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ORIGINAL ARTICLE

# Genomic diversity among sorghum genotypes with resistance to sorghum shoot fly, *Atherigona soccata*

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**Abstract** Host plant resistance is one of the important components for management of sorghum shoot fly, *Atherigona soccata*. The levels of resistance in cultivated germplasm are low to moderate, and therefore, it is important to identify sorghum genotypes with diverse mechanisms of resistance based on physico-chemical and or molecular markers. We assessed the genetic diversity of 15 sorghum genotypes with different levels of resistance/susceptibility to shoot fly, *A. soccata* using 93 sorghum simple sequence repeat (SSR) primer pairs and simultaneously characterized for 15 morpho-biochemical traits associated with shoot fly resistance. Of these 93 SSR primer pairs, amplification products from 79, thought to correspond to single-copy loci distributed across all ten sorghum chromosome pairs, showed good polymorphism across the 15 sorghum genotypes. The polymorphic information content (PIC) values of these 79 SSR markers ranged from 0.06 to 0.86. The Principal Coordinate Analyses (PCoA) and cluster analyses based on dissimilarity matrices derived from SSR based allelic variation (Neighbor-Joining distance) and variation in 15 morpho-biochemical traits (based on Gower's distance), revealed grouping of most susceptible genotypes in single cluster. The improved breeding lines grouped with resistant or susceptible genotypes, based on shared pedigree. Based on these results, three resistant accessions viz., IS 1054, IS 1057 and IS 4664 were found diverse to IS 18551, which is widely used as shoot fly resistance donor. These diverse sources, after further characterization for resistance mechanisms, can be used in breeding programs for improving shoot fly resistance.

**Keywords** SSR markers · Genetic diversity · PIC value · Sorghum · Shoot fly · *Atherigona soccata*

**Abbreviations**

| | |
|---|---|
| PCR | Polymerase chain reaction |
| SSRs | Simple sequence repeats |
| EST-SSRs | Expressed sequence tags-simple sequence repeats |
| QTL | Quantitative trait loci |
| PIC | Polymorphic information content |
| PCoA | Principal coordinate analyses |
| $H_T$ | Total gene diversity |
| DAE | Days after seedling emergence |

## Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop in Asia, Africa, Australia, and the Americas. It is the fifth most important cereal crop globally after wheat, rice, maize, and barley. It is grown in 86 countries covering an area of about 42 million ha, with an annual grain production of 58.7 million tons (FAO 2004). In India, sorghum is the third important cereal after rice and wheat, and is currently grown in 10.4 mha annually, with an average grain production of 8 million tons (FAS 2005). Sorghum grain is used as a staple food in Asia and Africa (Awika and Rooney 2004; Ratnavathi and Sashidhar 1998), and as a livestock feed in Australia and the Americas. Sorghum has also been identified as a potential source of bio-fuels (Antonopoulou et al. 2008). It is a model species for tropical grasses having the 'C4' photosynthetic

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pathway, and is a logical complement to the 'C3'grass, *Oryza sativa* (Kresovich et al. 2005; Paterson et al. 2009).

Genetic manipulation of sorghum since the 1960s has led to development of several high-yielding varieties and hybrids. However, average sorghum grain yields on farmers' field in India continue to be quite low (500–800 kg ha$^{-1}$), although the potential yields are as high as 10 tons ha$^{-1}$ (Sharma 1985). Several insect pests constrain sorghum yields in the semi-arid tropics. Nearly 150 insect species have been reported to damage this crop, of which sorghum shoot fly, *Atherigona soccata* (Rondani) (Diptera: Muscidae) is one of the most important sorghum insect pests in Asia, Africa, and the Mediterranean basin.

Over 30,000 germplasm accessions have been screened for resistance to shoot fly, *A. soccata* (Sharma et al. 2003), considerable progress has been made in transferring resistance into elite breeding lines (Sharma et al. 1992, 2005), and QTLs for components of host plant resistance have been identified (Folkertsma et al. 2003; Satish et al. 2009). A number of sorghum genotypes with resistance to shoot fly have been identified, but the levels of resistance are low to moderate (Jotwani 1978; Taneja and Leuschner 1985; Sharma et al. 2005). In the past, studies have been conducted on genetic variation in morphological and biochemical traits associated with resistance to this insect (Sharma and Nwanze 1997). However, this approach has its limitations as complex quantitatively inherited traits are difficult to understand solely on the basis of phenotype. For this reason, DNA-based methods have been employed in studies on sorghum genetic diversity, and in genetic improvement of this crop. In contrast to morphological and biochemical traits, molecular markers rely on DNA assay, and are not influenced by variation in shoot fly abundance and climatic conditions across seasons and locations. High-density genetic maps available for sorghum (Klein et al. 2000; Bowers et al. 2003; Mace et al. 2008), have recently been complemented by the aligned genome sequence (Paterson et al. 2009), permitting *in silico* mapping of many additional markers and genes (Yonemaru et al. 2009; Li et al. 2009; Ramu et al. 2009, 2010; Mace and Jordan 2010). There is considerable information available on genetic linkage maps and molecular markers associated with different traits in sorghum. The present studies were undertaken to study the genetic diversity among the sorghum lines with different levels of resistance/susceptibility to *A. soccata* to identify genetically diverse lines for use in sorghum improvement for resistance to *A. soccata*.

