Received: 27 May 2020 | Revised: 18 Jun 2020 | Accepted: 19 Jun 2020

Electronic Journal of Plant Breeding

Research Article

SSR Marker-based DNA fingerprinting and morphological characterization for varietal identification in popular sorghum varieties of Tamil Nadu

S. Santhiya[1*], K. Kowshiga[2], D. Abisha[2], C. Sarankumar[2], D. Kavithamani[1], B. Selvi[1] and N. Senthil[2]

[1]Department of Millets, Centre for Plant Breeding and Genetics,
[2]Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-03, Tamil Nadu, India
***E-Mail:** senthil_natesan@tnau.ac.in

Abstract
DNA fingerprinting of varieties is mandatory for registration of germplasm and notification of newly released varieties. The present study attempted to develop a DNA fingerprinting profile of newly released varieties of sorghum using publically available SSR markers along with morphological DUS descriptors. Twenty-one SSR markers were used for the identification of unique variety-specific fingerprints in nine varieties/cultures. Of them, 14 primers (66.7%) showed clear and unambiguous amplification which is good enough to identify unique banding patterns for specific cultivars (77.8%). The SSR markers Xtxp024, Xtxp231, Xtxp075 produced unique alleles in CO 32 whereas Xtxp354 produced an unique alleles in K12. The SSR marker Xtxp003, Xtxp201 produced unique alleles in CSV 33 MF which could serve as valid genotype-specific SSR markers in varietal purity test program. The varietal-specific SSR marker will supplement the DUS test and could play a major role in varietal identification, thus resolving disputes during the seed certification process.

Keywords
DUS test, SSR fingerprinting, varietal identification and protection

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), an annual diploid (2n=2x=20) ranks fifth among global cereal crops next to maize, rice, wheat and barley (FAOSTAT 2015) with a global production of about 57.10 million metric tonnes (USDA, 2019). More than half of world sorghum production is from the semi-arid regions of Africa and Asia and one of the dietary staples of the worlds poorest, especially in the semi-arid tropics. It is notable for its multiple economic uses like food, fodder, biofuel, and other industrial uses.

Elite cultivars coupled with High-quality seeds play an indispensable role in the production. Varietal identification is of prime importance worldwide from the perspective of Plant Variety Protection (PVP). Protection can be granted if the newly evolved cultivar satisfies Distinctiveness, Uniformity, and Stability (DUS) (Prajapati *et al.*2018). Since new cultivars were developed through hybridization between members of elite groups of genetically similar parents, the genetic variability among newly evolved cultivars were found to be even smaller (Rahman *et al*., 2009). This makes the unambiguous distinction of cultivars to be difficult from the rest based on DUS tests which consist of morphological and physiological characteristics. For varietal registration under “Protection of Plant Varieties and Farmer’s Rights (PPV&FR) Act, 2001” the newly evolved cultivar must fulfill the DUS test. Elite sorghum genotypes were first selected based on morphological characterization (Beta and Corke, 2001). However, from several studies, it is significant

that morphological markers alone are insufficient due to low level of heritability, low level of abundance and is highly influenced by the environment (Seetharam and Ganesamurthy, 2013, Verma *et al.* 2017, Bhusal *et al.* 2017, Prajapati *et al.* 2018).

However, Molecular markers based on fingerprinting allows precise, objective and rapid cultivar identification that are challenging to characterize due to the identical morphological characters or indistinct traits (Galovic *et al.,* 2006). It's clear from several studies that several types of molecular markers such as RAPD (Sorghum: Mehmood *et al*., 2008), AFLP (Wheat: Heun *et al*., 1997) and SSR marker (Sorghum: Bantte and Mogus, 2016) can be used for genetic fingerprinting.

However, the Simple Sequence Repeat (SSR) represents a high degree of polymorphism, reproducibility, co-dominance and multi allelic types of variation (Becher *et al.,* 2000) and hence widely used in genetic analysis and cultivar identification. Applicability of SSR markers in cultivar identification has been reported in Rice (Rahman *et al.,* 2009), Grapes (Dangl *et al.,* 2001) and Potato (Coombs *et al*., 2004).

