https://doi.org/10.17221/100/2019-CJGPB

Impact of parent inbred lines on heterosis expression for agronomic characteristics in sunflower

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Citation: Zakeri Haddadan A., Ghaffari M., Majidi Hervan E., Alizadeh B. (2020): Impact of parent inbred lines on heterosis expression for agronomic characteristics in sunflower. Czech J. Genet. Plant Breed., 56: 123–132.

Abstract: To study the impact of parent-inbred lines on the heterosis expression of the agronomic characteristics in sunflower hybrids, 24 sunflower hybrids along with the parent lines were evaluated for their agronomic characteristics as a randomised complete block design with three replications in the 2018–2019 growing seasons in Karaj, Iran. According to the results, the hybrids R29 × A346, R19 × A346, R29 × A40 had the highest achene yield (4 159, 4 143 and 4 108 kg/ha, respectively), but the highest heterosis was observed in R29 × A212 and R19 × A212 (182 and 181%, respectively) suggesting that the incidence of heterosis is related to the relative performance of both the parents and hybrids. The results confirmed the heterosis expression for most of the agronomic traits. The heterosis for the days to flowering and maturity were negative. All the mid-parent heterosis (MPH) for the plant height, head diameter, stem diameter and achene number were positive, while only the plant height was positive for the best parent heterosis (BPH). Almost all the MPH and BPH of the crosses for the achene and oil yield were positive, which indicates a considerable heterosis for the achene and oil yield. The results showed that the relative impact of the restorer (R)-lines was higher than the cytoplasmic mail sterile (CMS)-lines on the heterosis expression for the days to maturity, stem diameter, achene number per head and achene and oil yield. The CMS-lines had more of an impact on the heterosis expression for the plant height and the relative impact of the R-lines and CMS-lines were almost similar for the days to flowering, head diameter, achene weight and oil content. Due to the higher relative impact of the paternal lines on the heterosis expression for half of the studied characteristics in this study, choosing suitable parental lines will have a crucial role in breeding the sunflowers for a desired trait.

Keywords: heterosis; hybrid; principle component; restorer

The selection of parent-inbred lines to be-crossed to benefit from heterosis is one of the main challenges in the improvement of cross-pollinated crops as well as in sunflowers. The heterosis expression has been reported for most of the agronomic characteristics in the sunflower (Encheva et al. 2015). The study on the relationships between the parent lines and hybrid performance was an important interest for sunflower breeders. Skoric (1982) reported a significant correlation between the parental inbred lines and the related F_1 hybrids for the plant height, leaf number, leaf area, husk percentage, oil content and achene yield. Miller et al. (1982) concluded that about half of the variation of the hybrids for the oil content could be explained by the variation in the related female line. Manivannan et al. (2004) reported a significant relationship between the parent lines and the related hybrids for the flowering time, plant height, head diameter and achene yield.

Supported by the Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran.

https://doi.org/10.17221/100/2019-CJGPB

Table 1. The physico-chemical characteristics of the soil in the experimental field (the average over the two years)

Electrical conductivity (ds/m)	pH	Organic carbon	N	P	K	Clay	Silt	Sand	Soil texture
		(%)		(ppm)		(%)			
1.42	7.7	0.87	0.09	13.1	181	26	49	25	clay loam

Multivariate methods such as principle component analysis have been used to find relationships among the different aspects of a plant. Tersac et al. (1993) used a principle component analysis (PCA) to determine if there were any structure to the sunflower populations related to the country of origin. Vega et al. (2001) revealed two-dimensional structures among the genotypes and environments based on their interactions using a PCA analysis. Ghaffari et al. (2011) used a PCA as a reflector of combining the abilities in the sunflower and as an effective method for the determination of superior inbred lines. To test the effect of the parental lines on the grain yield and stability of the sunflower hybrids, Liović et al. (2012) reported that the greatest stability was shown by the restorer line oM7 in the cross combinations with the inbred lines cms1 and cms2, while the same restorer line exhibited a large genotype × environment interaction and low stability when crossed with cms3. The heterosis expression in relationship to the parent lines has been reported in limited studies. Getting information on the relationships between the parent lines and the heterosis expression for the agronomic characteristics in the related hybrid using multivariate methods such as a PCA could have practical applications in the appropriate selection of the parent lines in breeding sunflowers. The objective of this study was to identify any relationship between the parent lines and the heterosis expression in the F_1 crosses.

