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Oil and Protein Stability in Some *Camelina* (*Camelina sativa* L. Crantz) Genotypes

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ABSTRACT

Camelina sativa L. Crantz., which draws attention with its non-food use (biodiesel, animal feed etc.) against the increasing demand for oilseeds worldwide, is a good alternative plant. It is important to identify suitable and stable genotypes for regions along with high protein and oil content. The purpose of this study, camelina genotypes of Turkey in 3 different locations (Ankara, Ankara, Eskisehir) to investigate the quality characteristics in terms of genotype environment interactions in unirrigated and unfertilized conditions. 36 different genotypes, purified lines by negative selection, were analysed with 3 standard genotypes with augmented trial design. Environmental (E), genotype (G) and G × E interactions, which are sources of variation for protein and oil content, have been shown to be important. Oil and protein content were found ranged from 34.35%-37.88% and 25.76%-27.64% respectively. We have obtained important findings in our study to see the performance of *Camelina sativa*, and the possibility of alternative oil plants for these regions. İn terms of correlation of protein ratio and oil ratio, genotypes with high value that were least affected by each other were determined. The results showed that genotype selection by regions is important in terms of protein and oil ratio.

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Introduction

The natural growing region of *Camelina sativa*, which is also known by names like false flax, German sesame, Siberian oilseed, is Mediterranean and Central Asia (Mcvay and Lamb, 2008; Putnam et al., 1993). The plant's cultivation started in the Neolithic age and was used as an oil plant throughout the Iron Age. It is reported that it was grown in a wide area up to Southeast Europe and Southwest Asian steppes during the Roman Empire (Putnam et al., 1993). Today, it is a little bit cultivated in Canada (Downey, 1971; Robinson, 1987), Germany, Poland and the former Soviet Union. The plant is an annual plant that can be grown both winter and summer. In recent years, it has increased its usage mainly as biofuel raw material (Vollmann et al., 2007). There are 7 commonly known species of the Camelina Crantz genus, including the camelina plant. These; *C. sativa* (L.) Crantz., *C. laxa* C. A. Mey, *C. rumelica*, *C. microcarpa* Andrz. ex DC., C. *hispida* Boiss., *C. anomala* Boiss. & Hausskn. and *C. alpkoyensis* Yıld. (Güner et al., 2012). The cultivated species is *Camelina sativa* (Kurt and Seyis, 2008).

Camelina oil contains many natural antioxidants such as tocopherols, which make the oil stable and used as cooking oil. The amount of tocopherols in oil is 700 mg / kg (Kurt and Seyis, 2008). The most important feature of the camelina plant is the high rate of linolenic acid (38%) contained in camelina oil. Linolenic acid is one of the OMEGA-3 fatty acids, which is a quality fatty acid found only in fish oil and flax. In a study done in Ireland, it was found that linoleum oil contains around 35-40% linolenic acid, while this ratio is around 8% in rapeseed and soy. Therefore, it is seen that ketchup is important in meeting OMEGA-3 fatty acid rich edible oil demand (Crowley and Fröhlich, 1998). In addition, in a clinical study conducted in comparison with camelina oil and olive-rapeseed oil,

camelina oil contains 2.5 times more linolenic acid compared to rapeseed oil and 4 times more compared to olive oil, and accordingly, linoleum oil is 12%. It is stated that while decreasing 2, rapeseed oil decreases by 5.4% and olive oil by 7.7% (Karvonen et al., 2002). It provides high levels of OMEGA-3 fatty acid in meat and eggs produced by using the linens plant in the nutrition of eggs and broilers, and helps to produce healthier meat and eggs for human health (Rokka et al., 2002; Ryhänen et al., 2007). Camelina flour is similar in biological value to soy flour and contains 45-47% crude protein and 10-11% fiber (Korsrud et al., 1978). The plant is also an important source of biodiesel, and the oils obtained from the plant are used in machine lubrication in the industry. The high iodine value of the methyl ester of camelina oil allows the oil to be used in machine lubrication for a longer time without deterioration (Fröhlich and Rice, 2005).

