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HETEROSIS IN TOP-CROSS HYBRIDS OF DIVERSE CYTOSTERILE SOURCES OF SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

Standard heterosis was estimated for eight quantitative traits in top-cross hybrids of three diverse cytoplasmic male sterile (CMS) sources of sunflower *viz., Helianthus petiolaris* (CMS-PET1), *Helianthus petiolaris* ssp. *fallax* (CMS-PEF1) and *Helianthus annuus* ssp. *lenticularis* (CMS-ANL2) maintained under different nuclear backgrounds. The top-cross hybrids were derived by crossing the above three CMS sources with 12 male parents in a line x tester design. Significant heterosis over two standard checks in the desirable direction was observed for all traits. All three sources under study advanced the maturity in most of the hybrids. CMS-PET1 was found to be the best compared with the other sources as far as oil content was concerned. Mean performance of the hybrids for achene yield and other economically important characteristics indicated that the hybrids derived in the cytoplasmic background of CMS-ANL2 were the best followed by CMS-PET1 and CMS-PEF1 suggesting that CMS diversification in heterosis breeding programs would be rewarding in sunflower.

Key words: cytoplasmic male sterility, diversification, heterosis

INTRODUCTION

Availability of cytoplasmic male sterile (CMS) and fertility restoring sources and the highly cross-pollinating nature of sunflower has made the exploitation of heterosis possible on the commercial scale. This has resulted in the development and release of several hybrids suitable for different agroclimatic situations in India as well as in the world. In India, the first ever sunflower hybrid, BSH-1, was released from Bangalore (Seetharam, 1980). Owing to the several advantages of hybrids over open pollinated varieties, more than 70% of the sunflower area is under hybrids. The cytoplasm of all hybrids under cultivation in the world is derived from a single

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source, *Helianthus petiolaris* (Leclercq, 1969). This has resulted in genetic uniformity for the cytoplasmic background in the crop. Prevalence of genetic uniformity of this kind over a large area could result in genetic vulnerability of hybrids if the cytoplasm becomes susceptible to a new strain of disease or pest similar to what happened in maize when *Texas* cytoplasm became susceptible to *Helminthosporium maydis* in USA (Tatum, 1971 and Anonymous, 1972). Among several strategies available to overcome this problem, diversification of CMS source itself is possibly the cheapest and most effective method. During last 15 years, several new sources of CMS have been identified in wild sunflower populations (Leclercq, 1971; Anaschenko *et al.,* 1974; Whelan and Dedio, 1980; Heiser, 1982; Serieys, 1987; Jan and Rutger, 1988; Havekes *et al.,* 1991 and Miller *et al.,* 1992). But, the nature and extent of genetic differences and breeding value of most of these new sources have not been studied in sufficient detail to include them in heterosis breeding programs. Hence, in the present study, an attempt has been made to study the levels of heterosis in 36 top cross hybrids derived by crossing 3 diverse CMS sources with 12 male parents in a line x tester design.

MATERIALS AND METHODS

Three diverse cytoplasmic male sterile lines *viz.,* 234A, 265A and 274A, representing *Helianthus petiolaris* (CMS-PET1) (Leclercq, 1969), *Helianthus petiolaris* ssp. *fallax* (CMS-PEF1) (Serieys, 1987) and *Helianthus annuus* ssp. *lenticularis* (CMS-ANL2) (Heiser, 1982) maintained under the nuclear background of HA 234B, HA 265B and HA 274B, respectively, and 12 diverse male parents, *viz.,* Morden, Acc. Nos. 218, 398, 400, 401, 409, 438, 652, 666, 693, 781 and 1091, were used in the investigation. The CMS sources and testers were obtained from Project Coordinating Unit (Sunflower), UAS, GKVK, Bangalore, India.

