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Magnitude and Nature of Gene Effects Controlling Oil Content and Quality Components in Sunflower (*Helianthus Annuus* L.)

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Abstract: The present research aimed to study gene effects for oil content and fatty acid composition in sunflower. It involved a set of 92 hybrids developed by crossing four CMS lines with 23 perfect restorers. Experiment was conducted at experimental field area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The data was recorded on oil content and quality traits. The analysis of variance revealed significant differences among the traits studied. Among the lines; CMS 42A was observed to have higher significant positive *gca* effects for oil content, linoleic acid & linolenic acid and higher significant negative *gca* effects for palmitic acid and stearic acid, whereas, for oleic acid line, CMS 40A had higher positive *gca* effects. Among the testers, TSG 275 had higher significant positive *gca* effects for linolenic acid and significant negative *gca* effects for stearic acid. High positive *gca* effects for oleic acid and oil content were observed for TSG 331. The tester OPH 91 was good combiner with high positive *gca* effects for oleic acid and negative *gca* effects for palmitic acid, whereas, tester TSG 288 exhibited highest positive *gca* effects for linoleic acid. The best cross combinations; CMS 40A × TSG 259, CMS 607A × TSG 271 and CMS 40A × OPH 73 showed significant specific combining ability effects for oil content and cross CMS 40A × TSG 289 had significant specific combining ability for oleic acid and linoleic acid. The cross combination CMS 40A × TSG 259 is giving a significant jump of over 12% against the current commercial check for oil percentage and for other quality traits more than 50% over the standard check, which is significant for undertaking improvement of hybrid for oil quality.

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Keywords: sunflower, hybrids, combining ability, heterosis and line × tester

Introduction

Sunflower is an oilseed crop used for edible purpose and other industrial use. It is the fourth major source of edible oil after soybean worldwide and second in Europe after rapeseed (Arshad *et al.*, 2010). It is an excellent option for crop rotation and succession systems for various production regimes because of its large capacity of adaptation to different edaphic and climatic conditions (Carvalho, 2003). Sunflower seeds contain high level of oil content (40–50 %) (Lopez *et al.*, 2000). In conventional sunflower oil, 90 % of the total fatty acids content is comprised of oleic acid (C-18:1) & linoleic acid (C-18:2) and 8–10 % of mainly palmitic acid (C-16:0) and stearic acid (C-18:0). According to Friedt *et al.* (1994), in addition to conventional fatty acids, sunflower oil also contains several other fatty acids, but are present only in traces (C-14:0, C-16:1, C-14:1, C-20:0, C-22:0). Standard sunflower cultivars contain high linoleic acid, moderate oleic acid and low linolenic acid (Jabrino *et al.*, 2003). Oil rich in oleic acid is preferred for nutritional use while oil with higher content of linoleic acid is preferred by paint or fuel industry. Sunflower oil with high oleic acid content is nutritionally similar to olive oil which is considered superior to other types of seed oil (Doty, 1978). Grundy (1986) also suggested that a diet rich in mono unsaturated fatty acids i. e. oleic acid reduces cholesterol in blood plasma (reducing the risk of coronary heart diseases), has greater shelf life and high degree of oxidative stability. Main breeding objective of sunflower is to develop high yielding, disease resistant hybrids with high oil quality (Dudhe *et al.*, 2009). Combining ability analysis helps in identification of potential parents, superior cross combinations and to get the information regarding nature and magnitude of gene effects controlling quantitative traits. It is well known that, heterosis is attained by crossing inbred lines and since lines can be genetically related, therefore, appearance of heterosis is not bound to be observed in all hybrid combinations. Heterotic performance of hybrid combination depends upon combining ability of its parents (Kadkol *et al.*, 1984). A wide range of heterosis has been reported both for oil content and fatty acid composition in sunflower by various authors (Aslam *et al.*, 2010; Joksimović *et al.*, 2006; Khalil *et al.*, 2000; Sawargaonkar and Ghodke, 2008; Sujatha and Reddy, 2009). Kaya and Atakisi (2004) reported that superior hybrids have been obtained by crossing inbred CMS lines and restorer lines with high *gca* (general combining ability) values. Among the various biometrical techniques, line × tester is an efficient method for evaluation of large number of inbreds and for identifying the parents and hybrids with good *gca* and *sca* effects, respectively.

