Molecular Characterization of Sorghum Germplasm for Drought Tolerance Exhibiting Stay-green Trait

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(Received: 17 January 2012; Revised: 18 October 2012; Accepted: 18 November 2012)

The best characterized form of drought tolerance in sorghum during crop growth is the non-senescence or "stay-green" trait. To assess the genetic diversity available in sorghum germplasm for drought tolerance, 16 stay-green specific simple sequence repeat markers (SSR) were used of which 10 were polymorphic. A total of 47 scorable alleles were generated by these 10 primers of which 44 were polymorphic (93.6%). The number of alleles produced by different primers ranged from three to seven with an average of 4.7 alleles per primer**.** Similarity coefficients based on 10 polymorphic SSR markers ranged from 0.205 to 0.864. The dendrogram grouped the sorghum accessions into 11 major clusters and categorized the drought-resistant and drought-susceptible genotypes into separate clusters. Most of the genotypes in the cluster I and IV had better stay-green score ranging between 1.80 to 2.5. The study revealed that the presence of genetic diversity is high among the accessions and the stay-green specific primers sorted the genotypes based on their level of drought tolerance.

Key Words: Cluster, Drought tolerance, Polymorphism, Simple sequence repeats, Stay-green

Introduction

Drought response in sorghum has been characterized at both pre-flowering and post-flowering stages. Drought stress during the post-flowering stage needs serious consideration because the negative impact of post-flowering drought on yield can be very drastic. Yield reduction can result from the loss of yield associated with premature plant death, stalk rot, lodging and reduced seed size of post-flowering drought-susceptible cultivars. Post-flowering drought adaptation in sorghum is associated with the stay-green phenotype. Genotypes with resistance to post-flowering stress retain their leaves in an active photosynthetic state when subjected to water stress conditions during the grain-filling period. Such genotypes are described as possessing the stay-green trait which makes the plant resist premature senescence, retain green leaves, fill grain normally and resist lodging under conditions of post-flowering drought stress (Rosenow *et al*., 1977).

As the stay-green is expressed only in those tests in which terminal stress occurred, neither the efficiency nor the reliability of selection is high when only conventional breeding approaches are used for selection of this trait. In that scenario, the use of molecular markers could help improve selection efficiency for drought resistance. SSR markers have been used as a successful tool in genotyping and studying the genetic diversity of many plant species because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage as compared to other DNA markers. Stay-green trait and the SSR markers linked to them have been identified in sorghum (Crasta *et al.*, 1999; Tao *et al.*, 2000; Xu *et al.*, 2000; Kebede *et al.*, 2001; Subudhi *et al.*, 2002) which would facilitate a more efficient selection of genotypes for drought resistance in order to gain stable and optimal yield under drought conditions. Thus, the study was undertaken to assess the extent of genetic diversity available in sorghum for drought resistance by using stay-green specific, simple sequence repeat markers, Tamil Nadu.

Material and Methods

The experimental material for this study comprised 100 accessions of sorghum, which were adapted to different agroclimatic zones of Tamil Nadu (Table 1). The seeds were obtained from germplasm collection maintained at Millet Breeding Station, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Visual Rating of Stay-green

The stay-green trait was estimated visually on plot basis, on a scale of 1 to 5, based on the degree of leaf and

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Table 1. The 100 sorghum genotypes used in the study

