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Inheritance of Leaf and Seed Yield in F_1 Hybrids of Different Types of Tobacco (*Nicotiana tabacum* L.)

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Abstract **Original Research Article**

The paper studies four F_1 hybrids obtained by crossing five parental genotypes, four of which are oriental in the role of mother (three of the Prilep type: P-23, P 18-50/4, P 76/86, and one of the Basmak type - MS 8/1) and a large-leaf Burley type (B-1/91) in the role of father, for the traits: yield of green leaf mass per stalk and per hectare, yield of dry leaf mass per stalk and per hectare and yield of seed per plant and per hectare. The experiment was placed on the experimental field at the Scientific Tobacco Institute - Prilep by Random block system in four repetitions for the vegetative period et 2019 and 2020. All appropriate cultural practices were applied during the growing season. The aim of this work was to study the mode of inheritance of stated agronomic traits, to detect heterosis in F_1 generation and assess its economic viability. The most common mode of inheritance for the agronomic properties in the F_1 generation is the intermediate, then the partial-dominant. In the inheritance of the yield of green and dry leaf mass per stalk, in the two years of investigation there is no occurrence of heterosis, while in the inheritance of the seed yield per plant in all combinations there is the occurrence of positive heterosis. The highest heterotic effect has MS 8/1 x B-9/91. In analyzing the yield of green and dry leaf mass and seeds in tons per hectare, a different vision was obtained, because the calculations are made at different planting distances of genotypes. Thus, for the yield of green leaf higher value than the more productive parent showed P 76/86 x B-9/91, while for the yield of dry leaf higher values than the more productive parents showed P 18-50/4 x B-9/91 and P 76/86 x B-9/91. In terms of seed yield per hectare, hybrids did not exceed the parents. The mode of inheritance of the agronomic traits per stalk indicates a shorter and more reliable successive period of time in the selection activity for the creation of new, superior varieties of tobacco. The paper opens space for further selection research in direction at obtaining hybrids with a high heterotic effect in the inheritance of seed quantities, in accordance with new trends for alternative use of tobacco as a sustainable crop for seed oil, biofuel and biomass.

Keywords: tobacco (*Nicotiana tabacum* L), yield, inheritance, intermediate, partial dominance, dominance, heterosis.

INTRODUCTION

Tobacco leaves have been used by people for centuries for a pleasures in different ways, usually by smoking. Today there is a strong propaganda about its harmful effect on human health. Therefore, efforts are being made for alternative use of this crop in the direction of seed production, extraction of oil from it, as well as for providing renewable sources for biomass and biofuel. However, there is a huge number of smokers who, despite the harmfulness, do not give up the pleasure that this ancient crop gives them.

This paper covers genetic studies on the inheritance of the most important agronomic traits, green and dry leaf yield and seed yield, in F_1 offspring of hybrids obtained by crossing between varieties of different types of tobacco. A number of scientists of this agricultural crop have worked on the same topic for the last ten years, such as: Gixhari & Sulovari [9], conducted three years of investigations at two sites in a genetically diverse population of eight oriental tobacco and their one-way diallel hybrids and obtained a dominant and partial-dominant mode of inheritance and heterosis for leaf mass yield. Aleksoski & Korubin – Aleksoska [1], carried out three-year studies of green and dry mass yields in a one-way diallel of three oriental and one large-leaf variety and their six F_1 crosses and obtained positive and negative heterosis. Bucciarelli *et al.* [4], performed studies of two genotypes and found that Line 1917 gave a seed yield of 1.8 t/ha and a dry leaf yield of 1.93 t/ha, while the

