × (UCh V2 × Bulk Y-55) -1-5-1]-5-1-6-1	–
IS 18551	Land Race (Ethiopia)	–
SFCR 151	[(1011 E No 23-2 (PM 12645 × IS 2205))-5-1-2-2]	–
SFCR 125	(ICSV 705 × YT-3-47)-7-1-1-2	–
ICSV 91011	(IS 9608 × ([(SC 108-3 × Swarna) × IS 9327]-6-2-1))-3-6-2	–
CS 3541	IS 3675 × IS 3541	1974
MR 750	(SC 108-3 × CSV 4)-27-2-1	–
ICSV 745	((IS 3443 × DJ 6514)-1-1-1-1-1) × (E35-1 × US/B 487) -2-1-4-1-1-3)-4-1-1-1	–
Swarna	Selection from IS 3924	1968

mixture of black soil and farmyard manure (3: 1). Seven day-old seedlings were used for DNA extraction. Leaf stripes (5 cm long) of 10 plants for the parents, and of 5 plants of F_1 hybrids (final weight per sample 30 mg) were collected in eppendorf tubes containing two pre-chilled steel balls (at -20°C for about 30 minutes). DNA was isolated from the leaf-tissue using the mini-prep 3% CTAB method (Mace et al., 2003) in a 96-well format. The DNA quantity for each sample was assessed using Picogreen^{TM} (Juro Supply Gmbh, Switzerland) and inflorescence (Spectrafluor Plus, Tecan, Switzerland), and DNA concentrations were normalized at 2.5 ng/μl. The DNA quality of each sample was evaluated by running 1 μl of DNA on a 0.8% agarose gel.

Primer selection

The eight putative SSR markers (Table 2) linked with sorghum shoot fly resistance traits QTLs were selected from a joint genetic map constructed based on the segregation of 111 and 107 SSR markers in 296B × IS 18551 (258) and BTx 623 × IS 18551 (252) derived two Recombinant Inbred Line (RIL) populations, respectively, covering a map length of 2166 cM, assigned to 10 linkage groups (LGs) A through J (Sajjanar, 2002; Hash et al., 2003; Folkertsma et al., 2003), and used in the present studies to characterize the F_1 hybrids and their parents to gain an understanding on effects of cytoplasmic male-sterility on the expression of resistance to *A. soccata*. The QTL analyses

Table 2. Genetic variability at eight SSR loci in six sorghum groups, heterozygosity observed (H_{obs}), expected heterozygosity (H_{exp}), Nei's unbiased estimate (H_{unb}) based on small sample size correction, and Hardy-Weinburg Equilibrium (HWP) using TFPGA (ICRISAT, Patancheru 2004)

	IS 258			IS 264			IS 328			Xgap 1			Xtxp 141			Xtxp 258			Xtxp 289			Xtxp 65			Average all loci		
Groups	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	HWP	H_{obs}	H_{exp}	H_{unb}
I	0.000	0.390	0.00	0.000	0.420	0.00	0.250	0.514	0.00	0.050	0.509	0.00	0.050	0.421	0.00	0.350	0.634	0.00	0.000	0.388	0.00	0.050	0.219	0.01	0.094	0.437	0.448
II	0.261	0.448	0.02	0.422	0.562	0.53	0.565	0.528	0.34	0.512	0.511	1.00	0.442	0.510	0.52	0.630	0.665	0.01	0.455	0.375	0.25	0.255	0.261	1.00	0.443	0.482	0.489
III	0.594	0.721	1.00	0.406	0.442	0.67	0.581	0.493	0.42	0.400	0.331	0.56	0.867	0.741	0.04	0.588	0.555	1.00	0.433	0.362	0.29	0.849	0.617	0.00	0.590	0.533	0.541
IV	0.067	0.789	0.00	0.000	0.710	0.00	0.071	0.676	0.00	0.143	0.357	0.06	0.133	0.811	0.00	0.067	0.633	0.00	0.071	0.656	0.00	0.154	0.660	0.02	0.088	0.662	0.686
V	0.971	0.682	0.00	0.657	0.708	0.73	0.844	0.734	0.68	0.727	0.558	0.04	0.593	0.676	0.44	0.625	0.729	0.11	0.677	0.635	0.50	0.667	0.555	0.27	0.720	0.660	0.672
VI	0.833	0.781	1.00	0.520	0.621	0.22	0.696	0.661	0.68	0.375	0.322	0.55	0.840	0.788	0.66	0.640	0.614	0.24	0.750	0.690	1.00	0.760	0.711	1.00	0.677	0.649	0.662
Avgerage	0.45	0.635	0.34	0.334	0.577	0.36	0.501	0.601	0.35	0.368	0.431	0.37	0.488	0.658	0.28	0.483	0.638	0.23	0.398	0.518	0.34	0.456	0.504	0.38	0.435	0.570	0.583
Alleles		7			10			6			3			7			4			4			5			46	
Overall Fst		0.1059			0.1177			0.1096			0.1045			0.1044			0.1082			0.1044			0.1054			0.108	

