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# PRODUCTIONAL CHARACTERISTICS OF SOME ORIENTAL TOBACCO LINES RESISTANT TO BLACK SHANK (*Phytophthoraparasitica var. nicotianae*)

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## ABSTRACT

The aim of investigations was to evaluate morphological, productional and qualitative traits of 4 newly created fertile inbred lines and their resistance to black shank disease (*Phytophthoraparasitica*var. *nicotianae*), compared to the susceptible standard variety YV 125/3(∅). The investigated lines were obtained by intraspecific hybridization.The breeding process startedby crossing of introduced oriental tobacco varieties withstable domestic linesresistant to black shank (AA) which were used as mother components and susceptible lines and varieties (aa), used as father components. Selection of hybrid progenies was made using the Pedigree method. The investigated lines were also stable in plant height andin number, shape and sizeof the leaves. According to the results of field experiments, the newly created resistant lines have higherleaf number per plant (45-53 leaves) as compared to the variety YV 125/3∅ (37 leaves). They also achieved higher yields (33.66%- 68.80%), higher purchase price (14.3%- 27.7%) and greater economic effect (41.01% to 81.29%). Black shank is economically important disease of oriental tobacco throughout the world. In favorable conditions for its occurrence it can also cause severe damage in some microregionswith mass production of Yaka tobacco.The tobacco varieties and lines were investigated for their resistance to the causing agent of *Phytophthoraparasitica*var.*nicotianae*during 2010, with artificial inoculation in glasshouse conditions (Biological laboratory) of Tobacco Institute -Prilep. Of the four lines investigated, three were evaluated as highly resistant to black shank and they can be included not only in commercial production but also as sources of resistance in breeding programs.

**Keywords:**tobacco, disease, black shank, oriental tobacco varieties, lines, Yaka type

# ПРОИЗВОДНИ КАРАКТЕРИСТИКИ НА НЕКОИ ОРИЕНТАЛСКИ ЛИНИИ ТУТУН ОТПОРНИ НА ЦРНИЛКАТА (Phytophthoraparasitica var. nicotianae)

Со изведените истражувања си поставивме за цел врз основа на добиените експериментални резултати да се утврдат морфолошко-производните и квалитетните својства, како и отпорноста спрема болеста црнилката (Phytophthoraparasitica var. nicotianae) на 4 новосоздадени фертилни инбрид линии тутун во споредба со стандардната сорта ЈВ 125/3 (∅ неотпорна). Проучуваните линии се добиени по пат навнатревидова (меѓусортова) хибридизација. Селекциониот процес започна со вкрстување на странски ориенталски сорти, како и наши ориенталски стабилни линии тутун – отпорни на црнилката (АА), кои се користеа како мајчина компонента, а како таткова беа користени наши неотпорни ориенталски сорти и линии тутун (аа). Одбирањето од хибридните потомства е вршено по методот “Педигре”. Испитуваните линии се консолидирани и по однос на висината на растенија, бројот,формата и големината на листовите. Според добиените резултати од полските опити новосоздадените отпорни линии се одликуваат со поголем број на листови по растение( 45- 53 лист./растение) во вднос на ЈВ 125/3∅ (37 лист./растение), повисок принос kg/ha (од 33,66 до 68,80%), повисока откупна цена ( од 14,3 до 27,7%) и поголем економски ефект (41,01до 81,29%). Отпорноста на испитуваните сорти и линии тутун спрема причинителот на болеста црнилка (Phytophthoraparasitica var. nicotianae) беше проучувана во текот на 2010 година во услови на вештачка инокулација во заштитен простор-стакленик (биолошката лабораторија) на Научниот институт за тутун- Прилеп. Од проучуваните 4 линии тутун, три се оценети како високоотпорни линии на црнилката, овие линии освен што ќе можат да најдат примена во производството, ќе можат да се користат и како извори на отпорност во селекционите програми.

**Клучни зборови:** тутун, болест, црнилка, ориенталскисорти, линии, тип јака

## INTRODUCTION

The need for creating and introducing new productive oriental tobacco varieties with improved quality traits in comparison with the existing standard varieties is permanently growing and studies of this kind have a continuous character. In our country, however, little work has been done on creation of oriental tobacco varieties resistant to economically important diseases. The black shank (Phytophthoraparasitica) is economically very important disease on oriental tobaccos, which in the years with favorable conditions for its occurrence can cause a serious damage to the mass production of tobacco. A number of authors point out that in many areas the disease received epiphytotic dimensions and caused serious losses to tobacco industry (Mickovski 1984, 1988, Trancheva 2001,Tashkoski 2005,Dimitrieski *et al*. 2011, etc). The pathogen ability to persists in soil for a long time complicates the application of chemicals in control of this disease. Current world tendency is to reduce the use of chemicals by introducing resistant varieties in tobacco production (Palakarcheva 1986, cited by Trancheva 2000).

Realizing the serious threats of black shank and the damages it causes in certain areas, our research in recent years has been focused on creation of new resistant oriental tobacco varieties and lines (Gornik 1973, Trancheva 2001, Dimitrieski *et al*. 2012,). Thereby, several oriental lines with high resistance to black shank were obtained. Four of these lines of the type Yaka, in combination with the standard variety YV 125/3, were chosen to be the subject of our investigations on morphological and productive traits and the resistance to black shank.

## MATERIAL AND METHOD

Subject of this research were four newly created tobacco lines resistant to black shank: YK l. 123-82, YK l. 20-23/10, YK l. 22-82/10, YK l. 301/23 and standard YV 125/3.

The newly created resistant lines were obtained by intraspecies hybridization, using foreign resistant varieties and domestic non-resistant oriental varieties and lines as its components. They are genetically stable and consolidated in terms of plant height and leaf number, shape and size.

