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Genetic Variation of Sorghum (*Sorghum bicolor* L. Moench) Varieties Assessed by ISSR Markers

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ABSTRACT

Sorghum has multipurpose cultivated types and is widely recognized as a potential alternative source of bio-fuel because of its sugar content in the sugar stalk varieties. The objective of the study was to assess the genetic variation and relationship among different sorghum varieties. Inter Simple Sequence Repeat (ISSR) markers were used to assess the genetic diversity of 10 sorghum varieties, nine of Indians and one of Syrian origins. Out of 20 ISSR primers screened, 9 primers were selected for their polymorphic and repeatable fragments. 110 fragments were polymorphic out of total 130 while the percentage of polymorphic bands value ranged from 63.6.% of $(AC)_8$ T to 100% of $(AG)_8$ A with a mean of 84.61%. As a result poly (AG)-anchored primers were more polymorphic and reproducible than other di-nucleotides and tri-nucleotides motifs. The UPGMA clustering associated the varieties into two major clusters, separating Indian varieties from the Syrian one variety. Among the Indian varieties two sub-clusters were related to the yielding type and maturity date. Grouping of varieties by UPGMA cluster analysis correlated with the geographical origin, pedigree information, yielding type and agronomic traits indicating that ISSR markers could be realistically used to evaluate the genetic diversity and differentiation among sorghum varieties.

Key words: Sorghum, ISSR, Genetic variation, Cluster analysis.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench; 2n = 2x =20] is the world's fifth most commonly grown cereal crop after wheat, rice, maize and barley [32]. Sorghum has many types of cultivated varieties, such as grain genotypes, fodder, fiber and sugar genotypes and dual purpose genotypes. Sorghum belong to C4 plant characteristic for tolerate abiotic stresses more than many crops [15]. Recently, sorghum has received significant attention because of the newer use as a Biofuel feedstock [29].

Assessment of the genetic variability within cultivated crops and varieties has a strong impact on plant breeding strategies and conservation of genetic resources [8,40] and is particularly useful in the characterization of individuals, accessions and cultivars in germplasm collections and for the choice of parental genotypes in breeding programs [7,37]. In the past, indirect estimates of similarity based on morphological information have been widely used in many species including sorghum [2]. However, morphological variation does not reliably reflect the real genetic variation because of genotype-environment interactions and the largely unknown genetic control of poly-genetically inherited morphological and agronomic traits [42]. Molecular analyses in conjunction with morphological and agronomic evaluation of germplasm are recommended, because these provide complementary information and increase the resolving power of genetic diversity analyses [41].

DNA markers have proved valuable in crop breeding, especially in studies on genetic diversity and gene mapping. The commonly used polymerase chain reaction (PCR)-based DNA marker systems are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and more recently simple sequence repeats (SSRs) or microsatellites [47,19]. The major limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers for SSR polymorphism. ISSR-PCR is a technique that overcomes most of these limitations [26,52,18,50]. It is rapidly being used by the research community in various fields of plant improvement in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of crop species. [16]. In this study, the amount of genetic variation among sorghum cultivars has been assessed using ISSR markers tool.

Material and Methods

Plant Materials:

Ten varieties of sorghum were selected to evaluate the genetic diversity and relationship among them. Seeds of nine sweet sorghum and dual purpose

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varieties were obtained from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India and one grain local sorghum "Razinieh" was developed by General Commission For Scientific Agricultural Research (GCSAR), Syria. They have a wide range of morphological variation such as grain, sugar, dual-purpose, days to flowering, maturity, Plant height, etc. (Table. 1).

Table 1: list of names and characters of sorghum genotypes used in this study.

Variety No.	Name	Pedigree[1]	Agronomic group and morphological note[2]
1	ICSSH 31	ICSA38 × ICSV700	Sugar type, early maturity
2	ICSSH 25	ICSA 675 × ICSV 700	Sugar type, early maturity
3	ICSSH 30	ICSA 724 × SSV 74	Sugar type, high sugar, late maturity, tall
4	ICSV 25280	(ICSV93046×SSV84)-7-2-1-3	Sugar type, late maturity
5	ICSB 324	[{(IS18417×ICSB11)×ICSB45}×ICSB30]1-2-1-1	Dual purpose, late maturity
6	ICSB 474	(IS18432×ICSB6)11-1-1-2-2	Dual purpose, early maturity
7	ICSB 479	[(ICSB70×ICSV700)×PS19349B]5-4-1-2-2	Dual purpose, early maturity
8	ICSB 675	(E 36-1× ICSB 17)12-2	Dual purpose, dwarf, medium- maturity
9	ICSB 38	[(BT×623×MR862)B Lines bulk]-5-1-3-5	Dual purpose, dwarf, medium-maturity
10	Razinieh*	Bulk	Fodder, fiber, early maturity

[1]Pedigree Certificate ICRISAT 2009, India. [2]Reddy et al.2006. *Syrian variety.

