



GENETIC ANALYSIS OF EARLINESS, YIELD, OIL QUALITY-RELATED TRAITS, AND DNA-BASED HYBRID AUTHENTICATION IN SUNFLOWER

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SUMMARY

The study, conducted at the research area of Raja Wala farm, University of Agriculture, Faisalabad, Pakistan, assessed the sunflowers' (*Helianthus annuus L.*) early maturity and yield improvement. Experimental material came from the United States Department of Agriculture and the National Agricultural Research Centre. Cytoplasmic male sterile lines and restorers, grown in the field, had their data gathered regarding early maturity. Then, the crossing of selected lines employed the line × tester design. The following season, the resulting crosses and their parents' evaluation proceeded in a randomized complete block design (RCBD) using three replications. The crosses declared as best hybrids in terms of early maturity and yield were 7-A × 86-R, 11-A × 83-R, 23-A × 81-R, 25-A × 80-R, 25-A × 94-R, and 27-A × 80-R. These best hybrids further underwent oil content and quality analysis. The crosses 23-A × 81-R and 25-A × 80-R revealed good performance for oil contents (palmitic, stearic, linoleic, and oleic acids) and quality traits like early maturing with better yield. Using RAPD markers, the authenticity assessment of the best hybrids through the presence and absence of bands compared with parents ensued. These hybrids will be helpful in future breeding programs for the development of early maturing varieties with improved achene yield and quality, which is rare in Pakistan. This material will also help develop the required hybrids.

Keywords: sunflower, male sterility, line × tester, oil quality parameters, primers

Key findings: Genotypes 80-R, 81-R, 83-R, 86-R, 94-R, and 96-R proved early maturing. The hybrids 23-A × 81-R and 25-A × 80-R emerged as the best crosses for early maturity, yield, and oil quality-related traits.

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INTRODUCTION

Sunflower is a non-traditional oil seed crop. It comprises a high amount of decent quality edible oil, and it is the best fit in local cropping patterns of Pakistan. Sunflower has a huge potential to enhance local oil production to

meet the rising domestic demand for edible oil (Dudhe *et al.*, 2017; Muddassir and Al-Zahrani, 2022). With competition among other major crops, the growing area for sunflowers remains limited in Pakistan. The unavailability of locally developed, well-adopted varieties/hybrids is the main barrier to achieving an optimal yield

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of sunflower oil (Vikas *et al.*, 2015; Bashir *et al.*, 2022). The imported source of private seed companies fulfilled nearly all the seed requirements. Additionally, the threats of new insect pests and fungal diseases are always high from the imported seed (Geetha *et al.*, 2012; Safdar *et al.*, 2022).

Hence, its quality and yield in Pakistan are very low compared with other sunflower-growing countries. Bearing in mind the previous points provides an urgent need to develop the local hybrids, which have the potential to give high yield and quality edible oil. These will become available to the farmers at low cost according to their need to get the standard crop stand and decrease their production price. Furthermore, it will also lessen the load on foreign funds by reducing importing cost of edible oil (Mohan and Seetharam, 2005; Tyagi and Dhillon, 2016; Khan *et al.*, 2022).

In a systematic breeding program, it is crucial to have excellent inbred parents to increase the genetic diversity of sunflower genotypes. The development of heterotic hybrids is urgently needed to advance sunflower breeding by utilizing the excellent combining ability of inbred parents and the heterotic vigor present in genetically distant parental lines and testers (Zafar *et al.*, 2022). For good sunflower hybrid development, it is necessary to estimate the combining ability of new inbred for productivity attributes like early maturity, more seed production, and higher oil content (Yali, 2022).

Hybrids are most stable, resistant to diseases, tolerant to environmental conditions, high yielding, and more uniform in maturity (Andarkhor *et al.*, 2013). The discovery of cytoplasmic male sterility (CMS) and male fertility restoration system in 1970 was due to which hybrid sunflower became a reality (Memon *et al.*, 2015). The constricted genetic base of germplasm is imperative to attain a high-yielding hybrid. Heterosis application has permitted sunflowers to develop their status as one of the most significant oilseeds in the country (Zafar *et al.*, 2020; Demir, 2021). The imported hybrid seed of sunflower is unacclimatized with the environmental conditions of Pakistan. The development of hybrids that are adaptable to climatic conditions could increase productivity and oil quality. In addition, Pakistan needs early maturing hybrids that best fit the local cropping pattern (Zafar *et al.*, 2021a; Chaudhry *et al.*, 2022).

Sunflower is a highly open-pollinated crop, causing high chances of contamination.

Authentication serves as proof for a specific hybrid of two particular parents. Molecular markers are the way to prove the authentication (Raza *et al.*, 2018). The PCR-based DNA marker techniques often provide the means for generating needed information for genetic relatedness. Technical simplicity and speed are the reason for using RAPD methodology in several crops (Ibrar *et al.*, 2022). The use of this technique ensures that the ideal crosses made are free from contamination. The obtained information will help plant breeders develop better achene and oil-yielding local hybrids containing better oil quality (Faraghati *et al.*, 2022). It is also a hope that the project will produce effective breeding material and potential sunflower hybrids. The presented research aimed to develop short-duration, high-yielding, and quality sunflower hybrids and obtain the best-inbred lines under the existing environmental conditions.

MATERIALS AND METHODS

The research plan proceeded at Raja Wala farm of the University of Agriculture, Faisalabad, Pakistan, from spring 2019 to autumn 2020, with the collected material coming from USDA (USA) and NARC, Pakistan. The material of 40 lines included 20 CMS lines and 20 testers. Screening for early maturity continued in the spring of 2019.

Screening for early maturity

Sowing of the 40 lines, including 20 CMS lines, their maintainers, and 20 testers, occurred in the experimental field in RCBD with three replications. Data measurement for days to germination, days to flower initiation, days to flower completion, and days to maturity ensued. The following season (autumn 2019), the crossing of selected six female inbred lines (CMS), i.e., 7-A, 11-A, 19-A, 25-A, and 27-A, and six male lines (restorers), which were 80-R, 81-R, 83-R, 86-R, 94-R, and 96-R employed a line \times tester mode, followed by separate harvesting of the seed.

Sowing the seeds of 36 hybrids with their parental material was in the spring of 2020. The experiment had three replications in RCBD manners. The row-to-row and plant-to-plant distances were 75 cm and 25 cm, respectively. One line was approximately 54 cm long. The pathway between lines was at 10 cm. All suggested plant defense and agronomic practices for standard growth of crops were

consistent for all parental genotypes during the growth period. Approximately irrigating seven to eight times throughout the season included urea application three times, from the time of sowing till maturity, as required by the crop. A repeat of the same experiment materialized in the spring.

Data recording for yield and yield-related traits

Random tagging of plants from inbred lines and their F_1 progeny was simultaneous with data gathering. Recorded data were for the succeeding pre- and post-harvest parameters. Randomly choosing three plants from every entry in each replication occurred as data gathering continued. The collected data consisted of days to germination, days to flower initiation, days to flower completion, plant height (cm), number of leaves per plant, leaf area (cm²), internodal distance (cm), number of whorls of achene per head, head diameter (cm), days to maturity, 100 achene weight (g), and achene weight per head (g).