The remaining cake of oil after the oil extract of the camelina seed contains 10% oil, 45% protein, 13% fiber, 5% mineral substance, a small amount of vitamins and also contains certain secondary metabolites, glucosinolates, sinapines, tannins and phytate which are anti-nutrients if present at high levels. Camelina meal contains a low amount of glucosinolates (14.5–36.2 mmol kg-1) compared to other crucifers like rapeseed (100–120 mmol kg-1) and mustard (62.4–77.1 mmol kg-1) (Berhow et al., 2013). It is a cheap source of protein and lipid (rich in n-3 an n-6 fatty acids) for live-stock feed (Pilgeram et al., 2007). But, glucosinolates in meal are reduced either by heating it at 100°C for 30 min or by soaking it in water (Tyagi, 2002). On the other hand, since the amino acid distribution of the camelina meal is largely similar to that of the soybean meal, it has the feature of being an alternative to soy. The composition of amino acids in camelina protein is particularly suitable for feeding poultry. It is also stated that camelina meal is a high source of protein and energy for ruminant animals (Bertrand and Brühl, 2001; Schuster and Friedt, 1998).

Camelina ability to adapt to extreme conditions is high, and the plant is not too demanding in terms of nutrients. The high competition of the plant against weeds limits the use of chemical drugs. This situation is an important feature for the environment (Kurt and Seyis, 2008).

Although the camelina plant has many uses, there has not been enough scientific research on the plant in our country and in the world. After the importance of oil in terms of human health has emerged in recent years, it has attracted attention and characterization and adaptation studies have started in many countries, especially in Germany. It has attracted attention again in recent years due to its low environmental demand and high quality of fatty acids. The fact that it can be grown in marginal areas especially highlights the plant as an important alternative oil plant. Therefore, good genotypes should be obtained by breeding studies. İdeal genotypes are highly efficient as well as stable. For this reason, determine of stable varieties with multienvironmental trials are important in plant breeding for evaluating genotypes for stability and adaptability of genotype by environment (GE) interaction. (Montesinos-lópez et al., 2018).

Yan and Rajcan (2002) demonstrated that can be used a genotype x property (GP) biplot (with an application of the GGE biplot technique) to study genotype x property data. This application of GP biplot was an excellent for visualizing genotype x property data. Within the scope of this study, 36 different camelina lines were examined in terms of protein and oil content in 3 locations of Turkey and data presented to the use of breeders and scientists.

Material and Method

Material

The seeds of 36 camelina genotypes (*Camelina sativa* (L.) Crantz) used in the study, obtained from The Seed Bank of The Agricultural Research Service of The United States Department of Agriculture (USDA) (Table 1). Additionaly 3 control (C) genotyps (Line-1 (C1), PI 650149 (C2) and PI 650151 (C3)) were used in the trial. Field experiments were conducted during the 2014-2015 cropping season at the research and implementation area of Field Crops Agricultural Research Institute, Ankara, GAP Agricultural Research Institute, Şanlıurfa and Transitional Zone Agrıcultural Research Instıtute, Eskişehir in Turkey. Climatic data of these locations are shown in Figure 1.

Method

Field experiments were conducted during the 2014-2015 cropping season. The materials were examined in the Augmented trial pattern in three locations on October, 15 cm between rows, 5 cm above the row and 5 meters in row length, and were examined in terms of their oil and protein content. No irrigation and fertilization process were applied during the vegetation period.

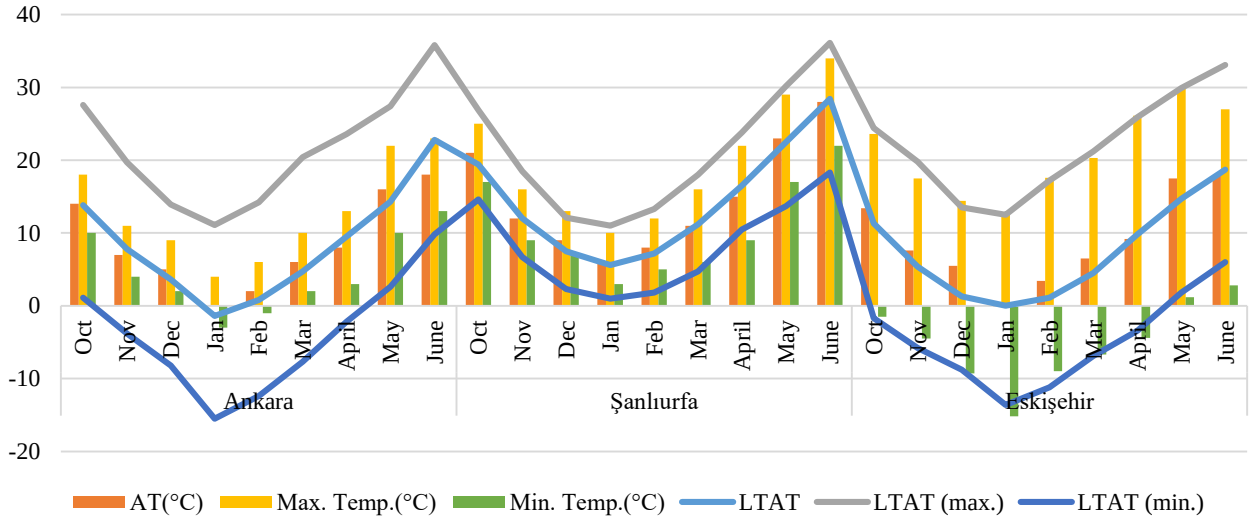
The oil content (%) was determined from the seeds of 10 plants selected randomly from the plant rows. Oil ratio analyzes were performed with the Soxhlet extractor (Soxtherm 2000 automatic, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) using the method reported by Bertrand and Brühl (2001). The homogenous sample, taken from each row of camelina seeds, was ground. Crude oil weight obtained from the 5 g sample taken was determined in proportion to the sample weight.

