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Biochemical, transcriptional and fluorescence spectroscopy analysis of fatty acids in seeds of camelina varieties in the organic intercropping system

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Intercropping involves the cultivation of two or more crops in the same field at the same time to improve yield per unit area by using resources or ecological processes that would otherwise not be used by a single crop. Camelina (*Camelina sativa* L.) is traditionally grown as an oilseed crop for producing vegetable oil and animal feed and is studied for its exceptional level of omega-3 fatty acids (up to 45%). The seeds contain 38 to 43% oil and 27 to 32% protein. The aim of this study is biochemical evaluation of the potential of this oil crop under organic cultivation of three varieties of camelina alone K1 (Luna), K2 (Lenka) and K3 (Local Bulgarian landrace) and co-cultivated with leguminous crops - pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.). Biochemical analysis showed that the percentage of oil content decreased in co-cropping with leguminous crops in variant K1 but increased in variants K2 and K3 from 33.1 - 35% to 37 - 38.9%. Fatty acid levels and gene expression profiles for the *fad6* gene associated with the synthesis of fatty acids (FA) were examined in three oilseed camelina cultivars that have utility for cultivar development in our spring camelina breeding program. The biochemical analyses of monounsaturated fatty acids as omega-9 oleic acid in camelina seeds show an increasing trend when intercropping was applied with vetch and pea. The most significant increase was observed in polyunsaturated fatty acids as linoleic C18:2 and linolenic acids C18:3 profiles in all three variants and co-cultivation with vetch. As a result of transcriptional analysis, the expression of *fad6* in camelina co-grown with vetch was 5 times higher compared to control plants grown alone. The application of a mobile fiber-optic system for qualitative biochemical and transcriptional analysis is demonstrated. The spectral distribution of the emission fluorescence signal depends on expression in camelina. In camelina grown with peas and grown alone, a clear correlation was observed. The intensity of the fluorescence signal directly depends on the content of monosaturated fatty acids. Correlations were observed, except in the spectral distribution of the fluorescence signal and in its intensity when growing camelina alone and in combination with peas. When grown alone, the distribution of the signal is better compared to representatives grown in combination with peas and vetch.

Keywords: *Camelina sativa L.,* biochemical analysis, transcriptional analysis, fluorescence spectroscopy analysis, intercropping

INTRODUCTION

Camelina (*Camelina sativa* (L.) Crantz is an oilseed crop from the *Brassicaceae* family, and it is important for food, feed, and industrial uses. *C. sativa* possesses several agronomic properties such as resistance to insects and easy adaptation to any climatic conditions, the only limitation for its cultivation being heavy clay or waterlogged soils. Seed yields and oil content were highly variable depending on the environment and it outdid rapeseed in trials under identical drought conditions [1].

Considering the climate changes, good drought tolerance has been pointed out as one of

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the advantages of this crop [2, 3]; especially during flowering, drought can reduce plant growth and development [4]. It shows good cold survival as it can germinate at low temperatures and the plants can tolerate freezing [5], although this has not been fully characterized for all stages of development and varieties. For some genotypes, the crop has been observed to survive low temperatures without damage [6].

The plant provides a high-value feed with sufficient residual lipid content (5–10%) that has a rich protein profile similar to soybean meal [7]. The main content of the seeds of this crop is oil (40%) [8, 9]. Many studies have shown that the oil plays a role in reducing cholesterol and inflammatory potential due to the increased content of α-linolenic (omega-3) and linoleic (omega-6) fatty acids, as well as bioactive compounds such as tocopherols and phenols [10]. The camelina crop is studied for its extremely high level (up to 45%) of omega-3 fatty acids, which is unusual in plant sources [7]. Over 50% of the fatty acids in cold-pressed camelina oils are polyunsaturated - omega-3 fatty acids, alpha-linolenic acid, gamma-tocopherol, and linoleic acid. The oil is also very rich in natural antioxidants, such as tocopherols, which makes it extremely stable and very resistant to oxidation and rancidity. It contains 1 - 3% erucic acid and the vitamin E content of camelina oil is approximately 110 mg/100 g [11]. It is very suitable for use as frying oil, as it has an almond-like taste and aroma.

Camelina has recently been grown because of its potential as a biofuel and biolubricant [5]. Studies show that camelina-based jet fuel reduces net carbon emissions by about 80% [12]. The US Navy selected it as the feedstock for its first aviation biofuel test and successfully operated an F414 static engine in October 2009 at Naval Air Station Patuxent River, Maryland [13]. Dutch organic farming company Waterland International and the Japanese Farmers' Federation reached an agreement in March 2012 to plant and grow camelina on 2,000 to 3,000 ha in Fukushima Prefecture. The health organization has approved oil as a healthy food. The plant is a valuable source of essential amino acids, especially sulfur-containing ones, which are generally lacking in leguminous crops, thus representing an alternative source of protein for both humans and farm animals.