In 2010 comparative trial was set up in randomized block design with four replications and transplanting was done at 40cm x 12cm spacing. Usual cultural practices, necessary for normal growth and development of oriental tobacco were applied on transplanted tobacco in field.

The necessary morphological measurements and phenological observations were also carried out. The quality of harvested and cured tobacco was estimated according to the Rulebook for unique measures for assessing the quality of raw tobacco.

Resistance of investigated tobacco varieties and lines to the causing agent of black shank disease was studied under conditions of artificial inoculation in a protected area (Biological Laboratory) of Tobacco Institute in 2010.Tobacco plants weretransplanted in pots on 14.06.2010, with 24plants for eachcultivar.

Pure culture of the fungus *Phytophthoraparasitica var. nicotianae* obtained from naturally infected tobacco plants was used as inoculum.The fungus was sown on potato-dextrose agar and incubated at a temperature of 25$^{o}$C in a period of 15 days.

Isolate P25, race 0 of the pathogen was used in the trial. Tobacco plants were inoculated with suspension prepared from the fungus culture of one petri-dish,mixed in 100ml distilled water.

Each plant was injured in the root system prior to inoculation. For easier infection, a knife was used to cut soil and root system around the stalk (Tashkoski, Pejcinovski 2002). After that, 30ml of the prepared suspension was added to eachplant by watering, and 30ml distilled water was added to control plants. Inoculation was performed on 13.07.2010.

First symptoms of the disease, expressed through wilting ofthe leaves, appeared 4days after inoculation. During the vegetation, several readings of the infected plants were made,and the last assessment was done on 01.09. 2010. The ratio between the numberof infected plants and the total number of observed plants was used to assess disease intensity of eachcultivar, expressedin percentages. The index of disease in investigated cultivars and in the check was used to calculate the index of resistance according to Abbott’s formula. Based on this index and by the scale of Kutova (cited by Trancheva, 2000), with minor corrections, all varieties are classified into 5 categories:

0 - highly resistant- **no visible infection**

1 – resistant-**10 % infected plants**

2 - – moderately resistant **-40 % infected plants**

3 - susceptible- **50% infected plants**

4 – highly susceptible- **50% - 100% infected plants**

## RESULTS AND DISCUSSION

According to the analyzed morphological proportions, the newly created lines are typical for Yaka tobacco (Table 1).

The highest values for plant height were recorded in line Yaka l. 20-23/10 (118 cm) and the lowest in the standard variety Yv. 125/3 (98 cm). The highest leaf number per plant was recorded in lines Yaka l. 20-23/10 and Yaka l. 22-82/10 (53) and the lowest in the standard Yv. 125/3 (37).

From the data presented in Table 1 it can be seen that standard variety Yv 125/3 has the lowest values for the biggest leaf, with an average size of 19,7 cm in length and 9.5 cm in width. Line Yk. l. 301/23 has the highest leaf length (25.1 cm) and Line Yaka l. 22-82 the highest width (11.8 cm).

The newly created lines resistant to black shank showed somewhat higher values for leaf size compared to the standard variety, but they are still within the frames typical for oriental tobacco.

**Table 1.Morphological characteristics of Investigated lines and the standard variety**

| Varieties Lines | Plant with inflorescence height cm | Leaf number per plant | Largest leaf size | |
|---|---|---|---|---|
| | | | Length | Width |
| Yv. 125/3∅ | 98 | 37 | 19,7 | 9,5 |
| Yk . l.123-82 | 103 | 45 | 20,9 | 10,3 |
| Yaka l.20-23/10 | 118 | 53 | 20,9 | 9,6 |
| Yaka l.22-82/10 | 112 | 53 | 21,4 | 11,8 |
| Yk. l. 301 /23 | 103 | 47 | 25,1 | 11,7 |

Data about productional characteristics of the newly created tobacco lines compared to the standard Yv. 125/3 are presented in Table 2. The lowest average yield was obtained in the standard variety (1628 kg/ha), and the highest in Yaka l. 20-23/10 (2748 kg/ha), which is 68.80% higher than the standard. The yields of the other three lines were 33.66% - 60.50% higher compared to the standard. With regard to yeld per hectare the newly created tobacco lines showed statistically significant differences at 1% level,compared to the snadard variety YV-125/3. The highest purchase price was recorded for Yaka l. 22-82/10 (2,08 €/kg), which is 11,82% higher than the standard Yv. 125/3 (1,86 €/kg). The other lines, showed equal or somewhat higher purchase price compared to the standard. In relation to the economic effect, relatively high differences were observed between the investigated lines and the standard (Table 2). Thus, the lowest gross income was achieved in the standard Yv.125/3 (3.028,08 €/ha), and the highest in Yaka l. 20-23/10 (5.496,00 €/ha), which is 81.50% higher compared to the standard. Economic effects of the other three lines, too, were 40.85% to 70,86% higher compared to the standard. Regarding the gross income three of the newly created lines showed statistical significance at 1% while in line YK 1.123-82 the significance level was 5%, compared to the snadard variety YV-125/3.

From the results of the comparative investigations it can be stated that the four newly created lines, due to their resistance to black shank and good quality, are a great contribution in the selection of Yaka tobacco. These perspective lines, as future varieties, will make a solid alternative ground in elimination of harmful effects caused by the common black shank in the tobacco producing regions and micro-regions of the type Yaka.

