

## EVALUATION OF MORPHOLOGY, PRODUCTIVE POTENTIAL, OIL CONTENT AND COMPOSITION OF PLANT GENETIC RESOURCES OF *CAMELINA SATIVA*

M. P. MARCHEVA

Agricultural University, BG-4000, Plovdiv, Bulgaria

### Abstract

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The increasing interest to *Camelina sativa* in the last decade is provoked by its various applications as source for biodiesel, edible oil or fodder. It regains interest the last decades with its good adaptation to various conditions and lower cost cultivation. Evaluation of morphological traits and productive potential of some plant genetic resources of the ex situ collection of the National gene bank of Bulgaria has been presented. Field trials of three cultivars and one local landrace in 3 succeeding years on two different soil types were conducted. Results are commented in order to establish possibilities for cultivation and expected yields of seed and oil and its fatty acid composition. Environmental and economic gain can be projected estimating the possible uses of the presented accessions or strategies for further breeding programs has been marked.

*Key words:* biodiesel, camelina, evaluation, fatty acids, plant genetic resources

### Introduction

*Camelina sativa* regain interest as attractive crop because of its good adaptation to various environmental conditions – soil type and fertility, water regime, temperature, low requirements to fertilization and pesticides (Crowley and Frohlich, 1998). It has various applications as feedstock for biodiesel, technical or edible oil and fodder. Camelina oil contained high percentages of linolenic (32.6 wt %), linoleic (19.6 wt %), and oleic (18.6 wt %) acids. Consequently, camelina oil methyl and ethyl esters (CSME and SEE) exhibited poor oxidative stabilities and high iodine values versus methyl esters prepared from canola, palm, and soybean oils (CME, PME, and SME). As blend components in ultra-low sulfur diesel fuel, CSME and CSEE were essentially indistinguishable from SME and soybean oil ethyl ester blends with regard to low temperature operability, kinematic viscosity, lubricity, and surface tension (Frohlich and Rice, 2005). Given these features, *C. sativa* will not displace or compete with other food-crops and will not affect food supply.

The by – products from oil extraction are similar to soybean

and canola meal and contain more than 40% proteins and has a moderately – low glucosinolate content. They can be used in heifer, chick, pig and fish feeding. In Europe camelina is being marketed in salad dressing, cooking oil (not suitable for deep-fat fry oil). It is also used in cosmetics, skin care products, soaps and soft detergents. Camelina performs well as companion crop in establishing legumes for green manure cover and for forage (Hunter, 2010).

Despite this potential, there are few camelina varieties that have been genetically improved and genetic and genomic characterization of camelina is still lagging behind. It has been proved that transgenic *Camelina sativa* can produce omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), widely recognized as functional food for prevention of cardiovascular disease and related metabolic conditions and primarily found in fish oils, without any impact of the total seed oil as stable trait in field conditions (Usher et al., 2015). The eicosapentaenoic acid oil from transgenic Camelina (ECO) could be used as a substitute for fish oil, however it is a hybrid oil containing both fish oil and vegetable oil (18:2 n-6) fatty acid signatures that resulted

\*E-mail: marina.marcheva@gmail.com

in similarly mixed metabolic and physiological responses. In addition, wild-type camelina is naturally rich in  $\alpha$  linolenic acid (ALA 18:3 n-3) (~ 45%) (Gunstone et al., 2007). It is the substrate fatty acid for the biosynthesis of n-3 LC-PUFA.

Molecular markers for genetic characterization in camelina, has been published by Vollmann et al. (2005) – RAPD, Ghamkhar et al. (2010) – ALFP and Manca et al. (2013) – SSR. Beside of providing a useful tool for germplasm identification and genetic diversity they will be very useful in genetic mapping and assisting plant breeders in early progeny selection.

## Materials and Methods

The aim is to evaluate the expression of the genetic potential of different accessions of *Camelina sativa* (L.) Crantz from the ex situ collection of the National Genebank of Bulgaria in IPGR Sadovo, two spring-seeded trials were conducted on two sites with different soil types – Chromic Luvisol and Fluvisol in three years from 2008 to 2010.