Camelina is approved as a food additive for cattle in the US, as well as an ingredient (up to 10% of the ration) in chicken feed for broilers and feed for laying hens. Several by-products have been extracted with significant crude protein content. Camelina is also used in cosmetics and cooking. The edible seeds can be sprinkled on salads or mixed with water to make an egg substitute.

According to Maitra *et al*. [14], intercropping is the agricultural practice of growing two or more different crops in the same field at the same time. The main objective of intercropping is the production of a higher yield per unit area by using resources or ecological processes that would not otherwise be used by a single crop. Multi-crop intercropping systems provide respective ecosystems with benefits due to their independent symbiotic capacity for nitrogen fixation (in the case of legumes) and nitrogen contribution to the next crop, remobilization of phosphorus by mycorrhizal and soil microorganisms, resulting in reduced nitrogen input and phosphorus with fertilizers and more rational use of energy, improving biodiversity and overall soil health.

From 2021, the Agricultural University in Plovdiv is a partner of several European countries in the SCOOP Project. This project is focused on organic farming systems intended to preserve the ecosystem and agricultural land integrity, biodiversity, as well as food and feed security. While camelina can potentially be grown anywhere in Europe, SCOOP companion crops will be identified locally according to the specific needs of stakeholders, the expectations of local farmers and traditional food. The purpose of the study is to evaluate the biochemical composition of oil, transcription of the *fad6* gene involved in the synthesis of very long fatty acids (VLFA) and fluorescence spectroscopy analysis of fatty acids in seeds of camelina cultivars grown on certified organic far alone and in intercropping with legumes peas (*Pisum sativum*) and vetch (*Vicia sativa*). In higher plants, polyunsaturated fatty acids (PUFAs) are synthesized by various FAD (fatty acid desaturases). FAD is a ubiquitous enzyme family and is responsible for introducing double bonds into the hydrocarbon chains of fatty acids [15]. They play an essential role in fatty

acid metabolism and maintain biological membranes in most creatures [16]. Biosynthesized α-linolenic acid is converted from linoleic acid by FAD genes. Linoleic acid and α-linolenic acid are so-called essential fatty acids (EFAs) in human bodies because of their ability to synthesize these compounds [16, 17]. Therefore, *fad6* is the key enzyme for producing linoleic acid and is also the speed-limiting enzyme for routes of ω-6 and ω-3.

Fluorescence spectral analysis allows non-invasive analysis, in a short time, with high sensitivity, without disturbing the integrity of the biological object. The optical properties of compounds in cereals and legumes are determined by energy structure of electrons in molecules, which includes both occupied and unoccupied electronic energy levels, as well as the energy levels of atomic vibrations in molecules. Fluorescence spectra of seeds of camelina cultivars allow obtaining important information for optimizing the yield and content of polyunsaturated fatty acids (PUFA) [18].

MATERIALS AND METHODS

Field experiments

Field trials were conducted on the certified organic field at the Agroecological Center of the Agricultural University - Plovdiv. The soil type is Mollic fulvisols – FAO, with a low humus content of 3.7% and neutral pH. A randomized complete block design was used for setting a small plot experiment on the effect of an intercropping system of camelina and protein crops, compared to a sole crop of the same species. Three genotypes of *Camelina sativa* – the Polish winter varieties Luna (K1) and Lenka (K2) and a local Bulgarian landrace (K3) were grown in two successive years -2022 and 2023 in a pure stand and combined with fodder pea (*Pisum sativum*) and vetch (*Vicia sativa*). In this study, we present the results from the spring cultivation in small plots of 10 m^2 (1.4 × 7.7 m). The sowing was executed with plot seeder Wintersteiger AG with 800 germinating seeds/m^2. Each variant of the sole crop or its combinations (Table 1) was set in three replications. Fertilization with approved for organic farming solid fertilizer - 30 kg/ha of active substance nitrogen was done before soil cultivation. Appropriate insecticides were necessary as we observed a serious infestation of cabbage flea beetle (*Phyllotreta cruciferae*) on the field at the beginning of the vegetation. No significant environmental or biotic stress factors were observed during the vegetation of camelina in both years.

