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Research Article

Genetic diversity studies among maintainers and restorers on milo and maldandi cytoplasm from minicore collection of sorghum using D^2 statistics

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Abstract
Sorghum is the important cereal crop around the world and hence understanding and utilizing the genetic variation in sorghum accessions are essential for improving the crop. The D^2 statistics was applied to assess the diversity among 41 B-lines and 43 R-lines of sorghum identified from minicore collection. The analysis of variance revealed significant differences among the genotypes for all the characters under study. The genotypes were grouped into 13 clusters, where cluster III comprised maximum of 14 genotypes followed by cluster IV with 7 genotypes each among R-lines whereas B-lines grouped into 6 clusters with cluster I with 22 genotypes followed by cluster II with 9 genotypes. Inter-cluster distance was maximum between the clusters IX and X with 648.07 among R-lines and in B-lines maximum intercluster distance was between I and VI with 1136.00 which indicated that genotypes included in these clusters may give heterotic response and thus better segregants. Regarding genetic divergence of the genotypes, panicle weight (32.07 %), SPAD readings at maturity stage (29.76) and 100 seed weight (20.49 %) contributed major share among R-lines and incase of B-lines panicle weight (49.49 %), primaries per panicle (25.13 %) and 100 seed weight (10.77 %) contributed high towards genetics. These traits can be utilized for selection of individual B-lines and individual R-lines for future crop improvement programme.

Key words
Sorghum, Intercluster, Minicore, Genetic divergence and D^2 statistics

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) has been cultivated in India for centuries, owing to its better adaptability, assured grain and fodder yields even in harsh climatic conditions. The objectives for sorghum breeding had been to enhance yield and quality of grain and stover for food and livestock use, biomass and stem sugar content for biofuel purposes (Reddy *et al*., 2008). Due to excellent grain and fodder quality, post-rainy sorghums are used as food and fodder for livestock. Although several hybrids have been developed and released for *rabi* season cultivation, the area covered under hybrids is almost negligible. This is because of lack of appropriate hybrids with acceptable grain quality adapted to *rabi* season (Prabhakar *et al*., 2014). One of the constraints in the development of hybrids in *rabi* sorghum was low heterosis due to narrow genetic base between the hybrid parental lines.

Grouping or classification of genotypes based on suitable quantitative traits is quite imperative to understand the usable diversity existing among them. Selection of suitable parental (maintainer and restorer) lines to develop heterotic combinations can be facilitated by determining genetic divergence among them. In the present study, Mahalanobis's D^2 statistic technique has been applied to assess the diversity among 41 B-lines and 43 R-lines identified on milo and maldandi cytoplasm from minicore collection of sorghum.

Materials and Methods

The present field experiment on sorghum (*Sorghum bicolor* L. Moench) was conducted at botanical garden, Dept of genetics and plant breeding, UAS, Dharwad in *rabi* 2015-16. All the recommended agronomic practices were followed to raise a good crop. The experiment was laid out in randomized block design with two replications and row of 4 m length and spacing of 45 x 15cm. The observations were recorded on five randomly selected plants for characters chlorophyll at flag leaf stage, chlorophyll at maturity, days to 50 % flowering, days to maturity, plant height, panicle length, panicle width, 100 seed weight, panicle weight, primaries per panicle and grain yield per plant.

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The data was subjected to statistical analysis. Wilk's criteria was used to test the significance of pooled differences in mean values for all the eleven characters. Genetic diversity was studied using Mahalanobis's (1936) D^2 statistic and clustering of genotypes was done according to Tocher's method.

Results

Analysis of variance revealed the significant differences among genotypes for all characters under study. Based on D^2 statistics and Tocher's method 43 R-lines were grouped into 13 clusters with a variable number of entries revealing the presence of considerable amount of genetic diversity in the material (Table 3 & 5.). Intracluster distance was maximum (81.82) in cluster IV which constituted seven exotic genotypes, where as cluster II comprising two genotypes showed lowest divergence among its constituents (0.49). Clusters VI, VII, VIII, X, XI, XII and XIII had no intracluster distance as each of them had only one genotype. Cluster III was the largest of all consisting of 14 genotypes (one from India and others are of exotic). This cluster was nearest to VI and highly divergent to cluster IX.

