Combining ability and gene action for agronomic traits and oil content in sunflower (*Helianthus annuus* L.) using F1 hybrids

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Abstract

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General and specific combining abilities as well as genetic variance components for different agronomic traits in sunflower (Helianthus annuus L.) inbred lines were estimated using "Line × Tester" analysis method. Six new restorer lines were crossed with four CMS lines as tester in 2006 growing season. The 24 F1 single cross combinations were planted in complete randomized block design with four replications in Khoy Field Research Station in 2007 growing season. There were significant differences between hybrids for all traits except for head diameter. Variances among CMS lines for all traits were greater than restorer lines indicating the possible existence of some degrees of maternal effect on some traits, particularly, seed and oil yields. Variances of "Lines × Tester" were not greater than variances for either CMS lines or testers for any traits. This implies the importance of additive effect on concerned traits. Life-cycle duration, 1000 seed weight, seed number head⁻¹ and oil yield were under control of both additive and dominant effects. Seed yield was mainly governed by dominant effects. However, plant height and oil content were controlled by additive effects. Over dominant effects for life-cycle duration and seed yield and complete dominant for plant height were also observed. Principle component analysis (PCA) revealed close positive relationship between seed yield, oil yield and seed number head⁻¹, whereas there were negative association between these traits and 1000 seed weight. Restorer line "R21" and CMS line "CMS356" had stronger effect on their corresponding hybrids. "R23 x CMS78" was identified as superior early maturity hybrid with high oil content and yield.

Key words: General combining ability, Specific combining ability, Line x Tester, Principle component analysis and Life-cycle duration.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops

in the world, but its growing area has been very variable and decreased from 105,000 ha in 1994 to 30,000 ha in Iran (Anonymous, 2010). Sunflower breeding programs at the Seed and Plant Improvement Institute, Iran, develop new hybrid cultivars to replace cultivars. open pollinated Iranian hvbrid cultivars have not been successful; however, some new hybrids have promising been developed in the sunflower breeding at Seed programs and Plant Improvement Institute. These breeding programs are basically focused on development of many parental lines.

Estimation of combining ability of new lines for identification of superior parents for hybridization is essential in sunflower breeding programs. General and specific combining abilities as well as gene action for different agronomic traits have been estimated by many researchers (Mihaljcevic, 1988; El-Hity, 1992; Orthegon- Morales et al., 1992; Hladni et al., 2006). Over dominance gene action is reported for plant height, head diameter, oil content, 100 seed weight and seed and oil yield (Gangappa et al., 1997). However, additive gene action for these traits has also been reported (Singh et al., 1989). Estimates of GCA and SCA indicating additive effects were more important for oil content (Bedov, 1985). Additive gene action

has the greatest effect on flowering (Alvarez et al., 1992).

Significant relationship between morphological characteristics in inbred lines and SCA was found for oil yield and seed number plant⁻¹ (Luczkiewicz and Kaczamarek, 2004). These relations could be used to evaluate inbred lines directly based on their agronomic performance instead of abilities. combining Biometrical methods such as principle component analysis (PCA) permit us to recognize differences in genetic structures between genotypes based on multivariate traits. Tersac et al. (1993) used PCA based on SCA to show the structure of sunflower populations by country of origin. De la Vega et al. (2001) also used PCA for revealing two dimensional structures among genotypes and their environments based on their interactions. They reported the effectiveness of PCA for revealing "genotype x environment" interactions. In this study "Line \times Tester" approach was employed to specific estimate general and combining abilities as well as genetic variance components for different in sunflower agronomic traits (Helianthus annuus L.) inbred lines.

Materials and methods

Parental inbred lines have been recently developed in sunflower breeding program at Khoy Field Station, Khoy, Iran. Khoy Field Station is located at 38° 32' N and 44° 58' E. Six new restorer lines were crossed with four cytoplasmic male sterile (CMS) lines in "line x tester" scheme to generate single cross hybrids in 2006 growing season and 24 F1 hybrids were grown, in randomized complete block design with four replications, in 2007. Each experimental plot consisted of three rows of four meters length with 60 \times 25 cm spacing between and within rows. Fertilizers were applied at the rates of 100: 70: 90 kg/ha of N: P: K, respectively. Field management practices were carried out following Ghaffari (2006). Data for hybrids subjected to "Line x Tester" analysis (Singh and Chaudhury, 2001) to estimate general combining ability (GCA), specific combining ability (SCA), and their respective variance Principle components. Component Analysis (PCA) was used to estimate the ordination of hybrids in two dimensional biplots (Kroonenberg, 1997) based on agronomic traits and combining abilities.

