# GENETIC ANALYSIS OF NICOTINE INHERITANCE IN ONE-WAY DIALLEL CROSSES OF DIFFERENT TOBACCO TYPES

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## ABSTRACT

Investigations are made with four parental genotypes, three of which are oriental (P-84, P 10-3/2 and YK-48), and one semi-oriental (FL) and their six one-way $F_1$ diallels and the same number of $F_2$, $BC_1(P_1)$ and $BC_1(P_2)$ crosses to estimate nicotine content in dry tobacco leaves. Crossings were made in 2010 and 2011 and the trial with parents and hybrids was set up in 2012 in the field of Tobacco Institute-Prilep, using randomized block design with four replicates. Usual cultural practices were applied during the growing season.

The aim of investigation is to make genetic analysis of the inheritance of nicotine content, which would give reliable directions in selection of low-nicotine tobacco genotypes.

The prevailing mode of inheritance in $F_1$ is partial dominance, but positive and negative heterosis is also observed; in $F_2$ intermediate mode is prevailing; in $BC_1$ it varies, in back-crossing with $P_1$ positive heterosis is present and in back-crossing with $P_2$ negative heterosis. In $F_1$ the additive (D) and dominant ($H_1$ and $H_2$) components of genetic variance are equal, while in the other generations the additive component has significantly higher magnitude than the dominant one. The interaction (F) value indicates that genes from the parent with lower nicotine content are predominant. The average degree of dominance shows complete dominance in $F_1$ and partial dominance in $F_2$ and $BC_1$, which means rapid fixation of the trait in the successive selection. The ratio between dominant and recessive alleles indicates that recessive genes prevail. Degree of heritability ($h^2$) confirms that nicotine is a highly heritable trait on which environmental factors have a limited effect.

***Key words:*** *tobacco (Nicotiana tabacum L.), nicotine, genetic analysis, mode of inheritance, components of genetic variance.*

## Introduction

Nicotine is alkaloid present in some species of the genus *Solanaceae,* especially in tobacco (*Nicotiana tabacum*), from which its name is derived. Its biosynthesis is performed in plant roots, but it is stored in the leaves. It is toxic and stimulating element that causes smoking dependence. It was isolated in 1828 by the German chemists Ch. W. Posselt and K.L. Reimann. Its chemical empiric formula was first determined by L. Melsen in 1843, and structural formula by G. Pinner in 1893. The first synthesis of nicotine was made by A. Pictet and P. Crepieux in 1904 (M.S. Abdullahi et al., 2014). In recent studies, the genetics of dependence from this alkaloid has been investigated. Vink J.M. et al. (2005) reports that 75% of the heritability fall on genetic factors, and 25% on the environment. This perception caused a need for further investigations on location and identification of specific genes that provoke nicotine dependence.

Nicotine inheritance is conditioned by a number of genes called minorgenes or polygenes. Its development and expression is greatly affected by environmental factors. Due to this, variability of this trait in pure lines is of environmental nature. Therefore, selectionists, make hybridization and thorough recombination of parental genes they allow selection of individuals with various amounts of nicotine from the second generation. However, the environmental and genetic variability are simultaneously present, which complicates the process of selection in this direction.

The aim of the paper is to make genetic analysis on the inheritance of nicotine content and thus to improve our knowledge on the mode of inheritance, gene type and heritability level, which will give reliable directions in selection of low-nicotine tobacco genotypes. Their implementation in fabrication will lead to a production of low-nicotine cigarettes with reduced health risks and with secure placement on the market.

**Material and methods**

Investigations included four tobacco varieties, of which three were oriental (Prilep P-84, P 10-3/2 and Yaka YK-48 and one semi-oriental (Floria FL-breeding line). They were used in 2010 to make one-way diallel and seed was obtained from the 6 crosses for $F_1$ generation. In 2011 a seed for $F_2$ by $F_1$ isolation was obtained and crosses were made to provide seed for six hybrids of $F_1$ and for the same number of hybrids for $F_2$, $BC_1$ ($P_1$) and $BC_1$ ($P_2$) generations. The trial with 28 variants (parents and crosses) was set up in 2012, in the field of Tobacco Institute-Prilep using randomized block design with four replicates. Usual agricultural practices typical for the region of Prilep were applied during the growing season.

