The impact of weather conditions in different years on the biochemical composition of linseed oil

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Влияние погодных условий разных лет на биохимический состав масла льна

G. A. POPOVA[1], N. B. ROGALSKAYA[1], N. V. KNYAZEVA[1], V. M. TROFIMOVA [1], T. V. SHELENGA[2], E. A. POROKHOVINOVA[2], N. B. BRUTCH[2]*

[1] Siberian Research Institute of Agriculture and Peat, branch of the Siberian Federal Scientific Center of Agro-Bio Technologies of the Russian Academy of Sciences, 3 Gagarina St., Tomsk 634050, Russia

[2] N.I. Vavilov All-Russian Institute of Plant Genetic Resources, 42, 44 Bolshaya Morskaya Street, St. Petersburg 190000 Russia
** n.brutch@vir.nw.ru*

Г. А. ПОПОВА[1], Н. Б. РОГАЛЬСКАЯ[1], Н. В. КНЯЗЕВА[1], В. М. ТРОФИМОВА[1], Т. В. ШЕЛЕНГА[2], Е. А. ПОРОХОВИНОВА[2], Н. Б. БРАЧ[2]*

[1] Сибирский научно-исследовательский институт сельского хозяйства и торфа – филиал Сибирского федерального научного центра агробиотехнологий Российской академии наук, 634050 Россия, г. Томск, ул. Гагарина, 3,

[2] Федеральный исследовательский центр Всероссийский институт генетических ресурсов растений имени Н.И. Вавилова, 190000 Россия, г. Санкт-Петербург, ул. Б. Морская, 42, 44
** n.brutch@vir.nw.ru*

Background. Linseed oil is a versatile product with varying biochemical composition. Linseed breeding is aimed at producing cultivars with different chemical properties in their oil. The crop is widespread but the environment has a great effect on its fatty acid biosynthesis.
Materials and methods. The impact of weather conditions on the variations in fatty acids composition was studied. Twenty linseed cultivars and accessions of diverse origin and with varying oil composition from the VIR collection were grown in Tomsk Province in 2016–2018. The content of 15 fatty acids (lauric, myristic, palmitic, palmitoleic, margaric, stearic, oleic, cis-vaccenic, linoleic, linolenic, arachidic, eicosenic, arachidonic, behenic, and lignoceric) was assessed in linseed oil using gas–liquid chromatography.
Results. The tested material presented a wide diversity of genes controlling different steps of fatty acids biosynthesis and genetic mechanisms involved in the responses to the changing environment. The analysis of variance proved that the content of oleic, linoleic, linolenic and lignoceric acids was controlled solely by the genotype. The content of palmitic and stearic acids was influenced by both the genotype and environment. The synthesis of lauric and cis-vaccenic acids was significantly affected by the weather. Practically all acids, except linoleic and linolenic ones, showed a very high random variation, which in our experiment included genotype × environment interaction. The accessions differed in the range of variation in their characters under different conditions. There was no definite regularity in the changes of fatty acid content in the tested genotypes during 3 years.
Conclusion. The assessed genotypes probably possess different regulatory mechanisms for fatty acid biosynthesis. Thus, they present a diverse stock for further investigations into fatty acid biosynthesis and for the development of new linseed cultivars widely adaptable to environmental conditions.

Key words: linseed oil, fatty acids, environmental conditions, genotype.

Актуальность. Масло льна используют для различных целей в зависимости от состава, и селекция культуры направлена на создание сортов с различными биохимическими характеристиками. Культура широко распространена в различных климатических зонах, условия оказывают сильное влияние на биосинтез жирных кислот. Исследование направлено на изучение воздействия погодных условий на изменения состава жирных кислот.
Материалы и методы. Двадцать сортов и линий масличного льна из коллекции ВИР, различающихся по происхождению и составу масла, выращивали в Томской области в 2016–2018 гг. Содержание лауриновой, миристиновой, пальмитиновой, пальмитолеиновой, маргариновой, стеариновой, олеиновой, вакценовой, линолевой, линоленовой, арахиновой, эйкозеновой, арахидоновой, бегеновой и лигноцериновой кислот оценивали методом газо-жидкостной хроматографии.
Результаты. Проанализированный материал представляет широкое разнообразие генов, контролирующих различные этапы биосинтеза жирных кислот, и генетических механизмов, участвующих в реакциях на изменяющиеся условия среды. Дисперсионный анализ показал, что содержание олеиновой, линолевой, линоленовой кислот контролируется только генотипом. Содержание пальмитиновой и стеариновой кислот зависит от генотипа и окружающей среды. На синтез лауреновой и цис-вакценовой кислот значимо влияют условия выращивания. Все кислоты, кроме линолевой и линоленовой, демонстрировали высокое случайное варьирование, которое в нашем опыте включало в себя взаимодействие генотипа со средой. Образцы различались по степени варьирования признаков в разных условиях. Определенной закономерности изменения содержания жирных кислот за три года эксперимента обнаружено не было.
Заключение. Изученные генотипы, возможно, имеют различные механизмы регуляции синтеза жирных кислот и представляют разнообразный материал для дальнейших теоретических исследований этой регуляции и, с другой стороны, для селекции новых сортов масличного льна с широкой адаптацией к условиям выращивания.

Ключевые слова: масло льна, жирные кислоты, погодные условия, генотип.