In recent years, fingerprinting the commercial sorghum cultivars based on molecular markers is of paramount significance for unambiguous and quick identification of similar or closely related varieties which could prevent the disputes arising due to varietal ownership. The applicability of SSR markers in Sorghum Fingerprinting has been reported by Bantte and Mogus, 2016 and Gangurde *et al., 2016*. The DNA fingerprinting is developed for the released sorghum varieties of Ethiopia (Bantte and Mogus, 2016). Therefore, this study intended to probe the appliance of the molecular marker in the context of DUS tests to disclose unique variety-specific fingerprints. This varietal fingerprint could be used for various varietal purity test programs of closely related sorghum cultivars and submission of fingerprint data for the crop variety registration.

MATERIALS AND METHODS

A total of eight elite sorghum cultivars were studied. These genotypes were chosen based on their significant role in the seed production system and are categorized based on their use in agricultural practices: grain sorghum (K8, K12, CO(S) 28, CO 30, CO 32), fodder sorghum (CSV 33MF) and pre-release cultures which are in the advanced stage of yield trails like MLT and ART (TNS 660, TNS 661) (Table 1). The popular sorghum varieties along with cultures were evaluated for varies agro-morphological traits based on 31 DUS descriptors provided by Protection of Plant Variety and Farmers Right Act (PPV&FRA, 2007).

A set of twenty-one informative SSR markers were used for identification of unique variety-specific fingerprint. These SSR markers were picked based on their maximum genome coverage. The SSR markers include Xtxp003, Xtxp024, Xtxp027, Xtxp030, Xtxp031, Xtxp038, Xtxp043, Xtxp051, Xtxp058, Xtxp075, Xtxp088, Xtxp145, Xtxp201, Xtxp231, Xtxp274, Xtxp285, Xtxp286, Xtxp287, Xtxp297, Xtxp312 and Xtxp354. The SSR markers PCR conditions validated by Shehzad *et al* 2008, Kong *et al.,* 1999 is used in the present study (Table 2).

Table 1. List of sorghum genotypes used in this study

Varieties/ cultures	Pedigree	Year of release	Special characters
K8	IS 12611 x SC 108	1989	Rainfed
K12	SPV 772 x S 35–29	2014	Dual-purpose variety
CO(S) 28	CO 25 × SPV 942	2001	High yielder, short duration, non-lodging, resistant to shoot borer
CO 30	APK 1 × TNS 291	2010	High dry matter digestibility, moderately resistant to shoot fly, resistant to downy mildew
CO 32	APK 1 × M 35-1	2020	Dual-purpose variety, high protein content, moderately resistant to shoot fly and stem borer
TNS 660	TNS 603 × EP 60	*	Short duration
TNS 661	TNS 603 × IS 18551	*	Moderately resistant to shoot fly
CSV 33 MF	EMS Mutant of COFS 29	2016	Forage sorghum, tall, thin stem, high tillering

*Pre-release cultures

Seedlings were raised under the greenhouse during November 2018. Genomic DNA from each cultivar was obtained from fresh leaf tissues of two-week-old seedlings based on the modified CTAB method (Grewal *et al*., 2013). DNA quality and quantity were obtained photometrically by Bio-Spectrometer, Kinetic (Eppendorf, Germany) and visually by agarose gel electrophoresis (0.8% agarose gel).

PCR amplification was performed in Eppendorf, Mastercycler Gradient, Germany. Polymerase Chain Reaction (PCR) was set out for 10µl comprising master mix (smART Prime) 7 µl, Forward primer 0.5 µl, reverse primer 0.5 µl, DNA 1 µl, and water 1 µl. The amplification profile comprised of Initial denaturation of template DNA at 94°C for 5 mins and subsequent 35 cycles each with Denaturation at 94°C for 1 min, Annealing at 55°C to 60°C

for 1 min, Extension at 72°C for 1 min. In the last cycle, the Final extension was provided at 72°C for 7 min. For the separation of PCR product, electrophoresis was carried out on a 3% agarose gel containing Ethidium bromide using 1X TBE buffer (pH 8.0). The amplified products were visualized under UV light source (Bio-Rad, CA and USA). Only clear and unambiguous SSR alleles were scored based on base pair (bp) size in each genotype.