## Materials and methods

### Experimental material

The experimental material consisted of 15 sorghum genotypes comprising of seven highly resistant germplasm accessions (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, and IS 2205), and three moderately resistant breeding lines (SFCR 125, SFCR 151, and ICSV 700) to shoot fly (Sharma et al. 2006),and five commercial cultivars (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) susceptible to *A. soccata* (Chamarthi et al. 2011). Of these, IS 18551 and Swarna served as resistant and susceptible checks, respectively. The origin and pedigrees of the test genotypes are given in (Table 1). The experiments were conducted at the research farm of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), at Patancheru, Andhra Pradesh, India, during the 2004–2005 rainy (Jul–Nov) and post-rainy (Oct–Mar) seasons.

### Evaluation of sorghum genotypes for resistance to shoot fly and morphological traits associated with shoot fly resistance

The test material was evaluated for resistance/susceptibility to shoot fly in replicated trials over three seasons (Chamarthi et al. 2008). Data on 14 different morphological and biochemical traits *viz.*, deadhearts incidence (%) [14 and 21 days after emergence (DAE)], oviposition incidence (%) [21 and 28 DAE], leaf glossiness, trichome density on abaxial and adaxial surfaces of the leaf blade, plumule and leaf sheath pigmentation, leaf surface wetness, tannins (%), total soluble sugars

**Table 1** Origin and pedigrees of 15 sorghum genotypes differing in their shoot fly reactions

| Genotypes | Pedigree |
|---|---|
| Shoot fly resistant | |
| IS 1054 | Maldandi 35-1, PI 248264 (Durra landrace, India) |
| IS 1057 | Bird resistant, PI 248267 (Durra landrace, India) |
| IS 2146 | Kaura, PI 221569 (Durra landrace, Nigeria) |
| IS 2205 | Jaglur (Durra-Bicolor landrace, India) |
| IS 2312 | Safra shahadasal Q2-2-88 (Durra landrace, Sudan) |
| IS 4664 | Dagri dahere (Durra landrace, India) |
| IS 18551 | Jijwejere 935 (Durra-Bicolor landrace, Ethiopia)[a] |
| SFCR 125 | (ICSV 705×YT-3-47)-7-1-1-2 |
| SFCR 151 | (1011 E No 23-2 (PM 12645×IS 2205))-5-1-2-2 |
| ICSV 700 | (IS 1082×SC 108-3)-1-1-1-1-1 |
| Shoot fly susceptible | |
| Swarna | Selection from IS 3924 (Durra-Caudatum landrace from India) |
| CK 60B | Selection from (Day milo×Blackhull kafir) |
| ICSV 745 | ((IS 3443×DJ 6514)-1-1-1-1-1)×(E 35-1×US/B 487)-2-1-4-1-1-3)-4-1-1-1 |
| 296B | IS 3922×Karad local |
| ICSV 112 | [(IS 12622 C×555)×((IS 3612 C×2219B)-5-1×E 35-1)]-5-2 |

[a] Shoot fly resistance donor parent in three sorghum RIL mapping populations

(TSS) (%), fat (%), Mg (%) and Zn (ppm) were recorded as per Chamarthi et al. (2011). This study reported significant correlation of these morphological and biochemical traits with shoot fly resistance among the 15 selected test genotypes used in current study.

### Genomic DNA isolation

For DNA extraction, the test material was planted in the greenhouse in small pots (10 cm dia) using a potting mixture of black soil and farmyard manure (3:1). Seven day-old seedlings were used for DNA extraction. Leaf strips (5 cm long) were collected in eppendorf tubes containing two pre-chilled steel balls (at −20°C for 30 min). DNA was isolated from the leaf tissue samples using a mini-prep 3% CTAB method (Mace et al. 2003). The quality and quantity of each DNA sample was determined based on agarose gel electrophoresis using uncut-λ DNA standards of known concentration and subsequently normalized to a working concentration of 5 ng/$\mu$l.

