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Research Article

Association between molecular diversity and hybrid performance in sunflower (*Helianthus annuus* L.)

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Abstract
The present study was aimed to assess the molecular genetic diversity in 14 parental lines (10 maintainers and 4 restorers) of sunflower crop using 51 SSR markers. A total of 147 alleles were produced with the range of 1-6 alleles per locus. The polymorphic information content (PIC) values ranged from 0.12(ORS1198 in LG5) to 0.74(ORS384 in LG15) with an average PIC of 0.51. A phylogenetic tree was constructed by neighbour- joining method which grouped the parents into four clusters. Among the maintainer and restorer combinations, the dissimilarity index was maximum between CMS 519B and CSFI 99 (0.67) which indicateed high divergence between them. Lower dissimilarity value was observed between RCR CMS 38B and LTRR 341 (0.40) suggested less divergence between them. Mahalanobis D^2 analysis was also carried out among the parental lines. The D^2 analysis grouped the parental lines into six clusters. Among them genotype pairs CMS104B vs RHA 1-1 and CSFI 99 vs LTRR341 had highest and lowest D^2 values respectively. Dissimilarity and D^2 values for parental combinations, mean performance, mid and better parental heterosis of 40 hybrids for yield and its component traits were subjected to simple correlation analysis. The D^2 analysis did not show any association with performance of hybrids. However, the dissimilarity values showed association with hybrid performance and heterosis for plant height and volume weight. Hence, the present study indicated that the selection of parental genotypes with high molecular diversity could result in improved performance of plant height and volume weight in sunflower hybrids. However further study is required with multi environment data sets.

Keywords: Sunflower, molecular diversity, hybrid, association, heterosis.

INTRODUCTION

Sunflower is an important oilseed crop in the world. Sunflower competes in the world oilseed complex with the other major oilseed crops such as soybean, groundnut, and rapeseed. It has about 46-52% oil content (Das *et al*., 2020). Ever since sunflower was introduced in 1969 in India, as a supplement to traditional oilseed crops, its area has shown a steady increase and presently, India has emerged as the second major sunflower producing country in Asia after China. It is a cross pollinated crop. The commercial success of the production was archived by hybrid breeding. Hybrid breeding in Sunflower was started economically after the discovery of CMS by Leclercq in 1960 and restorer genes by Kinman in 1970 (Fick and Miller, 1997). and currently, hybrid breeding program is well developed (Chandirakala *et al.*,2016; Nichal *et al*., 2017; Ghaffari and Shariati, 2018; Hilli *et al*.,2020; Karande *et al*., 2020).

The choice of the parents is a crucial step in the heterosis breeding program. The genetic diversity of parental lines is an important criterion in the manifestation of heterosis (Melchinger, 1999). The genetic diversity of plant material can be observed from morphological as well as molecular data. D^2 statistic is a powerful tool for the identification of genetically diversified parents which can be used in hybridization programs (Punitha *et al*., 2010). DNA markers *viz.,* RFLP, RAPD, SSR, ISSR *etc*., can be used to assess the diversity studies. Among them, SSR markers have great potential in genetic and breeding studies (Shoba *et al*., 2010; Ramanaiah and Kadirvel, 2021).

In recent years, molecular method of diversity research has been conducted in most of oilseed crops by SSR markers (Ramanaiah and Kadirvel, 2021; Yihan *et al*., 2022; Ahmed *et al*., 2022 in sunflower, Teklu *et al*.,2022 in sesame, Tomar *et al*., 2022 in groundnut and Kumar *et al*., 2022 in soybean) due to its codominant and highly reproducible characteristics. The relationship between molecular diversity with hybrid performance and heterosis was studied in several crops to access the reliability of markers-based prediction in hybrid performance (Xiao *et al*., 1996; Sureja *et al*., 2006; Gupta *et al.,* 2018 and Somashekhar *et al.*, 2020). Hence, an attempt was made to study the molecular genetic diversity among parental lines and their association with the hybrid performance in sunflower.

MATERIALS AND METHODS

This research work consists of 14 parents which includes ten maintainer lines *viz*., COSF 6B, COSF 10B, COSF 12B, COSF 13B, CMS 104B, CMS 207B, CMS 519B, ARM 248B and RCR CMS 38B and four restores *viz.,* IR 6, CSFI 99, RHA-1-1 and LTRR341. A field experiment was carried out at the Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore (11° N latitude and 77° E longitude). Forty hybrids were produced from these parents using a line x tester mating design. During July-September season, 2021, 40 hybrids and their parents were evaluated in a randomized complete block design with two replications. Morphological observations were taken on nine quantitative traits *viz.,* days to 50% flowering, plant height (cm), head diameter (cm), days to maturity, volume weight (g/100ml), hundred seed weight (g), seed yield /plant (g), oil content (%) and oil yield/plant (g) with randomly selected five plants in each entry in each replication. The oil content of the seeds was estimated by using a Near-infrared (NIR) spectroscopy instrument (Make: M/s ZEUTEC, Germany; Model: SPA 1.0) available at the Centre of Excellence in Molecular Breeding, TNAU, Coimbatore.

The DNA was extracted from young leaves of 14 parents by following CTAB method (Doyle and Doyle, 1987). The DNA quality and quantity were checked on 0.8% agarose gel, and DNA concentration was normalized to 10 ng/µl.

