Combining Ability of Agronomic Traits in Sunflower (*Helianthus annuus* L.) Using Line X Tester Analysis

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Abstract

Eight lines and six testers of sunflower were crossed using line x tester method. The 48 F1 single cross combinations along with their parents and two checks were planted in simple lattice design with two replications. The results of analysis of variance showed significant differences among the genotypes for all the traits including plant height, head diameter, life-cycle duration, grain yield, 1000 seed weight, oil content and oil yield. Line x tester analysis revealed significant effects of genotypes, hybrids and interaction effects of line x tester for all the studied traits. The testers including RF81-25 and RF81-30 with significant positive general combining ability (GCA) effects for grain yield and 1000 seed weight respectively and also RF-131/1 with significant negative GCA effect for plant height were considered as good combiners and also the lines including AF80-488/1/2/1 and AF80-488/2/1/1 were good combiners for grain yield and 1000 seed weight respectively. The crosses including AF80-460/2/1/1 x RF81-25 and AF8-6937 x RF81-30 had significant positive specific combining ability (SCA) effects for grain yield, oil content and oil yield. Estimating low narrow sense heritability for all the traits indicating the importance of non additive genetics effects for them, so for improving these traits hybrid method will be effective.

Keywords: Sunflower, General combining ability, Specific combining ability, Line x tester

1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the world. The importance of hybrid cultivars in sunflower has increased recently because of their higher seed yield compared with cross-pollinated varieties in many countries in the world. Hybrids of sunflower are more stable, highly self-fertile, with high yield performance, and more uniform at maturity (Kaya and Atakisi, 2004; Seetharam, 1979; Sujatha *et al.,*2002). Resistance to diseases and Orobanche has also increased the importance of hybrid varieties. The heterotic performance of a hybrid combination depends upon the combining abilities of its parents (Allard, 1960; Kadkol *et al.,* 1984). Kaya and Atakisi (2004) reported that superior hybrids have been obtained by crossing cytoplasmic male sterile inbred (CMS) female and restorer lines with high general combining ability (GCA) and specific combining ability (SCA) values. General combining ability refers to the average performance of parental lines as reflected in its hybrid combinations and specific combining ability refers to the average performance of a particular cross. Based on the combining ability analysis of different characters, higher SCA values refer to dominance gene effects and higher GCA effects indicate a greater role of additive gene effects controlling these characters in the plants. If both the GCA and SCA values are not significant, epistatic gene effects play an important role in determining these characters (Fehr, 1993).The importance of combining

ability studies lies in the assessment of parental lines and their hybrids showing significant additive and non-additive effect with respect to certain traits. In a systematic breeding program, it is essential to identify superior parents for hybridization and crosses to expand the genetic variability for selection of superior genotypes (Inamullah *et al*., 2006b). One crucial step in hybrid development is testing of inbred lines for their GCA.

The line x tester analysis is one of the efficient methods of evaluating a large number of inbreds as well as providing information on the relative importance of general combining ability and specific combining ability effects for interpreting the genetic basis of important plant traits. Recently, line x tester analysis has widely been used for combining ability tests, suggested by Singh and Chaudhary (2001). Kempthorne (1957) reported that line x tester analysis is an extension of top cross method in which several testers are used. Virupakshappa *et al*. (1997) stated that two testers were enough to efficiently test GCA of inbred 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 (Bajaj *et al*.,1997; El-Hity, 1992; Hladni *et al*., 2006;Mihaljcevic, 1988; Orthegon- Morales *et al.,* 1992). 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). The same importance of additive and dominance effects was reported for oil content (El-Hity, 1992; Fick, 1987).Significant negative GCA and SCA effects were found for plant height and life-cycle duration (Ghaffari *et al*.,2011; Khan *et al*.,2008)and also some researcher (Khan *et al*.,2009; Karasu *et al*.,2010) reported significant positive GCA and SCA effects for oil content, seed yield and yield associated traits.