The CMS lines and testers were planted in the crossing block during *kharif* 1994-95 at experiment plots of Regional Research Station, GKVK, Bangalore, India. To achieve synchrony in flowering, staggered planting of both males and females was carried out. The capitula of CMS lines were covered with cloth bag a day prior to the opening of ray florets in order to prevent undesirable pollination. Similarly, the capitula of testers were also covered to collect pollen. Pollen from the testers was collected in petri plates and applied to the lines using camel hair brush during morning hours. The pollination was repeated till sufficient numbers of seeds were obtained in each of the combinations. At maturity, the crossed seeds of all 36 combinations and selfed seeds of 12 testers were collected for future evaluation.

The lines, testers and hybrids were evaluated by growing them along with two standard checks, *viz.,* MSFH-17 and KBSH-1, in a randomized complete block design in three replicates during summers of 1995-96 and 1996-97 at the experiment plots of Regional Research Station, GKVK, Bangalore, India. Since the restoration behavior of the testers selected was not known, as a precautionary measure to provide pollen to all those hybrids which would become sterile, three pollen

Table 1: Analysis of variance for combining ability in sunflower

Source	df	Mean sum of squares							
		Days to 75% flowering	Days to maturity	Plant height (cm)	Head diameter (cm)	Hull content (%)	Test weight (g)	Seed yield/ plant (g)	Oil content (%)
Treatment	50	43.14**	15.79**	1269.83**	5.00**	174.60**	1.51**	145.51**	21.35**
Replication	2	4.06**	27.12**	2694.75**	10.16**	14.16	1.14**	227.77**	0.63
Parent	14	73.92**	20.26**	1714.17**	5.54**	76.36**	2.82**	68.73*	16.15**
Crosses	35	27.74**	14.39**	830.28**	3.68**	215.84**	0.95**	88.80**	14.62**
Parents vs.crosses	1	151.17**	2.12	10433.31**	43.81**	106.56**	2.72**	3205.43**	329.62**
Lines	2	191.50**	146.56**	4208.87**	23.52**	11.66	6.75**	506.75**	182.21**
Testers	11	35.69**	9.90	1210.98**	3.84	312.40	1.03	89.44	6.84
Line x testers	22	8.87	4.62*	332.79	1.80	186.13**	0.38*	50.48	3.27**
Error	100	5.57	2.38	240.54	1.63	14.35	0.21	34.72	0.56

* Significant at 0.05 level
** Significant at 0.01 level

sources, *viz.,* Morden, MSFH-17 and KBSH-1, of different maturity periods, were planted in alternate rows of the hybrids. The crop was raised under irrigated conditions following all recommended agronomic packages. At harvest, pollen sources that were planted in between the hybrids were first cut and discarded. Means over two years were used for all statistical analyses. Analysis of variance was carried out as suggested by Kempthorne (1957). The magnitude of heterosis over two standard checks (MSFH-17 and KBSH-1) was estimated for eight important characteristics, *viz.,* days to 75% flowering, days to maturity, plant height, head diameter, hull content, test weight, seed yield per plant and oil content, by recording observations on five randomly selected plants from each of the parents, hybrids and checks. The standard heterosis was estimated using the following formula:

$$\text{standard heterosis (\%)} = \frac{F_1\text{-standard parent}}{\text{standard parent}} \times 100$$

The checks, MSFH-17 and KBSH-1, are the two ruling hybrids all over India. MSFH-17 is a private sector hybrid known for high seed yield and KBSH-1 is a public sector hybrid known for both high seed yield and high oil content.

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant differences among the parents (lines and testers) for the eight characters evaluated, *viz.,* days to 75% flowering, days to maturity, plant height, head diameter, hull content, test weight, seed yield per plant and oil content (Table 1). Probably due to this, the crosses also were significantly different from each other for all the characters. Significant differences among the different sources of CMS lines were also evident for all characters except hull content. In contrast to this, the variance due to testers was not significant for all traits except days to 75% flowering, plant height and test weight, indicating similarity between them for majority of the characters. In spite of this, crosses derived from them were significantly different from each other as already mentioned. This is not surprising because variance due to lines x testers is significant for majority of characters which has rendered the crosses to differ significantly from each other. Further, significant variance due to parents vs. crosses indicated the presence of overall heterosis for all the characters except days to maturity.

