

# Agronomic performance and seed quality attributes of *Camelina* (*Camelina sativa* L. Crantz) in multi-environment trials across Europe and Canada

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## Abstract

*Camelina* (*Camelina sativa* L. Crantz) is considered a relatively new oilseed *Brassicaceae* in both Europe and North America, even though its history as a crop dates back to the Bronze Age. *Camelina* has recently received renewed interest from both the scientific community and bio-based industries around the world. The main attractive features of this species are: drought and frost tolerance, disease and pest resistance, a unique seed oil composition with high levels of n-3 fatty acids, a considerably high seed oil content, and satisfactory seed yields, in particular under low-input management and in limiting environments. Aiming at evaluating the feasible introduction of recently released *Camelina* breeding lines under different environmental conditions and their productive potential a multi-location trial was set up. The agronomic performance of nine improved genotypes of *Camelina* was evaluated in a wide range of environments in Europe (Greece, Italy, Poland) and in five locations across Canada, in two consecutive growing seasons (2015 and 2016). Sowing time was optimized for each location according to the different climatic conditions. *Camelina* proved to be a highly adaptable species, reaching seed yields of about 1 Mg DM ha<sup>-1</sup> under the most limiting conditions (i.e., low precipitation, poor soil quality, extremely high temperature at flowering). Growing environments characterized by mild temperatures and adequate rainfall (> 170 mm, during the growing season) resulted in higher average seed yields. The length of the growing cycle varied greatly between different locations (80–110 d), but the cumulative thermal time was quite stable (~1200 GDD, growing degree days). The advanced breeding line 787–08, which possesses up to 30% larger seed compared to the mean seed size of all other test entries, proved to be the most promising genotype across all locations in Europe and Canada, combining high seed yields (1.1–2.7 Mg DM ha<sup>-1</sup>) with improved yield stability. To the best of our knowledge, for the first time, *Camelina* lines with improved oil composition (i.e., increased oleic and  $\alpha$ -linolenic and lower linoleic acid contents) for feed, food and industrial applications were identified (789–02 and 887).

## KEYWORDS

Oilseed, Oil, Protein, Oil yield,  $\alpha$ -linolenic acid, Eicosenoic acid, TKW

## 1. INTRODUCTION

*Camelina* [*Camelina sativa* (L.) Crantz] is currently enjoying the attention of both research and industry in Europe and North America (Berti et al., 2016; Zanetti et al., 2013) due to its environmental adaptability, satisfactory seed yields, combined with a unique oil suitable for a multitude of bio-based applications (i.e., biofuels, jet fuel, oleochemical compounds, feed, and food). *Camelina* is native to Southeast Europe and Southwest Asia (Larsson, 2013; Radatz and Hondelmann, 1981) and has an ancient history dating back to 4000 BCE. Recently, *Camelina* has been sporadically cultivated, especially around its centre of origin, until the middle of the 20th century (Knörzer, 1978). Thereafter, more productive oilseeds, such as rapeseed (*Brassica napus* L. var. *oleifera*), became the primary source of vegetable oil in continental Europe. Interestingly, *Camelina* was “rediscovered” in the last decade and has gained considerable research attention, as demonstrated by the high number of recently published scientific papers (reviewed in Berti et al., 2016) and the considerable number of large EU projects (i.e., ICON; ITAKA, CORE, COSMOS), funded either within the FP7 (Framework Program 7) or Horizon 2020. In North America, *Camelina* has been identified as a promising oilseed crop in view of relatively low agricultural input requirements (Ehrensing and Guy, 2008; Obour et al., 2015; Robinson, 1987), resistance to common Brassica pests (Carcamo et al., 2007; Deng et al., 2004; Pachagounder et al., 1998; Singh and Sachan, 1997), and diseases (reviewed in: Séguin-Swartz et al., 2009; Vollmann and Eynck, 2015), as well as tolerance to drought (Hunsaker et al., 2011, 2013) and low temperature (Putnam et al., 1993). The environmental adaptability and the recent commercial availability of both winter and spring cultivars (Mirek, 1980) confer an enormous advantage to *Camelina* over other emerging oilseed crops, even those belonging to the same botanical family (*Brassicaceae*), such as Indian mustard (*B. juncea*),

Ethiopian mustard (*B. carinata*), or crambe (*Crambe abyssinica*), for the inclusion in traditional crop rotations primarily based on cereals (wheat and corn) or pulses (soybean) (Chen et al., 2015; Gesch et al., 2014). Different from other cultivated *Brassicaceae*, camelina has a unique seed oil composition (reviewed in: Righini et al., 2016; Vollmann and Eynck, 2015) with a high content of  $\alpha$ -linolenic acid (20 to >35%), eicosenoic acid (11–19%) and tocopherols (Vitamin E), (Abramovic et al., 2007; Zubr, 1997; Zubr and Matthäus, 2002) as well as a naturally low content of the undesirable fatty acid erucic acid (< 4%), rendering camelina oil well-suited for a variety of food, feed or non-food applications (Berti et al., 2016; Eynck and Falk, 2013; Faure and Tepfer, 2016; Murphy, 2016; Waraich et al., 2013; Zubr, 1997). On the other hand, some negative traits of camelina obviously exist that hinder readily adoption by farmers and there is a strong need to improve them through breeding; in particular, the small seed size (thousand seed weight ~1.0 g) can cause difficulties in stand establishment as well as for harvesting of the crop (Sintim et al., 2016). In the framework of an international project a close collaboration was built between Canada and Europe aiming at studying the agronomic potential of improved camelina lines under different climatic conditions in order to possibly select the most suitable genotype for each environment. In the present study, the productive performance (seed yield, seed oil and protein content, seed size and oil composition) of eight new spring camelina breeding lines and one cultivar was tested in Canada and Europe for two consecutive years.