Average number of alleles per locus = 5.75; Number of polymorphic loci = 8; Percent polymorphic loci (95%) = 100; Fst upper = 0.125; Fst lower = 0.084.

revealed presence of putative QTLs for all important shoot fly resistance traits, which accounted for 6 to 36% phenotypic variance. Of the 8 primer pairs used; Xtxp 258 [base pair size (bp) 190/230] and Xtxp 289 (bp 270/294) were associated with trichome density at linkage group F; Xgap 1 (bp 180/254) and Xtxp 141 (bp 154/169) were linked to deadheart incidence, leaf glossiness and trichome density at linkage group G; IS 328 (bp 144/166) and IS 264 (bp 153/207) were linked to leaf glossiness at linkage group I; and IS 258 (bp 170/193) and Xtxp 65 (bp 125/134) were linked to deadhearts and leaf glossiness at linkage group J. Forward primers were labeled with FAM (Xtxp 289 and Xgap 1 were labeled with 6-carboxyflorescein), HEX (Xtxp 65 and IS 264 with 4,7,2',4',5',7'-hexachloro-6-carboxyflorescein), or NED (Xtxp 141, IS 258, and IS 328 with 7'8"-benzo-2',4,7-trichloro-3-carboxyflorescein) (Perkin Elmar Applied Biosystems), allowing post-PCR pooling of the primer products into groups of three primer products each, with each primer product in a given group being labeled with a different dye. Primer Xtxp 258 was unlabeled.

Polymerase chain reaction (PCR) of selected SSR primers

PCR conditions for each of the eight SSR markers were optimized and PCR reactions were set up in volumes of 5 μl in 384-well PCR plates (ABGene). Each PCR reaction contained 2 to 4 pM of primer, 1 to 4 mM $MgCl_2$, 0.1 to 0.2 mM dNTP, 0.1 to 0.125 U Amplitaq Gold Polymerase (Perkin Elmer-Applied Biosystems), and 1x PCR buffer (Perkin Elmer-Applied Biosystems). Temperature cycling was done using the Gene-Amp PCR System 9600 (Perkin Elmer-Applied Biosystems) and touch-down PCR amplification; 15 minutes denaturation, followed by 10 cycles of 94°C for 10 sec, 61°C for 20 sec (ramp of 1°C per cycle) and 72°C for 30 sec, followed by 31 cycles of 94°C for 10 sec, 54°C for 20 sec, and 72°C for 30 sec. After completion of the 31 cycles, a final extension of 20 min at 72°C was included to try and minimize the +A overhang (Smith et al., 2000).

Electrophoresis of amplified SSR primers

Amplified products of primer Xtxp 258 were run on 6% non-denaturing polyacrylamide gels. Amplified DNA fragments revealed after silver staining (Fritz et al., 1999) were scored using '1' for presence, and '0' for absence of bands. PCR products of the labeled markers were pooled post-PCR, where 1 μl of the FAM-labeled product, I μl of HEX-labeled product, and 1.5 μl of the NED-labeled product were mixed with 7 μl of formamide (Perkin Elmer-Applied Biosystems), 0.3 μl of the ROX-labeled 400 HD size standard (Perkin Elmer-Applied Biosystems), and 4.2 μl of distilled water. DNA fragments were denatured and size fractioned using capillary electrophoresis on an ABI 3700 automatic DNA sequencer (Perkin Elmer-Applied Biosystems). The Genescan 3.1 software (Perkin Elmer-Applied Biosystems) was applied to size peak patterns, using the internal ROX 400 HD size standard. Genotyper 3.1 (Perkin Elmer-Applied Biosystems) was used for allele calling. Presence or absence of amplified fragments were converted into "1" and "0", respectively.

