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Evaluation of heterotic parental combinations based on early seed Vigor and SSR based molecular analysis in sorghum (*Sorghum bicolour* L.)

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Abstract
Seventeen SSR markers were used for the prediction of genetic relationship among parental lines and their respective F1 crosses based on heterosis analysis for various physiological seed quality traits in sorghum. A total of eleven parental line including five male sterile lines and six pollinator lines were used to make thirty crosses along with two checks and a detailed analysis was done based on comparison between SSR similarity matrix (SM) of parents and three types of heterosis (BP, MP and SP). Among lines, $11A_2$ gave more significant results with all pollinators in context with heterosis and similarity matrix. For seed vigor, significant and positive heterosis was exhibited 25 crosses ranged from 8.52 to 67.53 (BP) and 20 crosses ranged from 6.90 to 53.68 (MP). Only one cross i.e. ICSA 469 x SPV1616 (15.6) gave better results of heterosis over standard parent. Maximum heterobeltiosis was recorded in $11A_2$ x M35-1 for with parents were found to be very dissimilar genetically (SM 0.53). This study provides a good genetic base at seedling stage for the selection of parental lines for the development of potential heterotic combinations.

Keywords: heterosis, physiological quality, seed vigor, *Sorghum bicolour*, SSRs

Introduction
For a successful crop improvement programme, availability of adequate genetic variation is one of the most important requirements. In a particular crop population gene pool, an accurate assessment of this variation provides an adequate basis to construct efficient and effective crop breeding strategies for long-term selection of genetic gains. Assessment of the sufficient genetic diversity and range of genetic variation provides an objectively targeted utilisation of crop genetic resources for hybrid breeding programme by selection of more heterotic parental combinations. Due to presence of sufficient information about more diverse gene pool helped to design the evolutionary relationships which resulted in advanced sorghum [Sorghum bicolor (L.) Moench] improvement programme for the development of high-yielding varieties for various agroclimatic conditions.

Seed vigor is strongly associated with various physiological quality traits of a particular genotype and may be very useful for first stage selection of genetically potential genotypes on the basis of performance of seed vigor and associated attributes. In a hybrid breeding program, the major problem is the influence of environment on various morphological traits during the selection of genetically vigorous parents. It has been proved through various studies that field performance can be predicted in very early stages by seed vigor testing in controlled conditions. The seeds vigor may be defined as the sum total of genetic, physical, physiologic and sanitary attributes that affect the seed capacity to perform well in adverse environmental field conditions (Moterle *et al*., 2011) [21]. By using vigor and associated attributes, the problem of environment effect during selection of superior genotypes can be overcome. Few studies resulted that with help of genetic effects related to the seeds quality, it is possible to obtain genetic gain. According to Gomes *et al*. (2000) [12], the germination and vigor results indicated the hybrids are superior to the lineages regarding the physiological quality. In conventional plant breeding tools, various methods have been efficiently used to identify potential parents having superior yield potential. To exploit more genetic variation present in population, the major selection criteria for parental selection is based on individual performance, yield stability and adaptability. Selection is basically done through various phenotypic and morphological parameters. Combining ability tests are the traditional methods used to predict the hybrid contributions of sorghum parental lines (Bhatnagar *et al*., 2004; Fan *et al*., 2004) [5, 9].

However as per demand of present scenario, more specific selection techniques are needed for the further enhancement of genetic gain as maximum phenotypic variability has been exploited based on morphological markers. The use of molecular markers like SSRs has been proposed as a more efficient method of selecting parental inbred lines and superior hybrid combinations, which can reduce the number of multi-location trials of potential hybrids (Menkir *et al*., 2004; Barata and Carena, 2006) [17, 2]. In present investigation, an associative study of molecular markers and seed physiological attributes has been done based on heterosis estimates of various parental combinations.