In the present study, publicly available ORS markers mapped by Tang *et al*. (2002) were used. A total of 51 SSR primers were used for analyzing the diversity among parents. These markers were well distributed among the 17 linkage groups (LGs) in sunflower at the rate of 3 to 5 markers per LG. The Polymerase Chain Reaction (PCR) mixtures were prepared as per Subramaniyan *et al*. (2022). The DNA was amplified in a thermocycler (Applied Biosystems, Veriti) under the following conditions: Initially single cycle of 94°C for 3 min followed by touch down step (0.5°C decrease in the annealing temperature after each cycle) (Hube *et al*. 2005) in 20 cycles (of 94°C for 30 s, 63°C for 30 s, 72°C for 1 min) followed by 20 cycles of 94°C for 15 s, 55°C for 30 s, 72°C for 1 min with a final extension step of 72°C for 10 min. The PCR products were separated on 3% agarose gel and photographed using GELSTAIN 49 advanced gel documentation unit (M/s Medicare, India).

In each parent, the amplified bands of 51 markers were scored as present (1) or absent (0) for each unique allele. Polymorphism information content was estimated for each marker using the formula suggested by Botstein *et al*. (1980). The allelic data was subjected for estimating the genetic distance. A neighbour-joining tree (Saitou and Nei, 1987) was created using the DARwin 6.0 software package (Perrier and Jacquemoud-Collet, 2006). Data on the 14 parental lines were subjected into D^2 analysis as suggested by Mahalanobis (1928) and clustered by Tocher method as suggested by Rao (1952).

The mean value of nine biometrical traits of 40 hybrids and dissimilarity values and D^2 values of 40 parental combinations were used for correlation analyses. Simple correlation analysis was performed as per the standard method (Pearson, 1896) with MS Excel.

RESULTS AND DISCUSSION

In the present study, 51 markers were taken for molecular diversity analysis in a set of 14 sunflower parental lines (10 maintainer lines and 4 restorer lines). All the linkage groups had three polymorphic markers except the LG8 and LG10 which had two polymorphic markers (**Table 1**). A total of 147 alleles were produced from 51 SSR markers. The number of bands produced per primer ranged from 1 to 6 with an average of 2.88 alleles. The Polymorphic Information Content (PIC) values ranged from 0.12 (ORS1198 in LG5) to 0.74 (ORS384 in LG15) with an average PIC of 0.51 (Zia *et al*., 2014; Zeinalzadeh-Tabrizi *et al*., 2018). The monomorphic markers (ORS762 in LG8; ORS1130 in LG10) had zero PIC values. The highest PIC value (0.74) was recorded by the polymorphic markers *viz*., ORS384 and ORS565 and they were located in LG15 and LG17 respectively. Hence, these markers are highly suitable for distinguishing individuals and understanding the genetic diversity among them (Darvishzadeh *et al*., 2010; Zeinalzadeh-Tabrizi *et al*., 2018). The genetic dissimilarity indices ranged from 0.30 (COSF 12B vs COSF 10B) to 0.68 (RHA-1-1 vs CSFI 99) (**Table 2**). High dissimilarity indices were recorded between CSFI 99 *vs* RHA-1-1 (0.68) followed by CMS 519B *vs* CSFI 99 (0.67), CSFI 99 *vs* IR 6 (0.67) and CMS 104B *vs* CSFI 99 (0.66) which indicated high

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Table 1. List of SSR markers used for diversity analysis in sunflower

S.No	Markers	LG	Sequences	Allele size (bp)	Number of alleles	PIC	Remark
1	ORS822	1	F: CAATGCCATCTGTCATCAGCTAC R: AAACAAACCTTTGGACGAAACTC	140-170	3	0.55	Polymorphic
2	ORS965	1	F: TTGGATTACCTTGGATAGTCAGC R: CTTACCCTCCTCAGACCCTACCT	240-700	3	0.58	Polymorphic
3	ORS543	1	F: CCAAGTTTCAGTTACAATCCATGA R: GGTCATTAGGAGTTTGGGATCA	300-310	2	0.27	Polymorphic
4	ORS1264	2	F: TAGAAGCGGTTGGGTTGACAGTA R: TGAACTCGGTTGATTCTCTAGCC	260-400	3	0.51	Polymorphic
5	ORS1152	2	F: CCCAAGCTCCCTCTTCATCTTA R: TGTTCATAGCCTCATTCATGTTG	250-270	3	0.58	Polymorphic
6	ORS1073	2	F: AGAGTGTTGGTCTTGATTTGTGG R: AAAGAAAGGCGCACGTTAGTC	100-200	3	0.58	Polymorphic
7	ORS1040	3	F: CTGCTGATCGTTTCTTGGATAGA R: TGCTAATCCTTCTAATCAACTTCCAC	200-210	2	0.25	Polymorphic
8	ORS1080	3	F: TTTGTTTCTTTGTGTGGTTATCG R: GGGTGTTGTCGGAGGTATAAAGT	400-1000	2	0.38	Polymorphic
9	ORS488	3	F: CCCATTCACTCCTGTTTCCA R: CTCCGGTGAGGATTTGGATT	190-450	2	0.29	Polymorphic
10	ORS1197	4	F: CCCAGTACGTTACAGTCGTGTGTT R: CAAACAATCACGCAAGGGTTTA	200-370	3	0.41	Polymorphic
11	ORS558	4	F: AGGCATCGGTTCAAATGAGA R: CTTCCCACAAAGGGTAATCG	300-310	2	0.23	Polymorphic
12	ORS334	4	F: TTGGCACAATCTGAAACAAGA R: AATCAAACCGAAAGCCAAACT	230-300	3	0.45	Polymorphic
13	ORS1198	5	F: GGTCTTTTGTTTAGAGGCGTGAT R: CGTTTCCCCATTTACATCATCTT	140-150	2	0.12	Polymorphic
14	ORS1024	5	F: GGGAAGTGGGCTTGTCTATGTAT R: AACACACCGAAATCACCTATGAA	200-350	4	0.65	Polymorphic
15	ORS 1159	5	F: TTTCGTGATGGTGATTGATGATT R: CAGCAACTCTGACCGTTTCATTA	200-260	3	0.53	Polymorphic
16	ORS1193	6	F: GACGGTGATGGATGATAAAGAGA R: TTATTGTCACTCATCGAGAAATTGA	100-210	5	0.60	Polymorphic
17	ORS1219	6	F: CAAACTCTTGCATGTTATGTCCTTT R: GATCTATCTCAACCACCACTGATCT	160-170	2	0.36	Polymorphic
18	ORS725	6	F: TCCGACGACCAAAGAAACTT R: CACAATGAAGGGAAATGGAGA	490-510	3	0.57	Polymorphic
19	ORS1259	7	F: ACCCATGTTGATAGCCAACCTT R: TAGATTCCGAGGTGTGAGGGTAT	140-500	5	0.72	Polymorphic
20	ORS331	7	F: TGAAGAAGGGTTGTTGATTACAAG R: GCATTGGGTTCACCATTTCT	300-400	3	0.59	Polymorphic
21	ORS328	7	F: GACCTGTAGGCCAATATGAGACTT R: TTATACCGGTGTTGTATCGTATCC	200-270	2	0.37	Polymorphic
22	ORS185	8	F: AGCCGCTCCTAACTTGAACCA R: TCACCCTTAAACATCACCCACC	290-320	2	0.37	Polymorphic
23	ORS780	8	F: TGATTACAACCCTAATTCGCATAC R: GATACTGGTGGGACAGATGTTG	100-290	4	0.69	Polymorphic
24	ORS762	8	F: TGCACATGAGGGTATTCTTGTC R: TCGAGGAGAGTGTGACGTTG	210	1	0.00	Monomorphic
25	ORS805	9	F: CATGGATTATAAGAACGGGTGTT R: AATCCCAGGGGTAAAATTGC	320-600	2	0.37	Polymorphic
26	ORS739	9	F: CTCCGTCGCGTATGATAATG R: CAAGAAGTTGTTCACTCTTGATCC	120-190	3	0.43	Polymorphic