Data analysis

The binary SSR data was plotted on a mean distance similarity (MDS) scatter plot in order to screen for between-genotype differences for 144 F_1 hybrids, and their 36 parents. The NTSYSpc (Version 2.10d, Rohlf, 2000), with Winboot values as input, for Dice's dissimilarity coefficient (Dice, 1945) were used to construct UPGMA (Un-weighted Pair Group Method with Arithmetic Averages) dendrogram for the parents. The genotypic and phenotypic data was made (I = F_1 hybrids (n = 144); II – A- and B-lines (n = 24); and III = R-lines (n = 12)) to analyze pair-wise genotypic (Dice's coefficients) and phenotypic (Euclidean distances method) distances to understand the genetic and phenotypic relatedness of the hybrids with their parents, and the effects of CMS on the expression of resistance to sorghum shoot fly, *A. soccata*.

In addition, the molecular and phenotypic data of the 144 F_1 hybrids and their parents was divided into six groups, with each genotype assigned to one of the six groups based on their reaction to sorghum shoot fly [group I = shoot fly-resistant A-, B-, and R-lines (n = 21); II = shoot fly-resistant A-line x shoot fly-resistant R-line based hybrids (n = 49); III = shoot fly-resistant A-line x shoot fly-susceptible R-line based hybrids (n = 35); IV = shoot fly-susceptible A-, B-, and R-lines (n = 15); V = shoot fly-susceptible A-line x shoot fly-resistant R-line based hybrids (n = 35); and VI = shoot fly-susceptible A-line x shoot fly-susceptible R-line based hybrids (n = 25)]. The molecular data of the six groups were analyzed for average number of alleles per locus (allelic frequencies of at least 5%), number of

polymorphic loci, percentage of polymorphic loci, observed heterozygosity (H_{obs}), average expected gene diversity (H_{exp}), and average expected gene diversity corrected for small sample sizes (H_{unb}), genetic variability (Fst), and polymorphic information content (PIC). F-statistics for inter- and intra-group genetic variability, descriptive statistics for within and between group gene diversity using the program "Tools For Population Genetic Analyses" (TFPGA Version 1.3, Miller 1997, http://bioweb.usu.edu/mpmbio/index.htm) (Nei, 1978). The genotypic and phenotypic data divided into six groups were also analyzed for pair-wise genotypic distances by modified Nei's distance (Nei & Li, 1979) and phenotypic distances by Euclidean distances. The genotypic distances using Nei's distance were plotted on UPGMA.

Results

Cluster analyses of parents and their F_1 hybrids

The diversity analysis among the twelve A-, B-, R-lines, and their 144 F_1 hybrids using eight SSR markers associated with resistance to sorghum shoot fly indicated that the shoot fly-resistant parents and their hybrids were genetically less diverse than the shoot fly-susceptible parents and their hybrids. The dendrogram expressing the diversity among 36 parental genotypes used in the hybridization program (Figure 1) allowed the identification of 11 distinct groups at 50% dissimilarity coefficient. The shoot fly-susceptible genotypes Swarna, ICSV 745, and 296A & B were placed into separate groups. Amongst the 11 groups, 5 groups comprised of shoot fly-susceptible genotypes, and 3 groups of shoot fly-resistant genotypes. Three groups had both shoot fly-resistant and -susceptible genotypes. The shoot fly-resistant and -susceptible F_1 hybrids derived from these parents using line x tester mating design also showed similar grouping trend. The results suggested that there is greater diversity in the shoot fly-susceptible genotypes than in the shoot fly-resistant genotypes.

The pedigrees of the A-, B-, and R-lines (Table 1) indicated that BTx 623 was common as a female parent in five shoot fly-resistant, and two shoot fly-susceptible A- and B-lines. The shoot fly-resistant restorers ICSV 708 and PS 30710 had the common ancestry, while MR 750 and ICSV 700 had one parent in common. The higher intensity of repetition of parents in the development of shoot fly-resistant A-, B-, and R-lines narrowed their genetic base, resulting in low diversity in the shoot fly-resistant parents and their F_1 hybrids tested.

