



CYTOPLASMIC EFFECTS ON COMBINING ABILITY FOR AGRONOMIC TRAITS IN SUNFLOWER UNDER DIFFERENT IRRIGATION REGIMES

V. TYAGI* and S.K. DHILLON

Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

*Corresponding author's email: vikranttyagi97@gmail.com

Co-author's email address: sklb-pbg@pau.edu

SUMMARY

This study involved a set of 40 sunflower hybrids developed by crossing nine CMS analogues from different cytoplasmic sources and their maintainer (NC-41B) with 4 perfect restorers from PET-1 source. The analysis of variance revealed significant differences among the cytoplasmic sources for traits studied under both the environments, both the years and pooled over years. CMS ARG-3A (*Helianthus argophyllus*), DV-10A (*H. debilis ssp. vestitus*) and PRUN-29A (*H. praecox ssp. runyonii*) recorded significantly higher seed yield as well as oil content as compared to common maintainer NC-41B under both normal and stress environments respectively. DV-10A (*H. debilis ssp. vestitus*) had bigger head size in normal and higher 100 seed weight under stress environment as compared to NC-41B. Significant increase in seed yield and oil content for all CMS analogues as compared to conventional *H. petiolaris* source was observed which may be attributed to the effect of cytoplasmic genes or nuclear cytoplasmic interactions. CMS ARG-3A from *H. argophyllus* and CMS-XA (unknown source) may be designated as potential CMS sources for normal and water stress environments respectively. The CMS analogues E002-91A (*H. annuus*), ARG-3A (*H. argophyllus*) and ARG-6A (*H. argophyllus*) and tester RCR-8297 were recorded as very good combiners for seed yield under both the environments. CMS-XA × P124R, CMS-XA × P100R, PKU-2A × P124R, ARG-2A × P100R, ARG-3A × P124R, ARG-6A × P69R, DV-10A × P100R and PRUN-29A × RCR-8297 were identified with high SCA effects for seed yield per plant under both the environments. Non additive component of the genetic variance was observed for all the traits studied.

Key words: Combining ability, gene action, sunflower hybrids, CMS sources, water stress

Key findings: It is an important finding that the impact of different cytoplasmic sources on the expression of most of the traits particularly seed yield and oil content was in desirable direction and may play an important role in diversification of cytoplasm and the hybrid base for future breeding programmes in sunflower. The CMS analogues E002-91A (*H. annuus*), ARG-3A (*H. argophyllus*) and ARG-6A (*H. argophyllus*) and the tester RCR-8297 recorded very good combining ability for seed yield under both the environments. These parental lines may use to develop high yielding water use efficient hybrid which, having different cytoplasm than commercial hybrids.

Manuscript received: September 18, 2015; Decision on manuscript: June 27, 2016; Manuscript accepted: July 28, 2016.

© Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2016

Communicating Editor: Bertrand Collard

INTRODUCTION

Sunflower is moderately tolerant to water stress and its production is greatly affected by drought. Evidence indicates that drought stress

during the vegetative phase, flowering and seed-filling period in sunflower causes a considerable decrease in yield and oil content (Ali *et al.*, 2009). Breeding research for genetic enhancement in sunflower has been in

progress for more than 4 decades in India. The objective of sunflower breeding is to develop the high yielding hybrids along with quality oil and resistance to biotic and abiotic stress Dudhe *et al.*, (2009). Sunflower is one of the important crops in which extent of heterosis is very much associated with genetic differences between the parental lines Imran *et al.*, (2015) and Hladni *et al.*, (2006). Development and evaluation of CMS lines is prerequisite in sunflower breeding for development of high yielding sunflower hybrids. The discovery of cytoplasmic male sterility (CMS) in *Helianthus petiolaris* (PET-1) by Leclereq, (1969) and subsequent identification of fertility restoring genes have resulted in the development of commercial hybrids since 1972. However, all the sunflower hybrids that are commercially grown are based on a single source of CMS discovered by Leclereq leading to homogeneity and potential risk that was evident in case of maize (CMS-T). All the hybrids in maize had this (CMS-T) background and exclusively used for maize hybrid-seed production in the United States. It became susceptible to Southern corn leaf blight (SCLB), caused by race T of the fungus which caused SCLB epidemic in 1970 (Ullstrup, 1972). Diversification of CMS source is inevitable in heterosis breeding programs as the use of a single CMS source involves a potential risk if it becomes susceptible to a new strain of disease. Similarly in sunflower at present only one CMS source i.e. *Helianthus petiolaris* (PET-1) is being widely used for hybrid breeding programme. In order to diversify the cytoplasmic base, attempts have been made and several new cytoplasmic sources have been identified. Beneficial cytoplasmic nuclear interactions have been reported in various crops (Jan, 1992). A limited number of studies have reported the interactions between cytoplasm and nuclear genes in the expression of several qualitative and quantitative traits in sunflower. For example in sunflower a unique cytoplasmic nuclear interaction causing reduction in chlorophyll, photosynthetic rate has been reported by Jan, 1990 and positive effects on oil content have been reported by Serieys, (1992). A set of nine CMS lines developed from different CMS sources at Punjab Agricultural University, Ludhiana was evaluated for their performance and diversity for different traits under normal irrigated

environment (Tyagi *et al.*, 2013, 2015a) as well as under moisture-stress conditions (Tyagi *et al.*, 2015b; Tyagi and Dhillon 2016) at PAU, Ludhiana.

This investigation was therefore aimed to study the effect of cytoplasmic male sterility sources on combining ability for yield and component traits under normal irrigated and water stress environments to exploit them in hybrid development program.

MATERIALS AND METHODS

This investigation was carried out in the research fields of the oilseeds section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. The experiment was conducted during spring season in the two years 2011 and 2012 under two different irrigation regimes; Normal irrigation (N) and Control irrigation (S), under normal irrigated regime all the recommended irrigation supplied but in control irrigation regime the irrigation was stopped after the anthesis for stress environment.

