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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Relationship Between Heterosis and Genetic Distance Determined by SSR Markers in Oriental Tobacco

Oryantal Tütünde SSR Markörleriyle Belirlenen Heterosis ile Genetik Mesafe Arasındaki İlişki

Ahmet KINAY[1*], Ibrahim SAYGILI[2], Nejdet KANDEMIR[3]

Abstract

Hybrid cultivars could be beneficial to achieve high leaf yields while maintaining good quality properties in oriental tobacco. Identification of parents constitutes a major part of a hybrid breeding program. The aim of the present study was to determine the relationship between the genetic distance of parents determined by simple sequence repeats (SSR) markers and heterosis levels in hybrids produced from those parents for leaf yield and quality properties in oriental tobacco. Twenty-one hybrids produced by half diallel crossing among seven tobacco genotypes used in oriental tobacco production in Turkey were grown along with their parents in three locations in 2012 and 2013. Twenty-nine SSR markers were used to determine genetic distances among seven tobacco genotypes. A total of 80 alleles were produced by all twenty-nine markers. Average number of observed alleles per polymorphic marker was 2.96. Twenty-seven of 80 alleles were observed in only one of the seven parents. The polymorphic information content of markers varied from 0.215 to 0.810 (average 0.480). Mid-parent heterosis levels ranged from 18.03 to 42.00% for leaf number, between -19.75 and 38.06% for leaf width, between -17.51 and 36.25% for leaf length, between -34.38 and 76.12% for leaf yield, between -78.30 and 154.01% for sugar content and between -45.40 and 143.29% for nicotine content. Heterosis levels were correlated with genetic distances between parents for leaf number in Erbaa 2012 and Tokat 2012 locations, for leaf width in Erbaa 2013 location, for leaf length in Erbaa 2013 location, and for leaf yield in Tokat 2012 location only. SSR markers were very effective to determine genetic distance of oriental tobacco, and only two markers could distinguish all seven genotypes used in the study. The findings indicated that genetic distance determined by SSR markers used in the present study is not sufficient to predict hybrid performance in oriental tobacco.

Keywords: *Nicotiana tabacum* L., Genetic diversity, Hybrid, Microsatellite markers, Yield

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Öz

Hibrit çeşitler, oryantal tütünde iyi kalite özelliklerini korurken yüksek yaprak verimi elde etmek için faydalı olabilir. Hibrit çeşitleri oluşturacak ebeveynlerin belirlenmesi hibrit çeşit ıslah programlarında önemli bir yer tutmaktadır. Bu çalışmanın amacı, basit dizi tekrarları (simple sequence repeats, SSR) markörleri ile belirlenen ebeveynlerin genetik uzaklığı ile oryantal tütünde yaprak verimi ve kalite özellikleri için bu ebeveynlerden üretilen hibritlerdeki heterozis seviyeleri arasındaki ilişkiyi belirlemektir. Türkiye'de yoğun kullanılan yedi tütün genotipinden ve bu ebeveynlerden yarım diallel melezlemeyle ile elde edilen 21 hibrit ebeveynleriyle ile üç bölgede 2012 ve 2013 yıllarında yetiştirilmiştir. Yedi tütün genotipi arasındaki genetik uzaklıklarını belirlemek için yirmi dokuz SSR markörü kullanılmıştır. Yirmi dokuz markör toplamda 80 allel üretmiştir. Polimorfik markör başına ortalama gözlenen allel sayısı 2.96 olarak belirlenmiştir. Seksen allelin 27'si sadece bir ebeveynde gözlemlenmiştir. Markörlerin polimorfik bilgi içeriği 0.215 - 0.810 (ortalama 0.480) arasında değişmiştir. Ebeveyn ortalamasına göre hesaplanan heterozis değerleri yaprak sayısı için %18.03 - %42.00, yaprak eni için %-19.75 - %38.06, yaprak boyu için %-17.51 - %36.25, yaprak verimi için %-34.38 - %76.12, şeker oranı için %78.30 - 154.01 için ve nikotin içeriği için %-45.40 - %143.29 arasında değişmiştir. Genetik uzaklık ile heterozis arasındaki korelasyon, yaprak sayısında Erbaa 2012 ve Tokat 2012, yaprak eninde Erbaa 2013, yaprak boyunda Erbaa 2013 ve yaprak veriminde Tokat 2012 lokasyonlarında önemli bulunurken, diğer lokasyonlarda önemli bulunmamıştır. Çalışmada kullanılan yedi genotipi yalnızca iki markörün ayırt edebilmesinden dolayı, SSR markörlerinin oryantal tütünün genetik uzaklıkları belirlemek için çok etkili olduğu söylenebilir. Bu araştırmada kullanılan SSR markörleriyle belirlenen genetik uzaklık değeri doğrudan hibrit performansı tahmin etmek için yeterli olmamıştır.

Anahtar Kelimeler: *Nicotiana tabacum* L., Genetik çeşitlilik, Hibrit, Mikrosatellit markör, Verim

1. Introduction

Heterosis is a phenomenon producing higher performance in all crops irrespective of their pollination biology. It is commonly used in the improvement of cross-pollinating crops. Recently, it also has been a focus of attention in the breeding of self-fertilizing crops (Adhikari et al., 2020; Farooq et al., 2024). In classical hybrid breeding programs, the determination of parent combinations with the best performance is a tedious and costly procedure that can take years. It was assumed that the best way of exploiting heterosis was through the use of genetically diverse parents (Tomkowiak et al., 2020) and that molecular markers could be a useful tool to estimate heterosis levels (Marcón et al., 2019)

Tobacco is one of the most important non-food crops. The quality of cigarettes is mainly determined by the quality of tobaccos used in their blends (Xiao et al., 2007). Oriental tobaccos are used as regulators in cigarette blends throughout the world for their balanced nitrogen/carbohydrate contents and high aroma levels (Kinay and Kurt, 2022). Unlike their qualities, yields of oriental tobaccos are low. The use of hybrid cultivars could provide a way to increase leaf yields without lowering their quality (Kinay et al., 2020).

