ISSN 2709-3662 (Print)
ISSN 2709-3670 (Online)
https://doi.org/10.52587/JAF020202
Journal of Agriculture and Food
2021, Volume 2, No.2, pp. 9-22

Heterotic groups for identifying of suitable combiners in sunflower using top-cross

Mehdi Ghaffari[1]*, Farnaz Shariati[1], Habib Tashakkori Meymand[1], Nadia Safavi Fard[1]

Abstract

The study was conducted in order to identify the suitable parental inbred lines using top cross method for improvement of new sunflower F_1 single cross hybrids at research field of Seed and Plant Improvement Institute in Karaj, Iran during two Crop season (2018 and 2019). Experimental materials consisted of 31 restore lines and 43 cytoplasmic male sterile lines which were crossed with A1221 and R14 as the testers respectively. The developed F_1 hybrids were evaluated for GCA of three breeding objectives i.e. flowering time, plant height and grain yield during two years replicated trials. Cluster analysis revealed two heterotic groups in which the restorer lines; R22, R24 and R38 (Grain yield of 33, 32 and 31 g head^{-1} respectively) and three CMS lines; A32, A370 and A110 (Grain yield of 47, 44 and 43 g head^{-1} respectively) were identified as the suitable restorer and cytoplasmic male sterile line for improvement of new sunflower single cross hybrids. Evaluation of specific combing ability of the resulted combinations will reveal the efficiency of this selection in the following generation.

Keywords: Cluster analysis, Combining ability, Restorer line, Tester

Article History:

Received: 7th December, 2021; **Revised:** 28th December, 2021 **Accepted:** 31rh December, 2021

Oil Crops Research Department, Seed and Plant Improvement Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran; *Corresponding Author: Mehdi Ghaffari, ghaffari@areeo.ac.ir

Introduction

Sunflower with an annual production of about 27 Mha, following rapeseed and soybean, was the most important oil crop worldwide (FAO, 2019) that is well adapted to the different regions of Iran. Production of sunflower in Iran has been started since 1967 and increased to 105,000 ha in 1990s. Several high yielding single cross hybrids were developed in Iran such as as Mehr and Shafagh in 1987; Ghasem and Farrokh in 2010-2012; Shams in 2016 and finally Golsa and Zarrin in 2019 (Ghaffari et al., 2019; 2020). These hybrids caused continues per unit area yield during previous decades. Due to cross pollination, hybrid breeding is the main strategy for manifestation of heterosis in sunflower (Fehr, 1991; Ghaffari & Shariati, 2020). Hybrid cultivars are superior to open pollinated cultivars in terms of grain yield and uniformity (Seiler & Jan, 2010). The magnitude of heterosis is determined by the combining ability of the parent lines. General combining ability (GCA) is defined as the average performance of an inbred line in a series of crosses and generally inbred line with high GCA value had higher positive additive alleles, whereas specific combining ability (SCA) is the performance of inbred line in specific combination and breeding line with positive SCA values were more likely to give good hybrid (Sharma, 1998; Skoric, 2012).

Identification of an inbred line for hybrid breeding is major objective for commercial exploitation of heterosis. Breeding material may be tested through several breeding schemes such as diallel, top cross or poly cross methods. Top cross or polycross methods have several advantages due to simplicity and less laborious, which make it possible for rapid screening of inbred lines. Methods such as line tester analysis or diallel cross were used to estimate of SCA effect and gene action associated with traits of interest during latter stage of breeding program with lesser number of inbred lines (Kaya & Atakisi, 2004; Ortis et al., 2005, Ghaffari et al., 2011; 2016; Aghdam et al., 2019). Top cross method involved crossing large sets of inbred lines with few common testers. These common testers may expose the genetic load of inbred lines due to their broad genetic base (Singh & Chaudhary, 1985). Selection of useful inbred lines was most commonly based on measurements of general combining ability with one or more tester lines (Vear, 2010).

Grain yield is the main objective in sunflower improvement. Other traits such as phenology and plant characteristic as plant height are other objectives, which considered during inbreeding process. Development of inbred lines with better combining ability helped the breeders to develop high yielding hybrids (Fernandez et al., 2009).

Yield is quantitatively inherited trait which was highly influenced by environmental factors, that also depends on the genetic potential of the cultivar and contributions of other yield components, such as seed mass, head diameter and plant height (Kaya et al., 2007). Trait such as days to maturity were also likely to influence the seed yield to differential accumulation of degree days in hybrids differing for maturity (Kaya et al., 2009) Male lines had more influence on heterotic expression for economical traits such as days to maturity, stem diameter, achene number per head and achene and oil yield (Hadadan et al., 2020.

