Valiollah Rameeh* and Seyed Abbasali Andarkhor

Line × Tester Analysis for Duration of Flowering, Yield Components and Seed Yield in Sunflower (*Helianthus Annuus* L.)

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Abstract: The general (GCA) and specific (SCA) combining abilities among eight cytoplasmic male sterile lines and six restorers as testers were estimated using line ×tester method to assess the potential of the materials in the sunflower (*Helianthus annuus* L.) breeding program. Test cross progenies along with their parents were evaluated in two sowing dates. Both lines and testers had significant differences for all the traits except seeds per head. The lines: LA2 and LA7 were superior combiners with significant positive general combining ability (GCA) effects for seed yield and most of the yield components. Among the testers: RF1 and RF6 were good combiners for seed yield. Most of the lines and testers with significant positive GCA effects for seed yield had significant positive GCA effect for oil yield. LA7×RF6 had significant positive specific combining ability (SCA) effects for seed width, seed yield and oil yield. The crosses LA4×RF1 and LA7×RF6 had significant SCA effects for seed yield and oil yield. Estimating degree of dominance more than one for all the traits except seeds per head and percentage of kernel indicate the importance of non additive genetic effects for them; therefore for improving these traits, hybrid breeding will be more effective.

Keywords: general combining ability, hybrids, line ×tester, oil yield, sunflower

Introduction

Sunflower (*Helianthus annuus* L.) is one of the four most important oil crops in the world. Based on data from the National Sunflower Association, more than 25 million hectares of global croplands are planted in sunflower with 43.8 million metric tons

***Corresponding author: Valiollah Rameeh,** Agronomic and Horticulture Crops Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center AREEO, 48175556 Sari, Iran, E-mail: vrameeh@gmail.com
Seyed Abbasali Andarkhor, Agronomic and Horticulture Crops Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center AREEO, 48175556 Sari, Iran

of grain production (Chambo *et al.*, 2017). Sunflower is also a rich source of good quality edible oil with suitable fatty acid pattern. It can be successfully grown in different parts of the world due to its wide adaptability, photo-insensitive and thermoinsen-sitive nature. In sunflower, being the cross-pollinated crop, heterosis can be exploited for better seed yield and other yield components. 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. General combining ability (GCA) refers to the average performance of parental lines as reflected in its hybrid combinations and specific combining ability (SCA) refers to the average performance of a particular cross (Kadkol *et al.*, 1984). The heterotic performance of a hybrid combination depends upon the combining abilities of its parents. 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 the GCA and SCA effects. Due to high heterosis occurring generally in hybrids between genetically unrelated inbred lines, all crop breeders that use heterosis have the challenge to find good combiners. Breeding programs can take advantage from such information on combining ability to find best selection strategy for developing high yielding lines and hybrids (Skoric, 1992). The environmental conditions influence the evaluation of combining ability of sunflower genotypes (Petakov, 1996). Regarding combining ability analysis, SCA variance higher than GCA variance means that dominant genes have higher effects than recessive ones in determining the studied characters. Conversely, higher GCA variance indicates that additive gene effects play a more important role in determining these traits. If neither variance is significant, it implies the existence of epistatic gene effects (Marinković *et al.*, 2000; Skoric et al., 2000). Combining ability of important sunflower yield characteristics was evaluated by many researchers (Ghaffari *et al.*, 2011; Kanwala *et al.*, 2016). The line x tester analysis is one of the efficient methods for evaluating a large number of inbreds as well as providing information on the relative importance of general and specific combining ability effects for interpreting the genetic basis of important plant traits. Line × tester analysis has also been widely used for combining ability tests, suggested by Singh and Chaudhury (2001). Mather and Jinks (1982) reported that line × tester analysis is an extension of top cross method in which several testers are used. Virupakshappa *et al.* (1997) affirmed that two testers were enough to efficiently test GCA of inbred lines.

The objectives of this study were to estimate the GCA effects of parents and also SCA effects of crosses to identify superior combiners for desired traits including yield components and also to estimate genetic variance components of the traits in sunflower.

Materials and methods

The genetic materials for the study contained eight cytoplasmic male sterile (CMS) of sunflower (*H. annuus* L.) as female parents including LA1, LA2, LA3, LA4, LA5, LA6, LA7, LA8 and 6 testers restorers (male parents) viz., RF1, RF2, RF3, RF4, RF5 and RF6; and also their 48 F_1 crosses. The eight CMS lines were crossed with the 6 restorers/testers in a line × tester fashion during spring 2009 to obtain sufficient seed for evaluation in the following season. The 48 F1 crosses along with their 14 parents and two checks were evaluated based on lattice design with two 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 2010–2011 cropping seasons. Each plot 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 duration of flowering, head diameter (cm), seeds per head, seed yield (kg ha^{-1}), percentage of kernel and oil yield (kg ha^{-1}) were determined with the method as explained by Schneiter and Miller (1981). Oil content was estimated with the help of nuclear magnetic resonance spectrometry (NMR) (Madson, 1976).

