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## Analyses and evaluation of the main chemical components in different tobacco (*Nicotiana tabacum* L.) genotypes

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**SUMMARY:** The nicotine, reducing sugar and ion contents from the threshing of tobacco can re-used from the industry. The crude oil and fatty acid compositions of tobacco seeds can be considered as an alternative source of raw material for biodiesel. In this study, the nicotine, reducing sugar content, crude oil, fatty acid composition and ion content were determined in 29 genotypes and 1 cultivar of tobacco. The genetic diversity was determined among the tobacco cultivar and genotypes base on examined properties. The nicotine content varied between 0.10-0.87%, reducing sugar ranged from 9.70-21.30%, crude oil varied between 24.33-47.00% and fatty acid compositions was found in the range of 77.94-100%. Linoleic (13.92-75.04%) and butyric (0.33-64.98%) acids were the major components. Overall, the BSR-5 (52.56 mg/g) and ESR-5 (44.58 mg/g) genotypes exhibited the highest potassium contents and ESR-7 (6.54 mg/g) and ESR-8 (1.28 mg/g) genotypes had the lowest chlorine contents. As a result of this study, the highest nicotine content, reducing sugar and crude oil of tobacco were found in ESR-4, ESR-11 and BSR-5 genotypes, respectively. The dendrogram analysis divided the tobacco into two main groups and most of the same origin genotypes fell into the same group. The results indicated that the different tobacco leaves and seeds can be evaluated as an alternative source in the industry as cigarettes, biodiesel and different industrial applications such as cosmetic, oil paints and varnishes based on their chemical properties.

**KEYWORDS:** *Fatty acid; Nicotine; Sugar; Tobacco*

**RESUMEN:** *Análisis y evaluación de los principales componentes químicos de diferentes genotipos de tabaco (Nicotiana tabacum L.).* La nicotina, el contenido de iones y azúcares reductores pueden usarse en la selección en la industria del tabaco. La composición del aceite crudo y los ácidos grasos de las semillas de tabaco pueden evaluarse de manera alternativa para la industria de biodiesel. En este estudio, la nicotina, el contenido de azúcares reductores, el aceite crudo, la composición en ácidos grasos y el contenido de iones se determinaron en tabacos de 29 genotipos y 1 cultivar. La diversidad genética se determinó entre los cultivares de tabaco y los genotipos basándose en las propiedades examinadas. El contenido de nicotina varió entre 0,10-0,87%, el valor de azúcares reductores varió entre 9,70-21,30%, el aceite crudo osciló entre 24,33-47,00% y las composiciones de ácidos grasos oscilaron entre 77,94 y 100%. Los componentes principales fueron los ácidos linoleico (13,92-75,04%) y butírico (0,33-64,98%). En general, en los genotipos BSR-5 (52,56 mg/g) y ESR-5 (44,58 mg/g) mostraron el mayor contenido de potasio y los genotipos ESR-7 (6,54 mg/g) y ESR-8 (1,28 mg/g) el contenido más bajo de cloro. Como resultado de este estudio, se encontró un mayor contenido de nicotina, azúcares reductores y aceite crudo en los tabacos de los genotipos ESR-4, ESR-11 y BSR-5, respectivamente. El análisis mediante dendrograma mostró dos grupos principales y la mayor parte de los mismos genotipos de igual origen tuvieron lugar en el mismo grupo. Los resultados indicaron que las diferentes hojas y semillas de tabaco pueden evaluarse como una fuente alternativa en la industria como cigarrillos, biodiesel y diferentes industrias como cosmética, pinturas al óleo y barnices en función de sus propiedades químicas.

**PALABRAS CLAVE:** *Ácidos grasos; Azúcares reductores; Nicotina; Tabaco*

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## 1. INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is a commercial plant and its leaves are used for cigarette production and chewing. It is most widely grown for its leaves as a non-food crop in the world. The tobacco leaf is the most important part of the plant. For this reason, it is seen as a valuable cash crop (Regassa and Chandravanshi, 2016).

Nicotine is synthesized from the root of the plant and has pleasurable properties in low doses and toxic properties in high doses. The sugar contents in tobacco ranged from 2-10% (less) to 20-25% (higher). The combustion products of reducing sugars of the acidic substances fundamentally prevent the throat from burning and the pungency that alkaloids and volatile bases generate. Therefore, reducing sugars are seen as a positive factor for cigarette tobaccos (TEA, 2018).

Tobacco seeds were seen as a waste product of the tobacco leaf industries. The nutritional value of tobacco cured oil is better than groundnut and cotton seed oils and can be compared to safflower oil. In addition, in some European countries the refined tobacco seed oil is used as edible oil (Talaqani *et al.*, 1986).

The burning quality of the tobacco is primarily dependent on the characterization and quantities of the inorganic substances in the chemical combination. Excessive amounts of ion (eg 7-9%) in the chemical combinations make it difficult to burn the tobacco, thus reducing the smoking quality of tobacco. Among the ion contents, potassium and chloride are the most influential factors for combustion. Potassium salts have a positive effect on combustion, while chloride salts have negative effects. In addition, potassium plays an important role as a catalyst in the combustion of tobacco. Equal rates of potassium and calcium or a high potassium rate facilitate burning the chemical combination of the tobacco. On the other hand, the excess amount of calcium reduces burning even though the amount of chloride in the chemical combination is low (Er and Yıldız, 2014; TEA, 2018).

The goal of this work was to determine the chemical contents, namely nicotine, reducing sugar, crude oil, fatty acid composition and ion content in tobacco genotypes and cultivar under the Bolu ecological conditions. It serves to identify the components of tobacco thought to be influential in determining the quality parameters of tobacco leaves and seeds. We also determined

the fatty acid composition of the tobacco seeds because this knowledge has important taxonomic significance in plant classification and is useful for preserving seed purity in the tobacco manufacturing industry.

## 2. MATERIALS AND METHODS

Three tobacco cultivars (Akhisar 97, Burley 94 and Virginia 90) and 29 genotypes obtained from 9 different provinces in Turkey were used in this study. These tobaccos were grown in the 2015 and 2016 growing seasons, and except for the Burley 94 and Virginia 90 cultivars, all tobaccos adapted to the Bolu ecological conditions (Table 1).

Tobacco seeds were sown in pots (100 × 120 mm) with 3 replicates in a greenhouse. The soil of the pots was prepared with equal amounts of a sand-turf-soil mixture (1:1:1) in May of both years. During the growth period, all required agricultural practices were carried out such as weed control, monitoring for disease and pests. When the plants reached 10-15 cm height, they were transplanted to the trial area on July 3, 2015 and July 7, 2016 in open-field conditions.

The field experiments were conducted at the research area of the Department of Field Crops, Faculty of Agriculture, Bolu Abant İzzet Baysal University (BAIBU) (Turkey) (40° 44' 44" N, 31° 37' 45" E, 881 m altitude). Research area soils had clay-loam with 7.5 pH. The useful phosphor value, potassium ratio, organic matter content and salt content were 237.4 kg/ha, 380 kg/ha, 1.6 and 0.008%, respectively. During the vegetation period, average climatic data were recorded as 19.10 °C and 18.0 °C temperature; 259.1 mm and 208.8 mm rainfall; 71.8 and 70.86% humidity for 2015 and 2016, respectively (Yaldiz *et al.*, 2019).

The experimental design was a randomized complete block design with three replicates. Each experimental plot consisted of three rows with a row-to-row distance of 0.4 m and plant to-plant distance of 0.15 m and plot size 2.52 m<sup>2</sup>. The distance between the blocks was one meter. During the vegetation period in the experimental years, all required agricultural practices were conducted. 40 kg/ha DAP (diammonium phosphate) were applied to the plots as a base fertilizer. After transplanting, 20 kg/ha nitrogenous fertilizer as ammonium nitrate were applied to the plants. The harvest was done between September 10 and October 8 in the first year and between September 15 and October 12

TABLE 1. List of Tobacco Genotypes and Cultivar Used in the Current Study.

