



Genetic diversity for morphophysiological character studies in rainfed sorghum (*Sorghum bicolor* [L.] Moench) parental lines

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ARTICLE INFO	ABSTRACT
Received : 17 August 2022 Revised : 22 January 2023 Accepted : 06 March 2023 Available online: 25 June 2023 Key Words: Diversity Genetic Advance Heritability Parental lines Variability Coefficient of variance	The experimental trial was conducted at Sorghum Research Centre, Marathwada Agricultural University, Parbhani during <i>kharif</i> 2021 in randomized design with 3 replications. Eighteen parental (B & R) lines were evaluated to study genetic diversities and variability for fifteen yield contributing characters. Among all the eighteen genotypes of parental lines significant differences were observed for yield and its yield contributing traits except total chlorophyll content. Coefficient of variance at phenotypic level showed higher values than at genotypic level among all traits. Coefficient of variance for the traits like chlorophyll stability index (G=23.035, P=23.714), leaf area (G=21.064, P=21.673), 1000 grain weight (G=28.845, P=29.929), fodder yield (G=21.858, P=22.458), grain number per panicle (G=29.564, P=30.158), leaf dry weight (G=29.044, P=29.903) was observed high at both level. Traits like plant height (H=90, GAM=21.041), grain yield (H=86, GAM=29.074), leaf dry weight (H=94, GAM=23.664), leaf area (H=94, GAM=42.17), chlorophyll stability index (H=94, GAM=46.093), grain number per panicle (H=96, GAM=59.703), fodder yield (H=94, GAM=43.823), 1000 grain weight (H=92, GAM=52.270) and harvest index (H=84, GAM=32.321) were recorded high heritability with high genetic advancement. Genotype AKR 504, NR 39-15, KR 218, KR 219, PMS 100B, AKMS 90 B and INDORE 12 showed the better performance for all characters thus, should be used for development of hybrids and inbreds in breeding programmes by DUS (Distinctness, Uniformity and Stability) testing.

Introduction

Sorghum bicolor [L.] Moench is an angiospermic crop plant that belongs to poaceae family. It is often cross pollinated crop. In various regions it has several names like Jowar, Indian millet, great millet etc. It is rich in protein, carbohydrates, fat, vitamins, calcium and little quantity of iron. It is a C₄ plant having high water use efficiency and photosynthetic efficiency. It can withstand in extreme drought condition for long period and have ability to tolerate drought stress, therefore sorghum is also called as 'camel of desert'. After wheat and rice, Sorghum is the fifth most extensive and beneficial crop in the world, with its nutritional quality and potential use in agriculture. Most of the

poor and rural people lived in village prefer jowar bhakri than wheat chapati. According to Department of Agriculture, Cooperation & Farmers Welfare (2020-21), All India estimates for sorghum in terms of area, production and yield during 2020-21 was 1.66 million hectares, 1.75 million tones and 1053 kg/hectare. And according to U.S. Department of Agriculture the area and production of sorghum crop in world for year 2020-21 was 40 million hectare and 60 million tones respectively. Selection of the superior parents to be used in hybridization is one of the main decisions faced by the plant physiologists and plant breeders that will accelerate the exploitation of maximum variability in crops

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and production of superior new genotypes. (Bertanet *et al.*, 2007). For this reason, utilization of genetic variability and selection of genotypes is very necessary. Knowledge of interrelationship among all the characters which influence the yield of crop to greater extent enables the researcher to plan field trial accordingly. Efforts were made to assess the genetic variability in parental (B and R) lines of *kharif* sorghum for utilizing cytoplasmic genetic male sterility to develop high yielding hybrids. (Godbharle *et al.*, 2010)

Material and Methods

Analysis of variance was performed according to standard methods prescribed by panse and sukhatme (1985) for fifteen yield contributing characters of 18 parental genotypes. Experiment was laid out in randomized block design with three replications at Sorghum Research Station, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani during *kharif* 2021. Total 18 (7B and 11R) parental lines were used which were collected from IIMR Hyderabad, ICRISAT Hyderabad, Indore, Parbhani and Akola listed in table 1. Some of the parameters were analysed in the field at 50 % flowering stage of collected sample. Then those samples were taken to laboratory for analysis of relative water content by Barrs and Weatherley (1962) and chlorophyll stability index by Arnon (1949). Most of the samples were analysed at 50 % flowering and flowering stage those are as; Days to 50% flowering, days to maturity, plant height at physiological maturity, leaf dry weight at flowering, stem dry weight at flowering, leaf area at flowering, SPAD value at 50 % flowering (SPAD meter), total chlorophyll content, 1000 grain weight, grain number per panicle, grain yield, fodder yield and harvest index.

