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Unsaturated Fatty Acids Content, Their Relationship with Morphological Characteristics and Genetic Diversity of *Camelina sativa* Doubled haploid Lines

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ABSTRACT

Camelina (*Camelina sativa* L.) is an oilseed crop valued for its nutritional profile and biofuel potential. This study assessed the genetic diversity of 35 doubled haploid (DH) camelina lines based on unsaturated fatty acid composition and key agronomic traits. Substantial genotypic variation was observed for oil content and fatty acids such as stearic, oleic, linoleic, γ-linolenic, arachidic, eicosenoic, and erucic acids. Among the lines, one exhibited up to 41.90% seed oil content, while others showed high proportions of essential omega-3 and omega-6 fatty acids, including linolenic acid (>37%) and linoleic acid (>18%), along with reduced erucic acid (<2.4%). Most biochemical traits displayed moderate to high heritability and genetic advance, with linoleic acid showing the highest heritability (83.60%). Significant correlations were detected among traits, particularly between aerial biomass and seed weight (r = 0.97), and between plant height and pods per plant (r = 0.87). Principal component analysis revealed that the first two components explained 71.61% of the total variance, and cluster analysis delineated eight distinct subpopulations. The results highlight considerable genetic diversity within the DH population, which can be effectively utilized in breeding programs aimed at improving oil quality and agronomic performance in camelina.

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1. Introduction

Oilseeds are already important among crops, constituting the second largest food reserve in the world after cereals, with high reserves of fatty acids (Vafaei *et al.*, 2010). Over the past four decades, plant breeders have worked hard to develop the quality features that the industry demands, primarily related to the fatty acid composition of seed oil (Velasco and Fernández-Martínez, 2002).

Camelina sativa (false flax) or gold of pleasure, which is a member of the Brassicaceae family, is grown as an oilseed crop and is of interest for low-input biofuels production and as a potential source of omega-3 fatty acids, as well as for its value in drought stress tolerance (Freeborn, 2015). This crop is especially suited for arid and semiarid areas, making it a resilient choice amid the increasing challenges of climate change (Locatelli and Mazzilli, 2025). Due to the high amounts of antioxidants, omega-3 fatty acids and vitamin E, camelina plays an important role in boosting immunity, preventing cancer and improving health (Kahrizi *et al.*, 2015). It is reported that the fatty acid composition of camelina was largely unsaturated (over 90%), with a significant proportion (30–40%) of linolenic acid and a relatively small proportion (3–4%) of erucic acid. Researchers (Budin *et al.*, 1995) showed that camelina grains contain about 43% of the dry matter and the amount of UFA (unsaturated fatty acid) in the oil is about 90% and about 50% of polyunsaturated linoleic acid (18:2, n-6) and - α-linolenic acid (18 :3, n-3). The content of erucic acid (C22:1, n-9) in the oil is about 3%. Doubled haploid lines are pure genetic material created in many crops using some techniques, such as pollen or anther culture

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and have important applications in breeding experiments, e.g., in genetic (QTL) mapping studies. Using correlation statistics, cotton reports showed positive correlations (P<0.01) between 100-seed weight and oleic acid, between seed weight and protein content (P<0.05) (Bolek *et al.*, 2016). Highly significant correlations between stearic acid and palmitic acid (0.845**), between linoleic acid and erucic acid (-0.784**), and between linolenic acid and erucic acid (-0.894**) are reported because long-chain eicosenoic and erucic acids are undesirable in edible oils due to their negative effects on health (Tonguç and Erbaş, 2012).

Luo et al. (2019) examined the genetic diversity and population structure of 213 camelina accessions using principal coordinate analysis (PCoA) and AMOVA analysis and SNP markers and assessed genetic diversity along with the population structure that revealed two distinct subpopulations that trace the accessions and suggesting the genetic material for further breeding research. Gugel and Falk (2006) studied 19 *C. sativa* and three oilseed Brassica accessions in Canada and found that camelina accessions produced seed yields competitive with Brassica oilseeds but the seed size was significantly smaller and also average oil and protein contents of *C. sativa* in the range of 27 to 43% and also the predominant fatty acids named oleic, linoleic, linolenic and eicosenoic where such species can be introduced as a new oilseed crop, e.g. for Canada for oil and flour consumption. Such results suggest the existence of genetic diversity between camelina genotypes and the potential for next breeding programs.

This experiment was conducted to assess the genetic diversity of a population of doubled haploid lines based on an assessment of the oil and its fatty acid compositions (Table 1) and some morphological characteristics.

In *C. sativa*, less work is done in doubled haploidy, and in this study, oil and fatty acids and 5 morphological traits are evaluated to investigate the DH population generated for further studies. Due to the importance of non-saturated fatty acids that are essential for the body, this study has been carried out to assess the quantity of unsaturated fatty acids and fatty acids, especially essential fatty acids such as omega-3 and omega-6 in different lines.

2. Materials and methods

2.1. Genetic entries and agronomic traits evaluation

This study was conducted during the 2019 crop year at the research farm of Razi University's Agriculture and Natural Resources Campus, located at 34°19' N, 47°06' E, with an altitude of 1,319 m. The region is classified as having a cold and semi-arid climate based on its climatic conditions (Supplementary Table S1). The region experiences an average annual rainfall of 444.7 mm, with an average temperature of 14.3°C (Pashaie *et al.*, 2023). The annual maximum and minimum temperatures recorded are 44.1°C and -27°C, respectively.

Before starting the experiment, soil samples were collected from the field and sent to the Soil and Water Laboratory for physical and chemical analysis, the results of which (type of the farm soil) are presented in Supplementary Tables S1 and S2, as pointed out by (Pashaie *et al.*, 2023).

In this study, 35 Doubled haploid camelina lines from 29 different crosses (Table 2) were tested. For this, the lines were selected based on the morphological diversity derived from the previous study provided by the Bisotun Knowledge-Based Company, Kermanshah (Mafakher *et al.*, 2022). The desired lines were generated by anther culture method. For convenience, genotypes were coded from 1 to 35 (Table 2). This experiment was performed based on a randomized complete block design with three replicates.

Five three-meter lines were considered for each line, from which three lines and two meters in the middle of each line were taken with sufficient cultivation spacing and margin. The recommended seed rate for cultivation was seven kilograms per hectare. No fertilizer was applied during the cultivation period and weed control was carried out manually. Due to the climatic conditions, this plant was cultivated in autumn. The cultivation of this plant was carried out in early December, before the onset of cold. The harvest took place on June 20, 2019. For sampling, 5 plants were randomly selected from each line in each replicate, taking into account the marginal effect, where samples include a range of plants considering homogeneity of sampling, avoiding bias and fully representative of the plot's conditions. Traits: plant height, number of pods per plant, number of seeds per pod, shoot weight and seed weight per plant of each plot planted in three blocks were recorded and measured separately.

Table 1. Fatty acid composition of camelina seed, oil, cake, and meal (% of total fatty acids) (Juodka *et al.*, 2022).