Gene diversity and genetic variability

The TFPGA analysis for 144 hybrids and their parents using eight SSR markers revealed a total of 46 putative alleles (Table 2). All the 8 loci were 100% polymorphic at 95% level of significance. The division of 144 hybrids and their parents into six groups revealed a variability of 8.8 to 72.0% in the average observed heterozygosity among these groups. Group I and IV had the lowest heterozygosity, and did not follow the Hardy-Weinberg Equilibrium, since these groups included shoot fly-resistant and -susceptible inbred lines, respectively.

The overall $H_{obs,}$ H_{exp}, and H_{unb} confirmed that the gene diversity was higher in the groups comprising of shoot fly-susceptible female x -resistant or susceptible male-based hybrids. The maximum gene diversity (H_{obs}) was observed in group V (hybrids based on shoot fly susceptible female x resistant male parents) with loci IS 258. The Fst value between the six groups for all loci varied from 0.01 to 0.22. The genetic variability among and within groups ranged from 8.4 to 12.5 and 10.8 to 89.2%, respectively, suggesting the maximum variability within groups instead of between groups.

Allelic polymorphism

The PIC of eight loci in six groups are given in Table 3. Lowest allelic polymorphism was observed in group I with loci Xtxp 289 (19.8%), and highest in group VI with loci IS 258. The pooled PIC values across groups suggested lowest allelic polymorphism (33.9%) with Xtxp 289, while loci Xtxp 258 gave the highest polymorphism (66.8%).

Morphological and genetic distances between hybrids and their parents

Morphologically, the hybrids vs. A- and B-lines were 11.7%, hybrids vs. R-lines were 86.8%, and A- and B-lines vs. R-lines were 67.1% distinct. Similar dissimilarity pattern of hybrids vs. A- and B-lines, hybrids vs. R-lines, and A- and B-lines vs. R-lines was also observed based on molecular markers (Figure 2). Genetically, these groups were 5.1, 10.7, and 29.84% distinct, respectively. The results indicated that morphologically as well as genetically, the hybrids were more close to CMS lines than to the restorers.

