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# Assessment of Genetic Diversity of Cultivated Sunflower in Terms of Oil Content, Fatty Acid Compositions and Seed Traits

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**Abstract:** Sunflower (*Helianthus annuus* L.) cultivated accessions contains useful genes encoding different phenotypic characteristic through which the origin of sunflower oil could be hypothesized. Those genes could be later used for future breeding programs for providing better quality sunflower oil.

The objective of the current study is to discriminate genetic diversity of cultivated sunflower seeds collection through the statistical methods such as PCA (principal component analysis) and Pearson correlation analysis for two characters; seed oil content and fatty acid composition.

Materials and methods: In the present study, the genetic diversity of 107 cultivated accessions of *Helianthus annuus* L. was studied for fatty acid composition and oil content. Pearson correlation and Principal Component Analysis (PCA) were used to determine the correlation between the studied parameters. A dendrogram using Ward's method and the squared Euclidean distance coefficient was produced.

The results showed that the average seed oil content in the accessions was 29.51 % with a profile of 7.23 % palmitic acid (PAL), 5.04 % stearic acid (STE), 36.85 % oleic acid (OLE) and 50.85 % linoleic acid (LIN). The highest oil content was found in accession Hopi Dye (43.66 %). High levels of OLE were observed in the Csehszlovakiai "B" (60.14 %) and Vk-47 (55.73 %) accessions. On the other hand, Fuksinka 10 and Georgia accessions had the lowest mean PAL values

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(4.98 %) and STE (1.81 %), respectively. Palmitoleic acid (PALM) was identified in 29 accessions and linolenic acid (LIL) in 32 accessions, the highest in Gonondu (0.86 %) and Oleisty Borovskil (0.76 %), respectively. A significant negative relationship between OLE, STE and saturated fatty acids (SFA) with oil content was observed. The Pearson correlation of unsaturated fatty acids to saturated fatty acids ratio (UFA/SFA ratio) with oil content was positive and significant. In the PCA analysis, four major principal components (PCs) were identified, accounting for 87.19 % of the total variations. In PC1, PAL and STE (with positive coefficients) and UFA/SFA ratio (with negative coefficients), had the highest loadings, which determined 41.33 % of the total variations. In PC 2, OLE (with negative coefficient) and LIN (with positive coefficient), had the highest values. According to the dendrogram of the accessions, they were grouped into seven distinct clusters and the accessions in clusters 4 and 7 contained high UFA and low SFA values.

The findings of this study showed that there is a significant genetic diversity among the accessions, which can be used to maximize heterosis in sunflower breeding programs.

**Keywords:** genetic diversity, fatty acids, oil content, sunflower, cultivated accessions

# Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important and most cultivated crops for edible oil ranking fourth in the world (Duca *et al.*, 2013). This crop has high oil and protein content and as an annual oil seed plant has a wide range of adaptation (Afkari Bajehbaj, 2010) and is cultivated in a range of 40 °S to 55 °N and at 0 to 2500 meters above the sea level (depending on the latitude) (Jan and Seiler, 2007).

There are two kinds of sunflower in the world; the first type is for human consumption or as bird feed, known as “confection type”. The second type is an “oil-type sunflower” of high-quality edible oil which accounts for about 90 % of the world’s sunflower seed production (Hladni, 2016). The oil extracted from sunflower seeds is composed of about 90 % of the two unsaturated fatty acids (UFAs) including OLE (18: 1) and LIN (18:2), which is important in controlling blood cholesterol levels and preventing heart disease. The remaining 10 % contains SFAs (STE (18: 0) and PAL (16: 0) plus other fatty acids in a small amount (Gunstone, 2011). In contrast to other edible oils, sunflower oil has high levels of LIN that is resistant to oxidation during storage (Martínez-Force *et al.*, 2015).

Sunflower oil has the potential to be improved in terms of nutritional and economic characteristics (Vear, 2016). The use of of genetic diversity from diverse sunflower germplasms and wild sunflower populations is one of the options for breeders to increase oil content. By collecting, studying and selecting the best populations, the characteristics can be introgressed into cultivated sunflower (Seiler, 2007). Although the amount of seed oil of wild accessions are less than that of cultivated sunflowers, it is possible to bring the oil content to an acceptable level by backcrossing (Seiler *et al.*, 2010a).

In the commercial hybrids, the oleic acid values ranged between 10 and 50 %, depending on the climatic conditions of the field and the temperature of seed growth. A strong negative correlation between oleic and linoleic acids was reported (Fernandez-Martinez *et al.*, 1986; Vranceanu *et al.*, 1995).

The first genotype with a high oleic content was Pervenets variety obtained in the former Soviet Union after treating the seeds with dimethyl-sulphonate (Soldatov, 1976) The oleic acid content of this variety is of about 75 % on an average, although with individual plants this content ranges between 50 and 80 % (Miller and Zimmerman, 1983). and with individual seeds, the variation is often greater, between 19 and 94 % (Urie, 1985). The oil containing a high level of oleic acid is preferred for nutritional use whereas that having higher linoleic content is preferred by the paint or fuel industry. Standard sunflower hybrids contain high linoleic acid, moderate oleic acid and low linolenic acid (Sabrino *et al.*, 2003). Previously, both oil quality and content in sunflower have been well documented by several researchers (Burton *et al.*, 2004; Nolasco *et al.*, 2004). The fatty acid composition changes depending on genotypes and other factors such as environmental conditions, planting and harvesting date (Gupta and Rathore, 1994; Qadir *et al.*, 2006).

The present study examined the genetic diversity of 107 cultivated accessions of sunflower (collected from all over the world). Thus, the objective of the study was to analyze cultivated accessions to see how as Principle Component Analysis (PCA) and Pearson analysis would group and discriminate sunflower accessions from different geographical origins for oil content, fatty acid composition.

## Material and methods

The germplasm used in this study included 107 cultivated sunflower accessions from the USDA-ARS National Plant Germplasm System (NPGS), Beltsville, Maryland, USA (https://npgsweb.ars-grin.gov/gringlobal/search.aspx?) (Table 1).

