Screening of among seventeen sunflowers (*Helianthus annuus L.*) genotypes for oil content and the first flowering day using random amplified polymorphic DNA markers

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Background
Sunflower refers to the tribe Helianthus, subtribe Helianthinae, and family Asteraceae, which collectively contains 20 genera and 400 species. An important oilseed crop that yields edible oil is *Helianthus annuus* L.
Objective
The primary goal of the current study was to assess the genetic diversity of 17 genotypes of sunflower (*Helianthus annuus* L) To measure the oil content during the initial flowering period and to reach the highest percentage of oil can be obtained from the first flowering day.
Materials and methods
Five RAPD (random amplified polymorphic DNA) primers were used to detect the genetic diversity of the 17 sunflower hybrid genotypes obtained from Spain. Phylogenetic relationships of 17 sunflower genotypes were determined using three replications and 6 m lines on August 15, 2019, at the National Research Centre farm in Nubaria as part of a donation from the German corporation (strobe), Spain. To analyze the genetic diversity and phylogenetic linkages in sunflower germplasm, DNA fingerprinting and the Random Amplified Polymorphic DNA (RAPD) molecular marker approach were also used.
Results and conclusion
The oil content of 17 sunflower genotypes (*Helianthus annuus* L.) was assessed, with values ranging from 46 to 50%, with the highest values falling into five genotypes. However, the two genotypes were found to have the lowest oil percentage (46%). The early age and oil percentage differed among the varieties. In the Tornado and Elves genotypes, the longest and shortest days were 59 and 47, respectively. The means and standard errors for all statistical data are reported. Statistical significance was evaluated using the LSD. *P* values were considered statistically significant at *P* less than or equal to 0.05. According to the findings, RAPD primers generated 49 bands with a size range of 0.1–3 kb and an 87.75% polymorphism percentage. For RAPD, 43 polymorphic bands with distinct bands were observed. Morphological features and RAPD analysis separated the UPGMA Dendrogram into three groups. Jaccard's coefficient was used to analyze the genetic similarity matrix, and a morphological study revealed that Tornado and Elvas, both from Spain, shared the most genetic similarity (0.970). RAPD analysis and morphological features are useful in identifying genetic variants. Conclusion, according to our findings, *Helianthus annuus* L. has a significant variation ratio. Indicating substantial diversity across the 17 sunflower genotypes, the genetic similarity index calculated using pooled data from RAPD markers showed an extensive range from 0.645 to 0.986. This study may be a reference for future research on *Helianthus annuus* L. and may support breeding initiatives and species concepts.

Keywords:
genetic diversity, oil content, RAPD (Random amplified polymorphic DNA), sunflower (Helianthus annuus L.)

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Introduction

Helianthus annuus, a large annual forb of the genus Helianthus, is a common sunflower. It is frequently cultivated owing to its taste and fatty seeds. In addition to being used to make frying oil, it is also utilized in several industrial applications, such as bird food, as a

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household garden adornment, and as feed for cattle (as a meal or silage plant). Wild H. annuus is an annual shrub with many branches and several flower heads. However, domestic sunflowers frequently have only one sizable inflorescence (flower head) on top of an unbranched stem.

Sunflower hybrids maturing late in the spring season accumulated more photothermal units and had higher oil content. Hysun-33 and SMH-0907 showed the best performance in terms of growth-, yield-, and oil quality-related traits. The sunflower hybrid Hysun-33 performed best under the subtropical conditions of Haripur and possessed superior quality oil. Sunflower hybrid Hysun-33 is recommended for commercial-scale growth under subtropical climatic conditions [1].

Late-maturing sunflower hybrids produce more photothermal units and higher oil concentrations. Regarding the qualities linked to growth, yield, and oil quality, Hysun-33 and SMH-0907 performed the best. Hysun-33, a hybrid sunflower that produces exceptional amounts of oil, performed best in Haripur's subtropical climate. Hysun-33, a sunflower hybrid, is recommended for commercial-scale growth in subtropical climates [2].