Statistical and biometrical analysis

Evaluation of all characters under consideration for early maturity underwent analysis of variance (Steel *et al.*, 1997), with their means calculated. Assessing all characters of hybrids and parents under consideration for general and specific combining abilities, heterosis, and heterobeltiosis followed the method of Kempthorne (1957).

Oil quality evaluation

After the analysis, the best-performing hybrids proceeded for quality character evaluation, with the quality traits taken by an MPA spectrophotometer. The data underwent ANOVA (Steel *et al.*, 1997). Then, checking the overall results, noted the best hybrids concerning early maturity, yield, and quality. Further, validating the best crosses for DNA hybrid authentication followed.

DNA Hybrid authentication

Two hybrids emerged best after all the analyses. Then, these hybrids and their parents attained checking for their authenticity. DNA extraction of these hybrids and parents used the CTAB method by Doyle

and Doyle (1987). Using RAPD markers indicated the authenticity of the sunflower hybrids, with a total of 10 primers. Although, only one primer verifies the authentication. The authenticity confirmation was through the presence and absence of bands and comparison with the parents.

RESULTS AND DISCUSSION

The presented research, conducted at Raja Wala farm, University of Agriculture, Faisalabad, assessed the early maturing lines and the genetic base of 36 hybrids developed by crossing the CMS lines known as A-lines and restorers, commonly known as testers. Various estimates showed valuable results, which are as follows:

Early maturity of CMS lines

The cultivation of promising hybrids can significantly boost sunflower productivity. The advantageous crosses could result from crossing effective female lines with male testers. The presence of genetic variation is a prerequisite for developing high-yielding and better-adapted hybrids, synthetics, and composite sunflower cultivars (Zafar *et al.*, 2021b). In the presented study, lines were significantly different for all the studied traits related to early maturity (Table 1). The range for days to germination is from five to 14 days in the research material. Genotype 27-A had less number of days to germination. Genotype 7-A germinated in eight to nine days, 11-A in eight days, 19-A in seven days, and 23-A in six days. Similarly, more days to germination showed in line 31-A. Different studies reported five to 11 days for germination (Hernández and Larsen 2013; Qamar *et al.* 2015). Study findings revealed that genotypes showing fewer days to germination were early maturing. More days to germination are acceptable in case early maturity is not required. The range in this study for days to flower initiation is 54 to 64 days. In recorded literature, it was 55 to 65 (Hassan *et al.*, 2012; Hernández and Larsen, 2013). The reason for variation in days for flowering initiation in studied genetic material may refer to photoperiod because different genotypes respond differently to a specific photoperiod. The days-to-flower completion range is 67 to 76 days.

Table 1. Mean square values from analysis of variance for early maturity-related traits of lines and restorers.

SOV	D.F	L	R	L	R	L	R	L	R
		DG	DG	DFI	DFI	DFC	DFC	DM	DM
Replication	2	0.81	0.51	2.01	18.31	7.55	3.71	0.39	1.51
Lines	19	8.48**	9.48	102.66**	324.29	165.66**	274.79	71.17**	242.34
Error	38	0.699	0.72	1.98	6.24	2.33	3.71	1.01	2.65
Total	59								

DG = Days to Germination, DFI = Days to Flower Initiation, DFC = Days to Flower Completion and DM = Days to Maturity.

Different studies reported 69–78 and 85–95 days for flower completion and maturity (Qamar *et al.*, 2015; Rameeh and Andarkhor, 2017). Given the results of selecting six CMS lines, namely, 7-A, 11-A, 19-A, 23-A, 25-A, and 27-A, for further research as these lines were early maturing.

Early maturity of restorer lines

Analysis of variance revealed that various restorers differed significantly for all studied traits (Table 1). Faridi *et al.* (2015) also reported highly significant variations among sunflower genotypes for these traits. The days to germination range are five to 11 days in this research. Genotype 96-R had a few numbers days to germination and days-to-flower initiation. Similarly, more days-to-flower initiation and completion resulted for restorers 73-R. The range of days-to-flower initiation recorded in literature was 55 to 65 by Hassan *et al.* (2012) and Hernández and Larsen (2013). The range of days-to-flower completion recorded in literature was 68–78 (Qamar *et al.*, 2015; Rameeh and Andarkhor, 2017). Days to maturity ranged from 85 to 95 days in the research material. Genotype 96-R had less number of days to maturity compared with line 73-R, with more days. Highly significant differences in lines would help select those with the desirable trait. Considering the results, the chosen six restorers, namely, 80-R, 81-R, 83-R, 86-R, 94-R, and 96-R, need further research, as these lines were early maturing.

Evaluation of variability

The analysis of variance exhibited significant differences ($P \leq 0.05-0.01$) among all genotypes for studied traits. For all the characteristics, hybrids depicted relevant differences ($P \leq 0.05-0.01$), excluding days to maturity, achene weight per plant, and the number of leaves per plant. The line \times tester

interaction was significant ($P \leq 0.05-0.01$) for days to flower initiation, days to flower completion, number of leaves per plant, plant height, internodal distance, area of the leaf, and 100 achene weight, while the interaction was non-significant for head diameter, number of whorls, days to maturity, and achene weight per plant. Crosses vs. parents ($P \leq 0.05-0.01$) also showed highly significant results for all the traits except head diameter (Table 2). Substantial variation among genotypes for yield-related traits also resulted from studies by Sarwar *et al.* (2013) and Tyagi *et al.* (2020).

General combining ability effects

The parents 7-A, followed by 23-A and 27-A, had negative significant GCA estimates for days to germination, days to flower initiation and completion, days to maturity, plant height, and internodal distance (Table 3a). The 7-A gave the highest significantly positive GCA effects for achene weight per plant and leaf area. The 23-A had a significantly positive GCA effect for leaf area, head diameter, 100 seed weight, and number of whorls per head. Further, 27-A showed substantial positive effects for the number of leaves per plant and the number of whorls per head. Among testers, 80-R showed negatively significant results for days to germination, days to flower initiation, plant height, and internodal distance, whereas it exhibited positively significant results for all other traits (Table 3b). Genotype 83-R had significantly positive GCA effects for 100 achene weight leaf area, number of leaves per plant, and number of whorls per head. The 96-R showed highly significant positive results for the number of leaves per plant, leaf area, number of whorls per head, and head diameter.

Choosing appropriate parental lines is a key factor in any breeding program's success. Zafar *et al.* (2022) stated that the parents having better GCA effects for agronomic and

Table 2. F-values from analysis of variance for early maturity and yield-related traits.