MATERIAL AND METHODS

Physico-Chemical characteristics of soil in the experimental field is indicated in Table 1. Twenty-four sunflower hybrids were produced through crossing eight cytoplasmic male sterile (CMS) lines as the lines and three fertility restorer (R) inbred lines as the testers (Table 2) in line × tester fashion. All the

Table 2. The list and pedigree of the hybrids and related parent lines in the study

No.	Hybrid/line pedigree	Origin	Type	No.	Hybrid/line pedigree	Origin	Type
1	RGK19 × AGK28	Iran-SPII	SCH	19	RGK46 × AGK110	Iran-SPII	SCH
2	RGK19 × AGK40	Iran-SPII	SCH	20	RGK46 × AGK212	Iran-SPII	SCH
3	RGK19 × AGK110	Iran-SPII	SCH	21	RGK46 × AGK222	Iran-SPII	SCH
4	RGK19 × AGK212	Iran-SPII	SCH	22	RGK46 × AGK330	Iran-SPII	SCH
5	RGK19 × AGK222	Iran-SPII	SCH	23	RGK46 × AGK344	Iran-SPII	SCH
6	RGK19 × AGK330	Iran-SPII	SCH	24	RGK46 × AGK346	Iran-SPII	SCH
7	RGK19 × AGK344	Iran-SPII	SCH	25	AGK 28	Iran-SPII	CMS
8	RGK19 × AGK346	Iran-SPII	SCH	26	AGK 40	Iran-SPII	CMS
9	RGK29 × AGK28	Iran-SPII	SCH	27	AGK110	Iran-SPII	CMS
10	RGK29 × AGK40	Iran-SPII	SCH	28	AGK212	Iran-SPII	CMS
11	RGK29 × AGK110	Iran-SPII	SCH	29	AGK222	Iran-SPII	CMS
12	RGK29 × AGK212	Iran-SPII	SCH	30	AGK330	Iran-SPII	CMS
13	RGK29 × AGK222	Iran-SPII	SCH	31	AGK344	Iran-SPII	CMS
14	RGK29 × AGK330	Iran-SPII	SCH	32	AGK346	Iran-SPII	CMS
15	RGK29 × AGK344	Iran-SPII	SCH	33	RGK 19	Iran-SPII	RL
16	RGK29 × AGK346	Iran-SPII	SCH	34	RGK 29	Iran-SPII	RL
17	RGK46 × AGK28	Iran-SPII	SCH	35	RGK 46	Iran-SPII	RL
18	RGK46 × AGK40	Iran-SPII	SCH				

SPII – Seed and Plant Improvement Institute; SCH – single cross hybrid; CMS – cytoplasmic male sterile line; RL – restorer line

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lines and related single cross hybrids have been improved as part of the sunflower breeding project in the Seed and Plant Improvement Institute (SPII) in Iran. In order to estimate the heterosis expression in comparison to the mid-parent heterosis (MPH) and the better parent heterosis (BPH), the resulting single cross hybrids along with eleven parents were evaluated for their agronomic characteristics as a Randomised Complete Block Design (RCBD) with three replications for two years (the 2018 and 2019 growing seasons) in the SPII experimental fields in Karaj, Iran located at 35°55'N latitude and 50°54'E longitude. Each plot consisted of three rows, 5 m in length, and had a row spacing of 60 cm and a space between the seedlings of 25 cm. One-third of a nitrogen fertiliser with phosphate and potassium fertilisers based on 250 kg of urea, 150 kg of ammonium phosphate and 200 kg of potassium sulfate was used before planting, and the rest of the nitrogen fertiliser was used 2 and 4 weeks after germination. During the growing season, the days to flowering and maturity were noted according to the phenological stages defined by (Schneiter & Miller 1981), the plant height, head and stem diameter were measured on six plants in each plot at the physiological maturity (R9). The oil yield components (1 000 achene's weight, achene number/head, oil content and achene yield) were measured after harvesting 4 m of the inner row. The heterosis for these traits were estimated according to Wynne et al. (1970) using Equations (1) and (2)

$$\%BPH = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100 \quad (1)$$

$$\%MPH = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100 \quad (2)$$

where:

BPH – better parent heterosis
MPH – mid-parent heterosis
F_1 – single cross hybrid
BP – related best parent
MP – related mid-parents

A principle component analysis was used for the ordination of the entries in the two-dimensional bi-plots (Kroonenberg 1997) based on the mean of the parent-inbred lines, the crosses and the related heterosis. The statistical analysis was performed in Statgraphics (Ver. 16.1.11, 2007).

RESULTS AND DISCUSSION

According to the mean comparisons of the hybrids for the agronomic traits (Table 3), the values for the MPH and BPH are presented in Table 4 and 5, respectively. The hybrids R29 × A346, R19 × A346 and R29 × A40 had the highest achene yield (4 159, 4 143 and 4 108 kg/ha, respectively), among them R29 × A346 had more oil content (44.4%) and had the highest oil yield (1 847.7 kg/ha). The results confirmed the heterosis expression for most of the agronomic traits. There was a positive and negative heterosis for the traits in the different crosses, however most of the MPH and BPH for the days to flowering and maturity were negative, which indicated the earlier maturity of the hybrids when compared with the related parental lines. All the MPH for the plant height, head diameter, stem diameter and achene number were positive, while only the plant height was positive for the BPH. This shows higher values for these characteristics in the hybrids in comparison to the related parents. Except for R19 × A212, all the MPH and BPH of the crosses for the achene and oil yield were positive, which indicates considerable heterosis for the achene and oil yield in the sunflower which justifies the hybrid breeding in the sunflower.

