



Original article

## Tobacco Breeding for Leaves and Yield

Jane Aleksoski <sup>a,\*</sup>, Verica Ilieva <sup>b</sup> & Ana Korubin – Aleksoska <sup>a</sup>

<sup>a</sup> University "St Kliment Ohridski" – Bitola, Scientific Tobacco Institute – Prilep, Republic of North Macedonia

<sup>b</sup> Goce Delcev University – Stip, Faculty of Agriculture, Republic of North Macedonia

### Abstract

The aim of this study was to investigate the mode of inheritance of leaf number per stalk, area of the middle belt leaves and yield of dry leaf mass per stalk in four F1 tobacco hybrids. These tobacco hybrids were obtained by crossing five varieties, four of which are Oriental in the role of mother and one Broadleaf as a father. Investigations were made in 2020 and 2021. The most common mode of inheritance is negative dominance for the first trait, partial for the second trait and intermediate dominance for the third trait. There is no heterosis. The best results considering the size of the middle belt leaves and the yield of dry mass gave P-76/86 x B-1/91. The obtained mode of inheritance is an indicator of good selection of individuals in future generations and quick fixation and stabilization of the traits. The four hybrid combinations represent very interesting starting material for tobacco breeding.

**Keywords:** *Nicotiana Tabacum* L., Hybrids, Inheritance, F1 Generation, Quantitative Traits.

**Received:** 21 August 2023 \* **Accepted:** 03 December 2023 \* **DOI:** <https://doi.org/10.29329/ijjaar.2023.630.3>

---

\* **Corresponding author:**

Jane Aleksoski, University "St Kliment Ohridski" – Bitola, Scientific Tobacco Institute – Prilep, Republic of North Macedonia.  
Email: [yane\\_aleksoski@outlook.es](mailto:yane_aleksoski@outlook.es)

## **INTRODUCTION**

The production of Oriental tobacco stands as one of the most vital sectors in the economy of the Republic of North Macedonia. A significant portion of the tobacco's raw material is destined for international markets. The inclusion of our tobacco in the production of high-quality cigarette brands attests to its exceptional quality and delightful aroma. Consequently, investigations in tobacco genetics and selection are of great importance. Through the application of these scientific methods, breeders aim to develop more productive and superior quality varieties compared to the existing ones. The introduction of these superior varieties into tobacco production is expected to have a positive economic impact, enhancing the livelihoods of producers and boosting financial inflow into the country.

The primary objective of this paper is to investigate the inheritance patterns of three traits: the number of leaves per stalk, the area of the middle belt leaves and the yield of dry leaf mass per stalk within the F<sub>1</sub> generations obtained from various tobacco types. This study seeks to uncover potential heterotic effects and provide valuable data for further successive tobacco breeding activities.

## **MATERIALS and METHODS**

As materials for our study, we selected five genotypes from the gene bank of the Scientific Tobacco Institute – Prilep: Prilep P-23, Prilep P 18-50/4, Prilep P 76/86, Basmak MS 8/1 and Burley B-1/91. The broadleaf variety B-1/91 served as the parent-father. With its pollen, in 2019 and 2020 we made four F<sub>1</sub> hybrids: P-23 x B-1/91 (Figure 6), P 18-50/4 x B-1/91 (Figure 7), P 76/86 x B-1/91 (Figure 8) and MS 8/1 x B-1/91 (Figure 9). These parental varieties and their F<sub>1</sub> hybrids were planted in a randomized block system with four replications on an experimental field at STI-Prilep in 2020 and 2021. The working area covered approximately 291.6 m<sup>2</sup>, or a total area of 655.2 m<sup>2</sup>, including working surfaces and pathways.

The broadleaf variety and F<sub>1</sub> hybrids were planted at a spacing of 90 cm between rows and 50 cm between plants in a row, while the oriental varieties were planted at a spacing of 45 cm between rows and 15 cm between plants. The number of leaves per stalk and the dimensions of the leaves from the middle belt were assessed at the full stage of plant development, at the beginning of flowering. The data collected for the number of leaves per stalk were subjected to variance and statistical analysis, including the calculation of the standard deviation ( $\sigma$ ) and the coefficient of variability (CV).

