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Genetic diversity of elite sweet sorghum genotypes assessed by SSR markers

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Abstract

To determine genetic diversity among 47 elite sweet sorghum (*Sorghum bicolor* ssp. *bicolor* L.) genotypes, 46 simple sequence repeat (SSR) markers evenly distributed on all 10 chromosomes were selected. All SSR markers used were polymorphic among the genotypes studied. A total of 228 alleles were identified with an average of 4.96 alleles per marker. Furthermore, the genotypes studied showed medium genetic diversity. Clustering analysis grouped the 47 genotypes into 5 distinct clusters.

Additional key words: germplasm, genetic relationship, *Sorghum bicolor.*

Introduction

Sweet sorghum accumulates 10 - 25 % of sugar in stalk juice near the time of grain maturity. It is adapted to a wide range of climatic and soil conditions, including drought and high temperature. Most cultivated sweet sorghums are released as open-pollinated cultivars. Out-crossing in effectively homozygous lines is <10 %. However, hybrids have been developed between grain and sweet sorghums (Lu and Sun 2005). Detailed knowledge regarding genetic diversity and the relationship among breeding materials is essential for the development of new cultivars and molecular markers are excellent tools for this purpose. The various types of markers that have been used in many crops: fragment length polymorphism (RFLP; Banerjee *et al.* 1999, Deu *et al.* 2005), random amplified length polymorphism (RAPD; Uptmoor *et al.* 2003, Cordeiro *et al.* 2008), amplified fragment length polymorphism (AFLP; Menz *et al.* 2004, Saker *et al.* 2006), and simple sequence repeat (SSR; Schloss *et al.* 2002, Chimote *et al.* 2004, Akriditis *et al.* 2009). Among them, SSR loci are highly polymorphic and adaptable to automation. Numerous SSR markers in sorghum have been developed, mapped, and used in investigations of genetic diversity (Brown *et al.* 1996, Taramino *et al.* 1997, Bhattramakki *et al.* 2000, Kong *et al.* 2000, Schloss *et al.* 2002, Menz *et al.* 2004, Folkertsma *et al.* 2005). Recently, Ritter *et al.* (2007) investigated the genetic relationship between sweet and grain sorghums using AFLP, and Ali *et al.* (2008) assessed genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. China preserves a number of sweet sorghum cultivars, landraces and introduced accessions but their genetic diversity is unknown. In this study, we selected a set of SSR markers to identify selected elite sweet sorghum genotypes, and to assess the level of genetic diversity and genetic relationships among them.

Materials and methods

Common sweet sorghum (*Sorghum bicolor* ssp. *bicolor* L.) genotypes (45 including accessions, non-hybrid varieties, cultivars and breeding lines) used frequently in breeding programs in China, 2 sorghum sterile lines (Tx-623A and

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Abbreviations: AFLP - amplified fragment length polymorphism; CTAB - cetyltrimethyl ammonium bromide; di-SSR - SSR which contains a dinucleotide repeat motif; EST-SSR - SSR originated from expressed sequence tag; genome-SSR - SSR from the genomic library or anonymous DNA sequences; PIC - polymorphism information content; RAPD - random amplified length polymorphism; RFLP - restriction fragment length polymorphism; SSR - simple sequence repeat; UPGMA - unweighted pair group method with arithmetic mean.

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7050A) which were frequently used as the female parent of sweet sorghum hybrids were selected to evaluate the genetic diversity and the relationship among them (Table 1). These sorghum genotypes were acquired from Tianjin Agricultural University. Seeds of the sorghum genotypes analyzed were sown in small pots and genomic DNA was isolated from bulked leaf samples from 20 plants (14-d-old) by using cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). Finally, the quality and quantity of DNA were analyzed by running 1 % agarose gel electrophoresis containing λ-DNA standards.