SOV	DF	DG	DFI	DFC	PH	LP	LA	ID	NWAH	HD	D.M	100-SW	AWP
Replications	2	2.84	15.69**	18.63**	3.78	3.41	0.95	0.05	2.83	0.96	1.22	0.94	0.16
Genotypes	47	15.18**	16.40**	15.04**	35.45**	23.73**	24.51**	15.88**	32.06**	24.48**	14.00**	15.43**	25.86**
Crosses	35	15.45**	18.13**	17.91**	16.43**	7.60	17.73**	24.77**	14.10**	17.74**	4.46	14.51**	7.57
Lines (C)	5	23.61**	26.78**	10.15**	16.89**	27.12**	28.13**	23.08**	16.80**	47.45**	18.85**	1.75	33.08**
Testers (C)	5	12.49**	11.53**	11.13**	18.27**	11.65**	20.30**	12.85**	16.19**	12.90**	11.56**	12.72**	11.26**
Lines × Testers (C)	25	3.61	14.92*	19.01**	35.97**	18.89**	15.14*	23.48**	3.14	2.77	4.16	15.42**	5.73
Parent	11	4.71	14.49**	5.71	51.13	22.22**	25.92**	17.87**	14.53	17.44**	2.89	2.40	10.30**
Lines (P)	5	11.21**	1.00	0.80	1.96	15.20**	13.45**	16.53**	18.80**	2.31	2.37	2.20	10.20**
Testers (P)	5	8.83	8.50	21.10**	92.63**	18.74**	25.12**	16.67**	14.09	14.50**	3.97	2.35	10.16**
Lines (P) Vs Testers (P)	1	11.68**	21.93**	23.24**	339.46**	64.70	82.31**	20.50**	45.40	47.72	10.07**	3.61	11.48**
Crosses Vs Parents	1	30.87**	57.05**	22.59**	408.96**	344.94**	48.19**	22.88**	733.26**	327.71	0.17	70.88**	477.03**

* = Significant at 0.05 probability level; ** = Significant at 0.01 probability level; SOV = Sources of variation, DF = Degree of freedom, DG= Days to germination, DFI= Days to flower initiation, DFC= Days to flower completion, PH = Plant height, LP = Number of leaves/plants, LA= Leaf area, ID= Internodal distance, NWAH = Number of whorls of achene/head, HD = Head diameter, SW = 100 Seed weight, AWP = Achene weight/head, DM= Days to maturity.

Table 3a. General combining ability (GCA) effects of lines for early maturity and various yield-related traits.

	D.G	DFI	DFC	PH	LP	LA	ID	NWAH	HD	D.M	100-SW	AWP
7-A	-0.64 **	-2.36 **	-0.48	8.75 **	-1.22*	18.35	-1.46 **	0.80 *	-1.55 **	-1.49 **	-0.03	-18.01 **
11-A	0.36	1.42 **	1.80 **	5.77 *	1.28 **	-84.68 **	1.26 **	1.19 **	-2.60 **	-0.94 *	-0.28	-2.33
19-A	-0.97 **	-1.81 **	-0.81	-10.94 **	-1.17 **	-110.58 **	-0.92 **	-0.48	-2.68 **	-0.99 *	-0.06	1.07
23-A	-1.19 **	-3.19 **	-1.30 **	-5.10 *	1.17 **	95.48 **	-0.80 **	0.24	3.04 **	1.56 **	0.39*	5.60 *
25-A	0.03	-0.75	-1.31 **	4.00	-0.33	83.26 **	-0.51	-0.26	2.36 **	0.95 *	0.19	6.07 **
27-A	-0.03	-1.31*	-1.48**	-2.48	1.72**	64.87**	-0.49	1.48 **	1.42 **	-0.90 *	0.37 *	7.61 **
Standard Error	0.19	0.49	0.43	2.30	0.38	19.59	0.29	0.36	0.39	0.43	0.18	2.27

Table 3b. General combining ability (GCA) effects of testers for early maturity and various yield-related traits.

	D.G	DFI	DFC	PH	LP	LA	ID	NWAH	HD	D.M	100-SW	AWP
80-R	-0.59**	-1.77**	-0.80	-8.57 **	0.33	131.68 **	-0.75 *	1.63 **	0.97 *	0.84*	0.56 **	7.36**
81-R	-0.19	0.47	0.24	-14.80 **	0.50	-20.31	-0.30	-1.04 **	0.07	0.18	-0.17	0.24
83-R	-0.64**	-1.64**	-1.43**	-0.04	1.44**	67.80**	-0.66*	1.35*	0.68	-0.97*	0.45*	6.06*
86-R	-0.14	-0.42	0.02	-0.94	-0.11	-34.14	0.74 *	-0.43	-0.66	-0.27	-0.00	-2.53
94-R	0.53 **	0.08	-0.37	0.97	0.39	-33.37	0.05	-0.20	-0.82 *	0.18	-0.22	-1.36
96-R	-0.44**	-1.47**	-0.26	6.24 **	1.67**	76.06**	-0.99	1.31*	1.24*	0.86*	0.08	-0.04
Standard Error	0.19	0.49	0.43	2.30	0.38	19.59	0.29	0.36	0.39	0.43	0.18	2.27

* = Significant at 0.05 probability level; ** = Significant at 0.01 probability level; SOV = Sources of variation, DF = Degree of freedom, DG= Days to germination, DFI= Days to flower initiation, DFC= Days to flower completion, PH = Plant height, LP = Number of leaves/plants, LA= Leaf area, ID= Internodal distance, NWAH = Number of whorls of achene/head, HD = Head diameter, DM= Days to Maturity, SW = 100 Seed weight, AWP = Achene weight/head, DM= Days to maturity.

yield-related parameters could benefit breeding programs to develop high-yielding hybrids. The shorter crop growth cycle and the minimum number of days to maturity proved preferable in sunflower breeding programs (Memon *et al.*, 2015). Early maturing hybrids reduced the attack of insect pests and increased the profit of sunflower farmers. In agreement with the study findings, Archana *et al.* (2018) reported significantly negative GCA effects for days to maturity, while Ahmad *et al.* (2012) indicated significantly negative GCA for plant height. As a result, they regarded such parents as good general combiners for producing sunflower hybrids with reduced plant height.

Specific combining ability effects

Using SCA effects commonly, assessed the appropriateness of F₁ hybrids for desired economic characteristics (Zafar *et al.*, 2020). The results of SCA effects on early maturity and various yield-related traits appear in Table 4. The hybrids 7-A × 86-R, 11-A × 83-R, 23-A × 81-R, 25-A × 80-R, 25-A × 94-R, and 27-A × 80-R showed significant and negative SCA effects for days to germination, days to flower initiation and completion, days to maturity, and plant height. Hybrid 23-A × 81-R showed highly significant results for all the studied characters. The 25-A × 80-R exhibited significant results for 100 achene weight, number of leaves per plant, and leaf area. Moreover, 11-A × 83-R showed highly significant results for all the traits except head diameter. Likewise, 7-A × 86-R displayed highly significant results for all the traits except achene weight per plant. Additionally, 27-A × 80-R depicted significant positive results for the number of leaves per plant, number of whorls per plant, head diameter, achene weight per head, and 100 achene weight.

The genotypes exhibiting significant SCA effects have more leaves, increasing the photosynthetic efficiency. The increased photosynthetic rate positively impacts head diameter, number of seeds per achene, and yield (Zia-Ullah *et al.*, 2013). Ghaffari and Shariati (2018) found significant negative SCA effects for germination in sunflowers. Similar findings came from Khalid *et al.* (2017) and Ghaffari and Shariati (2018). The characters revealing positive SCA estimates suggested that these traits have dominant and over-dominant gene actions governing these (Zafar *et al.*, 2020). Andarkhor *et al.* (2013) reported higher positive SCA for 1,000-achene weight.

These results are consistent with the present findings.