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Table1. Continued..

S.No.	Markers	LG	Sequences	Allele size (bp)	Number of alleles	PIC	Remark
27	ORS1265	9	F: GGGTTTAGCAAATAATAGGCACA R: ACCCTTGGAGTTTAGGGATCA	190-220	2	0.37	Polymorphic
28	ORS1110	10	F: CATTCAAGGGGCTATTGTGTAAG R: GGTTTTGGAGAGGTCGATGTG	200-600	2	0.15	Polymorphic
29	ORS437	10	F: GACGTCTTCACAGTTCAAATAACG R: GCATCGACTCTGTTCTTCTCG	320-550	3	0.22	Polymorphic
30	ORS1130	10	F: AGCAACAACATCAACAATCAAGC R: CCCACTCGGGATCCTATATAAAC	230	1	0.00	Monomorphic
31	ORS621	11	F: CGCCTTATGCTGAGAGGAAA R: CCTGAAGCGAAGAAGAATCG	180-380	4	0.64	Polymorphic
32	ORS990	11	F: GGGAAACTTACTCCCTTGATGTT R: GCGACACTATTATTTCACTCACTTTC	130-140	2	0.38	Polymorphic
33	ORS630	11	F: TGTGCTGAGGATGATATGCAG R: GCACGACCCGGATATGTAAC	310-350	2	0.13	Polymorphic
34	ORS502	12	F: ATCCCAACAGACGCCATTAT R: AACATTGGAGGGAGCCAATA	100-270	2	0.35	Polymorphic
35	ORS358	12	F: CACTGACCTCACACTCATGCT R: GTTCTGTTCCTTACTCAGCTTTG	270-300	2	0.37	Polymorphic
36	ORS559	12	F: CAATGAATGCACGAAAGGTC R: TCTGCACATTATCTCCCTCTCTC	110-200	3	0.58	Polymorphic
37	ORS673	13	F: TGGTGCTACTCCATCCTTGA R: GGCATGTTCTCACCGTTCAT	160-500	4	0.68	Polymorphic
38	ORS317	13	F: TTTGGCAGTTTGGTGGCTTA R: GGTCGTATGCTTAATTCTTTCTCT	200-210	2	0.22	Polymorphic
39	ORS707	13	F: GCAGTCAATTCGTAGCATCG R: GCTGAAGCTGAAGACAGATCC	150-170	2	0.38	Polymorphic
40	ORS578	14	F: CTCTCAATCCCTAAAGTCCCCT R: TGGTGGATGTGGTTGTTGAT	200-600	4	0.6	Polymorphic
41	ORS694	14	F: CCTGGAACTGAACCGAGAAC R: GCCGTGAAACAGAGAGAGGA	160-200	3	0.57	Polymorphic
42	ORS307	14	F: CAGTTCCCTGAAACCAATTCA R: GCAGTAGAAGATGACGGGATG	110-130	2	0.26	Polymorphic
43	ORS812	15	F: TTGCACATGAGGGTAGATCG R: CCGGTGGTCGAATATGAGAG	180-800	3	0.47	Polymorphic
44	ORS384	15	F: CCTGGGATAGAGAGGTTGCTT R: CCACTCATGTTGTATTGGGAAT	130-500	6	0.74	Polymorphic
45	ORS668	15	F: TTTCGATTGGACTGTTGCTAAA R: CATGTGAGGGCATTTATGTCA	180-200	2	0.26	Polymorphic
46	ORS899	16	F: GCCACGTATAACTGACTATGACCA R: CGAATACAGACTCGATAAACGACA	300-340	4	0.54	Polymorphic
47	ORS902	16	F: GCAGAGTGGACAATTTCACTACAA R: CTTGGTTGTTGTTGCCTCGTAT	280-290	2	0.28	Polymorphic
48	ORS807	16	F: CCGATATTTTGACCGATATTTTGC R: TCTCACCCTTCATCTCCTTCC	190-260	6	0.73	Polymorphic
49	ORS565	17	F: TGGTCAACGGATTTAGAGTCAA R: TCCAGTTTGGTCTTGATTTGG	160-200	5	0.74	Polymorphic
50	ORS727	17	F: GGTGGCAAGTGGTGGTTAGA R: AAAGCTGGTCATCTCAAGGGTA	170-190	3	0.57	Polymorphic
51	ORS1245	17	F: GAAGTGGAGCAATGTTGGTGA R: CGCCAAGATATTAGTGTGATGATT	170-200	4	0.68	Polymorphic
	Average				2.88	0.51	

