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A Study of the Morphological and Agro-physiological Characteristics of *Camelina sativa* (L.) Doubled Haploid Lines

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ABSTRACT

The plant *Camelina sativa* (L.), a member of the *Brassicaceae* family, is an ancient oilseed crop. Due to its adaptation to vast areas of the world and its unique oil composition and properties, it is useful for the production of biofuels, jet fuel, bio-based products, feed, and food. The present study was performed to investigate the morphological and agro-physiological characteristics of this plant through Factor Analysis (FA). For this purpose, 136 doubled haploid line genotypes were assessed in the form of a randomized complete block design with three replications in the research field of Razi University, Kermanshah, Iran. FA, based on the principal component analysis method and varimax rotation, showed that two important factors make up about 74.97% of the total variety of characters. The Eigen values of these two factors were 9.76 and 3.72, respectively. The first and second factors assigned 53.99 and 20.98 percent, respectively, of the total variation. Factor 1, which was called the biological performance, included the seed yield, number of pods per plant, number of pods per main branch plant, number of pods per lateral branch, biological yield per five plants, plant height with roots, root weight, shoots weight, pod straw weight, number of lateral branches, length of lateral branch and length of the main branch. Factor 2, which was called the seed characteristics, covered seed length, seed perimeter, seed area, the weight of 1000 seeds, and the number of seeds in the pod. Using FA, two sets of traits were identified and named in *Camelina*. The traits in one category that affected the attributes of the other category were selected to be studied to help *Camelina* breed more precisely. Entering more traits and performing FA increased the accuracy of the categories.

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Introduction

Camelina [*Camelina sativa* (L.) Crantz] is a member of the *Brassicaceae* family. *Camelina sativa* (chromosome number 2n=40, genome size 750 Mbp) (Zanetti *et al*., 2021) is an ancient oilseed crop also known by different common names such as false flax, gold of pleasure, and German sesame. *Camelina* crop originated in Germany about 600 B.C. and later spread to Central Europe (Gore and Kurt, 2021; Mondor and Alvarez, 2022).

Camelina's adaptation to vast areas of the world, along with its unique oil composition and properties useful for the production of biofuels,

jet fuel, bio-based products, feed, and food, has resurfaced interest in this ancient crop (Vollmann *et al*., 2005; Pilgeram, 2007; Berti *et al*., 2016; Ghamarnia *et al*., 2022). The seed oil content is approximately 38–43% with the major fatty acids being linolenic, oleic, linoleic, and eicosenoic in proportions of 36.2–39.4%, 12.8–14.7%, 16.3–17.2%, and 14.0–15.5%, respectively (Mondor and Alvarez, 2022). Erucic acid and Glucosinolate in this plant are 3% lower than the other species (Berti *et al*., 2016). *Camelina* oil has higher levels of Poly-Unsaturated Fatty Acids (PUFAs), linoleate (18: 2), and linolenate (18: 3) compared to soybean and canola oils (Fallah *et al*., 2020; Rostami Ahmadvandi and Faghihi, 2021). *Camelina* plant has many morphological and geomorphological characteristics, and identification and measurement of the relationship between them are effective in improving and productivity of this plant (Gao *et al.*, 2022). In the present investigation, morphological and morpho-physiological traits Factor Analysis (FA) was conducted in doubled haploid lines in *Camelina* [*Camelina sativa* (L.) Crantz]. FA was used to facilitate a complex data set by representing the set of variables in terms of a smaller number of underlying variables as factors or bosomed variables (Chijioke and Osekita, 2021; Estakhr and Ranjbar, 2021). Therefore, the purpose of FA is to determine the main controlling variables among a series of traits, or in other words, to find the least number of variables, cover the most observed changes among the data series, and reduce the data dimensions (Zaluski *et al*., 2020; Ghobadi *et al*., 2021). The use of double haploids in this research is because doubled haploids are generally made from hybrids of crosses that come behind chromosome doubling. Therefore, each doubled haploid line could be a potential cultivar/variety (Rahman and De-Jiménez, 2016; Rostami Ahmadvandi et al., 2021). This method is useful for using smaller populations for the genetic study of quantitative traits. Producing a double haploid is a new technology in many plants breeding projects.

