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Research Article

Morphological Characterization and Determination of Genetic Diversity in Sorghum Accessions by SSR Markers

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Abstract
Sorghum ranks fifth in worldwide among the cereal's crops having 64.20 million tonnes annual production. it is important food, feed as well as forage crop and provides raw material for Fibbers, Biofuel, Starch and Alcohol. The present study was conducted for the determination of genetic diversity through morphological and molecular characterization. Genetic divergence pattern was assessed among 82 sorghum accessions by evaluating the 9 different parameters for growing season. Results of Analysis of Variance (ANOVA) revealed that all parameters had significant differences. The Principal Component Analysis showed that first three Principal Components (PCs) having eigen value >1 and shared 29.95% variability and 26.57% variation for all parameters. Correlation Analysis exhibited that leaf width, plant height, and panicle length had positive correlation with leaf length. Biplot analysis exhibited that plant height, leaf length, grain number/panicle and 100-grain weight had positive and positive coordinates while none of the parameters lies in negative and negative coordinates. The molecular relationship among 96 sorghum accessions was estimated through 16 polymorphic SSR markers techniques. Results revealed that 99 alleles were observed by SSRs. Maximum allelic frequency band was observed 1 at locus Sorg6 and Sorg11. Polymorphic information content (PIC) values showed that locus Sorg16 had maximum value (0.88) while maximum heterozygosity was 0.85 at locus Sorg5. Dendrogram was also constructed and results revealed that 30 morphotypes were produced. This study depicted that SSRs had discriminating power for the estimation of genetic diversity. In addition, the sorghum germplasm showed maximum diversity in Pakistan climate season.

Keywords: Sorghum, Biplot, Genetic Diversity, PCA

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1. INTRODUCTION
Pakistan is an Agro-based country and has a variety of important crops. There are a number of crops whose genetic diversity is required to be found out due to their importance in different fields. One such crop includes sorghum which is ranked the 5th most important cereal crop in the world. It is a drought resistant low input cereal grain grown throughout the world. It is one of the important crops that can be utilized for the production of bioethanol and electricity. It can produce 7000 litters of ethanol per hectare.

Sorghum is 5th important C4 cereal species among rice, wheat, maize and barley and its area under cultivation is about 257

thousand/ha with a production of 149 thousand tones (Economic Survey of Pakistan, 2016-2017). Alongside being a commercial food, feed, and forage crop, sorghum also gives raw material to produce fibber, biofuels, alcohols, dextrose syrup, starch and many other products. However, the average yield of Pakistan for both grain and fodder sorghum is quite low as compared to other sorghum growing countries.

Sorghum is mainly grown in semi-arid tropics of India and Africa, for being used as a staple food for poor people (Mehmood et al., 2008). It is mainly a self-pollinated crop with a basic chromosome number 10 (2n=2x=20) and with DNA content of 1.7 pg and genome size is about 735 Mbp (Dillon et al. 2007). According to Vijayakumar et al. (2014) sorghum produced worldwide is 64.20 million tonnes with a cultivated area of 41 million hectares.

Estimation of genetic variability among crops has a solid influence on plant breeding and conservation of genetic resources (Dean et al.,1999; Simioniuc et al.,2002). It is helpful for the characterization of genotypes and for the selection of parents in breeding programmed (Graner et al., 1992). In the past two decades, evaluation of genetic variation that depends upon morphological information has been extensively used in many plant species including sorghum.

Morphological traits are easy to measure in the field and mostly they give an explanation of floral morphology and vegetative parts that are essential for the survival of plants. Although this type of information in not reliable for further study because of genotype-environment interaction (Smith and Smith, 1992). Improvement in molecular studies led to the development of molecular markers which give the path to the estimation of genetic variability and genetic diversity without the influence of environment. So, in future, the molecular markers will help for the conservation of germplasm as well as for its characterization.

