

CORRELATION STUDIES OF SSR MARKER BASED GENETIC DISTANCE AND HETEROSIS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) one of the most important oilseed crops in India known for its quality oil. However, in recent years the area under cultivation is decreasing owing to crop being affected by biotic and abiotic stress. This situation necessitated development of higher heterotic hybrids involving diverse germplasm to break the yield plateau. An experiment was conducted at Main Agricultural Research Station, UAS - Raichur to evaluate 49 sunflower hybrids along with parents to determine the correlation between SSR based genetic distance (GD) and heterosis for nine quantitative traits. The 49 hybrids were derived by crossing seven CMS lines and seven restorers in line x tester design. Significant heterosis was recorded in hybrids for all nine traits studied. Genetic distance between pairs of tested CMS lines and testers ranged from 0.18 to 0.68. The correlation between genetic distance and heterosis was not significant for the most of characters studied. A highly significant positive correlation was observed between genetic distance and head diameter both at mid-parent ($r=0.48$; $p<0.01$) and better parent ($r=0.475$; $p<0.01$) heterosis level. However, significant negative heterosis was recorded between genetic distance and mid-parent heterosis for number of seeds per head ($r=-0.348$; $p<0.05$) and oil content ($r=-0.391$; $p<0.01$). The SSR markers included in the study are solely for their high PIC values. The poor correlation of GD with heterosis except for head diameter indicates the need to include the markers linked to yield contributing traits to help in to rely on marker based GD to predict hybrid performance. .

Key words: Sunflower, heterosis, genetic distance, correlation

INTRODUCTION

The cultivation of sunflower at commercial scale as an oilseed crop is worldwide. The largest traditional producer is Russia and other sunflower producing countries include Argentina, the European Union, USA, China, India, Turkey and South Africa. The world sunflower production is around 30 million tones and is being cultivated over an area of 20 million hectares. . In India, sunflower is being grown over an area of 0.69 million hectares with a production of 0.54 million tones with the productivity of 791kg per ha (Anon., 2015).

Sunflower being a highly cross pollinated is an ideal crop for exploitation of heterosis. The discovery of Cytoplasmic Male Sterility by Leclercq (1969) followed by fertility restoration system by Kinman (1970) provided the required breakthrough in the development of hybrids. Hybrids are also highly self fertile and resistant to diseases, thus resulting in enhanced seed set and seed filling (Seetharam, 1981). After sunflower being introduced to India as oil seed crop in early 1970's, the first sunflower hybrid BSH-1 was released during 1980 and thereafter several hybrids have been released. The exploitation of heterosis through hybrid breeding is one of the landmark achievements in plant breeding (Duvick, 2001) and particularly in sunflower (Seetharam, 1984). In last decade (2001-2010), 6 varieties and 11 hybrids have been released for commercial cultivation (Anon., 2014) in India.

In heterosis breeding programmes, a large number of experimental hybrids need to be and are routinely produced and tested to identify hybrid vigour. This requires huge resources and manpower. In

general, heterosis is considered as an expression of the genetic divergence among inbreds/parents used for crossing. Reliable prediction of single-cross performance is crucial in hybrid breeding as the evaluation of large number inbred lines in numerous cross combinations is difficult. Several prediction approaches have been suggested using phenotypic data with co-ancestry coefficients calculated from pedigree records or marker data (Schrage et al., 2009). The information on the genetic diversity and distance among the breeding lines and correlation between genetic distance and hybrid performance are important in determining breeding strategies, classifying the heterotic groups and predicting the hybrid performance.

Studies of genetic diversity in relation to hybrid performance have been undertaken in several crops. Investigations in corn, *Zea mays* L., have shown that the genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Schrage et al., 2006). Genetic diversity of different sunflower gene pools has been studied with enzymes (Tersac et al., 1993), RFLP markers (Hongtrakul, 1997) and SSR markers (Solodenko et al., 2005). However, the literature data on the predication of sunflower heterosis and hybrid performance by marker based genetic distance of the parental lines is scarce (Tersac *et al.* 1994, Cheres et al. 2000). The objective of the study was to identify the reliability of SSR markers to determine the genetic diversity and association between SSR based genetic diversity and heterosis for yield component traits in sunflower.