**Table 2 Productionalcharacteristics of Investigated lines and the standard variety**

| Varieties Lines | Yield | | Average purchase price | | Average economic effect | |
|---|---|---|---|---|---|---|
| | kg/ha | % | € /kg | % | € /ha | % |
| Yv. 125/3∅ | 1628 | 100,00 | 1,86 | 100,00 | 3.028,08 | 100,00 |
| Yk. L. 123-82 | 2176 | 133,66 | 1,96 | 105,38 | 4.264,96 | 140,85 |
| Yaka l.20-23/10 | 2748 | 168,80 | 2,00 | 107,52 | 5.496,00 | 181,50 |
| Yaka l.22-82/10 | 2259 | 138,76 | 2,08 | 111,82 | 4.698,72 | 155,17 |
| Yk. l.301/23 | 2613 | 160,50 | 1,98 | 106,44 | 5.173,74 | 170,86 |

LSD p=0,05=143kg/ha LSD p=0.05=1015€ /ha
P=0,01=203kg/ha p=0.01=1425€ /ha

According to the results on the resistance to black shank in conditions of artificial inoculation (Table 3), out of the 5 cultivars and lines of oriental tobacco included in investigations, 3 lines were highly resistant (Yaka l. 20-23/10, Yaka l. 22-82/10 and Yk l.301/23) (Fig. 2). These plants showed 100% resistance, i.e. no symptoms of disease appeared during the growing period, up to 01.09.2010.

After inoculation with suspension prepared from the fungus culture, line Yaka l.123-82 was estimated as moderately resistant (index 2).

The standard cultivar, (as non-resistant control) Yv. 125/3 (Fig. no. 1) was rated as highly susceptible (index 4) to the pathogen (Phytophthoraparasitica var. Nicotianae). In this cultivar, the percentage of infected plants after inoculation was 87.5%.

The above results point out to the existence of differences in the level of resistance. According to our findings from previously conducted research (Tashkoski, Gveroska, Dimitrieski, Miceska, 2008), these differences depend on the resistance of the investigated cultivars and virulence of the isolates.Thus, out of 13 cultivars investigated, only Rila82 showed the highest level of resistance, from 75% healthy plants in the more virulent isolates (P2 and P10) to 100% in the less virulent isolate (P13). Similar resistance was observed in Krumovgrad 58, which showed slightly higher susceptibility towards the more virulent isolate (P. 10).

In creation of black shank resistant cultivars, the following resistant lines canbe used in breeding programs as components in hybridization: Yakal.20-23/10, Yakal.22-82/10 and Yaka l.301/23.

**Table 3. Tobacco cultivars inoculated with a culture of *Pytophthoraparasitica var. nicotianae* - greenhouse 2010**

| **Cultivars-lines** | Inoculated plants | Total No. of infected plants | Infestation, % | Level of resistance | Index |
|---|---|---|---|---|---|
| YV 125/3 ∅ | 24 | 21 | 87,50 | 12,50 | 4 |
| Yk. l. 123-82 | 24 | 4 | 16,66 | 83,34 | 2 |
| Yk. l. 20-23/10 | 20 | 0 | 0,00 | 100,00 | 0 |
| Yk.l..22-82/10 | 24 | 0 | 0,00 | 100,00 | 0 |
| Yk.l..301/23 | 20 | 0 | 0,00 | 100,00 | 0 |

0 – highly resistant- **no visible infection**

1 – resistant-**10 % infected plants**

2 – moderately resistant –**up to 40 % infected plants**

3 - susceptible- **up to 50% infected plants**

4 – highly susceptible-**over 50%infected plants**


**Photo 1.** YV 125/3


**Photo 2.** Yaka l. 301/23

## CONCLUSIONS

- According to the analyzed morphological proportions, the newly created lines are typical for Yaka tobacco. Plants of the new resistant lines are somewhat higher compared to those of the standard variety.

- The highest number of leaves per plant (53) was achieved in lines Yk l. 20-23/10 and Yaka l. 22-82/10, but also in the other two lines the leaf number was higher compared to the standard YV 125/3 (37), which dimensions are adequate for this tobacco type.

- The investigated lines achieved higher yield per hectare. In relative amount it is 33.66% - 68.80% higher compared to the standard variety.

- The purchase price (den/kg) and economic effect (den/ha) in newly created perspective lines was 41.01% - 81.29% higher compared to the standard variety YV 125/3.

- In conditions of artificial inoculation, three of the four investigated lines of Yaka tobacco (Yaka l. 20-23/10, Yaka l.22-82/10 and Yaka l. 301/23) showed high resistance to black shank (index 0) and one line showed medium resistance (index 2), compared to the highly susceptible standard variety (index 4).

- A common conclusion can be drawn that 3 of the newly created lines of Yaka tobacco developed high resistance to black shank. Beside their application in production, they can be used as sources of resistance in selection of new varieties resistant to the disease.

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Original Scientific paper

# LEAD UPTAKE BY DIFFERENT ORGANS OF ORIENTAL TOBACCO GROWN IN THE REPUBLIC OF MACEDONIA

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## ABSTRACT

The study was conducted on 150 farms that grow oriental tobacco from three well known tobacco regions in Macedonia (Pelagonia, Southeastern Region and Vardar Valley). The total content of lead in soil samples was measured through decomposition by HF, $HClO_4$, and $HNO_3$ acids. A solution of 0.005 mol/L diethylentriaminepentaacetic acid (DTPA) and 0.1 mol/L triethanolamine at pH 7.3 was used for extraction of the element mobile form from soils. The digestion of plant samples was made with $HNO_3$ and $H_2O_2$ using microwave digestion system. Atomic emission spectrometry with inductively coupled plasma (ICP-AES) was used for the determination of Pb content in the soil and plant samples. A correlation analysis was conducted between pH, organic matter content, total and mobile form of lead in the soil, and the concentration of this element in different organs of tobacco.