Entry	Source	Entry	Source
B 35	NRCS, Hyderabad	AS 5779	Coimbatore
CO 26	Coimbatore	AS 5787	Coimbatore
AS 1	Palani	AS 6002	Perundurai
AS 5058	Gudiyatham	AS 6184	Kangeyam
AS 5055	Dindigul	AS 7759	Omalur
AS 321	Manapparai	AS 6329	Dharapuram
AS 557	Dindigul	AS 8747	Coimbatore
AS 1038	Cumbam	AS 6616	Kangeyam
AS 1041	Gobi	K 3	Kovilpatti
MS 73	Trichy	AS 8735	Voyalpad
CO 21	Coimbatore	LS 120	Cutralam
CO 22	Coimbatore	LS 161	Rajapalayam
CO 25	Coimbatore	AS 9772	Erode
M 35-1	NRCS, Hyderabad	AS 8007	Palayamkottai
AS 391	Salem	AS 5573	Dindigul
AS 668	Thirunelveli	AS 9841	Thirunelveli
AS 817	Coimbatore	AS 9857	Omalur
AS 5052	Karur	AS 9912	Salem
AS 3776	Coimbatore	AS 9916	Thirunelveli
LOCAL 3	Coimbatore	MS 7819	Salem
TENKASI 1	Tenkasi	MS 7826	Vellore
AS 2059	Coimbatore	MS 7837	Vellore
AS 2699	Coimbatore	MS 7818	Chittirapatti
AS 2752	Coimbatore	MS 7738	Omalur
AS 5078	Periyakulam	MS 5133	Perambalur
AS 3283	Kovilpatti	MS 5826	Thirumangalam
AS 3850	Madurai	IS 5379	Coimbatore
AS 5057	Vellore	MS 7703	Trichengode
AS 7742	Omalur	MS 7725	Salem
AS 3998	Thirunelveli	MS 7728	Salem
AS 9447	Thirunelveli	MS 7843	Vellore
AS 8021	Thirumangalam	MS 7863	Ariyalur
AS 4107	Perundurai	MS 7804	Vellore
AS 4153	Kovilpatti	MS 7806	Vellore
AS 4226	Gobi	Murunga-patti local	Murungapatti
AS 4242	Erode	Uppam cholam	Coimbatore
AS 4243	Erode	VS 1564	Vamban
AS 4265	Kovilpatti	AS 900	Manappari
AS 4289	Manamadurai	AS 3284	Kovilpatti
AS 4294	Karur	AS 3285	Coimbatore
AS 4567	Pollachi	AS 3289	Coimbatore
AS 4599	Kovilpatti	AS 9098	Thirunelveli
AS 4641	Pollachi	AS 9783	Dharapuram
AS 4647	Kovilpatti	MS 7861	Trichy
AS 4654	Ramnad	MS 7862	Trichy
AS 8038	Thirumangalam	MS 7816	Thiruman-galam
AS 4959	Kangeyam	VS 1560	Vamban
AS 8262	Thirumangalam	CO 24	Coimbatore
AS 8444	Gobi	CO 1	Coimbatore
AS 5773	Dindigul	ACM 6	Madurai

plant death at physiological maturity in the field under post-flowering drought stress as described by Wanous *et al*. (1991). A rating of 1 indicated essentially no leaf death, while a rating of 5 corresponded to complete plant death (leaves and stem).

Genotyping

The genomic DNA was isolated from 100 sorghum genotypes according to Gawel and Jarret (1991) and diluted to 25ng/l. Its concentration was estimated through Fluorometer (Model DYNA Quant 200, Hoefer, California, USA) and agarose gel electrophoresis. Sixteen stay-green specific SSR markers described by Crasta *et al*. (1999), Xu *et al*. (2000) and Haussmann *et al*. (2002) were used to survey the polymorphism between 100 genotypes and the list of primers surveyed is given in Table 2. The polymerase chain reaction (PCR) was performed in a 15 µl mixture containing 25 ng of genomic DNA, 50 ng each of forward and reverse primers (Sigma Aldrich, Bangalore, India), 0.25 mM dNTPs, 0.02 U *Taq* polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), 10X assay buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$) and 2.5 mM $MgCl_2$. Amplifications were performed on a thermal cycler (PTC-100TM, MJ Research Inc., USA). The standardized amplification profile was: initial denaturation temperature of 94°C for 15 min followed by 10 cycles of denaturation at 94°C for 10 sec; primer annealing at 61-52°C for 20 sec (the annealing temperature for each cycle is reduced by 1°C) and primer extension at 72°C for 30 sec followed by 35 cycles of denaturation at 94°C for 10 sec; primer annealing at 54°C for 20 sec and primer extension at 72°C for 30 sec. The last PCR cycle was followed by a final extension at 72°C for 20 min.

