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Heterosis studies for biochemical traits associated with aphid tolerance in experimental hybrids based on cms lines in safflower (*Carthamus tinctorius* L.)

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Abstract

Line x Tester analysis involving two females (CMS lines) and twenty males of safflower yielding forty hybrids was conducted to estimate the magnitude of heterosis, heterobeltiosis and standard heterosis over three checks *viz.*, PKV PINK, PBNS 12 and CO 1 for the biochemical traits associated with aphid tolerance and seed yield (under aphid infestation). There was substantial genetic variability present in the genotypes studied for seed yield and other biochemical traits associated with aphid tolerance. Considerable range of variability for heterosis over mid parent, better parent and standard check was observed for aphid infestation index (A.I.I.), phenol content and seed yield as compared to chlorophyll content. Further, the female parent AKS CMS 2A performed well in combination with male parents for all the traits as compared to AKS CMS 3A. The highest, significant heterosis over mid parent, better parent and best check in desirable direction for the trait A.I.I. and chlorophyll content index was observed in cross AKS CMS 2A x AKS/S-33. The highest magnitude of average heterosis, heterobeltiosis and useful heterosis for phenol content was observed in cross AKS CMS 2A x S-518. Further, the highest positive and significant heterosis, heterobeltiosis and standard heterosis in desirable direction were recorded for seed yield per plant (under aphid infestation) by the cross AKS CMS 2A x GMU 3876. Hence, these three crosses *viz.*, AKS CMS 2A X AKS/S-33, AKS CMS 2A X S-518 and AKS CMS 2A X GMU 3876 found to have immense value as far as the traits associated with aphid tolerance is concerned. These crosses may further be tested at various locations to find out best one as aphid tolerant hybrid for commercial cultivation.

Keywords: Safflower, heterosis, aphid tolerance, AII, phenols, seed yield

Introduction

Among most important oilseed crops in India, safflower has the high potential value due to nutritional and pharmaceutical properties of seed oil and petals. Safflower oil is a rich source in Poly Unsaturated Fatty Acid (Linoleic acid 78%), which plays an important role in reducing blood cholesterol level, has good dyeing property and therefore, used in manufacturing of paints, varnishes and linoleum. Safflower oil contains 55-81 *per cent* linoleic acid, 7.42 *per cent* oleic acid, 1-10 *per cent* stearic acid and 1-10 *per cent* palmitic acid with 90 *per cent* unsaturated and 10 *per cent* saturated fatty acids (Anonymous, 2002) [1]. Latha and Prakash (1984) [2] reported that the seeds of safflower contain almost 27.5 *per cent* oil, 15 *per cent* protein, 41 *per cent* crude fiber and 2.3 *per cent* ash.

More than 60 countries in the world grow safflower, but over half is produced in India mainly for vegetable oil market (Patil *et al.*, 1999) [3]. Safflower acreage and production around the world has been witnessing wide fluctuations since last two decades. Hence, there is an urgent need not only to stabilize it but also to enhance by developing high yielding hybrids. Heterosis breeding is an important way for improving crop productivity and quality in order to feed the ever-increasing human population, particularly in developing countries. With the availability of male sterile sources in safflower, exploitation of heterosis on commercial scale has become feasible and economical. Further, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. P.D.K.V.), Akola has developed cytoplasmic male sterile lines *viz.*, AKS CMS 2A and 3A (Deshmukh *et al.*, 2014) [4], which increases the ease in hybrid seed production considerably and hence, it is of great importance to plant breeders for exploitation of heterosis by developing high yielding hybrids. Most of the released varieties (CO-1) are highly susceptible to the attack by aphids [*Uroleucon compositeae* (Theobald)], recorded 86 aphids per 5 cm twig. Further, it causes 40-50 *per cent* seed yield losses and reduction in oil content of damaged seed up to 32 *per cent* and seed weight by 50.6 *per cent* (Mane *et al.*, 2012) [5].

In addition to the huge direct economic losses, deleterious effects of pesticides remain in the environment. Therefore, development of a cost effective and an environmentally friendly approach like improvement of cultivars resistant to aphids is necessary. Though, a large number of high yielding varieties and few hybrids have been released, in the country, however, to meet the future requirements, it is utmost necessary to breed resistant / tolerant varieties and hybrids having agronomically superior traits suiting to the need of farmers and its end users as well. The pest resistant cultivar or hybrid is the most suitable non-cash input for marginal farmers and the development of such cultivars or hybrids requires identification of the pest resistant/tolerant sources. Hence the present study was conducted to identify superior cross combinations (involving CMS lines), which will exhibit high magnitude of heterosis for the traits associated with aphid tolerance in addition to seed yield and it can be released for commercial cultivation after thorough testing.