Fatty Acid	Seed		Oil		Cake	
	References*					
	[a]	[b]	[c]	[d]	[e]	[f]
Myristic (C14:0)	-	-	0.15	0.11	0.08	0.26
Pentadecanoic (C15:0)	-	-	-	0.04	-	-
Palmitic (C16:0)	6.07	5.24	7.43	7.05	6.28–6.44	7.73
Margaric (C17:0)	-	-	-	0.06	-	-
Stearic (C18:0)	1.91	2.60	2.01	2.37	2.37–2.68	2.76
Arachidic (C20:0)	-	-	-	1.51	1.33–1.39	0.99
Heneicosanoic (C21:0)	-	-	-	0.02	-	-
Behenic (C22:0)	-	-	-	0.36	0.30–0.31	2.18
Lignoceric (C24:0)	-	-	-	0.21	-	2.55
SFA	-	7.84	9.59	11.73	-	-
Palmitoleic (C16:1n-7)	-	-	0.24	0.22	0.02–0.16	-
Hexadecenoic (C16:1n-9)	-	-	-	0.08	-	-
Heptadecenoic (C17:1n-9)	-	-	-	0.05	-	-
Vaccenic (C18:1n-7)	-	-	-	1.35	-	-
Oleic (C18:1n-9)	16.46	15.70	17.69	17.11	15.28–17.17	12.8
Eicosenoic (C20:1n-9)	12.99	14.61	-	12.28	14.04–15.34	8.85
Erucic (C22:1n-9)	5.02	2.04	-	3.20	2.38	2.31
Nervonic (C24:1n-9)	-	-	-	0.92	-	-
MUFA	-	20.62	17.93	35.21	-	-
Linoleic (C18:2n-6)	18.84	-	21.09	24.16	21.13–22.63	23.47
Linolelaidic (C18:2n-6 trans)	-	-	-	0.02	-	-
Octadecadienoic (C18:2n-6cis, trans)	-	-	-	0.04	-	-
γ—linolenic (C18:3n-6)	-	-	-	0.11	0.24–0.25	-
α—linolenic (C18:3n-3)	33.43	36.67	29.47	25.88	27.73–28.82	36.11
Octadecatetraenoic (C18:4n-3)	0.36	-	-	-	-	-
Eicosadienoic (C20:2n-6)	1.47	1.97	-	1.65	-	-
Eicosatrienoic (C20:3n-3)	-	-	-	0.84	0.98–1.17	-
Eicosatrienoic (C20:3n-6)	-	1.48	-	0.00	-	-
Arachidonic (C20:4n-6)	1.02	-	-	0.05	2.47	-
Eicosapentaenoic (C20:5n-3)	0.12	-	-	0.00	0.08–0.09	-
Docosadienoic (C22:2n-6)	-	-	-	0.30	-	-
Docosatetranoic (C22:4n-6)	0.33	-	-	0.03	-	-
Docosapentaenoic (C22:5n-3)	0.04	-	-	-	-	-
Docosahexaenoic (C22:6n-3)	0.34	-	-	-	-	-
n-6 PUFA	21.66	-	-	26.36	-	-
n-3 PUFA	34.29	-	-	26.72	-	-
PUFA/SFA	-	-	-	4.53	-	-
n-6/n-3	-	0.60	-	0.99	-	-
Linoleic/α-linolenic	-	-	0.72	-	-	-

*References: [a]: (Ciurescu *et al.*, 2016), [b]: (Jaśkiewicz *et al.*, 2014), [c]: (Anca *et al.*, 2019), [d]: (Juodka *et al.*, 2018), [e]: (Oryschak *et al.*, 2020), [f]: (Bulbul *et al.*, 2015). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 2. Specifications of doubled haploid camelina lines used in this research.

Cross No	(♀) Maternal parent		(♂) Paternal parent		Naming F1 by number
	Variety	Origin country	Variety	Origin country	
1	Voronezskij 349	Russia	Kirgizskij 1	Kazakistan	30
2	Omskij Mestnyj	Russia	Irkutskij Mestnyj	Russia	20, 24, 26, 31, 35
3	Przybrodzka	Poland	Hoga	Denmark	6, 9
4	Saratouskij	Russia	Bronowska	Poland	11, 17
5	Chulymskij	Russia	Omskij Mestnyj	Russia	3, 12, 18, 19
6	Krupnosemjannyj	Russia	Brzybrodzka II	Poland	34
7	Came	Germany	Volynskaja	Russia	25
8	Boha	Denmark	Volynskaja	Poland	10, 33
9	Came	Germany	Omskij	Russia	1, 15, 29
10	Svalöf	Sweden	Ukrajinskij	Russia	13
11	Calena	Germany	Blaine Greek	Greek	7, 16, 28
12	Zavolzskij	Russia	Sortandinskij	Russia	8, 21
13	VNIIMK 17	Russia	Borowska	Poland	22
14	Voronezh 349	Russia	Czestochowska	Poland	2, 23
15	Lindo	Germany	Ukrajinskaja	Russia	4, 5, 14

2.2. Oil content and fatty acid determination

The seeds of the camelina lines were completely pulverized by hand mill after cleaning them from external impurities. The extraction of oil from half a gram of seed powder was carried out for eight hours using a Soxhlet and normal hexane as the solvent (Jensen, 2007). The free fatty acids (18 fatty acid types in 3 classes) contained in the oil after removal of the solvent under vacuum conditions. The resulting fatty acids were then methylated. The methyl alcohol derivative of the fatty acids was isolated by the GC method after cooling to room temperature and removing the solvent. The extraction was repeated three times for each sample. Repeatability in gas chromatography extraction refers to the consistency of results and repeated measurements.

In this study, with repeated injections of a single sample, the results should remain the same and be repeated. Of course, to achieve high repeatability, key factors such as the correct selection of stationary and mobile phases, appropriate oven temperature, carrier gas flow rate, sample volume, and proper maintenance of the column and apparatus components must be controlled. To isolate and identify different types of saturated (SFAs) (lauric acid, C12:0; myristic acid, C14:0; palmitic acid, C16:0; stearic acid, C18:0; arachidic acid, C20:0; behenic acid, C22:0; lignoceric acid, C24.0) and unsaturated (USFAs) including mono (MUFA) (palmitoleic acid, C16:1; oleic acid, C18:1; eicosenoic acid, C20:1; erucic acid, C22:1; nervonic acid, C24:1) and poly (PUFA) (linoleic acid, C18:2; linolenic acid, C18:3; eicosadienoic acid, C20:2; eicosatrienoic acid, C20:3; docosadienoic acid, C22:2; docosatrienoic acid, C22:3), unsaturated fatty acid profiles of camelina seeds, its measurement in mg g^{-1} was performed using gas chromatography (Variant CP 3800) connected to an FID detector and equipped with a polar silica column (CP8945) (column length: 60 m, internal diameter: 0.25 mm, film thickness: 0.2 µm) (Supplementary Fig. S1).

Then the quantitative values were determined by comparing the area under the peak of the fatty acids of the sample and the fatty acid standard prepared from Sigma Aldrich at the National Institute of Genetic Engineering and Biotechnology as considered by Mafakher et al. (2022). The standard fatty acid mixtures are provided by TCI Chemicals Company, which is indexed by product number S0460 as standard mixture of fatty acid Methyl Esters, standard material of GC (https://www.tcichemicals.com/JP/en/p/S0460). The components of each sample were analyzed with the workstation software (V 6.4). The determination of the percent of oil yield considering oil contents of the seeds with the seed yield is performed by (Roussis *et al*., 2022). Moreover, Relina et al. (2022) determined the crude oil% as the multiplication of obtained oil by wight of absolutely dry milled seed.

2.3. Computation and statistical analyses

2.3.1. Analysis of variance (ANOVA)

The experiment was performed in a randomized complete block design with three replicates. Statistical analyses were performed using the R v.4.5.1 (correlation and multivariate analyses, drawing figures) and MINITAB 19 (normality and homogeneity of variances tests, ANOVA and mean comparison) software programs. After checking the normality of the data (using Anderson-Darling and Kolmogorov–Smirnov methods), data were subjected to analysis of variance and comparison of means was performed using Fisher's LSD test at a probability level of 1%.

2.3.2. Components of variance

Prior to analyses, the assumptions of analysis of variance were checked, including the normality of residuals, homogeneity of variance, and independence of observations. Normality is assessed using normal probability plots and methods such as the Ryan-Joiner or the Kolmogorov-Smirnov test. Homogeneity of variance was performed using Bartlett's or Levene's tests. In independence of observations, there is no relationship among the observations within each group or between the groups themselves, as performed using run charts and the Durbin-Watson test. The Durbin-Watson statistic ranges from 0 to 4, with a value of 2 indicating no autocorrelation. For genetic parameters estimation, key assumptions include: the normality, the lack of epistasis, no assortative mating, and no population stratification in genetic association studies.