SSR markers have been used to reveal genetic diversity in different tobacco types. Through using SSR markers, He et al. (2020), determined genetic diversity levels in Virginia tobaccos, Davalieva et al. (2010), in burley tobaccos, and Kurt et al. (2022; 2023), in oriental tobaccos. He et al. (2020), found that 91 SSR markers produced 304 alleles on 33 flue-cured tobacco genotypes. Yongliang et al. (2020), studied 33 tobacco cultivars with 22 SSR markers and found a total of 81 alleles. In another study by Darvishzadeh et al. (2014), on the other hand, a polymorphism ratio of 100% was found in 13 SSR markers studied on 70 oriental tobacco genotypes. Examining 29 markers in 319 tobacco genotypes collected from different regions of Turkey (Saygili et al., 2022), observed polymorphism for 86% of the markers and an average of four alleles per marker. Therefore, SSR markers could efficiently reveal high genetic diversity levels found in cultivated tobacco.

It has been proposed that genetic distance determined by DNA markers could be used to estimate heterosis. Heterosis was estimated by genetic distance based on DNA markers in cotton (Geng et al., 2021), corn (Palaniyappan et al., 2023), cabbage (Dong et al., 2021) and sunflower (Buti et al., 2013), However, there are also reports that heterosis was not associated with genetic diversity of parents in rapeseed (Tian et al., 2017), wheat (Liu et al., 1999), soybean (Cerna et al., 1997), corn (Dermail et al., 2020) and rice (Kwon et al., 2002). Two studies conducted on wheat, on the other hand, found that the genetic distance was correlated with heterosis, but was not enough to determine the best parental combinations (Corbellini et al., 2002; Dreisigacker et al., 2005). The level of correlation between genetic distance calculated by molecular markers and heterosis could vary depending upon marker type (Solomon et al., 2012) and genetic material (Kwon et al., 2002).

Hybrid breeding has been the topic of different studies. Along with new developments that allow a better understanding of the heterosis phenomenon, there have been recent efforts to facilitate hybrid breeding in self-pollinating crops. Determining the best parental combinations constitutes a major part of hybrid breeding programs. Conventionally, parents of hybrids are determined based on top-crosses and diallel crosses which take many years to produce and evaluate in field trials. A possible relationship between genetic distance and heterosis could make it easier to identify the best parental combinations for superior hybrid cultivars. The present study was carried out to examine the possible relationship between genetic distance determined by SSR markers and heterosis levels for yield and quality traits in hybrids produced between seven oriental tobacco genotypes commonly grown in Tokat-Central, Tokat-Erbaa, and Samsun-Bafra regions of Turkey.

2. Materials and Methods

2.1. Plant material

Seven tobacco cultivars/lines in traditional oriental tobacco areas in Northern Turkey and 21 F_1 hybrids produced by half diallel crosses among them were used. Detailed plant characteristics of genotypes are available in (Peksüslü et al., 2012) and (Kinay et al., 2019). Parents and hybrid combinations used in the study were given in *Table 1*. Hybrid seeds were produced on greenhouse-grown plants based on methods described by Wernsman and Matzinger (Wernsman and Matzinger, 1980). Hybrid seeds were planted in viols and seedlings were produced in a greenhouse.

Table 1. Parents and hybrids used in the study

No	Parents/Hybrids	No	Parents/Hybrids
1	Xanthi-2A	15	Nail x Taşova
2	Nail	16	Nail x Katerini
3	Gümüshacıköy	17	Nail x Canik
4	Tasova	18	Nail x Erbaa
5	Katerini	19	Gümüshacıköy x Taşova
6	Canik	20	Gümüshacıköy x Katerini
7	Erbaa	21	Gümüshacıköy x Canik
8	Xanthi-2A x Nail	22	Gümüshacıköy x Erbaa
9	Xanthi-2A x Gümüshacıköy	23	Taşova x Katerini
10	Xanthi-2A x Taşova	24	Taşova x Canik
11	Xanthi-2A x Katerini	25	Taşova x Erbaa
12	Xanthi-2A x Canik	26	Katerini x Canik
13	Xanthi-2A x Erbaa	27	Katerini x Erbaa
14	Nail x Gümüshacıköy	28	Canik x Erbaa

2.2. Field trials

Field trials were conducted in Tokat, Erbaa, and Bafra locations in the 2012 and 2013 growing periods. Climatic data of Tokat, Erbaa, and Bafra locations in experimental years were given in *Table 2*.

Table 2. Climatic datas of field trial years and long term

Months	Years	Average temperature (°C)			Average relative humidity (%)			Total precipitation (mm)		
		Tokat	Erbaa	Bafra	Tokat	Erbaa	Bafra	Tokat	Erbaa	Bafra
May	1975-2011	18.5	17.8	15.4	53.8	60.7	78.6	73.5	54.2	46.3
	2012	17.6	18.1	17.4	62.6	68.5	89.0	114.7	63.4	28.6
	2013	19.4	19.7	18.5	53.1	66.0	84.1	32.3	25.9	28.8
June	1975-2011	21.1	21.8	20.1	49.8	58.1	74.2	36.2	42.1	44.9
	2012	21.4	22.0	22.4	56.5	67.9	79.7	36.3	25.4	69.8
	2013	20.8	21.2	21.5	52.4	63.4	81.1	36.1	21.1	43.6
July	1975-2011	22.7	23.5	22.8	49.1	55.3	72.5	16.2	17.5	29.8
	2012	23.6	24.3	24.2	54.6	64.2	81.5	30.7	19.6	116.8
	2013	21.9	23.0	23.3	51.0	62.0	81.4	1.6	10.1	48.0
August	1975-2011	23.1	23.3	22.7	47.4	56.0	73.9	1.0	8.6	44.4
	2012	23.4	23.8	23.2	51.8	58.3	82.8	1.5	5.4	209.8
	2013	22.8	23.5	23.8	49.3	63.9	87.8	0.4	30.7	66.8
September	1975-2011	20.1	20.1	19.2	54.1	58.3	76.3	8.7	14.2	58.5
	2012	20.3	20.6	20.5	50.9	62.1	85.8	5.1	15.3	78.2
	2013	19.8	18.7	18.9	47.8	69.6	82.8	12.3	16.5	37.2

*Turkish State Meteorological Service

Soil samples were taken from the upper 30 cm of experimental soils. Five samples were taken in each location. Soil samples were analyzed in Tokat Gaziosmanpasa University (TOGU) Faculty of Agriculture Soil Science laboratories. Soil analysis results were given in *Table 3*. Field trials were carried out in Erbaa county of Tokat province (latitude 40º60′ N, longitude 36º62′ E, elevation 580 m), Bafra county of Samsun province (latitude 41º03′ N, longitude 35º04′ E, elevation 162 m), and central of Tokat province (latitude 39º51′ N, longitude 35º27′ E, elevation 623 m).

