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Association of SSR based genetic distances with heterosis in sunflower

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Abstract

Aim : Development of commercial hybrid of sunflower on basis of best inbred combination remains a key challenge to sunflower breeders. In the current investigation, heterosis of F_1 hybrids, parental genetic diversity and correlation between genetic distance and level of heterosis were estimated.

Methodology : Thirty five parental genotypes (3 CMS A lines and 32 R lines) and their hybrids were assessed for physio-morphological, yield and quality traits. Heterosis was measured as mid-parent and better parent heterosis. Among parents, SSR marker based genetic distances were calculated using DARwin software. Correlation between heterosis and genetic distances was carried out by Karl Pearson's simple correlation method.

Results : Range of genetic distances, based on SSR marker analysis, varied from 0.32-0.73. Genetic distance had significant positive correlation with the heterosis for oil content ($r = 0.22$ $p < 0.05$) and linoleic acid ($r = 0.32$ $p < 0.05$), but negative correlation was observed for days to maturity, test weight, volume weight, stearic acid and oleic acid. There was no significant correlation between genetic distance and heterosis for seed yield and other agronomic traits.

Interpretation : Although, genetic distance is poor predictor of heterosis, dependence of oil content on genetic distance among parental lines may be used for designing an effective breeding program for sunflower.

Key words: Dice co-efficient, Genetic distance, *Helianthus annuus*, Heterosis, SSR markers

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Introduction

Sunflower (*Helianthus annuus* L.) is an annual crop grown for its high quality edible oil rich in polyunsaturated fatty acids and vitamins (Gossal *et al.*, 1988). Due to the possibility of using its oil as raw material for manufacturing biodiesel, it is preferred by farmers, agriculture professionals and companies worldwide (Backes *et al.*, 2008). Exploitation of heterosis through hybrid breeding is a milestone in plant breeding (Duvick, 2001), therefore, heterosis must be utilized as sunflower hybrid cultivars are superior to open pollinating varieties in terms of uniformity and yield (Beg *et al.*, 1984). The objective of sunflower breeding therefore, is to produce hybrids with higher productivity, increased oil content and resistance to biotic and abiotic stresses. So, for successful hybrid breeding information about genetic distances among the parental lines is a prerequisite.

Estimation of genetic diversity in sunflower genotypes and identification of heterotic groups has been carried out on the basis of both morpho-physiological traits (Kholghi *et al.*, 2011) as well as molecular markers (Darvishzadeh *et al.*, 2010). As the production of hybrids is costly, prediction through heterosis is desirable. Through marker based screening of parental lines, the efficiency of hybrid breeding programs can be increased, which in turn results in prediction of high mean oil percentage before field evaluation (Gvozdenovic *et al.*, 2009). In several crops such as alfalfa (Riday *et al.*, 2003), maize (Schrag *et al.*, 2006) and wheat (Sud *et al.*, 2010) studies on parental lines diversity have been the basis for prediction of hybrids performance, showing different levels of correlation between F_1 hybrid performance and genetic distance of parental genotypes. Review of literature has revealed less work on the prediction of heterosis and hybrid performance using molecular markers based parental diversity in sunflower (Cheres *et al.*, 2000). In view of the above, the present investigation was carried out to analyze the parental genetic diversity using SSR primers and to find the association between genetic diversity among parental lines with mid and better parent heterosis in sunflower.

Materials and Methods

Experimental site: The present study was carried out in the Breeding Program of Oilseed Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Experimental details: Thirty five parental lines (32 R lines and 3 CMS A lines), developed in breeding program (Table-1) and selected on the basis of their agronomic performances, were crossed in a line $\times$ tester manner, during *kharif* 2014. To generate 96 crosses, the cytoplasmic male sterile lines and restorer lines were sown in the same plot for crossing efficiently.