Table 2. List of microsatellite primers used in the study

Marker	Forward Primer	Reverse Primer
Xtxp43	AGT CAC AGC ACA CTG CTT GTC	AAT TTA CCT GGC GCT CTG C
Xtxp296	CAG AAA TAA CAT ATA ATG ATG GGG TGA A	ATG CTG TTA TGA TTT AGA GCC TGT AGA GTT
Xtxp295	AAA TCA TGC ATC CAT GTT CGT CTT C	CTC CCG CTA CAA GAG TAC ATT CAT AGC TTA
Xtxp88	CGT GAA TCA GCG AGT GTT GG	TGC GTA ATG TTC CTG CTC
Xtxp1	TTG GCT TTT GTG GAG CTG	ACC CAG CAG CAC TAC ACT AC
Xtxp38	ACA AAC CGC GAC GAA GTA AC	ACA AGG CAA AGC ACA AAG C
Xtxp340	AGA ACT GTG CAT GTA TTC GTC A	AGA AAC TCC AAT TAT CAT CCA TCA
Xtxp208	AAG GCC GTG AGG ATG	AAG CAG CCA AGA GCA G
Xtxp8	ATA TGG AAG GAA GAA GCC GG	AAC ACA ACA TGC ACG CAT G
Xtxp23	AAT CAA CAA GAG CGG GAA AG	TTG AGA TTC GCT CCA CTC C
Xtxp231	GGA AAT CCA GGA TAG GGT	AGG CAA AGG GTC ATC A
Xtxp149	AGC CTT GCA TGA TGT TCC	GCT ATG CTT GGT GTG GG
Xtxp227	TGA AAG TTT TGG CAT TGA	TGT AGG ATA GCC CAG GTT
Xtxp278	GGG TTT CAA CTC TAG CCT ACC GAA CTT CCT	ATG CCT CAT CAT GGT TCG TTT TGC TT
Xtxp59	GAA ATC CAC GAT AGG GTA AGG	GAC CCA GAA TAG AAG AGA GG
Xtxp357	CGC AGA AAT ACG ATT G	GCT ATC TGG AGT AAC TGT GT

Fifteen microlitres of the reaction mixtures were size fractionated through 3% agarose gel by electrophoresis and stained with ethidium bromide. The SSR gel images were scanned using the Gel Doc 2000 Bio-Rad system (Bio-Rad Laboratories, Hercules, California, USA). The bands were sized and then binary coded by 1 or 0 for their presence or absence in each genotype. The scores were used to create a data matrix to analyze genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) as described by Rohlf (1990). Cluster analysis was based on similarity matrices obtained with the Unweighed pairgroup method (UPGMA) (Sokal and Michener, 1958). To measure the informativeness of the markers, the Polymorphism Information Content for each SSR was calculated according to the formula: PIC = $1-\sum pi^2$, where pi is the frequency of the i^{th} allele.

Results and Discussion

Of the 16 SSR primers surveyed, 10 primers were polymorphic and produced scorable, unambiguous markers. A total of 47 scorable alleles were generated by these 10 primers of which 44 were polymorphic (93.6%). The number of alleles produced by different primers ranged from three to seven with an average of 4.7 alleles per primer. The polymorphic loci clearly discriminated all the genotypes. A high level of polymorphism (93.6%) was observed among the 100 sorghum genotypes. The mean number of alleles per locus was 4.7, with allelic sizes in close agreement with reports in the literature (Brown *et al.*, 1996; Taramino *et al.*, 1997; Bhattramakki *et al.*, 2000; Agrama and Tuinstra, 2003).