Experimental material

Nine diverse CMS analogues were developed by backcrossing them with using a common maintainer at PAU, Ludhiana and 4 common perfect restorers for these CMS sources were identified. A total of 10 female lines comprising nine CMS analogues from different cytoplasmic sources along with NC-41B (common maintainer) from conventional source were crossed with 4 common restorer lines using line \times tester design and 40 crosses were attempted. All the agronomic practices were performed from sowing to harvest.

Experiment layout and observations

Parents along with hybrids were sown in the randomized block design with 3 replications each having paired rows of 4.5 m length with 60 cm and 30 cm inter and intra row spacing respectively. All the agronomic practices recommended for the region were followed to raise a good crop. The data for plant height, head diameter and seed yield per plant were recorded for 5 random plants in the field from each entry of each replication under open pollination. For 100-seed weight a sample of

100 filled seeds was drawn at random from the bulked seeds of 5 plants with an electronic balance. To determine oil percent in seeds, the wide line nuclear magnetic resonance (NMR) instrument Newport Analyzer MK 111 A was used.

Statistical analysis

The data recorded was statistically analyzed following standard procedures for the estimation of components of genetic variance for each irrigation level separately and for pooled over the different environments. Combining ability analysis was done following Line \times Tester analysis, as suggested by Kempthorne, (1957) using SPAR 2.0 developed at Indian Agricultural Statistics Research Institute, New Delhi, India. To study the effect of different cytoplasmic sources the mean values of 4 crosses from each source were pooled and their grand mean values were calculated to study the effect of different cytoplasmic sources and compared them with the conventional *H. petiolaris* source (NC-41B) under both the environments.

RESULTS

The analysis of variance for all the traits revealed significant differences among the sources under both the environments, individual and pooled over the years. Highly significant differences for female vs. male, females and males were recorded for all the traits under both normal and stress environment however, the mean square due to female vs. males were non-significant for 100 seed weight under normal environment while for head diameter and seed yield in stress environment. The differences among the parents, parents vs. crosses and crosses were observed to be highly significant for all the character indicating the existence of wider genetic differences among parents and crosses. The mean squares due to parent \times years, female \times years and male \times years interactions were recorded as highly significant for all the traits under both the environments except male \times years which were non-significant for head diameter, 100 seed weight and seed yield under normal environment and head diameter under stress environment.

The combining ability analysis, (pooled over the years) presented in Table 1 reveals that the mean squares due to years were highly significant for plant height, head diameter, seed yield and oil content under both the environments, while, mean squares for 100 seed weight highly significant under normal environment. Mean squares due to restorers were significant for all the traits under stress environment. The differences among the female, female \times male, female \times years and male \times years were observed to be highly significant for all the traits, mean square due to female \times male \times years was non-significant for plant height and head diameter under normal environment indicating the existence of wider genetic differences among parents.

Proportional contribution of parents and gene action

With respect to proportional contribution of parents and their interactions, the contribution of female parents was observed to be higher as compared to testers in the expression of all the traits under both the environments. However, interaction component (lines \times testers) had higher contribution for most of the traits i.e. head diameter, 100 seed weight, seed yield and oil content under both normal and stress environment respectively. However, the contribution of females was higher than the contribution of males as well as their interaction for plant height under both normal and stress environment (Table 1). Non additive component of the genetic variance had a predominant role in the inheritance of plant height. This is confirmed by GCA/SCA ratio for plant height, head diameter, 100 seed weight, seed yield and oil content in F_1 generation which was below the value of one under both normal and stress environment respectively (Table 1).

Effect of diverse cytoplasmic sources on performance of hybrids

The mean performances of different cytoplasmic sources were compared with the mean performance of cultivated source PET-1 to investigate the impact of different sources on hybrid performance under both the environments. The mean performance of hybrids computed over different sources with respect to yield and component traits pooled

Table 1. Analysis of variance for combining ability under normal irrigation and stress environment (individual and pooled over years).

Source of variation	df	Mean squares									
		Plant height (cm)		Head diameter (cm)		100 seed weight (g)		Seed yield (g)		Oil content (%)	
		N	S	N	S	N	S	N	S	N	S
Years	1	41.86**	11511.40**	210.31**	1343.91**	18.64**	0.11	2961.48**	372.00**	456.52**	39.43**
Rep./years	4	3.24**	271.07**	5.32**	4.27**	1.45**	1.05**	88.81**	30.93	1.14**	0.54**
Females	9	8843.68**	7463.88**	33.55**	25.82**	2.98**	3.53**	543.29**	841.16**	27.63**	79.32**
Males	3	5348.23**	8359.56**	11.16**	56.45**	4.15**	5.40**	976.08**	158.49**	18.94**	12.92**
Female × Male	27	1229.17**	1377.25**	20.84**	33.21**	1.85**	1.60**	379.31**	338.09**	16.63**	32.99**
Female × Years	9	1861.30**	3174.90**	18.13**	22.67**	2.39**	2.10**	145.52**	189.12**	17.34**	41.69**
Male × Years	3	1393.10**	1952.73**	10.57**	4.39**	7.52**	1.26**	343.09**	693.05**	3.20**	15.71**
F × M × Years	27	721.73	757.04**	14.06	15.64**	1.66**	1.41**	149.26**	130.24**	17.61**	39.02**
Error	146	11.93	15.98	0.32	0.37	0.30	0.16	13.03	13.30	0.14	0.16
Estimates of genetic components											
σ^2 Females		269.79	152.87	0.36	0.60	0.02	0.05	6.99	18.51	0.47	1.82
σ^2 Males		38.31	64.30	0.07	0.38	0.04	0.04	4.48	8.25	0.19	0.04
σ^2 Female × Males (SCA)		169.15	206.74	2.26	5.86	0.06	0.07	76.68	69.28	0.33	2.01
σ^2 GCA		87.04	82.94	0.02	0.17	0.02	0.04	5.00	2.61	0.24	0.41
σ^2 GCA/ σ^2 SCA		0.51	0.40	0.01	0.03	0.33	0.57	0.07	0.04	0.73	0.20
Proportional contribution (percent)											
Contribution of females		64.66	55.75	34.07	18.77	31.67	37.18	28.74	44.52	33.86	43.81
Contribution of males		8.38	13.38	2.43	8.80	9.44	12.17	11.06	1.80	4.98	1.53
Contribution of X		26.96	30.86	63.50	72.43	58.90	50.64	60.20	53.68	61.16	54.66