DNA-based molecular markers help to identify unique DNA profiles for the safety of seed purity test and characterize new varieties (Reif et al. 2003). These primers have high polymorphic behavior, and freedom from environmental influence (Perry, 2004). Furthermore, these molecular markers help to detect the presence and absence of favorable alleles between germplasm and estimation of genetic diversity more reliably and competently than phenotypic markers. Summarily, DNA based molecular marker system aids conventional breeding in different aspects, such as to (1) assess of genetic diversity, fingerprinting and establish heterotic patterns, (2) screening for helpful genes, (3) speed up backcross breeding programs via selection of genes of interest and (4) protect commercial cultivars through DNA fingerprinting (Xiao et al.1996).

There are different types of DNA based markers like (RFLPs), (RAPDs), (AFLPs), (SSR), and (SNPs). Between these molecular markers, SSRs are the best markers system due to their high throughput and high accuracy level for DNA barcoding and genetic diversity study in organisms (Gupta. and. Varshney, 2000). SSRs markers are relatively advance class of molecular markers based upon tandem repeats of short (2-6 bp) DNA sequences because of their locus specificity, co-dominant behavior (Litt and Lutty 1989). So, it is a useful tool for identification of plant species (Cregan.et al., 1999; Goulao. et al., 2001).

Simple sequence repeats molecular markers have multi-allelic character and high degree of polymorphic behavior, so they have been extensively used for the identification of sorghum genotypes (Li et al., 2005), rice (Rahman and Rahman 2009), pigeonpea (Khalekar et al., 2014), rose (Crespel et al., 2009), pear (Kimura et al., 2002), potato

(Coombs et al., 2004), rape (Louarn et al., 2007), soybean (Tantasawat et al., 2011). The sorghum genome sequence project identified 71,000 SSRs molecular markers in the genome (Paterson et al. 2009).

Therefore, keeping in view importance of sorghum as a valuable crop and SSRs as a powerful technique, the present study was conducted for diversity analysis of sorghum accessions by SSR markers to develop diverse genotypes and for genetic polymorphism in available sorghum germplasm.

2. MATERIALS AND METHODS

2.1. Experimental Site

The experiment was conducted in kharif season (April-2017) at the Institute of Plant Breeding and Biotechnology, MNS-University of Agriculture, Multan, Pakistan. The Plant material was comprised of 82 genotypes of sorghum collected from National and International resources. These genotypes were sown in RCBD design with 3 Replications and Plant to Plant distance 20 cm and Row to Row distance 60 cm for phenotypic characterization. All cultural practices were applied uniformly to all experimental units.

2.2. Data Recording

Different parameters were recorded at field i.e.: Plant height(cm), Number of leaves, Width of leaf(cm), Length of leaf(cm), Inter-node length(cm), Length of peduncle(cm), Weight of panicle, Grain number per panicle and 100-Grain weight per panicle.

2.3.Analysis of phenotypic data

In order to discriminate germplasm, the mean data for each parameter were subjected to ANOVA as to steel et al., (1997). Statistical software 8.1 were used to check the significance of all parameters. For the identification of morphological differences, Principal Coordinate Analysis (PCA) was showed on correlation matrix by XLSTAT. Cluster analysis was performed by UPGMA (Sneath and Sokal, 1973; Swofford and Olson, 1990).

2.4. Molecular Characterization:

Molecular characterization of sorghum genotypes by molecular markers system (SSR) was done in the Cloning Lab, MNSUM, Multan.

2.4.1. Plant Material

The Plant material that was used in this study consisted of 96 sorghum genotypes for morphological characterization.

2.4.2 DNA Extraction from Plant Material

Fresh leaf samples of 12-days old sorghum seedling were collected from field and stored at-20 for genomic DNA extraction through modified CTAB method followed by (Doyle et al., 1987). The extracted DNA was electrophoresed on a 0.8% agarose gel for quality assessment and quantified on Nanodrop (ND-2000) at 260 nm.

