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RESEARCH ARTICLE

Morphological and Molecular Diversity Analysis in Forage Sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract

The present investigation was carried out to find the diverse genotypes by using D^2 statistics and simple sequence repeat (SSR) markers. The genetic divergence assessed by Mahalanobis D^2-statistics grouped 22 genotypes into eight clusters. The maximum inter-cluster distance was observed between clusters V and VIII (645.77). Thus, genotypes included in these clusters may be utilized under an inter-varietal hybridization program for forage yield improvement. The molecular diversity analysis shows that the number of alleles for SSR primers ranged from two to four, with an average number of alleles per locus was 2.79. The similarity coefficients among all the 22 genotypes ranged from 0.185 to 0.875. The clustering pattern of the dendrogram generated by pooled molecular data of seventeen SSR loci generated two clusters, *viz.,* A and B, at a similarity coefficient of 0.33. The combined results for morphological and molecular diversity estimates showed that genotype "SSG-59-3" was found to be distinct from other genotypes.

Keywords: Cluster, Diversity, D^2-statistics, Sorghum, SSR.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) ranks first among the cereal fodder crops because of its growing ability in low fertile soil, faster growth habit, higher fodder yield, palatability and nutritious quality. It gives almost uniform green fodder yield throughout the year. It is mostly grown in the semi-arid tropics of Asia and Africa, where water availability is limited due to its drought-tolerant nature (Kumar *et al*., 2011).

To create forage sorghum as an enterprising and remunerative crop, there's a necessity to develop varieties or hybrids having faster growth, good vigor and high forage yield, as well as high protein content and low HCN content at the flowering stage. The collection, maintenance and evaluation of germplasm are the most important and primary steps of any crop improvement program. The best way to perceive the potential of the available germplasm is by analyzing its genetic diversity. Consideration of geographical diversity as a reasonable index of genetic diversity might result in inaccurate conclusions. Mahalanobis' D^2 statistics of multivariate analysis is recognized as a powerful tool in quantifying the degree of genetic divergence among the breeding material.

The information obtained from phenotypic characterization does not reflect the real genetic variation because of genotype × environment interaction. The use of molecular markers has numerous advantages in genetic diversity analysis as their expression is not affected by the environment (Gepts, 1993).

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Table 1: Experimental material used in the study

S. No.	*Genotypes*	*Origin*	*Types*
1	SRF-316	Surat, Gujarat	Single-cut forage type
2	MALWAN	Local landraces Gujarat	Dual purpose; Forage & Grain type
3	GFS-5	Surat, Gujarat	Single cut forage type
4	CSV-21	Surat, Gujarat	Single cut forage type
5	SRF-286	Surat, Gujarat	Single cut forage type
6	DS-1053	Deesa, Gujarat	Dual purpose; Forage & Grain type
7	DS-1168	Deesa, Gujarat	Dual purpose; Forage & Grain type
8	DS-1111	Deesa, Gujarat	Dual purpose; Forage & Grain type
9	DS-1146	Deesa, Gujarat	Dual purpose; Forage & Grain type
10	DS-1187	Deesa, Gujarat	Dual purpose; Forage & Grain type
11	UTML-529-10(8)	IIMR, Hyderabad	Forage type
12	PDJP-1612/621513	IIMR, Hyderabad	Forage type
13	PDJP-1614/621515	IIMR, Hyderabad	Forage type
14	DSF-168	Deesa, Gujarat	Single cut forage type
15	DSF-117	Deesa, Gujarat	Single cut forage type
16	GAFS-12	Anand, Gujarat	Single cut forage type
17	GFS-4	Surat, Gujarat	Single cut forage type
18	DSF-172	Deesa, Gujarat	Single cut forage type
19	SH1813	Hissar, Haryana	Multicult Forage type
20	S-652	Hissar, Haryana	Multicult Forage type
21	SSG-59-3	Hissar, Haryana	Multicult Forage type
22	SH-1488	Hissar, Haryana	Multicult Forage type

Several DNA-based markers have been successfully used, in which SSR is found to be the marker of choice for diversity analysis in sorghum because of their ability to produce informative multi-allelic loci and greater genotypic differentiation. SSRs are highly polymorphic (Anas and Yoshida, 2004) and possess wider genome coverage than restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. Their co-dominant nature, simple and quick operation, good stability and simplicity make SSR markers a versatile choice for genetic differentiation studies. The present investigation aimed to characterize forage sorghum genotypes concerning their morphological traits and molecular markers.

