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Genotypic and environmental variability of the total content of tocopherols in the seeds of inbred sunflower lines

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ABSTRACT

Breeding for high total content of tocopherols (vitamin E) in sunflower seed oil involves studying the genetics of this trait. Twenty-nine inbred lines of sunflower with different total tocopherol content (TTC) were selected from 60 samples of the original material and studied at VNIIMK, Krasnodar, in 2016-2019. TTC was determined in an average sample from a head of shelled seeds by direct hexane extraction and further measurement of the optical density of the colored solution on a spectrophotometer. Both the genotype and the growing conditions influence the TTC in the seeds. The average range of variation over four years was from 302 to 605 mg kg^{-1}. The largest differences, 2.6 times, between genotypes were found in 2018 with a range of variability from 258 to 667 mg kg^{-1}. Significant differences were found between the average values of 360 mg kg^{-1} in 2017 and 432 mg kg^{-1} in 2019. The genotype and the year of growing have the greatest influence on the TTC, measured as 65% and 8%, respectively, and the genotype-year interaction accounts for 14% of the effect. The TTC correlation coefficient in the parent-offspring series was 0.65–0.85, and the heritability coefficient in the studied lines was 42–72%. The data on the valuable genetic control of TTC in seeds can help predict the effectiveness of breeding to improve this trait.

Keywords: Vitamin E, Inheritance, Genotype, Oil, Seeds.
Article type: Research Article.

INTRODUCTION

Tocopherols are lipophilic antioxidants produced by photosynthetic organisms such as plants, some algae, and cyanobacteria. Their chemical structure consists of a chromanol ring and a phytildiphosphate-derived side chain (Falk & Munné-Bosch 2010). Four isoforms of tocopherols have been found in nature: α, β, γ, and δ, which differ in the number and position of methyl groups in the chromanol ring (Zhirong *et al.* 2010). A methyl group in the aromatic ring of tocopherols makes these molecules thermally stable and resistant to acids and alkalis. The α-form is the most biologically active of the four isoforms of tocopherol. Its activity is taken as 100%, and the activities of β-, γ- and δ-forms are 30%, 15%, and 5%, respectively. Therefore, human needs for vitamin E are limited primarily by the consumption of α-tocopherol (Hussain *et al.* 2013). Tocopherol is most known as antioxidant that breaks the chain and prevents the cyclic propagation of lipid peroxidation (Mustacich *et al.* 2007). Green tissues of plants contain mainly α-tocopherol, which protects photosynthetic membranes from reactive oxygen species, participates in the protection of plant cells from oxidative damage under abiotic stress (Munné-Bosch 2005). In contrast to leaves, the composition of seed tocopherols is much more diverse and depends on the plant species, however, γ-tocopherol is the most common form (DellaPenna & Pogson 2006; Chen *et al.* 2006). Vegetable oils are the main sources of vitamin E in the human diet. Although this vitamin is readily available, its amount consumed by human population is not enough (Mène-Saffrané 2018). The predominant form of tocopherol, both

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in sunflower vegetative tissues and in seeds, is α-tocopherol, which usually accounts for more than 95% of the total amount of tocopherols. This makes sunflower seeds and oil one of the foods rich in vitamin E (Padley *et al.* 1994). Due to their lipophilic nature, tocopherols make part of the extractable oil, where they play an important role in both its nutritional and technological properties (Kamal-Eldin & Appelqvist 1996). The final content of tocopherols in oil depends both on the degree of its purification and on many factors, such as the content of tocopherols in the seeds, their oil content (Tasan & Demirci 2005), the place of production of oilseeds, methods and conditions of oil extraction and purification conditions (Wen *et al.* 2020). Wen *et al.* (2020) reported the content of tocopherols in eight varieties of sunflower oil of different purification from 555 to 915 mg kg^{-1} (average 743 mg kg^{-1}) (Wen *et al.* 2020). Seeds start accumulating tocopherols already on day 9-12 after flowering, with its increase by day 33-36 (Del Moral *et al.* 2013; Dong *et al.* 2007). The biosynthesis of tocopherols is not related to the biosynthesis of fatty acids and triacylglycerols (Somerville *et al.* 2005). A negative correlation was found between the content of tocopherols in oil and the oil content of sunflower achenes (Marquard 1990; Demurin 1986), according to other data, no such correlation was found (Alpasan & Gunduz 2000). Thus, the amount of tocopherols in oil depends on both the oil content of the seeds and their tocopherol content.There has been reported on significant differences among sunflower genotypes in the TTC in the seeds. Velasco *et al.* (2010) studied 952 sunflower samples and determined the range of TTC variability in seeds from 119 to 491 mg kg^{-1} (Velasco *et al.* 2010). The same authors report on the TTC variation in the seeds of 36 hybrids from 314 to 1024 mg kg^{-1} (Velascol *et al.* 2002) Another study reported on the development of imi-1 and imi-2 lines with increased (410 and 341 mg kg^{-1}), as well as KG27 and VK561 lines with reduced (178 and 189 mg kg^{-1}) TTC in the seeds (Demurin *et al.* 2018). The influence of growing conditions on TTC in sunflower seeds has been also studied. The results have shown that the genotype and the growing conditions, as well as the genotype-environment interaction significantly influence the studied trait (Marquard 1990; Alpaslan & Gunduz 2000; Velascol *et al.* 2002). The effect of the genotype turned out to be predominant. Heritability was assessed in the parent-offspring series in the F_2-F_3 generation account for 0.59 for a reduced and 0.67 for an increased content of tocopherols (Del Moral *et al.* 2011).