Table 1: List of parental (B and R) lines used for study

B-line	Source	R-line	Source
AKMS 30B	Akola	AKR 456	Akola
AKMS 33B	Akola	AKR 504	Akola
AKMS 70B	Akola	AKR 524	Akola
AKMS 90B	Akola	KR 192-2	Parbhani
PMS 100B	Parbhani	KR 218	Parbhani
PMS 237B	Parbhani	KR 219	Parbhani
PMS 28B	parbhani	C 85	Hyderabad
		NR 10-15	Hyderabad
		NR 12-15	Hyderabad
		NR 39-15	Hyderabad
		INDORE 12	INDORE

Results and Discussion

Analysis of variance for fifteen characters in parental lines (B and R) of *kharif* sorghum and mean performances of fifteen characters studied in *kharif* sorghum parental (B and R) lines are presented in table 2 and 3. Traits like days to 50% flowering, days to maturity, plant height, leaf dry weight, stem dry weight, leaf area, relative water content, SPAD value, chlorophyll stability index, 1000 grain weight, grain no per panicle, grain yield, fodder yield and harvest index showed significant differences among all genotypes while total chlorophyll content (TCC) showed non significant difference. Differences among all the genotypes in respect of all yield contributing characters (traits) studied showed significant values at 5% level of significance indicating the presence of variability and diversities of these characters which provides ample scope for selection of superior and desirable genotypes for physiologists and breeders for further genetic improvement and for selection of parents for hybrid development. WASP-Web Agri Stat Package version 2.0 statistical software were used for analysis of variance and mean performance. Grain yield is important character to find out the best physiological trait by its productivity and to choose higher grain yield trait in selecting for crossing. The best performance for grain yield (q/ha) was observed in genotypes PMS 100B (29.88) q/ha, INDORE 12 (23.57) q/ha, KR 218 (23.06) q/ha, KR 192-2 (22.73) q/ha, KR 219 (22.73) q/ha, and AKR 504 (22.39) q/ha. While C-85 (17.26) q/ha showed lowest grain yield among all genotypes. Tirkey *et al.* (2021) has earlier reported similar result higher grain yield in PMS 100 B genotype while similar least yield was observed in PMS 237B reported by Gangaiah *et al.* (2020). Days to fifty percent flowering is recorded until 50 % of plant had atleast one open flower in plot. Less required the days to reach fifty percent flowering results less days to reach maturity within time. Genotypes AKR 524 (76.6), AKMS 70B (79.66) and PMS 100B (80.00) required less days to reach for fifty percent flowering while genotype INDORE 12 (83.00) required higher days to 50 % flowering. Rao *et al.* (2019) has earlier reported similar result required less days to reach 50 % flowering in genotypes AKMS 70B and AKR 524.

Table 2 : Analysis of variance for fifteen characters in parental lines (B and R) of *kharif* sorghum

S N	Sources of variation	Degrees of freedom	Dfif	Days to maturity	Plant height at physiological maturity (cm)	Leaf dry wt at flowering (g/m ²)	Stem dry wt at flowering (g/m ²)	Leaf area at flowering (cm ²)	RWC (%) at 50% flowering
1	Replication	2	4.57	1.500	29.685	10.167	7330.907	1352.519	16.074
2	Treatment	17	6.63*	12.431*	853.11*	428.049*	48893.028*	85931.983*	40.780*
3	Error	34	1.96	1.912	29.038	8.402	12007.770	1650.166	16.917

Contd..