Material and Methods

The experiments were conducted at the Instructional Dairy Farm of the G.B. Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar) India, during *Kharif* season in the year 2014-2015 and at the Maize laboratory, Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar)**.** The experimental materials for the present study consist of thirty F_1 crosses developed through line × tester mating design involving five diverse CMS lines (female) and six sorghum pollinator (male) lines. The experimental materials was obtained through the F1 crosses made in Kharif season of 2014-2015 in field experiment. The details of parental lines (lines and testers) and their F_1s have been presented in Table 1:

Table 1: Parentage, origin/source and important characteristic features of parental lines used for the study

Name of the Parental line	Parentage	Origin/Source	Tillering/ Non-Tillering
ICSA 467	-	ICRISAT	Non-tillering
ICSA 469	[(ICSB 37 x ICSV 702) x PS 19349B]3-3-4-2	ICRISAT	Non-tillering
ICSA 276	(ICSB 101 x TRL 74/C 57) x PM17467B]2-5-1-3-3	ICRISAT	Non-tillering
$11A_2$	Non-milo	DSR, Hyderabad	Non-tillering
MR $750A_2$	Non-milo	DSR, Hyderabad	Non-tillering
Pant Chari 5	CS 3541 x IS 6953	Pantnagar	Non-tillering
UPC 2	VIDISHA 60-1x ISC 953	Pantnagar	Non-tillering
CS3541	IS 3675 x IS3541	DSR, Hyderabad	Non-tillering
M 35-1	Selection from Maldandi landraces	Mahol	Non-tillering
JJ1041	-	Indore	Non-tillering
SPV1616	-	DSR, Hyderabad	Non-tillering
CSH-20MF (National)	2219A x UPMC-503	Pantnagar	Tillering
CSH-24MF (National)	ICSA 467 X PC6	Pantnagar	Tillering

The experiment was conducted in between paper method. The germination paper was moisture with water and 100 seeds were arranged in between of the moist germination paper as per the procedure of ISTA. The closed germination paper was placed in a germinator at 25 $\pm$ 1 ^{0}C for 10 days. Standard germination percent was calculates by evaluating the seedlings at regular interval and normal seedlings were counted on 10th day. The percentage of normal seedlings provided the germination percentage. The shoot length was measured with the help of a measuring scale for ten randomly selected seedlings on final count after eight days in each replication. The root length was calculated with the help of a measuring scale for ten randomly selected seedlings on final count after eight days in each replication. Seedling fresh weight was assessed after the final count in the standard germination test. Ten normal seedlings were randomly taken from each replication of germination test. The fresh seedlings were weighed and the average seedling weight was calculated. For the fresh weight, seedlings were dried in an oven for 72 hrs at 72 ^{0}C temperature. The dried seedlings were weighed and the average dry weight was calculated.The seedling vigour index was calculated by two different methods (Abdul-Baki and Anderson, 1973) [1]

Seedling Vigour Index-I= Standard germination percentage x Seedling length (cm)

Seedling Vigour Index-II= Standard germination percentage x Seedling dry weight (g)

Three types of heterosis were estimated for germination seed vigor and its components. The estimates of heterosis for various characters are presented in Table 4. For the estimation of standard heterosis, two released hybrids of multicut forage sorghum *viz.* CSH 20 MF and CSH 24 MF as checks or standard genotypes. However, out of these three checks, CSH 24 MF the released hybrid was found to be best for most of the characters. Therefore, CSH 24 MF was invariably used for estimation of standard heterosis for all the characters. Heterosis expressed as percentage increase or decrease of F_1s over better parent, mid parent and check parent was calculated as suggested by Fonseca and Petterson (1968) [10].

For molecular analysis, seventeen SSR primers were selected to diversify the sorghum genotypes (Table 2). DNA was extracted from fresh seedlings (eight days old) by the method described by Dellaporta *et al.,* (1989) [8]. For PCR amplification, a master mix without DNA template was prepared for different tubes to reduce pipetting error and redistributed in each PCR tube (18 µl each). PCR amplification was performed in a final volume of 20 µl reaction set up containing 2 µl of DNA, 1.2µl of dNTPs, 2.0 µl PCR buffer, 0.5 µl of forward primer, 0.5 µl of primer reverse primers, 0.4 µl of Taq DNA polymerase and 13.4µl of double distilled water. The reaction conditions were as follows: initial denaturation (94 ˚C for 5 min) followed by 35 cycles of denaturation (94 ˚C for 1 min), annealing at 55 ˚C for 2 min (temperature reduced by 1˚C for each cycle) and primer extension (72 ˚C for 2 min). This step was followed by final cycle of denaturation at 94 ˚C for 1 min, annealing at 55 ˚C for 1 min and extension at 72 ˚C for 7 min. PCR amplified DNA fragments were resolved by submerged horizontal electrophoresis in 1.5% agarose gel and visualized by staining with ethidium bromide. After completion of electrophoresis, image of the gel was viewed and saved in a gel documentation system (Alpha Imager EC).