Results and Discussion

There were significant differences between crosses for all traits except head diameter which implies the existence of genetic variability among crosses (Table 1). Variances among CMS lines, used as testers, were greater than restorers, which implies some degrees of maternal effects on some traits, particularly, seed and oil yields (Table 1). Variances of "Lines x Tester" were not greater than variances for either lines or testers for any traits (Table 1), indicating the importance of additive effects on the concerned traits (Table 2). Life-cycle duration, 1000 seed weight, seed number head⁻¹ and oil yield were under control of both additive and dominant effects. Seed mainly governed vield was by dominance effects. Plant height and oil content were under control of additive effects. Over dominance effects for life-cycle duration and seed yield, complete dominance for plant height and partial dominance for other traits were also observed (Table 2).

Combining ability effects are summarized in Table 3. Restores R50, R21, and R23 had the highest GCA for seed yield; however, none of them were significant (Table3). This could be due to high variability in

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experimental conditions or similarities for of genetic sources used development of these restorer lines. R21, R23, and R56 had significant positive GCA for seed number head⁻¹, 1000 seed weight and oil content, respectively (Table 3). This implies that these restorer lines posses favorable alleles with additive effects for these traits. R26 and R50 had negative significant GCA for life-cycle duration, indicating the presence of alleles with additive effects on earliness. R26 seemed to posses alleles with additive effects on plant height. Single branch restorer R50 was distinguished as a suitable line for being used in sunflower hybrid development program, because of desirable GCA for seed yield and lifecycle duration (Table 3).

Testers CMS52 and CMS148 had the highest GCA for seed yield but only CMS52 had significant GCA in desired (+) direction (Table 4). CMS356, CMS52 and CMS78 showed significant positive GCA for 1000 seed weight, seed number haed⁻¹ and oil content, respectively. CMS356 seemed to have alleles with additive effects on increasing life-cycle duration, while CMS52 had alleles for decreasing it (Table 4). CMS52 had a desirable and significant GCA for seed yield and life-cycle duration; therefore, it can be recommended as a valuable A-line in hybrid development programs.

F1 hybrids "R23 × CMS78" and "R56 × CMS356" showed high SCA for both seed yield and life-cycle duration (Table 5). These hybrids had seed yields of 4105 and 3022 kg ha-1, and life-cycle duration of 107 days, respectively (Table 6). Crossing R21, R26, and R50 with CMS52, and R21 with CMS148 resulted in higher seed yield (> 3500 kg ha-1) with shorter life-cycle (107 days), which are considered as high yielding early maturity hybrids. They are suitable for summer cropping.

Ordination by PCA was used to determine whether there was any structure associated with agronomic performance, GCA and SCA using multivariate characters.

According to combining abilities two principle components accounted for 62% of variation in the GCA and SCA of entries. Ordination in Biplot was based on discrimination of entries by multivariate analysis of GCA and SCA for measured characters (Fig. 1). Agronomic traits with favorable values for principle components could effectively discriminate entries to exert multivariate selection.

S. O. V.	d.f.	Life-cycle duration	Plant height	Head diameter	1000 seed weight	Seed No. head ⁻¹	Seed yield	Oil content	Oil yield
Replication	3	44.9**	339.26	48.244**	314.2**	35628.6	1162745.4*	50.32**	182658.6
Crosses	23	32.4**	349.49**	5.445	154.7**	79052.1**	1155345.3**	18.12**	278046.6**
Lines	5	39.8**	550.8**	2.932	171.9**	34051.2	470879.6	33.48**	123211
Testers	3	68.2**	605.8*	5.653	610.33**	349693.9**	3627592.4**	58.64**	1043106**
Lines × Testers	15	22.8**	231.1	6.241	57.8*	39923.9*	889051.3**	4.89**	176646.7*
Error	69	6.6	160.9	5.373	28.7	20324.9	361540.3	7.039	86292.98
C.V. (%)		2.42	6.86	12.47	7.42	21.73	19.29	5.87	20.80

Table 1. Summary of analysis of variance for agronomic traits in 24 sunflower F1 hybrids

* and **: Significant at the 0.05 and 0.01 levels of probability, respectively.

