



Agronomic and chemical properties of hybrid oriental tobacco (*Nicotiana tabacum*) lines and their stabilities

AHMET KINAY

Tokat Gaziosmanpasa University, Agriculture Faculty, Tokat 60 150, Turkey

Received: 19 August 2018; Accepted: 2 September 2019

ABSTRACT

The present study was conducted at six Basma tobacco production centres, viz. Esencay, Endikpinar, Uzumlu, Karayaka, Evciler and Yenice in 2016 and 2017. The aim was to find the best stable hybrid Basma tobacco lines with attributes desired by the growers and buyer firms. Four hybrid lines and their parents were used as the plant material of the study. Plant height, leaf width, leaf length, yield, grade index characteristics were investigated and nicotine, glucose, fructose, chlorogenic acid and rutin analyses were performed with an HPLC system. Genotype stabilities were determined with regard to yield and grade index. Attribute means, regression coefficient (b), regression constant (a), coefficient of determination (r^2), coefficient of variation (CV%) and standard deviation of regression (S^2d) were used as the stability parameters. As the average of six locations, plant height was 109.22 cm, number of leaves was 28.34, leaf width was 12.09 cm, leaf length was 22.71 cm, yield was 1612.2 kg/ha, grade index was 64.10%, nicotine content was 1.69%, glucose content was 4.54%, fructose content was 5.07%, chlorogenic acid content was 388.32 ppm and rutin content was 607.05 ppm. Hybrids had greater leaf width, leaf length, yield and chemical attributes than the parents. Significant differences were observed in all of the investigated parameters. H₂ (dry leaf yield: 1808.6 kg/ha, grade index 66.20%) hybrid was identified as the most stable and promising registration candidate hybrid.

Key words: Basma, Genotype × environment, Heterosis, HPLC, Quality, Yield

Highly aromatic oriental tobaccos are usually preferred in American blends. Current cultivars are not meeting the demands of both the growers and buyers of Basma tobacco resulting in yield and quality losses. Hybrid Basma tobacco production has not been performed worldwide.

Yi *et al.* (2005) carried out a breeding study with different burley tobacco cultivars and reported that resultant hybrids had 2.9% greater yields than the parents. In addition, hybrids also provided 5.8% increase in grower incomes because of increased quality of the tobaccos. Gixhari and Sulovari (2010) carried out a study with oriental tobaccos and reported that hybrids had 2.8-4.7% greater yields than their parents. All these previous hybrid tobacco breeding studies indicated that yield and quality could together be improved or high-yield oriental tobacco cultivars could be developed without any losses in quality.

Hybrid cultivars may yield better outcomes not only in regions with favourable environmental conditions, but also in the regions with poor environmental conditions. Physical and chemical characteristics of tobacco cultivars are largely dominated by genetic structure, agricultural

practices, soil texture and fertilization, climate, pests and diseases, lugs, harvest and drying conditions (Godoy *et al.* 2015). Changes in any one of these factors may result in substantial differences in chemical composition and thus taste of tobacco leaves. Therefore, in breeding new tobacco lines, these issues should be taken into consideration and resultant hybrids should be cultured in adapted ecologies.

Decreasing oriental-type tobacco production created an insufficiency in meeting the demands of world tobacco markets. It is quite hard to get desired yield and quality levels, therefore producers easily give up oriental tobacco production activities. Despite the existence of several Basma tobacco cultivars in the world, there is still a need for high yield and quality cultivars able to satisfy both the growers and tobacco industry. This study was conducted to develop a new hybrid Basma tobacco cultivar with superior characteristics. In this way, it will be possible to meet the demands of both Basma farmers and private sector buyers.

