RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (2) 811-819, 2026
http://doi.org/10.54910/sabrao2026.58.2.31
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

IDENTIFICATION OF GENES ASSOCIATED WITH OLEIC ACID THROUGH PHYLOGENY TREE AND CORRESPONDENCE ANALYSIS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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SUMMARY

The following study comprised identifying the genes associated with oleic acid through phylogeny tree and correspondence analysis in five cultivars of sunflower (*Helianthus annuus* L., the number of chromosomes, 2n = 34), carried out at the University of Kerbala, Kerbala, Iraq. Five sunflower cultivars (Argensun, Awess xxxxl, Coban, Agway XL-10, and Royal 2), as selected, had their seeds grown during the first 10 days of October 2024. The genomic DNA extraction from younger plant leaves used test kits, while measuring the concentration and purity of DNA used the NanoDrop ND-1000. The calculation of ratios of genetic dimensions and similarities among the sunflower cultivars also transpired, with the phylogeny tree and correspondence analysis performed using the PAST3 program. The sunflower cultivars exhibited successful DNA extraction, with notable variations in DNA concentration and purity, reflecting the main genetic differences among these genotypes. Since the sunflower genotypes had the highest concentration of DNA, the cultivars Royal-2 and Argensun were ideal for gene amplification and genetic diversity analysis. The Coban cultivar had the highest content of oleic acid, while the Argensun cultivar had the lowest amount of oleic acid.

Keywords: Sunflower (*H. annuus* L.), genes associated with oleic acid, phylogenetic trees, crops, genomic DNA, correspondence analysis, genetic dimensions and similarities

Communicating Editor: Prof. Dr. Zahoor Ahmed Soomro

Manuscript received: July 20, 2025; Accepted: October 18, 2025.

Citation: Al-Shimmery MMG, Al-Asadi QTH, Hussein HA, Rabee DA, Al-Samak R, Al-Ibrahemi N (2026). Identification of genes associated with oleic acid through phylogeny tree and correspondence analysis in sunflower (*Helianthus annuus* L.). *SABRAO J. Breed. Genet. 58 (2) 811-819. http://doi.org/10.54910/sabrao2026.58.2.31*.

Key findings: Significant changes in DNA content and purity occurred among the five sunflower (*H. annuus* L.) cultivars, reflecting the main genetic variants between these genotypes. Cultivars Royal-2 and Argensun were ideal for the genetic diversity analysis and gene amplification, and the sunflower genotypes exhibited the highest DNA concentration.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) has become one of the most important oilseed crops worldwide due to its high economic, nutritional, and environmental values. This plant belongs to the family Asteraceae, characterized by its unique ability to track with the movement of the sun during its growth stages, a phenomenon known as heliotropism (Kaul *et al*., 2000).

The sunflower is unique with its rapid growth and remarkable adaptability to diverse environmental conditions, making it one of the most widely cultivated crops across Europe, Asia, and the Americas. In sunflower breeding programs, a key objective is to increase the oleic acid content of seeds (Tang *et al*., 2002). Achieving this typically occurs by targeting genes involved in fatty acid biosynthesis, with the *FAD2-1* gene being the most significant. The *FAD2-1* gene mediates the conversion of oleic acid to linoleic acid, and mutations in this gene can result in the accumulation of more than 80% oleic acid in seed oil (Ibrar *et a*l., 2022).

In confirming that DNA is suitable for genetic applications, its extraction from the plant material first takes place. Afterward, measuring its concentration and purity follows using sophisticated molecular analysis methods. This is the crucial first step before subsequent genetic analyses, such as gene amplification using PCR technology and molecular marker analysis to identify similarities and differences among crop cultivars, including sunflower—determining the concentration and purity of the DNA (Kahraman *et al*., 2023).

With the following goals in mind, the relevant study concentrated on five sunflower cultivars (Argensun, Awess xxxl, Coban, Agway XL-10, and Royal 2): a) extracting DNA and utilizing spectrophotometry and electrophoresis to assess its concentration and purity (Zuil *et al*., 2012); b) examining the genetic connections among the five sunflower cultivars using appropriate molecular markers to evaluate genetic dimensions and ratios of genetic similarity (Martínez-Rivas *et al*., 2001); c) investigating the genetic relationships between the five cultivars of sunflower by utilizing suitable molecular markers to assess genetic dimensions and genetic similarity ratios (Meng *et al*., 2025); and d) identifying the sunflower varieties' genetic relatedness to facilitate upcoming breeding and hybridization initiatives. Understanding the genetic makeup of economically significant sunflower cultivars and supplying molecular information to help the enhancement of oil characteristics through precise genetic guiding could be possible from this study (Premnath *et al*., 2016).