## 2. MATERIAL AND METHODS

Nine different spring-type camelina lines (Table 1) were tested in a multi-location (three locations in Europe and five in Canada) and multi year (2015 and 2016) screening trial aimed at identifying the most suitable breeding line(s) for different environments. All genotypes, except for the cultivar Midas (Table 1), are advanced breeding lines developed at Linnaeus Plant Sciences, Inc. in Saskatoon (Canada). Midas was developed at the Saskatoon Research and Development Center of Agriculture and Agri-Food Canada (AAFC-SRDC). Plot size was 10 m<sup>2</sup> in all European trials and ranged from 7.4 m<sup>2</sup> (Saskatoon and Swift Current) to 18 m<sup>2</sup> (Vanguard) in Canada. At all sites, seeding was accomplished using a plot drill, apart from Greece where sowing was carried out manually. The trials were arranged as completely randomized blocks with three or four replicates. Characteristics of soil and climate at each study site are summarized in Table 2.

Table 1. Camelina lines tested in the screening trials in Europe and Canada for two consecutive growing seasons (2015 and 2016).

### 2.1. Experimental set up of trials in Europe

Accession number/Variety name	Study ID	Source
Midas	<i>Midas</i>	Agriculture and Agri-food Canada, Saskatoon, Canada
14CS0886	886	
14CS0887	887	
13CS0787-05	787-05	
13CS0787-06	787-06	Linnaeus Plant Sciences, Inc.,
13CS0787-08	787-08	Saskatoon, Canada
13CS0787-09	787-09	
13CS0787-15	787-15	
13CS0789-02	789-02	

The European trials were set up according to a commonly agreed upon experimental protocol, in order to be able to easily compare results. The three locations (Table 2), covering a large geographical area from 38 to 53° North latitude and from 11 to 23° East longitude, are highly representative of very different environments potentially suitable for growing camelina. Sowing took place between mid-March and mid-April, while harvesting occurred three to four months later depending on location (Table 3). The same sowing density (500 seeds m<sup>-2</sup>), row distance (0.15 m) and fertilization (ranging from 40 to 60 kg of N ha<sup>-1</sup>, depending on available soil N) were used in all locations. Nitrogen fertilizer was manually broadcasted at the beginning of stem elongation as urea. Trials were all rain fed, except in Greece where supplemental water was applied by means of a sprinkler irrigation system (20 and 40 mm of water in 2015 and 2016, respectively). Neither pesticide application nor chemical weed control were necessary during the growing season, and weeds were controlled by hand weeding.

## 2.2. Experimental set up of trials in Canada

Field trials in Canada were seeded at Fort St. John in the Peace River area of Northern British Columbia and four locations in the Prairie Provinces, Alberta (AB) and Saskatchewan (SK): Oyen (AB), Vanguard, Swift Current and Saskatoon (SK), (Table 2). Prior to seeding, soil fertility was determined and adjusted to 150 kg N ha<sup>-1</sup> (optimal level for canola) by applying urea. In 2015, trials were seeded between May 4 (VAN) and May 21 (OYE). In the second trial year, seeding occurred between early May (May 6, VAN) and early June (June 3, OYE). The seeding rate was 500 seeds m<sup>-2</sup> at all locations except for SAK (350 seeds m<sup>-2</sup>). Row spacing ranged from 0.2 m in Fort St. John to 0.3 m in Saskatoon. Weeds were controlled either entirely by hand weeding or a combination of hand weeding and pre-emergence application and incorporation of ethalfluralin (5%, at 17 kg ha<sup>-1</sup>), trifluralin (480 g l<sup>-1</sup>, at 1.7 l ha<sup>-1</sup>) or glyphosate (540 g l<sup>-1</sup>, at 0.61 l ha<sup>-1</sup>). Plots were either left standing in the field until they were completely ripe, swathed or treated with diquat and subsequently combined with a plot combine. Harvest dates in 2015 ranged from end of August (August 31, SAK and SWC) to mid-late October (October 19, OYE). The late harvest in OYE was caused by dry conditions in early summer and wet conditions in July and August. In 2016, plots were combined between mid-late August (August 18, SWC) to late September (September 27, OYE, Table 3).

Table 2. Locations, soil type and main climatic characteristics (20-yr historical data) of study sites in 2015 and 2016.

Location (country/province)		Site ID	Coordinates	Type of soil	Mean annual precipitation (mm)	Mean annual temp (°C)
Europe	Aliartos (Greece)	ALI	38°22'N, 23°6'E	Sandy loam	485	16.7
	Bologna (Italy)	BOL	44°33'N, 11°23'E	Silty clay loam	613	13.4
	Kętrzyn (Poland)	KET	53°58'N, 21°8'E	Sandy loam	683	8.0
Canada	*Fort St. John (British Columbia)	FSJ	56°15'N, 120°50'W	Silty clay loam	445	2.3
	Oyen (Alberta)	OYE	51°21'N, 120°28'W	Sandy loam	312	4.1
	Saskatoon (Saskatchewan)	SAK	52°7'N, 106°40'W	Clay loam	340	3.3
	Swift Current (Saskatchewan)	SWC	50°17'N, 107°47'W	Silt loam	393	4.1
	Vanguard (Saskatchewan)	VAN	49°54'N, 107°18'W	Clay	357	4.3

Table 3. Sowing and harvesting dates, Growing Degree Days (GDD), and main climatic conditions of surveyed growing seasons (2015 and 2016) for spring camelina lines across all trial locations in Europe and Canada.

\* in this location the second year harvest was not performed due to adverse environmental conditions.

\*GS= Growing season from seeding to harvest

Site ID	2015						2016					
	Sowing date	Harvest date	Mean min temp GS* (°C)	Mean max temp GS* (°C)	Cumulate precipitation GS* (mm)	GDD**	Sowing date	Harvest date	Mean min temp GS* (°C)	Mean max temp GS* (°C)	Cumulate precipitation GS* (mm)	GDD**
ALI	04/10	07/15	13.6	26.4	87.1***	1300	03/21	06/21	11.7	25.8	25.5***	1266
BOL	04/01	06/26	11.7	24.1	190.5	1117	03/17	06/29	11.0	22.3	225.8	1226
KET	04/14	07/27	8.5	19.6	207.6	933	04/07	08/21	10.5	20.8	427.9	1411
FSJ****	05/20	09/18	8.4	21.1	177.8	1193	-	-	-	-	-	-
OYE	05/21	10/19	7.9	22.9	112.5	1520	06/03	09/27	9.0	21.6	194.3	1205
SAK	05/12	08/31	10.0	23.4	196.2	1309	05/17	08/22	11.5	23.5	210.6	1227
SWC	05/05	09/01	9.0	23.7	127.6***	1350	05/17	08/18	10.5	22.6	207.9	1096
VAN	05/04	08/20	8.8	23.5	247.9	1219	05/06	08/20	9.4	21.5	308.6	1369

\*\*Base temperature for calculation 5°C (Gesch, 2014)

\*\*\* irrigated trials

\*\*\*\* in this location the second year harvest was not performed due to adverse environmental conditions.