PCR reactions were conducted in a 0.025 cm^3 reaction mixture containing 200 μM of each dNTP (*Biotech*, Tiangen, China), 1× PCR buffer [200 mM Tris-HCl, pH 8.4; 200 mM KCl; 100 mM $(NH_4)_2SO_4$], 1.5 or 2.0 mM $MgCl_2$, 0.2 or 0.4 μM of each primer (*Sangong*, Shanghai, China), 0.5 units of *Taq* polymerase (*Biotech*) and 20 ng of genomic DNA. DNA amplifications were performed in a *Mastercycler Gradient 5331* (*Eppendorf*, Hamburg, Germany) using the following program: an initial denaturation for 3 min at 94 °C; 35 or 40 cycles of denaturation for 30 s at 94 °C, 50 - 60 °C annealing for 40 s and extension at 72 °C for 50 s; a final extension for 7 min at 72 °C. PCR products were separated in 6 % denaturing polyacrylamide gels and silver stained as described Bassam *et al.* (1991).

For each allele, the most intense band or the top band of stuttered bands was considered to correspond to its size. Allele sizes were determined by comparison with a 50 bp DNA molecular mass ladder. The presence or absence of each allele was coded as 1 or 0, and scored in a binary data matrix. Where a PCR product was not obtained, data for the specific locus and genotype were treated as missing. In all cases, analysis tolerance of missing data was set to a 5 % per locus. Polymorphism (PIC) values provide an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus, but also the relative frequencies of those alleles in the population under study. The PIC for each SSR locus was determined as described Smith *et al.* (1997). Pair-wise genetic dissimilarities between individual genotypes were calculated using Jaccard's genetic dissimilarity coefficient, followed by cluster analysis with the *SAHN* subprogram using the unweighted pair group method with arithmetic mean (UPGMA) clustering method as implemented in the software package *NTSYS-PC* (Rohlf 2000). Mantel's test was performed to check the goodness of fit of a cluster analysis for the matrix on which it was based using the *NTSYS-PC* software with 1000 permuted data sets (Rohlf 2000). Similarly, the correlation between the Euclidean distance matrix based on SY and Jaccard's genetic dissimilarity matrix obtained with SSR data was tested for significance using a Mantel test. Allele sizes and 0/1 matrix are available to readers upon request.

Table 1. Sweet sorghum genotypes used in the study.

Genotype	Accession No.	Origin	Code
Xinliang-52		Shanxi, China	1
Tx623-A	GW002662	Texas, America	2
7050A		Liaoning, China	3
8142		Liaoning, China	4
8143		Liaoning, China	5
8147		Liaoning, China	6
8161		Liaoning, China	7
8169		Liaoning, China	8
81101		Liaoning, China	9
81102		Liaoning, China	10
BJ-285		Beijing, China	11
BJ-298		Beijing,China	12
BJ-299		Beijing, China	13
BJ-320		Beijing, China	14
BJ-338		Beijing, China	15
Luneng-2		Beijing, China	16
Luneng-3		Beijing, China	17
IS-4555	GW000686	India	18
Early honey	GW003097	Mississippi, America	19
Mal giunra	GW003267	Mississippi, America	20
MN-3460	GW003614	Mississippi, America	21
MN-3461	GW003615	Mississippi, America	22
MN-4251	GW003754	America	23
Saccaline-5	GW003968	Mississippi, America	24
Straightneck-1	GW003998	Mississippi, America	25
Straightneck-3	GW004000	Mississippi, America	26
B35	GW004073	India	27
W-454		Liaoning, China	28
W-455		Liaoning, China	29
W-456		Liaoning, China	30
W-457		Liaoning, China	31
W-461		Liaoning, China	32
Jiuliliang		Jilin, China	33
Yuan-341		Jilin, China	34
Jian-8-2		Jilin, China	35
Yuantian-1		Jilin, China	36
WPW		Tianjin, China	37
Sugar drip	GW001771	Mississippi, America	38
Honey-2	GW003167	Mississippi, America	39
Keller-Jin	GW003223	Shanxi, China	40
Cowley	GW003068	Texas, America	41
Wray	GW004069	Mississippi, America	42
Rio	GW003945	Mississippi, America	43
Roma	GW003948	America	44
Italian	GW000011	Australia	45
TS-175		Tianjin, China	46
TS-185		Tianjin, China	47