Inbred lines are generally crossed to determine their potential in hybrid breeding and many of the inbred lines may not be found valuable for hybrid breeding. Heterosis manifestation in a cross between two inbred lines was dependent on several factors such as genetic distance, and combining ability of inbred lines. Therefore, combining ability analysis was done to estimate the performance of inbred line during S4 generation, known as early testing of combining abilities after the first generation of self-pollination (Shein, 1978). Selection of suitable parent lines has a direct effect on expression of agronomic characteristics in sunflower improvement, so this study was carried out with the aim of screening of restorer and CMS inbred lines to identify the superior combiners for agronomic futures which will be used for single cross hybrid production in sunflower.

Methodology

The study was conducted at research field of Seed and Plant Improvement Institute in Karaj, Iran (35.84° N, 50.93° E; altitude of 1321m above sea level) during two growing season (2018 and 2019). Experimental materials consisted of 31 restore lines and 43 cytoplasmic male sterile lines (CMS) which evaluated for general combining ability by top-cross method. The restore lines were crossed with A1221 and the inbred lines which all were CMS line were crossed with R14 as testers to perform top-cross mating design. Both testers are parental lines of commercial hybrid; Ghasem. Pollen mixture of five restorer lines were used in crosses with CMS tester (A1221). Restorer tester (R14) was planted in four isolated cage each consisted of three rows 3 m long with a row spacing of 60 cm and within row spacing of 20cm). During flowering pollen mixture of testers were collected and used to cross with CMS lines. Sterile form is manually crossed with a pollen mixture from at least five isolated plants of a newly created line. The resulted F_1 hybrids were planted next year (2019) in randomized complete block design with three replications. Each plot consisted of three rows 3 m long with 60 cm rows intervals and 25 cm plant intervals. Seed bed were prepared with supplementary tillage before planting. One third of nitrogen fertilizer with phosphate and potassium fertilizers based on 250 kg hectare^{-1} of urea, 150 kg hectare^{-1} of ammonium phosphate and 200 kg hectare^{-1} of potassium sulfate were used before planting, and the rest of the nitrogen fertilizer was applied in two splits up to 8-10 leaf stage. Broadleaved weeds were controlled through pre-emergent herbicide trifluralin (2.5 liters hectare^{-1}). During the growing season, plants were evaluated for following traits; flowering time according to Schneider and Miller (1981), plant height and grain yield were recorded. For each line GCA and calculated as the following function:

$$\text{GCA (Line)} = \frac{x_{l.}}{r} - \frac{x_{..}}{rl}$$

(1)

Where xl. is the mean of F1 cross between l_{th} line (restorer or CMS line) and related tester; x.. is the mean of all F1 crosses between Restore/CMS lines with related testers. The software SPSS (Version 25) was used for analyzing of the data and STATGRAPHICS for plotting dendrogram lines using Wards method by squared Euclidian distances.

Results and Discussions

Combining ability of restorer and CMS lines were estimated for grain yield (g) following top cross. The results indicated that the restorer lines R22, R3, R24 had the highest grain yield (33.0, 32.3 and 32.0 g respectively) and had the highest GCA for this trait (Fig. 1). Grain yield and GCA of the check restorer line; R14 was lower than mean of all restorer lines. Among the CMS lines, A32, A370 and A110 had the highest grain yield (47.3, 44 and 43 g respectively). The GCA composition of these lines was also more than other lines (Fig. 2). The top-cross involving selected tester may substantially reduce the number of crosses required for estimating the GCA (Gopal et al., 2008). The selective testers were parental line of well-known commercial hybrids; Therefore, they may effectively identify the superior inbred lines.

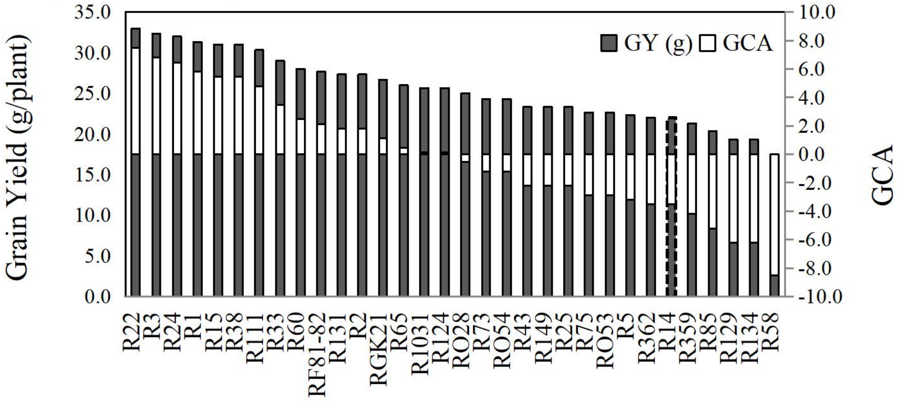

Figure 1. Grain yield and GCA of sunflower restorer lines. Gray and white columns represent grain yield and GCA for grain yield respectively. Dashed column represents the check restorer (R14)