*, ** Significant at 0.05 and 0.01 of probability. N: Normal environment, S: Stress environment.

over the years under the two environments are presented in Table 2 (normal and stress environment). The value for plant height, seed yield and oil content differed significantly with respect to all the sources under both the environments except ARG-6A (*H. argophyllus*) which recorded non-significant differences for these traits under stress environment. No significant effect on head diameter was observed as compared to control except for one source DV-10A (*H. debilis ssp. vestitus*) under normal environment while under stress environment 3 sources CMS-XA (unknown source), E002-91A (*H. annuus*) and PKU-2A (*H. annuus*) differed significantly from PET-1 source. The sources DV-10A (*H. debilis ssp. vestitus*) and ARG-3A (*H. argophyllus*) recorded significant differences for 100 seed weight from PET-1 under stress environment whereas, no significant differences were observed under normal environment (Table 2). All the CMS sources significantly differed from PET-1 (*H. petiolaris*) source with respect to seed yield and oil content under both the environments. ARG-3A (*H. argophyllus*), DV-10A (*H. debilis ssp. vestitus*) and PRUN-29A (*H. debilis ssp. runyonii*) recorded highly significant yield differences with NC-41B (*H. petiolaris*) under both normal and stress environments respectively (Table 2). In general the oil content of all the sources was in the lower range. However, it was significantly better than control NC-41B in both the environments.

Effect of different cytoplasmic sources on combining ability under different environments (pooled over years)

The importance of combining ability in selection of parents for hybridization has been emphasized by many workers in sunflower (Putt, 1996; Giriraj *et al.*, 1987). The potentiality of any line to be used as a parent in hybridization depends on its performance and the performance of F₁ hybrid derived from it and its own GCA effect. The estimates of the general combining ability (GCA) and specific combining ability (SCA) effects for the yield and component traits (pooled over the years) under two different water regimes is presented in Tables 3 to 7.

CMS analogues i.e. ARG-2A, ARG-3A, DV-10A, PHIR-27A and NC-41B were very good combiners for dwarf plant type as they had highly significant negative GCA effects under both the environments. CMS ARG-6A had good combining ability for tall plant type under both the environments, while, PARUN-29A was good combiner for tall plant type under normal environment and for dwarf plant type under water stress environment. CMS-XA, E002-91A and PKU-2A were recorded very good combiner for tall plant type under normal environment whereas, good combiner for dwarf plant type under water stress environment. The male parent P69R and P124R recorded highly significant negative GCA effects showing very good combining ability for dwarf plant type under both the environments. While, male parent RCR-8297 and P100R were observed as having very good combining ability for tall plant type under both the environments. Thirty cross combinations out of 40 were identified as having high SCA effects for dwarf plant type under both the environments. The cross combinations are CMS-XA × RCR-8297, E002-91 × RCR-8297 and PKU-2A × RCR-8297 having positive high SCA effects under water stress environment good for tall plant type whereas, highly significant negative SCA effects under normal environment good for dwarf plant type (Table 3).

With respect to head diameter female parents, PKU-2A, ARG-2A, ARG-6A, DV-10A and PRUN-29A source and NC-41B from PET-1 source were observed as very good combiner due to their highly significant positive GCA effects for head diameter under normal environment from different cytoplasmic sources (Table 4). Under stress environment the female parents CMS-XA, E002-91, PKU-2A and ARG-3A were observed as very good combiner for this trait. NC-41B and PKU-2A recorded very good combiner for this trait under both the environments. The male parent RCR-8297 recorded highly significant GCA effects for this trait under stress environment while P124R and P100R showed very good combining for this trait under normal environment. The cross combination NC-41B × P100R was only identified as having high SCA effects for head diameter under both the environments whereas, 4 cross combinations

Table 2. Effect of diverse cytoplasmic sources on seed yield and component traits under different irrigation regimes (pooled over the years).

No.	Sources	Plant height (cm)		Head diameter (cm)		100 seed weight (g)		Seed yield (g)		Oil content (%)	
		N	S	N	S	N	S	N	S	N	S
1	CMS-XA	135.4**	139.5**	16.9	17.1**	5.5	5.4	50.1**	47.0**	32.8**	30.6**
2	CMS-E002-91A	143.7**	154.1**	17.1	17.0**	5.5	5.3	53.2**	42.2**	29.9**	30.7**
3	CMS-PKU-2A	144.3**	139.5**	18.3	16.4*	5.6	5.2	51.6**	39.6**	31.2**	32.2**
4	CMS-ARG-2A	133.7**	124.7**	18.1	15.1	5.5	4.9	48.5*	36.6**	32.6**	31.9**
5	CMS-ARG-3A	146.4**	126.1**	17.7	16.1	5.3	5.5*	56.6**	45.2**	31.1**	33.6**
6	CMS-ARG-6A	135.3**	110.3	17.2	15.1	5.3	5.3	50.1**	35.9**	30.7*	30.2**
7	CMS-DV-10A	138.0**	112.7*	19.9*	14.4	5.2	5.6**	54.1**	43.1**	30.8*	31.0**
8	CMS-PHIR-27A	140.8**	113.6*	17.8	15.2	5.1	4.4	52.4**	39.6**	30.6*	29.1**
9	CMS-PRUN-29A	158.0**	127.2**	18.7	14.0	5.6	5.1	52.5**	49.4**	33.3**	31.5**
10	NC-41B (C)	107.2	98.7	19.1	15.7	5.7	5.2	46.2	24.9	29.5	25.7
SE		4.13	5.23	0.30	0.33	0.06	0.10	0.93	2.20	0.40	0.68
CD 0.05		9.34	11.84	0.68	0.75	0.13	0.24	2.11	4.97	0.90	1.53
CD 0.01		15.31	19.40	1.11	1.23	0.21	0.39	3.46	8.15	1.48	2.51

*, ** Significant at 0.05 and 0.01 of probability respectively. N: Normal environment, S: Stress environment, SE: Standard error, CD: Critical differences.