**Table 1:** Mean of fatty acid composition in 107 accessions of cultivated sunflower.

| Accession | Oil percentage | PAL | PALM | STE | OLE | LIN | LIL | UFA | SFA | UFA/SFA |
|---|---|---|---|---|---|---|---|---|---|---|
| PI 213175 | 33.36 | 6.31 | – | 4.00 | 36.31 | 54.59 | – | 90.89 | 10.31 | 8.82 |
| PI 256334 | 32.10 | 7.75 | 0.05 | 3.00 | 43.07 | 46.47 | – | 89.58 | 10.75 | 8.33 |
| PI 263178 | 27.20 | 5.59 | – | 5.99 | 38.04 | 52.32 | – | 90.36 | 11.58 | 7.80 |
| PI 432512 | 20.34 | 8.66 | – | 5.85 | 21.93 | 65.01 | – | 86.93 | 14.51 | 5.99 |
| PI 432519 | 32.93 | 7.89 | – | 5.79 | 31.64 | 55.84 | – | 87.48 | 13.68 | 6.39 |
| PI 496263 | 31.97 | 9.14 | – | 5.48 | 22.86 | 62.68 | – | 85.53 | 14.63 | 5.85 |
| PI 500688 | 34.08 | 5.53 | – | 4.53 | 35.76 | 54.39 | – | 90.16 | 10.07 | 8.95 |
| PI 507899 | 29.67 | 12.84 | – | 4.94 | 50.35 | 31.99 | – | 82.34 | 17.78 | 4.63 |
| PI 507901 | 34.03 | 6.69 | – | 4.98 | 30.68 | 58.42 | – | 89.09 | 11.67 | 7.63 |
| 371-3 S | 32.06 | 7.22 | – | 4.86 | 32.97 | 55.81 | – | 88.78 | 12.08 | 7.35 |
| 6 Sc Ug L6 | 22.55 | 8.68 | 0.06 | 7.18 | 36.79 | 47.93 | – | 84.78 | 15.87 | 5.34 |
| Advance | 25.95 | 7.90 | – | 3.64 | 51.75 | 37.59 | – | 89.34 | 11.54 | 7.74 |
| Aftab-Parast | 33.73 | 6.58 | – | 6.07 | 22.99 | 65.56 | – | 88.55 | 12.65 | 7.00 |
| Aguapei | 26.91 | 5.81 | – | 5.17 | 50.77 | 37.50 | 0.51 | 88.78 | 10.98 | 8.08 |
| Ames 101 | 31.67 | 7.32 | – | 4.10 | 37.87 | 51.41 | – | 89.27 | 11.43 | 7.81 |
| Ames 10101 | 28.18 | 6.71 | 0.08 | 4.76 | 39.85 | 46.12 | 0.09 | 86.13 | 11.48 | 7.50 |
| Ames 21671 | 34.05 | 6.87 | – | 4.05 | 33.64 | 55.92 | – | 89.57 | 10.92 | 8.20 |
| Ames 2350 | 35.53 | 8.84 | – | 3.94 | 29.32 | 58.07 | – | 87.39 | 12.78 | 6.84 |
| Arge Pehuen | 26.25 | 9.25 | 0.73 | 6.49 | 37.51 | 44.77 | – | 83.01 | 15.74 | 5.27 |
| Armavirsky | 21.83 | 6.78 | 0.06 | 6.55 | 46.72 | 40.82 | 0.75 | 88.35 | 13.33 | 6.63 |
| Arrowhead | 30.88 | 6.81 | – | 4.11 | 38.17 | 49.16 | – | 87.33 | 10.91 | 8.00 |
| Aycicegi | 33.47 | 5.95 | 0.13 | 3.79 | 38.14 | 52.40 | 0.16 | 90.83 | 9.74 | 9.32 |
| B-7422 | 24.23 | 6.72 | 0.08 | 5.39 | 45.73 | 42.45 | – | 88.25 | 12.11 | 7.29 |
| Bekecsi “B” | 31.23 | 5.47 | – | 4.15 | 39.41 | 50.66 | – | 90.07 | 9.63 | 9.36 |
| Black Sayar | 34.53 | 6.59 | – | 4.51 | 31.99 | 57.39 | 0.49 | 89.86 | 11.10 | 8.10 |

(*continued*)

**Table 1:** (*continued*)

| Accession | Oil percentage | PAL | PALM | STE | OLE | LIN | LIL | UFA | SFA | UFA/SFA |
|---|---|---|---|---|---|---|---|---|---|---|
| Cakinslij 321 | 32.90 | 8.96 | 0.12 | 4.52 | 31.79 | 54.67 | 0.13 | 86.70 | 13.49 | 6.43 |
| Cca82-2 | 23.19 | 6.10 | – | 4.56 | 52.85 | 36.47 | – | 89.32 | 10.65 | 8.38 |
| Chang Ling | 27.33 | 5.96 | – | 3.72 | 48.09 | 40.03 | – | 88.12 | 9.68 | 9.11 |
| Cinza 42 | 41.50 | 6.64 | – | 2.95 | 25.00 | 65.37 | – | 90.37 | 9.59 | 9.42 |
| Co-Pb 68 | 36.28 | 5.31 | 0.51 | 2.96 | 38.04 | 52.80 | – | 91.36 | 8.26 | 11.06 |
| Csehszlovakiai “B” | 18.53 | 7.69 | – | 4.85 | 60.14 | 28.82 | – | 88.96 | 12.54 | 7.10 |
| D-75-10 | 14.81 | 6.71 | – | 7.64 | 51.69 | 35.24 | – | 86.93 | 14.36 | 6.06 |
| D-75-4 | 28.17 | 7.88 | – | 7.05 | 25.94 | 58.54 | – | 84.48 | 14.93 | 5.66 |
| Dark Stripe | 32.02 | 8.80 | 0.52 | 4.95 | 33.11 | 53.99 | 0.06 | 87.69 | 13.75 | 6.38 |
| Egnazia | 32.01 | 8.23 | – | 5.22 | 29.33 | 57.42 | – | 86.75 | 13.45 | 6.45 |
| Enisej | 32.28 | 6.28 | – | 4.71 | 34.24 | 55.88 | – | 90.12 | 10.99 | 8.20 |
| France “E” | 25.86 | 6.66 | – | 5.32 | 42.51 | 45.64 | – | 88.15 | 11.98 | 7.36 |
| Fuksinka 10 | 31.22 | 4.98 | – | 3.67 | 42.14 | 48.08 | – | 90.22 | 8.65 | 10.43 |
| Geogia | 40.39 | 6.83 | – | 1.81 | 34.61 | 57.77 | – | 92.38 | 8.64 | 10.69 |
| Gigant 549 | 29.23 | 7.79 | – | 3.98 | 43.14 | 45.89 | – | 89.02 | 11.77 | 7.56 |
| Giza | 29.74 | 5.50 | – | 3.80 | 45.23 | 46.21 | – | 91.44 | 9.30 | 9.83 |
| Gonondu | 34.56 | 5.89 | 0.86 | 3.68 | 38.15 | 51.12 | 0.09 | 90.22 | 9.57 | 9.42 |
| Guayacan Inta | 30.33 | 6.77 | 0.48 | 5.18 | 36.56 | 52.35 | – | 89.39 | 11.95 | 7.48 |
| Hatzor Ayala | 31.86 | 8.48 | 0.14 | 5.00 | 31.96 | 54.27 | 0.27 | 86.65 | 13.47 | 6.43 |
| Havaupai | 31.74 | 5.94 | – | 4.71 | 35.48 | 53.90 | 0.17 | 89.55 | 10.65 | 8.40 |
| Hemas | 18.69 | 5.71 | – | 7.84 | 42.80 | 43.81 | – | 86.61 | 13.55 | 6.39 |
| Hopi | 38.35 | 5.70 | – | 3.90 | 26.57 | 63.87 | – | 90.44 | 9.61 | 9.41 |
| Hopi Dye | 43.66 | 7.07 | – | 2.90 | 20.74 | 69.53 | – | 90.27 | 9.97 | 9.05 |
| Impira Inta | 30.73 | 9.31 | 0.33 | 6.29 | 25.64 | 58.66 | – | 84.63 | 15.60 | 5.43 |
| Jdanovsky6432Nd3 | 31.35 | 7.38 | 0.05 | 4.47 | 36.77 | 52.25 | 0.05 | 89.12 | 11.85 | 7.52 |