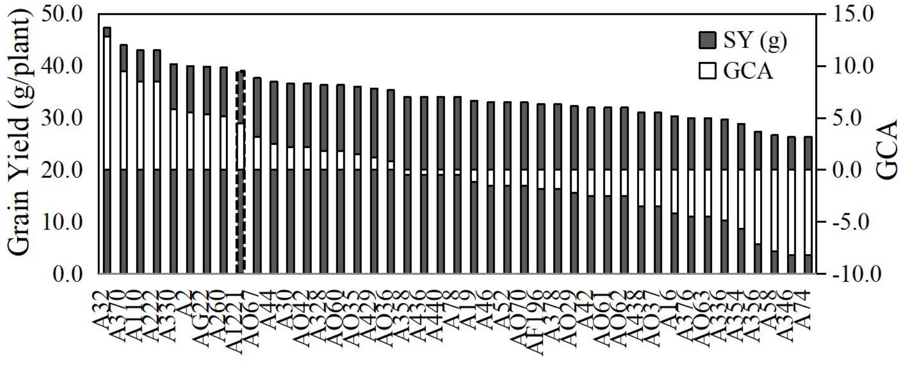

Figure 2. Grain yield and GCA of sunflower CMS lines. Gray and white columns represent grain yield and GCA for grain yield respectively. Dashed column represents the check restorer (A1221)

Cluster analysis based on wards method and using combining abilities for days to flowering, plant height and grain yield has created four clusters with similar characteristics from the 32 restorer lines (Fig. 3). This method can eliminate the number of restorer lines and may predict potential inbred lines giving optimum heterosis in the F_1 generation (Jocic et al., 2015). This method has been exploited for the determination of heterotic groups among sunflower accessions through morphological traits (Ibrar et al., 2015). The classes had 6, 7, 18 and 3 members respectively. Plant height had more effect on segregating of the first class so this class differentiated breeding lines with the highest plant height (193 cm) (Fig. 4). The second class differentiated by lower grain yield, the third by early flowering (56 days) and the fourth class by late flowering and higher grain yield (32 g per plant).

These characteristics were reflected in mean GCA of groups for the traits. As shown in Fig. 4 the first group had the highest positive GCA for plant height and the fourth group for higher GCA for days to flowering and grain yield. The third class was characterized with negative GCA for days to flowering and plant height. Results indicated that restorers in this group were suitable for development of early maturing hybrids with lower plant height. For improvement of higher yielding hybrids, the restorers in forth class will be useful. There are only three restorers in forth class and using all of them in crossing blocks is possible without difficulty. However, selection of the restorer from third class needs to consider individual characteristics of each line. The mean data for each inbred restorer in table 1 demonstrated that the restorers R65 and RO54 (53 days to flowering) were good choice for improvement of early maturity and restorers R3, R25 and R21 were good combiners for reduction of plant height.

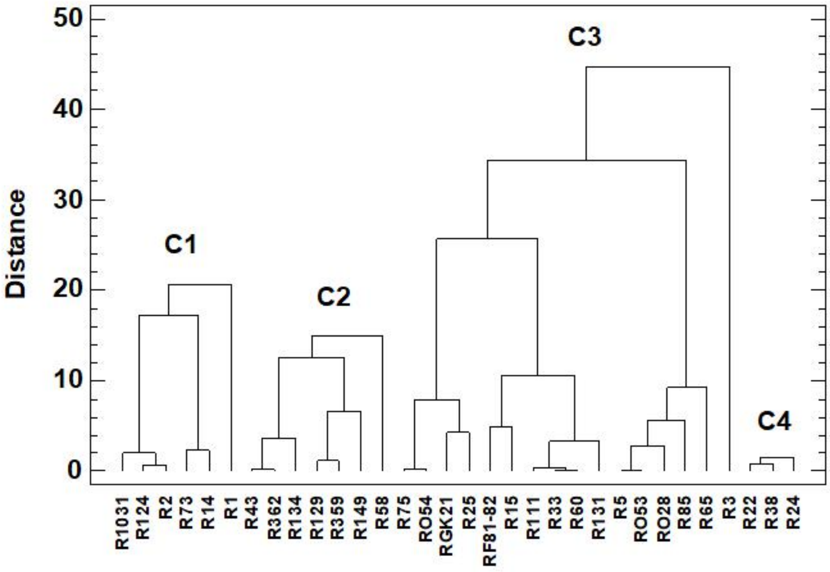

Figure 3. Dendrogram for clustering sunflower restorer lines using Wards method. C denotes to the differentiated classes.

Regarding the mean value for the traits and related GCA it is concluded that the restorer line R65 and RO28 with 53 days to flowering had the lowest negative GCA and suitable for development of early flowering breeding material (Table 1). Reduction in plant height is major breeding objective of sunflower, therefore, restorer lines R3 with 158 cm of plant height and considerable negative GCA for plant height, which may make it suitable candidate for reduction of plant height in F_1 hybrids.