MATERIALS AND METHODS

The concerned study regarding identification of genes associated with oleic acid through phylogeny tree and correspondence analysis in five sunflower (*H. annuus* L.) cultivars (Argensun, Awess xxxxl, Coban, Agway XL-10, and Royal 2) transpired at the University of Kerbala, Kerbala, Iraq. Its location is 25 km from the city center at a longitude of 32.67 and a latitude of 44.16 in a mixed clay soil. Five sunflower cultivar seeds underwent selection and growing in pots (0.5 L) with an average of 10 seeds per pot during the first 10 days of October 2024 (Table 1) (Al-Masaoodi *et al*., 2025). Fresh leaf samples collected from all sunflower cultivars had their genomic DNA extracted (Al-Yassiry *et al*., 2024; Al-Ibrahemi *et al*., 2025).

Genomic DNA extraction

The kit prepared by the American company ZYMO served to extract the DNA from the leaves of young sunflower plants. The DNA

Table 1. The studied sunflower cultivars with their source and origin.

Cultivar	Source	Origin
Argensun	College of Agriculture, Karbala University	Argentina
Awess xxxxl	College of Agriculture, Karbala University	Argentina
Coban	College of Agriculture, Karbala University	Turkey
Agway XL-10	College of Agriculture, Karbala University	China
Royal 2	College of Agriculture, Karbala University	Argentina

concentration and purity measurement used the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA) at wavelengths of 260 and 280 nanometers.

Genetic dimensions and similarities among cultivars

According to the genetic distance ratios among the studied sunflower cultivars based on SSR markers using the Dice similarity index equation, relying on the packages, the study used the PAST-3 program on the computer (Ibrar *et al*., 2018).

Genes associated with oleic acid

The use of SSR markers helped identify the genes associated with high oleic acid content in five sunflower cultivars' seed oil. The purpose is to benefit from them in a breeding and selection program based on genetic markers determined through double and single bands for each cultivar. Such an approach prevents the traditional, labor-intensive, and lengthy method, as well as effects from environmental conditions (Singchai *et al*., 2013).

Phylogeny tree and correspondence analysis

After calculating the similarity and difference ratios according to the previous laws, the next phase begins to calculate genetic relatedness and draw the phylogenetic tree, with the similar genetic structures clustered together in one cluster. The UPGMA method application helps draw the genetic relationship, being the more commonly used, relying on NTSYSps version 2 (Rohlf, 2002), with the PAST-3 program used on the computer.

RESULTS AND DISCUSSION

DNA purity and concentration

Based on the spectrophotometric measurement using the NanoDrop apparatuses, variations in the DNA concentration and purity appeared among the five sunflower cultivars (Table 2). Sunflower cultivar Royal 2 recorded the highest concentration (234.76 ng/µL), followed by cultivar Argensun (231.76 ng/µL), indicating the extraction quality and ability to be beneficial in multiple molecular applications. Cultivars Agway XL-10 and Coban showed purity ratios (>2.0), indicating the possibility of an RNA contamination, which can be resolved using the RNase enzyme with an additional purification step. The lowest DNA purity recorded resulted in the cultivar Royal 2 (1.756), which was within the minimum acceptable range; however, it may require additional purification if used in precise applications like quantitative PCR.

In the context of genetic analysis, despite the absence of detailed molecular fingerprint data, the observed differences in the primary DNA characteristics among the sunflower cultivars may reflect underlying genetic disparities associated with the genetic makeup of each genotype. In ascertaining the level of genetic relatedness and genetic distance among the agricultural cultivars, these results can be an initial indicator of the prevalent genetic variation to undergo subsequent verification using genetic diversity analysis techniques, such as SSR and RAPD (Khan *et al*., 2015). The extracted DNA also emerged to be of acceptable quality and appropriate for use in molecular procedures, including the polymerase chain reaction (PCR), which necessitates a very pure DNA sample

Table 2. Purity and concentration of DNA in the sunflower cultivars.