The most frequently used methods among the few effective methods for identifying genetic distinction across and within plant populations are random amplified polymorphic DNA (RAPD) and ISSR [3,4]. More polymorphic loci (56.88%) were found using RAPD primers than ISSR primers (52.24%). A dendrogram created using RAPD, ISSR primers, and pooled data was connected to one another, demonstrating the effectiveness of both marker methods in researching the genetic diversity of sunflowers. The restorer lines EC 623023, R 16, and seed parental lines CMS 234 B and PET 2-7-1B were shown to be genetically the most diversified and fell into various groups based on RAPD, ISSR, and pooled data analysis [5].

The genetic similarity index, which was calculated, using combined data from SSR and RAPD markers, ranged from 0.18 to 0.74, demonstrating considerable heterogeneity among 14 sunflower genotypes [6].

The ability of RAPD markers to establish correlations between different genotypes and demonstrate how yield characteristics are associated at the genetic level and characterization of barley germplasm, breeding programs, and conservation efforts all depend on an understanding of the genetic diversity among barley species. ISSR analysis and physical characterization are techniques used to identify genetic variations [7,8]. RAPD and ISSR are two of the most popular and efficient techniques [9,10]. RAPD and ISSR are simpler to employ than other molecular marker systems because neither technique requires prior knowledge of the target sequences. The present study covered multiple variables to assess the value of sunflower crops under rainfed/famine conditions [11]. The current study crossed four separate CMS lines with ten testers to assess the value of the sunflower crop under rainfed/famine situations [12].

In this study, RAPD markers for oil content and the first blooming day were used to examine the chemical reactions of 17 distinct sunflower varieties (*Helianthus annuus*).

Materials and methods

Plant materials

Seventeen imported hybrids were cultivated by a German company (Strube), Spain branch, at the National Research Center Farm in Nubaria, on August 15, 2019, on lines of 6 m/line, and the distance between clicks was 25 cm, in three replications.

Planting seeds in pots

This study was conducted at the greenhouse of the National Research Centre, Dokki, in the Giza Governorate of Egypt. Each pot contains 50 seeds from one of the following seventeen hybrid sunflower varieties: Tornado, Elvas, ST 9093, Domingo ST, Serigio ST, Marciano ST, Lmidor, Lmitop, PF100, Pro 229 SU, Casto ST, Rmfo ST, Orfeo ST, Fausto ST, Gonzalo ST, Ramiro ST, and Telmo ST. The soil was then sterilized in an autoclave. Three replicates of each pot were planted in the comparison and treatment experiments. The greenhouse side was irrigated and monitored for 2 months, and samples were taken from fresh leaves of the plants for the two sunflower trials.

Determination of oil content and early flowering rate

The average for the early trait was calculated using data from the first flower's appearance date and analysis, according to [13], as well as the percentage of oil in each hybrid's seeds extracted with petroleum ether using the Soxhlet method, as per [14]. To evaluate genetic diversity and polymorphism data, 17 sunflower genotypes (*Helianthus annuus* L.) were chosen (Table 1). The young leaves of the plants were

Table 1 Genotypes days to first flower and oil percentage among seventeen sunflower genotypes

Genotypes	Days to first flower	Oil%
Tornado	47.00	50.00
Elvas	47.00	50.00
ST 9093	49.00	46.00
Domingo ST	48.00	48.00
Serigio ST	50.00	50.00
Marciano ST	50.00	48.00
Lmidor	50.00	48.00
Lmitop	59.00	48.00
PF100	52.00	47.00
Pro 229 SU	53.00	47.00
Casto ST	49.00	50.00
Rmfo ST	47.00	46.00
Orfeo ST	50.00	48.00
Fausto ST	50.00	49.00
Gonzalo ST	49.00	50.00
Ramiro ST	50.00	48.00
Telmo ST	50.00	49.00
LSD 5%	1.45	0.70
LSD 1%	2.00	0.97

removed, immediately washed with double-distilled water, wrapped in aluminum foil, and stored at −20°C until DNA extraction.