At the time of flowering, to prevent open pollination all the heads in lines and testers were covered with cloth bags. Pollen from restorer lines (R lines) was collected separately with the help of a camel hair brush in petri dishes during morning hours (9:00 to

11:00 a.m.) and used to pollinate each of the cytoplasmic male sterile lines (A lines) separately and the cloth bag was replaced after pollination. Pollination was carried out till all the florets in the capitulum showed signs of drying. F_1 seeds from female head were collected, dried and threshed. Hybrid evaluation was done with well filled seeds that were separated out from each cross. Randomized complete block design was used for evaluating a set of 96 hybrids along with parents (in a plot of 1.2×3 m) with three replications during spring 2015. Crop was raised according to the recommended package of practices.

Data were recorded for morpho-physiological traits like days to 50% flowering (DF), days to maturity (DM), plant height (PH), head diameter (HD), stem girth (SG), seed yield/plant (SY), number of filled seeds/head (NF), autogamy per cent (A), test weight (TW), volume weight (VW), hull content (HC), biological yield (BY), harvest index (HI), oil content (OC) and fatty acid composition *viz.*, palmitic acid (PA), stearic acid (SA), oleic acid (OA) and linoleic acid (LA) of thirty five parental lines and corresponding cross combinations. Five randomly selected plants were critically observed for recoding data of all morpho-physiological traits whereas oil content was measured using nuclear magnetic resonance (NMR) – MQC-5 analyzer (Oxford, London) supplied with preloaded “easy cal” software and Gas Liquid Chromatography (GLC) was used for estimating per cent fatty acids.

Estimation of heterosis: Heterosis was calculated following the method of Hayes *et al.* (1955). Heterosis over mid-parent (MP) and better parent (BP) was estimated using mean performances for each trait.

DNA isolation: For DNA extraction, young leaves of 30-day-old seedlings grown in growth chamber were collected. From these young leaves DNA of all 35 parental lines (Table 1) was extracted using Cetyl Trimethyl Ammonium Bromide method (Murray and Thomson, 1980).

Assessment of genetic diversity: In total, fifty two SSR markers were selected from relevant literature available, from which thirty four markers showed polymorphism (Table 2). These polymorphic microsatellites were then utilized for diversity analysis among thirty five parental lines. Temperature regime for particular SSR primer pair was adjusted on the basis of their nucleotide sequence. Temperature profile for the amplification varied from 41.9°C to 57.6°C. The formula provided by Mazers *et al.* (1991) was used to calculate melting temperature (T_m) whereas annealing temperature of primer ranged 2°C below melting temperature. After amplification of DNA product in PCR, 2.5% electrophoresis gel prepared in 0.5X TBE buffer was used for resolution.

Statistical analyses: Data recorded for five random plants were pooled and average of all three replications for each trait was used for further analysis. Heterosis over mid-parent and better parent was estimated as per cent deviation of F_1 hybrid

performance from the mid-parent and better parent as suggested by Matzinger *et al.* (1962) and Fonseca and Patterson (1968). Data of SSR analysis was presented in matrix of binary trait so as to assess polymorphism between the parental lines. For each microsatellite, (SSR) marker total number of alleles was recorded in all the genotypes by giving the number to amplified alleles as 1, 2, 3 and so on. Presence of band was recorded as 1 and absence was recorded as 0 in a binary matrix.

For analyzing genetic diversity among 35 parental lines, computer software programme - DARwin suggested by Perrier and Jacquemond-Collet (2006) was used. Dissimilarity matrix for SSR primers to find out genetic relationships was constructed using Dice coefficient of associations. Dendrogram was constructed using unweighted pair group method with arithmetic mean (UPGMA). Genetic distances measured according to SSR primers analysis were correlated with MPH and BPH. Correlation between the extent of heterosis with parental divergence was carried out following Karl Pearson's simple correlation coefficient method as suggested by Mortant (1923). Significance of correlation was determined using table of Snedecor (1959).