The surface area of the leaves was calculated by multiplying the mean values of the length by the mean values of the width and by the coefficient  $k=0.6354$ . The dry leaf mass was calculated following tobacco manipulation.

The mode of inheritance for these components was determined based on the statistical significance of the F<sub>1</sub> generation concerning the averages of both parent varieties.

***Parental genotypes:***

Prilep P-23 - Kosta Nikoloski and Milan Mitreski are authors of this variety. Belongs to the oriental sun-cured tobaccos (Figure 1). The characteristics of this variety are described by Korubin – Aleksoska (2004).

Prilep P 18-50/4 - creation by Ana Korubin – Aleksoska. The variety belongs to the group of oriental sun-cured tobaccos (Figure 2).

Prilep P-76/86 - is an oriental sun-cured variety, created by Dimche Chavkaroski and his collaborators (Figure 3). It is distinguished by a long vegetation period (from planting to flowering 85-95 days). A description of the variety can be found in Korubin – Aleksoska (2004).

Basmak MS 8/1 - created by a group of authors, headed by Dusko Boceski. It belongs to the basmak sun-cured type, which was created from the Jakali type from Greece (Figure 4). The morphological traits of the genotype are described by Korubin – Aleksoska and Ayaz Ahmad (2016).

Burley B-1- 9 - Dimche Cavkaroski and his collaborators are the authors of the variety. Belongs to the group of broadleaf air-cured tobacco (Figure 5). A description of the variety can be found in Korubin – Aleksoska (2004).



**Figure 1.** Prilep P-23



**Figure 2.** Prilep P 18-50/4



**Figure 3.** Prilep P-76/86



**Figure 4.** Basmak MS 8/1



**Figure 5.** Burley B-1/91



**Figure 6.** P-23 x B-1/91 (F<sub>1</sub>)



**Figure 7.** P 18-50/4 x B-1/91(F<sub>1</sub>)



**Figure 8.** P-76/86 x B-1/91 (F<sub>1</sub>)



**Figure 9.** MS 8/1 x B-1/91 (F<sub>1</sub>)

***Climatic and soil conditions in the area of investigations:***

When conducting scientific research on quantitative traits from the perspective of selection and genetics, it is imperative to consider the environmental conditions in which the studies were conducted.

The climatic parameters for 2020 and 2021 exhibited significant differences. In 2020, the average temperature (May-September) was 22.15°C, with a minimum temperature of 15.6°C, a maximum temperature of 28.8°C, and a relative humidity of 61.2%. The total rainfall in this period amounted to

400.6 mm. Conversely, in 2021, the average temperature was 18.9°C, the minimum temperature was 13.1°C, the maximum temperature reached 24.1°C, and the relative humidity was 52.2%. The total rainfall during the same period measured 174 mm (<https://en.climate-data.org/europe/macedonia/prilep/prilep-37313/>). Notably, in 2021, the temperature from May to September was lower, air humidity decreased and there was approximately 43% less rainfall.

Our research was conducted on the experimental field at the Scientific Tobacco Institute - Prilep, utilizing a deluvial (colluvial) soil type.

## **RESULTS and DISCUSSION**

### ***Number of leaves per stalk:***

One of the most extensively examined quantitative traits by tobacco breeders is the number of leaves per stalk, as it holds a direct correlation with the yield.

Among the parental genotypes, B-1/91 is characterized by the lowest number of leaves (30.3), while P-76/86 exhibits the highest count (54.4). In the hybrids, the least number of leaves is observed in P-23 x B-1/91 (29.2), and the highest in P 18-50/4 x B-1/91 (34.3). The standard deviation ranges from 1.2 (in P 18-50/4 and P-76/86 x B-1/91) to 2 (for B-1/91). The coefficient of variability spans from 2.2% (in P-76/86 x B-1/91) to 4.5 (for B-1/91). Notably, all variants have a coefficient of variability below 10, indicating that the tested variants are stable and consistent.