## 2.3. Meteorological data

Main meteorological data, including air temperature (minimum and maximum) and precipitation, were collected by weather stations located nearby each experimental location for the two growing seasons (Table 3). Since the test lines did not present significant differences regarding their phenological development, growing degree days (GDD) were calculated for each location and growing season (Table 3) as follows:

$$GDD = \sum[(T_{\max} + T_{\min})/2 - T_{\text{base}}]$$

Where  $T_{\max}$  and  $T_{\min}$  are daily maximum and minimum air temperature, respectively, and  $T_{\text{base}}$  is the base temperature for which a value of 5 °C was adopted (Gesch, 2014).

#### 2.4. Surveyed parameters in the field and laboratory analyses

At the European locations, seed yield was assessed at full maturity (i.e., seed moisture  $\leq 12\%$ ) by manually harvesting the central portion of each plot ( $\sim 6 \text{ m}^2$ ), followed by threshing. Residual seed moisture content after threshing and cleaning was determined on a representative seed sub-sample from each plot by oven drying at  $105 \text{ }^\circ\text{C}$  until reaching constant moisture levels, and weighed. At the Canadian locations, trimmed plots were combined with a plot combine, the seed cleaned and dried at  $35 \text{ }^\circ\text{C}$  for 48 h and the weight of the seed determined (seed moisture content 2–3%). Seed yields presented here are adjusted to dry matter (DM). All qualitative parameters of camelina seeds were analyzed in the same lab in Canada. Thousand kernel weight (TKW) was determined on representative seed samples derived from each plot using an Elmor C1 seed counter (Elmor Ltd., Schwyz, CH). Seed oil and protein contents were determined on representative whole seed samples from each plot by near infrared spectroscopy (NIRS) at Agriculture and Agri-Food Canada, Saskatoon using a FOSS NIR Systems Model 6500 (FOSS, Hillerød, DM), (Petisco et al., 2010; Velasco et al., 1999). Protein contents are reported as a percentage,  $\text{N} \times 6.25$ , calculated on a whole seed dry matter (zero moisture) basis. Oil contents are reported as a percentage on a whole seed dry matter (zero moisture) basis. To determine fatty acid composition of the seed oil, 25–30 camelina seeds from each plot, considered a representative sample, were methylated overnight in tightly screw capped glass tubes at  $80 \text{ }^\circ\text{C}$  in 2 ml 1 M methanolic HCl (Supelco) and 0.5 ml hexane. After cooling, 2 ml 0.9% NaCl and 1.5 ml hexane were added to each tube. Samples were vortexed and allowed to settle. Gas chromatography of fatty acid methyl esters in the hexane layer was conducted using an Agilent 6890N GC fitted with a DB-23 capillary column (0.25 mm  $\times$  30 m, 0.25  $\mu\text{m}$  thickness; J & W, Folsom, CA, USA) as described previously (Kunst et al., 1992; Puttick et al., 2009).

#### 2.5. Statistical analysis

Prior to analysis of variance (ANOVA), the Bartlett's Test ( $P \leq 0.05$ ) was used to verify the homoscedasticity of data. If the variance was homogeneous, data were subjected to a two-way ANOVA considering “year” as a random factor. ANOVAs were applied only on data available for all locations in both years. For this reason, all results from Fort Saint John (FSJ) were excluded from ANOVAs, due to impossible harvest in 2016 (see Section 3.1), likewise, productive data (seed and oil yields) from Vanguard (VAN) were excluded from the ANOVA, due to adverse meteorological condition (see section 3.1). When the analysis of variance revealed significant differences among treatments ( $P \leq 0.05$ ), the Newman-Keuls test was used to separate means.

### 3. RESULTS

#### 3.1. Weather conditions and crop development

Precipitation and temperature patterns were generally consistent with historical data at all locations in Europe (Table 3). In 2016, shortly before harvest an intense hail storm occurred in Italy (BOL) causing a yield reduction of about 40%. With the exception of the extremely southern location (Greece, ALI), in both years all European sites benefited from a cumulative amount of rainfall ( $> 170 \text{ mm}$ ) able to meet the water requirement of camelina, as given by Hergert et al. (2016). Thermal time (Table 3) from planting to harvest was similar across years and locations with an average of 1209 GDD, a value in line with those reported by Gesch (2014). Both the lowest and highest GDD values were observed in Poland (KET, Table 3). Thus, in 2015, the growth cycle of camelina was completed after 933 GDD (i.e., a value inline with typical environmental conditions of the site); however, the second season, characterized by exceptionally high precipitation in July and August, showed a prolonged maturation phase resulting in 1411 GDD.

Generally, the weather conditions at the Canadian locations were appropriate to allow adequate camelina development. In most of the Prairie Provinces, the first growing season (2015) experienced an early summer drought that, however, only partially affected the performance of the crop. In SWC prolonged drought after seeding resulted in poor emergence; in order to prevent complete loss of the stand, rescue irrigation (15 mm) was applied one month after sowing, causing delayed secondary emergence resulting in a prolongation of the entire growing cycle, which is reflected by the increased GDD value (Table 3). It is also worth noting that in OYE a significant delay in harvest, due to adverse climatic conditions, directly translated into a significant increase in GDD values. The second growing season (2016) presented precipitation amounts more in line with typical values for each site, and likewise temperatures at each site demonstrated the same tendency. VAN experienced an intense hail storm at the beginning of July, when camelina was in the middle of flowering, which caused significant yield losses. Nevertheless, seed qualitative traits were not affected. Trials established in FSJ experienced unusually early snow fall before harvest; therefore, data from this location could not be

included in the analysis. In summary, for the second growing season only data for four Canadian locations were available. Generally, the length of the growing cycle for camelina (GDD) was more stable across locations in 2016, with a mean of 1230 GDD needed to reach maturity, similar to that surveyed across European sites.