Genetic variances

Days to flower completion, leaves per plant, and 100 seed weight, showed SCA/GCA variances and degree of dominance lesser than one, indicating the presence of additive gene action. All other traits showed SCA/GCA variances and degrees of dominance greater than one, signifying the presence of non-additive gene action (Table 5). These results were similar to Archana *et al.* (2018), who found that SCA was more than GCA for the yield-related components. Azad *et al.* (2016) found an over-dominant effect on plant height, leaf area, 100-seed weight, and head diameter. Faridi *et al.* (2015) reported that SCA was more important than GCA for seed yield and 100-achene weight. Meanwhile, Ghaffari and Shariati (2018) found significant additive genetic variance for head diameter and 100 seed weight. Mojghan *et al.* (2012) indicated a greater proportion of GCA variance for yield characters.

Heterosis manifestation

The results regarding the heterotic manifestation of 36 hybrids are in Table 6. The crosses 7-A × 80-R, 7-A × 81-R, 7-A × 86-R, 11-A × 81-R, 11-A × 83-R, 19-A × 80-R, 19-A × 81-R, 19-A × 83-R, 25-A × 80-R, 25-A × 94-R, and 27-A × 80-R showed negative significant heterotic values on mid- and better parents for days to germination, days to flower initiation, days to flower completion, plant height, internodal distance, and days to maturity. Hybrids 19-A × 86-R, 19-A × 94-R, and 19-A × 96-R showed negative values over mid-parents and better parents for days to flower initiation, days to flower completion, and days to maturity. The 23-A × 86-R, 23-A × 94-R, 25-A × 96-R, and 27-A × 94-R showed significant positive values over mid-parents and better parents for days to flower initiation, plant height, number of leaves per plant, number of whorls per head, head diameter, and achene weight per plant.

Hybrids 7-A × 80-R, 7-A × 81-R, 7-A × 83-R, 7-A × 94-R, 11-A × 81-R, 19-A × 81-R, 19-A × 83-R, 19-A × 86-R, 23-A × 83-R, 25-A × 80-R, 25-A × 83-R, 25-A × 94-R, and 27-A × 80-R displayed significant negative heterosis on mid-parent and better parent for days to flower germination, days to flower initiation, days to flower completion, plant height, and

Table 4. Specific combining ability (SCA) effects for early maturity and various yield related traits.

Hybrids	DG	DFI	DFC	PH	LP	LA	ID	NWAH	HD	D.M	100-SW	AWP
7-A × 80-R	0.42	-1.25	-0.80	-14.36 *	-1.72	-154.92 **	-1.12	-0.24	-1.96 *	3.71 **	0.32	2.96
7-A × 81-R	0.08	0.58	0.09	11.86 *	-1.89	79.52	0.97	-1.24	1.04	0.38	-1.09 *	-3.95
7-A × 83-R	1.03 *	1.69	2.09	14.05 *	1.39	-10.77	1.94 **	-0.63	0.11	0.66	-0.81	-4.97
7-A × 86-R	-1.54**	-4.86**	-3.02**	-11.42*	3.72**	159.93**	-1.42*	2.15**	2.55**	-3.51**	1.54 **	14.86*
7-A × 94-R	-0.31	-0.36	-1.30	0.03	0.56	32.49	-1.20	1.59	1.47	-1.95	0.00	4.76
7-A × 96-R	-0.58	1.19	0.93	-2.17	0.94	83.61	-0.61	-0.63	-1.21	-1.29	0.03	-3.66
11-A × 80-R	0.42	3.31 **	1.59	40.75 **	-2.56 **	64.41	2.89 **	1.70	-1.11	1.16	-1.07 *	-4.12
11-A × 81-R	-0.25	-1.86	-1.85	-14.34 *	-1.72	-88.55	-2.00 **	2.04 *	-1.39	-2.18 *	-0.14	-11.53 *
11-A × 83-R	-1.64**	-3.08**	-3.15**	-13.31*	4.89 **	127.55*	-1.64*	2.69**	2.81*	-2.56**	1.61**	20.15 **
11-A × 86-R	-0.64	-2.31	-1.96	-17.00 **	-1.78	-61.84	0.23	-2.57 **	-0.90	0.27	-0.31	-6.49
11-A × 94-R	0.36	1.19	0.76	-0.78	0.39	10.96	0.33	-0.80	1.55	0.49	0.88	0.08
11-A × 96-R	0.75	0.75	0.31	-4.31	0.78	47.48	-0.81	0.31	1.04	0.82	0.04	1.92
19-A × 80-R	0.08	-1.47	0.54	-41.00 **	-4.44 **	-147.73 **	-3.18 **	-2.30 *	-0.28	-1.12	0.28	-0.80
19-A × 81-R	0.08	-0.97	-0.57	-4.93	0.06	-144.82 **	-0.46	-0.30	1.02	-0.79	-0.66	-7.74
19-A × 83-R	0.03	1.47	0.43	0.29	-0.00	100.94 *	0.71	0.98	0.47	0.16	0.22	4.04
19-A × 86-R	0.03	0.58	-0.69	14.33 *	1.67	58.43	0.58	1.43	-0.16	-0.01	0.04	-1.96
19-A × 94-R	0.03	1.08	1.70	20.39 **	0.83	90.42	1.85 *	1.20	-0.87	0.88	-0.01	1.77
19-A × 96-R	-0.25	-0.69	-1.41	10.92	1.89	42.76	0.50	-1.02	-0.19	0.88	0.13	4.68
23-A × 80-R	0.92	6.19 **	4.43 **	7.90	2.56 **	255.04 **	1.77 *	0.31	0.58	1.66	-1.59 **	-18.09 **
23-A × 81-R	-1.42**	-3.03**	-3.65**	-14.83*	5.39 **	174.63**	-1.25**	2.65**	2.72*	-2.66*	1.74 **	34.31 **
23-A × 83-R	-1.47 **	-3.53 **	-4.35 **	4.77	-0.67	45.12	-1.28	1.59	0.19	-2.73 *	1.26 **	11.25*
23-A × 86-R	0.86	-0.75	0.54	16.43 **	-0.67	-97.27 *	-0.58	-0.30	-0.07	-0.90	-0.16	7.45
23-A × 94-R	0.86	1.42	1.59	-19.42 **	-4.17 **	-210.57 **	-0.15	-2.52 **	-2.55 **	3.32 **	-1.61 **	-17.52 **
23-A × 96-R	-0.75	-4.36 **	-2.85 **	1.15	-2.44 *	-66.94	0.18	0.26	0.13	-2.01	0.36	-4.91
25-A × 80-R	-1.92**	-4.19 **	-3.96 **	-14.79**	4.72 **	133.46**	-2.04**	1.81 *	2.17*	-3.73 **	1.82**	13.01 *
25-A × 81-R	0.42	1.31	0.93	5.46	-1.11	30.77	1.04	0.15	1.00	0.94	-0.05	-3.76
25-A × 83-R	0.36	-0.58	-1.07	-13.64 *	-4.17 **	-10.08	-0.02	-0.57	0.16	-0.79	-0.23	-8.72
25-A × 86-R	1.36 **	4.86 **	3.81 **	-13.44 *	-1.17	23.99	0.24	-1.80 *	-2.40 *	2.71 *	-1.38 **	-6.92
25-A × 94-R	-1.64 **	-3.97 **	-3.46 **	-11.48 *	2.33 *	191.06**	-2.94**	2.31*	2.69*	-2.40 *	1.74 **	11.18 *
25-A × 96-R	0.42	2.58 *	3.76 **	5.35	-0.61	-102.28 *	-0.29	-0.91	-1.62	3.27 **	-0.89	-4.78
27-A × 80-R	-1.90**	-2.58 *	-3.80**	-11.93*	2.44*	166.66**	-1.83*	2.30*	3.58*	-2.68*	1.24 **	17.04*
27-A × 81-R	0.08	-0.08	0.76	12.77 *	-0.72	48.46	0.39	-1.30	-3.39 **	0.99	0.21	-7.34
27-A × 83-R	0.69	2.03	1.76	-1.16	-1.44	-152.76 **	-0.71	-0.69	-1.73	3.27 **	-1.04 *	-9.26
27-A × 86-R	-0.97 *	-0.53	-0.69	9.11	1.22	106.62 *	-0.49	2.09 *	2.98 **	-0.56	0.27	3.07
27-A × 94-R	0.69	0.64	0.70	-11.71 *	0.06	-14.36	0.11	-0.80	-1.30	-0.34	-1.01 *	-0.26
27-A × 96-R	0.42	0.53	-0.74	-10.94	-0.56	-4.63	1.03	1.98 *	1.85	-1.68	0.33	6.75
S. E	0.46	1.20	1.07	5.65	0.95	47.99	0.73	0.88	0.96	1.06	0.46	5.56