Materials and Methods

Morphological diversity analysis

The experimental material comprising 22 forage sorghum genotypes was given in Table 1, along with their place of origin and single-cut/multi-cut type. These genotypes were evaluated in randomized block design with three replications at Sorghum Research Station, Sardarkrushinagar Dantiwada Agricultural University, Deesa during *Kharif* – 2020.

Each genotype was sown in one row of three-meter length with an optimum inter-row spacing of 30 cm. The observations were recorded both as visual assessment for days to 50% flowering and measurement on five randomly selected plants for plant height (cm), number of leaves per plant (no), stem girth (mm), length of leaf blade (cm), width of leaf blade (cm), leaf: stem ratio, dry fodder yield per plant (g), brix content (%), hydrocyanic acid content (ppm), crude protein content (%) and green fodder yield per plant (g). The replication-wise mean values were used for statistical analysis. The morphological divergence analysis was carried out by D^2 statistics as proposed by Mahalanobis (1936) and described by Rao (1952). Grouping of genotypes into different clusters was done by tocher's method. The intra-cluster and inter-cluster distances were calculated by the formula given by Singh and Chaudhary (1977) using TNAUSTAT software.

Molecular diversity analysis

The molecular analysis was carried out at the Department of Genetics and Plant Breeding, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Fresh, healthy leaves were collected from all the genotypes at the time of flowering for DNA extraction. High molecular weight genomic DNA was isolated from 22 sorghum genotypes by

Table 2: Analysis of variance (ANOVA) showing mean sum of squares for twelve wtraits in forage sorghum

S. No	Characters	Mean sum of squares		
		Treatment	Replication	Error
	Degree of freedom (df)	21	2	42
1.	Days to 50% flowering	68.02**	11.56	10.53
2.	Plant height (cm)	1356.29**	856.49	323.00
3.	Number of leaves per plant (no)	1.79**	2.00	0.607
4.	Stem girth (mm)	9.69**	1.35	1.35
5.	Leaf length of blade (cm)	166.46**	22.03	46.06
6.	Leaf width of blade (cm)	2.76**	0.02	0.49
7.	Leaf: Stem ratio	0.01**	0.00007	0.001
8.	Dry fodder yield per plant (g)	1421.81**	14.98	66.91
9.	Brix (%)	4.51**	0.12	0.51
10.	Hydrocyanic acid content (ppm)	155.58**	2.97	1.95
11.	Crude protein (%)	3.99**	0.06	0.21
12.	Green fodder yield per plant (g)	9433.12**	109.50	393.47

*Significance at 5% probability level **Significance at 1% probability level

following the cetyl trimethyl ammonium bromide (CTAB) extraction method as described by Doyle and Doyle (1987) with some modifications. The list of primers selected for the study is shown in Supplementary Table 2. About 17 SSR primers after their optimization and screening were selected and used.

Scoring and statistical analysis of data

The amplified products of each primer were scored separately based on the presence or absence of a band across 22 sorghum genotypes, *i.e.,* the use of binary codes 1 and 0 for the presence or absence of a band, respectively. The data was entered into a binary matrix and subsequently analyzed using the NTSYSpc version 2.20 software package (Rohlf, 2000).

The similarity coefficient among the 22 sorghum genotypes was calculated by using Jaccard's similarity coefficient by SimQual function. The dendrogram, including all the 22 genotypes, was constructed by using the un-weighted pair group method with arithmetic mean (UPGMA) method by SAHN clustering function of NTSYSpc (version 2.20). The diversity parameters, such as the number of alleles per locus, heterozygosity and polymorphism information content, were calculated using power marker software. The software program Alpha Ease FC version 4.0.0 (Alpha Innotech Corporation, USA) was used for determining the molecular weight (MW) of bands separated on the gel. The polymorphism information content (PIC) values measure the informativeness of a given DNA marker for the 22 sorghum genotypes. Heterozygosity (H) is a parameter indicating the average frequency of a heterozygous individual's occurrence.