As it provides information on the importance of *gca* and *sca* (specific combining ability) effects, thus helps in interpreting the genetic basis of important plant traits (Khan *et al.*, 2008). The present study was therefore aimed to study gene effects for oil content and quality parameters through line × tester analysis in sunflower. It also focused on the extent of heterosis for oil content along with the nature of the gene action involved in the inheritance of important oil quality traits in ninety two hybrids obtained from four CMS lines and twenty three restorer lines.

Material and methods

A set of four cytoplasmic male sterile lines *viz*. CMS 40A, CMS 42A, CMS 47A and CMS 607A and twenty three restorer lines *viz*. TSG 22, TSG 259, TSG 263, TSG 271, TSG 275, TSG 277, TSG 289, TSG 290, TSG 292, TSG 294, TSG 310, TSG 317, TSG 331, TSG 255, TSG 288, OPH 71, OPH 78, OPH 87, OPH 91, OPH 73, 95-C-1, P122R, and P123R were planted during *kharif* 2016. Four CMS lines were crossed with twenty three restorer lines to synthesize a set of ninety two hybrids in line × tester fashion in the experimental area of Oilseed Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. During the spring season 2017, the developed ninety two hybrids along with their parents and check PSH 1962 were sown in randomized block design with three replications in the same plot (1.2×3 m^2) for the evaluation of combining ability effects of parents & hybrids and evaluation of heterotic effects for oil content and fatty acid composition (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid). A random sample of open pollinated seeds of all the genotypes from all the three replications was used for oil content estimation using a benchtop pulsed nuclear magnetic rasonance (NMR)-MQC-5 analyser (oxford, London) (ISO 10565) and fatty acid estimation was done using Gas Liquid Chromatography (GLC) (Appelqvist, 1968). The mean values of inbred lines and F_1 hybrids were used to calculate the values of combining abilities and to assess the gene effects using line × tester method (Singh and Choudhary, 1976). Heterosis was estimated in three different ways, mid parent heterosis, better parent heterosis and standard heterosis. Mid parent heterosis was estimated as superiority of F_1 over the mean value or average of the two parents expressed in terms of percentage. Better parent heterosis also known as heterobeltiosis was estimated as superiority of hybrid over parent which was having desirable values. Better parent heterosis was also expressed in terms of percentage. Standard heterosis refers to the superiority of F_1 over the standard commercial check hybrid expressed in terms of percentage. It is also called as economic heterosis or useful heterosis. In the present investigation, PSH 1962 was considered as standard check.

Results and discussion

From the analysis of variance for combining ability, significant differences were observed for all the characters under study that indicated considerable genotypic variation among parents and hybrids. The mean squares due to lines (females) were significant for all the characters except for oleic acid, linoleic acid and linolenic acid whereas mean squares due to testers (males) were highly significant for all characters except for linolenic acid. The differences due to female x male interaction were highly significant for oil content as well as fatty acids (Table 1).

Table 1: Analysis of variance for experimental design for oil content and fatty acids.

Source of variation	d.f.	OC	PA	SA	OA	LA	LiA
Replicates	2	1.44	0.00	0.01	0.02	0.28	0
Genotypes	118	17.06**	1.70**	1.88**	245.95**	232.97**	0.04**
Parents	26	8.52**	1.18**	1.49**	188.57**	232.97**	0.04**
Line (L)	3	23.80**	0.53**	3.13**	157.46**	160.48**	0.01**
Testers (T)	22	6.65**	1.31**	1.33**	199.97**	207.15**	0.03**
L vs T	1	3.95*	0.11*	0.00	31.12**	37.57**	0.03**
Parents vs Crosses	1	3.28	1.16**	5.78**	10.70**	0.06	0.05**
Crosses	91	19.65**	1.84**	1.94**	264.94**	246.31**	0.05**
Error	236	0.97	0.02	0.01	0.55	0.86	0.01

*Significant at 5% probability level
**Significant at 1% probability level
Oil content (OC), Palmitic acid (PA), Stearic acid (SA), Oleic acid (OC), Linoleic acid (LA), Linolenic acid (LiA)