Table 3. Properties of experimental locations and soils in Tokat, Erbaa and Bafra

	2012			2013		
	Tokat	**Erbaa**	**Bafra**	**Tokat**	**Erbaa**	**Bafra**
Altitude (m)	623	580	162	623	580	162
Latitude	39º51′	40º60′	41º03	39º51′	40º60′	41º03
Longitude	35º27	36º62	35º04	35º27	36º62	35º04
Texture	Clayed loam	Sandy clayed loam	Clayed	Clayed	Clayed	Clayed
pH	8.01 Slightly alkaline	7.87 Slightly alkaline	8.05 Slightly alkaline	7.10 Neutral	6.98 Neutral	8.34 Moderately alkaline
EC (dS/m)	0.29	0.23	0.57	0.80	1.10	0.48
$CaCO_3$ (%)	14.33 Rich	10.66 Rich	20.87 Excess	3.93 Moderate	3.71 Moderate	4.6 Moderate
Organic matter (%)	6.24 High	4.41 High	2.03 Moderate	3.86 Good	1.82 Insufficient	3.8 Good
P_2O_5 (kg/ha)	31.4 Insufficient	35.5 Insufficient	23.8 Very insufficient	73.5 Insufficient	93.8 Sufficient	175.0 Sufficient
K_2O (kg/ha)	589.4 Sufficient	429.8 Sufficient	502.4 Sufficient	309.9 Sufficient	491.4 Sufficient	471.9 Sufficient

Plant stands were established via seedling transplanting to fields. Planting dates of seedlings in field conditions for each trial were given in *Table 4*. Field trials were carried out in Randomized Complete Blocks Design with three replications. Each plot consisted of four rows of 4 m long. A 1 m space was left between plots and 1.5 m between blocks. Based on local tobacco growing practices, planting density was 45 x 12 cm in Tokat and Erbaa while 50 x 12 cm in Bafra. Experimental soils were fertilized with 60 kg/ha nitrogen, 40 kg/ha phosphorus (P_2O_5), and 60 kg/ha potassium (K_2O) **(**Kinay and Kurt, 2022**)**. All fertilizers were given to soil-prepared plots just before planting. During the growing period, maintenance procedures such as hoeing and disease/pest management practices were carried out. Field trials were conducted under rainfed conditions (no irrigation). Leaves that reached harvest maturity were harvested on dates given in *Table 4*.

During the vegetation period, leaf width, length, and number measurements were made on ten plants per plot (Saygili et al., 2021). Leaves were put on strings manually in the Tokat and Erbaa locations, while a machine was used for this purpose in the Bafra location. Stringed leaves were wilted for one or two days and taken to drying areas. Dried leaves were weighed and leaf yields were determined on a 17% moisture basis. Samples were taken from the second main hands for reducing sugar and nicotine assays (Kinay and Kurt, 2022), which were carried out in the Analysis Laboratory of Field Crops Department of Tokat Gaziosmanpasa University Faculty of Agriculture.

Table 4. Planting and harvest dates of field trials

Locations	**Planting dates**		**Harvest dates**					
	2012	**2013**	**2012**			**2013**		
			First	**Second**	**Third**	**First**	**Second**	**Third**
Erbaa	12 May	06 May	25 June	14 July	04 Aug.	26 June	14 July	15 Aug.
Bafra	22 May	11 May	12 July	27 July	13 Aug.	27 June	16 July	13 Aug.
Tokat	16 May	08 May	28 June	17 July	02 Aug.	28 June	12 July	07 Aug.

2.3. DNA extraction and polymerase chain reaction

DNA marker analyses were conducted in the Agricultural Biotechnology Laboratories of TOGU, Faculty of Agriculture. Genomic DNA was isolated from plants with 3-5 leaves using Turkuaz DNA preparation kit (Keskin et al., 2014). The quality and quantity of isolated DNAs was measured using 1% agarose gels and

spectrophotometer (Thermo, Biomate 3). DNA concentration was adjusted to 50 ng/µl. PCR was carried out in 40 µl volumes. PCR reaction contained 1X PCR buffer, 250 nM of each primer, 0.2 mM of each nucleotide, 1.5 mM $MgCl_2$, 50–100 ng genomic DNA, and 0.5 units Taq-DNA polymerase (Biobasic). A typical PCR procedure was as follows: 5 minutes at 94 °C, 40 cycles of 30 seconds at 94 °C, 30 seconds at 50-60 °C (depending on the primer annealing temperature), 30 seconds at 72 °C, and 5 minutes at 72 °C. Amplicons of SSR markers were run on a 3% Metaphor Agarose gel (Lonza cat no: 50180) using 1xTBE buffer in 90 V for at least two hours or more depending on amplicon size and differences among alleles. Amplicons were visualized on a gel imaging system (Vilber Lourmat CN-08) through ethidium bromide (10%) added to the gel (Bahar et al., 2019).

2.4. SSR markers

A total of 29 SSR markers were studied. Primers with PT prefix used in the study were selected among primers reported by Moon et al. (2009), Bindler et al. (2007), or Bindler et al. (2011), while the ones with TM and TME prefixes were selected from Tong et al. (2012) based on high Polymorphism Information Content (PIC) values (≥0.7). PIC is a measure of the informativeness of the DNA markers for genetic characterization (Serrote et al., 2020). Higher PIC value means the better utilizing the discriminatory power of a markers.