* = Significant at 0.05 probability level; ** = Significant at 0.01 probability level; SOV = Sources of variation, DF = Degree of freedom, DG= Days to germination, DFI= Days to flower initiation, DFC= Days to flower completion, PH = Plant height, LP = Number of leaves/plants, LA= Leaf area, ID= Internodal distance, NWAH = Number of whorls of achene/head, HD = Head diameter, DM= Days to maturity, SW = 100 Seed weight, AWP = Achene weight/head.

Table 5. Estimates of variance due to GCA(σ^2_{GCA}), SCA (σ^2_{SCA}), additive (σ^2_A), dominance (σ^2_D), ratio of SCA to GCA ($\sigma^2_{SCA}/\sigma^2_{GCA}$), and degree of dominance ($(\sigma^2_D/\sigma^2_A)^{1/2}$)

Traits	Genetic Components							
	$\sigma^2_{GCA} = \frac{\{(1+F)\}}{4} \sigma^2_A$	(a) with F=0, σ^2_A	(b) with F=1, σ^2_A	$\sigma^2_{SCA} = \frac{\{(1+F)\}}{2} \sigma^2_D$	(a)with F=0, σ^2_D	(b)with F=1, σ^2_D	$\sigma^2_{SCA}/\sigma^2_{GCA}$	$(\sigma^2_D/\sigma^2_A)^{1/2}$
D.G	0.02	0.08	0.04	0.49	1.98	0.49	24.5	4.53
DFI	0.10	0.43	0.21	6.18	24.74	6.18	61.8	6.95
DFC	-0.007	-0.02	-0.01	4.57	18.30	4.57	-652.85	-762.33
PH	1.29	5.19	5.19	256.66	1026.64	256.66	198.96	123.63
LP	-0.06	-0.25	-0.12	6.63	26.54	6.63	-110.5	-89.64
LA	370.45	1481.83	740.91	10285.08	41140.34	10285.08	27.76	23.13
ID	0.03	0.15	0.07	1.28	5.14	1.28	42.66	29.18
NWAH	0.04	0.18	0.09	1.76	7.07	1.76	44	32.70
HD	0.30	1.23	0.61	2.00	8.03	2.00	6.66	5.45
D.M	0.01	0.07	0.03	3.51	14.05	3.51	351	175.6
100-SW	-0.01	-0.04	-0.02	0.88	3.55	0.88	-88	-73.88
AWP	2.54	10.19	5.09	105.36	421.44	105.36	41.48	34.47

DFI= Days to flower initiation, DFC= Days to flower completion, PH = Plant height, LP = Number of leaves/plants, LA= Leaf area, ID= Internodal distance, NWAH = Number of whorls of achene/head, HD = Head diameter, 100-SW = 100 Seed weight, AWP = Achene weight/head.

Table 6. Heterotic manifestation in hybrids for early maturity and yield-related traits.

Hybrids	D.G		DFI		DFC		PH		LP		LA		ID		NWAH		HD		DM		100-SW		AWP	
	Het.	Bet. P. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.
7-A x 80-R	-15.56	-24.00	-10.93	-13.30	-4.19	-7.62	-2.61	-28.48	4.41	0.00	-16.10	-40.86	-9.30	-22.78	25.76	18.57	3.25	-10.05	2.43	-0.00	37.86	22.93	343.9	246.7
7-A x 81-R	-20.00	-28.00	-7.22	-8.24	-2.36	-4.61	34.92	19.52	12.70	0.00	57.36	48.34	24.79	13.09	11.63	7.46	25.10	17.29	-1.32	-2.96	-1.15	-17.83	239.1	162.1
7-A x 83-R	-4.55	-12.50	-10.70	-14.80	-1.17	-4.09	75.46	68.45	15.56	9.86	-8.55	-32.74	70.89	54.46	50.00	25.81	25.65	19.83	-1.14	-1.89	-12.62	-14.01	176.3	85.56
7-A x 86-R	-17.95	-20.00	-11.05	-11.80	-4.46*	-1.93	-14.36	-7.19	24.19	8.45	51.65	-3.71	-22.94	-40.19	36.13	30.65	22.77	19.23	-2.49	-2.67	44.41	35.67	316.9	216.4
7-A x 94-R	-0.00	-5.00	-7.12	-8.43	-2.19	-2.90	39.35	24.61	31.09	9.86	32.70	18.58	11.64	5.86	44.35	33.87	35.37	31.22	-2.67	-3.04	0.63	-0.62	278.5	169.8
7-A x 96-R	-5.56	-15.00	-3.49	-6.74	3.23	0.48	58.03	55.64	31.03	7.04	46.98	31.20	15.44	6.13	29.91	22.58	13.73	9.43	-0.97	-1.92	12.58	8.28	189.7	107.5
11-A x 80-R	-8.33	-12.00	1.62	-0.00	1.85	-1.35	29.66	-8.91	10.61	8.96	1.61	-23.22	23.08	8.11	63.64	28.57	16.40	-10.94	-1.83	-2.19	13.83	10.77	375.6	351.6
11-A x 81-R	-16.67	-20.00	-6.04	-6.04	-2.35	-4.15	21.76	1.48	24.59	13.43	-14.54	-18.58	-11.10	-16.66	55.14	23.88	21.38	-1.37	-5.54	-5.88	28.21	15.38	282.3	258.0
11-A x 83-R	-19.15	-20.83	-10.05	-13.27	0.23	-2.27	69.65	64.48	41.98	38.81	39.94	-36.45	-29.88	-13.64	92.68	88.10	43.10	17.93	-3.91	-5.15	20.57	11.84	531.7	389.2
11-A x 86-R	-9.52	-17.39	-6.44	-8.24	0.00	-0.96	13.79	-12.46	23.33	10.45	-19.92	-23.75	37.74	30.36	46.39	24.56	25.29	4.81	-1.87	-3.68	11.94	8.70	299.4	265.1
11-A x 94-R	17.07	4.35	0.85	-1.65	3.63	2.39	45.37	22.21	42.61	22.39	0.01	-0.89	21.75	19.66	65.59	45.28	51.60	33.48	-1.31	-2.94	23.02	11.18	342.0	271.3
11-A x 96-R	23.08	4.35	1.15	-3.30	5.19	1.91	65.02	51.66	42.86	19.40	9.67	8.56	7.46	2.26	68.42	45.45	40.00	16.23	0.00	-2.94	18.55	12.41	381.1	306.8

Table 6 (cont'd).