### 3.2. Camelina productive performance

The genotype by location interaction was significant only for some qualitative traits of oil composition (C18:2 and C20:1), while location, and to a lesser extent, genotype presented significant effects on camelina productivity (seed yield, oil and protein content, oil yield, TKW, oil composition), demonstrating how climatic conditions strongly modulate camelina productive performance (Table 4). Seed and oil yields were the most variable traits, showing the highest variation coefficient (CV = 0.35), while qualitative seed traits appeared less affected (CV = 0.11). Mean seed yields (Fig. 1) varied significantly across locations: the highest seed yields were measured in SAK (Canada) and BOL (Italy). When considering single growing season data, the highest mean seed yield over all nine tested entries was reached at SWC in 2015 (2.22 Mg DM ha<sup>-1</sup>, data not presented), and the lowest was observed at OYE in the same year (1 Mg DM ha<sup>-1</sup>). Seed yield also varied significantly among tested genotypes ( $P \leq 0.05$ , Fig. 2), even though less than across locations. Line 787-08 was the highest-yielding entry at almost all sites, with an average seed yield of 1.79 Mg DM ha<sup>-1</sup>. In contrast, line 887 was the least productive ( $P \leq 0.05$ ) line with only 1.49 Mg DM ha<sup>-1</sup>, but significant difference emerged only when compared to 787-08. The latter also showed the lowest CV for seed yield (0.26 vs. 0.33, 787-08 vs. mean CV of all trials, respectively), confirming its superior yield stability. Oil yield mirrored seed yield, and it was generally higher in Canada than in Europe (Fig. 1). Comparing locations (Fig. 1), the highest oil yield occurred in SAK and SWC (Canada), while the lowest was observed in ALI (Greece). Despite similar seed yields, SWC (Canada) showed a higher oil yield potential than BOL (Italy) due to a higher seed oil content (Fig. 3). This might be related to environmental conditions suited to a prolonged maturation phase in Canada. Protein content was also significantly ( $P \leq 0.05$ ) affected by the location (Fig. 3) and, to a lesser extent, by genotype (Fig. 4). As expected, seed oil and protein contents were negatively correlated ( $r = -0.84$ ,  $P \leq 0.05$ ), with generally higher seed oil contents at the Canadian locations and increased protein contents at the European trial sites (Fig. 3). Comparing the different genotypes (Fig. 4), 787-15 and 787-09 were characterized by increased seed oil content. The genotype 787-09 also showed the second highest seed production (Fig. 2), after 787-08. The tested camelina lines showed a significant positive, but negligible, correlation between seed oil content and seed yield ( $r = 0.14$ ,  $P \leq 0.05$ ).

Seed weight (TKW) represents one of the major constraints limiting camelina establishment; also for this trait both location and genotype effects were significant ( $P \leq 0.05$ ). In contrast to the situation for all other evaluated parameters, genotype had a greater effect on seed weight than growing location (on average 36% difference between the heaviest and the lightest genotype, vs. on average 29% difference across locations). KET (Poland) and OYE (Canada) were identified as the locations (Fig. 5) producing larger seeds ( $P \leq 0.05$ ), while ALI (Greece) produced significantly smaller seeds. Among tested genotypes, two lines, 787-06 and 787-08, were identified as large-seeded (Fig. 6), being able to produce seeds with a TKW of ~1.9 g in certain years and environments (FSJ, Canada in 2015, data not presented).

For both location and genotype the effect on FA composition of camelina oil was found to be significant. Equally, principal FAs (i.e., C18:1, oleic, C18:2, linoleic, C18:3,  $\alpha$ -linolenic and C20:1, eicosenoic acids) and FA groups (saturated fatty acids, SFA, mono-unsaturated fatty acids, MUFA, and poly-unsaturated fatty acids, PUFA) showed significant differences ( $P \leq 0.05$ ) in response to environmental conditions (Table 5), with camelina grown at locations characterized by lower temperatures during seed filling, such as OYE (Canada) and KET (Poland), being able to accumulate higher quantities of  $\alpha$ -linolenic acid and PUFAs than those grown at the warmer Mediterranean sites, like ALI (Greece) and BOL (Italy). Interestingly, oleic and linoleic acid contents were more variable than  $\alpha$ -linolenic and eicosenoic acid contents, demonstrating higher CVs (0.16 and 0.20 for oleic and linoleic acid, respectively, compared to 0.09 and 0.05 for  $\alpha$ -linolenic and eicosenoic acid, respectively). Among the tested camelina genotypes, lines 887 and 789-02 presented significantly different fatty acid profiles (Table 6), characterized by increased contents of oleic and  $\alpha$ -linolenic acids and

reduced amounts of linoleic acid. This trait remained stable in response to different growing environments and resulted in a significantly lower content of PUFAs (Table 5) in these two lines compared to all other genotypes.