Introduction

Linum usitatissimum L. is one of the oldest and most important industrial crops. It is grown all over the world. For many centuries, mainly fiber flax was cultivated in Russia. But now its area is greatly reduced and replaced by linseed because of its unique traits and wide-scale applications in various industries and medicine (Kapinos et al., 2014). So, an increase in linseed production appeared to be an answer to the demands of the world market. In 2001, linseed in Russia occupied only 8.7 thousand hectares. According to the data of the Federal State Statistics Service for 2019, the sowing area of linseed in Russia farms of all categories reached 814.7 thousand hectares. In one year, the linseed cultivation area increased by 9.3% (by 69,100 ha). In 5 years, the increase by 63.6% (by 316,700 ha) was observed; in 10 years, by 458.3% (by 668,800 ha). Significant part of linseed is now produced in Siberia, where this crop is not traditional. In Omsk Province, in 2019, linseed was cultivated over the area of 124,100 ha (15.2% of the total area under linseed); in Chelyabinsk Province, 92,000 ha (11.3%); in Altai Territory, 89,000 ha (10.9%); in Kurgan Province, 62,400 ha (7.7%). Although the main oilseed crop in terms of its sowing area in Novosibirsk Province is sunflower (Kapinos et al., 2014), its yield is not high: in 2008–2012 it was 0.4 t/ha. The average yield of linseed in those years was 0.76 t/ha. This figure also exceeded the yield of rapeseed (0.69 t/ha), cultivated in more humid areas. Such results may be explained by a relatively high drought tolerance of linseed, cultivated in areas with different water availability (Antonova et al., 2012). So, the choice of the linseed crop for cultivation in Siberia should be recognized as rational (Kapinos et al., 2014).

Linseed oil is used for various purposes, depending on its chemical composition. Main differences among modern linseed cultivars can be found in the content of linoleic (LIO) and linolenic (LIN) acids. Traditional linseed oil contains 50% of LIN acid or more. But this kind of oil is not stable – after pressing, it turns rancid quickly (within 3 months). However, it is this acid that provides the oil with a wide range of pharmacological properties (Thompson, Cunnane, 2003) and the ability to dry quickly, which is necessary for the paint industry. Nowadays, many cultivars with low LIN acid content – 2–3% (solin) – are being bred. The latter type of cultivars is usually distinguished for a lot of LIO acid. The ratio between LIO and LIN acids determines the main utilization purposes of the oil: industrial or nutrition and medicine. Oleic (OLE) acid also makes an important contribution to oil quality. Other fatty acids can be found in linseed oil in low and very low concentrations. However, as these acids form a sequential chain of biosynthesis, evaluation of their intraspecific diversity plays an important role in discovering their genetic control and biosynthesis regulation. Such knowledge can also be used in breeding (Brutch et al., 2016a).

The synthesis of fatty acids in plants starts on the basis of acetyl-CoA. After carboxylation, malonyl-CoA is formed. Then the acetyl-CoA carboxylate complex initiates the development of malonyl-acyl carrier protein (ACP), which is the initial substrate of fatty acid synthesis (Durrett et al., 2008). The newly formed malonyl-CoA joins the growing carbon chain. Thus, by sequentially adding two-carbon fragments, myristoil- and palmitoyl-ACPs are synthesized (Tai, Jaworski, 1993). The formation of a carbon chain is catalyzed by the acetyl-CoA carboxylase complex (Nikolau et al., 2003). Termination of fatty acid elongation is catalyzed by acyl-ACP thioesterases, which hydrolyze ACP to produce free fatty acids. Desaturation of carbon chains is controlled by the system of desaturase enzymes (Somerville et al., 2000).

The research into flax is quite often dedicated to the evaluation of genes, controlling the formation of stearic acid and its unsaturated derivatives. The elongase responsible for the formation of stearic acid (STE), synthesized from palmitic acid (PAL), is controlled by the *FAB1* gene. Successive formation of double bonds in the STE acid chain is carried out by various desaturases. First, the double bond is formed at the 9th position in the carbon chain, generating OLE through stearoyl-ACP desaturases, which are encoded by the *SAD1* and *SAD2* genes. In LIO, another double bond is added at the 6th position under the effect of fatty acid desaturases-2, encoded by the *FAD2A* and *FAD2B* genes. The *FAD3A*, *FAD3B*, and *FAD3C* genes control desaturase-3, which forms the third double bond in position 3 of LIN (Vrinten et al., 2000; Banik et al., 2011). All the mentioned genes have been sequenced. They have multiple alleles that carry deletions and point mutations (Thambugala et al., 2013; Khadake et al., 2009; Krasowska et al., 2007, etc.). The desaturase-2 genes in flax are recognized as the main ones that determine the fatty acid composition of the oil (Fofana et al., 2006) and the product of the *FAD2B* gene has a much stronger effect on the trait than that of *FAD2A*. The *FAD3A* and *FAD3B* genes have a high degree of homology (> 95%). However, *FAD3B* has a greater effect on LIN synthesis than the *FAD3A* gene. The reason for this is that the product of the *FAD3A* gene has increased enzymatic activity, and the *FAD3B* gene shows a higher level of expression (Banik et al., 2011).

Contradictory results were obtained for the expression levels of all six desaturase genes in genotypes differing in fatty acid composition. D. Thambugala and S. Cloutier (2014) found that the expression of desaturase genes did not differ significantly between genotypes with different fatty acid compositions. But A. Rajwade et al. (2014) discovered a gene-specific and temporal expression pattern for all flax desaturases and also correspondence of their differential expression profiles with the variation of fatty acid accumulation in the two groups of genotypes: with low and high LIN content. Probably those controversial results were based on the genotypes with different degrees of diversity in the tested samplings.

