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Determining the Genetic and Agronomic Variations in Lines From Samsun Tobacco Growing Areas

Samsun Tütün Üretim Alanlarındaki Hatlarda Genetik ve Agronomik Varyasyonların Belirlenmesi

Dursun KURT[1], Ahmet KINAY[2], İbrahim SAYGILI[3], Nejdet KANDEMİR[4]

[1]Vocational School of Bafra, Ondokuz Mayıs University, Samsun
• **dursun.kurt@omu.edu.tr** • ORCID > 0000-0001-6697-3954

[2]Faculty of Agriculture, Tokat Gaziosmanpasa University, Tokat
• **ahmet.kinay@gop.edu.tr** • ORCID > 0000-0003-4554-2148

[3]Faculty of Agriculture, Tokat Gaziosmanpasa University, Tokat
• **ibrahim.saygili@gop.edu.tr** • ORCID > 0000-0003-0449-4872

[4]Polatlı Faculty of Sciences and Letters, Ankara Hacı Bayram Veli University, Ankara
• **nejdet.kandemir@hbv.edu.tr** • ORCID > 0000-0002-9658-2193

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Sorumlu Yazar / Corresponding Author: Dursun KURT

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DETERMINING THE GENETIC AND AGRONOMIC VARIATIONS IN LINES FROM SAMSUN TOBACCO GROWING AREAS

ABSTRACT

Tobaccos grown in Samsun are known all over the world. Samsun tobaccos are grown using nonregistered populations traditionally maintained by local farmers. The present study was conducted to determine the genetic and agronomic variations in Samsun tobaccos. Fifty-eight lines were collected from the tobacco growing areas in Samsun. These lines were analyzed using 18 SSR markers. Polymorphic information content (PIC) values of markers ranged between 0.0 and 0.702. Forty-two alleles were obtained from 18 SSR markers. The average number of alleles per SSR locus was 2.33. Forty-eight lines were different for at least one SSR locus, indicating a high level of variation. The forty-eight lines were evaluated in two field trials along with local varieties Nail and Canik for agronomic characteristics such as plant height, number of leaves, leaf width, leaf length, leaf yield, grade index, leaf sugar, and nicotine contents. A high level of variation was also evident for agronomic characters. Lines No: 2, 11, 13, 21, 28 and 41 were notable for their superior agronomic characteristics. Some of these lines could be directly registered as new cultivars, but they should be further evaluated in future field trials in multiple environments. These lines could also be used as parents for the development of new cultivars.

Keywords: Grade Index, Leaf Yield, Nicotine, Oriental, SSR Marker, Sugar Content.

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SAMSUN TÜTÜN ÜRETİM ALANLARINDAKİ HATLARDA GENETİK VE AGRONOMİK VARYASYONLARIN BELİRLENMESİ

ÖZ:

Samsun'da yetiştirilen tütünler tüm dünyada tanınmaktadır. Samsun tütünleri bölge çiftçileri tarafından devam ettirilen, tescil edilmemiş popülasyonlar kullanılarak da yetiştirilmektedir. Bu çalışma Samsun tütünlerindeki genetik ve agronomik varyasyonları belirlemek amacıyla yürütülmüştür. Samsun'da tütün yetiştirilen alanlardan elli sekiz hat toplanmıştır. Bu hatlar, on sekiz SSR markörü kullanılarak analiz edilmiştir. Markörlerin polimorfik bilgi içeriği (PIC) değerleri 0.0 ile 0.702 arasında değişmiştir. On sekiz SSR markörü ile 42 allel belirlenmiştir. SSR lokusu başına ortalama allel sayısı 2.33 olarak belirlenmiştir. Kırk sekiz hattın en az bir SSR lokusu bakımından farklı olduğu belirlenmiş ve bu durum varyasyon seviyesinin yüksek olduğuna işaret etmektedir. Kırk sekiz hat, lokal çeşitler Nail

ve Canik ile birlikte bitki boyu, yaprak sayısı, yaprak genişliği, yaprak uzunluğu, yaprak verimi, randıman, yaprak şekeri ve nikotin içeriği gibi tarımsal özellikler açısından iki tarla denemesinde değerlendirilmiştir. Tarımsal karakterlerde de yüksek düzeyde varyasyonlar belirlenmiştir. Hat 2, 11, 13, 21, 28 ve 41 üstün agronomik özellikleri bakımından ön plana çıkmıştır. Bu hatların bazıları doğrudan yeni çeşitler olarak tescil ettirilebilir, ancak hatlar birden fazla lokasyonlarda yürütülecek tarla denemelerinde daha ileri düzeyde değerlendirilmelidir. Bu hatlar, yeni çeşitlerin geliştirilmesi için ebeveyn olarak da kullanılabilir.

Anahtar Kelimeler: Nikotin, Oryantal, Randıman, SSR Markör, Şeker İçeriği, Yaprak Verimi.

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1. INTRODUCTION

More oriental tobacco is grown in Turkey than in any other country in the world. This type of tobacco is used to enhance the smoking character of cigarette blends due to their high aromatic content. Oriental tobaccos grown in Samsun Province of Turkey are known worldwide as Samsun tobaccos with high aromatic and quality characteristics (An et al., 2013). In the region, tobacco production is carried out using traditional populations, i.e. landraces, rather than pure line cultivars. Landraces are cultivars developed and maintained by farmers (Acquaah, 2012) and contain a very high level of genetic variation (Kyratzis et al., 2019). Therefore, they are of great value as plant genetic resources since they constitute a large gene pool for future genetic improvement programs (Ceccarelli, 1994). Tobacco populations in Samsun region are an important genetic resource for oriental tobacco. However, little is known about their genetic variation level as well as their agronomic performance.

Variations needed to develop a new cultivar could be found in modern cultivars, landraces, and wild species. By crossing the elite materials within a market class, modern cultivars have been widely used, and crop genetic bases have become increasingly narrower (Moon et al., 2009a). Kandemir et al. (2010) pointed out that landraces are better than wild relatives for the improvement of quality traits because wild species may contain deleterious genes that come along with the gene of interest. Wild relatives have been mainly used as sources of disease-resistance genes (Moon et al., 2009a). In order to use landraces for plant breeding, they need to be characterized. New cultivars with high performance can be developed through direct selection of superior lines, or new alleles in landraces could be used in plant breeding programs.

Oriental tobaccos are famous for their quality characteristics and the high aroma of their small leaves with low sugar and nicotine contents. Dry leaves of tobac-

co populations in Samsun region are orange-colored, with medium-sugar content of 8-12% (Peksuslu et al., 2012). They are used to improve the aroma of blends. Tobaccos with different characteristics are grown in the region, and there are many tobacco populations known as Samsun tobacco grown in this region. Tobaccos from the region are known by a variety of names in the literature. Among them are Samsun SM-1 (Aleksoska et al., 2014), Samsum (Bindler et al., 2007) Samsum Maden, TI 981 Samsun, Turkish Samsun (Fricano et al., 2012), Samsun (Tong et al., 2012), Samsun katenizi (Darvishzadeh et al., 2013), Samsun 959 and Samsun dere (Darvishzadeh et al., 2014). This variation results in a mixture of genotypes in the production area with different quality characteristics, thereby compromising the fine quality of Samsun oriental tobacco.