DNA Extraction and PCR Amplification:

Leaves of two week-old seedlings were collected from five plants, for each of the 10 genotypes. Genomic DNA was extracted from young sorghum leaves with Genomic DNA purification kit according to the manufacturer's instructions (Fermentas, Germany). The DNA was RNA se-treated and subsequently the DNA quality checked in 0.8% agarose gel and the DNA concentration was determined with Eppendorf BioPhotometer plus™ (Eppendorf, Germany).

Twenty ISSR primers were screened on the ten genotypes to determine their potential for clear polymorphisms and reproducibility. Reactions were carried out in a 25 µl reaction volume using GoTaq® Green Master Mix following the manufacturer's instructions (Promega, USA). The PCR reaction performed with initial cycle of 12 min. at 94^{o}C, followed by 35 cycles of 30s at 94^{o}C, 30s at 48^{o}C, 1 min. at 72^{o}C and ended with 12 min at 72^{o}C. The amplified products were separated on 2% agarose gel in 1X TBE buffer with 90 V for 2h and visualized by staining with ethidium bromide. Sizes of bands were estimated in relation to 1Kb DNA ladders (Fermentas, Germany). The banding patterns obtained from ISSR were scored as present (1) or absent (0), each of which was treated as an independent character.

Data Analysis:

The unweighted pair group method with arithmetic averages (UPGMA) and Squared Euclidean distances of the STATISTICA program were used to construct the matrices and the dendrogram [46]. This distance was computed in the program according to Sneath and Sokal (Sneath and Sokal, 1973); i.e., Distance $(x, y) = 3_i (x_i - y_i)^2$.

The principal coordinates analysis (PCA) was applied to plot the relationship between distance matrix elements with respect of their first two principal coordinates using GenAlEx software (Peakall and Smouse, 2001).

Results and Discussions

Screening of Primers and Genetic Diversity Analysis:

Out of the 20 ISSR primers screened, 9 primers were selected based on the preliminary test for their capacity to amplify polymorphic and repeatable fragments (Table 2.). Six of them contained the di-nucleotides; $(GA)_8$, $(AC)_8$, $(TG)_8$, $(AG)_8$ and $(AG)_7$ anchored with T or C or A nucleotides at the 3′ end; one $(CA)_8$ was anchored with G nucleotide at the 5′ end. Three of them composed of tri-nucleotides $(CTC)_5$ and $(CAG)_5$ anchored with T or G at the 3′ end. The amplified PCR products were easily distinguished in 2% agarose gel (Fig. 1). Sum of 130 DNA fragments were scored ranging in size from 250 to 2000 bp with an average of 14.4 fragments per primer, 110 of the fragments were polymorphic. The selected primers produced polymorphic bands with Percentage of Polymorphic Bands (PPB%) value ranging from 63.63% to 100% with a mean of (84.61%). Our results showed that among the 9 selected primers, the highest number of polymorphic bands observed in the case of (AG)-anchored primers $(AG)_7$ ACC and $(AG)_8$ A with 92 and 100% respectively.

Table 2: Polymorphism detected by 9 ISSR pirmers employed in the genetic diversity studies on ten sorghum varieties.

Primer	Sequence (5'---3')	Amplified bands	Polymorphic bands	Percentage of polymorphic bands %
ISSR1	(GA)8 T	16	13	81.25
ISSR2	(AC)8 T	11	7	63.63
ISSR3	(TG)8 C	7	5	71.42
ISSR4	(CTC)5	12	9	75.00

ISSR5	(AG)8 A	15	15	100.00
ISSR6	(AG)7 ACC	14	13	92.85
ISSR7	(CAG)5 T	17	15	88.26
ISSR8	(CAG)5 G	20	17	85.00
ISSR9	G(CA)8	18	16	89.80
Average		130	110	84.61

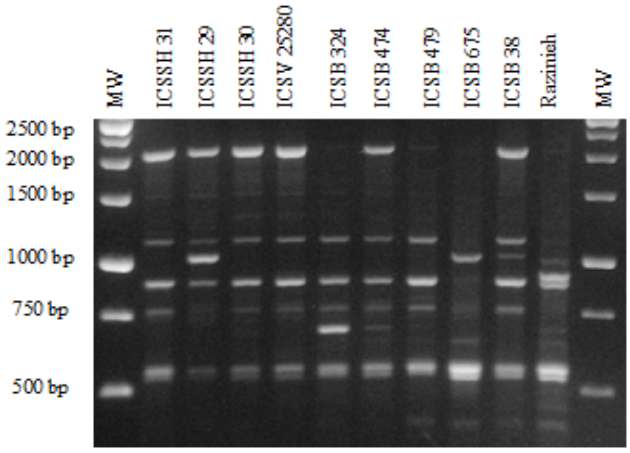

Fig. 1: An example of PCR amplification profile generated from genomic DNA of 10 sorghum varieties with ISSR6, resolved on 2% agarose gel. M-marker = 1 Kb.