**Keywords:**lead, oriental tobacco, uptake, soil

## ИЗВЛЕКУВАЊЕ НА ОЛОВОТО ОД РАЗЛИЧНИ ОРГАНИ НА ОРИЕНТАЛЕН ТУТУН ОДГЛЕДУВАН ВО РЕПУБЛИКА МАКЕДОНИЈА

Студијата е спроведена на 150 локалитети од три познати реони во Македонија каде се одгледува ориенталентутун (Пелагонија, Југоисточниоти Вардарскиот тутуно- производен реон). Вкупната содржина на олово во примероците од почва е определена по нивното целосно разложување со HF, $HClO_4$, и $HNO_3$. За екстракција на подвижната форма на олово во испитуваните примероци почва е употребен раствор од 0.005 mol/Lдиетилентриаминпентаоцетна киселина и 0.1 005 mol/L триетаноламинсо pH 7.3. Разложувањето на растителните примероци е извршена со $HNO_3$ и $H_2O_2$ и примена на микробранов систем за разложување. Содржината на оловото во растителните и почвените примероци е определена со примена на атомската емисиона спектрометрија со индуктивно спрегната плазма (ICP-AES). Корелациони анализи се направени помеѓу pH на почвата, содржината на органска материја во почвата, содржината на вкупната и подвижна форма на олово во почвата и содржината на оловото во различните органи на ориенталскиот тутун.

**Клучни зборови:**олово,ориентален тутун, извлекување, почва

## INTRODUCTION

The toxic impact of Pb on the vital processes of almost all plants is well known (Rose et al. 2001; Ghaedi et al. 2007). It is confirmed that tobacco (*Nicotina tabacum*) can easily accumulate lead (Gondola and Kadar 1994). This crop is intermediate reservoir through which trace elements from soils, and partly from waters and air, move to final consumers, humans. Trace element content of the tobacco plants are variable and depend on many factors such as: soil properties, the climate, application of soil improvers and many more. Most of these properties, as well as environmental surroundings influence on the trace element uptake by tobacco plants (Adamu et al. 1989; Radojičić and Cvetković 2004; Golia et al. 2007). One of the parameter that hasthe strongest impact on the intensity of metal accumulation is soil pH (Bell et al. 1992; Golia et al. 2007; Golia et al. 2009; Zaprijanova 2010). According to these authors trace element content in tobacco is influenced individually, or interactively and by several other parameters such as: organic matter content, clay content, etc.

Focus of this study has been given on exploring the relationship between soil properties, total and mobile forms of Pb in soil and it content in different parts of tobacco (root, stem, flower leaves and seed) from the oriental variety group of tobacco. This plant is used as indicator to provide information of the quality of the soil in which is grown and to determine the availability of the studied element for tobacco uptake.

## MATERIAL AND METHOD

Two years survey (2010-2011) was conducted in the well-known tobacco growing regions in Macedonia: Pelagonia region (PR), Southeastern region (SER) and Vardar Valley (VV) from 19 municipalities at 150 sampling sites (Figure 1). Soil composite samples from pedological profiles at fixed depth (0-30 cm) were taken from fields of each mentioned municipality. Samples were collected from 150 sites in each field with two replicates. Samples were taken from cultivated soil after tobacco harvesting, and from uncultivated soils in the nearest vicinity. Samples pretreatment was done in accordance to ISO 11464:2006. First the samples were air-dried and after that crushed and sieved through a 2-mm sieve.

The following soil properties were determined: mechanical composition (Đamić, 1996), pH (ISO10390:2005), total nitrogen (ISO11261:1995), organic matter (OM) with the wet oxidation method (Đamić, 1996), and calcium carbonate equivalent volumetrically (ISO 10693). Electroconductivity was measured in a saturation extract, extractable phosphorus and potassium according to the ammonium lactate method, while the cation exchange capacity (CEC) was measured by the method described by Sumner and Miller (1996). Soil samples were digested with mixture of several acids ($HNO_3$, HF, $HClO_4$ and HCl) for total digestion (ISO 14869-1). Plant available fraction of Pb was determined by extraction method using buffered solution of diethylentetraaminepentaacetic acid (DTPA) at pH 7.3 (ISO 14870). Extracts were collected after filtration through Whatman No. 42 filter paper.

Tobacco samples (root, stems, leaves, blossoms and seeds) were selected from plants at the same sites where soils were sampled. Tobacco leaves from three primings were collected with the total of 450 representative samples from 150 sampling locations. Oriental tobacco samples were washed carefully to remove any adhering soil particles and rinsed with redistilled water. The plant material was dried and homogenized to a constant weight after drying at 75 $^oC$ for 12 hours. Plant samples (0.5000 g) were digested in Teflon vessels with $HNO_3$ and $H_2O_2$ using the Mars microwave digestion system (CEM, USA) (for elemental analysis. The plant samples were digested at 180$^o$C. After cooling the digested samples were quantitatively transferred into 25 mL calibrated flasks(Bačeva et al. 2012).

The investigated element was analyzed by the application of atomic emission spectrometry with inductively coupled plasma - AES-ICP (Varian, 715-ES). For the calibration a commercial standard mix solution (11355 - ICP Multi Element Standard IV, Merck) was used. The optimal instrumental conditions are given by Balabanova et al. (2010).