Results and Discussion

Morphological diversity analysis

The analysis of variance for all the twelve traits is shown in Table 2. The mean sum of squares due to treatments showed highly significant differences among the genotypes for all the traits, which indicates the wide spectrum of variation among them. The mean performance of 22 genotypes studied for 12 traits in three replications is presented in Supplementary Table 1. A wide range of variation among the genotypes was found for all the traits *viz*., days to 50% flowering (57.33–78.67), plant height (157.33–243.00 cm), number of leaves per plant (6.6–10.2), stem girth (5.37–14.00 mm), leaf length of blade (45.73–81.64 cm), leaf width of blade (4.35–8.75 cm), leaf: stem ratio (0.18–0.46), dry fodder yield per plant (42.51–135.49 g), brix content (7.56–12.23%), hydrocyanic acid content (11.82–37.06 ppm), crude protein (6.65– 11.03%) and green fodder yield per plant (92.39–351.27 g). The wide range of variations in forage yield and components were also reported earlier by Jadhav *et al*. (2011) and Rana *et al*. (2016).

For improving complex traits like forage yield, the selection of parents having wide divergence for various characters is of prime importance which can be assessed by D^2 statistics developed by Mahalanobis (1936). The greater distance between two clusters indicates greater divergence and vice versa. The genotypes falling in the same cluster indicate that they are more closely related than those belonging to other clusters. In view of this, Mahalanobis D^2 statistics was used to assess the genetic divergence in different forage sorghum genotypes to identify superior

Table 3: Distribution of 22 genotypes of forage sorghum in different clusters on the basis of D^2 statistics

Cluster	*Number of genotypes*	*Name of genotype*
I	5	DS-1187, UTML-529-10(8), PDJP-1612/621513, GAFS-12 and GFS-4
II	8	MALWAN, CSV-21, SRF-286, DS-1053, DS-1111, DSF-168, S-652 and SH-1488
III	3	SRF-316, GFS-5 and DSF-117
IV	2	DS-1168 and DS-1146
V	1	PDJP-1614/621515
VI	1	SSG-59-3
VII	1	SH-1813
VIII	1	DSF-172

Table 4: Average Intra-cluster (Diagonal) and Inter-cluster (off-diagonal) distance of 8 clusters of forage sorghum

Cluster	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
I	52.07	79.06	116.99	170.83	144.29	128.40	167.45	299.40
II		64.12	148.54	117.35	145.86	98.55	222.77	321.47
III			69.27	306.21	322.91	262.62	97.97	131.43
IV				63.33	93.88	149.10	472.11	581.65
V					0	218.76	520.00	645.77
VI						0	272.27	387.32
VII							0	116.86
VIII								0

genotypes which can be utilized for future breeding programmes.

In the present study, the clustering was done based on the relative magnitude of D^2 values by the tocher's method. With the help of D^2 values, eight clusters were formed from 22 genotypes with 12 traits of forage sorghum. The composition of clusters is given in (Table 3).

The results indicated that a maximum number of eight genotypes appeared in cluster II followed by five genotypes in cluster I, three genotypes in cluster III, two genotypes in cluster IV and each genotype in clusters V, VI, VII and VIII. This indicated that there is a presence of diversity among the 22 genotypes studied. Each of the following genotypes PDJP-1614/621515, SSG-59-3, SH-1813 and DSF-172 occupied their position individually in separate clusters, which shows there is a presence of distinguished traits from the rest of the genotypes studied. The average intra and inter-cluster distance between all possible pairs of eight clusters are presented in Table 4 and Fig. 1.