Plant material

During the flowering stage, 30 plants per variant and replication were marked according to stage. During maturation pods were collected at 10-day intervals, 10 up to 30 days after flowering (DAF), as shown in Figure 1. Seeds were harvested in liquid nitrogen and stored at −80°C for gas chromatographic analysis and RNA extraction in triplicate as was published by Petkova *et al*. [19].

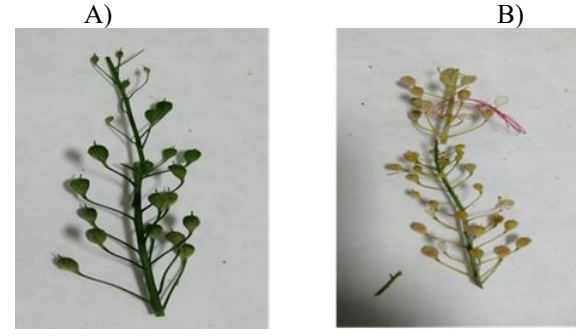

Figure 1. Development stages of seeds maturation A) 10 DAF and B) 30 DAF.

Determination of oil and fatty acid profile by gas chromatography

At 30th DAF, oil was extracted from seeds with n-hexane using accelerated solvent extraction according to the methods of Haagenson [19, 20] for oil content determination. One g of camelina seeds was dried for 4 h at 70°C. The seeds were ground in a coffee grinder with 3.5 g of diatomaceous earth, and samples were loaded into 10 ml stainless steel cells. Any remaining extraction cell void volume was filled with diatomaceous earth before extraction. The solvent containing extracted oil was collected in pre-weighed vials, and the solvent was evaporated to dryness with a stream of dry air (dew point of -70°C). Extracted samples were air-dried, and reground for a second extraction and the total oil recovery from the two extractions was recorded. Oil is reported as a percentage of seed dry weight. The seeds of camelina varieties (2 g) were subjected to quantitative and

qualitative analysis of the composition of fatty acids. The extraction of fatty acids from the seeds was performed with a 1 ml solution of esterification buffer (75 ml of hexane, 20 ml of chloroform and 5 ml of sodium methoxide in methanol) [21]. GC analysis was performed with a Hewlett-Packard gas chromatograph (model: HP5890 fitted with a 30 m FFAP capillary column (0.25 mm narrow aperture and 0.5 µm thick film). The fat content was determined by the residual method with a Soxhlet apparatus.

Real-time PCR analysis

Total RNA was extracted from 100 mg of control camelina leaves and leaves from camelina intercropped plants. First-strand cDNAs were reverse-transcribed from 1 µg each total RNA using a Hi-cDNA synthesis kit (Himedia, Mumbai, India). Gene-specific primers for *fad6* gene (*fad6*-Fw – 5'-ATCACATAAGCCCAAGCATACCG-3' and *fad6*-Rv – 5'-TCGTCTTCATCAACCGCCATTT-3') expression by Primer 5 software (Applied Biosystems, Foster City, CA, USA). Each 25 µlPCR reaction contained 12.5 µl 2 × SYBR Green Mastermix (- iTaq Univer SYBR Green SMX 500, BioRad, Hercules City, CA, USA), 0.5 µl 10 µM each primer, 1 µl of each first-strand cDNA template, and 10 µl dd H_2O. The PCR reaction was performed in a real-time PCR detector (Bio-Rad Laboratories, Hercules City, CA, USA) with Opticon Monitor 3 software (Bio-Rad Laboratories). The following thermal cycling profile was used: 95°C for 10 min; 35 cycles of 95°C for 15 s and 56°C for 1 min; and 95°C for 15 s, 60°C for 1 min, and 95 °C for 15 s [22]. As an internal control to normalize all data actin expression was used [23]. Experiments were carried out with three independent biological replicates each containing three technical replicates [24]. The difference in mRNA expression was estimated using threshold cycles, by the $2^{-\Delta\Delta CT}$ method of Livak and Schmittgen [25].

Spectral measurements

The mobile fiber-optical spectral installation is designed specifically for the study of fluorescence signals for the rapid analysis of camelina seed samples. It includes the following components (Figure 2):

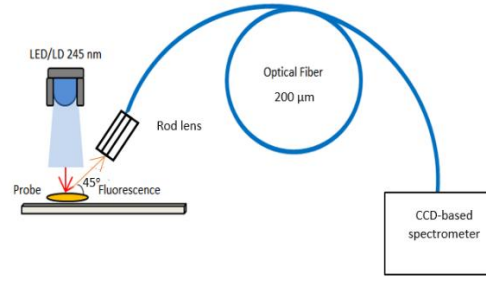

Figure 2. Mobile experimental installation used for fluorescence spectroscopy.