The nicotine content was determined spectrophotometrically, on dry fermented leaf samples. The data obtained for investigated generations were processed by variational-statistical method. The mode of inheritance was evaluated according to the test-significance of the mean values of the progeny in relation to the parental average (Borojević, 1981). Genotypic variance components were estimated by the method of Mather and Jinks (1974).

During tobacco growing season, from May to September in 2012, the mean monthly temperature was 20,3$^0$ C, mean monthly relative humidity was 53 % and there were 26 days with 186 l/m² rainfall. Presented climate data were obtained from the meteorological station located in the Experimental field of Tobacco Institute-Prielp.

**Results and discussion**

The lowest nicotine content among parental genotypes was recorded in the semi-oriental breeding line Floria FL (0,41), followed by Prilep P-84 (1,35), YK-48 (1.63) and Prilep P 10-3/2 (1.79).

Our investigations revealed the presence of all modes of nicotine inheritance in the four generations studied. Partial dominance was prevailing in $F_1$ generation, positive heterosis was recorded in YK-48 x FL and negative heterosis in P-84 x FL. In $F_2$ generation the most common mode of inheritance was intermediate inheritance. In $BC_1$ ($P_1$) partial-dominant type was prevailing and in (P 10-3/2 x YK-48) x P 10-3/2 positive heterosis ocurred. In $BC_1$ ($P_2$) negative heterosis was prevailing in (P-84 x FL) x FL, (P 10-3/2 x YK- 48) x (YK- 48), (P 10-3/2 x FL) x FL and (YK- 48 x FL) x FL (Table 1).

**Table 1. Inheritance of nicotine content in one-way diallel of tobacco**

| Diallel crosses | Parents and genotypes (nicotine content, %) | | | | | |
|---|---|---|---|---|---|---|
| | $P_1$ | $P_2$ | $F_1$ | $F_2$ | $BC_1$ ($P_1$) | $BC_1$ ($P_2$) |
| 1. P-84 x P 10-3/2 | 1,35 | 1,79 | $1{,}68_{pd}$ | $1{,}60_{i}$ | $1{,}68_{pd}$ | $1{,}55_{i}$ |
| 2. P-84 x YK-48 | 1,35 | 1,63 | $1{,}55_{pd}$ | $1{,}51_{i}$ | $1{,}51_{i}$ | $1{,}42_{pd}$ |
| 3. P-84 x FL | 1,35 | 0,41 | $\mathbf{0{,}15_{-h}}$ | $0{,}45_{-d}$ | $1{,}37_{+d}$ | $\mathbf{0{,}20_{-h}}$ |
| 4. P 10-3/2 x YK-48 | 1,79 | 1,63 | $1{,}67_{pd}$ | $1{,}80_{+d}$ | $\mathbf{1{,}89_{+h}}$ | $\mathbf{1.35_{-h}}$ |
| 5. P 10-3/2 x FL | 1,79 | 0,41 | $1{,}39_{pd}$ | $0{,}99_{i}$ | $1{,}42_{pd}$ | $\mathbf{0{,}21_{-h}}$ |
| 6. YK-48 x FL | 1,63 | 0,41 | $\mathbf{1{,}83_{+h}}$ | $1{,}14_{i}$ | $1{,}37_{pd}$ | $\mathbf{0{,}19_{-h}}$ |

**Table 2.Genetic components of variance for the character nicotine content in dry tobacco leaves in $F_1$, $F_2$, $BC_1$ ($P_1$) and $BC_1$ ($P_2$) generation**

| Components of the genetic variance | Generation | | | |
|---|---|---|---|---|
| | $F_1$ | $F_2$ | $BC_1$ ($P_1$) | $BC_1$ ($P_2$) |
| D | 0,47 | 0,45 | 0,47 | 0,46 |
| $H_1$ | 0,48 | 0,03 | 0,40 | 0,27 |
| $H_2$ | 0,05 | 0,12 | 0,33 | 0,23 |
| F | - 0,09 | - 0,50 | - 0,25 | - 0,05 |
| E | 0,0003 | 0,018 | 0,003 | 0,004 |
| $H_2 / 4H_1$ | 0,026 | 1 | 0,206 | 0,213 |
| $\sqrt{H1/D}$ | 1,011 | 0,258 | 0,922 | 0,766 |
| Kd / Kr | 0,827 | -0,365 | 0,552 | 0,867 |
| $h^2$ | 0,999 | 0,961 | 0,994 | 0,988 |

In the progeny of $F_1$ generation, dominant component $H_1$ is approximately equal to the additive component D of the genetic variance, while the corrected component $H_2$ is smaller than D. In $F_2$, $BC_1$ ($P_1$) and $BC_1$ ($P_2$), the value for D is significantly higher than that for $H_1$ and $H_2$, indicating that in the inheritance of nicotine content, the main part of genetic variance belongs to the additive component.