G105 variety gave a seed yield of 1.47 t/ha and a dry leaf yield of 1.51 t/ha. The weight of 1000 seeds in Line 1917 is 73 mg, and in G105 69 mg. Imtiaz *et al*. [11], in an experiment conducted in Khan Gari, Mardan - Pakistan, with seven Virginia flue-cured genotypes and their 42 two-way diallel crosses found heterosis with high heterotic effect with the possibility of its utilization for the weight of green leaf mass per stalk. Grisa *et al*. [10], in their research on alternative tobacco production in Italy, they studied the yield of seeds of the Solaris variety and the extraction of oil from it, guided by the idea that tobacco could indeed become a new industrial crop, that offers opportunities for its use for biomass and biofuel. 31 to 34 g seeds per plant were recorded, ie 1.1-1.8 t/ha from one harvest, with oil yield up to 0.59 t/ha. From two seed harvests they determined a total seed yield of 4.5 t/ha, from which 1.48 t oil / ha can be obtained. Kinay & Yilmaz [12], experiments with hybrids obtained by one-way diallel crosses between varieties: Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa, in the Turkish province of Tokat, found an average heterosis of 28.4%. The heterotic effect on dry leaf yield was 4%. Dyulgerski & Docheva [5], analyzed ten introduced Burley tobacco varieties for biological, economic, and chemical traits and found that Kentucky 908 and Banquet 102 could be used in selection programs as yield donors. Ganachari *et al*. [7], examined six flue-cured tobaccos and their 30 crosses at ZAHRS (Zonal Agricultural and Horticultural Research Station), College of Agriculture - Shivamogga, Karnataka - India, to assess heterosis for dry leaf yield and its components. The authors identified five hybrids that showed a highly significant heterotic effect. Ganachari *et al*. [8], in their research on six flue-cured tobaccos and their 30 diallel crosses, in ZAHRS, Karnataka, India, got heterosis with a highly significant heterotic effect. Aleksoski [2], in a one-way diallel of four small-leaf aromatic and one large-leaf flue-cured variety, and their 10 F_1 crosses, revealed the existence of all possible inheritance modalities and positive heterosis for leaf mass yield. Dyulgerski [6], in eight newly created Burley hybrid combinations of the first generation for yield, he assessed that all of them are better than the standard variety Pliska 2002, which indicates the possibility of using heterosis in Berley tobacco. Qaizar *et al.* [14], conducted investigations on seven flue-cured varieties and lines and their complete diallel, in the Mardan and Mansehra regions of Pakistan, and found positive and negative heterosis in the inheritance of some agronomic and biochemical traits. The best hybrids with favorable significant heterotic effect for leaf yield were: KHG24 x Spt G 28; KHG21 x NC606 and Spt G 126 x KHG24. Kinay *et al*. [13], in a semi-diallel of seven genotypes and 21 F_1 hybrids, tested in two locations in the Black Sea region of Turkey, obtained positive heterosis for yield, who had no economic viability.

The aim of this research is to study the mode of inheritance and to detect a possible heterotic effect on the yield of green and dry leaf mass and the seed yield per stalk and per hectare in F_1 generation obtained by crossing varieties of different types, which will provide important guidance for future selection programs for tobacco breeding in the direction of increasing leaf and seed yield.

MATERIAL AND METHODS

For genetic studies on inheritance of green and dry leaf yield and seed yield, five varieties of tobacco were selected, four of which in the role of mother: three of the type Prilep (P-23, P 18-50/4 and P 76/86) and one of the Basmak type (MS 8/1), and the Burley variety B-9/91 in the role of father.

In 2018, in field conditions, with manual castration and pollination, four crosses were made: P-23 x B-9/91, P 18-50/4 x B-9/91, P 76/86 x B-9/91 and MS 8/1 x B-9/91.

In 2019, at the Experimental field at the Scientific Tobacco Institute - Prilep, we set up an experiment with nine genotypes (five parents and four F_1 hybrids), according to a Randomized block system in four replications. Oriental genotypes are planted at a distance of 45 x 15 cm (45 cm between rows, and the distance between plants in a row is 15 cm), large-leaf variety B-9/91 in 80 x 50 cm, and F_1 hybrids in 60 x 30 cm. The same year we collected seeds for the second generation and again made the same crosses from which we collected seeds for the first generation.