S N	Sources of variation	Degrees of freedom	SPAD value at 50% flowering	TCC (mg/ml)	CSI (%)	1000 grain wt (g)	Grain no/panicle	Grain yield (q/ha)	Fodder yield (q/ha)	HI (%)
1	Replication	2	12.519	0.000	5.352	0.310	2602.463	2.743	12.878	4.019
2	Treatment	17	22.623*	0.002 ^{NS}	851.198*	24.229*	511247.849*	30.358*	1124.83*	33.02*
3	Error	34	6.362	0.001	16.646	0.603	6816.110	1.531	20.508	1.920

* Significant at 5% level, ^{NS}non significant

A similar or different value in days to fifty percent flowering was observed by Rao *et al.* (2016, 2017, 2018, 2019), Gangaiah *et al.* (2020) also observed significant differences among genotypes. They observed B line AKMS 33B and R line AKR 456 and NR 12 15 to be the quickest flowering. Days to maturity tells the number of days required from transplanting to reach its physiological maturity. Less no of days to fifty percent flowering results in less days to reach maturity. Genotypes AKR 524 (117.33), AKMS 70B (120.33), required less days to reach physiological maturity while genotypes NR 10-15 (126.33), INDORE 12 (125.33), required higher days to reach physiological maturity. Rao *et al.* (2019) has earlier reported similar result required less days to reach physiological maturity in B line containing genotype AKMS 70B and R line containing AKR 524. A different trend in days to fifty percent flowering was reported by Rao *et al.* (2016, 2017, 2018). Gangaiah *et al.* (2020) reported significant differences and observed B line AKMS 33B and R line AKR 456 and NR 12 15 to be the quickest maturity as well.

Plant height is very important character as it is correlated with the lifespan, mass of seeds, days required for maturity and have the ability to compete with other plants in population having higher length for light, CO₂. Plant height at physiological maturity was observed superior highest in genotypes AKR 504 (190cm) and PMS 100B (186.33cm), higher in KR 192-2 (176.67cm) and KR 219 (169.33cm), moderate in AKMS 30B (158.67cm) and AKMS 70B (157.67cm) and lowest height was observed in genotype AKMS 90B (134.67cm) and KR 218 (138cm). Similar result

superior plant height in R line containing genotype AKR 504, KR 192-2 and B line containing PMS 100B was earlier reported by Rao *et al.* (2019) and Gangaiah *et al.* (2020). A similar or different value was reported for plant height in inbred lines by Chavhan *et al.* (2022). Leaf dry weight indicates the biomass of leaf and ability to photosynthesize for storing food. The best performance for leaf dry weight was observed in genotype AKR 504 (62.66) g/m², followed by AKMS 70B (56.33) g/m², NR 39-15 (53.66) g/m², AKMS 90B (52.00) g/m² and INDORE 12 (51.66) g/m², while the lowest dry weight observed in AKR 524 (19.67) g/m². Rao *et al.* (2019) has earlier reported similar result higher leaf dry weight for R line containing genotype NR 39-15. Results for leaf dry weight is in accordance with earlier worker Chavhan *et al.* (2022), Gangaiah *et al.* (2020).

Stem dry weight is dependent on the weight of dry leaf biomass. Increase in photosynthesis in leaf increases in biomass of leaf and transport of organic solutes from leaf to stem results in increase in dry matter of stem. The best performance for stem dry weight was observed in genotype KR 192-2 (1108.667) g/m², followed by AKR 504 (982.67) g/m², NR 10-15 (927.0) g/m² and lowest in genotype AKMS 90B (635.67) g/m². These results are in accordance with the earlier findings by Gangaiah *et al.* (2020). Leaf area of plant is important to determine environmental impacts on plant biomass production and describes the measurement of canopy structure of plant. The highest leaf area was observed in genotypes AKR 504 (1115.33) cm² and NR 39-15 (1058.0) cm², while lowest leaf area observed in KR 218

Table 3: The Mean performances of fifteen characters studied in *kharif* sorghum parental (B and R) lines

