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Variability and inheritance of high stearic acid content in the seed oil of sunflower inbred lines

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Abstract: The main purpose of the paper included research on the variability of stearic acid content in the seed oil of the accessions of the V.S. Pustovoit All-Russian Research Institute of Oil Crops (VNIIMK) sunflower genetic collection and to determine the mode of inheritance of the trait in F_1 generation for choosing a hybrid breeding strategy. Gas-liquid chromatography of methyl esters and infra-red spectrometry were used for the estimation of fatty acid profiles. The inbred lines of I_5 seeds LG31, LG32, LG33, LG34 and LG35 have been developed with high stearic acid content from 11.6 to 22.6%. Lines LG31 and LG32 showed relatively high values of oleic acid in the range of 56.7–70.5%. Lines LG33 and LG34 belonged to a group with a content of oleic acid in the range of 11.4–19.9%. An inbred line LG35 possessed increased content of stearic acid up to 11.6% on the low oleic background. The recessive type of inheritance of the mutations with high content of stearic acid in sunflower seed oil was verified. Both complete and incomplete dominance degree of standard content of stearic acid was found. Intermediate inheritance in F_1 of this character was firstly distinguished in the cross of standard with high stearic acid line LG35.

Keywords: fatty acid composition; inheritance; stearic content.

Introduction

Traditional sunflower oil contains four main fatty acids – palmitic $C_{16:0}$, stearic $C_{18:0}$, oleic $C_{18:1}$ and linoleic $C_{18:2}$ (6, 4, 30 and 60%, respectively). There is a possibility of developing various types of sunflower oil based on the mutant

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lines: low saturated (<7%), high palmitic (>25%), high stearic, HS (>25%), high oleic, HO (>85%), high linoleic, HL (>75%) and some of their combinations (Fernández-Martínez et al. 2007; Osorio et al. 1995). For the food industry, there is evidence that stearic acid belongs to desirable saturated fatty acid (Bootello et al. 2012; Valenzuela et al. 2011) because it does not affect the amount of cholesterol in the human blood (Anushree et al. 2017; Mensink 2005).

Results from the study by Kelly et al. (2001) indicate that stearic acid (19 g/day) in the diet has favourable effects on thrombogenic and atherogenic risk factors in males. The authors recommend that the food industry consider enriching foods with stearic acid instead of palmitic acid and trans fatty acids. Thus, stearic acid is nontoxic and biocompatible to the human body.

A healthy diet requires stable and semi-solid oils at room temperature with a long shelf life and without trans fats. Palm oil is widely used in the food industry due to its low cost, high oxidative stability index, long shelf life and lack of trans fats. However, increased palm oil production is damaging ecosystems and threatening human health. Excessive consumption of palmitic acid increases the level of "bad" cholesterol and the risk of cardiovascular disease. Stearic acid was shown to have no negative effect on health (Kelly et al. 2001; Van Rooijen and Mensink 2020).

In 1999, Spanish scientists carried out research on the inheritance mode of the high stearic acid content of about 25% in the CAS-3 mutant line of sunflowers. It was shown that this trait is managed by recessive alleles at two loci *Es1* and *Es2*. The *Es1* locus has a greater influence on the $C_{18:0}$ content than *Es2*. A low stearic acid value showed partial dominance (Pérez-Vich et al. 1999, 2004). In 2006, the hybridization of the super high stearic line (about 40%) CAS-14 and the line with the normal content of this acid was made, and the data were obtained that a very high stearic acid content is determined by another recessive gene *Es3* (Pérez-Vich et al. 2006). All mutations of high stearic acid content were obtained using chemical mutagenesis (ethyl methanesulfonate, sodium azide). From a biochemical point of view, the oil from high-stearic sunflower seeds is a new source of saturated fats (Fernandez-Moya et al. 2002; Pleite et al. 2006; Salas et al. 2014).

A high stearic (16.3%) high oleic (70.7%) sunflower hybrid of HSHO type was developed by traditional breeding methods without the use of genetic engineering. The seeds of the HSHO hybrid are currently produced by the company Advanta Semillas S.A.I.C., Argentina, under the trade name Nutrisun (Anushree et al. 2017).