2.4.3. Optimization of Polymerase Chain Reaction conditions

The PCR conditions were optimized for concentration of Template, 10X PCR Buffer, MgCL2, dNTPs, Forward and Reverse primer and Taq polymerase. Annealing temperature for all SSRs primers were optimized through gradient PCR. The amplification was accomplished in Gradient thermal cycler, following the program: Initial Denaturation 95°C for 5 min, 35 cycles, Annealing 50-60°C and Final Extension of 3 min at 72°C

2.4.4. Polymerase Chain Reaction Amplification of SSR markers.

Once high-quality DNA of known concentration is in hand, a series of different PCR are performed for testing specific elements or genes in test samples. Firstly, the isolated DNA is subjected to a control reaction using primer specific for housekeeping genes or microsatellite for that crop species to test quality of DNA for amplification. Secondly, specific PCR is conducted using specific primer for marker/reporter genes which are used in

identification of genes and diversity analysis of crops. If samples are found positive for any of these elements, the type and source of genes are validated through genes and even specific PCR, respectively. PCR was performed for 16 SSR markers in 0.2 ml PCR tubes.

2.4.5. Gel Scoring

The bands formed by 16 SSR markers were determined by Gel Electrophoresis on 2% Agarose Gel, Run in 2X TBE Buffer, visualized under a UV Transilluminator and snapped using OmniDoc Gel Documentation system.

2.5 Analysis of Genotypic Data

2.5.1. Phylogenetic Analysis

The PCR product obtained after SSR analysis were scored on the basis of presence (1) and absence (0) of bands for all genotypes. Ambiguous bands that could not be distinguished clearly were not scored. Genetic diversity and cluster analysis were accomplished by NTSYSPC software. Dendrogram were constructed by unweighted pair group method with arithmetic average (UPGMA). The polymorphism information content (PIC) was considered with formula used by (Botstein et al., 1980).

2.5.2. Diversity Analysis

The Power Marker programming (Liu and Muse, 2005) was utilized to figure essential component of microsatellite (SSRs) and the variable parameters including, Number of alleles per locus, Allele frequency, Genetic Diversity, Heterozygosity and the PIC value.

Results

3. Morphological Characterization

3.1. Analysis of Variance (ANOVA)

The Analysis of Variance (ANOVA) showed significant differences for 82 sorghum genotypes among all parameters viz., Plant height (PH), Leaves number per plant (LN), Leaf width (LW), Inter-nodal length (INL), Panicle length (PL), Panicle weight (PW), Leaf length (LL) Grain number per panicle (GN/P), and 100-Grain weight (GW). The Analysis showed the presence of genetic variability between 82 sorghum genotypes for all parameters. Table 3.1 exhibited that 82 genotypes differ significantly ($p<0.05$) for all traits

Table 3.1. Mean square values of 9 morphological traits of sorghum germplasm

SOV	DF	LN	LL	LW	INL	PL	PH	PW	GN	100-GW
REP	2	7.6	925.83	109	72.93	25.03	228.13	34.43	1349	3.5
Genotypes	81	70.80**	24.57**	4.51	3.54**	3.22**	210.79**	15.23**	94.46**	1.78**
Error	162	2.37	28.54	1.48	13.74	2.64	23.59	15.14	3933	3.08

Degree of freedom (DF), Plant height (PH), Leaves number per plant (LN), Leaf length (LL), Leaf width (LW), Inter-nodal length (INL), Panicle length (PL), Panicle weight (PW), Grain number per panicle (GN/P), and 100-grain weight (100-GW), Highly significant (**)

3.2. Estimation of Correlation among different yield contributing parameters different yield contributing parameters

The correlation between yield related parameters is important because it helps us to find those parameters which have positive correlation with yield. The correlation between different parameters is discussed in Table no 3.2.

