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Effects of Heterosis on Agronomically Important Traits of Oriental Tobacco (*Nicotiana tabacum* L.) Hybrids

Ahmet KINAY[1]* Güngör YILMAZ[1]

[1]Gaziosmanpasa University, Faculty of Agriculture, Department of Field Crops, Tokat
*Corresponding author: ahmet.kinay@gop.edu.tr

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Abstract: This experiment was conducted to determine heterosis effects in crosses between different oriental tobacco varieties in Tokat Province of Turkey. Hybrids were produced from half-diallel crosses between Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa tobacco varieties in 2012. The experiment was conducted based on 7 parents (P_1 and P_2) and their 21 F_1 populations during 2013 and 2014 tobacco growing seasons in the condition of Kazova/Tokat. Heterosis effects with regards to the plant height, leaf width, leaf length, number of leaves, leaf yield, nicotine and sugar rates were calculated. The average increases due to the heterosis behavior were 28.4% and 4.4% for cured leaf yield and plant height, respectively. In addition, hybrids had about 16.6% less sugar and 10% more nicotine in cured leaves.

Key words: Heterosis, *Nicotiana tabacum* L., nicotine, sugar, tobacco, yield

Oriental Tütün (*Nicotiana tabacum* L.) Hibritlerinde Heterosisin Önemli Tarımsal Ürün Özelliklerine Etkisinin İncelenmesi

Özet: Bu araştırma, farklı oriental tütün tipleri arasındaki melezlerin heterosis üzerine etkilerini belirlemek için Türkiye'de Tokat ilinde yürütülmüştür. Hibrit hatlar, Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik ve Erbaa genotiplerinden yarım diallel melezleme ile 2012 yılında elde edilmiştir. Çalışma 2013-2014 yılları tütün vejatasyon sürecinde Kazova/ Tokat şartlarında 7 ebeveyn (P_1 ve P_2) ve onların 21 F_1 populasyonu kullanılarak yürütülmüştür. Araştırmada, bitki boyu, yaprak sayısı, yaprak eni, yaprak boyu, yaprak verimi, nikotin ve şeker oranları incelenmiştir. Çalışma sonucunda melezler kuru yaprak veriminde ortalama %28,4 ve bitki boyunda ortalama %4,4 heterosis etkisi göstermiştir. Hibritler ortalama olarak ebeveynlere göre kuru yaprakta %16,6 daha az şeker oranına sahip olurken %10 daha fazla nikotin içeriğine sahip olmuşlardır.

Anahtar kelimeler: Heterozis, *Nicotiana tabacum* L., nikotin, şeker, tütün, verim

Introduction

Tobacco is an annual crop plant that belongs to genus *Nicotiana* of *Solanaceae* family (Goodspeed, 1954, Bürün and Emiroğlu, 1988). Tobacco has a special place in international as well as domestic trades and consumption. Tobacco products have been subject of public debates in recent years throughout the world due to their negative health considerations. Tobacco economy worldwide constitutes a 600-650 billion dollar market size, and Turkey has a tobacco economy of 25-30 billion dollars (Anonymous, 2013a; Anonymous, 2013b; Anonymous, 2013c; Anonymous, 2013d; Anonymous, 2015).

Hybrid tobacco breeding is used different tobacco types but not used oriental tobacco in the world. There is a little search hybrid oriental tobacco breeding. Butorac et al. (2004) carried out half diallel crosses in Croatia between four Burley tobacco cultivars (Saturn, TN 86, Bs 92 and Bols

100). The highest yield was obtained from Saturn x TN 86 hybrid (3,38 t/ha), plant height from Bs 92 x Bols 100 hybrid (189 cm), leaf length from TN 86 x Bs 92 hybrid (69,5 cm) and leaf width from Bs 92 x Bols 100 hybrid (41 cm). Drastic changes were not observed for leaf numbers between hybrids and parents. A diallel crossing program was conducted in Israel using nine oriental tobacco cultivars in field conditions (Marani and Sachs, 1966). In this research, higher cured leaf yields were obtained from hybrids compared to their parents. Except for total alkaloid content and combustibility, significant heterosis effects were not reported for many traits such as leaf numbers, plant height and days to flowering.

