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Components of genetic variance for yield and nicotine inheritance in tobacco

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Abstract

Investigations are made with four tobacco varieties (3 oriental and 1 semi – oriental), with their six one – way F_1 and the same number of F_2 crosses to estimate dry mass yield per stalk and nicotine content in dry tobacco leaves. Crossings were made in 2010 and 2011 and the trial with sixteen varieties (parents and hybrids) was set up in 2012 and 2013 in the field of the Tobacco Institute – Prilep, using randomized block design with four replicates. Common agricultural practices were applied during the growing season. The aim of the investigations was to study the genetics of inheritance of dry yield and nicotine content in dry leaves through analysis of the components of genetic variance, in order to obtain reliable directions in the selection of new higher – yielding and low – nicotine tobacco genotypes. The prevailing mode for inheritance of yield was the intermediate, and for nicotine it was partial dominance, followed by the intermediate mode. In some combinations heterotic effect for nicotine content was observed. In both generations and for both traits, the additive genetic component had higher values than the dominant one (with the exception of F_1 generation for the inheritance of nicotine, in which both components had equal effect), indicating that a major part of genetic variance is governed by the additive gene action. The interaction shows that in both F_1 and F_2 generations the genes of the parent with lower nicotine content are overdominant. In F_1 genes of the parent with lower yield, and in F_2 those of the parent with higher yield are prevailing. The ratio between dominant and recessive alleles indicates the dominance of the latter. The heritability values point to the limited impact of environmental factors, which means that yield and nicotine are highly heritable traits.

Keywords: Tobacco (*Nicotiana tabacum L.*), yield, nicotine, mode of inheritance, components of genetic variance, heritability (h^2).

Introduction

Plant breeding and the creation of new superior varieties is the desired outcome of selectors. The main goal in the development of new genotypes is to increase the yield, to improve the quality traits and resistance in all cultures, and for the tobacco used for enjoyment to have desirable levels of nicotine.

Nicotine is an 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 a 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 information caused a need for further investigations on location and identification of specific genes that provoke nicotine dependence.

Yield and nicotine inheritance are 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 the traits in pure lines is of environmental nature. Therefore, when selectionists make hybridization and thorough recombination of parental genes they allow selection of individuals with higher yield and 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 this paper is to study the inheritance of yield and nicotine and to analyze the genetic variance, and therefore to improve our knowledge of the mode of inheritance, gene type and heritability level. This will give reliable guidance in the selection of getting more productive low – nicotine tobacco genotypes. Their implementation in fabrication will lead to a production of low – nicotine cigarettes with reduced risks for human health and with secure placement on the market.

In implementing the selection programs of this type, it is necessary to have a good understanding of the available genetic potential of the parental genotypes. Therefor genetic analysis should be performed on the parental pairs and their offspring, which will provide reliable guidance for further successive selection.

Materials and Methods

Investigations included four tobacco varieties, of which three were oriental (Prilep P – 84 – Figure 1, Prilep P 10 – 3/2 – Figure 2, and Yaka YK – 48 – Figure 3) and one semi – oriental (Floria FL – breeding line – Figure 4). They were used in 2010 to make one – way diallel and seed were 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 again for six hybrids of F_1 generations. The trial with 16 variants (parents and crosses) was set up in 2012 and 2013, in the field of the Tobacco Institute – Prilep using randomized block design with four replicates. Usual agricultural practices were applied during the growing season.

Dry mass yield was measured at each harvest during manipulation of cured tobacco. To estimate dry mass yield per stalk, tobacco weights from each plot were added and then divided with the number of stalks. The nicotine content was determined spectrophotometrically, on dry fermented leaf samples. The obtained data for the investigated generations was processed by the variational – statistical method. The mode of inheritance was evaluated by the test – significance of the mean values of the progeny in relation to parental average (Borojević, 1981). Components of the genetic variance were calculated by the method of Mather & Jinks (1974).