In the classic crop breeding program, to obtain a nearly complete homozygous plant (99.2%) with favorite traits, in total, about eight generations were required in (self) correction (Rahman and De-Jiménez, 2016). Doubled haploid demonstrated its benefits in achieving perfect homozygosity (100%) of all traits. This was a much faster way to develop pure genetically modified lines in one generation (Xu *et al*., 2007). In the traditional reciprocal population of two parents, a simple trait was controlled by a dominant allele segregated in the F2 with a 1:2:1 ratio for homozygous dominant, heterozygous, and homozygous recessive, respectively. However, the same trait was isolated in the doubled haploid population to 1:1 for dominant homozygous and recessive homozygous, respectively (Kahrizi and Mohammadi, 2009). For multiple genes, the technology of doubled haploid proved genotypic recombination for homozygosity for many loci in one generation (Rahman and De-Jiménez, 2016; Xu *et al*., 2007; Zanetti *et al*., 2021). Germplasm collections of *C. sativa* and its wild relatives were preserved by several institutions; however, the number of accessions available was relatively small, reflecting the partial role of *Camelina* as a product in the past (Vollmann and Eynck, 2015). Thus, a total of 801 *C. sativa* accessions were listed in the European catalog of plant germplasm collections (EURISCO, http://www.eurisco.ecpgr.org), 137 accessions were held at Plant Gene Resources of Canada (PGRC, http://pgrc3.agr.gc.ca/), and 44 were listed in the USDA National Plant Germplasm System (http://www.ars-grin.gov/npgs/) till May 2016.

In the production of new genetic materials and improved cultivars, genetic diversity is a key factor (Vollmann *et al*., 2005). Genetic diversity serves as a way for populations to adapt to changing environments. Hybridization and the creation of doubled haploids are methods for generating genetic diversity in plants. The following objects were considered in this research.

A) Introduction and investigation of the diversity of morphological and agrophysiology traits in doubled haploid lines *Camelina*.

B) Investigation of correlation coefficients between yield and morphological and agrophysiology traits in doubled haploid lines *Camelina*.

C) Investigation of correlation coefficients between yield and other characters in *Camelina*.

D) Detection of relationships between the

selected traits of doubled haploid line Camelina through FA with varimax rotation.

Materials and Methods

In this study, 136 doubled haploid lines of *Camelina sativa* seeds were obtained from previous research (Fallah, 2021) at Razi University and evaluated under dry farming conditions in the experimental field of the faculty of agriculture, Razi University, Kermanshah, Iran (47° 9_ N, 34° 21_ E and 1319 m above sea level), during the cropping season of the years 2018-2019. This experiment was performed in a randomized complete block design with three replications. Each genotype was planted in 3 rows at a length of 1 meter. The row spacing was 20 cm and the seed density was 400 seeds per square meter. Cultivation was done in the 2018-2019 cropping season. The soil texture of the experimental farm was silty clay. The climate of the cool temperate northern Zagros Mountains farm and rainfall at the time of the tests (609.5 mm) were estimated. In the first stage of the statistical analysis, the variations in the selected parameters of 136 doubled haploid lines *Camelina* was determined. In the second stage, FA with varimax rotation was conducted in SAS Software version 9.1. At the time of harvest, Yield (Y) was quantified from 5 spikes per pot. The morphological and agro-physiological features were measured, too (Table 1).