Estimated correlation determined that leaf number is positive correlated with leaf length, plant height and panicle length while it is negatively correlated with inter-nodal length and panicle weight. Leaf length is positive correlated with leaf width, plant height, panicle length while it showed negative correlation with inter-nodal length and panicle weight. Leaf width is positive correlated with panicle length. Inter-nodal length shows no correlation among plant height, panicle length, panicle weight, grain number/panicle and 100-grain weight. Plant height is positive correlated with panicle length and grain number/panicle However, panicle length with panicle weight, grain

Table 3.2. Correlation matrix among the morphological traits.

	LN	LL	LW	INL	PH	PL	PW	GN/P
LL	0.74**							
LW	0.07	0.22**						
INL	-0.30**	-0.36**	0.02					
PH	0.72**	0.50**	0.03	-0.04				
PL	0.20**	0.21**	0.21**	0.02	0.30**			
PW	-0.19**	-0.25**	0.02	0.08	-0.11	0.04		
GN/P	0.04	-0.01	-0.10	0.11	0.32**	0.08	-0.12	
100-GW	0.01	0.08	0.11	-0.03	0.02	0.06	0.05	-0.06

*= p<0.05 **= p<0.01

number/panicle, grain weight and, panicle weight with grain number/panicle, grain

weight and grain number/ panicle with grain weight showed no correlation.

3.3. Biplot analysis

Biplot analysis characterized that variables were enforced as vector; relative length of the vector characterized as the relative proportion of the variability in each variable. The genotypes that were plotted faraway from origin exhibited to less closeness when contrasted with the genotypes plotted close

with the main issue. The traits like plant height, grain number, and leaf number, were well characterized and showed high variability during growing season (Figure 3.1 and Figure 3.2) on the other hand, parameters i.e., Panicle length and 100-Grain Weight showed less variability as compared other parameters. Quantitative characters like Plant Height, Leaf Length, Panicle Length, Grain Number and 100-Grain Weight were assigned at positive (+) and positive (+) coordinate in biplot analysis in the growing season. Parameters Leaf Length and Leaf width were shown at second positive negative coordinate. On the other hand, parameter Internodal Length and Panicle Weight deviated to 1st positive negative coordinate for growing season. The maximum variation found among the traits showed the diverse pattern of sorghum genotypes.

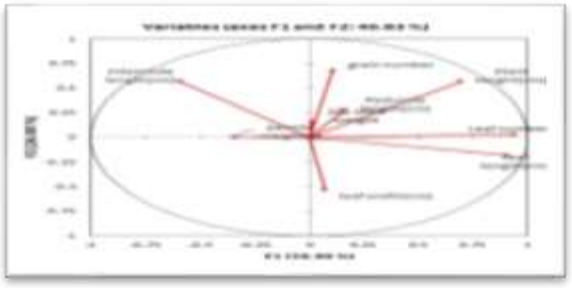

Fig 3.1: Biplot analysis associated to different morphological parameters in sorghum

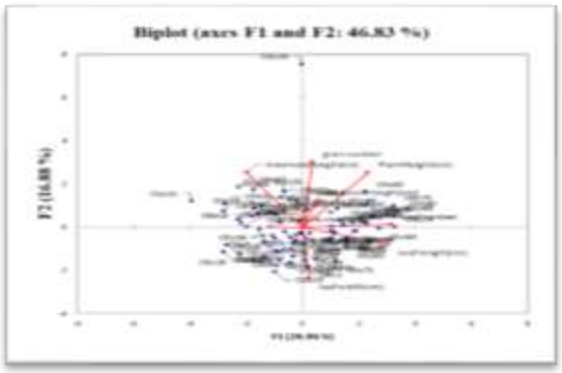

Fig 3.2: Biplot analysis associated to different morphological parameters in sorghum

3.4. Cluster Analysis

UPGMA evaluated 82 sorghum genotypes. (Figs. 3.3 and 3.4). UPGMA divided clusters into 12 groups (C1, C2, C3, C4, C5, C6, C7, C8. C9, C10, C11, and C12). It generated 29 morpho-types of 82 sorghum genotypes. Whereas, Cluster C3, C4, C5, C7, C9, C10, C12 accommodated by 1 genotype only. Cluster C1 compromised of 13 genotypes. Cluster C2 was made up of 31 genotypes.