Material and Methods

- 1. Plant material:** Genetically diverse parents on the basis of their distinguishing characters including tolerance and susceptibility to aphid infestation i.e. two CMS lines *viz.*, AKS CMS 2A and AKS CMS 3A (Cytoplasmic male sterile lines developed by Oilseed Research Unit, Dr. P.D.K.V., Akola) as females and 20 males *viz.*, S-518, CW-99, CCC-B2, GILA, AKS/NS-1, AKS-322, AKS-325, AKS/S-33, GMU-3923, GMU-3965, GMU 3924, GMU 3876, GMU 3863, GMU 3963, GMU 3325, GMU 6877, GMU 6881, GMU 6835, AKS 08R and AKS 10R were deliberately selected. The crosses were effected in line x tester (L x T) scheme to obtain F₁ seeds of 40 crosses at the experimental field of Oilseeds Research Unit, Dr. P.D.K.V., Akola. Utilizing CMS based system; only hand pollination using pollens from protected flowers from male parents was performed with the protected flowers of CMS based females in the morning hours. The parental seeds were multiplied by selfing.
- 2. Field trial:** A field trial of 65 genotypes, consisting 20 parents, 40 F₁s and three checks *viz.*, PKV PINK, PBNS 12 and CO 1 was raised in Randomized Block Design (RBD) with two replications during *rabi* 2017-18 at the field of Oilseeds Research Unit, Dr. PDKV, Akola. Each genotype was planted in a single row of 2 m length with 45 cm spacing between and 20 cm within rows. All the cultivation practices were followed as per recommendations for safflower cultivation. Further, infester rows of susceptible variety *viz.*, "CO-1" were sown one month before sowing of screening block. Sowing of screening block was executed in such a way that crop should attain age of 35-45 days at peak incidence of aphids. Infester plants were uprooted and distributed across the screening block uniformly when the main crop had attained age of 35-40 days. Necessary plant protection measures were undertaken to avoid wilt and *Alternaria blight*.
- 3. Observations:** The observations were recorded on randomly selected five competitive plants per plot per replication in parents, F₁s and checks for following traits.
 - 1. Aphid Infestation Index (A.I.I.):** The observations of foliage drying grade due to aphids were recorded (1-5 scale) when 'CO-1' was completely killed (Anonymous,

2016) [6].

The A.I.I. was estimated by using following formula

$$A.I.I. = \frac{(1 \times a) + (2 \times b) + (3 \times c) + (4 \times d) + (5 \times e)}{a + b + c + d + e}$$

Where,

1 to 5 = Different foliage drying grades.

a to e = Actual number of plants falling in each of the 5 corresponding foliage drying grades.

- 2. Total phenols:** Total phenol content in safflower leaves was estimated as per the procedure described by Bray and Thorpe (1954) [7].
- 3. Chlorophyll content index:** It was determined by using chlorophyll meter, model SPAD-250 plus. For recording the observations; three leaves were randomly collected from observational plants and used for estimation of chlorophyll. Then the mean values of chlorophyll index of three leaves were calculated.
- 4. Seed yield per plant:** (under aphid infestation) in gram was recorded on randomly selected five plants per genotype per replication and averages were worked out.
- 5. Statistical analysis:** The data were subjected to analysis of variance for mean performance (Panse and Sukhatme, 1987) [8] and magnitude of heterosis over mid parent (MP), better parent (BP) and standard check (SC) was estimated as per the standard procedure given by Hays *et al.* (1955) [9] and Turner (1953) [10].

Results and Discussion

It was revealed that, mean squares due to genotypes (treatments) were significant for all the traits studied (Table 1). This indicated the presence of substantial genetic variability for these characters. Further, partitioning of genotypic (treatments) variance into components *viz.*, parents, crosses and parents v/s crosses revealed that the parent differed among them-self significantly for all the characters. Similarly, crosses also showed significant differences for all the traits.