Table 1. Studied traits and their abbreviations in this study

Row	Traits	Abbreviation
1	Seed Yield (g/5 Plant)	S. Y
2	Number of Pod per Plant	N. P. P
3	Number of Pod per Main Branch Plant	N. P. M. B
4	Number of Pod per Lateral branch Plant	N. P. L. B
5	Biological Yield per Five Plants	B. Y
6	Plant Height with roots	P. H
7	Root Length	R. L
8	Root Weight	R. W
9	Shoots Weight	SH. W
10	Pod Straw Weight	P. S. W
11	Number of Seeds per Pod	N. S. P
12	Number of Lateral Branch	N. L. B
13	Length of Lateral Branch	L. L. B
14	Length of Main Branch	L. M. B
15	Seed Length	S. L
16	Seed Perimeter	S. P
17	Seed Area	S. A
18	Weight of 1000 Seeds	W.S.

Results

The variance analysis indicated very significant differences across the genotypes for all the characters assessed (Table 2), offering the presence of a significant amount of variability among the genotypes of *Camelina* evaluated in the present study. Table 3 indicates the correlation coefficients of the variables.

Table 2. Analysis of variance (mean squares) for the studied traits in *C. sativa* doubled haploid lines.

S.O.V.	df	S. Y	N P P	NP M B	N P L B	B Y	P H
Replication	2	19.39	137.19	67.33	12.76	747.49	8.75
Genotype	135	51.27**	766.68**	326.76**	133.85*	725.47**	40.40**
Error	270	6.08	57.29	23.96	17.65	80.23	13.089
CV%	-	31.22	11.47	10.79	20.39	27.25	7.56
S.O.V	**df**	**R L**	**R W**	**SH W**	**P S W**	**N S P**	**N L B**
Replication	2	17.59*	1.65	61.70	1.35	41.91	2.91
Genotype	135	16.29**	4.62**	135.55**	24.23**	39.34**	7.36**
Error	270	4.10	0.63	18.51	2.90	10.49	1.19
CV%	-	17.78	30.98	28.99	27.49	29.26	18.21
S.O.V	**df**	**L L B**	**L M B**	**S L**	**S P**	**S A**	**W S**
Replication	2	6.41	2.74	0.004	0.016	0.019	0.004
Genotype	135	179.85**	90.64**	0.07**	0.49**	0.20**	0.083**
Error	270	16.76	8.71	4.10	8.71	0.03	0.001
CV%	-	15.33	11.84	6.28	5.34	11.71	3.51

* and **: Significant at 5% and 1% probability levels, respectively.

Table 3. Correlation coefficients between the studied traits in *C. sativa* doubled haploid lines.

	S. Y	NPP	NPMB	NPLB	BY	PH	RL	RW	SHW	PSW	NSP	NLB	LLB	LMB	SL	SP	SA	WS
S. Y	1																	
NPP	.824**	1																
NPMB	.772**	.959**	1															
NPLB	.766**	.896**	.732**	1														
BY	.936**	.849**	.794**	.792**	1													
PH	.686**	.714**	.729**	.570**	.732**	1												
RL	.362**	.427**	.460**	.304**	.403**	.485**	1											
RW	.813**	.794**	.760**	.712**	.833**	.650**	.556**	1										
SHW	.891**	.818**	.767**	.760**	.920**	.775**	.389**	.769**	1									
PSW	.902**	.784**	.699**	.785**	.915**	.653**	.341**	.796**	.896**	1								
NSP	.125	.174*	.184*	.129	.089	.004	.120	.125	.103	.048	1							
NLB	.791**	.702**	.639**	.681**	.787**	.565**	.348**	.725**	.797**	.828**	.003	1						
LLB	.785**	.761**	.662**	.788**	.825**	.690**	.333**	.707**	.858**	.858**	.031	.776**	1					
LMB	.738**	.845**	.817**	.746**	.773**	.796**	.343**	.652**	.813**	.736**	.066	.587**	.795**	1				
SL	-.077	-.133	-.159	-.069	-.054	-.014	-.079	-.026	-.059	.042	-.241**	-.011	.018	-.088	1			
SP	-.073	-.113	-.134	-.062	-.057	.028	-.067	-.036	-.053	.050	-.283**	.001	.025	-.051	.945**	1		
SA	-.100	-.145	-.168	-.084	-.088	.009	-.080	-.052	-.076	.015	-.275**	-.017	.004	-.074	.905**	.969**	1	
WS	-.042	-.148	-.191*	-.057	.000	.021	-.093	.007	-.013	.078	-.316**	-.018	.047	-.089	.784**	.780**	.763**	1

* and **: Significant at 5% and 1% probability levels, respectively.