2.5. Data analysis

Bands were analyzed using BioCapt v.11.02 software. Dendrogram was prepared using the UPGMA algorithm in POPGENE v.1.32 (Yeh et al., 2000). Genetic distance between the lines was calculated according to Nei (1972). SSR marker polymorphism rates were determined using PIC values, which were calculated according to the following formula: $PIC = 1 - \sum P^2_i$ where P_i is the frequency of i^{th} allele (Anderson et al., 1993). Alleles with frequencies of less than 5% were defined as rare alleles (He et al., 2020). Since only seven genotypes were used in the present study, alleles found in only one genotype were considered as rare allele. Heterosis was calculated as mid-parent heterosis using the following formulae: MPH = [Vh - (Vp+Vm)/2] / (Vp+Vm)/2*100, where Vh: hybrid's value for a trait, Vp: paternal value, Vm: maternal value (Lamkey and Edwards, 1997).

3. Results and Discussion

Twenty-nine SSR markers were used to determine genetic distances between seven tobacco genotypes. Twenty-six of the markers were polymorphic and three were monomorphic (*Table 5*). Thus, the polymorphism rate was 90% (26/29). A polymorphic PT30274 marker profile was given in *Figure 1a*. Number of alleles produced by each marker was given in *Table 5*. One marker produced six alleles, while two markers produced five alleles, three markers four alleles, nine markers three alleles, eleven markers two alleles, and three markers one allele (*Table 5*). Thus, totally 80 alleles were produced by all twenty-nine markers. Average number of alleles per marker was 2.76 (mean for polymorphic markers were 2.96). Thirty percent of 80 alleles (27) were observed in only one of seven parents. PIC values of polymorphic markers varied from 0.215 to 0.810 (average 0.480).

A dendrogram was prepared based on SSR marker data. Three main groups appeared in dendrogram. Erbaa genotype was clearly different from others and constituted a group alone, while Katerini and Tasova genotypes were close to each other and formed the second group. The other four genotypes grouped together, making the third group (*Figure 1b*). Genetic distances of genotypes relative to each other were determined based on Nei's coefficient (Nei, 1972). The highest genetic distance (1.639) was observed between Katerini and Erbaa genotypes, followed by Erbaa and Nail (1.476) and Erbaa and Gümüshaciköy (1.252). The lowest value was obtained between Tasova and Katerini (0.637). Erbaa genotype had the highest genetic distances from other genotypes, which meant that it carries alleles different from other genotypes grown in the region.

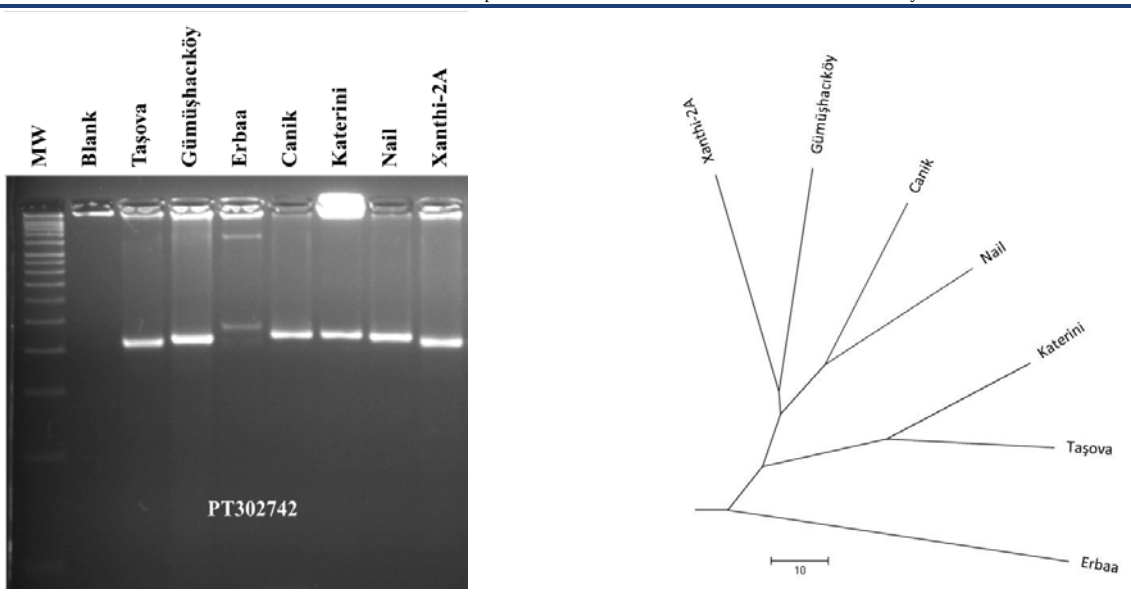
Twenty-six of the 29 SSR markers studied were informative. Even just two SSR markers (PT20172 and PT30265 or PT30274) yielded enough information to distinguish all parents studied. The polymorphism rate of 90% observed in the present study is higher than the 80% reported by Davalieva et al. (2010) for 10 tobacco cultivars including Virginia, burley, and oriental tobacco types using 30 SSR markers. In previous studies polymorphism rate values from 80 to 100% (Gholizadeh et al., 2012; Kurt et al., 2023; Saygili et al., 2021). On tobacco, reported average allele numbers of SSR markers ranged from 2.69 to 3.68 (Gholizadeh et al., 2012; He et al., 2020). The average allele number of polymorphic markers in this study (2.9) was similar to or slightly lower than what was reported in previous studies. Considering the genotype numbers in other studies, the allele numbers determined in only seven genotypes in the present study showed that genetic diversity is high among the genotypes.

Preferred SSR markers proved to be useful in determining the existing diversity. PIC value, which expresses the discriminatory power of SSR markers (Serrote et al., 2020), is an important criterion for selecting markers to be used for genetic diversity analyses. The markers used in the present study were selected among the markers with high PIC values. This is why the PIC values were high despite the small number of genotypes in the present study. In the studies dealing with genetic variation in tobacco, PIC values ranged from 0.39 to 0.781 (Davalieva et al., 2010; Saygili et al., 2021; Tong et al., 2012). Thus, a high level of genetic variation was observed in the present study. In addition, the power of SSR markers as a tool to distinguish tobacco genotypes was also confirmed.