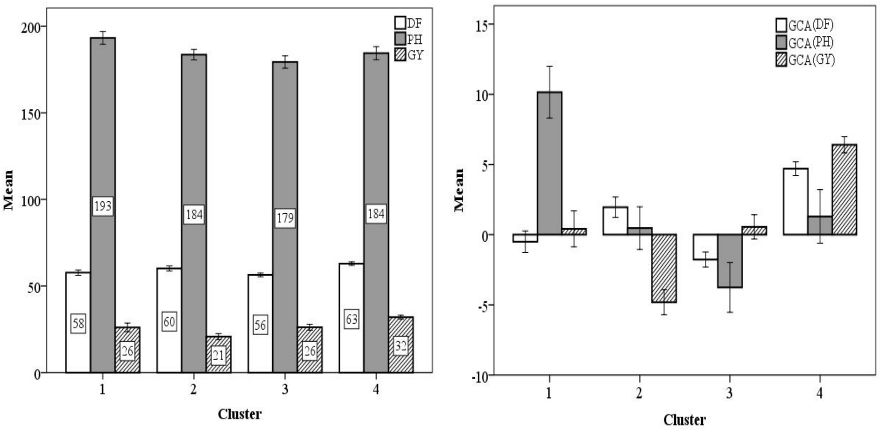

Figure 4. Mean values for agronomic traits (left) and related GCA values (right) for each of clusters created by cluster analysis. Bars represent ±SE for each related mean. DF; Days to flowering, PH; Plant height and GY; Grain yield.

A positive correlations of plant height, with achene yield plant^{-1} in sunflower has been reported earlier, showing that highly productive sunflower hybrids were taller (Shankar et al., 2006). However, taller plant canopy make it susceptible to lodging and delay maturity. The results in this study demonstrated that the restorers in first cluster with higher plant height resulted to the lowest grain yield. This show that plant height alone is not a criterion for selection for higher grain yield and genetic potential of a genotype was a main determinant of grain yield.

In the case of grain yield, it is complicated due to the quantitative inheritance of this trait. Due to the lower level of both broad and narrow sense heritability of grain yield in sunflower (Fick and Miller, 1997, Skoric, 2012), the yield of a hybrids was not generally well correlated with the yield of its inbred parents and it was highly influenced by the environment and specific reactions of hybrid combinations according to parental lines. However, the top cross results are expressed in F_1 hybrids and was not recorded on self-pollinated inbred lines. In fact, all the top-cross are new single cross hybrids and heterosis was estimated through grain yield. With this consideration all the restorer lines in forth cluster (R22, R24 and R38) were expressed as good combiner for getting higher grain yield in F_1 generation. All of restorers (R22, R24 and R38) were the late flowering lines in this study. The results are in accordance with Zakeri Hadadan et al. (2020) who reported a close relationship between the flowering days and the BPH in F_1 crosses.

The high correlation between flowering and maturity time showe that flowering time may be chosen to discriminate sunflower germplasm instead of creating redundant data from both traits .

CMS lines were also clustered into four groups (Fig. 5). The first class involved the lowest (five) while the second had the highest number (17) of CMS lines. First cluster was differentiated mainly by late flowering (62 days), and higher plant height (186 cm), the second by early flowering, the third by higher grain yield (42 g) and the last cluster by lower grain yield (32 g) and higher plant height (185 cm) (Fig. 6). Comparison of means of clusters r for GCA of the evaluated traits revealed a better picture of classes that is in concordance with the mean value of the traits (Fig. 6). Positive GCA for days to flowering and plant height in first cluster, negative GCA for days to flowering and plant height and positive GCA for plant height in forth cluster was observed. The highest positive GCA for grain yield was observed for third cluster. The results show that the second class is suitable for exploiting of early flowering lines. Due to the higher heritability of flowering time (Ghaffari, 2016), the selected CMS lines could be used for improvement of early mature hybrids. However, the lines in this group had lowest GCA for grain yield which may affect the grain yield of the related hybrids. The mean value for the GCA of each CMS line has been revealed that the CMS lines in third group as A32 and A370 are the best combiner for grain yield considering that they had the highest grain yield per head (47.3 and 44 g) (Table 2). Interestingly the later inbred; A370 also expressed a desired GCA for plant height (-11.5). A110 in this cluster had a suitable GCA; although not the best, for all of the traits; negative GCA for days to flowering (-2.6), and plant height (-12.1) and positive GCA for grain yield (8.5). Therefore, the results express A370 as the best combiner among the all CMS lines. There were other lines with the lowest GCA for flowering time as A429 and A354 but due to lower GCA for grain yield are not suggested for hybrid production.