SN	Genotypes	Dff	Days to maturity	Plant height at physiological maturity (cm)	Leaf dry wt at flowering (g/m ²)	Stem dry wt at flowering (g/m ²)	Leaf area at flowering (cm ²)	RWC (%) at 50% flowering	SPAD value at 50% flowering	TCC (mg/ml)	CSI (%)	1000 grain wt (g)	Grain no/panicle	Grain yield (q/ha)	Fodder yield (q/ha)	HI (%)
1	AKMS 30B	81.67	122.00	158.67	56.33	710.67	1001.33	79.00	55.00	0.11	96.33	9.59	1191.33	17.85	80.81	18.00
2	AKMS 33B	80.67	122.00	149.00	46.00	894.67	589.33	84.67	48.00	0.14	68.00	11.45	1195.00	18.01	67.34	21.00
3	AKMS 70B	79.67	120.33	152.67	44.67	679.00	725.33	88.67	56.33	0.11	87.67	6.04	2191.33	20.20	67.34	22.67
4	AKMS 90B	81.67	125.00	134.67	52.00	635.67	820.33	87.00	58.33	0.14	93.67	6.49	764.67	17.93	67.34	20.33
5	PMS 100B	80.00	121.67	186.33	35.67	801.00	615.00	85.33	57.00	0.09	71.67	8.27	1184.67	29.88	106.90	21.67
6	PMS 237B	80.67	121.67	147.67	34.67	646.33	619.00	88.33	56.00	0.09	41.00	5.67	564.67	17.85	67.34	20.33
7	PMS 28B	82.67	124.00	142.33	26.67	674.67	731.67	93.33	53.67	0.11	47.33	8.34	1275.67	19.61	69.87	21.67
8	AKR 456	82.33	124.33	160.33	29.33	848.00	785.67	83.67	52.67	0.15	84.33	8.80	1426.00	17.93	65.66	21.00
9	AKR 504	81.33	123.33	190.00	62.67	982.67	1115.33	83.67	58.33	0.13	81.33	8.17	1512.33	22.39	63.97	25.33
10	AKR 524	76.67	117.33	144.67	19.67	726.33	631.33	86.67	57.00	0.14	55.33	9.21	1329.33	19.70	88.38	17.67
11	KR 192-2	81.33	123.33	176.67	24.33	1108.6	678.00	87.67	57.67	0.13	71.33	8.67	1621.67	22.73	111.95	16.33
12	KR 218	82.33	124.33	138.00	33.67	844.00	583.67	93.33	58.67	0.10	81.33	10.20	1964.67	23.06	106.90	17.33
13	KR 219	80.00	122.33	169.33	40.67	894.67	922.67	81.00	58.00	0.14	90.00	10.07	1263.00	22.73	105.22	17.33
14	C 85	82.33	123.00	134.33	44.00	716.67	831.33	83.67	56.67	0.07	85.00	16.70	1374.00	17.26	110.27	13.00
15	NR 10-15	81.33	126.33	160.33	44.67	927.00	840.00	89.33	53.33	0.09	65.00	10.27	1275.00	19.53	115.32	14.00
16	NR 12-15	81.67	123.00	141.67	32.67	816.00	754.67	84.67	58.33	0.14	78.33	9.45	1333.33	18.27	92.59	16.00
17	NR 39-15	82.33	122.67	141.00	53.67	737.33	1058.00	87.67	58.00	0.08	50.33	14.68	1326.33	18.69	106.90	14.67
18	INDORE 12	83.00	125.33	150.00	51.67	873.33	1020.67	87.00	54.33	0.11	55.33	13.07	2170.67	23.57	85.86	21.00
	General mean	81.20	122.89	154.31	40.72	806.48	795.74	86.37	55.96	0.11	72.41	9.73	1386.87	20.40	87.78	18.85
	SE (m)	0.810	0.798	3.111	1.674	63.266	23.453	2.375	1.456	0.019	2.356	0.448	47.666	0.714	2.614	0.800
	CD at 5%	2.326	2.294	8.941	4.809	181.80	67.397	6.824	4.185	N/A	6.769	1.288	136.977	2.062	7.546	2.299
	C.V	1.727	1.125	3.492	7.118	13.587	5.105	4.762	4.507	28.95	5.635	7.981	5.953	6.066	5.159	5.185

Table 4: Mean and genetic variability parameters for fifteen characters in parental (B and R) lines of *kharif* sorghum