Table 5. Information about SSR markers used

SSR	Annealing Temperature (°C)	Repeat motifs	Observed allele number	Rare allele number	PIC	Polymorphism
PT20172	55	CTT	6	5	0.810	Polymorphic
PT20242	55	AGG	3	1	0.530	Polymorphic
PT30034	55	TAA	4	2	0.641	Polymorphic
PT30099	55	CGA	4	3	0.502	Polymorphic
PT30114	55	TA	3	1	0.530	Polymorphic
PT30132	55	TA	2	1	0.215	Polymorphic
PT30137	55	TAA	2	0	0.370	Polymorphic
PT30159	55	TA	2	1	0.215	Polymorphic
PT30265	55	TA	5	3	0.740	Polymorphic
PT30274	55	GAA	5	3	0.740	Polymorphic
PT30350	55	TAA	3	0	0.580	Polymorphic
PT30364	58	TAA	3	0	0.580	Polymorphic
PT30375	55	TAA	4	1	0.685	Polymorphic
PT30392	55	TAA	3	1	0.502	Polymorphic
PT30449	55	TA	3	0	0.580	Polymorphic
PT40005	55	GAA	2	1	0.215	Polymorphic
PT40015	55	GA	2	0	0.325	Polymorphic
PT50182	55	TA	2	1	0.215	Polymorphic
PT53303	55	GA	1	0	0.000	Monomorphic
PT61056	55	TA	3	1	0.502	Polymorphic
TM10013	60	ATA	2	0	0.370	Polymorphic
TM10181	60	AGA	3	0	0.534	Polymorphic
TM10211	60	ACA	2	0	0.370	Polymorphic
TM10654	60	TA	2	1	0.530	Polymorphic
TM10821	60	TA	2	0	0.370	Polymorphic
TM10976	57	AAT	3	1	0.502	Polymorphic
TM11110	60	AAC	2	0	0.370	Polymorphic
TM11359	60	ACA	1	0	0.000	Monomorphic
TME0293	60	TCA	1	0	0.000	Monomorphic

The number of leaves, leaf width and length, dried leaf yield, sugar, and nicotine contents of dried leaves were determined in 21 hybrids developed in a half-diallel crossing program among seven parents. Percent heterosis values calculated based on mid-parent heterosis were given in *Table 6-11*. Mid-parent heterosis levels calculated in each location varied based on different agronomic traits studied. Heterosis levels of crosses varied from -18.03 to 42.00% (average -1.50%) for leaf number (*Table 6*), from -19.75 to 38.06% (average 8.43%) for leaf width (*Table 7*), from -17.51 to 36.25% (average 5.65%) for leaf length (*Table 8*), from -34.38 to 76.12% (average 20.46%) for dried leaf yield (*Table 9*), from -78.30 to 154.01% (average -6.02%) for sugar content (*Table 10*) and from -45.40 to 143.29% (average 3.82%) for nicotine content (*Table 11*). These results meant that heterosis values varied considerably by environment.



(a) (b)

Figure 1. (a) The SSR marker profile of genotypes using PT30274 marker. The first lane shows molecular weight marker (50 bp). Length of bands in Taşova is 212 bp, Gümüşhacıköy and Nail 215 bp, Erbaa 239 bp, Canik and Katerini 218 bp, Xanthi-2A 209 bp.; (b) Dendrogram showing genetic distances among parents based on 29 SSR markers.

Table 6. Mid-parent heterosis levels of different hybrids (%) in six growing environments for leaf number

Hybrid	Genetic distance	Leaf number						
		E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	0.974	-1.91	-11.7	-0.45	-11.24	-7.27	-5.76	-6.39
Xanthi-2A X Gümüşhacıköy	0.792	-2.34	10.89	-2.19	8.69	0.31	1.53	2.82
Xanthi-2A X Taşova	1.061	2.82	-1.11	-6.23	-1.06	0.95	-3.36	-1.33
Xanthi-2A X Katerini	0.956	-0.79	-5.35	0.86	-4.04	-3.98	-9.09	-3.73
Xanthi-2A X Canik	0.773	-2.5	3.59	6.84	6.11	-4.81	-2.5	1.12
Xanthi-2A X Erbaa	0.997	10.68	14.41	26.55	-3.48	13.79	2.33	10.7
Nail X Gümüşhacıköy	0.657	-11.9	-5.62	8.83	-7.64	-14.84	-2.14	-5.55
Nail X Taşova	0.879	-5.99	-7.31	5.81	-14.33	-12.25	-5.48	-6.59
Nail X Katerini	1.08	0.3	-6.13	1.79	-17.73	-7.94	-11.4	-6.85
Nail X Canik	0.639	-10.14	-6.65	2.53	-10.32	-8.97	-1.51	-5.84
Nail X Erbaa	1.476	1.84	-7.12	10.7	-10.43	10.2	-2.43	0.46
Gümüşhacıköy X Taşova	1.331	0.59	-6.9	-1.76	5.14	-3.71	1.96	-0.78
Gümüşhacıköy X Katerini	1.08	-13.63	1.63	-10.84	-7.66	-9.11	-7.24	-7.81
Gümüşhacıköy X Canik	1.08	-7.58	-0.17	-8.25	13.89	-10.65	4.8	-1.33
Gümüşhacıköy X Erbaa	1.252	6.7	9.18	10.65	1.99	10.44	3.05	7
Taşova X Katerini	0.619	-10.02	-3.61	-13.54	-12.72	-5.59	-13.84	-9.89
Taşova X Canik	0.956	-7.89	0.39	-6.64	6.87	-4.04	-8.63	-3.32
Taşova X Erbaa	1.169	5.25	7.14	16.45	3.39	19	5.1	9.39
Katerini X Canik	1.179	-8.4	-0.29	-10.34	-14.04	-11.69	-9.84	-9.1
Katerini X Erbaa	1.639	-1.51	0.96	-7.51	-18.03	4.74	-5.28	-4.44
Canik X Erbaa	1.051	14.26	3.68	25.36	4.68	16.3	-4.96	9.89
Average	1.03	-2.01	-0.48	2.32	-3.9	-1.39	-3.56	-1.5