DNA extraction

Total DNA for five alleles was extracted from 1 g of fresh 6 days old seedlings using Biokits according to the manufacturer's instructions (Table 2). The DNA was stained with ethidium bromide (0.1 g/ml) to determine its quality after electrophoresis in a 2% agarose gel at 100 V for an hour in 1xTBE buffer.

Electrophoresis and RAPD analysis

Polymerase chain reaction (PCR) DNA amplification of the sunflower germplasm was carried out using five optimized RAPD primers. RAPDs were used to ascertain the genetic diversity among the sunflower cultivars. The three phases of the PCR were as follows: denaturation at 95°C for one minute; annealing at 36°C for one minute; and extension at 72°C for 2 min for 35 cycles. The PCR program was programmed to maintain results at 4°C. Using a micro centrifuge, 6 l of loading dye (0.50% xylene cyanol and 0.50% bromophenol blue) was added to the products. A 2% agarose gel with a voltage of 100 V was used to electrophorese the PCR result, and amplified bands were detected on a gel documentation system Bio-Rad (Hercules) [15].

Data analysis only distinct, clear, and reproducible bands were used for data processing. Each band was regarded as a distinct area. Data were graded according to [16] as (1) for existence and (0) for absence for each cultivar to distinguish between positive and negative markers. The similarity coefficients generated by the SPSS application version 10 (UPGMA) were utilized to generate a dendrogram using the UN weight pair group technique with an arithmetical average [17,18].

Statistical analyses

Using the computer statistical analysis program MSTAT-C [19], data were statistically analysed in accordance with [20]. To compare the variations in treatment means, the least significant difference (LSD) test at a probability level of 0.05 was manually calculated. To create the genetic similarity matrix, RAPD bands were scored using (1) for bands that were present and (1) for bands that were lacking. The popgen32 software (version 1.44) was used to analyze genetic similarities [21]. to gauge the genetic separation between sunflower genotypes. By counting the trustworthy visible bands, the quantity of amplifying fragments (QAFs), number of polymorphic fragments (QPFs), number of monomorphic fragments (QMFs), and percentage of polymorphic fragments (PPFs) were determined. The polymorphism information content (PIC) was calculated in accordance with [22]. For the molecular link between 17 genotypes, a dendrogram based on the unweighted pair group analysis with arithmetic average (UPGMA) was produced using popgen32 software Version 1.44. The groupings are shown in the dendrogram. The polymorphic genetic information (PIC) of each marker was calculated using the formula PIC = 1 Pi 2, where Pi is the band frequency of the gene [23].

Table 2 Random amplified polymorphic DNA markers, sequence of primer, total number (TB) and number of polymorphic bands (PB),Monorphic bands (MB), unique bands and percentage of polymorphism (PP)

Primer code	Primer sequences	Total bands	Polymorphic bands	Monorphic bands	Unique bands	% polymorphism
*P*1	CGCAAGACCT	9	7	2	2	77.78
*P*2	TCGGCGTCAA	8	8	0	3	100
*P*3	CCAGCAGCTT	10	9	1	2	90
*P*4	GACTGCACAC	12	10	2	3	83.33
*P*5	ACGCAGGCAC	10	9	1	2	90
Total	49	43	6	12	87.75	

Results and discussion

Agro-flowering traits and oil content variation

The sunflower genotypes and important changes in oil content are shown in Table 1. It is critical to understand how plant height, maturity, blooming traits, and oil content are related to the oil yield. In general, phenotypic correlations associated with genotypic correlations were weaker. The lower values of phenotypic correlations may be due to minimal environmental influence on the connection of traits at the gene level [24]. The relationship between the interval before bloom initiation and oil yield is inverse, as shown in Table 1.