By the present time, a lot of research has been carried out to evaluate the impact of various environmental factors on the fatty acid composition of oils in different plants species. Great part of such research was aimed at testing desaturase activity changes in different environments. Several experiments helped to discover that high temperatures reduced the activity of desaturases by initiating the degradation of these proteins (Dar et al., 2017). For *Arabidopsis*, the changes in gene expression levels and protein content with temperature were observed in the cases of Δ9, Δ12, and Δ15 desaturases (Vega et al., 2004; Teixeira et al., 2010; Teixeira et al., 2009). Significant natural variation was also found for the temperature responsiveness of v-6 desaturation which is controlled by a separate QTL (Menard et al., 2017).

This experiment was dedicated to the assessment of the effect produced by different weather conditions in Tomsk Province on the variation of fatty acid composition in genetically diverse linseed cultivars and lines in order to select versatile material for cultivation, breeding and further genetic analyses.

Material and methods

Twenty linseed cultivars and accessions of diverse origin and with a wide range of oil quality from the VIR genetic collection (Table 1) were grown in the field in Tomsk Province, Russian Federation, in 2016–2018 on the plots of 1 m^2. The seeding rate was 8 g/m^2, with inter-row spacing of 12.5 cm. Harvesting was conducted at the stage of yellow ripening. The chemical composition of seed oil was analyzed using gas–liquid chromatography. The analyses were performed for the content of 15 fatty acids: lauric, myristic, PAL, palmitoleic, margaric, STE, OLE, cis-vaccenic, LIO, LIN, arachidic, eicosenic, arachidonic, behenic, and lignoceric.

Mathematical processing of the experimental results was performed using generally accepted statistical methods. The effect of the genotype and weather conditions of the year on the fatty acid content was assessed using two-factor analysis of variance in Excel for Windows. Correlations were calculated using the Bravais–Pearson formula.

Tomsk Province occupies the southeastern part of the West Siberian Lowland and borders on Kemerovo, Novosibirsk, Omsk and Tyumen Provinces, and Krasnoyarsk Territory. The climate in the province is continental cyclonic (Evseeva, 2001). In the annual cycle, the predominance of a negative sum of air temperatures over positive ones is observed. Despite the location of this area in the zone of excessive moisture, droughts and dry spells may occur in some years. The main source of moisture is rainfall, the greater portion of which (up to 80%) occurs during the warm period, with a maximum in summer. The years 2016–2018 of the experiment differed significantly in weather conditions (Figure). In 2016, May and June were characterized by temperatures and precipitation lower than normal levels. July was hot and wet, while August was warm and dry. That year, harvesting was done in the middle of August. In 2017, May had normal temperatures but excessive precipitation. June was colder than usual but more humid. July had normal temperatures but less rainfall. August was characterized by a standard temperature and lots of rains. Harvesting took place in the beginning of August. The year 2018 had low temperatures in May and June and normal ones in July and August. Precipitation was abundant in May but deficient in the remaining season. Harvesting was done in the last third of August.

Table 1. **Linseed cultivars and accessions from VIR grown in Tomsk Province in 2016–2018**

Таблица 1. Сорта и линии из коллекции ВИР, выращенные в Томской области в 2016–2018 гг.

VIR catalogue No.	Name of the accession	Origin
k-5579	cv. 'Voronezhsky 1308'	Russia
k-5831	cv. 'VIR 1650'	Russia
k-8156	cv. 'Severny'	Russia
k-8409	cv. 'Kinelsky 2000'	Russia
k-8438	cv. 'Aisberg'	Ukraine
k-8451	cv. 'Shanxi'	China
k-8599	cv. 'Walaga'	Australia
k-8605	cv. 'Amon'	Czech Republic
k-8606	cv. 'Omega'	Canada
k-8677	cv. 'Istok'	Russia
i-623760	accession IDG 4101	Czech Republic
i-0151239	line l-1 derived from accession k-3730, China	Russia
i-0139791	line l-2-3 derived from accession k-6210 NP (RR) 38, India	Russia
k-8587	line l-1-1 derived from cv. 'L. Dominion', k-6272, England	Russia
i-0148214	line l-1 derived from cv. 'Minerwa' k-6298, USA	Russia
k-8589	line l-2 derived from cv. 'Bolley Golden' k-6392, USA	Russia
i-0139804	line l-1 derived from cv. 'Currong' k-6608, Australia	Russia
i-0139808	line l-1 derived from cv. 'Mermilloid' k-6634, Czech Republic	Russia
k-8597	line l-1-2 derived from cv. 'Eyre' i-601679 Australia	Russia
i-620805	line No. 854	England