Characters	Range		Mean	GCV	PCV	Heritability %	Genetic advance	Genetic Advance value % means
	Min	Max						
Dfif	76.67	83	81.20	1.536	2.311	44.170	1.708	2.103
Days to maturity	117.33	126.33	122.89	1.524	1.894	64.717	3.103	2.525
Plant height at physiological maturity (cm)	134.33	190	154.32	10.740	11.294	90.440	32.469	21.041
Leaf dry wt at flowering (g/m ²)	19.67	62.67	40.72	29.044	29.903	94.334	23.664	58.110
Stem dry wt at flowering (g/m ²)	635.67	1108.67	806.48	13.749	19.330	50.591	162.469	20.145
Leaf area at flowering (cm ²)	583.67	1115.33	795.74	21.064	21.673	94.452	335.567	42.170
RWC (%) at 50% flowering	79	93.33	86.37	3.265	5.774	31.981	3.286	3.804
SPAD value at 50% flowering	48	58.67	55.96	4.160	6.134	46.006	3.253	5.813
TCC (mg/ml)	0.07	0.15	0.12	11.480	31.160	13.573	0.010	8.713
CSI (%)	41	93.67	72.41	23.035	23.714	94.354	33.374	46.093
1000 grain wt (g)	5.67	16.7	9.73	28.845	29.929	92.889	5.572	52.270
Grain no/panicle	564.67	2191.33	1386.87	29.564	30.158	96.101	828.108	59.703
Grain yield (q/ha)	17.26	29.88	20.40	15.200	16.364	86.278	5.931	29.074
Fodder yield (q/ha)	63.97	115.32	87.78	21.858	22.458	94.725	38.467	43.823
HI (%)	13	25.33	18.85	17.081	18.596	84.373	6.093	32.321

(583.661) cm², AKMS 33B (589.33) cm², followed by PMS 100B (615.0) cm², PMS 237B (619.0) cm². These results are in accordance with the earlier findings by Chavhan *et al.* (2022), Gangaiah *et al.* (2020). Rao *et al.* (2019) has earlier reported similar result highest leaf area in R line containing genotypes NR 39-15 and AKR 504.

Relative water content means that how much amount of water leaf can hold, also is an indicator of plant water status in crops. The best genotypes for relative water content (RWC) was observed in PMS 28B and KR 218 (93.33%), followed by NR 10-15 (89.33%), AKMS 70B (88.67%) and lowest values observed in AKMS 30B (79.0%) respectively. Chavhan *et al.* (2022) reported high water content in inbred line PVK 1025 and lowest in CSV 39.

SPAD is accurate and non destructive method of measurement of leaf chlorophyll concentration widely used in estimation of status of chlorophyll in crop plants. Significant differences were observed among the genotypes for SPAD at 50 % flowering. Among KR 218 (58.67), AKMS 90B (58.33), NR 12-15 (58.33), AKR 504 (58.33) observed superior higher values while least in genotype AKMS 33B (48.00). These results are in accordance with the earlier findings by Gangaiah *et al.* (2020), Rao *et al.* (2019), Chavhan *et al.* (2022).

Differences in total chlorophyll content (TCC) was observed non significant for all the genotypes.

Chlorophyll stability index also is an indicator of plant that indicates the ability of tolerance/resistance capacity of plants when an external abiotic stress like temperature, drought is influenced on it. Chlorophyll stability index (CSI) was observed significant differences among all the genotypes. Highest stability was observed in genotype AKMS 30B (96.33%), AKMS 90B (93.67%), KR 219 (90.00%) followed moderately by AKMS 70B (87.67%), C 85 (85.00 %), AKR 504 (81.33%) and lowest stability observed in PMS 237B (41.00%) and PMS 28B (47.33%). Similar results significant differences among all the genotypes in inbred lines were earlier reported by Chavhan *et al.* (2022). They observed high stability in CSV 34, PDKV Kalyani and lowest in CSV 31.

Test weight is the weight of 1000 grain seeds and is used in grain quality indicator. The test weight i.e; 1000 grain weight was observed higher in C 85

(16.70 g), NR 39-15 (14.68g), INDORE 12 (13.07g) and lowest weight in AKMS 90B (6.49g), AKMS 70B (6.04g) and PMS 237B (5.67g). A similar results higher 1000 grain weight in C 85 and least in PMS 237B was reported by Gangaiah *et al.* (2020). Chavhan *et al.* (2022) reported higher 1000 grain weight in inbred line PDKV Kalyani, AKSV 346 and lowest weight in SPV 2510, AKSV 318.