No	Region-Code	Genotypes/cultivar names	Obtained place or person	Geographic coordinates	
				Latitude	Longitude
1	AR-1	Akhisar 97	Manisa/Cultivar	38°55'3.5904" N 27°50'11.8320" E	
2	AR-2	Akhisar-Sarılar	Manisa/Akhisar/Sarılar/Ufuk Özcan	39°6'26.9100" N 28°0'8.7084" E	
3	MR-1	Agonya-Yarış Village	Çanakkale/Agonya/Yarış Village/Anıl Özyurt	39°47'1.6332" N 27°15'51.7320" E	
4	ESR-1	B. Çelikhan 97	Adıyaman/Çelikhan	38°1'59.0520" N 38°14'13.9128" E	
5	BSR-1	Bafra Gökçeada	Samsun/Bafra/Gökçeada/Şefik Kara	41°32'45.9204" N 35°45'41.4324" E	
6	BSR-2	Bafra Paşaşeyh	Samsun/Bafra/Paşaşeyh/Cemil Yüksel	41°28'50.8260" N 35°44'18.0888" E	
7	MR-2	Balıkesir (AARI, 42986)	AARI	-	
8	MR-3	Balıkesir (AARI, 64073)	AARI	-	
9	ESR-2	Bitlis Mutki Erler Village-1	Bitlis/Mutki/Erler Village/Sait Sülün	38°28'46.7472" N 41°43'56.5536" E	
10	ESR-3	Bitlis Mutki Erler Village-2	Bitlis/Mutki/Erler Village/Yusuf Kesim	38°28'46.7472" N 41°43'56.5536" E	
11	MR-4	Bursa (AARI, 42884)	AARI	-	
12	MR-5	Bursa (AARI, 78215)	AARI	-	
13	ESR-4	Bitlis (AARI, 42076)	AARI	-	
14	ESR-5	Bitlis (AARI, 80111)	AARI	-	
15	ESR-6	Eski Tütün-Hatay	Hatay	36°12'1" N 36°10'34" E	
16	ESR-7	Hatay (AARI, 42126)	AARI	-	
17	ESR-8	Hatay (AARI, 42128)	AARI	-	
18	ESR-9	Hatay (AARI, 42132)	AARI	-	
19	AR-3	Manisa (AARI, 64062)	Aegean Agricultural Research Institute	38°36'50.5188" N 27°25'46.4232" E	
20	ESR-10	Muş (AARI, 42094)	AARI	-	
21	AR-4	Salihli Kale Village	Manisa/Salihli/Kale Village/ İbrahim Zeybek	38°43'18.6780" N 28°8'17.9700" E	
22	BSR-3	Samsun (AARI, 49184)	AARI	-	
23	BSR-4	Samsun (AARI, 49188)	AARI	-	
24	BSR-5	Samsun (AARI, 49219)	AARI	-	
25	BSR-6	Samsun (AARI, 49224)	AARI	-	
26	BSR-7	Samsun Tekkeköy Hamzalı	Samsun/Tekkeköy/Hamzalı/ Mustafa Anıl	41°12'19.8756" N 36°32'15.2844" E	
27	BSR-8	Samsun Terme Akçay	Samsun/Terme/Akçay/Mümin Bayram	41°8'0.7332" N 37°9'22.2264" E	
28	BSR-9	Samsun Tekkeköy Balcalı	Samsun/Tekkeköy/Balcalı/Alı Doğru	41°9'14.8932" N 36°34'5.4624" E	
29	BSR-10	Samsun Tekkeköy Kahyalı	Samsun/Tekkeköy/Kahyalı/ Osman Kul	41°10'21.5508" N 36°32'21.6564" E	
30	ESR-11	Yayladağı Sebenoba	Hatay/Yayladağı/Sebenoba/Ayşe Şahin	36°2'48.2424" N 36°1'15.0384" E	

AARI: Aegean Agricultural Research Institute, AR: Aegean Region, MR: Marmara Region, ESR: Eastern and Southeastern Anatolia Region, BSR: Black Sea Region.

(3 times) in the second year. The harvest was done in the early morning or coolness of the evening (after 17:00 pm) for both two years (Er and Yildiz, 2014). After harvesting, the leaves were dried under laboratory conditions.

## 2.1. Analyses of tobacco leaves

### 2.1.1. Nicotine extraction method

The nicotine extraction is given in Table 2a. A homogenized tobacco sample was weighed and extracted with *n*-hexane, distilled water and NaOH. Then the sample was shaken in vortex and the upper phase was removed and diluted with *n*-hexane and placed in the GC-MS instrument (ARGEFAR, 2016).

### 2.1.2. Extraction method of reducing sugar

Homogenized tobacco leaves were weighed and extracted with distilled water and methanol. Then the sample was shaken in a vortex and the upper phase was removed and filtered into a vial. The amount reducing sugar was calculated as g/100 g in the solution by subjecting it to the HPLC-RI system (Table 2a) (ARGEFAR, 2016).

### 2.1.3. Analysis of ion content

The results from the ion content analysis of tobacco genotypes are shown in Table 2b. 0.5 g of the weighed plant sample was soaked in 50 ml of sterile distilled water in an ultrasonic bath for 30 minutes. Then, the extracts were filtered using

TABLE 2. Device parameters used in nicotine and reducing sugar contents (2a) and optimum operation conditions for Dionex ics 1100 ion chromatography (2b).

TABLE 2a		
	Nicotine Analysis	Reducing Sugar Analysis
Equipment	Shimadzu GC-MS	HPLC RI
Column	RTX-CL- Pesticide 2	NH2 5 µm 4.6x250 mm
Heat program	80 °C; 45 °C increase to 200 °C; 20 °C increase to 230 °C; after 30 °C increase to 300 °C 5 min. isothermal	-
Injection type	Splittles	-
Sensor	MS	RID
Injection volume	2 mL	10 µl
Carrier gas	Helium: 1 mL/min	-
Injector heat	250 °C	-
Mobile Phase	-	HPLC purity Acetonitrile-Water
RID Detector Temperature	-	30 °C
Flow rate	-	1.500 ml/min

TABLE 2b		
Operation conditions	Anion	Cation
Mobile phase	9 mM Na <sub>2</sub> CO <sub>3</sub>	20 mM Methanesulfonic acid
Column	Ionpac AS9-HC (250x4 mm)	Ionpac CS12-A (250x4 mm)
Guard Column	Ionpac AG9-HC (50x4 mm)	Ionpac CG12-A (50x4 mm)
Supressor	ASRS-4 mm	CSRS-4mm
Supressor current	45 mA	65 mA
Detector	Conductivity Detector	Conductivity Detector
Pressure (psi)	2000-3000	2000-3000
Oven temperature	30 °C	30 °C
Background conductance	<30 µS	0.5-2 µS
Flow Rate	1.00 mL/min	1.00 mL/min
Injection volume	500 µL	1000 µL
Rate of data transfer	5.0 Hz	5.0 Hz
Duration	30 min	15 mins

0.22 µm cellulose acetate filter paper and prepared for analysis. Before sample analysis, the standard Dionex was used for calibration. The ion contents in the samples were determined by using Dionex ICS 1100 Series ion chromatography (Wang *et al.*, 2016).

## 2.2. Analyses of tobacco seeds

### 2.2.1. Isolation of seed crude oil

Tobacco seeds were ground and measured as 5 g. Then, they were extracted at 80 °C by a Soxhlet extractor for 8 h, using n-hexane as solvent. After oil extraction the solvent was removed by a rotary evaporator (Yaldiz and Camlica, 2019).

### 2.2.2. Determination of fatty acid composition

Quantitative determinations of FAMES were conducted according to Yaldiz and Camlica (2019) using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Tokyo, Japan) with a flame ionization detector (FID) and Rtx-2330 capillary column (60 m × 0.25 mm) of 0.2 µm. The detector temperature was set at 240 °C. The GC oven temperature was programmed at 140 °C for 5 min. Then the temperature was increased up to 260 °C at a rate of 4 °C/min and kept constant at 260 °C for 20 min. Helium (1 ml/min) was used as carrier gas. The FAMES were determined by comparing their retention times with reference standards (mixture FAME Mix, SUPELCO, which included 37 FAMES). Methyl undecanoate (Sigma Aldrich Chemical Co., St. Louis, MO, USA) was used for FAMES quantity as the internal standard. The obtained total results from FAMES were expressed as percentages.

## 2.3. Statistical analysis

The nicotine, reducing sugar, ion contents and seed crude oil determinations were repeated three times, and their analyses were performed within each replicate sample three times with standard deviation (SD). Significant differences among the genotypes were determined by the one-way ANOVA with means separation by the Least Significant Difference (LSD) test at the 0.01 level. dendrogram was used to show the hierarchy of clusters and to determine the genetic variability among tobacco cultivars and genotypes based on

chemical components and FAC by the XLSTAT program (Yaldiz and Camlica, 2019).