Hybrids	D.G		DFI		DFC		PH		LP		LA		ID		NWAH		HD		DM		100-SW		AWP	
	Het.	Bet. P. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het	Bet. Het.	Het.	Bet. Het.	Het	Bet. Het.	Het	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.	Het.	Bet. Het.
19-A x 80-R	-24.44	-32.00	-10.14	-12.77	-3.24	-6.28	-23.01	-45.87	-13.67	-18.92	-19.37	-50.67	-48.34	-58.81	22.69	4.29	14.09	-7.52	-3.51	-4.74	69.03	55.28	363.8	310.1
19-A x 81-R	-24.44	-32.00	-8.64	-9.89	-4.23	-5.99	16.23	-3.03	14.73	0.00	-6.35	-30.54	-8.30	-22.63	22.41	5.97	28.33	11.14	-3.17	-3.70	36.23	35.58	291.7	250.8
19-A x 83-R	-22.73	-29.17	-9.92	-14.29	-4.43	-6.82	58.59	53.95	2.90	-4.05	11.11	-30.16	36.37	33.27	73.63	61.22	31.39	15.57	-2.26	-2.62	29.41	8.55	339.9	294.6
19-A x 86-R	-12.82	-15.00	-5.68	-6.21	-1.93	-2.87	23.18	-5.16	21.26	4.05	36.85	-4.50	31.83	23.92	47.17	36.84	21.51	8.65	-1.32	-2.25	38.59	21.01	313.2	278.9
19-A x 94-R	-0.00	-5.00	-3.43	-4.52	1.21	0.00	48.57	25.03	24.59	2.70	55.24	10.98	28.24	12.76	52.94	47.17	23.91	17.17	-0.00	-0.75	20.45	-1.24	321.6	318.4
19-A x 96-R	-5.56	-15.00	-5.54	-8.47	-1.23	-4.31	63.59	50.52	27.73	2.70	42.49	1.79	9.81	-6.13	36.54	29.09	22.77	8.87	0.96	-1.12	38.71	18.62	364.6	364.5
23-A x 80-R	18.18	4.00	11.29	7.45	4.85	1.79	8.03	-25.33	45.45	35.38	91.04	19.53	21.92	-17.62	45.61	18.57	52.40	22.38	2.78	1.09	10.55	6.50	358.1	278.0
23-A x 81-R	-	-	-5.64*	-11.65	-3.23*	-4.38	-18.95	-	74.77	73.21	127.4	75.49	-	-	36.94	13.43	69.75	45.50	-1.68	-1.74	91.74	83.33	1119.	894.8
23-A x 83-R	-11.63	-20.83	-9.43	-14.29	-8.37	-10.45	72.70	62.93	26.67	18.75	39.26	-10.25	39.65	13.05	93.02	88.64	67.53	45.81	-2.26	-2.26	44.36	26.32	456.9	288.9
23-A x 86-R	36.84	36.84	1.14	1.14	2.65	1.43	30.99	-1.20	41.28	37.50	46.43	5.77	46.84	11.60	48.51	31.58	60.65	42.12	0.95	0.38	24.60	13.77	663.9	510.8
23-A x 94-R	51.35	47.37	6.32 *	5.71	3.86 *	2.38	26.05	3.58	30.77	21.43	21.31	-10.05	29.05	-6.55	42.27	30.19	53.49	43.48	6.06	5.66	-22.18	-33.54	278.1	182.1
23-A x 96-R	25.71	15.79	-2.64	-5.14	-0.49	-3.81	64.63	47.46	38.61	25.00	66.13	23.09	29.55	-8.06	55.56	40.00	63.01	43.02	0.96	-0.75	35.14	20.69	456.2	317.1
25-A x 80-R	-22.73	-32.00	-11.42	-15.43	-8.71	-13.00	6.99	-23.08	32.35	26.76	23.45	-15.15	-19.06	-30.00	50.88	22.86	46.36	21.97	-	-4.81*	88.60	74.80	838.5	663.0
25-A x 81-R	-4.55	-16.00	-1.42	-4.40	-1.19	-4.61	30.87	12.63	15.87	2.82	78.49	61.51	3.39	-4.69	31.53	8.96	54.60	38.04	2.46	0.37	59.81	59.05	583.5	449.5
25-A x 83-R	-2.33	-12.50	-10.08	-15.82	-5.69	-9.55	53.63	52.46	-9.63	-14.08	12.97	-19.19	18.33	5.19	74.42	70.45	56.33	41.85	0.38	-0.75	23.74	4.61	387.5	236.8
25-A x 86-R	26.32	26.32	5.20	4.00	5.65	4.88 *	11.78	-11.56	14.52	0.00	53.96	28.01	20.00	15.56	36.63	21.05	35.21	24.81	5.57	4.96	8.64	-4.35	476.7	354.6
25-A x 94-R	-8.11	-10.53	-8.72	-9.25	-5.42	-5.88	48.36	28.84	39.50	16.90	79.14	54.06	-8.57	-11.72	62.89	49.06	70.44	66.74	-	-4.76	64.66	36.02	648.2	451.3
25-A x 96-R	25.71	15.79	3.86	2.34	7.54	5.94	65.55	57.79	22.41	0.00	31.04	12.57	-3.55	-9.77	45.45	30.91	41.24	29.25	7.57	6.95	19.20	2.76	470.8	322.8
27-A x 80-R	-24.44	-32.00	-8.99	-11.17	-5.80	-8.97	7.25	-26.58	18.80	16.18	23.57	-14.95	-19.03	-32.16	30.36	4.29	43.45	19.55	-3.39*	-3.28	87.90	86.40	675.1	608.9
27-A x 81-R	-11.11	-20.00	-4.16	-4.95	-1.65	-3.69	40.74	13.14	18.70	7.35	69.64	53.86	0.35	-10.63	19.27	-2.99	22.60	9.46	0.56	0.37	57.21	44.00	482.1	425.4
27-A x 83-R	-0.00	-8.33	-6.13	-10.20	-1.87	-4.55	72.47	60.05	3.03	0.00	-22.54	-44.51	14.78	5.60	69.05	69.05	38.98	26.11	3.00 *	2.23	1.08	-7.89	357.2	244.3
27-A x 86-R	-12.82	-15.00	-4.52	-5.59	-1.21	-1.92	29.40	-3.54	27.27	13.24	62.03	34.99	16.40	16.40	55.56	35.09	62.92	50.38	-0.19	-1.49	42.21	35.51	562.4	484.2
27-A x 94-R	26.32	20.00	-1.14	-2.79	0.49	-0.48	35.84	10.11	29.31	10.29	39.23	19.99	6.67	-0.69	45.26	30.19	62.92	41.09	0.38	-0.74	-0.70	-11.80	465.2	360.0
27-A x 96-R	22.22	10.00	-0.29	-3.91	0.50	-2.40	58.87	40.11	23.89	2.94	43.40	23.46	14.29	3.23	58.76	40.00	56.91	43.58	-0.19	-2.60	41.48	31.72	566.0	445.3