Cultivar	Purity	Concentration	Qualitative assessment of purity
Argensun	1.899	231.76	Pure good
Awess xxxxl	1.799	169.56	Accepted
Coban	2.056	151.56	Molecular contaminant
Agway XL-10	2.134	173.76	Clear pollutant
Royal 2	1.756	234.76	Simple protein contamination

Table 3. The genetic distance among the sunflower cultivars.

Cultivar	Argensun	Awess xxxxl	Coban	Agway XL-10	Royal 2
Argensun	0				
Awess xxxxl	0.3567	0			
Coban	0.4245	0.5355	0		
Agway XL-10	0.6354	0.4566	0.5436	0	
Royal 2	0.4765	0.5436	0.3765	0.4567	0

devoid of inhibitors. The range of DNA concentrations in sunflowers was 120.93 to 488 nanograms/microliter, which was adequate for carrying out most molecular tests (Kumar *et al*., 2008). Given the differences in the chemical makeup of the leaves, some genetic compositions (2.12), which have relatively high purity values, may be more successful at eliminating pollutants and proteins. It is typical that the internal chemical composition of plant tissues, particularly the content of phenols and sugars, affects the efficiency of the isolation process in various crop plants (Porebski *et al*., 1997). Therefore, it seemed that the analysis of genetic markers could reveal precise relationships, contributing to the improvement of breeding strategies among the different crop cultivars. The difference between high-concentration and less-pure cultivars can reach further scrutiny to develop breeding programs aimed at generating more compatible and higher-quality molecular plants (Singh and Sengar, 2015).

Genetic distance among cultivars

Based on the Nei Royal 2-10 coefficient, the lowest genetic distance (0.3567) was evident between the genetic compositions of the two sunflower cultivars Awess xxxxl and Argensun, revealing similarity between these genotypes (Table 3). This genetic proximity allows us to differentiate between the crop genotypes during cultivation compared with their other traits. It gives a wider scope for the breeder to search for the best options for propagating genetically distant cultivars with high productivity and quality specifications and exclude genetically related and close cultivars from the local germplasm. The highest genetic distance (0.6354) occurred between the sunflower cultivars Argensun and Agway XL-10, which may be due to the differences in their genetic origins and the genetic improvement programs applied to the crop genotypes over time (Singh, 2011). Crop cultivars that are genetically distant from each other share the least number of bands due to differences in nucleotide sequences in the genome of those cultivars (Yu *et al*., 2012). A past study revealed the genetic similarity coefficient among the sunflower genotypes ranged from a maximum of 0.88 to a minimum of 0.25 (Darvishzadeh *et al*., 2010).

The distance between two groups of genotypes indicates the degree of relationship between them. Groups close to each other cluster in branches that are also close to each other, as the genetic similarity ratio for all genetic cultivars ranged between 0.3319 and 0.6466 based on the genetic distance values. This reveals a high genetic variation between the cultivars and compositions, recognizing them as important genetic resources. Crop

Table 4. Investigation of some genes associated with oleic acid in the sunflower cultivars.

Cultivar	ORS 878	ORS 086	ORS 296	ORS 310	ORS 899	ORS 598	Allele
Argensun	-	+	+	-	+	-	1
Awess xxxxl	+	+	-	-	-	+	5
Coban	+	+	-	+	+	-	7
Agway XL-10	+	-	+	-	-	-	3
Royal 2	+	-	+	+	+	-	4
	4	3	3	2	3	1	

cultivars recording the highest genetic distance can be options as parental genotypes in hybridization programs to further improve the genetic makeup of cultivars in various crop plants (Israt *et al*., 2014).