DNA markers are good tools for genetical characterization. Many different types of DNA markers have been developed since the 1980s. Highly polymorphic, reliable, inexpensive, and easy-to-use microsatellite markers, or SSRs, are widely used for genetic fingerprinting purposes (Bindler et al., 2007; Davalieva et al., 2010; Moon et al., 2009a; Nunome et al., 2009; Thakur et al., 2013). Moon et al. (2009b) reported 92% genetic variation in 702 materials from the US *Nicotiana tabacum* germplasm collection scored with 70 SSR markers. Thirteen SSR markers in 70 genotypes produced a total of 35 alleles and a polymorphism rate of 100% (Darvishzadeh et al., 2014) SSR markers can be used to isolate genotypes from landraces, and better characterization can be achieved by eliminating identical lines and conducting field trials with larger plot sizes or more powerful experimental designs.

The aims of the present study were (i) to identify different genotypes among the tobacco populations traditionally grown in Samsun province using SSR markers (ii) to evaluate agronomic performance of the identified lines in field trials; and (iii) finding superior ones with good yield and quality traits. Use of the superior lines could help improve the quality of the tobacco leaves produced. Besides, these lines could be used in future oriental tobacco improvement programs.

2. MATERIALS AND METHODS

2.1. Plant Material

The plants were sampled from the production fields in Samsun province of Turkey where oriental tobacco is produced using traditional farmer populations. Single plants were selected based on different morphological traits such as number of leaves, leaf color, leaf size and leaf texture (Figure 1). Outcrossing was prevented in these plants by enclosing the flowers with a paper bag. Self-pollinated seeds were harvested from 58 lines. These lines, along with Nail and Canik local varieties, were planted in viols containing 50% peat and 50% perlite in a greenhouse. Nail is

nonregistered and Canik is a registered variety of oriental tobacco commonly used in the production of oriental tobacco in Samsun province.

Figure 1. Geographical data for the locations where landraces were collected

2.2. SSR Analysis

Tobacco plants with two or three leaves were kept in the dark at 18°C for five days to decrease the phenol and sugar content of their leaves. DNA was extracted from leaf tissue using a genomic DNA purification kit (Keskin et al., 2014). The quantity and quality of Genomic DNA were detected on a 1% agarose gel and with a spectrophotometer (Thermo BioMate^{TM}). The concentration of DNA was adjusted to 50 ng μl^{-1}.

A total of 18 SSR markers were studied. The markers with PT letters were selected from Moon et al. (2009a, b) and Bindler et al. (2007, 2011), while the ones with TM and TME letters were adopted from Tong et al. (2012) (Table 1) according to high PIC values. Polymerase Chain reactions (PCR) were conducted in 40 µl volume, which included 50 ng of genomic DNA, 250 nM each of the two primers, 0.2 mM each of the nucleotides, 1.5 mM $MgSO_4$, 10 X PCR buffer and 0.5 units of Taq-DNA polymerase (Biobasic). PCR cycling was as follows: 5 min. at 94 °C, then 32 cycles of 45 sec. at 94 °C, 45 sec. at 55-60 °C (depending on the primer), 45 sec. at 72 °C, and 5 min. of final extension at 72 °C.

Table 1. Some general information about the SSR markers used in the study

SSR	LG	Forward Primer	Reverse Primer	AT (°C)	Size (bp)	RM
PT20172	3	ACACCTCCTTCTTCCTGC	CCAAAATGGTTCACTGGA	55	203	CTT
PT20242	12	TCCAAAGTTGGACCAGAA	GTCCTACATGGGGCTCTT	55	200	AGG
PT30014	11	TGCCGTGTAAATTTCATTTGG	AGGATTCCTAACGTGTATTATGTTCT	55	205	TA
PT30034	22	GACGAAACTGAGGATATTCCAAA	TGGAAACAAAGCCATTACCC	55	216	TAA
PT30114	2	ATCCCACATAGGCCTCACAC	GTCCGGTGCACTAAACTTCC	55	144	TA
PT30137	13	TTTGGTGAGGTGTTACGATAAAGA	TCCACACCAAACATCAACTTT	55	219	TAA
PT30274	17	TGACAGCTAAGCTAATAACAGTAAATG	GGACTTTGGAGTGTCAAATGC	55	213	GGA
PT30364	22	CACTTTCAAGTTCGTCACGC	ATATGTTGACGACGACCCGT	58	173	TAA
PT40005	24	TGATCACACTTGATAGCCTAAAGAA	CGCACGACCTATACCCATTT	55	250	GAA
PT40015	8	CAAGGAATGGAAGAGAGGCA	TTTGAAACAGCACCAATCCA	55	170	GA
PT50182	1	TGCTTTGGTATAATTTATTTCTTACG	GCTGGTCAAAGAGAGGTGTCA	55	150	TA
PT53303	7	GTAAGGTGTCCGGAGCTGAA	ACATAAATGCAACGCATGGA	55	200	GA
PT61056	3	TCCAATCTTTACACAATTAGTCGTTC	TGGCTTCTCTGTCTAGGGAGG	55	200	TA
TM10013	-	TGGAATTCCGGTTATGTCCT	TTGAAATAGCGCGTACCCTAA	60	141	ATA
TM10181	-	GTGGTTTGATCTTCTTCCCATT	GGAATTAACCACCACCATGC	60	118	AGA
TM10211	-	ATCCGGACGAGGCTATCTCT	GCAGGGGTAAGGTCTGCAT	60	115	ACA
TM10821	8	GCAAACATCTCAGGATTCCAC	GGCCTCTGGATCTGGTATGA	60	132	TTA
TME0293	11	AAGGAGGAGCAGGACCAACT	TGGAGCCATTTATTGTCAAGC	60	132	TCA

AT: Annealing temperature, LG: linkage group, RM: Repeat motif

Amplicons produced by SSR markers were run on 3% MetaPhor agarose gels (Lonza cat no: 50180) in 1% TBE buffer. DNA was visualized with ethidium bromide added to gel using a gel image system (Vilber Lourmat CN-08). PCR band profiles were scored using BioCapt v.11.02 software. A dendrogram was constructed with POPGENE v.1.31 using the UPGMA algorithm (Yeh et al., 1997). The total number of alleles and rare alleles (frequency ≤ 0.05) were determined (Moon et al., 2009b). PIC values were calculated according to the following formula: $PIC = 1 - \sum P^2_i$, where Pi is the frequency of the i^{th} allele (Anderson et al., 1993).