Results and Discussion

In the present study, reliable correlation between genetic distance and heterosis was observed for some economically important traits. Diversity among lines at molecular level was positively associated with days to maturity, quality oil and oil content, hence, hybrids developed using those diverse lines would be of short duration and produce higher quantity of oil, rich in fatty acids. Heterosis is the superiority of F_1 hybrid, as F_1 hybrids are higher yielding than parental lines for some of the traits, or they excel both the parents for multiple traits. Therefore, heterosis for one or multiple traits, especially for traits such as early maturity, seed yield, oil content etc., is of keen importance. In the present study, the hybrids produced by crossing three different maternal lines (CMS 67A, CMS 47A and CMS 11A) to thirty two common paternal R-lines were divided into three groups, i.e., 67A group, 47A group and 11A group. Hybrids within the group may be considered as unique genetic models and allow us to analyze contribution of maternal parents into heterosis.

The data presented in Table (3) shows mean values of heterotic effects for each maternal group, overall mean and range of MPH and BPH for all the examined traits. Hybrids belonging to 67A maternal group had maximum heterotic effects for seed yield (154.42) and least for days to maturity (-4.76), hence, these would be early maturing as well as high yielding. Reddy and Nadaf (2013) reported that majority of hybrids involving CMS 17A and CMS-1030A as female parent recorded highest heterotic effects for days to 50% flowering, days to maturity, head diameter, test weight and seed yield. In contrast to this, 11A produced hybrids with maximum heterotic effects for oil content (9.17) with lesser percentage of stearic acid (11.23) which is undesirable and high amount of linoleic acid (2.36) an important polyunsaturated fatty acid. The cross combination 67A $\times$ P184R (77.39gm) with 239.9% MPH and 217.5% BPH was the best performing hybrids for seed yield per plant. Whereas, for another important trait, i.e., oil content 11A $\times$ P178R had maximum heterosis over mid-parent (33.34%) as well as better parent (31.95%), however 47A $\times$ P197R had highest per se performance with 46.3% oil content. While considering quality parameters 11A $\times$ P187R (60.6%) and 47A $\times$ P187R (64.3%) performed well for per cent linoleic acid and oleic acid, respectively. Sapkale *et al.* (2016) reported similar findings where they studied 50 sunflower hybrids for heterosis and reported that hybrid CMS-148 $\times$ SVR-467 (21.19%) exhibited positive and significant heterosis for seed yield per plant over check Phule Raviraj. The overall average heterotic effects over mid-parent observed in the present study were highest for biological yield per plant (160.52%) followed by seed yield per plant (142.17%). Although the amount of heterosis varied from trait to trait, the overall average MPH was in the desired direction, except for hull content (8.58), harvest index (-4.78%) and linoleic acid (-5.93%). Like MPH, biological yield per plant also showed highest BPH (112.70%), followed by seed yield per plant (100.10%).

Traits viz. harvest index, oleic acid and linoleic acid showed better parent heterosis in negative direction which is undesirable for these traits. The results are in line with the findings of Dutta *et al.* (2011) and Rathi *et al.* (2016) regarding increase over mid-parent and better parent heterosis for seed yield and oil content. It was observed that crossing different maternal lines with common paternal lines results in different levels of heterosis

Table 1 : List of genotypes evaluated in the study

R lines							
1	P172R	10	P184R	19	P196R	28	P207R
2	P173R	11	P185R	20	P197R	29	P209R
3	P174R	12	P187R	21	P198R	30	P211R
4	P175R	13	P188R	22	P200R	31	P212R
5	P176R	14	P189R	23	P201R	32	P213R
6	P177R	15	P190R	24	P202R	A lines	
7	P178R	16	P192R	25	P203R	33	67A
8	P179R	17	P194R	26	P205R	34	47A
9	P180R	18	P195R	27	P206R	35	11A