Table 3. Estimation of combining ability effects and mean performance of hybrids for plant height under normal and water stress environment (pooled over years).

No.	Female/Male		RCR-8297		P69R		P124		P100R		GCA	
			N	S	N	S	N	S	N	S	N	S
1	CMS-XA	Mean	156.33	150.00	126.75	119.71	131.42	128.42	143.34	143.41	-15.23**	3.64**
		SCA	-11.28**	6.08**	-29.20**	-13.99**	-24.05**	-10.83**	-15.19**	-2.73**		
2	E002-91	Mean	161.37	158.50	149.04	123.25	155.42	140.88	150.71	152.04	-6.95**	18.31**
		SCA	-6.26**	9.50**	-27.11**	1.13	-16.68**	5.46*	-10.08**	2.26		
3	PKU-2A	Mean	165.67	157.54	137.59	131.08	122.79	135.88	131.75	152.88	-6.27**	3.63**
		SCA	-6.83**	12.42**	-22.47**	-6.64**	-19.64**	-16.68**	-9.58**	-10.60**		
4	ARG-2A	Mean	147.29	150.00	102.83	104.71	116.93	138.63	131.55	141.33	-16.95**	-11.17**
		SCA	-11.28**	-0.06	-38.07**	-30.22**	-18.01**	-20.65**	-16.41**	-10.74**		
5	ARG-3A	Mean	141.29	167.50	100.92	151.38	125.83	134.63	136.33	132.25	-4.18**	-9.73**
		SCA	-0.93	-4.13*	-10.47**	-31.52**	-20.38**	-14.61**	-21.78**	-7.49**		
6	ARG-6A	Mean	131.08	147.33	90.38	105.04	93.42	135.67	126.38	153.08	21.42**	9.71**
		SCA	-12.86**	-11.05**	-37.88**	-38.67**	-19.76**	-36.61**	-9.46**	-14.25**		
7	DV-10A	Mean	134.58	155.55	90.08	106.15	101.50	153.83	124.51	136.33	-7.38**	-17.89**
		SCA	-8.00**	-8.68**	-37.22**	-38.87**	-9.02**	-31.13**	-19.37**	-15.51**		
8	PHIR-27A	Mean	123.17	147.92	109.50	122.63	102.00	137.96	119.58	154.75	-9.80**	-22.26**
		SCA	-12.52**	-16.42**	-27.48**	-25.70**	-18.41**	-30.79**	-8.48**	-18.86**		
9	PRUN-29A	Mean	130.88	180.67	124.55	151.92	110.21	143.55	143.33	156.00	7.42**	-8.58**
		SCA	6.85**	-11.19**	-10.15**	-15.48**	-15.10**	-25.22**	-7.74**	-2.74		
10	NC-41B (C)	Mean	109.25	103.29	66.38	93.90	86.54	107.21	132.66	124.42	-43.41**	-37.11**
		SCA	-38.91**	-25.87**	-44.46**	-54.96**	-36.60**	-41.28**	-26.42**	-9.98**		
	GCA		9.36**	11.27**	-7.85**	-5.32**	-4.49**	-10.24**	2.97**	4.29**		

*, ** - Significant at 0.05 and 0.01 of probability respectively. N: Normal environment, S: Stress environment.

Table 4. Estimation of combining ability effects and mean performance of hybrids for head diameter under normal and water stress environment (pooled over years).

No.	Female/Male		RCR-8297		P69R		P124		P100R		GCA	
			N	S	N	S	N	S	N	S	N	S
1	CMS-XA	Mean	17.46	16.01	17.37	15.99	16.50	17.41	16.96	18.06	-0.93	1.73**
		SCA	-14.63	22.17**	-14.71	21.53**	-7.15	15.47**	-3.66	18.65**		
2	E002-91	Mean	17.05	16.60	17.16	15.76	16.66	17.86	17.13	18.21	-0.69	1.66**
		SCA	-11.47	19.30**	-15.96	20.11**	-4.75	16.56**	-2.88	19.84**		
3	PKU-2A	Mean	18.58	18.51	17.21	18.22	16.12	18.13	13.83	18.16	0.46**	1.10**
		SCA	-1.28	30.02**	-2.82	20.45**	-3.30	12.82**	-3.13	-3.25		
4	ARG-2A	Mean	16.67	17.89	13.81	17.69	13.84	17.93	16.00	19.05	0.34**	-0.26
		SCA	-4.59	16.62**	-5.64	-3.36	-4.37	-3.17	1.60	11.94**		
5	ARG-3A	Mean	17.28	16.37	15.71	18.20	16.71	17.98	14.67	18.10	-0.13	0.75**
		SCA	-12.68	20.95**	-2.93	9.93**	-4.10	16.92**	-3.48	2.67		
6	ARG-6A	Mean	14.04	18.92	12.66	15.96	15.66	17.31	18.04	16.79	0.79**	-0.66
		SCA	0.92	-1.74	-14.91	-11.42	-7.71	9.61**	-10.44	26.25**		
7	DV-10A	Mean	15.72	18.60	13.08	16.99	14.20	26.75	14.46	17.08	1.78**	-0.90
		SCA	-0.80	9.97**	-9.41	-8.47	42.66**	-0.64	-8.92	1.18		
8	PHIR-27A	Mean	16.71	19.07	14.09	16.52	14.16	17.76	15.66	17.83	0.00	-0.18
		SCA	1.69	16.91**	-11.92	-1.40	-5.30	-0.89	-4.91	9.56**		
9	PRUN-29A	Mean	13.54	19.29	14.42	18.71	12.67	17.70	15.37	19.15	0.92**	-1.34
		SCA	2.85*	-5.24	-0.22	0.89	-5.60	-11.34	2.14	7.58**		
10	NC-41B (C)	Mean	14.76	14.72	12.79	19.60	13.79	21.29	21.36	20.83	1.31**	0.34**
		SCA	-21.51	3.29*	4.53**	-10.49	13.56**	-3.49	11.07**	49.49**		
GCA			-0.43	1.12**	-0.15	-0.43	0.32**	-0.66	0.26**	-0.03		