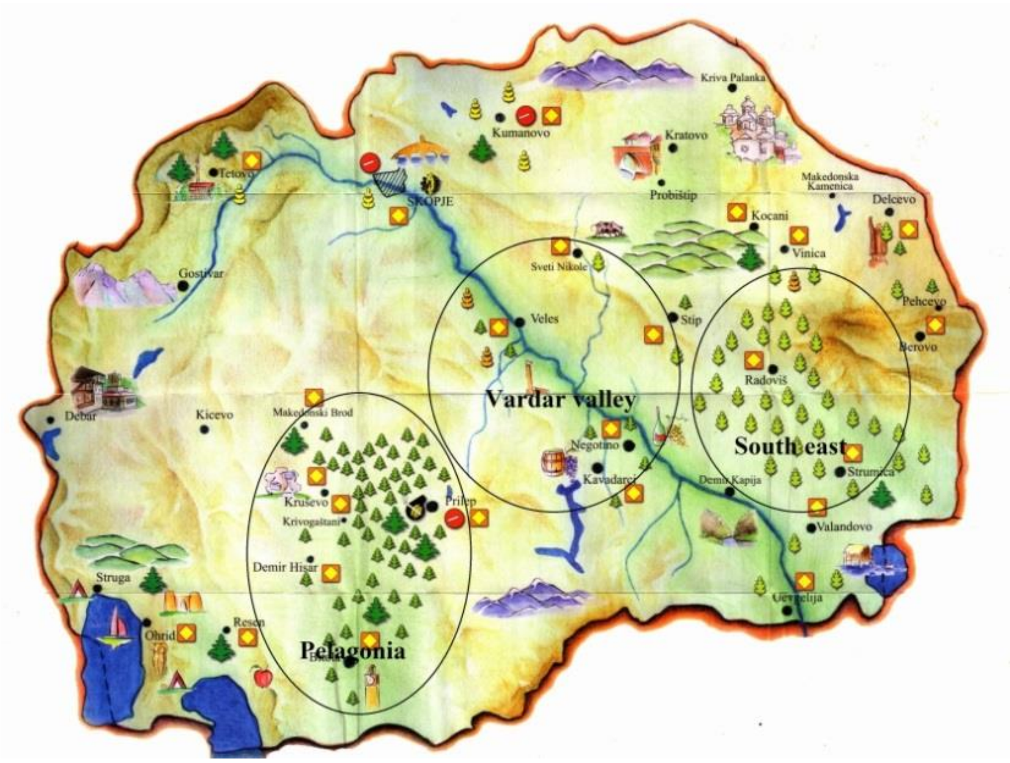


Figure 1. The location of the investigated tobacco growing regions in Macedonia (Pelagonia, South-eastern Region and Vardar Valley) provided by the Ministry of Agriculture, Foresty and Water Economy, Economic Chamber of Macedonia and Scientific Tobacco Institute – Prilep, 1999

## RESULTS AND DISCUSSION

Tobacco quality is highly influenced on the soil physical and chemical properties. Descriptive statistics of analyzed soil properties are given in Table 1. According to texture, the analyzed soilsamples vary from silt loam (18.8 % clay) to silt clay loams (77.6 % clay). Clayed soils were found only in the municipality of Krivogaštani (Pelagonia region). Average values of soil pH were 6.5 and 6.7 for PR and SER, respectively. Average OM content is generally low to moderate, according to soil texture (Table 1). Available phosphorus and potassium concentrations varied differently. Extreme concentrations of available phosphorus (60-198 mg/100 g) were recorded in some sampling spots from municipalities of Novaci, Demir Hisar and Prilep (PR), Studeničani, Veles and Caška (VV). In the same sampling spots the anomalies for available phosphorus were detected (Prilep and Veles); concentrations of available potassium of 60.93 mg/100 g and 63.64 mg/100 g were recorded, respectively. None of the examined soil samples displayed a low capacity for nutrient storage (CEC < 10 $cmol_c$/kg).

All soils had an adequate CEC for agricultural production. According to EC most of the samples are non-saline, except for some samples from Vardar Valley production region from municipality of Veles (EC > 360 μS/cm).

**Table 1. Basic soil properties, descriptive statistics of three tobacco production areas**

| | Pelagonia Region | | | South-eastern Region | | | Vardar Valley Region | | |
|---|---|---|---|---|---|---|---|---|---|
| Soil properties | Mean | $s_a$ | Min-Max | Mean | $s_a$ | Min-Max | Mean | $s_a$ | Min-Max |
| OM, % | 1.5 | 0.5 | 0.8-3.2 | 1.3 | 0.3 | 0.7-2.1 | 1.6 | 0.3 | 1-2.1 |
| TN*, % | 0.08 | 0.04 | 0.02-0.4 | 0.06 | 0.02 | 0.01-0.11 | 0.07 | 0.02 | 0.05-0.1 |
| pH | 6.5 | 0.6 | 5-8.3 | 6.7 | 0.5 | 5.5-8 | 7.7 | 0.8 | 6.5-8.6 |
| Clay, % | 37.7 | 11.1 | 18.8-78 | 36.4 | 11.9 | 19.4-64 | 45.9 | 7.2 | 32-56 |
| Available P, mg/100 g | 19.7 | 32.2 | 0.5-198 | 12.5 | 23.5 | 1.5-154 | 24.5 | 25.3 | 1.8-64 |
| Available K, mg/100 g | 20.4 | 7.4 | 3.2-64 | 21.8 | 4.6 | 13-32 | 31.7 | 14.3 | 22-63 |
| CEC, $cmol_c$/kg | 10.6 | 2.6 | 6-19 | 9.7 | 2.3 | 6.5-16 | 11.8 | 1.9 | 8.2-15 |
| EC, μS/cm | 83 | 56 | 23-360 | 79 | 55 | 27-264 | 215 | 109 | 42-362 |

*TN – total nitrogen, $s_a$-standard deviation, Min-minimum, Max-maximum

The total lead content in soils varies from 10 mg/kg to 30 mg/kg (Table 2). According to production region distributions, samples had similar Pb concentration (Table 2). Although Veles region is known for its lead and zinc industrial activity in the nearest past (Stafilov et al. 2010), concentration of Pb in soils from all production regions is not exceeding the contamination limit of 85 mg/kg for optimal value and 530 mg/kg for intervention value (The new Dutch list, (http://www.contaminatedland.co.uk/std-guid/dutch-l.htm#KEYWORD-ONE).

As it can be seen from data presented in Table 2, the mobile lead content varies from 0.5 mg/kg to 4.4 mg/kg. The highest values were recorded in soils with the highest total Pb content, respectively.