Inheritance of yield and nicotine are controlled by a large number of genes i.e. polygenes and its manifestation, to certain limits, is affected by environmental factors. For this reason, data on meteorological conditions in the investigation period are necessary. From May to September 2012, during tobacco growing season, the mean monthly temperature was 20,3 ^{0}C, the mean monthly relative humidity 53% and number of rain days 26, with precipitation of 186 mm. In the same period in 2013 the mean monthly temperature was 19,4 ^{0}C, mean monthly relative humidity 52% and number of rain days 34, with precipitation of 153 mm. The presented climate parameters were obtained from the Meteorological station located in the experimental field of the Tobacco Institute – Prilep.

Figure 1. Prilep P – 84

Figure 2. Prilep P 10 – 3/2

Figure 3. Yaka YK – 48

Figure 4. Floria FL

Results and Discussion

The highest dry mass yield per stalk among parental genotypes was observed in FL – breeding line (27,52 g – 2012, 28,41 g – 2013), followed by YK – 48 (17,73 g – 2012, 18,95 g – 2013), P – 84 (17,33 g – 2012, 18,27 g – 2013), and the lowest – yielding variety was P 10 – 3/2 (14,15 g – 2012, 13,89 g – 2013). The highest yields among hybrids are those where one of the parents is FL. Our investigation revealed prevailing of partial dominance in inheritance of this trait, and it is followed by Intermediate. In the crosses P 10 – 3/2 x YK – 48 and P – 84 x YK – 48 in F_2 the inheritance is dominantly positive. There is no occurrence of heterosis (Table 1).

The lowest nicotine content among parental genotypes was recorded in FL (0,41% – 2012, 0,43% – 2013), followed by P – 84 (1,35% – 2012, 1,42% – 2013), YK – 48 (1,63% – 2012, 1,59% – 2013) and P 10 – 3/2 (1,79% – 2012, 1,84% – 2013). The investigations revealed the presence of all modes of nicotine inheritance. 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 (Table 2).

Table 1. Mode of inheritance for dry mass yield per stalk in one – way diallel of tobacco

Diallel crosses	Parents and genotypes (dry mass yield per stalk, g)							
	2012				2013			
	P_1	P_2	F_1	F_2	P_1	P_2	F_1	F_2
P-84 x P 10-3/2	17,33	14,15	16,85 pd	16,55 pd	18,27	13,89	17,05 pd	16,86 pd
P-84 x YK-48	17,33	17,73	17,54 i	17,51 i	18,27	18,95	18,72 pd	18,93 +d
P-84 x FL	17,33	27,52	25,77 pd	24,42 pd	18,27	28,41	24,98 pd	24,12 i
P 10-3/2 x YK-48	14,15	17,73	17,19 pd	17,35 +d	13,89	18,95	18,07 pd	18,24 pd
P 10-3/2 x FL	14,15	27,52	23,26 pd	20,08 i	13,89	28,41	24,14 pd	22,78 i
YK-48 x FL	17,73	27,52	24,91 pd	24,24 pd	18,95	28,41	25,23 pd	25,05 pd

Table 2. Mode of inheritance for nicotine content in one – way diallel of tobacco

Diallel crosses	Parents and genotypes (nicotine content, %)							
	2012				2013			
	P_1	P_2	F_1	F_2	P_1	P_2	F_1	F_2
P-84 x P 10-3/2	1,35	1,79	1,68 pd	1,60 i	1,42	1,84	1,70 pd	1,67 i
P-84 x YK-48	1,35	1,63	1,55 pd	1,51 i	1,42	1,59	1,49 i	1,53 pd
P-84 x FL	1,35	0,41	0,15 - h	0,45 -d	1,42	0,43	0,17 - h	0,30 -d
P 10-3/2 x YK-48	1,79	1,63	1,67 pd	1,80 +d	1,84	1,59	1,72 i	1,78 pd
P 10-3/2 x FL	1,79	0,41	1,39 pd	0,99 i	1,84	0,43	1,23 i	0,89 pd
YK-48 x FL	1,63	0,41	1,83 +h	1,14 i	1,59	0,43	1,78 +h	1,05 i

Genetic analysis in Table 3 shows that the dominant component (H_1 and H_2) in F_1 and F_2 progenies is lower than the additive one (D) in two years of research (H_1 = 2,24 and H_2 = 1,865 < D= 39,925 in F_1; H_1 = 3,255 and H_2 = 2,265 < D= 40,58 in F_2), which indicates a stronger effect of recessive genes in the inheritance of dry mass yield per stalk.