Table 2. List of SSR markers, Primer Sequence, Repeat Motif, and Annealing Temperature

Marker name	Chromosome no.	Repeat motif	Forward primer	Reverse primer	Annealing T (°C)	Amplicon Size(bp)
Xtxp003	2	$(CT)_8$+$(CT)_{36}$	AGCAGGCGTTTA TGGAAG	ATCCTCATACTGCAGGACC	50	200-235
Xtxp024	4	$(TC)_{21}$	TTGTGTAGTCCATCCGATGC	TTCTAAGCCCACCGAAGTTG	60	145-160
Xtxp027	4	$(AG)_{37}$	AACCTTGCCCTATCCACCTC	TATGATGAATCAAGGGAGAGG	45	332
Xtxp030	10	$(AAT)_{25}$	AAAAAGGACCGCAGCTG	CTGGTCTCCACCATCCGTAG	60	290-300
Xtxp031	3	$(CT)_{25}$	TGCGAGGCTGCC CTACTAG	TGGACGTACCTATTGGTGC	60	222
Xtxp038	3	$(AG)_{17}$	ACAAACCGCACGAAGTAAC	ACAAGGCAAAGCACACAAAGC	60	437
Xtxp043	1	$(CT)_{28}$	AGTCACAGCACACTGCTTGTC	AATTTACCTGGCGCTCTGC	60	170
Xtxp051	4	$(TG)_{11}$	TCTCGGACTCAAGAGCAGAGG	GGACAGCAGCGGCTTCAG	60	225-230
Xtxp058	1	$(AG)_{13}$+ $(GA)_{16}$	TTCCCTTGCTGTTGCTTGTG	TTCCCTTGCTGTTGCTTGTG	55	145-160
Xtxp75	1	$(TG)_{10}$	CGATGCCTCGAAAAAAAAACG	CCGATCAGAGCGTGGCAGG	50	140-170
Xtxp088	1	$(AG)_{31}$	CGTGAATCAGCGAGTGTTGG	TGCGTAATGTTCCTC	53	150 -190
Xtxp145	1	$(AG)_{22}$	GTTCCTCCTGCCATTACT	CTTCCGCACATCCAC	60	200-230
Xtxp201	2	$(GA)_{36}$	GCGTTTATGGAAGCAAAAT	CTCATAAGGCAGGACCAAC	60	225-265
Xtxp231	3	-	GGAAATCCAGGATAGGGT	AGGCAAAGGGTCATCA	55	150-178
Xtxp274	9	$(TTC)_{19}$	GAAATTACAATGCTACCCCT AAAAGT	ACTCTACTCCTTCCGTCCACAT	60	280-320
Xtxp285	3	$(CTT)_{11}CTC(CTT)_{16}$	ATTTGATTCTTCTTGCTTTGCC TTGT	TTGTCATTTCCCCCTTCTTTCTTTT	60	205-260
Xtxp286	2	$(GCA)_4$ ACA $(GCA)_5$ A$(CAA)_5$+ $(AAC)_9$	AGCAGCAGGCAGCAACAG	GCGTGGTCTTTGTGGTTC	55	190-220
Xtxp287	6	$(AAC)_{21}$	GCAAGCGAGCTGACTTATGTAAC GAGA	CAAAGTGCTACTAAACCTATGCAGGGTGAA	60	330-360
Xtxp297	2	$(AAG)_{24}$	GACCCATATGTGGTTTAGTCGC AAAG	GCACAATCTTCGCCTAAATCAACAAT	55	170-400
Xtxp312	5	$(CAA)_{26}$	CAGGAAAATACGATCCGTGCC AAGT	GTGAACTATTCGGAAGAAGTTT GGAGGAAA	60	90-185
Xtxp354	8	$(GA)_{21}$+$(AAG)_3$	TGGGCAGGGTATCTAACTGA	GCCTTTTTCTGAGCCTTGA	60	130-170

RESULT AND DISCUSSION

Morphological markers have been frequently used in the genus Sorghum for descriptive purposes and are used in plant variety protection for distinguishing the individual varieties based on its distinctness, uniformity, and stability (DUS) test (Prajapati *et al.2018*). Characterization and evaluation of the accessions are the pre-requisites for the utilization of the available diversity in breeding perspective. Hence, the sorghum varieties are characterized to identify cultivar specific traits (Table 3) and cultivar specific SSR fingerprint which could be used for variety identification.