MATERIALS AND METHODS

In this study, 4 Basma hybrid lines, viz. Nail × Katerini (H₁), Xanthi-2A × Katerini (H₂), Katerini × Erbaa (H₃) and Canik × Erbaa (H₄) and their parents, viz. Nail (P₁), Katerini (P₂), Xanthi-2A (P₃), Erbaa (P₄) and Canik-10821 (P₅) which have been prominent since 2011 were used. Experiments were conducted at six locations, viz. Esencay, Endikpinar, Uzumlu, Karayaka, Evciler and Yenice that best

*Corresponding author e-mail: ahmet.kinay@gop.edu.tr

represent the Basma production centers in 2016-17. There were no significant differences between the climate data (temperature and relative humidity) for the experimental years between long-term climate data, but experimental locations had about 35% less precipitations in both years than the long-term averages. Soil samples were taken from 0-30 cm soil profile and analyzed at university laboratories. Soils of 2016 locations were clay in texture, strong alkaline, poor in organic matter, sufficient in phosphorus in Uzumlu location and rich in phosphorus in the other two locations. Soils of locations in 2017 were sandy-clay in texture, slight-medium alkaline, medium in organic matter, rich in potassium and poor in phosphorus. Different from the other locations, Karayaka soils were sandy-loam in texture.

Experiments were conducted under farmer's conditions without irrigation. Seedlings were placed into vials in controlled greenhouse conditions. Experiments were conducted in randomized block design with 3 replications. Each plot was 4 m long and composed of 4 plant rows. Experimental plots were treated with 60 kg/ha nitrogen, 40 kg/ha phosphorus (P_2O_5) and 60 kg/ha potassium (K_2O). All fertilizer treatments to plots were performed right before the plantation and fertilizers were incorporated into the soil. Dried leaves were weighed and set at 17% moisture. Then, yields were calculated and organoleptic observations were performed (Kurt and Ayan 2014). Samples were taken from the weighed tobaccos for chemical analyses and they were ground at zero moisture. Agronomic characteristics (plant height, leaf width, leaf length), yield, grade index and chemical contents (nicotine, glucose, fructose, invert sugar (glucose+fructose), chlorogenic acid, rutin and chlorogenic acid+rutin) were investigated. Extractions were performed with 1% acetic acid and acetonitrile for nicotine; with 1% acetic acid and methanol for glucose and fructose; with water and acetonitrile for chlorogenic acid and rutin. Extracts were analyzed with the aid of an HPLC system equipped with DAD detector for nicotine, chlorogenic acid and rutin and with RI detector for glucose and fructose (Moghbel *et al.* 2015, Cirak *et al.* 2016).

Resultant data were subjected to variance analyses in accordance with randomized block design through combining the years and locations and means were compared with Duncan's multiple range test. Genotype stabilities were investigated for yield and grade index parameters. The genotypes with a regression coefficient (b) close to 1, parameter mean greater than the general mean, a positive regression constant (a), a high coefficient of determination (r^2), a low coefficient of variation (CV%) and standard deviation of regression (S^2d) were assumed to be stable (Albayrak 2005).

RESULTS AND DISCUSSION

Yield and quality are assessed together in new cultivar breeding studies of oriental tobaccos. Tobacco dry leaf yield and quality are influenced by several factors. Plant genetics and growing ecology are the primary ones.

Plant heights of the parents and hybrids varied

between 34.4-161.9 cm and genotypes (G) and genotype \times environment (G \times E) interaction were found to be significant ($P<0.01$). Effects of dominant genes on plant height and hybrid power were mentioned in previous studies (Butorac *et al.* 2004), but such a case was not observed in present study. Number of leaves varied between 20.5-41.0 and again genotypes and G \times E interaction were found to be significant ($P<0.01$). While the greatest number of leaves was obtained from P_5 cultivar, average of parent was greater than the average of hybrids. There are some studies indicating existence of hybrid power in number of leaves, low hybrid powers (Butorac *et al.* 2004, Gixhari and Sulovari 2010) and no hybrid power in number of leaves. The leaf lengths of six environment varied between 12.7-33.8 cm and differences in leaf lengths were found to be significant at $P<0.01$ level. The greatest leaf length was obtained from P_5 cultivar because of type-effect (Table 1). Parallel to study of Dylugerski and Dimanov (2012) indicating leaf lengths of between 7.7-7.5 and hybrid power of 4.7%, an average 3.8% hybrid power was observed in present study.