MATERIALS AND METHODS

The studies were conducted at the central experimental base of VNIIMK, Krasnodar, in 2016-2019. The experiment involved 60 lines of the breeding and genetic collection of various origins. The VNIIMK genetic collection lines were LG26, LG27, LG28. The VNIIMK breeding lines were VA760 B, VA93, VK101, VK276, VK580, VK591, VK639B, VK653B, VK680, VK700, VK732, VK776, VK780, VK787, VK788, VK789, VK794, VK900, VK902, VK905, VK906, VK914, VK915, VK917, VK918 Rf, VK919 Rf, VK920 Rf, VK921 Rf, VK922 Rf, VK925, VK927, VK929, VK930 Rf, VK931, VK935, VK939 Rf, VK941 Rf, L1066, L690, L1079, L1401, SL_{05}4154B, SL_{06}2546 B, SL_{07}006, SL_{07}108, SL_{07}383, SL_{08}1858B, SL_{13}2232 B. Lines of the Department of Agriculture (USA) were HA413, HA421, HA422, HA424, HA89. Collection samples include K3436, P453, R14. Field experiments were performed in compliance with the generally accepted methodology for four years, from 2016 to 2019. The plants were isolated before flowering, self-pollinated and harvested individually, each genotypes produced five heads of seeds. The years of the experiments varied in meteorological conditions, but in general they were favorable for obtaining reliable data. The average summer air temperature was the highest, 25.5°C, in 2016 and the lowest, 23.9°C, in 2019. In 2017 and 2018, the air temperature rose to an average of 24.4 and 25.1°C, respectively. Moisture deficit was observed in June 2018 and 2019 during budding, which is a critical period for sunflower. Precipitation in summer 2016 was 310 mm, in 2017 - 277 mm, in 2018 - 223 mm, in 2019 - 277 mm, with a climatic norm of 232 mm for this period. TTC in seeds was determined by the modified method. For this purpose, 1 g of sunflower seed kernels from the average sample of the head was finely ground in a pirouette. A 200 mg sub-sample was taken from the average seed sample. 5 ml of hexane was added into a measuring tube (10 cm^3 with a ground stopper) with a sub-sample, and left for at least 8 hours with periodic stirring. After 8 hours, 1 ml of the hexane layer was taken from the test tube, 3 ml of ethyl alcohol, and 0.5 ml of a 0.25% solution of α-dipyridyl in absolute alcohol and 0.5 ml of 0.1% ferric chloride ($FeCl_3$) in absolute alcohol were successively added. After keeping in the dark for 20 minutes, the optical density of the colored solution was measured on a PE-5400vi spectrophotometer. In parallel, the optical density of the solution was determined in blank.

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The TTC was calculated by the formula, considering that the reaction product of 1 g of α-tocopherol in 1 liter of alcohol under the above conditions has an optical density of 39.7 with a layer thickness of 1 cm. In this case, the content of tocopherols in a sample from ground seed kernels (X) in mg kg^{-1}, was calculated by the formula:

$X = D \times V / 0.0397 \times H \times 5$,

where: D is the concentration of α-tocopherol in the analyzed solution; V is the volume of the solution, ml; H is the weight of the sub-sample, mg. The results were processed in Excel statistical applications. Heritability in parent-offspring generations was assessed by the squared correlation coefficient according to Rokitskii (Rokitskii 1987).

RESULTS

The TTC variability in the seeds of 60 inbred lines was from 289 mg kg^{-1} in $SL_{07}383$ to 622 mg kg^{-1} in $SL_{08}1858$ (average 413 mg kg^{-1}; Fig. 1). Thus, the min and max values were twofold, with a range of 332 mg kg^{-1} and a fairly high CV = 19%. A continual distribution of the variation series was observed, the highest frequency was in groups with a TTC range of 331 to 450 mg kg^{-1}.