In 2020, an experiment was set up using the same method, in which the same set of nine genotypes was planted. The paper presents two-year results from the first generation.

Our investigations focuses on: yield of green and dry leaf mass per stalk and per hectare and yield of seeds per plant and per hectare. The yield of green leaf mass was measured after each harvest, the weight of all harvests from each plot was added and 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.

Processing of results

The obtained results are processed statistically. The mode of inheritance of traits is determined on the basis of test significance of the mean value of the F_1 generation relative to the average of both parents, according to Borojevic [3]. Intermediate mode of inheritance (i) occurs when the mean value of a trait in the hybrid 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 hybrids 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 that of the parent with a lower mean value.

Meteorological data

A more realistic vision for the inheritance of quantitative traits is obtained by displaying data on climatic conditions during the tobacco vegetation in 2019 and 2020.

In 2019: The average monthly air temperature from May to September was 22.43 °C (May 15.77 °C, June 22.77 °C, July 24.26 °C, August 27.39 °C, September 21.97 °C). The average monthly maximum air temperature from May to September was 27.2 °C (May - 20.0 °C, June - 28.0 °C, July - 29.0 °C, August - 32.0 °C, September - 27.0 °C). The average monthly minimum air temperature from May to September was 14.8 °C (May - 8.0 °C, June - 16.0 °C, July - 14.0 °C, August - 22.0 °C, September - 14.0 °C). The average monthly relative air humidity from May to September was 58.7 % (May - 71.30 %, June - 67.17 %, July - 59.42 %, August - 42.61 %, September – 53 %). The total rainfall from May to September was 404.8 mm (May - 124.1 mm, June - 139.9 mm, July - 91.8 mm, August - 9.5 mm, September - 39.5 mm). One watering was performed in July, and two waterings in August with a field irrigation rate of 300 m^3/ha of water.

In 2020: The average monthly air temperature from May to September was 22.15 °C (May 16.97 ^{0}C, June 20.3 ^{0}C, July 24.77 ^{0}C, August 25.48 ^{0}C, September 23.23 ^{0}C). The average monthly maximum air temperature from May to September was 28.8 °C (May - 25 °C, June - 27 °C, July - 31 °C, August - 31 °C, September - 30 °C). The average monthly minimum air temperature from May to September was 15.6 °C (May - 9 °C, June - 14 °C, July - 20 °C, August - 20 °C, September - 15 °C). The average monthly relative air humidity from May to September was 61.18 % (May - 69.8 %, June - 70.4 %, July - 59.2 %, August - 56.7 %, September - 49.8 %). The total rainfall from May to September was 400 mm (May - 64 mm, June - 73 mm, July - 97 mm, August - 134 mm, September - 32 mm).

In July and August, one watering was performed with the same watering norm.

The results for temperature and relative are humidity move within the optimal limits for normal development of tobacco and obtaining quality tobacco raw material.

Soil conditions

The soil with its mechanical composition and nutrient content is the substrate on which tobacco grows and develops. Our investigations were performed on the Experimental field at the Scientific Tobacco Institute - Prilep on deluvial (colluvial) soil type. This soil is characterized by low humus content and total nitrogen, moderately acidic to neutral reaction, low to extremely low supply of 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 three spring plows were carried out. The basic fertilization was performed with the second spring plowing.

RESULTS AND DISCUSSION

Yield is a priority and its increase is an eternal aspiration for every breeder. Usually yield and quality do not go in the same direction. Therefore, the duty of the breeder is to increase the yield in his creations, without compromising its quality. This paper covers genetic studies on the inheritance of leaf and seed yield in tobacco.

The highest yield of green leaf mass per stalk among the parent genotypes in 2019 and 2020 is the large-leaf aer-cured variety B-9/91, and among the oriental parents the variety P 76/86. The most productive F_1 hybrid is P 76/86 x B-9/91, and the least productive is P-23 x B-9/91.