Variability estimates, including genotypic and phenotypic variances, heritability, genotypic and phenotypic coefficients of variation, and genetic advance, were calculated. as mentioned by Alam et al. (2022). The genotypic (δ_g^2) and phenotypic (δ_p^2) components of variance and their related CV (coefficients of variation) were computed for the traits

observed as $\delta_g^2 = \frac{MSg-MSe}{r}$, $\delta_p^2 = \delta_g^2 + \delta_e^2$ and $\delta_e^2 = \frac{MSe}{r}$, where *MSg*, and *MSe* are mean squares of genotypes and error, respectively; *r* as replication number.

Broad sense heritability (h_b^2) and the genetic advance in the form of percentage of mean was also calculated. It was estimated as the ratio $\frac{\delta_g^2}{\delta_p^2} \times 100$. The categorization of heritability values is as low, moderate and high: 0-30% (low); 30-60%(moderate); 60% and above (high). The expected genetic advance percentage (GA) was estimated as $GA = k.\delta_p.h^2$, where k: standardized selection differential constant (2.06) at 5% selection intensity, δ_p denotes the phenotypic standard deviation. Genetic gain (GG) was computed as: $GG = \frac{GA}{\bar{X}} \times 100$ where GA denotes genetic advance.

SI or selection index for each trait was computed as: $SI = K.\sigma_p$. In addition, mean values have been used for genetic analysis to determine phenotypic coefficient of variation (or variability) (PCV), genotypic coefficient of variation (GCV), and environmental coefficient of variation (ECV), as follows (Equations 1-3).

(1) $$GCV(\%) = \frac{\sqrt{\delta_g^2}}{\mu} \times 100$$

(2) $$PCV(\%) = \frac{\sqrt{\delta_p^2}}{\mu} \times 100$$

(3) $$ECV(\%) = \frac{\sqrt{\delta_e^2}}{\mu} \times 100$$

Where: δ_g^2 refers to genotypic variance, δ_p^2 refers to phenotypic variance and μ for the sample mean. The GCV and PCV values can be ranked from low to high, as: 0-10% (Low); 10-20% (moderate); >20% (High).

2.3.3. Statistical multivariate analyses

The multivariate analyses, determining the number of clusters, PCA and cluster analysis using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method on scaled data, were performed with programes specially R v4.5.1 (https://cran.r-project.org/) environment.

3. Results and discussion

3.1. ANOVA and mean comparisons

Considering agronomic characteristics, the mean square of lines for pods per plant (PPP) and seeds per pod (SPP) were the only significant ones. For PPP, doubled haploid lines 9 (84.67) and 11 (34.00) had the highest and lowest values, respectively. For SPP, lines 15 (16.70) and 34 (8.80) showed the highest and lowest values, respectively. The nonsignificant effects of genotypes for aerial weight can be attributed to nonsignificant of traits like plant height and seed weight. The significant difference between camelina DH lines has reported by Fereidooni et al. (2024) under supplementary irrigation and rainfed conditions in Kermanshah, Iran province, during the crop year 2020-2021. They reported lines DH5 and DH3 as the highest grain yielder (2221 and 2106 kg ha^{-1}, respectively) and lines DH1, DH2, DH3, DH5, DH6, DH10, DH7 and the check cultivar had more drought resistance. The current experiment shows the high range of these pure lines, which can be applied in hybrid crosses or QTL mapping programs of camelina. This is true for biochemical traits, which will be discussed later in this paper.

The results of the analysis of variance (Table 3) showed that there was a significant difference between the studied genotypes (DH lines) in terms of oil content and fatty acids: stearic (C18:0), oleic (C18:1), linoleic (C18:2), γ-linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1), eicosadienoic (C20:2), erucic (C22:1) but not significant for C12.0 (lauric acid), C14.0 (myristic acid), C16.0 (palmitic acid), C16.1 (palmitoleic acid), C22.0 (behenic acid), C24.0 (lignoceric acid), C24.1 (nervonic acid), C20.3 (eicosatrienoic acid), C22.2 (docosadienoic acid) and C22.3 (docosatrienoic acid).

According to the results obtained in this experiment (Tables 4-6), in the case of oil content, DH line 25 had the highest oil content (41.90%) and line 16 had the lowest oil content (34.60%). For fatty acids C18:0 (stearic acid, 3.33), C18:1 (oleic acid, 32.13), C18:2 (linoleic acid, 18.89), C18:3 (linolenic acid, 37.21), C20:0 (arachidic acid, 1.98), C20:1 (eicosenoic acid, 15.32), C20:2 (eicosadienoic acid, 2.17) and C22:1 (erucic acid, 3.33), DH lines 26, 12, 33, 27, 27, 20, 13 and “2 and 20” with the highest levels of fatty acids, with line 15 having the lowest erucic acid content (2.37%). In general, the highest level of detrimental

erucic acid in these lines was 3.33% of the seed oil. Among other fatty acids with doubled haploid content, linolenic acid and arachidic acid had the lowest values.

In this area, a two-year field experiment was conducted by Yildiz et al. (2024) to assess the yield performance and fatty acid composition of 33 camelina genotypes in Mediterranean climate conditions. The results showed that drought-resistant genotypes 28 (yielding 3,120 kg ha^{-1}) and 9 (yielding 2,735 kg ha^{-1}) performed well. Additionally, genotypes 28 (with 3.09% erucic acid), 9 (with 2.66%), and 1 (with 2.73%) were identified as the preferred options based on their mean erucic acid content. The above findings indicate the existence of the necessary diversity between lines that allows them to be considered as a probable source in breeding programs, e.g., for a mapping population, e.g., in QTL mapping (Arminian *et al.*, 2011). However, for better screening of doubled haploid lines, we consider the means of each line (only showing mean comparisons for biochemical traits) in Tables 4-6 with significcant effect in ANOVA table (Table 3). Achieving a DH population from parents with contrasting traits will provide a broad range of genetic variation, facilitating the identification of genes associated with these traits.

3.2. Correlation study

In this experiment, the correlation (Pearson) between oil content, fatty acids and agronomic traits was calculated using R v4.5.1 and Minitab v.19, at 1% statistical significance level. According to Fig. 1 and Supplementary Tables S3-A and S3-B, the highest correlation coefficient occurred between aerial weight (AWT) and seed weight (SW, r=0.97, p≤0.01), between plant height and pods per plant (r=0.87, p≤0.001), respectively (Fig. 1; Supplementary Tables S3-A and S3-B). This shows that Aerial biomass can significantly increase the number of pods per plant, indicating higher grain production and oil or fatty acids composition, in this or other crops. Wang et al. (2025) showed that medium to large seeds exhibited enhanced growth in the field, including improvements in morphological and photosynthetic parameters in soybean.

Considering biochemical characteristics, the largest correlations existed between linoleic and linolenic acid (r=-0.72, p≤0.001), between linoleic and eicosadienoic acids (r=0.53, p≤0.001), between stearic and arachidic acids (r=0.48, p≤0.001), between oleic and linoleic acids (r=-0.43, p≤0.05), between arachidic acid and eicosadienoic (r=-0.42, p≤0.05), and between number of seeds per pod and eicosenoic acid (r=0.39, p≤0.001), respectively. Erucic acid (C22:1) as an anti-nutrition factor, had no significant correlations with other traits. In current experiment, the robust negative correlation of linoleic: linolenic acids, and also the strong positive relationship of stearic: arachidic, and linoleic: eicosadienoic acids are confirmed by Soorni et al. (2022).

Table 3. Descriptive statistics and mean squares for agromorphology, oil and fatty acid contents (saturated/unsaturated fatty acids (S/U FAs) among camelina DH lines in this study.