Heterosis for aphid infestation index (A.I.I.) over mid parent ranged between -34.12 (AKS CMS 2A x AKS/S-33) to 20.97 (AKS CMS 2A x GILA) per cent (Table 2 and 3). Out of 40 crosses, 11 crosses showed negative and significant heterosis, out of which the cross AKS CMS 2A x AKS/S-33 (-34.12%) showed highest negative and significant heterosis over mid parent (Table 4) followed by AKS CMS 2A X AKS 322 (-32.53%), AKS CMS 2A x AKS 325 (-27.50%) and AKS 2A X CCC-B2 (-24.14%). Whereas, the range for heterobeltiosis was the -45.10 (AKS CMS 2A x AKS 322 and AKS CMS 2A X AKS/S-33) to 32.61 (AKS CMS 3A x GMU 3924) per cent. Out of 40 crosses, 18 crosses showed negatively significant heterobeltiosis, out of which the cross AKS CMS 2A x AKS 322 and AKS CMS 2A X AKS/NS-1 (-45.10%) showed negative and highest significant heterobeltiosis followed by AKS CMS 2A x GMU AKS 325 (-43.14%) and AKS 2A X CMS GMU 3924 (-41.18%). However, none of the crosses showed positive and significant heterobeltiosis. Standard heterosis ranged from -45.10 to 47.06 per cent, -42.00 to 50.00 per cent and -60.81 to 1.35 per cent over the checks PKV PINK, PBNS-12 and CO-1, respectively in AKS

CMS 2A x AKS/S-33 (lowest) and AKS CMS 2A x GILA (highest) cross. However, nineteen crosses showed negative and significant standard heterosis, out of which the cross AKS CMS 2A x AKS/S-33 (-45.10%) followed by AKS CMS 2A x AKS 325 (-43.14%) and AKS CMS 2A x GMU 3924 (-41.18%) showed negatively significant standard heterosis for A.I.I. over the check PKV PINK. Whereas, none of the crosses showed negative and significant useful heterosis over the check PBNS-12 and CO-1 for this trait.

Heterosis for phenol content ranged from -14.04 (AKS CMS 2A x GILA) to 26.83 (AKS CMS 2A x S-518) per cent. Out of 40 crosses, none of the crosses exhibited negative and significant heterosis. Whereas, seven crosses AKS CMS 2A X S-518 (26.83%), AKS CMS 2A X AKS 325 (25.09%), AKS CMS 3A X AKS 325 (22.23%), AKS CMS 3A X S-518 (20.77%), AKS CMS 2A X GMU 6881 (20.73%), AKS CMS 3A X AKS 322 (17.91%) and AKS CMS 2A X CCC-B2 (17.67%) showed positive and significant heterosis for this trait. The heterosis over better parent ranged from -25.69 (AKS CMS 2A x GILA) to 25.36 (AKS CMS 2A x S-518) per cent. Whereas, twenty two crosses showed negatively significant heterobeltiosis, out of which the cross AKS CMS 2A x GILA (-25.69%) showed highest, negative and significant heterobeltiosis followed by AKS CMS 3A X GILA (-23.21%) for this trait. Further, three crosses showed significant positive heterobeltiosis for this trait. The range for standard heterosis was -24.96 to 29.61 per cent, -21.64 to 35.34 per cent and -6.24 to 83.49 per cent over the checks PKV PINK, PBNS 12 and CO-1, respectively in AKS CMS 2A X GILA (lowest) and AKS CMS 2A X S-518 (highest). Whereas, eight cross showed positive and highly significant standard heterosis over the check PKV PINK i.e. AKS CMS 2A x S-518 (29.61%), AKS CMS 2A X AKS 325 (25.30%) and AKS CMS 2A X GMU 3876 (24.96%). Three crosses AKS CMS 2A x GMU 3963 (36.02%), AKS CMS 2A X S-518 (35.34%) and AKS CMS 2A X AKS 325 (30.85%) showed positive and highly significant heterosis over the check PBNS-12. Three crosses *viz.*, AKS CMS 2A X GMU 3963 (84.41%), AKS CMS 2A X S-518 (83.49%) and AKS CMS 2A X AKS 325 (77.40). recorded positive and highly significant standard heterosis over the check CO-1.