The main purpose of this paper included research on the variability of stearic acid content in the seed oil of accessions of the VNIIMK sunflower genetic collection and to determine the mode of inheritance of the trait in the F_1 generation for choosing a hybrid breeding strategy.

Materials and methods

The research was carried out in the experimental field, greenhouse and biochemical laboratory of VNIIMK, Krasnodar, Russia in 2018–2020. The accessions of sunflower genetic collection were used.

Plants of a segregating population of a high stearic specimen of sunflowers were grown under field conditions. The sowing scheme was 70 × 35 cm with a plant density of 40,000/ha. At the beginning of flowering, the plants were isolated with individual mesh bags and self-pollinated. Hybridization was performed under individual mesh bags using manual emasculation.

In each generation of inbreeding (I_1–I_5) and the F_1 seeds, the content of stearic acid was assessed in a bulk sample and individual seeds. The profiles of fatty acids were estimated in the laboratory of biochemistry using the method of gas-liquid chromatography of methyl esters on a Chromatek–Kristall 5000 device and IR spectrometry on an MATRIX-I Bruker Optics apparatus as standard.

The degree of dominance of the trait of stearic acid content was calculated using the formula developed by Mather and Jinks as the ratio h/d (Mather and Jinks 1982). Statistical processing was performed using ANOVA applications in Excel.

Results and discussion

A specimen of the genetic collection with high stearic acid content in the seed oil was found in 2018. This genotype was segregated after self-pollination for the branching, male fertility restoration, high oleic content and high stearic acid content traits.

After using the IR spectrometry method, 400 sunflower seeds of the I_2 and I_3 generations were analyzed in 2019. From the analyzed seeds, 17 ones with a high level of stearic acid were selected for planting under greenhouse conditions in the winter period of 2019–2020. As a result, seven HSHO lines of the I_4 generation were obtained, two of which were branched and five possessed single-head. The other three belonged to HSLO (high stearic low oleic) lines. In 2020, I_4 seeds were sown in the field for selection based on morphological characteristics and further self-pollination.

Thus, four lines of I_5 seeds LG31, LG32, LG33 and LG34 have been developed (Table 1). Estimation of profiles of fatty acids in the oil of individual seeds of the lines confirmed high stearic acid content from 18.6 to 22.6%. Moreover, these lines differed in the content of oleic acid. The HSHO type was presented by lines LG31 and LG32 with relatively higher values of oleic acid at the level of 56.7–70.5%. Lines LG33 and LG34 belonged to a group of HSLO with a content of oleic acid in the range of 11.4–19.9%.

Table 1: The profiles of fatty acids in the seed oil of sunflower inbred lines.

Line	Content of fatty acid, %				
	Palmitic	Stearic	Oleic	Linoleic	Others
LG31	4.8	18.6	70.5	2.2	3.9
LG32	6.0	19.3	56.7	13.8	4.2
LG33	6.0	22.6	11.4	57.3	2.7
LG34	6.0	21.6	19.9	49.3	3.2
LG35	5.8	11.6	42.4	37.9	2.3
VK101 st	5.6	6.1	46.5	39.8	2.0
VK580 st	4.8	3.2	38.7	51.8	1.5
VK680 st	3.4	3.1	90.1	1.3	2.1
LSD_{05}	0.3	1.4	5.4	5.3	–

An inbred line LG35 was another specimen with high stearic acid content at the level of 11.6% which derived from the line VK276 after directed selection for the trait. Three lines VK101, VK580 and VK680 were used as a standard with normal stearic acid content varied from 3.1 to 6.1% (Table 1). Besides, the last genotype was high in oleic acid content up to 90.1%.

The high stearic acid content of 18.6% in the seed oil of the line LG31 in the cross with VK680 showed in F_1 the mean value of 3.1% of the trait. This matched the complete dominance degree of the wild type with the value of h/d coefficient of –1.00 on the high oleic genetic background. The high stearic acid content of 22.6% of another line LG33 in the cross with VK580 revealed in F_1 seeds the mean value of 4.6% of the character. This corresponded to the incomplete dominance degree of the wild type with the value of h/d coefficient of –0.92 on the low oleic background (Figure 1).