MATERIAL AND METHODS

Seven CMS lines (CMS-2A, CMS-821A, CMS-850A, R-10-46-2A, CMS-4A, CMS-6A, CMS-10A) and seven R-lines (R-GM-39, R-GM-41, R-GM-49, R-GM-69, 83-Br, R-393, R2F01120B) were crossed in L x T fashion during kharif-2013-14. The resultant 49 hybrids along with parents were evaluated for nine yield and yield contributing characters in RCBD design with three replications. Heterosis, expressed as per cent increase or decrease of derived F₁ over mid parent (average heterosis) and better parent (heterobeltiosis) was calculated for each character as per the method of Turner (1953) and Hayes *et al.* (1956).

Table 1: list of sunflower SSR primers used for the study

1. ORS-287	16. ORS-324	31. ORS-677
2. ORS -290	17. ORS-332	32. ORS-769
3. ORS-296	18. ORS-333	33. ORS-780
4. ORS-300	19. ORS-339	34. ORS- 807
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13 ORS-321	28. ORS-578	43. ORS- 1220
14. ORS-322	29. ORS- 628	44. ORS- 1245
15. ORS-323	30. ORS- 671	

The genomic DNA of 14 parental lines was extracted by following modified CTAB method. Forty four sunflower SSR primers were used for PCR amplification using gradient thermocycler. The amplified products were separated using 3.5% agarose gel electrophoresis. DNA polymorphism between two inbreds was estimated by comparison of amplified fragments. Jaccard similarity coefficient (j) was calculated according to Staub et al., (2000). Genetic distance (GD) among all parental lines was estimated as per formula $GD=1-j$ given by Spooner et al., (1996).

The values of genetic distances as measured by SSR markers were correlated with mid-parent heterosis and better parent heterosis to estimate their relationship using Pearson's coefficient of correlation. Correlations were done for hybrid combinations from each tester and lines separately. Significance of correlation was determined using the table of Snedecor (1959).

RESULTS AND DISCUSSION

The 49 sunflower hybrids derived by crossing seven CMS and seven restorers in LxT fashion were evaluated for yield and yield component traits along with parents. High degree of variation was observed for all characters studied in both parents and hybrids. The mean values of the hybrids were significantly higher than the parental lines for plant height, head diameter, 100 seed weight and seed yield per plant (Table 2).

Table 2: Mean values and coefficient of variation (V) for the sunflower parental lines and their hybrids.

Character	Female parent		F1 Hybrid		Restorer lines	
	Mean	V (%)	Mean	V (%)	Mean	V (%)
Plant height (cm)	102.38	29.9	144.90	9.98	107.5	15.07
Days to 50% flowering	64.00	2.77	65.50	1.94	63.00	5.59
Head Dia (cm)	14.76	1.85	17.50	1.49	11.04	1.20
No. of leaves	20.46	3.78	27.32	2.59	21.34	2.23
100 seed wt (g)	3.27	0.71	3.94	0.48	2.39	0.33
No. of seeds/head	1206.6	127.7	1179	117.0	1349	148.9
Volume wt (g/100ml)	36.36	2.06	40.06	1.74	37.66	2.83
Seed yield/pl (g)	30.13	2.59	35.42	2.98	27.30	4.06
Oil content (%)	34.65	3.98	37.81	1.38	34.70	2.95

The heterosis level for most of the traits studied was significantly superior viz., plant height, head diameter, 100 seed weight, seed yield per plant (Table 3). The highest level of mid-parent heterosis observed for 100 seed weight(39.28) followed by plant height (38.70) and head diameter (35.78). Whereas the highest level of better parent heterosis observed for plant height (34.65) followed by 100 seed weight(28.04) and head diameter (24.60).

Table 3: Mean and range of heterosis for nine quantitative traits in 49 sunflower hybrids

Trait	Mid-parent Heterosis		Better Parent Heterosis	
	Mean	Range	Mean	Range
Plant height (cm)	38.70**	6.8 – 88	34.65**	-5 – 61
Days to 50% flowering	3.25	-10.41 – 15.40	2.45	- 11.90 – 7.90
Head Dia (cm)	35.78**	8.35 – 79.03	24.60**	-2.73 – 46.51
No. of leaves	31.00	-2.75 – 37.43	28.20	-14.51 – 30.51
100 seed wt (g)	39.28*	11 – 45.40	28.04*	6.01 – 36.38
No. of seeds/head	-3.13	-11.77 – 11.37	-10.60	-23.20 – 11.54
Volume wt (g/100ml)	8.80	6.56 – 20.53	6.44	4.41 – 12.51
Seed yield/pl (g)	24.40*	6.96 – 58.16	19.50*	1.42 – 55.78
Oil content (%)	6.70	-4.51 – 18.80	5.81	-11.39 – 12.36

