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Assessment of transferability of sorghum *(Sorghum bicolor)* EST-SSR markers among its wild species and other members of Gramineae family*

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SSR markers are the most popular molecular markers for crop improvement and are frequently used in fingerprinting cultivars, genetic diversity studies, mapping QTL and marker-assisted breeding of crop plants. With the availability of large numbers of expressed sequence tags (ESTs) in the public databases, the development of SSR markers has been accelerated to a great extent through *in silico* approaches with lower cost and effort. Mining of SSR markers from ESTs has been reported in an array of crop plants, including sorghum (Srinivas *et al.* 2008).

Comparative genetic mapping of cereal crops has shown that both gene content and/or order are largely conserved over the evolutionary history of the grasses (Gale and Devos 1998). EST-SSRs, being derived from the conserved expressed component of the genome are expected to show greater transferability between species and genera (Varshney *et al.* 2005). However, the conserved nature of the EST-SSRs may limit their polymorphism within the species from which they were derived. A relatively high sequence similarity and transferability among members of the Gramineae family have been reported (Kantety *et al.* 2002, Saha *et al.* 2004). These markers are useful for assaying functional diversity, comparative mapping and evolutionary studies. The main objective of the present study was to assess the transferability of *Sorghum bicolor* derived EST-SSR markers to its related wild species, and to other members of Gramineae family.

Eighteen genotypes were used in the study (Table 1). A set of 14 random sorghum (*S. bicolor*) EST-SSRs, viz Stgnhsbm 1, 3, 4, 5, 9, 17, 18, 21, 22, 27, 30, 32, 33 and Stgnhsbm 34 (Srinivas *et al.* 2008) were used for the assessment of their intra- and inter- specific/genera transferability.

The genomic DNA was extracted using the CTAB method and PCR were performed as described in Srinivas *et al.*, (2008). Number of alleles, allele frequencies, and polymorphic information content (PIC) for each marker was calculated using Powermarker VER. 3.0 (Liu and Muse 2005). Genetic distance matrix was computed using the binary data matrix and the distance matrix was subjected to cluster analysis based on neighbour-joining (Saitou and Nei 1987) algorithm using Darwin 5.0 (Perrier *et al.* 2003). To confirm transferability of sorghum EST derived markers, BLASTN (Altschul *et al.,* 1990) analysis of SSR-ESTs (http://www.ncbi.nlm.nih.gov/blast/) with a homology filter of expectation value _ 1.00E-09 was conducted against GenBank data base of rice, maize, sugar cane, pearl millet and oats. Hits with significant E- value were aligned using ClustalW (Thompson *et al.* 1994) to observe the conservation of SSR motif.

A total of 94 SSR alleles were detected using 14 EST-SSR primer pairs, with an average of 3 alleles/locus in sorghum, and 6.71 alleles in Gramineae as a whole. The number of alleles/primer pair varied from 1 (Stgnhsbm1 and 32) to 6 (Stgnhsbm33) in sorghum, while it was 3 (Stgnhsbm1) to 10 (Stgnhsbm 5, 17 and 33) in Gramineae. The allele size ranged from 95 to 370 bp in sorghum and 95 to 410 bp in Gramineae members. The PIC values varied from zero (Stgnhsbm1 and 32) to 0.74 (Stgnhsbm34) with an average of 0.40 in sorghum, whereas, it ranged between 0.41 (Stgnhsbm1) and 0.85 (Stgnhsbm5, 17 and 34) with a mean 0.69 in Gramineae. All the 14 markers were transferable in *S. halepense* while 13 markers were PCR amplified in *S. usumbarense*, parasorghum and *S. propinquum* (92.8% transferability) indicating robust transferability of *S. bicolor* EST-SSRs across sorghum species. Of the 14 markers studied, 6 markers (Stgnhsbm 3, 4, 5, 9, 33 and 34) were polymorphic (42.8%) within cultivated sorghums (*S. bicolor*) and 12

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(85.7%) were polymorphic between cultivated and wild sorghums. The markers Stgnhsbm1 and Stgnhsbm32 were monomorphic in both cultivated and wild sorghums.