The reciprocal crosses of VK101 × LG35 indicated no difference in stearic acid content between them with values of 8.4 and 8.5% in the F_1 seeds. In this case, the intermediate inheritance of the trait was observed with the value of h/d coefficient of –0.13 and –0.16 on the low oleic genetic background (Figure 2).

It is interesting to note that inheritance in F_1 of stearic acid content in crosses with large differences between parent lines (P_2–P_1) of 15.5 and 19.4% fitted dominance of normal low stearic content (Figure 1) whereas the cross with a relatively smaller parent difference of 5.5% matched with intermediate inheritance (Figure 2).

Spanish scientists proposed a classification of stearic acid phenotypic classes: standard (5%), mid (9%), mid (17%), high (25%) and very high (40%) with genes *Es1*, *Es2* and *Es3* involved (Pérez-Vich et al. 2004, 2006). Considering this,

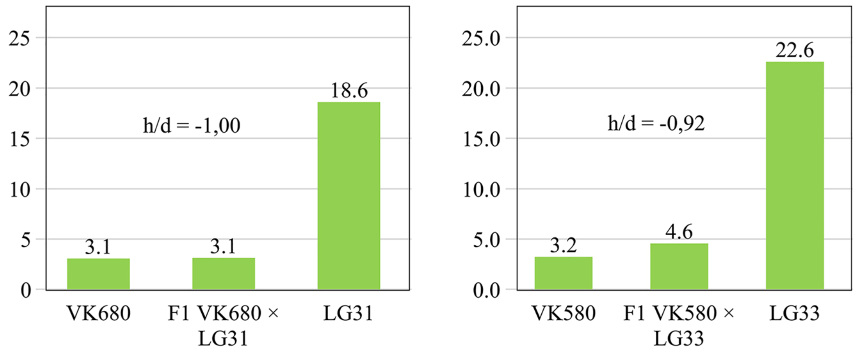

Figure 1: Phenotypes of F_1 and their parents for stearic acid (%) in the crosses.

our inbred lines LG35 with 12% of stearic acid content, and LG31 and LG32 with 19% correspond to the mid-stearic class whereas LG33 and LG34 with 23 and 22% are rather classified as high stearic. There is no very high stearic genotype in our genetic collection.

The recessive type of inheritance of the mutations with the increased level of stearic acid in sunflower seed oil was generally verified in our research. There was the observation of complete dominance degree along with incomplete dominance of standard level of stearic acid (wild type). Moreover, intermediate inheritance in F_1 of this character was first described.

Further segregation analysis in F_2 and backcrosses will provide an opportunity for comparison of the number of genes controlling the trait in the lines of our genetic collection.

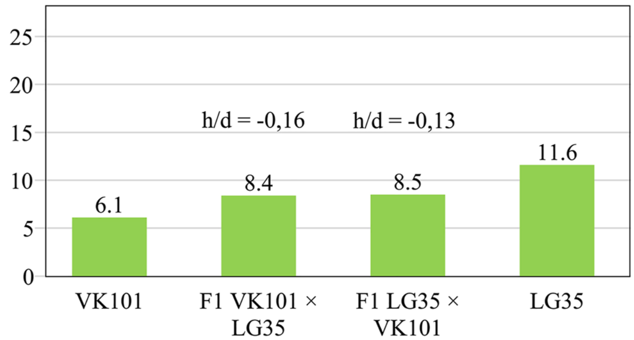

Figure 2: Phenotypes of F_1 and their parents for stearic acid (%) in the reciprocal crosses.

Conclusions

Sunflower (*Helianthus annuus* L.) genotypes with high stearic acid content in the seed oil may be useful for food and industrial applications. The inbred lines LG31, LG32, LG33, LG34 and LG35 were successfully developed with a different stable level of stearic acid content from 11.6 to 22.6%. Due to the recessive nature of increased stearic acid levels, the strategy of sunflower hybrid breeding should include the development of both female and male parents with high stearic acid content in the seed oil.

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