The evaluation of three varieties – Sortadinskiy (Poland), Hoga (Netherlands), Roye (Poland) and one local landrace from Bulgaria has been conducted on basic field conditions – natural soil fertility with minimal cultivation inputs. Experiments were arranged as lattice designs in two replicas on 10 m<sup>2</sup> plots. The trials were sown in March following wheat crop. Seeding rates were 4 kg of pure, live seeds per hectare. No fertilization was used as well as treatment with any pesticides. Harvesting in the beginning of June was conducted by standard plot combine directly without desiccation. Morphological and agronomical traits has been determined on 30 plants from each variant and replication – PH (plant height, cm); H 1 ( height to the first branch, cm); NB (number of branches), NP (number of pods per plant), LP (length of pod, cm); WP (width of pod, cm); LW ratio; SP (number of seeds per pod), W1000 (weight of 1000 seeds, g); WS (weight of seeds in one plant, g); seed yield (kg ha<sup>-1</sup>), Seed moisture (%); Oil content (%); Oil yield (l ha<sup>-1</sup>). Adjusted entry means from the lattice analysis were used for the combined analyses of variance; LSI (least significant increase, one-tailed t-statistic) was used as a statistical testing procedure (Petersen 1994).

Determination of oil content was performed in Department of Chemical Technology, Plovdiv University. The seeds (20 g sample) were air-dried (10% humidity). Camelina oil was extracted from finely ground seeds with hexane in a Soxhlet apparatus for 8 h. After extraction the solvent was removed in a rotary vacuum evaporator and the oil was determined by weight. Fatty acid composition of triacylglycerols and sterol esters was determined by gas chromatography (GC) of fatty acid methyl esters (FAME) (ISO 5508 (1990)).

## Results and Discussion

### *Climate and soil conditions*

The evaluation of *Camelina sativa* accessions is made in the Institute of Plant Genetic Resources (IPGR) – Sadovo. The accessions in *ex situ* collection are characterized and evaluated in field conditions in experimental plots of 10 m<sup>2</sup>. The experimental field is located in the Thracian Plain of South Central Bulgaria at 141 m asl, 42°07'N and 24°56'E. The climate is transcontinental with hot and dry summer and critical drought period in spring. Average annual rainfall around 345 m<sup>3</sup>/m<sup>2</sup>, unevenly distributed in crop vegetation, high summer temperatures (up to 38°C) and cold winters without snow cover are typical for the region. High air temperature accompanied by low air humidity and winds are observed in the end of May and June and often cause extreme and rapid drying of seeds and plants in the last vegetation period (Uzundzhaliyeva, 2014).

The Botanical garden is located on Chromic Luvisol (by FAO) – sandy loam with moderate humus horizon and low humus content. The soil pH reaction is acid to poor acid. The soil reserve of mobile phosphorus and acid hydrolysable mineral nitrogen is poor and good for mobile potassium. Mobile manganese, copper and zinc are sufficient but molybdenum supply is unfavorable. The experimental field is situated on Fluvisol (by FAO) with poor humus horizon, characterized with light mechanical structure and low moisture preservation ability. A moderate to low humus content and neutral soil pH reaction with good reserve of mobile potassium, moderate to low content of mobile phosphorus and very low nitrogen supply has been determined (Koinov et al., 1998).

### *Agronomical performance*

In the conditions of Southeastern Bulgaria *Camelina sativa* has very short vegetation period – 75 to 80 days. Sown in March, the accession flowers simultaneously in the beginning of May and reach maturity in early June. Camelina is used an annual crop from mustard family with similar but tenderer appearance and aroma then canola. The plant grows up to a height from 47 cm (Sortadinskiy) to 81 cm (Hoga) (Table 1). The stems are single, usually branched above, glabrous, sometimes with few simple and branched hairs. The leaves are alternate, sessile, lanceolate, entire (Roye) or slightly (Hoga) toothed, sparsely hairy. The inflorescences are elongated racemes borne on ascending pedicels. The flowers are tetramerous, light yellow in colour. Each plant forms 36 (Sortadinskiy) to 177 (Roye) pods which are leathery, pear-shaped and contain many seeds (up to 17 in the local landrace) that are oblong, brown, deeply grooved and 2–3 mm long (Table 1 at the end).