All the 14 primer pairs produced amplification at least in one of the six grass species studied. The amplification of primer pairs was 100% (sugarcane), 92.8% (maize), 85.7% (finger millet), 85.7% (rice), 78.5% (pearl millet) and 78.5% (oats) indicating varied inter-generic transferability. This could be due to conservation of primer binding sites across Gramineae species studied. Highest polymorphism was 58% (rice) and lowest was 33.3% (pearl millet and oats).

NCBI database was searched for the identification of homologous sequences using BLASTn search (NCBI) analysis. Homologous sequences for all the 14 sorghum SSR-ESTs could be detected in maize (100%), while 13 homologous sequences of rice (92.8%) were detected (Table 2). Sequence comparison of *in silico* transferable EST-SSRs of different species should allow understanding of conservation of SSR motif during the evolution process. With respect to 14 sorghum EST-SSRs, 11 (78%) in maize and 2 (14%) in rice had either partially intact or disrupted SSR motif in homologous position (Table 2). For the remaining instances, either SSR motif was not present or if detected, found in non-homologous position.

The allelic data was used to detect genetic relationships among the five species of sorghum, and six members of Gramineae family using cluster analysis with DICE dissimilarity co-efficients. The highest and lowest dissimilarity coefficients of sorghum and its wild species was 0.11 and 0.25, while it was 0.17 with maize and 0.27 with sugarcane, suggesting the degree of genetic similarity among the species and genera. Dendrogram based on the dissimilarity matrix revealed seven groups, corresponding to different Gramineae species (Fig 1). Sorghum and its wild species were clustered together, but separated from maize, finger millet, rice, pearl millet, oats and sugarcane. Genotypes within species were grouped together for all the species studied.

Several studies have shown that EST-SSRs developed for a species could be used in related plant species and their success of cross-species amplification depends on the evolutionary relatedness of the species studied (Dayanandan *et al.* 1997). In recent years, it has been shown for several plant species that EST-derived SSRs show a considerable degree of transferability to related species (Cordeiro *et al.* 2001; Varshney *et al.* 2005) in contrast to genomic SSRs that show lesser cross amplification (Sourdille *et al.* 2001). High transferability rate of *S. bicolor*-markers into its wild species in present study indicated that all *bicolor* derived markers can be readily applied in sorghum wild species to link and introgress useful genes and traits into cultivated sorghums, besides enriching the genome information of wild species for their genome analysis. This needs to be further validated using more number of EST-SSRs and wild species. Dillon *et al.* 2005 also found high cross species amplification of *S. bicolor*-derived SSR markers in 25 Sorghum species. The high transferability rate observed in this study using sorghum EST-SSRs into maize, rice, finger millet, sugarcane, pearl millet and oats, is comparable to earlier studies in cereals (Saha *et al.* 2004, Holton *et al.* 2002), and is in accordance with the relative phylogenetic affinities between sorghum and the other target cereal species (Kellogg 1998 and Wang *et al.* 2005). Amplification of sorghum markers in other cereals offers an opportunity for using them in a variety of studies in related species. Further, these markers can be also used as flanking markers for synteny based targeted mapping of QTL in less studied crops which has been validated for sorghum stay green QTLs (Srinivas *et al.* 2008).

The level of polymorphism detected across species or genera mainly depends on the genetic divergence of species tested and primers used. As expected, a higher level of polymorphism was found among the different species (85.2%) of sorghum than within *bicolor* species (42.8%), rice (58%), sugarcane (42.9%), finger millet (38.5%), in pearl millet (33.3%) and oats (33.3%) indicating that the EST-SSR markers are variable in their polymorphism across genera.

Table 1 Gramineae species and genotypes used for screening of sorghum EST-SSR markers

Common name	Scientific name	Genotype used	Genome (2n)	Predominant pollination mode
Sorghum and species	*Sorghum bicolor* (L.) Moench	BTx623, IS10284 and IS26866	20	Self
	S. halepense (L.) Pers.	IS18850	20	Self
	S. usumbarense	IS18903	20	Self
	Para sorghum	IS18942	20	Self
	S. propinquum (Kunth) Hitchc	IS18933	20	Self
Maize	*Zea mays* L.	Aparanjali	20	Cross
Finger millet	*Eleusine coracana* (L.) Gaertn.	GPU-28 and IE2912	40	Self
Sugarcane	*Saccharum* sp.	Cane-01 and 02	60	Self
Rice	*Oryza sativa* L.	IR24 and Jaya	24	Self
Pearl millet	*Pennisetum glaucum* (L.) R.Br.	Takarama and RIB335174	14	Cross
Oats	*Avena sativa* L.	Kent and H58	42	Self