Due to same trend of variation of data for two years all genetic parameters were calculated based on two-year means. Data for hybrids was 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 detected by the equations as follows:

1. Estimation of GCA effects of parents:
   a. Lines: $GCA = (X_{i}../fr) - (X\ldots/fmr)$
   b. Testers: $GCA = (X_{.j}./mr) - (X\ldots/fmr)$
2. Where, f = number of CMS lines (female parent), t = number of testers (male parent), r = number of replications, Xi = total of the F_1 resulting from crossing ith lines with all the testers, $X_{.j}$ = total of all crosses of j^{th} tester with all the lines; X … = total of all crosses.
3. Estimation of SCA effects of the crosses:

$SCA = S_{ij} - (X_{ij}/r) - (X_{i}../fr) - (X_{.j}./mr) + (X\ldots/fmr)$

Where X_{ij} = total of F_1 resulting from crossing i^{th} lines with j^{th} testers.

Results and discussion

Analysis of variance

Most of the traits except seed and oil yields were not affected by sowing dates. Lines had significant difference for duration of flowering, head diameter, seeds per head, seed yield, percentage of kernel and oil yield but testers had significant difference for all the traits except seeds per head (Table 1). Variances among CMS lines used as lines were greater than the restorers (testers) for duration of flowering and seeds per head implies some degrees of maternal effects on these traits. Significant mean squares of the line ×tester and non-significant ratio of GCA to SCA mean squares and high degree dominance estimates for all the traits except percentage of kernel indicate the importance of non-additive genetic effects for controlling these traits except percentage of kernel (Table 2). 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 seed length, 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 (Bedove, 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-

Table 1: Combined analysis of variance for yield and yield components of eight sunflowers lines and six testers.

S.O.V	df	Duration of flowering	Head diameter	Seeds per head	Seed yield	Percentage of kernel	Oil yield
Sowing date (S)	1	204.188	17.34	2,063,381	225.75**	221.794	63.746**
Error 1	2	12.209	2.034	127,133	0.178	22.822	0.003
Tester (T)	5	7.692**	5.436*	382,275	1.478**	47.505**	0.374**
S*T	5	2.564**	5.571**	239,232**	6.868**	45.975**	1.89**
Line (L)	7	2.822**	9.185**	113,757.6**	3.786**	73.094**	0.783**
S*L	7	3.693**	5.441**	38,464.33	8.353**	24.112	1.483**
L*T	35	3.881**	6.108**	60,681.4**	1.477**	14.209	0.253**
S*L*T	35	2.786**	3.817**	40,530.9**	2.047**	25.165**	0.359**
Error2	94	0.118	1.708	21,253.45	0.176	12.561	0.034

*,** Significant at $p<0.05$ and 0.01, respectively.

Table 2: Variance components for yield and yield components of eight sunflowers lines and 6 testers.

Variance components	Duration of flowering	Head diameter	Seeds per head	Seed yield	Percentage of kernel	Oil yield
Variance of GCA	0.238	0.042	20,099.598	0.00006	2.081	0.008
V_A for lines (L)	0.476	0.084	40,199.196	0.00013	4.162	0.015
Variance of GCA Testers (T)	0.088	0.256	4423.019	0.192	4.907	0.044
V_a for Testers	0.177	0.513	8846.039	0.385	9.814	0.088
Variance of GCA for Testers	0.098	0.086	13,381.064	0.083	3.292	0.023
V_A for Parents	0.197	0.172	26,762.129	0.165	6.584	0.047
V_d	1.882	2.200	19,713.976	0.651	0.824	0.110
GCA/SCA	0.127	0.019	1.020	0.0001	2.525	0.069
GCA-T/SCA-T	0.047	0.117	0.224	0.296	5.955	0.403
GCA-L/SCA-L	0.052	0.039	0.679	0.127	3.995	0.212
Degree of dominance	3.15	3.59	*0.86*	1.99	0.35	1.53

GCA: general combining ability, SCA: specific combing ability.

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.

General combining ability of parents

Combining ability effects of lines and testers are presented in Table 3. The restorer lines as testers including RF1, RF2 and RF6 had significant positive GCA effects for duration of flowering, therefore these lines will have increasing effects for the trait in most of cross combinations. RF3, RF4 and RF6 with significant positive GCA effects for head diameter were good combiners for improving this trait (Table 3). RF6 as superior combiner for seeds per head has also significant positive GCA effect for seed yield. None of testers had significant positive GCA effect for percentage of kernel. RF1 with significant positive GCA effect for seed yield was best combiner for oil yield.