The same way as like R-lines 41 B-lines were also grouped into 6 clusters based on D^2 values obtained (Table 4 & 6). The intracluster distance was maximum (118.29) in cluster IV which constituted five exotic genotypes, where as cluster I comprising 22 genotypes showed lowest divergence among its constituents (61.03), Clusters V and VI had no intracluster distance as each of them had only one genotype. The intercluster distances revealed that maximum divergence occurred between clusters I and VI (1136.00) which indicated that genotypes included in these clusters may give heterotic response followed with better segregants (Santosh *et al.*, 2015 and Dojjad *et al.,* 2016), while close proximity existed between clusters V and VI (87.98). Cluster I comprised of 22 genotypes, of which six were indigenous and others were from Ethiopia (1), Sudan (1), Zimbabwe (2), South Africa (2).

Among the R-lines characters contributing towards divergence were panicle weight (32.07 %) (Table 1) followed by SPAD readings at maturity (29.76 %), 100 seed weight (20.49 %) primaries per panicle (12.44 %) and grain yield per plant (2.80 %). Very little contribution was made by the remaining characters. Among the B-lines characters contributing towards divergence were panicle weight (49.49 %) (Table 2.) followed by primaries per panicle (25.13 %), 100 seed weight (10.77 %), SPAD readings at maturity (4.74 %), and grain yield per plant (3.59 %). Very little contribution was made by the remaining characters. Similar results were reported by Mahajan *et al*., (2010).

Cluster means (R-lines) in respect of 11 characters were presented in Table 7. The genotypes in cluster XIII was the earliest to flower and maturity followed by VI, while cluster XII flowered late. The genotypes in the remaining clusters were intermediate. The genotypes in cluster XII were tallest. Cluster V has low mean value for plant height. The cluster means for panicle length ranged from 13.08 (cluster XIII) to 28.75 (cluster VII). High panicle width was recorded in cluster II (11.43). High panicle weight was recorded by cluster IX (50.14). The highest mean for number of primaries per panicle were recorded by cluster II with only two genotypes (45.00). Next in order were XII and I. The highest mean value for 100 seed weight were recorded by cluster V (5.00) followed by cluster I (3.89). Regarding grain yield per plant, the genotypes in the cluster IX registered highest mean value while cluster II was the lowest yielder.

Cluster means (B-lines) in respect of 11 characters were presented in Table 8. The genotypes in cluster V was the earliest in flowering and maturity followed by II, while cluster I flowered late. The genotypes in the remaining clusters were intermediate. The genotypes in cluster VI were tallest. Cluster I has low mean value for plant height. The cluster means for panicle length ranged from 13.60 (cluster V) to 33.02 (cluster VI). High panicle width was recorded in cluster IV (9.85). High panicle weight was recorded by cluster III (52.13). The highest mean for number of primaries per panicle were recorded by cluster VI with only one genotype (43.50). Next in order were V and IV. The highest mean value for 100 seed weight were recorded by cluster V (3.92) followed by cluster II (3.58). Regarding grain yield per plant, the genotypes in the cluster VI registered highest mean value while cluster I was the lowest yielder.

Discussion

The data on intercluster distances were used to select genetically diverse and agronomically superior genotypes (Sameer kumar *et al*., 2010). The genotypes exceptionally good with one or more characters were seemed to be desirable. Inter-crossing of divergent groups would lead to greater opportunity for crossing over, which releases hidden

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potential variability by disrupting the undesirable linkages (Thoday, 1960). The progeny derived from such diverse crosses are expected to have wide spectrum of genetic variability, providing a greater scope for isolating transgressive segregants in advanced generations. Hence, these genotypes might be used in a multiple crossing programme to recover transgressive segregants (Shivani and Sreelakshmi, 2015).

Relation between genetic diversity and geographical diversity:

In case of diversity studies among R-lines and B-lines large number of genotypes allocated to single cluster even though many of the lines have diverse geographical origin. Even the indigenous lines does not allocated in to same cluster. Rao *et al.* (1989) reported in bajra that the genotypes of different geographical origin share same group because of their similar pedigree or similar allelic constitution. These results indicate geographical origin cannot be taken as criteria in estimating diverse nature. So crossing between lines of different geographical origin may/may not give desired results.

Among 13 clusters of R-line group 7 clusters were solitary with single genotype where as in maintainer group 2 solitary clusters out of 6 were observed. Sinha and Kumaravadivel (2016) reported in sorghum that diverse genotypes with other genotypes fall into separate solitary clusters. Crossing between genotypes of such diverse solitary clusters could give desirable transgressive segregants.