Leaf widths varied between 6.1-19.5 cm and differences in leaf widths were found to be significant. Dylugerski and Dimanov (2012) mentioned yield-improving effect of leaf width and other researchers (Butorac *et al.* 2004) mentioned about hybrid power in leaf widths because of dominance. Leaf widths of hybrids were 13% greater than the leaf widths of parents. Such a case was also reflected on yields and hybrids had 14% greater yields than the parents. High heterosis in leaf yields indicate the effects of dominant genes on this attribute. Butorac *et al.* (2004) and Gixhari *et al.* (2010) reported similar findings. Different response of genotypes to the environment is mostly related to ecology, climate and soil factors. Present experiments were conducted at six locations with altitudes of between 232-609 m. Differences in altitudes may alter average temperature, relative humidity, CO_2 content and total precipitation. The changes in ecology may then alter day-night temperature differences, thus net photosynthesis rates, light intensity and durations, dry matter accumulation and genetic potentials. Yield values varied in a broad range between 502.6-2992.9 kg/ha ($P<0.01$). The greatest yield values were obtained from H_1 and H_2 genotypes and yields of hybrids were 18% greater than the yields of the parent. A grade index value varied between 40-100% and the greatest performance in two years and at six locations was obtained from the P_2 . The H_2 and H_3 genotypes including P_2 were also placed into the same statistical group and had greater grade index than the other hybrids (Table 1).

In all chemical characteristics directly influencing the quality, hybrids had greater values than the parents and G and G \times E interaction were found to be significant. While stable genotypes with the greatest yield and performance are put forward agronomically, market demands are considered to decide about chemical characteristics. Nicotine demands of leaf-tobacco buying companies vary between 2.2-2.7%. Although present nicotine contents varied between 0.5-3.49%, average nicotine contents were

Table 1 Agronomic and technological properties of the tobacco parent (P) and hybrids (H) at six locations

Genotypes	Plant height (cm)		Number of leaves		Leaf width (cm)		Leaf length (cm)		Yield (kg/ha)		Grade index (%)			
H ₁	106.86	c	25.74	f	13.87	a	23.48	ab	1820.3	a	60.69	cd		
H ₂	103.95	c	26.40	df	13.57	a	23.26	b	1808.6	a	66.20	b		
H ₃	104.91	c	27.03	de	13.60	a	23.27	b	1642.0	b	66.47	b		
H ₄	117.96	b	30.37	b	10.97	de	22.81	b	1684.5	b	61.84	c		
P ₁	116.92	b	28.62	c	12.52	b	22.91	b	1539.8	cd	66.76	b		
P ₂	85.52	d	30.27	b	11.72	c	20.53	c	1526.4	cd	71.43	a		
P ₃	106.31	c	26.13	ef	10.84	de	20.95	c	1383.3	e	66.08	b		
P ₄	114.12	b	27.28	d	11.27	cd	22.72	b	1484.7	d	59.55	cd		
P ₅	126.44	a	33.25	a	10.48	e	24.49	a	1612.2	bc	57.88	d		
Mean of hybrids	108.42		27.39		13.00		23.21		17.41		63.80			
Mean of parent	109.86		29.11		11.36		22.32		15.09		64.34			
Mean	109.22		28.34		12.09		22.71		16.12		64.10			
LSD _{0.05}	3.87		0.98		0.70		1.04		9.46		2.79			
CV%	5.35		5.21		8.78		6.94		8.87		6.60			
	<i>Nicotine (%)</i>		<i>Glucose (%)</i>		<i>Fructose (%)</i>		<i>Invert Sugar (%)</i>		<i>Chlorogenic acid (ppm)</i>		<i>Rutin (ppm)</i>		<i>Chlr+Rut (ppm)</i>	
H ₁	1.62	b	4.63	c	5.69	a	10.33	b	417.79	b	646.34	bc	1064.13	b
H ₂	1.81	b	4.77	bc	5.58	a	10.36	b	408.46	bc	582.28	d	990.74	c
H ₃	1.71	b	5.10	b	5.00	bc	10.10	b	375.58	cd	598.17	d	973.75	c
H ₄	1.81	b	4.66	c	5.12	b	9.77	bc	409.90	bc	645.50	bc	1055.40	b
P ₁	1.63	b	6.88	a	5.76	a	12.63	a	503.56	a	695.57	a	1199.13	b
P ₂	1.74	b	3.54	e	4.82	bc	8.36	e	320.96	e	523.95	e	844.91	d
P ₃	2.08	a	4.08	d	4.60	c	8.68	de	341.25	de	609.62	cd	950.87	c
P ₄	1.61	b	2.89	f	4.06	d	6.95	f	285.06	f	493.28	e	778.33	e
P ₅	1.23	c	4.28	d	4.96	bc	9.24	cd	432.37	b	668.73	ab	1101.09	a
Mean of hybrids	1.74		4.79		5.35		10.14		402.93		618.07		1021.00	
Mean of parent	1.66		4.33		4.84		9.17		376.64		598.23		974.87	
Mean	1.69		4.54		5.07		9.60		388.32		607.05		995.37	
LSD _{0.05}	0.22		0.33		0.39		0.65		34.42		38.95		61.13	
CV%	19.42		11.05		11.68		10.30		13.40		9.70		9.28	