*, ** - Significant at 0.05 and 0.01 of probability respectively. N: Normal environment, S: Stress environment.

under normal environment and 23 under water stress environment out of 40.

The female lines ARG-6A was observed as very good combiner for 100 seed weight under both the environments. While ARG-3A was recorded as very good combiner under stress environment only however, male parent P69R was very good general combiner as recorded highly significant positive GCA effects under both the environments. The cross combination NC-41B \times P69R was identified with high SCA effects for 100 seed weight under both the environments whereas, 6 cross combinations had high significant SCA effects for this trait under water stress environment only (Table 5).

For seed yield the CMS analogues E002-91A (*H. annuus*), ARG-3A (*H. argophyllus*) and ARG-6A (*H. argophyllus*) having highly significant positive GCA effects were recorded as very good combiner, whereas, NC-41B from PET-1 with negative GCA effects was poor combiner for seed yield under both the environments. CMS analogues CMS-XA (Unknown) and PRUN-29A (*H. praecox ssp. Runyonii*) were recorded as very good combiner because these had highly significant GCA effects for seed yield under water stress environment (Table 6). The testers RCR-8297 was recorded very good combiner under both the environments, while P100R appeared to be very good general combiner for seed yield per plant under normal environment only. The hybrid combinations CMS-XA \times P124R, CMS-XA \times P100R, PKU-2A \times P124R, ARG-2A \times P100R, ARG-3A \times P124R, ARG-6A \times P69R, DV-10A \times P100R and PRUN-29A \times RCR-8297 were identified with high SCA effects for seed yield per plant under both the environments.

CMS analogues CMS-XA, ARG-2A and PRUN-29A source were recorded as very good combiner for oil content under both the environments. CMS analogues E002-91, PKU-2A and DV-10A were recorded very good combiners for this trait under water stress environment only. The male parent RCR-8297 and P69R were recorded as very good combiner under stress environment whereas P100R was recorded very good combiner under normal environment while, P100R very good combiner for this trait under normal environment. The twenty one cross combinations were identified with high SCA

effects for oil content under both the environments (Table 7).

DISCUSSION

The analysis of variance for all the traits revealed significant differences among the sources under both the environments, individual and pooled over the years. The combining ability analysis, (pooled over the years) presented in Table 1 reveals that the mean squares due to years were highly significant under both the environments. Highly significant means square due to years for plant height, head diameter, seed yield and oil content in combining ability analysis revealed that the performance for these traits under both normal as well as water stress conditions is subjected to change with the change in climate conditions presenting over the years. The results are in accordance with earlier workers (Pavani *et al.*, 2006; Shankar *et al.*, 2007; Mohansundaram *et al.*, 2010; Meena *et al.*, 2013; Faridi *et al.*, 2015, Shinde *et al.*, 2016 and Dhillon and Tyagi 2016). With respect to proportional contribution of parents and their interactions, the contribution of females towards hybrids was higher than the testers for all traits under both the environments. The interaction component (lines \times testers) had higher contribution for most of the traits i.e. head diameter, 100 seed weight, seed yield and oil content under both normal and stress environment. Higher contribution of line \times tester interaction in the expression of various traits was also reported by Marinkovic *et al.* (1993) and Shinde *et al.* (2016). In this study, analysis of gene action expressed the higher proportion of SCA than GCA for plant height, head diameter, 100 seed weight, seed yield and oil content indicating more importance of non-additive genes action (Table 1). Non additive gene effects for oil content has also been reported by Shekar *et al.* (1998); Parameshwari *et al.* (2004); Imran *et al.* (2015) and Shinde *et al.* (2016). The mean performance of hybrids computed over different sources with respect to yield and component traits pooled over the years under the 2 environments were significantly different among the CMS sources (Table 2). A number of previous studies by Baldani *et al.* (1991); Serieys (1999) have reported the positive and negative influence of cytoplasm types in

Table 5. Estimation of combining ability effects and mean performance of hybrids for 100 seed weight under normal and water stress environment (pooled over years).