Values near 1 show that the two elements behave the same. Conversely, values close to -1 show that the two elements are behaving in reverse *i.e*., if one increased, the other decreased. A value close to 0 showed that the elements are independent of each other (Shrestha, 2021).

Results showed that all the study factors have a significant positive correlation with seed yield, except the number of seeds in the pod, seed length, seed perimeter, seed area, and weight of 1000 seeds. Seed yield was highly positively correlated with the number of pods per plant (0.824), biological yield per five plants (0.936), root weight (0.813), shoots weight (0.891), and pod straw weight (0.902).

The outcomes of FA are shown in Table 4. The first 2 factors are momentous, which accounted for more than 74% of the total variance. The contribution of factor 1 was 27.54%, and the contribution of factor 2 was 20.70%.

Table 4. FA and variance explained by principal components using varimax rotation in 136 doubled haploid lines in *C. sativa* (L.)

Factor	Initial Eigenvalues			Squared Loadings Sums Extraction			Squared Loadings Sums Rotation		
	Total	% Variance	%Cumulative	Total	% Variance	Cumulative %	Total	% Variance	%Cumulative
1	9.769	54.274	54.274	9.769	54.274	54.274	9.719	53.994	53.994
2	3.727	20.705	74.979	3.727	20.705	74.979	3.777	20.986	74.979

Extraction Method: Principal Component Analysis.

Table 4 indicates the importance of each factor. It should be mentioned that, because factor structures form more suitable factorial coefficients turned over through the varimax method, the factorial coefficient was considered to be greater than 0.5, neglecting the related sign as the significant coefficient.

Table 5 shows a principal factor matrix after orthogonal rotation for these two factors. The content of the table or factor loadings demonstrates the loading of every factor.

Table 6 indicates that two basic factors (groups) accounted for 74.94% of the whole variability in the related structure. The first factor had the highest coefficient for the seed yield, number of pod per plant, number of pod per main branch plant, number of pod per lateral branch, biological yield per five plants, plant height with roots, root weight, shoots weight, pod straw weight, number of lateral branches, length of the lateral branch, and length of the main branch which accounted for 53.99% of the total variability in the dependent structure. The suggested name for this factor is biological yield. The secondary factor had the highest coefficient for seed length, seed perimeter, seed area, the weight of 1000 seeds, and the number of seeds in the pod, which accounted for 20.98% of the total variability in the dependent structure and is called the seed characteristics.

Table 5. Rotated (Varimax rotation) factor loadings for the estimated variables of 136 doubled haploid lines in *C. sativa* (L.)

Variable	Factor	
	1	2
Seed Yield (g/5 Plant)	0.924	-0.064
Number of Pod per Plant	0.925	-0.150
Number of Pod per Main Branch Plant	0.868	-0.186
Number of Pod per Lateral Branch Plant	0.858	-0.069
Biological Yield per Five Plant	0.950	-0.037
Plant Height with roots	0.806	0.025
Root Length	0.478	-0.102
Root Weight	0.871	-0.027
Shoots Weight	0.941	-0.036
Pod Straw Weight	0.925	0.072
Number of Seeds in Pod	0.095	-0.394
Number of Lateral Branch	0.831	0.018
Length of Lateral Branch	0.890	0.051
Length of Main Branch	0.864	-0.071
Seed Length	-0.014	0.950
Seed Perimeter	0.001	0.970
Seed Area	-0.029	0.955
Weight of 1000 Seeds	0.002	0.880

Discussion

Correlation coefficient evaluation across different characters makes it possible to determine more accurately the selected indirect selection indices and remove the inactive characters (Kiani *et al*., 2022). According to the

results, all the study factors have a significant positive correlation with seed yield, except the number of seeds in the pod, seed length, seed perimeter, seed area, and weight of 1000 seeds. Kiani *et al*. (2022) reported that plant height and the number of seeds per pod have a positive and significant correlation, which is consistent with the results of this study. In the research of Kiani *et al*., (2022), the correlation of seven traits was considered, while in the present study, eighteen traits were investigated. Based on the results of Meamari et al. (2016), the simple correlation coefficient was negative and significant between the seed yield and the pod length. This founding is consistent with the result of the present study.