Table 2: Range of SSR loci scored, number and size of exclusive loci amplified in the sorghum genotypes

SR. No.	Primer code	No. of loci amplified	No. of monomorphic loci	No. of polymorphic loci	Percentage polymorphism	Range of amplified loci (bp)	PIC value
1.	Xisep 0101	7	2	5	71	100-200	0.795
2.	Xisep 0983	5	1	4	80	200-220	0.698
3.	Xisep 0841	17	2	15	88	200-1100	-0.878
4.	Xisep 0449	2	0	2	100	200-230	0.304
5.	Xisep 0523	5	0	5	100	100-200	-0.134
6.	Xisep 0805	2	0	2	100	200-250	0.498
7.	Xisep 1202	2	0	2	100	200-250	0.484
8.	Xisep 0747	3	0	3	100	200-250	0.519
9.	Xisep 0809	2	0	2	100	200-350	0.000
10.	Xisep 0829	5	1	4	80	100-210	0.000
11.	Xisep 1014	4	0	4	100	100-230	0.000
12.	Xisep 0131	5	2	3	60	200-260	0.782
13.	Xisep 0444	4	0	4	100	100-180	0.726
14.	Xisep 1140	2	0	2	100	100-120	0.000
15.	Xisep 0203	2	0	2	100	100-200	0.000
16.	Xisep 0327	2	0	2	100	100-200	0.000
17.	Xisep 1012	4	1	3	75	100-250	-0.265
Total		73	9	64			3.529
Average		4.2941	0.52941	3.7647	91		0.2075

On the basis of absence and presence of SSRs band and statistical data, similarity matrix coefficient among the eleven sorghum accessions were calculated by following Jaccard's similarity index (1998).

$$\text{Similarity Index (SI)} = \frac{\text{Number of matching bands in two lanes compared}}{\text{Total number of bands}}$$

All the numerical taxonomic analysis with respect to SSRs (DNA fragment analysis) was performed using the NTSYS-pc software (Rohlf, 1992) [24].

Results

ICSA467 x JJ1041 (SM 0.52) performed better for root length (BP 27.02 and MP 21.62) and vigor index I (BP 65.19 and MP 40.23) while ICSA467 x SPV1616 (SM 0.53) gave positive and significant heterosis only for vigor index I (BP 14.27). Cross, ICSA467 x PC5 (SM 0.56) showed positive and significant heterosis for many characters, i.e. shoot length (BP 42.39 and MP 28.37), root length (BP 21.48 and MP 20.20), fresh weight (BP 22.84) and vigor index I (BP 31.22 and MP 25.93). ICSA467 x UPC2 (SM 0.64) performed better having positive and significant heterosis only for standard germination per cent (BP 4.38 and MP 4.38) while ICSA467 x M35-1 (SM 0.71) revealed better results for standard germination per cent (BP 14.3 and MP 8.14), shoot length (BP 38.9 and MP 28.92), rootlength (MP 32.58) and vigor index I (BP 65.19 and MP 40.23). For ICSA467 x CS3541 (SM 0.75), positive and significant heterosis was recorded only for standard germination per cent (BP 9.30) and vigor index I (BP 17.80).

Table 3: Similarity matrix of SSR markers for Jaccard's Coefficient in sorghum parental genotypes

	M35-1	ICSA 276	ICSA 467	CS 3541	UPC 2	JJ 1041	MR 750A2	PC5	SPV 1616	ICSA 469	11A2
M35-1	1.0										
ICSA276	0.78	1.0									
ICSA467	0.71	0.86	1.0								
CS3541	0.61	0.75	0.75	1.0							
UPC2	0.59	0.67	0.64	0.76	1.0						
JJ1041	0.52	0.55	0.52	0.56	0.64	1.0					
MR750A2	0.55	0.63	0.60	0.61	0.67	0.84	1.0				
PC5	0.43	0.56	0.56	0.68	0.63	0.56	0.64	1.0			
SPV 1616	0.51	0.61	0.53	0.63	0.65	0.46	0.59	0.73	1.0		
ICSA469	0.52	0.65	0.60	0.64	0.69	0.47	0.57	0.69	0.90	1.0	
11A2	0.53	0.61	0.56	0.63	0.73	0.51	0.59	0.71	0.89	0.88	1.0