The estimates of heterosis over two standard checks, *viz.,* MSFH-17 and KBSH-1, for eight quantitative characters are presented in Table 2.

It is evident from the table that all hybrids irrespective of their CMS background were either significantly earlier or on-par with the checks for days to 75% flowering and maturity as indicated from significant negative heterosis or non-significant heterosis, respectively. This suggested the possibility of synthesizing early maturing hybrids simply by changing the source of CMS.

As far as plant height was concerned, change in the CMS background did not seem to alter the plant height of most hybrids as indicated from non-significant heterosis over the checks. However, the hybrids 265A x Morden and 274A x 398 derived from CMS-PF and CMS-I, respectively, were significantly shorter than the checks.

The check MSFH-17 itself was found to be superior to all hybrids irrespective of their source of CMS background except the hybrid 274A x 398 for head diameter. However, when compared with the other check, KBSH-1, a few hybrids from each of CMS-PET1 and CMS-ANL2 were significantly superior for this character although the magnitude of heterosis was lower. Nevertheless, the hybrid 274A x 398 exhibited significant positive heterosis to the extent of 32.09%. Majority of the hybrids possessing CMS-PEF1 in their female parents were inferior to KBSH-1.

The hybrids involving CMS-PET1 and CMS-PEF1 as their female parents were better than those involving CMS-ANL2 for hull content over standard check MSFH-17 as evident from more hybrids manifesting significant negative heterosis in CMS-F and CMS-PF. Whereas, most of the hybrids regardless of their source of cytoplasm expressed significant heterosis over KBSH-1 in the desirable direction (negative). It is well documented that hull content and oil content are inversely related in sunflower. In this context, the hybrids evaluated in the present study hold promise of synthesizing hybrids with lower hull content and hence with higher oil content.

Although significant variation was observed among the hybrids for test weight, most of the hybrids synthesized in the backgrounds of CMS-PET1 and CMS-ANL2 were superior to both MSFH-17 and KBSH-1 indicating their significant influence on the character. Conversely, CMS-PF appeared to inherit negative alleles for this character as evidenced from significant negative heterosis in all hybrids.

As regards the seed yield per plant, the change in the source of cytoplasm in the synthesis of hybrids did not seem to influence the character in the desirable direction when compared with MSFH-17. However, the hybrids 234A x 398 (CMS-PET1) and 274A x 398 and 274A x 652 (CMS-PEF1) exhibited as high as 26.42 and 39.86 and 30.19% heterosis, respectively, over KBSH-1.

Irrespective of the source of cytoplasm, almost all hybrids excelled MSFH-17 with respect to oil content. However, CMS-PF with different testers did not appear to influence the oil content favorably as indicated by significant negative heterosis over KBSH-1. Nevertheless, a few hybrids derived from CMS-PET1 and CMS-ANL2 expressed significant positive heterosis over KBSH-1.

Table 2: Estimates of heterosis over standard checks for eight quantitative characters in sunflower