Two genotypes belong to Cluster C6. The C8 comprised of 6 genotypes and C11 consist of 23 genotypes. The class centroids for all 12 clusters and their description is shown in Table 3.3.

Table. 3.3. Characterization of all twelve class centroids based on cladogenesis studies

Class	LN	LL	LW	INL	PH	PL	PW	GN	100-GW
1	13	41.29	8.21	12.28	121.19	9.89	23.31	213.62	2.03
2	12.32	42.69	9.04	11.29	106.11	7.12	12.58	152.6	2
3	10	60.96	10.41	7.62	86.36	7.62	37	920	2.42
4	8	45.72	12.87	10.16	91.44	12.7	16	515	2.76
5	4	17.78	7.86	6.43	60.96	11.43	21	410	4.15
6	8.5	29.21	10.96	14.61	88.86	6.1	20	844.5	2.98
7	8	22.86	10.41	13.97	101.6	7.87	50	50	1.33
8	7.83	29.76	8.24	21.8	119.08	7.66	10	64.67	2.14
9	4	17.78	7.04	22.86	60.79	8.38	28	11	4.55
10	13	30.56	5.5	22.86	244.26	8.64	12	2600	2.95
11	24.33	60.9	8.2	10.03	161.97	8.32	10.96	271.9	2.22
12	24	63.5	10.49	9.14	157.48	8.64	20	6	8.39

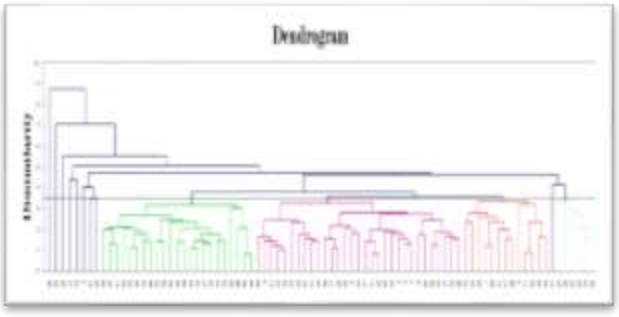

Fig 3.3: Cataloging of 82 sorghum genotypes into different morphotypes by UPGMA cluster analysis

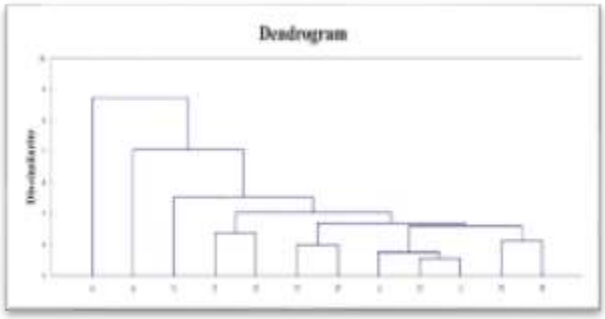

Fig 3.4: Classification of 82 sorghum genotypes on the base of Homology studies

3.5. Principal component analysis (PCA)

PCA of different parameters was used to put out redundancy in data set. Scree plot analysis classified the total variance of all parameters into 9 principal components (PCs) (Fig 3.5). For growing season, 3 principal components (PC1, PC2 and PC3) have eigen value >1 is shown in Table 3.4. The cumulative variation found 46.1% in three principal components (PCs). The PC1 shown 29.95% of the total variability in all 9 parameters followed by PC2 (46.83%) and for PC3 (61.73%). Different quantitative parameters counted more than 50% of variation factor in PC1 i.e. leaf number (57%) and leaf length (55%). PC1 indicated strong and positive correlation with plant height (42%). PC1 showed weak and positive correlation with panicle length (10%). On the other hand, PC1 showed negative correlation with intermodal length (INL) and panicle weight (PW).