### PCR amplification of SSR markers

Primer pairs for the SSR markers used were previously defined by Brown et al. (1996) for the *Xgap* series; Taramino et al. (1997) for the *SbKAFGK1* and *XSbA* series; Kong et al. (2000) and Bhattramakki et al. (2000) for the *Xtxp* series from Texas A&M University; Schloss et al. (2002) for the *Xcup* series of EST-SSRs from mapped cDNA probe sequences; Ramu et al. (2009) for the *Xisep* series of EST-SSRs from ICRISAT; and Mace et al. (2009) for the *Xgpsb* and *XmSbCIR* series from GenoPlante and CIRAD. A total of 93 SSR primer pairs were tested, of which 79 SSRs (Table 2) were found to provide scorable amplification products sufficiently polymorphic for assessment of genetic diversity and molecular characterization of the sorghum genotypes tested. The polymerase chain reaction (PCR) for SSR amplification was performed in 5 µl reaction volumes with final concentrations of 5 ng DNA, 2 mM $MgCl_2$, 0.1 mM of dNTPs, 1x PCR buffer, 0.2 µM of each primer, and 0.1 U of *Taq* DNA polymerase (AmpliTaq Gold®, Applied Biosystems, USA) in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) with the following touch-down PCR protocol: initial denaturation at 94°C for 15 min (to activate *Taq* DNA polymerase), followed by 10 cycles of denaturation at 94°C for 15 s, annealing at 61°C for 20 s (temperature reduced by 1°C for each cycle), and extension at 72°C for 30 s. This was followed by 34 cycles of denaturation at 94°C for 10 s, annealing at 54°C for 20 s, and extension at 72°C for 30 s with the final extension of 20 min at 72°C. Amplified PCR products were separated by capillary electrophoresis using an ABI Prism 3700 DNA Sequencer. For this purpose, forward primers were labeled 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) to allow post-PCR pooling of the amplified products. PCR products were pooled with 0.5 µl of the FAM-labeled product, 0.5 µl of the HEX labeled product, and 1 µl of the NED labeled product being mixed together with 0.075 µl of the ROX-labeled 400 HD size standard (Applied Biosystems) and Hi-Di$^{TM}$ formamide (Applied Biosystems) to a total volume of 15 µl. DNA fragments were denatured for 5 min at 94°C in the PCR machine (Perkin Elmer 9700, Applied Biosystems), and size fractioned using capillary electrophoresis. Repeatability of each PCR and capillary electrophoresis run was verified by including a control sample (BTx623) in every assay.

### Data analysis

All of 79 SSR markers showing high reproducibility, with high consistency in size of the amplified product of the control sample (BTx623) between PCR and ABI runs. Therefore, all 79 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 (http://www.icrisat.org/gt-bt/Allelobin.htm) utilizes the algorithm developed by Idury and Cardon (1997). Using the "binned" data set, PowerMarker v3.25 (Liu and Muse 2005) was used to 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 (Smith et al. 2000) and gene diversity. DARwin v5.0 (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 Neighbor-Joining trees (Saitou and Nei 1987) using DARwin v5.0.

Pair-wise genetic dissimilarity values based Gower's distance (Gower and Legendre 1986) were worked out using morphological and biochemical traits data (SAS 9.1). The dissimilarity indices obtained were used to perform principal coordinate analyses using DARwin v5.0 (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 10000. 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 v5.0.