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Table 7. Mid-parent heterosis levels of different hybrids (%) in six growing environments for leaf width

Hybrid	Leaf width						
	E12	**E13**	**B12**	**B13**	**T12**	**T13**	**Ave.**
Xanthi-2A X Nail	-1.32	-1.62	28.94	19.02	6.48	15.22	11.12
Xanthi-2A X Gümüşhacıköy	9.42	-2.21	15.9	17.84	6.22	15.18	10.39
Xanthi-2A X Taşova	-2.51	-7.27	-18	-3.57	2.19	-10.36	-6.58
Xanthi-2A X Katerini	20.73	0.18	25.56	14.57	7.34	9.93	13.05
Xanthi-2A X Canik	10.44	-7.35	32.35	3.62	6.15	6.77	8.67
Xanthi-2A X Erbaa	8.02	2.39	8.61	5.04	0.4	5.01	4.91
Nail X Gümüşhacıköy	2.97	-2.22	22.4	11.77	11.83	-0.69	7.68
Nail X Taşova	-10.45	-1.36	2.56	7.24	3.94	7.95	1.65
Nail X Katerini	12.26	0	38.06	3.01	12.69	19.53	14.26
Nail X Canik	2.38	-6.37	22.79	1.4	10.79	19.04	8.34
Nail X Erbaa	1.96	11.66	19.93	17.67	7.67	18.1	12.83
Gümüşhacıköy X Taşova	-3.3	4.54	14.07	14.36	3.51	12.13	7.55
Gümüşhacıköy X Katerini	17.71	1.93	19.35	6.45	8.04	8.43	10.32
Gümüşhacıköy X Canik	17	1.58	9.41	2.43	5.41	3.71	6.59
Gümüşhacıköy X Erbaa	14.71	10.88	28.81	23.78	5.53	16.87	16.76
Taşova X Katerini	-0.28	-0.03	7.67	7.7	12.03	13.98	6.85
Taşova X Canik	-2.37	-9.42	-14.1	8.07	7.83	4.67	-0.89
Taşova X Erbaa	1.68	5.05	0.34	8.51	4.43	-5.35	2.44
Katerini X Canik	19.37	10.75	27.16	13.21	19.13	29.14	19.79
Katerini X Erbaa	20.85	6.13	24.14	0.36	7.53	15.38	12.4
Canik X Erbaa	22.62	-0.95	19.79	2.4	4.44	5.38	8.95
Average	7.71	0.77	15.99	8.81	7.31	10	8.43

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Table 8. Mid-parent heterosis levels of different hybrids (%) in six growing environments for leaf length

Hybrid	Leaf length						
	E12	**E13**	**B12**	**B13**	**T12**	**T13**	**Ave.**
Xanthi-2A X Nail	-1.16	-6.14	23.48	8.84	4.48	6.8	6.05
Xanthi-2A X Gümüşhacıköy	11.55	-5.83	10.78	7.65	7.97	9.24	6.89
Xanthi-2A X Taşova	-2.01	-8.7	-17.5	0.19	-2.46	-10.28	-6.8
Xanthi-2A X Katerini	20.08	-0.95	26.62	10.3	7.17	8.19	11.9
Xanthi-2A X Canik	6.41	-4.82	-2.7	0.54	3.34	6.57	1.56
Xanthi-2A X Erbaa	13.93	0.77	11.15	1.03	1.78	-1.05	4.6
Nail X Gümüşhacıköy	4.16	-5.94	20.63	8.42	10.44	-4.43	5.55
Nail X Taşova	-7.57	-5.91	2.5	4.21	3.07	6.93	0.54
Nail X Katerini	10.44	-2.99	36.25	1.12	12.21	14.63	11.94
Nail X Canik	-1.05	-6.09	18.22	-0.09	7.57	15.24	5.63
Nail X Erbaa	-2.66	0.63	14.55	7.22	6.15	10.05	5.99
Gümüşhacıköy X Taşova	-5.8	-0.41	12.47	9.71	2.21	5.56	3.96
Gümüşhacıköy X Katerini	15.96	-0.91	13.83	9.56	7.34	9.99	9.3
Gümüşhacıköy X Canik	6.71	-2.22	1.64	-0.66	3.19	1.35	1.67
Gümüşhacıköy X Erbaa	15.6	7.87	25	21.24	8	11.44	14.86
Taşova X Katerini	-2.26	-2.55	10.08	4.11	6.37	14.31	5.01
Taşova X Canik	-5.15	-7.86	-13	4.45	2.69	3.77	-2.51
Taşova X Erbaa	5.13	0.22	2.55	6.66	3.74	-9.27	1.5
Katerini X Canik	8.87	2.63	19.12	3.01	12.88	23.11	11.6
Katerini X Erbaa	24.18	2.4	20.63	4.76	9.07	16.55	12.93
Canik X Erbaa	16.71	-1.06	14.98	-1.14	4.16	5.49	6.53
Average	6.29	-2.28	11.97	5.29	5.78	6.87	5.65

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Leaf width and length are other traits with relatively high heterosis levels (8.43 and 5.65%, respectively). Nicotine content and leaf number had relatively low levels of heterosis, while the sugar content of dried leaves had negative heterosis (-6.02%). Since oriental tobaccos are mainly used to improve the quality of tobacco blends, quality is of primary importance in oriental tobaccos. Sugar content is an important quality trait in tobacco. Higher sugar content means higher quality. Despite relatively stable sugar and nicotine contents in oriental tobacco hybrids, dried leaf yields increased by about 20% in hybrids. These findings clearly showed the importance of heterosis to increase tobacco yields without deteriorating the quality.