The PC2 showed (46.83%) variability for all quantitative parameters. PC2 contributed strong and positive correlation with plant height (PH) and intermodal length (INL) for (46%) and grain number (GN) for 55%. On the other hand, PC1 contributed weak and positive correlation for panicle length (PL) and 100-GW i.e., 23% and 13% respectively. Leaf length and leaf width showed negative correlation for PC2.

The PC3 contributed strong and positive correlation for Panicle length (PL) 58%, 100-grain weight (100-GW) 52%, and showed 42% contribution for leaf width (LW) and Panicle weight (PW). Negative correlation was observed for leaf number (LN), intermodal length (INL) and plant height PH).

Table 3.4. PCA associated with different quantitative traits in sorghum genotypes

Parameters	PC_1	PC_2	PC_3
LN	0.57	0.02	-0.04
LL	0.55	-0.15	0
LW	0.04	-0.43	0.42
INL	-0.36	0.46	-0.13
PH	0.42	0.46	-0.06
PL	0.1	0.23	0.58
PW	-0.21	0	0.42
GN	0.06	0.55	0.13
100-GW	0.01	0.13	0.52
Eigen Value	2.7	1.52	1.34
Total variance (%)	30	16.9	14.9
Cumulative variability (%)	30	46.8	61.7

leaves number per plant (LN), Leaf length (LL), Leaf width (LW), inter-nodal length (INL,) plant height (PH), panicle weight (PW) panicle length (PL), grain number per panicle (GN/P), and 100-grain weight (100-GW), Principal component analysis (PC)

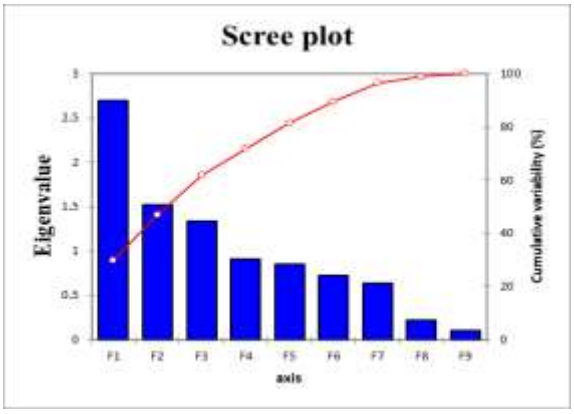

Fig 3.5: Analysis through scree plot of sorghum genotypes

3.6. Molecular characterization

3.6.1. Estimation of Genetic Diversity by SSR Markers

All 16 SSR markers were examined and genetic variation was determined and generated multiple fragments in 96 genotypes of sorghum. A total 99 alleles were analyzed by simple sequence repeats primers with an average of 6 alleles per marker with range of 3 to 11 per locus (Table 3.5)

On the other hand, polymorphic information content (PIC) showed that Sorg16 had maximum value of 0.88 while marker Sorg6 was found to be monomorphic having PIC value 0 as compared to all other available markers. A value of 0.55 was noted as a mean value for all sixteen markers as shown in Table 4. While major allele frequency (MAF) was noticed maximum 1 for Sorg6 and Sorg11 while minimum MAF 0.57 was noticed for Sorg2 marker. MAF were also recorded varied from 0.57 to 1 with an average of 0.85. The genetic diversity index value was found maximum 0.49 for Sorg2 and Sorg4 marker while minimum value 0 was noticed for Sorg6 marker. Genetic diversity index value was ranged from 0.00 to 0.4965 with an average of 0.25.

Likewise, maximum heterozygosity value was found 0.85 for Sorg5 while minimum heterozygosity value of 0 was noticed for Sorg6 with an average of 0.54 among all markers. Molecular markers which amplified PCR product were characterized as polymorphic or monomorphic. From 16 markers, Sorg6 was found monomorphic while other 15 markers were polymorphic. The results showed that these sixteen SSRs markers could be for the analysis of diversity for the detection of polymorphic studies on sorghum crop.