**Table 2** SSR markers used for assessing genetic diversity of sorghum genotypes (ICRISAT, Patancheru, India)

| SSR marker locus | Linkage group | Allele no. | Minimum allele size (bp) | Maximum allele size (bp) | Gene diversity value | Heterozygosity value | PIC value |
|---|---|---|---|---|---|---|---|
| *Xgpsb017* | SBI-05 | 3 | 189 | 195 | 0.42 | 0.00 | 0.37 |
| *Xgpsb027* | SBI-10 | 6 | 167 | 193 | 0.77 | 0.00 | 0.74 |
| *Xgpsb067* | SBI-08 | 4 | 188 | 198 | 0.56 | 0.00 | 0.50 |
| *Xgpsb089* | SBI-01 | 4 | 164 | 172 | 0.44 | 0.00 | 0.41 |
| *Xgpsb123* | SBI-08 | 3 | 308 | 314 | 0.43 | 0.00 | 0.39 |
| *Xgpsb128* | SBI-02 | 2 | 264 | 286 | 0.48 | 0.00 | 0.36 |
| *Xgpsb148* | SBI-07 | 3 | 130 | 144 | 0.37 | 0.07 | 0.32 |
| *XmSbCIR223* | SBI-02 | 4 | 121 | 133 | 0.51 | 0.00 | 0.46 |
| *XmSbCIR238* | SBI-02 | 6 | 90 | 106 | 0.77 | 0.00 | 0.74 |
| *XmSbCIR240* | SBI-08 | 4 | 125 | 139 | 0.65 | 0.07 | 0.60 |
| *XmSbCIR246* | SBI-07 | 3 | 114 | 120 | 0.24 | 0.00 | 0.23 |
| *XmSbCIR248* | SBI-05 | 2 | 110 | 120 | 0.23 | 0.00 | 0.20 |
| *XmSbCIR262* | SBI-10 | 2 | 232 | 236 | 0.42 | 0.07 | 0.33 |
| *XmSbCIR276* | SBI-03 | 2 | 248 | 252 | 0.12 | 0.00 | 0.12 |
| *XmSbCIR283* | SBI-10 | 4 | 132 | 160 | 0.54 | 0.07 | 0.50 |
| *XmSbCIR286* | SBI-01 | 5 | 127 | 145 | 0.67 | 0.07 | 0.63 |
| *XmSbCIR300* | SBI-07 | 3 | 122 | 128 | 0.64 | 0.00 | 0.57 |
| *XmSbCIR306* | SBI-01 | 2 | 139 | 141 | 0.50 | 0.00 | 0.37 |
| *XmSbCIR329* | SBI-01 | 4 | 129 | 135 | 0.51 | 0.00 | 0.46 |
| *XSbAGA01* | SBI-10 | 6 | 86 | 100 | 0.78 | 0.00 | 0.75 |
| *XSbAGB02* | SBI-07 | 5 | 114 | 140 | 0.61 | 0.07 | 0.57 |
| *XSbAGE03* | SBI-02 | 2 | 76 | 78 | 0.23 | 0.00 | 0.20 |
| *SbKAFGK1* | SBI-05 | 4 | 130 | 148 | 0.54 | 0.21[a] | 0.48 |
| *Xcup02* | SBI-09 | 4 | 210 | 225 | 0.47 | 0.07 | 0.44 |
| *Xcup07* | SBI-10 | 3 | 191 | 269 | 0.54 | 0.00 | 0.45 |
| *Xcup14* | SBI-03 | 3 | 222 | 228 | 0.46 | 0.07 | 0.41 |
| *Xcup28* | SBI-04 | 3 | 152 | 164 | 0.56 | 0.00 | 0.50 |
| *Xcup53* | SBI-01 | 3 | 205 | 217 | 0.66 | 0.07 | 0.59 |
| *Xcup60* | SBI-01 | 2 | 151 | 163 | 0.12 | 0.00 | 0.12 |
| *Xcup61* | SBI-03 | 2 | 215 | 218 | 0.39 | 0.00 | 0.31 |
| *Xcup62* | SBI-01 | 2 | 188 | 191 | 0.39 | 0.00 | 0.31 |
| *Xcup63* | SBI-02 | 2 | 152 | 164 | 0.12 | 0.00 | 0.12 |
| *Xcup69* | SBI-02 | 2 | 236 | 251 | 0.50 | 0.00 | 0.37 |
| *Xgap010* | SBI-04 | 4 | 250 | 302 | 0.47 | 0.07 | 0.44 |
| *Xgap034* | SBI-08 | 2 | 195 | 197 | 0.32 | 0.00 | 0.27 |
| *Xgap072* | SBI-06 | 4 | 207 | 213 | 0.60 | 0.00 | 0.53 |
| *Xgap084* | SBI-02 | 6 | 201 | 239 | 0.77 | 0.07 | 0.73 |
| *Xgap206* | SBI-09 | 9 | 127 | 163 | 0.86 | 0.00 | 0.85 |
| *Xgap342* | SBI-07 | 6 | 274 | 286 | 0.74 | 0.07 | 0.71 |
| *Xisep0228* | SBI-04 | 3 | 215 | 223 | 0.59 | 0.00 | 0.51 |
| *Xisep0607* | SBI-10 | 3 | 206 | 215 | 0.66 | 0.13[b] | 0.58 |
| *Xisep0608* | SBI-10 | 3 | 228 | 237 | 0.64 | 0.00 | 0.57 |
| *Xisep0632* | SBI-08 | 2 | 208 | 212 | 0.44 | 0.00 | 0.35 |
| *Xisep0948* | SBI-04 | 2 | 217 | 239 | 0.50 | 0.00 | 0.37 |
| *Xisep1014* | SBI-09 | 4 | 214 | 240 | 0.44 | 0.00 | 0.41 |
| *Xtxp006* | SBI-06 | 7 | 81 | 115 | 0.80 | 0.00 | 0.77 |
| *Xtxp010* | SBI-09 | 5 | 152 | 168 | 0.67 | 0.00 | 0.61 |
| *Xtxp012* | SBI-04 | 7 | 192 | 214 | 0.78 | 0.07 | 0.75 |