2.3. Field Experiments

Forty-eight lines, which had at least one different allele at any SSR loci investigated, were evaluated in field trials along with local varieties, Nail and Canik. Field trials were conducted under rainfed conditions in Gökçekent village (41^0 32' 42" N and 35^0 48' 36" E, altitude 43 m) (Figure 1) in 2014 and 2015 years. Soil analysis results of the experimental areas are given in Table 2. Figure 2 depicts the experimental area's climate data.

Table 2. Soil properties of experimental lands

	2014	2015
Texture	Clayed	Clayed
pH	7.68	7.86
EC (dS m^{-1})	1.34	1.30
$CaCO_3$ (%)	19.13	18.26
Organic matter (%)	1.75	1.60
P_2O_5 (kg ha^{-1})	101	119
K_2O (kg ha^{-1})	1855	1395

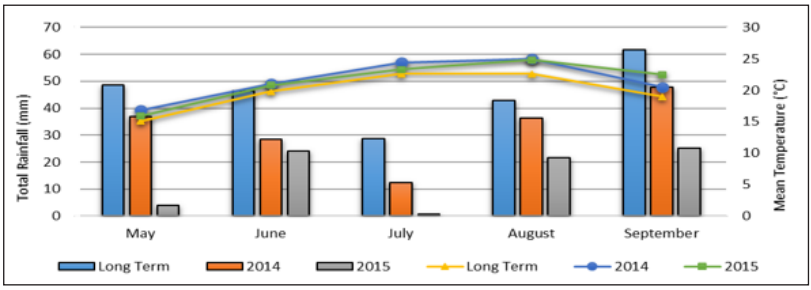


Figure 2. Climatic data of the experimental area

Seedlings grown in viols were transplanted to plots consisting of four rows. The distance between the rows was 45 cm, and the row length was 5 m. The plant distance on the rows was 15 cm. Transplantation was conducted on May 19, 2014 and May 21, 2015. Fertilizers (60 kg ha-1 N, 40 kg ha-1 P2O5) were applied before the transplanting of seedlings. Weeds were controlled manually twice during the growing period. When plants reached the flowering stage, the plant height, and number, width and length of leaves were determined in ten plants. Leaf harvesting was completed in three harvests, and leaves were dried under the sun. The grade index was calculated based on the American Grading system. Dried leaf yield, nicotine and glucose contents in leaves were calculated based on 17% moisture content (Kurt, 2020).

Nicotine and glucose contents were investigated in dried leaves. Samples were taken from dried leaves for chemical analyses and they were ground to a fine powder at zero moisture. Extractions were performed with 1% acetic acid (16 ml) and acetonitrile (4 ml) for nicotine, and with 5% acetic acid (6 ml) and methanol (4 ml) for glucose. The sample was vortexed for five minutes and incubated in an ultrasonic water bath for 30 minutes. After centrifuging at 3000 RPM for 10 minutes, the preparation was filtered through a 45 µm filter. Extracts were analyzed in an

HPLC system equipped with a DAD detector for nicotine content and with a RI detector for glucose content (Kinay and Kurt, 2021). Acetonitrile, methanol and ultra-distilled water were used as the mobile phase. Chemical contents were calculated from resultant chromatograms previously subjected to standard calibrations (r2; 0.999). Extraction recovery ratios indicating the reliability of analyses were obtained as 101% for nicotine and 106% for glucose.

The experimental design was randomized complete blocks with three replications. Due to non-homogenous variance of the traits based on Bartlett's homogeneity test (Steel et al., 1997), years were analyzed separately. Arcsine transformation was applied to percent values. Post hoc comparisons were performed among the means using Duncan's multiple range test. MSTAT-C statistical analysis software was used for all data from field trials (Freed and Eisensmith, 1986). Principal component analyses (PCA) were conducted using Minitab V17 software.

3. RESULT AND DISCUSSION

3.1. Genetic Diversity

Fourteen of the 18 SSR markers investigated were polymorphic (Table 3). The polymorphism rate of markers used in the study was 78% (14/18). In various other studies, polymorphism rates were 80% in 10 tobacco genotypes from different types (such as Virginia, Burley and oriental) (Davalieva et al., 2010), 100% in different types of tobaccos collected in Iran and Yugoslavia, Russia, Turkey, Greece and Bulgaria (Darvishzadeh et al., 2014) and 100% in 702 genotypes of *Nicotiana tabacum* (Moon et al., 2009b). The observed polymorphism rate of SSR markers in the present study (78%) may seem lower than in other studies. However, this rate was obtained from a single type of tobacco rather than from different types of tobaccos in other studies.

The eighteen SSR markers studied produced 42 alleles in 58 genotypes (Table 3). Three markers (TM10821, PT20242 and TM10181) produced four alleles, four markers (PT20172, PT30274 PT61056 and PT10013) produced three alleles; and seven markers (PT30034, PT30137, PT30114, PT40005, PT50182, PT53303 and TM10211) produced two alleles. Number of rare alleles in 18 SSR markers was only two. The rare alleles were detected in TM10821. The number of alleles per polymorphic marker was 2.71. Gholizadeh et al. (2012) reported the number of alleles per marker as 3.47 in 72 Flue-cured Virginia tobacco genotypes using 30 SSR markers. In 25 SSR loci, 135 flue-cured Virginia tobaccos had a total of 85 alleles (3.40 alleles per marker) (Ganesh et al., 2014). Darvishzadeh et al. (2013) found two or three alleles per marker (mean: 2.69) in 100 tobacco genotypes, half of which originated from Iran. The results of the present study were in accordance with those of Darvishzadeh et al. (2013) dealing with only one type of

tobacco from a single region. The fact that only two rare alleles were found in the present study showed that tobaccos grown in the region had the same alleles in the SSR loci investigated. None of the genotypes had a heterozygous marker profile. This finding implicated that the genetic variation observed was not due to the heterozygosity of the plants sampled.