The protein ratio of homogenous sample taken from camelina seeds obtained from each row was made by Dumas method (Velp Scientifica NDA-701) according to AOAC 992.23: Crude Protein in Cereal Grains and Oilseeds method. In the calculation of the protein, the nitrogen factor was taken as 6.25.

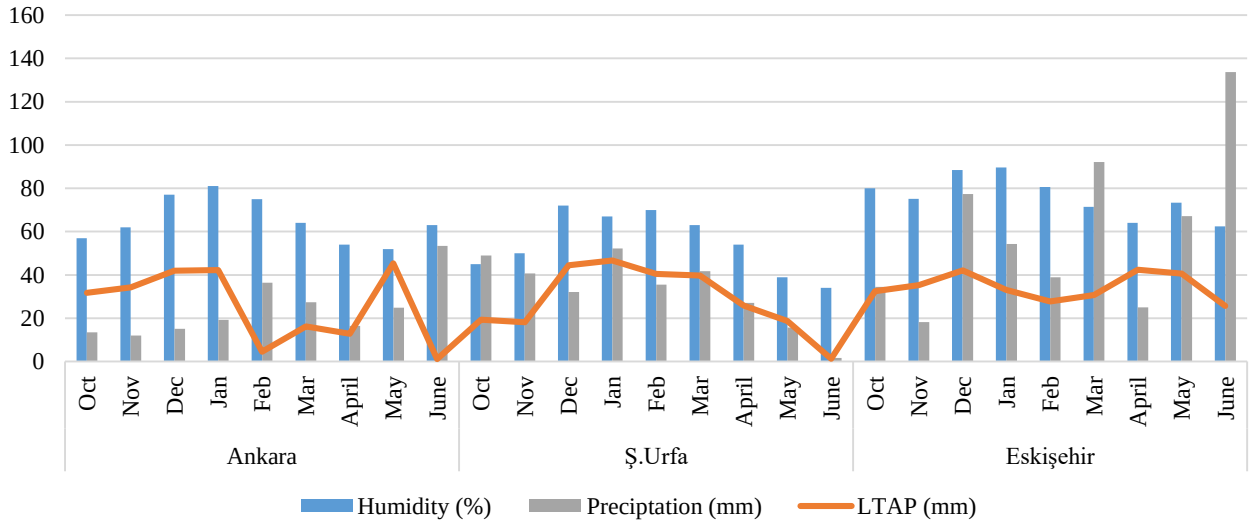
Data were analysed using the statistical analysis software JMP Pro 13 (SAS Institute, NC, USA, 2013), while the bi-plot graph was produced with GEA-R statistical software for visual evaluation of protein and oil performance and stability of genotyps across environments. Properties values means were compared using the Duncan test with XLSTAT statistical software.

Findings and Discussion

Field trials were established in Ankara, Şanlıurfa and Eskişehir locations in October 2014. Harvesting was in the middle of June in Eskişehir and Ankara, in the middle of May in Şanlıurfa in 2015. Oil content and protein content are determined.