Practical utility of divergent pairs in different categories:

The divergent pairs in maintainer (B-lines) group can be used for creating variability and isolating better 'B' lines on diverse sources of male sterility (Table 9.). The B x B combinations with high D^2 values were IS 4360 X IS 2413 (D^2 value = 1156.87) and IS 23521 X IS 13893 (D^2 value = 1023.65). In the restorer group (R-lines), maximum diversity was observed in combination IS 28313 x IS 31651 (D^2 value = 1059.68) and IS 28313 X IS 25989 (D^2 value = 1043.65). Since these were restorers on two cytoplasms, segregating generation can be used for selecting genotypes which commonly restores fertility on both cytoplasms.

Development of broad based heterotic populations

Most of the available B and R-lines are genetically related. As a result, level of realized yield heterosis has been limited. Therefore, development of heterotic groups is expected to realize maximum heterosis. Heterosis relies more on exploitation of diverse yield influencing loci which act as good combiners in hybrid development (Reddy *et al*., 2003). So heterotic grouping helps in increase of proportion of favourable alleles and also genetic diverse nature of such population.

In the present study the traits like panicle weight, number of primaries per panicle which were contributing for genetic divergence in B-lines were in complementation with traits panicle weight and 100 seed weight contributing for genetic divergence in R-lines. Development of separate B-gene pool and R-gene pool by selecting lines based on traits contributing to divergence and intermating within B-lines and within R-lines will increase genetic diversity between the two B and R population. The crosses between such new developed B and R-lines will definitely enhance heterosis.

In the present study the pair wise combinations based on high D^2 value of maintainer group were IS 4360 X IS 2413 (1156.87), IS 23521 X IS 13893 (1023.65), IS 23521 X IS 13893 (998.25) and IS 3971 X IS 23521 (984.38). These combinations helps in isolation of B-lines in segregating generations with high genetic diversity.

In the present study the pair wise combinations based on high D^2 value of restorer group were IS 28313 X IS 31651 (1059.68), IS 28313 X IS 25989 (1043.65), IS 28313 X IS 4581 (1010.32), IS 28313 X IS 26025 (997.54) and IS 28313 X IS 29269 (986.27). These combinations helps in isolation of R-lines in segregating generations with high genetic diversity.

The above mentioned B and R-lines with high D^2 values with other genotypes shows highly diverse nature. Such lines has to be given due importance in creating B-gene pool and R-gene pool.

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Table 1. Percentage contribution of eleven characters towards genetic divergence in rabi sorghum of 43 genotypes (restorers on either of milo/maldandi cytoplasms)

S.No	Characters	Contribution %
1	Panicle weight (g)	32.07
2	SPAD readings at maturity stage	29.76
3	100 seed weight (g)	20.49
4	Primaries/panicle	12.44
5	Grain yield per plant (g)	2.80
6	Panicle width (cm)	1.22
7	SPAD readings at flag leaf stage	0.73
8	Plant height (cm)	0.37
9	Days to 50% flowering	0.20
10	Days to maturity	0.15
11	Panicle length (cm)	0.10

Table 2. Percentage contribution of different characters towards genetic divergence in 41 genotypes (maintainers on either of milo/maldandi cytoplasm) of minicore collection in rabi sorghum

S.No	Characters	Contribution %
1	Panicle weight (g)	49.49
2	Primaries per panicle	25.13
3	100 seed weight (g)	10.77
4	SPAD readings at maturity stage	4.74
5	Grain yield per plant (g)	3.59
6	Panicle width (cm)	2.56
7	SPAD readings at flag leaf stage	2.31
8	Days to maturity	0.77
9	Panicle length (cm)	0.26
10	Days to 50%flowering	0.13
11	Plant height (cm)	0.10

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Table 3. Average intra and intercluster distances for 43 genotypes (restorers on either of milo/maldandi cytoplasms) in minicore collection of sorghum

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	42.4	163.63	156.39	163.51	74.28	139.56	89.9	206.61	191.95	291.43	159.19	148.02	127.74
II		0.49	228.37	335.99	223.96	104.01	246.86	166.27	526.07	173.17	292.08	137.63	150.20
III			72.70	153.68	202.31	107.74	124	129.42	327.06	185.74	130.47	169.81	140.30
IV				81.82	202.18	285.10	214.05	360.2	189.38	448.34	294.82	293.69	330.38
V					16.57	234.08	183.82	281.57	308.59	382.9	252.13	295.57	149.81
VI						0.00	93.46	31.75	416.44	39.37	72.90	46.61	49.55
VII							0.00	115.66	195.05	213.01	39.56	87.36	105.42
VIII								0.00	515.38	40.57	68.39	113.81	62.62
IX									65.17	648.07	311.52	325.33	467.19
X										0.00	127.54	109.07	111.55
XI											0.00	98.15	95.71
XII												0.00	136.05
XIII													0.00