## 3. RESULTS AND DISCUSSIONS

The data presented in Table 3 presents the significant differences in Nicotine Contents (NC), Reducing Sugar Contents (RSC) and Seed Crude Oil (SCO) among the tobacco genotypes and cultivar.

### 3.1. Nicotine content (NC)

NC is the most important factor in the tobacco industry especially in cigarette blends, despite the fact that it has some negative effects on the human body as healthy and protective. The quality parameter of the cigarette blend is determined by the NC. There were statistically significant differences among the tobacco genotypes and cultivar in terms of the NC (Table 3). The NC of tobacco genotypes and cultivar varied between 0.10-0.87%. The highest NC was found for the ESR-4 genotype when compared to the others, followed by ESR-5 (0.78%) and BSR-5 (0.59%). Cultivar 'Akhisar 97' was found to be the highest of the 20 genotypes at 0.41%. It was found that MR-4 (0.10%) and MR-3 (0.16%) genotypes contained lower nicotine ratios than the others. While the highest values were obtained for ESR-4 and ESR-5, the lowest values were obtained from MR-2, 3, 4, and 5 genotypes. Compared to the average NC of the tobacco-growing regions, AR, MR, ESR, and BSR were found at 0.33, 0.22, 0.46, and 0.37%, respectively. The results showed that the tobacco genotypes of ESR were higher than the others. This situation can be explained by the ecological conditions of Bolu, which are similar to a continental climate. It was noted that the characterization of quality and aroma of the tobacco depended on the soil, climate conditions and low amount of nitrogen (Bilgin *et al.*, 1993). It was also reported that Aegean tobacco had very low nicotine contents (Delibacak *et al.*, 2014). Abdallah (1986) reported that high levels of nicotine gave the hard and burner features, and low levels of nicotine led to poor taste and physiological dissatisfaction. These tobaccos can be used because of their low nicotine level and their rich flavor (Otan and Aпти, 1989). Although in low doses nicotine has a stimulating effect, increasing activity, alertness and memory, it also increases the heart rate and blood pressure and causes anorexia (Bastida and Beltran, 2011).

TABLE 3. Nicotine, sugar and crude oil contents in evaluated tobacco genotypes and cultivar.

No	Genotypes/ Cultivar	Nicotine Content (%)	Reduced Sugar Content (%)	Crude oil (%)
1	AR-1	0.41±0.01f	16.90±0.04e	46.33±1.53ab
2	AR-2	0.34±0.01hij	13.80±0.09k	33.33±1.15hi
3	MR-1	0.36±0.01ghı	11.80±0.08o	26.33±2.08lm
4	ESR-1	0.41±0.01f	14.30±0.05ij	42.00±1.73cde
5	BSR-1	0.36±0.01ghı	20.80±0.05b	44.00±2.00a-d
6	BSR-2	0.42±0.01ef	13.80±0.01k	34.67±1.15ghı
7	MR-2	0.24±0.01mn	18.40±0.08d	31.67±3.51jkl
8	MR-3	0.16±0.01o	19.40±0.01c	25.33±3.06lm
9	ESR-2	0.22±0.01n	<b>9.70±0.10q</b>	<b>24.33±2.08m</b>
10	ESR-3	0.36±0.01ghı	16.10±0.01f	44.67±1.15a-d
11	MR-4	<b>0.10±0.05p</b>	15.50±0.09g	41.33±2.52c-f
12	MR-5	0.22±0.01n	18.40±0.01d	26.33±1.53lm
13	ESR-4	<b>0.87±0.01a</b>	14.10±0.10jk	45.33±1.15abc
14	ESR-5	0.78±0.01b	<b>9.70±0.01q</b>	37.33±1.15fgh
15	ESR-6	0.33±0.01ijk	15.40±0.02g	43.67±1.53a-d
16	ESR-7	0.46±0.01de	13.40±0.04l	29.00±3.61jkl
17	ESR-8	0.42±0.02ef	14.60±0.01hi	45.33±0.58abc
18	ESR-9	0.50±0.05d	21.00±0.10ab	35.67±0.58ghı
19	AR-3	0.29±0.01kl	17.20±0.03e	38.67±1.15efg
20	ESR-10	0.38±0.01fgh	<b>9.70±0.03q</b>	34.67±1.15ghı
21	AR-4	0.36±0.01ghı	15.30±0.11g	25.33±3.06lm
22	BSR-3	0.34±0.01hij	13.00±0.04m	40.67±0.58def
23	BSR-4	0.40±0.05fg	10.80±0.02p	34.67±0.58ghı
24	BSR-5	0.59±0.01c	14.00±0.02jk	<b>47.00±2.65a</b>
25	BSR-6	0.32±0.01ı-l	12.30±0.10n	46.33±1.53ab
26	BSR-7	0.28±0.01lm	12.70±0.05m	46.67±1.15a
27	BSR-8	0.30±0.05jkl	14.90±0.01h	41.67±0.58cde
28	BSR-9	0.41±0.01f	14.10±0.10jk	28.33±0.58klm
29	BSR-10	0.23±0.01n	14.00±0.50jk	32.67±2.08ij
30	ESR-11	0.34±0.01hij	<b>21.30±0.03a</b>	42.33±1.53b-e
	<b>Average</b>	<b>0.37</b>	<b>14.88</b>	<b>36.85</b>
	<b>SD</b>	<b>0.16</b>	<b>3.22</b>	<b>7.54</b>
	<b>LSD (1%)</b>	<b>0.03</b>	<b>0.33</b>	<b>4.07</b>
	<b>CV (%)</b>	<b>5.52</b>	<b>1.02</b>	<b>5.03</b>

AR: Aegean Region, MR: Marmara Region, ESR: Eastern and Southeastern Anatolia Region, BSR: Black Sea Region.

Different letters in the same column indicate significant differences ( $P < 0.01$ ).

SD: Standard Deviation, LSD: Least Significant Difference, CV: Coefficient Variation.



These tobaccos can be used in the tobacco industry for protect human health because of their low NC.

Ekren and Sekin (2008) noted that NC was found from 0.12 to 1.06% in tobacco from the Akhisar region. 1.8-2.1% (Kurt and Ayan, 2014), 0.73-0.95% (Yagac, 2015) were also found.

The results were similar to those of other researchers, except for Kurt and Ayan (2014). The differences can be explained by the fact that the tobacco genotypes and cultivar showed differences depending on their adaptation, genetic properties and survival in different places and the soil contents as organic matter can affect the NC of tobacco (Griesser *et al.*, 2015).

### 3.2. Reducing sugar content (RSC)

Reducing sugar contents can provide mildness when smoking tobacco and was found to positively affect its quality (Abdallah 1986). Differences in RSC were found to be statistically significant among the tobacco genotypes and cultivar. As seen in Table 3, the RSC of tobacco genotypes and cultivar ranged from 9.70 to 21.30%. The RSC of ESR-9 and ESR-11 were higher than the other genotypes and cultivar. The ESR-2, ESR-5 and ESR-10 genotypes were found to be lower than the others at 9.70%. When compared among the regions, the highest RSC was found in MR at 16.70% followed by AR (15.43%), ESR (14.48%) and BSR (14.04%). The cultivar was found to 16.90% above the average of the regions.

The desirable amount of RSC is between 8-13% (Camas *et al.*, 2007). The closest values to the desired ones were seen in 8 genotypes between 9.70-13.0%. The values were found in BSR genotypes (BSR-2, 3, 4, 5), followed by ESR genotypes (ESR-2, ESR-5 and ESR-10) and MR-1 in MR genotypes. Based on the RSC values (Table 3), these genotypes are different from the others. Therefore, they are suitable for the production of the RSC used in the tobacco industry for a quality product.

It is noted that the RSC of tobacco varied between 11.30-21.83% (Yagac, 2015) and 7.81-33.71 (Ekren and Sekin, 2008). Our results have been compared to those of other researchers and it was determined that they showed similar values as reported by researchers.

### 3.3. Ion content (IC)

Ions are non-combustible substances and are only present in the ash. It is known that, due to the

increase in potassium from these substances, the ability of the tobacco to burn improves, while the chloride salt affects it negatively (Er and Yildiz 2014).

The present study was conducted for the evaluation of IM such as Potassium ( $K^+$ ), Magnesium ( $Mg^{2+}$ ), Calcium ( $Ca^{2+}$ ), Chloride ( $Cl^-$ ), Phosphorus ( $PO_4^{3-}$ ) and Sulfate ( $SO_4^{2-}$ ), and in the seeds of tobacco genotypes and cultivar. Significant differences were found among tobacco genotypes and cultivar in terms of IC (Table 4).