A principle component analysis was used to find out any structure between the performance of the parent lines, hybrids and the related crosses. The relative impact of the PCA components on the variability in the sunflower characteristics indicated the efficiency of this method in differentiation of the genotypes according to their F_1 expression and heterosis (Table 6). Regarding the days to flowering, two PCA components efficiently differentiated the crosses. The first component was affected positively by the MPH and BPH. In the second component, the CMS-lines had a higher efficiency in differentiating the hybrids (Table 7). Both the R and CMS-parent lines had almost a similar negative impact on the heterosis expression for the days to flowering. There was a close relationship between the flowering days in the F_1 crosses and the BPH, which is a proof for the heterosis expression for this trait (Figure 1A). There are inconsistent reports about the heterosis modality for the flowering time in the sunflower. Ashok et al. (2000) reported a negative one while Seetharam et al. (1980) reported a positive heterosis for the flowering time in the sunflower. The flowering time is mainly under control of the additive gene action (Ghaffari

https://doi.org/10.17221/100/2019-CJGPB

et al. 2011 and Ghaffari 2016), so selection of this during inbreeding could result in the improvement of the inbred lines with a different flowering date.

Three components accounted for 86.6% of the variability in the days to maturity (Table 6). The first component was positively affected by most CMS-lines, while the second component was negatively affected by the R-lines (Table 7), so these components could efficiently discriminate the hybrids according to these lines. There was a close relationship between the days to maturity of the R-lines and BPH, so these lines had more of an impact on the heterosis expression for the days to maturity, however, the CMS-lines had a more intense relationship with the F_1 crosses (Figure 1B). The growth duration is mainly under the control of the additive effects (Bajaj et al. 1997), although there are reports pointing to the involvement of the non-additive effects on this feature (Ghaffari et al. 2011). The expression of the significant negative heterosis for some hybrids such as R19 × A212 and R19 × A330 (–5.2) and positive heterosis for R46 × A212 (4.9) indicated that crossing between the appropriate parent lines could result in a desirable heterosis in a negative or positive direction and, in this study, the effect of the R-lines was more pronounced than the CMS-lines in the expression of the BPH.

For the plant height, the R-lines along with the BPH and MPH efficiently discriminated the hybrids according to PC1, while the PC2 discrimination was more affected by the CMS-lines (Table 7). A close relationship between the plant height of the F_1 crosses and the BPH verified the heterosis expression for this trait which is in accordance with Encheva et al.

Table 3. The mean comparison of the agronomic traits for the sunflower hybrids

Hybrids	Days to flowering	Days to maturity	Plant height	Head diameter	Stem diameter (mm)	Achene weight (g)	Achene No./head	Oil content (%)	Achene yield	Oil yield
			(cm)						(kg/ha)	
R19 × A28	59.6	111.4	207.5	22.3	28.8	65.7	949.7	41.0	3 126.9	1 281.8
R19 × A40	60.3	113.3	214.7	21.3	28.6	70.0	971.5	43.8	3 550.2	1 555.1
R19 × A110	59.3	108.8	181.8	21.0	25.7	65.1	569.6	42.9	1 379.1	592.1
R19 × A212	59.8	109.3	204.1	20.4	26.1	65.3	1 022.0	46.3	3 444.4	1 594.1
R19 × A222	60.3	107.0	201.6	20.1	25.6	66.7	975.5	43.0	3 365.0	1 447.7
R19 × A330	59.4	109.4	204.7	19.7	26.0	65.6	921.5	42.2	3 049.5	1 285.3
R19 × A344	61.6	108.7	208.9	19.5	24.1	64.8	818.3	41.7	2 571.4	1 071.3
R19 × A346	59.8	108.1	215.0	19.6	26.5	66.5	1 164.6	44.2	4 142.8	1 831.4
R29 × A28	60.7	108.9	212.7	19.7	24.8	70.2	892.6	42.1	3 206.3	1 348.9
R29 × A40	60.8	111.0	214.4	21.8	28.0	74.7	1 039.5	42.6	4 108.5	1 748.9
R29 × A110	60.6	109.3	187.6	19.7	23.8	64.9	1 037.1	46.8	3 523.8	1 647.7
R29 × A212	62.1	107.6	212.8	19.0	24.6	67.6	969.7	44.3	3 457.6	1 533.0
R29 × A222	59.7	108.3	189.1	18.9	22.4	67.3	809.3	44.7	2 677.2	1 196.7
R29 × A330	61.1	109.3	224.9	21.1	27.3	81.8	885.4	38.7	3 775.1	1 459.6
R29 × A344	60.0	107.0	215.8	19.2	23.8	61.7	807.5	39.7	2 333.3	927.1
R29 × A346	59.9	106.4	215.6	19.7	24.9	65.4	1 183.6	44.4	4 158.7	1 847.7
R46 × A28	59.6	109.2	195.5	19.0	23.0	64.6	1 015.9	47.2	3 365.0	1 588.5
R46 × A40	60.4	109.7	206.9	22.0	26.1	71.5	1 056.8	43.2	3 984.1	1 722.2
R46 × A110	60.3	107.6	168.8	22.1	26.4	62.9	1 152.2	45.7	3 841.2	1 757.0
R46 × A212	60.7	106.3	204.3	20.4	25.7	67.1	1 097.0	45.7	3 880.6	1 772.4
R46 × A222	59.6	109.2	206.5	20.6	27.0	69.2	1 066.9	44.3	3 888.8	1 723.7
R46 × A330	61.8	112.0	204.4	19.6	26.7	68.0	1 051.3	44.5	3 761.9	1 673.8
R46 × A344	61.0	108.1	196.0	19.9	25.9	65.9	731.5	40.8	2 174.6	886.3
R46 × A346	61.2	106.9	201.3	19.6	26.6	71.1	989.9	41.5	3 722.2	1 543.2
LSD 5%	2.7	3.6	20.4	3.3	4.0	10.6	249.6	6.1	1 285.4	558.8
LSD 1%	3.6	4.8	27.2	4.4	5.3	14.1	331.5	8.1	1 707.0	742.0