Results

In a preliminary experiment, 102 SSR markers were chosen based on genome position (10 sorghum linkage groups or chromosomes), repeat units, and the number of alleles from SSR loci previously reported (Brown *et al.* 1996, Taramino *et al.* 1997, Kong *et al.* 2000, Schloss *et al.* 2002). PCR conditions for these SSR markers were optimized by adapting the annealing temperature, $MgCl_2$ concentration, primer concentration, and cycle number

Table 2. Characteristic of the 46 SSR markers. Chr - chromosome location (linkage group); common alleles - number of alleles per locus with frequency ≥ 5 %, PIC - polymorphism information content.

Locus	Chr	Repeat motif	Origin	Number of alleles [locus^{-1}]	Common alleles	Size range [bp]	PIC
Sb5-256	1	(AG)8	genome clone	3	3	170-174	0.2644
Sb6-57	1	(AG)18	genome clone	5	5	292-318	0.6492
Xcup33	1	(AT)7	EST-RFLP-Probe	2	1	275-277	0.0815
Xcup53	1	(TTTA)5	EST-RFLP-Probe	2	2	189-197	0.4563
Xcup60	1	(CGGT)4	EST-RFLP-Probe	2	2	159-167	0.2748
Xcup62	1	(GAA)6	EST-RFLP-Probe	2	2	192-195	0.3608
Xtxp32	1	(AG)16	genome clone	13	6	132-162	0.8334
Xcup26	2	(CT)6	EST-RFLP-Probe	3	3	228-236	0.5518
Xcup29	2	(AT)6	EST-RFLP-Probe	3	3	197-203	0.5138
Xcup63	2	(ATGCG)4	EST-RFLP-Probe	2	2	144-151	0.1901
Xcup69	2	(GGATGC)4	EST-RFLP-Probe	3	3	247-259	0.5360
Xcup74	2	(TG)9	EST-RFLP-Probe	3	3	234-240	0.5903
Xtxp19	2	(AG)5(AG)10	genome clone	9	7	233-279	0.8542
Sb5-236	3	(AG)20	genome clone	9	5	173-187	0.7257
Xcup11	3	(GCTA)4	EST-RFLP-Probe	3	3	168-174	0.4993
Xcup14	3	(AG)10	EST-RFLP-Probe	3	3	213-221	0.5287
Xcup61	3	(CAG)7	EST-RFLP-Probe	2	2	205-208	0.1901
Xtxp31	3	(CT)25	genome clone	10	8	200-242	0.8574
Sb1-10	4	(AG)27	genome clone	4	4	256-304	0.5591
Sb4-121	4	(AC)14	genome clone	5	5	221-237	0.6021
Xcup05	4	(GA)8	EST-RFLP-Probe	7	5	216-244	0.7533
Xcup20	4	(AT)6	EST-RFLP-Probe	3	3	226-232	0.4599
Xcup71	4	(CA)7	EST-RFLP-Probe	2	2	126-124	0.4509
Sb5-85	5	(AG)12	genome clone	6	5	211-225	0.6949
SbKAF1	5	(AAC)9	genome clone	4	3	130-149	0.6292
Xtxp15	5	(TC)16	genome clone	5	4	199-223	0.7614
Xtxp23	5	(CT)19	genome clone	7	5	180-192	0.8085
Sb4-72	6	(AG)16	genome clone	4	4	189-199	0.5410
Xcup12	6	(TG)7	EST-RFLP-Probe	4	3	125-133	0.4174
Xcup37	6	(AG)9	EST-RFLP-Probe	3	3	209-215	0.5360
Xtxp17	6	(TC)16(AG)12	genome clone	7	4	168-194	0.7198
SbAGB02	7	(AG)35	genome clone	6	4	97-121	0.5052
Xcup57	7	(TAGC)5	EST-RFLP-Probe	5	4	174-190	0.6360
Xcup68	7	(TGAT)5	EST-RFLP-Probe	2	2	219-220	0.3096
Xcup70	7	(TTGTT)5	EST-RFLP-Probe	4	4	146-162	0.5858
Sb6-34	8	(AC)(CG)15	genome clone	4	3	194-208	0.5310
Xcup47	8	(GA)21	EST-RFLP-Probe	6	4	240-260	0.6990
Xtxp18	8	(AG)21	genome clone	10	6	220-266	0.8330
Sb5-206	9	(AC)13	genome clone	15	7	106-162	0.8873
SbAGE03	9	(AG)34GA(CA)4	genome clone	3	3	66- 96	0.2553
Xcup02	9	(GCA)6	EST-RFLP-Probe	4	2	199-214	0.2943
Xtxp10	9	(CT)14	genome clone	6	4	142-158	0.7103
Xcup13	10	(CCGG)5	EST-RFLP-Probe	2	2	208-212	0.3585
Xcup49	10	(GGAT)6	EST-RFLP-Probe	7	7	152-170	0.7945
Xcup50	10	(ACAGG)5	EST-RFLP-Probe	5	5	148-173	0.7252
Xtxp20	10	(AG)21	genome clone	9	7	189-243	0.8311