A study on data revealed that the inter-cluster distance D^2 values ranged from 79.06 to 645.77. The maximum inter-cluster distance was observed between clusters V and VIII (645.77) followed by clusters IV and VIII (581.65), cluster V and VII (520.00), cluster IV and VII (472.11), cluster VI and VIII (387.32), cluster III and V (322.91), cluster II and VIII (321.47) and cluster III and IV (306.21). The least inter-cluster distance was observed between clusters I and II (79.06) followed by clusters IV and V (93.88), clusters III and VII (97.97), clusters

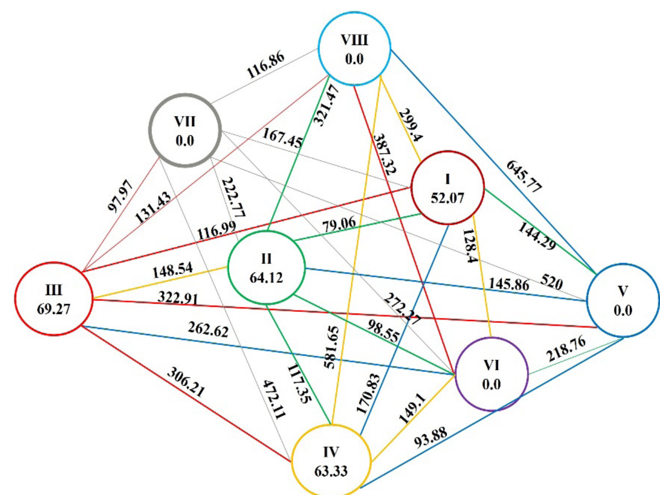


Fig.. 1: Cluster diagram showing interrelationship among eight clusters using D^2 analysis

II and VI (98.55), clusters VII and VIII (116.86), cluster I and III (116.99), cluster II and IV (117.35), cluster I and VI (128.40), cluster III and VIII (131.43), cluster I and V (144.29), cluster II and V (145.86), cluster II and III (148.54), cluster IV and VI (149.10), cluster I and VII (167.45), cluster I and IV (170.83), cluster V and VI (218.76), cluster II and VII (222.77), cluster III and VI (262.62), cluster VI and VII (272.27) and cluster I and VIII (299.40).

The intra-cluster distance was ranged from 0.0 to 69.27. The maximum intra-cluster distance of 69.27, followed by

Table 5: Percent contribution of different characters towards total genetic divergence of forage sorghum

S. No	*Character*	*Number of times character ranked first*	*Percent contribution*
1	Days to 50% flowering	4	1.73
2	Plant height (cm)	2	0.87
3	Number of leaves per plant (no)	0	0
4	Stem girth (mm)	3	1.30
5	Length of leaf blade (cm)	1	0.43
6	Width of leaf blade (cm)	0	0
7	Leaf:Stem ratio	8	3.46
8	Dry fodder yield per plant (g)	16	6.93
9	Brix (%)	9	3.90
10	Hydrocyanic acid content (ppm)	133	57.58
11	Crude protein (%)	30	12.99
12	Green fodder yield per plant (g)	25	10.82

64.12, 63.3 and 52.07, was observed in clusters III, II, IV and I, respectively. In contrast, the least intra-cluster distance (*i.e.*, 0.0) was observed in clusters V, VI, VII and VIII.

In the present investigation, the inter-cluster distance was higher than the intra-cluster distance which indicated the wide genetic diversity among the different accessions of different clusters than those of the same cluster. Similar results were reported by Elangovan *et al*. (2014), Meena *et al*. (2016) and Ahalawat *et al*. (2018). The maximum inter-cluster distance observed between clusters V and VIII indicates that the genotypes included in these clusters are more diverse and may generate high heterotic responses in a breeding program. Similar results were reported by Mohanraj *et al*. (2006).

The contribution of different traits towards divergence is shown in Table 5. HCN content (57.58%) was the main contributor to the total genetic divergence, followed by crude protein content (12.99%) and green fodder yield per plant (10.82%). The traits *viz*., the number of leaves per plant and width of leaf blade showed no contribution toward total genetic divergence. HCN content contributed more towards divergence and a similar result was reported by Damor *et al*. (2017). Meena *et al*. (2016) also showed that the number of leaves per plant had no contribution towards divergence.

