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Determination of seed yield, quality and fixed oil components of different basil (Ocimum basilicum L.) genotypes: Evaluation of fatty acid profile by PCA biplot analysis

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Research Article

Abstract − Basil (*Ocimum basilicum L.*) plants are generally grown for different properties and produce seed with a consid-erable amount at the end of the growing season. This study was carried out to determine the seed yield, quality and fixed oil components of eight different basil (B) genotypes with purple (PB) and green (GB) leaf color ob-tained from different countries. In other words, seed yield and quality, fixed oil components, the seed yield, 1000 seed weight, oil content, oil yield, crude protein, ash, linoleic acid, linolenic acid, oleic acid, palmitic acid, stearic acid, hexadecatrienoic acid properties, ethyl linolate and trace oil contents were investigated. Seed yield, 1000 seeds weight and oil yield were significant statistically among the genotypes. While the best result in terms of seed and oil yield was obtained from GB1, the highest 1000 seeds weight was recorded in GB4. Palmitic acid and trace oil contents of genotypes were significant statistically. The highest palmitic acid content was detected in GB2, whereas the maximum trace oil contents were obtained from GB5. In results of principal components analysis (PCA) purple and green basil types demonstrated different features in terms of fixed oil components. GB2, GB4 and GB5 basil genotypes which have green leaf types were superior according to the PCA. GB2 genotype obtained from Hungary was the better in terms of seed yield and fixed oil components among the purple and green basil genotypes used in this research.

Keywords − *Basil, fixed oil, , genotype, Ocimum basilicum L., seed*

1. Introduction

Ocimum basilicum L. (basil) grown up to 60 cm height is an annual plant. The roots of these plants are thin and grow by branching. Basil leaves are different oval and elongated forms and the basil flowers are at the top of the main axis. Basil seeds are oval and nut-shaped and maintain the germination vigour (Dachler & Pelzmann, 1999). Basil flowers bloom from bottom to top. Therefore, first matured seeds are at the bottom of the flower. Due to periodic ripening and delayed harvest, seeds matured about 20-25% separate as a by-product and then the basil plants are harvested. Another option to obtain seed is waiting till plant fully matures (Domokos & Perédi, 1993). The origin of basil plants is unknown. However, since many basil plants are similar to the species grown in Africa, it is assumed to be of African origin. On the other hand, it is estimated that India and Asia can also be its origin (Small, 2006). Basil cultivation was widely performed in Turkey, Iran, Japan and China (Sadeghi, Rahnavard, and Ashravi, 2009).

Basil seeds have been traditionally used in the treatment of indigestion, ulcer, diarrhea, sore throat and kidney ailments (Rezapour, Tarzi, and Movahed, 2016). Also, gum obtained from basil seeds has been used for many purposes such as a fiber source, pharmaceutical drug additive, suspending agent, anti-diabetic agent, growing plant seedlings and biodegradable film (Tabasi & Razavi, 2017). Fixed oils are glycerol esters commonly found in both animals and plants (Asil & Bozdoğan-Konuşkan, 2021). These oils have

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been widely used in the food, lubrication, soap, paint, polish and fuel industries (Behera, Nagarajan, and Rao, 2004; Kadam, Yadav, Shivatare, Bhilwade, and Patil, 2012). Seed oils differ significantly in fatty acid composition at different taxonomic and typical differentations (Zhang, Zhang, Zhang, and Kitajim, 2015). Fatty acid compositions in the seed oils can be found at different levels among the species and genotypes and within species (Motojest, Ogunlaja, and Amos, 2011; Zhang et al., 2015; Idris et al., 2020). Despite this popularity, few current studies have evaluated the seed oil yield and compositions of basil (Angers et al., 1996; Sadeghi et al., 2009; Tarchoune et al., 2013; Mostafavai et al., 2019; Idris, Nour, Ali, Erwai Ishag, and Nour, 2020). Therefore, this study aimed to investigate the seed and oil yield, chemical characteristics and fatty acid compositions of different basil genotypes to rise the economical usage.