Class	Traits	C:D*	Mean	SE	C.V%	Min.	Max.	Mean Squares			
								Genotype (DF=34)	Error (DF=68)	1-α	P-val.
Oil	Oil (%)		38.23	0.21	3.47	34.60	41.90	4.87*	1.23	0.955	0.045
UFA/ω3FA	Linolenic acid	C18:3	34.70	0.18	5.50	32.86	37.21	4.22**	0.51	0.993	0.007
UFA	Linoleic acid	C18:2	17.02	0.22	8.20	14.89	18.89	4.98***	0.31	0.999	0.001
PUFA	Eicosadienoic acid	C20:2	1.58	0.05	7.20	1.24	2.17	0.51*	0.13	0.954	0.046
MUFA	Eicosenoic acid	C20:1	14.24	0.06	2.90	13.44	15.32	0.79*	0.18	0.965	0.035
MUFA/ω9FA	Oleic acid	C18:1	14.11	0.21	4.90	12.13	16.66	2.85**	0.40	0.990	0.010
MUFA/ω9FA	Erucic acid	C22:1	2.96	0.05	2.80	2.37	3.33	0.46*	0.10	0.969	0.031
SFA	Stearic acid	C18:0	2.54	0.06	4.70	2.09	3.33	0.37*	0.08	0.969	0.031
SFA	Arachidic acid	C20:0	1.44	0.05	19.98	1.12	1.98	0.35*	0.07	0.974	0.026
Agro-Morphology	PH		64.50	1.65	15.17	35.47	89.70	287.42^{ns}	356.81	0.310	0.690
	PPP		58.96	1.64	16.44	34.00	84.67	281.98^{ns}	152.19	0.774	0.226
	SPP		12.92	0.29	13.42	8.80	16.70	9.02**	1.20	0.991	0.009
	AWT		6.43	0.34	31.56	2.40	11.20	12.35^{ns}	18.78	0.201	0.799
	SW		1.49	0.08	30.48	0.65	2.54	0.62^{ns}	0.57	0.493	0.507

ns(P>0.05); *(P<0.05); **(P<0.01); ***(P<0.001); SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid; PH: plant height, PPP: pod per plant; SPP: seed number per pod; AWT: aerial weight (biomass); SW: seed weight; Omega-3/9 fatty acid (ω3/9FA) are essential types of polyunsaturated fatty acid (PUFA) that features a double bond located three carbons from the end of its molecular chain. $LSD_{(0.025, 68)}$=1.668. Note: Only fatty acids are shown in this table that their genotypes effect in the ANOVA table are significant.

Table 4. Sorted average of DH lines of camelina according to oil (fat) a nd MUFA fatty acid composition (%) sing LSD method (1% level)

DHline	Oil (%) of D.M.	DH	C18.1	DH	C20.1	DH	C22.1
DH25	41.90^{a}	DH18	16.66^{a}	DH20	15.32^{a}	DH2	3.33^{a}
DH6	39.88^{ab}	DH3	16.66^{a}	DH16	14.60^{ab}	DH20	3.33^{a}
DH10	39.63^{abc}	DH16	15.61^{ab}	DH17	14.57^{ab}	DH12	3.30^{a}
DH11	39.21^{bcd}	DH11	15.61^{ab}	DH25	14.50^{ab}	DH14	3.27^{a}
DH31	39.21^{bcd}	DH15	15.61^{ab}	DH19	14.49^{abc}	DH11	3.20^{ab}
DH7	39.18^{bcd}	DH1	15.61^{ab}	DH15	14.48^{abc}	DH19	3.20^{ab}
DH17	38.94^{bcde}	DH35	15.44^{abc}	DH21	14.48^{abc}	DH1	3.20^{ab}
DH13	38.89^{bcde}	DH2	15.34^{abc}	DH27	14.46^{abc}	DH21	3.19^{ab}
DH3	38.87^{bcde}	DH17	15.34^{abc}	DH2	14.46^{abc}	DH3	3.19^{ab}
DH5	38.87^{bcde}	DH29	15.24^{bc}	DH23	14.42^{abc}	DH24	3.17^{ab}
DH23	38.87^{bcde}	DH4	14.46^{bcd}	DH31	14.41^{abc}	DH6	3.17^{ab}
DH21	38.85^{bcde}	DH19	14.46^{bcd}	DH6	14.41^{abc}	DH25	3.16^{abc}
DH2	38.73^{bcde}	DH8	14.36^{bcd}	DH14	14.39^{bc}	DH7	3.16^{abc}
DH22	38.73^{bcde}	DH23	14.36^{bcd}	DH32	14.38^{bc}	DH22	3.13^{abcd}
DH1	38.58^{bcde}	DH26	14.21^{cd}	DH7	14.38^{bc}	DH33	3.13^{abcd}
DH15	38.58^{bcde}	DH10	14.11^{cd}	DH33	14.36^{bc}	DH4	3.13^{abcd}
DH27	38.26^{bcdef}	DH25	14.11^{cd}	DH8	14.36^{bc}	DH13	3.12^{abcd}
DH35	38.25^{bcdef}	DH13	13.74^{de}	DH18	14.29^{bcd}	DH31	3.10^{abcd}
DH30	38.25^{bcdef}	DH27	13.67^{def}	DH30	14.21^{bcd}	DH27	3.09^{abcd}
DH14	38.25^{bcdef}	DH30	13.65^{def}	DH5	14.21^{bcd}	DH9	3.09^{abcd}
DH28	38.13^{bcdef}	DH32	13.60^{def}	DH22	14.18^{bcd}	DH34	3.08^{abcde}
DH18	37.94^{bcdef}	DH31	13.58^{def}	DH24	14.17^{bcd}	DH17	3.07^{abcde}
DH33	37.94^{bcdef}	DH34	13.54^{def}	DH26	14.16^{bcd}	DH18	3.06^{abcde}
DH29	37.94^{bcdef}	DH28	13.36^{defg}	DH11	14.16^{bcd}	DH26	3.05^{abcdef}
DH26	37.90^{bcdef}	DH7	13.35^{efg}	DH1	14.16^{bcd}	DH8	3.05^{abcdef}
DH9	37.75^{bcdef}	DH22	13.35^{defg}	DH12	14.14^{bcd}	DH32	2.54^{bcdef}
DH19	37.62^{bcdef}	DH9	13.33^{defg}	DH4	14.07^{bcd}	DH5	2.54^{bcdef}
DH24	37.48^{cdef}	DH24	13.33^{defg}	DH29	14.07^{bcd}	DH23	2.54^{bcdef}
DH34	37.48^{cdef}	DH12	13.32^{defg}	DH3	14.06^{bcd}	DH28	2.48^{cdef}
DH8	37.00^{def}	DH5	13.31^{defg}	DH28	14.06^{bcd}	DH10	2.48^{cdef}
DH32	36.72^{efg}	DH20	13.31^{defg}	DH10	13.71^{bcd}	DH16	2.47^{def}
DH12	36.72^{efg}	DH6	12.42^{efg}	DH35	13.71^{bcd}	DH35	2.46^{def}
DH4	36.72^{efg}	DH21	12.42^{efg}	DH13	13.58^{cd}	DH30	2.45^{def}
DH20	36.12^{fg}	DH33	12.34^{fg}	DH9	13.44^{d}	DH29	2.40^{ef}
DH16	34.60^{g}	DH14	12.16^{g}	DH34	13.44^{d}	DH15	2.37^{f}
LSD (1%) = 2.40		LSD (1%) = 1.37		LSD (1%) = 0.92		LSD (1%) = 0.68	

DH: doubled haploid; D.M.: dry matter; MUFA: mono-unsaturated fatty acid.

Overall, the negative correlation between α-linolenic acid (ALA) and linoleic acid (LA) may be influenced by several key factors: 1) biochemical competition: direct competition of ALA and LA for access to Δ-6 desaturase, 2) genetic regulation: overexpression of the FAD3 (fatty acid desaturase 3) gene may result in reduced levels of LA, 3) environmental adaptation: in cold environments, the level of ALA increases while the level of LA may decrease, 4) breeding trade-offs: oils with high ALA content tend to have low LA content. Practically, achieving a balanced ratio of LA to ALA in breeding is important.