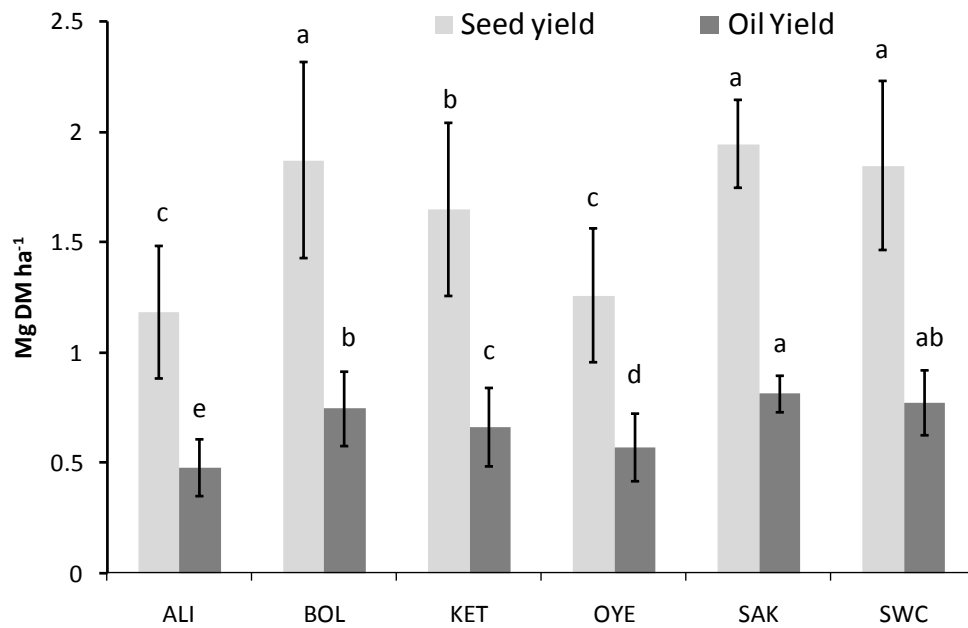


Figure 1. “Main effect: environment” for seed and oil yield (Mg DM ha<sup>-1</sup>) for nine camelina breeding lines grown at six different locations for two consecutive growing seasons (2015 and 2016). Different letters within each parameter: significant different values ( $P \leq 0.05$ , Newman-Keuls test). Vertical bars: standard deviation.

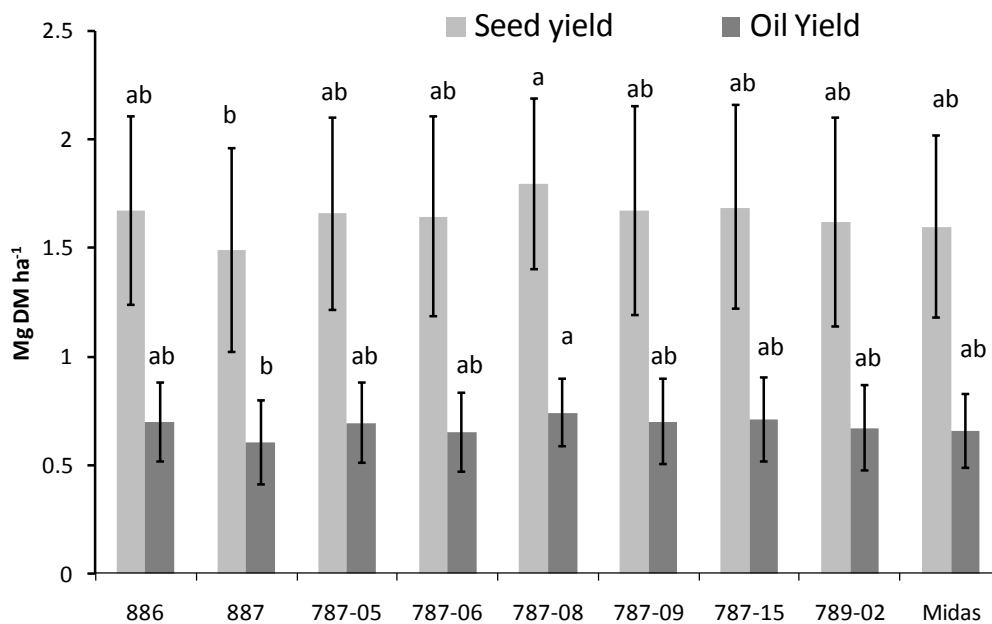


Figure 2. “Main effect: genotype” for seed and oil yield (Mg DM ha<sup>-1</sup>) obtained for nine camelina breeding lines grown at six different locations for two consecutive growing seasons (2015 and 2016). Different letters within each parameter: significant different values ( $P \leq 0.05$ , Newman-Keuls test). Vertical bars: standard deviation.

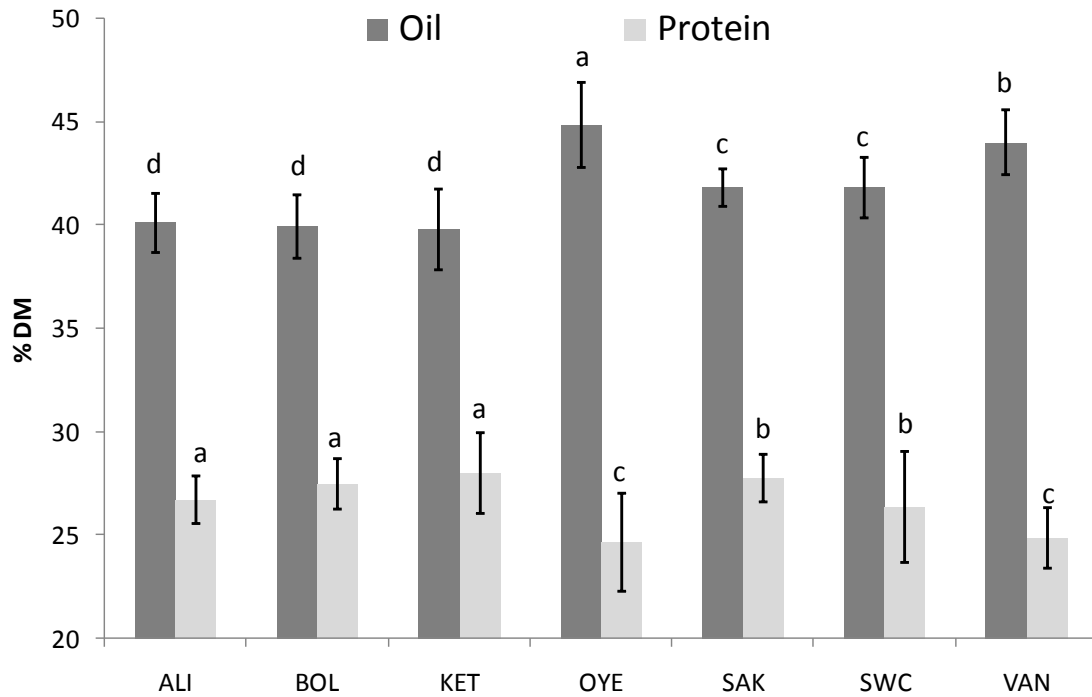


Figure 3. “Main effect: environment” for seed oil (dark grey histograms) and protein (light grey histograms) content (% DM) for nine camelina breeding lines grown at seven different locations for two consecutive growing seasons (2015 and 2016). Different letters within each parameter: significant different values ( $P \leq 0.05$ , Newman-Keuls test). Vertical bars: standard deviation.