Table 3. The results of marker analysis

SSR Marker	Results	Number of alleles	Frequency of alleles (%)				PIC
			A	B	C	D	
PT20242	Polymorphic	4	47.5	31.1	8.2	13.1	0.653
PT20172	Polymorphic	3	60.7	11.5	27.9	0.0	0.541
PT30034	Polymorphic	2	85.7	14.3	0.0	0.0	0.245
PT30137	Polymorphic	2	54.1	45.9	0.0	0.0	0.497
PT30114	Polymorphic	2	42.6	57.4	0.0	0.0	0.489
PT30274	Polymorphic	3	12.9	40.3	46.8	0.0	0.602
PT30364	Monomorphic	1	100.0	0.0	0.0	0.0	0.000
PT30449	Monomorphic	1	100.0	0.0	0.0	0.0	0.000
PT40005	Polymorphic	2	73.8	26.2	0.0	0.0	0.387
PT40015	Monomorphic	1	100.0	0.0	0.0	0.0	0.000
PT50182	Polymorphic	2	68.9	31.1	0.0	0.0	0.429
PT53303	Polymorphic	2	85.2	14.8	0.0	0.0	0.252
PT61056	Polymorphic	3	77.0	11.5	11.5	0.0	0.380
PT10013	Polymorphic	3	17.7	53.2	27.4	0.0	0.610
TM10181	Polymorphic	4	22.6	11.3	41.9	24.2	0.702
TM10211	Polymorphic	2	91.8	8.2	0.0	0.0	0.150
TM10821	Polymorphic	4	3.3	4.9	49.2	42.6	0.573
TME0293	Monomorphic	1	100.0	0.0	0.0	0.0	0.000
Mean or Rate	77.8%	2.33	-	-	-	-	0.362

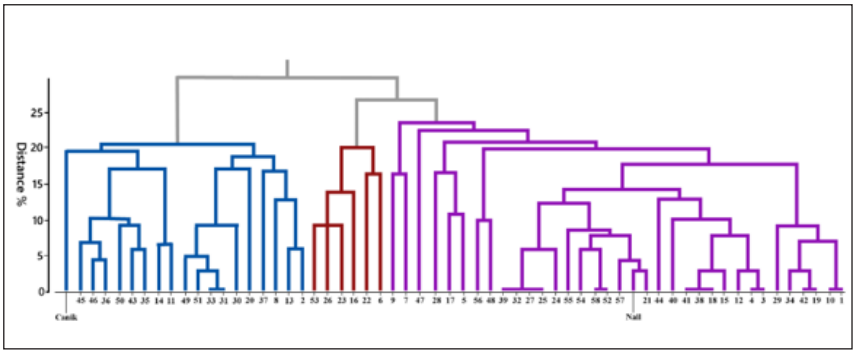
PIC: Polymorphic information content

Polymorphic information content (PIC) is an informative accounting of DNA markers. Higher PIC values indicate higher distinguishing power of markers. The PIC values of 18 markers evaluated in the present study ranged from 0.0 to 0.702 (mean: 0.361). The highest PIC value was obtained from TM10181 (0.702) and the lowest from TM10211 (0.150). The mean PIC values of SSR markers in the previous studies were 0.39 by Davalieva et al. (2010) using 30 SSR in 10 genotypes, 0.59 by Fricano et al. (2012) using 49 SSR in 312 genotypes, 0.48 by Ganesh et al. (2014) using 25 SSR in 135 genotypes and 0.736 by Moon et al. (2009b) using 70 SSR in 702 genotypes. In these studies, the number of genotypes and tobacco types were

higher than those in the present study. Mean PIC values obtained in the present study (0.361) can be considered high compared to that of Davalieva et al. (2010) using 30 SSR and 10 tobacco genotypes from different types (oriental, Virginia and Burley). Our findings indicated that use of only seven SSR markers (TM10181, PT30274, PT20242, PT10013, TM10821, PT20172 and PT10013) could distinguish different oriental tobacco genotypes produced in Samsun region.

A dendrogram was created to visualize the relationships among genotypes using SSR marker profiles (Figure 3). Lines were separated into three distinct groups on the dendrogram (with similarities of lower than 25%). Group 1 had the lines No: 11, 14, 35, 43, 50, 36, 45, 46, 2, 8, 13, 20, 30, 31, 33, 37, 49, 51 and Canik local variety. Group 2 had the lines No: 6, 16, 22, 23, 26 and 53. Group 3 was the largest and had the lines No: 1, 3, 4, 5, 7, 9, 10, 12, 15, 17, 18, 19, 21, 24, 25, 27, 28, 29, 32, 34, 38, 39, 41, 42, 44, 47, 48, 52, 54, 55, 56, 57, 58 and the local variety Nail. The following genotypes were identical: 1 and 10; 19 and 42; 3 and 4; 18, 38 and 41; 52 and 58; 25, 27, 32 and 39; 31 and 33 (Figure 3). Since local varieties Canik and Nail are commonly grown in Samsun region, some of the lines studied could have simply been the plants of Canik or Nail. But the most distinctive genotype in group 1 was Canik. The closest line to Nail was line 21. Genotype 21 differed by TM10013 marker. Of the 58 lines examined, 48 were different for at least one SSR locus. Identifying 48 different genotypes among 58 plants collected from a small area (only 270 square kilometers in total) indicated that the genetic variation of tobaccos grown in the region was very high.

Figure 3. Dendrogram based on SSR marker data



3.2. Variations in Agronomic Traits

The investigation of traditional populations in comparison to a known or commonly grown genotype is important for evaluating their variations. For this purpose, agronomic variations in 48 collected lines were evaluated together with local varieties Nail and Canik which are widely used in the Samsun region and known worldwide (Ding et al., 2007; Cai et al., 2015). Some lines were identified with better agronomic performance than Nail or Canik, and these superior lines could be evaluated in future studies.

As leaves of oriental tobacco are usually harvested by hand, plant height is an important characteristic in terms of ease of harvest. Leaf harvesting is easier in plants which have a tall stature. Significant differences were observed for plant height in both years ($p<0.01$). The highest plant height was obtained from the line 8 (170.7 cm) in 2015 (Table 4). The 37.7 cm difference in plant height between the two years may have been caused by factors such as precipitation and temperature. Previous studies showed similar yearly changes in plant height of oriental tobaccos (Kurt and Ayan, 2014). Eleven of the lines studied had longer plants than the control genotypes. The differences between the lines indicated a high level of variation for this trait.

The number of leaves in tobacco directly affects the yield obtained in a unit area. Significant differences were found for the number of leaves in both years ($p<0.01$). The number of leaves of the lines varied between 16.9 and 40.1 in the first year and between 24.1 and 41.7 in the second year. Lines 2, 13 and 20 in 2014, and lines 2, 8, 13, 20, 21, 22, 23, 24, 26, 29, 43, 45, 52 and 57 in 2015 had more leaves than control genotypes (Table 4). Considering the results of the two years together, lines 2, 13 and 20 produced more leaves than Nail and Canik varieties. The range of the variation for the number of leaves among the lines was higher than the variation observed for oriental tobaccos in previous studies carried out in this region (Camas et al., 2009a, b; Kurt and Ayan, 2014; Kurt, 2020). These findings might reveal the influence of genotypic variations on number of leaves. Significant differences were detected for leaf width in both years ($p<0.01$). Although the leaf width values of the lines were not statistically different from Nail and Canik varieties in 2014, lines 3, 7, 11, 22, 23, 42, 46, 48, 53 and 57 had higher leaf width than Nail and Canik in 2015 (Table 4). Leaf width is a character significantly affected by the environment. Kurt (2021) found that the leaf width of the same line varied between 7.3 and 16.1 cm in four different environments including the Samsun region.