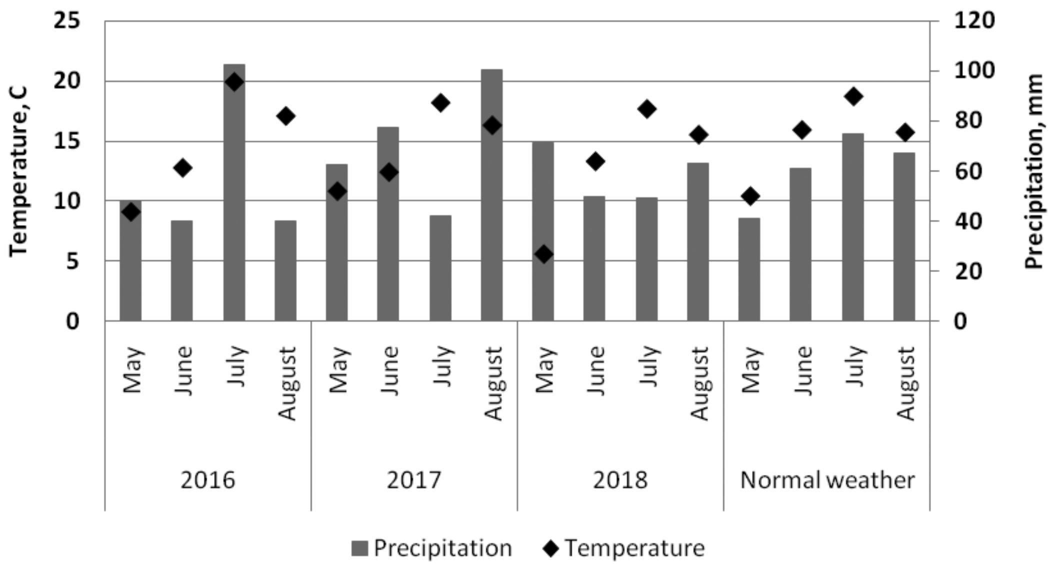

Figure. **Mean monthly temperatures and precipitation amounts in Tomsk Province in 2016–2018 compared to the normal weather (official data)**

Рисунок. **Среднемесячные температуры и суммы осадков в Томской обл. в 2016–2018 гг. в сравнении с нормой (официальные данные)**

Results and discussion

The tested genotypes significantly differed in their fatty acid composition and its stability under different weather conditions. Major differences were observed in the content of **linolenic acid**. The highest amount of it, over 60% on average for 3 years, was found in cvs. 'Severny' (k-8156) and 'Kinelsky 2000' (k-8409) from Russia, 'Aisberg' (k-8438, Ukraine), 'Shanxi' (k-8451, China), and in the accessions i-0151239 (derived from k-3730), k-8587 (from 'L. Dominion', Northern Ireland), i-0139808 (from 'Mermilloid', Czech Republic), i-0148214 (from 'Minerwa', USA) and k-8589 (from 'Bolley Golden', USA) (Table 2). The lowest content of LIN (2.74%) was detected in the seed oil of accession No. 854 (i-620805, England). Some accessions were of specific interest, because they demonstrated a rather rare medium content of LIN. They formed two groups. The first one synthesized about 20% of LIN: cv. 'Istok' (k-8677, Russia), cv. 'Walaga' (k-8599), Australia) and the accession k-8597 (derived from cv. 'Eyre', Australia). All these genotypes demonstrated low LIN contents under the conditions of Leningrad Province (Brutch et al., 2016a, b). The second group synthesized about 40% of LIN: cvs. 'Voronezhsky 1308' (k-5579, Russia) and 'Omega' (k-8606, Canada). The latter cultivars, on the contrary, were bred as high-LIN ones. Such results show that a complex of environmental peculiarities can play a very important role in fatty acid synthesis in linseed.

The genotypes tested in Tomsk Province also dramatically differed in the stability of LIN synthesis. The most stable values in different years were observed in the high-LIN accessions: cv. 'Kinelsky 2000', and the line i-0139808 (CV < 2%). The most variable were the low-LIN genotypes: cv. 'Walaga', the accessions k-8597 and i-620805, with CVs about 70–85%. Unlike the other two genotypes, the Eyre accession produced the maximum amount of LIN in the hot 2016. However, it should be mentioned that in July 2016, during seed development, the rainfall was in abundance. All these genotypes have different mutant alleles of the *fad3b* gene (Brutch et al., 2016a) which most probably have different sensitivity to environmental conditions. Other cultivars and accessions did not have definite correlations between the level of LIN and temperature during the ripening period. Nevertheless, the analysis of variance showed that the amount of LIN significantly depended only on the genotype (92.56%) (Table 3). This result can be explained by the fact that the difference between extreme values of LIN content reached on average 23 times.

Besides, considerable differences among the genotypes were found in the content of LIO, the predecessor of LIN. That is why genotypes abundant in LIN lacked LIO. The maximum amount of LIO – 63% on average for 3 years – was recorded for cv. 'Amon' (k-8605, Czech Republic). Accession i-620805 also had a lot of this acid (58%). The lowest level of LIO (12%) was found in cv. 'Kinelsky 2000'. As in the case with LIN, the genotypes differed in the stability of the character's expression. The most stable among them were: the low-LIO accession i-0139791 (derived from accession k-6210 NP (RR) 38, India), with CV = 0.9%; cv. 'Severny', CV = 1.15%; and the high-LIO accession i-620805, CV = 2.55%. It is interesting that the latter genotype had high variation in the LIN content, which was low in its oil. The analysis of variance showed that the amount of LIO also significantly depended only on the genotype (93.42%) (see Table 3).

The content of **oleic acid**, a predecessor of LIO, was low, as expected. Its higher level was found in the oil of 'Walaga' – 26% on average for 3 years. This cultivar had at the same time medium amounts of LIO (40%) and LIN (24%). Even more OLE (29%) was detected in accession i-620805. However, unlike cv. 'Walaga', this genotype synthesized 58% of LIO and 3% of LIN. The lowest levels of OLE were demonstrated by accession IDG 4101 (i-623760, Czech Republic) and the accession i-0151239: 12% and 10%, respectively. Accession i-620805, as in the case with LIO, showed the most stable expression of this character (CV = 4.19%). The most variable ones appeared to be the accession i-0148214 with CV = 37.48%, 'Shanxi' (k-8451, China) with 37.27%, and accession i-623760 with 30.99%. The analysis of variance showed that the amount of OLE mainly depended on the genotype (67.39%). Meanwhile, the effect size of random variation, which in our experiment included genotype × environment interaction, was rather high (31%).