Kara and Esendal (1997) studied the mechanism of inheritance of some quantitative characters in a population consisted of six parental tobacco cultivars/lines (Basma, Canik, 320-4A, 20-24B, 40-18B and 30-35) and their 15 half-diallel crosses by using Jinks-Hayman diallel procedure. According to the results, the analysis indicated the importance of both additive and dominant components of genetic variance for all traits, except for total nitrogen, but the additive component for plant height and number of leaves and the dominant component for leaf lenght and leaf widht were predominant, and total nitrogen was gowerned by the genes with dominant effect. Narrow sense heritability estimate was high for total alkaloid, number of leaves, ash content and plant height.

New tobacco varieties with better yield and quality traits have been developed using plant breeding. Most of these varieties are standard pureline cultivars while some are hybrids. In Turkey, on the other hand, very limited breeding studies have been carried out so far despite the needs for new varieties in recent years. The present study was carried out to establish a start point to develop new hybrids of oriental type tobaccos produced in Turkey.

Materials and Methods

Parent cultivars of known agronomic and technological features (Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa) constituted the material of the present study. Plants of these cultivars were crossed in half diallel fashion and all 21 hybrid combinations excluding reciprocals were produced between them. Enough seeds were produced from these hybrids by hand pollination and from parents by isolation.

Field study was carried in Kazova/Tokat conditions in 2013 and 2014 growing seasons using seven parents and 21 hybrid combinations. Experimental design was randomized complete block design with three replicates. Each plot consisted of four rows which were 4 m long. Row spacing was 45 cm and plant spacing within rows was 12 cm, as parallel to common practice observed in the region and a total of 136 seedlings were used in each plot.

Equal amounts of nitrogen (60 kg/ha N), phosphorus (40 kg/ha P_2O_5) and potassium (60 kg/ha K_2O) were applied homogenously to the plots. All fertilizers were given to prepared plots and mixed with the soil before the transplanting of seedlings (Kınay, 2010). There were 1 m spaces between plots and 1,5 m between the blocks. Study was conducted under rainfed conditions.

Seedlings were grown in viols in a greenhouse. Transplanting was carried out when the plants had 4 to 6 leaves and 12-15 cm height on May 15th, 2013 and May 5th, 2014. During the growth period, crop maintenance practices such as hoeing for weeds, stripping off the lowest leaves, disease and pest control were conducted. Leaves that reached the harvest maturity were hand harvested on three different dates. Harvested leaves stringed. After wilting for one or two days, leaves were taken to drying areas. After drying, stacks were made into string of tobacco. During the vegetation period, observations were made for plant height, leaf width and length, leaf number. Organoleptic examinations were made on dried leaves. Nicotine ratio was determined by the spectrophotometric method (Willits et al., 1950). The reducing sugar content was determined by spectrophotometric method based on the change in method Lindsay (Sekin, 1979). Data obtained were subjected to analysis of variance according to randomized complete block design, and means were compared using Duncan's

multiple comparison test. All statistical analyses were conducted using MSTAT software (Freed et al., 1989).

Results and Discussion

Plant height, leaf and quality characteristics in tobacco cultivars and hybrids were given in Table 1. The results of the study showed that hybrids produced and grown in the study had better leaf quality than Xanthi-2A, commonly grown cultivar in the region.

The highest yield increase was obtained from Gümüşhacıköy x Erbaa hybrid by 51,5%. Except for only one hybrid (Xanthi-2A x Taşova), all hybrids had higher yields than their respective parents. Based on the expertise value, twelve hybrids (Xanthi-2A x Nail, Xanthi-2A x Taşova, Xanthi-2A x Katerini, Xanthi-2A x Canik, Xanthi-2A x Erbaa, Nail x Taşova, Nail x Katerini, Nail x Erbaa, Gümüşhacıköy x Taşova, Taşova x Katerini, Taşova x Erbaa and Katerini x Erbaa) were found to have acceptable physical quality properties. Reducing sugar contents varied from 2,6 to 8,7% and nicotine contents from 0,9 to 3,0%. Leaf numbers ranged from 28,0 (Nail x Gümüşhacıköy and Nail x Taşova) to 39,2 (Canik), leaf width from 9,4 cm (Canik) to 14,4cm (Nail x Katerini) and leaf length from 19,2 (Xanthi-2A x Taşova) cm to 23,8 (Taşova x Katerini). Statistically significant ($p<0,05$ and $p<0,01$) variations were observed in all variables studied (Table 1).