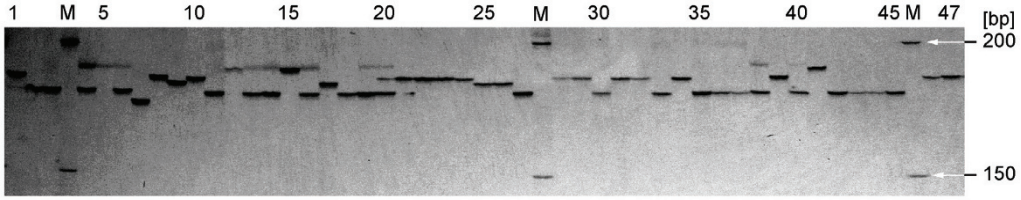

Fig. 1. Fingerprint patterns generated using SSR primer pair Xcup57 from the genomic DNA of the 47 sweet sorghum genotypes. M - 50 bp ladder; *lanes* 1 - 47 corresponding to the code of the 47 sweet sorghum genotypes in Table 1.

based on a panel of 12 sweet sorghum genotypes. Finally, 46 sorghum SSR primer pairs generating good patterns were chosen for genotyping the selected sweet sorghum genotypes. The selected SSR markers were well-distributed on all 10 chromosomes according to the chromosomal nomenclature of Kim *et al*. (2004) (Table 2). The mean map distance between neighboring SSR markers was approximately 14.6 Mbp (cM) and the number of SSR loci sited on each chromosome was generally proportional to its estimated physical length (Mbp). Among this set of SSR loci, 25 (54 %) were expressed sequence tagged-SSR (EST-SSR), and 31 (67 %) were di-repeats.

All 46 SSR markers employed were polymorphic (Fig. 1) and the profiles generated using them were collectively able to discriminate among 47 sweet sorghum genotypes. A total of 228 putative alleles with different fragment sizes were found. The average number of alleles per SSR marker was 4.96, ranging from 2 alleles (a total of 9 SSR loci including Xcup53) to 15 alleles (Sb5-206) (Table 2). When we considered only alleles with frequencies of at least 5 % and defined alleles with frequencies of less than 5 % as rare, the average number of common alleles per SSR marker was reduced to 3.85, ranging from 1 allele for marker Xcup33 to 7 alleles for marker Xtxp31. These results indicate the presence of a relatively large proportion of rare alleles among the sweet sorghum genotypes studied. The mean of the PIC value over the 46 SSR markers averaged 0.5619, ranging from 0.0815 for marker Xcup33 to 0.8873 for marker Sb5-206.