Molecular fingerprinting is of utmost importance in protecting the novelty of a newly evolved plant variety. In the present study 21 SSR primer pairs were used to score a set of eight cultivars. Among them, 14 primers (66.7%) showed clear and unambiguous amplification, which is good enough to identify unique allelic patterns for specific cultivars (77.8%). Further, two sorghum cultivars K8 and TNS 661 could not be distinguished, that indicated their lower genetic variability possibly due to close relatedness and a limited number of polymorphic SSR markers. The cultivars K8, TNS 660 and CSV 33 MF could be easily differentiated during the early growth stages from other varieties based on greyed purple pigmentation in coleoptile as well as leaf sheath. The SSR marker Xtxp297 produced unique alleles in TNS 660 (200bp) and CSV 33 MF (190bp) and hence it can be used for differentiation of these two cultivars. On the other hand, Xtxp003 and Xtxp201 can be used for differentiating CSV 33 MF (205bp, 180bp) from TNS 660 (Fig. 1b). Hence the markers Xtxp003, Xtxp201 can be designated as genotype-specific SSR markers for identifying CSV 33 MF in varietal purity testing programs. Bhusal *et al*. (2017) reported the presence of greyed purple pigmentation on seedling could be correlated with tannin content in seeds. It is proven by various studies that the presence of purple pigmentation on leaf sheath had a positive correlation (r = 0.56) with shoot fly dead hearts (%) (Mayilsamy *et al.* 2017).

Table 3. Cultivar specific distinguishing DUS traits of sorghum

S.NO	TRAITS	SCORE*	K8	K 12	CO S 28	CO 30	CO 32	TNS 660	TNS 661	CSV 33MF
1	Seedling: Anthocyanin colouration of coleoptile	1-2	2	1	1	1	1	2	1	2
2	Leaf sheath: Anthocyanin colouration	1-2	2	1	1	1	1	2	1	2
3	Plant: time of panicle emergence	1-9	7	3	3	5	3	5	5	7
4	Lemma: Arista formation	1,5	1	1	1	1	1	1	1	5
5	Stigma: anthocyanin colouration	1,5	5	1	1	1	1	5	5	5
6	Stigma: yellow colouration	1,5	5	5	1	1	1	5	5	5
7	Stigma: Length	3-9	5	3	3	3	3	3	3	5
8	Flower with pedicel: Length of flower	1-9	7	5	5	5	5	5	5	9
9	Anther: Length	3-7	3	5	5	5	5	3	3	3
10	Anther: Colour of dry anther	1-4	4	2	2	2	2	3	4	4
11	Glume: colour	1-6	4	3	3	3	3	5	4	6
12	Plant: Total height	1-9	5	5	5	5	7	3	3	7
13	Stem diameter	3-7	5	3	3	3	3	5	5	3
14	Leaf: Length of blade	3-9	7	7	7	7	5	7	7	9
15	Leaf: Width of blade	3-9	9	7	7	5	7	5	7	3
16	Panicle: Length without peduncle	1-9	7	3	3	5	5	5	5	9
17	Panicle: Length of branches	3-9	7	5	5	5	3	5	5	9
18	Panicle : Density at maturity	1-9	5	7	7	7	7	7	7	1
19	Panicle : Shape	1-5	3	3	3	3	3	3	3	5
20	Neck of panicle : Visible length above sheath	1-9	1	9	3	9	9	3	3	7
21	Glume : Length	1-9	3		7	5	5	1	1	9
22	Grain: Threshability	1-7	5	1	1	1	1	1	1	7
23	Caryopsis: Colour after threshing	1-5	3	3	3	3	3	3	3	5
24	Grain: Shape(in dorsal view)	1-3	3	3	3	3	2	3	3	2
25	Grain: Shape in profile view	1-3	2	2	2	3	2	3	3	2
26	Grain: Size of mark of germ	1-9	5	5	5	5	5	5	5	3
27	Grain: Texture of endosperm	1-9	5	3	3	3	3	3	3	5
28	Grain: Colour of vitreous albumen	1-3	1	1	1	1	1	1	1	2

* - Based on Guidelines for the conduct of test for Distinctiveness, Uniformity, and Stability on Sorghum (*Sorghum bicolor* (L.) Moench), PPV & FRA. 2007