Heterosis levels of hybrids for different traits varied considerably based on locations and years. Such a variation implies that hybrids could have high performance under specific conditions. Of the different traits studied, the highest heterosis level was obtained for dried leaf yield (20.46%). Similarly, other studies found that leaf yields had the highest heterosis levels of all traits studied (Butorac et al., 2004). Besides, the highest heterosis levels were obtained for yield per area in other crops such as wheat (Gimenez et al., 2021), rapeseed (Ali et al., 1995; Rao et al., 2023), corn (Dermail et al., 2020), and sunflower (Buti et al., 2013). These results indicate that heterosis could produce higher yields in all crops whether they are self or cross-pollinating.

Table 9. Mid-parent heterosis levels of different hybrids (%) in six growing environments for dried leaf yield

Hybrid	Dried leaf yield						
	E12	**E13**	**B12**	**B13**	**T12**	**T13**	**Ave.**
Xanthi-2A X Nail	-2.69	10.47	36.85	-3.95	17.34	14.08	12.02
Xanthi-2A X Gümüşhacıköy	-0.93	20.49	76.12	23.75	33.76	41.33	32.42
Xanthi-2A X Taşova	-0.95	1.49	-10.3	15.63	3.32	-9.16	0
Xanthi-2A X Katerini	25.8	14.98	12.31	25.79	22.88	23.38	20.86
Xanthi-2A X Canik	5.73	9.96	-5.17	7.84	3.6	19.39	6.89
Xanthi-2A X Erbaa	-0.64	4.59	41.09	16.23	46.62	12.91	20.13
Nail X Gümüşhacıköy	16.68	6.19	50.57	3.4	28.76	21.61	21.2
Nail X Taşova	-9.56	19.14	10.49	9.73	11.63	20.64	10.35
Nail X Katerini	3.45	3.86	53.87	21.52	19.97	28.07	21.79
Nail X Canik	-3.55	20.99	14.65	26.45	52.28	42.49	25.55
Nail X Erbaa	1.87	10.95	42.21	9.93	59.23	34.85	26.5
Gümüşhacıköy X Taşova	7.06	20.6	43.63	32.15	37.71	41.63	30.47
Gümüşhacıköy X Katerini	21.04	14.04	43.56	8.57	48.18	23.02	26.4
Gümüşhacıköy X Canik	29.52	32.32	1.53	20.83	54.21	36.28	29.12
Gümüşhacıköy X Erbaa	17.58	27.84	26.88	18.58	69.22	38.37	33.08
Taşova X Katerini	2.33	2.79	-18.1	62.98	8.3	33.2	15.25
Taşova X Canik	-0.42	1.94	-34.4	17.42	31.98	21.88	6.4
Taşova X Erbaa	30.33	-0.72	22.15	-5.6	41.86	-14.79	12.21
Katerini X Canik	13.47	5.13	15.84	50.92	17.04	24.81	21.2
Katerini X Erbaa	8.9	14.61	32.79	19.78	60.37	24.33	26.8
Canik X Erbaa	31.33	15.72	53.79	14.86	63.07	7.21	30.99
Average	9.35	12.26	24.3	18.9	34.82	23.12	20.46

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Correlation coefficients between genetic distances and heterosis levels in different locations were given in *Table 12*. Significant positive correlations were found between genetic diversity levels of parents and heterosis of hybrids for leaf number in the Erbaa 2012 ($P<0.05$) and the Tokat 2012 ($P<0.05$) locations. Heterosis for leaf width was positively and highly significantly ($P<0.01$) correlated with the genetic diversity level of parents in the Erbaa 2013 location. Heterosis level for leaf length had a highly significant positive correlation ($P<0.01$) with genetic diversity only in the Erbaa 2013 location. Dried leaf yields were positively correlated with genetic diversity only in the Tokat 2012 location ($P<0.05$). Sugar and nicotine contents of dried leaves, on the other hand, had no significant correlations with genetic diversity in any of the locations.

Table 10. Mid-parent heterosis levels of different hybrids (%) in six growing environments for sugar content in dried leaves

Hybrid	Sugar content in dried leaves						
	E12	**E13**	**B12**	**B13**	**T12**	**T13**	**Ave.**
Xanthi-2A X Nail	18.74	36.73	-51.2	-10.4	9.04	4.65	1.26
Xanthi-2A X Gümüşhacıköy	10.99	58.18	-33.2	31.34	59.16	-9.32	19.53
Xanthi-2A X Taşova	11.41	-24.28	-7.71	30.89	71.28	-7.05	12.42
Xanthi-2A X Katerini	23.26	30.45	-32.3	5.97	-0.45	-7.21	3.28
Xanthi-2A X Canik	11.25	-6.07	154	-14.23	18.41	-0.21	27.19
Xanthi-2A X Erbaa	14.09	10.23	31.36	-13.67	17.49	-18.66	6.81
Nail X Gümüşhacıköy	-16.2	5.07	-77	-2.13	-25.5	-57.47	-28.87
Nail X Taşova	-2.65	25.34	-37.5	-1	-26.01	25.94	-2.65
Nail X Katerini	-19.61	-31.08	-46.7	-24.97	-69.59	20.47	-28.58
Nail X Canik	-1.13	2.82	-6.74	-40.53	-70.61	3.94	-18.71
Nail X Erbaa	22.7	5.45	-9.47	-5.77	-78.3	2.96	-10.4
Gümüşhacıköy X Taşova	9.61	18.26	-37.6	12.08	36.64	-25.41	2.26
Gümüşhacıköy X Katerini	3.33	-7.56	-61.6	1.6	-27.26	-47.53	-23.17
Gümüşhacıköy X Canik	9.96	18.19	-17.9	-37.17	-44.04	27.89	-7.18
Gümüşhacıköy X Erbaa	3.83	-18.37	-36.8	-30.19	-26.65	-2.64	-18.47
Taşova X Katerini	-4.97	-1.95	-40.7	6.53	-28.28	-36.06	-17.56
Taşova X Canik	69.62	-18.02	11.17	-15.71	-16.78	-55.3	-4.17
Taşova X Erbaa	45.82	-1.88	-30.6	66.03	12.55	-24.02	11.32
Katerini X Canik	-0.61	-16.93	-52.2	-33.21	-16.34	-29.76	-24.84
Katerini X Erbaa	10.32	-5.4	-64.6	-32.73	-20.51	-16.6	-21.59
Canik X Erbaa	26.7	-7.97	-7.64	17.87	-18.28	-36.21	-4.25
Average	11.74	3.39	-21.7	-4.26	-11.62	-13.7	-6.02