Table 2 BLAST and similarity searches of sorghum SSR- ESTs with rice and maize genomic sequences

Marker	EST accession no.	SSR motif	Rice		SSR motif in rice EST	Maize		SSR motif in maize EST	Putative function
			BLAST Search	E-value		BLAST search	E- value		
Stgnhsbm1	AW286961	$(AGC)_5$	Os01g0700000	1e-57	$(AGC)_2A(G)_4ACC$	AY104851	0	$(AGC)_4AG$	Unknown protein
Stgnhsbm3	BG464180	$(CT)_6$	Os01g0702700	2e-43	Not homologus	NM_001155516.1	2e-10	Not homologus	Putative myb-related protein
Stgnhsbm4	CF432983	$(TG)_5$	Os01g0708100	6e-31	Not homologus	BT063638	0	$(TG)_3CGTG$	N-myristoyl transferase
Stgnhsbm5	CN126720	$(CT)_7$	Os01g0709400	7e-172	Not homologus	BT064135	0	$(C)_3(TC)_4C(CT)_2$	Acid phosphatase
Stgnhsbm9	CD223691	$(CAG)_7$	Os01g0729600	9e-120	$G(CAG)_2CG(GC)_2$ ACGA	BT067332.1	0	----$GCA(C)_2AGC(G)_2$ C(AG)2C	Putative aminotransferase
Stgnhsbm17	CF760991	$(TA)_8$	Os01g0825700	2e-45	Not homologus	EU946450	9e-132	----$(TA)_5$	Putative VHS2 protein
Stgnhsbm18	CN147567	$(GCT)_5$	No hits			BT061500	2e-25	$(GCT)_2$ACT----	Unknown protein
Stgnhsbm21	CN127248	$(CGC)_{13}$	Os01g0620100	0	Not homologus	AY105674	0	$(CGC)_4C(GCC)_2GC$---- ----GACGTCACTAG	SEC13 protein homolog YGL100w- like
Stgnhsbm22	CB925228	$(GAC)_6$	Os01g0628700	3e-55	Not homologus	EU954634	7e-97	Not homologus	Cytochrome P450- like
Stgnhsbm27	CF434308	$(GA)_8$	Os01g0667600	4e-124	Not homologus	EU972697	6e-167	$(GAAG)_2(GA)_4$	Putative GTP binding protein Rab11b
Stgnhsbm30	CN133175	$(AG)_6$	Os01g0689800	0	Not homologus	EU973012	0	$(AG)_5AC$	Cgi67 serine protease-like
Stgnhsbm32	CF430486	$(GA)_{12}$	Os09g0422000	2e-142	Not homologus	EU955507	0	Not homologus	Putative hydroxycinnamoyl transferase
Stgnhsbm33	CD209273	$(AT)_6$	Os09g0425900	4e-79	Not homologus	EZ064484	6E-140	$(AT)_3$	Senescence- associated protein DH
Stgnhsbm34	CF482416	$(CCA)_5$	Os09g0434200	5e-149	Not homologus	EU954580	0	$(C)_4$ACCAC-ACCACA---	Putative ABI3-interacting protein2, AIP2