Variance	Life cycle duration	Plant height	Head diameter	1000 seed weight	Seed No. head ⁻¹	Seed yield	Oil content	Oil yield
Additive	3.13*	34.72*	-0.19	33.33**	15194.86*	116018.49+	4.12**	40651.16*
SE	1.24	12.40	0.12	9.94	5568.92	58699.98	1.03	16712.78
Dominance	4.03**	17.53	0.22	7.28*	4899.76*	131877.70**	0	22588.43*
SE	0.99	10.47	0.29	2.55	1764.04	38865.08	0.26	7787.033934
Dominance	1.61	1.00	0.66*	0.66*	0.80	1 51	0.00	1.05
degree	1.01	1.00	0.00*	0.00*	0.00	1.31	0.00	1.05

Table 2. Genetic variance components for agronomic traits in 24 sunflower F1 hybrids

* and **: Significant at the 0.05 and 0.01 levels of probability, respectively.

Restorer	Life cycle duration	Plant height	Head diameter	1000 seed weight	Seed No. head ⁻¹	Seed yield	Oil content	Oil yield
R19	0.07	-6.11	-0.32	-0.62	-50.91	-254.66	-1.28*	-158.63*
R21	-0.61	7.96*	0.58	-3.37	72.74*	114.51	-0.34	44.43
R23	2.51**	1.97	-0.43	5.41**	-28.18	82.43	1.14	82.07
R26	-1.55*	-7.72*	0.39	0.94	-31.40	-112.57	0.29	-41.77
R50	-1.49*	1.54	0.13	0.94	30.39	217.85	-1.82*	36.76
R56	1.07	2.37	-0.35	-3.31	7.38	-47.57	2.01**	39.20
SE	0.59	2.90	0.53	1.22	32.54	137.22	0.61	67.04

Table 3. General combining ability for agronomic traits in sunflower restorer lines

* and **: Significant at the 0.05 and 0.01 levels of probability, respectively.

CMS	Life cycle duration	Plant height	Head diameter	1000 Seed weight	Seed No. head ⁻¹	Seed yield	Oil content	Oil yield
CMS78	-0.70	2.19	-0.35	-2.41	21.10	23.96	1.31*	54.65
CMS52	-1.32*	3.47	0.69	-2.05	87.16**	318.68*	0.60	154.93*
CMS148	-0.45	-7.46**	-0.33	-3.09	67.96	211.18	0.35	97.01
	2.47					-		
CMS356	**	1.81	-0.01	7.53**	-176.21	553.82**	-2.26	-306.57**
SE	0.46	2.24	0.41	0.95	25.20	106.29	0.47	51.93

Table 4. General combining ability for agronomic traits in sunflower CMS lines

* and **: Significant at the 0.05 and 0.01 levels of probability, respectively.

Combining ability for seed number head⁻¹ (SN), oil yield (OY) and seed yield (SY) had the highest values in principle component 1; therefore, this component could discriminate entries according to the traits (Fig. 2). R23 × CMS78 with the highest SCA for SY was located further up in positive direction of its vector (Fig. 1). Two other hybrids with high SCA located near the vector but with different distance from the vector. Hybrid R21 \times CMS52 had the highest SCA.

On the other hand, the lowest SCA of SY belonged to $R23 \times CMS52$ and logically it is located further down in the opposite direction of its vector