**Table 2. Total and DTPA extractable Pb in soil from different tobacco growing areas (given in mg/kg)**

| | LOD* | Pelagonia Region | | | South-eastern Region | | | Vardar Valley | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mg/kg | $X_g$ | $s_a$ | Min-Max | $X_g$ | $s_a$ | Min-Max | $X_g$ | $s_a$ | Min-Max |
| Total Pb | 10 | 14 | 4.8 | 10-30 | 15 | 5.5 | 10-29 | 14 | 4.5 | 10-22 |
| Mobile Pb | 0.5 | 0.5 | 0.17 | 0.5-1.0 | 0.9 | 0.5 | 0.5-2.2 | 2.2 | 1.1 | 0.9-4.4 |

*LOD- limits of detection, $X_g$-geometrical mean, $s_a$-standard deviation, Min-minimum, Max-maximum

Distribution of Pb content in different tobacco organs are given in Figure 2. Highest accumulation with average value of 1.5 mg/kg was recorded in leaves from the first harvesting zone (first priming). According to Tso (1990) and Kabata-Pendias (2011) lead concentration in tobacco leaves and plant material may reach up to 200 mg/kg. According to regulative in Macedonia, maximum permitted concentrations for Pb in tobacco leaves are 10 mg/kg. Availability of 6.2 % of the studied element was calculated as a ratio ($c_{DTPA}/c_{total\ x100}$) of available concentration (DTPA extraction) and total concentration of Pb in soil.

Using determinated concentrations in different parts (root, stem, flower, leaves and seed) of tobacco plant and total element content of examined soils the Biological Accumulation Factor (BAF) was calculated. This factor is defined as the ratio between total content of the elements in all parts of studied plant and corresponding soil. Calculated average values for Pelagonia Region and South-eastern Region are 0.16 meaning tobacco is capable to accumulate small amounts of this element. Higher average values of BAF of 0.21 were obtained from samples of Vardar Valley region.

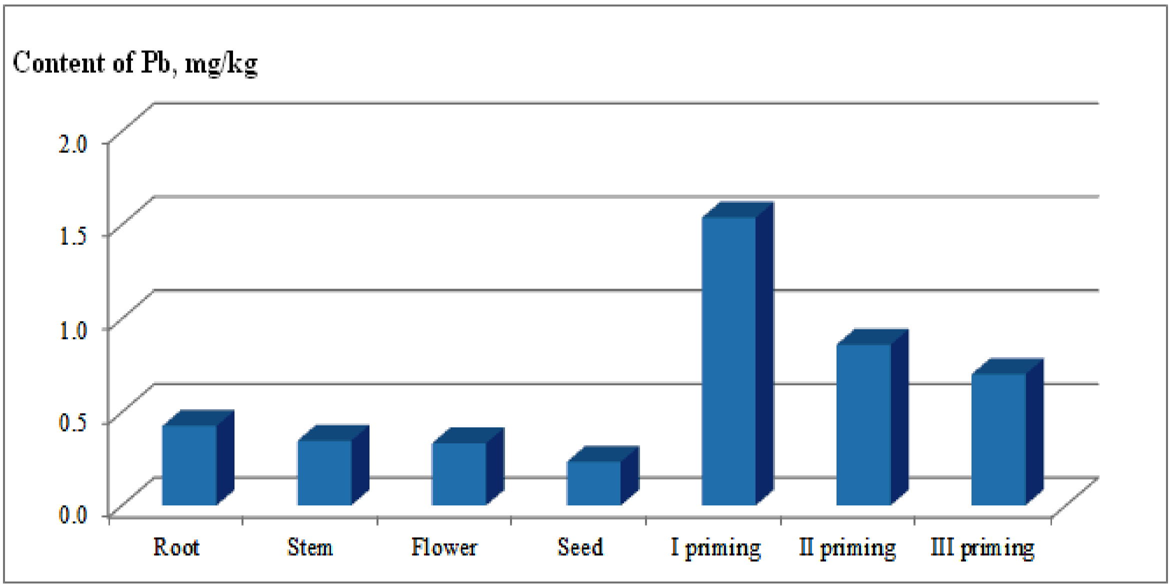


Figure 2. Pb content in different tobacco organs

The correlation dependencies among important parameters of soil and total quantities of Pb in soil and tobacco leaves as well DTPA extract were analyzed using correlation analysis (Pearson correlation, two-tailed). The obtained results are given in Table 3. As it can be seen no significant correlation was observed among Pb content of tobacco leaves and soil parameters. Gondola and Kadar (1994), Golia et al. (2009) and Zaprijanova et al. (2010) observed correlations among soil properties and element concentration in tobacco harvesting zones, finding significant negative correlation between pH and organic matter of soil and Pb content in oriental tobacco. All these authors found a statistically important relation between the mobile forms of Pb extracted from the soil and their concentration in tobacco leaves. This is in accordance with the results of our study where significant correlation was observed between most of the soil properties and mobile form of Pb.