Table 2. Average fatty acid content and its variation in linseed accessions grown in Tomsk Province, Russia, in 2016–2018

Таблица 2. Среднее содержание и коэффициент вариации жирных кислот у образцов масличного льна, выращенных в Томской области России в 2016–2018 гг.

VIR number[1]	12:0 Lauric	14:0 Myristic	16:0 Palmitic	16:1 Palmitoleic	17:0 Margaric	18:0 Stearic	18:1 Oleic	18:1 c 11 Cis-vaccenic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenic	20:4 Arachi-donic	22:0 Behenic	24:0 Lignoceric
5579	0.01[2]	0.02	4.54	0.04	0.02	4.40	20.29	0.55	25.70	44.10	0.09	0.14	0.03	0.04	0.04
	44.61[3]	*30.62*	*10.03*	*39.94*	*8.75*	*9.58*	*12.91*	*66.92*	*26.62*	*17.57*	*21.89*	*12.27*	*80.93*	*21.96*	*17.96*
5831	0.01	0.02	4.16	0.04	0.02	3.92	15.50	0.65	16.66	58.66	0.08	0.16	0.02	0.05	0.04
	31.08	*19.31*	*4.49*	*34.23*	*34.10*	*14.12*	*27.18*	*64.56*	*20.34*	*11.69*	*36.67*	*31.22*	*96.12*	*50.98*	*31.59*
8156	0.03	0.02	4.27	0.02	0.01	4.00	13.32	0.64	12.61	63.99	0.06	0.96	0.02	0.03	0.03
	134.09	*86.66*	*9.98*	*89.06*	*93.47*	*20.07*	*27.57*	*73.51*	*1.15*	*5.07*	*94.67*	*152.21*	*114.40*	*96.61*	*58.43*
8409	0.01	0.02	4.46	0.02	0.04	4.35	14.50	0.62	11.99	63.68	0.08	0.12	0.02	0.04	0.05
	21.14	*5.05*	*7.25*	*45.46*	*112.54*	*11.75*	*17.69*	*60.89*	*13.25*	*1.93*	*23.42*	*28.53*	*87.39*	*36.27*	*24.74*
8438	0.01	0.02	4.23	0.02	0.01	4.64	13.23	0.25	13.74	63.54	0.08	0.10	0.02	0.05	0.04
	53.09	*23.62*	*8.64*	*25.83*	*14.99*	*11.42*	*6.13*	*44.51*	*15.75*	*4.48*	*32.83*	*21.31*	*61.09*	*58.72*	*20.99*
8451	0.01	0.02	4.76	0.02	0.01	4.94	12.49	0.61	12.37	64.47	0.07	0.08	0.04	0.06	0.04
	124.30	*91.52*	*14.75*	*20.09*	*86.61*	*5.68*	*37.27*	*2.68*	*7.15*	*6.23*	*17.57*	*36.67*	*93.53*	*20.09*	*40.03*
8599	0.02	0.03	4.83	0.03	0.01	4.21	25.98	0.71	39.77	24.17	0.07	0.07	0.03	0.03	0.05
	103.83	*27.90*	*11.22*	*24.47*	*35.84*	*11.34*	*41.65*	*49.75*	*32.08*	*69.32*	*35.50*	*44.69*	*110.94*	*97.10*	*12.17*
8605	0.02	0.02	4.85	0.03	0.01	4.10	19.76	0.80	62.99	7.22	0.05	0.06	0.01	0.03	0.04
	36.22	*57.25*	*15.18*	*17.08*	*68.35*	*6.48*	*14.79*	*13.30*	*7.52*	*40.59*	*27.43*	*9.42*	*173.21*	*19.39*	*15.34*
8606	0.03	0.01	4.33	0.05	0.01	3.74	14.89	0.79	31.94	43.99	0.05	0.07	0.03	0.05	0.03
	61.91	*92.36*	*13.52*	*72.33*	*87.97*	*5.41*	*22.29*	*5.45*	*5.36*	*9.84*	*61.29*	*35.43*	*93.06*	*48.42*	*86.60*
8677	0.06	0.01	4.47	0.03	0.01	4.09	16.57	0.91	49.01	24.63	0.05	0.07	0.01	0.03	0.05
	139.37	*86.82*	*12.01*	*23.01*	*86.62*	*14.68*	*14.66*	*24.64*	*13.22*	*34.07*	*8.84*	*22.41*	*86.94*	*86.74*	*11.15*
i-623760	0.03	0.02	4.53	0.03	0.01	5.02	11.57	0.92	28.30	49.33	0.06	0.06	0.01	0.04	0.06
	80.75	*58.48*	*4.81*	*11.43*	*27.85*	*12.69*	*30.99*	*20.33*	*6.09*	*10.50*	*56.20*	*37.39*	*123.17*	*59.03*	*16.20*