For ICSA469, among all the pollinators, SPV1616 having highest similarity matrix (SM 0.90) gave best results for most of the characters i.e. germination per cent (BP 5.88 and MP 4.15), shoot length (BP 32.63), dry weight (BP 27.5 and SP 15), vigor index I (BP 15.24 and MP 13.39) and vigor index II (MP 21 and SP 15.6) followed by PC5 (SM 0.69) for germination per cent (BP 1.47), shoot length (BP 41.05 and MP 30.37), root length (BP 21.09), fresh weight (BP 23.60 and MP 14.72) and vigor index I (BP 31.72 and MP 14.54) while M35-1 having lowest similarity with ICSA469 (SM 0.52) reported for negative and significant heterosis for most of the characters. For ICSA469, among all the pollinators, SPV1616 having highest similarity matrix (SM 0.90) gave best results for most of the characters i.e. germination per cent (BP 5.88 and MP 4.15), shoot length (BP 32.63), dry weight (BP 27.5 and SP 15), vigor index I (BP 15.24 and MP 13.39) and vigor index II (MP 21 and SP 15.6) followed by PC5 (SM 0.69) for germination per cent (BP 1.47), shoot length (BP 41.05 and MP 30.37), root length (BP 21.09), fresh weight (BP 23.60 and MP 14.72) and vigor index I (BP 31.72 and MP 14.54) while M35-1 having lowest similarity with ICSA469 (SM 0.52) reported for negative and significant heterosis for most of the characters.

Table 4: Magnitude of heterosis over better parent (BP), mid- parent (MP) and standard check (SD)

crosses	Germination %			Shoot length (cm)			root length (cm)		
	BP	MP	SP	BP	MP	SP	BP	MP	SP
ICSA467XPC5	1.47	1.47	-2.81	42.39**	28.37*	-3.72	21.48*	20.20*	-4.22
ICSA467XUPC2	4.38*	4.38*	1.4	4.96	-7.08	-21.27*	-1.68	-10.58	-22.83**
ICSA467XCS3541	9.30**	3.29	-0.70	21.48	17.04	-11.11	-3.56	-14.68	-21.51*
ICSA467XM35-1	14.3**	8.14**	-1.7	38.9*	28.92*	-0.94	51.91	32.58**	-7.27
ICSA467XJJ1041	-1.8	-2.36	-5.6**	19.7	16.87	-10.16	27.02*	21.62*	-8.44
ICSA467XSPV1616	-3.2	-0.18	-4.2	19.3	2.61	-8.33	15.25	8.47	-29.65**
ICSA469XPC5	1.47*	-1.42	-2.8	41.05**	30.37*	-4.61	21.09*	6.50	-2.49
ICSA469XUPC2	-2.5	-2.7	-5.2**	6.14	-6.03	-20.38*	-8.38	-21.62*	-35.55**
ICSA469XCS3541	7.75*	4.12*	-2.1	21.48	19.98	-11.11	-13.61	-15.17	-32.19**
ICSA469XM35-1	10.6*	1.5	-4.9*	-5.14	-9.85	-32.38**	0.00	-25.39**	-38.96**
ICSA469XJJ1041	3.29	2.5	-0.7	23.48	20.49	-7.38	26.53*	25.00*	-10.98
ICSA469XSPV1616	5.88*	4.15*	1.40	32.63**	18.63	9.27	11.08	-3.05	-32.19**
ICSA276XPC5	1.8	1.0	-2.46	31.47*	24.65*	-11.11	-0.64	-1.91	-22.02*
ICSA276XUPC2	2.6	0.37	-4.5*	25.0	12.22	-3.88	13.55	-4.18	-22.02*
ICSA276XCS3541	4.6*	0.93	-4.9	31.58*	29.95*	-3.72	27.76*	18.47	-10.12
ICSA276XM35-1	12.2*	5.3**	-3.5	27.2	24.04*	-9.27	45.83**	27.59*	-10.98
ICSA276XJJ1041	4.5*	2.7	-2.8	21.6	20.21	-6.50	16.07	13.26	-20.29*
ICSA276XSPV1616	-3.9*	-5.0**	-4.9	23.5	7.69	-2.77	-4.16	-28.50**	-41.50**
11A2XPC5	6.9*	6.0**	2.4	35.5*	28.55*	-8.33	49.67	39.58**	5.23
11A2XUPC2	6.1**	6.9**	3.16	-2.2	-8.89	-19.44	15.03	4.88	-9.30
11A2XCS3541	11.6**	10.34**	1.4	22.7	19.77	-10.16	14.81	5.08	-21.16*
11A2XM35-1	20.4**	12.8**	3.5	61.1**	57.00**	14.83	16.66	8.40	-28.78**
11A2XJJ1041	6.9**	6.7**	2.4	42.3**	39.10**	12.05	55.25**	48.29**	11.90
11A2XSPV1616	1.4	2.33	0.35	35.7**	15.17	1.83	55.25**	34.88**	-5.90
MR750A2XPC5	6.8**	5.2**	-0.7	36.9*	28.23*	-7.38	9.85	1.12	-24.56**
MR750A2XUPC2	3.9*	1.77	1.05	15.24	4.78	-9.27	-25.40**	-28.62**	-29.80
MR750A2XCS3541	7.36**	4.52*	-2.4	15.18	8.35	-15.72	8.58	6.85	-14.39
MR750A2XM35-1	16.8**	12.2*	0.35	28.60*	23.78*	-8.33	-12.50	-17.64	-46.59**
MR750A2XJJ1041	5.4**	2.67	1.4	29.35*	29.35**	1.83	1.12	-16.54	-27.11**
MR750A2XSPV1616	3.9*	3.22	1.4	54.29*	30.88**	15.72	1.41	-5.76	-38.09**