below these demanded values. Such a deficiency can be eliminated with nitrogenous fertilizers (Karaivazoglou *et al.* 2006) and planting density (Bilalis *et al.* 2015). A similar case is also valid for glucose, fructose and reduced sugar and markets usually demand parallel values with nicotine or demand high sugar contents. Glucose, fructose and reduced sugar (glucose + fructose) contents varied respectively between 1.2-13.2%, 0.4-10.9% and 1.5-23.8%. The greatest sugar content was observed in parent Nail. All of the hybrids had greater values than the parents and they were all placed into the same statistical group (Table 1). Present sugar contents were generally within desired values, but they can easily be improved with various agricultural practices. Besides nicotine and reduced sugars, designating taste and smoking characteristics, secondary metabolites directly effective in color, taste and aroma should also be taken into consideration in product design and formation processes. Of these secondary metabolites, polyphenols are

the most significant ones and chlorogenic acid and rutin are the most common polyphenols in tobaccos (Wang *et al.* 2008). Xie *et al.* (2011) investigated chlorogenic acid and rutin contents of oriental tobaccos in China and reported these values respectively as 1560 ppm and 4240 ppm. In present study, chlorogenic acid contents varied between 110.3-948.2 ppm, rutin contents varied between 163.3-1287.5 ppm and total of these two polyphenols varied between 273.6-2235.7 ppm. Tobaccos are quite sensitive to genetic and environmental factors, thus present polyphenol contents were generally lower than the earlier reports (Ji *et al.* 2013). Hybrids had greater polyphenol values than the parents (Table 1).

With regard to stability of the genotypes, it was observed that all of the hybrids had yield values greater than the general average. Since H₄ hybrid had a regression coefficient (b) far from 1, H₃ hybrid had a negative regression constant (a), H₁ and H₂ hybrids were found to be stable