3.3. Genetic parameters evaluation

As already mentioned, the genetic parameters for oil content and fatty acid components were calculated in the current experiment, which will be discussed later at the end of this experiment.

3.3.1. Variability

In this context, linolenic acid showed the highest phenotypic (1.927), genotypic (1.311) and environmental (0.583) variance and selection index (Table 7). The phenotypic coefficient of variation (or variability) (PCV), genotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) were calculated (Table 7). According to Table 7, the fatty acids eicosadienoic acid (33.55, 23.68 and 23.68) and arachidic acid (20.40, 15.63 and 13.77) had the highest PCV, GCV and ECV, respectively. According to Fallah et al. (2023) selection based on the traits with high ECV is not effective. GCV and PCV values were categorized as low if they were less than 10%, moderate if between 10% and 20%, and high if greater than 20% (Deshmukh *et al.*, 1986) as cited by Terfa and Gurmu (2020).

Table 5. Sorted average of DH lines of camelina according to PUFA fatty acid composition (%) using LSD method (1% level).

DH line	C18.2	DH	C18.3	DH	C20.2
DH33	18.89^{a}	DH27	37.21^{a}	DH13	2.17^{a}
DH28	18.81^{a}	DH17	36.82^{ab}	DH20	2.10^{ab}
DH20	18.76^{a}	DH3	36.82^{ab}	DH32	2.09^{ab}
DH6	18.76^{a}	DH34	36.22^{abc}	DH3	2.07^{abc}
DH12	18.73^{ab}	DH21	35.85^{abcd}	DH23	2.07^{abc}
DH32	18.56^{ab}	DH7	35.85^{abcd}	DH11	2.05^{abcd}
DH35	18.45^{abc}	DH24	35.61^{bcd}	DH35	2.05^{abcd}
DH11	18.45^{abc}	DH10	35.61^{bcd}	DH33	2.03^{abcd}
DH29	18.34^{abcd}	DH1	35.37^{bcde}	DH12	2.00^{abcde}
DH23	18.18^{abcde}	DH15	35.37^{bcde}	DH19	1.52^{abcde}
DH9	18.18^{abcde}	DH19	35.02^{cdef}	DH21	1.49^{abcde}
DH13	17.69^{abcde}	DH5	35.02^{cdef}	DH31	1.49^{abcde}
DH8	17.53^{bcdef}	DH13	34.75^{cdefg}	DH1	1.49^{abcde}
DH22	17.53^{bcdef}	DH25	34.68^{cdefg}	DH2	1.47^{abcde}
DH14	17.28^{cdefg}	DH31	34.67^{defg}	DH29	1.47^{abcde}
DH25	17.24^{defg}	DH35	34.63^{defgh}	DH9	1.47^{abcde}
DH21	17.23^{defg}	DH11	34.63^{defgh}	DH22	1.47^{abcde}
DH7	17.23^{defg}	DH26	34.50^{defgh}	DH10	1.45^{abcde}
DH30	17.11^{efgh}	DH2	34.50^{defgh}	DH30	1.45^{abcde}
DH26	17.00^{efgh}	DH16	34.50^{defgh}	DH26	1.44^{abcde}
DH18	16.42^{fghi}	DH14	34.39^{defghi}	DH6	1.44^{abcde}
DH4	16.42^{fghi}	DH9	34.37^{defghi}	DH18	1.41^{abcde}
DH31	16.32^{ghi}	DH23	34.37^{defghi}	DH7	1.40^{abcde}
DH27	16.23^{ghij}	DH18	34.33^{defghi}	DH27	1.40^{abcde}
DH19	15.91^{hijk}	DH4	34.33^{defghi}	DH25	1.39^{abcde}
DH5	15.91^{hijk}	DH8	34.01^{efghi}	DH5	1.39^{abcde}
DH10	15.79^{ijk}	DH22	34.01^{efghi}	DH4	1.38^{bcde}
DH24	15.79^{ijk}	DH30	33.88^{efghi}	DH24	1.38^{bcde}
DH2	15.56^{ijk}	DH33	33.87^{efghi}	DH17	1.36^{bcde}
DH16	15.56^{ijk}	DH29	33.68^{fghi}	DH8	1.35^{bcde}
DH17	15.41^{ijk}	DH12	33.45^{ghi}	DH28	1.35^{bcde}
DH3	15.41^{ijk}	DH32	33.37^{ghi}	DH14	1.34^{bcde}
DH15	15.05^{jk}	DH28	33.09^{hi}	DH16	1.30^{cde}
DH1	15.05^{jk}	DH6	32.86^{i}	DH15	1.27^{de}
DH34	14.89^{k}	DH20	32.86^{i}	DH34	1.24^{e}
LSD (1%) = 1.21		LSD (1%) = 1.55		LSD (1%) = 0.78	

PUFA: poly-unsaturated fatty acid.

Table 6. Sorted average of DH lines of camelina according to SFA fatty acid composition (%) using LSD method (1% level).

DH line	C18.0	DH	C20.0
DH26	3.33^{a}	DH27	1.98^{a}
DH29	3.22^{ab}	DH25	1.97^{ab}
DH27	2.86^{abc}	DH15	1.97^{ab}
DH10	2.86^{abc}	DH6	1.87^{abc}
DH31	2.86^{abc}	DH26	1.83^{abcd}
DH33	2.86^{abc}	DH24	1.81^{abcde}
DH15	2.84^{abcd}	DH9	1.80^{abcdef}
DH4	2.84^{abcd}	DH10	1.80^{abcdef}
DH35	2.82^{abcde}	DH30	1.80^{abcdef}
DH23	2.82^{abcde}	DH8	1.77^{abcdef}
DH11	2.80^{abcdef}	DH29	1.76^{abcdef}
DH25	2.80^{abcdef}	DH14	1.39^{bcdefg}
DH32	2.80^{abcdef}	DH7	1.36^{cdefg}
DH34	2.80^{abcdef}	DH32	1.30^{cdefg}
DH21	2.79^{abcdef}	DH16	1.30^{cdefg}
DH24	2.77^{abcdef}	DH31	1.30^{cdefg}
DH6	2.70^{cdefg}	DH11	1.30^{cdefg}
DH8	2.55^{cdefg}	DH12	1.30^{cdefg}
DH7	2.33^{cdefg}	DH35	1.29^{cdefg}
DH30	2.30^{cdefg}	DH34	1.29^{cdefg}
DH9	2.30^{cdefg}	DH19	1.29^{cdefg}
DH12	2.29^{cdefg}	DH1	1.29^{cdefg}
DH20	2.28^{cdefg}	DH21	1.29^{cdefg}
DH2	2.23^{defg}	DH17	1.27^{defg}
DH17	2.23^{defg}	DH4	1.27^{defg}
DH19	2.22^{defg}	DH5	1.26^{defg}
DH5	2.20^{efg}	DH13	1.26^{defg}
DH1	2.20^{efg}	DH33	1.24^{efg}
DH16	2.20^{efg}	DH3	1.24^{efg}
DH13	2.19^{fg}	DH2	1.23^{efg}
DH14	2.18^{fg}	DH18	1.22^{fg}
DH3	2.14^{g}	DH23	1.14^{g}
DH18	2.13^{g}	DH22	1.13^{g}
DH22	2.10^{g}	DH28	1.12^{g}
DH28	2.09^{g}	DH20	1.12^{g}
LSD (1%) = 0.62		LSD (1%) = 0.58	

SFA: saturated fatty acid; DH: doubled haploid; Means with shared alphabetically letters are not significantly different (P>0.05).