(*continued*)

**Table 1:** (*continued*)

| Accession | Oil percentage | PAL | PALM | STE | OLE | LIN | LIL | UFA | SFA | UFA/SFA |
|---|---|---|---|---|---|---|---|---|---|---|
| Jupiter | 30.71 | 8.41 | – | 3.15 | 49.07 | 38.15 | 0.59 | 87.80 | 11.56 | 7.60 |
| Karlik | 36.23 | 7.79 | – | 4.70 | 24.78 | 63.54 | – | 88.33 | 12.49 | 7.07 |
| Kenya White | 31.21 | 8.30 | 0.68 | 6.04 | 27.74 | 56.90 | – | 85.31 | 14.33 | 5.95 |
| Kortus | 32.13 | 8.94 | 0.18 | 4.68 | 31.70 | 53.14 | 0.15 | 85.17 | 13.62 | 6.25 |
| Kosim | 31.69 | 7.64 | 0.06 | 5.79 | 28.08 | 58.54 | 0.23 | 86.91 | 13.43 | 6.47 |
| Krzynowloski | 27.59 | 7.36 | – | 5.61 | 36.75 | 50.53 | – | 87.28 | 12.97 | 6.73 |
| KrzynowloskiMiejs | 26.50 | 5.75 | – | 4.97 | 44.51 | 45.48 | – | 89.98 | 10.72 | 8.39 |
| Kvuglik A-41 | 35.13 | 6.67 | – | 4.79 | 27.90 | 60.87 | 0.24 | 89.01 | 11.46 | 7.77 |
| L-2625-1 | 28.10 | 7.16 | – | 5.85 | 35.00 | 52.98 | – | 87.97 | 13.00 | 6.77 |
| Liao 2 | 25.76 | 8.31 | – | 6.33 | 35.58 | 50.45 | – | 86.03 | 14.64 | 5.87 |
| Lovaszpatonal | 28.65 | 9.17 | – | 5.50 | 33.87 | 52.04 | – | 85.91 | 14.67 | 5.86 |
| Mandan #1 | 32.71 | 6.87 | – | 3.71 | 38.31 | 51.51 | – | 89.82 | 10.58 | 8.49 |
| Mandan #2 | 34.65 | 6.77 | – | 3.17 | 37.81 | 52.84 | – | 90.65 | 9.95 | 9.12 |
| Manfredi Inta | 31.03 | 5.88 | – | 3.46 | 44.73 | 45.25 | 0.38 | 90.36 | 9.34 | 9.68 |
| No. 1879 | 28.28 | 6.02 | – | 4.88 | 41.34 | 48.66 | – | 90.01 | 10.90 | 8.26 |
| No. 2 | 35.30 | 6.83 | – | 5.45 | 22.63 | 65.33 | – | 87.96 | 12.28 | 7.16 |
| No. 2770 | 26.77 | 6.71 | – | 5.75 | 37.87 | 49.48 | – | 87.35 | 12.46 | 7.01 |
| No. 3332 | 32.55 | 7.47 | – | 5.10 | 29.10 | 57.73 | – | 86.84 | 12.57 | 6.91 |
| No.5 | 24.18 | 7.93 | 0.16 | 5.78 | 35.61 | 38.31 | 0.46 | 74.54 | 13.71 | 5.44 |
| No. 9588 | 27.26 | 5.68 | – | 4.41 | 46.38 | 44.23 | – | 90.62 | 10.09 | 8.98 |
| Novosadski Br.4 | 28.80 | 6.25 | – | 5.69 | 35.75 | 53.87 | – | 89.62 | 11.94 | 7.51 |
| Ns-B-16-63 | 28.30 | 9.07 | – | 5.78 | 32.78 | 52.62 | – | 85.40 | 14.85 | 5.75 |
| Odesskij 113 | 28.91 | 5.67 | – | 4.65 | 41.30 | 48.62 | – | 89.92 | 10.32 | 8.71 |
| Oleisty Borovskil | 25.25 | 7.09 | – | 6.81 | 36.99 | 48.84 | 0.76 | 86.58 | 13.91 | 6.23 |
| Peredovik304Ussr6 | 31.61 | 7.42 | – | 6.01 | 25.98 | 58.64 | 0.35 | 84.97 | 13.43 | 6.33 |

(*continued*)