The mode of inheritance for this trait is negative dominant, with partial dominance observed only in P 18-50/4 x B-1/91. Heterosis is notably absent.

Partial dominance in inheritance of leaf number per stalk and absence of heterosis found: Korubin – Aleksoska (2000) in the crosses of three oriental varieties, Korubin – Aleksoska (2001) in ten oriental genotypes, Gixhari and Sulovari (2010) in a semi-diallel of eight oriental genotypes. Different way of inheritance and a weak heterotic effect received Aleksoski (2010) in a one-way diallel of three oriental and one Burley variety. Dyulgerski and Radoukova (2019) in seven hybrids of the Berlay type in the F1 generation found the dominance of the parents with a larger number of leaves.

Heterosis with a positive heterotic effect on the trait found: Butorac et al. (1999) in F1 offspring of four Burley varieties, Lalitha et al. (2006) in crosses on six lines and six testers, Dimanov and Dyulgerski (2012) at ten crosses of local and introduced Burley varieties (high heterotic effect is detected), Aleksoski et al. (2013) in hybrids of four parent genotypes of tobacco of different types (the heterosis had a weak heterotic effect), Ramachandra et al. (2015) in hybrids obtained from six lines of different types of tobacco and eight testers.

### **Area of the middle belt leaf of the stalk:**

The parental genotypes exhibit variations in leaf area from the middle belt of the stalk, with the smallest area recorded for P 18-50/4 (173 cm<sup>2</sup>) and the largest for B-1/91 (1203.5 cm<sup>2</sup>). In the F1 hybrids, the smallest leaf area is observed in MS 8/1 x B-1/91 (847 cm<sup>2</sup>), while the largest is seen in P-76/86 x B-1/91 (1270 cm<sup>2</sup>). Notably, the standard deviation and the coefficient of variability are not calculated for this trait because the values are derived from the application of a formula that incorporates mean values of leaf length and width with repetitions.

The mode of inheritance for this trait is partially dominant, with positive dominance found only in P-76/86 x B-1/91. Heterosis is not observed.

Leaf area has been studied by many authors, because the value of this trait correlates with the yield. The most common way of inheritance is the partially dominant and intermediate. Similar results were obtained by: Aleksoski (2010) in a one-way diallel of four parental genotypes of Oriental and Burley origin; Gixhari and Sulovari (2010) in a one-way diallel of eight oriental genotypes; Aleksoski et al. (2013) in a diallel of four parent genotypes of tobacco of different types; Aleksoski (2018) in a diallel of four oriental varieties, etc.

Positive heterosis in inheriting of leaf area received: Korubin – Aleksoska (2000) in diallel of three oriental and one semi-oriental variety (a positive heterotic effect appeared in two crosses where one parent is the introduced variety Pobeda-2); Lalitha et al. (2006) in hybrids of six line and six testers (the resulting heterotic effect was low to moderate in both directions); Aleksoski (2010) in a one-way diallel of four parental genotypes - three oriental and one Burley (the weak heterotic effect had no economic justification); Gixhari and Sulovari (2010) in a diallel of eight parent oriental genotypes; Aleksoski et al. (2013) in six diallel crosses of four parent tobacco genotypes of different types; Aleksoski (2018) in hybrids of four oriental varieties.

### **Yield of dry leaf mass per stalk:**

The investigations for the yield of dry leaf mass are always present in programs for the creation of new more productive varieties and improving of existing ones.

Among the parental genotypes, MS 8/1 exhibits the lowest yield (15.5 g/stalk), whereas B-1/91 demonstrates the highest yield (170.5 g/stalk). In the F1 hybrids, the lowest yield is observed in P-23 x B-1/91 (72 g/stalk), while the highest yield is found in P-76/86 x B-1/91 (104 g/stalk).

The mode of inheritance for this trait is intermediate, with partial dominance observed only in P-23 x B-1/91. Heterosis is not detected.

The dry leaf mass per stalk has been studied by many breeders. The most common way of inheritance is the partially dominant and intermediate.