Average leaf yields of all hybrids and parent cultivars were 1,94 and 1,51 t/ha, respectively, which means an average of 28,48% increase. Average plant height of hybrids was 4,41% higher than that of parent cultivars. Compared to parent cultivars, slight increases were observed in hybrids for leaf width, leaf length and leaf number. In terms of quality features, reducing sugar contents decreased by 16,67% and nicotine content increased by 10,00% in hybrids compared to parent cultivars.

Nail x Canik (2,26 t/ha) Katerini x Erbaa (2,20 t/ha) and Nail x Erbaa (2,17 t/ha) were the highest yielding hybrids under Tokat conditions. Of the parents that constitute these hybrids, Katerini gave 1,73 t/ha leaf yield followed by Nail (1,59 t/ha), Xanthi-2A (1,55 t/ha) and Erbaa (1,39 t/ha). These results showed a clear heterosis effect for leaf yields.

Heterosis effects of tobacco cultivars and hybrids on plant height, leaf and quality characteristics were shown in Table 2. The results of this research, except for only one hybrid (Xanthi-2A x Taşova), all hybrids showed positive heterosis effect for leaf yields. Gümüşhacıköy x Erbaa (51,50%), Nail x Erbaa (45,80%), Nail x Canik (45,10%), Gümüşhacıköy x Canik (44,60%), Gümüşhacıköy x Taşova (%44,50) and Katerini x Erbaa (41,10%) were the highest heterosis effect for leaf yields. Although heterosis effect of expertise value ranged from -36,80% (Xanthi-2A x Gümüşhacıköy) to 31,40% (Canik x Erbaa), all hybrids have acceptable physical quality properties (Table 2).

Based on the result of this research, the highest heterosis was obtained in Gümüşhacıköy x Erbaa hybrid for yield, Canik x Erbaa for quality. Furthermore, tobacco cultivars that can meet the requirements of tobacco farmers and industry were determined. Based on the results, Xanthi-2A x Nail, Xanthi-2A x Katerini, Xanthi-2A x Canik, Xanthi-2A x Erbaa, Nail x Taşova, Nail x Katerini, Nail x Erbaa, Taşova x Katerini and Katerini x Erbaa hybrids were found to have superior yield and quality traits.

It has long been known that selfing results in homozoygosity, and consequently a loss in vigor of plants. Heterozygous plants produced from inbred lines developed from consecutive selfings, on the other hand, feature an increase in performance called heterosis (Hayes et al., 1995). Heterosis concept was introduced by Shull in 1914 who started analyzing yield and developmental performance of inbred lines and hybrids between them. Heterosis is the improved vigor, strength, yield, growth rate and resistance against diseases, pests and adverse environmental conditions observed in F_1 hybrids compared to their parents' average values. Despite various possible explanations proposed for heterosis such as dominance, over-dominance, heterozygosity, epistatic effects, nucleus/cytoplasm

interactions, a clear conclusion has not been reached so far (Schnable and Springer, 2013). Heterosis, which manifests itself in different forms, could be formed by all these effects and many others which are not clear yet.

Table 1. Plant height, leaf number, leaf width-length, leaf yield, leaf sugar and nicotine content in tobacco cultivars and hybrids

Çizelge 1. Tütün çeşit ve hibritlerinde bitki boyu, yaprak sayısı, yaprak eni-boyu, yaprak verimi, yaprak şeker ve nikotin içeriği

