



HETEROTHIC EFFECT OF SOME QUANTITATIVE TRAITS IN F₁ DIALLEL HYBRIDS OF VARIOUS TOBACCO TYPES

Jane Aleksoski^{1*}

¹Scientific Tobacco Institute – Prilep, University St. Kliment Ohridski – Bitola
Kicevski pat bb, 7500 Prilep, Republic of North Macedonia

*Corresponding author: yaleksoski@gmail.com

Abstract

The mode of inheritance and heterotic effect were studied in ten F₁ crosses obtained by one-way diallel crossing between five parental genotypes: MV-1, P 76/86, Adiyaman, Basma-Djebel and P 66 9 7. The following quantitative traits were studied: the number of leaves per stalk, length of leaves from the middle belt of the stalk and yield of green leaf mass per stalk and per hectare. The trial was set up in the experimental field of Scientific Tobacco Institute-Prilep, using a randomized block design with four replications in the period 2018-2019. Traditional cultural practices were applied during the growing season of tobacco in the field.

The aim of this research was to study the mode of inheritance of the quantitative traits, to detect heterosis in the F₁ generation and to assess its economic viability.

Analysis of variance determined statistically significant differences in traits between parents and hybrids in the two-year investigation. The most common way of trait inheritance is partial-dominant, then intermediate. The negative heterotic effect on the number of leaves per stalk has P 76/86 x P 66 9 7. The hybrids MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7 have a positive heterotic effect on the length of the leaves. Oriental hybrids, where one of the parents is the variety P 66 9 7, have positive heterosis in the yield of green leaf mass per stalk. The investigation provides very useful guidance for future successive selection activities.

Key words: *Nicotiana tabacum* L., F₁ crosses, inheritance, dominance, heterosis

INTRODUCTION

The mode of inheriting traits is an important factor for determining the directions and duration in different selection programmes. Heterotic effect or F₁ vigour is a phenomenon that occurs in the first generation, where the offspring shows higher (or lower) values of particular trait from both parents. It is widely used in many crops where high-yielding hybrids are developed. In tobacco, F₁ hybrids with heterotic effect are rarely used due to their economic unviability. However, there are possibilities for their use, such as resistance to economically significant diseases.

The topic of this paper is focused on the mode of inheriting of the most important quantitative traits in F₁ offspring of hybrids obtained by diallel crossing between varieties of different tobacco types. In the last ten years, a number of authors – breeders have worked on

this subject: Aleksoski (2010), in a two-year study of four parental genotypes and their six diallel F₁ hybrids, obtained a different way of inheriting of leaf length and a weak heterotic effect without economic justification. Gixhari & Sulovari (2010), conducted three-year research at two locations in a genetically diverse population of eight oriental tobaccos and their one-way diallel hybrids and obtained a dominant and partial-dominant mode of inheritance and heterosis in leaf size and yield. Aleksoski & Korubin – Aleksoska (2011), conducted three-year studies for green and dry mass yields in a one-way diallel on three oriental and one large-leaf variety and their six F₁ crosses and obtained positive and negative heterosis. Dimanov & Dyulgovski (2012), found heterosis with varying strength in number of leaves in ten F₁ crosses obtained from parent pairs of local and introduced Burley

tobacco varieties. Dyulgerski & Dimanov (2012), performed examinations of the dimensions of 7-8 leaves and 13-14 leaves in the P_1 , P_2 and F_1 populations at ten crosses originating from local and introduced varieties of Burley tobacco and obtained a high heterotic effect of significant economic importance. In an experiment in Khan Gari, Mardan, Pakistan, with seven Virginia flue-cured genotypes and their 42 two-way diallel crosses, Imtiaz et al. (2014), found heterosis with high heterotic effect, with the possibility of using it on leaf number per stalk, leaf length and weight of green leaf mass per plant. Dyulgerski & Radoukova (2015), studied the mode of inheritance, the coefficient of inheritance and the expression of heterosis and transgression in seven Burley-type crosses and seven Virginia-type crosses of local and introduced origin in F_1 and F_2 offspring and found a dominant and partial - dominant inheritance in the length of the leaves, always in the direction of the parent with longer leaves, and the resulting heterosis