Genetic Relationship Among Cultivars:

A dendrogram was constructed with the ten varieties using UPGMA method by STATISTICA software to analyze the genetic distances. The resulting dendrogram grouped the ten varieties in two major clusters at the genetic distance level of 0.59; one for the Syrian variety and the other for the Indian varieties (Table 3). This result was also supported by principal coordinate analysis (Fig. 3). Interestingly, group two divided into two sub-clusters distinctly separated among sugar-types (sub group I) and dual purpose types (sub group II) of genotypes and each sub group subdivided into groups according to phylogeny and agronomic traits. Moreover, the varieties in subgroups I and II subdivided into sub clusters that were mostly related to maturity. Subgroup I is composed of four sweet sorghum varieties ICSSH31, ICSSH29, ICSSH30 and ICSV25280 [35]. The two sugar varieties ICSSH31 and ICSSH29 grouped together at a genetic distance 0.18. While, varieties ICSSH30 and ICSV25280 clustered together at higher genetic distance 0.29. The subgroup II is composed of five dual-purpose varieties ICSB324, ICSB474, ICSB479, ICSB675 and ICSB38 [36]. The variety ICSB324 distinctly split up at genetic distance 0.35. While the two varieties ICSB474 and ICSB479 clustered together at lower genetic distance level 0.27 The varieties ICSB675 and ICSB38 clustered together at lower genetic distance 0.25.

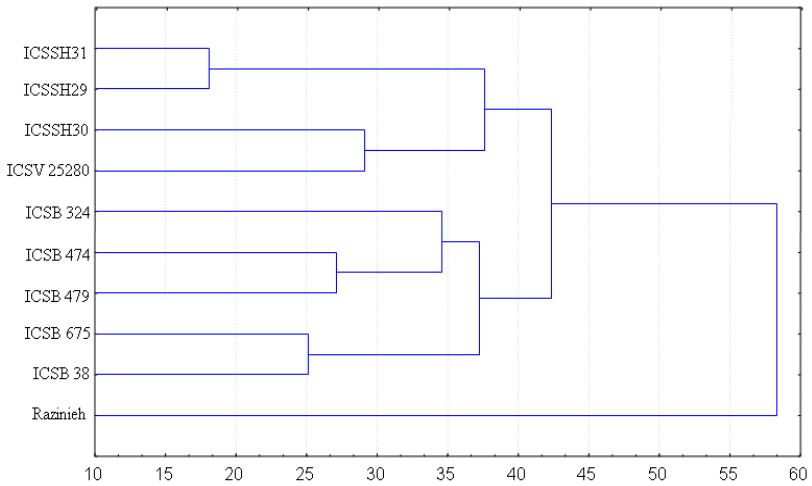

Fig. 2: Dendrogram showing grouping of 10 varieties of sorghum based on the genetic distance derived from ISSR markers using UPGMA analysis.

Table 3: Genetic Distance estimated among the ten varieties of sorghum.

Varieties	ICSSH 31	ICSSH 29	ICSSH 30	ICSV 25280	ICSB 324	ICSB 474	ICSB 479	ICSB 675	ICSB 38	Razinieh
ICSSH 31	0	18	35	34	37	46	41	48	45	66
ICSSH 29		0	39	42	37	38	41	40	43	62
ICSSH 30			0	29	38	49	42	49	46	65
ICSV 25280				0	35	40	41	44	45	68
ICSB 324					0	37	32	37	46	59
ICSB 474						0	27	32	35	52
ICSB 479							0	35	38	55
ICSB 675								0	25	46
ICSB 38									0	51

The two sugar varieties ICSSH31 and ICSSH29 grouped together at a genetic distance 0.18. While, varieties ICSSH30 and ICSV25280 clustered together at higher genetic distance 0.29. The subgroup II is composed of five dual-purpose varieties ICSB324, ICSB474, ICSB479, ICSB675 and ICSB38 [36]. The variety ICSB324 distinctly split up at genetic distance 0.35. While the two varieties ICSB474 and ICSB479 clustered together at lower genetic distance level 0.27 The varieties ICSB675 and ICSB38 clustered together at lower genetic distance 0.25.