Due to estimation of genetic parameters of the traits are related the genetic material under study, the objectives of this study were to evaluate sunflower hybrids for their combining ability for different traits and to identify the best lines and testers for important agronomic traits and also the way of genetic control of the traits.

2. Materials and Methods

The experimental materials were contained 8 cytoplasmic male sterile lines (CMS,female parents) of sunflower (*Helianthus annuus* L.) viz., AF80-488/1/2/1, AF80-427/2/1/1, AF80-463/1/1/1, AF80-460/2/1/1, AF80-6920, AF80-438/1/2/2, AF80-6937, AF80-533/1/1/1 and , 6 testers/restorers (male parents) viz., RF81-25, RF81-150/1, RF81-65, RF81-053/2, RF81-131/1 and RF81-30 and also their 48 F1 crosses. The eight CMS lines were crossed with the 6 restorers/testers in a line x tester fashion during spring 2009 to obtain sufficient seed for evaluation in the following season. The 48 F1crosses along with their 14 parents and 2 checks were evaluated based on lattice design with 2 replications at Dashtenaz Agronomy Research Station located in Sari, Iran (53°, 11′ E longitude and 36° 37′ N latitude, 10.5 m above sea level) during spring 2010. Each plot was consisted of four rows 5.5 m long and 60 cm apart. The distance between plants on each row was 20 cm. Sowing was done by dibbling two seeds per hill to ensure uniform stand which was later thinned to one plant per hill at V2 stage as explained by Schneiter and Miller (1981). Fertilizers were applied at the rates of 100: 70: 90 kg/ha of N: P: K, respectively. Harvesting and threshing were done manually. Data were taken on 10 randomly selected plants of each entry from each replication on the following traits. The traits including plant height, head diameter, life-cycle duration, grain yield, 1000 seed weight, oil content and oil yield were determined with the method as explained by Schneiter and Miller (1981). A sample of 1000-filled seeds (at 8% moisture content) was drawn at random from the bulked seed of 10 random plants and weighed with an electronic balance. Oil content was estimated with the help of nuclear magnetic resonance spectrometry (NMR) (Madson, 1976). 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 components. The estimates of general combining ability and specific combining effects of parents and hybrids were obtained as under.

2.1 Estimation of GCA effects

Lines: $GCA = (X_{i.}/fr) - (X_{...}/fmr)$ (a)

Testers: $GCA = (X_{.j}/mr) - (X_{...}/fmr)$ (b)

where f= number of CMS lines (female parent)

        t = number of testers (male parent)

        r = number of replications

        X_i = Total of the F_1 resulting from crossing ith lines with all the testers

X.j = Total of all crosses of jth tester with all the lines

X = Total of all crosses

2.2 Estimation of SCA effects

SCA=Sij-(Xij/r)-(Xi../fr)-(X.j./mr)+(X…/fmr)

where Xij = Total of F_1 resulting from crossing ith lines with jth testers

3. Results and Discussion

Significant mean squares of treatment was observed for all the traits including plant height, head diameter, life-cycle duration, grain yield, 1000 seed weight, oil content and oil yield, indicating significant genetic variation for these traits (Table 1). Variances among CMS lines, used as lines, were greater than the restorers (testers) for the traits including head diameter, life-cycle duration, grain yield, and oil content which implies some degrees of maternal effects on these traits. Significant mean squares of the line x tester and non significant ratio of GCA to SCA mean squares and low narrow sense heritability estimates for all the traits indicating the importance of non additive genetic effects for controlling these traits (Table 2). 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 by Singh *et al.*, (1989). Estimates of GCA and SCA indicating additive effects were more important for oil content (Bedov, 1985). The same importance of additive and dominance effects was reported for oil content (El-Hity, 1992; Fick, 1975).