https://doi.org/10.17221/100/2019-CJGPB

(2015), who reported a considerable heterosis for the plant height. The CMS-lines had more of an impact on the heterosis expression for the plant height than the R-lines (Figure 1C). The plant height is under control of both the additive and non-additive gene action (Tabrizi et al. 2012). Due to the inheritance of the cytoplasm via the female parent (Acquaah 2012), the results indicated that the cytoplasm-originated factors from the CMS-lines in this study had a considerable effect on the plant height of the F_1 crosses.

In the case of the head diameter, PC1 discriminated the hybrids positively by the CMS-lines and negatively by MPH and BPH. PC2 and PC3 was positively affected by the head diameter of the R-lines and F_1 crosses, respectively (Table 7). There was no general relationship between the F_1 crosses and the heterosis expression for the head diameter (Figure 1D), which is an indication of the absence of the heterosis for this trait, however, R19 × A212 and R29 × A212 expressed a considerable positive heterosis for that trait (40.7 and 31.1, respectively). Encheva et al. (2015) also reported a positive heterosis for the head diameter of some crosses. The close angle of the R-lines and BPH vectors indicated the affectability of the BPH by the male parents more, which show the importance of the restorer lines in the heterosis expression in the F_1 crosses.

Regarding the stem diameter, PC1 and PC2 was more positively affected by the MPH and F_1 crosses, respectively, while PC3 was negatively affected by the CMS-lines (Table 7). The close relationship between the stem diameter of the F_1 crosses with the BPH and MPH (Figure 1E), was a sign of the heterosis expression for this trait. The R-lines had a larger effect on the F_1 and BPH. Due to the involvement of the additive gene action on the inheritance of the stem diameter (Tabrizi et al. 2012), the selection for the stem diameter could be effective in the improvement of the sunflower hybrids with thick stems, which is important to the lodging tolerance.

Table 4. The mid-parent heterosis (MPH) in percent for the agronomic traits in the sunflower hybrids

Hybrids	Days to flowering	Days to maturity	Plant height	Head diameter	Stem diameter	Achene weight	Achene No./head	Oil content	Achene yield	Oil yield
R19 × A28	–2.6	–1.2	9.6	21.0	17.8	10.4	85.7	–1.0	74.7	75.3
R19 × A40	–2.0	–0.2	11.1	12.9	13.3	13.3	56.1	2.2	49.0	56.5
R19 × A110	–1.8	–2.1	12.1	17.6	9.8	11.5	11.6	–3.1	–9.9	–12.0
R19 × A212	–4.0	0.5	19.0	28.6	21.1	44.5	59.4	1.6	113.5	120.3
R19 × A222	–1.6	–2.3	12.7	11.5	10.3	19.3	32.4	–2.0	33.9	30.0
R19 × A330	–4.2	–1.8	9.2	13.5	9.4	19.4	40.5	–1.2	40.4	39.5
R19 × A344	0.0	–1.0	15.3	15.8	7.6	26.3	39.1	1.6	47.8	54.7
R19 × A346	–3.0	–1.0	17.4	21.1	13.0	9.7	96.8	1.3	89.4	93.2
R29 × A28	–1.3	–1.4	20.0	20.0	19.8	17.0	105.1	1.3	99.8	107.8
R29 × A40	–1.2	–0.4	19.9	21.1	23.1	20.0	81.4	1.6	77.7	85.4
R29 × A110	–0.1	–1.0	25.7	20.7	16.3	13.6	79.6	2.0	64.8	68.5
R29 × A212	–1.7	0.5	34.9	33.7	33.0	54.0	69.7	0.3	148.8	149.6
R29 × A222	–1.6	–0.9	18.3	14.5	13.1	23.0	26.4	0.8	23.5	22.9
R29 × A330	–2.5	–1.0	24.7	26.0	24.0	40.2	49.1	–4.5	75.6	65.1
R29 × A344	–0.8	–0.9	28.5	22.6	18.8	26.2	51.7	0.1	53.2	58.4
R29 × A346	–2.5	–0.9	28.7	31.2	21.1	10.9	127.0	2.5	109.7	116.3
R46 × A28	–1.4	–2.0	7.8	1.6	0.8	–3.3	90.6	6.0	51.5	63.9
R46 × A40	–0.7	–1.8	10.6	6.0	4.3	1.0	62.6	1.1	40.7	44.0
R46 × A110	0.4	–2.4	9.8	10.6	7.8	–3.6	71.6	–0.3	40.5	41.0
R46 × A212	–2.0	–0.8	21.6	15.0	15.0	18.0	64.6	0.6	83.1	85.5
R46 × A222	–0.8	–1.2	16.3	4.2	9.6	4.9	38.4	–0.9	30.7	29.0
R46 × A330	–1.2	–0.5	10.9	3.9	7.2	4.7	51.0	1.1	40.0	42.6
R46 × A344	0.8	–1.2	13.2	6.9	7.9	7.6	27.7	0.1	13.3	13.5
R46 × A346	–0.7	–1.4	15.2	9.5	9.3	0.4	71.3	–2.2	48.5	43.5