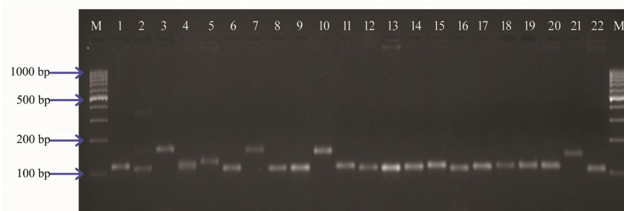
Molecular Diversity Analysis

SSR marker analysis of 17 SSR primer pairs *viz*., msbCIR238, msbCIR240, msbCIR276, msbCIR300, msbCIR329, Xcup14, Xcup53, Xtxp12, Xtxp15, Xtxp57, Xtxp67, Xtxp136, Xtxp141, Xtxp265, Xtxp289, Xtxp317 and Xtxp358 generated a total of 42 alleles among 22 forage sorghum genotypes. The results were depicted in Table 6 and the SSR amplification profile of primer msbCIR240 is given in Fig. 2. The number of alleles ranged from two to four, with an average number of 2.79 alleles per locus. This result was closely related to the findings of Agrama and Tuinstra (2003) and Akansha *et al*. (2020). The maximum number of four alleles was recorded in Xtxp289 and msbCIR240, followed by three alleles in msbCIR238, Xcup53, Xtxp12, Xtxp15, Xtxp67, Xtxp141, and Xtxp265, and a minimum number of two alleles in msbCIR300, msbCIR329, Xcup14, Xtxp57, and Xtxp317. The primers msbCIR276, Xtxp136 and Xtxp358 were found to be monomorphic. Kondombo *et al*. (2010) also reported the primer Xtxp136 was monomorphic.

The genotypes were subjected to Jaccard's coefficient analysis to find the similarity between all possible pairs of genotypes. The results indicated that the similarity coefficients among all the 22 genotypes ranged from 0.185 to 0.875. The genotypes SRF-316 and DS-1168 were found to be most dissimilar, with a similarity coefficient of 0.185. The study of results from pair wise combinations indicated that the genotypes GFS4 and SH-1813 were highly related to each other as the value of similarity coefficient 0.875 was higher as compared to other genotypic combinations.

The molecular size of the amplified PCR products ranges from 91bp (msbCIR238) to 348bp (Xtxp289). The PIC value ranged from 0.15 (msbCIR329) to 0.64 (Xtxp289), with an average of 0.42.

To study the phylogenetic/evolutionary relationship among different genotypes, the dendrogram was constructed by using 17 SSR markers through NTSYS-pc software (Fig. 3). Based on the dendrogram, two main clusters were formed from 22 parental genotypes, *i.e.*, cluster A and cluster B, which were formed at a similarity coefficient of 0.33. Cluster A is subdivided into sub-clusters A_1, A_2 and A_3. The sub-cluster A_1 includes seven genotypes *viz*., SRF-316, DSF-168, CSV-21, UTML-529-10(8), DSF-117, DSF-172



Where,

M=Ladder	6.DS-1053	12.PDJP-1612/621513	18.DSF-172
1.SRF-316	7.DS-1168	13.PDJP-1614/621515	19.SH1813
2.MALWAN	8.DS-1111	14.DSF-168	20.S-652
3.GFS-5	9.DS-1146	15.DSF-117	21.SSG-59-3
4.CSV-21	10.DS-1187	16.GAFS-12	22.SH1488
5.SRF-286	11.UTML-529-10(8)	17.GFS-4	

Fig. 2: SSR amplification profile of primer msbCIR240 in 22 forage sorghum genotypes