**Table 2** (continued)

| SSR marker locus | Linkage group | Allele no. | Minimum allele size (bp) | Maximum allele size (bp) | Gene diversity value | Heterozygosity value | PIC value |
|---|---|---|---|---|---|---|---|
| *Xtxp015* | SBI-05 | 6 | 218 | 248 | 0.74 | 0.00 | 0.70 |
| *Xtxp020* | SBI-10 | 5 | 182 | 222 | 0.73 | 0.00 | 0.69 |
| *Xtxp021* | SBI-04 | 3 | 188 | 198 | 0.34 | 0.00 | 0.31 |
| *Xtxp023* | SBI-10 | 7 | 175 | 189 | 0.77 | 0.07 | 0.73 |
| *Xtxp027* | SBI-04 | 9 | 295 | 327 | 0.87 | 0.00 | 0.86 |
| *Xtxp031* | SBI-03 | 8 | 201 | 237 | 0.81 | 0.07 | 0.79 |
| *Xtxp040* | SBI-07 | 2 | 154 | 157 | 0.44 | 0.00 | 0.35 |
| *Xtxp047* | SBI-08 | 3 | 260 | 266 | 0.55 | 0.00 | 0.48 |
| *Xtxp057* | SBI-06 | 5 | 259 | 271 | 0.65 | 0.00 | 0.61 |
| *Xtxp065* | SBI-05 | 3 | 127 | 133 | 0.55 | 0.00 | 0.46 |
| *Xtxp075* | SBI-01 | 5 | 150 | 178 | 0.72 | 0.00 | 0.67 |
| *Xtxp088* | SBI-01 | 8 | 105 | 163 | 0.78 | 0.07 | 0.76 |
| *Xtxp095* | SBI-06 | 5 | 51 | 99 | 0.70 | 0.00 | 0.66 |
| *Xtxp114* | SBI-03 | 3 | 249 | 258 | 0.60 | 0.00 | 0.54 |
| *Xtxp136(Kaf3)* | SBI-10 | 2 | 257 | 260 | 0.06 | 0.07 | 0.06 |
| *Xtxp141* | SBI-10 | 5 | 154 | 184 | 0.71 | 0.07 | 0.67 |
| *Xtxp145* | SBI-06 | 5 | 231 | 261 | 0.73 | 0.00 | 0.69 |
| *Xtxp210* | SBI-08 | 4 | 185 | 205 | 0.75 | 0.00 | 0.70 |
| *Xtxp215* | SBI-03 | 3 | 165 | 169 | 0.24 | 0.00 | 0.23 |
| *Xtxp262* | SBI-05 | 3 | 166 | 170 | 0.56 | 0.07 | 0.50 |
| *Xtxp265* | SBI-06 | 6 | 209 | 227 | 0.80 | 0.07 | 0.77 |
| *Xtxp273(Pbbf)* | SBI-08 | 4 | 235 | 253 | 0.69 | 0.00 | 0.64 |
| *Xtxp278* | SBI-07 | 2 | 263 | 269 | 0.23 | 0.00 | 0.20 |
| *Xtxp289* | SBI-09 | 5 | 267 | 321 | 0.59 | 0.00 | 0.55 |
| *Xtxp295* | SBI-07 | 6 | 166 | 176 | 0.74 | 0.07 | 0.72 |
| *Xtxp304* | SBI-02 | 9 | 214 | 313 | 0.83 | 0.00 | 0.81 |
| *Xtxp312* | SBI-07 | 7 | 138 | 219 | 0.77 | 0.07 | 0.73 |
| *Xtxp320* | SBI-10 | 4 | 293 | 305 | 0.65 | 0.00 | 0.59 |
| *Xtxp321* | SBI-08 | 7 | 212 | 236 | 0.78 | 0.13[b] | 0.75 |
| *Xtxp340* | SBI-01 | 5 | 181 | 199 | 0.65 | 0.00 | 0.61 |
| *Xtxp354* | SBI-08 | 7 | 155 | 167 | 0.76 | 0.07 | 0.73 |
| Minimum | | 2 | 51 | 78 | 0.06 | 0.00 | 0.06 |
| Maximum | | 9 | 308 | 327 | 0.87 | 0.21 | 0.86 |
| Mean | | 4.2 | – | – | 0.57 | 0.03 | 0.52 |
| Total | | 332 | – | – | – | – | – |

[a] The high "heterozygosity" value for this SSR marker, the primer pair which was derived from a multi-copy kafirin seed storage protein gene, suggested that this primer pair detects polymorphism at more than one genetic locus in the sorghum nuclear genome

[b] Two additional SSR markers that detected polymorphism at more than one genetic locus, based on their high "heterozygosity" values

## Results

### Genetic diversity based on SSR markers

Of the 93 microsatellite (SSR) primer pairs used, 79 showed good polymorphism between the sorghum genotypes tested. Primer pairs for seven SSR loci for which map positions have previously been reported (*Xgap001*, *Xisep0110*, *Xisep0314*, *Xisep0128*, *Xtxp287*, *Xtxp327*, *Xtxp343*) failed to produce detectable amplicons, five SSRs (*Xgpsb118*, *Xisep0310*, *Xcup52*, *Xtxp339* and *Xtxp059*) were monomorphic across this small set of cultivated sorghum genotypes, and PCR products from two SSR primer pairs (*Xisep0443* and *Xisep1008*) had high "heterozygosity" values,

suggesting that they each detect polymorphism at more than a single genetic locus. The list of the remaining 79 SSR markers, which appear to detect single-copy (or at least low copy) loci, with PCR products exhibiting scorable polymorphism is given in Table 2. Individual PCR products were pooled based on their expected size ranges, and separated by capillary electrophoresis using an internal size standard. A total of 332 alleles were detected with an average of 4.2 alleles per marker, and 2 to 9 alleles per marker with an average of 0.52. Heterozygosity ranged from 0.00 to 0.21 (mean 0.03). Among the 79 retained markers, SbKAFGK1 showed maximum observed heterozygosity value (0.21) across 15 genotypes. The polymorphic information content (PIC) values ranged from 0.06 to 0.86. The highest level of polymorphism was detected with the primer pair for *Xtxp027* (0.86), followed by *Xgap206* (0.85). Lowest polymorphism was observed with the primer pair for *Xtxp136 (Kaf3)* (0.06) (Table 2).

Factorial analysis of the 15 sorghum genotypes using DARwin v5.0, based on a simple matching dissimilarity matrix constructed from allele sizes for 79 SSR markers, placed the sorghum genotypes into 5 divergent groups on first two axes (A=IS 18551, IS 2205, IS 2312 and IS 2146; B=IS 1054, IS 1057, IS 4664 and ICSV 700; C=296B and SFCR 125; D=Swarna, CK 60B, ICSV 745 and ICSV 112; and E=SFCR 151 (Fig. 1). The first axes accounted for 35.5% of total variation detected by these 79 SSRs. Neighbor-joining cluster analyses of 15 sorghum accessions placed these genotypes in three major clusters. All shoot fly-resistant and moderately-resistant genotypes were grouped together in cluster IIa and IIb. Most of the agronomically elite, shoot fly-susceptible breeding products (both hybrid parental lines and pure line varieties) having the appearance of intermediate racial backgrounds were placed in cluster I, while ICSV 700 was placed separately in cluster III (Fig. 2).