(a)



(b)

Figure 1. Monthly average of meteorological data of the experimental farm during growing season (2014-2015) and long-term values ((a): average temperature (AT), maximum temperature (Max. Temp.), minimum temperature (Min. Temp.), long term average temperatures (LTAT max. and LTAT min.); (b): humidity, precipitation and long-term average preciptation (LTAP)

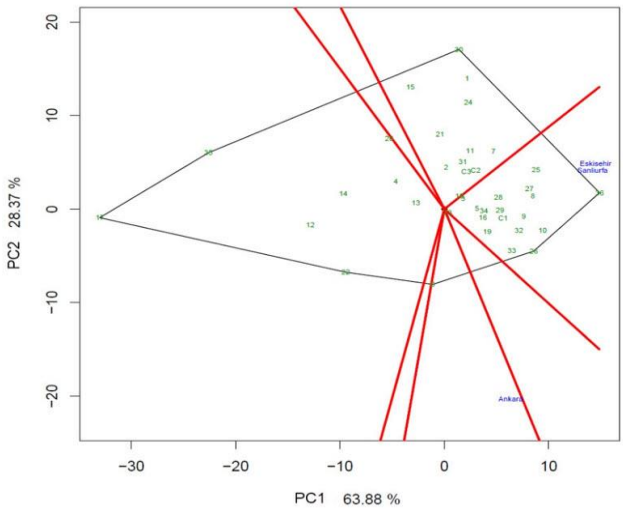


Figure 2. Polygon view of GGE-biplot plot for camelina genotypes and environments in oil ratio.

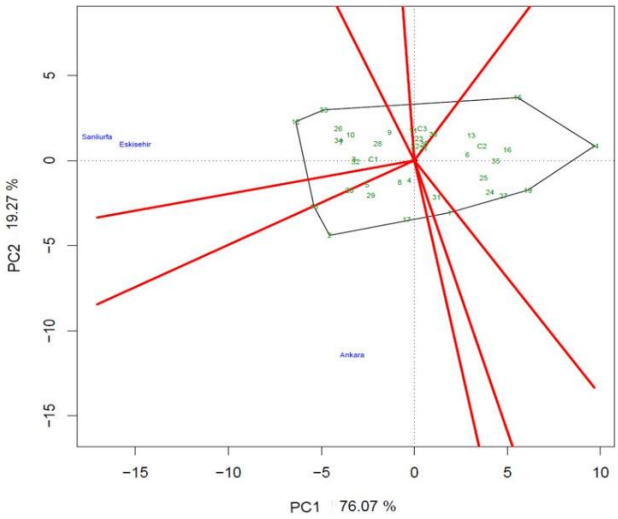


Figure 3. The polygon of the GGE-biplot plot for camelina genotypes and environments in protein ratio.

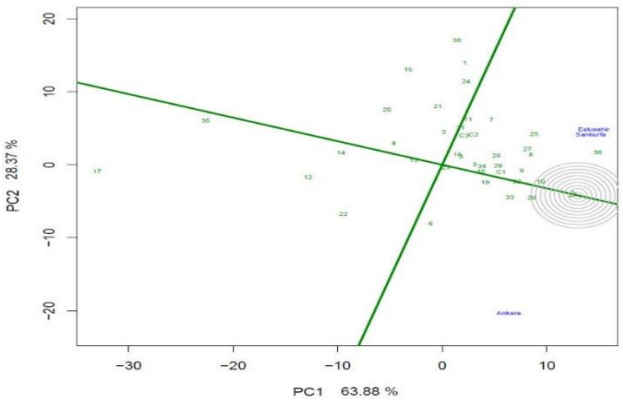


Figure 4. GGE-biplot chart based on comparison of genotypes with ideal genotype in oil ratio.

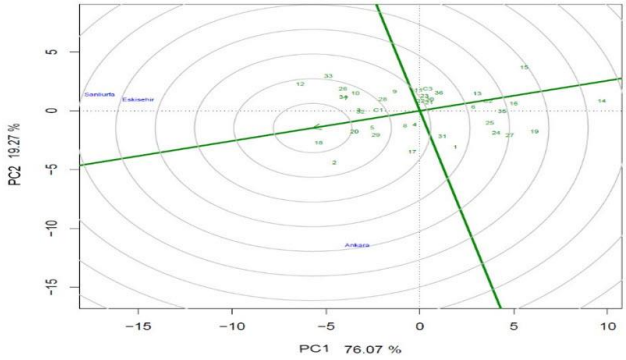


Figure 5. GGE-biplot graph based on comparison of genotypes with ideal genotype in protein ratio

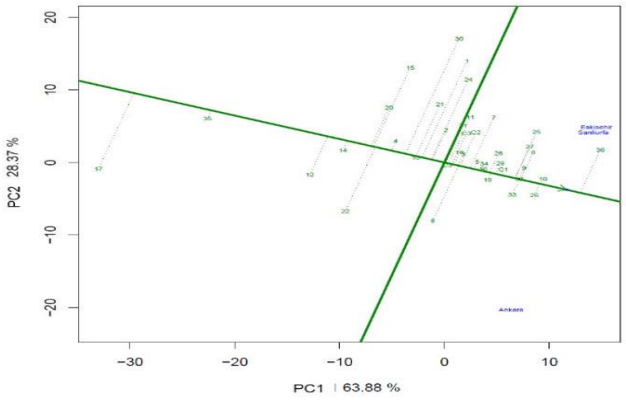


Figure 6. Average environmental coordination view of the DDE-biplot chart in the oil ratio.