In this study, the concentration of  $K^+$  varied from 8.1 to 52.6 mg/g plant. The  $K^+$  concentrations in the leaves were different among all the different genotypes. The highest value was found for BSR-5 and the lowest value was found for BSR-7. Krishnamurthy and Ramakrishnayya (1993) reported that the tobacco plant had a higher K requirement compared to other cultivated plants. They also noted that color, texture, thickness, elasticity, and burning capacity were affected by dry leaves and the high  $K^+$  content in the dried leaves was generally considered to be the highest quality criterion. The obtained  $K^+$  values were higher than those reported by Irget *et al.*, (1999) (0.87-2.24%). Our tobaccos were found to be rich in terms of  $K^+$ . Therefore, especially BSR-5 and ESR-5 genotypes were found to be more suitable for the burning capacity of tobacco.

The  $Mg^{2+}$  concentrations of the tobacco genotypes and cultivar varied between 6.04 and 0.61 mg/g plant (Table 4). The highest value was determined for BSR-3, and the lowest value was determined for the AR-4 genotype.  $Ca^{2+}$  content varied from 0.27 to 37.4 mg/g. While the highest values were obtained for ESR-10 (37.4 mg/g) and BSR-1 (34.7 mg/g), the lowest values were obtained for the ESR-4 (0.27 mg/g) and BSR-10 (10.7 mg/g) genotypes.  $Cl^-$  was present in the range of 1.28-32.3 mg/g. The highest concentration was present in the BSR-6 genotype, followed by the BSR-3 (29.2 mg/g) genotype. In the present study, the concentration range of  $PO_4^{3-}$  was 4.29-9.44 mg/g, as shown in Table 4. The highest level of that form of  $PO_4^{3-}$  was found in the ESR-7 genotype (9.44 mg/g), followed by the BSR-6 genotype (8.38 mg/g).  $SO_4^{2-}$  concentrations ranged from 2.61 to 7.40 mg/g. Its maximum content (7.40mg/g) was present in ESR-5, and its minimum content (2.61 mg/g) was present in BSR-9. The highest amounts of  $K^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  were found in the MR genotypes, the highest  $PO_4^{3-}$  and  $SO_4^{2-}$  in the ESR genotypes and the highest

TABLE 4. Ion Contents in Tobacco Genotypes and Cultivar (mg/g).

Tobacco genotypes/ cultivar	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>
MR-1	21.24±0.02R	5.32±0.01D	24.72±0.01M	18.2±0.01L	6.31±0.01O	3.82±0.00O
AR-1	30.38±0.03H	0.94±0.00Z	15.41±0.00a	11.97±0.01T	4.75±0.02a	2.77±0.00c
AR-2	23.84±0.01N	5.04±0.00F	32.61±0.01D	22.35±0.05E	6.15±0.01Q	3.77±0.02R
ESR-1	29.16±0.04J	1.78±0.00V	19.11±0.01T	14.13±0.03R	6.86±0.01J	4.89±0.00J
BSR-1	21.46±0.04Q	5.15±0.00E	34.74±0.03C	22.04±0.03F	8.33±0.00C	4.89±0.00J
BSR-2	14.02±0.02Y	3.63±0.00M	30.89±0.01G	14.8±0.00O	5.46±0.00S	3.3±0.00Y
MR-2	27.83±0.03L	2.48±0.00P	18.36±0.05U	9.34±0.00b	6.02±0.00R	2.64±0.01d
MR-3	28.82±0.02K	4.66±0.01H	25.08±0.03L	19.15±0.06J	5.35±0.00T	4.97±0.00I
ESR-4	-	-	0.27±0.00d	10.29±0.02a	6.42±0.00N	4.07±0.00N
ESR-5	44.58±0.02B	2.23±0.00Q	25.71±0.01J	23.78±0.02D	8.15±0.00E	7.4±0.01A
ESR-2	22.05±0.05P	5.69±0.00C	29.92±0.02H	21.69±0.01G	7.05±0.01H	5.82±0.00E
ESR-3	36.17±0.03F	0.86±0.00a	19.52±0.01R	14.26±0.05Q	6.54±0.00L	3.26±0.00Z
MR-4	18.64±0.02T	4.22±0.01K	29.88±0.01I	14.42±0.01P	6.5±0.02M	5.76±0.00F
MR-5	42.64±0.00C	3.53±0.01N	20.54±0.00P	20.75±0.00H	8.3±0.00D	6.08±0.00D
ESR-6	37.05±0.05E	1.43±0.01Y	21.48±0.01O	14.12±0.02R	7.32±0.00G	4.11±0.00M
ESR-7	16.79±0.00U	4.57±0.00J	24.15±0.00N	6.54±0.00c	9.44±0.00A	4.39±0.01K
ESR-8	24.86±0.04M	2.2±0.00R	16.00±0.01Y	1.28±0.00d	6.19±0.00P	3.73±0.00S
ESR-9	10.89±0.01b	4.6±0.00I	31.4±0.00F	10.62±0.02Z	6.72±0.00K	5.02±0.00H
AR-3	37.00±0.00E	0.75±0.00b	15.6±0.01Z	14.39±0.02P	5.34±0.00T	3.65±0.00T
ESR-10	22.42±0.01O	6.55±0.00A	37.43±0.02A	20.33±0.02I	7.02±0.00I	6.19±0.00C
AR-4	12.22±0.01Z	0.61±0.00c	16.69±0.01V	13.78±0.02S	4.92±0.00V	5.09±0.00G
BSR-3	20.91±0.01S	4.85±0.00G	19.21±0.01S	24.73±0.03C	7.57±0.00F	4.17±0.00L
BSR-4	29.58±0.02I	6.04±0.00B	35.02±0.01B	29.21±0.01B	6.3±0.00O	3.48±0.00U
BSR-5	52.56±0.07A	3.65±0.01L	20.08±0.01Q	18.52±0.02K	7.06±0.00H	3.79±0.00Q
BSR-6	-	-	-	32.31±0.01A	8.38±0.00B	7.07±0.00B
BSR-7	16.3±0V	2.51±0.00O	25.05±0.03L	11.02±0.01V	6.03±0.00R	3.8±0.00P
BSR-8	31.22±0.02G	2.13±0.00U	10.71±0.02c	17.57±0.03M	4.78±0.00Z	3.39±0.00V
BSR-9	11.79±0.01a	2.14±0.00T	31.88±0.01E	10.88±0.02Y	4.29±0.00b	2.61±0.00e
BSR-10	8.08±0.02c	2.15±0.00S	25.33±0.02K	11.37±0.02U	5.2±0.01U	3.12±0.00a
ESR-11	40.72±0.01D	-	10.98±0.02b	16.98±0.02N	4.81±0.00Y	2.82±0.00b
<b>Average</b>	26.19	3.32	23.03	16.36	6.45	4.33
<b>LSD (%1)</b>	0.05	0.01	0.03	0.05	0.01	0.01
<b>CV (%)</b>	0.10	0.10	0.07	0.14	0.09	0.08
<b>SD</b>	11.01	1.74	8.33	6.51	1.25	1.26

AR: Aegean Region, MR: Marmara Region, ESR: Eastern and Southeastern Anatolia Region, BSR: Black Sea Region.

Different letters in the same column indicate significant differences ( $P < 0.01$ ).

SD: Standard Deviation, LSD: Least Significant Difference, CV: Coefficient Variation.



Cl<sup>-</sup> was seen in the BSR tobaccos. The change in the same ion contents in the tobacco genotypes among the regions can be explained by genetic differences and tobacco producers may have used the same tobacco genotype seeds in the same region.

Ca<sup>2+</sup>, Mg<sup>2+</sup> and Cl<sup>-</sup> contents were found between 2.08-6.32%, 0.20-0.98%, and 0.1-0.50%, respectively (Irget *et al.*, 1999). The obtained data was found somewhat similar to other researchers.

### 3.4. Seed crude oil (SCO)

Significant differences were found among the tobacco genotypes and cultivar in terms of SCO, as shown in Table 3. The SCO values ranged from 24.33-47.0%. The analysis showed that SCO was the highest in BSR-5 (47.0%), followed by BSR-6 (46.33%) genotypes and AR-1 cultivar (46.33%). The lowest SCO values were found for the ESR-2 (24.33%), MR-3 (25.33%) and AR-4 (25.33%) genotypes. When the SCO of the tobacco genotypes and cultivar were compared among the regions, SCO was found at 32.44% in AR, 30.20% in MR, 38.06% in ESR, and 39.20% in BSR. In general, the average SCO of the tobacco genotypes and cultivar was over 30%, with the highest average SCO determined for the BSR genotypes. So, BSR-5, BSR-6 and the AR-1 cultivar had highest crude oil contents and they can be used in biodiesel production as an alternative fuel source (Fornasier *et al.*, 2018).