Interaction (F) in F_1 generation has a negative value ($\bar{x}$ = –3,01), which is a sign of the prevalence of genes from lower yielding – parents, while in F_2 this value is positive ($\bar{x}$ = 2,73), indicating the dominance of genes from higher yielding – parents.

The low values for E ($\bar{x}$ = 0,105 in F_1, $\bar{x}$ = 0,075 in F_2), indicate that the environment has limited impact on this property.

The value of $H_2/4H_1$ in both generations is less than 0,25 ($\bar{x}$ = 0,209 in F_1), $\bar{x}$ = 0,187 in F_2), which is an indication of asymmetrical distribution of dominant and recessive alleles.

The average degree of dominance ($\sqrt{H1/D}$) in F_1 and F_2 is lower than 1 ($\bar{x}$ = 0,715 in F_1), $\bar{x}$ = 0,715 in F_2), which indicates partial dominance in inheritance of this trait.

The dominant/recessive alleles ratio (Kd/Kr) in F_1 is lower than 1 ($\bar{x}$ = 0,715), which denotes that the inheritance of this trait is controlled by recessive alleles, and in F_2 it is higher than 1 ($\bar{x}$ = 1,289), indicating the prevalence of dominant alleles.

There is high heritability – h^2 (percentage of genetic variance) in both generations ($\bar{x}$ = 99,5% in F_1, $\bar{x}$= 99,6% in F_2), which means that the investigated trait is highly inheritable.

Genetic analysis in Table 4 shows that in the progeny of F_1 generation, dominant component H_1 is approximately equal to the additive component D of the genetic variance (H_1 = 0,485 ≈ D = 0,43), while the corrected component H_2 is smaller than D (H_2 = 0,05 < D = 0,43). In F_2, the value for D is significantly higher than that for H_1 and H_2 (H_1 = 3,255 and H_2 = 2,265 < D = 40,58), indicating that in the inheritance of nicotine content, the main part of the genetic variance belongs to the additive component.

Interaction F has negative value in both generations ($\bar{x}$ = – 0,16 in F_1, $\bar{x}$ = – 0,4 in F_2),

which indicates the prevalence of genes from parents with lower nicotine content.

Values for E are very low ($\bar{x}$ = 0,0003 in F_1, $\bar{x}$ = 0,0165 in F_2), 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_1 is less than 0,25 ($\bar{x}$ = 0,03), which is an indication of asymmetrical distribution of the dominant and recessive alleles. The value of $H_2/4H_1$ in F_2 is higher than 0,25 ($\bar{x}$ = 0,85), indicating symmetrical distribution of the dominant and recessive alleles.

The average degree of dominance $\sqrt{H1 / D}$ in both generations is higher than 1 ($\bar{x}$ = 1,066 in F_1, $\bar{x}$ = 1,313 in F_2), indicating complete dominance.

The ratio between dominant and recessive alleles (Kd/Kr) in investigated generations is lower than 1 ($\bar{x}$ = 0,705 in F_1, $\bar{x}$ = 0,089 in F_2), which indicates the dominance of recessive genes in inheritance of the trait.

Heritability h^2 is high (F_1 ≈ 100 %, F_2 = 95%), 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.

Conclusion

The inheritance of dry mass yield per stalk is partially dominant and intermediate. There is no occurrence of heterosis.

Genetic analysis shows that the presence of the additive component of the genetic variance in inheritance of dry mass yield per stalk is higher than the dominant one (D > H_1 and H_2), which indicates higher a participation of recessive genes in inheritance of this trait. From interaction it can be concluded that in F_1 progeny genes from the lower yielding-parents prevail, while F_2 progeny is controlled by the genes from parents with higher yields. Low values of E show limited impact of the environment on the modification of this trait.