Table 6: Results of SSR analysis in twenty-two genotypes of forage sorghum

S. No.	*Primer*	*Molecular band size (bp)*	*No. of alleles*	*PIC*	*Heterozygosity*
1.	msbCIR238	91-112	3	0.58	0.00
2.	msbCIR240	108-171	4	0.60	0.00
3.	msbCIR276	235	1	0.00	0.00
4.	msbCIR300	106-114	2	0.35	0.00
5.	msbCIR329	114-135	2	0.15	0.00
6.	Xcup14	202-217	2	0.26	0.00
7.	Xcup53	180-204	3	0.38	0.00
8.	Xtxp12	163-200	3	0.48	0.00
9.	Xtxp15	196-228	3	0.25	0.10
10.	Xtxp57	230-264	2	0.38	0.00
11.	Xtxp67	163-194	3	0.44	0.06
12.	Xtxp136	250	1	0.00	0.00
13.	Xtxp141	132-170	3	0.56	0.09
14.	Xtxp265	179-205	3	0.37	0.00
15.	Xtxp289	261-348	4	0.64	0.05
16.	Xtxp317	153-168	2	0.38	0.00
17.	Xtxp358	210	1	0.00	0.00
		Average	2.79	0.42	0.02

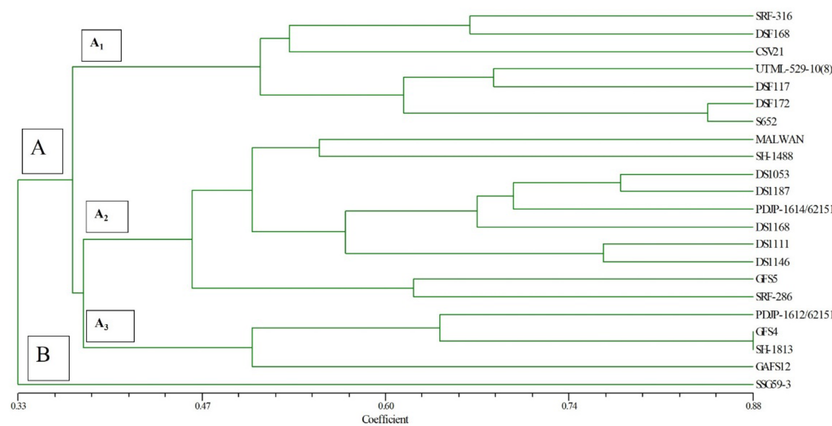


Fig. 3: Dendrogram showing clustering of 22 forage sorghum genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from SSR-based PCR analysis

and S652. The sub-cluster A_2 includes ten genotypes *viz*., MALWAN, SH-1488, DS-1053, DS-1187, PDJP-1614/621515, DS-1168, DS-1111, DS-1146, GFS-5 and SRF-286. The sub-cluster A_3 includes four genotypes *viz*., PDJP-1612/621513, GFS-4, SH-1813 and GAFS-12. The sub-cluster B includes single genotype SSG-59-3. Based on molecular data of the present investigation, it was observed that genotype SSG-59-3 is the most diverse genotype, as it was found placed in a separate cluster from the rest of the genotypes.

Conclusion

In the present investigation, the diversity of twenty-two genotypes of forage sorghum was estimated through morphological and molecular data (*i.e.,* SSR marker). A total of eight distinct clusters were formed through D^2 analysis whereas two main clusters (A & B) with three subclusters, *i.e.,* A_1, A_2, A_3 in the main cluster A, were formed through NTSYSpc version 2.20 program, which revealed that grouping of genotypes through Mahalanobis D^2 analysis was not utterly similar to a grouping of genotypes based on the molecular data through NTSYSpc version 2.20 program. It may be due to the gene concerned with morphological traits is stage-specific, while molecular analysis of the genome represents evolutionary variation that may be functional or non-functional.

Based on combined results for morphological and SSR

genetic diversity estimates, genotype "SSG-59-3" was found to be distinct from other genotypes and can be exploited to harness their unique features in breeding programs.