- Laser diode (LED) with emission radiation of 245 nm with a supply voltage in the range of 3V. It is enclosed in a hermetically sealed TO39-type metal case. The emitter current consumption is 0.02A, and its voltage drop is in the range of 1.9 to 2.4V. -6V is the minimum emitter voltage value.
- Rod lens consists of two connected Schott and Corning lenses with anti-reflective coatings that have different dispersion coefficients. It is of the achromatic doublet type. The chromatic aberration of one lens compensates for that of the other. This is due to the values of their radii. The forming optic has a diameter tolerance of - 0.005 mm.
- The multimode optical fiber has a core diameter of 200 µm. The optical fiber is of the type FG200LEA. It has a step index of the attenuation coefficient.
- The area of the quartz glass is 4 cm^2. Its optical properties include being transparent to visible light and ultraviolet and infrared rays. For this reason, it is observed that there are no inhomogeneities that scatter the light. The optical and thermal properties of quartz glass are superior to those of other types of glass due to its purity. Quartz glass has a very low light absorption coefficient.
- The sensitivity of the CMOS detector is in the range of 200 nm to 1100 nm. $\delta\lambda = 5$ is its resolution. Unlike widely used sensors, the profile of the detector sensor projections used in this study along the X and Y axes is designed to generate very small amounts of data.

The sample fluoresces after being irradiated by the LED. The emission signal is obtained at 45° from Rod Len, and the emission signal is generated. It is then transmitted through the optical fiber to the detector.

The fiber optic set-up used in this study has the following three unique advantages:

• Rod Len was used in the construction of the system. This lens was chosen for its high light transmission coefficient, which is due to the complete filling of the air gaps between the individual lenses included in its composition.

• An optical fiber and a Rod Len are precisely connected in a duralumin housing. This design achieves the optimum in laser diode imaging and fiber optic compilation, ensuring low levels of signal intensity loss.

• The emission fluorescence signal is obtained at 45°.

RESULTS AND DISCUSSION

Determination of total fat and fatty acid composition

Oil content in camelina seeds varies between genotypes and environmental conditions from 35 to 45% [26-29]. Our results correspond to these reports but remain far from the higher level of total crude fats. The low level of fertilization in our trial was chosen for better discrimination of the effect of the companion protein crop on the overall benefits of intercropping. While the yield of both crops is beneficial for the farmers [30], the seed oil content varies and is closer to the findings of Juodka that the crude fat content in seeds of camelina grown in Lithuania was around 36.84% [31]. Genotype reactions to poor nutrition regimes remain significant not only for the productivity of grains but also for their quality.

Differences between varieties are up to 4.8% - from 34.4% in the sole crop of Luna up to 39.2% for the local Bulgarian landrace. K3 has better productivity [30] and a higher percentage of total crude fats in all variants with a slight decrease (only 1.1%) for the camelina pea combination. This intercropping is more suitable for K2 cultivation where an increase of 1% is observed. The oil content of the Luna seeds is higher when accompanied by vetch (37.9%) than for the variant of camelina and fodder pea (33.5%).

Camelina oil has saturated fatty acids (FA) such as palmitic acid (PA: 16:0) and stearic acid (SA; 18:0) and unsaturated – monounsaturated oleic acid (OA; 18:1) and polyunsaturated as linoleic acid (LA; 18:2) and α-linolenic acid (ALA; 18:3). This omega-3 fatty acid is needed as a substitute for the human eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [32]. The results of the percentage content of fatty acids with a carbon chain length of C12 to C24 in the oil obtained from the seeds of three varieties of camelina K1 (Luna), K2 (Lenka) and K3 (Local) grown as sole-crop and compared to intercropping with leguminous crops in our certified organic field are presented on Table 2.

Lauric acid C12:0 is not contained in K3 but is present in low concentrations (0.1%) in the oils of all the other variants. The amount of myristic acid C14:0 does not exceed 0.1% in all tested seeds, which makes the oil less harmless to human health. The percentage content of palmitic acid C16:0 is higher in K1 (6.0%) and remains up to 5.7% in the other variants. Fat is an essential macronutrient of the human diet and vegetable oils represent a more consumed fat [33]. The effects of saturated fatty acids (C12:0 till C18:0) have been the focus on the reduction of cardiovascular diseases, obesity, and cancer prevention [34, 35].

Table 1. Total crude fat content in the mature seeds of three varieties of camelina K1 (Luna), K2 (Lenka) and K3 (Local Bulgarian landrace) grown alone and co-cultivated with leguminous crops - pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.), %.