had no economic importance. Ramachandra et al. (2015), obtained hybrids superior to the control variety in number and length of leaves, in genetic studies on the yield and quality of 62 genotypes (six lines of different types of tobacco, eight testers and their crosses), at the Agricultural Research Station, Nipaniat Belgaum, Karnataka - India. Shah et al. (2017), found highly significant differences in the number of leaves per stalk in a two-year study of ten Virginia flue-cured hybrids. The field experiment was set up in agro-ecological conditions at Khan Gari in Mardan, Pakistan.

The aim of these investigations was to study the mode of inheritance and to detect possible heterosis in the number of leaves per stalk, length of the leaves from the middle belt and yield of green leaf mass per stalk and per hectare. The F_1 generation obtained by diallel crosses of tobacco varieties from different types will give us an important guidance for future selection programs in tobacco breeding.

MATERIAL AND METHODS

In order to investigate the quantitative traits of tobacco, in 2016, five varieties of different tobacco types were selected with previous studies of the available assortment at the Scientific Tobacco Institute - Prilep: MV-1 is a large-leaf variety of the Virginia type, P 76/86 is an oriental variety of the Prilep type, Adiyaman is an oriental variety of the Kabukalak type, Basma-Djebel (Basma-Dzebel or Basma-Cebel) is an oriental variety of the type Basma, P 66 9 7 is an oriental variety of the Prilep type.

In 2017, ten one-way diallel crosses were made in field conditions, by applying the diallel method of crossing, using hand castration and pollination method: MV-1 x P 76/86, MV-1 x Adiyaman, MV-1 x Basma-Djebel, MV-1 x P 66 9 7, P 76/86 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7, Adiyaman x Basma-Djebel, Adiyaman x P 66 9 7 and Basma-Djebel x P 66 9 7.

In 2018, the trial with 15 genotypes (5 parents and 10 F_1 hybrids) was set up in the experimental field of STI-Prilep, using randomized block system in four replications. The same year, seeds for the second generation were collected and again diallel crosses between the listed parents for the first generation were made.

In 2019, a trial was set up using the same method, in which the same set of 15 genotypes was planted. The total area of the trial (working

area-405m² + area of paths-477 m²) was 882 m². 826 plants (770 plants + 56 plants as protection) were planted to fill the surface of one repetition. A total of 3304 plants (stalks) were planted in the experiment.

This paper places the analysis based on: number of leaves per stalk, length of leaves from the middle belt and yield of green mass per stalk and hectare. Measurements of leaf number and length were taken during flowering, in late July and August, when the population is in lush growth. Due to the uniformity of the homozygous parent genotypes and the heterozygous F_1 offspring, 20 stalks of each variant were measured in one replication, i.e., 80 plants in the four replications (1200 plants in total). The yield of green leaf mass was measured after each harvest, the weight of all harvests from each plot was added and then it was divided by the number of plants from which the tobacco was harvested, which gave us the weight of a green leaf per stalk. The yield of green leaf mass per hectare was obtained by multiplying the weight of the green leaf per stalk by the number of plants planted on the surface of one hectare.

Statistical data processing was performed using the measurements of the subject traits of each variant. The mode of inheritance of traits is determined on the basis of test-significance from

the mean value of the F₁ generation in relation to the average of both parents. Intermediate mode of inheritance (i) occurs when the mean value of a trait in the offspring is equal to the parent average. There is a partial-dominant mode (pd) when the mean value of the hybrid offspring approaches one of the parent varieties. Dominance in inheritance (d) - positive or negative, occurs when the mean value of the cross coincides with

the mean value of one of the parents (+d - when the parent with a higher mean value dominates, -d - when the parent with a lower mean value dominates). Positive heterosis (+h) occurs in the hybrid with a significantly higher value than that of the parent with a higher mean value, while negative heterosis (-h) occurs in the hybrid with a significantly lower value than the one from the parent with a lower mean value.