2. Materials and Methods

2.1. Plant Material

The origins of the eight different green and purple basil genotypes used in this study were given in Table 1. One purple and three green basil genotypes were obtained from Garafarm Corporation from Hungary. The Arapgir genotype (purple basil) marked geographically in Turkey was obtained from Malatya province. Genotypes originating from France were provided via Turkish Vilmorin Seed Corporation.

Table 1

Knowledge about the basil genotypes used in this study

Abbreviations	Genotypes	Colour of Leaves	Leaf Types	Origin
PB1	Arapgir	Purple	Elongation	Turkey
PB2	Piros	Purple	Oval	Hungary
PB3	Midnight	Purple	Oval	France
GB1	Fahéj illatú	Green	Elongation	Hungary
GB2	Magas	Green	Elongation	Hungary
GB3	Törpe	Green	Oval	Hungary
GB4	Compact	Green	Elongation	France
GB5	Large Sweet	Green	Oval	France

2.2. Growing the Basil Seedlings, Experimental Design, Climatic Conditions and Soil Properties

Purple and green basil seeds obtained from different countries were sown in viol including 1:1 peat and perlite mediums on 7 March in 2020. First seedling emergences were determined five days after sowing. Seedlings reaching a period of 4-5 leaves were planted in experimental field at Hatay Mustafa Kemal University on 22 March 2020. The experimental design was laid out the randomized completed blocks with three replications. The plants were planted by setting 40 cm space among the plants in rows and 50 cm space among the rows in experimental field. The row length was 5 m. Before planting, the field was fertilized with 3 kg nitrogen and 5 kg phosphorus. Plants were irrigated with a drip irrigation system according to their water needs during the growing period. Plants were again fertilized with 3 kg nitrogen at pre-flowering stage. The climatic conditions were given in Table 2. While precipitation decreased significantly in all months compared to the LYA (long year averages) climatic conditions, the temperature increased considerably. The soil of the experimental field was clay-loam texture and slightly alkaline reaction (pH: 7.60), also the organic matter content was 2.30% (Yılmaz, Hür, and Ertekin, 2018; Ertekin, Atış, and Yılmaz, 2020).

2.3. LYA: Long Year Averages (Anonimous, 2020)Harvest and Yield

Twenty basil plants randomly selected from each parcel were harvested on 15 September 2020 when the seeds were fully mature. Samples filled separately in sacks were laid out on the drying table at room

Table 2

The climatic conditions of the province of Hatay where the experiment was conducted

Months	Year and LYA	Precipitation (mm)	Temperature (°C)	Relative Humidity (%)
March	2020	49.4	14.9	82.1
	LYA	143.3	13.0	-
April	2020	38.2	18.1	75.0
	LYA	103.9	17.2	-
May	2020	13.8	23.2	63.4
	LYA	81.1	21.2	-
June	2020	0.4	25.2	67.4
	LYA	32.0	24.8	-
July	2020	0.0	29.5	68.3
	LYA	16.0	27.2	-
August	2020	0.0	29.6	64.7
	LYA	18.2	27.8	-
September	2020	0.0	29.6	65.6
	LYA	41.1	25.7	-

LYA: Long Year Averages (Anonimous, 2020)

temperature conditions. The dried samples were collected after one week and filled in sacks. To remove seeds from flowers, the plants in the sacks were chewed and hammered. The seeds removed from flowers were cleaned from plant residues via sieves. Seeds for each genotype and replicate were counted and weighed four times with groups of a hundred. 1000 seed weights of genotypes were determined in this way. The seed yield of genotypes was calculated over twenty plants randomly selected and harvested with the number of plants grown per decare.