Table 1. Mean value, GCA and advantage of restorers over tester for agronomic traits

R-Line	Cluster	DF	GCA (DF)	PH	GCA (PH)	GY	GCA (GY)
R1031	1	60.3	2.2	193.0	9.6	25.7	0.1
R1	1	57.0	-1.1	201.3	18.0	31.3	5.8
R124	1	58.0	-0.1	190.0	6.6	25.7	0.1
R2	1	59.3	1.2	189.3	6.0	27.3	1.8
R73	1	56.3	-1.8	195.3	12.0	24.3	-1.2
R14	1	55.3	-2.8	190.7	7.3	22.0	-3.5
R43	2	58.7	0.6	186.3	3.0	23.3	-2.2
R129	2	61.3	3.2	185.0	1.6	19.3	-6.2
R134	2	58.7	0.6	184.8	1.5	19.3	-6.2
R149	2	63.7	5.6	185.0	1.6	23.3	-2.2
R359	2	61.0	2.9	180.3	-3.0	21.3	-4.2
R362	2	59.0	0.9	187.7	4.3	22.0	-3.5
R58	2	58.7	0.6	176.0	-7.4	17.0	-8.5
R75	3	57.7	-0.4	180.3	-3.0	22.7	-2.9
RF81-82	3	60.3	2.2	182.7	-0.7	27.7	2.1
RGK21	3	57.3	-0.8	172.3	-11.0	26.7	1.1
R111	3	57.3	-0.8	180.0	-3.4	30.3	4.8
R131	3	55.7	-2.4	184.7	1.3	27.3	1.8
R15	3	58.7	0.6	186.7	3.3	31.0	5.5
R25	3	59.3	1.2	171.3	-12.0	23.3	-2.2
R3	3	55.3	-2.8	158.3	-25.0	32.3	6.8
R33	3	57.0	-1.1	182.3	-1.0	29.0	3.5
R5	3	54.7	-3.4	179.0	-4.4	22.3	-3.2
R60	3	57.3	-0.8	182.3	-1.0	28.0	2.5
R65	3	53.0	-5.1	187.3	4.0	26.0	0.5
R85	3	55.0	-3.1	184.3	1.0	20.3	-5.2
RO28	3	53.0	-5.1	179.0	-4.4	25.0	-0.5
RO53	3	54.3	-3.8	180.3	-3.0	22.7	-2.9
RO54	3	57.0	-1.1	179.0	-4.4	24.3	-1.2
R22	4	63.7	5.6	186.0	2.6	33.0	7.5
R24	4	63.0	4.9	180.7	-2.7	32.0	6.5
R38	4	62.0	3.9	186.7	3.3	31.0	5.5

DF; Days to flowering, PH; Plant height, GY; Grain Yield

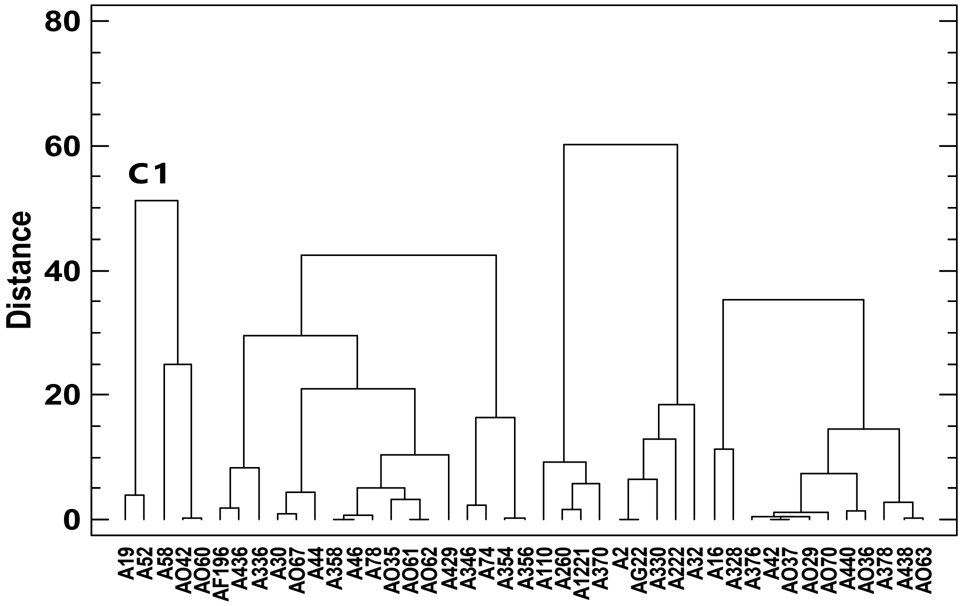

Figure 5. Dendrogram for clustering sunflower restorer lines using Wards method. C denotes to the differentiated classes.

The construction of heterotic groups was performed by including all the R and CMS lines and GCA effects for flowering time, plant height and grain yield. Two main cluster was formed as the two heterotic groups most of the R-lines located in the first cluster and the most of A-lines in the second. The three suitable R-lines that identified as the suitable R-line combiner were in the first while the preferred A-lines as A32, A110 and A370 located in the second cluster. As heterosis is the state of maximal heterozygosity, heterozygosity of the F_1 generation for the highest number of alleles is obtained by crossing genetically different inbred lines (Jocic et al., 2015). Crossing genetically distant inbred lines in different heterotic groups will achieve higher heterozygosity in F1 generation (Vear, 2010). The higher heterosis was predictable with crossing among these R and A lines. Other lines in these groups may also be selected as the parental lines for production of new F_1 hybrids. However, attempt of these crosses may be dependent over devoting of resources to handle breeding material in experimental field.