https://doi.org/10.17221/100/2019-CJGPB

Table 5. The better parent heterosis (BPH) in percent for the agronomic traits in the sunflower hybrids

Hybrids	Days to flowering	Days to maturity	Plant height	Head diameter	Stem diameter	Achene weight	Achene No./head	Oil content	Achene yield	Oil yield
R19 × A28	–3.8	–1.2	32.1	11.7	28.2	–7.1	169.9	–7.8	92.4	103.0
R19 × A40	–2.2	0.9	36.6	–5.0	13.5	–3.5	72.3	–1.5	31.3	45.6
R19 × A110	0.6	–2.4	34.2	7.1	12.0	–4.0	–0.6	–8.6	–46.0	–49.3
R19 × A212	–6.8	–5.2	47.4	40.7	30.3	71.5	75.5	2.5	181.2	192.8
R19 × A222	–0.5	–1.8	28.3	–5.2	14.1	14.8	17.2	–4.5	7.6	2.2
R19 × A330	–7.3	–5.2	30.2	0.8	9.3	16.1	38.3	–5.2	22.4	23.1
R19 × A344	3.9	2.2	32.9	7.3	10.6	38.3	44.5	–6.3	47.3	69.6
R19 × A346	–5.3	2.9	36.7	21.7	20.1	–9.2	164.0	–0.6	98.3	106.3
R29 × A28	–2.2	–2.4	89.4	–1.1	10.2	–0.8	156.6	–2.1	97.3	113.6
R29 × A40	–1.6	–0.6	90.8	–2.7	10.7	2.9	84.4	–1.0	52.0	63.7
R29 × A110	2.8	–1.8	67.1	0.3	3.9	–4.4	81.0	–0.5	38.0	41.2
R29 × A212	–1.1	–3.6	89.5	31.1	47.8	98.7	66.5	–1.8	182.3	181.6
R29 × A222	–1.6	–0.6	68.3	–10.5	–0.4	15.8	–2.8	–0.8	–14.4	–15.6
R29 × A330	–2.7	–2.1	100.2	8.0	14.4	44.5	32.9	–10.0	51.5	39.7
R29 × A344	1.1	0.6	92.2	5.5	9.8	31.6	42.6	–7.6	33.6	46.8
R29 × A346	–4.8	–4.8	91.9	22.7	13.0	–10.8	168.3	3.4	99.0	108.2
R46 × A28	–1.6	–3.0	33.1	–4.7	0.9	–8.7	192.1	4.6	99.1	108.0
R46 × A40	–0.5	–2.4	40.8	–1.7	3.3	–1.5	200.3	–4.2	47.4	61.2
R46 × A110	2.3	–3.3	24.6	12.7	15.3	–7.2	101.1	–2.6	50.4	50.6
R46 × A212	0.0	4.9	47.6	20.7	12.4	–0.8	88.4	1.1	129.6	132.1
R46 × A222	–1.6	0.3	40.5	–2.6	18.2	2.1	28.2	–1.8	24.4	21.6
R46 × A330	1.6	0.6	39.0	0.6	11.9	0.5	57.8	–1.4	50.9	60.3
R46 × A344	2.8	1.6	33.3	9.8	13.2	–2.6	29.2	–9.7	24.5	16.1
R46 × A346	0.5	1.9	36.9	16.1	16.5	–3.0	124.4	–8.1	78.2	73.9

For achene weight, the first PC was mainly affected by the MPH while the second one was affected by the achene weight of the F_1 crosses (Table 7). Both heterosis types had an intense relationship with the achene weight of the F_1 crosses, but no specific relationship was detected between the parent lines and the heterosis, however, the CMS-lines had a close relationship with the achene weight of the F_1 crosses (Figure 1F). Gangappa et al. (1997) reported the major role of overdominance, while Bajaj et al. (1997) reported the importance of the additive effects on the inheritance of the achene weight in the sunflower. The observation of the considerable heterosis for the specific cases (Table 5), such as R29 × A212 (98.7%)