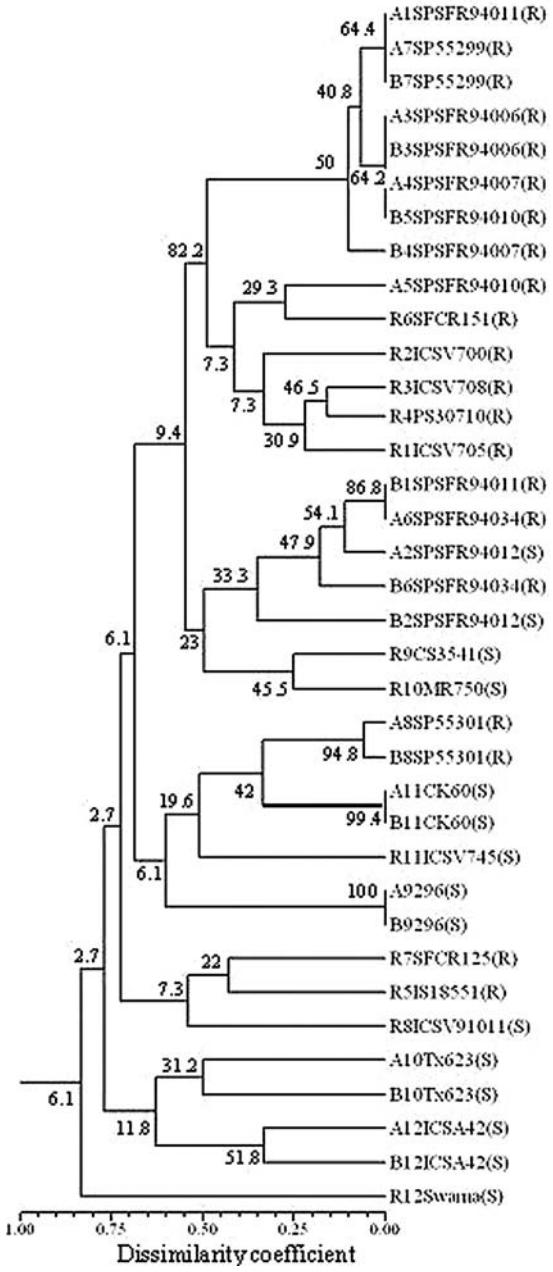

Figure 1. UPGMA dendrogram of 12 cytoplasmic male-sterile (A1 to A12), maintainer (B1 to B12), and restorer (R1 to R12) lines of sorghum using eight micro satellite markers. The letters in the parenthesis are reaction to shoot fly i.e., R = resistant, and S = susceptible. The values on the nodes of dendrogram are bootstrap values.

Table 3. Polymorphic Information Content (PIC) of eight SSR loci in six sorghum groups based on traits associated with resistance to shoot fly and their reaction to sorghum shoot fly using TFPGA (ICRISAT, Patancheru 2004)

	PIC values of 8 SSR loci							
Groups	IS 258	IS 264	IS 328	Xgap 1	Xtxp 141	Xtxp 258	Xtxp 289	Xtxp 65
I	0.466	0.632	0.635	0.429	0.632	0.590	0.198	0.215
II	0.471	0.636	0.644	0.433	0.638	0.616	0.240	0.314
III	0.669	0.492	0.530	0.424	0.704	0.498	0.439	0.597
IV	0.667	0.726	0.708	0.371	0.712	0.582	0.547	0.584
V	0.522	0.647	0.642	0.502	0.646	0.677	0.293	0.532
VI	0.790	0.659	0.635	0.393	0.761	0.540	0.655	0.661
Pooled	0.538	0.662	0.667	0.473	0.659	0.668	0.339	0.546

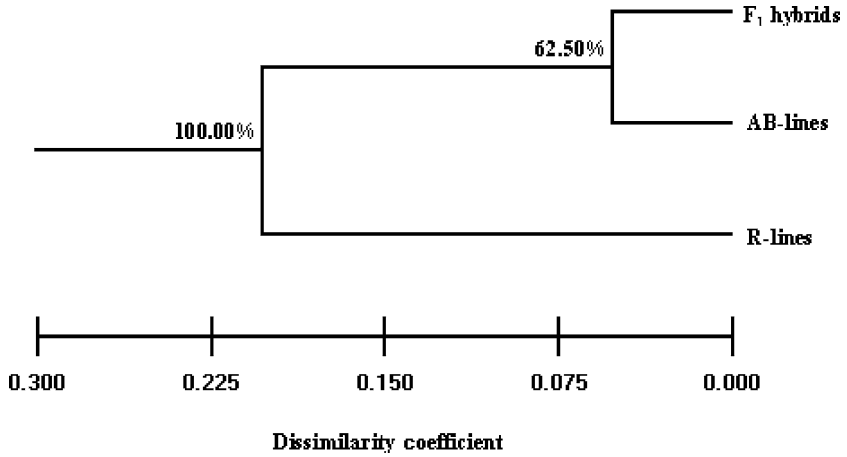

Figure 2. UPGMA dendrogram of three groups of 144 F_1 hybrids and their parents (24 AB-lines and 12 R-lines) based on QTLs associated with deadhearts, leaf glossiness, and trichome density. The values on the nodes of dendrogram are bootstrap values.

Morphological and genetic distances between shoot fly-resistant and -susceptible parents and the hybrids

The field observations on shoot fly resistance, and molecular data on eight SSR markers revealed that Group I (resistant A-, B-, and R-lines) vs. IV (susceptible A-, B-, and R-lines) were 73.2% morphologically and 38.5% genetically distinct from each other (Table 4). More morphological and genetic distinctness was encountered in certain resistant and susceptible cross combinations than their resistant and susceptible parents. Morphologically, group I (resistant A-, B-, and R-lines) vs. II (resistant × resistant crosses) were 98.1% similar to each other, while group IV (susceptible A-, B-, and R-lines) vs. VI (susceptible × susceptible cross) were 97.7% similar to each other. Similar closeness pattern of I vs. II and IV vs. VI was also observed based on molecular markers (Figure 3). Morphologically (94.0%) and genetically (47.1%), the maximum distance was observed between group I (resistant A, B-, and R-lines) vs. VI (susceptible × susceptible crosses). Morphologically, group II (resistant × resistant cross) was closer to III (resistant × susceptible crosses) than to group V (susceptible × resistant crosses). Genetically, group II (resistant × resistant crosses) was closer to group V (susceptible × resistant crosses), followed by group III (resistant × susceptible crosses). Genetically and morphologically, group III (resistant × susceptible crosses) was closer to group IV (susceptible A, B-, and R-lines) than to groups V (susceptible × resistant crosses) and VI (susceptible × susceptible crosses).