Climatic and soil conditions in the area of investigations

A more realistic vision for the inheritance of quantitative traits is obtained by displaying

data on climatic conditions during the tobacco vegetation in 2018 and 2019 (Table 1).

Table 1. Weather conditions in Prilep presented by monthly averages for 2018, 2019.

Year	Climatic parameters	May	June	July	August	September	\bar{x}
2018	Avg. temp. (°C)	19.80	22.40	24.40	23.90	20.10	22.12
	Min. temp. (°C)	11.00	12.90	15.80	15.30	12.00	13.40
	Max. temp (°C)	22.30	25.90	27.50	29.80	23.60	25.82
	Humidity (%)	81.00	80.00	78.00	75.00	80.00	78.80
	Precipitation /Rainfall (mm)	18.00	20.00	21.00	20.00	90.00	$\Sigma = 169.00$
2019	Avg. temp. (°C)	15.77	22.77	24.26	27.39	21.97	22.43
	Min. temp. (°C)	8.00	16.00	14.00	22.00	14.00	14.80
	Max. temp (°C)	20.00	28.00	29.00	32.00	27.00	27.20
	Humidity (%)	71.30	67.17	59.42	42.61	53.00	58.70
	Precipitation / Rainfall (mm)	124.10	139.90	91.80	9.50	39.50	$\Sigma = 404.80$

Source: <https://en.climate-data.org/europe/macedonia/prilep/prilep-37313/>

The results of the temperature and relative humidity are within the optimal limits for normal development of tobacco and obtaining quality tobacco raw material. In July and August, one irrigation was performed with a watering norm of 300 m³/ha of water.

The soil with its mechanical composition and nutrient content is the substrate on which tobacco grows and develops. Our investigations were performed in the experimental field of Scientific Tobacco Institute - Prilep on deluvial soil type. This soil is characterized by low humus

and total nitrogen content, moderately acidic to neutral reaction, low to extremely low supply with readily available phosphorus and medium to good potassium supply. Throughout its depth, the soil is carbonate-free. Taking into account the stratigraphy of the profile and the agrochemical traits of the soil for the performance of the profile, it was properly prepared. One autumn and spring plowing were carried out along with basic tillage. The basic fertilization was performed with the spring plowing, using 250 kg / ha NPK fertilizer 8:22:20.

RESULTS AND DISCUSSION

The number of leaves is a trait that is directly related to yield. Because of that, it is the most common subject of research in all selection programs for tobacco breeding.

In 2018 the most common way of inheriting of the number of leaves per stalk in the F_1 generation is the partial-dominant. Intermediate inheritance occurs only in Adiyaman x P 66 9 7. The hybrid P 76/86 x P 66 9 7 has heterosis with a negative heterotic effect, which means that the offspring in the F_1 generation has less leaves

than both parents. In 2019 we found an identical scheme for this trait. This indicates the fact that these are stable homozygous parents whose offspring forms the first investigated generation. The number of leaves is a highly inherited trait on which environmental factors have limited influence.

Table 2 shows the mean values of the number of leaves per stalk in the parental genotypes and their diallel F_1 offspring, as well as the mode of inheritance of this trait in 2018 and 2019.

Table 2. Mode of inheritance of the number of leaves per stalk in diallel F_1 hybrids in 2018 and 2019.