	Days to 75% flowering		Days to maturity		Plant height (cm)		Head diameter (cm)	
Hybrid	MSFH-17	KBSH-1	MSFH-17	KBSH-1	MSFH-17	KBSH-1	MSFH-17	KBSH-1
234 A x Morden	-7.77**	-9.64**	-3.16*	-3.16*	-5.56	-17.13	-7.73**	1.00
234 A x 218	-3.11	-5.08**	-2.11	-2.11	-0.49	-12.67	-11.59**	-3.23**
234 A x 398	0.53	-1.52	-1.05	-1.05	22.12	7.17	-2.50*	6.72**
234 A x 400	-2.07	-4.06*	-2.11	-2.11	10.11	-3.37	-11.59**	-3.23**
234 A x 401	-5.18**	-7.11**	-2.11	-2.11	5.96	-7.01	-14.09**	-5.97**
234 A x 409	-3.11	-5.08**	0.00	0.00	17.40	3.02	-6.59**	2.24*
234 A x 438	3.11	1.02	0.00	0.00	18.76	4.22	-5.91**	2.99**
234 A x 652	-4.13**	-6.09**	-6.32**	-6.32	3.27	-9.38	-16.36**	-8.46**
234 A x 666	-4.13**	-6.09**	-1.05	-1.05	1.15	-11.24	-5.68**	3.23**
234 A x 693	-3.62	-5.58**	-2.11	-2.11	7.02	-6.08	-16.59**	-8.71**
234 A x 781	-3.62	-5.58**	-2.11	-2.11	-13.73	-24.29	-12.95**	-4.73**
234 A x 1091	-3.11	-5.08**	0.00	-0.00	8.39	-4.88	-8.86**	-0.25
265 A x Morden	-12.44**	-14.21**	-7.02**	-7.02**	-16.56	-26.77*	-9.77**	-1.24
265 A x 218	-9.84**	-11.68**	-5.26**	-5.26**	-6.36	-17.82	-14.77**	-6.72**
265 A x 398	-2.07	-4.06*	-6.32**	-6.32**	2.87	-9.72	-7.95**	0.75
265 A x 400	-8.29**	-10.15**	-4.21**	-4.21**	-11.04	-21.93	-15.45**	-7.46**
265 A x 401	-6.22**	-8.12**	-4.21**	-4.21**	-0.97	-13.10	-16.36**	-8.46**
265 A x 409	-2.58	-4.57*	-3.16*	-3.16*	-0.57	-12.75	-14.55**	-6.47**

Table 2: Estimates of heterosis over standard checks for eight quantitative characters in sunflower (continued)

	Days to 75% flowering		Days to maturity		Plant height (cm)		Head diameter (cm)	
Hybrid	MSFH-17	KBSH-1	MSFH-17	KBSH-1	MSFH-17	KBSH-1	MSFH-17	KBSH-1
265 A x 438	3.11	-3.55	-4.21**	-4.21**	0.93	-11.43	-20.00**	-12.44**
265 A x 652	-9.33**	-11.17**	-7.02**	-7.02**	-4.77	-16.43	-14.09**	-5.97**
265 A x 666	-13.46**	-15.23**	-6.32**	-6.32**	-2.16	-14.14	-10.23**	-1.74
265 A x 693	-11.39**	-13.20**	-7.02**	-7.02**	0.31	-11.97	-3.64**	5.47**
265 A x 781	3.64	1.52	-2.11	-2.11	4.68	-8.14	-15.45**	-7.46**
265 A x1091	-3.62	-5.58**	-5.26**	-5.26**	-10.11	-21.22	-22.05**	-14.68**
274 A x Morden	-1.55	-3.55	-1.05	-1.05	-3.80	-15.58	-1.82	7.46**
274 A x 218	-3.11	-5.08**	-4.21**	-4.21**	-0.88	-13.02	-7.27**	1.49
274 A x 398	7.26**	5.08**	0.00	0.00	-34.70**	18.21	20.68**	32.09**
274 A x 400	0.53	1.52	-1.05	-1.05	11.88	-1.82	-9.32**	-0.75
274 A x 401	2.08	0.00	-1.05	-1.05	13.02	-0.81	-4.55**	4.48**
274 A x 409	3.11	1.02	0.00	0.00	8.17	-5.08	0.00	9.45**
274 A x 438	3.64	1.52	0.00	0.00	26.05*	10.62	2.05	11.69**
274 A x 652	0.00	-2.03	-1.05	-1.05	14.61	0.58	-4.55**	4.48**
274 A x 666	-2.07	-4.06	-1.05	-1.05	3.31	-9.34	-9.55**	-1.00
274 A x 693	-1.02	-3.05	-2.11	-2.11	5.70	-7.25	-6.14**	2.74**
274 A x 781	0.53	-1.52	-1.05	-1.05	11.92	-1.78	-4.32**	4.73**
274 A x1091	-0.51	-2.54	-2.11	-2.11	-1.28	-13.37	-9.55**	-1.00