Table 4. Agronomic characteristics of the genotypes examined

Genotype	Plant height (cm)		Number of leaves		Leaf width (cm)		Leaf length (cm)	
	2014**	2015**	2014**	2015**	2014**	2015**	2014**	2015**
2	114.3 a-e	165.4 ab	40.1 a	41.2 ab	8.58 abc	9.27 n-v	15.36 h-q	20.73 f-l
3	111.9 a-g	135.5 g-j	25.9 f-p	29.5 k-s	8.96 abc	11.95 b-g	15.44 h-q	22.26 d-i
5	93.7 h-p	119.0 l-p	22.5 l-t	28.2 l-u	10.20 ab	10.27 h-q	21.19 a	18.71 k-o
6	85.5 l-t	124.3 i-o	21.9 l-u	27.7 m-u	10.32 ab	9.09 o-v	18.11 b-e	20.06 g-m
7	73.49 st	126.9 i-o	21.7 l-u	27.9 m-u	8.82 abc	12.48 bcd	15.38 h-q	22.31 d-i
8	115.9 a-d	170.7 a	29.6 c-h	35.3 d-j	9.68 abc	8.90 p-v	16.17 f-o	22.49 d-i
9	88.0 j-s	125.8 i-o	21.0 o-u	30.9 j-o	9.13 abc	8.53 s-v	13.76 q	15.74 o-r
10	88.6 j-s	129.1 h-m	22.2 l-u	30.6 k-p	8.98 abc	11.18 d-k	14.86 l-q	21.12 e-k
11	103.1 d-j	157.6 a-d	28.9 c-i	33.8 e-k	9.85 abc	11.88 b-g	19.17 b	26.29 abc
12	99.1 e-m	126.3 i-o	23.4 j-t	24.8 tu	9.78 abc	8.78 r-v	15.87 g-p	15.39 o-r
13	122.4 ab	158.5 a-d	35.7 ab	41.7 a	10.29 ab	8.86 q-v	17.19 c-i	18.82 j-o
14	89.4 j-r	139.0 f-i	20.6 p-u	33.6 e-k	9.09 abc	10.42 h-o	16.75 d-l	21.12 e-k
15	91.8 i-q	119.2 l-p	23.0 k-t	25.1 stu	9.50 abc	9.39 m-v	15.00 l-q	16.38 n-r
16	96.2 g-n	128.2 h-n	21.6 m-u	31.0 j-o	9.80 abc	10.73 g-m	16.39 e-n	23.48 c-g
17	81.2 n-t	110.8 p-t	19.6 r-u	24.7 tu	8.85 abc	12.57 a-d	15.27 i-q	20.72 f-l
20	125.8 a	147.7 c-g	33.4 bc	39.7 a-d	8.82 abc	10.98 e-l	15.18 k-q	23.49 c-g
21	102.1 d-k	150.1 c-f	27.2 d-l	37.8 a-e	8.62 abc	13.96 a	15.61 h-q	25.48 a-d
22	101.2 d-l	159.8 a-c	31.9 bcd	39.0 a-d	9.38 abc	11.37 c-j	16.49 d-m	24.35 a-e
23	109.2 b-h	147.7 c-g	26.8 d-n	35.8 c-i	8.42 bc	11.87 b-g	14.63 m-q	21.20 e-k
24	83.4 m-t	158.3 a-d	18.0 tu	35.9 c-i	8.39 bc	9.91 k-t	14.25 opq	18.53 k-o
25	93.8 h-p	136.6 g-j	28.8 c-j	32.7 f-l	9.43 abc	10.65 g-n	18.72 bc	20.91 e-k
26	97.3 f-n	136.6 g-j	24.7 h-s	36.1 c-h	9.68 abc	10.89 f-l	15.01 l-q	23.94 b-f
28	90.9 i-r	134.9 g-k	23.9 i-s	31.3 i-o	9.71 abc	9.36 m-v	16.00 f-p	16.50 n-r
29	106.7 c-i	153.0 b-e	31.5 b-e	40.3 abc	9.38 abc	11.50 c-i	16.46 d-m	23.24 c-h
30	70.3 t	146.3 d-g	20.1 q-u	31.5 h-n	9.25 abc	8.63 s-v	15.46 h-q	19.95 h-m
31	77.6 q-t	102.5 st	25.1 g-r	26.1 p-u	8.11 c	10.35 h-o	15.47 h-q	20.83 f-l
34	99.2 e-m	128.4 h-n	23.3 k-t	30.4 k-q	9.61 abc	8.81 r-v	14.70 m-q	17.46 l-q
35	112.8 a-f	115.2 n-s	31.8 bcd	27.3 n-u	9.38 abc	8.46 uv	17.17 c-i	15.89 o-r
36	97.1 f-n	131.3 h-l	25.2 g-q	25.4 r-u	8.64 abc	10.11 i-r	17.78 b-f	17.00 m-r
37	94.9 h-o	131.4 h-l	21.3 n-u	33.0 f-k	9.11 abc	10.33 h-p	16.40 d-n	23.02 c-h
40	79.7 o-t	114.6 o-s	19.4 stu	24.3 u	9.26 abc	9.41 m-v	14.10 pq	14.87 pqr
41	86.7 k-s	136.9 f-j	20.7 p-u	26.9 o-u	9.25 abc	12.57 a-d	17.06 c-k	26.87 ab
42	90.7 i-r	131.2 h-l	22.8 l-t	30.0 k-r	8.46 abc	12.38 b-e	14.22 pq	24.62 a-d
43	101.0 d-l	100.1 t	26.4 e-o	24.2 u	10.43 a	9.72 l-v	16.72 d-l	16.84 m-r
44	92.1 i-q	117.9 m-q	23.3 k-t	25.1 stu	9.22 abc	9.08 o-v	15.28 i-q	13.87 r
45	96.1 g-n	159.3 a-d	28.3 c-k	36.9 b-f	9.58 abc	9.96 j-s	15.34 h-q	22.20 d-j
46	91.6 i-r	140.5 e-h	20.5 p-u	32.3 g-m	8.50 abc	13.01 ab	16.97 c-k	25.62 a-d
47	108.8 b-h	116.3 m-r	30.7 b-f	24.9 tu	9.06 abc	9.22 n-v	17.22 c-h	16.28 n-r
48	78.1 p-t	117.3 m-r	20.8 p-u	25.0 stu	8.94 abc	11.57 c-h	15.98 f-p	19.40 i-n
49	119.6 a-c	114.8 o-s	30.4 c-g	25.8 r-u	9.61 abc	9.12 o-v	17.72 b-g	13.53 r
50	98.8 e-m	116.2 m-r	23.0 k-t	24.1 u	9.24 abc	8.78 r-v	17.59 b-g	13.97 r
51	111.1 a-g	105.7 q-t	23.8 i-s	23.9 u	8.78 abc	9.85 k-u	15.33 h-q	14.44 qr
52	89.4 j-r	121.9 k-p	25.1 g-r	37.1 a-f	10.43 a	10.30 h-p	18.28 bcd	18.28 k-p
53	84.5 m-t	135.5 g-j	25.8 f-p	27.2 n-u	9.84 abc	12.79 abc	17.12 c-j	24.31 a-e
54	88.1 j-s	104.4 rst	21.9 l-u	24.6 u	9.44 abc	8.30 v	15.33 h-q	14.10 qr
55	97.0 f-n	135.6 g-j	23.8 i-s	29.2 k-t	8.60 abc	8.83 q-v	14.81 l-q	15.73 o-r
56	75.8 rst	140.9 e-h	16.9 u	31.1 j-o	9.01 abc	8.50 tuv	15.22 j-q	15.59 o-r
57	102.0 d-k	135.3 g-j	31.3 b-e	36.6 c-g	8.68 abc	12.31 b-f	14.83 l-q	27.49 a
Canik	89.7 j-r	126.8 i-o	26.9 d-m	29.9 k-r	9.78 abc	8.78 r-v	16.28 e-n	14.64 qr
Nail	92.4 i-q	128.1 h-n	19.3 stu	25.9 q-u	8.78 abc	9.72 l-v	14.50 n-q	14.78 qr
Mean	94.6	132.3	25.3	30.7	9.25	10.32	16.10	19.70