crosses	Fresh wt. (g)			dry wt. (g)			Vigor index I			Vigor index II		
	BP	MP	SP	BP	MP	SP	BP	MP	SP	BP	MP	SP
ICSA467XPC5	22.84**	3.64	-9.36*	80.00	17.39	-10.00	31.22**	25.93**	-6.56**	82.66	19.20	-12.77
ICSA467XUPC2	1.90	-10.62*	-19.66**	19.33	10.00	-26.66	5.49	-5.37*	-21.11**	11.93	-13.45	-25.96
ICSA467XCS3541	-7.91	-23.11**	-21.53**	-27.90	-77.77	-48.33	17.80**	1.75	-17.09**	42.74	-5.54	-48.91
ICSA467XM35-1	-18.33**	-18.33**	-17.41**	48.38	-1.07	-23.33	65.19**	40.23**	-5.96*	69.12	4.88	-25.05
ICSA467XJJ1041	9.19	-1.11	-8.80	-13.33	-29.09	-35.00	18.28**	16.33**	-14.41**	-14.80	-30.76	-38.80
ICSA467XSPV1616	-14.46**	-17.36**	-22.47**	15.00	-2.12	-23.33	14.27**	4.77	-22.89**	11.43	-1.63	-26.66
ICSA469XPC5	23.60**	14.72**	-8.80	63.33	34.24	-18.33	31.72**	14.54**	-6.20*	65.46	31.54	-20.98
ICSA469XUPC2	10.21	3.68	-13.10**	40.54	10.00	-13.33	-4.20	-16.6**	-32.16**	37.15	30.33	-17.87
ICSA469XCS3541	-11.87*	-19.08**	-11.04*	-21.53	-66**	-15.00	8.52*	5.22	-23.62**	13.13	17.67	-17.19
ICSA469XM35-1	8.79	-0.50	-7.30	35.48	13.51	-30.00	7.16	-18.3**	-38.99**	49.56	13.59	-33.71
ICSA469XJJ1041	-10.31	-13.04**	-25.09**	-8.10	-17.07	-43.33	27.31**	25.93**	-9.84**	-6.41	-14.90	-43.96
ICSA469XSPV1616	-0.62	-6.05	-9.92	27.5*	19.44	15*	15.24**	13.39**	-11.05**	28.94	21**	15.6*
ICSA276XPC5	33.24**	12.54**	-1.68	90.00	20.00	-5.00	14.08**	11.28**	-18.76**	93.46	21.00	-7.61
ICSA276XUPC2	18.52**	6.28	-6.55	25.33	53.62	-11.66	22.70**	3.86	-17.20**	26.87	12.30	-16.08
ICSA276XCS3541	4.43	-10.73**	-7.30	24.32	-66.3*	-23.33	25.62**	25.23**	-11.59**	21.24	51.77	-27.40
ICSA276XM35-1	-23.00**	-23.07**	-22.28**	9.67	-29.16	-43.33	52.40**	31.74**	-13.24**	22.76	-27.10	-45.59
ICSA276XJJ1041	-4.70	-11.82**	-20.41**	-13.33	-21.21	-35.00	24.35**	20.00**	-16.09**	-12.31	-18.79	-37.01
ICSA276XSPV1616	13.18*	9.69*	-3.55	67.50	61.44	11.66	-7.74*	-14.6**	-26.47**	60.54	52.90	5.64
11A2XPC5	35.78**	23.27**	0.18	62.16	79.10	0.00	43.05**	42.66**	1.30	70.49	89.69	2.08
11A2XUPC2	17.10**	2.60	-7.67	21.33	22.07	-21.66	14.96**	4.92	-11.26**	21.72	-2.25	-19.49
11A2XCS3541	-2.51	-12.40**	-5.43	-1.85	-63.4*	-11.66	26.35**	23.69**	-14.74**	14.97	50.08	-10.63
11A2XM35-1	19.19**	11.44**	5.80	67.74	52.94	-13.33	67.53**	49.31**	-4.63	49.22	71.51	-10.64
11A2XJJ1041	-1.12	-10.54*	-17.41**	48.88	25.23	11.66	58.81**	53.68**	14.91**	58.76	33.82	14.03
11A2XSPV1616	-2.68	-7.91	-11.79*	7.50	-18.09	-28.33	31.38**	27.20**	-1.74	9.06	-15.93	-28.22
MR750A2XPC5	16.24*	0.439	-14.23**	43.33	2.38	-28.33	23.34**	20.02**	-16.77**	48.71	8.38	-28.98
MR750A2XUPC2	6.65	2.51	-15.91**	17.33	41.37	-31.66	-12**	-12**	-19.00**	3.76	-0.91	-31.36
MR750A2XCS3541	-23.33**	-29.53**	-22.47**	-45.16	-77**	-43.33	17.81**	12.37**	-17.08**	54.11	-17.91	-44.84
MR750A2XM35-1	-25.29**	-26.84**	-27.52**	-3.22	-29.41	-50.00	26.69**	15.95**	-27.87**	12.58	-21.80	-50.10
MR750A2XJJ1041	3.81	2.77	-13.29**	6.97	4.54	-23.33	21.83**	6.90**	-11.84**	7.67	7.25	-22.66
MR750A2XSPV1616	-1.05	-2.08	-12.17**	27.02	22.07	-21.66	25.61**	18.19**	-11.04**	32.02	25.79	-20.94