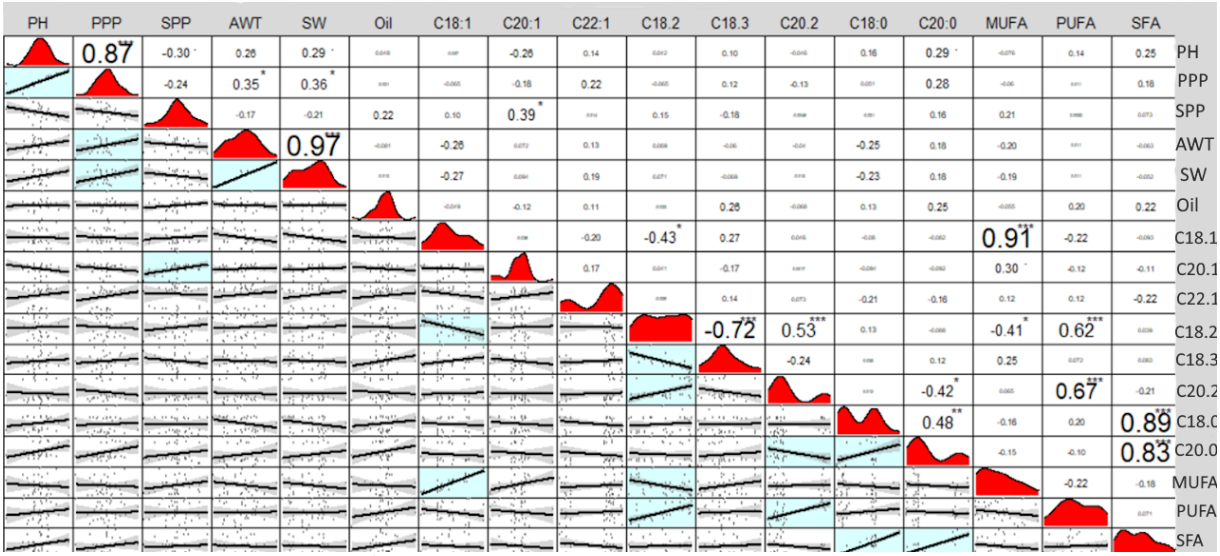

Figure 1. Correlation trend between oil percent, fatty acids and agro-morphological traits studied in this experiment. * (P<0.05), **(P<0.01). PH: plant height; PPP: pods per plant; SPP: seeds per pod; AWT: aerial weight; SW: seed weight per plant.

3.3.2. Heritability

As shown in Table 7, heritability was highest for linoleic (83.60%) and linolenic (70.28%) acids, and lowest for eicosenoic acid (32.25%), respectively. In a population of DH lines of camelina assessed by Fallah et al. (2019), the highest heritability occurred for arachidic (96.49%), eicosadienoic (98.92%) and eicosatrienoic (98.59%) acids, respectively. Heritability estimates range from 0 to 0.20 (20%) as low, 0.40 to 0.80 as moderate to high, and higher than 0.80 as very high (Vinkhuyzen *et al.*, 2013). As stated by Limbongan et al. (2024), heritability values play a crucial role in plant selection, particularly in breeding lines, as they gauge the progress of selection efforts. High heritability values indicate that the genetics being tested still show considerable diversity. Furthermore, these values suggest that continued breeding could lead to significant improvements in these traits.

3.3.3. Genetic advance and gain

In terms of genetic advance (GA), the fatty acids linolenic acid (2.01%) and linoleic acid (1.99%) each had the highest values (Table 7). The genetic gain (GG) was highest for eicosadienoic (34.18%) and arachidic (23.61%) acids, respectively. Fallah et al. (2019) in camelina DH lines, reported that eicosenoic acid (9.06%) and linolenic acid (3.12%) showed the highest GA, respectively. For genetic gain, lauric acid (90.97%) and eicosenoic acid (62.21%) showed the highest values, respectively. Thus, the high values of GA in linolenic acid (C18.3) in current and also in Fallah et al. (2019) researches, makes this fatty acid important. One form of linolenic acid is called α-linolenic acid (ALA), an omega-3 and an essential fatty acid. It has roles, for instance, in mental and brain health.

3.4. Multivariate statistical analyses

The genotypes (doubled haploid lines) were subjected to multivariate analysis including biplot and cluster categorization (Figs. 2-5). Overall, the first two principal components accounted for 71.61% of the variation using the Horn parallel analysis and the Elbow method (Figs. 2 and 3). The Horn parallel analysis is used to select the number of principal components. Elbow Point finds the elbow point on the variance curve declared by each successive PC. These methods can be used to determine the number of PCs to retain.

The results attributed to the PCA supported the differentiation of all camelina lines based on oil and fatty acid composition (Fig. 3). As previously mentioned, principal component analysis (Figs. 2-4) and correlation analysis (Fig. 1) between genotypes were performed to determine relationships for observed traits.

Table 7. The phenotypic (PCV), genotypic (GCV) and environmental (ECV) coefficients of variation, heritability, genetic advance and genetic gain for biochemical traits in this study.

Trait			Mean	Variance components			PCV	GCV	ECV	SI	h^2	GA	GG
				δ_p^2	δ_g^2	δ_e^2	(%)	(%)	(%)		(%)	(%)	(%)
Oil (%)			38.23	2.46	1.22	1.22	4.10	2.89	2.89	3.23	50.00	1.62	4.24
Fatty acids	MUFA	Oleic	14.11	0.976	0.651	0.319	7.00	5.72	4.00	2.04	67.00	1.36	9.64
		Erucic	2.96	0.198	0.114	0.095	15.03	11.40	10.42	0.92	54.54	0.50	16.89
		Eicosenoic	14.24	0.348	0.183	0.159	4.14	3.00	2.80	1.22	32.25	0.39	2.74
	PUFA	Linoleic	17.02	1.339	1.139	0.227	6.80	6.27	2.80	2.38	**83.60**	**1.99**	11.69
		Linolenic	34.70	**1.927**	**1.311**	**0.583**	4.00	3.30	2.20	**2.86**	**70.28**	**2.01**	5.79
		Eicosadienoic	1.58	0.281	0.140	0.140	**33.55**	**23.68**	**23.68**	1.09	49.22	0.54	**34.18**
	SFA	Stearic	2.54	0.148	0.074	0.064	15.15	10.72	9.96	0.79	50.00	0.40	15.75
		Arachidic	1.44	0.086	0.051	0.039	**20.40**	**15.63**	**13.77**	0.60	56.00	0.34	**23.61**

PCA showed significant differences between the genotypes of all biochemical traits. In a PCA biplot including loading plots, opposite array paths show negative correlations and positive path arrows show the positive correlation between traits. In the PCA biplot (Fig. 3), the fatty acids linoleic (C18.2) acid and linolenic (C18.3) acid showed oppositely directed arrows and negative correlation coefficients (r=-0.72, p≤0.01). Overall, the PCA biplot confirmed the correlation coefficients between oil and fatty acids (Figs. 1 and 3).

The only most notable correlation coefficients between agromorphological traits and fatty acids were: plant height and pod per plant with arachidic acid (C20:0) (r=0.29, p≤0.01; r=0.28, p>0.05 ns, respectively), seed per pod with eicosenoic acid (r=0.39, p≤0.01), pod per plant with erucic acid (C22.1) (r=0.22, p>0.05) (Fig. 1).

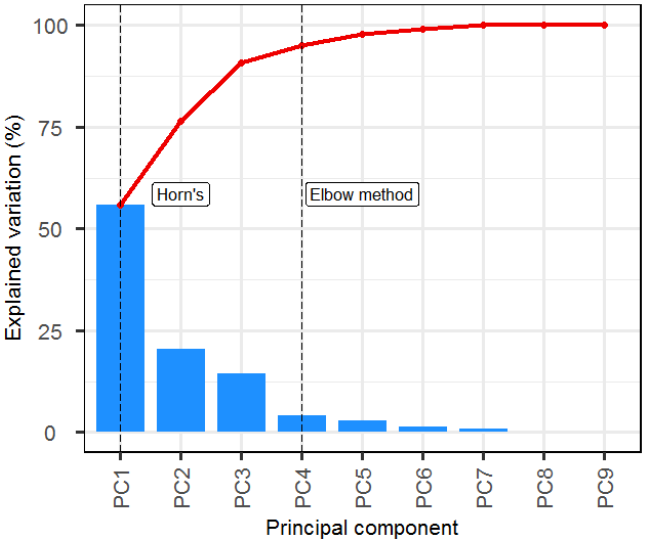

Figure 2. Scree plot showing the Horn's parallel analysis and Elbow method results to determine the number of PCs to retain in which two components accounted for 71.61% of the variation.