Table 4. continued

Genotype	Yield (kg ha^{-1})		Grade index score (%)		Nicotine content (%)		Glucose content (%)	
	2014**	2015**	2014**	2015**	2014**	2015**	2014**	2015**
2	1483 a	1703 d	71.7 abc	66.7 abc	1.31 mn	0.97 op	6.71 bcd	8.84 b
3	1416 ab	1572 e	73.3 ab	68.3 abc	1.46 hij	1.25 j-m	6.57 c-f	6.18 j-m
5	716 z	1345 j	55.0 e-h	50.0 efg	1.21 o-r	1.22 lmn	5.36 o	6.39 g-m
6	1148 i-l	1273 lmn	63.3 b-f	58.3 c-f	1.33 mn	1.04 op	4.53 q	7.86 cd
7	903 rst	1292 l	71.7 abc	66.7 abc	1.20 o-r	1.22 k-n	5.04 p	6.22 j-m
8	1055 mno	1595 e	71.7 abc	66.7 abc	1.16 qr	1.00 op	6.04 i-m	6.82 e-l
9	953 pqr	857 wx	51.7 fgh	61.7 b-e	1.62 de	1.60 bcd	6.23 g-j	6.75 e-l
10	1064 mno	1194 op	58.3 d-h	53.3 d-g	1.53 fgh	1.23 k-n	6.19 h-l	6.51 g-m
11	920 qrs	1173 pq	78.3 a	73.3 a	1.64 d	1.26 i-m	5.97 j-n	8.67 b
12	1466 a	1398 i	56.7 e-h	51.7 efg	1.19 pqr	1.70 ab	6.07 i-l	6.90 e-j
13	1196 g-j	1836 a	68.3 a-d	63.3 abcd	1.51 f-i	1.10 no	5.88 k-n	6.69 e-m
14	860 s-v	1233 no	78.3 a	73.3 a	2.02 b	1.23 k-n	6.74 bcd	7.08 efg
15	1245 e-h	1297 kl	55.0 e-h	51.7 d-g	1.37 klm	1.36 g-k	6.76 bcd	5.02 op
16	1213 f-i	1427 hi	71.7 abc	66.7 abc	1.50 f-i	1.10 no	6.18 h-l	7.30 cde
17	1115 j-m	1233 no	65.0 b-e	60.0 c-f	1.18 pqr	1.34 g-l	6.72 bcd	6.64 e-m
20	890 r-u	1190 p	78.3 a	73.3 a	1.23 opq	1.24 klm	6.74 bcd	6.01 mn
21	1306 cde	1781 bc	73.3 ab	68.3 abc	1.31 mn	1.27 i-m	6.07 i-l	7.09 efg
22	928 qrs	1808 ab	71.7 abc	66.7 abc	1.98 b	1.50 def	6.02 j-m	7.91 c
23	1110 k-n	1673 d	76.7 a	71.7 ab	1.19 pqr	1.38 f-j	5.75 mn	6.76 e-l
24	1075 l-o	1264 lmn	46.7 h	46.7 g	1.55 efg	1.67 abc	6.01 j-m	6.66 e-m
25	1346 bcd	1355 j	65.0 b-e	60.0 c-f	1.78 c	0.95 p	7.10 a	6.31 h-m
26	1181 h-k	1484 g	73.3 ab	68.3 abc	1.41 jk	1.04 op	6.90 ab	7.36 cde
28	1304 cde	1258 lmn	50.0 gh	51.7 efg	2.17 a	1.40 f-i	4.44 q	5.16 op
29	1366 bc	1439 h	71.7 abc	66.7 abc	1.16 qr	1.28 i-m	6.29 f-j	6.89 e-j
30	755 xyz	939 tu	71.7 abc	66.7 abc	2.19 a	1.22 k-n	6.54 c-g	6.81 e-l
31	887 r-u	983 s	65.0 b-e	60.0 c-f	1.41 jkl	1.39 g-j	5.20 op	6.15 klm
34	1314 cde	977 st	51.7 fgh	60.0 c-f	1.54 fg	1.73 ab	6.33 f-i	4.74 p
35	1202 ghi	885 vw	56.7 e-h	61.7 b-e	1.22 opq	1.55 cde	4.68 q	5.09 op
36	891 r-u	1290 l	58.3 d-h	51.7 d-g	1.33 mn	1.43 e-h	6.76 bcd	6.50 g-m
37	834 t-x	1259 lmn	58.3 d-h	53.3 d-g	1.18 pqr	1.35 g-l	6.69 b-e	6.51 g-m
40	802 v-y	843 x	53.3 e-h	58.3 c-f	1.46 hij	1.73 a	6.19 h-k	7.00 e-h
41	997 opq	1339 j	61.7 c-g	56.7 c-g	1.44 ijk	1.19 mn	6.48 d-h	9.80 a
42	744 yz	1172 pq	58.3 d-h	53.3 d-g	1.53 fgh	1.31 h-m	6.83 abc	6.02 mn
43	792 v-z	1024 r	48.3 h	56.7 c-g	1.37 klm	1.38 f-j	5.37 o	7.08 efg
44	959 pqr	923 uv	51.7 fgh	56.7 c-g	1.33 lmn	1.51 def	5.99 j-m	7.25 def
45	853 s-w	1758 c	71.7 abc	66.7 abc	1.23 opq	1.02 op	7.06 a	5.98 mn
46	994 opq	1135 q	65.0 b-e	60.0 c-f	1.50 f-i	1.27 i-m	6.01 j-m	6.48 g-m
47	1108 k-n	1282 lm	56.7 e-h	48.3 fg	1.27 no	1.47 d-g	5.30 op	5.43 no
48	811 u-y	1244 mn	55.0 e-h	50.0 efg	2.00 b	0.99 op	6.48 d-h	6.87 e-k
49	1275 d-g	1334 jk	51.7 fgh	51.7 efg	1.62 de	1.18 mn	5.01 p	5.24 op
50	770 w-z	890 vw	56.7 d-h	53.3 d-g	1.49 ghi	1.34 g-l	5.16 op	6.27 i-m
51	1292 c-f	889 vw	51.7 fgh	58.3 c-f	1.37 klm	1.25 j-m	6.18 h-l	6.98 e-i
52	1150 i-l	1527 f	61.7 b-g	56.7 c-g	1.57 ef	1.01 op	6.47 d-h	6.12 lm
53	1018 op	1359 j	68.3 a-d	63.3 a-d	1.39 j-m	0.97 op	6.66 b-e	7.04 efg
54	779 v-z	894 vw	56.7 e-h	58.3 c-f	1.38 klm	1.39 f-i	5.69 n	6.81 e-l
55	1245 e-h	1199 op	58.3 d-h	53.3 d-g	1.75 c	1.36 g-k	6.39 e-h	6.76 e-l
56	792 v-z	1348 j	58.3 d-h	50.0 efg	1.14 r	1.74 a	5.87 lmn	6.54 f-m
57	1343 bcd	1573 e	71.7 abc	66.7 abc	1.51 f-i	1.67 abc	6.57 c-f	6.22 j-m
Canik	1029 nop	1358 j	55.0 e-h	51.7 d-g	1.26 nop	1.72 ab	5.08 op	6.48 g-m
Nail	847 s-w	996 rs	56.7 e-h	58.3 c-f	1.28 no	1.18 mn	6.71 bcd	6.81 e-l
Mean	1055	1282	62.5	59.7	1.46	1.31	6.0	6.66