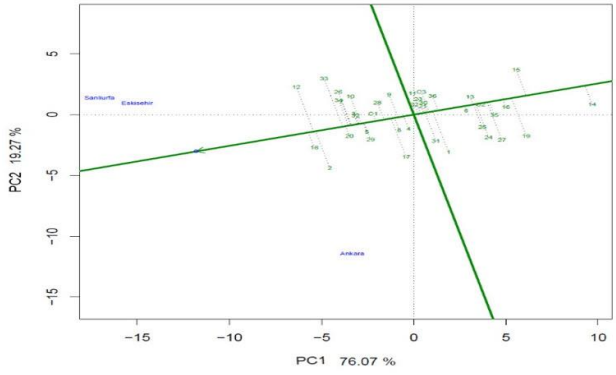


Figure 7. Average environmental coordination view of GGE-biplot graph in protein ratio

Analysis of variance revealed significant (P<0.01) effects for all sources of variation (environment, genotype and G × E interaction) for protein ratio and oil ratio (Table 2). The interaction between genotype and environment (G × E interaction showing that genotypes do not give the same response in different environments) is important in terms of guiding breeders in the selection of regional genotypes (Ilker et al., 2018; Silveira et al., 2016). There was a statistically significant differences of oil and protein content values in Ankara, Şanlıurfa and Eskişehir location.

The highest oil rates were determined in Ankara, Şanlıurfa and Eskişehir locations with the rates of 43.53%, 46.07%, 46.94%, respectively, in genotype 6, 36 and 36. The highest protein ratios were determined in Ankara, Şanlıurfa and Eskişehir locations with the rates of 31.05%, 34.01%, 32.22% in genotype 2, 34 and 12, respectively. In terms of both of the characters examined, the accessions were in different groups at different locations. This emerged as a result of the genotype environment relationship

Polygon charts showing genotype (G) and genotype x environment interaction (GE) in experiments in multiple environments are shown in Figure 2 and Figure 3. In terms of oil ratio, it is seen that genotype 36 was more prominent in Eskişehir and Şanlıurfa, these two locations affect the genotypes similarly, and genotype 6 was prominent in the Ankara location (Figure 2). In terms of protein ratio, it was determined that genotype 12 and genotype 33 in Eskişehir and Şanlıurfa regions and genotype 2 in Ankara location were found to be prominent (Figure 3).

The GGE biplot in Figure 4 and Figure 5 show that genotyps possitions according to ideal enviroments. The central circle is calculated and as ideal environment is considered. It is seen that the closest environments to the ideal environment, in terms of oil ratio (Figure 4) and protein ratio (Figure 5) are Eskişehir and Şanlıurfa locations. Likewise, it was observed that the ideal genotypes, in terms of oil ratio were genotype 26 and genotype 10 (Figure 4), and the ideal genotypes, in terms of protein ratio were 20 and 18 genotypes (Figure 5).

The ideal test environment should have the larger PC1 (expressing the main genotypic effect) score and the smaller absolute PC2 (more representative of the whole environment) score. Although it is not really an ideal environment, the ideal test environment can be used as a reference in multiple environment trials (Kaya et al., 2006). When the yield performance and stability of genotypes are evaluated with the average environmental coordination method, an average environment is defined by this method (Yan and Hunt, 2001). Scores of PC1 and PC2 average of all circles intersect is indicated by a small circle. By drawing a line through this mean circumference point and the biplot origin, the average circumference abscissa of the mean perimeter coordination is formed. Absis means that while the average circumferential direction is unidirectional, genotypes' oil ratios (Figure 6) and protein ratios (Figure 7) increase in this direction. The other line drawn as ordinate expresses that the stability decreases in both directions from the origin point and the effect of genotype x environment interaction increases. Considering this situation, it was seen that the oil ratio, high value and stability were reached in the genotype 26 and genotype 10, while the protein ratio was reached only in genotype 10 (Figure 6 and Figure 7).