Fodder yield consists of weight of dry matter biomass and depend on various factors. The best performance for fodder yield was found in genotypes NR 10-15 (115.32q/ha), KR 192-2 (111.95q/ha), C 85 (110.27q/ha) and poor performance was observed in PMS 237B, AKMS 90B, AKMS 70B (67.34q/ha), AKR 456 (65.66q/ha) and AKR 504 (63.97q/ha). The best performance for fodder yield in genotype PVK 1025, SPV 2510 and poor in AKSV 318 was earlier reported by Chavhan *et al.* (2022). Harvest index is the ratio of grain yield to its biological yield and used to describe dry matter partitioning in crop plants. Genotypes AKR 504 (25.33%), AKMS 70B (22.67%) showed highest harvest index and lowest in C 85 (13.00%) followed by NR 12-15 (16.00%), NR 39-15 (14.67%), NR 10-15 (14.00%) and KR 192-2 (16.33%). Results are in accordance with Chavhan *et al.* (2022) reported highest index in inbred lines SPV 2504 and lowest in PDKV Kalyani.

Genotypic and phenotypic coefficient variance matrix.

The results of estimated genetic parameters are represented in table no 4.

Genotypic and phenotypic coefficient variance matrix indicates whether the traits are influenced by environmental factors or genetic factor which leads to select appropriate trait in order for breeding programme for crop improvement. Coefficient variance matrix for both level (phenotypic and genotypic) were classified into three level <10 as low, 11-21 as moderate and > 21 high in accordance with Sivasubramanian and Menon (1973). Coefficient variance matrix at phenotypic level showed higher values than at genotypic level among all traits. Similar results high phenotypic variance than genotypic variance was earlier reported by Godbharle *et al.* (2010) and Chavhan *et al.* (2022). Traits like grain no per panicle, leaf dry

weight, 1000 grain weight, fodder yield, chlorophyll stability index (CSI), leaf area were recorded high coefficient variance matrix at both genotypic and phenotypic level. Similar results higher values of GCV and PCV for grain no per panicle, leaf dry weight, 1000 grain weight were earlier reported by Chavhan *et al.* (2022). Moderate coefficient variance matrix at both genotypic and phenotypic were observed for plant height at physiological maturity, dry weight of stem and grain yield. Similar results observed moderate values of GCV and PCV for plant height by Chavhan *et al.* (2022) in his earlier findings. While low coefficient variance matrix at both genotypic and phenotypic were recorded for traits like days to fifty percent flowering, days to maturity, relative water content (RWC) and SPAD value. Similar results observed low values of GCV and PCV for days to fifty percent flowering, days to maturity, relative water content (RWC) and SPAD value by Chavhan *et al.* (2022) in his earlier findings. Higher values of phenotypic matrix indicate that the traits are influence by several environmental factors, hence traits showing higher genotypic values should be select and use in crop improvement programme.

Heritability and genetic advance

Heritability is transmission of characters from its parents to its offsprings. Heritability estimation helps in selection of best genotypes from diverse genetic group. High heritability indicates that traits are low influenced by environmental factor. The heritability percentage to determine its level of heredity classified as low (0-31), medium (31-61) and high (61 and above) in accordance with Johnson *et al* (1955). Grain yield, plant height at maturity, grain number per panicle, leaf dry weight at flowering, chlorophyll stability index, 1000 grain weight, fodder yield, leaf area and harvest index recorded high heritability and high genetic advance.

Similar results high heritability with high genetic advance for grain yield and fodder yield were earlier reported by Tirkey *et al.* (2021). These traits are beneficent and important to researcher for better selection in crop improvement programme. High percentage of heritability with low genetic advance were observed in days to reach physiological maturity. Similar results high heritability with low genetic advance for days to reach physiological maturity was earlier reported by Chavhan *et al.* (2022). Traits which showing high heritability percentage are likely to inherit maximum genes or characters from its parent to offsprings. So, traits which showing high heritability percentage should used for hybrid seed development.

Conclusion

From the above suggestion it is concluded that the variability and diversities in parental lines helps in selecting the appropriate genotype need for production of hybrids or new cultivars. Therefore the study of physiological parameters for genetic diversity in parental sorghum lines is very essential in order to choose better quantitative and qualitative trait (drought resistant, heat resistant, stress tolerance, water logged resistant) for exploitation of inbred and hybrids in hybrid development programme. As sorghum is the fifth most important crop in the world for its various uses for consumption, feed and fodder, ethanol production etc, the development of hybrid through selection of desirable trait for increased productivity with qualitative features enhance in crop improvement globally all over the world.

Conflict of interest

The authors declare that they have no conflict of interest.

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