Tobacco seed oil was reported as 24.56-41.93% (Mohammad and Tahir 2014), 29.82% (Abbas Ali *et al.*, 2008). The obtained data were found similarly those reported by other researchers.

### 3.5. Fatty acid compositions (FAC)

Table 5 shows the FAC of the tobacco genotypes and cultivar. Linoleic (18:2), oleic (18:1), palmitic (C16:0) and butyric acids (C4:0) were the four most abundant acids among the 25 FAC (13.92-75.04, 0.46-17.80, 5.55-19.11 and 0.33-64.98%, respectively).

Linoleic acid was found to be the main component and predominated the oils of tobacco (13.92-75.04%), except for the MR-5 and ESR-6 genotypes. The highest linoleic acid was found in ESR-3 (75.04%), followed by the AR-1 cultivar (67.84%) and AR-4 (67.44%) genotype. The lowest linoleic acid was determined for BSR-8

(13.92%) and ESR-9 (24.54%). The high content of linoleic acid in tobacco seed oil is very important for the production of oleo-chemicals (Abbas Ali *et al.*, 2008). It can be used as a surfactant, dispersant, bio lubricant, in cosmetics and a variety of synthetics in the formulation of protective coating and in preparations of other long chain compounds (Awola *et al.*, 2010). The content of linoleic acid was higher than that reported (4.2-9.23%) by Mohammad and Tahir (2014) and found similar to the 6.45-77.48% reported by Poltronieri (2016). Therefore, especially the ESR-3 and AR-1 genotypes are promising for linoleic acid to be obtained at high levels and these genotypes can be used in oleo-chemical production and other areas such as cosmetic and surfactant. It can also be used in making quick-drying oils such as oil paints and varnishes (Chiririwa *et al.*, 2014). In addition to this, Kirkova *et al.*, (2016) noted that the linoleic acid content must be lower to obtain better oil as quality. Therefore, the BSR-8 and ESR-9 genotypes can be evaluated for the best oil quality.

High concentrations of oleic (0.46-17.80%) and palmitic acids were detected (5.55-19.12%; except for ESR-1 genotype) in all the tobacco studied oils. The highest oleic acid was found in the BSR-3 genotype with 17.80%, followed by ESR-9 (17.35%) and AR-2 (16.35%). The lowest oleic acid was found in ESR-8 (0.46%). The highest palmitic acid was determined in MR-5 (19.12%) and the lowest palmitic acid was found in the BSR-7 genotype.

The effects of oleic acid are mediated by preventing the reduction in palmitic acid-mediated activated protein kinase (AMPK) activity, resembling the action of metformin (Palomer *et al.*, 2018). Palmitic and oleic acids were determined at 21.33-25.667% and 17.00-26.667% by Mohammad and Tahir (2014). The main fatty acids (FA) in tobacco were found to be linoleic acid (60-80%), oleic acid (10-20%) and palmitic acid (8-20%) (Giannelos *et al.*, 2002; Abbas Ali *et al.*, 2008; Stanisavljević *et al.*, 2009; Bucciarelli *et al.*, 2013; del Piano *et al.*, 2014).

The palmitic and oleic acid results were found to be different from those obtained by Mohammad and Tahir (2014); but they were close to those reported by other researchers. It has been reported that climatic conditions affect the properties of plants such as growth, yield and biochemical contents (Zandalinas *et al.*, 2018).

TABLE 5. Fatty Acid Compositions of Tobaccos (%).

Fatty acid compositions	RT (min)	AR-1	AR-2	MR-1	ESR-1	BSR-1	BSR-2	MR-2	MR-3	ESR-2	ESR-3	MR-4	MR-5	ESR-4	ESR-5	ESR-6	ESR-7	ESR-8
Butyric acid (C4:0)	4.709	11.77	14.86	5.33	7.13	17.68	27.95	16.29	6.69	20.63	0.33	24.66	58.61	3.15	17.84	64.98	20.07	23.82
Caproic acid (C6:0)	5.215	-	0.99	0.08	0.51	-	0.61	-	0.70	-	0.11	-	1.41	0.41	-	3.47	-	-
Caprylic acid (C8:0)	5.99	-	0.50	0.20	0.14	-	-	-	-	-	-	-	-	0.11	-	-	-	-
Palmitic acid (C16:0)	17.324	6.86	13.71	11.7	-	7.34	7.81	6.44	13.75	6.99	7.7	7.15	19.12	11.17	7.32	11.43	6.27	6.53
Palmitoleic acid (C16:1)	18.461	-	-	0.12	11.11	-	-	-	-	-	0.14	-	-	0.10	-	-	-	-
Heptadecanoic acid (C17:0)	19.199	0.12	-	0.23	0.18	-	0.22	-	-	-	0.13	0.12	-	0.18	-	-	-	0.12
<i>cis</i> -10-heptadecanoic acid (C17:1)	20.15	-	-	0.51	0.29	-	-	-	-	-	0.07	-	-	-	-	-	-	-
Stearic acid (C18:0)	21.048	2.90	7.17	4.26	4.65	2.38	1.91	2.68	5.46	2.81	2.79	2.44	13.53	4.44	2.80	7.02	2.55	2.21
Elaidic acid (C18:1n9t)	21.88	-	-	0.08	-	-	0.46	-	-	-	0.06	7.22	-	0.04	-	-	-	6.87
Oleic acid (C18:1n9c)	22.02	8.18	16.35	13.22	12.57	8.58	7.00	7.85	16.26	7.59	9.02	0.51	5.53	15.98	9.25	4.81	7.48	0.46
Linoleic acid (C18:2n6c)	23.531	67.84	40.45	57.94	59.43	58.6	47.76	60.51	47.79	59.41	75.04	55.32	-	59.18	60.43	-	61.97	57.94
Arachidic acid (C20:0)	24.526	0.21	0.62	0.42	0.35	-	-	-	-	0.18	0.22	0.17	-	0.35	0.21	-	-	0.17
$\gamma$ -linolenic acid (C18:3n6)	25.158	0.98	0.33	0.65	0.78	1.26	0.88	0.92	-	0.89	1.45	0.86	-	0.54	0.86	-	0.90	1.07
<i>cis</i> -11-eicosenoic acid (C20:1)	25.416	-	-	-	0.51	-	-	-	-	-	0.04	-	-	0.07	-	-	-	-
Linolenic acid (C18:3n6)	26.189	-	0.27	0.12	-	-	0.28	-	-	-	0.04	-	-	-	-	4.56	-	-
Henicosanoic acid (C21:0)	26.765	-	0.33	0.15	-	-	0.53	-	1.32	-	0.07	-	-	0.04	-	-	-	-
<i>cis</i> -11,14-eicosadienoic acid (C20:2)	27.752	0.14	0.4	0.92	-	-	0.66	-	0.7	-	0.26	-	1.80	0.43	-	1.79	-	-
Behenic acid (C22:0)	27.896	-	-	0.19	-	-	0.24	-	1.19	-	0.04	-	-	-	-	-	-	-
Arachidonic acid (C20:4n6)	29.198	-	0.28	0.10	-	-	0.66	-	0.8	-	0.04	-	-	-	-	-	-	0.14
Tricosanoic acid (C23:0)	29.683	-	-	-	0.15	-	1.13	-	-	-	0.11	-	-	0.06	-	1.95	-	-
Lignoceric acid (C24:0)	30.475	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>cis</i> -13,16-docosadienoic acid (C22:2)	29.73	-	-	0.19	-	-	-	-	1.65	-	0.06	-	-	-	-	-	-	-
<i>cis</i> -5, 8, 11, 14, 17-eicosapentaenoic acid (C20:5n3) EPA	30.771	-	0.43	0.51	0.24	-	0.57	-	-	-	0.10	-	-	0.19	-	-	-	-
Nervonic acid (C24:1)	32.315	-	-	0.08	0.25	-	-	-	0.70	-	-	-	-	0.29	-	-	-	-
<i>cis</i> -4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6n3) DHA	33.391	0.10	1.74	1.89	1.71	-	-	-	-	-	1.66	0.34	-	2.33	-	-	-	0.28
USFA (%)		77.24	60.24	76.34	86.88	68.44	58.27	69.28	67.9	67.89	87.98	64.25	7.33	79.15	70.54	11.16	70.35	66.76
TSFA (%)		21.86	38.18	22.55	13.12	27.4	40.42	25.41	29.11	30.61	11.51	34.54	92.67	19.92	28.17	88.84	28.89	32.85
MUFA (%)		8.18	16.35	13.89	13.61	8.58	7.46	7.85	16.96	7.59	9.2	7.73	5.53	16.38	9.25	4.81	7.48	7.33
PUFA (%)		69.06	43.89	62.33	62.16	59.86	50.81	61.43	50.94	60.3	78.65	56.52	1.8	62.66	61.29	6.35	62.87	59.43
Total (%)		99.1	98.42	98.89	100	95.84	98.68	94.69	97.01	98.5	99.49	98.79	100	99.07	98.71	100	99.24	99.61

TABLE 5. (Continued).