№	Genotype	Camelina	Camelina	Camelina	Average for the genotype
		sole crop	pea	vetch	
1	Luna K1	34.4	33.5	37.9	35.3
2	Lenka K2	35.1	36.1	35.1	35.4
3	BG landrace K3	39.2	37.9	38.9	38.7
4	Average for the crop system	36.2	35.8	37.3	36.5

Table 2. Fatty acid composition (FAC) of the camelina oil from sole-crop and intercropping camelina Polish varieties K1 – Luna, K2 – Lenka and K3 – local Bulgarian landrace with pea and vetch, %.

FAC, %	1	2	3	4	5	6	7	8	9
	K1	K2	K3	K1+ pea	K2+ pea	K3+ pea	K1+ vetch	K2+ vetch	K3+ vetch
12:0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
14:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:0	6.0	5.7	5.7	5.7	5.5	5.7	5.6	5.5	5.7
16:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
18:0	1.9	2.1	2.3	1.9	2.0	2.3	1.9	2.1	2.3
9-18:1	14.1	17.3	18.3	14.9	17.2	17.2	14.6	17.7	18.3
11-18:1	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
18:2	20.6	19.9	18.4	21.0	19.7	18.5	20.5	19.5	19.0
18:3	31.5	30.2	32.9	31.7	31.0	34.1	32.5	30.9	35.6
20:0	1.5	1.5	1.4	1.4	1.5	1.3	1.4	1.5	1.4
20:1	15.1	15.3	14.2	14.8	15.3	13.9	15.0	15.2	16.0
20:2	1.9	1.6	1.3	1.9	1.6	1.4	1.9	1.5	1.3
20:3	1.1	0.9	0.9	1.1	1.0	1.0	1.1	1.0	0.9
22:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
22:1	3.7	3.1	2.5	3.4	3.1	2.5	3.4	3.0	2.5
24:0	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.1
24:1	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6

Polyunsaturated fatty acids include oleic, linoleic, and α-linolenic acids. Oils rich in oleic acid are suitable for heat treatment and have a long shelf life. In the present experiment, variation in oleic acid content is due mostly to genotype (around 14% for K1 up to 18.3% for K3) and to a lesser extent to the combination with vetch or fodder pea in our organic field. The cropping system has a lower impact on the linoleic C18:2 content and variations are mainly due to the variety. The most significant increase was observed in α-linolenic acids C18:3 profiles of the local Bulgarian landrace K3 when cultivated with vetch (up to 35.6%) and pea (34.1%) compared to the sole crop (32.9%). Its content is better also for the intercropping of the other two varieties Luna (K1) with vetch and Lenka (K2) with pea. Linolenic acid is a very valuable acid, but it oxidizes easily, which lowers the quality of food products during their storage. The oil with a high content of C18:3 was used for technical purposes and combustion.

The vetch as companion crop for K3 has a positive effect on the increase of eicosenoic acid C20:1. (Table 2).

Fad6 gene expression in the development seeds of camelina

Two PCR reactions were performed with 2 sample pairs of primers. The purified RNA does not contain DNA; therefore, the obtained amplification curves are not due to contamination and the RNA is of the necessary quality to run the PCR reactions. Actin was chosen as the "housekeeping" gene because it is expressed equally in all stages of camelina seeds development. As a result, *fad6* expression in camelina K3 variety grown intercropping with vetch was 5 times higher compared to control self-grown plants (Fig. 3). A correlation was observed between levels and *fad6* gene expression across seed formation of K3 in the nine different cultivation plots. Positive correlation was detected in C18:3 and C20:1 fatty acids.

Fluorescence spectroscopy analysis of seeds of camelina

From the stated circumstances, it can be concluded that fluorescence spectroscopy finds application for the analysis of camelina. This allows obtaining essential information about changes in the chemical composition and expression of camelina plants under organic cultivation and co-cultivation with peas and figs. The three main advantages of fluorescence spectroscopy are that the method is fast, can be performed *in situ* under uncontrolled conditions, and does not require consumables when performing the analyses.

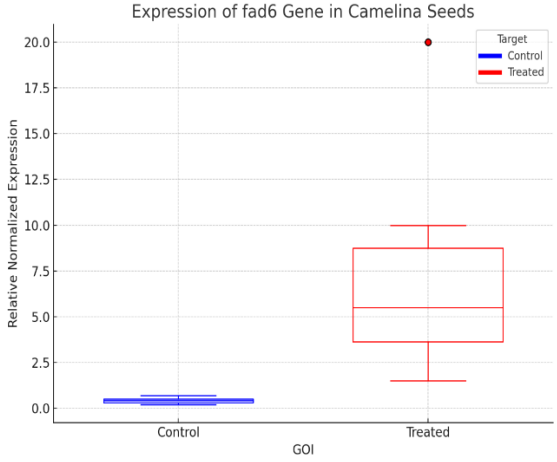

Figure 3. Comparison of *fad6* gene expression level between control self-grown plants and K3 variety grown in intercropping with vetch.