At the time of peak flowering, K8 can be uniquely differentiated from other varieties based on the presence of anthocyanin pigmentation and yellow coloration in stigma. The varieties, K8 and CSV 33 MF have medium stigma length while others have a short stigma. Meanwhile, Xtxp312 can be used for differentiating CSV 33 MF (145bp) from K8 (Fig. 1a). Based on anther length, K8, TNS 660, TNS 661 and CSV 33 MF can be categorized as short whereas K12, CO (S) 28, CO 30 and CO 32 as medium. The medium-sized anther type varieties were further differentiated using the SSR marker. The SSR marker Xtxp145 differentiated K12 (210bp) and CO 30 (220bp) from CO (S) 28 and CO 32 by the presence of unique alleles whereas, Xtxp354 and Xtxp024 can be used for further differentiation of K12 (210bp) and CO 32 (160bp). Henceforth the SSR markers Xtxp354 and Xtxp024 can be used as a genotype-specific marker in varietal purity testing program. Absence of lemma arista formation (awn) reduced the evapotranspiration rate (Ayana and Bekele, 1998) which is noticed in all the tested varieties except CSV 33 MF. The presence of awn in CSV 33 MF acts as a defensive mechanism as bird scarers.

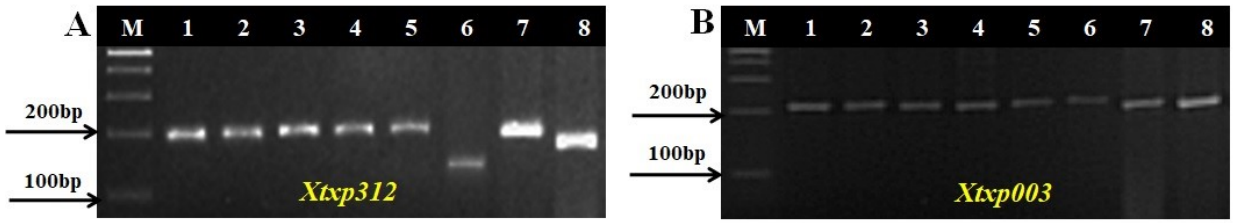

Fig. 1. DNA fingerprinting of sorghum varieties/cultures using sorghum SSR markers.

Note: M- Ladder (100bp); 1-CO 30; 2- CO(S) 28; 3- TNS 660; 4- TNS 661; 5- CO 32; 6- CSV 33 MF; 7-K8; 8-K12.

At physiological maturity, the genotypes K8, TNS 660, TNS 661 could be differentiated based on medium stem diameter (2 - 4 cm) while other varieties have small stem girth (< 2 cm). The larger stem girth denotes their resistance to lodging through enhanced culm strength. Broader leaf varieties like K8, K12, CO (S) 28, TNS 661 and CO 32 could ultimately be a high yielder because of more photosynthetic area. The SSR markers Xtxp274 can be used further for differentiating CO (S) 28 (310bp) from K8, K12, TNS 661 and CO 32 whereas Xtxp075 can be used for differentiating CO 32 (175bp) from K8, K12, CO (S) 28 and TNS 661. The dwarf genotypes could be used for developing desired plant types whereas genotypes with increased plant height are prone to lodging. But, beneficial as fodder, biomass fuel and thatching (Bhusal *et al*. 2017). From this perspective TNS 660 and TNS 661 were short (76 - 150 cm) whereas, CO 32 and CSV 33 MF were long (226 - 300cm). The SSR marker Xtxp231 can be used for differentiating CO 32 (210bp) from CSV 33 MF.

Fig. 2. Panicle shape of the sorghum varieties/ culture and fodder sorghum.

Note: 1-K8; 2-K12; 3-TNS 661; 4- CO 30; 5- CO 32; 6- CSV 33 MF

Panicle shape, compactness and length are important characters in determining grain yield and could be used as morphological marker for quicker varietal identification. CSV 33 MF can be uniquely differentiated from other varieties based on panicle shape like pyramidal type, whereas other varieties have symmetric type (Fig. 2). To further differentiate symmetric types, Xtxp058 can be used for differentiating K12 (190bp). The SSR marker Xtxp030 can be used for differentiating CO (S) 28 (305bp), CO 30 (290bp) and CO 32 (290bp). The open panicles could perform better in high rainfall and humid areas by avoiding mould and ergot diseases (Singh *et al.,* 1997). Moreover, K8 possess semi-loose panicle and CSV 33MF has a very loose panicle and rest of genotypes K12, CO (S) 28, CO 30, TNS 660, TNS 661 and CO 32 have semi-compact panicles.