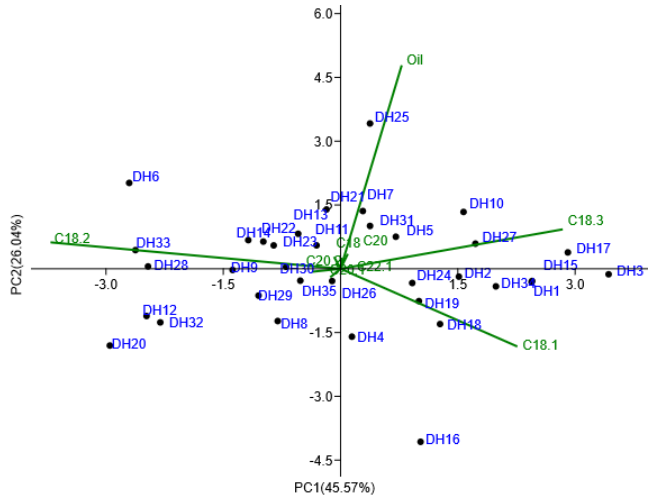

Figure 3. Principal component analysis (PCA) based on camelina oil and fatty acids components.

An interesting approach in multivariate statistics and PCA is to plot the coefficients of each principal component corresponding to each trait graphically. This was done in this study (Figs. 4A and 4B) as a loading plot, which is a plot of the component loadings for selected principal components/eigenvectors and labeling variables that account for the variation propelling along this. For example, as can be seen from Fig. 4 (loading plot), the fatty acids C18:2, and C20:2 had the most negative coefficients, respectively, and the fatty acids C18:3, C18:1 and oil each had the most positive coefficients in PC1. This trend can also be observed in other components. As a result, oil content had the largest and positive effect in PC2 and C18:1 had the most negative coefficient on PC2. These two components are sufficient to explain 71.61% of the variance for biochemical traits in this research. From another point of view, the correlation coefficients between traits and each principal component were estimated, correlating principal components with continuous variables and testing the significance of these correlations (Fig. 5).

In the next step, a heatmap with a dendrogram was developed to facilitate showing similarities and differences in seed oil quantity (content) and fatty acid composition of camelina seeds (Fig. 5). Clustering of observations (DH lines) and variables (oil properties) using UPGMA method confirmed the pattern of DH lines in the PCA biplot (Fig. 3).

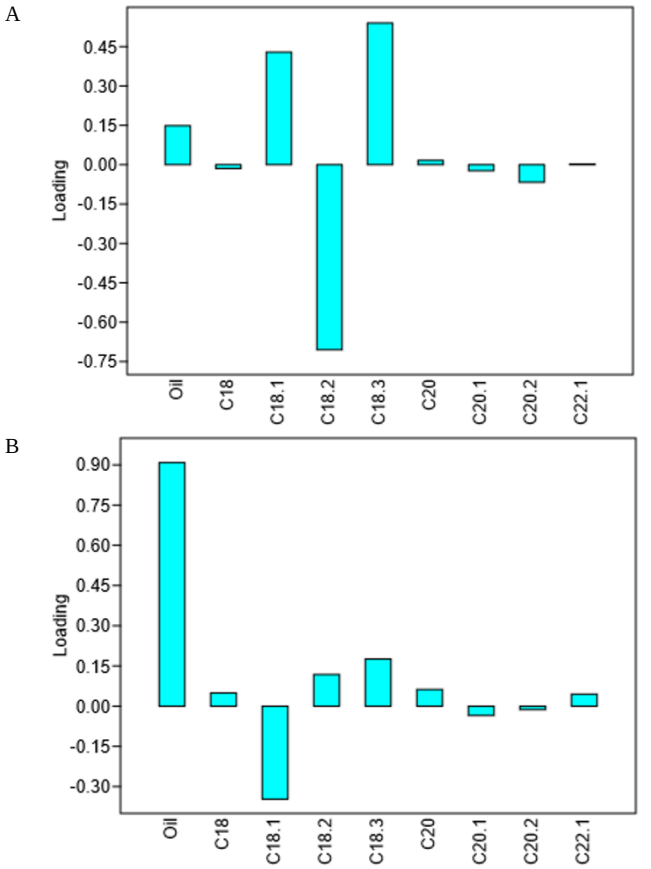

Figure 4. Loading plot showing the coefficients of each principal component with related traits. A) for PC2, B) for PC1.

The heatmap of genotypes and traits was created and as shown in this heatmap (Fig. 5), three main groups were identified. The genotypes 5, 6, 11, 13, 23, 25, 26, 28, 29, 30, 32, 35 proportionally represented a large group, which was mainly characterized by a higher oil content, SFAs and PUFAs. The second group of genotypes (12, 20, 33) was formed with a high amount of MUFAs and PUFAs and a lack of SFAs. The remaining genotypes formed the third group. In this

study, DH lines 2, 3, 5, 13, 18, 20, 22, 23, 28 and 33 contained the lowest levels of erucic acid. A genotype can be high in oil or a particular fatty acid, but also low in erucic acid, which is a prominent case and such a genotype can be used in breeding and feeding programs. These results demonstrated the existence of high genetic variation between doubled haploid camelina lines, which can be exploited in the next programs and also in the genetic mapping of loci controlling such biochemical traits.

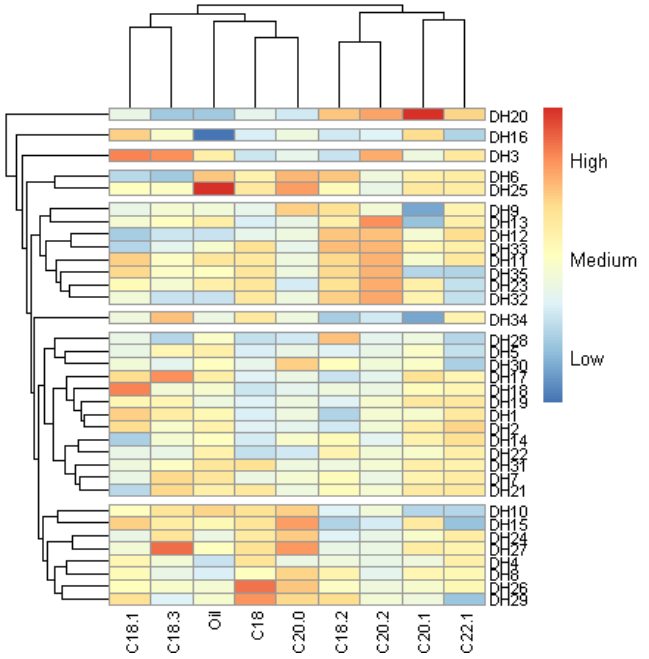

Figure 5. Heatmap representing the association of oil, fatty acids compositions with doubled haploid lines of camelina in the experiment using UPGMA algorithm.

As mentioned (Table 7), in this experiment, the fatty acids eicosadienoic acid and arachidic acid showed the highest PCV, GCV, and ECV values, respectively. The genetic advances were highest for linoleic and linolenic acids. The gain for eicosadienoic and arachidic acids was highest, indicating the presence of additive genes and selection response. These traits are considered a good benchmark for camelina selection. Although there is further research to genetically study the agronomic properties of camelina, reports on the genetic parameters of camelina fatty acid composition are limited. Accordingly, Jewett (2014) considered good information on camelina agronomic properties and oil and fatty acid composition and mentioned a concentration range of 30–40% for the most valuable linolenic fatty acid in camelina. Velasco et al. (2025), reported a high heritability of linolenic fatty acid (0.86) in quinoa and Marwede et al. (2004) pointed out the heritability of oil content ranged from 0.56 to 0.90 in canola.