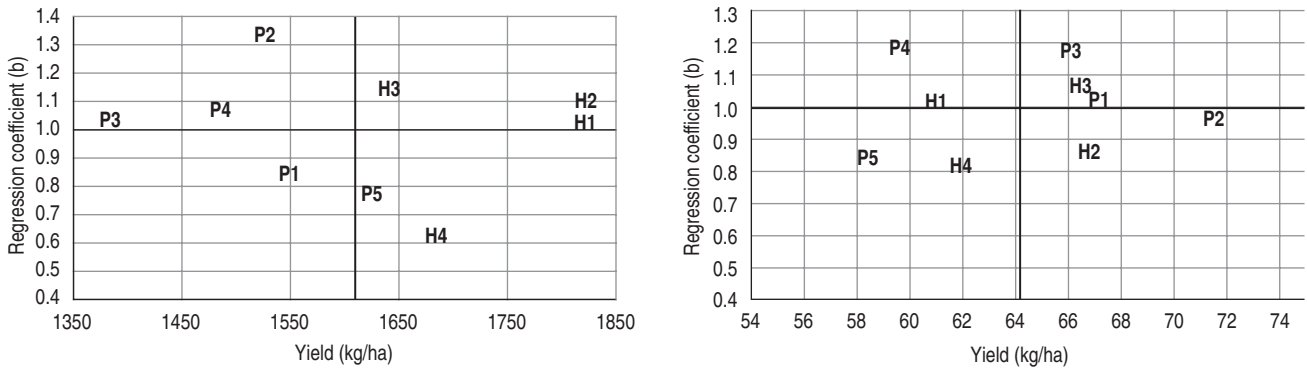


Fig 1 Stability conditions of genotypes according to yield, grade index and regression coefficient.

Table 2 Stability parameters for yield and grade index

Genotypes	mean	b	a	r ²	CV	S ² d
<i>Yield (mean; 1612.2 kg/ha)</i>						
H ₁	1820.3	1.02	18.23	0.97	6.58	144.67
H ₂	1808.6	1.10	3.27	0.95	8.66	245.23
H ₃	1642.0	1.15	-21.69	0.97	7.51	152.24
H ₄	1684.5	0.63	67.03	0.82	10.81	331.24
P ₁	1539.8	0.85	16.59	0.97	6.33	94.95
P ₂	1526.4	1.33	-62.45	0.81	26.21	1600.57
P ₃	1383.3	1.04	-28.82	0.96	9.71	180.63
P ₄	1484.7	1.08	-25.53	0.91	14.07	436.27
P ₅	1612.2	0.79	33.37	0.90	9.89	254.65
<i>Grade Index (mean; 64.10%)</i>						
H ₁	60.69	1.01	-3.59	0.97	5.12	9.66
H ₂	66.20	0.86	10.97	0.93	6.35	17.65
H ₃	66.47	1.07	-2.22	0.98	4.24	7.93
H ₄	61.84	0.82	8.98	0.71	15.72	94.52
P ₁	66.76	1.03	0.72	0.79	14.29	91.03
P ₂	71.43	0.97	9.21	0.86	9.91	50.16
P ₃	66.08	1.18	-9.77	0.94	7.98	27.83
P ₄	59.55	1.19	-16.79	0.81	17.36	106.87
P ₅	57.88	0.86	2.54	0.94	7.35	18.12

and they were reported to exhibit well yield performance at every kind of environmental conditions. The r², CV and S²d values of these two hybrids also support the stability of them (Table 2, Fig 1).

With regard to grade index, H₁ and H₄ hybrids and P₄ and P₅ parents had values below general average. With a negative regression constant (a), H₁ and H₃ hybrids and P₃ and P₄ parents required well environmental conditions. The parents P₁ and P₂ with low r² values were remarkable. H₂ hybrid seemed to be more stable with regard to grade index (Table 2, Fig 1). Besides stability, agronomic, morphological and technological characteristics should also be taken into consideration in line selection studies.

As the average of the locations, the greatest yields were obtained from H₁ and H₂ hybrids and the greatest grade

index values were achieved in H₂ and H₃ hybrids. With regard to chemical characteristics, hybrids were able to meet market demands. H₂ hybrid was identified as the most stable hybrid with regard to yield and grade index. Cultivar registration will be proposed for this hybrid prominent with these characteristics. This hybrid with superior attributes over the other genotypes will be a registered hybrid tobacco cultivar able to be used in tobacco culture of primary oriental tobacco producer countries of the world such as Turkey, Greece, Bulgaria and Macedonia.