The fodder sorghum variety CSV 33 MF had a very long glume cover compared to all the other grain sorghum varieties in the present study. The grain sorghum exhibits lesser glume coverage and could be easily threshable, while fodder sorghum exhibits higher glume coverage indicating difficulty in threshing (Verma *et al.,* 2017).
Moreover, the cultivar TNS 660 had greyed red glume color and CSV 33 MF had greyed purple glume color. Likewise, K8 and TNS 661 had greyed orange glume. Darker Glume color was found to be associated with grain mould resistance (Audilakshmi *et al.,* 1999) in many of the sorghum varieties tested. The corneous endosperm was found to be correlated with grain mould resistance (Jambunathan *et al.*1992 and Mukuru, 1992). The genotypes K12, CO (S) 28, CO 30, TNS 660, TNS 661 and CO 32 grains had 75% corneous endosperm. Hence, the glume color and endosperm texture can be used as a morphological marker for selecting parents in resistance breeding program. In addition to this, CSV 33 MF can be easily differentiated from the rest based on seed characters like caryopsis color after threshing (greyed orange), size of mark of germ (small) and color of vitreous albumen (greyed orange).

The discriminating morphological and DUS criteria can be efficiently used for varietal identification and grouping of varieties/cultures as grain or forage type. The SSR marker profile of Xtxp24, Xtxp231, Xtxp075, Xtxp354, Xtxp003 and Xtxp201 can be used for identification of specific cultivar. The unique variety-specific fingerprint obtained from the study can be used for varietal registration under the PPV and FR Act for obtaining plant varietal protection. This will also be used in varietal identification for consumer protection and resolving disputes in seed certification.

ACKNOWLEDGEMENT

The Core projects for research activities at Colleges and Research stations of TNAU- Phase-I (CPMB/CBE/PBT2012/CP004) funded by the Agricultural Department (AU), Government of Tamil Nadu. The financial support assisted by Department of Millets, TNAU to carry out DUS characterization under the URP project on CPBG/CBE/PBG/SOR/2018/001 - Collection and characterization of sorghum germplasm are kindly acknowledged.

REFERENCE

Audilakshmi, S., Stenhouse, J. W., Reddy, T. P., and Prasad, M. V. R. 1999. Grain mould resistance and associated characters of sorghum genotypes. *Euphytica*, **107**(2), 91-103.

Ayana, A., and Bekele, E. 1998. Geographical patterns of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea: qualitative characters. *Hereditas*, **129**(3), 195-205. [Cross Ref]

Bantte, K., and Mogus, Y. 2016. DNA Fingerprinting and Genetic Relationship among Ethiopian Sorghum [*Sorghum bicolor* (L.) Moench] Lines. *Int.J.Plant Breed.Genet.***10**, 38-44. [Cross Ref]

Becher, S. A., Steinmetz, K., Weising, K., Boury, S., Peltier, D., Renou, J. P. and Wolff, K. 2000. Microsatellites for cultivar identification in Pelargonium. *Theor Appl Genet*, **101**(4), 643-651. [Cross Ref]

Beta, T. and Corke, H. 2001. Genetic and environmental variation in sorghum starch properties. *J. of Cereal Sci.,* **34**:261-268. [Cross Ref]

Bhusal, N., Pahuja, S. K., Vats, A. K., Srivastava, A., and Kumar, R. S. 2017. Morphological characterization of forage sorghum genotypes for its various DUS traits. *J. Appl. Nat. Sci.,* **9**(2), 912-919. [Cross Ref]

Coombs, J. J., Frank, L. M., and Douches, D. S. 2004. An applied fingerprinting system for cultivated potato using simple sequence repeats. *American J. of Potato Res.*, **81**(4), 243-250. [Cross Ref]

Dangl, G. S., Mendum, M. L., Prins, B. H., Walker, M. A., Meredith, C. P., and Simon, C. J. 2001. Simple sequence repeat analysis of a clonally propagated species: a tool for managing a grape germplasm collection. *Genome*, **44**(3), 432-438. [Cross Ref]

FAOSTAT, 2015. FAO statistical database for food. Food and Agriculture Organization of the United Nations, Rome, Italy.

