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Original article

Phenotypic Variability and Correlation of Fatty Acids in Seed Oil of Sunflower Genetic Collection Lines of VNIIMK

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Abstract

Studying the full profile of fatty acids in seed oil from 52 lines of the VNIIMK sunflower genetic collection was the goal of this work. The composition of ten fatty acids in the seed oil was analyzed using gas-liquid chromatography of fatty acid methyl esters. The content of fatty acids (%) in the seed oil was: palmitic acid on average 6.3 with a range from 3.0 to 27.7; palmitoleic 0.3 (0.03-5.4); stearic 3.9 (1.1-10.4); oleic 51.5 (14.0-91.4); linoleic 32.2 (0.8-71.1); linolenic 0.05 (0.01-0.1); arachidic 0.3 (0.1-0.6); eicosenic 0.2 (0.1-0.3); begenic 1.0 (0.4-2.2); lignoceric acid 0.3 (0.1-0.6). The coefficient of variation ranged from 27% for lignoceric acid to 59% for palmitic acid, with an average CV of 40%. Of the 45 studied pairs of connections between fatty acids, reliable correlation coefficients were established for 20 pairs of characteristics, 10 positive (from 0.917 to 0.281) and 10 negative (from -0.974 to -0.315) values. The highest positive value of the correlation coefficient was noted for the palmitic-palmitoleic acid pair, and the most negative for the oleic-linoleic acid pair. The discovered wide variation in the content of each fatty acid in the lines of the sunflower genetic collection will allow the lines to be used both in researches on biochemical genetics and in practical breeding for oil quality.

Keywords: fatty acid, variability, correlation, oil

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INTRODUCTION

Sunflower is one of the main oilseeds in the world, ranking fourth in terms of production with 9.2% of the world's vegetable oils (Pilorgé 2020). Traditional sunflower oil contains up to 85–90% unsaturated (monounsaturated (MUFA) and polyunsaturated (PUFA) and 10–15% saturated (SFA) fatty acids (Vegetable oils ... 2002). The main acids included in the oil are palmitic, stearic, oleic and linoleic. Their biosynthesis takes place in three successive stages: C16:0 elongates to C18:0, the latter is desaturated to C18:1, which is then desaturated to C18:2 (Serrano-Vega et al. 2005; Rozhon et al. 2023). Minor fatty acids included in sunflower oil include palmitoleic, linolenic, arachidic, eicosenoic, behenic, lignoceric fatty acids, and the content of each of them ranges from 0.01 to 3%.

Expansion of the sunflower genetic collection and breeding work became possible with the discovery of mutations in the content of oleic, palmitic and stearic fatty acids. In 1976, for the first time, as a result of chemical mutagenesis, the Pervenets variety was created, the seeds of which had an increased content of oleic acid of about 85% (Soldatov 1976). Then, high-palmitic (C16:0) and high-stearic (C18:0) mutants were chemically induced. The high palmitic line CAS-5 had 25% C16:0 and a high content of palmitoleic acid (C16:1) up to 5%. The high-stearic line CAS-3 contained more than 25% of C18:0, and the line CAS-14 was characterized by a very high content of C18:0 up to 37% (Ivanov et al. 1988; Osorio et al. 1995; Fernandez-Moya et al. 2002). In 2013, using X-rays, a new high-oleic mutation was induced, which was more thermostable compared to the mutation from Pervenets. Line NM1 contained on average up to 92.5% oleic acid (Alberio et al. 2015). Another high oleic mutation has been described recently (Rozhon et al. 2023).

At VNIIMK, lines with a mid oleic acid content that do not carry the *Ol* mutation were selected and described (Demurin et al. 2020). New high-stearic acid donors with stearic acid content from 11 to 25% on a linoleic and high-oleic background have also been obtained (Demurin et al. 2022).

Studying the full profile of fatty acids in seed oil from the lines of the VNIIMK sunflower genetic collection was the goal of this work.