A literature survey was conducted to discover a similar research published by Slavova [24]. It turned out that until now the described experimental approach for analysis of camelina crops under organic cultivation and co-cultivated with pea and vetch is not known to have been implemented to conduct our experiment. Due to this fact, we claim that for the first time, we applied fluorescence spectroscopy for the analysis of different camelina plant samples (alone and in intercropping variants) after harvest.

Figure 4 A) represents the spectral distribution of the emission fluorescence signal of K1, K1+pea and K1+ vetch. A significantly stronger intensity of K1+vetch at 435 nm is observed, which is due to increased expression compared to K1 and K1+pea. The same result is confirmed by Figures 4 B and 5 A for the remaining two varieties K2 and K3, respectively.

The level of distribution of the emission signal in Figures 4 and 5 is dependent on the fatty acid content. The results of these three graphs show that camelina with a higher fatty acid content is best obtained if grown in combination with vetch. The highest level of fatty acid content is observed in K1+vetch. This result is presented in Figure 5B. Luna variety co-cultivated with vetch is the best of the three combinations for optimal obtaining of the highest fatty acid content.

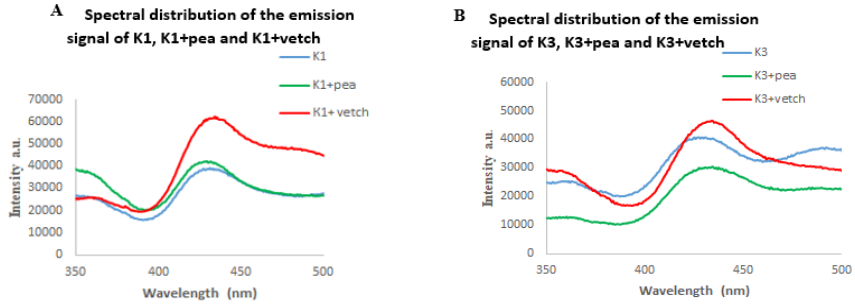

Figure 4. Spectral distribution of the emission signal of A) K1, K1+pea and K1+vetch and B) K3, K3+pea and K3+vetch

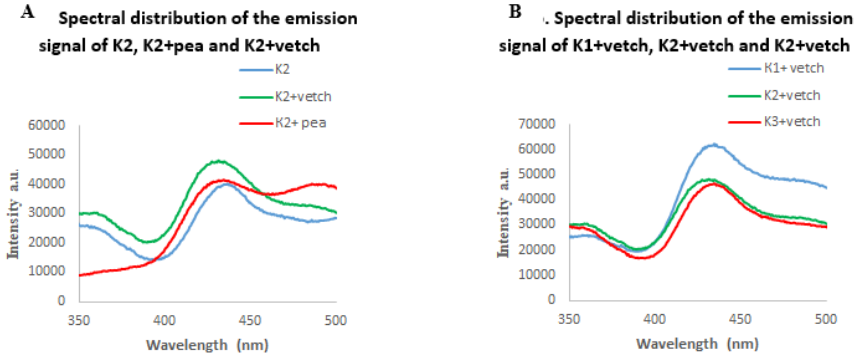

Figure 5. Spectral distribution of the emission signal of A) K2, K2+pea and B) K2+vetch and K1+vetch,

K2+vetch and K3+vetch

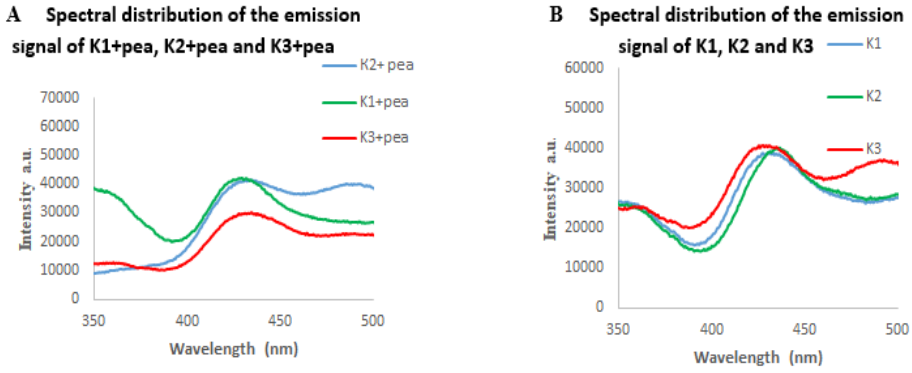

Figure 6. Spectral distribution of the emission signals of A) K1+pea, K2+pea and K3+pea and B) K1, K2, and K3.