Fatty acid	compositions	RT (min)	ESR-9	AR-3	ESR-10	AR-4	BSR-3	BSR-4	BSR-5	BSR-6	BSR-7	BSR-8	BSR-9	BSR-10	ESR-II	SD
Butyric acid (C4:0)		4.709	19.32	14.27	23.56	12.62	9.91	12.22	4.91	18.24	35.54	34.82	5.70	19.04	5.20	14.71
Caproic acid (C6:0)		5.215	0.52	-	-	-	0.73	-	0.06	-	-	1.24	0.58	1.71	0.88	0.83
Caprylic acid (C8:0)		5.99	0.97	-	-	-	0.27	-	0.10	-	-	-	0.20	0.90	0.20	0.32
Palmitic acid (C16:0)		17.324	18.49	6.91	6.99	7.12	14.53	6.71	10.7	5.69	5.55	12.88	11.68	19.05	10.65	4.10
Palmoleic acid (C16:1)		18.461	-	-	-	-	-	-	0.11	-	-	-	0.12	-	0.19	4.15
Heptadecanoic acid (C17:0)		19.199	-	-	-	-	0.25	0.12	0.18	-	-	-	0.22	-	0.21	0.05
<i>cis</i> -10-heptadecanoic acid (C17:1)		20.15	0.56	-	-	-	0.26	-	0.30	-	-	0.85	0.53	0.61	0.43	0.22
Stearic acid (C18:0)		21.048	8.88	2.98	2.48	0.12	-	2.71	4.17	1.97	1.96	8.34	4.28	6.76	4.36	2.76
Elaidic acid (C18:1n9t)		21.88	-	-	-	2.78	-	-	-	-	-	-	-	-	13.28	4.86
Oleic acid (C18:1n9c)		22.02	17.35	8.67	8.20	8.05	17.8	8.06	13.41	6.25	6.11	13.22	13.18	14.11	0.66	4.84
Linoleic acid (C18:2n6c)		23.531	24.54	64.95	56.37	67.43	45.95	67.57	60.28	45.79	48.45	13.92	57.83	32.83	56.54	13.36
Arachidic acid (C20:0)		24.526	0.73	0.19	0.17	0.20	0.44	0.18	0.40	-	-	0.82	0.40	0.69	0.54	0.21
$\gamma$ -linolenic acid (C18:3n6)		25.158	-	0.91	0.93	1.00	0.43	1.03	0.59	-	0.90	-	0.66	-	0.67	0.25
<i>cis</i> -11-eicosanoic acid (C20:1)		25.416	-	-	-	-	-	-	0.06	-	-	-	-	-	-	0.23
Linolenic acid (C18:3n6)		26.189	-	-	-	-	-	-	0.11	-	-	1.41	0.13	-	0.43	1.46
Henecosanoic acid (C21:0)		26.765	0.43	-	-	-	-	-	0.13	-	-	1.97	0.17	-	0.26	0.61
<i>cis</i> -11,14-eicosadienoic acid (C20:2)		27.752	1.10	-	-	-	0.69	-	0.64	-	-	4.38	0.92	1.95	1.11	1.03
Behenic acid (C22:0)		27.896	-	-	-	-	-	-	-	-	-	-	0.20	-	0.30	0.42
Arachidonic acid (C20:4n6)		29.198	0.53	-	-	-	-	-	0.10	-	-	1.60	0.11	-	0.21	0.47
Tricosanoic acid (C23:0)		29.683	0.52	-	-	-	0.21	-	0.14	-	-	2.55	-	-	0.06	0.90
Lignoceric acid (C24:0)		30.475	0.66	-	-	-	-	-	0.31	-	-	-	-	-	-	0.25
<i>cis</i> -13,16-docosadienoic acid (C22:2)		29.73	-	-	-	-	-	-	-	-	-	-	0.19	-	0.24	0.67
<i>cis</i> -5, 8, 11, 14, 17-eicosapentaenoic acid (C20:5n3) EPA		30.771	0.58	-	-	-	0.39	-	0.27	-	-	2.01	0.51	0.91	0.56	0.48
Nervonic acid (C24:1)		32.315	0.48	-	-	-	0.26	-	0.08	-	-	-	0.28	-	0.57	0.21
<i>cis</i> -4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6n3) DHA		33.391	1.46	-	-	-	2.29	-	1.40	-	-	-	1.88	1.43	1.88	0.72
USFA (%)			46.61	74.53	65.50	79.26	68.07	76.66	77.35	52.04	55.46	37.39	76.34	51.85	76.75	18.81
TSFA (%)			50.53	24.35	33.2	20.06	26.35	21.94	21.1	25.9	43.05	62.61	23.42	48.15	22.66	19.01
MUFA (%)			18.4	8.67	8.2	10.83	18.32	8.06	13.85	6.25	6.11	14.07	13.99	14.72	14.93	4.15
PUFA (%)			28.21	65.86	57.3	68.43	49.75	68.6	63.4	45.79	49.35	23.32	62.22	37.13	61.63	17.83
Total (%)			97.14	98.88	98.7	99.32	94.42	98.6	98.46	77.94	98.51	100	99.76	100	99.41	4.04

AR: Aegean Region, MR: Marmara Region, ESR: Eastern and Southeastern Anatolia Region, BSR: Black Sea Region, RT: Retention Time,

USFA: Unsaturated Fatty Acid, TSFA: Total Saturated Fatty Acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated Fatty Acids,

SD: Standard Deviation.

Butyric acid can be used in different industries, and there has recently been a great interest in using it as a precursor to biofuels (Dwidar *et al.*, 2012). Moreover, it has many uses in the pharmaceutical and chemical industries. It was well known for its anticancer effects and it induces morphological and biochemical changes in various cells leading to the concomitant suppression of neoplastic properties (Cao *et al.*, 2011). Butyric acid was found between 0.33-64.98% in all the tobaccos. The highest butyric acid was found for ESR-6 (64.98%), followed by the MR-5 (58.61%) and BSR-7 (35.54%) genotypes. The lowest butyric acid was found in ESR-3 with 0.33%. ESR-6, MR-5 and BSR-7 genotypes can be cultivated to obtain high butyric acid contents for use in biofuel.

A correlation was seen between butyric acid and linoleic acid. When linoleic acid was not detected, such as in ESR-6 and MR-5, butyric acid was found at the highest levels in these genotypes. Elaidic and cis-11, 14 heneicosanoic acids were the minor fatty acids in these tobaccos, constituting 0.04-7.22 % and 0.04-1.97%, respectively. Linoleic acid and butyric acid were found at the highest FA rates among the others at 54.0% and 18.57%, respectively.

The FA of tobacco seed crude oil can be divided into two main components as saturated fatty acids (SFA) and unsaturated fatty acids (USFA). The total SFA and USFA in tobacco seed oil were found to be 33.65 and 64.26%, respectively. While the 11 FA compositions were found as SFA, 14 FA compositions were determined as USFA. SFA compositions varied from 11.51 to 88.84% in ESR-3 and ESR-6 from the ESR genotypes. The USFA compositions were found between 11.16-87.98% in ESR-3 and ESR-6 in the ESR genotypes. Among the USFA, linoleic acid (54.0%) and oleic acid (9.52%) were the most abundant while palmitic and butyric acids (18.71%) were most abundant among the SFA (Table 5).

The total FA compositions ranged from 77.94-100% in the tobacco genotypes and cultivar. The highest total FA compositions were found in the ESR-1 and BSR-8 genotypes (100%), followed by MR-5, ESR-6 and BSR-10 with 99.99%. The lowest total FA compositions were seen in BSR-6 (77.94%), followed by the BSR-3 (94.42%) and MR-2 (94.69%) genotypes.