The morphological data for 15 morphological and biochemical traits were used for dissecting the diversity in the selected shoot fly resistant and susceptible accessions. A dissimilarity matrix based on Gower's genetic distance was used for plotting the tree and PCoA. The PCoA (Fig. 3) revealed clear distinction of shoot fly resistant and susceptible accessions, with susceptible accessions grouped in single cluster (Cluster C), and resistant accessions in two clusters (Cluster A and B). There is a concurrence of this grouping with SSRs based factorial analysis. The Gower's dissimilarity distance values for this data set ranged from 0.42 to 0.91. The tree based on hierarchical clustering grouped the accessions into three clusters, with two clusters for resistant (Cluster I and II) and one for susceptible (Cluster III) accessions (Fig. 4). The groupings were mostly similar, except where shared pedigrees over ride the trait based clustering (e.g. for 296B and SFCR125). Further to this, the scores for morphological traits *viz*., glossiness score, trichome density, leaf surface wetness score, and biochemical traits *viz*., tannins (%), Zn (ppm) along with the shoot fly resistance measure of oviposition (II), and deadhearts incidence (I and II) information were associated with the SSR-based tree constructed using DARWin v5.0. 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 to the

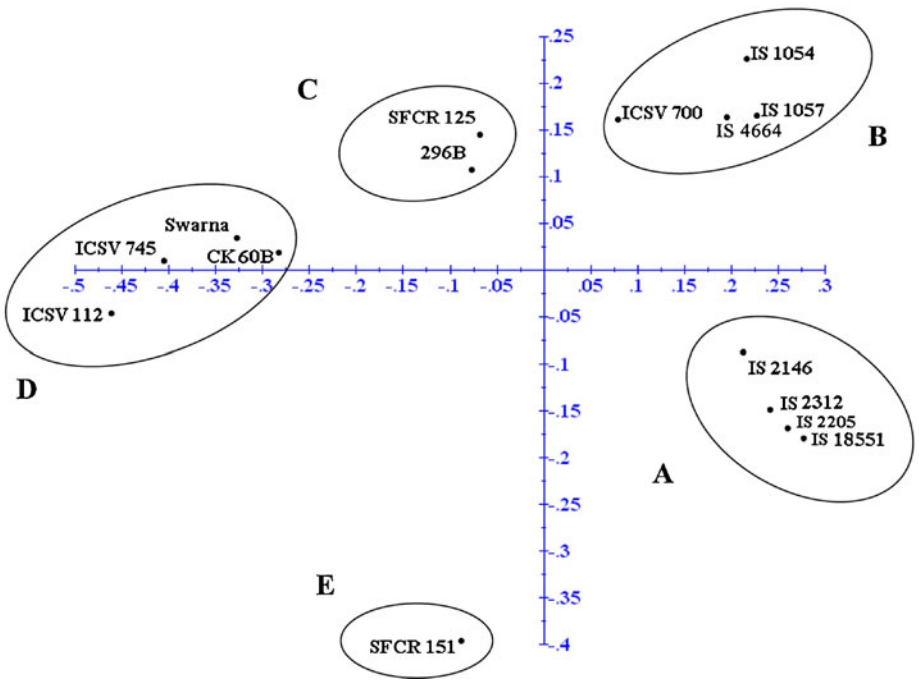


**Fig. 1** Principal Coordinate Analysis (PCoA) of 15 sorghum genotypes based on a simple matching dissimilarity matrix of allele size data using 79 sorghum SSR primer pairs

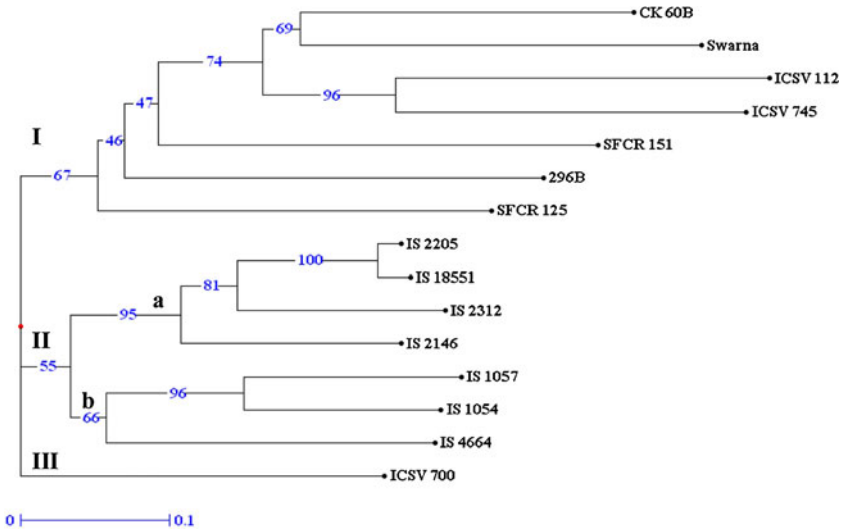


**Fig. 2** Neighbor-joining tree of 15 sorghum genotypes, based on a simple matching dissimilarity matrix of allele size data produced using 79 sorghum SSR primer pairs