Table 6. Factor loadings for the approximated variables of 136 doubled haploid lines in *C. sativa* (L.)

Characters	Loading	Total Communality Percentage	Name of Suggested Factor
(Factor 1)	11.131	%53.99	Biological Yield
Seed Yield (g/5 Plant)	0.924		
Number of Pod per Plant	0.925		
Number of Pod per Main Branch Plant	0.868		
Number of Pod per Lateral Branch Plant	0.858		
Biological Yield per Five Plants	0.950		
Plant Height with roots	0.806		
Root Length	0.478		
Root Weight	0.871		
Shoots Weight	0.941		
Pod Straw Weight	0.925		
Number of Lateral Branch	0.831		
Length of Lateral Branch	0.890		
Length of Main Branch	0.864		
(Factor 2)	4.149	%20.98	Seed Characteristics
Seed Length	0.950		
Seed Perimeter	0.970		
Seed Area	0.955		
Weight of 1000 Seeds	0.880		
Number of Seed in Pod	-0.394		
Cumulative Variance		%74.97	

FA consisted of the diminution of a major number of associated variables to a much smaller number of clusters of variables, called factors (Zalusk *et al*., 2020). After calculating the first-factor loading, the process was repeated on the remaining matrix to detect further factors. When the contribution of a factor to the total percentage of the trace was lower than 10%, the procedure was stopped (Shrestha, 2021). In the research of Zaluski *et al*., (2020), ten genotypes of Spring *Camelina* from Poland were investigated and three factors were introduced in the FA of the morphological traits. According to Zaluski *et al*., (2020) the first factor included plant mass weight, seed weight per plant, and the number of seeds per plant, which accounted for 32.9% of the total variance. The second factor, which accounted for 23.1% of the total variance, included the traits of plant height, seed yield, and stubble weight. The third factor of this research, the weight of 1000 seeds, was introduced with an explanation of 14.8% of the total variance. As shown in Table 6, and emphasized by Zaluski *et al*. (2020), seed-related traits, such as the weight of 1000 seeds and seed dimensions (seed length, seed area, and seed circumference) introduce a factor. The next factors that are introduced in the *Camelina* plant usually refer to morphological traits such as the height and weight of the plant and the number of seeds in the plant. The

difference between the present research and Zaluski *et al*. (2020) can be found in the number of investigated traits. In the present study, 74.97% of the total variance was justified by examining 18 morphological traits while in the research of Zaluski *et al*. (2020), only 8 morphological traits were subjected to FA. Considering the general purpose of FA, which is to find the existence of the internal correlation between the observable traits through factors that are not observable, reducing the number of input traits in it causes the effects to be hidden. Increasing the measured traits in the FA will clarify the hidden relationship between the traits. After exploitation, the matrix of factor loading was reported to a varimax orthogonal rotation, as applied by Kaiser (1958). The effect of rotation was to accent the larger loadings in every factor and to repress the smaller loadings coefficients to amend the chance of achieving a meaningful biological interpretation of each factor. By varimax rotation, which magnifies the variance across the factors, the factors which vindicate a greater percentage of variations among the characters have had more serious effects which must be evaluated (Tadesse and Bekele, 2001). The arrangement of commonality and the value of the variance of a variable accounted by the common factors were estimated simultaneously by the highest correlation coefficient in each array as offered by Seiller and Stafford (1985). Hence, the effective characters on each factor are recognized and the factors are named according to the most effective characters (Tadesse and Bekele, 2001; Shrestha, 2021).

Conflicts of interest

The authors have declared no conflicts of interest.

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