Table 2. Genetic dissimilarity coefficient values between 14 parents in sunflower

Parents	COSF 6B	COSF 10B	COSF 12B	COSF 13B	CMS 104B	CMS 112B	CMS 207B	CMS 519B	ARM 248B	RCR CMS 38B	IR 6	CSFI 99	RHA-1-1	LTRR 341
COSF 6B	0	0.38	0.43	0.49	0.4	0.45	0.54	0.56	0.49	0.52	0.53	0.65	0.53	0.52
COSF 10B		0	0.3	0.38	0.45	0.51	0.52	0.51	0.55	0.54	0.56	0.65	0.59	0.5
COSF 12B			0	0.37	0.49	0.52	0.54	0.54	0.54	0.56	0.55	0.64	0.56	0.54
COSF 13B				0	0.5	0.57	0.57	0.59	0.58	0.54	0.52	0.63	0.5	0.48
CMS 104B					0	0.31	0.47	0.5	0.46	0.46	0.53	0.66	0.56	0.46
CMS 112B						0	0.49	0.57	0.52	0.57	0.55	0.64	0.58	0.55
CMS 207B							0	0.53	0.62	0.54	0.57	0.66	0.61	0.58
CMS 519B								0	0.57	0.51	0.64	0.67	0.58	0.57
ARM 248B									0	0.45	0.56	0.63	0.61	0.44
RCR CMS 38B										0	0.52	0.63	0.47	0.4
IR 6											0	0.67	0.41	0.49
CSFI 99												0	0.68	0.64
RHA-1-1													0	0.4
LTRR 341														0

divergence between them. In general, the restorer inbred CSFI 99 had more divergence with other parental lines. Low dissimilarity indices were recorded between COSF 15B vs COSF 12B (0.30) followed by COSF 12B vs CMS 104B (0.31), COSF 12B vs COSF 13B (0.37) and RCR CMS 38B vs LTRR 341(0.40) which indicated less divergence between them. Similar results were reported by various scientists (Zia *et al*., 2014; Zeinalzadeh-Tabrizi *et al*., 2018; Yihan *et al*., 2022; Ahmed *et al*., 2022) in sunflower.

A phylogenetic tree was constructed by neighbour-joining method which grouped the parents into four clusters (**Fig. 1**). Among the four clusters, cluster I had 5 genotypes, clusters III and IV had four genotypes each and cluster II had one genotype. The clustering pattern

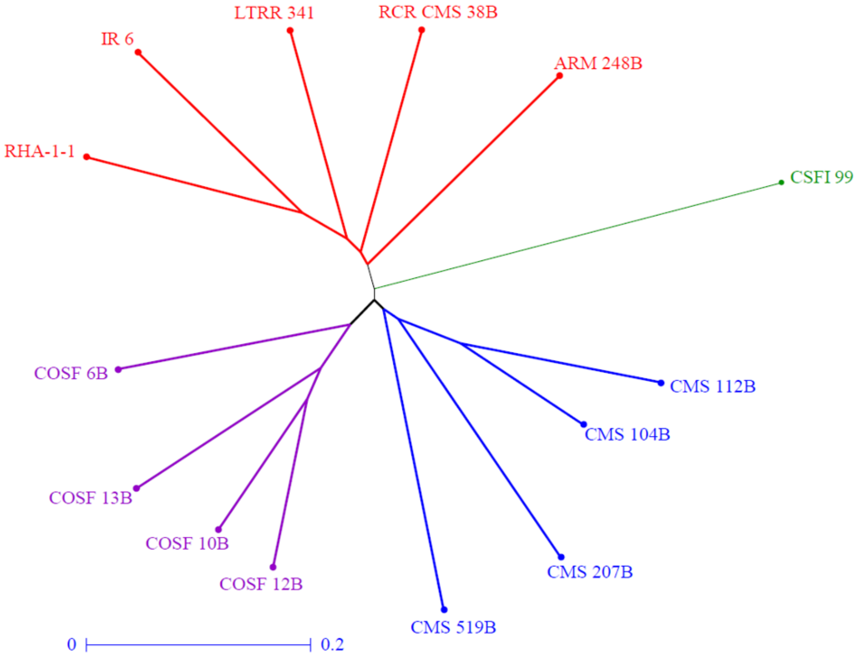

Fig. 1. Neighbour-joining tree of 14 sunflower parental genotypes based on SSR marker data

indicated that three out of four restorer inbreds were grouped into one cluster while the restorer inbred CSFI 99 was grouped separately from other parental lines. Likewise, the COSF series of maintainer inbreds were grouped into a single cluster. It might be due to the similar selection pressure for various traits during the breeding programme. This result was in accordance with the study conducted by Lochner, (2011) in sunflower in which the SSR cluster analysis grouped the A and B lines (female) into one cluster and the restorers (male) were grouped in a separate cluster.