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Supplementary Table 1: Mean performance of twenty-two genotypes for different characters in forage sorghum

S No.	*Genotypes*	*Days to 50 per cent flowering (days)*	*Plant height (cm)*	*Number of leaves per plant (no)*	*Stem girth (mm)*	*Leaf length of blade (cm)*	*Leaf width of blade (cm)*
1	SRF-316	76.33	157.3	8.53	11.55	69.53	7.27
2	MALWAN	66.00	214.7	8.20	10.17	61.00	6.21
3	GFS-5	76.67	162.1	8.67	9.34	52.42	6.36
4	CSV-21	70.00	198.0	8.20	9.76	61.40	6.49
5	SRF-286	78.67	214.7	9.07	11.22	61.47	6.01
6	DS-1053	69.67	243.0	9.13	9.10	57.40	6.67
7	DS-1168	75.67	236.0	10.20	8.28	54.13	6.05
8	DS-1111	67.00	224.7	8.07	8.56	59.80	6.55
9	DS-1146	71.67	215.3	9.07	9.01	63.80	6.49
10	DS-1187	70.67	199.7	7.87	8.37	61.60	6.37
11	UTML-529-10(8)	69.67	185.3	7.33	8.15	65.47	5.76
12	PDJP-1612/621513	57.33	180.6	6.60	5.37	45.73	4.67
13	PDJP-1614/621515	72.67	200.0	7.73	8.36	66.93	6.21
14	DSF-168	75.67	190.0	8.70	9.06	63.22	5.62
15	DSF-117	69.33	211.7	8.20	9.57	54.73	6.26
16	GAFS-12	68.67	212.0	7.33	8.47	68.13	4.51
17	GFS-4	66.00	205.8	7.93	5.79	53.27	4.35
18	DSF-172	76.67	204.4	9.34	14.00	81.64	8.75
19	SH-1813	69.33	199.0	8.13	9.76	58.40	6.07

20	S-652	70.67	196.0	8.40	10.70	67.20	6.46
21	SSG-59-3	69.00	233.0	8.20	9.61	62.80	5.00
22	SH-1488	73.33	200.7	8.07	10.00	67.47	6.85
	Mean	70.94	203.81	8.32	9.28	61.71	6.14
	Maximum	78.67	243	10.2	14	81.64	8.75
	Minimum	57.33	157.33	6.6	5.37	45.73	4.35
	S.Em. (±)	2.64	14.67	0.84	0.94	5.54	0.57
	CD at 5%	7.53	41.86	2.40	2.68	15.81	1.63
	CV (%)	4.57	8.82	12.35	12.53	10.99	11.4

Supplementary Table 1: Continued

S No.	*Genotypes*	*Leaf: stem ratio*	*Dry fodder yield per plant (g)*	*Brix content (%)*	*Hydrocyanic acid content (ppm)*	*Crude protein content (%)*	*Green fodder yield per plant (g)*
1	SRF-316	0.35	109.86	11.07	31.4	8.93	255.84
2	MALWAN	0.24	103.90	11.56	24.7	8.40	219.59
3	GFS-5	0.27	77.96	9.87	26.0	10.68	214.01
4	CSV-21	0.23	62.61	12.23	18.4	8.58	155.85
5	SRF-286	0.37	75.25	9.02	19.9	9.45	196.83
6	DS-1053	0.27	90.96	10.35	24.9	9.45	208.19
7	DS-1168	0.33	82.08	9.59	11.8	10.68	176.87
8	DS-1111	0.24	66.14	9.71	14.1	9.10	146.52
9	DS-1146	0.46	85.69	10.67	14.5	8.75	169.29
10	DS-1187	0.29	72.53	10.26	21.9	8.05	147.62
11	UTML-529-10(8)	0.25	61.72	8.43	26.7	9.10	136.37
12	PDJP-1612/621513	0.25	53.21	7.56	22.2	7.53	104.26
13	PDJP-1614/621515	0.33	42.51	7.69	13.4	10.15	92.39
14	DSF-168	0.23	57.06	10.23	15.8	9.10	146.75
15	DSF-117	0.21	99.83	9.51	33.8	10.85	208.26
16	GAFS-12	0.25	62.85	8.98	28.7	9.45	161.86
17	GFS-4	0.18	75.58	9.84	21.0	9.45	162.46
18	DSF-172	0.23	135.49	9.46	37.1	10.33	351.27
19	SH-1813	0.22	95.25	11.10	35.0	7.53	220.97
20	S-652	0.23	101.75	11.24	21.2	11.03	221.91
21	SSG-59-3	0.24	86.92	8.53	17.7	6.65	199.89
22	SH-1488	0.26	97.14	11.01	19.9	9.28	243.75
	Mean	0.27	81.65	9.91	22.73	9.22	188.23
	Maximum	0.46	135.49	12.23	37.06	11.03	351.27
	Minimum	0.18	42.51	7.56	11.82	6.65	92.39
	S.Em. (±)	0.029	6.68	0.58	1.14	0.37	16.19
	CD at 5%	0.08	19.06	1.66	3.25	1.06	46.20
	CV (%)	13.35	10.02	7.17	6.12	4.98	10.54