Table 2. The end

Таблица 2. Окончание

VIR number[1]	12:0 Lauric	14:0 Myristic	16:0 Palmitic	16:1 Palmitoleic	17:0 Margaric	18:0 Stearic	18:1 Oleic	18:1 c 11 Cis-vaccenic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenic	20:4 Arachi-donic	22:0 Behenic	24:0 Lignoceric
i-0151239	0.02	0.02	4.52	0.02	0.01	4.46	10.46	1.01	17.79	61.47	0.05	0.08	0.01	0.03	0.05
	117.41	*40.59*	*5.35*	*26.97*	*20.72*	*19.54*	*18.13*	*23.09*	*53.19*	*13.21*	*14.40*	*28.10*	*88.22*	*48.90*	*32.14*
i-0139791	0.01	0.02	4.30	0.02	0.00	4.14	21.06	0.84	13.61	55.82	0.04	0.07	0.00	0.02	0.04
	12.74	*32.37*	*7.92*	*16.97*	*173.21*	*6.95*	*10.03*	*12.95*	*0.90*	*4.60*	*34.15*	*32.97*	*94.74*	*91.68*	*42.85*
8587	0.01	0.02	3.55	0.02	0.02	3.93	15.53	0.69	14.65	61.35	0.05	0.09	0.02	0.03	0.03
	69.93	*28.28*	*11.79*	*28.78*	*46.81*	*14.08*	*23.15*	*24.34*	*8.79*	*5.95*	*16.99*	*71.87*	*36.97*	*42.19*	*29.18*
i-0148214	0.01	0.01	3.54	0.02	0.01	3.60	14.67	1.01	14.17	62.79	0.04	0.07	0.01	0.02	0.02
	85.85	*87.84*	*8.27*	*26.25*	*36.79*	*12.31*	*37.48*	*18.15*	*10.33*	*6.97*	*48.25*	*32.97*	*89.39*	*87.18*	*87.33*
8589	0.01	0.02	3.95	0.03	0.01	4.12	16.11	0.81	12.57	62.18	0.05	0.07	0.01	0.03	0.05
	55.06	*46.75*	*8.08*	*3.34*	*88.52*	*9.05*	*24.87*	*21.18*	*11.61*	*6.91*	*29.53*	*11.74*	*29.31*	*25.39*	*4.10*
i-0139804	0.02	0.02	4.10	0.03	0.01	5.42	16.16	0.65	18.52	54.77	0.08	0.10	0.02	0.04	0.05
	121.22	*32.95*	*15.29*	*62.62*	*34.14*	*3.35*	*18.06*	*23.21*	*40.85*	*13.04*	*27.86*	*40.44*	*99.77*	*54.75*	*22.71*
i-0139808	0.01	0.02	4.30	0.03	0.01	5.76	12.29	0.68	13.81	62.83	0.07	0.07	0.01	0.03	0.06
	58.36	*22.10*	*7.28*	*59.31*	*132.16*	*4.14*	*16.73*	*44.69*	*13.08*	*1.94*	*38.27*	*25.76*	*173.21*	*44.10*	*15.77*
8597	0.04	0.04	4.89	0.04	0.02	5.16	24.10	0.85	45.43	19.03	0.06	0.23	0.01	0.03	0.08
	138.33	*57.87*	*5.71*	*68.74*	*42.98*	*2.34*	*23.84*	*32.92*	*17.16*	*68.82*	*30.50*	*107.91*	*86.66*	*38.14*	*39.83*
l-3 from i -620805	0.05	0.02	4.95	0.05	0.01	4.17	29.28	0.71	57.68	2.74	0.08	0.11	0.03	0.04	0.07
	61.19	*94.50*	*1.51*	*60.27*	*126.55*	*2.47*	*4.19*	*30.97*	*2.55*	*85.13*	*55.52*	*98.66*	*152.18*	*115.30*	*39.27*
LSD	**0.01**	**0.003**	**0.19**	**0.004**	**0.004**	**0.27**	**2.36**	**0.08**	**7.75**	**9.59**	**0.01**	**0.09**	**0.005**	**0.005**	**0.007**

Note: 1 – names and origin of the genotypes are listed in Table 1; 2 – average amount of fatty acids in the oil (first line); 3 – CV% (second line)

Примечание: 1 – названия и страны происхождения образцов приведены в таблице 1; 2 – среднее содержание жирных кислот в масле (первая строка); 3 – CV% (вторая строка)

Table 3. The effect of the genotype and weather conditions in the years of testing (2016–2018) on the fatty acid composition of linseed oil in Tomsk Province according to the two-factor analysis of variance

Таблица 3. Влияние генотипа и погодных условий в годы эксперимента на жирнокислотный состав масла масличного льна, выращенного в 2016-2018 гг., по результатам двухфакторного дисперсионного анализа.

Fatty acid		Effect size, %		
		Year	Genotype	Random variation
12:0	Lauric	23.7*	28.7	47.7
14:0	Myristic	2.5	24.9	72.6
16:0	Palmitic	17.5*	53.5*	29.0
16:1	Palmitoleic	4.8	33.2	62.0
17:0	Margaric	1.2	33.7	65.1
18:0	Stearic	5.2*	66.9*	27.9
18:1	Oleic	1.6	67.4*	31.0
18:1 c 11	Cis-vaccenic	9.3*	37.3	53.4
18:2	Linoleic	0.0	93.4*	6.6
18:3	Linolenic	0.2	92.6*	7.3
20:0	Arachidic	8.8	36.4	54.8
20:1	Eicosenic	3.2	33.3	63.5
20:4	Arachidonic	8.8	21.8	69.4
22:0	Behenic	9.7	25.3	65.0
24:0	Lignoceric	3.4	53.5*	43.0

* the effect of the factor is significant (P0 < 0.01)
* влияние фактора достоверно (P0 < 0.01)

Cis-vaccenic acid is one of the isomers of OLE. It can be found in linseed oil in appreciable concentrations of about 1%. The lowest amount (0.25% on average for 3 years) was found in cv. ‘Aisberg’. But the accessions i-0151239 and cv. ‘Minerwa’ formed 4 times more cis-vaccenic acid – about 1%. This character was most stable in cvs. ‘Shanxi’ and ‘Omega’, with CV = 2.68% and 5.45%, respectively. The Russian cultivars ‘Voronezhsky 1308’, ‘VIR 1650’ (k-5831, Russia), ‘Severny’ and ‘Kinelsky 2000’ produced an unstable content of this acid: CV = 60.89–73.51%. The analysis of variance showed that the amount of cis-vaccenic acid was significantly controlled only by weather conditions. But the effect size of random variation, which in our experiment included genotype × environment interaction, was quite high (53%).