Genes associated with oleic acid

The use of several primers sorted out the sunflower cultivars with high and low oleic acid content (Table 4). The primer ORS878 showed the presence of high oleic acid alleles in four sunflower cultivars (Awess xxxxl, Coban, Agway XL-10, and Royal 2), while the primer ORS598 gave an allele in only one cultivar (Argensun). The cultivar Coban exhibited the highest content of oleic acid (7), while the cultivar Argensun had the least content of oleic acid (1). One of the monounsaturated fatty acids (MUFA) with significant nutritional and economic value is oleic acid (C18:1), which helps in enhancing the oxidative stability of vegetable oils. Its consumption is also ideal from a health perspective due to its positive role in reducing low-density lipoprotein (LDL) cholesterol levels in the blood. The genetic expression of particular enzymes involved in the pathways for fatty acid biosynthesis, specifically the enzymes SAD (Stearoyl-ACP Desaturase) and FAD2 (Fatty Acid Desaturase 2), is the primary determinant of oleic acid levels in oilseed crops (Cahoon and Shanklin, 2000). Given that it produces an enzyme that adds a second double bond to the carbon chain, converting oleic acid into linoleic acid (C18:2), and the *FAD2* gene is the primary regulator of oleic acid levels. Thus, it is strategically crucial to partially or completely inhibit the *FAD2* gene to raise the amount of oleic acid in edible oils (Haun *et al*., 2014).

Additionally, the *SAD* gene contributes to the conversion of stearic acid (C18:0) to oleic acid; therefore, elevated activity of this gene causes an initial rise in oleic acid production before conversions mediated by FAD2 in soybean and other oilseed crops (Kinney, 1996). This is consistent with the study that identified high oleic acid genes in sunflowers. Therefore, it is preferable to expand the scope of the study to include more wild cultivars and strains to build a broader database of genetic relationships and genetic diversity within the genus *Helianthus* and other oilseed crops (Nagarathna *et al*., 2011).

Phylogeny tree

In the presented study, the phylogenetic tree drawn for five cultivars of sunflower had the genetic distance between the genotype groups indicate the degree of relationship between them (Figure 1). The groups close to each other gather in branches near each other within the same group, with the same pattern used in genetic diversity studies of sunflower and other oilseed crops (Darvishzadeh *et al*., 2010). The analysis revealed two main groups: a large group containing two subgroups—the first group included two sunflower cultivars, viz., Awess xxxxl and Agway XL-10, while the second group included three cultivars, i.e., Coban, Argensun, and Royal-2. The phylogenetic tree showed some sunflower cultivars came together, and this may be due to their common origins, as in two cultivars, Awess xxxxl and Agway XL-10, which trace back to Chinese origins. The other three sunflower cultivars, Coban, Argensun, and Royal-2, which were in the second group, have Argentine origins. The cultivars from different

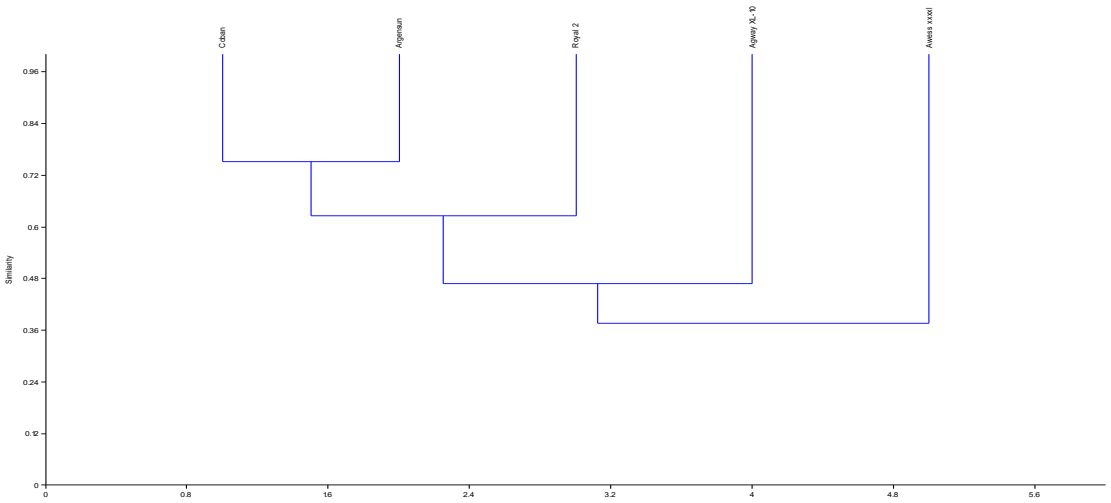

Figure 1. Dendrogram illustrates the genetic tree among the five sunflower genotypes.