2.4. Chemical Analyzes, Oil Extraction and Determination of Oil Components

100 g seed obtained from genotypes was ground in a mill with 1 mm sieve diameter (Ertekin, Çeliktaş, Can, and Kızılşimşek, 2017; Kizilsimsek, Ozturk, Yanar, Ertekin, Ozkan, and Kamalak, 2017; Ertekin, Atış, Yılmaz, Can, and Kızılşimşek, 2019; Ertekin and Kızılşimşek, 2020). Nitrogen (N) content of seeds was analyzed by the Kjeldahl method (AOAC, 2019). Crude protein (CP) contents were calculated as N×6.25. Ash content was determined by burning in the muffle furnace at 550 °C for 4 hours (AOAC, 2005). Oil extraction of seeds was performed extraction method via soxhlet extractor according to the AOAC (2005). Also, oil ratio and oil yield of genotypes was calculated. After taking 100 µl of oils obtained from basil seeds and adding 3 ml of N-Heptane and 400 µl of 2N methanolic KOH solution, esterification was applied and the components of the oils were analyzed by GC / MS. Determination of fixed oil components was carried out under the following conditions with Thermo Scientific ISQ Single Quadrupole model gas chromatograph device. A column with a TR-FAME MS model, 5% Phenyl Polysilphenylene-silohexane, 0.25 mm inner diameter × 60 m length, 0.25 µm film thickness was used. Helium (99.9%) was used as carrier gas at a flow rate of 1 mL min^{-1}. The ionization energy was set at 70 eV and the mass range m/z 1.2-1200 amu. Scan Mode was used for data collection. Temperatures of MS transfer line, MS ionization, injection port and initially column were 250, 220, 220 and 120 °C, respectively. The 1 µL of the esterified sample was taken by the autosampler and placed in the injection port. Samples in initially column temperature were kept for 1 min. Column temperature increased by 10 °C per minute to 175 °C and samples were kept there for 10 min. Column temperature rose by 5 °C per minute to 210 °C and the samples were kept there for 5 minutes. Then, the column temperature increased by 5 °C per minute to 230 °C and the analysis was concluded by waiting for 6 minutes here. Total analysis time was 38.5 minutes. The structure of each compound was identified with the Xcalibur program using mass spectra (Türkmen & Koçer 2021).

2.5. Statistical Analyses

Analysis of variance was performed to compare the seed yield, seed chemical characteristics and fixed oil components of different basil genotypes. Significance among the means was evaluated by using the LSD test ($p \leq 0.05$). Also, standard error means of the data were presented. Principle component analysis (PCA) was carried out using XLSTAT to examine relationships between basil genotypes and fixed oil components detected via GC/MS.

3. Results and Discussion

3.1. Yield and Chemical Characteristics

Means of properties of seed yield, 1000 seeds weight, oil ratio, oil yield, crude protein and ash content were given in Table 3. Basil genotypes had significant effect on the seed yield, 1000 seeds weight and oil yield, but not on the oil ratio, crude protein and ash. Seed yield ranged from 178.89 to 606.67 kg da^{-1}. The highest seed yield was obtained from GB1, whereas the lowest value was detected in PB2. 1000 seeds weight varied between 1.03 and 1.48 g. While the highest 1000 seeds weight was determined in GB4, the lowest value was found in GB3. Oil yield values ranged from 24.36 to 97.34 kg da^{-1}. The maximum oil yield was achieved in the GB1, while the minimum value was recorded in PB3. The oil ratio values varied between 12.17 and 17.08%. The maximum oil ratio was found in PB1, whereas the lowest was determined in GB5. Crude protein contents of genotypes ranged from 15.70-19.30%. The highest crude protein ratio was obtained from GB2, while the lowest value was detected in GB5. The ash contents of genotypes varied between 8.99 and 10.46%. The maximum ash content was recorded in PB2, whereas the minimum ash content was achieved in GB2. The seed yield, 1000 seeds weight and oil yield properties, as affected by different basil genotypes, were determined to investigate yield and these features changed highly among the genotypes. Egata, Geja, and Mengesha (2017) reported that the seed yield per plant varied between 1.88 and 14.03 among the Ethiopian sweet basil genotypes. In addition, another study described that the seed yield per plant ranged from 9.26 to 44.84. (Gowda, Dorajeerao, Madvai, and Suneetha, 2019). Nassar, El-Segai, and Mohamed (2013) reported that the 1000 seeds weight was 1.396 g in basil. Results obtained

Table 3

Effects of genotypes on seed yield, 1000 seeds weight, oil ratio, oil yield, crude protein and ash contents