Cultivars or hybrid	Plant height (cm)*	Leaf number (number)**	Leaf width (cm)**	Leaf length (cm)*	Leaf yield (t/ha)**	Leaf sugar content (%)**	Leaf nicotine content (%)**
Xanthi-2A	91,1 def	29,4 g-j	11,1 e-h	20,4 cde	1,55 jkl	3,6 e-f	2,8 ab
Nail	101,7 a-f	31,6 c-ı	11,9 c-h	21,0 a-e	1,59 ıjk	8,7 a	2,1 cde
Gümüşhaciköy	96,1 b-f	29,7 f-j	10,2 hı	20,4 cde	1,34 l	5,2 b-e	0,9 g
Taşova	90,8 ef	29,9 f-j	11,2 d-h	20,6 b-e	1,47 kl	3,7 e-ı	2,9 ab
Katerini	91,2 def	34,4 bc	12,9 a-d	19,9 de	1,73 hıj	4,3 d-ı	2,3 b-e
Canik	119,0 a	39,2 a	9,4 ı	21,1 a-e	1,52 jkl	5,4 bcd	2,0 def
Erbaa	93,6 c-f	30,8 d-j	10,9 f-ı	20,1 cde	1,39 kl	2,6 ı	1,2 g
Xanthi-2A x Nail	100,1 a-f	28,7 ıj	12,7 b-e	21,8 a-e	1,82 fgh	6,6 b	2,5 a-e
Xanthi-2A x Gümüşhacıköy	98,6 b-f	30,0 e-j	11,8 c-h	22,1 a-e	1,99 b-g	5,4 bcd	1,9 ef
Xanthi-2A x Taşova	84,1 f	29,5 g-j	10,7 ghı	19,2 e	1,46 kl	4,6 c-h	2,6 abc
Xanthi-2A x Katerini	98,2 b-f	29,8 f-j	12,9 a-d	21,7 a-e	2,02 b-f	3,8 d-ı	2,8 ab
Xanthi-2A x Canik	105,2 a-e	33,1 c-f	10,9 f-ı	21,6 a-e	1,72 hıj	4,9 c-f	2,5 a-e
Xanthi-2A x Erbaa	99,2 a-f	32,6 c-g	11,3 d-h	20,3 cde	1,87 d-h	3,0 ghı	2,9 ab
Nail x Gümüşhacıköy	104,1 a-f	28,0 j	11,8 c-h	21,4 a-e	1,83 e-h	4,3 d-ı	1,5 fg
Nail x Taşova	98,6 b-f	28,0 j	12,3 b-g	21,8 a-e	1,78 ghı	5,9 bc	2,4 a-e
Nail x Katerini	98,2 b-f	29,8 f-j	14,4 a	23,1 abc	2,05 a-e	4,1 d-ı	2,3 b-e
Nail x Canik	116,4 ab	33,5 cd	12,2 b-g	23,2 abc	2,26 a	3,9 d-ı	1,9 def
Nail x Erbaa	107,4 a-e	32,5 c-h	12,8 a-d	22,2 a-e	2,17 abc	2,9 ghı	2,4 a-e
Gümüşhacıköy x Taşova	96,5 b-f	29,5 g-j	11,5 d-h	21,3 a-e	2,03 b-f	4,5 c-h	1,4 fg
Gümüşhacıköy x Katerini	99,9 a-f	29,4 g-j	12,5 b-f	21,9 a-e	2,01 b-f	3,0 ghı	2,2 cde
Gümüşhacıköy x Canik	111,6 a-d	33,3 cde	10,3 hı	21,1 a-e	2,07 a-d	4,6 c-g	1,1 g
Gümüşhacıköy x Erbaa	111,9 abc	32,4 c-h	11,7 c-h	22,2 a-e	2,07 a-d	3,3 f-ı	1,1 g
Taşova x Katerini	97,4 b-f	29,0 hıj	13,6 ab	23,8 a	1,93 d-h	2,7 ı	3,0 a
Taşova x Canik	101,7 a-f	32,4 c-h	11,0 f-ı	21,4 a-e	1,89 d-h	2,9 ghı	2,1 cde
Taşova x Erbaa	97,3 b-f	34,1 cd	11,1 e-ı	20,2 cde	1,57 ıjk	2,9 hı	3,0 a
Katerini x Canik	107,4 a-e	32,8 c-g	13,8 ab	23,6 ab	1,96 c-g	3,8 d-ı	2,5 a-d
Katerini x Erbaa	98,9 a-f	32,5 c-h	13,3 abc	22,7 a-d	2,20 ab	2,8 ı	2,6 abc
Canik x Erbaa	107,9 a-e	37,2 ab	10,7 ghı	21,5 a-e	1,91 d-h	3,0 ghı	1,9 def
Cultivar mean	**97,6**	**32,2**	**11,1**	**20,5**	**1,51**	**4,8**	**2,0**
Hybrid mean	**101,9**	**31,3**	**12,1**	**21,8**	**1,94**	**4,0**	**2,2**
General mean	**100,9**	**31,5**	**11,8**	**21,5**	**1,83**	**4,2**	**2,2**
CV (%)	3,1	2,9	4,1	2,7	4,6	4,7	3,2

[a-b]: The values in the same column with different letters are statistically different (**p<0,01, *p<0,05)

[a-b]:Aynı sütünda farklı harflerle gösterilen değerler arasındaki farklar istatiksel olarak önemlidir (**p<0,01, *p<0,05)