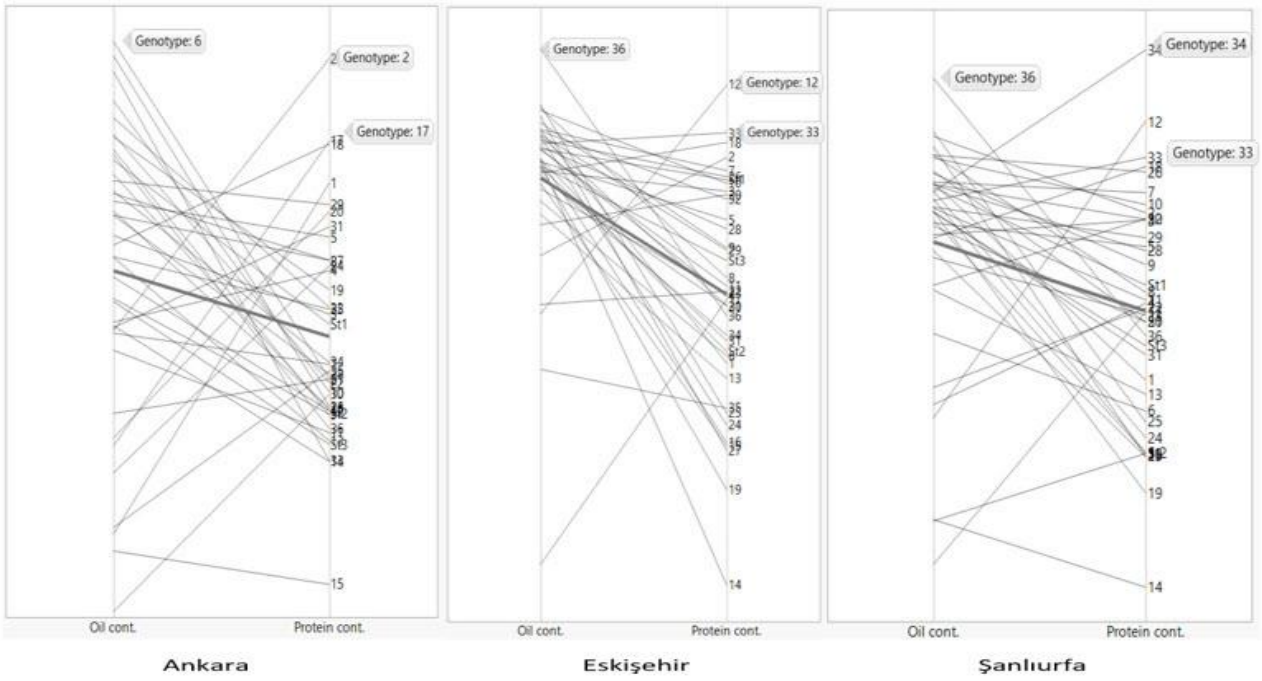


Figure 8. Parallel coordinate plot for oil and protein content.

Table 1. Materials used in the research and the countries they belong to.

Accessions ID	Genotype No.	Origin	Accessions ID	Genotype No.	Origin
PI 650141	1	America, Minesota	PI 650155	19	Poland
PI 650164	2	Austria	PI 650158	20	Poland
PI 597833	3	Denmark	PI 650159	21	Poland
PI 650142	4	Denmark	PI 650162	22	Poland
PI 650144	5	Denmark	PI 650153	23	Russia
PI 650150	6	Denmark	PI 650154	24	Russia
Ames 31220	7	Georgia	PI 650156	25	Russia
Ames 31224	8	Georgia	PI 650157	26	Russia
Ames 31231	9	Georgia	PI 650160	27	Russia
Ames 31232	10	Georgia	PI 650161	28	Russia
Einfact (Leindotter)	11	Germany	PI 650166	29	Russia
PI 633193	12	Germany	PI 652885	30	Slovenia
PI 633194	13	Germany	PI 652886	31	Slovenia
PI 650145	14	Germany	PI 304269	32	Sweden
PI 650148	15	Germany	PI 304270	33	Sweden
PI 650149	16	Germany	PI 304271	34	Sweden
PI 311735	17	Poland	PI 650147	35	Sweden
PI 311736	18	Poland	PI 650151	36	Swiss

In our study, although the correlation between protein ratio and oil ratio is not statistically significant, the correlation between protein ratio and oil ratio of genotypes in 3 locations is seen in the parallel coordinate plot graph in Figure 8. In oil plants, the ratio of protein in the seed is as important as the oil ratio. Genotype 17 in Ankara location and Genotype 33 in Eskişehir and Şanlıurfa locations were the least negative effects of protein and oil ratios on each other.