distribution of these traits in each group. A major role of glossiness score, trichome density, leaf surface wetness and tannins (%) for clustering was observed, with the racial differentiation (for land race accessions) and/or shared pedigree (for improved lines) defining the within-cluster differentiation. The Mantel's test further revealed a significant association (Standard Normal variate, g=9.72; Mantel's coefficient, Z=15.53; and correlation coefficient, r=0.95) between the SSR- and morphological-based dissimilarity matrices at $p$= 0.05 for the critical value of g=2.575.

The morphological traits when overlaid over SSR-based tree, clearly distinguished most susceptible genotypes from other accessions. The non-glossy, non-trichomed genotypes with more leaf wetness and high oviposition incidence (%) compared with shoot fly resistant genotypes were present in single cluster (Cluster I).

## Discussion

Apart from RAPD, AFLP, RFLP markers, SSRs and DArT markers are very popular for detecting population structure among germplasm accessions, provided that sufficiently broad germplasm samples and large numbers of markers are used. Dhillon et al. (2006) did not observe any differences between shoot fly-resistant and -susceptible parents and their hybrids using a small sample of SSR loci reported to be associated with shoot fly resistance in the sorghum accession IS 18551 (Folkertsma et al. 2003; Satish et al. 2009).

Of the 93 microsatellites primer pairs used in the present study, 79 detected easily scorable PCR product polymorphism between the 15 sorghum accessions studied. Heterozygosity values (calculated in a manner confounding amplification of two PCR product bands from single SSR

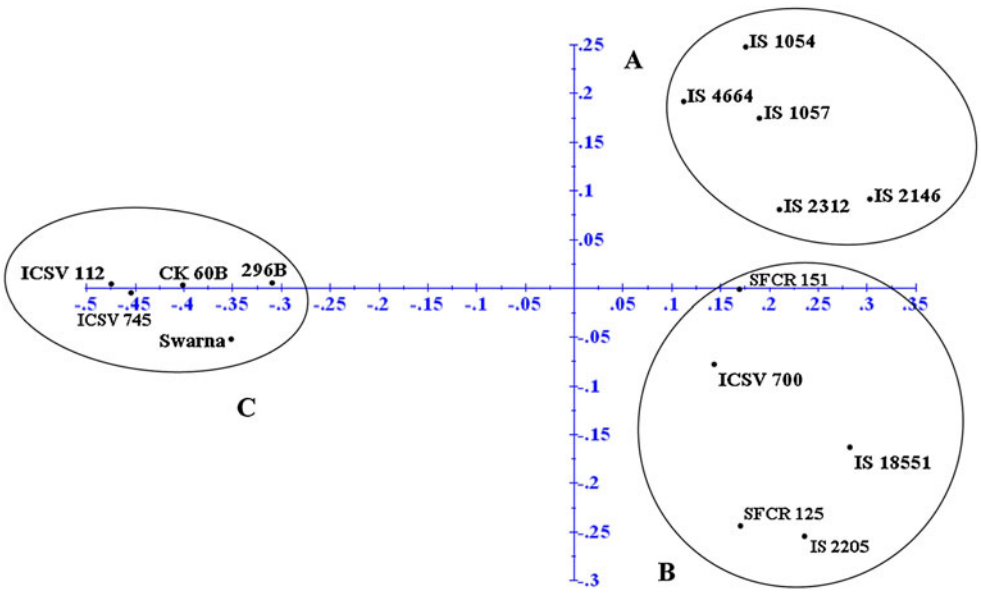


**Fig. 3** Principal Coordinate Analysis (PCoA) of 15 sorghum genotypes based on morphological traits using the Gower's dissimilarity indices with resistant and susceptible reaction to shoot fly

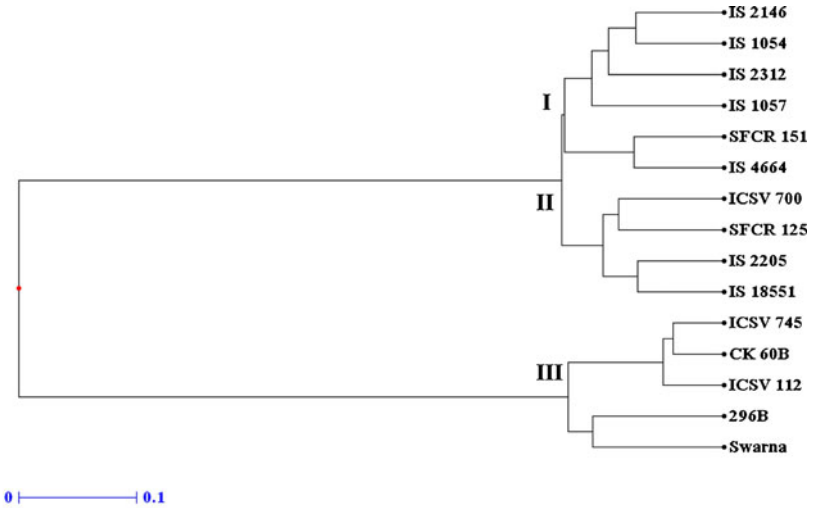


**Fig. 4** Tree constructed based on morphological traits using the Gower's dissimilarity indices and Ward's minimum variance method of clustering (with 40% threshold equality), for 15 sorghum accessions expressing resistant and susceptible reaction to shoot fly