Genotypes	Seed Yield (kg/da)	1000 seeds weight (g)	Oil Yield (kg/da)	Oil ratio (%)	Crude protein (%)	Ash (%)
PB1	256.67±58.97^{b}	1.37±0.03^{ab}	43.59±9.63^{bc}	17.08±0.23	18.21±0.45	9.34±0.12
PB2	178.89±17.88^{b}	1.21±0.06^{c}	28.59±2.61^{c}	16.02±0.35	16.42±0.57	10.46±0.48
PB3	181.11±15.44^{b}	1.13±0.03^{cd}	24.36±1.95^{c}	13.46±0.25	16.93±0.22	9.74±0.12
GB1	606.67±173.60^{a}	1.33±0.04^{b}	97.34±21.12^{a}	16.76±2.06	18.19±1.06	9.56±0.72
GB2	300.00±42.21^{b}	1.43±0.06^{ab}	41.15±0.78^{bc}	14.23±1.86	19.30±1.03	8.99±0.21
GB3	228.89±41.16^{b}	1.03±0.03^{d}	34.19±7.62^{bc}	14.73±0.62	15.88±0.31	9.68±0.13
GB4	406.67±24.57^{ab}	1.48±0.01^{a}	60.05±4.82^{b}	14.81±1.18	18.55±1.19	9.53±0.25
GB5	382.22±103.79^{ab}	1.34±0.04^{b}	45.65±11.67^{bc}	12.17±0.56	15.70±1.06	10.18±0.34
Mean	317.64	1.29	46.86	14.91	17.40	9.69
Significance	*	***	**	ns	ns	ns
P value	0.04	<.0001	<.01	0.13	0.07	0.25
LSD	249.87	0.12	30.99	3.60	2.59	1.15

ns: non-significant; *:0.05≥p>0.01; **:0.01≥p>0.001; ***: 0.001≥p; ^{abcd}Row means with common superscripts do not differ.

from this study about seed yield and 1000 seeds weight was to similar literature reports. Also, while the GB1 was superior in term of seed yield, the GB4 was the highest in terms of 1000 seeds weight. There was no literature report about basil seed oil yield. Safflower seed yield varies between 60 and 100 kg da^{-1} (Kolsarıcı, Gür, Başalma, İşler, and Kaya, 2005). Seed oil yield results obtained from this study was similar to safflower seed oil yield. Nour, Elhussein, Osman, and Nour (2009) reported that the oil ratio of 14 different basil genotypes was between 8.8-30%. Moreover, a study determined that the oil ratio was between 12.4 and 21.6% (Kakaraparthi, Srinivas, Kumar, and Kumar, 2015). Sarfraz, Anjum, Khan, Arshad, and Nadeem (2011) found that the crude protein content of basil was 11.4. The crude protein content of genotypes used in this study was higher than the results of Sarfraz et al. (2011). Sarfraz et al. (2011) reported that the ash content of basil was 6.3. Ash content obtained from this study was higher than the findings of Sarfraz et al. (2011). The main reason for these results may be the difference in genotypes.