The same rank is obtained for the yield of dry leaf mass per stalk in the two years of investigations, because it is the same genome of heredity.

MS 8/1 is characterized by the highest yield of seeds per plant, and with the lowest P 18-50/4. Among the F_1 hybrids MS 8/1 x B-9/91 has the highest and P-23 x B-9/91 the lowest seed yield per plant.

Inheritance of green leaf mass yield per stalk in 2019 and 2020 in F_1 offspring is intermediate. Only P-23 x B-9/91 has partial dominance. These inheritance modalities point to fast and reliable selection in the process of creating new superior varieties.

An identical inheritance occurs for the dry mass yield per stalk in the two years of investigations. The biennial analysis of seed inheritance by stalk in all crosses informs of positive heterosis. This knowledge is the basis for further studies in the field of hybridization and evaluation of the heterotic effect.

Table 1. shows the mean values for the yield of green leaf mass per stalk, dry leaf mass per stalk and seed per plant in the parent genotypes and their F_1

offspring, as well as the mode of inheriting the traits in 2019 and 2020.

Table-1: M**ode of inheritance for the yield of green leaves, dry leaves and seeds per stalk of F_1 hybrids in 2018 and 2019**

No.	Genotypes and F_1 hybrids		Yield per stalk (g) 2019			Yield per stalk (g) 2020		
			Green leaves	Dry leaves	Seeds	Green leaves	Dry leaves	Seeds
			$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
1.	P-23	P1 (♀)	113.14	19.13	11.32	127.66	20.86	10.55
2.	P 18-50/4	P1 (♀)	123.11	20.14	10.41	125.59	20.48	10.30
3.	P 76/86	P1 (♀)	142.10	22.17	10.88	144.82	22.20	11.00
4.	MS 8/1	P1 (♀)	102.71	16.52	11.36	104.19	16.39	11.38
5.	B-9/91	P2 (♂)	1296.53	179.69	10.78	1302.07	184.29	10.98
6.	P-23 x B-9/91	F_1	502.82 pd	75.18 pd	16.27 +h	513.82 pd	75.58 pd	16.55 +h
7.	P 18-50/4 x B-9/91	F_1	594.56 i	88.82 i	16.48 +h	603.07 i	89.41 i	17.11 +h
8.	P 76/86 x B-9/91	F_1	726.84 i	103.86 i	17.05 +h	737.15 i	106.04 i	16.86 +h
9.	MS 8/1 x B-9/91	F_1	558.53 i	82.93 i	23.24 +h	555.75 i	80.53 i	23.52 +h
		LSD $_{0.05}$ =	33.66	8.39	1.65	25.75	5.59	0.74
		$_{0.01}$ =	60.58	15.11	2.97	46.34	10.07	1.32

The ranking of parental genotypes and F_1 hybrids in terms of yield of green leaf, dry leaf and seed per hectare coincides with the ranking of parental genotypes in terms of yield of green leaf, dry leaf and seed per stalk. The differences are in mode of inheritance of the traits, and this is due to the different distance of the *planting* (explained in Materials and Methods). So, the number of plants in hectare is different, therefore the outcome in determining the inheritance of the trait in hybrids is different. Essentially the true vision for inheritance of traits is given by yield per stalk. Yield per hectare gives the quantity that can be obtained from the genotype, which is directly related to economic justification.

Inheritance of green and dry leaf mass yield per hectare in 2019 and 2020 in F_1 offspring is partial-dominant and positive-dominant. There is a positive heterosis for the yield of green leaf mass per hectare in P 76/86 x B-9/91, while for the yield of dry leaf mass per hectare in P 76/86 x B-9/91 and P 18-50/4 x B-9/91.

Inheritance of seed yield per hectare is intermediate. Only in MS 8/1 x B-9/91 there is a partial dominance. This means that the yield of seed per hectare in hybrids at a seedling distance of 60 cm x 30 cm, does not exceed the yield from the higher yielding parent who has a seedling distance of 45 cm x 15 cm. The fact that there is strong heterosis for seed yield per stalk in all F_1 hybrids gives hope and inspiration for further research to obtain new hybrids in which the heterotic effect will persist in the analysis of seed yield per hectare.