**Table 1:** (*continued*)

| Accession | Oil percentage | PAL | PALM | STE | OLE | LIN | LIL | UFA | SFA | UFA/SFA |
|---|---|---|---|---|---|---|---|---|---|---|
| Pervenets | 31.04 | 8.90 | – | 3.96 | 36.67 | 48.45 | – | 85.12 | 12.86 | 6.62 |
| Record | 34.97 | 8.57 | – | 4.30 | 28.59 | 58.79 | – | 87.38 | 12.87 | 6.79 |
| Relax Hybrid | 32.10 | 8.55 | – | 4.88 | 31.77 | 55.00 | 0.23 | 87.00 | 13.42 | 6.48 |
| Rogress | 32.22 | 9.16 | 0.12 | 4.84 | 31.30 | 54.85 | 0.15 | 86.41 | 14.00 | 6.17 |
| Romsun V3355 | 27.73 | 7.48 | – | 4.80 | 41.80 | 47.02 | – | 88.83 | 12.28 | 7.23 |
| Rosman N-2-2004 | 15.22 | 8.99 | – | 10.72 | 30.43 | 51.07 | – | 81.50 | 19.71 | 4.14 |
| Seneca | 32.28 | 5.78 | 0.06 | 4.17 | 38.41 | 51.35 | 0.33 | 90.15 | 9.95 | 9.07 |
| Sepasol | 24.24 | 7.03 | – | 5.78 | 42.83 | 44.65 | – | 87.48 | 12.81 | 6.83 |
| Skorospelyi | 28.59 | 6.42 | 0.03 | 3.82 | 46.75 | 43.80 | – | 90.59 | 10.24 | 8.85 |
| Smena | 29.58 | 6.81 | – | 6.24 | 31.90 | 56.23 | 0.48 | 88.61 | 13.05 | 6.79 |
| Spannace | 25.26 | 6.66 | – | 7.33 | 31.94 | 54.48 | – | 86.42 | 13.99 | 6.18 |
| Start | 29.16 | 5.53 | – | 4.98 | 39.76 | 51.12 | – | 90.88 | 10.52 | 8.64 |
| Stepnyak | 26.82 | 5.55 | – | 4.95 | 43.01 | 45.56 | – | 88.57 | 10.50 | 8.43 |
| Sundak | 28.63 | 6.88 | 0.16 | 4.29 | 43.68 | 45.11 | 0.08 | 89.04 | 11.17 | 7.97 |
| Sunrise | 29.89 | 5.84 | – | 4.15 | 37.93 | 46.26 | – | 84.18 | 9.99 | 8.43 |
| Szaratovszkij Rann | 30.98 | 6.62 | – | 5.21 | 31.32 | 52.96 | 0.34 | 84.62 | 11.83 | 7.15 |
| Tchernianka Select | 29.70 | 6.78 | – | 4.54 | 38.57 | 48.88 | 0.05 | 87.50 | 11.32 | 7.73 |
| Ussr Mayak ‘66 | 23.10 | 6.64 | – | 6.21 | 44.49 | 44.31 | 0.18 | 88.98 | 12.86 | 6.92 |
| Ussr Mayak 8931 | 24.00 | 7.88 | 0.07 | 6.11 | 42.10 | 45.18 | 0.16 | 87.51 | 14.00 | 6.25 |
| Vir 019 | 31.10 | 7.51 | – | 6.58 | 24.59 | 62.76 | – | 87.35 | 14.08 | 6.20 |
| Vir 160 | 33.00 | 8.44 | – | 4.78 | 29.05 | 56.91 | – | 85.96 | 13.22 | 6.50 |
| Vir 847 | 31.89 | 8.93 | 0.12 | 4.96 | 30.95 | 54.51 | 0.15 | 85.73 | 13.89 | 6.17 |
| Vk-12 | 28.01 | 6.75 | – | 4.36 | 44.65 | 45.59 | – | 90.25 | 11.11 | 8.12 |
| Vk-32 | 23.02 | 11.02 | – | 7.75 | 31.28 | 52.12 | – | 83.41 | 18.76 | 4.44 |
| Vk-47 | 23.32 | 6.98 | – | 3.82 | 55.73 | 32.79 | – | 88.51 | 10.79 | 8.20 |

(*continued*)

**Table 1:** (*continued*)

| Accession | Oil percentage | PAL | PALM | STE | OLE | LIN | LIL | UFA | SFA | UFA/SFA |
|---|---|---|---|---|---|---|---|---|---|---|
| Vniimk 1646 4Mot | 32.37 | 8.48 | – | 5.63 | 26.74 | 60.51 | – | 87.26 | 14.11 | 6.18 |
| Vniimk 6540 | 26.36 | 7.87 | 0.44 | 7.12 | 31.68 | 52.44 | 0.28 | 84.84 | 15.00 | 5.66 |
| Vniimk 8883 | 27.49 | 5.53 | – | 4.97 | 43.79 | 44.57 | 0.64 | 89.00 | 10.50 | 8.47 |
| Voshod | 23.79 | 6.21 | 0.04 | 5.45 | 47.02 | 41.47 | 0.15 | 88.68 | 11.66 | 7.60 |
| W.Y.I/7 | 29.00 | 8.00 | – | 5.85 | 32.44 | 54.78 | – | 87.23 | 13.86 | 6.29 |
| Yawne | 24.38 | 8.80 | 0.15 | 4.55 | 49.09 | 38.19 | – | 87.43 | 13.35 | 6.55 |
| Zaria | 23.72 | 6.25 | 0.04 | 5.81 | 44.75 | 43.83 | – | 88.62 | 12.06 | 7.35 |
| Mean | 29.51 | 7.24 | 0.06 | 5.05 | 36.85 | 50.86 | 0.09 | 87.86 | 12.29 | 7.37 |
| LSD at 5% | 2.80 | 0.95 | 0.08 | 1.05 | 1.43 | 1.25 | 0.13 | 1.98 | 1.44 | 0.90 |

OP: Oil percentage (%), PAL: palmitic acid (%), PALM: palmitoleic acid, STE: stearic acid, OLE: oleic acid, LIN: linoleic acid, LIL: linolenic acid, UFA: unsaturated fatty acids, SFA: saturated fatty acids and UFA/SFA: unsaturated to saturated fatty acid ratio.

The seeds were kept at the temperature of 5 °C and low moisture (less than 20 %). The characteristics of the accessions, including the name, the oil content and fatty acid composition are presented in Table 1.

The study was conducted during the growing season of 2015–2016 at the National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran, located at latitude 35° 74′N and longitude 51° 16′E with a cold and humid climate with an average annual rainfall of 269.9 mm and an average monthly temperature of 17.4 °C.

Oil extraction with Soxhlet was performed using the Seiler (1992) method and using the eq. (1), the oil percentage was calculated.

$$\text{Oil percentage of seeds} = (\text{Weight of extracted oil}/\text{Weight of seeds}) \times 100 \quad (1)$$

Fatty acid composition was determined from 20 achenes from each accession using a Gas Chromatograph (Young Lin model ACME6100 with a column 60 meters in length and 0.25 inches in diameter) using Helium as a carrier gas as described by Baldini *et al.* (2002). Powdered seed samples of 10 to 20 mg were mixed with 200 µl of methanol saturated with KOH and one mL of hexane left undisturbed for 20 minutes until the methyl esterification was completed. Then, 1 µl of the upper phase with 1–100 split was injected into the GC device. The temperature of the column, detector and injection was 180, 280 and 220 °C, respectively.