Table 2 : Detail about SSR primers used for estimating parental diversity

Primer	Ta	No. of alleles	Forward Primer (5'-3')	Reverse Primer (5'-3')
HA 2505	41.9	2	GTGTCATGACTCGGT	GGACAATGTGATTGC
HA 2682	49.1	2	CACAATCGTTTCTTTCCAAAA	ACCCATATGCCACTCATAA
HA 2879	42.7	2	CATACCGTTCTTGTTT	CAACCTCCTAGGTCA
HA 3513	47.6	2	TGACCCATTCAACTTCTTAA	TCATGGTTCTGATGAGAAT
HA 3555	48.3	2	GATATCTCTCATAAGTGCCG	GGTCTTGATGATGACGAA
HA 3638	55.2	2	GACATAATCACTAGTTGTTGGTGC	CTCCTCCACCTCAACAATTTT
HA 3651	51.0	2	GGAATTATCCATTGTAGGTTTGG	GGATGATTGATTAATTGAGGG
HA 3691	56.6	2	GAATGAAGCATGTGGAAGGCGG	GTGGAGGTGATGATGGTATGAG
HA 3878	49.8	2	TTTGTTTAGCATCATCATC	GAGACCCTAACCATTAACATGA
HA 4057	50.7	2	AAACCCCTCCGACTTATCTC	TAAAGAGAGAGCCCCAACAG
HA 4136	52.8	4	CCTATTCCTGATAATCTACTAAGC	GGTAGCATGCTTACATTAAGATG
HA 4142	53.6	2	GAGTCGACATTTTCGGAAATCG	CTTCATCTTCTGACACCCAAC
HA 4149	54.3	2	CAAAAACCTCTCTCCGTTGGC	GACTCCAAAGTCCACCAAAATC
ORS 10	53.5	2	CTCCACCACACAATCTCCG	TGTGGAGTTTGCCAGAACTG
ORS 1088	54.1	2	ACTATCGAACCTCCCTCCAAAC	GGATTTCTTTTCTCTTTGTGGTG
ORS 123	51.4	3	GAAAACCCATGCAGGCATAC	ACATCCATCACAGTCCATTTTG
ORS 149	57.6	2	GCTCTCTATCTCCCTTGACTCG	TGCTCTAAGATCTCAGGCGTGC
ORS 154	51.4	2	GCACCTTTGGTGAGGAGATA	TGCATCAGTAGCTATTGTCTAT
ORS 317	51.9	4	TTTGGCAGTTTGGTGGCTTA	GGTCGTATGCTTAATTTCTTCTCT
ORS 331	51.9	2	TGAAGAAGGGTTGTTGATTACAAG	GCATTGGGTTTACCATTCTT
ORS 35	51.7	3	TTTTCTCAGCTTCAGTTTCAGC	TTTGGATCAAGAAGTTCATGGC
ORS 382	52.8	2	ACCGATGGACGATGATCCTA	TGCACCTGGAATGGCTAGAT
ORS 443	52.1	2	GGAAGTAGGAAGAACAAAGAGCA	TTGGAGTGTGAATTTGGATTTG
ORS 47	55.0	2	TGAGATTCCTCGTACTTCATCTG	CCATAGGATGCATAGGAAGAGG
ORS 476	51.1	2	GCGTCGAGTCATTGGGATAA	TGCAAACGCATCTACGATATT
ORS 591	52.6	2	GTTGCAGGAAAGGTCGAAAG	TTTATTCTACGCAGACAAGACG
ORS 607	52.4	2	GCCTGCACATGAGAATGACA	CTAATACAAAGCAACCCGACAA
ORS 613	52.5	2	GTAACCCCTAGGTCAATTTGCAG	ATCTCCGGAACATTCTCG
ORS 70	52.5	2	GACCCTGGTCACCGAAGTTA	ATCTGAAATCGGACAAGATTCA
ORS 708	52.8	2	GCACCTCAACATCACGAATCC	CAACACCTTGGGAACACCTT
ORS 721	50.7	3	ATTCCATTGCGATTCGTT	TCCTCAACGCTTCACACAAG
ORS 809	53.0	2	GCCGGTTCTCTCATTCTTCTC	TCAGAAACAAACCACCACCA
ORS 822	54.3	2	CAATGCCATCTGTCATCAGCTAG	AAACAAACCTTTGGACGAAACTC
ORS 928	52.1	2	CATGGTTATTTGGTTTGGGTTT	GCTATTATCATGTCTTGTCTTTT

for different agronomic traits in F_1 . SSR markers known as microsatellites are commonly used molecular markers for characterization of genotypes. Being highly mutable loci, which could be present at various sites in a genome (Tautz, 1989; Morgante and Olivieri, 1993), SSRs are widely used for analyzing polymorphism among the genomes of same or different origins. Thirty four SSRs (Table 2) used in the present investigation revealed polymorphism of great extent between parental lines. Fig. 1 (A and B) shows a great extent of diversity among parental lines using ORS70 and HA4136, respectively. The number of alleles varied from 2 to 4 for each locus with 2.20 average alleles per locus.