Conclusions

This study was performed in order to identify suitable R and A-lines combiner for production of new sunflower F_1 single cross hybrids using top-cross method. The crosses in first year involved parental lines such as A1221 and R14; both were the parental lines of commercial hybrid, as the A line and testers for R-lines respectively. The obtained F_1 hybrids were evaluated for GCA of three breeding targets; flowering time, plant height and grain yield. Cluster analysis revealed two heterotic groups for these traits in which restorer lines; R22, R24 and R38 in first croup and three CMS lines; A32, A370 and A110 in the second group were identified as the suitable R-line and CMS-line combiners for improvement of new sunflower single cross hybrids. Evaluation of specific combing ability of the resulted combinations will reveal the efficiency of this selection in following generation.

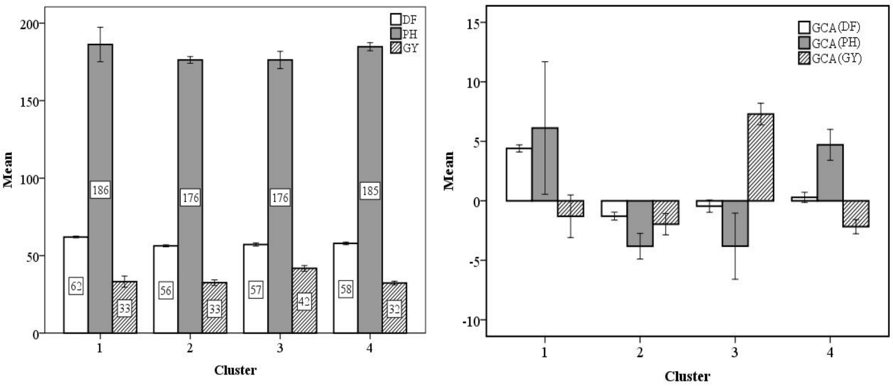

Figure 6. Mean values for agronomic traits (left) and related GCA values (right) for each of clusters created by cluster analysis. Bars represent ±SE for each related mean. DF; Days to flowering, PH; Plant height and GY; Grain yield.

Table 2. Mean values, GCA and advantage of restorers over tester for agronomic traits

A-Line	Cluster	DF	GCA (DF)	PH	GCA (PH)	SY	GCA (SY)
A19	1	62.3	4.7	202.7	22.9	33.3	-1.2
A52	1	61.7	4.1	195.0	15.2	33.0	-1.5
A58	1	63.0	5.4	171.7	-8.1	26.7	-7.8
AO42	1	61.3	3.7	182.0	2.2	36.7	2.2
AO60	1	61.7	4.1	179.7	-0.1	36.3	1.8
AF196	2	58.0	0.4	173.3	-6.5	32.7	-1.8
A30	2	58.3	0.7	176.3	-3.5	36.7	2.2
A336	2	56.7	-0.9	167.3	-12.5	29.7	-4.8
A346	2	57.3	-0.3	183.7	3.9	26.3	-8.2
A354	2	54.5	-3.1	174.5	-5.3	28.8	-5.7
A356	2	55.0	-2.6	173.0	-6.8	27.3	-7.2
A358	2	56.3	-1.3	175.7	-4.1	34.0	-0.5
A429	2	54.0	-3.6	175.0	-4.8	35.7	1.2
A436	2	59.0	1.4	168.3	-11.5	34.0	-0.5
A44	2	56.3	-1.3	173.3	-6.5	37.0	2.5
A46	2	56.7	-0.9	177.0	-2.8	33.0	-1.5

A-Line	Cluster	DF	GCA (DF)	PH	GCA (PH)	SY	GCA (SY)
A74	2	55.7	-1.9	179.7	-0.1	26.3	-8.2
A78	2	56.3	-1.3	180.0	0.2	34.0	-0.5
AO35	2	55.3	-2.3	181.7	1.9	36.0	1.5
AO61	2	55.3	-2.3	179.0	-0.8	32.0	-2.5
AO62	2	55.3	-2.3	179.7	-0.1	32.0	-2.5
AO67	2	57.3	-0.3	179.3	-0.5	37.7	3.2
A110	3	55.0	-2.6	167.7	-12.1	43.0	8.5
A2	3	57.3	-0.3	184.7	4.9	40.0	5.5
A222	3	55.0	-2.6	182.3	2.5	43.0	8.5
A260	3	56.3	-1.3	166.3	-13.5	39.7	5.2
A32	3	59.0	1.4	180.3	0.5	47.3	12.8
A330	3	59.3	1.7	183.0	3.2	40.3	5.8
A370	3	58.0	0.4	168.3	-11.5	44.0	9.5
AG22	3	56.8	-0.8	185.7	5.9	39.8	5.3
A1221	3	57.7	0.1	168.3	-11.5	39.0	4.5
A16	4	54.7	-2.9	192.7	12.9	30.3	-4.2
A328	4	56.3	-1.3	193.7	13.9	36.3	1.8
A376	4	57.7	0.1	183.7	3.9	30.0	-4.5
A378	4	59.7	2.1	186.7	6.9	32.7	-1.8
A42	4	58.3	0.7	181.3	1.5	32.0	-2.5
A438	4	60.0	2.4	181.3	1.5	31.0	-3.5
A440	4	58.3	0.7	185.3	5.5	34.0	-0.5
AO29	4	57.7	0.1	184.0	4.2	32.3	-2.2
AO36	4	57.0	-0.6	186.3	6.5	35.3	0.8
AO37	4	58.0	0.4	182.0	2.2	31.0	-3.5
AO63	4	59.3	1.7	180.7	0.9	30.0	-4.5
AO70	4	57.7	0.1	180.0	0.2	33.0	-1.5