Restorer	CMS	Life cycle	Plant height	Head	1000 seed	Seed No.	Seed yield	Oil	Oil yield
		duration		diameter	weight	head ⁻¹		content	
	CMS78	-0.11	-1.51	1.18	1.13	-79.13	-308.12	-1.38	-172.54
	CMS52	-0.99	10.06*	-0.91	0.52	18.04	60.49	0.86	50.80
R19	CMS148	3.64**	1.31	0.53	1.94	59.81	406.32	-0.79	152.33
	CMS356	-2.53*	-9.89	-0.79	-3.56	1.28	-158.68	1.32	-31.98
	CMS78	-1.93	-3.96	-1.02	-7.25	-23.97	-403.96	1.46	-139.56
	CMS52	0.95	-2.46	1.92*	-1.60	149.51*	649.66*	-1.12	250.58*
R21	CMS148	0.57	2.09	-1.54	2.94	30.39	257.16	-0.46	109.74
	CMS356	0.41	4.30	0.64	5.94*	-155.94*	-502.85*	0.12	-222.15
	CMS78	-2.05*	-7.74	0.65	2.35	145.60*	881.47**	0.15	411.84**
	CMS52	-1.18	10.14*	-1.69	-0.88	-120.49*	-598.26*	0.74	-252.21
R23	CMS148	-2.05*	0.01	0.22	-0.22	-55.88	-222.43	0.11	-89.18
	CMS356	5.28**	-2.44	0.83	-1.22	30.76	-60.76	-1.00	-71.83
	CMS78	0.51	2.45	0.42	-0.56	-65.97	-350.21	0.80	-149.18
	CMS52	-0.11	2.32	-0.47	-0.54	44.58	235.07	1.07	148.78
R26	CMS148	0.26	-2.55	-0.35	-2.37	68.75	200.91	-0.35	71.64
	CMS356	-0.66	-2.25	0.41	3.50	-47.39	-85.76	-1.52	-72.63
	CMS78	2.45*	7.86	-1.07	1.94	-8.67	6.04	-0.48	-17.65
	CMS52	-0.43	-16.36**	0.54	-0.66	13.36	29.66	-0.40	3.84
R50	CMS148	-2.05*	-0.01	1.91*	-3.00	-37.44	-337.85	0.60	-132.12
	CMS356	0.03	8.49	-1.38	1.75	32.75	302.16	0.28	144.53
	CMS78	1.14	2.86	-0.16	2.44	32.12	174.80	-0.55	65.00
	CMS52	1.76	-3.74	0.62	3.21	-105.02	-376.60	-1.16	-203.87
R56	CMS148	-0.36	-0.89	-0.76	0.75	-65.63	-304.10	0.90	-114.49
	CMS356	-2.53*	1.74	0.30	-6.37	138.52*	505.91*	0.81	251.98*
SE		1.02	5.02	0.92	2.12	56.35	237.68	1.05	116.12

Table 5. Specific combining ability for agronomic traits in 24 sunflower F1 hybrids

*and **: Significant at the 0.05 and 0.01 levels of probability, respectively

Restorer	CMS	Life cycle	Plant height	Head diameter	1000 seed	Seed No.	Seed yield	Oil	Oil yield
		duration			weight	head ⁻¹		content	
	CMS78	106.00	179.63	19.10	70.38	547.26	2578.35	43.842	1136.02
	CMS52	104.50	192.48	18.05	70.13	710.49	3241.68	45.37	1459.63
R19	CMS148	110.00	172.80	18.48	70.50	733.06	3480.02	43.477	1503.25
	CMS356	106.75	170.88	17.48	75.63	430.36	2150.01	42.965	915.35
	CMS78	103.50	191.25	17.80	59.25	726.07	2851.68	47.617	1372.05
	CMS52	105.75	194.03	21.78	65.25	965.61	4200.02	44.328	1862.47
R21	CMS148	106.25	187.65	17.30	68.75	827.29	3700.02	44.738	1663.71
	CMS356	109.00	199.13	19.80	82.38	396.79	2175.01	42.702	928.24
	CMS78	106.50	181.48	18.45	77.63	794.72	4105.02	47.798	1961.08
	CMS52	106.75	200.63	17.15	74.75	594.69	2920.02	47.675	1397.31
R23	CMS148	106.75	179.58	18.05	74.38	640.10	3188.35	46.798	1502.43
	CMS356	117.00	186.40	18.98	84.00	482.57	2585.01	43.065	1116.19
	CMS78	105.00	181.98	19.05	70.25	579.93	2678.35	47.59	1276.23
	CMS52	103.75	183.13	19.20	70.63	756.54	3558.35	47.15	1674.47
R26	CMS148	105.00	167.33	18.30	67.75	761.51	3416.68	45.475	1539.42
	CMS356	107.00	176.90	19.38	84.25	401.20	2365.01	41.693	991.57
	CMS78	107.00	196.65	17.30	72.75	699.02	3365.02	44.205	1486.29
	CMS52	103.50	173.70	19.95	70.50	787.11	3683.35	43.573	1608.07
R50	CMS148	102.75	179.13	20.30	67.13	717.11	3208.35	44.32	1414.19
	CMS356	107.75	196.90	17.33	82.50	543.13	3083.35	41.39	1287.25
	CMS78	108.25	192.48	17.73	69.00	716.80	3268.35	47.96	1571.39
	CMS52	108.25	187.15	19.55	70.13	645.72	3011.68	46.638	1402.79
R56	CMS148	107.00	179.08	17.15	66.63	665.91	2976.68	48.443	1434.26
	CMS356	107.75	190.98	18.53	70.13	625.89	3021.68	45.74	1397.14
LSD5%		3.53	17.81	3.23	7.72	195.7	819.5	3.74	414.4