Discussion

Exploration of the genetic diversity within the sorghum gene pool has become a necessity to broaden the

Table 4. Group distances between six sorghum parents and hybrid groups based on the reaction to shoot fly damage using eight microsatellite markers (genetic) and field observations (morphological) using deadhearts at 14 DAE, leaf glossiness, and trichome density on both upper and lower leaf surfaces (ICRISAT, Patancheru 2004)

Groups compared	Genetic distances	Pairwise Fst-values	Morphological distances
I vs. II	0.0115	0.011	0.0190
I vs. III	0.1282	0.092	0.5109
I vs. IV	0.3853	0.180	0.7317
I vs. V	0.1092	0.070	0.6425
I vs.VI	0.4707	0.222	0.9401
II vs. III	0.1312	0.096	0.4347
II vs. IV	0.3720	0.185	0.6476
II vs. V	0.0883	0.060	0.5177
II vs. VI	0.4684	0.224	0.8196
III vs. IV	0.1502	0.066	0.0215
III vs. V	0.2222	0.111	0.0323
III vs. VI	0.1561	0.081	0.0675
IV vs. V	0.2335	0.067	0.0484
IV vs. VI	0.0226	0.026	0.0227
V vs. VI	0.3058	0.107	0.0515

DAE = Days after seedling emergence.

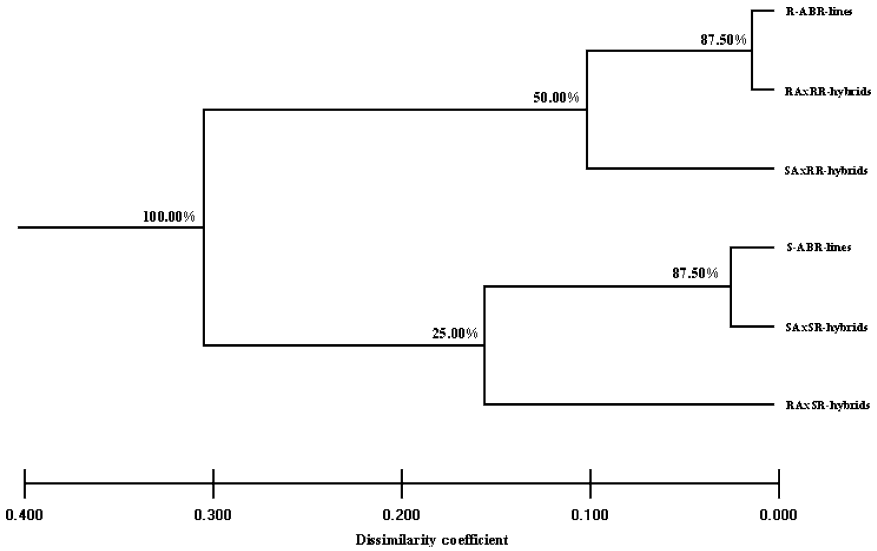

Figure 3. UPGMA dendrogram of six groups of shoot fly resistant- and susceptible parents and their F_1 hybrids in different cross combinations (grouping based on phenotypic data on deadhearts) based on markers associated with deadhearts, leaf glossiness, and trichome density. R-ABR-lines = Shoot fly-resistant cytoplasmic male-sterile (A), maintainer (B), and restorer (R) lines; S-ABR-lines = Shoot fly-susceptible ABR-lines; RA = Resistant-A-lines; SA = Susceptible-A-lines; RR = Resistant-R-lines; SR = Susceptible-R-lines. The values on the nodes of dendrogram are bootstrap values.