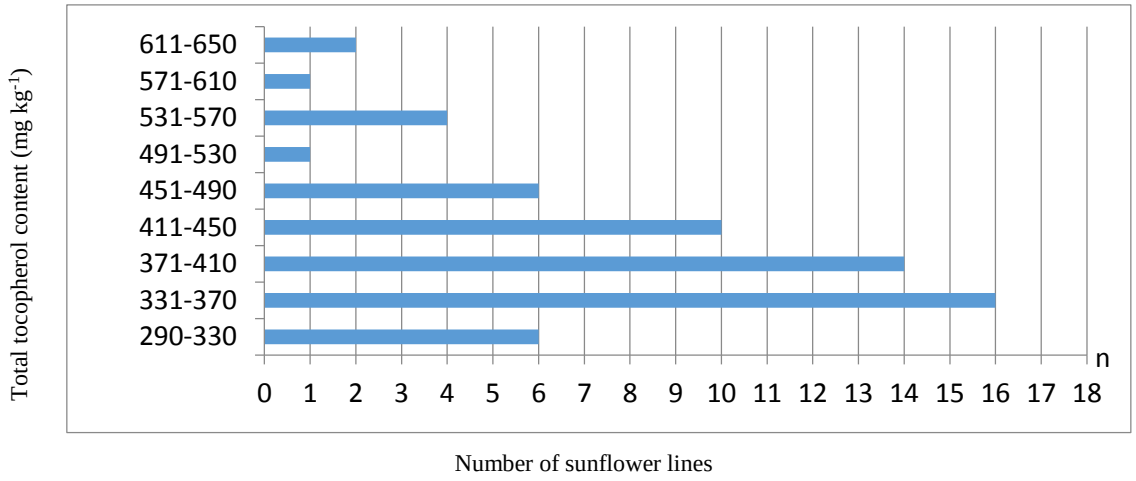

Fig. 1. TTC variability in seeds of sunflower lines (n = 60), Krasnodar, 2016.

For further study of TTC in seeds, 29 samples with increased and decreased trait were selected. Disruptive selection caused a characteristic two-peak variation series and the continuous distribution (Fig. 2). The highest frequency was observed in periods with TTC genotypes below average, from 290 to 380 mg kg^{-1}.

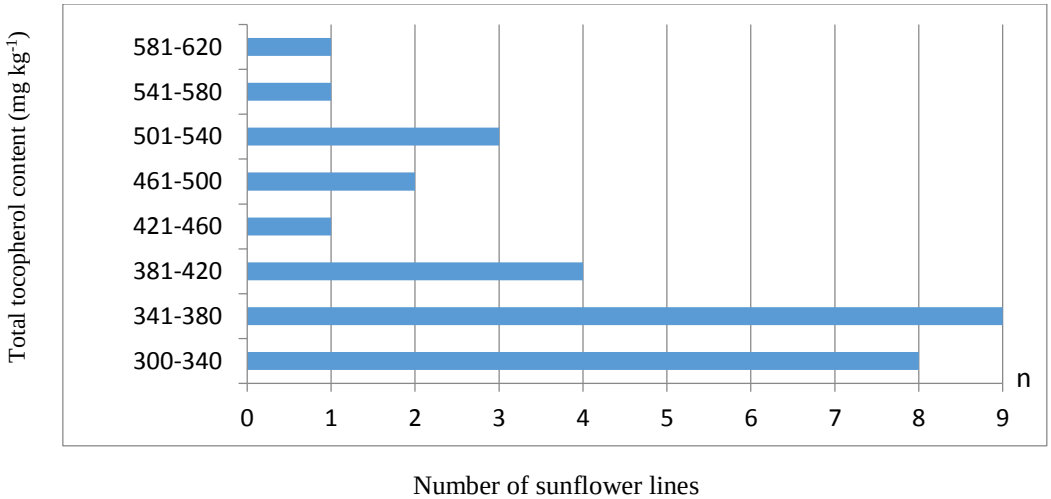

Fig. 2. TTC variability in seeds of sunflower lines (n=29), Krasnodar, 2016-2019.

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A four-year experiment from 2016 to 2019 showed a wide range of TTC variability in seeds of 29 sunflower lines, from 226 mg kg^{-1} for R14 in 2017 to 667 mg kg^{-1} for VK925 in 2018. The range of variation was 441 mg kg^{-1}, i.e. threefold. Weather variations led to significant environmental variability in the content of tocopherols in seeds in the studied lines in a four-year experiment. 2017 turned out to be unfavorable for the accumulation of tocopherols in seeds, which can be judged by the average value of 360 mg kg^{-1}, and by the limits of 226-575 mg kg^{-1}, which were lower than in the rest of the test years. At the same time, the highest coefficient of variation of TTC equal to 26% was in 2017 (Table 1). In 2016, the studied trait varied from 289 to 622 mg kg^{-1} (average 420 mg kg^{-1}), CV = 24%. The range of variation was 333 mg kg^{-1}. In 2018, the range of TTC variability in the lines was the largest for the years of the study - from 258 to 667 mg kg^{-1} (average 388 mg kg^{-1}).