The average dissimilarity based on all 46 SSR markers among the 47 genotypes was 0.7313 ranging from 0.0426 for the genotypes pairs Jiuliliang/WPW to 0.8916 for the genotypes pairs Straightneck-3/8161 and BJ-298/8161 (data not shown). This indicated that there was a high amount of variation among the genotypes. Mantel test demonstrated that there was a significant and positive correlation between the two distances matrices from fermented sugar yield (SY) and SSR data (r = 0.23; P < 0.002), indicating that there was a weak correlation between SY and SSR data in the genotypes studied.

The UPGMA dendrograms of the sweet sorghum genotypes based on the dissimilarity matrix were truly representative of their similarity matrices since the cophenetic correlation values were 0.837 (P < 0.002) (Fig. 2). According to the cluster plot, all genotypes studied except 8161 could be classified into five groups (I, II, III, IV, and V), which included 12, 11, 5, 8, and 10 genotypes, respectively. Most genotypes with high SY, including a few of the more commonly used cultivars such as Keller-Jin, Cowley, Wray, Rio, Roma and Luneng-2, were clustered into group I, while groups IV and V contained mainly genotypes with low SY.

It could be inferred from the pedigree and background information of the studied genotypes (Table 2) that some genotypes were closely related genetically, including

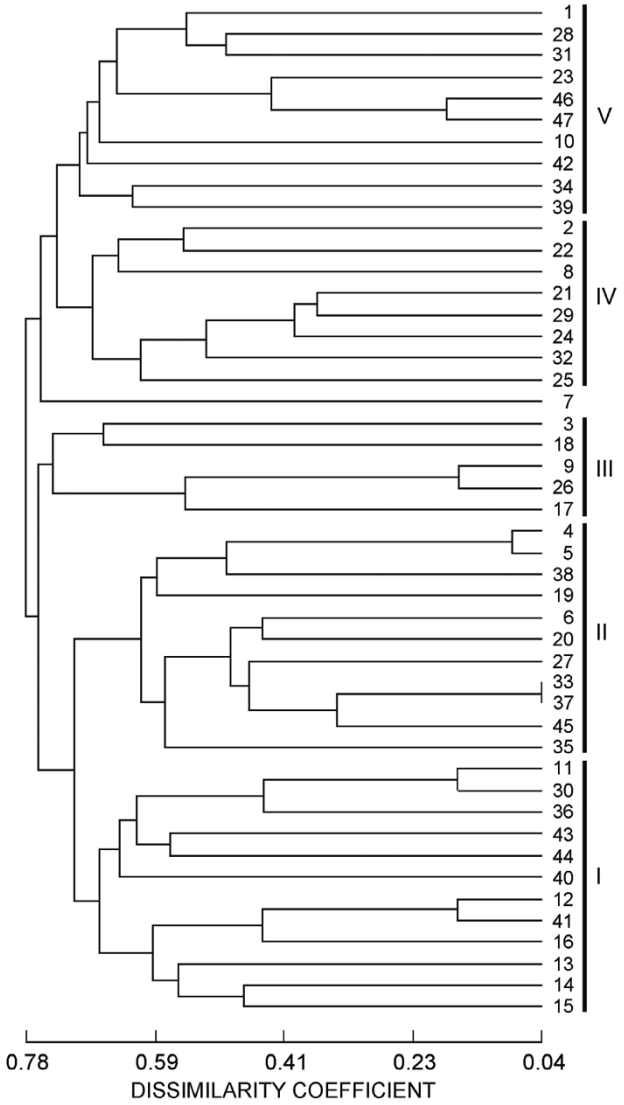

Fig. 2. UPGMA dendrogram of 47 sweet sorghum genotypes based on 46 SSR markers.