Parents	Number of leaves per stalk				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	27.65	37.90 ^{pd}	28.09 ^{pd}	25.02 ^{pd}	32.75 ^{pd}
P 76/86		60.09	35.09 ^{pd}	28.60 ^{pd}	48.55 ^{-h}
Adiyaman			29.89	25.86 ^{pd}	44.62 ⁱ
Basma-Djebel				18.07	29.84 ^{pd}
P 66 9 7					54.81
2019					
MV-1	28.52	39.32 ^{pd}	28.88 ^{pd}	24.62 ^{pd}	33.93 ^{pd}
P 76/86		59.56	36.68 ^{pd}	27.54 ^{pd}	49.72 ^{-h}
Adiyaman			30.82	26.46 ^{pd}	46.08 ⁱ
Basma-Djebel				17.26	27.51 ^{pd}
P 66 9 7					56.44

In the inheritance of the leaves' length from the middle belt of the F_1 offspring in 2018, all the modalities are present.

There is intermediate inheritance in Adiyaman x P 66 9 7. There is a partial-dominant way of inheritance in MV-1 x Basma-Djebel, MV-1 x P 66 9 7, P 76/86 x Adiyaman and Adiyaman x Basma-Djebel. Positive dominance occurs in MV-1 x P 76/86. Positive heterosis in inheritance of this trait occurs in MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-

Djebel x P 66 9 7, which means that the F_1 generation has longer leaves than the leaves of the both parents.

The same mode of inheriting in crosses was obtained in 2019, which means that the trait is highly inherited and it is a varietal characteristic.

Table 3 shows the mean values of the leaf length of the middle belt of the stalk in the parental genotypes and their diallel F_1 offspring, as well as the mode of trait inheritance in 2018 and 2019.

Table 3. Mode of inheritance of the length of the leaves from the middle belt of the stalk in diallel F₁ hybrids in 2018 and 2019.

Parents	Length of the leaves from the middle belt of the stalk (cm)				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	50	48.51 ^{+d}	55.22 ^{+h}	45.12 ^{pd}	46.23 ^{pd}
P 76/86		23.62	31.55 ^{pd}	25.04 ^{+h}	24.47 ^{+h}
Adiyaman			35.75	30.53 ^{pd}	30.26 ⁱ
Basma-Djebel				20.57	24.39 ^{+h}
P 66 9 7					23.01
2019					
MV-1	52.57	51.86 ^{+d}	56.57 ^{+h}	43.78 ^{pd}	47.05 ^{pd}
P 76/86		23.44	35.22 ^{pd}	24.36 ^{+h}	24.16 ^{+h}
Adiyaman			37.29	32.05 ^{pd}	29.28 ⁱ
Basma-Djebel				20.74	23.73 ^{+h}
P 66 9 7					22.49

Table 4. Mode of inheritance of green mass yield per stalk in diallel F₁ hybrids in 2018 and 2019.

Parents	Green mass yield per stalk (g)				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	970.94	716.14 ^{pd}	742.64 ^{pd}	567.41 ⁱ	667.67 ^{pd}
P 76/86		168.95	165.25 ^{pd}	163.45 ^{+d}	177.58 ^{+h}
Adiyaman			153.81	144.79 ^{+d}	168.07 ^{+h}
Basma-Djebel				63.59	148.85 ^{+h}
P 66 9 7					137.12
2019					
MV-1	990.42	751.47 ^{pd}	757.34 ^{pd}	581.72 ^l	683.86 ^{pd}
P 76/86		172.45	174.54 ^{+d}	177.28 ^{+d}	182.49 ^{+h}
Adiyaman			150.38	135.15 ^{pd}	176.80 ^{+h}
Basma-Djebel				69.84	153.35 ^{+h}
P 66 9 7					129.39

In 2018, the most common way of inheritance of the yield of green leaf mass per stalk in F_1 offspring is the partial-dominant in the direction of the parent with higher yield, followed by the positive-dominant. Intermediate mode is found only in MV-1 x Basma-Djebel. Crosses where one parent is P 66 9 7 have positive heterosis, which means that they are more productive than the parent with higher yield (with the exception of MV-1 x P 66 9 7 in which there is partial dominance).