The heterosis for chlorophyll content index ranged from -8.61 (AKS CMS 2A x S-518) to 12.66 (AKS CMS 2A x GMU 6881) per cent. Whereas, three crosses exhibited positive and highly significant heterosis over mid parent i.e. AKS CMS 2A x GMU 6881 (12.66%) followed by AKS CMS 2A x AKS/S-33 (11.53%) and AKS CMS 2A x AKS 322 (07.39%). However, the cross AKS CMS 2A x S-518 (-8.61%) exhibited highest significant negative heterosis. The heterobeltiosis ranged from -12.81 (AKS CMS 3A x GILA) to 10.84 (AKS CMS 2A x AKS/S-33) per cent. Out of 40 crosses, 3 crosses showed positive and significant heterosis over better parent out of which the cross AKS CMS 2A X AKS/S-33 (10.84%), showed positive and highly significant heterobeltiosis followed by AKS CMS 2A X GMU 6881 (10.66%) and AKS CMS 2A X GILA (10.03%). Whereas, ten crosses showed significant and negative heterobeltiosis, out of which the cross AKS CMS 3A x GILA (-12.81%) showed highest negative and significant heterobeltiosis followed by cross AKS CMS 2A x S-518 (-9.27%). Standard heterosis ranged from -12.27 to 11.64 per cent, -10.97 to 13.29 per cent and -2.31 to 24.31 per cent over the checks PKV PINK, PBNS 12 and CO-1, respectively in the crosses AKS CMS 3A X GILA (lowest) and AKS CMS 2A X AKS/S-33 (highest). Whereas, four

crosses showed positive and significant standard heterosis over the check PKV PINK, out of which the crosses AKS CMS 2A x AKS/S-33 (11.64%), AKS CMS 2A x AKS 322 (11.02%) and AKS CMS 2A X GMU 6881 (10.08%) exhibited positive and significant standard heterosis over the check PKV PINK. Whereas, two crosses AKS CMS 3A X GILA (-12.27%) and AKS CMS 3A X GILA (-10.50%) showed negative and significant standard heterosis over the check PKV PINK. However, the three cross AKS CMS 2A x AKS/S-33 (13.29%) followed by AKS CMS 2A x AKS/S-33 (12.66%) and AKS CMS 2A x GMU 6881 (11.71%) showed positive and highly significant standard heterosis over the check PBNS-12. Out of 20 crosses, one cross AKS CMS 2A x AKS/S-33 (24.31%) showed positive and highly significant standard heterosis over the check CO-1.

The heterosis for seed yield per plant ranged from -29.08 (AKS CMS 2A x GILA) to 35.04 (AKS CMS 2A x GMU 3876) per cent. Three crosses *viz.*, AKS CMS 2A x GMU 3876 (35.04%), AKS CMS 2A x AKS 325 (13.09%) and AKS CMS 2A x GMU 3863 (16.86%), exhibited highly significant and positive heterosis. The range of heterobeltiosis for seed yield was -48.35 (AKS CMS 2A x GILA) to 27.61 (AKS CMS 2A x GMU 3876) per cent. Out of 40 crosses, three crosses showed positive and significant heterosis over better parent *viz.*, AKS CMS 2A x GMU 3876 (27.61%) followed by AKS CMS 2A x GMU 3924 (16.91%) and AKS CMS 2A x AKS 325 (9.38%). Whereas, two crosses AKS CMS 2A X GILA (-48.35%) and AKS CMS 2A X GILA (-46.34%) showed negatively significant heterosis over better parent. Standard heterosis ranged from -56.96 to 19.48 per cent over the check PKV PINK, -54.99 to 24.96 per cent over PBNS 12 and -59.33 to 12.92 over CO-1 in cross AKS CMS 2A X GILA (lowest) and AKS CMS 2A X GMU 3876 (highest). Two crosses AKS CMS 2A x GMU 3876 (19.48%, 24.96% and 12.92%) and AKS CMS 2A x GMU 3924 (4.74%, 9.55% and 4.03%) exhibited significant heterosis in desirable direction over both checks PKV PINK, PBNS-12 and CO-1. Whereas, two cross showed negatively significant useful heterosis over the checks PKV PINK and PBNS-12. Further, seven crosses were exhibited highly significant and negative standard heterosis over the checks CO-1.