Comparative estimates of variances due to *gca* and *sca* for oil content and oil quality traits revealed that palmitic acid, stearic acid and linolenic acid had *gca: sca* ratio less than unity which indicates that these parameters were predominantly under the control of non-additive gene action. Whereas, for oil content, oleic acid and linolenic acid, ratio was more than unity, which indicated that additive type of gene action was involved in expression of these traits (Table 2). The involvement of additive gene action w.r.t. oil content has earlier been reported by Ortegon-Morales *et al.* (1992), Rojas and Fernández-Martínez (1998), Mijić *et al.* (2008) and Salem and Ali (2012), whereas, Škorić *et al.* (2000), Karasu *et al.* (2010) and Hladni *et al.* (2011) observed non-additive gene action for oil content. As in our study, Sakthivel (2003) and Ortis *et al.* (2005) also reported non-additive gene action for palmitic acid and Madhavilatha *et al.* (2004) and Ortis *et al.* (2005) observed non-additive gene action for stearic acid.

Table 2: Analysis of variance for combining ability for oil content and fatty acids.

Source of variation	d.f	Mean sum of squares					
		OC	PA	SA	OA	LA	LiA
Replicates	2	0.48	0.01	0.00	0.05	0.42	0.00
Line Effect	3	19.65**	1.84**	7.99**	106.54	148.43	0.08
Tester Effect	22	157.16**	2.02	2.54*	492.76**	453.35**	0.04
Line × Tester Eff.	66	30.18**	3.05*	1.47**	196.20**	181.75**	0.05**
Error	182	9.89**	1.44**	0.01	0.55	0.95	0.01
Genetic components							
σ^2 Females		2.26	0.03	0.12	1.54	2.14	0.01
σ^2 Males		2.43	0.25	0.21	41.02	37.70	0.01
σ^2 Females × σ^2 Males		2.97	0.47	0.49	65.22	60.27	0.02
σ^2 gca		4.58	0.12	0.13	7.39	7.41	0.01
σ^2 sca		2.97	0.47	0.26	14.77	14.81	0.02
σ^2 gca/σ^2 sca		1.54	0.26	0.49	65.22	60.27	0.19
Heritability (Narrow Sense) %		58.12	20.59	0.53	0.23	0.25	16.02
Contribution (%) of							
Females		26.36	3.61	31.62	44.96	44.5	20.91
Males		37.12	39.92	54.84	53.71	53.52	73.25
Females × Males		36.52	56.47	13.54	1.33	1.99	5.84

*Significant at 5 % probability level
**Significant at 1 % probability level
Oil content (OC), Palmitic acid (PA), Stearic acid (SA), Oleic acid (OC), Linoleic acid (LA), Linolenic acid (LiA)

Combining ability

When *gca* effects of lines and testers are to be considered for the oil content, highest significant positive *gca* effects were observed for CMS 42A, CMS 47A, TSG 277, 95-C-1 and TSG 331 which suggested these lines and testers to be good combiners. The female parents; CMS 42A, CMS 607A for palmitic acid & CMS 42A, CMS 47A, CMS 40A for stearic acid and male parents OPH 91, OPH 78 for palmitic acid & TSG 22, TSG 275 and OPH 71 for stearic acid were good general combiners as negative *gca* effects are desirable. In case of oleic acid; the female parent CMS 40A and male parents TSG 331 & OPH 91 were recorded to be very good general combiners. The CMS lines; CMS 42A, CMS 47A and restorers; TSG 288, TSG 271, TSG 289 and OPH 73 were recorded as good

combiners for the linoleic acid and the CMS lines; CMS 42A and CMS 47A and tester; TSG 275 has the significant high positive significant *gca* value for the linolenic acid (Table 3).

Table 3: GCA effects of female and male parents for different morpho-physiological, yield and quality traits.