Variety Sortadinskiy (Poland) is highly variable in different years and soil conditions. Its stem is relatively shorter (63 cm)

**Table 1**  
**Morphological characteristics of *Camelina sativa* accessions, evaluated in IPGR Sadovo in 2008 to 2010**

Variety		soil	PH	HB	NB	NP	LP	WP	L : W	SP	W1000	WS
Sortadinskiy	2008	CL	47.46	21.1	7.8	79	6.2	3.4	1.82	12.2	3.4	1.51
		F	51.25	26.5	5.5	73.25	7	4.75	1.47	12.5	1.2	0.78
	2009	CL	68	34.11	7.78	123.45	6.4	4.5	1.42	13.71	1.2	0.8
		F	72.5	33.29	7.25	57.18	6	4.2	1.43	10.38	3.26	1.83
	2010	CL	73.1	32.8	7.7	36.2	5.8	3.6	1.61	11.1	1.4	1.42
average	F	66	44.1	5.3	85.5	5.9	3.9	1.51	13.7	1.2	0.66	
Hoga	2008	CL	63.05	31.98	6.89	75.76	6.22	4.06	1.55	12.27	1.94	1.16
		F	79.2	45.7	7.7	108.5	6.1	4	1.53	12	4.8	1.6
	2009	CL	80.1	44.8	7.5	98.1	6	3.7	1.62	12.3	5	1.2
		F	80.2	44.7	8.7	118.1	5.8	4.1	1.41	11.9	4.8	0.76
	2010	CL	79.3	44.4	8.8	96.9	6.1	4.4	1.39	12.1	3.44	1.15
average	F	81.3	46.6	5	41.1	6.3	3.7	1.7	12.8	4	1.11	
Roye	2008	CL	78.1	44.4	4.6	121.2	6.7	3.7	1.81	13	5.6	0.65
		F	79.7	45.1	7.05	97.32	6.17	3.93	1.58	12.35	4.61	1.08
	2009	CL	64.3	32.5	8.1	82.3	6	4.1	1.46	14.7	3.4	1.7
		F	66.7	33.1	8.5	71.5	5.9	4.1	1.44	13.9	2.9	1.56
	2010	CL	63.9	30.6	8.2	68.6	6.1	4.2	1.45	15.1	0.8	0.34
average	F	68.5	33.8	9.4	86.8	5.8	4.1	1.41	14.2	3.68	1.37	
Local landrace	2008	CL	69.8	33.7	7.6	39.2	6.9	3.8	1.82	13.7	0.8	2.2
		F	69.3	34.6	8.1	177.6	7	3.5	2	14.9	0.8	1.68
	2009	CL	67.08	33.05	8.32	87.67	6.28	3.97	1.6	14.42	2.06	1.48
		F	65.7	34.5	8	80.3	6	4.2	1.43	16.2	1.7	1.9
	2010	CL	68.3	32.1	8.1	78.6	5.9	4.3	1.37	15.1	2.5	2.12
average	F	63.4	30.4	8	69.2	6	4.2	1.43	14.7	1.5	1.1	
Local landrace	2008	CL	65.5	32.5	8.4	88.3	5.9	4.2	1.4	15.9	2.1	1.4
		F	69.2	34.1	8.6	67.8	6.7	3.9	1.72	16.8	1.9	2.5
	2009	CL	67.8	35.2	8.6	88.6	6.9	3.9	1.77	15.1	2	1.98
		F	66.65	33.13	8.28	78.8	6.23	4.12	1.52	15.63	1.95	1.83
	2010	CL										
average	F											

and forms the first branches at 32 cm above the soil surface which is comfortable for 3 mechanical harvesting. The plants have lowest number of branches, pods and number of seeds per pod. The seeds are also smallest with average 1000 seed weight 1.94 grams.

The Netherlands' variety Hoga is the tallest one in our conditions but no logging was observed. Its height is relatively stable in various environmental conditions (78 to 81 cm). The first branch is settled up on 45 cm. The plant is branched and forms around 100 pods with 11–13 seeds. The accession has the biggest seeds with average 1000 seed weight from 3.44 up to 5.6 gram in 2010. Serious shattering of the pods was observed every year and they were significant yield losses. The collected seeds per plant were smaller than the other accessions and did not exceed 1.6 g (average 1 g).

Relatively high (67 cm) and stable in our conditions is variety Roye (Poland). It is suitable for mechanic harvesting with around 8 branches, first of which branches above 33 cm. The total number of pods can reach 177 per plant, each of one has around 14 seeds with 1000 seeds weight up to 3.68 g (average 2 g).