Cowley/Luneng-2, Rio/Keller-Jin, Roma/TS-175, and Rio/Wray, in which the former genotype of each couple was one of the parents of the latter. Moreover, MN-3460/MN-3461 was selected from MN 856 and TS-175/TS-185, both belonged to the Sudan grass type. However, according to our SSR data, the Cowley/Luneng-2, Rio/Keller-Jin, MN-3460/MN-3461, and TS-175/TS-185 were placed in the same groups, while Roma/TS-175 and Rio/Wray fell into different groups. Our results also showed that two coupled genotypes without pedigree and background information, Jiuliliang/WPW (dissimilarity of 0.0426) and 8142/8243 (dissimilarity of 0.0851), were closely related. In addition, the two sterile lines, Tx-623A and 7050A, fell into group III and group IV, respectively.

Discussion

Differences in genetic diversity reported in previous studies using SSR can be associated with sample size and the sample origin and background. For instance, the high genetic diversity revealed by Grenier *et al.* (2000) and Menz *et al.* (2004) may be attributable to the fact that the former sampled a large number of sorghum accessions (around 200) while the latter selected 50 sorghum inbreds belonging to different races or with different genetic backgrounds. In this study, the mean number of alleles per SSR locus (4.96) detected in sweet sorghum genotypes was higher than that detected by Ali *et al.* (2008) and Schloss *et al.* (2002) (3.22 and 3.4 respectively), similar to that described by Agrama and Tuinstra (2003) and Smith *et al.* (2000) (4.3 and 5.9 respectively), but lower than that by Kamala *et al.* (2006), Menz *et al.* (2004) and Grenier *et al.* (2000) (8.8, 7.8 and 15.4, respectively). Similarly, the gene diversity observed among the genotypes studied (mean PIC value = 0.5619) was higher than that of Ali *et al.* (2008) and Schloss *et al.* (2002) (0.4 and 0.46, respectively), similar to that of Agrama and Tuinstra (2003) and Smith *et al.* (2000) (0.62 and 0.58, respectively), and was lower than that of Grenier *et al.* (2000) and Kamala *et al.* (2006) (0.80 and 0.81, respectively).

Another factor that influenced the reported diversity level is the use of EST-SSRs. An evaluation of the distribution of different classes of SSRs in the genomes of wheat, *Arabidopsis*, maize, and rice revealed that the frequency of microsatellites was significantly higher in ESTs than in genomic DNA across all species (Morgante *et al.* 2002). However, a few studies have shown that EST-SSRs were less polymorphic than genome-SSRs in wheat (Eujayl *et al.* 2002), rice (Cho *et al.* 2000) and grape (Scott *et al.* 2000). Our results from sweet sorghum genotypes were similar to these studies in that the average number of alleles per SSR marker and the average number of common alleles per SSR marker for 25 EST-SSRs (3.4 and 3.0) was significantly lower than that for 21 genome-SSRs (6.9 and 4.9), with a T value of 4.7664 ($P < 0.01$) and a T value of 4.5254 ($P < 0.01$), respectively, indicating that EST-SSRs provided less genetic diversity information than genome-SSRs. This was mainly because the majority of genome-SSRs is likely to have neither genomic function nor close linkage to transcribed sequences, and may not be subject to selection pressure. The combination of SSRs used in our study was nearly neutral in that 54.3 % were EST-SSRs (25/46) and 45.6 % were genome-SSR (21/46). Thus, the medium level of genetic diversity revealed by our study may reflect the true level of diversity found in SSRs.