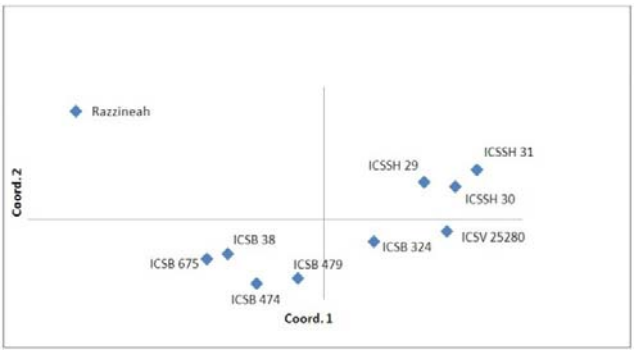

Fig. 3: Principle coordinate analysis of 10 sorghum varieties.

Discussion:

The genetic diversity among the ten genotypes was considerably high based on the PPB value (84.61%), much higher than what was detected by morphological traits, allozymes, or microsatellites [9,1]. The diversity level among sorghum genotypes revealed by ISSR-PCR analysis has a great significances in sorghum breeding programs [33,13]. The potential of ISSR markers to generate genetic information through polymorphic fragments depends on the inter- microsatellite frequency and their distribution in the genome wide scale of the species [27]. Our results showed that among the 9 selected primers, the highest number of polymorphic bands observed in the case of (AG)-anchored primers $(AG)_7$ ACC and $(AG)_8$ A with 92 and 100% respectively. As a result, di-nucleotides repeat poly (AG)-anchored ISSR primers are more polymorphic and reproducible than the other di-nucleotides and tri-nucleotides. Suggesting that the polymorphism rate would be higher when the motifs are composed of $(AG)_n$-anchored sequences. This result is compatible with Li. [24] who found that targeted unit di-nucleotides are more abundant in sorghum and suggests that the frequency of poly (AG) in the sorghum genome is higher than that of poly (CT), poly (AT), poly (CG), poly (AC) and poly (GT). This result is also consistent with [10] who found that poly $(AG)_n$-anchored primers are more suitable to be used in analyzing the genetic relationship of sorghum species.

The resulting dendrogram grouped the ten varieties in two major clusters consists of Syrian variety 'Razinieh' distinctively separated from other varieties, a result compatible with the fact that 'Razinieh' is geographically isolated from other varieties and has a different agronomic and morphological traits from all the Indian varieties tested in this study and is known as a fiber and fodder type (Table 1). The dendrogram in this present study indicated very clear pattern of clustering according to the regions where the Syrian cultivar and the Indian cultivars are grown. Similar results were obtained in barley [39,11,3,21]. The higher levels of genetic variation found in this study may be due to the fact that geographically isolated populations accumulate genetic differences as they adapt to different environments [44].

The sugar content of stem in sorghum is considered a strong trait for genetic differentiation among cultivated varieties of sorghum in group II. Moreover, the varieties in subgroups I and II subdivided into sub clusters that were mostly related

to maturity. [49] and [5] reported that ordination of ISSR data revealed discrete groups based on molecular, morphological, yielding and phylogeny data. Sugar-stem trait determines the commercial value of the crop and plays a role in flavour characteristics and consumer acceptability in food products. Recently, renewable carbohydrate in sweet sorghum has become of interest for biological transformation into ethanol for use as fuel or fuel additive [23]. The sugar accumulation is mainly influenced by varieties [25]. While Maturity date is one of the key characteristics, because it plays a large role in crop management, biotic and abiotic effects on sorghum such as drought tolerance, diseases, pests, fertilization and harvest date [38,22,48]. Many reports indicated a significant correlation between ISSR analyses and maturity, agronomic and yielding [6,14,20,4,12,17].

The genetic closeness 0.18 among the two varieties ICSSH31 and ICSSH29 can be explained by the fact, that both of them slope of the same progenitor ICSV700 [31] (Table 1) and are known for early maturity [45]. Varieties ICSSH30 and ICSV25280 clustered together at higher genetic distance 0.29, both of them are known for late maturity and their ability to survive under severe drought [51]. The variety ICSB324 distinctly split up at genetic distance 0.35. Interestingly, this variety is known for late maturity. While the two varieties ICSB474 and ICSB479 clustered together at lower genetic distance level 0.27 both of them are knows for early maturity [34]. The varieties ICSB675 and ICSB38 clustered together at lower genetic distance 0.25 wherein the two varieties were distinguished with plant height trait (dwarfness) and medium maturity [34].

In conclusion, results from the ISSR analysis were correlated with the morphological and agronomic characteristics and geographical location of 10 varieties. The data indicate that ISSR technique is useful to determine genetic diversity and genetic relationship among sorghum varieties, providing a scientific basis for genetic breeding, differentiation and new cultivar selection.

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