No.	Female/Male		RCR-8297		P69R		P124		P100R		GCA	
			N	S	N	S	N	S	N	S	N	S
1	CMS-XA	Mean	5.20	4.64	5.47	5.80	5.27	5.87	5.46	5.70	-0.20	0.04
		SCA	-30.07	-4.17	-12.61	0.69	-11.60	-2.96	-14.23	0.55		
2	E002-91	Mean	5.20	5.79	5.93	5.38	5.13	5.29	5.05	5.41	-0.23	0.01
		SCA	-12.76	-4.21	-19.03	9.21**	-20.36	-5.60	-18.49	-7.03		
3	PKU-2A	Mean	5.21	5.70	5.25	5.99	5.38	4.89	4.82	5.87	-0.08	-0.15
		SCA	-14.13	-4.11	-9.76	-3.24	-26.32	-0.87	-11.60	-11.17		
4	ARG-2A	Mean	4.72	5.64	4.91	5.33	4.84	5.82	5.14	5.10	-0.23	-0.41
		SCA	-15.09	-13.08	-19.74	-9.53	-12.36	-10.82	-23.21	-5.43		
5	ARG-3A	Mean	5.64	4.84	5.55	5.10	5.48	5.12	5.33	6.02	-0.43	0.19**
		SCA	-27.07	3.93*	-23.18	2.16	-22.87	0.91	-9.30	-1.87		
6	ARG-6A	Mean	5.30	5.84	4.34	4.77	4.86	4.56	6.76	6.16	0.51**	0.41**
		SCA	-12.02	-2.49	-28.11	-20.15	-31.34	-10.51	-7.18	24.55**		
7	DV-10A	Mean	6.32	5.11	5.10	5.40	4.93	5.32	5.99	5.00	-0.29	-0.04
		SCA	-22.99	16.45**	-18.69	-6.06	-19.88	-9.16	-24.70	10.22**		
8	PHIR-27A	Mean	4.69	5.00	4.41	5.68	4.27	5.45	4.36	4.39	-0.57	-0.88
		SCA	-24.74	-13.72	-14.53	-18.86	-17.92	-21.38	-33.92	-19.80		
9	PRUN-29A	Mean	4.61	5.99	6.06	6.42	4.66	4.04	5.10	5.89	-0.11	-0.20
		SCA	-9.79	-15.16	-3.31	11.65	-39.09	-14.13	-11.24	-6.05		
10	NC-41B (C)	Mean	4.34	5.02	6.42	7.00	4.41	5.39	5.60	5.18	-0.05	-0.12
		SCA	-24.39	-20.04	5.43**	18.29**	-18.80	-18.78	-21.94	3.05*		
GCA			-0.14	-0.11	0.31**	0.37**	-0.02	-0.14	-0.15	-0.11		

*, ** Significant at 0.05 and 0.01 of probability respectively. N: Normal environment, S: Stress environment.

Table 6. Estimation of combining ability effects and mean performance of hybrids for seed yield under normal and water stress environment (pooled over years).

No.	Female/male		RCR-8297		P69R		P124R		P100R		GCA	
			N	S	N	S	N	S	N	S	N	S
1	CMS-XA	Mean	51.15	56.13	37.54	30.18	52.80	48.28	59.05	53.32	-1.30	6.60**
		SCA	-2.14	7.46**	-9.10	-15.76	4.76**	2.37*	6.47**	5.93**		
2	E002-91	Mean	56.99	55.42	51.71	36.43	51.72	47.02	52.30	29.92	1.75**	1.82**
		SCA	0.66	11.52**	2.03	-4.72	0.64	5.88**	-3.32	-12.69		
3	PKU-2A	Mean	47.67	40.63	42.32	34.12	57.80	45.97	58.58	37.58	0.16	-0.80
		SCA	-7.07	-0.64	-5.78	-4.41	8.31**	7.46**	4.54**	-2.40		
4	ARG-2A	Mean	64.97	39.63	26.29	31.00	41.93	27.92	60.95	47.67	-2.90	-3.82
		SCA	13.28**	1.38	-18.75	-4.51	-4.51	-7.57	9.97**	10.70**		
5	ARG-3A	Mean	55.51	51.33	51.90	38.77	57.90	47.63	61.10	42.97	5.17**	4.80**
		SCA	-4.25	4.46**	-1.20	-5.36	3.39**	3.52**	2.06	-2.62		
6	ARG-6A	Mean	62.17	42.70	42.34	21.53	44.02	31.08	51.91	48.18	7.21**	3.78**
		SCA	0.37	-3.16	5.46**	4.99**	0.99	4.77**	-6.82	-6.60		
7	DV-10A	Mean	66.27	48.62	43.52	37.30	49.30	37.98	57.28	48.64	0.21	-0.04
		SCA	1.67	-4.62	-4.63	-1.99	-0.25	-1.29	3.20**	7.90**		
8	PHIR-27A	Mean	57.29	39.08	47.08	37.60	50.63	33.00	54.72	48.65	1.00	-0.79
		SCA	1.71	-2.20	-1.85	-0.94	0.30	-5.52	-0.15	8.65**		
9	PRUN-29A	Mean	62.36	53.88	46.45	56.52	48.72	41.35	52.49	45.68	1.07	8.98**
		SCA	6.70**	2.83**	-2.56	8.21**	-1.69	-6.94	-2.45	-4.09		
10	NC-41B (C)	Mean	42.02	29.62	45.72	20.63	46.97	26.27	50.06	23.03	-5.24	-15.49
		SCA	-7.33	3.03**	3.03**	-3.21	2.87**	2.44*	1.43	-2.27*		
GCA			3.15**	1.70**	-3.50	-1.05	-2.10	-1.06	2.44**	0.41		

*, ** Significant at 0.05 and 0.01 of probability respectively, N: Normal environment S: Stress environment.

Table 7. Estimation of combining ability effects and mean performance of hybrids for oil content under normal and water stress environment (pooled over years).