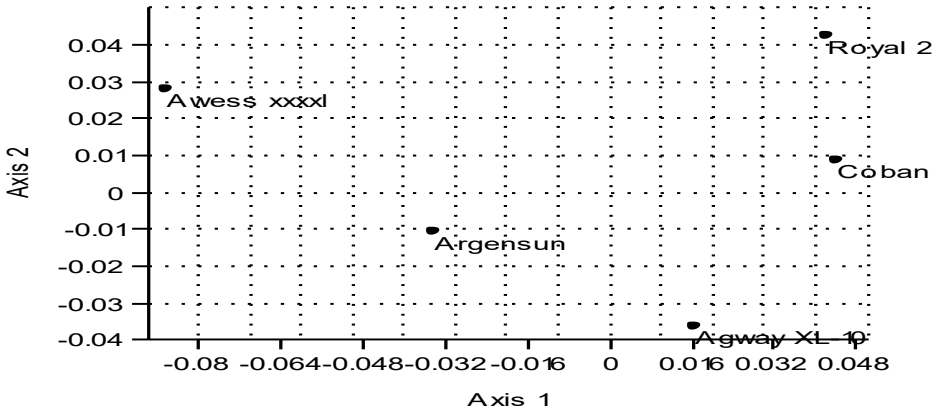

Figure 2. Dendrogram illustrates the correspondence analysis among the five sunflower genotypes

ancestors in the same location may refer to their genetic adaptation to the prevailing environmental conditions at the existing location; thus, the genetic material seemed similar, and that led to its reflection.

The number of shared bundles between the genotypes may also have a common genetic base, and thus, it was possible for genes to flow between different geographic distributions (Mishra *et al*., 2014). The distinction between cultivars from the different countries and different origins was ascribable to the efficiency of the SSR markers in detecting genetic diversity (Singh, 2011). Their ability to construct the phylogenetic tree indicates their importance in determining the genetic relationship among genotypes and selecting parental genotypes for breeding based on marker-assisted selection and searching for high oleic acid content in sunflower genotypes (Singchai *et al*., 2013).

Correspondence analysis

Ensuring the relationship between five sunflower cultivars (Argensun, Awess xxxxl, Coban, Agway XL-10, and Royal-2) and their various physiological characteristics had a correspondence analysis carried out (Figure 2). It was possible to distinguish among the

different impact patterns of each sunflower genotype on the examined plant traits because the results demonstrated an obvious distribution of the genotypes in two major dimensions (Axis 1 and Axis 2). Sunflower cultivars Royal-2 and Coban were close on the far right of the first dimension (Axis 1), which indicates their plant responses were extremely similar. These two products might have positively influenced vegetative growth traits like plant height and leaf count because of their effectiveness in encouraging early plant growth. In complex agricultural data, correspondence analysis can successfully reveal nonlinear relationships between the genotypes and their traits (Greenacre, 2016).

However, the cultivar Awess xxxxl was unique because it was at the far right of the first dimension and at the bottom of the second dimension (Axis 2). An explanation stated sunflower genotypes that differed on one axis but shared another were only partially related in the pattern of influence, and this difference in effect supports their explanation. This implies that although it shares some characteristics with cultivars Royal-2 and Coban, it differs in other elements, having more association with productive traits, such as the grain dry weight and the number of clusters (Lebart *et al*., 1984). Conversely, the cultivar Agway XL-10, with a location in the center of the graph, suggested a moderate impact on the number of characteristics.

The usage of low-concentration and generic products had frequent citation as an explanation for this posture, which indicated the sunflower cultivar Agway XL-10 has consistently influenced the majority of the attributes without substantially changing any one of them (Husson *et al*., 2011; Rabee *et al*., 2023). Conversely, the cultivar Argensun stood out from the other genotypes at the extreme left of the figure. This dimension could refer to the cultivar Argensun's generally mild influence, given that it only affects one feature while leaving the others unchanged. In correspondence analysis, these genotypes were commonly effective for occasional use to identify transactions with inconsistent performance (Abdi and Williams, 2010).

CONCLUSIONS

DNA extraction was successful from the five cultivars of sunflower (*H. annuus* L.), with significant variations in DNA concentration and purity, reflecting the primary genetic differences between these genotypes. The cultivars Royal-2 and Argensun were ideal for the genetic diversity analysis and gene amplification because the sunflower genotypes had the highest concentration of DNA.

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