Among the CMS lines, LA7 and LA8 with significant positive GCA effect for duration of flowering have increasing effect for this trait. LA2 with significant positive GCA effect for most of yield components including head diameter, oil yield had also significant positive GCA effect for seed yield (Table 3). Although some of lines such as LA7 and LA8 had significant positive GCA effects for most of yield components but these lines had not significant positive GCA effect for

Table 3: General combining ability effects for yield and yield components of eight sunflowers lines and six testers.

Parents	Duration of flowering	Head diameter	Seeds per head	Seed yield	Percentage of kernel	Oil yield
RF1	0.529**	−0.019**	2.985	0.22**	−1.82**	0.18**
RF2	0.242**	−0.331**	−1.952	−0.23**	0.66	−0.07*
RF3	−0.283**	0.531**	0.085	0.13	1.14	0.04
RF4	−0.833**	0.131*	−6.302**	−0.09	0.96	−0.07*
RF5	0.017	−0.594**	−2.515	−0.25**	0.24	−0.12**
RF6	0.329**	0.281**	7.698**	0.22**	−1.19	0.03
Error for testers	0.061	0.231	1.715	0.07	0.63	0.03
LA1	−0.196**	0.673	−0.281	0.59**	0.60	0.24**
LA2	−0.096	0.240**	1.035	0.45**	0.40	0.23**
LA3	0.004	−0.577*	−2.848	−0.23*	0.85	−0.04
LA4	−0.512**	1.006	0.485	0.19*	−1.85*	0.02
LA5	−0.129	−0.677**	−8.931**	−0.45**	0.74	−0.15**
LA6	−0.063	−0.177*	−4.981*	−0.52**	0.61	−0.26**
LA7	0.438**	0.073**	5.869**	−0.02	2.05**	0.08*
LA8	0.554**	−0.560**	9.652**	−0.01	−3.40**	−0.12**
Error for lines	0.070	0.267	1.980	0.09	0.72	0.04

*, ** Significant at $p<0.05$ and 0.01, respectively.

seed yield, therefore these lines can be used for indirect improvement of seed yield. Significant negative GCA effects were detected for plant height and life-cycle duration in sunflower (Ghaffari *et al.*, 2011; Khan *et al.*, 2008).

Specific combining ability of the crosses

Specific combining ability (SCA) effects of crosses are presented in Table 4. Among the combinations, thirty five percentage of cross combinations had significant positive SCA effects for duration of flowering. Most of the combinations with significant positive SCA effects for duration of flowering had at least one parent with significant positive GCA effects for this trait.

Although, GCA and SCA mean squares were significant for head diameter but most of the crosses had non-significant SCA effect for this trait, therefore, epistasis genetic effects had more important role for controlling this trait. LA7 × RF2, LA8 × RF3 and LA6 × RF6 with at least one parent with significant positive GCA effect for Head diameter had significant positive SCA effect for this

Table 4: Specific combining ability effects for yield and yield components of eight sunflowers lines and six testers.