Grisan *et al*. [10] carried out studies on the yield of seeds et the Solaris variety and extraction of oil from it, prompted by the need for alternative tobacco production. Their results are drastically higher - 31 to 34g seeds per plant were recorded. This information is another incentive for selective activity in the direction of increasing the seed yield of tobacco.

Table 2. shows the mean values for the yield of green leaf mass, dry leaf mass and seed per hectare in the parent genotypes and their F_1 offspring, as well as the mode of inheriting the traits in 2019 and 2020.

Table-2: M**ode of inheritance for the yield of green leaves, dry leaves and seeds per hectare of F_1 hybrids in 2018 and 2019**

No.	Genotypes and F_1 hybrids		Yield per hectare (t) 2019			Yield per hectare (t) 2020		
			Green leaves	Dry leaves	Seeds	Green leaves	Dry leaves	Seeds
			$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
1.	P-23	P1 (♀)	16.761	2.834	1.677	18.913	3.090	1.563
2.	P 18-50/4	P1 (♀)	18.238	2.984	1.542	18.606	3.034	1.526
3.	P 76/86	P1 (♀)	21.052	3.284	1.612	21.455	3.289	1.630
4.	MS 8/1	P1 (♀)	15.216	2.447	1.683	15.435	2.428	1.686
5.	B-9/91	P2 (♂)	32.413	4.492	0.269	32.552	4.607	0.274
6.	P-23 x B-9/91	F_1	28.050 pd	4.193 pd	0.908 i	28.660 pd	4.216 pd	0.923 i
7.	P 18-50/4 x B-9/91	F_1	33.160 +d	4.954 +h	0.919 i	33.640 +d	4.987 +h	0.954 i
8.	P 76/86 x B-9/91	F_1	40.540 +h	5.793 +h	0.951 i	41.120 +h	5.915 +h	0.940 i
9.	MS 8/1 x B-9/91	F_1	30.600 +d	4.626 +d	1.296 pd	30.270 pd	4.492 +d	1.312 pd

CONCLUSION

- The inheritance of green leaf mass per stalk in F_1 hybrids in 2019 and 2020 is intermediate, with the exception of P-23 x B-9/91 where there is partial dominance. The exposed mode of inheritance indicates fast and reliable successive selection in the process of creating new superior varieties.
- A completely identical mode of inheritance occurs for the yield of dry leaf mass per stalk, because it is the same genome, carrier of the inheritance of the trait.
- The biennial analysis of the inheritance of seed per stalk in all crosses informs about positive heterosis. This knowledge gives opportunity for further studies in the field of hybridization and evaluation of the heterotic effect.
- Inheritance of green and dry leaf mass yield per hectare in 2019 and 2020 in F_1 offspring is partial-dominant and positive-dominant. There is a positive heterosis for the yield of green leaf mass per hectare in P 76/86 x B-9/91, while for the yield of dry leaf mass per hectare in P 76/86 x B-9/91 and P 18-50/4 x B-9/91.
- Inheritance of seed yield per hectare is intermediate, with the exception of MS 8/1 x B-9/91 where partial dominance occurs.
- The inheritance of the yield is identical in the two years of investigation, which indicates the fact that these are traits that are a varietal feature, and on which environmental factors have a limited impact, but also that there is a professional approach to the overall work.
- The inadequacy of inheritance for yield per stalk with yield per hectare comes from different *planting* distances in genotypes and hybrids.
- The results of this paper are an excellent original source material for further successive selection activity in the direction of obtaining varieties with higher yield of leaf mass, and varieties with higher seed yield.
- The paper reveals the possibility for seed production and oil extraction from it, in accordance with the new trends for alternative use of tobacco in providing renewable sources for biomass and biofuel.

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