The range of variability was 409 mg kg^{-1}, CV = 25%. In 2019, the range of variation was less than in previous years of the experiment - 308 mg kg^{-1}. At the same time, the variability of the trait ranged from 284 to 593 mg kg^{-1} with the highest average of 432 mg kg^{-1} and the lowest CV = 17%. Significant differences were between the average TTC for 2017 and 2016, as well as between 2017 and 2019, the difference between them exceeds LSD_{05} = 58 mg kg^{-1} (Table 1).

Table 1. Total content of tocopherols in the seeds of self-pollinated sunflower lines, mg kg^{-1} Krasnodar, 2016-2019.

Trait	Year				Mean
	2016	2017	2018	2019	
Mean	420	360	388	432	400
LSD_{05}	67	59	43	64	58
Min	289	226	258	284	302
Max	622	575	667	593	605
S	99	94	96	74	82
CV, %	23	26	25	17	21

The studied samples had genotypes more susceptible to changes in the conditions of the growing year. This is R14, with a variability of 226–456 m kg^{-1} and CV = 28%, $SL_{06}2546$ ranged from 283 to 442 mg kg^{-1} with CV = 23%. HA89 varied from 233 to 387 m kg^{-1} and CV = 21%. At the same time, there were genotypes more stable in the studied trait. This is LG27 with a variability of 505-545 mg kg^{-1} and CV = 3%. L1401 ranged from 399 to 434 mg kg^{-1} with CV = 4%. HA421 varied from 339 to 378 mg kg^{-1} with CV = 5%. To find the contribution of different genotypes and variations in growing conditions, as well as the effects of genotype-environment interaction in the total variance of the studied trait, the results were evaluated by analysis of variance. Differences in weather conditions and diversity of genotypes, as well as the effects of genotype-environment interaction were significant at the 5% level of the influence on the studied trait (Table 2). At the same time, the TTC is influenced to a greater extent by the genotype, which accounts for 64.6% of the total value. The influence of the growing year (7.6%) was two times less than the effects of genotype-environment interaction, estimated at 14.4%.

Table 2. Results of the analysis of variance of total tocopherol content in sunflower lines, Krasnodar, 2016-2019.

Source of variation	SS	df	MS	F factor	F_{05}	Share of influence, %
Year	2754.4	3	918.1	43.8	2.64	7.6
Genotype	23456.4	28	837.7	39.9	1.52	64.6
Interaction	5214.2	84	62.1	3.0	1.33	14.4
Internal	4867.9	232	21.0			13.4
Total	36292.9	347				100

Genotypes retained phenotypic ranks in different years of the study. The correlation coefficient of the total tocopherol content in the parent-offspring series was 0.65-0.85 and turned out to be significant at the 5% significance level (Table 3). This means that the heritability of TTC, estimated as the squared correlation coefficient in the studied lines, was 42–72%.

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Table 3. Coefficient of correlation of the total tocopherol content in the seeds of parent-offspring self-pollinated sunflower lines, n=29, Krasnodar, 2016-2019

Year	2017	2018	2019
2016	0,85*	0,80*	0,69*
2017		0,82*	0,65*
2018			0,73*

* significant at p = 0.05.

DISCUSSION

The results of the study indicate significant variability in TTC in the seeds of inbred sunflower lines, on average 302-605 mg kg^{-1} over the studied years. The range of variability was 303 mg kg^{-1}. This is consistent with the earlier reports of other authors on the large differences in TTC in sunflower seeds (Rokitskii 1978). Despite the fact the conditions of the growing year and the genotype-environment interaction had a significant influence on TTC (7.6% and 14.4%, respectively), the studied samples slightly changed their ranks over the years. Thus, TTC in sunflower seeds is largely due to the genotype (64% in our study), which is also consistent with the early results of the experiments in other material of other researchers (Demurin *et al.* 2018; Del Moral *et al.* 2011). The authors of previous studies report on the isolated genotypes with significant differences in TTC in the seeds of sunflower lines and on the preservation of these differences under different growing conditions (Rokitskii 1978). Our experiments also found genotypes with an increased TTC retained for four years. As the studied trait is largely subject to genetic control (up to 72%) and does not have a negative correlation with the oil content of seeds, we may suggest the effectiveness of creating sunflower hybrids with high vitamin E content in oil.

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