MATERIALS AND METHODS

The study was carried out at the central experimental base of the Federal State Budgetary Institution Federal Scientific Center (VNIIMK), Krasnodar, including field experiments and laboratory tests. 52 inbred sunflower lines from a genetic collection of different origins and possessing hereditarily controlled traits were selected as material for the study.

In the field conditions of 2022, the constant lines of the collection were propagated in the breeding nursery of the genetics laboratory using forced self-pollination of plants under individual perforated bags made of agrospanbond. The resulting seeds were used in laboratory analysis in 2023 based on the instrumentation of the biochemistry laboratory.

Analysis of the composition of ten fatty acids in seed oil was carried out using gas-liquid chromatography of methyl esters of fatty acids on a Khromatek-Kristall 2000 device with an automatic dispenser DAZH-2M on a capillary column SolGelWax 30 m × 0.25 mm × 0.5 μm in a flow of carrier gas - helium, with speed 22 cm/s, with temperature programming within 170-230 °C. The preparation of methyl esters and their chromatography were carried out in accordance with regulatory methods (GOST 31663-2012; GOST R 31665-2012).

To determine the fatty acid composition of average samples, 15 achenes per sample were used. The achenes were cleared of husks, and the seeds (kernels) were crushed using a mortar and pestle or a laboratory mill. A sample of 0.2 g was taken and 3 ml of hexane was added. The tubes were kept at room temperature for 60 minutes with periodic shaking. Afterwards, the upper hexane layer was removed and 0.5 ml of a solution of KOH(3N) in methanol was added. Next, the contents were mixed well using a Vortex Mixer. The test tubes were left for 15 minutes to separate the contents, after which the liquid was poured into clean test tubes through glass funnels with filter paper and 20 mg of Na_2SO_4. Next, 0.6 ml of sample was taken into chromatographic vials. Chromatograms were processed using the Chromatek Analyst program.

Mathematical processing of the results was carried out using the R software environment version 4.2.3 (R Core Team, 2023) and the standard “stats” package. Data visualization was carried out using the packages “ggplot2” (Wickham, 2016) and “ggcorrplot” (Kassambara, 2022).

RESULTS AND DISCUSSION

The content of palmitic acid (C16:0) in the seed oil averaged 6.3%, varied from 3.0 to 27.7% and CV = 59%. Two lines were distinguished by a mutational increased value of palmitic acid: VK850 - 27.7% and VK805 - 17.7% on a linoleic and oleic background, respectively. The same lines showed a maximum content of palmitoleic acid (C16:1) up to 5.4% with an average value for all lines of 0.3% and a minimum of 0.03% (Fig. 1 and Fig. 2).

The amount of stearic acid (C18:0) varied from 1.1% in VK805 to 10.4% in VK276 with an average value of 3.9% and CV = 43%.

The average oleic acid (C18:1) value for the entire collection was 51.5% with CV = 38%. Eight lines with the high oleic *Ol* mutation showed the maximum amount of this fatty acid from 85.4% in LG26 to 91.4% in RIL41. Mid-oleic lines had intermediate values of the trait from 66.3% in LG27 to 69.7% in VK805. The minimum oleic acid content of 14.0% was observed in the VK850 line.

Linoleic acid (C18:2) showed an average value of 36.2% for all lines in the collection with CV = 51%. The limits were 0.8 and 71.1% for VK876 and K3159, respectively.

The content of linolenic acid (C18:3) was very low, on average 0.05% and varied from 0.01 in VK876 to 0.11 in VK850 with CV = 40%.

Arachidic acid (C20:0) varied from 0.1 to 0.6% in RIL41 and BK276, respectively, with CV = 34%. The average value was 0.3%.

Eicosenoic acid (C20:1) varied from 0.1 to 0.3% in VK850 and VK464, respectively, with CV = 33%. The average was 0.2%.

Behenic acid (C22:0) varied from 0.4 to 2.2% in lines K3159 and KG19 with CV = 34%. The average value was 1.0%.