DFI= Days to flower initiation, DFC= Days to flower completion, PH = Plant height, LP = Number of leaves/plant, LA= Leaf area, ID= Internodal distance, NWAH = Number of whorls of achene/head, HD = Head diameter, 100-SW = 100 Seed weight, AWP = Achene weight/head.

days to maturity. The cross, 7-A × 80-R, 23-A × 83-R, 25-A × 80-R, 25-A × 83-R, and 27-A × 80-R, showed highly significant negative results over mid-parent and better parent for days to flower completion. Highly significant negative heterosis resulted in 7-A × 86-R, 19-A × 80-R, and 23-A × 81-R over mid- and better parent for plant height. The 11-A × 83-R, 11-A × 94-R, 11-A × 96-R, 25-A × 80-R, 23-A × 81-R, 23-A × 83-R, 23-A × 86-R, 23-A × 94-R, 23-A × 96-R, 25-A × 80-R, 25-A × 94-R, and 27-A × 80-R showed highly significant positive heterosis over mid- and better parents for the number of leaves per plant.

Overall, 7-A × 86-R, 11-A × 83-R, 23-A × 81-R, 25-A × 80-R, 25-A × 94-R, and 27-A × 80-R showed the desired negative heterotic values than mid- and better parents for early maturity-related traits, the height of the plant, and intermodal, showing significant positive and desirable results for all other characters. Mojhgani *et al.* (2012); Mehdi *et al.* (2014); Encheva *et al.* (2015); Rathi *et al.* (2016); Zia *et al.* (2016); Kulkarni and Supriya (2017); Dheya and Hussain (2017) and Lakshman *et al.* (2020) found the similar findings. Hence, this study declares these hybrids as the best hybrids.

Table 7. Mean square values from analysis of variance for quality parameters.

SOV	D.F	OC	PA	SA	LA	OA
Replication	2	5.25	0.166	1.76	6.16	2.27
Lines	5	269.28	21.34	6.3	314.01	20.51
Error	10	4.38	1.23	0.45	4.03	0.56
Total	17					

OC = Oil Contents, PA = Palmitic Acids, SA = Stearic Acids, Linoleic Acids, and OC = Oleic Acids.

Quality-related traits

The hybrids that showed the best performance in early maturity and yield in spring proceeded to evaluate for quality parameters to obtain the best hybrids. Significant variations were present among all lines for oil contents, palmitic, stearic, and oleic acids (Table 7). Khalid *et al.* (2017); Depar *et al.* (2017); Ghaffari and Shariati (2018), and Sher *et al.* (2022) also reported highly significant variations among sunflower genotypes for oil contents. Oil contents ranged from 33% to 42% in this research material (Figure 1). Palmitic acid ranged from 5% to 8%. Low palmitic acid showed in 23-A × 81-R and high palmitic acid in 7-A × 86-R, significantly different from all other hybrids (Figure 2). A low amount of palmitic acid is required, as it is a saturated fatty acid and not required so much by the body. Hybrid 23-A × 81-R gave the lowest values for stearic acid and was significantly different from other hybrids (Figure 3). Linoleic acid ranged from 59% to 75% in this research. High linoleic acid emerged in 23-A × 81-R (Figure 4). Linoleic acid is an essential polyunsaturated fatty acid. This material showed good content of linoleic acid. The oleic acid ranged from 25% to 30% in this study (Figure 5).

The hybrids 23-A × 81-R and 25-A × 80-R were already best for early maturity and yield. Now, these hybrids showed the best results in quality. These two hybrids gave the best results for oil quality, oleic acid, and linoleic acid and fewer amounts for palmitic and stearic acids. Therefore, 23-A × 81-R and 25-A × 80-R were the best hybrids for early maturity, good yield, and good quality.

DNA hybrid authentication by RAPD primer

The identified hybrids were through 10 random amplified polymorphic DNA (RAPD) markers. Out of these 10, six primers showed the best amplification and, thus, confirmed that the hybrids are of the specific parents. PCR of the DNA of hybrids proceeded in the thermal cycler. Six RAPD primer pairs showed confirmation of the hybrids obtained by the crosses. All the crosses showed the same banding pattern as their parents. Therefore, all these hybrids proved to be the original cross of their parental lines. The primers B14 and B16 showed the best results (Figures 6 and 7). Bhosle *et al.* (2015) reported similar results for hybrid authentication.

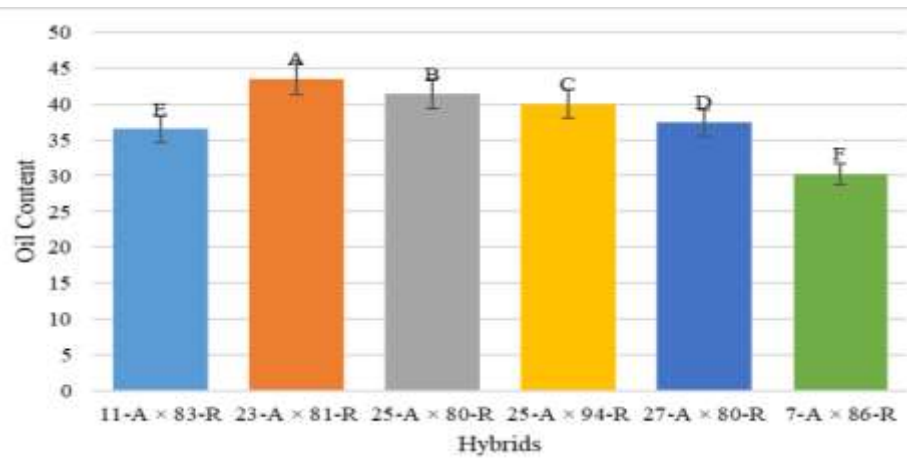


Figure 1. Mean performance of different Hybrids for Oil Content.

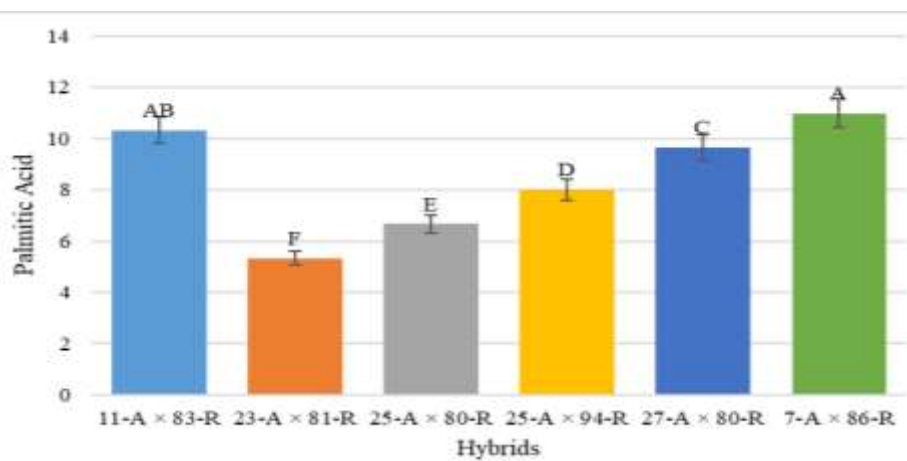


Figure 2. Mean performance of different Hybrids for Palmitic Acid.