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Molecular markers have been commonly searched as a possible tool to estimate hybrid performance in various crops (Dermail et al., 2020; Dong et al., 2021; Geng et al., 2021; Palaniyappan et al., 2023). In the present study, genetic distance was significantly associated with heterosis levels for leaf number in the Erbaa 2012 and Tokat 2012 locations, for leaf length in the Erbaa 2013 location, and for leaf yield in the Tokat 2012 location, while no significant correlations were found between genetic distance and heterosis levels for other traits in other locations. There are studies in literature concluding that heterosis levels could be estimated using DNA markers in cotton (Palaniyappan et al., 2023), corn (Palaniyappan et al., 2023) and cabbage (Dong et al., 2021). However, there are also reports that diversity levels determined by DNA markers were not associated with heterosis levels in rapeseed (Tian et al., 2017), wheat (Liu et al., 1999), soybean (Cerna et al., 1997), corn (Dermail et al., 2020) and rice (Kwon et al., 2002). In addition, two other studies found that genetic distance between wheat cultivars was correlated with heterosis, but was not enough to determine best parental combinations (Darvishzadeh et al., 2014; Ali et al., 1995). As suggested by Kwon et al. (2002), these conflicting results may indicate that genetic diversity and heterosis associations vary based on genetic material used. Some authors found that genetic diversity and heterosis associations could also be affected by type of DNA markers employed (Solomon et al., 2012). The present study adds to the complexity of marker - heterosis associations by showing that associations could change based on environment.

Table 11. Mid-parent heterosis levels of different hybrids (%) in six growing environments for nicotine content in dried leaves

Hybrid	Nicotine content in dried leaves						
	E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	-18.98	20.62	5.9	0.32	11.91	-7.35	2.07
Xanthi-2A X Gümüşhacıköy	-11.86	-7.68	-24.8	11.12	-19.29	12.92	-6.6
Xanthi-2A X Taşova	2.83	-38.51	-3.2	-22.11	1.7	-16.39	-12.61
Xanthi-2A X Katerini	-13.87	4.05	-2.55	12.76	13.98	7.64	3.67
Xanthi-2A X Canik	-3.56	-2.83	-2.54	11.03	-15.93	14.89	0.18
Xanthi-2A X Erbaa	20.52	31.4	1.14	42.88	54.8	31.36	30.35
Nail X Gümüşhacıköy	27.92	-40.04	13.32	-31.09	22.71	-18	-4.2
Nail X Taşova	-7.36	-3.69	-20.4	9.94	-18.65	7.42	-5.46
Nail X Katerini	-11.7	12.19	13.51	-9.04	22.52	-4.74	3.79
Nail X Canik	-12.01	8.41	-15.4	-13.79	-1.62	-9.72	-7.35
Nail X Erbaa	3.91	-26.23	-8.08	-17.38	143.29	-7.07	14.74
Gümüşhacıköy X Taşova	-19.77	-0.15	22.58	-17.96	-31.94	-21.79	-11.51
Gümüşhacıköy X Katerini	-31.62	56.12	-30.6	0.36	18.66	46.81	9.96
Gümüşhacıköy X Canik	-24.73	-33.06	-8.22	-0.21	-28.03	-29.36	-20.6
Gümüşhacıköy X Erbaa	-17.26	-19.75	-44.9	-1.46	16.84	-10.51	-12.83
Taşova X Katerini	-6.11	-11.95	26.73	9.91	12.67	17.8	8.18
Taşova X Canik	-10.73	-11.88	22.56	35.56	-45.4	15.98	1.02
Taşova X Erbaa	-32.41	80.4	1.64	34.18	37.23	50.88	28.65
Katerini X Canik	-21.81	25.57	38.82	16.17	21.55	16.16	16.08
Katerini X Erbaa	-10.3	13.47	14.37	22	67.01	36.78	23.89
Canik X Erbaa	-2.55	51.13	3.09	14.89	44.71	1.99	18.88
Average	-9.59	5.12	0.15	5.15	15.65	6.46	3.82

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Table 12. Correlation coefficients between genetic distance and mid-parent heterosis levels for different traits in tobacco hybrids

Location	Correlation coefficient					
	Leaf number	Leaf width	Leaf length	Dried leaf yield	Sugar content in dried leaves	Nicotine content in dried leaves
Erbaa 2012	0.52*	0.29	0.31	0.21	0.22	-0.28
Erbaa 2013	0.17	0.68**	0.67**	0.13	-0.26	0.22
Bafra 2012	0.13	0.07	0.16	0.23	-0.15	-0.02
Bafra 2013	-0.02	0.12	0.25	-0.12	-0.10	0.13
Tokat 2012	0.50*	-0.25	-0.03	0.51*	-0.03	0.42
Tokat 2013	0.24	0.16	0.09	-0.06	0.14	0.09

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

4. Conclusions

It was revealed that yields of oriental tobacco could be improved in hybrids without deteriorating their superior quality. SSR markers were very effective to determine genetic diversity of oriental tobacco, and only two markers (PT20172 and PT30265 or PT30274) could distinguish all seven genotypes used in the study. In other words, these the alleles identified by these two marker sets (PT20172 and PT30265 or PT20172 and PT30274) were able to indicate that all genotypes studied were genetically different. Heterosis levels were correlated with genetic distances calculated by SSR markers of parents for some traits in some environments. However, SSR markers cannot be used as reliable tools to estimate heterosis levels in oriental tobacco hybrids.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Kinay, A., Saygılı, İ., Kandemir, N.; Design: Kinay, A., Saygılı, İ., Kandemir, N.; Data Collection or Processing: Kinay, A., Saygılı, İ.; Statistical Analyses: Saygılı, İ.; Literature Search: Kinay, A., Saygılı, İ., Kandemir, N.; Writing, Review and Editing: Kinay, A., Saygılı, İ., Kandemir, N.

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