Lignoceric acid (C24:0) varied from 0.1 to 0.6% in lines RIL200 and K3619 with CV = 27%. The average value is estimated at 0.3%.

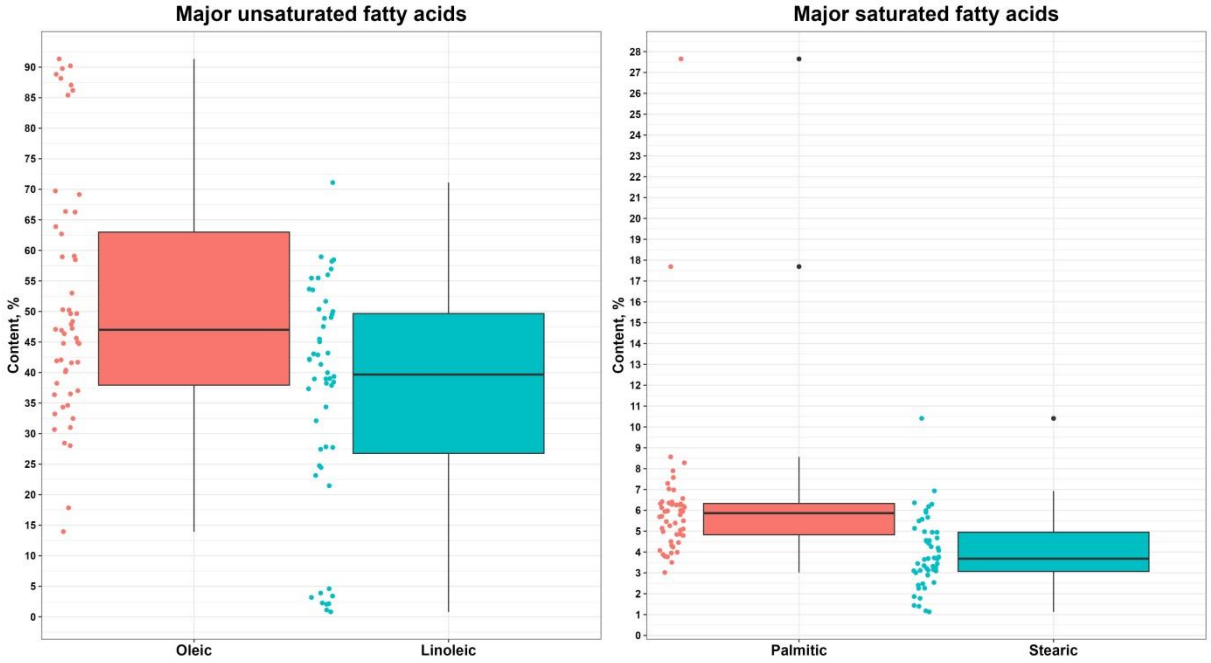

Figure 1. Major fatty acids in seed oil of sunflower genetic collection lines (quartile distribution)