Supplementary Table 2: List of SSR primers used in the study

S No.	*Name*	*Seq. (5′-3′)*	*Tm value*	*% GC content*	*Bases*
1	msbCIR238F	AGAAGAAAAGGGGTAAGAGC	55.25	45.00	20
2	msbCIR238R	CGAGAAACAATTACATGAACC	53.97	38.10	21
3	msbCIR240F	GTTCTTGGCCCTACTGAAT	54.51	47.37	19
4	msbCIR240R	TCACCTGTAACCCTGTCTTC	57.30	50.00	20
5	msbCIR276F	CCCCAATCTAACTATTTGGT	53.20	40.00	20
6	msbCIR276R	GAGGCTGAGATGCTCTGT	55.97	55.56	18
7	msbCIR300F	TTGAGAGCGGCGAGGTAA	55.97	55.56	18
8	msbCIR300R	AAAAGCCCAAGTCTCAGTGCTA	58.39	45.45	22
9	msbCIR329F	GCAGAACATCACTCAAAGAA	53.20	40.00	20
10	msbCIR329R	TACCTAAGGCAGGGATTG	53.69	50.00	18
11	Xcup14F	TACATCACAGCAGGGACAGG	59.35	55.00	20
12	Xcup14R	CTGGAAAGCCGAGCAGTATG	59.35	55.00	20
13	Xcup53F	GCAGGAGTATAGGCAGAGGC	61.40	60.00	20
14	Xcup53R	CGACATGACAAGCTCAAACG	57.30	50.00	20
15	Xtxp12F	AGATCTGGCGGCAACG	54.30	62.50	16
16	Xtxp12R	AGTCACCCATCGATCATC	53.69	50.00	18
17	Xtxp15F	CACAAACACTAGTGCCTTATC	55.92	42.86	21
18	Xtxp15R	CATAGACACCTAGGCCATC	56.67	52.63	19
19	Xtxp57F	GGAACTTTTGACGGGTAGTGC	59.82	52.38	21
20	Xtxp57R	CGATCGTGATGTCCCAATC	56.67	52.63	19
21	Xtxp67F	CCTGACGCTCGTGGCTACC	63.14	68.42	19
22	Xtxp67R	TCCACACAAGATTCAGGCTCC	59.82	52.38	21
23	Xtxp136F	GCGAATAGCATCTTACAACA	53.20	40.00	20
24	Xtxp136R	ACTGATCATTGGCAGGAC	53.69	50.00	18
25	Xtxp141F	TGTATGGCCTAGCTTATCT	52.35	42.11	19
26	Xtxp141R	CAACAAGCCAACCTAAA	47.95	41.18	17
27	Xtxp265F	GTCTACAGGCGTGCAAATAAAA	56.53	40.91	22
28	Xtxp265R	TTACCATGCTACCCCTAAAAGTGG	61.01	45.83	24
29	Xtxp289F	AAGTGGGGTGAAGAGATA	51.41	44.44	18
30	Xtxp289R	CTGCCTTTCCGACTC	50.57	60.00	15
31	Xtxp317F	CCTCCTTTTCCTCCTCCTCCC	63.73	61.90	21
32	Xtxp317R	TCAGAATCCTAGCCACCGTTG	59.82	52.38	21
33	Xtxp358F	CAAGGACAAGATTCATTTTAAGGG	57.59	37.50	24
34	Xtxp358R	TCACACCTCACAAAATAAAAGTGC	57.59	37.50	24