A fatty acid standard included PAL (C16:0), STE (C18:0), OLE (C18:1), LIN (C18:2), LIL (C18:3) and PALM (C16:1). The fatty acid peaks were identified by comparing the methyl ester fatty acid peaks and the retention time of the standards with peak samples. Auto Chro software was used to calculate the total area of the peaks and the level of each fatty acid expressed as a percentage of the total area. Mean percentage of fatty acids and seed oil was determined by three replicates for all of them.

All ANOVA, LSD, correlation analysis, PCA and grouping using Ward's method and Euclidean second power interval analyzes were done using SAS software version 9.2 and Minitab version 16.

## Results

The oil content and fatty acid composition of the in 107 sunflower accessions are shown in Table 1. The oil content varied from 14.81 % in the D-75-10 to 43.66 %

in the Hopi Dye accession. The composition of fatty acids in the extracted oil was significantly different among the accessions ($P \leq 0.01$). The most important identified fatty acids were PAL, STE, OLE, and LIN. In contrast, PALM and LIL were detected only in 29 and 32 accessions, respectively, while in 64 accessions, both of these fatty acids were not identified.

Among SFAs, PAL varied from 4.98 % in the Fuksinka 10 to 12.84 % in the PI 507899 accession, while STE ranged from 1.81 % in Georgia to 10.72 % in Rosman N-2-2004. UFAs included OLE, LIN, PALM, and LIL, and among them, OLE and LIN were identified in all accessions. LIN was highest in Hopi Dye (69.53 %) and Aftab-Parast (65.56 %), with the lowest in Csehszlovakiai "B" (28.82 %) and PI 507899 (31.99 %). Hopi Dye and PI 432512 accessions with 20.74 and 21.93, respectively, had the lowest values of OLE; while the highest OLE was observed in the accessions Csehszlovakiai "B" (60.14 %) and Vk-47 (55.73 %). The LIL acid was highest in Oleisty Borovskil (0.76 %), while completely absent in 70 % of the accessions. In contrast, PALM acid was not detected in 72 % of the accessions with the highest (0.86 %) found in the Gonondu accession. UFAs were highest in Georgia (92.38 %), Giza (91.44 %) and Co-Pb 68 (91.36 %), while the lowest was in No.5 (74.54 %). Similarly, the highest UFA/SFA ratio was observed in Co-Pb 68 (11.06) and Georgia (10.69), while the lowest ratio was found in Rosman N-2-2004 (4.14). The SFAs varied from 8.26 % in Co-Pb 68 to 19.71 % in the Rosman N-2-2004 accession (Table 1).

Since OLE is one of the most important UFAs in sunflower oil, the distribution of the accessions with respect to OLE are as follow. Table 1 The accessions with less than 30 % were classified as low OLE accessions with 21 accessions. Fifty-two accessions were identified as mid-OLE (having an OLE of 30–40 %). Finally, accessions with more than 40 % OLE acid were classified as OLE-high including 34 accessions.

Correlation between fatty acids and oil percentage (OP) had a positive and significant correlation with LIN and UFA/SFA ratio, while its correlation with OLE, STE and SFA was negative and significant (Table 2). PAL was positively correlated with SFA and STE and its correlation with OLE, UFA, and UFA/SFA ratio was negative ($P \leq 0.01$). STE acid also had similar correlations with PAL. Analysis of OLE correlation showed that its correlation with LIN and SFA was highly negative while the correlation of UFA and UFA/SFA ratio with OLE was positive ($P \leq 0.05$). PALM and LIL had no significant correlation with any of the fatty acids. It should be noted that there was a significant negative correlation between SFA and UFA (Table 2).

The results of the PCA are shown in Table 3. The four main (PC) explained 87.19 % of the total variation among the accessions. Most of the variance was explained by the PC1 (41.33 %), while the second, third, and fourth components

**Table 2:** Pearson correlation between fatty acid composition in 107 accessions of cultivated sunflower.

| | OP | PAL | PALM | STE | OLE | LIN | LIL | UFA | SFA | UFA/SFA |
|---|---|---|---|---|---|---|---|---|---|---|
| OP | 1.00 | | | | | | | | | |
| PAL | −0.07 | 1.00 | | | | | | | | |
| PALM | 0.09 | 0.12 | 1.00 | | | | | | | |
| STE | −0.55** | 0.28** | 0.03 | 1.00 | | | | | | |
| OLE | −0.57** | −0.28** | −0.07 | −0.18* | 1.00 | | | | | |
| LIN | 0.61** | 0.08 | 0.01 | 0.006 | −0.95** | 1.00 | | | | |
| LIL | 0.03 | −0.08 | 0.001 | 0.08 | 0.08 | −0.14 | 1.00 | | | |
| UFA | 0.09 | −0.64** | −0.14 | −0.57** | 0.24* | 0.07 | −0.13 | 1.00 | | |
| SFA | −0.39** | 0.81** | 0.09 | 0.79** | −0.29** | 0.05 | −0.002 | −0.76** | 1.00 | |
| UFA/SFA | 0.34** | −0.78** | −0.07 | −0.76** | 0.29** | −0.03 | −0.05 | 0.80** | −0.96** | 1.00 |

OP: Oil percentage (%), PAL: palmitic acid (%), PALM: palmitoleic acid, STE: stearic acid, OLE: oleic acid, LIN: linoleic acid, LIL, LIL: linolenic acid, UFA: unsaturated fatty acids, SFA: saturated fatty acids and UFA/SFA: unsaturated to saturated fatty acid ratio. * Significant correlation at the 0.05 level. ** Significant correlation at the 0.01 level.