The link was not significant at the genotypic level but was significant at the phenotypic level. This finding is in line with that of [25,26], who discovered a negative association between the number of days until 50% flowering and yield. In addition, plant height, oil content, and number of days until bloom initiation had negative genotypic and phenotypic connections. However, days to flower commencement exhibited a substantial positive correlation with days to flower conclusion, flowering time, and days to maturity at both genotypic and phenotypic levels. It was shown that days to bloom completion were negatively linked with oil content and output at both phenotypic and genotypic levels. A slight, unfavorable genotypic correlation between oil content, yield, and flowering time was observed. This suggests that shortening the flowering period may ultimately result in an increase in oil yield and oil content. At both phenotypic and genotypic levels, there was a substantial positive correlation between the number of days to maturity and oil yield.

The longest number of days was 59 and the shortest was 47 in the Rmfo ST, Tornado, and Elves genotypes (Table 1). Similar results were reported in [27]. In agreement with earlier research, plant height demonstrated a favorable and significant genetic connection with oil yield and content. The association coefficient of oil content with plant height and oil yield was positive and significant at both the genotypic and phenotypic levels (Table 1). [28] also reported similar results in their independent investigation. The effect of the independent parameters (flowering characteristics and oil content) on the dependent variable, or oil yield, was examined using path analysis. The division of the direct and indirect effects of various features on oil yield was made easier by analysis. This research will aid breeders in locating traits that might be utilized as selection criteria in breeding programs for sunflowers.

Cluster analysis of flowering traits and oil content

Phylogenetic tree analysis

According to the dendrogram generated by UPGMA cluster analysis of the two quantitative morphological traits of the 17 flowering accessions, the accessions were divided into three separate clusters on the phenogram, as shown in (Fig. 1). The genetic similarity score ranged between 0.645 and 1.00. Two genotypes, Tornado and Elvas, were found in Cluster I and were segregated at a phylogenetic distance of 0.75. Cluster II was composed of seven accessions. At taxonomic distances of 0.8 and 0.84, this cluster was further divided into two subclusters: subcluster A contained the two accessions Serigio ST and Gonzalo ST, which were separated at taxonomic level 0.90; sub-cluster B contained the five accessions Orfeo ST, which was separated at a taxonomic distance of 0.93 from the two grouped accessions Lmidor and Lmitop. The eight accessions comprised Cluster III. This cluster was further divided into two sub-clusters at a taxonomic distances of 0.86 and 0.90. Subcluster C contained three accessions Pro 229 SU that were separated at a taxonomic level of 0.86, from two accessions Ramiro ST and Domingo ST that were separated at a taxonomic distance of 0.97, whereas subcluster D contained five accessions Telmo ST and Fausto that were separated at taxonomic level 0.