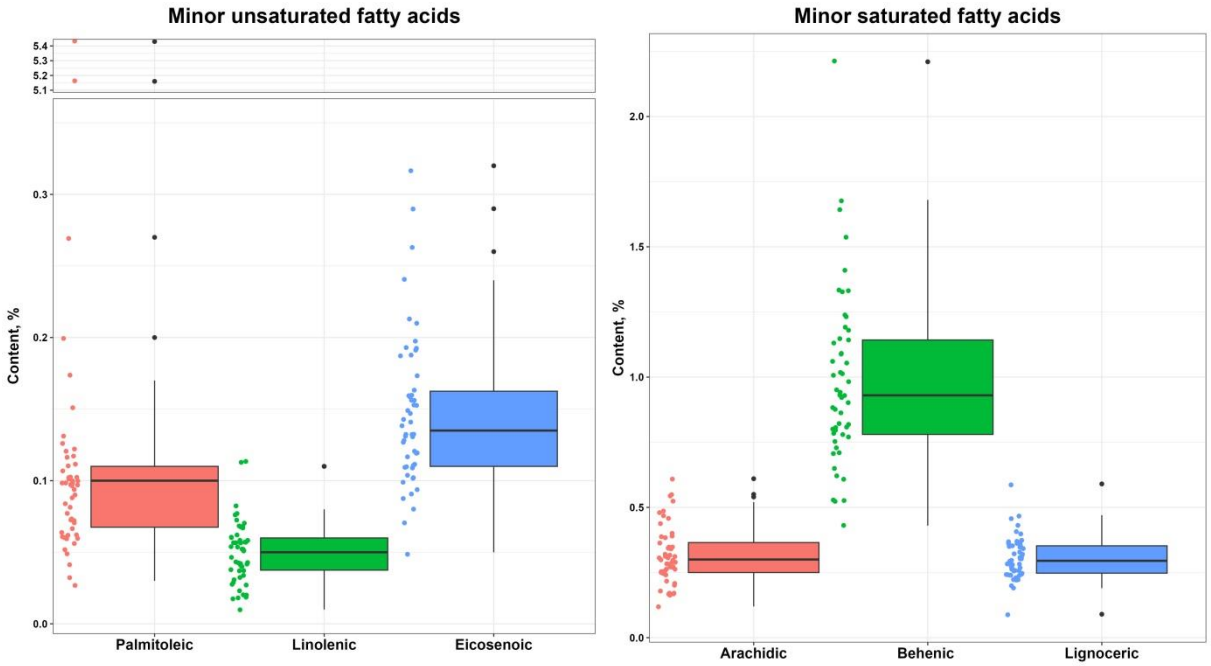

Figure 2. Minor fatty acids in seed oil of sunflower genetic collection lines (quartile distribution)

A significant positive correlation was established for pairs of traits: palmitic - palmitoleic (0.917), stearic - arachidic (0.908), oleic - eicosenic (0.709), palmitic - linolenic (0.484), arachidic - behenic (0.484), linoleic - linolenic (0.377), stearic - behenic (0.377), behenic - lignoceric (0.361), palmitoleic - linolenic (0.351) and oleic - lignoceric (0.281) (Fig. 3).

On the other hand, a significant negative correlation was found: oleic - linoleic (-0.974), linoleic - eicosenic (-0.647), stearic - eicosenic (-0.447), oleic - linolenic (-0.441), palmitic - oleic (-0.397), arachidic - eicosenic (-0.336), palmitic - eicosenic (-0.325), palmitoleic - stearic (-0.324), stearic - linolenic (-0.321) and linoleic - lignoceric (-0.315).

The maximum positive values of the correlation coefficient for palmitic and palmitoleic acids, as well as stearic and arachidic acids, probably reflect an increase in the content of the substance that follows in biosynthesis with an increase in the content of the precursor, namely C16:0-C16:1 and C18:0-C20:0, respectively.

The maximum negative value of the correlation coefficient for oleic and linoleic acids, observed by many researchers, is obviously explained both by the genetic block in the biosynthesis of C18:1-C18:2, and by the strong influence of temperature on this desaturation reaction. In addition, since these two acids are the main ones, reaching a total of 90% in the composition of fatty acids of the oil, the negative correlation between their percentage content can be caused by an arithmetic reason.

Figure 3. Correlation coefficient for the content of fatty acids in seed oil in the lines of the sunflower genetic collection

CONCLUSION

A wide variation in the content of each of the 10 fatty acids was found in 52 lines of the sunflower genetic collection. The coefficient of variation varied from 27% for lignoceric acid to 59% for palmitic acid, with an average CV of 40%. Of the 45 studied pairs of connections between fatty acids, significant correlation coefficients were established for 20 pairs of characteristics, 10 positive (from 0.917 to 0.281) and 10 negative (from -0.974 to -0.315) values. All the data obtained can be used in practical breeding for the quality of oil in sunflower seeds.

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