All the 14 parental lines were analysed for phenotypic diversity through D^2 analysis. The D^2 values e ranged between 65.96 (CMS 112A *vs* CSFI 99) to 2598.85 (CMS 104A vs RHA-1-1) (**Table 3**). The D^2 analysis grouped the parental lines into six clusters (**Table 4**). Among them, genotype pairs CMS104B *vs* RHA-1-1 and CSFI 99 *vs* LTRR341 had highest and lowest D^2 values respectively. Among the restorer inbreds, RHA-1-1 was grouped into a separate cluster. Likewise, the maintainer inbred CMS 207B was also grouped into a separate cluster. Other restorer inbreds CSFI 99 and LTRR 341 were clustered along with other maintainer lines. Likewise, the restorer IR 6 was clustered along with maintainer inbred CMS 519B.

The dissimilarity values, D^2 values, and mean performance of hybrids for yield and its component traits are presented in **Table 5**. Among the parental combinations, the dissimilarity values ranged from 0.4 (RCR CMS 38A *vs* LTRR 341) to 0.67 (CMS519A *vs* CSFI 99). The D^2 values ranged between 65.96 (CMS 112A *vs* CSFI 99) to 2598.85 (CMS 104A *vs* RHA-1-1). Mid-parent heterosis and better parent heterosis of 40 hybrids for yield and its component traits are presented in **Table 6 and 7**.

The mean value of nine biometrical traits, mid-parental heterosis, better parental heterosis, D^2 values and dissimilarity values of 40 hybrids were subjected to

Table 3. D^2 values between 14 parents in sunflower

Parents	COSF 6B	COSF 10B	COSF 12B	COSF 13B	CMS 104B	CMS 112B	CMS 207B	CMS 519B	ARM 248B	RCR CMS 38B	IR 6	CSFI 99	RHA-1-1	LTRR 341
COSF 6B	0.00	983.43	417.70	565.27	460.62	115.23	261.70	186.90	60.99	305.42	211.79	140.79	1100.58	119.34
COSF 10B		0.00	137.34	154.65	2342.28	1284.71	434.67	1168.35	1345.98	2001.40	821.31	1135.42	239.62	986.79
COSF 12B			0.00	117.42	1406.79	627.82	151.74	655.53	667.49	1142.09	457.42	518.79	333.49	420.20
COSF 13B				0.00	1826.95	887.15	361.86	646.50	863.04	1505.37	334.00	872.14	143.96	752.75
CMS 104B					0.00	186.40	921.06	745.21	241.97	59.88	1141.23	307.19	2598.85	344.89
CMS 112B						0.00	342.45	325.39	40.20	115.88	527.52	65.96	1468.83	84.85
CMS 207B							0.00	366.36	378.86	663.48	396.03	239.74	772.29	180.06
CMS 519B								0.00	217.02	452.63	126.76	403.69	1142.70	374.04
ARM 248B									0.00	143.03	369.84	128.09	1455.74	121.11
RCR CMS 38B										0.00	843.08	182.64	2201.41	226.80
IR 6											0.00	604.94	739.92	511.70
CSFI 99												0.00	1449.64	39.38
RHA-1-1													0.00	1283.11
LTRR 341														0.00

Table 4. Clustering pattern of parents by D^2 analysis

Clusters	Number of genotypes	Name of the genotypes				
I	5	CSFI 99	LTRR 341	CMS 112B	ARM 248B	COSF 6B
II	2	CMS 104B	RCR CMS 38B			
III	3	COSF 12B	COSF 13B	COSF 10B		
IV	2	CMS 519B	IR6			
V	1	RHA-1-1				
VI	1	CMS 207B				

Table 5. Dis-similarity and D^2 values and Mean performance of hybrids for yield and its component traits in sunflower