*, ** Significant at 5% and 1% levels of probability, respectively

For ICSA469, among all the pollinators, SPV1616 having highest similarity matrix (SM 0.90) gave best results for most of the characters i.e. germination per cent (BP 5.88 and MP 4.15), shoot length (BP 32.63), dry weight (BP 27.5 and SP 15), vigor index I (BP 15.24 and MP 13.39) and vigor index II (MP 21 and SP 15.6) followed by PC5 (SM 0.69) for germination per cent (BP 1.47), shoot length (BP 41.05 and MP 30.37), root length (BP 21.09), fresh weight (BP 23.60 and MP 14.72) and vigor index I (BP 31.72 and MP 14.54) while M35-1 having lowest similarity with ICSA469 (SM 0.52) reported for negative and significant heterosis for most of the characters. For vigor index I, among all the pollinators crossed with ICSA276, maximum significant and positive heterosis i.e. 52.40 (BP) and 31.74 (MP) was recorded for M 35-1 (SM 0.78) followed by CS3541 (SM 0.75) having BP 25.62 and MP 25.23, JJ1041 (SM 0.55) having BP 24.35 and MP 20.00, UPC 2 (SM 0.67) having BP 22.70 and PC5 (SM 0.56) having BP 14.05 and MP 11.28.

Among all the pollinators crossed with $MR750A_2$, maximum significant and positive heterosis i.e. 26.69 (BP) and 15.95 (MP) was recorded for M35-1 having lowest similarity matrix i.e. 0.55. For $11A_2$, PC5 (SM 0.71) gave significant and positive heterosis for standard germination per cent (BP 6.9 and MP 6.0), shoot length (BP 35.5 and MP 28.55), root length (MP 39.58), fresh weight (BP 35.78 and MP 23.27) and vigor index I (BP 43.05 and MP 42.66). $11A_2$ x UPC2 (SM 0.73) exhibited good results for standard germination per cent (BP 6.1 and MP 6.9), fresh weight (BP 17.10) and vigor index I (BP 14.96). $11A_2$ x CS3541 (SM 0.63) found to be better for standard germination per cent (BP 11.6 and MP 10.34) and vigor index I (BP 26.35 and MP 23.69) while $11A_2$ x M35-1 (SM 0.53) performed better for standard germination per cent (BP 20.4 and MP 12.8), shoot length (BP 61.1 and MP 57.00), fresh weight (BP 19.19 and MP 11.44) and vigor index I (BP 67.53 and MP 49.31). $11A_2$ x JJ1041 (SM 0.51) gave significant and positive heterosis for standard germination per cent (BP 6.9 and MP 6.7), shoot length (BP 42.3 and MP 39.10), root length (MP 55.25 and MP 48.29) and vigor index I (BP 58.81 and MP 53.68) while $11A_2$ x SPV1616 (SM 0.89) performed better for vigor index I (BP 31.38 and MP 27.20).

Discussion and Conclusion

On the basis of above findings, it can be assumed that for most of the crosses molecular diversity resulted in more heterotic combinations for seed vigor and other associated characters, although some destructions were also observed. There was no definite pattern was observed between molecular diversity and heterosis for various traits. This may be due to because sorghum exhibits comparatively low heterozygosity due to its inbreeding behaviour but its gene pool maintains a high level of allelic variation (Ghebru *et al.