3.2. Fixed Oil Components

Means of components of oil obtained from genotypes were given in Table 4. Our results indicated that the basil genotypes had a significant effect on the palmitic and trace oil acids, but not on the others. Linoleic acid component fixed oils obtained from different basil genotypes ranged from 21.05 to 25.62%. The maximum linoleic acid was achieved in PB1, while the minimum value was recorded in GB5. Purple basil genotypes gave generally better linoleic acid content than green basil genotypes. Linolenic acid determined as the major component varied between 47.77 and 51.00%. The highest linolenic acid content was detected in GB2, whereas the lowest value was determined in GB3. The oleic acid component ranged from 10.56 to 15.72%. The highest value was obtained from GB1, while the lowest was determined in PB1. Palmitic acid contents of basil fixed oils ranged from 8.31 to 9.78%. The maximum palmitic acid content was obtained from GB4 while the lowest value was detected in PB2. The stearic acid component varied between 2.10 and 3.23%. The maximum stearic acid content was achieved in PB2, whereas the minimum value was recorded in GB5. The hexadecatrienoic acid content of genotypes ranged from 0.50 to 1.01%. The highest hexadecatrienoic acid content was obtained from PB3, while the lowest value was found in GB1. The ethyl linoleate content varied between 0.50 and 0.79%. The maximum ethyl linoleate was detected in GB5, whereas the minimum ethyl linoleate was recorded in GB3. The trace oil acids varied between 0.78 and 1.37%. The highest value among the trace oil acids was determined in GB5 but PB1, PB3 and GB2 were statistically in the same group. The lowest value among the trace oil acids was recorded in GB3. Fixed oil components of different basil genotypes were investigated to determine the fixed oil quality. Angers, Morales, and Simon (1996) reported that the linoleic acid content of basil genotypes was between 18.3 and 21.7%. On the other hand, Domokos and Perédi (1993) found that linoleic acid content varied between 17 and 25%. Furthermore, Matthaus, Vosmann, Pham, and Aitzetmüller (2003) reported that the linoleic acid content in basil was 22% and 24.89%, respectively. Although results of linoleic acid content obtained from this study were similar to literature findings, some genotypes gave the higher linoleic acid content than literature results. Many researchers reported that the linolenic acid content in basil ranged from 50 and 63% (Domokos & Perédi, 1993; Angers et al., 1996; Matthaus et al., 2003). Linolenic acid contents of the genotypes used in this study were lower than the literature reports except for the GB2 basil genotype. Angers et al. (1996) found that the oleic acid content was between 18.3 and 21.7%. Also, Domokos & Perédi (1993) and Matthaus et al. (2003) determined that the oleic acid content was 9-15%, 15% and 7.43, respectively. Oleic acid content of GB1 basil genotype used in this study was higher than the results of these researches. Domokos & Perédi, (1993) reported that the palmitic acid content in basil was between 6 and 9%. Palmitic acid contents of the GB2, GB4 and GB5 basil genotypes used in this study were higher than the Domokos & Perédi (1993)'s results. Angers et al. (1996) detected that the stearic acid content in basil ranged from 2.0 to 2.8%. Except for the GB2, GB4 and GB5, stearic acid content in the study was higher than the literature results in five basil genotypes.

3.3. Principal Components Analysis for Fixed Oil Components

The eigenvalues and eigenvectors obtained as a result of the principle components analysis related to the fix oil components of different basil genotypes examined in this study were given in Table 5 and Figure

Table 4

Effects of genotypes on fixed oil components in different purple and green basil

Genotypes	Linoleic acid (%)	Linolenic Acid (%)	Oleic Acid (%)	Palmitic Acid (%)	Stearic Acid (%)	Hexadecatrienoic Acid (%)	Ethyl Linoleate (%)	Trace (%)
PB1	25.62±0.74	48.91±1.26	10.56±2.12	8.91±0.44^{bcd}	2.90±0.30	0.55±0.06	0.60±0.05	1.09±0.14^{abc}
PB2	25.35±0.75	48.37±0.66	11.91±2.06	8.31±0.27^{d}	3.23±0.14	0.60±0.23	0.57±0.10	0.91±0.12^{bc}
PB3	24.90±0.84	48.81±1.52	10.84±2.00	8.85±0.42^{bcd}	2.95±0.42	1.01±0.44	0.61±0.06	1.06±0.08^{abc}
GB1	24.67±1.21	45.51±1.42	15.72±1.76	8.51±0.05^{bcd}	2.89±0.32	0.50±0.24	0.63±0.09	0.81±0.10^{c}
GB2	21.70±1.92	51.00±2.10	11.46±2.10	9.78±0.19^{a}	2.77±0.12	0.67±0.20	0.62±0.13	1.28±0.06^{ab}
GB3	23.29±0.83	47.77±1.43	14.19±2.63	8.40±0.14^{cd}	2.86±0.52	0.78±0.44	0.50±0.23	0.78±0.08^{c}
GB4	22.38±1.44	48.56±0.79	14.55±1.24	9.25±0.30^{abc}	2.15±0.53	0.57±0.27	0.75±0.06	0.83±0.17^{c}
GB5	21.02±1.23	49.77±0.95	14.33±1.95	9.27±0.12^{ab}	2.10±0.26	0.61±0.09	0.79±0.15	1.37±0.21^{a}
Mean	23.62	48.59	12.95	8.91	2.73	0.66	0.63	1.02
Significance	ns	ns	ns	*	ns	ns	ns	*
P value	0.11	0.31	0.54	0.03	0.28	0.87	0.50	0.03
LSD	3.71	4.17	6.35	0.87	1.02	0.75	0.29	0.38

ns: non-significant; *:0.05≥p>0.01; ^{abcd}Row means with common superscripts do not differ.