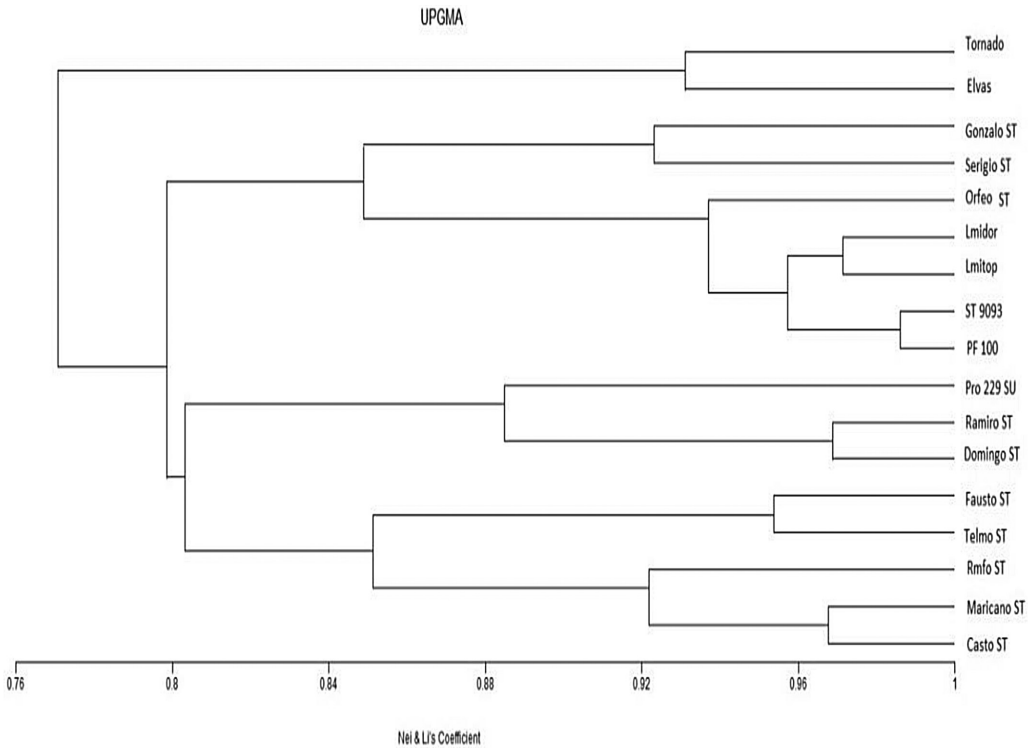
This can be explained by examining how these genotypes have changed over time in various agroclimatic zones, which suggests significant heterogeneity in how they respond to selection pressure, as detailed by many authors [29,30]. The morphological dendrogram (Fig. 1) demonstrates how cluster analysis found a tenuous connection between the genotypes' regional origins and their segregation.

Molecular results

Assessment of RAPD markers

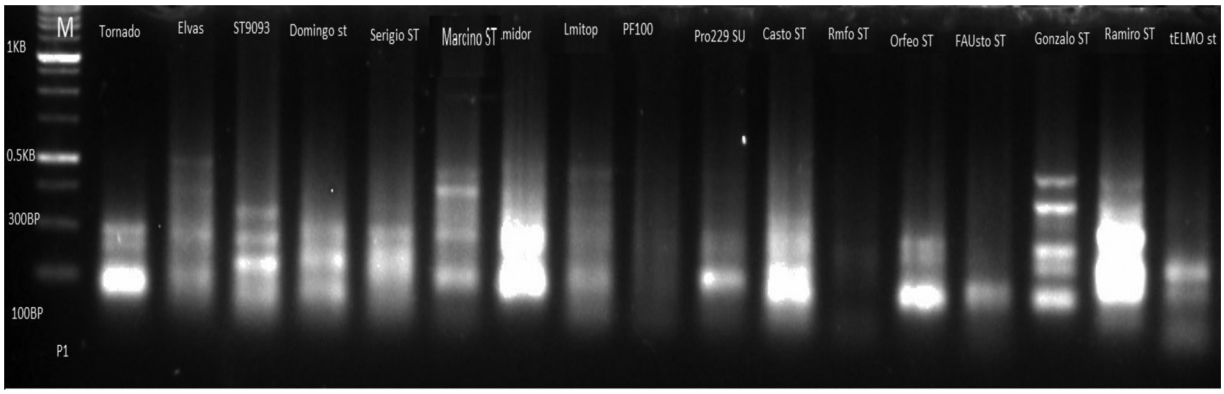
The 17 sunflower accessions were amplified by PCR, yielding 49 distinct bands, 12 of which had an 87.75% polymorphism rate. Of these, 43 were polymorphic traits. The total size of the PCR-amplified fragments ranged from 100 bp to 1000 bp (Table 2). The banding patterns of various sunflower genotypes are shown for primers P1, P2, P3, P4, and P5 (Figs 2 and 3). Nine bands were formed by marker P1, of which two were unique and seven were polymorphic, yielding a 77.78% polymorphism rate. However, the latter two bands were monomorphic. Most bands (12) were formed

Figure 1



Dendrogram unweighted pair group analysis with arithmetic average for seventeen sunflower genotypes using MVSP.

Figure 2



DNA random amplified polymorphic DNA assays using P1 for seventeen sunflower genotypes.