Forty four sunflower SSR primers were used to study genetic diversity among fourteen parental lines. Out of 44 primers used three primers failed to amplify and ten primers showed monomorphic amplification bands. The remaining 31 primers showed polymorphism with an average polymorphism of 39.65 % (PIC=39.65%). The number of amplified products ranged from 1 to 3 with an average of 1.21 bands per primer and 1.13 bands per primer were polymorphic.

The frequency of SSR polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands. The binary data was used to compute pair wise similarity coefficient (Jaccard, 1908). The genetic similarity computed considering data of SSR markers showed a wide range from 0.32 to 0.82 indicating the presence of high variability among 14 sunflower genotypes.

The highest similarity was observed between the parental genotype CMS 821A and CMS 2A (0.82) while the lowest similarity was observed between the parental genotypes viz., R-GM-41 and R-10-46-2A (0.32), R-GM-49 and CMS A6 (0.32).

Genetic diversity is the extent to which the heritable material differs within a group of plants, which is a result of evolution, including domestication and plant breeding. Assessing genetic diversity of cultivated crop plants is important to select proper genotype for hybridization programme. The sunflower genetic diversity and co-ancestry analysis have been carried out using RAPD (Arias *et al.*, 1995). The placement of individual cultivars into different accessions based on morphological attributes do not necessarily reflect the real genetic relationship.

The recent advances in molecular biology have provided the descriptors based on protein and DNA as an aid to plant breeding programme. Genetic diversity caused by sexual reproduction i.e. hybridization, selection and mutation results in genome changes from one base pair to entire chromosome. The molecular markers that are not influenced by environmental changes provide an opportunity to examine the genetic relationship between accessions more precisely. This can help in the rationalization of existing germplasm collections and allow future collection strategies towards specific objective. Molecular markers can be used as a valuable tool for identification of parental lines and varieties for protection of plant breeder's right. DNA markers also help in studying the evolutionary/phylogenetic relationship between inbreds and varieties.

The relation between SSR based genetic diversity among inbred lines and their hybrids performance dependent on the trait of interest examined. Correlation coefficient between genetic distance,

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The relation between SSR based genetic diversity among inbred lines and their hybrids performance dependent on the trait of interest examined. Correlation coefficient between genetic distance,

parental means and hybrid performance in terms of heterosis were not significant for most of the characters studied. However, significant correlation was observed for GD with both mid-parent ($r=0.48$) and better parent ($r=0.475$) heterosis. For both number of seeds per head and oil content, correlation between GD and mid-parent heterosis was significantly negative ($r=-0.348$ & $r=-0.391$).

The correlation between genetic distance and heterosis levels expressed was not significant for most of the traits studied except for head diameter, number of seeds per head and oil content. The SSR markers included in the study are solely for their high PIC values. The poor correlation of GD with heterosis except for head diameter indicates the need to include the markers linked to yield contributing traits to help in to rely on marker based GD to predict hybrid performance. (Charcosset *et al.*, 1991 and Bernardo *et al.*, 1992).

Tersac *et al.* (1994) described relationships between heterosis and enzymatic polymorphism of 39 sunflower populations. The correlation coefficients for all enzyme systems were too low to be used as predictors of the general combining ability, but when enzymatic systems were analyzed separately, four of them turned out to be useful markers for breeding purposes. Zeid *et al.* (2004) pointed out that the lack of association between heterosis and genetic dissimilarities for inter group hybrids may be explained by the absence of crosses between related parents i.e. by the absence of variation for parental relatedness: all crosses have unrelated parents.

In the present study the GD showed poor correlation with both mid-parent and better parent heterosis except for head diameter. Similar reports were done in previous studies on pepper, alfalfa, wheat and rapeseed (Diers *et al.* 1996, Geleta *et al.* 2004, Zeid *et al.* 2004, Riday *et al.* 2003).

CONCLUSION

The conclusion on the use and reliability of SSR based genetic distance to predict hybrid performance in terms of heterosis depends on use of large number of specific markers linked to yield contributing traits. A higher accuracy would also be possible by identification of molecular markers linked to combining ability.

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