**Means with different letters in each column are significantly different (P<0.01) according to Duncan test

Significant differences were observed for leaf lengths in both years (p<0.01). The leaf lengths of the lines varied between 13.76 and 21.19 cm in 2014 and between 13.5 and 27.5 cm in 2015. Lines 5, 11, 25, and 52 had higher leaf lengths than standard genotypes in both years (Table 4). In other studies with oriental tobaccos conducted under similar conditions, leaf length varied from 15.7 to 18.6 cm (Kurt and Ayan, 2014) and from 24.8 to 25.6 cm (Kurt, 2021). The leaf length values

observed in the present study were similar to the ones reported in those studies. However, the range of leaf length values in the present study (13.7-27.5 cm) was higher, indicating the high variation level among our lines in terms of leaf length.

Tobacco is a crop grown for its dried leaves. Dried leaf yield has been the character that interests plant breeders and tobacco producers. The highest dry leaf yield was obtained from the line 13 as 1836 kg ha-1 in 2015 and the lowest from the line 5 as 716 kg ha-1 in 2014 ($p<0.01$). Twenty of the lines examined in 2014 and 12 in 2015 had higher dried leaf yields than the standards Nail and Canik (Table 4). Since oriental tobacco is grown under rainfed conditions, adaptations of the lines with similar dried leaf yields under both low and high precipitation conditions are better (Kurt et al., 2020). Therefore, lines 2, 3, 12, 13, 16, 21, 26 and 57, which yielded higher dry leaf than the standards in both years, could be stated to have better adaptations to the region than the other lines. In other studies, carried out in the region, leaf yields varied between 940 and 1370 kg ha-1 (Kurt and Ayan, 2014) and between 900 and 1500 kg ha-1 (Camas, 1998). Thus, the dried leaf yields of our lines were higher than those of lines or cultivars used in previous studies, and it could be concluded that the lines evaluated in the present study have the potential for high dried leaf yields and could be useful in future tobacco breeding programs to improve dried leaf yields in changing climate conditions. Oriental tobaccos are known for their superior quality properties (Kinay and Yilmaz, 2016). Physical quality in tobacco is measured using a grade index score. The highest grade index scores were obtained from the lines 11, 14 and 20 as 78.3% in 2014 ($p<0.01$). In 2014, 18 lines had higher grade index scores than the standards, while in 2015, lines 11, 14, 20 and 23 had statistically higher grade index scores than the standards (Table 4). Grade index scores of oriental tobaccos grown in the same region as the present study were reported to vary between 24.2 and 69.3% by Kurt (2021) and between 58 and 80% by Kurt and Ayan (2014). Thus, grade index scores appeared to vary greatly by the genotype used. Oriental tobaccos should have grade index scores of at least 60%. The grade index scores of most lines evaluated in the present study were over 60% in both years, which indicated the satisfactory quality characteristics of the lines evaluated.

The first measure of chemical quality in oriental tobacco is the nicotine content of the dried leaves. The proportions of oriental tobaccos in cigarette blends are determined by the nicotine content of the leaves. The nicotine content of the lines varied between 1.14 and 2.19% in 2014 and between 0.95 and 1.70% in 2015 ($p<0.01$). In the first year, 28 lines had higher nicotine contents than the standard genotypes. In the second year, on the other hand, lines 12, 34, 40 and 56 had nicotine contents similar to Canik standard variety, while 21 lines had higher nicotine contents than Nail standard variety (Table 4). Nicotine contents of dried oriental tobacco leaves were reported to vary from 1.5 to 3.5% by Camas et al. (2009a) and from 2.1 to 3.3% by Yilmaz and Kinay (2011). Kurt (2021) found nicotine contents

of oriental tobaccos as 0.96-2.06% in other regions and 0.47% in Samsun region. Thus, in this region where the same tobacco genotypes had less nicotine contents compared to other regions, nicotine contents of the lines evaluated in the present study were considerably high.

Leaf glucose content is an important measure of chemical quality in tobacco. The leaf glucose contents of the lines varied between 4.44 and 7.10% in the first year and between 4.74 and 9.80% in the second year. In the first year, line 25 had a higher leaf glucose content than Canik local variety while 38 lines had higher leaf glucose contents than Nail. In the second year, on the other hand, lines 2, 6, 11, 16, 22, 26, 41 and 44 had higher leaf glucose contents than the two standard varieties (Table 4). Glucose is one of the most important soluble sugars (Roomer et al., 2012). In general, tobaccos with high sugar contents are considered to be of better quality (Hasebe and Subara, 1999). Leaf glucose content in oriental tobacco leaves were reported to be 2.0% (Ramusino et al., 1994), 2.98% (Kurt, 2021) and 4.2% (Kinay and Yilmaz, 2016). The glucose content values in the present study were higher than those reported in previous studies. The lines examined contained significant variations in leaf glucose content, and most of them had good quality in terms of leaf glucose contents.