The local landrace from Bulgaria is the most stable genotype in the experiment. The plant is medium high (65 to 71 cm) with first branches formed on 30–35 cm above the surface. It has around 8 branches per plant and can reach total of 88 pods with 10–16 seeds per pod. The 1000 seeds weight is average – 1.1 to 2.5 grams and the plant productivity can reach 2.50 grams.

Harvesting with standard plot combine directly without desiccation at seed moisture below 8 percent has been performed in the early June. Mean values of the results for seed and oil yields per hectare for each accession from all years and replica are presented on Table 2. Significant loss of seed yield in all plots and genotypes has been observed because of the serious shattering of pods. Obviously the absence of mineral fertilizers and any cultivation practices as treatments with pesticides has result in lower yielding capacity which is much below the reported camelina achievements in other countries (Frohlich and Rice, 2005; Hunter and Roth, 2010). At the provided minimal nutrition regime better performance is observed in variety Roye (Poland) – 745 kg ha<sup>-1</sup> and the local landrace (Bulgaria) – 680

kg ha<sup>-1</sup>. Variety Hoga (Netherlands) has the biggest genetic potential for seed yield but major part of the seeds cannot be collected because of the serious shattering of pods and thus its yields do not exceed 510 kg ha<sup>-1</sup>. As the safety guidelines call for camelina to be stored at no more than an eye-popping low 8 percent humidity all samples were harvested below these values. For safe storage 6 percent is ideal, while at 10 percent there is big possibility for spontaneous combustion.

The oil content of camelina seed vary from 33.1 (Hoga) up to 38.8 percent (Roye) which is more than the reported by Moser and Vaught (2010). With average seed yield of 745 kg ha<sup>-1</sup> at 7.5 % seed moisture the productive potential for oil yield of Roye is 289.2 l ha<sup>-1</sup>. The local landrace has similar results – at 36.9% of oil it can produce 250.9 l ha<sup>-1</sup>.

Those results are acceptable considering the low cost and could be obtained in organic farming systems on poor or unusable fields, unappropriated for other crop cultivation.

Cultivation practices aiming better nutrition and prevention of the shattering of seed pods and losses of great amounts of seeds and oil by appropriate date and harvest machinery or breeding new varieties with better shattering resistance and harder pods' shell can result in much better achievements. Additional applying of mineral fertilization even in small rates could

significantly increase the yield of seeds and oil.

The analyses of fatty acid composition of evaluated *Camelina sativa* accessions, differs from the reported results for other researches (Moser and Vaughn, 2010; Putnam, 1993). Monounsaturated fatty acids (MFA) prevail in all genotypes (Table 3). Highest composition is detected for oleic acid (18:1). In the local landrace from Bulgaria it value reach 25.9%. Similar data are found in Roye (25.8%) and Sortadinskiy (25.6%). The recorded values exceed Putnam's observations of 17%. Similar difference is found for eicosenoic acid (C 20:1). The second MFA has significantly higher amount in our camelina seeds then the reported by other authors (Putnam, 1993). The first Polland variety Sortadinskiy produces maximum 19.1% of it and the second one – Roye – has the smallest record – 17.2%. Erucic acid (C22:1) varies strongly and in Hoga and Bulgarian landrace it reach just 0.4% and 0.5% but in the Polland varieties is up to 5.3% of the oil content.

Polyunsaturated fatty acids (PFA) in camelina oil has been analyzed – linoleic (C 18:2),  $\alpha$ -linolenic (ALA) (C 18:3) and eicosadienoic acid (C 20:2). All has smaller percent of the total acid composition then other researchers report (Putnam, 1993). Linoleic acid reaches maximum 18.5% in the local landrace and 18.4% in variety Hoga. The Netherland's variety has also

**Table 2**

**Seed and oil yield of *Camelina sativa* accessions, evaluated in IPGR Sadovo in 2008 to 2010**

Variety	Origin	Seed yield, kg ha <sup>-1</sup>	Seed moisture, %	Oil content, %	Oil yield, l ha <sup>-1</sup>
Sortadinskiy	Poland	674	7.8	36	242.6
Hoga	Netherlands	510	8	33.1	168.8
Roye	Poland	745	7.5	38.8	289.1
Local landrace	Bulgaria	680	7.5	36.9	250.9