**Table 3:** Eigenvectors and percent explained variation by the first four principal components of fatty-acid profile of 107 cultivated accessions of sunflower.

| Traits | PC 1 | PC 2 | PC 3 | PC 4 |
|---|---|---|---|---|
| Oil percentage | −0.14 | −0.41 | −0.37 | 0.30 |
| Palmitic | 0.39 | −0.05 | −0.29 | 0.11 |
| Palmitoleic | 0.09 | −0.02 | −0.76 | −0.12 |
| Stearic | 0.38 | 0.14 | 0.32 | −0.09 |
| Oleic | −0.19 | 0.59 | −0.15 | −0.10 |
| Linoleic | 0.07 | −0.63 | 0.23 | 0.05 |
| Linolenic | −0.04 | 0.24 | 0.06 | 0.93 |
| Unsaturated fatty acids | −0.39 | −0.05 | 0.15 | −0.06 |
| Saturated fatty acids | 0.49 | 0.06 | 0.02 | 0.02 |
| Unsaturated /saturated fatty acids | −0.49 | −0.06 | −0.02 | −0.05 |
| Eigen value | 4.13 | 2.49 | 1.08 | 1.02 |
| Individual percentage | 41.33 | 24.89 | 10.79 | 10.17 |
| Cumulative percentage | 41.33 | 66.23 | 77.02 | 87.19 |

explained 24.89, 10.79, and 10.17 % of variations, respectively. PC1 had high positive coefficients for PAL, STE and SFA, and a high negative coefficient for UFA/SFA ratio. Accordingly, using the PC1, accessions can be selected that have high SFAs values. PC2 had high positive loadings for high OLE and high negative for oil percentage and LIN. In other words, the accessions selected using PC2 will have high OLE values, while their seed oil and LIN content will be low. PC3 had a high negative coefficient for PALM and PC4 with a high negative coefficient for LIL.

The spatial distribution of sunflower accessions is shown in Figure 1 in relation to the first two PC. Using the main components in axis 1 and 2, 107 accessions were divided into four distinct groups. The accessions in area A had high OLE values and low SFAs, among which the Csehszlovakiai “B”, VK-47 and Cc82-2 were distinct from the others. In area B, accessions were found to have high values in terms of OLE, PAL and STE, among which the PI 507901, Rosman N-2-2004 and Vk-32 accessions. The accessions located in Area C have lower values of OLE, PAL and STE, while the oil percentage and LIN are high in Hopi Dye, Cinza 42, Hopi, Geogia and Co-Pb 68’s accessions.

The grouping of the accessions was done using Ward’s method and squared Euclidean distance (Figure 2). Discriminant analysis was used to accurately construct the groups in the dendrogram and determine the number of clusters. Based on Wilk’s-Lambda statistic, the accessions were divided into 7 groups (Table 4). The first major cluster (A) consists of seven accessions divided into two

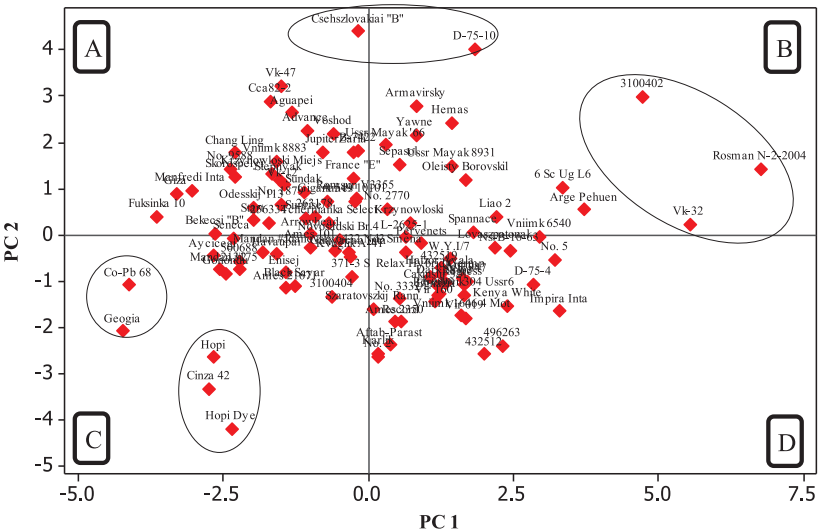


**Figure 1:** The ordination of 107 cultivated accessions of sunflower on principal component (PC) axes 1 and 2 using cluster analysis of the fatty acid profile.

subgroups. Sub-group A-1 contained only one accession, No. 5. Subgroup A-2 included six accessions including Dark Stripe, Kenya White, Impira Inta, Guayacan Inta, Vniimk 6540 and Arge Pehuen. The accessions in cluster A had high values for PALM (0.47 %) and LIL (0.11 %). In terms of oil content, PAL, STE and LIN also had a relative superiority compared to the accessions mean. In general, the accessions of this cluster were rich in SFAs, while the levels of OLE, UFA and UFA/SFA ratio were low. In cluster A, the accessions of the Dark Stripe and Kenya White were the most similar located at a distance of 3.73.

The second main cluster (B) was divided into four subgroups B-1, B-2, B-3 and B-4, consisting of 4, 12, 9 and 9 accessions, respectively. Subgroup B-1 consisted of accessions Szaratovszkij Rann, Kosim, Peredovik304, Ussr6, and Smena. The subgroup B-2 was subdivided into two sub-sections, with accessions of Ames 2350, Record, Vir 160, Egnazia, No. 3332 and Pervenets belonging to sub-group B-2-1. Subgroup B-2-2 included Cakinslij 321, Rogress, Vir 847, Kortus, Relax Hybrid Germp and Hatzor Ayala. Sub-group B-3 included Karlik, No. 2, Aftab-Parast, PI 432512, PI 496263, Vniimk 1646 4 Mot, Vir 019, D-75-4 and Spannace. Accessions of PI 432519, W.Y.I/7, L-2625-1, Krzynowloski, No. 2770, Lovaszpatonal, Ns-B-16-63, Liao 2 and 6 Sc Ug L6In constituted subgroup B-4. Accessions in cluster B had the highest LIN (56.33 %) and the lowest OLE