Underlined figures indicate intracluster distances

Table 4. Average intra and intercluster distances for 41 genotypes (maintainers on either of milo/maldandi cytoplasm) in sorghum of minicore collection

Clusters	I	II	III	IV	V	VI
I	61.03	158.28	346.61	178.4	841.68	1136
II		69.64	158.58	188.25	440.55	639.5
III			69.86	187.59	179.33	330.32
IV				118.29	536.25	760.74
V					0.00	87.98
VI						0.00

Underlined figures indicate intracluster distances

Table 5. Distribution of 43 genotypes (restorers on either of milo/maldandi cytoplasm) of sorghum in different clusters

Cluster No	Total No of genotypes in the cluster	Genotypes included in the clusters	Origin
I	4	IS 27887 (A_1ML), IS 26737 (A1), IS 29654 (A_1ML) and IS 23590 (A_1)	South Africa (2), China (1) and Ethiopia (1)
II	2	IS 24463 (A_1) and IS 22720 (A_1ML)	South Africa (1) and Somalia (1)
III	14	IS 4581 (A_1ML), IS 19975 (A_1), IS 31651 (A_1ML), IS 19389 (A_1ML), IS 26025 (A_1ML), IS 32439 (A_1ML), IS 11619 (A_1), IS 28313 (A_1ML), IS 29772 (A_1), IS 602 (A1), IS 26617 (A_1ML), IS 15744 (A_1), IS 19262 (A_1) and IS 23891 (A_1)	India (1), Senegal (1), Zaire (1), Bangladesh (1), Mali (1), Ethiopia (1), Yemen (1), Zimbabwe (1), U.S.A (1), Madagascar (1), Cameroon (1), Sudan (1) and Yemen (1)
IV	7	IS 28614 (A_1ML), IS 25989(A_1ML), IS 30451 (A_1), IS 29269 (A_1ML), IS 22720 (A_1), IS 24462 (A_1ML) and IS 21645 (A_1)	Yemen (1), Mali (1), China (1), Swaziland (1), Somalia (2) and Malawi (1)
V	2	IS 20743 (A_1) and IS 20679 (A_1)	U.S.A (2)
VI	1	IS 24348 (A_1)	India (1)
VII	1	IS 29627 (A_1)	South Africa (1)
VIII	1	IS 24175 (A_1ML)	Tanzania (1)
IX	4	IS 17941 (A_1ML), IS 995 (A_1ML), IS 19450 (A_1ML) and IS 15945 (A_1)	India (1), U.S.A (1), Botswana (1) and Cameroon (1)
X	1	IS 29269 (A_1ML)	Swaziland (1)
XI	1	IS29654 (A_1ML)	China (1)
XII	1	IS24492 (A_1)	South Africa (1)
XIII	1	IS 4581 (A_1ML)	India (1)

A_1 – refers to milo cytoplasm, ML – refers to maldandi cytoplasm

Table 6. Distribution of 41 genotypes (maintainers) of sorghum in different clusters

Cluster No	Total No of genotypes in the cluster	Genotypes included in the clusters	Origin
I	22	IS9745 (ML), IS16382 (ML), IS23586 (A_1ML), IS28389 (A_1ML), IS29335 (ML), IS20195 (A_1ML), IS18039 (A_1ML), IS24348 (ML), IS30572 (ML), IS2382 (ML), IS29187 (A_1ML), IS24218 (A_1ML), IS32787 (ML), IS23644 (A_1ML), IS9745 (ML), IS17980 (A_1ML), IS16151 (ML), IS12883 (A_1ML), IS29914 (ML), IS13893 (ML) and IS3971 (A_1ML)	Cameroon (3), Ethiopia (1), Gambia (1), India (6), Niger (1), Somalia (1), South Africa (2), Sudan (2), Swaziland (2), Tanzania (1), Yemen (1) and Zimbabwe (1)
II	9	IS14010 (A_1ML), IS14010 (A_1ML), IS29091 (ML), IS20632 (A_1ML), IS29606 (ML), IS19445 (A_1ML), IS29441 (A_1ML), IS6421(ML) and IS24139 (A_1)	Botswana (1), India (1), Lesotho (1), South Africa (3), Tanzania (1), U.S.A (1) and Yemen (1)
III	3	IS15931 (ML), IS23521 (A_1ML) and IS10969 (A_1ML)	Cameroon (1), Ethiopia (1) and U.S.A (1)
IV	5	IS8012 (ML) , IS31446 (ML), IS25910 (A_1), IS2389 (ML) and IS11026 (A_1)	Ethiopia (1), Japan (1), Mali (1), Uganda (1) and Yemen (1)
V	1	IS4360 (ML)	India (1)
VI	1	IS12804 (ML)	Turkey (1)