1 and in Table 6, respectively. When the eigenvalues were investigated, eigenvalue of Factor 1 (F1) and Factor 2 (F2) was over 1. Also, F1 and F2 explained the variation at the rate of 79.06 (Table 5 and Figure 1). Correlation analysis methods were used to select the influential features to indirect selection of best genotypes (Yaldiz & Camlica, 2020). In this context, principle components analysis (PCA) is a suitable multivariate technique to determine the genotypes. To detect the appropriate genotype in terms of fixed oil components, PCA method was used according to the literature evaluations (Leilah & Al-Khateeb, 2005; Golparvar, Ghasemi-Pirbalouti, and Madani, 2006). According to the PCA, GB1, GB4 and GB5 genotypes were superior in terms of fixed oil components.

Table 5

Eigenvalues of principal components analysis for fixed oil contents obtained from different basil genotypes

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	3.94	2.39	0.80	0.42	0.26	0.15	0.04
Variability (%)	49.20	29.86	10.02	5.23	3.31	1.83	0.55
Cumulative %	49.20	79.06	89.08	94.31	97.63	99.45	100.00

As the eigenvectors (Table 6), it was found that the components of palmitic acid and ethyl linoleate for F1 and oleic acid and ethyl linoleate for F2 should be evaluated. In the biplot analysis graph of basil genotypes presented in Figure 2, the F1 axis (49.20%) was formed with palmitic acid and ethyl linoleate, and the F2 axis (29.86%) with oleic acid and ethyl linoleate contents. In the positive direction of the F1 axis, it was observed that the GB5 genotype was superior in terms of ethyl linoleate component. Also, in the positive direction of F2 axis, it was determined that the GB4 was excellent in term of oleic acid content. GB2 and GB5 genotypes gave the good results in term of palmitic acid content. Basil genotypes circled in the principal component analysis biplot graph were found superior in terms of fixed oil contents.

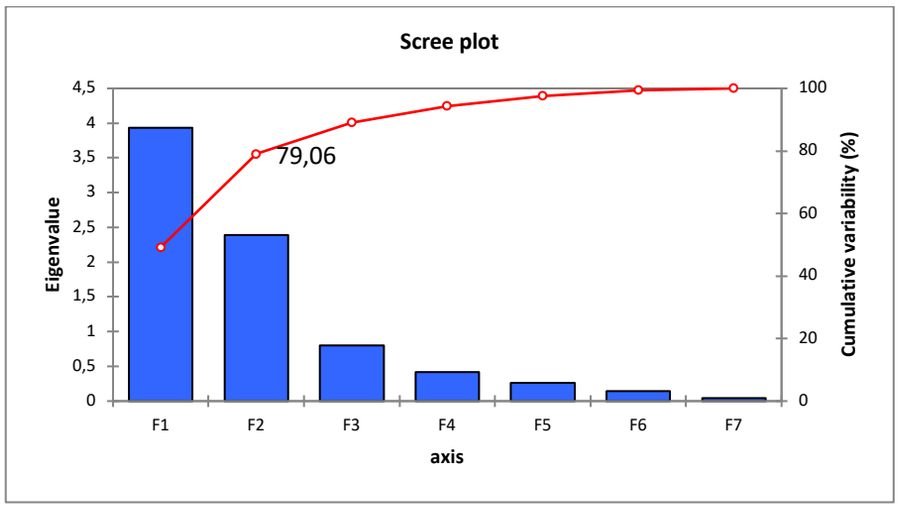

Figure. 1. Principle component factors based on eigenvalues line chart

Table 6

Eigenvectors of principle components analysis for fixed oil contents obtained from different basil genotypes