While it is expected that using a larger number of markers will provide a more precise estimate of genetic relationships, the distribution of these markers over the genome is equally important. Diversity studies based on a set of markers with poor coverage of the genome can give a different classification for a given germplasm. For example, many of AFLP markers using *EcoR* I and *Mse* I mapped by Menz *et al.* (2002) are located on the centromeric regions of each chromosome, and consequently these regions have a high "weight" in the classification of the germplasm. With the creation of saturated genetic maps for many crop species, the ability to select molecular markers that adequately cover the entire genome without under- or over-representation of any region is now feasible. More accurate estimates of dissimilarity among the studied genotypes could be achieved by selecting markers distributed uniformly across the genome. This study selected 46 SSR markers well-distributed on all 10 chromosomes of sorghum, thus providing a good estimate of genetic relationships among the studied genotypes.

In conclusion, the genotypes reported here displayed medium genetic diversity. Both the genetic relationship information and heterozygosity information among the sweet sorghum genotypes revealed by this study could be very helpful in future sweet sorghum breeding programs to improve sugar yield and maintain broad genetic diversity. Moreover, it is very important for sweet sorghum germplasm conservation that these genotypes could be identified unequivocally using selected SSR markers.

References

Agrama, H.A., Tuinstra, M.R.: Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. - Afr. J. Biotechnol. **2**: 334-340, 2003.

Akriditis, P., Mylona, P.V., Tsaftaris, A.S., Polidoros, A.N.: Genetic diversity assessment in Greek *Medicago truncatula* genotypes using microsatellite markers. - Biol. Plant. **53**: 343-346, 2009.

Ali, M.L., Rajewski, J.F., Baenziger, P.S, Gill, K.S., Eskridge, K.M., Dweikat, I.: Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. - Mol. Breed. **21**: 497-509, 2008.

Banerjee, H., Pai, R.A., Sharma, R.P.: Restriction fragment length polymorphism and random amplified polymorphic DNA analysis of chickpea accessions. - Biol. Plant. **42**: 197-208, 1999.

Bassam, B.J., Caetano-Anolles, G., Gresshoff, P.M.: Fast and sensitive silver staining of DNA in polyacrylamide gels. - Anal. Biochem. **196**: 80-83, 1991.

Bhattramakki, D., Dong, J.M., Chhabra, A.K., Hart, G.E.: An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. - Genome **43**: 988-1002, 2000.

Brown, S.M., Hopkins, M.S., Mitchell, C.V., Senior, M.L., Wang, T.Y., Duncan, R.R., Gonzalez-Candelas, F., Kresovich, S.: Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench). - Theor. appl. Genet. **93**: 190-198, 1996.

Chimote, V.P., Chakrabarti, S.K, Pattanayak, D., Naik, P.S.: Semi-automated simple sequence repeat analysis reveals narrow genetic base in Indian potato cultivars. - Biol. Plant. **48**: 517-522, 2004.

Cho, Y.G., Ishii, T.S., Temnykh, X., Chen, L., Lipovich, S.R., Mc-Couch, W.D., Park Ayres, N., Cartinhour, S.: Diversity of microsatellites derived from genomic libraries and GenBank sequenes in rice (*Oryza sativa* L.). - Theor. appl. Genet. **100**: 713-722, 2000.

Cordeiro, A.I., Sanchez-Sevilla, J.F., Alvarez-Tinaut, M.C., Gomez-Jimenez, M.C.: Genetic diversity assessment in Portugal accessions of *Olea europaea* by RAPD markers. - Biol. Plant. **52**: 642-647, 2008.

Deu, M., Rattunde, F., Chantereau, J.: A global view of genetic diversity in cultivated sorghums using a core collection. - Genome **49**: 168-180, 2005.

Doyle, J.F., Doyle, J.L.: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. - Focus **12**: 13-15, 1990.

Eujayl, I., Sorrells, M.E., Baum, M., Wolters, P., Powell, W.: Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. - Theor. appl. Genet. **104**: 399-407, 2002.

Folkertsma, R., Frederick, H., Rattunde, W., Chandra, S., Raju, G., Hash, C.: The pattern of genetic diversity of Guinea-race *Sorghum bicolor* (L.) Moench landraces as revealed with SSR markers. - Theor. appl. Genet. **111**: 399-409, 2005.