A_1 – refers to milo cytoplasm, ML – refers to maldandi cytoplasm

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Table 7. Cluster means for eleven characters of 43 genotypes (restorers on either of milo/maldandi cytoplasm) in rabi sorghum

Cluster No	No of genotypes	SPAD readings		Days to 50% flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	Primaries per panicle	100 seed weight (g)	Yield per plant (g)
		At flag leaf stage	At maturity stage									
I	4	47.31	36.57	70.00	129.75	181.13	13.20	6.21	28.65	32.66	3.89	22.17
II	2	54.72	30.00	66.50	143.50	174.00	20.13	11.43	15.30	45.00	3.47	6.41
III	14	48.92	36.23	71.00	132.29	177.75	24.38	6.90	27.99	24.85	2.53	18.53
IV	7	49.82	46.99	80.63	137.25	199.81	24.61	7.60	36.73	30.65	2.65	24.64
V	2	50.57	41.73	78.50	141.50	166.50	19.94	7.63	29.63	26.46	5.00	20.12
VI	1	46.14	25.00	65.50	118.50	192.50	20.95	6.05	27.45	31.74	2.89	20.39
VII	1	52.24	30.20	72.00	136.50	210.00	28.75	7.94	34.10	27.00	3.01	15.20
VIII	1	43.55	24.00	80.00	130.50	182.00	22.93	9.65	17.29	24.87	2.69	10.82
IX	4	47.99	41.85	80.25	136.13	202.88	20.14	7.27	50.14	32.75	2.83	41.69
X	1	37.90	18.29	81.00	137.50	187.50	23.70	4.65	21.43	28.80	2.77	18.82
XI	1	40.87	23.80	74.00	142.50	200.00	22.25	5.85	39.50	20.00	2.83	33.13
XII	1	51.47	21.35	90.00	152.50	233.00	25.45	5.05	28.29	38.10	2.67	20.93
XIII	1	53.75	27.15	64.50	120.00	192.50	13.08	4.90	21.92	23.74	3.76	15.99

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Table 8. Cluster means for eleven characters of 41 genotypes (maintainers on either of milo/maldandi cytoplasm) in rabi sorghum

Cluster No	No of genotypes	SPAD readings		Days to 50% flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	Primaries per panicle	100 seed weight (g)	Yield per plant (g)
		At flag leaf stage	At maturity stage									
I	22	51.55	37.45	74.71	134.50	190.88	22.46	6.41	20.65	28.74	3.10	22.23
II	9	50.73	34.29	73.83	131.39	209.61	23.04	7.21	41.65	29.23	3.58	31.33
III	3	45.39	45.59	75.50	135.83	210.50	26.38	8.32	52.13	35.18	3.38	43.03
IV	5	49.06	41.17	73.40	135.30	191.30	24.80	9.85	31.89	39.30	2.86	27.93
V	1	53.20	45.84	68.00	129.00	230.50	13.60	5.85	78.10	42.50	3.92	66.02
VI	1	52.64	38.65	71.50	143.50	239.50	33.02	7.90	92.60	43.50	1.78	66.66

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Table 9. Highly divergent pairs in different categories

Genotypic pairs	D^2 values
Maintainer group	
IS 4360 (M) X IS 2413(M)	1156.87
IS 23521 (M) X IS 13893(M)	1023.65
IS 23521 (M) X IS 13893(M)	998.25
IS 3971 (M) X IS 23521 (M)	984.38
Restorer group	
IS 28313 (A1, ML) X IS 31651 (A_1, ML)	1059.68
IS 28313 (A1, ML) X IS 25989 (A_1, ML)	1043.65
IS 28313 (A1, ML) X IS 4581 (A_1, ML)	1010.32
IS 28313 (A1, ML) X IS 26025 (A_1 ML)	997.54

A_1 refers to restorer on milo cytoplasm, ML refers to restorer on maldandi cytoplasm and M refers to maintainer on milo and maldandi**.**