The amounts of unsaturated and saturated fatty acids reported in the present work are close to those reported as 73.95-26.1% (Zalatanov *et al.*, 2000) and 85.2 and 14.8% (Giannelos *et al.*, 2002), respectively.

### 3.6. Genetic diversity of tobacco cultivar and genotypes

Dendrogram analyses were carried out among the chemical components as NC, RSC, SCO and 25 FACs in two different figures in 29 tobacco genotypes and 1 cultivar. The dendrogram analyses were divided into two main groups in terms of NC, RSC, SCO and FAC as A and B (Figures 1a and b). All figures consisted of 2 sub-groups as A1 and B1. While group A was formed of 16 genotypes and one cultivar, group B contained 13 genotypes. Sub-group A1 comprised 11, including cultivar (AR-1), 4 ESR, 4 BSR, 1 MR and 1 AR genotypes. Sub-group A2 included 6 genotypes (5 ESR, 5 BSR, 2 AR and 1 MR genotypes). 3 genotypes fell into sub-group B1 (ESR 4, ESR 5 and BSR 5) and sub-group B2 had 10 genotypes as 4 BSR, 3 ESR, 2 AR and 1 AR genotypes (Figure 1a). When figure 1b was examined, it was seen that the FACs of the tobacco cultivar and genotypes were divided into four sub-groups as A1, A2, B1 and B2. Sub-group A1 and B1 had only one FAC as lignoceric acid and arachidic acid, respectively. While group A2 had 13 FACs including one of the major acids, butyric and linoleic acid; B2 had FACs. Figures 1a and 1b were evaluated together and the major FACS were determined in group A and B1 subgroup and minor FACs were observed in B2 in Figure 1b was formed based on linoleic acid. Most of the saturated acids were found in group A and unsaturated acids were detected in group B in Figure 1b.

## 4. CONCLUSIONS

This study focused on the chemical components of 29 tobacco genotypes and 1 cultivar as nicotine, sugar, crude oil, fatty acid composition and ion content. The cultivar 'AR-1' was found to be higher than the other genotypes in terms of NC, RSC and SCO. Among the genotypes, ESR-4, ESR-5 and BSR-5 had better results for the examined properties except for IC. IC as K<sup>+</sup> was found in high contents in the genotypes, especially the BSR-5 genotype. This genotype can be characterized as having high quality because high K<sup>+</sup> and low Cl<sup>-</sup> contents in tobaccos have a positive effect. Generally, the BSR-5 genotype was determined to have high quality in terms of the chemical components of tobacco genotypes. From the quality perspective, tobacco seed fatty

acid compositions classified as butyric, oleic, palmitic and linoleic acid were found as the major components in the tobacco genotypes and cultivar. They can be used in paint industries and cosmetics as potential raw materials. For human use, the highest values in terms of butyric and

linoleic acid were found in ESR-6 and ESR-3. Hence, this study would be helpful for tobacco producers and users to know the chemical contents in tobacco products and to determine the chemical contents in tobacco genotypes and cultivar.

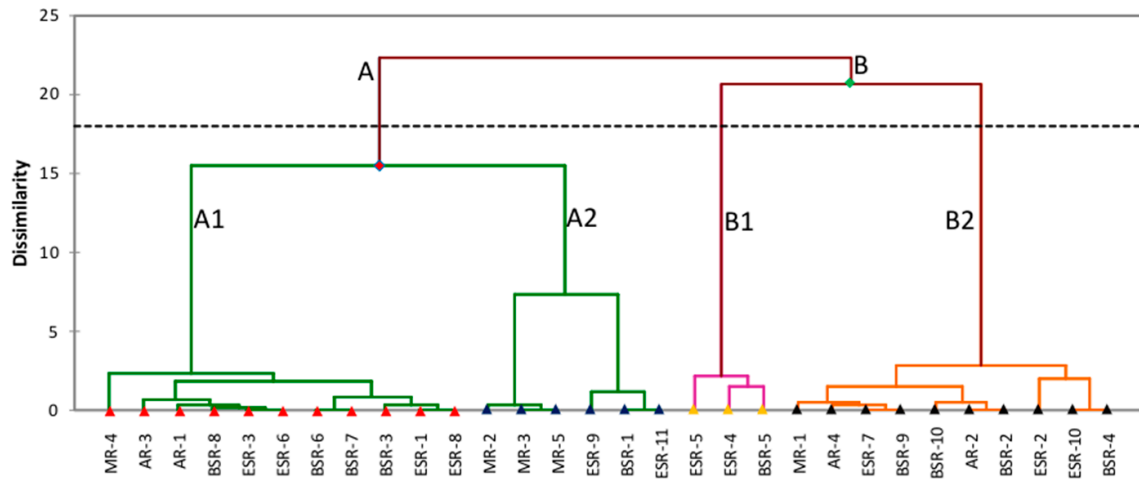


FIGURE 1a. AR: Aegean Region, MR: Marmara Region, ESR: Eastern and Southeastern Anatolia Region, BSR: Black Sea Region.

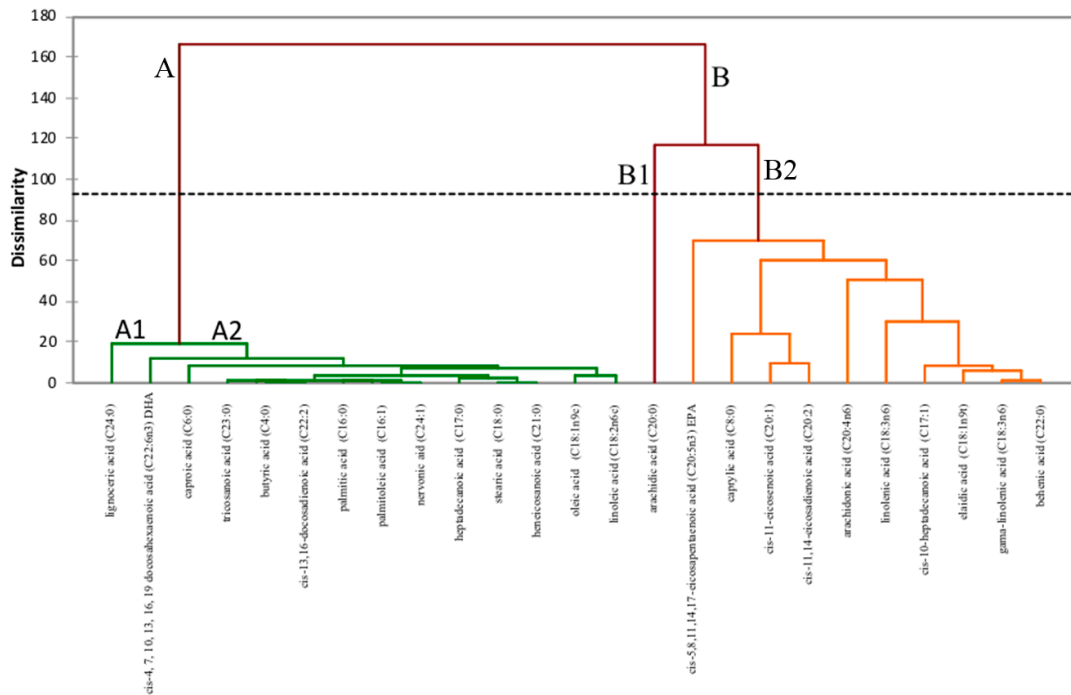


FIGURE 1b

FIGURE 1. Dendrogram analysis of tobacco cultivar and genotypes based on nicotine, reducing sugar contents and crude oil (Figure 1a) and fatty acid compositions (Figure 1b)