The average degree of dominance points to a partial dominance in the inheritance of this trait. Ratio between dominant and recessive alleles shows that recessive alleles prevail in the first generation and dominant alleles in the second generation. Heritability in both generations has

Table 3. Genetic components of variance for dry mees yield per stalk in F_1 and F_2 generation

Components of the genetic variance	Generations					
	F_1 2012	F_1 2013	$\bar{x}$	F_2 2012	F_2 2013	$\bar{x}$
D	37,52	42,33	39,925	37,59	43,57	40.58
H_1	1,47	3,01	2,24	1,94	4,57	3.255
H_2	1,25	2,48	1,865	1,71	2,82	2.265
F	- 2,89	- 3,13	-3,01	2,51	2,95	2,73
E	0,12	0,09	0,105	0,10	0,05	0.075
$H_2 / 4H_1$	0,213	0.206	0,2095	0,220	0.154	0.187
$\sqrt{H1 / D}$	0,1979	0.2667	0,2323	0,2272	0.3239	0.2755
Kd/Kr	0,674	0.756	0,715	1,345	1.233	1.289
h^2	0,9942	0.996	0,9951	0,9945	0.998	0.9963

Table 4. Genetic components of variance for nicotine content in dry tobacco leaves in F_1 and F_2 generation

Components of the genetic variance	Generations					
	F_1 2012	F_1 2013	$\bar{x}$	F_2 2012	F_2 2013	$\bar{x}$
D	0,47	0,39	0,43	0,45	0,37	0,41
H_1	0,48	0,49	0,485	0,03	0,05	0,04
H_2	0,05	0,05	0,05	0,12	0,14	0,13
F	- 0,09	- 0,23	- 0,16	-0,05	- 0,75	- 0,4
E	0,0003	0,0003	0,0003	0,018	0,015	0,0165
$H_2 / 4H_1$	0,026	0,0255	0,0257	1	0,7	0,85
$\sqrt{H1 / D}$	1,011	1,121	1,066	0,258	0,368	1,313
Kd/Kr	0,827	0,583	0,705	0,646	-0,468	0,089
h^2	0,999	0,999	0,999	0,927	0,974	0,951

high values, indicating that this is a highly heritable trait.

The inheritance of nicotine content in **F_1** generation is partially dominant and intermediate. Positive heterosis appears in YK-48 x FL and negative heterosis in P-84 x FL - because it is a low-nicotine hybrid. In F_2 the intermediate mode of inheritance is prevailing, while the 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 F_1 generation, the dominant component H_1 is approximately equal to the additive component D, while the corrected H_2 is lower than D. In F_2 the values for D are significantly higher than those for H_1 and H_2, which indicates that a major part of the genetic variance in the inheritance of nicotine content belongs to the additive component. This suggests that the inheritance of this trait is controlled by the recessive genes. The interaction in the 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 average degree of dominance shows complete dominance in F_1, and in F_2 generations this trait is inherited with partial-dominance. The value obtained from the ratio between total number of dominant and recessive alleles confirms the dominance of recessive genes in the inheritance of this trait. High heritability values show that the inheritance of the nicotine content is a highly heritable trait.

From the data the mode of inheritance and the genetic analysis of variance from diallel crosses can be separated P-84 x FL, P 10-3/2 x FL and YK-48 x FL. Starting from their F2 population, future successive selection in the shortest period can get stable higher-yields and low-nicotine varieties.

References

Abdullahi, M.S., R.U. Akpokerie, T.P. Adedokun, 2014. Determination of Nicotine Content in Some Cigarette (Aspen, Excel and Benson & Hedges) Brands Sold Within Kontagora Market, Nigeria. Global Advanced Research Journal of Food Science and Technology, l. 3(1): 001-007.

Borojević, S., 1981. Principi i metode oplemenjivanja bilja. Ćirpanov, Novi Sad.

Changchun, C., 2009. Genetic analysis of nicotine content of burley tobacco by using DH population. Acta Tabacaria Sinica, 15(4): 55-67.

Mather, K., J.L. Jinks, 1974. Biometrical genetics. Champan and Hall, London.

Vink, J.M., G. Willemsen, D.I. Boomsma, 2005. Heritability of Smoking Initiation and Nicotine Dependence. Behavior Genetics, 35(4): 397 – 406.