Table 6. Mean of agronomic traits in 24 sunflower F1 hybrids

followed by R21 x CMS356 and R21 x CMS78 (Fig. 1).

This finding is in agreement with De la Vega et al. (2001) reports for of discrimination genotypes and environment based on their Genotype × Environment interactions. Acute angle for SY, OY and SN indicated positive relationship between these traits. 1000 seed weight (SW), lifecycle duration (GP) and head diameter (HD) were also closely associated (Fig. 2). Therefore, selection for one of these traits should be accompanied by the associated traits, and this would provide the opportunity to exert multitraits selection in sunflower breeding programs. Oil content (OC) is not associated with seed yield (SY) and oil yield (OY) and there is strong negative association for 1000 seed weight (SW) with life-cycle (GP) and head diameter (HD) because of the obtuse angle of their vectors. Among parental lines, CMS356 had the highest GCA for 1000 seed weight (SW) and was located on the same direction of its vector by the further distance form the origin (Fig 2). R56 and CMS78 with high SCA for oil content (OC) were located on the same direction of their vectors. Genotypes with values close to the mean of entries were located near the origin (Fig. 1).

Agronomic traits with low weight in principle components could not efficiently discriminate the entries. This was true for R56 x CMS148 due to its low SCA for plant height (PH). Considering means of agronomic traits as; seed number head⁻¹ (SN), oil yield (OY) and seed yield (SY), had the highest negative weights in principle component 1, whereas in principle component 2 positive weight of oil content (OC) and plant height (PH), and negative weight of head diameter (HD) were the highest. Negative relationships for seed number head⁻¹ (SN) and 1000 seed weight (SW) were expected (Fig.2). R21 \times CMS52 and $R23 \times CMS78$ located further up in the positive direction of seed yield (SY) vector, because of their higher seed yields. Hybrids with low seed yield located in the opposite direction of biplot (Fig. 2).

Ordination of entries might be influenced by the presence of multitraits effects. For example, the order of $R26 \times CMS52$ with lower seed yield in comparison with $R21 \times CMS148$ was not in agreement with this statement, and in fact it was influenced by lifecycle duration (GP). Hence, multitraits could make problems in the order of entries as to make the selection difficult for breeders, but PCA biplots generate equilibrium (order) due to different traits which could be used for efficient selection. For example, if one wants to select an early maturity hybrid with higher oil yield and oil content $R23 \times CMS78$ would be a suitable selection (Fig 2). Association and discrimination behavior of biplot in Fig. 2 is the same, as mentioned for Fig. 1, but the orders of the entries were slightly different. Means of agronomic traits could more effectively discriminate between genotypes than

SCA. CMS 356 had a strong increasing effect on all combinations with 6 restorer lines. R56 \times CMS356 with higher SCA for seed yield (SY) had a seed yield close to the mean of entries and SCA could not show its real performance. Therefore, biplots provide more useful information for sunflower breeders and PCA can be performed based on agronomic traits determination data for of real performance of hybrids.



Fig. 1. Biplot for the 1st and 2nd principle components for general and specific combining abilities.



Fig. 2. Biplot for the 1st and 2nd principle components for mean of agronomic traits.

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