Galovic, V., Mladenovic-Drinic, S., Navalusic, J., and Zlokolica, M. 2006. Characterization methods and fingerprinting of agronomical important crop species. *Genetika*, **38**(2), 83-96. [Cross Ref]

Gangurde, S., Ghorade, R., Moharil, M., Ingle, K., and Wagh, A. 2016. Microsatellite Based DNA Fingerprinting of Sorghum Tellite Based DNA Fingerprinting of Sorghum [*Sorghum bicolor* (L.) Moench] Hybrid Csh-35 with its parents. *The Bioscan*, **12**(1): 215-219.

Grewal, A., A. Sharma, S. Phuja and S. Khatak. 2013. DNA extraction optimization in Sorghum (*Sorghum bicolor (L.*) Moench) for marker analysis *World J. of Pharmacy and Pharm. Sci.*, **2**(6): 5046-5051.

Heun, M., Schäfer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B., and Salamini, F. 1997. Site of Einkorn wheat domestication identified by DNA fingerprinting. *Science*, **278**(5341), 1312-1314. [Cross Ref]

Jambunathan, R., Kherdekar, M. S., and Stenhouse, J. W. 1992. Sorghum grain hardness and its relationship to mold susceptibility and mold resistance. *J. of Agric. Food Chem.*, **40**(8), 1403-1408. [Cross Ref]

Kong, L., Dong, J., and Hart, G. E. (2000). Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple-sequence repeats (SSRs). *Theor Appl Genet*, **101**(3), 438-448. [Cross Ref]

Mayilsamy, M. 2017. Characterization and genetic diversity analysis in sorghum (*Sorghum bicolor* (l.) Moench) germplasm for morphological traits associated with shoot fly resistance and yield contributing traits

Mehmood, S., Bashir, A., Ahmad, A., Akram, Z., Jabeen, N., and Gulfraz, M. 2008. Molecular characterization of regional *Sorghum bicolor* varieties from Pakistan. *Pak. J. Bot*, **40**(5), 2015-2021.

Mukuru, S. Z. 1992. Breeding for grain mold resistance. *Sorghum and millets disease: a second world review. ICRISAT, Patancheru, Andhra Pradesh*, **502**(324), 273-285.

PPV & FRA. 2007. "Guidelines for the conduct of test for Distinctiveness, Uniformity, and Stability on Sorghum (*Sorghum bicolor* (L.) Moench)." *Pl. Var. J.India* **1** (1):1-17.

Prajapati, D. K., Pahuja, S. K., Verma, N. K., and Chaudhary, S. 2018. Morphological characterization of sorghum [*Sorghum bicolor* (L.) Moench] germplasm for DUS traits. *Int.J.Curr.Microbiol.App.Sci*, **7**(2), 2058-2071. [Cross Ref]

Rahman, M. S., Molla, M. R., Alam, M. S., and Lutfur, R. 2009. DNA fingerprinting of rice (*Oryza sativa* L.) cultivars using microsatellite markers. *Australian J.Crop Sci.*, **3**(3), 122-128.

Seetharam, K., and Ganesamurthy, K. 2013. Characterization of sorghum genotypes for yield and other agronomic traits through genetic variability and diversity analysis. *Ele. J. Pl.Breed.*, **4**(1), 1073-1079.

Singh, F., Rai, K. N., Reddy, B. V., and Diwakar, B. 1997. Development of cultivars and seed production techniques in sorghum and pearl millet. Training manual. Training and Fellowship Program and Genetic Enhancement Division, ICRISAT.118

Shehzad, T., Iwata, H., and Okuno, K. 2009. Genome-wide association mapping of quantitative traits in sorghum (*Sorghum bicolor* (L.) Moench) by using multiple models. *Breed. sci.*, **59**(3), 217-227. [Cross Ref]

USDA. 2019. "https://apps.fas.usda.gov/psdonline/circulars/production.pdf."

Verma, R., Ranwah, B. R., Bharti, B., Kumar, R., Kunwar, R., Diwaker, A., and Meena, M. 2017. Characterization of Sorghum germplasm for various qualitative traits. *J. Appl. Nat. Sci.,* **9**(2), 1002-1007. [Cross Ref]