Table 6. The relative impact of the principle component analysis components on the variability of the sunflower characteristics

	Days to flowering		Days to maturity		Plant height		Head diameter		Stem diameter		Achene weight		Achene No./head		Oil content		Achene yield		Oil yield	
C	EV	CV%	EV	CV%	EV	CV%	EV	CV%	EV	CV%	EV	CV%	EV	CV%	EV	CV%	EV	CV%	EV	CV%
1	2.9	58.2	1.8	35.7	2.9	58.5	2.5	51.2	2.2	45.2	2.77	55.5	2.91	58.2	2.58	51.73	2.4	47. 6	2.36	47.2
2	1.2	83.4	1.3	62.5	1.4	88.3	1.3	78.9	1.4	74.2	1.18	79.2	1.11	80.4	1.29	77.72	1.3	73.7	1.37	74.7
3	0.7	98.2	1.2	86.6	0.5	99.3	1.0	99.4	1.1	96.9	0.89	97.0	0.86	97.7	1.02	98.22	1.1	94.8	1.04	95.7
4	0.1	99.9	0.7	99.9	0.03	99.9	0.02	99.9	0.1	99.9	0.14	99.9	0.09	99.7	0.08	99.99	0.2	99.6	0.19	99.5
5	0.0	100.0	0.0	100.0	0.00	100.0	0.01	100.0	0.01	100.0	0.01	100.0	0.01	100.0	0.00	100.0	0.01	100.0	0.02	100.0

C – PCA components; EV – Eigenvalue; CV – cumulative variance

https://doi.org/10.17221/100/2019-CJGPB

and R19 × A212 (71.5%), indicated that the heterosis breeding could result in the improvement of this trait in the sunflower.

Considering the achene number, the first PC was mainly affected by both the MPH and BPH while the second PC was affected by achene number of the R-lines (Table 7). The achene number of the F_1 crosses was mainly affected by the R-lines and there was a close relationship between the achene number of the F_1 crosses and the BPH (Figure 1G), a demonstration of the heterosis existence as also reported by Jan et al. (2005). The first PC of the oil content was mainly affected by the F_1 crosses and both the MPH and BPH (Table 7), which indicated the heterosis existence for the oil content which has also been confirmed by Volotovich et al. (2008). The second PC was negatively affected by the CMS-lines while the third PC by the R-lines. There was a close relationship between the oil content of the F_1 crosses and the BPH (Figure 1H), which confirmed heterosis expression for this trait. The PC analysis revealed the higher similarity in the oil content in the parent

Table 7. The weight of the different parameters on the components of the principle component analysis for the agronomic traits of the sunflower

Parameter	PC1	PC2	PC3	Parameter	PC1	PC2	PC3
Days to flowering				**Achene weight**			
F_1	0.334	0.590		F_1	0.06	0.70	
CMS-line	–0.346	0.715		CMS-line	–0.45	0.51	
R-line	–0.347	–0.359		R-line	–0.33	–0.47	
MPH	0.577	–0.100		MPH	0.59	0.13	
BPH	0.560	–0.030		BPH	0.56	–0.08	
Days to maturity				**Achene number/head**			
F_1	0.48	0.38	0.55	F_1	0.39	0.25	
CMS-line	0.71	0.12	–0.06	CMS-line	–0.46	0.06	
R-line	0.21	–0.59	0.40	R-line	–0.03	0.93	
MPH	–0.41	0.57	0.44	MPH	0.56	–0.20	
BPH	–0.22	–0.38	0.57	BPH	0.55	0.14	
Plant height				**Oil content**			
F_1	0.323	–0.56		F_1	0.59	–0.20	0.08
CMS-line	–0.01	–0.78		CMS-line	0.23	–0.79	–0.19
R-line	–0.53	–0.09		R-line	0.05	–0.15	0.96
MPH	0.53	0.26		MPH	0.48	0.54	0.05
BPH	0.57	–0.02		BPH	0.59	0.09	–0.13
Head diameter				**Achene yield**			
F_1	0.22	–0.09	0.91	F_1	0.46	0.48	–0.26
CMS-line	0.53	–0.43	0.06	CMS-line	–0.16	0.13	–0.91
R-line	0.31	0.72	0.11	R-line	–0.21	0.78	0.25
MPH	–0.53	–0.38	0.20	MPH	0.61	–0.27	–0.08
BPH	–0.52	0.35	0.32	BPH	0.58	0.23	0.14
Stem diameter				**Oil yield**			
F_1	–0.01	0.65	–0.56	F_1	0.45	0.529	–0.24
CMS-line	–0.44	–0.12	–0.66	CMS-line	–0.18	0.19	–0.89
R-line	–0.39	0.57	0.38	R-line	–0.19	0.75	0.32
MPH	0.62	–0.09	–0.29	MPH	0.60	–0.27	–0.11
BPH	0.51	0.47	0.07	BPH	0.59	0.19	0.13