<Table 1>

<Table 2>

Combining ability effects are summarized in Table 3. The restorer lines including RF81-150/1 and RF81-131/1 and also the CMS lines such as AF80-427/2/1/1, AF80-460/2/1/1, AF80-6920 and AF80-438/1/2/2 had significant negative GCA effects and were considered as good combiners for reducing plant height (Table 3). This implies that these lines posses favorable alleles with additive genetic effects for plant height. For head diameter, only AF80-488/1/2/1 had significant positive GCA effect, so this line can use for increasing this trait. RF81-131/1 had negative significant GCA for life-cycle duration, indicating the presence of alleles with additive genetic effects on earliness. RF81-25, AF80-488/1/2/1 and AF80-427/2/1/1 with significant GCA effects for seed yield were considered as superior combiners for seed yield increasing. The testers including RF81-25 and RF81-150/1 and also the lines viz., AF6920 and AF6937 had a desirable and significant positive GCA effects for oil content and also all of these genotypes except AF6920 had significant positive GCA effects for oil yield. Significant GCA effect of 1000 seed weight was exhibited by RF81-30 and AF80-460/2/1/1, therefore, they can be recommended as valuable R-line and A-line in hybrid development programs, respectively. Significant negative GCA effects were detected for plant height and life-cycle duration (Ghaffari *et al.*,2011; Khan *et al.*,2008).

<Table 3>

Data presented in Table 4 for SCA effects of the crosses showed that out of 48 F1 crosses, 12 crosses exhibited significant negative SCA effects for plant height, indicating the non additive genetic effects for decreasing of this trait. The crosses including AF80-427/2/1/1*RF81-053/2, AF80-438/1/2/2*RF81-150/1, AF80-533/1/1/1*RF81-25, AF80-463/1/1/1*RF81-25 and AF80-463/1/1/1*RF81-30 with significant negative SCA effect for plant height were considered as suitable combinations. Significant positive SCA effect of head diameter were exhibited by AF80-533/1/1/1*RF81-150/1, AF-6937*RF81-65 and AF80-533/1/1/1*RF81-65, therefore these combinations can be good candidate for seed yield improving. Out of F1 48 crosses, 8 crosses displayed significant negative SCA effects for life-cycle duration, indicating the presence of non additive genetic effects for earliness. The crosses including AF80-438/1/2/2*RF81-25, AF-6937*RF81-150/1, AF-6937*RF81-131/1 and AF80-463/1/1/1*RF81-30 with significant negative SCA effects for life-cycle duration were detected as good combinations. Out of 48 crosses, 8 crosses displayed significant positive SCA effects for seed yield. The cross combinations including AF80-460/2/1/1*RF81-25, AF80-463/1/1/1*RF81-150/1, AF80-427/2/1/1*RF81-053/2, AF80-6920*RF81-131/1 and AF-6937*RF81-30 with significant positive SCA effects for seed yield were suitable combinations for this trait and some of these crosses had also significant positive SCA effects for 1000 seed weight. The crosses including AF80-460/2/1/1*RF81-25, AF80-460/2/1/1*RF81-150/1, AF80-427/2/1/1*RF81-053/2, AF80-438/1/2/2*RF81-30 and AF-6937*RF81-30 had significant positive SCA effects for oil content and oil

yield and also these combinations can be superior candidate for improving high oil content genotypes. In earlier studies (Khan *et al*.,2009; Karasu *et al*.,2010) were reported significant positive SCA effects for oil content, seed yield and yield associated traits.

<Table 4>

4. Conclusion

Due to variances among CMS lines which used as lines, were greater than restorers (testers) for the traits including head diameter, life-cycle duration, grain yield, and oil content which implies some degrees of maternal effects on these traits. Non significant ratio of GCA to SCA mean squares and low narrow sense heritability estimates for all the traits indicating the importance of non additive genetic effects for controlling these traits. Among the combinations with significant positive SCA effects for yield and yield associated traits, at least one parent had significant positive GCA effect for these traits, therefore for improving these traits, GCA effects of parents can be considered as suitable criteria for SCA prediction of the crosses.