**Table 3. Correlation coefficients of Pb content in tobacco leaves and soil properties**

| | Pb soil | Pb DTPA | Pb I priming | Pb II priming | Pb III priming |
|---|---|---|---|---|---|
| Pb DTPA | 0.26 | 1 | | | |
| Pb I priming | -0.09 | -0.21 | 1 | | |
| Pb II priming | -0.12 | -0.10 | 0.15 | 1 | |
| Pb III priming | 0.07 | -0.10 | 0.08 | 0.11 | 1 |
| OM | 0.20 | **0.43** | -0.12 | -0.10 | 0.03 |
| TN | 0.07 | **0.43** | 0.06 | -0.03 | 0.03 |
| pH | 0.02 | **0.45** | -0.16 | -0.08 | -0.16 |
| Clay | 0.22 | **0.42** | -0.10 | -0.10 | 0.11 |
| $P_2O_5$ | 0.00 | -0.01 | 0.09 | 0.00 | 0.00 |
| $K_2O$ | 0.00 | 0.21 | -0.08 | -0.08 | 0.00 |
| CEC | 0.25 | **0.50** | -0.13 | -0.12 | 0.09 |
| EC | 0.09 | **0.62** | -0.17 | -0.07 | 0.06 |

Bold numbers present significant correlation at 0.01 levels

## CONCLUSIONS

According to soil properties, generally all analyzed samples are a good base for production of the high-quality oriental tobacco. Moreover, some adjustments are needed that can largely be achieved through appropriate fertilization rate recommendations. The soil concentration of lead in all analyzed samples pointed out levels which are typical of agricultural and low anthropogenic pressure areas. Contents of lead in all plant samples are under limits considered critical. According to results of correlation analysis there are no statistically significant dependences determined between the soil parameters and the lead concentration in the plant organs of oriental tobacco. Regression dependencies of some significance are observed between the mobile lead in the soil and most of the soil parameters except available forms of phosphorus and potassium.

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Original Scientific paper

# INHERITANCE OF THE LEAF SIZE IN VIRGINIA TOBACCO CROSSES

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## ABSTRACT

The character and extent of the genetic interactions were determined by applying hybridological analysis as well as by the number of genes differentiating between the initial parent forms and expressions of heterosis and transgression referring to the feature size of the leaves, the objective being the selection of desired genotypes. For this purpose, populations were investigated to $P_1$, $P_2$, $F_1$ and $F_2$ of six hybrid combinations. Data from hybridological analysis showed that in the studied samples of Virginia tobacco, inheritance of the length and width of leaves of the middle harvesting zone is overdominant and always in direction of the parent with the higher values. Heterosis and transgression appear reliable means of increasing the width of the leaves from the middle harvesting zone in Virginia tobacco. Middle and high values are determined for inheritability, so the selection of this sign will be effective in the early generations.

**Keywords:**Virginia tobacco, size of leaves, genetic analysis, inheritance, hereditability, transgression, heterosis.

## НАСЛЕДУВАЊЕ НА СВОЈСТВОТО ГОЛЕМИНАТА НА ЛИСТОВИТЕ КАЈ ВИРЏИНИСКИТЕ КРСТОСКИ ТУТУН

Со примена на хибридолошка анализа, одредувани се природата и степенот на генетски интеракции, како и на извесен број на гени кои се разликуваат од почетните родителски форми и експресиите на хетерозис и трансгресија коишто се однесуваат на својството големина на листот, со цел да се изврши селекција на саканите генотипови. За таа цел, кај популациите беа испитувани $P_1$, $P_2$, $F_1$и $F_2$на шест хибридни комбинации. Податоците од хибридолошката анализа покажуваат дека кај проучуваните примероци од вирџиниски тутуни, наследувањето на должината и ширината на листовите од средниот бербен појас е супердоминантно и секогаш во правец на родителот со повисоки вредности. Хетерозисот и трангресијата се покажаа како релевантни средства за зголемување на ширината на листовите од средниот појас кај тутунот од типот вирџинија. Средни и високи вредности се одредени за наследноста, па така селекцијата на овој признак ќе биде ефективна во раните генерации.

**Клучни зборови:** вирџиниски тутун, големина на листовите, генетска анализа, наследност, херитабилност, трансгресија, хетерозис.

## INTRODUCTION

The dimensions of the tobacco leaves are crucial for both yield and the percentage of first class (Dyulgerski, 2011; Kochoska and Risteski, 2011). As a result of studies on Virginia tobacco it has been found that the length of the leaves is inherited overdominantlyand dominantly to parent with longer leaves (Chinchev, 1979). Masheva (2007, 2008) found that the length of the leaves successively involves additive, additive-additive and dominant-dominant gene effects, and the sign determiningthe width is a fundamental part of the dominant gene effects. Mehta and others (1985) in his studies of Virginia tobacco found that the expression of the leading signs of leaf size, both length and width are for additive genetic effects. In tobacco for cigars, the inheritance of those signs with the highest proportion are additive genetic effects, both in length and width in succession leaves (Espino and Gill, 1980; Torrecila and Varroso, 1980). Epistacies strong interactions was observed in the inheritance of length of leaf from the middle zone (Dyulgerski, 2011).

Ibrachim and Avratovscukova (1982) and Peksuslu et al. (2002) obtained a high heritability in the broad sense - over 80% in length of the leaves. Amarnath (1987) found higher values for length and width of leaves. Such factors in the broad sense heritability - 83 and 96% for length and width of leaves were reported in other studies (Nizam Uddin and Newaz, 1983).

The aim of this study is through hybridological analysis to determine the nature and extent of gene interaction, number of genes that differ in parental forms, inheritability effect of selection and manifestations of heterosis and transgression concerning the length and width of leaves of middle harvesting zone (13-14 leaf) in tobacco Virginia crosses.

## MATERIAL AND METHODS

For realizing the objective in training and experimental field of ITTI - Markovo village in the period 2008-2011, populations of six crosses were investigated to $P_1$, $P_2$, $F_1$ and $F_2$, including Virginia tobacco varieties introduced from the U.S. In terms of plant height, arithmetic mean ($\bar{x}$), the average error ($\pm \mathbf{S}\bar{x}$), degree of dominance (d/a) were determined by the formula of Mather and Jinks (1985) and the heterosis effect in terms of better parental form (HP) by Omarova (1975). Investigations were made on transgression (Tr), number of genes that differ in parental forms (N), dominance (D), epistacy (E), coefficient of heritability of the trait ($h^2$), coefficient of efficiency of the selection of genotypes in phenotypic expression of the trait (Pp) by Sobolev (1976).