genetic base of the sorghum breeding material, especially for improving the level of resistance to insect pests. DNA markers have proven to be a robust and cost effective technology for assessment of genetic diversity in sorghum (Deu et al., 1994; Oliviera et al., 1996; Yang et al., 1996; Ghebru et al., 2002). Recently, SSR markers have been developed for sorghum, which are quite useful for cultivar identification, and resistance to biotic and abiotic stresses (Dean et al., 1999; Dje et al., 2000; Grenier et al., 2000; Smith et al., 2000). The SSR markers linked to QTLs associated with shoot fly-resistance traits such as leaf glossiness, trichome density, and deadhearts (Hash et al., 2003) characterized the parents and their hybrids effectively. Most of the shoot fly-resistant material originated from *durra* race, and this might have caused the loss of genetic diversity in the shoot fly-resistant parents and their hybrids. The shoot fly-resistant female × resistant or susceptible male-based hybrids and their parents formed the biggest group, having the QTLs mapped earlier for shoot fly resistance. The hybrid Tx 623A × IS 18551 and Tx 623B formed distinct groups indicating greater diversity in the shoot fly-susceptible CMS and its maintainer line Tx 623B. Genotype IS 18551 (shoot fly-resistant check) has been used for making two Recombinant Inbred Line (RIL) populations (BTx 623 × IS 18551 and 296B × IS 18551) to map QTL associated with resistance to shoot fly. Agrama et al. (2002) identified 9 QTLs associated with resistance and/ or tolerance to green bug, *Schizaphis graminum* (Rondani) biotypes I and K. Both resistance and tolerance to green bug biotypes I and K are controlled by QTL linked to four SSR (Sb5-214, Sb1-10, SbAGB03, and SbAGA01) and one RAPD (OPB12-795) markers, and these markers appeared to be linked to biotype non-specific resistance and tolerance genes.

This is the first report on the molecular characterization of shoot fly-resistant and -susceptible hybrids and their parents using the SSR loci employed for mapping QTL for shoot fly resistance in sorghum. The PIC values suggested lowest allelic polymorphism among shoot fly-resistant parents with loci Xtxp 289, and highest polymorphism among the hybrids based on susceptible females × susceptible males with loci IS 258. The genetic diversity in hybrids based on the shoot fly-susceptible female × -resistant or -susceptible male parents was greater than in hybrids based on shoot fly-resistant and -susceptible parents (Table 2). The pair-wise and overall genetic variability was lowest between shoot fly-resistant parents and the hybrids based on them, and highest in hybrids derived from shoot fly-susceptible parents. The self-pollinated species, *Hordeum spontaneum* and *Gliricidia sepium* have less within-population variation (43 and 40%, respectively) (Dawson et al., 1993), while the cross-pollinated species have higher (72 to 100%) within population variation (Huff et al., 1993). The present investigation suggested lower (10.8%) variability between groups in sorghum (which has nearly 10–20% cross pollination), and higher variability (89.2%) within groups. Although, the loss of genetic diversity in a diallel crosses is possible (Reif et al., 2003), but associations between single cross hybrid performance and genetic similarities between and within groups can help to identify heterotic groups in sorghum (Menz et al., 2004).

Morphologically, hybrids based on shoot fly-resistant female and male parents has been found with highest frequency of producing resistant hybrids (Dhillon, 2004), and are closer to the hybrids based on resistant females × susceptible males than to the hybrids derived from susceptible females × resistant males. However, SSR markers revealed a reverse trend, suggesting the need for fine mapping of QTL associated with resistance to shoot fly. It is expected that a higher number of markers will provide a more precise estimate of genetic relationship, but the distribution of these markers over the genome is equally important (Menz et al., 2004). The clusters of F_1 hybrids and their parents based on SSR markers and morphological variability followed similar pattern, and suggested that CMS influences on the expression of resistance to sorghum shoot fly. The gene frequencies in the hybrids were dependent on the gene frequencies in the CMS lines, because the gametes are produced in Hardy-Weinberg equilibrium (Witcombe & Hash, 2000). The frequency of genotypes having resistance-alleles at several loci increased greatly in both seed parents and hybrids when the overall frequency of resistance-alleles in the maintainer lines increased. These studies also demonstrated that molecular markers for QTL associated with insect resistance could also be used to characterize the homologous traits.

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