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Table 1. Mean squares from analysis of variance for agronomic traits of 8 sunflowers lines and 6 testers

SOV	DF	Plant height	Head diameter	Growth duration	Grain yield	Oil content	Oil yield	1000 seed weight
					M.S			
Rep	1	500.070**	6.99*	12.23**	167001.58 ns	0.01 ns	1052.26 ns	170.98*
Var	63	1307.760**	7.54**	25.35**	5154996.66**	44.29**	1106235.80**	217.55**
C vs P vs H	2	23193.38**	73.31**	80.54**	76553985.63**	8.73*	16005085.54**	2365.70**
C vs (P, H)	1	1423.92**	0.07 ns	3.11ns	10629017.18**	11.23**	2432666.22**	1114.95**
P vs H	1	44962.84**	146.54**	157.98**	142478954.08**	6.22 ns	29577504.86**	3616.45**
Checks (C)	1	484.01**	1.05 ns	1.16 ns	715584.74 ns	1.60 ns	93207.17 ns	529.80**
Parent (P)	13	745.75**	9.56**	37.51**	4162427.35**	68.30**	613187.02**	303.85**
Hybrid (H)	47	549.41**	4.32**	20.151**	2485737.83**	40.07**	630171.19**	95.62**
Line	7	828.87 ns	3.72 ns	48.05**	5848254.75**	72.54*	1310557.01**	120.58 ns
Tester	5	1310.46**	3.03 ns	9.61 ns	4957498.69**	62.33 ns	1687992.45**	229.47*
LXT	35	384.82**	4.62**	16.07**	1460125.749**	30.41**	342976.71**	71.51**
Error	63	44.57	1.60	1.17	218047.940	2.51	45818.87	30.47

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

Table 2. Genetic variance components for agronomic traits in 48 sunflower F1 hybrids

Genetic components	Plant height	Head diameter	Growth duration	Grain yield	Oil content	Oil yield	1000 seed weight
Vg	631.59	2.96	12.08	2468474.36	20.89	530208.46	93.54
Vp	653.88	3.77	12.67	2577498.33	22.14	553117.91	108.77
Cov.Hs(Vgca)	7928.11	0.08	0.91	281625.07	2.64	82592.71	7.39
Va	15856.22	0.17	1.82	563250.14	5.29	165185.43	14.78
Vd(Vsca)	56009.30	1.51	7.45	621038.91	13.94	148578.91	20.52
Hb^2	0.97	0.77	0.95	0.95	0.94	0.95	0.86
Hn^2	0.24	0.04	0.14	0.21	0.23	0.29	0.13
Vgca/Vsca	0.14	0.0	0.12	0.45	0.19	0.55	0.36
% of lines	0.15	12.85	35.51	35.04	26.95	30.97	18.78
% of testers	0.17	7.46	5.07	21.21	16.54	28.49	25.52
% of LXT	99.69	79.68	59.41	43.74	56.49	40.53	55.69

Table 3. General combining ability for agronomic traits in sunflower restorer(lines) and CMS lines(testers)

Female(Testers) and male(lines) parents		Plant height	Head diameter	Growth duration	Grain yield	Oil content	Oil yield	1000 seed weight
RF81-25	T1	13.07**	0.25	-0.21	887.33**	2.67**	524.04**	2.04
RF81-150/1	T2	13.93**	0.33	0.27	200.76	1.76**	141.26*	0.41
RF81-65	T3	2.01	-0.01	0.82**	76.13	0.48	16.77	-3.39*
RF81-053/2	T4	2.45	0.16	0.59*	-664.63**	-2.18**	-407.13**	-0.20
RF81-131/1	T5	-5.55**	-0.86**	-1.36**	-522.95**	-0.86*	-258.08**	-4.69**
RF81-30	T6	1.95	0.12	-0.10	23.35	-1.87**	-16.86	5.82**
AF80-488/1/2/1	L1	7.05**	1.30**	1.15**	932.88**	-0.78	367.09**	-0.10
AF80-427/2/1/1	L2	-6.78**	-0.12	0.39	592.75**	0.88	287.97**	2.92
AF80-463/1/1/1	L3	7.80**	-0.31	1.50**	50.35	0.81	46.76	-3.08
AF80-460/2/1/1	L4	-7.36**	0.09	-4.74	-1175.69**	-2.10**	-530.65**	5.24**
AF6920	L5	-4.53**	-0.47	0.65*	-87.15	1.45**	10.13	1.58
AF80-438/1/2/2	L6	-11.45**	-0.24	-0.14	-818.41**	-0.66	-405.31**	-4.49**
AF-6937	L7	9.14**	0.06	0.01	246.95	4.27**	303.80**	-1.20
AF80-533/1/1/1	L8	6.14**	-0.31	1.18**	258.32	-3.89**	-79.78	-0.88
S.E(for lines)		1.67	0.32	0.27	116.74	0.40	53.51	1.38
S.E(for testers)		1.93	0.37	0.31	134.80	0.46	61.79	1.59