Current information on the content of camelina fatty acids in this experiment confirmed the results of Fallah et al. (2019) in their experiment in camelina DH population, which reported lines having 35.81-36.67 of linolenic and lines with 22.08-23.00 percent of linoleic acids. Moreover, Yildiz et al. (2024) conducted a two-year field experiment in camelina. Assessing yield and fatty acids in Mediterranean conditions, reported at most 6. (%) of palmitic acid, 2.70 (%) of stearic acid, 18.91 (%) of oleic, 20.94 (%) of linoleic, 39.63 (%) of alpha-linolenic, 3.69/1.88 (higher/lower%) of erucic acid. Further, Zubr (2003), who gave the mean oil content in camelina seeds as 43.3 0.22%/DM and the α-linolenic acid content as 37.8 0.17%; and also oleic acid as 13.4 0.13%; linoleic acid as 14.8 0.12%; gondoic acid (20:1 n-9) as 15.4 0.16%; erucic acid as 2.76 0.07%/total FA. Palmitic acid is a fatty acid that is most commonly found in plants, microorganisms and animals. In this study, DH lines can be used in future camelina breeding experiments due to the high proportion of essential fatty acids in this particular oil crop compared to similar oil crops. The fatty acids are often found in other crops, such as cottonseed and oilseed rape. For example, In this regard, Qin et al. (2022) reported a notable genetic diversity and correlation of agronomic traits in a population consisting of 482 safflower genotypes.

One of the great strengths of this experiment is that it covers a relatively large number of genotypes, which validates the results, such as the correlation between traits and the results of multivariate statistics. In most experiments, the number of genotypes used is limited, which impairs the validity of the results. Since few experiments with camelina have examined the relationships between oil and its constituents, as well as morphological characteristics, this research may be valuable in this regard.

Of course, according to Table 3, there were lower coefficient of variation values for fatty acid compositions (excluding arachidic acid) compared to agro/morphological traits, indicating the presence of less variation between lines for most fatty acids, making it possible to find better lines as parents of hybrid crosses in the case of morphological traits and arachidic fatty acid. And also the presence of a lower

CV. for most fatty acids, represents lineage homozygosity for these traits and higher CV. for morphological traits represents environmental influence on such traits. In this regard, Ogunniyan and Olakojo (2014) concluded that the lower values of coefficient of variation for traits studied in doubled haploid lines, the lines can be expected to be subjected to inbreeding depression, leading to fixation of recessive genes, thus increasing homozygosity within the lines and also encouraging use of these parameters when selecting good parents for use crossings or lines for further improvement.

In this experiment, the means for each trait were normally distributed and there were small amounts of standard errors (SE) across the traits, confirming the results of Ogunniyan and Olakojo (2014), who concluded that the lines in such a case showed almost the same pattern of gene actions and indeed selection between inbred lines can be made for further improvement. As Binodh et al. (2008) found, information about character association is an important point for effective and rapid selection in plant improvement. As noted in this experiment, the nonsignificant correlation between erucic acid and other traits could be a reason for the suitability of recent doubled haploid lines. Also, an increase in aerial weight leads to an increase in seed number; in other words, more biomass weight permits more seed weight. This implies that camelina seed number or seed yield can be selected indirectly by considering aerial weight. In this context, Stefansson and Storgaard (1969) reported a strong negative correlation between oleic acid and other fatty acids and a positive correlation between linoleic and linolenic fatty acids in the rapeseed crop. Tonguç and Erbaş (2012) concluded that selection against erucic acid increases linoleic acid and linolenic acid in the species studied, as seen in *Sisymbrium loeselii*, *D. sophia*, and *C. draba*.

In another experiment, Maestri et al. (1998) analyzed the relationship between seed size, protein and oil content, and fatty acid composition of soybean and showed that oil content did not appear to have a decisive relationship with seed size, but there was a striking positive correlation between seed size and stearic acid and oleic acid and negative with linoleic fatty acid and recommend that it is worth noting that seed size and its relationship to some fatty acids should be considered in soybean breeding programs. In addition, Su et al. (2020) in the plant *Toona sinensis* (*A. Juss.*) Roem studied the relationship between seed morphology, thousand-seed weight traits, and fatty acid compositions and reported that seed length was significantly positively correlated with PUFA and between thousand-seed weight with MUFA and that seeds with greater width and length contain many more fatty acids that could serve as sources for breeding programs and the oil processing industry. This could be achieved through breeding for better genotypes and crop management. Achieving more potent camelina genotypes leads to increases in oil content, fatty acid composition and also agronomic traits such as seed number and ultimately grain yield.

In terms of genetic advance and heritability, it is evident that the percentage of phenotypic variance that is attributed to genetic variance is called heritability. As previously mentioned, the traits linoleic acid and linolenic acid showed the highest heritability and genetic advancement in this study (83.6/1.99; 70.28/2.01, respectively). Therefore, it is likely that higher heritability of these two traits is due to the additive effect of genes and/or minimal environmental influence on such traits, hence the selection of these traits will be reasonable. The selection for a character is most effective when it has both high heritability and high genetic advances (Larik *et al*., 2000). Of course, high heritability is not generally associated with major genetic advances. Therefore, heritability coupled with genetic advance is considered to predict the impact of selecting superior crop varieties (Ogunniyan and Olakojo, 2014). A very high heritability of oil content (0.89) and plant height (0.68) was examined by Gehringer et al. (2006), for camelina it was also lower (0.3112 for the number of branches per plant) and higher (0. 8521, days to 50% flowering) by Prakash et al. (2021) and also high heritability, highlighting the strong genotype dependence of the underlying traits and the potential for improvement through breeding. Furthermore, in this context, Ogunniyan and Olakojo (2014) studied the genetic variation, genetic advance and heritability for agronomic traits of inbred lines of corn, such that the heritability for agronomic traits was more than 80% and genetic advance for number of ears per plant was 72.03.

In this experiment, linoleic and linolenic acid showed the highest rate of heritability and genetic advance, suggesting the presence of additive effects of

genes for such traits and therefore improvement of such traits as selection indicators in camelina may be useful and effective. In this regard, genetic advance represents the degree of gain obtained in a character under particular selection pressure, and its high scores, along with higher heritability, are the best case for selection and also indicate the presenting the additive genes of traits and reliable breeding programs through selection (Nwangburuka and Denton, 2012; Ogunniyan and Olakojo, 2014). But in the case of oleic, erucic and arachidic acids, the genes are likely to have non-additive effects due to high heritability and lower genetic advance, and higher inheritance is due to environmental factors, so selection for such traits is unlikely to make sense.

Also, eicosenoic acid and eicosadienoic acid had low score in heritability (32.25%), and arachidic the lowest genetic advance (0.34), so they are likely to be strongly influenced by environmental factors and selection for them will not be profitable. In addition, there was enough variation in unsaturated fatty acids between the lines and the choice can be made depending on the purpose of the work. In particular, the unsaturated linolenic and linoleic fatty acids, which are omega-3 and omega-6 respectively, are selected for the nutritional uses of lines rich in unsaturated fatty acids. Multivariate statistical methods could distinguish the pure doubled haploid lines in this study and on this basis these lines can be used as parents of crosses and as a pure population for QTL mapping.

4. Conclusion

In this research, we found doubled haploid lines with the highest agronomic values, oil content, desirable omega-3 and omega-6 fatty acids, high heritability and genetic gain and low levels of erucic acid. These can be considered the best genetic material for further research and breeding programs including: GWAS, QTL mapping, marker-assiated selection, improving nutritional/industrial traits e.g., high-oleic/ linoleic/linolenic or low-erucic/glucosinolate lines.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors wholeheartedly reviewed and approved the manuscript, ensuring its readiness for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors contributed equally to study design, work, statistical analysis, and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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