3.6.2. Genetic Diversity and Cluster Analysis

On the bases of 99 alleles genetic similarity Co-efficient was assessed for each group of 96 genotypes of sorghum which ranges from 0.25 to 1.00. The dendrogram clearly distinguish that 29 morphotypes was produced in all the 96 sorghum genotypes as shown in (Fig 3.6) Genetic diversity ranges from 0.0 to 0.49 and maximum genetic diversity was found at locus Sorg2 and Sorg4 followed by Sorg1 (0.40).

Table 3.5: Major allele frequency (MAF), Allele Number (AN), Genetic diversity (GD), Heterozygosity (HET) and Polymorphic

information content (PIC) for 16 primers used in this study

Marker	MAF	AN	GD	HET	PIC
Sorg1	0.69	5	0.4	0.7	0.57
Sorg2	0.57	6	0.49	0.51	0.77
Sorg3	0.82	4	0.29	0.73	0.68
Sorg4	0.61	7	0.49	0.64	0.37
Sorg5	0.97	5	0.06	0.85	0.51
Sorg6	1.00	6	0.00	0.00	0.00
Sorg7	0.86	6	0.19	0.54	0.65
Sorg8	0.83	6	0.28	0.42	0.7
Sorg9	0.85	4	0.25	0.65	0.22
Sorg10	0.88	3	0.22	0.72	0.52
Sorg11	1.00	6	0.55	0.45	0.55
Sorg12	0.88	5	0.22	0.43	0.45
Sorg13	0.97	9	0.06	0.62	0.86
Sorg14	0.83	8	0.28	0.16	0.24
Sorg15	0.95	11	0.1	0.72	0.82
Sorg16	0.92	8	0.15	0.45	0.88
Total	13.63	99	4.03	8.59	8.79
Mean	0.85	6.19	0.25	0.54	0.55

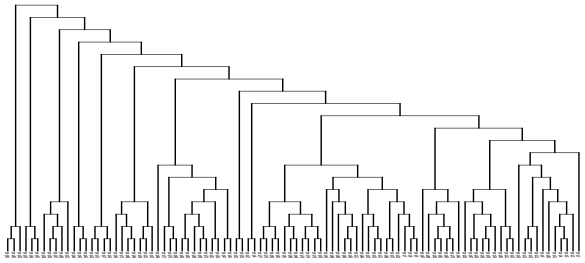

Fig.3.6: Dendrogram of 96 sorghum genotypes through cluster analysis of genetic similarity assessments produced by Li coefficient established on 16 SSRs primers.

4. Discussion

Characterization on morphological bases is the chief step to discover and to divide genetic diversity analysis for available genotypes (Rakshit et al. 2012). Current study described the broad information to the estimation the sorghum germplasm by exploring different tools. ANOVA shown that all 9 parameters were highly significant. Significant variation was found among sorghum genotypes for quantitative parameters i.e., Leaf number, leaf length, leaf width, plant height, panicle weight, panicle length, grain number, and 100-grain weight of the germplasm reported (Noor et al., 2012; Khan et al., 2007; Jain et al., 2011; Jadhav et al., 2011; Chohan et al., 2003).

Multivariate statistical software offers authentic information to find out the morphological diversity among germplasm (Shrestha, 2013). The current study determined that three principal component (PCs) shared 29.95% contribution and total variability was observed 16%. Principal coordinate grouping for germplasm can be valuable for the estimation of genotypes (Rakshit and Patil, 2013).

Biplot analysis estimated that plant height, grain number/panicle, and leaf length showed high variability as compared to panicle length and 100-grain weight. On the other hand, plant height, leaf number, panicle length, grain number and 100-grain weight were fall positive and positive coordinate while leaf length and leaf number were fall at 2nd positive negative coordinate.