DF; Days to flowering, PH; Plant height, GY; Grain Yield

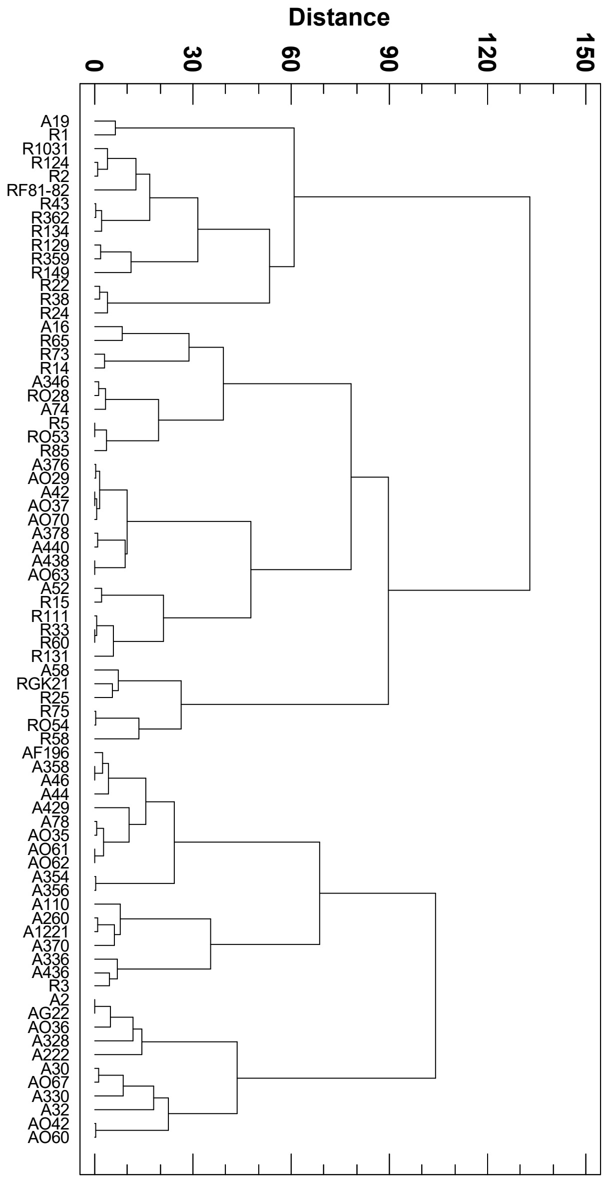

Figure 7. Dendrogram for clustering sunflower restorer and CMS inbred lines using Wards method. C denotes to the differentiated classes. The circles show the suitable R-line and CMS-line combiners for improvement of new sunflower single cross hybrids. C denotes to the differentiated classes.

Acknowledgements

This manuscript was extracted from the results of project No.: 0-03-03-065-980472. The authors thank to the Seed and Plant Improvement Institute, Karaj, Iran for financial support and providing research facilities.

References

Aghdam, M.Z., Kojouri, F.D., Ghaffari, M. and Ebrahimi, A., (2019). Genetic Analysis of Morpho-Physiological Characteristics of Sunflower under Stress and Non-Stress Drought Conditions. *Agrivita*, 41(3), 461-473.

FAO (2019) Agricultural production year book. Rome. Italy. Available at: http://faostat3.fao.org

Fehr, W.R., (1991). *Principles of Cultivar Development;* Theory and Technique, Vol. 1, Mcmillan. U.S.A.

Dominquez J, Fernandez-Martinez J.M. (1987). Evaluation of inbred testers in sunflower hybrid

breeding. *Helia*, 10:15–19.

Fernandez MJ, Perez-Vich B, Velasco L. (2009). Sunflower. In: Vollmann J, Rajcan I (ed) Oil crops. Handbook of plant breeding, vol 4, pp, 155–232.

Fick G.N. & Miller J.F. (1997). Sunflower Breeding. In: A. A. Schneiter (ed.). Sunflower science and technology, Agronomy Monograph. ASA Inc. Madison. WI., pp. 395–439.