PC1, PC2 and PC3 – the first, second and third principle components; CMS-line – cytoplasmic mail sterile line; R-line – restorer line; MPH – mid-parent heterosis; BPH – best parent heterosis

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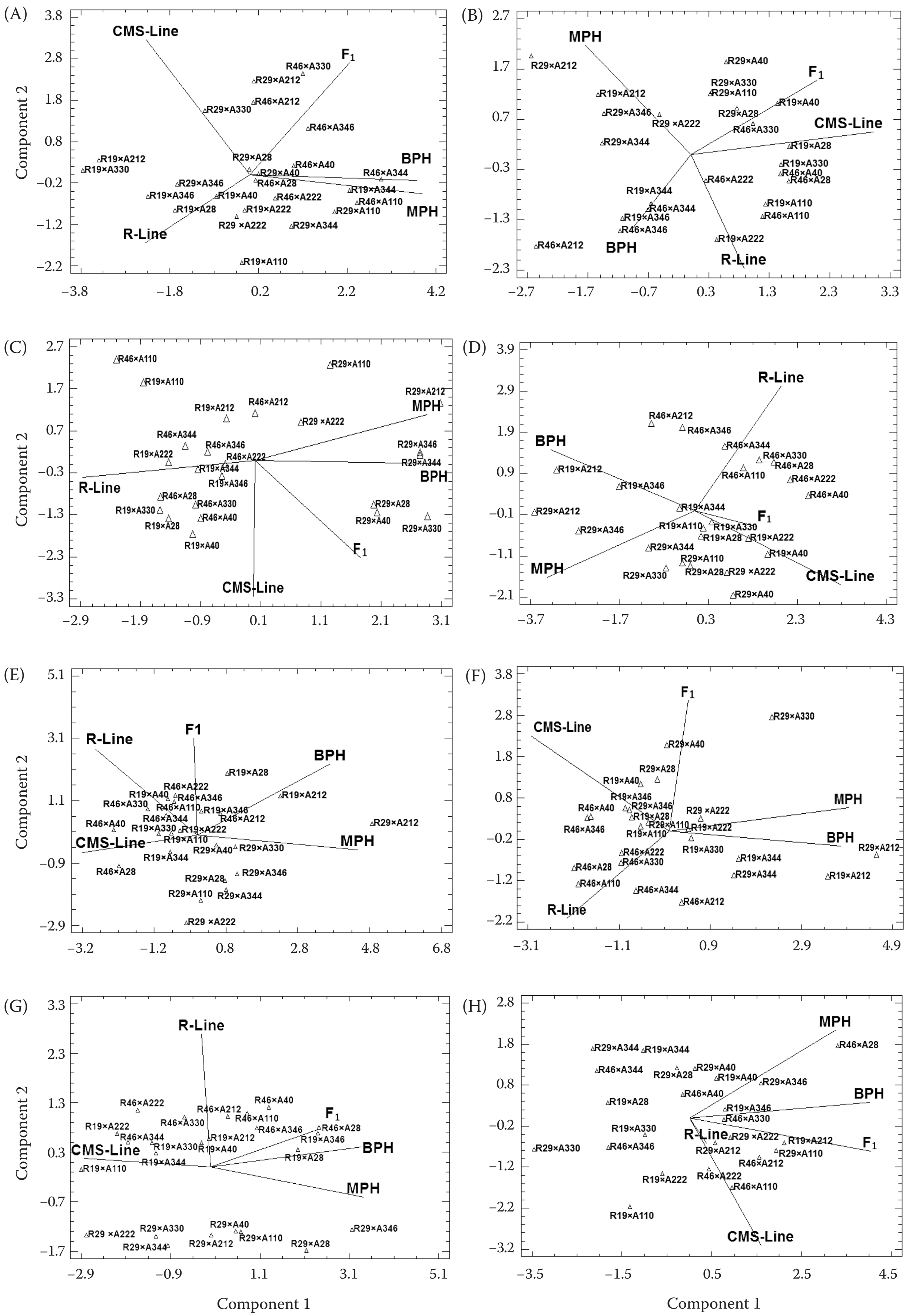

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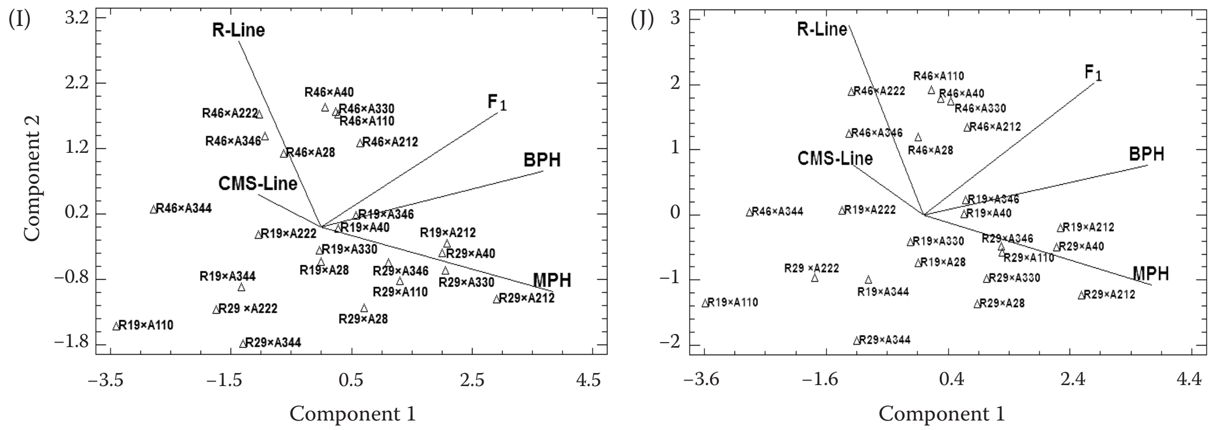