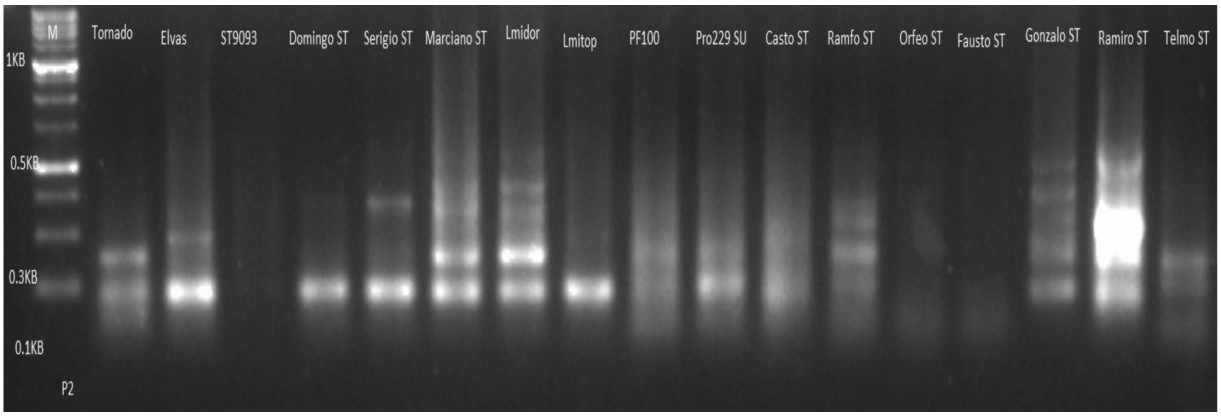
by P4, with a polymorphism proportion of 83.33%. Of these bands, two were monomorphic, three were unique, and ten were polymorphic. Eight bands were generated using the P2 primer, three of which were unique, with eight bands totaling 100% polymorphism and zero monomorphism. Ten bands were produced by P3 accession, with two being unique, one monomorphic, nine polymorphic, and 90% polymorphic. The P5 primer generated ten bands, two of which were unique, nine of which were polymorphic, indicating a 90% polymorphism percentage, one of which was monomorphic.

Geographic isolation and regional climatic differences may partially explain diversity, according to a prior study on the genetic similarities of sunflower genotypes using cluster analysis [31,32]. Environmental influences affect morphological markers, which can be difficult to research, time-consuming, and subject to uncertain interpretation.

Genetic diversity and relationships

(Figure 1) shows the dendrogram created by clustering seven RAPD markers from seven different sunflower species using the UPGMA algorithm. Three distinct

Figure 3



DNA banding using P2 for seventeen sunflower genotypes.

clusters were visible in the phenogram, with genetic similarity scores ranging from 0.645 to 1.00. The maximum genetic similarity coefficient for the Domingo and Serigio ST genotypes was 0.986, indicating a high level of genetic similarity. The accessions Ramiro ST and Lmitop, both of which were towed, had the lowest closeness ratio of 0.645%.

The use of PCR-based molecular indicator technology for genetic variety, association, and cultivar identification is becoming increasingly popular because it has several benefits over simply utilizing conventional markers. In this instance, genetic diversity was evaluated using the RAPD method and phylogenetic connections between sunflower species were ascertained using DNA fingerprinting [33]. High polymorphism was found between Egyptian sunflower accessions using RAPD-based genetic diversity analysis; a related finding was previously documented for rice [34] and oranges [35]. Accessions are routinely distinguished using RAPD markers, which are occasionally more effective than the RAPD markers. Numerous articles [36] have stated that the RAPD approach is an efficient tool for genotypic assessment of a variety of plant species. This has been advocated in older and more recent studies.