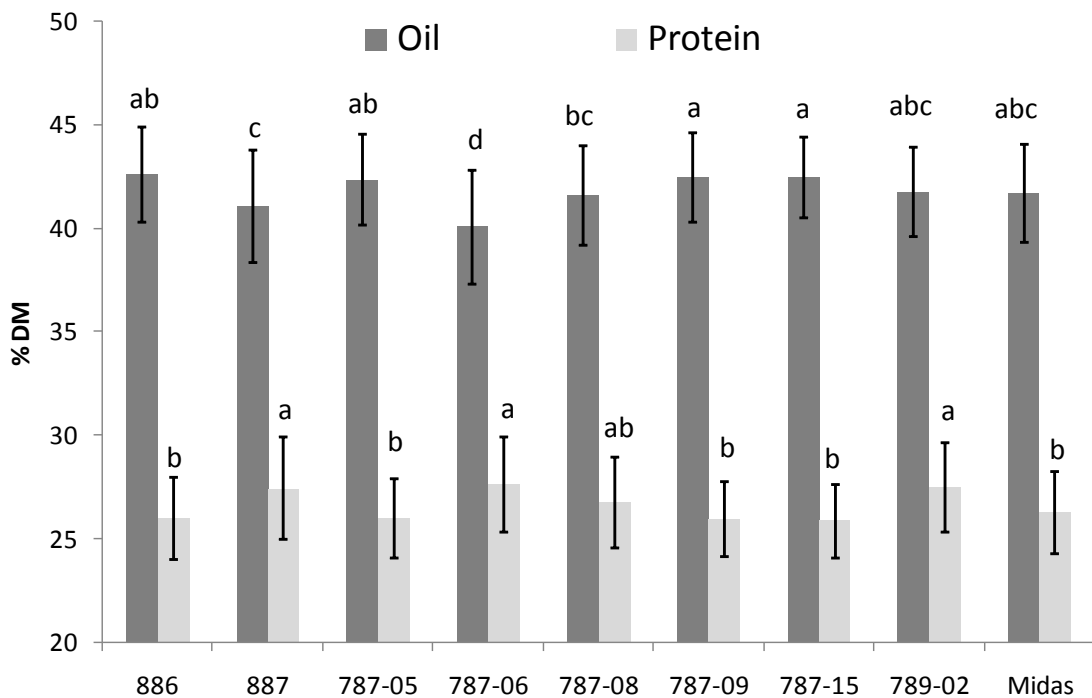


Figure 4. “Main effect: genotype” for seed oil (dark grey histograms) and protein (light grey histograms) content (% DM) obtained for nine camelina breeding lines grown at seven different locations for two consecutive growing seasons (2015 and 2016). Different letters within each parameter: significant different values ( $P \leq 0.05$ , Newman-Keuls test). Vertical bars: standard deviation.

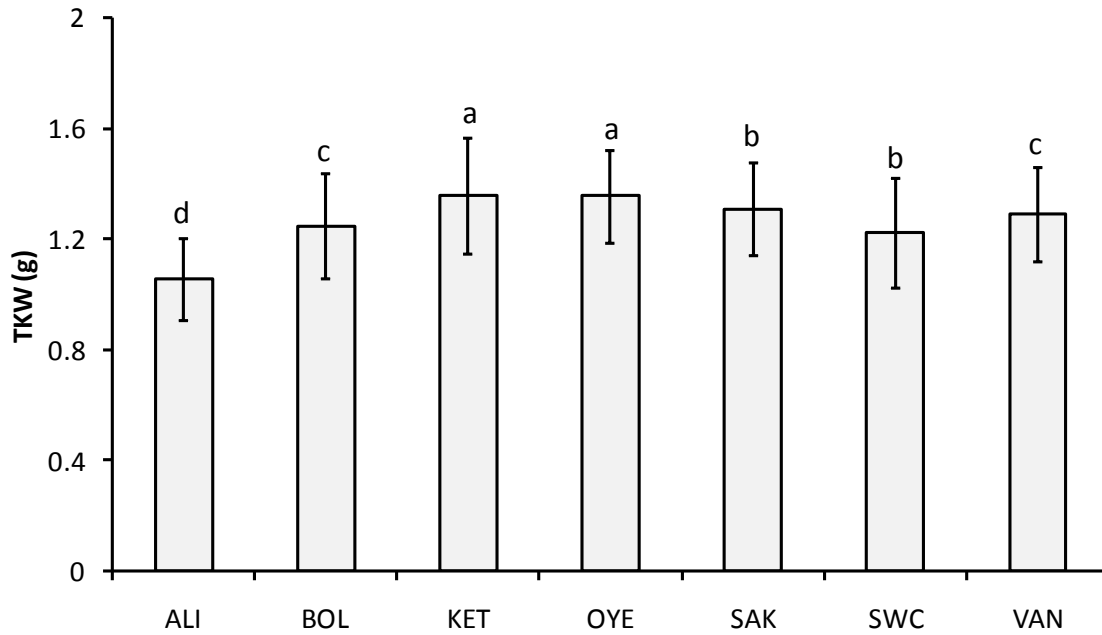
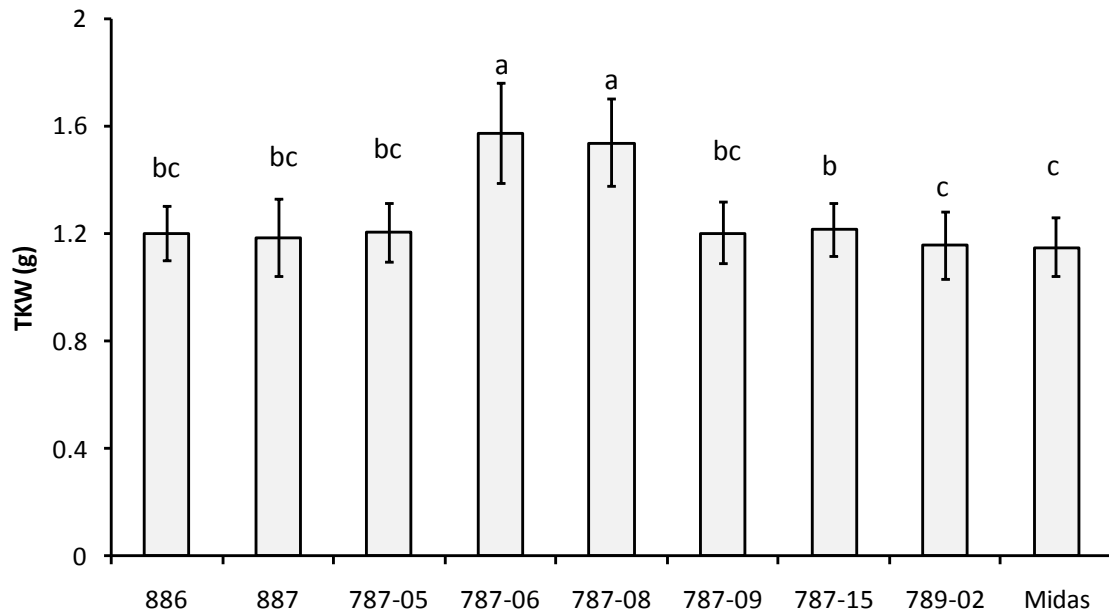