Lines x testers	Days to 50% flowering	Plant height (cm)	Head diameter (cm)	Days to maturity	Volume weight (g/100ml)	100 seed weight (g)	Seed yield / plant (g)	Oil content (%)	Oil yield / plant (g)	Dis-similarity	D^2 values
COSF 6A x IR 6	58.50	169.00	11.55	90.50	43.30	4.18	52.97	45.14	23.91	0.53	211.79
COSF 6Ax CSFI 99	53.00	174.95	15.50	84.50	42.54	5.42	62.54	39.75	24.87	0.65	140.79
COSF 6A x RHA-1-1	50.50	151.50	13.38	79.50	40.00	5.91	75.46	34.72	26.23	0.53	1100.58
COSF 6A x LTRR 341	54.50	126.30	12.35	85.50	29.30	3.37	40.12	35.31	21.24	0.52	119.34
COSF 10A x IR 6	61.00	185.10	15.48	91.00	43.35	4.87	51.71	39.32	28.92	0.56	821.31
COSF 10A x CSFI 99	49.50	177.83	14.72	81.50	42.00	6.25	69.73	39.68	27.15	0.65	1135.42
COSF 10A x RHA-1-1	45.50	168.70	17.41	76.00	41.15	7.06	51.69	40.55	19.66	0.59	239.62
COSF 10A x LTRR 341	52.00	165.40	14.20	83.00	44.10	4.88	72.88	42.26	31.80	0.50	986.79
COSF 12A x IR 6	57.50	187.68	18.64	89.50	39.25	4.53	88.59	39.68	33.64	0.55	457.42
COSF 12A x CSFI 99	51.00	185.45	14.00	82.50	47.25	5.32	53.94	40.90	23.02	0.64	518.79
COSF 12A x RHA-1-1	43.00	131.93	12.49	76.50	36.40	5.10	50.43	39.55	23.51	0.56	333.49
COSF 12A x LTRR 341	51.50	182.00	14.33	83.50	40.40	4.98	79.45	40.85	32.34	0.54	420.20
COSF 13A x IR 6	55.50	183.00	18.06	87.50	37.55	4.66	84.53	38.87	32.89	0.52	334.00
COSF 13A x CSFI 99	49.00	178.02	17.24	79.00	46.30	5.79	69.06	41.68	28.74	0.63	872.14
COSF 13A x RHA-1-1	47.00	154.05	13.69	78.00	42.85	5.69	55.89	40.66	21.50	0.50	143.96
COSF 13A x LTRR 341	53.50	154.00	15.36	86.00	35.10	4.90	69.85	36.57	25.55	0.48	752.75
CMS 104A x IR 6	60.00	176.20	14.40	93.50	41.65	4.12	52.23	38.90	20.27	0.53	1141.23
CMS 104A x CSFI 99	50.00	162.00	16.23	82.00	40.35	3.96	63.80	35.97	23.11	0.66	307.19
CMS 104A x RHA-1-1	48.50	149.45	13.40	80.00	40.55	5.28	34.29	35.92	12.35	0.56	2598.85
CMS 104A x LTRR 341	56.00	162.82	15.65	86.50	41.70	5.83	63.04	40.17	25.27	0.46	344.89
CMS 112A x IR 6	57.50	171.30	15.41	88.00	39.70	4.05	79.58	40.87	32.56	0.55	527.52
CMS 112A x CSFI 99	54.50	175.70	18.55	85.50	35.45	4.68	78.70	36.22	28.71	0.64	65.96
CMS 112A x RHA-1-1	49.00	167.82	14.59	81.50	36.95	4.93	74.87	37.87	28.30	0.58	1468.83
CMS 112A x LTRR 341	49.00	157.42	16.68	80.50	39.90	7.55	48.10	36.35	17.47	0.55	84.85
CMS 207A x IR 6	53.00	167.02	17.37	84.00	39.20	4.50	55.84	38.26	21.42	0.57	396.03
CMS 207A x CSFI 99	51.50	159.90	15.36	83.50	38.30	4.74	59.00	35.09	20.77	0.66	239.74
CMS 207A x RHA-1-1	49.50	143.20	16.38	80.00	37.35	6.02	85.52	37.37	31.94	0.61	772.29
CMS 207A x LTRR 341	58.00	158.39	16.91	90.00	32.95	5.22	82.61	34.72	28.71	0.58	180.06
CMS 519A x IR 6	60.50	191.35	19.63	93.00	42.45	4.95	74.66	35.83	26.75	0.64	126.76
CMS 519A x CSFI 99	57.00	199.98	15.03	88.50	43.40	5.11	60.90	35.54	21.65	0.67	403.69
CMS 519A x RHA-1-1	55.50	166.20	14.63	87.50	36.35	4.95	56.83	29.38	16.72	0.58	1142.70
CMS 519A x LTRR 341	54.50	174.10	17.50	88.50	35.00	5.35	58.45	33.58	19.63	0.57	374.04
RM 248A x IR 6	59.00	183.80	17.76	90.00	39.90	4.64	80.87	40.09	30.41	0.56	369.84
RM 248A x CSFI 99	53.00	174.00	16.75	86.00	38.20	6.25	77.75	40.51	31.60	0.63	128.09
RM 248A x RHA-1-1	57.00	168.84	22.70	89.00	37.10	8.45	31.22	31.94	9.91	0.61	1455.74
RM 248A x LTRR 341	53.00	129.32	13.59	83.50	32.65	6.97	32.09	31.52	9.63	0.44	121.11
RCR CMS 38A x IR 6	58.00	178.00	19.24	89.50	42.55	5.56	87.19	38.52	33.54	0.52	843.08
RCR CMS 38A x CSFI 99	51.50	171.57	13.68	85.00	41.55	5.12	64.99	36.62	23.83	0.63	182.64
RCR CMS 38A x RHA-1-1	50.50	159.95	20.19	83.00	36.80	5.45	65.53	37.28	24.40	0.47	2201.41
RCR CMS 38A x LTRR 341	57.50	166.10	16.47	89.00	35.40	5.91	84.47	35.40	29.84	0.40	226.80

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Table 6. Mid-parental heterosis of hybrids for yield and its component traits in sunflower