**Table 3**

**Fatty acid composition of *Camelina sativa* accessions, evaluated in IPGR Sadovo in 2008 to 2010, in percents %**

Fatty Acid Composition	Composition in Genotype / Origine, in %			
	Sortadinskiy Polland	Hoga Netherland	Roye Polland	Landrace Bulgaria
C12:0	0.1	0.2	0.1	0,1
C14:0	0.3	0.3	0.3	0.3
C14:1	1.4	1	0.8	1.2
C16:0	12.5	11.2	11.7	12
C16:1	0.3	0.3	0.2	0,3
C17:0	0.1	0.1	0.1	0,1
C18:0	4.2	3.7	4.1	4,1
C18:1	25.6	24.3	25.8	25,9
C18:2	15	18.4	16.8	18.5
C18:3	11.6	17.8	15.4	14.2
C20:0	2.3	2.4	1.8	2,1
C20:1	19.1	17.6	17.2	18,5
C20:2	1.3	1.3	1.4	1,3
C22:0	0.6	0.7	0.5	0,6
C22:1	5.3	0.4	3.6	0.5
C24:0	0.3	0.3	0.2	0,3

higher composition of ALA – 17.8% but still it cannot be compared with the 31% bound by Putnam. The content of eicosadienoic acid is around 1.3–1.4% in the seeds of all camelina accessions. From the saturated fatty acids major component in camelina seed is palmitic acid (C 16:0) with 11.2% (Hoga) to 12.5% (Sortadinskiy), followed by stearic acid (C 18:0) 3.7% (Hoga) up to 4.2% (Sortadinskiy).

## Conclusions

In the conditions of Southeastern Bulgaria *Camelina sativa* has very short vegetation period – 75 to 80 days. The plants grow up to 81 cm (Hoga), forms its first branches at 30–45 cm above the surface. Harvesting with standard plot combine directly without desiccation at seed moisture below 8 percent is possible in the early June. No logging has been observed, but very serious shattering of the pods at full maturity occurs in all years, soil types and genotypes. At the provided minimal nutrition regime better performance is observed in variety Roye (Poland) – 745 kg ha<sup>-1</sup> and the local landrace (Bulgaria) – 680 kg ha<sup>-1</sup>. Variety Hoga (Netherlands) has the biggest genetic potential for seed yield but major part of the seeds cannot be collected because of the serious shattering of pods and thus its yields do not exceed 510 kg ha<sup>-1</sup>. The oil content of camelina seed can reach 38.8 percent (Roye). With average seed yield of 745 kg ha<sup>-1</sup> at 7.5% seed moisture the productive potential for oil yield of Roye is 289.2 l ha<sup>-1</sup>. The local landrace has similar results – at 36.9% of oil it can produce 250.9 l ha<sup>-1</sup>. The analyses of fatty acid composition of evaluated *Camelina sativa* accessions, differs from the reported results for other researches. Highest composition is detected for MFA – oleic acid – 25.9% (the local landrace), Poland variety Sortadinskiy produces maximum 19.1% eicosenoic acid, Erucic acid varies strongly – 0.4% (Hoga) and 0.5% (Bulgarian landrace) up to 5.3% of the oil content (Poland varieties). Polyunsaturated fatty acids have smaller percent of the total acid composition then other researchers report. Linoleic acids reach maximum 18.5% in the local landrace and 18.4% in variety Hoga. The Netherland's variety has also higher composition of ALA – 17.8% but still it cannot be compared with the 31% bound by Putnam. The content of eicosadienoic acid is around 1.3–1.4% in the seeds of all camelina accessions. From the saturated fatty acids major component in camelina seed is palmitic acid (C 16:0) with 11.2% (Hoga) to 12.5% (Sortadinskiy), followed by stearic acid (C 18:0) 3.7% (Hoga) up to 4.2% (Sortadinskiy). Successful cultivation of *Camelina sativa* in Bulgaria could be achieved after improvement of nutrition regime by mineral or organic fertilization, optimization of practices for prevention of the shattering of seed pods and losses of

great amounts of seeds and oil by appropriate date and harvest machinery or breeding new varieties with better shattering resistance and harder pods' shell.

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