Crosses	Duration of flowering	Head diameter	Seeds per head	Seed yield	Percentage of kernel	Oil yield
LA1 × RF1	0.87**	0.65	−34.52	0.13	−1.49	−0.02
LA2 × RF1	−0.23	−0.61	85.20	−0.27	−2.39	−0.20*
LA3 × RF1	−0.33	−0.10	−139.04	0.22	3.12	0.01
LA4 × RF1	0.39*	0.52	60.00	−0.39	1.11	0.07
LA5 × RF1	−0.70**	0.90	−11.42	0.15	−2.08	0.00
LA6 × RF1	−0.36*	−1.10	16.98	−0.05	2.88	−0.04
LA7 × RF1	−0.26	−0.45	36.71	0.46*	0.92	0.25**
LA8 × RF1	0.62**	0.19	−13.91	−0.25	−2.07	−0.07
LA1 × RF2	0.46**	−0.74	22.34	0.36	0.39	0.17
LA2 × RF2	1.56**	−0.20	−83.38	−0.57**	−1.02	−0.22*
LA3 × RF2	−0.04	0.21	130.54	1.30**	−0.44	0.50**
LA4 × RF2	0.48**	−1.07	−42.83	−0.54*	−2.82	−0.14
LA5 × RF2	−0.41*	0.81	106.73	0.03	2.53	0.01
LA6 × RF2	−1.48**	−0.09	−268.3**	−0.80**	−1.32	−0.31**
LA7 × RF2	0.02	−1.14	153.61*	−0.03	1.00	−0.14
LA8 × RF2	−0.59**	2.20**	−18.71	0.25	1.69	0.13
LA1 × RF3	−0.82**	0.00	−111.69	0.43*	0.91	0.22*
LA2 × RF3	0.08	0.94	11.59	0.05	−0.46	−0.01
LA3 × RF3	−0.52**	−1.25	−13.22	−0.05	−0.42	0.01
LA4 × RF3	1.00**	−0.33	136.80	0.96**	0.91	0.38**
LA5 × RF3	0.12	−2.05**	13.18	−0.34	−1.40	−0.14
LA6 × RF3	0.05	−0.15	−117.08	−0.35	2.01	−0.08
LA7 × RF3	0.05	0.70	35.65	−0.64**	−1.82	−0.26**
LA8 × RF3	0.03	2.14**	44.77	−0.07	0.27	−0.13
LA1 × RF4	−1.45**	2.57**	−99.37	0.06	0.47	0.00
LA2 × RF4	1.67**	0.85	46.81	−0.62**	0.72	−0.22*
LA3 × RF4	1.10**	0.25	173.56*	0.50*	−3.02	0.18*
LA4 × RF4	1.10**	−1.00	69.77	0.08	−0.67	0.00
LA5 × RF4	0.48**	−2.96**	−36.28	−0.12	−2.31	−0.05
LA6 × RF4	0.18	1.13	124.25	−0.05	0.55	0.06
LA7 × RF4	0.78**	−0.94	117.46	0.16	−0.42	0.00
LA8 × RF4	0.48**	0.38	−42.16	0.66**	−2.80	0.30**
LA1 × RF5	−1.80**	−1.61*	−96.15	−0.69**	−0.25	−0.34**
LA2 × RF5	0.32	−0.02	−74.24	0.18	1.53	0.09
LA3 × RF5	0.75**	0.88	−11.21	0.22	0.15	−0.01
LA4 × RF5	−0.85**	1.73*	−49.50	−0.44*	−0.56	−0.16
LA5 × RF5	0.13	−1.54*	31.55	−0.04	1.81	0.07
LA6 × RF5	0.07	−0.35	−122.70	−0.69**	−0.48	−0.25**
LA7 × RF5	0.47**	0.09	95.99	0.01	2.24	0.08
LA8 × RF5	−0.13	0.50	113.83	−1.79**	−2.09	−0.72**

(*continued*)

Table 4: (*continued*)

Crosses	Duration of flowering	Head diameter	Seeds per head	Seed yield	Percentage of kernel	Oil yield
LA1 × RF6	1.39**	−0.08	41.55	0.59**	0.57	0.03
LA2 × RF6	−1.00**	−0.50	−81.06	0.61**	−1.29	0.25**
LA3 × RF6	−0.06	0.20	206.06**	0.48*	−0.69	0.26**
LA4 × RF6	−0.06	0.15	−246.2**	0.57**	1.13	0.31**
LA5 × RF6	−0.68**	−0.01	−7.42	0.22	0.62	0.05
LA6 × RF6	−1.45**	2.57**	−99.37	0.06	0.47	0.00
LA7 × RF6	1.67**	0.85	46.81	−0.62**	0.72	−0.22*
LA8 × RF6	1.10**	0.25	173.56*	0.50*	−3.02	0.18*

*, ** Significant at $p<0.05$ and 0.01, respectively.

trait and therefore were suitable combinations for improving Head diameter. LA7 × RF1, LA3 × RF4, LA3 × RF6 and LA8 × RF6 had significant positive SCA effect for seeds per head. The cross combinations including LA4 × RF1, LA3 × RF2, LA2 × RF4, LA5 × RF5 and LA7 × RF6 with significant positive SCA effects for seed yield were suitable combinations for this trait. The crosses including LA4 × RF1, LA4 × RF2, LA2 × RF4, LA6 × RF6 and LA7 × RF6 had significant positive SCA effects for oil content and oil yield and also these combinations can be a superior candidate for improving high oil content genotypes. Earlier studies (Khan *et al.*, 2009; Karasu *et al.*, 2010) reported significant positive SCA effects for oil content, seed yield and yield associated traits.

Conclusion

Among the traits, seeds per head and percentage of kernel with less than one of degree dominance and therefore importance of additive genetic effects can be improved by selection breeding method. Variances among CMS lines were greater than the restorers (testers) for duration of flowering and seeds per head implies some degrees of maternal effects on these traits. Among the combinations with significant positive SCA effects for yield and most of 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. The crosses viz. LA4 × RF1, LA3 × RF2, LA2 × RF4, LA5 × RF5 and LA7 × RF6 with significant positive SCA effects for seed yield were superior combinations for this trait. The combinations such as LA4 × RF1, LA4 × RF2, LA2 × RF4, LA6 × RF6 and LA7 × RF6 with positive SCA effects for oil content and oil yield were considered as superior candidates for improving these traits.

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