Table 3. Cluster composition of 100 genotypes used in SSR analysis

Clusters	No. of accessions	Accessions
I	26	B35, TENKASI1, CO22, CO21, CO24, AS4289, VS1560, CO1, K3, MS73, AS5078, AS8444, VS1564, AS8038, AS5773, M. Local, U. Chloam, AS1038, MS7728, Local 3, AS6329, LS120, AS1041, MS7837, MS7843, AS2699
II	11	AS5058, AS9916, AS5779, MS5826, AS3284, CO25, AS2059, AS5052, AS3998, AS3283, MS7818
III	5	AS7742, AS8021, AS8262, AS6184, AS7759
IV	12	AS4294, AS6002, AS900, AS8735, MS7826, AS3289, AS9098, AS9783, AS4959, MS7819, MS5133, IS5379
V	2	AS9912, MS7806
VI	5	AS5055, AS321, AS557, AS4599, MS7804
VII	3	AS4107, AS4153, ACM6
VIII	5	AS668, AS4641, AS4242, AS4647, AS4654
IX	4	AS3776, AS4226, MS7861, MS7703
X	18	CO26, M35-1, AS1, MS7725, MS7862, AS817, AS4243, AS7738, AS2752
XI	9	AS8007, AS5573, MS7816, AS4265, MS7863, AS3285, AS391, AS5787, AS4567

Similarity index values derived from the polymorphic data gave the extent of genetic relatedness among accessions (data not shown).The similarity coefficients based on 10 polymormphic SSR markers ranged from 0.205 to 0.864. Among the 100 accessions, B35 and Tenkasi 1 showed the highest similarity index (0.864), while the genotypes AS 4289 and CO 26, showed the least similarity index (0.205). The genotypes AS 4289 and CO 26 varied considerably in drought and yield component traits and these genotypes could be useful for hybridization, since hybrid vigor has a positive relation with genetic distance (Xiao *et al*., 1996). Hybridization between these two lines will provide progenies with greater variation at molecular level. The similarity coefficients were used as input data for cluster analysis using NTSYSpc2 program and the resulting dendrogram is shown in Figure 1.

The dendrogram grouped the sorghum accessions into 11 major clusters (Table 3). Cluster I comprises maximum number of genotypes (26) followed by cluster X (18), cluster IV (12), cluster II (11) and cluster XI (9). Cluster VIII, cluster III, and cluster VI had five genotypes each. Cluster IX, cluster VII and cluster V had four, three and two genotypes, respectively. Cluster analysis categorized the drought-resistant and drought-susceptible genotypes into separate clusters. These genotypes which were grouped into different clusters, were compared for their visual stay-green ratings, made during the field level studies to know the level of expression of stay-green genes under molecular level.

Most of the genotypes in the cluster I and IV had the stay-green score ranging between 1.80 to 2.5. Cluster IV genotypes are likely to have the same level of *per se* non-senescence as do the cluster I genotypes, despite being grouped into different clusters. These sorghum accessions may be suitable for growing under drought -prone tracts and rainfed areas. Most of the genotypes in the Clusters II, III, VII, IX and XI appeared to have moderate level of stay-green with a score ranging between 2.6 to 3.5. Cluster V, VI, VIII and X contained the most senescent genotypes which had the stay-green score ranging between 3.6 to 5. The current study included a wide range of genotypes representing more than one location but with same geographic origin. Hence, the clustering pattern of the accessions showed that though the genotypes represent a single geographical origin, there was greater molecular diversity among them. This is in agreement with the findings of Jeya Prakash (2006).

The stay-green specific primers used in this study "sorted the genotypes based on their level of drought tolerance" discriminating the genetic diversity among sorghum accessions. Hence, the genotypes which were sorted as stay-green in this study can be used as the parents in the hybridization programme for drought tolerance.

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