A difference in the emission fluorescence signal of K1, K2 and K3 is presented in Figure 6 B. The spectral distribution for samples K1 and K2 is similar, but some differences in the intensities of the emission signal occurred. The difference in the emission wavelength shift between them and K3 is due to the different varietal characteristics. K3 has a more intense shift due to the tendency to increase the total crude fat content.

Figure 6 A presents the observed differences in the spectral distribution of the fluorescence signal and in its intensity when growing camelina alone and in combination with peas. Stability in signal propagation is observed in self-cultivation compared to camelina co-cultivated with pea and vetch. The presented spectral distributions of all tested materials are considered unique for each specific genotype. This can be considered as a basis for using the used installation for recognizing available camelina seeds in a non-invasive way with high accuracy. In this respect, it could be expected that seeds can be distinguished to confirm a closely related origin or genetically distantly related forms, the presence of inhomogeneous or stabilized selection material, and/or spectral definition of seeds of unknown origin. To confirm the application of fluorescence spectroscopy for the quantitative determination of fatty acids, it is necessary to deepen the studies on correlations in fatty acid measurements with spectral fluorescence analysis to confirm the possibility of using the dependence between the quantitative content of fatty acids in camelina seeds and the spectral distribution and intensity of the fluorescence signal obtained by spectral analysis of seeds of a selected genotype.

CONCLUSIONS

The analyses of our results show variation between genotypes, with a higher total crude fat content observed in the local Bulgarian landrace (K3) compared to the Polish varieties Luna (K1) and Lenka (K2). A clear tendency for improvement in oil content in camelina seeds was established when applying intercropping with fodder pea or vetch. Vetch proved to be a better companion for Luna, while fodder pea was more beneficial for Lenka. Therefore, a personalized decision for intercropping should be made based on the specific camelina variety.

Polyunsaturated fatty acids dominated the oil profile in all variants. Intercropping camelina with vetch enhanced the levels of α-linolenic acid (C18:3) and eicosenoic acid (C20:1) in K3 compared to its sole-crop variant. The same trend of increased α-linolenic acid was also observed in Luna when co-cultivated with vetch. For Lenka (K2), the fatty acid values were similar in both intercropping combinations. These findings were further supported by gene expression analysis, where the *fad6* expression in K3 intercropped with vetch was five times higher than in control plants grown in sole cropping. A positive correlation was observed between *fad6* expression levels and the content of α-linolenic

acid (C18:3) and eicosenoic acid (C20:1) in K3 across the different cultivation plots.

Fluorescence spectral analysis of camelina seeds revealed a correlation between the spectral characteristics (intensity and distribution of the fluorescence emission signal) and the content of monounsaturated fatty acids. Specifically, signal intensity was higher in seeds with elevated monounsaturated fatty acid content. For instance, K1 grown with vetch showed a stronger fluorescence signal at 435 nm, corresponding to higher FA levels. Notably, camelina grown in sole cropping showed more stable and clearly distributed emission signals compared to intercropped variants, suggesting that intercropping can influence seeds' biochemical composition and thus their spectral profiles.

In the future, this observed correlation between fluorescence spectral characteristics and fatty acid content could be further developed into a rapid, non-invasive method for screening camelina seed quality and biochemical composition under different organic cropping systems.