Canvin (1965) observed seed development at different temperatures in oilseed plants such as rape, safflower, sunflower, flax and castor bean plants; found that the oil content of sunflower, safflower and castor bean was not affected by the temperature, and that the oil content of rapeseed and flax seeds was higher at low temperatures. Ayerza (2009) reported that when Chia genotypes were tested in 5 different ecosystems, protein and oil ratios differ in some locations. Popovic et al. (2016) stated that in their study on soybean genotypes in 2008, 2009 and 2010, protein and oil ratios changed over the years. They found that the amount of both protein and oil was higher in 2008 and 2009 compared to 2010. Similarly, the absence of a correlation between of protein and oil content in our study and the increase of both in some environments show that environmental factors are more determinant than genotypic factors in terms of these properties.

Table 2. Results of analysis of variance for protein and oil content field trial data conducted with 36 camelina genotypes in 3 environments.

Source	DF	MS Protein Content	Oil Content
Model	131	7.210	38.778
Enviroment (E)	2	30.859**	125.403**
Blocks [E]	15	1.093*	4.093**
Genotype (G)	38	12.682**	69.785**
E × G	76	3.129**	21.441**
Error	30	0.453	0.327
C.V. (%)		2.503	1.565

DF: Degree of freedom, MS: Mean squares

Table 3. Corrected values obtained from Ankara, Şanlıurfa and Eskişehir locations and their groups.