locus due to heterozygosity, within-sample heterogeneity at that single locus, and amplification products from more than a single locus per primer pair) ranged from 0.00 to 0.21, and the kafirin seed storage protein gene-derived SSR marker SbKAFGK1 (Taramino et al. 1997) exhibited the highest "heterozygosity" value. However, it is likely that the presence of >1 PCR product band in some sorghum genotypes in this case was caused by the primer pair amplifying products at more than one kafirin locus. For two of the 79 SSR markers used (TXP321 and ISEP0607, the latter derived from an expressed gene tag sequence), also showed high (0.13) "heterozygosity" values, suggesting that they too detect two SSR loci, and not just one. Maximum polymorphism was detected by the primer pair *Xtxp027* (PIC=0.86), followed by *Xgap206* (0.85). Caniato et al. (2007) used 15 SSR primer pairs that detected total of 130 alleles across 47 sorghum lines, with 2 to 12 alleles detected per primer pair, and an average of 8.7 alleles per primer pair. The average number of alleles per primer pair observed in the present study (4.2) was fairly less than that reported for the elite sorghum lines (5.9) (Smith et al. 2000), sorghum landraces (8.7) from Southern Africa (Uptmoor et al. 2003), and sorghum hybrids and their parents with different levels of resistance to shoot fly (5.8) (Dhillon et al. 2006), probably due to the very small set of sorghum lines genotyped here. Using 3 selected SSR primer pairs, Djè et al. (1999) observed high total gene diversity ($H_T$) (0.84) across 60 sorghum germplasm samples from five regions in Morocco, while Uptmoor et al. (2003) reported $H_T$ of 0.60 using 25 SSR primer pairs with 23 sorghum landraces from southern Africa. Smith et al. (2000) reported mean PIC values of 0.58 for SSR primer pairs across 50 elite sorghum inbreds, while Abu Assar et al. (2005) detected 117 polymorphic bands with a mean of 7.3 alleles per SSR primer pair (PIC values ranged from 0.46 to 0.87, with a mean of 0.70) among 96 sorghum genotypes. In the present study, similar results were obtained with respect to gene diversity (range 0.06 to 0.87) and PIC values (range 0.06 to 0.86). Generally, PIC and gene diversity values, which are very closely related, increase with an increase in heterozygosity score. However, this trend was not consistent in the present studies for SSR markers SbKAFGK1, ISEP0607 and TXP321. All three had heterozygosity values ≥0.13 (which suggests that these primer pairs each detect SSRs at more than a single genetic locus), but different PIC values (0.48, 0.58 and 0.75, respectively).

The comparison of PCoA analysis based on dissimilarity matrix derived from SSR marker allelic variation (Fig. 1) and dissimilarity matrix derived from morphological traits based Gower's distance (Fig. 3), revealed grouping of most susceptible genotypes in a cluster and resistant land races in other cluster (with exception of 296B, ICSV700, SFCR125 and SFCR151). Similar comparison of tree diagrams of SSR-based clustering (Fig. 2) and morphological traits based clustering (Fig. 4), revealed grouping of susceptible genotypes in single cluster. The improved lines, which are breeding products from attempts to introgress shoot fly resistance into better agronomic backgrounds, were distributed across remaining clusters along with some shoot fly resistant land race accessions. This clustering of improved lines with shoot fly resistant/susceptible accessions could be driven by shared blood during improvement of these elite lines for shoot fly resistance by pedigree breeding. Leaf glossiness, trichome density, leaf surface wetness and tannins played a major role in placing the test genotypes in different clusters, with racial differentiation (for land race accessions) and/or shared pedigree (for improved lines). SSRs markers placed the test genotypes in five groups, while morphological traits placed the test material in three broad groups. All the susceptible genotypes were placed in the same group, both by the SSRs and morphological traits (except 296B). SSRs placed the shoot fly resistant lines in four groups, and thus providing further evidence for diversity among the shoot fly resistant genotypes. Sharma et al.

(2002) also reported similar results for sorghum germplasm grain mold-resistant and -susceptible genotypes.

This study helped to understand the genetic diversity among the shoot fly susceptible and resistant genotypes; and its relation to morphological and biochemical traits associated with shoot fly resistance. The dissimilarity matrices based on variation in morphological traits and SSR allelic variation, were compared to identify the diverse source of shoot fly resistance. The shoot fly resistant accessions IS 1054, IS 1057 and IS 4664 were found to be most diverse than IS 18551, an Ethiopian landrace accession widely used as donor for shoot fly resistance. The genotypes placed in different groups can be used in sorghum improvement to breed for resistance to sorghum shoot fly, *A. soccata*.

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