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

Table4. Specific combining ability effects for agronomic traits in 48 sunflower F1 hybrids

Crosses			¹Plant height	Head diameter	Growth duration	Grain yield	Oil content	Oil yield	1000 seed weight
AF80-488/1/2/1*RF81-25	T1	L1	4.76	0.31	1.16	-554.16	-1.35	-300.13	-1.03
AF80-427/2/1/1*RF81-25	T1	L2	22.09**	-1.20	-2.00*	-468.61	-3.28**	-370.66*	2.97
AF80-463/1/1/1*RF81-25	T1	L3	-14.49**	0.17	-1.00	-122.78	-2.07	-162.88	2.32
AF80-460/2/1/1*RF81-25	T1	L4	13.68**	1.72	4.16**	1557.93**	5.01**	861.78**	-2.56
AF6920*RF81-25	T1	L5	-7.16	0.12	1.66*	-262.26	-1.47	-176.92	-3.44
AF80-438/1/2/2*RF81-25	T1	L6	7.26	-0.96	-4.44**	-558.73	2.86*	-133.36	-1.80
AF-6937*RF81-25	T1	L7	-11.82*	-0.26	1.41	353.74	0.59	262.29	5.44
AF80-533/1/1/1*RF81-25	T1	L8	-14.32**	0.10	-0.96	54.86	-0.29	19.88	-1.90
AF80-488/1/2/1*RF81-150/1	T2	L1	4.76	-1.62	-1.36	305.39	0.85	239.94	3.14
AF80-427/2/1/1*RF81-150/1	T2	L2	-0.91	-1.28	5.54**	-1081.98**	2.77*	-371.89*	-3.18
AF80-463/1/1/1*RF81-150/1	T2	L3	8.51	-0.98	0.41	1210.12**	-1.65	490.78**	-1.08
AF80-460/2/1/1*RF81-150/1	T2	L4	18.68**	0.54	0.61	744.19*	2.89*	393.33*	2.38
AF6920*RF81-150/1	T2	L5	-10.16*	1.18	-0.74	-819.42*	1.78	-300.56	-1.77
AF80-438/1/2/2*RF81-150/1	T2	L6	-19.24**	1.45	1.04	-988.92**	0.86	-394.78*	0.76
AF-6937*RF81-150/1	T2	L7	-5.32	-1.38	-5.22**	-90.59	-7.23**	-347.16*	-5.18
AF80-533/1/1/1*RF81-150/1	T2	L8	3.68	2.09*	-0.27	721.21*	-0.27	290.34	4.93
AF80-488/1/2/1*RF81-65	T3	L1	-9.68*	0.82	-0.85	567.17	-0.30	281.61	7.94*
AF80-427/2/1/1*RF81-65	T3	L2	12.16*	1.03	-1.09	536.14	-2.71*	110.24	-2.61
AF80-463/1/1/1*RF81-65	T3	L3	-1.93	-1.75	1.86*	213.80	1.65	188.15	-4.13
AF80-460/2/1/1*RF81-65	T3	L4	-6.76	-1.16	-0.79	385.22	1.00	140.33	6.18
AF6920*RF81-65	T3	L5	20.91**	-2.37*	-0.29	-96.97	-1.25	-65.24	11.41**
AF80-438/1/2/2*RF81-65	T3	L6	-5.68	-0.82	-3.48**	-63.88	0.61	17.99	-1.75
AF-6937*RF81-65	T3	L7	-4.76	2.02*	2.46**	-1311.59**	3.63**	-481.92**	-4.38