Interaction F has negative value in all generations, which indicates the prevalence of genes from parents with lower nicotine content.

Values for E are very low, which indicates that the impact of the external environment on the changes of this trait is minimal.

The value of $H_2/4H_1$ in $F_2$ is higher than 0,25, indicating symmetrical distribution of dominant and recessive alleles, while in other generations the values are smaller, which is a sign of asymmetrical distribution.

The average degree of dominance $\sqrt{H1 / D}$ in $F_1$ is higher than 1, indicating complete dominance. In other generations this value is lower than 1, which is an indication of partial dominance in the inheritance of this trait.

The ratio between dominant and recessive alleles (Kd / Kr) in investigated generations is lower than 1, which indicates the dominance of recessive genes in inheritance of the trait.

Heritability, i.e. percentual representation of genetic variance among the four generations is high ($F_1 \approx 100$ %, $F_2 = 96$ %, $BC_1$ ($P_1$) = 99 % and $BC_1$ ($P_2$) = 99 % ), which indicates that the investigated trait is highly heritable.

Changchun (2009) reported high heritability in the inheritance of nicotine in $F_1$ population of Burley 37 ($P_1$) and Burley 21 ($P_2$). He informed that this trait is governed by low frequency polygenes, but that two pairs of major genes are the most important. Thus, major genes are responsible for 45,63 % of the total nicotine heritability in dry leaves, polygenes for 18,45 % and effective polygenes for 4,85 %. The author also confirmed the low impact of environmental factors in manifestation of this trait.

**Conclusions**

The nicotine content in $F_1$ progeny is inherited by partial-dominance, positive heterosis appears in YK-48 x FL and negative heterosis in P-84 x FL - because it is low-nicotine hybrid. In $F_2$ intermediate mode of inheritance is prevailing, while dominant mode is present in two hybrids (in P 10-3/2 x YK- 48 the parent with higher nicotine content is dominant and in P- 84 x FL the parent with lower nicotine content is dominant). In back-cross $BC_1$ generations three modes of inheritance occur: intermediate, partial-dominance and dominance. Positive heterotic effect occurs with $BC_1$ ($P_1$) in (P 10-3/2 x YK- 48 ) x P 10-3/2, and negative with $BC_1$ ($P_2$) in: (P-84 x FL) x FL, (P 10-3/2 x YK-48 ) x YK-48 , (P 10-3/2 x FL) x FL and (YK-48 x FL) x FL.

In $F_1$ generation, dominant component $H_1$ is approximately equal to the additive component D, while the corrected $H_2$ is lower than D. In $F_2$, $BC_1$ ($P_1$) and $BC_1$ ($P_2$) the values for D are significantly higher than those for $H_1$ and $H_2$, which indicates that major part of the genetic variance in inheritance of nicotine content belongs to the additive component. This suggests that the inheritance of this trait is controlled by recessive genes.

The F component (interaction) in investigated generations has a negative value, indicating dominance of the genes from parents with lower nicotine content.

The low values for E indicate a very low impact of the environment on the changes of this trait.

The value of $H_2 / 4H_1$ shows that distribution of dominant and recessive alleles is symmetrical only in $F_2$, while in other generations the allele distribution is asymmetrical.

The average degree of dominance $\sqrt{H1 / D}$ shows complete dominance in $F_1$, and in all other generations this trait is inherited with partial-dominance.

The value obtained from the ratio between total number of dominant and recessive alleles (Kd / Kr) confirms the dominance of recessive genes in inheritance of this trait.

High heritability values show high percentual representation of the genetic variance, which indicates that the inheritance of the nicotine content is a highly heritable trait.

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