These results were closer to those of Darvishzadeh *et al.* (2010) and Zeinalzadeh-Tabrizi *et al.* (2018) who observed 2.32 number of allele per locus in their study for characterizing 28 genotypes on the basis of molecular markers, resulting in too low

alleles per locus. However, contrasting results were observed by Zia *et al.* (2014) and Suresha *et al.* (2017) who reported 6.13 and 20.66 alleles per locus respectively. Amplified product size ranged from 50 to 350 bp. High genetic dissimilarity was revealed by dissimilarity matrix among all parental lines. CMS 11 A was found to be highly diverse with 0.59 mean genetic dissimilarity coefficient whereas range of genetic distances was 0.32 (67A × P188R) to 0.73 (11A × P174R). Genotypes having highest genetic distance can be used for hybridization as they are more diverse from each other.

With prospective to develop hybrids with desirable attributes, the breeders prefer genetically distant parents for hybridization, because it has been previously established in crop species such as in wheat (Sud *et al.*, 2010), rapeseed (Tian *et al.*, 2017) and maize (Fareghi *et al.*, 2018) that higher the divergence

Fig. 1 : DNA amplification pattern of 35 parental lines of *Helianthus annuus*. using (A) ORS70 and (B) HA4136 SSR primers.

Table 3: Mean values and range of heterosis (%) for morpho-physiological, yield and quality traits of F1 hybrids

Traits	Mid-parent heterosis					Better parent heterosis				
	Mean				Overall range	Mean				Overall range
	67A group	47A group	11A group	Overall		67A group	47A group	11A group	Overall	
DF	-6.22	-2.82	-2.90	-3.98	-15.87 – 3.34	-8.26	-6.82	-4.71	-6.59	-20.43 – 0.46
DM	-4.76	-4.04	-3.98	-4.26	-0.45 – (-0.17)	-6.20	-5.04	-4.99	-5.41	-11.76 – (-0.33)
PH	25.40	28.92	19.37	24.56	-11.42 – 52.94	18.29	22.29	13.06	17.88	-16.00 – 41.49
HD	35.42	27.87	26.60	29.96	-2.78 – 81.93	19.44	18.42	19.26	19.04	-11.90 – 66.78
SG	10.88	18.11	4.50	11.17	-14.29 – 43.21	4.90	8.13	1.18	4.74	-21.15 – 41.46
SY	154.42	140.39	131.70	142.17	-5.21 – 379.03	114.96	97.73	87.60	100.10	-7.06 – 217.55
NF	66.80	85.13	104.15	85.36	-4.25 – 252.57	45.97	47.24	59.99	51.07	-9.90 – 193.37
A	0.10	3.47	4.98	2.85	-7.97 – 18.20	-2.96	-1.44	-0.42	-1.61	-13.94 – 13.38
TW	50.94	35.23	17.85	34.67	-25.84 – 148.38	31.21	24.71	8.59	21.50	-35.69 – 63.15
VW	7.63	8.15	2.95	6.24	-11.33 – 26.61	4.47	5.13	0.16	3.26	-13.42 – 17.58
HC	-0.56	6.96	19.35	8.58	-25.84 – 49.70	-5.24	2.56	9.79	2.37	-35.90 – 42.86
BY	150.74	162.34	168.50	160.52	-0.64 – 495.99	115.60	118.12	104.37	112.70	-34.48 – 299.35
HI	1.84	-4.81	-11.36	-4.78	-48.21 – 71.73	-5.22	-10.24	-14.91	-10.12	-53.95 – 32.59
OC	0.94	3.60	9.17	4.57	-12.36 – 33.34	-6.43	-0.98	-0.22	-2.54	-23.83 – 31.95
PA	-5.09	8.48	-1.84	0.52	-42.79 – 41.75	-12.98	0.75	-8.49	-6.90	-49.39 – 46.00
SA	60.22	17.35	-11.23	22.12	-48.00 – 164.83	33.28	4.32	-30.58	2.34	-58.40 – 123.91
OA	7.94	16.72	-3.45	7.07	-42.76 – 64.82	-0.48	9.33	-13.67	-1.60	-49.35 – 53.56
LA	-7.21	-12.83	2.26	-5.93	-51.81 – 30.51	-13.96	-18.37	-8.53	-13.62	-35.93 – 34.28