Lines x testers	Days to 50% flowering	Plant height (cm)	Head diameter (cm)	Days to maturity	Volume weight (g/100ml)	100 seed weight (g)	Seed yield / plant (g)	Oil content (%)	Oil yield / plant (g)
COSF 6A x IR 6	-2.9	20.71	-9.09	-2.43	28.58	15.73	121.17	21.1	161.31
COSF 6Ax CSFI 99	-1.85	18.49	6.02	-2.87	11.57	22.35	55.61	-1.2	55
COSF 6A x RHA-1-1	-1.94	20.35	13.92	-4.22	11.58	11.04	140.4	-9.22	119.14
COSF 6A x LTRR 341	-4.8	-15.21	-10.99	-4.74	-19.45	-31.08	-7.2	-7.82	31.09
COSF 10A x IR 6	5.63	58.07	23.89	1.68	45.59	28.07	66.11	7	143.59
COSF 10A x CSFI 99	-3.88	42.55	2.15	-2.69	22.72	35.03	47.2	-0.05	44.64
COSF 10A x RHA-1-1	-7.14	63.81	50.93	-4.7	28.79	27.87	34.02	7.5	33.83
COSF 10A x LTRR 341	-5.02	31.2	3.92	-4.05	35.8	-3.89	44.56	11.86	68.03
COSF 12A x IR 6	0.44	48.89	50.5	0.56	18.42	14.61	194.51	6.57	189.08
COSF 12A x CSFI 99	0	38.71	-2.1	-0.9	25.68	11.31	16.45	1.75	24.2
COSF 12A x RHA-1-1	-11.34	17.86	9.32	-3.47	3.06	-10.01	34.41	3.5	62.67
COSF 12A x LTRR 341	-5.07	34.8	5.72	-2.91	12.71	-4.73	60.94	6.72	73.03
COSF 13A x IR 6	-4.72	49.14	46	-3.31	25.17	15.92	257.35	5.74	270.64
COSF 13A x CSFI 99	-5.77	36.57	20.69	-6.78	34.4	19.46	73.1	4.94	82.27
COSF 13A x RHA-1-1	-5.05	41.87	19.98	-3.41	33.18	-0.87	79.74	7.74	83.88
COSF 13A x LTRR 341	-3.17	16.97	13.44	-1.71	7.34	-7.55	62.67	-3.25	60.44
CMS 104A x IR 6	-3.61	12.7	1.19	0	35.45	-6.42	81.83	8.57	92.41
CMS 104A x CSFI 99	-10.71	-1.22	0.5	-6.55	14.63	-24.31	41.89	-7.28	32.59
CMS 104A x RHA-1-1	-9.35	5.07	0.98	-4.48	23.16	-13.77	-5.18	-2.41	-7.51
CMS 104A x LTRR 341	-5.49	-1.51	1.62	-4.42	24.66	2.6	31.31	8.94	43.65
CMS 112A x IR 6	-2.54	17.89	12.52	-3.83	35.84	0.43	197.38	14.06	232.53
CMS 112A x CSFI 99	3.32	14.87	18.83	-0.29	5.27	-3.6	83.02	-6.63	72.07
CMS 112A x RHA-1-1	-2.49	27.92	14.57	-0.31	17.68	-14.22	118.92	2.89	124.47
CMS 112A x LTRR 341	-12.5	2.05	12.21	-9.04	24.98	42.16	4.46	-1.41	3.7
CMS 207A x IR 6	-10.92	28.53	29.92	-8.45	43.72	39.92	63.75	15.77	92.5
CMS 207A x CSFI 99	-3.29	16.21	0.49	-2.91	20.72	17.35	17.2	-2.53	15.22
CMS 207A x RHA-1-1	-2.46	23.62	31.99	-2.44	26.83	22.07	105.87	9.84	129.04
CMS 207A x LTRR 341	2.65	14.02	16.3	1.41	9.92	16.32	54.74	1.87	57.92
CMS 519A x IR 6	-2.42	39.97	41.89	-0.8	54.64	61.37	297.02	16.49	366.75
CMS 519A x CSFI 99	2.24	38.53	-4.57	0.57	36.05	31.36	73.78	5.42	71.45
CMS 519A x RHA-1-1	4.23	35.56	13.63	4.17	22.7	3.5	116.54	-7.41	95.55
CMS 519A x LTRR 341	-7.63	19.51	16.63	-2.48	16.09	23.09	53.45	5.61	53.53
RM 248A x IR 6	-2.48	25.63	41.57	-3.49	37.35	33.24	261.92	15.17	287.08
RM 248A x CSFI 99	-2.3	13.02	15.84	-1.71	14.03	45.06	101.5	7.26	114.24
RM 248A x RHA-1-1	10.14	27.72	95.94	6.59	18.82	62.58	4.82	-10.75	-7.19
RM 248A x LTRR 341	-7.83	-16.71	-0.95	-7.48	2.83	46.51	-22.92	-12.07	-35.44
RCR CMS 38A x IR 6	-4.13	18.47	32.55	-2.45	34.65	34.5	190.73	13.82	229.42
RCR CMS 38A x CSFI 99	-5.07	8.66	-16.74	-1.16	15.26	3.28	40.58	-0.49	39.53
RCR CMS 38A x RHA-1-1	-2.42	17.49	48.95	1.22	8.96	-6.76	75.07	7.08	87.69
RCR CMS 38A x LTRR 341	0	4.33	5	0.28	3.21	9.34	71.43	1.51	73.16

Table 7. Better parental heterosis of hybrids for yield and its component traits in sunflower