The dendrogram of this study, which was created by combining UPGMA and RAPD based on the genetic similarity matrix, demonstrated that group structure was somewhat influenced by regional distribution. Combining genes from the same region, as seen in (Fig. 2), which also includes the genes for Tornado, Elvas, ST 9093, Domingo ST, Serigio ST, Marciano ST, Lmidor, Lmitop, Pro 229 SU, Casto ST, Rmfo ST, Orfeo ST, Fausto ST, Gonzalo ST, Ramiro ST, and Telmo ST. Sunflowers have also shown a trend of spatially related clustering using molecular markers [37,38]. In contrast to earlier research on barley and Aegilops, molecular clustering of sunflower does not correspond to its origin [39,40]. Additionally, ten polymorphic bands were discovered using five RAPD primers, demonstrating a high level of polymorphism (100%), and the results showed highly polymorphic profiles. The polymorphism rate in Moroccan sunflowers was lower (77.78%) [41,42].

Proximity similarity index among 17 Genotypes of sunflower based on banding patterns of 7 random amplified polymorphic DNA primers using MVSP.

Conclusion

Molecular marker-based and morphological clusters, in addition to the associated studies, revealed different hierarchical patterns of genetic variability between the genotypes [43]. The two approaches were shown to be equally useful for determining the level of relatedness and broad trends in genetic diversity among the tested sunflower genotypes, notwithstanding their variances [44]. Molecular markers, on the other hand, are more effective and illuminating in identifying sunflower genotypes and offer reliable tools for evaluating intraspecific connections [45,46,47].

Conclusively, our study demonstrated the effectiveness of the RAPD marker system in diversity analysis of sunflower genotypes. As a result, we analyzed the amount of oil present during the first few days of blooming, and the amount of oil that may be obtained starting on the first flowering day. These genotypes were shown to have strong seed yield

Case	Tornado	Elvas	ST 9093	Dominigo ST	Serigio ST	Marciano ST	Lmidor	Lmitop	PF100	Pro 229 SU	Casto ST	Rmfo ST	Orfeo ST	Fausto ST	Gonzalo ST	Ramiro ST	Telmo ST
Tornado	1.000																
Elvas	0.970	1.000															
ST 9093	0.941	0.971	1.000														
Dominigo ST	0.912	0.943	0.972	1.000													
Serigio ST	0.925	0.957	0.958	0.986	1.000												
Marciano ST	0.831	0.866	0.870	0.899	0.912	1.000											
Lmidor	0.813	0.818	0.824	0.824	0.836	0.923	1.000										
Lmitop	0.820	0.794	0.800	0.800	0.813	0.839	0.918	1.000									
PF100	0.754	0.730	0.738	0.738	0.750	0.774	0.852	0.931	1.000								
Pro 229 SU	0.794	0.800	0.806	0.806	0.788	0.750	0.762	0.833	0.900	1.000							
Casto ST	0.794	0.800	0.806	0.806	0.788	0.719	0.730	0.800	0.867	0.968	1.000						
Rmfo ST	0.800	0.806	0.812	0.841	0.824	0.758	0.708	0.774	0.839	0.906	0.938	1.000					
Orfeo ST	0.800	0.806	0.841	0.841	0.824	0.758	0.708	0.710	0.774	0.844	0.875	0.909	1.000				
Fausto ST	0.844	0.848	0.853	0.853	0.866	0.800	0.750	0.754	0.754	0.794	0.825	0.862	0.954	1.000			
Gonzalo ST	0.762	0.800	0.806	0.836	0.848	0.813	0.730	0.667	0.700	0.742	0.774	0.813	0.875	0.889	1.000		
Ramiro ST	0.769	0.806	0.841	0.870	0.853	0.818	0.738	0.645	0.677	0.750	0.781	0.818	0.879	0.862	0.969	1.000	
Telmo ST	0.754	0.794	0.831	0.862	0.844	0.774	0.689	0.655	0.655	0.733	0.767	0.806	0.806	0.754	0.867	0.903	1.000

performance and component attributes with considerable oil effects.

Seventeen sunflower parents were categorized into three major groups based on genetic diversity. Future breeding programs should make use of these genotypes.

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Conflicts of interest

There are no conflicts of interest, according to the authors.

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