Figure 5. “Main effect: environment” for thousand kernel weight (TKW, g) obtained for nine camelina breeding lines grown at seven different locations for two consecutive growing seasons (2015 and 2016). Different letters within each parameter: significant different values ( $P \leq 0.05$ , Newman-Keuls test). Vertical bars: standard deviation.

Figure 6. “Main effect: genotype” for thousand kernel weight (TKW, g) obtained for nine camelina breeding lines grown



at seven different locations for two consecutive growing seasons (2015 and 2016). Different letters within each parameter: significant different values ( $P \leq 0.05$ , Newman-Keuls test). Vertical bars: standard deviation.



Table 4. “Main effect: environment” for oil composition (i.e., average values for principal fatty acids and fatty acid groups) for nine camelina breeding lines grown at seven different locations for two consecutive growing seasons (2015 and 2016). Different letters within each column: significant different values ( $P \leq 0.05$ , Newman-Keuls test).

Site ID	C18:1	C18:2	C18:3	C20:1	SFA	MUFA	PUFA
<i>ALI</i>	16.9 a	20.9 a	28.7 f	13.9 c	10.6 a	36.0 a	52.8 b
<i>BOL</i>	16.6 a	19.4 b	30.8 e	13.3 e	10.4 a	35.2 ab	53.6 b
<i>KET</i>	13.9 c	16.9 f	35.0 a	13.9 bc	10.1 a	33.2 d	55.9 a
<i>OYE</i>	15.5 b	17.2 e	34.8 a	14.0 ab	7.3 c	34.0 c	55.7 a
<i>SAK</i>	16.8 a	18.7 c	31.7 d	13.5 d	10.4 a	35.2 ab	53.5 b
<i>SWC</i>	14.3 c	17.9 d	33.8 b	14.1 a	10.4a	33.4 cd	55.4 a
<i>VAN</i>	17.0 a	18.0 d	32.6 c	13.9 c	9.7 b	35.1 b	53.1 b

Table 5. “Main effect: genotype” for oil composition (i.e., average values for principal fatty acids and fatty acid groups) for nine camelina breeding lines grown at seven different locations for two consecutive growing seasons (2015 and 2016). Different letters within each column: significant different values ( $P \leq 0.05$ , Newman-Keuls test).

Study ID	C18 :1	C18 :2	C18 :3	C20 :1	SFA	MUFA	PUFA
886	14.8 c	19.7 a	31.9 d	13.9 ab	9.8 ab	33.8 c	55.4 a
887	21.3 a	13.6 d	33.5 b	14.1 a	9.6 ab	39.8 a	49.3 c
787-05	14.6 c	19.5 a	32.1 d	14.0 ab	9.8 ab	33.7 c	55.5 a
787-06	14.1 d	19.9 a	32.3 d	12.8 d	10.3 a	31.7 d	54.8 a
787-08	13.6 d	18.5 b	32.9 c	14.0 ab	10.2 a	33.3 c	55.3 a
787-09	14.8 c	20.0 a	31.9 d	13.9 ab	9.8 ab	33.8 c	55.4 a
787-15	14.8 c	19.9 a	31.6 d	13.9 ab	9.8 ab	33.8 c	55.4 a
789-02	19.5 b	14.9 c	34.6 a	13.9 b	9.4 b	37.7 b	51.9 b
Midas	14.9 c	19.6 a	32.2 d	13.6 c	9.9 ab	33.5 c	55.6 a