In 2019, the vision for complete identity is changed by the cross P 76/86 x Adiyaman (with positive dominance) and Adiyaman x Basma-Djebel (with partial dominance). If we take into account that the yield is a variable quantity that is greatly influenced by environmental factors,

then the obtained values are very reliable and reflect the professional setting of the experiment and timely activities for its cultivation and analysis.

Table 4 shows the mean values of the yield of green leaf mass per stalk in the parent genotypes and their diallel F_1 offspring, as well as the mode of inheritance of this trait in 2018 and 2019. In 2018, the mode of inheritance of the yield of green leaf mass per hectare in F_1 offspring is partially-dominant and positively dominant. Negative heterosis is present in crosses where one parent is MV-1 (with the exception of MV-1 x Basma-Djebel in which there is partial dominance in the direction of the weaker parent). The occurrence of positive heterosis has the Oriental crosses where one parent is P 66 9 7.

Table 5. Mode of inheritance of green mass yield per hectare in diallel F_1 hybrids in 2018 and 2019.

Parents	Green mass yield per hectare (t)				
	MV-1	P 76/86	Adiyaman	Basma-Djebel	P 66 9 7
2018					
MV-1	21.576	15.914 ^{-h}	16.503 ^{-h}	12.609 ^{pd}	14.837 ^{-h}
P 76/86		25.030	24.482 ^{pd}	24.215 ^{+d}	26.307 ^{+h}
Adiyaman			22.786	21.450 ^{+d}	24.899 ^{+h}
Basma-Djebel				9.420	22.051 ^{+h}
P 66 9 7					20.314
2019					
MV-1	22.009	16.699 ^{-h}	16.830 ^{-h}	12.927 ^{pd}	15.197 ^{-h}
P 76/86		25.548	25.858 ^{+d}	26.264 ^{+d}	27.035 ^{+h}
Adiyaman			22.278	20.022 ^{pd}	26.193 ^{+h}
Basma-Djebel				10.347	22.718 ^{+h}
P 66 9 7					19.170

In 2019, there are changes in the way of inheritance of the yield of green leaf mass per hectare and the yield of green leaf mass per stalk (P 76/86 x Adiyaman - positive dominance and Adiyaman x Basma-Djebel - partial dominance).

The analysis of the heredity of the yield of green leaf mass per stalk and the yield of green leaf mass per hectare indicates drastic differences and therefore it is necessary to indicate the reasons. From the shown heredity in the crosses where the parent pairs are of oriental

type, the reliability of the results is confirmed as a reflection of the professional approach to the overall work. Namely, all oriental hybrids showed the same inheritance pattern in yield per stalk and yield per hectare. But this is not the case with hybrids where one parent is the large-leaf variety MV-1.

Here, instead of partial dominance, negative heterosis occurs (with the exception of MV-1 x Basma-Djebel, where instead of intermediate, partial dominance occurs).

The reason for this outcome is in the different planting distance of the plants in the experiment. Oriental parents and oriental hybrids are planted with a row spacing of 45 cm and plant spacing of 15 cm, while the large-leaved MV-1 and its crosses are planted with a row spacing of 90 cm and plant spacing of 50 cm, which means that the calculation of yield per hectare is different,

depending on the plant composition of the genotype.

Table 5 shows the mean values of the yield of green leaf mass per hectare in the parent genotypes and their diallel F₁ offspring, as well as the mode of inheritance of the trait in 2018 and 2019.

CONCLUDING REMARKS

Inheritance of the number of leaves per stalk in F₁ generation in 2018 and 2019 is partial-dominant (only Adiyaman x P 66 9 7 has intermediation). The hybrid P 76/86 x P 66 9 7 showed a negative heterotic effect.

All the modalities in the two years of investigations can be found in the length of the leaves from the middle belt of the stalk. Positive heterosis in this trait occurs in MV-1 x Adiyaman, P 76/86 x Basma-Djebel, P 76/86 x P 66 9 7 and Basma-Djebel x P 66 9 7.