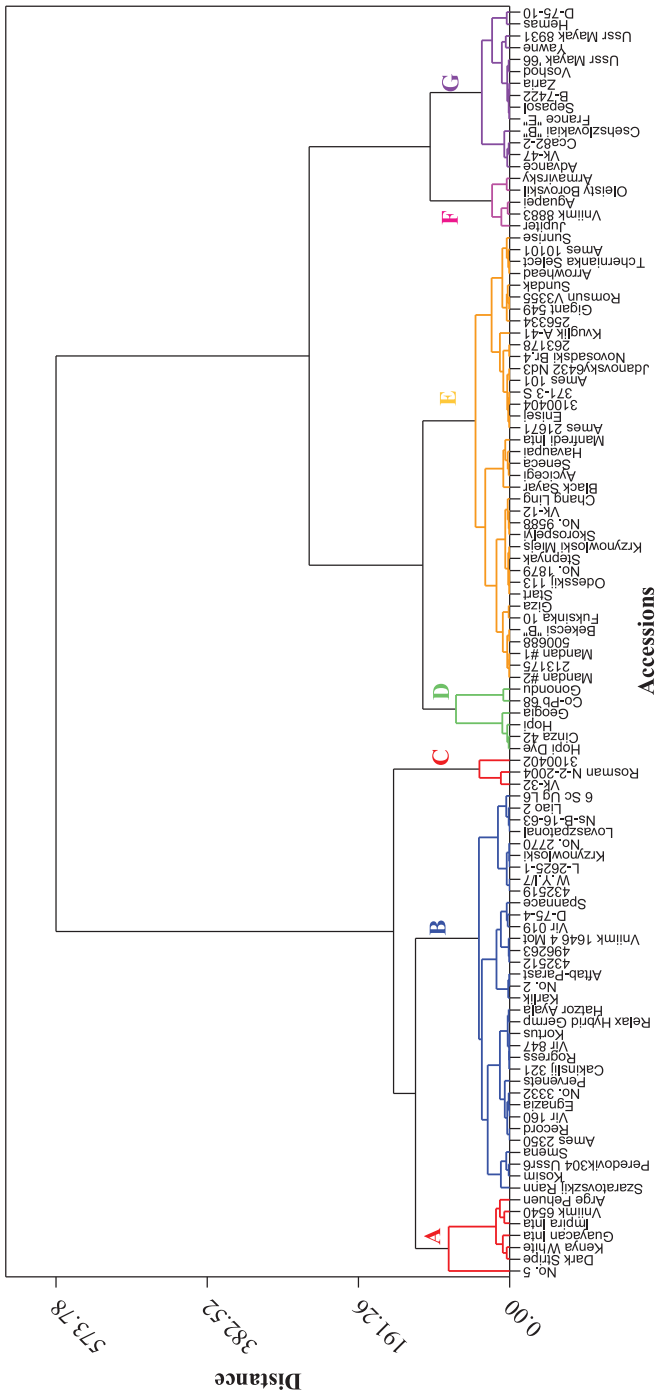


**Figure 2:** Cluster analysis of 107 wild accessions of cultivated accessions of sunflower in terms of oil percentage and fatty acid compositions using Ward method and squared Euclidean distance.

**Table 4:** Results of Wilk's Lambda statistic for determination of cluster numbers.

| No. of clusters | Wilk's Lambda | Chi-square | df | Sig. |
|---|---|---|---|---|
| 2 | 0.001 | 697.147 | 56 | 0.000 |
| 3 | 0.006 | 509.776 | 42 | 0.000 |
| 4 | 0.029 | 345.635 | 30 | 0.000 |
| 5 | 0.103 | 222.639 | 20 | 0.000 |
| 6 | 0.255 | 134.104 | 12 | 0.000 |
| 7 | 0.545 | 59.441 | 6 | 0.000 |
| 8 | 0.986 | 1.394 | 2 | 0.498 |

(30.17 %) among the seven clusters. Relative to the overall mean, this cluster had a positive deviation in terms of oil content, PAL, STE, LIN and SFA, and negative deviations in terms of PALM, LIL, UFA and UFA/SFA ratios. Therefore, these accessions contain more SFAs than UFAs. The third main cluster (C) included three accessions of Vk-32, PI 507899 and Rosman N-2-2004, which had the highest levels of saturated fatty acids (PAL, STE and SFA) among the clusters, while oil percentage, of UFA and UFA/SFA ratio were the lowest. These accessions also lacked PALM and LIL. The fourth cluster (D) that contained 6 Hopi Dye, Cinza 42, Geogia, Hopi, Co-Pb 68 and Gonondu accessions, had the highest values in terms of oil percentage, UFA and UFA/SFA ratio and their PAL, STE, LIL and SFA values were the lowest of the seven clusters.

The 5th cluster (E) contained 38 accessions, which showed a positive deviation in terms of oil content, OLE, UFA and UFA/SFA ratio, while in terms of other parameters, they had negative deviations. The E cluster was divided into two subgroups E-1 and E-2. Subgroup; E-1 was divided into three subgroups E-1-1 (included Mandan # 2, 213,175, Mandan # 1, PI 500688, Bekecsi B, Fuksinka 10, and Giza), subgroup E-1-2 (included Start, Odesskij 113, No. 1879, Stepnyak, Krzynowloski Miejs, Skorospelyi, No. 9588, Vk-12 and Chang Ling); and subgroup E-1-3 (consisted of Black Sayar, Aycicegi, Seneca, Havaupai and Manfredi Inta). The subgroup E-2 was also subdivided into two subgroups E-2-1 and E-2-2. Nine accessions including Ames 21671, Enisej, PI 507901, 371-3 S, Ames 101, Jdanovsky6432 Nd3, Novosadski Br.4, 263,178 and Kvuglik A-41 were placed in the subgroup E-2-1; while the sub-group E-2-2 included eight accessions of PI 256334, Gigant 549, Romsun V3355, Sundak, Arrowhead, Tchernianka Select, Ames 10101 and Sunrise.

The accessions of Jupiter, Vniimk 8883, Aguapei, Oleisty Borovskil and Armavirsky were placed in cluster 6 (F) with the lowest PALM (0.012 %) and the highest LIL (0.65 %) among other clusters, and the high percentage of OLE.

In addition, accessions of this cluster showed a positive deviation for STE, UFA and UFA/SFA ratio, while their derivation from total means was negative in terms of oil content, PAL, LIN, and SFA.

Finally, in the 7th cluster (G), the G-1 subgroup included Advance, Vk-47, Cca82-2, and Csehszlovakiai "B" accessions. Subgroup (G-2), included the accessions France E, Sepasol, B-7422, Zaria, Voshod, Ussr Mayak '66, Yawne, Ussr Mayak 8931, Hemas and D-75-10. The accessions in the cluster G had the highest OLE (48.10 %) and the lowest LIN (40.03 %). These accessions, which lacked PALM and LIL, had a higher mean for STE, UFA and SFA than the total mean. Also, their mean oil percentage, PAL and UFA/SFA ratio was less than the total average.

## Discussion

In the present study, high oil concentration of 43.66 % in the accession Hope Dye from France and 41.50 % for the Cinza 42 from Colombia were observed approximately equal to the value in sunflower hybrids (44–48 %). Usually, high levels of LIN in sunflower oil occur in areas where plants have not experienced high temperatures during flowering, achene filling and maturation (Hu *et al.*, 2010). In the present study, the highest LIN was found in Hopi Dye accessions from Arizona, USA, Aftab-Parast from Afghanistan and Cinza 42 from Colombia.