#### 4. DISCUSSION

From the data obtained in this study, conclusions can be drawn with regards to the adaptability and the productive potential of this species. Overall, the growing season of camelina was confirmed to be relatively short; the average thermal time (GDD) needed to reach maturity was inline with values reported by Gesch (2014), confirming stability of this trait in camelina, which may make its introduction into typical crop rotations easier. The short-season nature of camelina is a very attractive feature, particularly for short-season environments such as the Canadian Prairies. Yield performance of camelina was mostly affected by environment and to a lesser extent by genotype. Over all locations, genotypes and growing seasons the mean seed yield was  $1.66 \text{ Mg DM ha}^{-1}$ , which is higher than the value of  $1.45 \text{ Mg DM ha}^{-1}$  reported in a recent review by Berti et al. (2016), thus confirming the improved production potential of the tested genetic material. Furthermore, it is worth noting that in locations characterized by milder temperatures (mean temperature of about  $15\text{--}17^\circ\text{C}$ ) and precipitation of more than 170 mm during the growing cycle, such as Saskatoon (Canada) and Bologna (Italy), but also in Vanguard (Canada) in which data were available for one year only (2015, data not presented), seed yields reached almost  $3 \text{ Mg DM ha}^{-1}$ , a value often cited in the literature as upper limit, especially when evaluating spring camelina genotypes (Berti et al., 2011; Hergert et al., 2016; Masella et al., 2014; Schillinger et al., 2012). Interestingly, the interaction effect genotype  $\times$  location was not significant for any of the investigated traits. It is interesting to note that the genotype 787-08, characterized by an increased seed size (+30% compared to the average TKW of all other lines), performed well (high and stable yields) at all sites. This result contrasts with the conclusions by Vollmann et al. (2007) who pointed out that varieties with increased seed weight exhibited inferior productive performance compared to small-seeded ones. The generally small seed size of camelina can be identified as a major factor hampering the adoption of camelina by both producers and processors. Increasing the seed size of this crop, through breeding, will improve emergence at greater seeding depth, combinability and increase efficiency of the crushing process. The slightly positive, but significant, correlation between seed yield and seed oil content confirms findings by Geheringer et al. (2006), who found that a major QTL for oil content is co-localized with a QTL for seed yield. The negative correlation between seed oil content and seed weight was confirmed to be significant, as reported by Vollmann et al. (2007). However, in the present study the trend was only slightly negative ( $r = -0.10$ ), suggesting that breeding has been effective in improving the previously described strong negative correlation in older material. The overall mean value for seed oil content (41.8%), with limited CV (0.06), obtained in this multi-year-location-variety study, appears elevated (Blackshaw et al., 2011; Geheringer et al., 2006; Jiang et al., 2014), considering the differences across environmental conditions, thus demonstrating not only the ability of this species to accumulate lipids, but also the excellent potential of new improved genetic material. Since

seed oil yield is mainly driven by seed production, line 787-08 confirmed to be the highest yielding entry, able to reach an average oil yield, over all locations and the two growing seasons, of 0.80 Mg DM ha<sup>-1</sup>. The above mentioned value would present a realistic threshold for making camelina an economic option for farmers (Mupondwa et al., 2016), at least in the most productive locations. Obviously, in less productive environments, mainly characterized by uneven precipitation patterns and increased temperatures during flowering and/or seed filling, the potential productivity of camelina needs to be compared with average yields of other, alternative crops.

The uniqueness of camelina is not only linked to the reported wide environmental adaptability but is also strongly related to the composition of its oil, which is suitable for copious and innovative bio-based applications (Faure and Tepfer, 2016; Li and Sun, 2015). From a nutritional point of view  $\alpha$ -linolenic acid (C18:3) is the most relevant fatty acid (Berti et al., 2016; Pecchia et al., 2014), which is naturally stabilized by an increased content of vitamin E (Ibrahim and El Habbasha, 2015). As expected,  $\alpha$ -linolenic acid content, and more generally the amount of PUFAs, varied significantly across test environments and these differences appeared to be mainly associated with differences in temperatures during seed filling (Rodríguez-Rodríguez et al., 2013); in particular cooler temperatures after flowering and during seed ripening were generally associated with increased PUFA contents. The observed variation in oleic and linoleic acid contents corroborates results reported by Vollmann et al. (2007). Interestingly, we identified two genotypes, 887 and 789-02, with reduced contents (about -30%) of linoleic acid (C18:2). These lines may present an interesting resource for improving the oil quality of camelina through conventional breeding as the reduction in linoleic acid was accompanied by a concomitant increase in oleic (C18:1, about 50%) and, to a lesser extent, an increase in  $\alpha$ -linolenic acid contents. The unique FA profiles of the above-mentioned genotypes not only render their oil superior for food and feed applications through an improved n-6-/n-3 fatty acid ratio, but also make them of greater interest for the oleochemical industry. This makes lines 887 and 789-02 attractive germplasm for the introgression of improved seed oil quality traits into high yielding varieties such as 787-08.

Eicosenoic acid (C20:1) is a unique source of C11 intermediates for oleochemical applications and camelina represents one of the few plant species yielding considerable amounts of this FA (Gunstone and Harwood, 2007). The total amount of eicosenoic acid in camelina oil is still limited (about 12-15%) and, as reported in previous studies (Vollmann et al., 2007; Zubr and Matthäus, 2002) and confirmed in our work, varies little across environments and genotypes (mean CV for eicosenoic acid content in this study = 0.04). When analyzing the correlation between FAs for each line grown under different environments and years, the unique composition of line 789-02 was confirmed, with a significant and positive correlation ( $r=0.51$ ,  $P\leq 0.05$ ) between  $\alpha$ -linolenic and eicosenoic acid contents. This finding might inspire innovative studies aiming at possibly indirectly increasing eicosenoic content in camelina through modulating  $\alpha$ -linolenic acid content, which was shown to be strongly influenced by environmental conditions (i.e., temperature), as reported by Zubr and Matthäus (2002).

## 5. CONCLUSIONS

The presented dataset is derived from wide-ranging multi-location trials in terms of differences among growing conditions (from northern to southern Europe, to western Canada). Results of the present study confirm that camelina is suited to a broad set of environmental conditions (i.e., soil types and climate). Despite lower potential productivity than rapeseed, both in terms of oil and seed yield, camelina can be an attractive oil crop for its peculiar fatty acid composition, and in particular, for the high content of eicosenoic acid (very attractive for bio-based applications, such as a source of medium chain FAs (C10-14)). Nevertheless, important technological barriers may limit the deployment and market uptake of camelina such as seed size, harvest mechanization, full valorization of co-products after oil extraction, and a stable market price. Among the tested genotypes, line 787-08 has been identified as the best choice in terms of seed and oil yields, but also productive stability across tested environments; in addition, this line is also characterized by increased seed size, a valuable trait for farmers. The relatively short growing cycle and the possibility to grow camelina as a winter crop will greatly facilitate its introduction into conventional cropping systems as an alternative main crop or intercrop.

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