The highest, significant and negative heterosis over mid parent, and better parent for the trait A.I.I. was observed in cross AKS CMS 2A x AKS/S-33 and over best check AKS CMS 2A x AKS/NS-33. The highest magnitude of heterosis over mid parent and better parent for phenol content was observed in cross AKS CMS 2A x S-518, whereas, cross AKS CMS 2A x S-518 exhibited highest positive and significant useful heterosis over the best check i.e. PKV PINK. In case of chlorophyll content index. the cross AKS CMS 2A x AKS/S-33 showed highest and positive heterosis over mid parent and better parent, and check i.e. PKV PINK. The highest positive and significant heterosis and heterobeltiosis in desirable direction were recorded for seed yield per plant (under aphid infestation) by the cross AKS CMS 2A x GMU 3876 and the same cross showed highest and significantly positive standard heterosis over the checks i.e. PKV PINK, AKS 207 and PBNS-12. There were no reports found in the literature on heterosis for traits associated with aphid tolerance in safflower. However, Jadhav and Gawande (2015)^[11] reported that the crosses, ICCV 2 x Gulak1, ICCV2 x ICC 506 and JAKI 9218 x ICC 506, found to be most promising and identified on the basis of heterosis response and mean performance of the parents and crosses. These diverse crosses

have immense practical value and can be exploited in further breeding of chickpea for pod borer tolerance. It is well known fact that the heterosis phenomenon is the result of non-additive gene action for the respective trait and in addition to additive gene effects, importance of non-additive gene effects was also been reported for pod borer resistance in chickpea by Narayamma *et al.*, (2013)^[12] and Singh and Paroda (1989)^[13].

Out of two females, it was observed that the crosses involving AKS CMS 2A found to be more promising as compared to AKS CMS 3A for all the traits studied. However, amongst male parents, AKS/S-33 performed very well when crossed with AKS CMS 2A for A.I.I. and chlorophyll content index and S-518 and GMU 3876 with same female parent for the

trait phenol content and seed yield per plant (under aphid infestation). Hence, these three crosses *viz.*, AKS CMS 2A X AKS/S-33, AKS CMS 2A X S-518 and AKS CMS 2A X GMU 3876 found to be promising as far as the traits associated with aphid tolerance is concerned. These crosses may further be tested at various locations to find out best one as aphid tolerant hybrid. Further, for more authentications, it is necessary to find out another biochemical and morphological basis of resistance / tolerance in further studies and selection of crosses should not rest only on the per se performance of parents and heterosis for seed yield but the performance of parents and their hybrids for various traits associated with aphid tolerance should also be considered.

Table 1: Analysis of variance for combining ability for traits associated with aphid tolerance in safflower

Sources of variation	Mean sum of squares				
	d. f.	Aphid Infestation Index	Phenol content	Chlorophyll content	Seed yield (under aphids infestation)
Replications	1	0.178	0.131	7.610	0.798
Treatments	61	0.660**	0.089**	12.548**	5.166**
Parents	21	0.601**	0.059**	8.352*	4.580**
Crosses	39	0.677**	0.107**	24.469**	5.142**
Parents v/s crosses	1	0.112**	0.122**	25.760*	12.332**
Error	61	0.049	0.023	4.226	3.446

(* , ** Significant at 0.05 and 0.01 probability level, respectively)

Table 2: Estimates of heterosis (H₁), heterobeltiosis (H₂) and standard heterosis (H₃) for traits associated with aphid tolerance in some selected crosses of safflower.

Sr. No.	Genotypes		Aphid infestation index (A.I.I.)	Phenol Content	Chlorophyll content index	Seed yield	
1	AKS CMS-2A X S-518	H1	-12.94	26.83**	-8.61*	-3.99	
		H2	-27.45 **	25.36*	-9.27*	-7.24	
		H3	A	-27.45**	29.61**	-8.42	-17.08
			B	-26.00**	35.34**	-7.07	-13.28
			C	-50.00**	83.49**	1.97	-21.64
2	AKS CMS-2A X AKS-325	H1	-27.50**	25.09**	5.86	13.09**	
		H2	-43.14**	24.07*	5.15	9.38**	
		H3	A	-43.14**	25.30*	6.03	-2.45**
			B	-42.00**	30.85**	7.59	2.03**
			C	-60.81**	77.40**	18.06**	-7.81**
3	AKS CMS-2A X AKS/S-33	H1	-34.12**	10.58	11.53**	-8.86	
		H2	-45.10**	6.17	10.84*	-12.24	
		H3	A	-45.10**	16.51	11.64**	-21.01
			B	-44.00**	21.67	13.29**	-17.39
			C	-62.16**	64.96**	24.31**	-25.35
4	AKS CMS-2A X GMU-3924	H1	-22.08*	10.69	2.71	-20.93**	
		H2	-41.18**	0.16	0.50	16.91**	
		H3	A	-41.18**	24.92*	4.47	4.74**
			B	-30.23**	30.45**	6.01	9.55*
			C	-59.46**	76.86**	16.32**	4.03*
5	AKS CMS-2A X GMU-3876	H1	-20.48*	12.86	4.00	35.04**	
		H2	-35.29**	3.75	3.41	27.61**	
		H3	A	-35.29**	24.96*	4.05	19.48**
			B	-34.00**	30.49**	5.59	24.96**
			C	-55.41**	76.91**	15.86**	12.92**
6	AKS CMS-2A X GMU-3863	H1	00.00	-7.2	3.05	16.86*	
		H2	-3.64	-10.09	2.51	0.73	
		H3	A	-3.92	-9.02	1.98	-16.06
			B	6.05	-5.18	3.48	-12.21
			C	-28.38**	28.56	13.54**	-20.67
7	AKS CMS-3A X S-518	H1	-17.51**	20.77*	3.56	9.36	
		H2	-28.26**	17.86	3.40	8.27	
		H3	A	-35.29**	21.85*	4.37	-3.21
			B	-34.00**	27.24*	5.91	1.23
			C	-31.08**	72.51**	16.20**	-8.53
8	AKS CMS-3A X AKS-322	H1	-15.38	17.94*	-2.10	9.52	
		H2	-28.26**	10.78	-5.14	8.31	