Ghaffari, M. (2016). Genetic analysis of seed yield related traits under optimum and limited irrigation in sunflower. 19thInternational Sunflower Conference. *Edirne, Turkey*.

Ghaffari M., Farrokhi E. & Mirzapour M. (2011). Combining ability and gene action for agronomic traits and oil content in sunflower (*Helianthus annuus* L.) using F1 hybrids. *Crop Breeding Journal* 1 (1), 73 – 84.

Ghaffari, M., & Shariati, F. (2018). Combining ability of sunflower inbred lines under drought stress. *Helia*, *41*(69), 201-212.

Ghaffari, M., Davaji, A. M.N.R., & Ghadimi, F.N. (2019). Oil yield determinant of sunflower in climatically different regions of Iran. *Bulgarian Journal of Agricultural Science*, *25*(1), 67–71.

Ghaffari, M., Shariati, F., & Fard, N. S. (2020). Heterosis Expression for Agronomic Features of Sunflower. *Journal of Agriculture and Food*, *1*(1), 1-10.

Ghaffari, M., Andarkhor, S. A., Homayonifar, M., Ahmadi, S. A. K., Shariati, F., Jamali, H., & Rahmanpour, S. (2020). Agronomic attributes and stability of exotic sunflower hybrids in Iran. *Helia*, *43*(72), 67–81.

Gopal, J., Kumar, V., & Luthra, S. K. (2008). Top-cross vs. poly-cross as alternative to test-cross for estimating the general combining ability in potato. Plant breeding, 127(5), 441–445.

Haddadan, A.Z., Ghaffari, M., Hervan, E.M., & Alizadeh, B. (2020). Impact of parent inbred lines on heterosis expression for agronomic characteristics in sunflower. *Czech Journal of Genetics and Plant Breeding*, *56*(3), 123–132.

Ibrar, D., Khan, M. A., Mahmood, T., Ahmad, R., Hafiz, I. A., Jan, S. A., & Ahmad, R. (2018). Determination of heterotic groups among sunflower accessions through morphological traits and total seed storage proteins. *International Journal of Agriculture & Biology*, 20(9), 2025–2031.

Jocic, S., Miladinovic, D., & Kaya, Y. (2015). Breeding and genetics of sunflower. In *Sunflower* (pp. 1-25). AOCS Press.

Kaya, Y., & Atakisi, I.K. (2004). Combining ability analysis of some yield Characters of sunflower (*Helianthus annuus* L.). *Helia* 27: 75–84.

Kaya Y., Evci G., Durak S., Pekcan V., & Gucer T. (2007). Determining the relationships between yield and yield attributes in sunflower. *Turkish Journal of Agriculture and Forestry* 31:237–244

Kaya, Y., Evci, G., Durak, S., Pekcan, V., & Gucer, T. (2009). Yield components affecting seed yield and their relationships in sunflower (*Helianthus annuus* L.). *Pak. J. Bot*, *41*(5), 2261–2269.

Miller, J. F., Hammond, J.J., & Roath, W.W. (1980). Comparison of Inbred vs. Single-cross Testers and Estimation of Genetic Effects in Sunflower 1. *Crop Science*, *20*(6), 703–706.

Ortis, L., Nestares, G., Frutos, E., & Machado, N. (2005). Combining ability analysis for agronomic traits in sunflower (*Helianthus annuus* L). *Helia* 28: 125–134.

Schniter, A.A., & Miller, J.F., (1981) Description of sunflower growth stage. *Crop Science,* 21, 901–903.

Sharma, J.R. (1998). Statistical and Biometrical Techniques in Plant Breeding, New Age International Limited Publishers, New Delhi, pp. 129–137.

Seiler G. & Jan C.C. (2010). Basic Information. In: Jinguo, H., Seiler, G. and Chittaranjan, K. Genetics, Genomics and Breeding of Crop Plants, CRC Press, pp, 1–50.

Shein S.E. (1978). An evaluation of early generation testing for general combining ability in sunflower. In: Proceedings of the 8th international sunflower conference, Minneapolis, pp, 432–436.

Singh, R.K., & Chaudhury, B.D. (1985). Biometrical techniques in breeding and genetics. Kalyani publishers, New delhi, India.

Skoric, D. (2012). Sunflower breeding. 165-354. In: Skoric, D., Seiler, G. J., Zhao, L., ChaoChien, J., Miller, J. F. and Charlet, L. D. (eds) Sunflower genetics and breeding. International monograph. Serbian Academy of Sciences and Arts Branch in Novi Sad.

Vear, F. (2010). Classic Genetics and Breeding, In: J. Hu, J. Seiler, C. Kole (Ed.) Genetics, Genomics and Breeding of Sunflower, CRC Press, New York; 51–78.

Citation of Article

Ghaffari, M., Shariati, F., Meymand, H.T., & Fard, N.S. (2021). Heterotic groups for identifying of suitable combiners in sunflower using top-cross. *Journal of Agriculture and Food, 2(2)*, 9–22.