S. No.	Source	OC	PA	SA	OA	LA	LiA
Female parents							
1	40 A	−2.11**	0.20**	−0.08**	1.69**	−1.81**	−0.04**
2	42 A	1.44**	−0.21**	−0.29**	−1.09**	1.52**	0.04**
3	47 A	0.52**	0.04*	−0.13**	−0.75**	0.78**	0.02**
4	607 A	0.15	−0.03*	0.49**	0.15	−0.49**	−0.02**
	CD (0.05)	**0.23**	**0.03**	**0.02**	**0.18**	**0.23**	**0.01**
	CD (0.01)	**0.31**	**0.04**	**0.03**	**0.23**	**0.29**	**0.01**
Male parents							
1	TSG 22	0.65*	−0.26**	−0.81**	−2.26**	3.27**	0.02*
2	TSG 259	−1.08**	1.01**	0.58**	−9.08**	7.54**	−0.09**
3	TSG 263	0.38	0.14**	−0.33**	2.08**	−1.89**	−0.03**
4	TSG 271	−1.37**	0.05	0.25**	−6.46**	6.20**	−0.07**
5	TSG 275	0.87**	0.43**	−0.69**	−4.00**	4.12**	0.12**
6	TSG 277	3.08**	−0.58**	0.54**	2.84**	−2.11**	0.05**
7	TSG 289	−2.73**	0.28**	−0.38**	−5.72**	5.74**	0.04**
8	TSG 290	−1.20**	0.34**	0.29**	−1.47**	0.84**	−0.03**
9	TSG 292	−4.79**	0.09*	0.22**	−2.13**	1.83**	−0.05**
10	TSG 294	−0.33	0.46**	0.67**	−5.92**	4.74**	0.02*
11	TSG 310	0.61*	0.23**	0.43**	−1.80**	1.04**	0.07**
12	TSG 317	1.17**	−0.34**	−0.32**	10.76**	−10.15**	0.01
13	TSG 331	1.52**	−0.34**	0.11**	15.503**	−15.32**	0.01
14	TSG 255	−0.07	0.18**	0.69**	6.05**	−6.89**	−0.07**
15	TSG 288	0.28	0.32**	−0.34**	−7.95**	7.97**	−0.03**
16	OPH 71	−0.24	0.48**	−0.55**	−3.77**	3.84**	−0.03**
17	OPH 78	0.08	−1.03**	0.36**	−3.71**	4.29**	0.05**
18	OPH 87	−0.51	0.51**	−0.27**	2.57**	−2.93**	0.08**
19	OPH 91	0.79**	−1.13**	−0.26**	10.20**	−8.86**	0.02*
20	OPH 73	−0.36	−0.003	0.08**	−5.48**	5.31**	0.07**
21	95-C-1	1.77**	−0.12**	0.48**	5.87**	−6.16**	−0.11**
22	P 122 R	0.46	−0.35**	−0.45**	1.16**	−0.37	−0.02**
23	P 123 R	1.00**	−0.39**	−0.33**	2.74**	−2.05**	−0.001
	CD (0.05)	**0.56**	**0.08**	**0.04**	**0.42**	**0.55**	**0.02**
	CD (0.01)	**0.74**	**0.10**	**0.07**	**0.56**	**0.69**	**0.02**

*Significant at 5% probability level

**Significant at 1% probability level

Oil content (OC), Palmitic acid (PA), Stearic acid (SA), Oleic acid (OC), Linoleic acid (LA), Linolenic acid (LiA)

While studying specific combining ability of individual cross combination for oil content, twenty one hybrids were recorded with significant positive *sca* effects. Among these, CMS 40A × TSG 259, CMS 607A × TSG 271 and CMS 40A × OPH 73 were recorded with highest positive *sca* effects. A total of eighteen, forty one and thirty nine hybrids were reported as having significant positive *sca* effects for oleic acid, linoleic acid and linolenic acid, respectively. Hybrids; CMS 40A × TSG 288, CMS 40A × TSG 289, CMS 42A × OPH 91, CMS 40A × OPH 73 and CMS 47A × OPH 91 exhibited highest significant *sca* effects for oleic acid, hybrids; CMS 40A × TSG 289, CMS 40A × OPH 87 and CMS 607A × OPH 91 for linoleic acid and hybrids CMS 40A × OPH 87, CMS 42A × TSG 288 and CMS 42A × TSG 310 for linolenic acid. A large number of hybrids were identified as having significant negative *sca* effects for saturated fatty acids (thirty for palmitic acid and thirty seven for stearic acid). Out of these combinations; CMS 42A × OPH 91 and CMS 40A × TSG 259 had the highest significant negative *sca* effects for palmitic acid and stearic acid, respectively (Table 4). Results of our combining ability collaborate with the findings of Nasreen *et al*. (2014).