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## REFERENCES

- Abbas Ali M, Abu Sayeed M, Roy RK. 2008. Comparative study of on characteristics of Seeds oils and Nutritional composition of seeds from different varieties of tobacco cultivated in Bangladesh. *Asian J. Biochem.* **3** (4), 203-212. <https://doi.org/10.3923/ajb.2008.203.212>
- Abdallah F. 1986. Is Tobacco Quality Measurable? Trans. K. Ketenci. Monopoly Institute Istanbul.
- ARGEFAR. 2016. Center for Drug Research & Development and Pharmaceutical Applications, Ege University. <https://argefar.ege.edu.tr/eng-3641/html>, 21.08.2018.
- Awola VG, Ogunniyi DS, Odetoye TE. 2010. Refining, modification and characterization of tobacco seed oil for improved potentials for industrial use. *Nigerian J. Pure Appl. Sci.* 2168-2174.
- Bastida G, Beltran B. 2011. Ulcerative colitis in smokers, non-smokers and ex- smokers. *World J. Gastroenterol.* **17**, 2740-2747. <https://doi.org/10.3748/wjg.v17.i22.2740>
- Bilgin AE, Müftüoğlu Y, Ustralı A. 1993. Calibration of Commercial Tobacco Requirements and Phosphorus-Potash Analysis Methods of Oriental Tobacco Under Field Conditions in Aegean Region. T. C. Ministry of Agriculture and Rural Affairs General Directorate of Rural Services Menemen Research Institute Publications. Publication No: 1995, İzmir. 1993.
- Bucciarelli S, Civitella D, Fecondo G, Ghiann G, Sorrentino C, Del Piano L, Raimo F, Stanisci V. 2013. Influence of plant density and leaf priming on seed and seed oil yield tobacco. Proc. CORESTA Meeting, Agronomy/Phytopathology, Brufa di Torgiano (PG), Italy.
- Cao Y, Li HQ, Zhang J. 2011. Homogeneous synthesis and characterization of cellulose acetate butyrate (CAB) in 1-Allyl-3-methylimidazolium chloride (AmimCl) ionic liquid. *Indust. Engineer. Chem. Res.* **50** (13), 7808-7814. <https://doi.org/10.1021/ie2004362>
- Camas N, Karaali H, Ozcan H. 2007. Effects of different fertilizer doses on yield, quality and technological properties of pressed tobacco genotype in Erbaa-Taşova Conditions. 2007 TTL Foreign Trade. Research Report.
- Chiririwa H, Hapanyengwi A, Muzenda E. 2014. Tobacco seed oil as an economically viable alternative for the tobacco industry. International Conference on Chemical Engineering and Advanced Computational Technologies, November, 24-25, 36-39. <https://doi.org/10.15242/ICE.E1114017>
- Delibacak S, Ongun AR, Ekren S. 2014. Influence of soil properties on yield and quality of tobacco plant in Akhisar region of Turkey. *Eurasian J. Soil Sci.* **3**, 286-292.
- Dwidar M, Park JY, Mitchell RJ, Sang B. 2012. The future of butyric acid in industry. *Scient. World J.* 1-9. <https://doi.org/10.1100/2012/471417>
- Ekren S, Sekin S. 2008. Investigation of chemical and expertise characteristics of Akhisar region tobaccos and relations with the leaf yield. *Ege J. Agric. Res.* **45** (3), 165-173.
- Er C, Yıldız M. 2014. Pleasure plant. Ankara University Publications, No: 419.
- Fornasier F, Gomez JFC, de Carvalho FS, Schneider RdCdS, Costa ABd, Moraes JAR, Bravo dCAG. 2018. Biodiesel production from energy tobacco. *Orbital: Electronic J. Chem.* **10** (2), 123-132.
- Giannelos PN, Zannikos F, Stourna S, Lois E, Anastopoulos G. 2002. Tobacco seed oil as an alternative diesel fuel: physical and chemical properties. *Ind. Crops Prod.* **16**, 1-9. [https://doi.org/10.1016/S0926-6690\(02\)00002-X](https://doi.org/10.1016/S0926-6690(02)00002-X)
- Griesser M, Weingart G, Schoedl-Hummel K, Neumann N, Becker M, Varmuza K, Liebner F, Schuhmacher R, Forneck A. 2015. Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera* cv. Pinot noir). *Plant. Physiol. Biochem.* **88**, 17-26. <https://doi.org/10.1016/j.plaphy.2015.01.004>
- Irget ME, Oktay M, Hakerlerler H, Atıl H, Cakici H. 1999. A research on the nutrition and soil-plant relationships of Virginia (flue-cured) tobacco grown in Düzce. *J. AARI* **9** (2), 125-142.
- Kirkova S, Srbinoska M, Ivanova S, Georgieva A. 2016. Determination of fatty acid composition

- of seed of oriental tobacco. *Tobacco* **66** (1-6), 53-58. <https://doi.org/10.13140/RG.2.2.18885.14560>
- Krishnamurthy V, Ramakrishnayya BV. 1993. Yield and quality of fcv tobacco as affected by potassium nutrition. pp. 78-102. In: Plant nutrition effects on production and quality of tobacco. Potash and Phosphate Inst. of Canada. India Programme Sector-19, Haryana.
- Kurt D, Ayan AK. 2014. Effect of the different organic fertilizer sources and doses on yield in organic tobacco (*Nicotiana tabacum* L.) production. *J. Agric. Faculty Gaziosmanpasa Univer.* **31** (2), 7-14.
- Mohammad MT, Tahir NA. 2014. Evaluation of chemical compositions of tobacco (*Nicotiana tabacum* L) genotypes. *Seeds Annual Res. Review Biol.* **4** (9), 1480-1489. <https://doi.org/10.9734/ARRB/2014/8211>
- Otan H, Aпти R. 1989. Tobacco. T.C. T.O.K.İ.B. Ege Agricultural Research Institute Publications, No: 83. Menemen-İzmir.
- Palomer X, Pizarro-Delgado J, Barroso E, Vázquez-Carrera M. 2018. Palmitic and oleic acid: The yin and yang of fatty acids in type 2 diabetes mellitus. *Trends Endocrinol. Metabol.* **29** (3), 178-190. <https://doi.org/10.1016/j.tem.2017.11.009>
- Piano L del, Abet M, Raimo F, Modestia F, Sicignano M, Enotrio T. 2014b. Caraterizzazione morfologica e qualitativa di accessioni di *Nicotiana tabacum* L. per la produzione di olio da seme. Proc. X National Congress on Biodiversity, Rome, Italy.
- Poltronieri P. 2016. Tobacco seed oil for biofuels. Pp. 161-185. In: Poltronieri P and D'Urso O.F. Biotransformation of Agricultural Waste and By-Products. The Food, Feed, Fibre, Fuel (4F) Economy. Elsevier, Oxford, UK. 2016. ISBN: 9780128036228. <https://doi.org/10.1016/B978-0-12-803622-8.00006-9>
- Regassa R, Chandravanshi BS. 2016. Levels of heavy metals in the raw and processed Ethiopian tobacco leaves. *Springer Plus* **5**, 232 <https://doi.org/10.1186/s40064-016-1770-z>
- Stanisavljević IT, Veličković DT, Todorović ZB, Lazić ML, Veljković VB. 2009. Comparison of techniques for extraction of tobacco seed oil. *Europ. J. Lipid Sci. Technol.* **111** (5), 513-518. <https://doi.org/10.1002/ejlt.200800232>
- Talaqani TE, Shafik J, Mustafa FK. 1986. Fatty acid composition of the seed oil of certain tobacco varieties cultivated in Northern Iraq. *Indian J. Agric. Chem.* **19**, 147-154.
- TEA. 2018. Tobacco Experts Association. Chemical structure of tobacco leaf in terms of blending. <http://www.tutuneksper.org.tr>, 21.08. 2018.
- Wang M, Fu Y, Liu H. 2016. Nutritional quality and ions uptake to PTNDS in soybeans. *Food Chem.* **192**, 750-759. <https://doi.org/10.1016/j.foodchem.2015.07.002>
- Yagac C. 2015. Determination of yield and quality characters of tobacco cultivars of Aegean Region in Denizli conditions. MsC Namik Kemal University Graduate School of Natural and Applied Sciences Department of Field Crops.
- Yaldiz G, Çamlica M. 2019. Variation in the fruit phytochemical and mineral composition, and phenolic content and antioxidant activity of the fruit extracts of different fennel (*Foeniculum vulgare* L.) genotypes. *Ind. Crops Prod.* **142**, 111852. <https://doi.org/10.1016/j.indcrop.2019.111852>
- Yaldiz G, Camlica M, Ozen F. 2019. Biological value and chemical components of essential oils of sweet basil (*Ocimum basilicum* L.) grown with organic fertilization sources. *J. Sci. Food Agric.* **99**, 2005-2013. <https://doi.org/10.1002/jsfa.9468>
- Zalatanov M, Menkov N. 2000. Phospholipid and fatty acid composition of tobacco seeds. *Rostlinna Vyroba* **46**, 439-441.
- Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A. 2018. Plant adaptations to the combination of drought and high temperatures. *Physiol. Plant.* **162**, 2-12. <https://doi.org/10.1111/ppl.12540>