----indicate either deletion or insertion of one or more nucleotide base

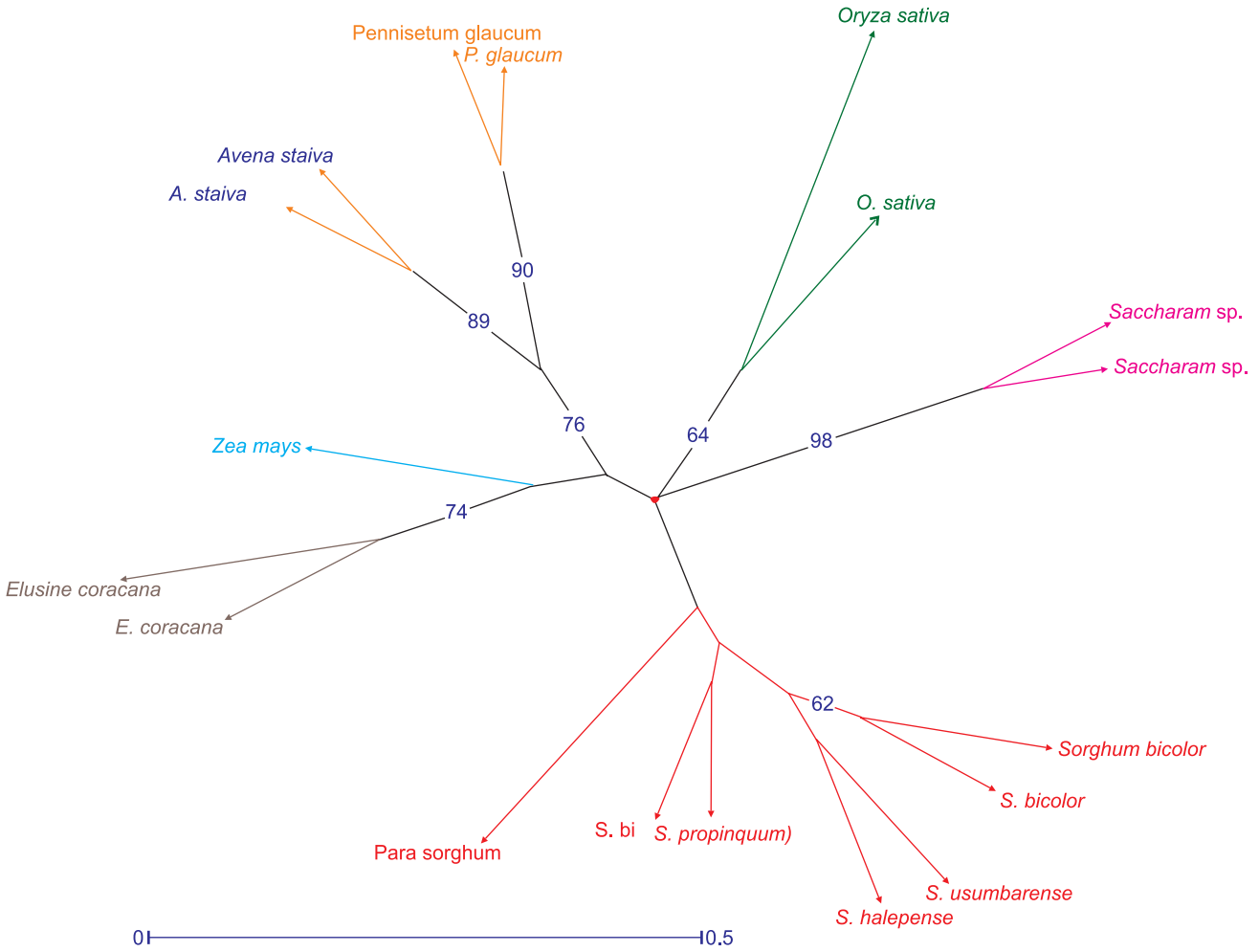

Fig 1 Neighbour-joining phenogram depicting relationships among 18 grass species. Numbers along the branches denote bootstrap support (shown only for values greater than 50)

The low level of polymorphism observed within Gramineae species is expected as EST-SSRs are located in the highly conserved portion of the genome and therefore display low level of polymorphism (Cho *et al.* 2000).

SUMMARY

EST-SSR markers are the popular markers due to their rapid *in silico* development and high cross-species and genera transferability. We assessed sorghum EST-SSR markers for their transferability into its wild species and other members of Gramineae family. The inter specific transferability of sorghum EST-SSRs ranged between 92.8% (*S. usumbarense*, *S. propinquum*, para sorghum) and 100% (*S. halepense*) while inter-generic transferability ranged from 78.5% (oats and pearl millet) to 100% (sugarcane). Six out of 14 markers were polymorphic among *S. bicolor* genotypes while 12 were polymorphic between wild species of sorghum. Dendrogram revealed seven groups differentiating *Gramineae* members. *In silico* comparison of SSR-ESTs of sorghum, maize and rice showed either intact or disrupted SSR motif in homologus position. This small set of sorghum EST-SSR markers with high transferability across species and genera will be highly useful for assessing the functional diversity, comparative mapping and other applications in crops with very limited markers.

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