Table 4: Top five selected hybrids on the basis of SCA effects for oil content and fatty acids.

S. No.	Hybrids	SCA effects	GCA effects	
			P1	P2
Oil content (%)				
1	40 A × TSG 259	3.00**	−2.11**	−1.08**
2	607 A × TSG 271	2.89**	0.15	−1.37**
3	40 A × OPH 73	2.88**	−2.11**	−0.36
4	42 A × TSG 277	2.67**	1.44**	3.08**
5	40 A × TSG 263	2.66**	−2.11**	0.38
Oleic acid (%)				
1	40 A × TSG 288	15.70**	1.69**	−7.95**
2	40 A × TSG 289	14.67**	1.69**	−5.72**
3	42 A × OPH 91	12.97**	−1.09**	10.20**
4	40 A × OPH 73	12.91**	1.69**	−5.48**
5	47 A × OPH 91	12.20**	−0.75**	10.20**
Linoleic acid (%)				
1	40 A × TSG 289	18.07**	−1.81**	5.74**
2	40 A × OPH 87	15.24**	−1.81**	−2.93**
3	607 A × OPH 91	13.95**	−0.41**	−8.86**
4	47 A × TSG 289	11.47**	0.78**	5.74**
5	607 A × TSG 331	10.75**	−0.49**	−15.32**

Based on the significant differences among the lines, it is implicated that there is possibility of genetic improvement w.r.t. oil content, palmitic acid and stearic acid in the present material used for this study. In case of testers, we can go for genetic improvement of the traits such as oil content, palmitic acid, stearic acid, oleic acid and linoleic acid, since, significant differences were present. Likewise, for line × tester, we can take up genetic improvement for all the characters. The traits governed by additive gene action such as oil content, oleic acid and linolenic acid suggests that genetic improvement of parents is possible for these traits *via* selection, whereas, for traits governed by non-additive gene action *viz*. palmitic acid, stearic acid and linoleic acid improvement can be expected in cross combinations. The non-additive gene action for oil content has been reported previously by Tyagi *et al*. (2012), Dhillon and Tyagi (2016) and Tyagi and Dhillon (2016) in different traits.

From the estimation of *gca* effects, the good general combiners could be selected for all the parameters. Although, no single line or tester is a good combination for all the traits. Among the lines, CMS 42A was observed to have higher significant positive *gca* effects for oil content, linoleic acid & linolenic acid and higher significant negative *gca* effects for palmitic acid and stearic acid, whereas, for oleic acid, line; CMS 40A has higher positive *gca* effects. Among the testers; TSG 275 has significant high positive *gca* effects for linolenic acid and significant negative *gca* effects for stearic acid. High positive *gca* effects for oleic acid and oil content were found in TSG 331. The tester OPH 91 was good combiner with high positive *gca* effects for oleic acid and negative *gca* effects for palmitic acid, whereas, tester TSG 288 exhibited highest positive *gca* effects for linoleic acid. High positive *gca* and *sca* effects for oil content had also been reported by Tyagi and Dhillon (2016) and Tyagi *et al*. (2018).

On the basis of *sca* effects for cross combinations, no single hybrid was selected for all the traits. Cross combination; CMS 40A × OPH 73 was a good combiner for oil content, oleic acid and palmitic acid.

Heterosis

Out of the total 92 hybrids, 48 cross combinations revealed significant positive heterosis over mid parent, 20 combinations over better parent and 8 combinations over the standard check for oil content. Variation w.r.t. heterosis was observed to be high for all the quality parameters *viz*. palmitic acid, stearic