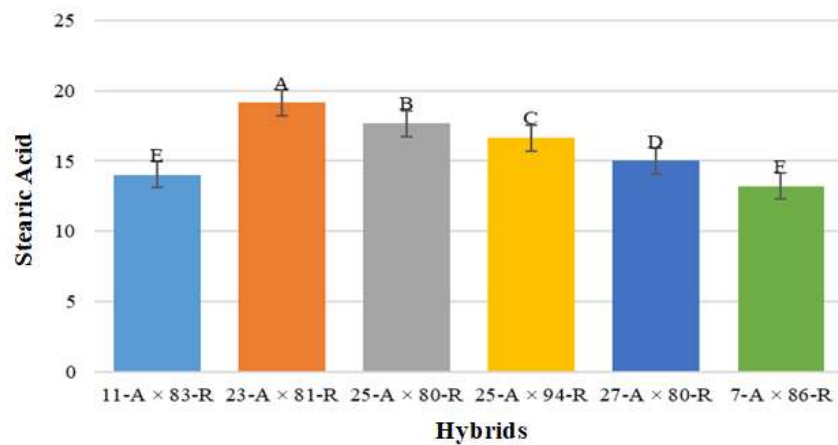


Figure 3. Mean performance of different Hybrids for Stearic Acid.

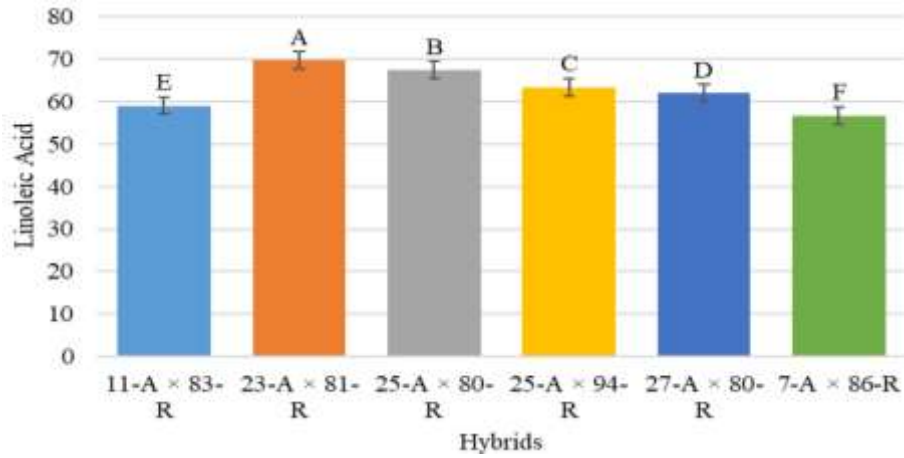


Figure 4. Mean performance of different Hybrids for Linoleic Acid.

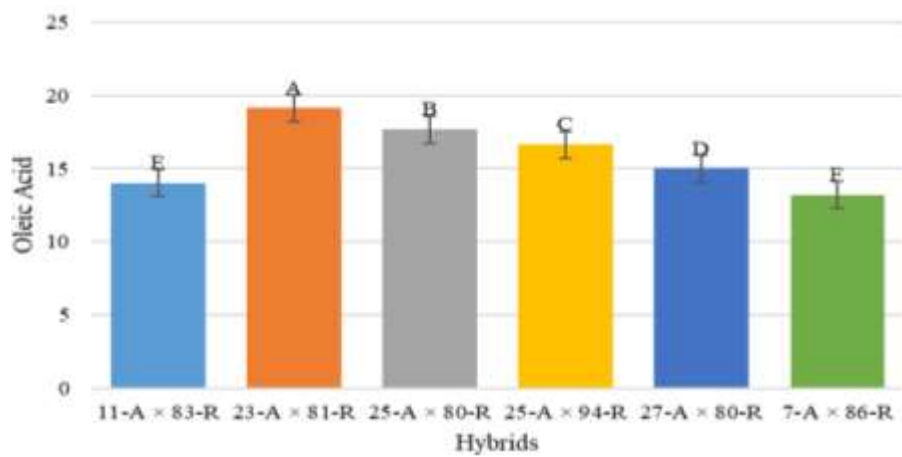


Figure 5. Mean performance of different Hybrids for Oleic Acid.

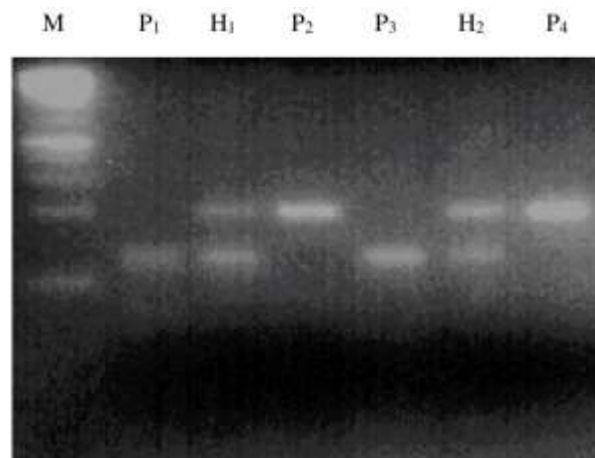


Figure 6. Hybrid identification of sunflower by using RAPD primer B14.

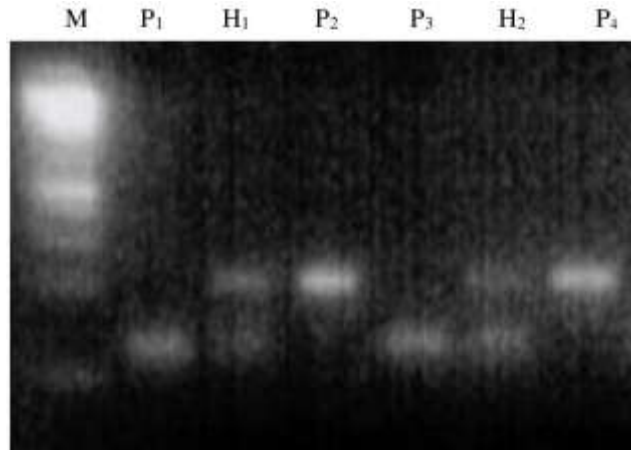


Figure 7. Hybrid identification of sunflower by using RAPD primer B16.

CONCLUSIONS

Overall, hybrids 23-A × 81-R and 25-A × 80-R showed best for the oil contents and quality, and they were already early maturing with better yield. These hybrids underwent RAPD analysis to check their authenticity as hybrids, as there were high chances of contamination in the field. Complying with DNA extraction, gel electrophoresis, DNA quantification, and PCR helped discover the final results. Results showed that the hybrids were authentic and had the same parents' crossed in the field, with no contamination found. The data will benefit the plant breeders to develop early maturing and improved achene yield with better quality local hybrids.

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