Stearic acid is a predecessor of all unsaturated C18 fatty acids and, in general, has a lower concentration than theirs, except for cis-vaccenic acid. The highest amount of STE, on average for 3 years, was found in the accession i-0139808 (5.76%). The lowest content (3.60%) was demonstrated by the accession i-0148214. Variations of this acid’s content in seeds of the tested genotypes were rather stable in different years of evaluation. The lowest CVs (less than 2.5%) were recorded for two low-LIN accessions: k-8597 and i-620805. The highest variation (CV = 20.07%) was exhibited by cv. ‘Severny’. Nevertheless, the analysis of variance witnessed that both the genotype and environment played a significant role in the expression of this character. The effect sizes of the genotype and weather conditions were 67% and 5%, respectively. Besides, the effect size of random variation, which in our experiment included genotype × environment interaction, was relatively high (28%).

Both short-chain and long-chain fatty acids were found in low concentrations in the oils of all tested genotypes. Nevertheless, significant differences were observed among them. A majority of the genotypes had very little **lauric acid** (about 0.02% on average for 3 years). The minimal amount of it (0.01%) was seen in cvs. ‘Voronezhsky 1308’, ‘VIR 1650’ and ‘Kinelsky 2000’; ‘Aisberg’; ‘Shanxi’; the accessions i-0139791, k-8587, i-0148214, k-8589, and i-0139808. Meanwhile, cv. ‘Istok’ and accession i-620805 had 0.06 and 0.05%% of this acid, respectively. The major part of the evaluated genotypes had high variation of lauric acid content. Cvs. ‘Severny’ and ‘Istok’ as well as the accession k-8597 had CVs higher than 130%. The most stable content of lauric acid (CV = 12.7%) was recorded for the accession i-0139791. It is important that the highest level of lauric acid in most of the genotypes was detected in the driest 2018. Sometimes, in 2016 and 2017, lauric acid was found in concentrations less than 0.005%. So, it is not surprising that the analysis of variance showed that only the environment had a significant effect on this character. Besides, the effect size of random variation, which in our experiment included genotype × environment interaction, accounted for almost half of the total variation (48%).

Greater part of the tested genotypes formed about 0.02% of **myristic acid**. The leader in the content of this acid was the accession k-8597, with 0.04% on average for 3 years. The most stable level of myristic acid in different years of evaluation (CV = 5.1%) was demonstrated by cv. 'Kinelsky'. Cvs. 'Severny' and 'Istok', 'Shanxi', 'Omega', and accession i-620805 were the most variable ones in the content of this acid, with CVs about 90%. The main effect size for myristic acid content variation (73%) was random, which in our experiment included genotype × environment interaction. In the case of myristic acid, it was impossible to select the most favorable year for its synthesis. It depended on the tested genotype.

The oil of the studied genotypes contained **palmitic acid** in concentrations similar to that of STE acid, ranging between 3.5 and 5% (the accessions i-0148214 and i-620805, respectively). Nevertheless, it was about 100 times higher than the amounts of lauric or myristic acids. In general, PAL content exhibited low variation within each genotype grown in different years. This character was most stable in the accession i-620805, with CV = 1.5%. The most variable PAL content was registered for cv. 'Amon' and the accession i-0139804 (from cv. 'Currong', Australia), with CVs of about 15%. Nevertheless, weather conditions significantly (17%) affected the amount of PAL, judging from the results of the two-way analysis of variance. But the main part of variation in this character was determined by genotype-specific differences (53%). At the same time, the effect size for PAL content (29%) had random variation, which in our experiment included genotype × environment interaction.

The amount of **palmitoleic acid**, synthesized on the basis of PAL, was in the tested genotypes more or less similar to that of lauric and myristic acids. Half of the tested accessions had the lowest amount of this acid (0.02%). The highest level of palmitoleic acid (0.05%) was found in cv. 'Omega' and accession i-620805. The most stable amount of palmitoleic acid in different years (CV = 3.34%) was observed in the accession k-8589. Cv. 'Severny' (Russia) appeared to be the most variable one, with CV = 89.06%. The analysis of variance showed that neither the genotype nor environmental conditions produced significant influence on the character's expression. The main effect size for this fatty acid (62%) had random variation, which in our experiment included genotype × environment interaction.

Margaric acid was found in extremely low concentrations: no more than 0.024%. Practically no margaric acid (0.003%) was synthesized in the accession i-0139891. The tested genotypes showed extreme diversity in environmental stability of the character's expression. The most stable amount of margaric acid was formed in cv. 'Voronezhsky 1308', with CV = 8.75%. Cv. 'Kinelsky 2000', the accessions i-0139791 and i-0139808, and i-620805 demonstrated coefficients of variation exceeding 100%. The analysis of variance showed that the presented results were similar to those for palmitoleic acid. Neither the genotype nor environmental conditions had a significant effect of the expression of this character. The main effect size for this fatty acid (65%) had random variation, which in our experiment included genotype × environment interaction.