REFERENCES

Albayrak S, Tongel O and Guler M. 2005. Stability analysis and determination of seed yield and yield componets of candidate vetch (*Vicia sativa* L.) varieties in middle black sea region. *Anadolu Journal of Agricultural Science* **20**(1): 50–5.

Bilalis D J, Travlos I S, Portugal J, Tsioros S, Papastilianou Y, Papatheohari Y, Avgoulas C, Tabaxi I, Alexopoulou E and Kanatas P J. 2015. Narrow row spacing yield and decreased nicotine content in sun-cured tobacco (*Nicotiana tabacum* L.). *Industrial Crops and Products* **75**: 212–5.

Butorac J, Beljo J and Gunjaca J. 2004. Study of inheritance of some agronomic and morphological traits in burley tobacco by graphic analysis of diallel cross. *Plant Soil Environment* **50**: 162–6.

Cirak C, Radusiene J, Jakstas V, Ivanauskas L, Seyis F and Yayla F. 2016. Secondary metabolites of seven *Hypericum* species growing in Turkey. *Pharmaceutical Biology* **54**(10): 2244.

Dyulgierski Y and Dimanov D. 2012. Study on heterosis behaviour related to the leaves size by the tobacco of burley variety group. *Acta Agriculturae Serbica* **34**(17): 75–77.

Gixhari B and Sulovari H. 2010. Nature of inheritance and heterosis estimated on some morphological quantitative characters that influence the tobacco yield. *Studiiși Cercetari de Biologie* **18**: 46–55.

Gixhari B, Vrapi H and Hobdari V. 2010. Heterosis and combining ability for quantitative bluemould (*Peronospora tabacina* Adam) resistance in tobacco. *Studiiși Cercetari de Biologie* **19**: 100–4.

Godoy L M F, Davis G and Morris J W. 2015. *High Yielding Tobacco with Oriental Tobacco Characteristics*, United States Patent Application Publication (US2015/0136152), pp 1-21. United States

Ji X, Wei Y, Liu G and Chen H. 2013. Quantitative determination of polyphenols in tobacco leaves by HPLC. *Journal of Food, Agriculture and Environment* **11**(1): 868–75.

- Karaivazoglou N A, Tsotsolis N C and Tsadilas C D. 2006. Influence of liming and form of nitrogen fertilizer on nutrient uptake, growth, yield and quality of virginia (flue-cured) tobacco. *Field Crops Research* **100**: 52–8.
- Kurt D and Ayan A K. 2014. Effect of the different organic fertilizer sources and doses on yield in organic tobacco (*Nicotiana tabacum* L.) production. *Journal of Agricultural Faculty of Tokat Gaziosmanpasa University* **31**(2): 7.
- Moghbel N, Ryu B and Steadman K J. 2015. A reversed-phase HPLC-UV method developed and validated for simultaneous quantification of six alkaloids from *Nicotiana* spp. *Journal of Chromatography B* **997**: 142–4.
- Wang H Y, Zhao M M, Yang B, Jiang Y M and Rao G H. 2008. Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. *Food Chemistry* **107**: 1399.
- Xie F, Yu A, Hou D, Liu H, Ding L and Zhang S. 2011. Rapid and sensitive analysis of eight polyphenols in tobacco by rapid resolution liquid chromatography. *American Journal of Analytical Chemistry* **2**: 929–34.
- Yi W, Guo-ping L, Zong-you X, Wen-chang H, Yiong-bil Z, Jun-jiel Z, Ru-bing X, Yun-feil Z, Gua-hua C and Chuan-liang Z. 2005. Breeding of a new burley variety eyan 4 and its characteristics. *Journal of Chinese Tobacco Science* **26**(3): 39–45.