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REFERENCES

1. E.J. Murphy, in: T.A. McKeon, D.G. Hayes, D.F. Hildebrand, R.J. Weselake (eds.), *Industrial Oil Crops*, AOCS Press, Urbana, IL, USA, 2016, p. 230.
2. J. Zubr, *Ind. Crops Prod*., **6**, 119 (1997).
3. R.K. Gugel, K.C. Falk, *Can. J. Plant Sci.,* **86,** 1058 (2006).
4. J. Vollmann, A. Damboeck, A. Eckl, H. Schrems, P. Ruckenbauer, in: J. Janick (ed.), *Progress in New Crops*, ASHS Press, Alexandria, VA, USA, 1996, p. 362.
5. D.T. Ehrensing, S.O. Guy, Camelina, Oregon State University Extension Service, Corvallis, OR, USA, EM 8953-E (2008).
6. A. Francis, S.I. Warwick, *Can. J. Plant Sci*., **89**, 810 (2009).
7. D.H. Putnam, J.T. Budin, L.A. Field, W.M. Breene, In: J. Janick, J.E. Simon (eds.), New Crops, Wiley, New York, NY, USA, 1993, p. 322.
8. V.K.S. Shukla, P.C. Dutta, W.E. Artz, *J. Am. Oil Chem. Soc*., **79**, 969 (2002).
9. C. Boyle, L. Hansen, C. Hinnenkamp, B.P. Ismail, *J. Am. Oil Chem. Soc*., **95**, 1062 (2018).
10. J.G. Crowley, A. Fröhlich, The composition and use of camelina, Teagasc, Dublin, Ireland, 1998.
11. R. Hofer (ed.), Sustainable solutions for modern economics, Royal Society of Chemistry, Cambridge, UK, 2009.
12. S. De Jong, K. Antonissen, R. Hoefnagels, L. Lonza, M. Wang, A. Faaij, M. Junginger, *Biotechnol. Biofuels*, **10**, 64 (2017).
13. K.A. McVay, P.F. Lamb, Camelina Production in Montana, Field Crops No. D-16, Montana State University Extension, Bozeman, MT, USA,2007.
14. S. Maitra, J.B. Palai, P. Manasa, D.P. Kumar, *Int. J. Agric. Environ. Biotechnol*., **12**, 45 (2019).
15. A.K. Singh, S.K. Raina, M. Kumar, L. Aher, M.B. Ratnaparkhe, J. Rane, A. Kachroo, *Plant Mol. Biol.*, **110**, 218 (2022).
16. S. Zhu, Z. Zhu, H. Wang, L. Wang, L. Cheng, Y. Yuan, D. Li, *Sci. Hortic*., **239**, 170 (2018).
17. H.G. Damude, A.J. Kinney, *Plant Physiol*., **147**, 968 (2008).
18. X. Zhu, D. Wang, N. Li, X.S. Sun, *ACS Omega*, **2**, 8004 (2017).
19. M. Petkova, W.S. Chao, L. Cook, M. West, M. Rahman, M.E. Foley, *Plant Breed. Seed Sci*., **72** ,32 (2015).
20. D.M. Haagenson, R.L. Brudvik, H. Lin, D.P. Wiesenborn, *J. Am. Oil Chem. Soc*., **87** , 1358 (2010).
21. M.D. Zlatanov, G. Angelova-Romova, R. Antova, R.D. Dimitrova, B. Momchilova, M. Nikolova-Damyanova, *J. Am. Oil Chem. Soc*., **86,** 875 (2009).
22. S. Lu, A.P. Smith, D. Moore, N.M. Lee, *Mol. Cell. Probes*, **24**, 320 (2010).
23. X. Zhao, Y. Shen, W. Hu, J. Chen, T. Wu, X. Sun, W. Chen, *Mol. Immunol*., **68**, 647 (2015).
24. V. Slavova, E. Ropelewska, K. Sabanci, *Eur. Food Res. Technol*., **249**, 3245 (2023).
25. K.J. Livak, T.D. Schmittgen, *Methods*, **25**, 408 (2001).
26. Y. Jiang, C.D. Caldwell, K.C. Falk, R.R. Lada, D. MacDonald, *Agron. J*., **105**, 1852 (2013).
27. S.S. Malhi, E.N. Johnson, L.M. Hall, W.E. May, S. Phelps, B. Nybo, *Can. J. Soil Sci*., **94**, 47 (2014).
28. B. Moser, *Lipid Technol*., **22**, 273 (2010).
29. E. Ropelewska, K.J. Jankowski, *OCL*, **27**, 46 (2020).
30. M. Marcheva, J. Jordanov, G. Stanchev, P. Zorovski, in: Book of Abstracts of V. Balkan Agricultural Congress, Edirne, Turkey, 23 (2023).
31. R. Juodka, R. Nainienė, V. Juškienė, R. Juška, R. Leikus, G. Kadžienė, D. Stankevičienė, *Animals*, **12,** 295 (2022).
32. A.C. Rustan, C.A. Drevon, *eLS* (2005).

33. J. Aranceta, C. Perez-Rodrigo, *Br. J. Nutr*., **107**, S22 (2012).
34. G. Assmann, P. Buono, A. Daniele, E. Della Valle, E. Farinaro, G. Ferns, V. Krogh, D. Kromhout, L. Masana, J. Merino, *Nutr. Metab. Cardiovasc. Dis*., **24**, 1300 (2014).
35. N.A. Berger, *Ann. N.Y. Acad. Sci*., **1311**, 76 (2014).