Figure 1. The bi-plot of the principle component analysis representing the relationships between the parent lines and the heterosis expression for: the days to flowering (A), the days to maturity (B), the plant height (C), the head diameter (D), the stem diameter (E), the achene weight (F), the achene No./head (G), the oil content (H), the achene yield (I), the oil yield (J) CMS – cytoplasmic male sterile; R – restorer; F_1 – single cross hybrid; BPH – best parent heterosis; MPH – mid-parent heterosis; the triangles represent the position of the hybrids on the bi-plot.

lines (Figure 1G). Both parent lines had a similar impact on the oil content of the F_1 crosses and the BPH which indicates the importance of both parents in the improvement of the oil content. Although the oil content is mainly under control of the additive gene action (Ashok et al. 2000) and its selection could enhance the oil content of the inbred lines, however, considering the overall negative heterosis in this study, it is concluded that these crosses failed to incorporate the desirable alleles for increasing the oil content in the related hybrids.

Three components efficiently accounted for 94.8% of the variability in the achene yield (Table 6). The MPH, R-lines and CMS-lines had more of an effect on these components, respectively (Table 7). The close angle between the achene yield of the F_1 crosses and the BPH (Figure 1I) confirmed the heterosis expression for this trait. The hybrids R29 × A212 and R19 × A212 (with an achene yield of 3 458 and 3 444 kg per ha, respectively) showed the highest amount of heterosis for this trait (182.3 and 181.2%, respectively) and most of the crosses expressed heterosis to some extent. This is in accordance with Encheva et al. (2015) who reported a significant heterosis for the achene yield of the sunflower. Due to the involvement of the overdominance in expressing the achene yield of the sunflower (Gangappa et al. 1997; Ashok et al. 2000), the heterosis breeding is a major approach for the improvement of the achene yield in the sunflower. The heterosis (BPH) expression in the hybrids with a higher achene yield; R29 × A346 (99%) and R19 × A346 (98%) was lower than the other ones, implying the fact that the heterosis expression is dependent on the potential of both parent lines and the F_1 hybrids, not solely on the F_1 hybrids. The R-lines had a higher impact on the achene yield of the F_1 crosses and the BPH. The crosses were related to the restorers R29 and the CMS-lines A212 and A330 leading up to the expression of the higher heterosis for the achene yield (Table 4 and 5).

In the case of the oil yield, the results were similar to that of the achene yield. The highest oil yield was observed in R29 × A346 and R19 × A346 (1 848 and 1 831 kg/ha, respectively), but the highest heterosis was recorded in R19 × A212 (193%) and R29 × A212 (182%) for the BPH (Table 5) suggesting that the difference between the parents and the related hybrids has crucial role in the amount of the heterosis expression. The crosses obtained from the restorer line; R29 was differentiated with a higher MPH. The MPH and R-lines had the highest positive effect on PC1 and PC2 while the CMS-lines had a negative effect on PC3 (Table 7). The proximity of the F_1 crosses and the BPH vectors (Figure 1J) confirmed the appearance of the heterosis for the oil yield. There was a close relationship between the oil yield of the R- and CMS-lines. The R-lines had more of an impact on the oil yield of the crosses and the expression of the BPH in comparison to the CMS-lines (Figure 1J), so it seems to be more important in the improvement of the oil yield in the heterosis breeding of the sunflower.

https://doi.org/10.17221/100/2019-CJGPB

The results of this study confirmed the heterosis expression for most of the agronomic features of the sunflower. The results showed that the relative impact of the R-lines was high in the heterosis expression for the days to maturity, stem diameter, achene number per head and achene and oil yield. The CMS-lines had more of an impact on the heterosis expression for the plant height. The relative impact of the R-lines and CMS-lines were almost similar on the heterosis expression for the days to flowering, head diameter, achene weight and oil content. Due to the higher relative impact of the paternal lines on the heterosis expression for half of the studied characteristics in this study, choosing the suitable parental lines will play a crucial role in the sunflower breeding for a desired trait.

Acknowledgements. The authors would like to thank the Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran and the Seed and Plant Improvement Institute, Karaj, Iran for supporting this study.

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Received: October 20, 2019
Accepted: February 2, 2020
Published online: April 1, 2020