Lines x testers	Days to 50% flowering	Plant height (cm)	Head diameter (cm)	Days to maturity	Volume weight (g/100ml)	100 seed weight (g)	Seed yield / plant (g)	Oil content (%)	Oil yield / plant (g)
COSF 6A x IR 6	-7.14	15.2	-11.43	-5.24	13.95	5.83	72.54	10.6	91.36
COSF 6Ax CSFI 99	-7.83	17.73	-4.32	-6.11	11.2	10.27	25.89	-2.61	26.92
COSF 6A x RHA-1-1	-12.17	3.27	2.61	-11.67	5.26	-11.79	135.22	-14.92	109.88
COSF 6A x LTRR 341	-5.22	-16.48	-16.04	-5	-22.89	-42.18	-28.06	-13.48	6.68
COSF 10A x IR 6	-3.17	38.86	22.66	-4.71	43.54	12.34	14.76	-1.11	61.2
COSF 10A x CSFI 99	-5.71	19.67	-9.14	-2.98	9.8	27.06	40.36	-0.19	38.53
COSF 10A x RHA-1-1	-13.33	60.55	37.96	-8.98	22.11	5.3	14.71	2	9.59
COSF 10A x LTRR 341	-8.77	9.37	-3.47	-7.26	26.91	-16.15	30.68	6.3	59.72
COSF 12A x IR 6	-8.73	40.8	50.32	-6.28	6.25	-2.27	106.22	-2.6	92.59
COSF 12A x CSFI 99	-0.97	24.8	-13.58	-1.79	23.53	8.14	8.57	0.38	17.45
COSF 12A x RHA-1-1	-16.5	11.05	0.73	-7.27	-1.46	-23.88	17.39	-2.92	34.61
COSF 12A x LTRR 341	-9.65	20.35	-2.58	-6.7	9.37	-14.43	42.46	0.26	62.41
COSF 13A x IR 6	-11.9	37.28	46	-8.38	22.51	-2.31	180.74	-2.31	175.42
COSF 13A x CSFI 99	-8.41	19.8	6.42	-7.6	21.05	17.7	39.01	4.75	46.67
COSF 13A x RHA-1-1	-12.15	37.42	10.67	-8.77	27.15	-15.15	74.22	2.19	80.03
COSF 13A x LTRR 341	-6.14	1.84	4.42	-3.91	1.01	-15.89	25.25	-8.09	28.33
CMS 104A x IR 6	-4.76	-1.78	-10.5	-2.09	29.55	-25.56	29.76	2.56	32.79
CMS 104A x CSFI 99	-18.7	-9.7	0.15	-10.38	5.49	-28.55	28.42	-9.28	17.94
CMS 104A x RHA-1-1	-21.14	-16.69	-16.72	-12.57	20.33	-21.27	-14.81	-5.3	-19.1
CMS 104A x LTRR 341	-8.94	-9.24	-2.73	-5.46	20	0.09	13.04	5.89	26.9
CMS 112A x IR 6	-8.73	8.9	2.6	-7.85	35.26	-15.54	119.11	7.75	136.33
CMS 112A x CSFI 99	-0.91	11.7	14.51	-2.29	-7.32	-4.78	58.41	-8.65	46.52
CMS 112A x RHA-1-1	-10.91	6.69	-2.86	-6.86	9.64	-26.42	106.14	-0.15	105.44
CMS 112A x LTRR 341	-14.04	0.08	11.05	-10.06	14.82	29.64	-13.75	-4.17	-12.28
CMS 207A x IR 6	-15.87	25.3	20.88	-12.04	33.56	37.46	9.49	13.42	30.21
CMS 207A x CSFI 99	-8.04	7.6	-5.19	-5.11	0.13	-3.66	15.69	-11.49	5.97
CMS 207A x RHA-1-1	-11.61	13.11	13.99	-9.09	10.83	-10.22	67.69	4.72	94.16
CMS 207A x LTRR 341	1.75	4.74	14.96	0.56	-5.18	-10.31	48.13	-3.03	44.2
CMS 519A x IR 6	-3.97	36.56	28.3	-2.62	44.63	51.38	265.8	6.21	360.72
CMS 519A x CSFI 99	-6.56	34.58	-7.22	-3.8	13.46	3.97	22.58	-10.35	10.46
CMS 519A x RHA-1-1	-9.02	18.61	-4.38	-4.89	7.86	-26.12	77.15	-17.67	46.11
CMS 519A x LTRR 341	-10.66	15.13	14.38	-3.8	0.72	-8.16	4.81	-6.23	-1.43
RM 248A x IR 6	-6.35	15.38	39.62	-5.76	35.95	25.58	194.18	11.72	206.97
RM 248A x CSFI 99	-8.62	9.23	3.4	-5.49	-0.13	27.06	56.5	2.17	61.27
RM 248A x RHA-1-1	-1.72	5.99	78.46	-2.2	10.09	26.12	-2.68	-10.99	-13.42
RM 248A x LTRR 341	-8.62	-18.82	-7.65	-8.24	-6.04	19.76	-42.46	-12.17	-51.66
RCR CMS 38A x IR 6	-7.94	6.46	15.49	-6.28	25.7	11.32	103.81	13.46	130.4
RCR CMS 38A x CSFI 99	-11.21	2.61	-17.89	-3.41	8.63	2.51	30.82	-7.64	21.59
RCR CMS 38A x RHA-1-1	-12.93	-4.34	21.19	-5.68	8.71	-18.66	53.18	4.47	67.61
RCR CMS 38A x LTRR 341	-0.86	-0.66	-1.14	-0.56	1.87	1.55	51.46	-1.13	49.87

Table 8. Correlations of D^2 and dissimilarity values with hybrid performance and heterosis

Characters	D^2 values			Dissimilarity values		
	Mean performance	Mid-parent heterosis	Better parent heterosis	Mean performance	Mid-parent heterosis	Better Parent heterosis
Days to 50% flowering	-0.14	0.13	-0.25	-0.13	0.16	0.03
Plant height (cm)	-0.06	0.08	-0.25	0.39**	0.29*	0.32*
Head diameter (cm)	0.09	0.22	0.01	0.13	0.01	0.01
Days to maturity	-0.13	0.29*	-0.18	-0.09	0.12	0.1
Volume weight (g/100ml)	0.08	0.08	0.15	0.31*	0.22	0.03
100 seed weight (g)	0.1	-0.21	-0.27*	-0.03	0.23	0.25
Seed yield / plant (g)	-0.17	-0.09	-0.06	0.01	0.01	0.05
Oil content (%)	-0.13	-0.11	-0.01	-0.01	-0.09	-0.15
Oil yield / plant (g)	-0.19	-0.11	-0.09	-0.02	-0.01	0.02

*, ** Significance at 5 and 1 % level respectively.

correlation analysis (**Table 8**). The D^2 values did not show any association with hybrid performance for all traits. Hence, the results indicated that mean performance of hybrids could not be predicted based on phenotypic diversity analysis. However, D^2 values were correlated significantly with mid-parental heterosis for days to maturity and better parent heterosis for hundred seed weight. Hence, the diverse parental combinations at phenotypic level may produce more mid parental or better parent heterosis for these traits. In contrast, the dissimilarity indices had significant and positive association with hybrid performance for plant height (0.39) and volume weight (0.31). Also, dissimilarity indices recorded significant and positive association with mid (0.29) and better parent (0.32) heterosis for plant height. These results indicated that diverse parental combinations at the molecular level may produce hybrids with increased performance for plant height and volume weight. Similar superior performance of hybrids can be expected for mid and better parent heterosis for plant height. This result in in accordance with the findings of Xiao *et al*. (1996) in rice; Sureja *et al*. (2006) in ash gourd and Gupta *et al*. (2018) in pearl millet and Somashekhar *et al*. (2020) in cotton. Hence, the molecular level diversity could be correlated with heterotic expression of two traits only and not for oil yield and other component traits. This study may be extended further with more number of parental lines and molecular markers over various environments for utilization of these results in the heterosis breeding program of sunflower.

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