LIN and OLE are two important fatty acids in sunflower oil. The introgression of sunflower with different fatty acid profiles and a stable linoleic concentration could facilitate the expansion of commercial sunflower production into the southern latitudes and increases the available genetic diversity for improving cultivated sunflower (Seiler *et al.*, 2010b).

OLE, another important UFA in sunflower oil varied among the accessions in the present study with the highest levels found accessions originating from the Eastern European region. On the other hand, the lowest OLE was identified in accessions originating from Arizona, USA (Hopi Dye and PI 432512).

There was also a high diversity in the concentration of PAL and STE among 107 accessions (4.98–12.84 %).

In the current study, there was a negative and significant correlation between LIN and OLE acid, meaning that in the cultivated accessions of *H. annuus*, high levels of LIN (greater than 65 %) were associated with low OLE values (less than 22 %). On the other hand, the high correlation between these two fatty acids suggests that there is a possibility for negative simultaneous selection in the breeding programs of sunflower. These results are consistent with the results reported by Tahmasebi-Enferadi *et al.* (2004) and Seiler

(2007). Also, the negative correlation between SFAs and UFAs suggests that the choice for UFAs levels simultaneously reduces levels of SFAs.

Four PCA groups were identified with a decreasing number of variables. In the first group, PAL, STE, SFA and UFA/SFA ratio were the best choice because they had the largest coefficients. For the PCA, the OLE, seed oil percentage and LIN are the best choices, while the PALM and LIL acids had high loadings for the PC3 and PC4, respectively. As a result, from 10 initial variables (which have significant internal correlations), four variables can be replaced, which are components of the original weight and are independent of each other.

The spatial distribution of 107 accessions used in this study and dendrogram suggest that there is a significant genetic variation in terms of oil content and fatty acids composition. It also suggests that some plant varieties that are morphologically different may be genetically related. The sunflower core collection developed by Brothers and Miller (1999) consisted of 112 sunflower cultivars, grouped into 10 clusters. The distinct accessions may have a high breeding value, and the populations in the same clusters represent members of a heterotic group. Seiler *et al.* (2010a) stated that the greatest diversity could be obtained by selecting in segregated populations, the accessions in different clusters to be used as parents. Therefore, the information obtained from genetic variation in the present study will be useful in improving the quality and quantity of sunflower oil.

## Conclusion

The results of this study showed that 107 cultivated accessions of *Helianthus annuus* are genetically different for oil content, fatty acids composition. The accessions of Csehszlovakiai "B", Vk-47, Cca82-2, Advance and D-75-10 (with the highest OLE acid) and the accessions of Geogia, Giza and Co-Pb 68 (with the highest levels of UFAs) may be useful for commercial sunflower improvement. In addition, the cross between accessions in different clusters may lead to heterosis for improving the quality of sunflower oil.

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# References

Afkari Bajehbaj, A., 2010. Effect of water limitation on grain yield of Sunflower (*Helianthus annuus* L.) cultivars. Journal of Food, Agriculture and Environment 1: 132–135.

Baldini, M., Giovanardi, R., Tahmasebi-Enferadi, S., Vannozzi, G.P., 2002. Effects of water regime on fatty acid accumulation and final fatty acid composition in the oil of standard and high oleic sunflower hybrids. Italian Journal of Agronomy 6(2): 119–126.

Brothers, M., Miller, J., 1999. Core subset for the cultivated sunflower collection. *In:* Paper presented at the Proceedings of 21st Sunflower Research Workshop. Fargo, ND.

Burton, J.W., Miller, J.F., Vick, B.A., Scarth, R., Holbrook, C.C., 2004. Altering fatty acid composition in oil seed crops. Advances in Agronomy 84(1): 273–306.

Duca, M., Port, A., Şestacova, T., Siniauskaya, M., Aksyonova, E., Davydenko, O., 2013. Microsatellite marker application in sunflower (*Helianthus annuus* L.) fingerprinting. Biotechnology and Biotechnological Equipment 27(3): 3772–3775.

Fernandez-Martinez, J., Munoz, J., Jimenez-Ramirez, A., Dominguez- Jimenez, J., Alcantara, A., 1986. Temperature effect on the oleic and linoleic acid of three genotypes in sunflower. Grasas Aceites 37(3): 327–333.

Gunstone, F., 2011. Vegetable Oils in Food Technology: Composition, Properties and Uses, John Wiley & Sons, Oxford, UK.

Gupta, S.S.D., Rathore, V.S., 1994. Influence of sowing dates on yield and oil quality in sunflower. Journal of Agronomy and Crops Science 172(2): 137–144.

Hladni, N., 2016. Present status and future prospects of global confectionery sunflower production. *In:* Paper presented at the Proceedings of the 19th International Sunflower Conference.

Hu, J., Seiler, G., Kole, C., 2010. Genetics, Genomics and Breeding of Sunflower, CRC Press, Clemson, SC, USA.

Jan, C., Seiler, G., 2007. Sunflower. Genetic Resources, Chromosome Engineering, and Crop Improvement 4: 103–165.

Martínez-Force, E., Dunford, N.T., Salas, J.J., 2015. Sunflower: Chemistry, Production, Processing, and Utilization, Academic Press and AOCS Press, Urbana, IL, USA.

Miller, J.F., Zimmerman, D.C., 1983. Inheritance of high oleic fatty acid content in sunflower. *In:* Proc. Sunfl. Research Worshop, Fargo N.D. 26 January.

Nolasco, S.M., Aguirrezabal, L.A.N., Crapiste, G.H., 2004. Tocopherol oil concentration in field-grown sunflower is accounted for by oil weight per seed. Journal of the American Oil Chemists Society 81(11): 1045–1051.

Qadir, G., Ahmad, S., Hassan, F.U., Cheema, M.A., 2006. Oil and fatty acid accumulation in sunflower as influenced by temperature variation. Pakistan Journal of Botany 38: 1137–1147.

Sabrino, E., Tarquis, A.M., Diaz, M.C., 2003. Modelling the oleic acid content in sunflower oil. Agronomy Journal 95(2): 329–334.

Seiler, G., 2007. Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower. Industrial Crops and Products 25(1): 95–100.

Seiler, G., Jan, C-C., 2010a. Basic information. *In:* Hu, J., Seiler, G., Kole, C. (eds.) Genetics, Genomics and Breeding of Sunflower. CRC publication, Clemson, SC, USA, pp. 1–40.

Seiler, G.J., 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops Research 30(3–4): 195–230.