* 2002) [11]. It has been suggested that the parents having more diversity positively correlated with heterosis of F1 crosses. Therefore, the extent of genetic diversity between parents has been suggested as a possible measure of the heterosis (Zhang *et al*., 1994) [26]. However, strong association has rarely been observed between heterosis and genetic diversity among parents (Rao *et al*., 2004) [22]. In previous studies on rice (Hua *et al*., 2002) [14], wheat (Corbellini *et al*., 2002) [7] and grain sorghum (Jordan *et al*., 2003) there were also non-significant associations between genetic distance and hybrid performance for various morphological characters. Morphological characters are highly influenced by environmental conditions; therefore exact prediction of genetic and phenotypic behaviour is affected many times. But in studies based on physiological characters in controlled conditions which have been found directly associated with field performance can provide more relevant information. A significant relationship between genetic diversity and hybrid vigor Boppenmaier *et al*. (1992) [6] and Mosar and Lee (1994) [20] reported by in maize and oats, respectively.

In present investigation, very good estimates of heterosis were observed for most of the characters in a wide range of F1 crosses. Positive and significant heterosis for standard germination per cent was ranged from 1.47 to 20.4 (BP) over 21 crosses and 4.12 to 12.2 over 14 crosses. For Seedling vigor, estimations of heterosis were ranged from 8.52 to 67.53 (BP) over 25 crosses and 6.90 to 53.68 over 20 crosses among a total of 30 crosses. These findings indicate that there was a good association of molecular diversity studies and overall hybrid vigor performance of F1 crosses. Only one cross exhibited better results of heterosis over standard parent i.e. ICSA 469 x SPV1616 (15.6) for seedling vigor. The similar findings were also reported by Miranda *et al*. (2003) [19]. In hybrid breeding programs, selection of parental lines is the most important and difficult task to predict the performance of hybrids. Although the relatedness, and consequently genetic distance, can be obtained from pedigree data (Helms *et al*., 1997) [13] by molecular marker systems it is considerably more specific to estimate the genetic distance, between genotypes (Milbourne *et al*., 1997; Virk *et al*., 1999; Barth *et al*., 2002) [18, 25]. A positive correlation between genetic distance and heterosis has been reported for oilseed rape (*Brassica napus*; Riaz *et al*., 2001) [23] and maize (Barbosa *et al*., 2003) [3].

It may be suggested that genetic bases at seedling stage can be a good indicator for the selection of parental line to be used in future breeding programme for development of hybrid. There is need of a detailed characterization of crop germplasm and an in-depth comprehension of the genetic basis of heterosis to develop strategies for the utilization of molecular markers in hybrid vigor prediction.

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