	F1	F2	F3	F4	F5	F6	F7
Linoleic Acid	-0.43	-0.14	-0.32	0.53	-0.17	0.09	-0.21
Linolenic Acid	0.36	-0.42	-0.04	-0.24	-0.16	-0.53	0.27
Oleic Acid	0.04	0.61	0.26	-0.21	0.30	0.16	0.10
Palmitic Acid	0.45	-0.15	-0.08	-0.17	-0.43	0.73	-0.07
Stearic Acid	-0.42	-0.30	-0.19	-0.24	0.26	0.34	0.68
Hexadecatrienoic Acid	-0.07	-0.39	0.85	0.30	0.04	0.14	0.06
Ethyl linoleate	0.40	0.26	-0.10	0.65	-0.04	-0.01	0.57
Trace	0.37	-0.32	-0.21	0.15	0.77	0.13	-0.27

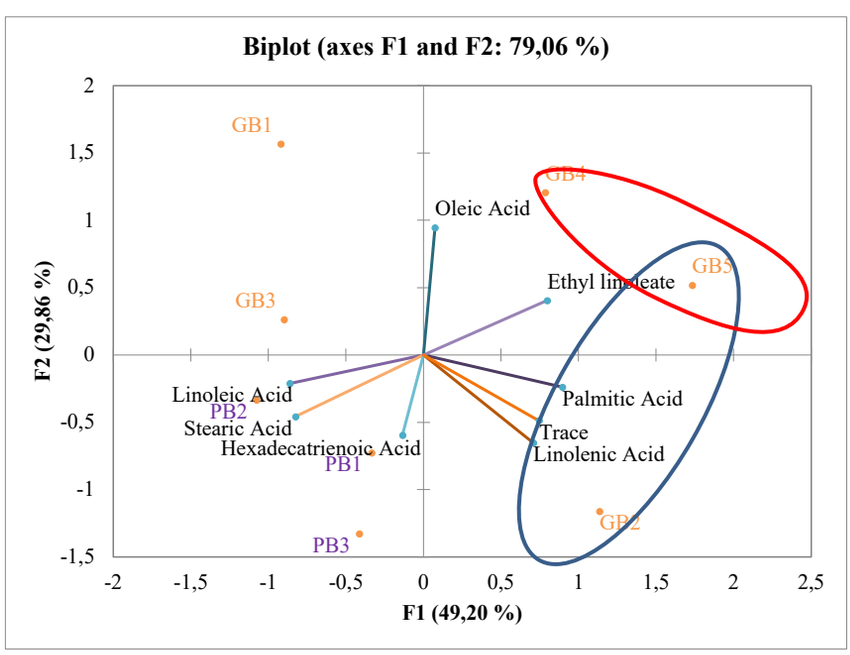

Figure. 2. Biplot distribution graph of principal components analysis for fixed oil contents obtained from different basil genotypes

4. Conclusion

This study aimed to investigate seed yield and quality and fixed oil components of different basil genotypes including purple and green plant types. The best seed yield was determined in GB1 which was obtained from Hungary and had green and elongation leaf types. It was found significant differences in terms of oil components among the basil genotypes. Purple and green basil types demonstrated different features in terms of fixed oil components. In results of principle components analysis, GB2, GB4 and GB5 basil genotypes which have green leaf type were superior. As a result of this study, we deduced that the GB2 genotype obtained from Hungary was the better in terms of seed yield and fixed oil components among the purple and green basil genotypes used in this research. The genotype of GB2 is not used due to its high linolenic acid, its oil quick oxidation and time to time oil bitterness. However, it can be recommended to be consumed directly as food and included in diets because this fatty acid is an omega 3 fatty acid.

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Author Contributions

Musa TÜRKMEN: Planned the study, contributed to the administer of the study, designed and performed the analyses, determined the final version of the article.

Yılmaz EREN: Planned the study, designed and performed the analyses, collected the data

Yusuf Ziya AYGÜN: Planned the study, designed and performed the analyses, collected the data

Esra Nermin ERTEKİN: Planned the study, designed and performed the analyses, Performed statistical analysis, wrote the paper

Conflicts of Interest

The authors declare no conflict of interest.

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