The amount of **arachidic acid** ranged, on average for 3 years, between 0.04% in the accessions i-0148214 and i-0139791, and 0.09% in cv. 'Voronezhsky 1308'. The most stable expression of this character (CV = 8.84%) was observed in cv. 'Istok'. Unstable expression (CV = 94.67%) was recorded for cv. 'Severny'. As with most of long-chain fatty acids, the amount of arachidic acid did not significantly depend on the peculiarities of genotypes or environments. According to the analysis of variance, the main effect size of this fatty acid's variation (55%) was random, which in our experiment included genotype × environment interaction.

Eicosenic acid, formed on the basis of arachidic acid, usually had slightly higher concentrations than its predecessor. Only cv. 'Severny' generated almost 1% of eicosenic acid, which was approximately 10 times more than the other accessions. More than half of the tested genotypes synthesized less than 0.1% of eicosenic acid. A stable level of eicosenic acid synthesis was demonstrated by cv. 'Amon' and the accession k-8589: CV = 9.42 and 11.74%, respectively. Very unstable results (CV = 152.21%) were obtained for cv. 'Severny'. The analysis of variance showed no significant effect of the genotype and environment on the amount of eicosenic acid, but the effect size of random variation, which in our experiment included genotype × environment interaction, reached 63%.

The maximum amount of **arachidonic acid** (0.04%) was recorded for cv. 'Shanxi'. This acid in the accession i-0139791, on average for 3 years, did not reach 0.005%. The amount of arachidonic acid was very unstable in different years. The best results for this indicator was shown by the accessions k-8589 and k-8587, with CV = 29.11 and 36.97%, respectively. The highest variation was observed in cv. 'Amon' and the accession i-0139808, both having CV = 173.21%. The analysis of variance showed no significant effect of the genotype and environment on the amount of arachidonic acid, but the effect size of random variation, which in our experiment included genotype × environment interaction, reached 69%.

The highest content of **behenic acid** (0.06%) was found in cv. 'Shanxi', while the lowest content (0.02%) in the accessions i-0139791 and i-0148214. More or less stable results were obtained for cvs. 'Voronezhsky 1308', 'Shanxi' and 'Amon', with CVs of about 20%. Accession i-620805 appeared to be very unstable (CV = 115.30%). The analysis of variance showed no significant effect of the genotype and environment on the amount of arachidonic acid, but the effect size of random variation, which in our experiment included genotype × environment interaction, reached 65%.

The accession k-8597 was the richest in **lignoceric acid**, forming 0.08% of it, on average for 3 years. Having a lot of behenic acid, the accession i-0148214 had the lowest concentration of lignoceric acid (0.02%). Stable results in different years were shown by the line k-8589, with CV = 4.10%. Oppositely, the most unstable content of lignoceric acid was characteristic of cv. 'Omega' and the line i-0148214, having CVs of 86.60 and 87.33%, respectively. Lignoceric acid appeared to be the only long-chain fatty acid that demonstrated a significant effect of the genotype on the level of its synthesis (54%). At the same time, the effect size of random variation, which in our experiment included genotype × environment interaction, reached 43%.

As fatty acids in plants are products of one biosynthetic pathway, correlations between their contents could be expected. In our experiment only some stable correlations, detected in all three years of evaluation, were recorded. The contents of OLE and LIN had strong and very strong negative correlations (r = –0.60…–0.84). This relationship was expectedly based on a very strong negative correlation between the contents of LIO and LIN (r = –0.95…–0.99). In linseed, about 95% of the synthesized STE is exposed to desaturation. From 10 to 30% of molecules form only one double bond, generating PAL and cis-vaccenic acids. So, 60–80% of molecules have two or three double bonds, and that is why the amounts of LIO and LIN have a strong negative correlation. Correlations were also found for arachidic acid: medium ones with arachidonic acid (r = +0.56…+0.62) and strong correlations with behenic acid (r = +0.70…+0.86), because it is the predecessor of both arachidonic and behenic acids.

Conclusion

Analyses of the described results showed that the tested material provided a wide diversity of not only genes controlling different steps of fatty acid biosynthesis but also genetic mechanisms involved in the responses to changes in environmental conditions. The analysis of variance showed that the contents of OLE, LIO, LIN and lignoceric acids were controlled solely by the genotype. The contents of PAL and STE acids were influenced by both the genotype and environment. The synthesis of lauric and cis-vaccenic acids was significantly affected by the environment. Practically all fatty acids, except LIO and LIN, had very high random variations, which in our experiment included interactions between the genotype and weather conditions. In addition to that, the genotypes differed in their range of a character's variation under different conditions. No strict regularity was found in the changes of fatty acid content for the tested genotypes during three years of experiments. It means that the tested cultivars and accessions probably have different mechanisms regulating fatty acid biosynthesis. Thus, they present diverse material for further theoretical research into fatty acid biosynthesis regulation and, on the other hand, for breeding of new linseed cultivars with wide adaptability to environmental conditions.

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Прозрачность финансовой деятельности / The transparency of financial activities

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ORCID

Popova G.A. https://orcid.org/0000-0001-9293-5812
Rogalskaya N.B. https://orcid.org/0000-0002-7601-5319
Knyazeva N.V. https://orcid.org/0000-0002-4995-5735
Trofimova V.M. https://orcid.org/0000-0001-9293-5812
Shelenga T.V. https://orcid.org/0000-0003-3992-5353
Porokhovinova E.A. https://orcid.org/0000-0002-8328-9684
Brutch N.B. https://orcid.org/0000-0003-2253-6263