A PCA analysis was carried out to show the agronomic variations among 48 pure lines along with Canik and Nail varieties and to determine the proportional importance of each traits within the total variation. The first two principal components (PC) (eigen value greater than one) accounted for 65.8% of the total variability. In the first PC, which explained 47.1% of the total variance, the predominant characters were grade index, leaf length, number of leaves and plant height. PC2, which accounted for 18.7% of the overall variation, explained 18.7.0% of the overall variation. Plant height and number of leaves contributed positively to this PC while leaf width had a negative impact (Figure 4a). All the characters which turned out to be significant in PCA and made considerable contributions were physical characters. The producers, who were the original developers of the local varieties, may have imposed their preferences through physical characters.

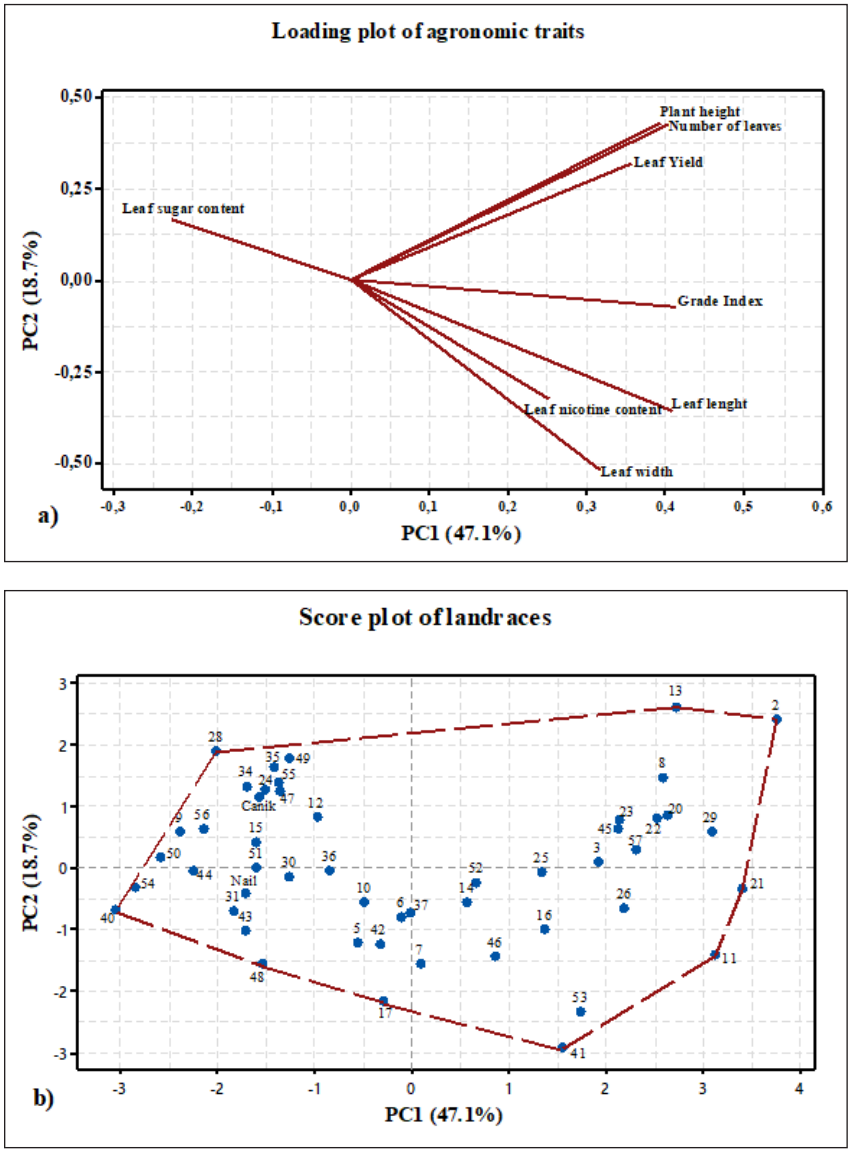


Figure 4. Loading plot (a) and score plot (b) of first two principal component

Therefore, it is an expected situation that physical characters constituted a major part of the overall variation observed among the lines. Indeed, Zakova and Benkova (2006) and Yadav et al. (2018) mentioned that variations in local varie-

ties were in harmony with producer preferences. Score plot was drawn to evaluate together the overall agronomic variations of the genotypes examined (Figure 4b).

The highest values of dried leaf yield and number of leaves were obtained from line 2, while the highest leaf length and grade index score were observed in line 11, the highest nicotine in line 28 and the highest sugar content in line 41. The lowest values were obtained from line 28 for sugar contents, from line 40 for number of leaves, leaf length and dried leaf yield. Lines 17 and 48 had low values for most of the characters evaluated, while line 13 had high values in half of the characters examined, and average values in other half, and line 21 had high values for almost all characters. In a score plot, the most responsive genotypes can be identified by drawing a polygon with endpoints in which genotypes with extreme values are located. These genotypes may be either the best or worst performing ones in some or all locations where they are examined (Yuksel and Akcura, 2012). According to these results, an overall evaluation can be made to reveal the lines with high and low performance for the involved character, or to identify the lines with superior overall performance.

4. CONCLUSION

Evaluation of the genetic variation level has been a major aim in crop improvement. Landraces have always been of great importance for both expanding shrinking genetic variations and selecting high-performance lines. In the present study, tobacco genotypes grown in a specific region were examined for both DNA and agronomic variations. Forty-eight different genotypes were identified among 58 single plants selected based on their appearance. The results showed that there is high variation in the oriental tobacco material used in the tobacco production area of Samsun. High variations were also determined in agronomic characters. The vast majority of the lines studied performed better than the local varieties Canik or Nail for most characters. The results of the present study revealed agronomic variations in the oriental tobacco production material, which is generally known as Samsun type of oriental tobacco. In order to use the variations detected by this study for tobacco breeding, the lines should be evaluated in multiple field trials. Lines 2, 11, 13, 21, 28, and 41 were considered promising due to their superiority in some or all agronomic characteristics. Direct selection of lines with high performance could contribute to the tobacco production in the region where good quality Samsun tobaccos are grown. The present study provides a detailed characterization of Samsun oriental tobacco genotypes for tobacco breeders. The lines examined could be directly used in tobacco production or they can be harnessed as gene donors for agronomic traits to be used in plant breeding programs.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study: DK (25%), AK (25%), IS (25%), NK (25%)

Data Acquisition: DK (35%), AK (35%), IS (30%)

Data Analysis: DK (25%), AK (25%), IS (25%), NK (25%)

Writing up: DK (20%), AK (20%), IS (20%), NK (40%)

Submission and Revision: DK (25%), AK (25%), IS (25%), NK (25%)

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