CONCLUSIONS

All three sources under study advanced the maturity in most of the hybrids. The hybrid 274A x 398 was significantly later than the checks. It is worth mentioning here that the same hybrid also exhibited significant positive heterosis over KBSH-1 for head diameter and test weight. Further, it is not surprising to observe the manifestation of significant positive heterosis by this hybrid for seed yield, as head diameter and test weight are directly related to seed yield. CMS-PET1, which is used widely all over the globe in heterosis breeding, conclusively proved to have significant positive influence on oil content compared with the other two sources of cytoplasm used in the present study. Both CMS-PET1 and CMS-ANL2 showed some promise for developing hybrids with increased seed yield. CMS source derived from *Helianthus petiolaris* ssp. *fallax* did not show encouraging results for most of the characters at least with the set of testers used in this study. The source of cytoplasm coupled with the nuclear genotype under consideration did have influence on the performance of hybrids for different characters in general. Development of alloplasmic lines and study of their hybrids may reveal more information on the effect of cytoplasm on various characters.

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HETEROSIS EN LOS HIBRIDOS TOP-CROSS DEL GIRASOL CREADOS DE DIVERSAS FUENTES CITOESTERILES

RESUMEN

La heterosis standard fue investigada para ocho caracteristicas cuantitativas en los hibridos top-cross del girasol creados de tres diversas fuentes de la esterilidad masculina citoplasmica (CMS), *Helianthus petiolaris* (CMS-PET1), *Helianthus petiolaris* ssp. *fallax* (CMS-PEF1) y *Helianthus annuus* ssp. *lenticularis* (CMS-ANL2) cruzados con las lineas consanguineas con bases de pipa diferentes. Los hibridos top-cross creados por el cruzamiento de tres fuentes de CMS antes citadas con 12 padres masculinos en el esquema linea x testador. La heterosis significativa en la direccion deseada fue notada para todas las caracteristicas cuando los hibridos top-cross eran comparados con dos controles standard. La fuerte CMS-PET1 fue mejor que otras fuentes con respecto al contenido de aceite. Los valores medios de hibridos con respecto al rendimiento de semillas y otras caracteristicas importantes economicamente han mostrado que los mejores hibridos eran esos con la base de CMS-ANL2, pues esos con las bases de CMS-PET1 y CMS-PEF1. Eso indica que el uso de diversas fuentes de CMS en los programas de seleccion que se basan en la utilizacion de heterosis puede ser util en la seleccion del girasol.

HÉTEROSIS DANS LES HYBRIDES TOP-CROSS DE TOURNESOL (*Helianthus annuus* L.) OBTENUS DE DIVERSES SOURCES CYTOSTÉRILES

RÉSUMÉ

Huit caractéristiques quantitatives de l'hétérosis standard ont été examinées dans des hybrides top-cross de trois différentes sources cytoplasmiques mâles de tournesol (CMS), l'*Helianthus petiolaris* (CMS-PET1), l'*Helianthus petiolaris* ssp. *fallax* (CMS -PEF1) et *l'Helianthus annuus* ssp. *lenticularis* (CMS-ANL2) croisées avec des lignes inbred à différentes bases nucléaires. Les hybrides top-cross ont été obtenus par le croisement des trois sources CMS mentionnées ci-dessus avec 12 parents mâles selon le schéma du testeur de ligne x. Une hétérosis significative dans la direction souhaitée a été observée pour toutes les caractéristiques quand les hybrides top-cross ont été comparés avec tous les contrôles standard. La source CMS-PET1 s'est montrée meilleure que les autres pour ce qui concerne le contenu en huile. La performance moyenne des hybrides pour ce qui est du rendement en graines et d'autres caractéristiques significatives du point de vue économique a démontré que les meilleurs hybrides étaient ceux qui avaient une base CMS-ANL2, puis, ceux à base CMS-PET1 et CMS-PEF1. Ceci montre que l'utilisation de différentes sources CMS dans les programmes de sélection basés sur l'utilisation de l'hétérosis peut être avantageuse dans la sélection du tournesol.