Grenier, C., Deu, M., Kresovich, S., Bramel-Cox, P.J., Hamon, P.: Assessment of genetic diversity in three subsets constituted from the ICRISAT sorghum collection using random *vs*. non-random sampling procedures: B. Using molecular markers. - Theor. appl. Genet. **101**: 197-202, 2000.

Kamala, V., Brame, P.J., Sivaramakrishnan, S., Chandra, S., Kannan, S., Harikrishna, S., Manohar, R.D.: Genetic and phenotypic diversity in downy-mildew-resistant sorghum (*Sorghum bicolor* (L.) Moench) germplasm. - Genet. Resour. Crop Evol. **53**: 1243-1253, 2006.

Kim, J.S., Klein, P., Klein, R., Price, H., Mullet, J., Stelly, D.: Chromosome identification and nomenclature of *Sorghum bicolor*. - Genetics **169**: 1169-1173, 2004.

Kong, L., Dong, J., Hart, G.E.: Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple-sequence repeats (SSRs). - Theor. appl. Genet. **101**: 438-448, 2000.

Lu, Q., Sun, Y.: The Genetic Improvement of Hybrid Sorghum. - China Agriculture Science Press, Beijing 2005.

Menz, M.A., Klein, R.R., Mullet, J.E., Obert, J.A., Unruh, N.C., Klein, P.E.: A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. - Plant mol. Biol. **48**: 483-499, 2002.

Menz, M.A., Klein, R.R., Unruh, N.C., Rooney, W.L., Klein, P.E., Mullet, J.E.: Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. - Crop Sci. **44**: 1236-1244, 2004.

Morgante, M., Hanafey, M., Powell, W.: Microsatellites are preferentially associated with no repetitive DNA in plant genomes. - Natur. Genet. **30**: 194-200, 2002.

Ritter, K.B., Lynne, C.L., McIntyre, C., Godwin, I.D., Jordan, D.R., Chapman, S.C.: An assessment of the genetic relationship between sweet and grain sorghums, within *Sorghum bicolor* ssp. *bicolor* (L.) Moench, using AFLP markers. - Euphytica **157**: 161-176, **2007**.

Rohlf, F.J.: NTSYSpc: Numerical Taxonomy and Multivariate System. Version 2.1 Exeter Software. - Applied Biostatics Inc., New York 2000.

Saker, M.M., Adawy, S.S., Mohamed, A.A., El-Itriby, H.A.: Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis. - Biol. Plant. **50**: 198-204, 2006.

Schloss, S.J., Mitchell, S.E., White, G.M., Kukatla, R., Bowers, J.E., Paterson, A.H., Kresovich, S.: Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L). Moench. - Theor. appl. Genet. **105**: 912-920, 2002.

Scott, K.D., Eggler, P., Seaton, G., Rossetto, M., Ablett, E.M., Lee, L.S., Henry, R.J.: Analysis of SSRs derived from grape ESTs. - Theor. appl. Genet. **100**: 723-726, 2000.

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., Kresovich, S., Ziegle, J.: An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. - Theor. appl. Genet. **95**: 163-173, 1997.

Smith, J.S.C., Kresovich, S., Hopkins, M.S., Mitchell, S.E., Dean, R.E., Woodman, W.L., Lee, M., Porter, K.: Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. - Crop Sci. **40**: 226-232, 2000.

Taramino, G., Tarchini, R., Ferrario, S., Lee, M., Pe′, M.E.: Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. - Theor. appl. Genet. **95**: 66-72, 1997.

Uptmoor, R., Wenzel, W., Friedt, W., Donaldson, G., Ayisi, K., Ordon, F.: Comparative analysis on the genetic relatedness of *Sorghum bicolor* accessions from Southern Africa by RAPDs, AFLPs and SSRs. - Theor. appl. Genet. **106**: 1316-1325, 2003.