AF80-533/1/1/1*RF81-65	T3	L8	-4.26	2.24*	2.18**	-229.89	-2.63*	-191.15	-12.67**
AF80-488/1/2/1*RF81-053/2	T4	L1	-11.11*	-0.47	-0.49	0.43	-4.12**	-221.43	0.85
AF80-427/2/1/1*RF81-053/2	T4	L2	-30.28**	1.54	-3.33**	1315.84**	2.66*	747.66**	7.49
AF80-463/1/1/1*RF81-053/2	T4	L3	11.14*	1.23	1.08	41.03	1.12	85.01	4.45
AF80-460/2/1/1*RF81-053/2	T4	L4	-15.20**	0.22	-4.17**	-768.71*	7.02**	-177.21	-2.94
AF6920*RF81-053/2	T4	L5	13.97**	0.38	-1.06	-797.51*	1.32	-265.67	3.02
AF80-438/1/2/2*RF81-053/2	T4	L6	10.39	0.07	2.80**	438.87	-2.31*	112.03	-0.89
AF-6937*RF81-053/2	T4	L7	13.30**	-0.14	3.57	340.62	-4.65**	-36.05	-8.80*
AF80-533/1/1/1*RF81-053/2	T4	L8	7.80	-2.83**	1.60*	-570.56	-1.05	-244.36	-3.18
AF80-488/1/2/1*RF81-131/1	T5	L1	9.89*	1.45	1.39	-137.02	2.10	36.13	-1.66
AF80-427/2/1/1*RF81-131/1	T5	L2	1.72	-1.01	0.04	-486.40	-1.84	-288.60	-6.43
AF80-463/1/1/1*RF81-131/1	T5	L3	18.14**	1.15	2.85**	258.94	0.38	134.76	1.61
AF80-460/2/1/1*RF81-131/1	T5	L4	-17.20**	-2.19*	0.28	-508.21	-3.64**	-337.40*	0.90
AF80-6920*RF81-131/1	T5	L5	-10.53*	1.34	0.78	1265.44**	-0.76	516.08**	-1.92
AF80-438/1/2/2*RF81-131/1	T5	L6	-5.11	1.09	2.75**	632.33	-4.75**	75.24	2.76
AF-6937*RF81-131/1	T5	L7	2.80	0.92	-4.44**	-589.15	4.05**	-153.19	4.64
AF80-533/1/1/1*RF81-131/1	T5	L8	0.30	-2.76**	-3.64**	-435.94	4.46**	16.98	0.10
AF80-488/1/2/1*RF81-30	T6	L1	1.39	-0.50	0.15	-181.81	2.83*	-36.13	-9.25*
AF80-427/2/1/1*RF81-30	T6	L2	-4.78	0.92	0.83	185.00	2.39	173.24	1.77
AF80-463/1/1/1*RF81-30	T6	L3	-21.36**	0.19	-5.20**	-1601.11**	0.57*	-735.82**	-3.17
AF80-460/2/1/1*RF81-30	T6	L4	6.80	0.88	-0.09	-1410.42**	-12.28**	-880.83**	-3.96
AF6920*RF81-30	T6	L5	-7.03	-0.64	-0.35	710.72*	0.38	292.31	-7.30
AF80-438/1/2/2*RF81-30	T6	L6	12.39*	-0.83	1.33	540.34	2.73*	322.88*	0.91
AF-6937*RF81-30	T6	L7	5.80	-1.16	2.22**	1296.97**	3.61**	756.04**	8.27*
AF80-533/1/1/1*RF81-30	T6	L8	6.80	1.15	1.10	460.30	-0.23	108.31	12.72**
S.E			4.72	0.90	0.77	330.19	1.12	151.36	3.90

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