4.1. Correlation Among Different Parameters

Association among quantitative parameters offers a chance for breeder to the selection desirable traits (Abubakar and Bubuche, 2013). In current study, correlation studies estimated that Leaf Length, Plant Height, Panicle Length have positive association with Leaf Number and showed negative correlation with Inter nodal length and Panicle Weigh. Leaf length is in positive correlation with Leaf Width, Plant Height, Panicle Length while it exhibited negative correlation with Inter nodal length and Panicle Weight and Leaf Width is positively correlated with Panicle length. Inter-nodal length has no correlation among Plant Height, Panicle Length, Panicle Weight, grain number/panicle and 100-grain weight. Plant Height is positive correlated with Panicle Length and grain number/panicle while Panicle Length, panicle weight and grain number/ panicle showed no correlation among the traits. Same correlation pattern was observed in the earlier readings

(Abubakar and Bubuche, 2013; Jain et al., 2011).

4.2. Allelic Diversity at SSRs Loci

Molecular marker used for this study was microsatellite, which is also called as Simple Sequence Repeats (SSR). Different studies related to genetic diversity can be achieved by markers system and through morphological traits viz. Amplified Fragment length Polymorphism (AFLP), Random amplified polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP), Single nucleotide polymorphism (SNP) and Simple sequence repeats (SSR) reported by (Geleta et al., 2006; Ritter et al., 2007).

The total number of alleles observed in this study were maximum as compared with Folkertsma et al. (2005) who reported total 123 alleles from 21 SSR loci among 100 sorghum accessions. Ghebru et al (2002) observed 208 a total 208 alleles from 15 SSR markers on 28 sorghum Eritrean landraces and 32 world sorghum lines accessions. While total no of alleles was found minimum from as compared with Perumal et al. (2007) who reported 97 polymorphic alleles from 7 SSR markers among 46 sorghum lines. Moreover, the result in the study indicate the presence of high genetic diversity among sorghum collections. The mean no of alleles per locus (6.19) observed in sorghum accessions was higher than that reported by Ng`Uni et al. (2011), Muui et al. (2016), Tesfamichael Abraha et al. (2014), Wang et al. (2013), Ali et al. (2008), Schloss et al. (2002), Pei et al., 2010), Agrama and Tuinstra (2003), and Smith et al. (2000) with mean allele per locus of 3.3, 3.22, 3.4, 4.4, 4.96, 4.3, 5.09, 5.05 and 4.8, respectively. However, it is lower than Missihoun et al. (2015), El-awady et al. (2008), Cuevas and Prom, (2013) with mean allele number per locus of 7, 7.3 and 14, respectively.

The average PIC value (0.55) that is higher than diversity value (0.46, 0.47,0.33,0.49) that was reported by Schloss et al. (2002), Nemera Geleta and Labuschagne, (2006), Missihoun et al. (2015) and Muui et al, (2016), respectively. However, the observed average PIC value is lower than that of Ceuvas and prom, (2013), Agrama and Tuinstra, (2004), who determined average PIC values of 0.78 and 0.62, respectively.

The genetic diversity was found 0.55 and 0.48 that was related to Ji et al. (2011), Folkertsma et al. (2005), Alina et al. (2014) 0.49, Madhusudhana et al. (2012) 0.52 and Habib et al. (2013) 0.53 genetic diversity, while it was maximum as compared with Schloss et al. (2002), Agrama and Tuinstra (2003) and Smith et al. (2000). On the other hand, high level of genetic diversity was observed (0.63) by 41 SSR markers in sorghum genotypes (Wang et al. 2013).

In conclusion, this study acknowledged maximum diversity and allele number among sorghum accessions in Pakistan. The data also depicted the power of SSRs markers for germplasm conservation and crop improvement. Many authors have specified that SSR marker system is highly cost-effective (Smith et al. 2000). Overall, the investigating sorghum accessions showed significant genetic variation, representing that the accessions could be used for the development of desired traits among the genotypes by breeding and selection programs.

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