		H3	A	-35.29**	24.07*	1.77	-5.00
			B	-34.02**	29.56*	3.27	-0.64
			C	-55.41**	75.65**	13.31**	-10.22
9	AKS CMS-3A XAKS-325	H1		-20.00	22.23*	1.24	12.19
		H2		-34.78**	21.64*	1.13	18.26
		H3	A	-41.18**	20.85	1.98	-0.76
			B	-40.02**	26.20*	3.48	3.79
			C	-59.46**	71.10**	13.54**	-6.22
	SE(d)	H1		0.19	0.13	1.78	1.60
		H2,H3		0.22	0.15	2.055	1.85
	CD (0.05)	H1		0.38	0.26	3.60	3.25
		H2,H3		0.44	0.31	4.15	3.75
	CD (0.01)	H1		0.52	0.36	4.82	4.35
		H2,H3		0.60	0.41	5.56	5.02

(* , ** Significant at 0.05 and 0.01 probability level, respectively)

Table 3: Range of heterosis (%) for traits associated with aphid tolerance in safflower

Sr. No.	Traits	Range of heterosis (%) over		
		Mid parent	Batter parent	Standard check*
1	Aphid infestation index	-34.12 to 20.97	-45.10 to 32.61	-45.10 to 47.06
2	Phenol content	-14.04 to 26.83	-25.69 to 25.36	24.96 to 29.61
3	Chlorophyll content	-8.61 to 11.53	-9.27 to 10.84	-10.50 to 11.64
4	Seed yield	-29.08 to 35.04	-48.35 to 27.61	-56.96 to 19.48

(*best check among three checks (PKV PINK, PBNS-12 and C0-1) for concerned trait)

Table 4: Crosses with maximum heterosis in desirable direction for traits associated with aphid tolerance in safflower

Sr. No.	Traits	Maximum beneficial heterosis (%) over mid parent		Maximum beneficial heterosis (%) over batter parent		Maximum beneficial heterosis (%) over standard check*	
		Crosses		Crosses		Crosses	
		H ₁	H ₂	H ₁	H ₂	H ₃	H ₃
1	Aphid infestation index (A.I.I)	AKS CMS 2A X AKS /S-33	-34.12**	AKS CMS 2A X AKS/S-33	-45.10**	AKS CMS 2A X AKS/S-33	-45.10**
2	Phenol content	AKS CMS 2A X S-518	26.83**	AKS CMS 2A X S-518	25.36**	AKS CMS 2A X GMU 3963	29.61**
3	Chlorophyll content index	AKS CMS 2A X AKS/S-33	11.53**	AKS CMS 2A X AKS/S-33	10.84**	AKS CMS 2A X AKS/S-33	11.64**
4	Seed yield	AKS CMS 2A X GMU 3876	35.04**	AKS CMS 2A X GMU 3876	27.61**	AKS CMS 2A X GMU 3876	19.48**

(*best check among three checks (PKV PINK, PBNS-12 and C0-1) for concerned trait)

(* , ** Significant at P=0.05 and P=0.01 probability level, respectively)

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