acid, oleic acid, linoleic acid and linolenic acid whereas, for oil content, less variation was recorded. The highest significantly positive better parent and standard heterosis for the oil content was observed for the cross CMS 40A × TSG 259 with values of 14.93 % and 12.13 %, respectively, whereas, cross; CMS 607A × TSG 294 (17.49 %) had highest significantly positive heterosis over mid parent. Sawargaonkar and Ghodke (2008) and Sujatha and Reddy (2009) also reported high heterosis in sunflower for oil content. Coming onto heterosis for fatty acid composition, which is important w.r.t. improving the oil quality, for oleic acid, forty nine hybrids had significant positive heterosis over standard check, out of which CMS 40A × TSG 288 was observed with the maximum value of 46.25 %. Forty four hybrids having positive heterosis over mid parent and 24 hybrids having positive heterosis over better parent were observed and combination CMS 42A × TSG 255 was recorded with highest positive significant heterosis over mid parent (84.22 %) and over better parent (58.83 %). Khalil *et al.* (2000), Joksimović *et al.* (2006), Aslam *et al.* (2010) and Shamshad *et al.* (2016) also reported that heterotic and heterobeltiotic effects for oleic acid were highly significant for most of the F_1 hybrids. The cross combination; CMS 607A × TSG 288 has the highest positive mid parent (76.56 %), better parent (72.25 %) and standard (61 %) heterosis for the linoleic acid. Total hybrids with positive heterosis over mid parent, better parent and standard check for the linoleic acid were 39, 20 and 38, respectively. For linolenic acid, 36 hybrids with significant positive heterosis for mid parent, 29 hybrids with significant positive heterosis over better parent and 26 hybrids with significant positive heterosis over standard check were reported. Highest mid parent & better parent heterosis was observed for cross CMS 40A × TSG 310 and highest standard heterosis for cross CMS 40A × TSG 22. In case of palmitic acid, highest significant negative mid parent (–68.82 %), better parent (–70.64 %) and standard heterosis (–74.81 %) was observed in case of cross CMS 40A × TSG 288. Highest significantly negative mid parent, better parent and standard heterosis observed with values of –47.95 %, –52.48 % and –62.65 %, respectively were observed in a combination CMS 42A × TSG 263 out of 18, 33 and 62 hybrids (with significant negative heterosis) (Table 5).

Estimation of heterosis assists in identification of superior combinations. Higher magnitude of heterosis is of utmost importance for a trait with respect to current commercial cultivar. The cross combination CMS 40A × TSG 259 is giving a significant jump of over 12 % against the current commercial check for oil percentage and for other quality traits more than 50 % over the standard check, which is significant for undertaking improvement of hybrid for oil quality.

Table 5: Top five hybrids on the basis of *per se* performance, standard heterosis, *sca, gca* and mean values of parents for oil content and fatty acids.

S. No.	Hybrid	*Per se* mean performance of hybrid	Standard heterosis	SCA effects	GCA effects		Mean performance of parents	
					P1	P2	P1	P2
Oil content (%)								
1	40 A × TSG 259	47.96	12.13**	3.10**	−2.11**	−1.08**	41.74	40.17
2	40 A × TSG 292	45.94	7.41**	−1.71**	−2.11**	−4.79**	41.74	37.65
3	40 A × OPH 78	45.53	6.45**	0.14	−2.11**	0.08	41.74	40.78
4	40 A × OPH 91	45.51	6.43**	1.57	−2.11**	0.79**	41.74	37.49
5	40 A × TSG 22	44.19	3.30**	2.43	−2.11**	0.65**	41.74	42.05
Oleic acid (%)								
1	40 A × TSG 288	78.84	46.25**	15.70**	1.69**	−7.95**	56.12	59.58
2	42 A × TSG 288	78.42	45.48**	−10.33**	−1.10**	−7.95**	32.03	59.58
3	40 A × TSG 292	76.10	41.17**	4.38**	1.69**	−2.13**	56.12	76.54
4	40 A × TSG 292	74.53	41.17**	4.39**	−1.09**	−2.13**	56.12	76.54
5	40 A × TSG 290	73.54	19.58**	−3.73**	−0.75**	−1.47**	56.12	68.17
Linoleic acid (%)								
1	607 A × TSG 288	58.49	61.00**	−2.94**	−0.49**	7.97**	32.29	33.97
2	40 A × TSG 310	54.24	49.32**	−5.89**	−1.81**	1.04**	37.10	26.90
3	42 A × TSG 263	53.17	46.37**	4.65**	1.52**	−1.89**	58.98	24.90
4	42 A × P123R	47.18	29.87**	2.69**	1.52**	−2.05**	58.98	40.05
5	42 A × OPH 71	47.16	29.82**	1.77**	1.52**	3.84	58.98	26.70

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