Table 4 : Correlation coefficient of Genetic distance (GD) with Mid-parent heterosis (MPH) and Better parent heterosis (BPH) for morpho-physiological, yield and quality traits

	DF	DM	PH	HD	SG	SY	NF	A	TW	VW	HC	BY	HI	OC	PA	SA	OA	LA
GD vs MPH	0.05	-0.23**	-0.15	-0.11	-0.15	0.06	0.15	0.05	-0.19*	-0.17*	0.28**	0.01	0.04	0.22*	0.12	-0.42**	-0.28**	0.32**
GD vs BPH	0.02	-0.24*	-0.1	0	-0.04	0.03	0.06	-0.01	-0.09	-0.16	0.18*	-0.12	0.18*	0.08	0.15	-0.41**	-0.32**	0.23**

r(0.05)= 0.17; r(0.01)= 0.21; DF: days to 50% flowering; DM: days to maturity; PH: plant height; HD: head diameter; SG: stem girth; SY: seed yield/ plant; NF: number of filled seeds/ head; A: Autogamy per cent; TW: test weight; VW: volume weight; HC: hull content; BY: biological yield; HI: harvest index; OC: oil content; PA: palmitic acid; SA: stearic acid; OC: oleic acid; LA: linoleic acid

between genotypes, higher is the heterosis for some traits. Earlier studies done by sunflower breeders demonstrated that the genetic distance can not accurately predict hybrid performance unless DNA markers used in the analysis were linked to the genes affecting the trait (Charcosset *et al.*, 1991 and Bernardo, 1992). The estimates of simple correlations (*r*) of genetic distance (GDs) with *F₁* performance for eighteen morpho-physiological, yield and quality traits are presented in Table (4). The correlation coefficient between genetic distance and mid-parent heterosis was significant for the traits like days to maturity (-0.23), test weight (-0.19), volume weight (-0.17), hull content (0.28), oil content (0.22), stearic acid (-0.42), oleic acid (-0.28) and linoleic acid (0.32) while for better parent days to maturity (-0.24), hull content (0.18), harvest index (0.18), stearic acid (-0.41), linoleic acid (0.23) and oleic acid (-0.32) was significant.

Usatov *et al.* (2014) also observed significant positive correlation between the GD among parental lines and MPH for seed yield. Whereas studies of Tian *et al.* (2017), Fareghi *et al.* (2018) and Nie *et al.* (2019) on various crop species such as rapeseed, corn and wheat showed low correlation of GD with heterosis for various traits. However, no correlation was observed by Gvozdenovic *et al.* (2009) between GD and heterosis in sunflower.

The observations of the present study showed that genetically diverse lines at molecular level results in the early maturing hybrids as correlation coefficient between GD and heterosis was significantly negative for days to maturity as shown in Table 4. While positive correlation was observed for other important traits such as oil content and linoleic acid illustrating

enhanced oil yield with quality fatty acid. Hence, significant correlation between genetic distances and heterosis for days to maturity (-0.24), oil content (0.22), stearic acid (-0.42) and linoleic acid (0.32) reveals that genetically diverse parental lines can be used for developing early maturing short duration hybrids having high percentage of oil, rich in polyunsaturated fatty acids.

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