Table 2. Heterosis effect of tobacco cultivars and hybrids on plant height, leaf number, leaf width-length, leaf yield, quality degree, leaf sugar and nicotine content

Çizelge 2. Tütün çeşitleri ile melezlerinin bitki boyu, yaprak sayısı, yaprak eni-boyu, yaprak verimi, yaprak kalitesi, yaprak şeker ve nikotin içeriği üzerine heterosis etkisi

Hybrids	Plant height	Leaf number	Leaf width	Leaf length	Leaf yield	Leaf sugar content	Leaf nicotine content	Quality degree
Xanthi-2A x Nail	3,9	-6,0	10,4	5,5	15,7	7,2	-0,1	-1,5
Xanthi-2A x Gümüşhacıköy	5,3	1,4	10,3	8,6	37,8	23,5	-1,2	-36,8
Xanthi-2A x Taşova	-7,6	-0,5	-3,9	-6,1	-3,2	26,1	-8,1	-20,0
Xanthi-2A x Katerini	7,8	-6,5	7,2	7,6	23,1	-3,7	10,3	-23,1
Xanthi-2A x Canik	0,2	-3,7	6,4	4,4	11,9	10,1	1,6	7,7
Xanthi-2A x Erbaa	7,4	8,4	2,5	0,5	27,4	-1,2	40,9	-1,8
Nail x Gümüşhacıköy	5,3	-8,7	6,4	3,7	25,1	-38,3	-3,1	-16,0
Nail x Taşova	2,4	-8,9	5,8	4,8	16,2	-4,2	-3,2	-4,8
Nail x Katerini	1,8	-9,7	15,7	13,3	23,8	-36,2	5,0	-10,3
Nail x Canik	5,5	-5,4	14,4	10,6	45,1	-44,0	-6,6	-2,2
Nail x Erbaa	10,0	4,0	12,3	7,9	45,8	-48,0	43,4	20,8
Gümüşhacıköy x Taşova	3,2	-1,0	7,6	3,8	44,5	3,1	-26,7	-5,5
Gümüşhacıköy x Katerini	6,7	-8,2	8,2	8,5	31,2	-36,7	34,7	-16,0
Gümüşhacıköy x Canik	3,7	-3,4	4,6	1,9	44,6	-12,6	-27,6	8,1
Gümüşhacıköy x Erbaa	18,0	6,9	10,6	9,6	51,5	-14,9	1,0	0,0
Taşova x Katerini	7,1	-9,7	13,0	17,4	20,5	-32,3	14,1	1,6
Taşova x Canik	-3,1	-6,2	6,3	2,8	26,7	-34,8	-12,7	-8,0
Taşova x Erbaa	5,5	12,3	-0,2	-0,7	9,9	-7,4	44,8	9,4
Katerini x Canik	2,2	-10,8	23,7	15,4	20,9	-21,7	18,5	-24,4
Katerini x Erbaa	7,1	-0,3	11,1	13,3	41,1	-18,7	48,7	0,0
Canik x Erbaa	1,5	6,1	4,9	4,3	31,2	-25,8	20,0	31,4

In the present study, each of the parents used to produce hybrids has some superior features. Higher number of leaves, thick leaf structure, larger leaf size and better physical and chemical properties are desired in tobacco. Combining these features in a germplasm is highly desirable. The most efficient way of combining these features is hybrids produced between these parents. Because of their large genetic base, hybrids have better resistance to negative environmental conditions. Tobacco hybrids produced in the present study have higher number of larger and thicker leaves, and consequently, higher leaf yields. In addition, varying levels of improved tissue characteristics, aroma, nicotine and reducing sugar contents were observed in some hybrids depending upon the parents used to produce them. Tobacco crop has considerable acreages in the world and in Turkey with quite different types of plants. Therefore, considerable amount of variation that can be employed efficiently to develop new cultivars exist in tobacco crop. In addition, because of its plant characteristics suitable for efficient plant breeding programs tobacco is a model plant for plant breeding as well as some other scientific studies.

The present study showed promising results with hybrids between oriental tobacco cultivars in terms of higher yield and maintained, or even improved, quality features. Hybrid combinations produced from other oriental tobacco cultivars or genotypes might produce better yield and quality performance. Therefore, additional hybrid studies involving new genotypes are needed.

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