Genotyps Numbers	Ankara		Şanlıurfa		Eskişehir	
	Oil content (%)	Protein content (%)	Oil content (%)	Protein content (%)	Oil content (%)	Protein content (%)
1	23.400^{kl}	28.810^{ac}	40.090^{c-h}	25.920^{l-q}	40.960^{c-g}	25.880^{i-p}
2	31.720^{f-j}	31.050^{a}	42.750^{bc}	30.020^{b-g}	31.780^{t}	30.570^{a-c}
3	35.600^{c-g}	26.450^{c-j}	37.120^{i-l}	29.840^{b-h}	37.990^{l-p}	29.800^{a-f}
4	32.060^{f-j}	27.230^{b-h}	27.400^{o}	27.800^{f-m}	39.620^{g-m}	27.400^{d-m}
5	37.000^{b-g}	27.840^{b-e}	37.790^{h-l}	29.190^{c-j}	38.660^{j-o}	29.150^{b-g}
6	43.530^{a}	25.290^{e-k}	31.470^{n}	25.160^{n-r}	35.790^{qr}	26.050^{h-o}
7	31.610^{f-j}	25.550^{d-k}	40.140^{c-h}	30.510^{b-e}	41.040^{c-g}	30.270^{a-d}
8	37.310^{a-g}	27.420^{b-f}	41.680^{b-e}	28.070^{e-m}	42.580^{bc}	27.830^{c-k}
9	39.110^{a-e}	24.630^{h-k}	40.630^{b-g}	28.750^{d-k}	41.530^{b-f}	28.510^{b-i}
10	41.110^{a-c}	25.050^{f-k}	41.520^{b-e}	30.220^{b-f}	42.420^{bcd}	29.980^{a-e}
11	30.910^{g-j}	24.320^{i-k}	38.640^{f-k}	27.880^{f-m}	39.540^{g-n}	27.640^{c-l}
12	33.900^{d-h}	24.710^{g-k}	26.650^{o}	32.260^{ab}	27.520^{u}	32.220^{a}
13	34.700^{c-h}	24.170^{jk}	33.960^{m}	25.570^{m-r}	34.83^{rs}	25.530^{j-p}
14	31.910^{f-j}	23.800^{kl}	20.830^{p}	20.840^{s}	38.730 $^{i-o}$	20.830^{q}
15	22.700^{kl}	21.590^{l}	36.220^{k-m}	24.040^{qr}	37.090^{o-q}	24.000^{n-p}
16	38.100^{a-f}	24.720^{g-k}	37.960^{g-l}	24.120^{qr}	38.830^{h-o}	24.080^{n-p}
17	27.010^{i-k}	29.560^{ab}	18.260^{q}	27.580^{g-n}	9.160^{w}	27.340^{d-m}
18	35.210^{c-g}	29.510^{ab}	36.930^{j-l}	31.150^{b-d}	37.830^{m-p}	30.910^{ab}
19	39.630^{a-d}	26.890^{c-i}	38.460^{f-l}	23.150^{r}	38.280^{k-p}	23.000^{pq}
20	27.330^{i-k}	28.290^{bc}	34.230^{m}	29.870^{b-h}	34.050^{s}	29.720^{a-f}
21	28.330^{h-k}	25.280^{e-k}	37.620^{h-l}	27.340^{h-o}	37.440^{o-q}	27.190^{e-m}
22	39.730^{a-d}	25.220^{e-k}	28.390^{o}	27.670^{f-n}	28.210^{u}	27.520^{d-l}
23	36.600^{b-g}	24.760^{g-k}	35.810^{lm}	27.510^{g-n}	36.680^{pq}	27.470^{d-l}
24	25.900^{j-l}	27.320^{b-g}	39.630^{d-j}	24.510^{p-r}	40.500^{e-j}	24.470^{m-p}
25	34.730^{c-h}	26.540^{c-j}	43.020^{b}	24.890^{o-r}	42.840^{b}	24.740^{l-p}
26	42.940^{ab}	24.790^{f-k}	41.670^{b-e}	31.020^{b-d}	40.240^{e-j}	30.130^{a-e}
27	36.430^{b-g}	27.420^{b-f}	42.160^{b-d}	24.040^{qr}	41.980^{b-e}	23.890^{op}
28	36.430^{b-g}	25.370^{e-k}	39.970^{d-h}	29.090^{c-k}	39.790^{f-l}	28.940^{b-h}
29	37.840^{a-f}	28.420^{bc}	38.700^{f-k}	29.410^{c-i}	40.650^{d-h}	28.460^{b-j}
30	20.230^{l}	25.010^{f-k}	40.750^{b-f}	27.340^{h-o}	40.570^{e-i}	27.190^{e-m}
31	31.830^{f-j}	28.020^{b-d}	38.460^{f-l}	26.540^{k-q}	38.280^{k-p}	26.390^{g-o}
32	40.410^{a-d}	26.570^{c-j}	40.000^{d-h}	29.860^{b-h}	40.900^{c-g}	29.620^{a-f}
33	42.310^{ab}	23.810^{kl}	39.080^{e-j}	31.370^{bc}	39.980^{f-k}	31.130^{ab}
34	37.410^{a-g}	25.580^{d-k}	39.580^{d-j}	34.010^{a}	37.710^{n-p}	26.520^{g-o}
35	23.660^{kl}	25.430^{d-k}	20.750^{p}	24.120^{qr}	23.460^{v}	24.860^{k-p}
36	38.900^{a-e}	24.390^{ijk}	46.070^{a}	27.000^{i-p}	46.940^{a}	26.960^{f-n}
C1	38.683^{a-e}	26.270^{c-k}	39.817^{d-i}	28.250^{e-l}	39.600^{g-m}	30.058^{a-e}
C2	33.017^{e-i}	24.645^{h-k}	39.157^{e-j}	24.148^{qr}	38.875^{h-o}	26.143^{h-o}
C3	32.900^{e-i}	24.108^{jk}	38.383^{f-l}	26.760^{j-p}	38.367^{k-p}	28.217^{b-j}
Std. Dev.	5.299	1.819	5.538	2.662	5.465	2.378
Minimum	20.230	21.590	18.260	20.840	9.160	20.830
Maximum	43.530	31.050	46.070	34.010	46.940	32.220
Means	34.354	25.758	37.380	27.271	37.879	27.643

Likewise, Gurmu et al., (2010) in their study with soy genotypes in 6 environments in 2007, stated that 2 genotypes out of 3 genotypes were not stable in terms of protein, that is, they were greatly affected by environmental conditions. Our findings in Camelina genotypes show that environmental conditions can increase the amount of protein and oil together.

Conclusion

In this study, it was observed that the environmental conditions in which the camelina plant grows affected the oil and protein ratio in a statistically significant way. Since oil plants are also a source of feed stuff, the high ratio of protein as well as the high oil ratio in the seed is important, so the selection of genotypes with good oil and protein ratio should be considered. This study showed that the negative relationship between the oil and the protein ratio in camelina seeds appeared in all environments. In addition, in this study, *Camelina sativa* genotypes with the least negative correlation of oil and protein ratio were determined (Genotype 17 and Genotype 33). Genotypes took place in different sequences in different environmental conditions in terms of oil and protein ratio. This situation shows that it is insufficient to carry out improvement studies in a single location. Selection studies in countries such as Turkey which is very different climate zones should be carried out in very different climatic zones as possible and environment-specific varieties must be developed. Instead of stable varieties, environmentally specific varieties should be recommended.

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