Inheritance of the number of leaves per stalk and the length of the leaves from the middle belt of the stalk in F₁ hybrids shows identical values for both years of research, which indicates the fact that the parents in the diallel are stable and homozygous and the environmental factors have a limited impact on these traits.

The mode of inheritance of the yield of green mass per stalk is partially-dominant in the direction of the parent with higher yield and positively-dominant (only in MV-1 x Basma-Djebel there is an intermediate mode). Positive heterosis occurs in Oriental crosses where one parent is P 66 9 7.

The mode of inheritance of the yield of

green leaf mass per hectare is partially-dominant and positively dominant. Negative heterosis is present in hybrids where one of the parents is MV-1 (with the exception of MV-1 x Basma-Djebel where there is partial dominance in the direction of the weaker parent). The occurrence of positive heterosis has the Oriental hybrids where one of the parents is P 66 9 7.

The picture of the heritability of the yield detects small differences in relation to the two years of research, which indicates the fact that it is a trait that is greatly influenced by the environmental factors, but also that there is a professional approach to the overall work.

From the analysis of the inheritance of the yield of green leaf mass per stalk and the yield of green leaf mass per hectare, it is concluded that there are no differences with the oriental hybrids, but there are differences with the hybrids where one of the parents is MV-1, and that is a consequence of the different planting distance of plants in the experiment.

The results of this paper are good original source material for further successive selection activity.

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ХЕТЕРОТИЧКИ ЕФЕКТ НА НЕКОИ КВАНТИТАТИВНИ КАРАКТЕРИСТИКИ КАЈ ДИЈАЛЕЛНИТЕ F₁ ХИБРИДИ ОД РАЗЛИЧНИ ВИДОВИ ТУТУН

Јане Алексоски

Научен институт за тутун – Прилеп, Универзитет „Св. Климент Охридски“ – Битола,
„Кичевски пат“ б.б., 7500 Прилеп, Република Северна Македонија

*Контакт автор: yaleksoski@gmail.com

Резиме

Трудот опфаќа проучувања за начинот на наследување и хетеротичниот ефект кај десет F₁ крстоски добиени со еднонасочни дијалелни вкрстувања помеѓу пет родителски генотипови: MV-1, P 76/86, Adiyaman, Vasma-Djebel и P 66 9 7. Анализирани се следниве квантитативни карактеристики: број на листови по страк, должина на листовите од средниот појас на стракот и принос на зелена лисна маса по страк и по хектар. Опитот беше поставен на експериментално поле при Научен институт за тутун – Прилеп, по рандомизиран блок-систем со четири повторувања во периодот 2018-2019 година. За време на вегетациониот период во полето беа применети традиционални културни практики.

Целта на овие истражувања беше да се проучи начинот на наследување на квантитативните особини, да се открие хетерозис кај F₁ генерацијата и да се процени неговата економска исплатливост.

Со анализата на варијансата се утврдија статистички значајни разлики во особините помеѓу родителите и нивните хибриди во двегодишното истражување. Најчестиот начин на наследување на особините е парцијално доминантниот, потоа интермедијарниот. Негативен хетеротичен ефект за бројот на листови по страк има кај P 76/86 x P 66 9 7. Хибридите MV-1 x Adiyaman, P 76/86 x Vasma-Djebel, P 76/86 x P 66 9 7 и Vasma – Djebel x P 66 9 7 имаат позитивен хетеротичен ефект за должината на листовите. Ориенталните хибриди, каде еден од родителите е сортата P 66 9 7, имаат позитивен хетерозис за приносот на зелената лисна маса по страк. Истражувањата нудат многу корисни насоки за идните суцесивни селекциони активности.

Клучни зборови: *Nicotiana tabacum* L., F₁ крстоски, наследност, доминантност, хетерозис.