A partially dominant mode of yield inheritance was obtained by Korubin – Aleksoska (2001) in a diallel of three oriental and one semi-oriental variety and Gixhari and Sulovari (2010) in a one-way diallel of eight oriental genotypes.

Heterosis with a positive heterotic effect on the trait found: Butorac et al. (1999) in F<sub>1</sub> generation of four Burley varieties; Gixhari and Sulovari (2010) in a diallel of eight parental oriental genotypes; Dyulgerski (2019) on eight Berley newly created hybrid combinations of the first generation, Kinay and Yilmaz (2016) in seven hybrids obtained by one-way diallel crosses between oriental varieties. The heterotic effect for dry mass yield was 4%. Kinay et al.(2020) in 21 F<sub>1</sub> half-diallel hybrids of seven oriental tobaccos mostly from the Black Sea region of Turkey.

Table 1 shows the mean values for the number of leaves per stem, area of the middle belt leaf and the yield of dry leaf mass per stalk in parents and F<sub>1</sub> hybrids for 2020 and 2021.

**Table 1.** Mode of inheritance of quantitative traits in parents and F<sub>1</sub> hybrids of tobacco

№	Parents and F <sub>1</sub> hybrids		Quantitative traits										
			Number of leaves per stalk					Area of the middle belt leaf			Yield of dry leaf mass per stalk		
			2020	2021	$\bar{x}$	$\sigma$ (±)	CV (%)	2020	2021	$\bar{x}$ (cm <sup>2</sup> )	2020	2021	$\bar{x}$ (g)
1.	P-23	P1-♀	43.5	42.5	43	1.4	3.5	184	172	178	20	19	19.5
2.	P 18-50/4	P1-♀	46.4	44.4	45.4	1.2	3.6	176	170	173	20	21	20.5
3.	P-76/86	P1-♀	53.6	55.2	54.4	1.5	3.6	174	191	182.5	23	25	24
4.	MS 8/1	P1-♀	40.3	42.1	41.2	1.5	4.1	202	203	202.5	15	16	15.5
5.	B-1/91	P2-♂	32.4	28.2	30.3	2.0	4.5	1197	1210	1203.5	169	172	170.5
6.	P-23 x B-1/91	F <sub>1</sub>	29.7	28.7	29.2 <sup>-d</sup>	1.5	2.4	1080	1088	1084 <sup>pd</sup>	70	74	72 <sup>pd</sup>
7.	P18-50/4xB-1/91	F <sub>1</sub>	34.8	33.8	34.3 <sup>pd</sup>	1.5	2.3	937	917	927 <sup>pd</sup>	89	86	87.5 <sup>i</sup>
8.	P-76/86 x B-1/91	F <sub>1</sub>	30.8	32.2	31.5 <sup>-d</sup>	1.2	2.2	1245	1295	1270 <sup>+d</sup>	98	110	104 <sup>i</sup>
9.	MS 8/1 x B-1/91	F <sub>1</sub>	32.2	30.4	31.3 <sup>-d</sup>	1.7	3.2	892	802	847 <sup>pd</sup>	84	72	78 <sup>i</sup>

## CONCLUSION

Based on our extensive research on parental genotypes and their F<sub>1</sub> hybrids, as well as the mode of inheritance regarding the number of leaves per stalk, area of the leaves from the middle belt and dry leaf yield per stalk, the following conclusions can be drawn:

- The varieties that are the subject of these studies are characterized by a high degree of stability and uniformity, as a result of their homozygosity. The parents in the role of mother and the parent in the role of father, differ significantly in the investigated traits.

- Inheritance of the number of leaves per stalk is negative dominant (only in P 18-50/4 x B-1/91 it is partially dominant).
- Inheritance of the leaf area in the middle belt of the stalk is partially dominant (only in P-76/86 x B-1/91 there is positive dominance).
- Inheritance of the dry leaf mass yield per stalk is intermediate (only in P-23 x B-1/91 there is partial dominance).
- There is no occurrence of a heterotic effect in the F<sub>1</sub> population in all studied morphological traits, in the two years investigations.
- The best results for leaf area and dry leaf yield per stalk were given by P-76/86 x B-1/91.
- With these investigations we obtained F<sub>1</sub> hybrid offspring, with which we provided material for further breeding activity.
- The results obtained with these studies are useful achievements in the genetics and tobacco breeding and they have primary importance for science and practice in the process of creating new superior varieties.