No.	Female/Male		RCR-8297		P69R		P124		P100R		GCA	
			N	S	N	S	N	S	N	S	N	S
1	CMS-XA	Mean	30.93	31.61	31.12	36.27	28.64	31.52	31.60	31.60	1.40**	0.22**
		SCA	0.62**	2.90**	5.28**	3.09**	0.53*	0.61	0.61**	3.57**		
2	E002-91	Mean	29.44	26.40	30.68	28.92	31.98	31.74	30.75	32.57	-1.45	0.36**
		SCA	-4.59	1.41**	-2.07	2.65**	0.75**	3.95**	1.58**	2.72**		
3	PKU-2A	Mean	31.07	29.79	32.06	32.39	30.04	30.83	35.69	31.59	-0.21	1.86**
		SCA	-1.20	3.04**	1.40**	4.03**	-0.16	2.01**	0.60*	7.66**		
4	ARG-2A	Mean	30.67	32.97	30.40	30.97	30.64	33.45	36.04	33.19	1.29**	1.58**
		SCA	1.98**	2.64**	-0.02	2.37**	2.46**	2.61**	2.20**	8.01**		
5	ARG-3A	Mean	34.49	31.85	33.55	30.02	33.13	30.02	33.15	32.58	-0.24	3.23**
		SCA	0.86**	6.46**	-0.97	5.52**	-0.97	5.10**	1.59**	5.12**		
6	ARG-6A	Mean	30.62	29.24	29.72	31.84	29.44	30.32	30.82	31.49	-0.37	-0.45
		SCA	-1.75	2.59**	0.85**	1.69**	-0.67	1.41**	0.50*	2.79**		
7	DV-10A	Mean	29.50	32.72	32.26	31.59	30.52	28.71	31.64	30.07	-0.75	0.68**
		SCA	1.73**	1.47**	0.60*	4.23**	-2.28	2.49**	-0.92	3.61**		
8	PHIR-27A	Mean	29.11	32.77	30.15	29.06	27.77	29.10	29.52	31.24	-0.81	-1.22
		SCA	1.78**	1.08**	-1.93	2.12**	-1.89	-0.26	0.25	1.49**		
9	PRUN-29A	Mean	31.80	32.09	29.80	32.60	31.46	32.48	32.97	36.00	1.94**	1.15**
		SCA	1.10**	3.77**	1.61**	1.77**	1.49**	3.43**	5.01**	4.94**		
10	NC-41B (C)	Mean	29.31	31.32	28.73	29.31	29.10	27.83	15.46	29.57	-1.85	-4.71
		SCA	0.33	1.28**	-1.68	0.70*	-3.16	1.07**	-1.42	-12.57		
GCA			-0.27	0.34**	-0.14	0.32**	-0.28	-0.35	0.68**	-0.31		

*, ** Significant at 0.05 and 0.01 of probability respectively, N: Normal environment S: Stress environment.

sunflower. Our study supported these past findings and reveals that there was significant increase in seed yield per plant and oil content per cent as compared to conventional source (PET-1). This may be attributed to effect of cytoplasmic genes or nuclear cytoplasmic interactions and this positive interaction can be exploited effectively for developing high yielding hybrids based on diverse sources both for normal and water stress environment. However, it is an important finding that the impact of different cytoplasmic sources on the expression of most of the traits particularly seed yield was in desirable direction and may play an important role in diversification of cytoplasm and the hybrid base for future breeding programmes. On the basis of this study, it is suggested that among the CMS analogues, CMS E002-91A, ARG-3A (*H. argophyllus*) and ARG-6A (*H. argophyllus*) and among the testers, RCR-8297 recorded very good combiners for seed yield under both the environments and may be used for development of water use efficient hybrid in sunflower. Hybrid combinations CMS-XA × P124R, CMS-XA × P100R, PKU-2A × P124R, ARG-2A × P100R, ARG-3A × P124R, ARG-6A × P69R, DV-10A × P100R and PRUN-29A × RCR-8297 were identified with high SCA effects for seed yield per plant under both the environments. Previously a number of different research workers i.e. Kandhola *et al.* (1995), Reddy and Madhavi Latha (2005), Haldni *et al.* (2006), Parameshwarappa *et al.* (2008), Imran *et al.* (2015), Faridi *et al.* (2015), Aleem *et al.* (2015), Shinde *et al.* (2016) and Dhillon and Tyagi (2016) have reported a large number of good combining lines for yield, while studying different genetic materials of sunflower.

CONCLUSIONS

It is concluded from this study that sufficient genetic variability was present in the CMS sources with respect to studied traits which can be exploited in further breeding programs for development of water efficient sunflower hybrids having diverse cytoplasmic background. According to SCA and GCA variances, the traits, plant height, head diameter, 100 seed weight, seed yield and oil content has been under the control of non-

additive gene action. Hence to improve yield and oil content, heterosis breeding is suggested for using these diverse CMS sources in sunflower breeding programs. Among the lines, CMS analogues E002-91A, ARG-2A (*H. argophyllus*) and ARG-3A (*H. argophyllus*) and tester RCR-8297 recorded very good combining ability under both the environments for seed yield. These lines may be useful if considered in future heterosis breeding programs. Cross combinations; CMS-XA × P100R, ARG-2A × P100R, ARG-6A × P69R, DV-10A × P100R and PRUN-29A × RCR-829 showed tremendous SCA performance for seed yield and related traits under both the environments.

ACKNOWLEDGEMENTS

This study is a part of PhD thesis, (“Effect of Alien Cytoplasm on Heterosis and Combining Ability of Yield, Quality and Water Use Efficiency Traits in Sunflower (*Helianthus annuus* L.)”). I am thankful to Department of Science and Technology (DST), New Delhi, India for providing INSPIRE fellowship during this study. The authors are grateful to the Indian Institute of Oilseeds Research, Hyderabad, India for providing the base material.

REFERENCES

- Aleem MU, Sadaqat HA, Saif-ul-Malook, Asif M, Qasrani SA, Shabir MZ, Hussain MA (2015). Estimation of gene action for achene yield in sunflower (*Helianthus annuus* L.). *American-Eurasian J. Agric. & Environ. Sci.* 15 (5): 727-732.
- Ali MA, Nawab NN, Rasool G, Saleem M (2009). Estimates of variability and correlation for some quantitative traits in *Cicer arietinum*. *J Agric Soc Sci*, 4: 177-79.
- Baldini M, Megale P, Benvenuti A (1991). Stability analysis, cytoplasmic effects and possible utilization on three male sterility sources in sunflower (*Helianthus annuus* L.). *Ann Bot.*, 49: 27-36.
- Dhillon SK, Tyagi V (2016). Combining ability studies for development of new sunflower hybrids based on diverse cytoplasmic sources. *Helia*, DOI 10.1515/helia-2015-0005.
- Dudhe MY, Moon MK, Lande SS (2009). Evaluation of restorer lines for heterosis studies on sunflower, (*Helianthus annuus* L.). *J. Oilseeds Res.*, 26: (Special issue) 140-142.