### **Acknowledgement**

I would like to thank all the employees of the Scientific Tobacco Institute - Prilep who helped in the realization of this research.

### **REFERENCES**

- Aleksoski, J. 2010. Estimation of the heterotic effect in F<sub>1</sub> generation of various tobacco genotypes and their diallel cross. *BIOTECHNOL. & BIOTECHNOL.* 24(2): 407-411.
- Aleksoski, J., Dimitrieski, M., Korubin – Aleksoska, A. 2013. Investigations of heritability as an indicator of the inheritance of quantitative characters in tobacco. *Тутун / Tobacco.* 63(7-12): 54-62.
- Aleksoski, J. 2018. The effect of Backcross Method in tobacco breeding. *Journal of Agriculture and Plant Sciences, JAPS.* 16(1): 9-19.
- Butorac, J., Vasilj, D., Kozumplik, V., Beljo, J. 1999. Quantitative parameters of some Burley tobacco traits. *Rostlinna Vyroba.* 45(4): 149-156.
- Dimanov, D., Dyulgierski, Y. 2012. Heterosis behaviour with regards to the height and number of the leaves by tobaccos of burley variety group. *Acta Agriculturae Serbica.* XVII(33): 53-58.
- Dyulgierski, Y. 2019. Estimation of new hybrids in first generation of Burley tobacco. *Bulgarian Journal of Crop Science.* 56(2): 20–25.
- Dyulgierski, Y., Radoukova, T. 2019. Hybridological analysis of the number and sizes of leaves in hybrid combinations of burley tobacco. *Agricultural Sciences – Plant breeding.* 72(1): 136-142.
- Gixhari, B., Sulovari, H. 2010. Nature of inheritance and heterosis estimated on some morphological quantitative characters that influence the tobacco yield. *Studii și Cercetări (SCSB), Universitatea "Vasile Alecsandri" din Bacău, Romania.* XVIII: 46-50.



- Kinay, A., Yilmaz, G. 2016. Effects of heterosis on agronomically important traits of oriental tobacco (*Nicotiana tabacum* L.) hybrids. Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi. 11 (1): 89-94.
- Kinay, A., Yilmaz, G., Kandemir, N. 2020. Yield and quality properties of some Oriental tobacco (*Nicotiana tabacum* L.) hybrids. GENETIKA. 52(2): 735-750.
- Korubin – Aleksoska, A. 2000. Mode of inheritance for the more important morphological traits of tobacco varieties and their diallel hybrids. ТУТУН / Tobacco. 50(1-3): 3-12.
- Korubin – Aleksoska, A. 2001. Study of heredity through the genotypic components of variance in some tobacco varieties. ТУТУН / Tobacco. 51(1-2): 3-8.
- Korubin – Aleksoska, A. 2004. Tobacco varieties from Tobacco Institute - Prilep. NITP, Republic of Macedonia: University “St. Kliment Ohridski” – Bitola.
- Korubin – Aleksoska, A., Ayaz Ahmad, M. 2016. Basmak - a new type of tobacco in the Balkans. Journal of Agriculture and Veterinary Science (IOSR-JAVS). 9(8): 12-17.
- Lalitha, D. D., Lakshminarayana, R., Atluri, J.B. 2006. Heterosis for seed and other quantitative characters in Tobacco (*Nicotiana tabacum* L.). Indian Journal of Agricultural Research. (40):10–17.
- Ramachandra, R.K., Nagappa, B.H., Anjenaya Reddy, B. 2015. Heterosis studies on yield and quality parameters in bide tobacco (*Nicotiana tabacum* L.). J.Bio.Innov. 4(4): 126-134.