- Faridi R, Khan FA, Saif-ul-Malook, Ashraf S, Arshad S, Annum N, Saleem (2015). Gene action study for morphological traits in sunflower (*Helianthus annuus* L.). *American-Eurasian J. Agric. & Environ. Sci.*, 15 (5): 769-775.
- Giriraj K, Shanta RH, Seenappa K. (1987). Combining ability of converted male sterile lines of sunflower (*Helianthus annuus* L.). *Indian J Gen*, 47: 315- 17.
- Haldni N, Skoric D, Kraljevic-Balalic M, Satic Z, Jovanovic DE (2006). Combining ability for oil content and its correlations with other yield components in sunflower (*Helianthus annuus* L.). *Helia* 44: 101-110.
- Imran M, Saif-ul-Malook, Saeed AQ, Nawaz MA, Ahabaz MK, Asif M, Ali Q (2015). Combining ability analysis for yield related traits in sunflower (*Helianthus annuus* L.). *American- Eurasian J. Agric. & Environ. Sci.*, 15(3): 424-436.
- Jan CC (1990). In search of cytoplasmic male-sterility and fertility restoration genes in wild *Helianthus* species. In: Proc. Sunflower Research Workshop, Fargo, ND. 8–9 Jan. 1990, Natl. Sunflower Assoc., Bismarck, ND. pp. 3–5.
- Jan CC (1992). Cytoplasmic-nuclear gene interaction for plant vigour in *Helianthus* species. *Crop Sci*, 32: 320-23.
- Kandhola SS, Behl RK, Punia MS (1995). Combining ability in sunflower. *Ann Biol*, 11: 103-106.
- Kempthorne O (1957). *An introduction to genetical statistics* (ed.) John Wile and Sons, Inc., New York, U.S.A. pp. 458-71.
- Leclercq P (1969). Une sterilité cytoplasmique chez tournesol. *Annales del Amelioration des Plantes*, 19: 99-106.
- Marinkovic R, Skoric D, Dozet B, Jovanovic D (1993). Line x tester analysis of the combining ability in sunflower (*Helianthus annuus* L.). *Indian J. Genet.*, 53 (3): 299-304.
- Meena CR, Meena HP, Sinha B (2013). Fertility restoration, combining ability effects and heterosis in sunflower (*Helianthus annuus* L.) using different CMS sources. *J. Oilseeds Res.*, 30 (1): 60-64.
- Mohansundaram K, Manivannan N, Vindhiya VP (2010). Combining ability analysis for yield and its components in sunflower (*Helianthus annuus* L.). *Electron. J. Plant Breed*. 1 (4): 864-868.
- Parameswari C, Muralidharan V, Subbalakshmi B, Manivannan N, (2004). Genetic analysis yield and important traits in sunflower (*Helianthus annuus* L.) hybrids. *J. Oilseed Res.*, 21(1): 168-170.
- Pavani E, Bharathi M, Reddy AV, Latha KM (2006). Combining ability studies in sunflower (*Helianthus annuus* L.) *J. Oilseeds Res.*, 23 (2) 168-170.
- Putt ED (1966). Heterosis, combining ability and predicted synthetics from a diallel cross in sunflower (*Helianthus annuus* L.). *Can. J. Pl. Sci.*, 46: 59-67.
- Reddy A, Madhavalatha K. (2005). Combining ability for yield and yield components in sunflower. *J. Res. ANGRAU*, 33: 12-17.
- Serieys H (1992). Cytoplasmic effects on some agronomical characters in sunflower. Proceedings of the 13th International Sunflower Conference, 2: 1245-1250.
- Serieys H (1999). Identification, study and utilization in breeding programmes of new CMS sources: FAO progress report (1996-1999). *Helia* 22: 71-84.
- Shankar VG, Ganesh M, Ranganatha ARG, Suman A, Sridhar V (2007). Combining ability studies in diverse CMS sources in sunflower (*Helianthus annuus* L.). *Indian J. Agric., Res.*, 41 (3): 171-176.
- Shekar GC, Jayaramaiah H, Virupakshappa K, Jagadeesh BN, (1998). Combining ability of high oleic acid in sunflower. *Helia* 21(28): 7-14.
- Shinde SR, Sapkale RB, Pawar RM (2016). Combining ability analysis for yield and its components in sunflower (*Helianthus annuus* L.). *International Journal of Agricultural Sciences*. 12(1), 51-55.
- Tyagi V, Dhillon SK (2016). Water-use-efficient cytoplasmic male sterility analogs in sunflower. *Journal of Crop Improvement*. DOI:10.1080/15427528.2016.1188336.
- Tyagi V, Dhillon SK, Bajaj RK, Kaur J (2013). Divergence and association studies in sunflower (*Helianthus annuus* L.). *Helia* 36(58):77–94.
- Tyagi V, Dhillon SK, Bajaj RK, Gupta S (2015a). Phenotyping and genetic evaluation of sterile cytoplasmic male sterile analogues in sunflower (*Helianthus annuus* L.). *Bangladesh Journal of Botany* 44 (1):23–30.
- Tyagi V, Dhillon SK, Gill BS (2015b). Morphophysiological expression in cms analogues of sunflower (*Helianthus annuus* L.) under water stress environment. *Electronic Journal of Plant Breeding* 6(4):1150–56.
- Ullstrup AJ (1972). The impacts of the southern corn leaf blight epidemics of 1970–1971. *Annual Review of Phytopathology* 10:37–50.