## RESULTS AND DISCUSSION

In our investigations of Virginia tobacco samples, inheritance of both the length and width of leaves is overdominant and always in direction of the parent with the higher values (Table1 and 2).

The manifestation of heterotic effect in the length of the ground leaves is insignificant (Table 1). Heterosis of significant values was observed in the first generation of Hybrid 726 (C 358 x NC 729), where heterosis effect slightly exceeds 5%.

The values of the coefficient of transgression showed insignificant values in terms of length, and its expression has significant values only in Hybrid 726, where in the available homozygous progenies plants may be selected with 1-2 cm greater length (Table 3). Heterosis and related trasngression as genetic phenomena have insignificant influence on its determining ground of leaf length from the middle harvesting zone surveyed in our Virginia tobacco crosses.

Unlike the length to width values of the leaves, significant heterosis was observed in five of the six crosses (Table 2). Most pronounced was it in Hybrid 715 (C 730 x C 358), with over 10%.

Similar results are obtained for the acts of transgression, which in all cases is positive (Table 4). With the exception of Hybrid 725, with other crosses available in homozygous progenies, plants may be selected with 2-3 cm wider leaves. Heterosis and transgression appear reliable means of increasing the width of the leaves from the middle harvesting zone in Virginia tobacco.

Data from hibridological analysis showed that the number of genes that differ in parental forms and influence the manifestation of the trait leaf length varies within narrow limits - from 9 to 13 (Table 3). Manifestation of symptoms is strongly affected by the negative epistacy interactions, which significantly reduces the appearance of dominant genes and their number also fluctuates within narrow limits.

The number of genes and their effect on the appearance of leaf width is too small - from 2 to 4 (Table 4). There is a strong expression of dominant gene effects, while that of epistacy is insignificant**.**

There are high values for inheritability the length of the leaves, indicating that environmental conditions did not strongly influence the determining factors of the trait. In all crosses tested heritability coefficient is above 50% (Table 3). There is therefore a high proportion of the effect of genotype on the manifestation of the studied indicators. Selection of this character will be effective in the early generations.

Lower than those in the length of leaves, but relatively high values of heritability coefficient were obtained with respect to the width of leaves (Table 4). In this case the selection will be effective in the early hybrid generations.

**Table 1.Biometric data on length of the 13-14 leaf (cm)**

| **Parents/crosses** | **$P_1$** $\bar{x} \pm S\bar{x}$ | **$P_2$** $\bar{x} \pm S\bar{x}$ | **$F_1$** $\bar{x} \pm S\bar{x}$ | **$F_2$** $\bar{x} \pm S\bar{x}$ | **d/a** | **HP** |
|---|---|---|---|---|---|---|
| Hybrid 714 (C 730 x C 254) | 61,2±0,17 | 60,7±0,23 | 63,8±0,21 | 62,3±0,26 | 11,4 | 104,2 |
| Hybrid 715 (C 730 x C 358) | 61,2±0,17 | 62,3±0,19 | 64,7±0,24 | 63,6±0,22 | 2,4 | 103,9 |
| Hybrid 719 (RG 8 x C 358) | 62,6±0,21 | 62,3±0,19 | 63,0±0,18 | 62,5±0,20 | 3,67 | 100,6 |
| Hybrid 725 (C 340 x C 358) | 62,1±0,15 | 62,3±0,19 | 63,7±0,25 | 62,4±0,21 | 1,4 | 101,8 |
| Hybrid 726 (C 358 x NC 729) | 62,3±0,19 | 61,4±0,14 | 65,6±0,28 | 63,7±0,25 | 8,33 | 105,3 |
| Hybrid 727 (C 358 x C 254) | 62,3±0,19 | 60,7±0,23 | 64,2±0,15 | 63,3±0,20 | 2,5 | 103 |

**Table 2.Biometric data on width of the 13-14 leaf (cm)**

| Parents/crosses | $P_1$ $\bar{x} \pm S\bar{x}$ | $P_2$ $\bar{x} \pm S\bar{x}$ | $F_1$ $\bar{x} \pm S\bar{x}$ | $F_2$ $\bar{x} \pm S\bar{x}$ | d/a | HP |
|---|---|---|---|---|---|---|
| Hybrid 714 (C 730 x C 254) | 30,4±0,16 | 30,8±0,14 | 33,7±0,18 | 33,1±0,16 | 15,5 | 109,4 |
| Hybrid 715 (C 730 x C 358) | 30,4±0,16 | 31,3±0,17 | 34,8±0,22 | 33,7±0,20 | 3,5 | 111,1 |
| Hybrid 719 (RG 8 x C 358) | 30,5±0,14 | 31,3±0,17 | 33,5±0,15 | 33,1±0,21 | 2,4 | 107 |
| Hybrid 725 (C 340 x C 358) | 32,1±0,13 | 31,3±0,17 | 33,0±0,18 | 32,4±0,16 | 3,25 | 102,8 |
| Hybrid 726 (C 358 x NC 729) | 31,3±0,17 | 31,9±0,19 | 34,4±0,23 | 33,6±0,18 | 2,7 | 107,8 |
| Hybrid 727 (C 358 x C 254) | 31,3±0,17 | 30,8±0,14 | 33,9±0,12 | 33,3±0,16 | 11 | 108,3 |

**Table 3. Genetic characteristic of length of the 13-14 leaf**

| Crosses | Tr | N | D | E | $h^2$ | Pp |
|---|---|---|---|---|---|---|
| Hybrid 714 (C 730 x C 254) | 0,48 | 12,74 | 6,647 | -19,65 | 0,63 | 16,86 |
| Hybrid 715 (C 730 x C 358) | 0,34 | 9,11 | 8,732 | -23,38 | 0,56 | 13,57 |
| Hybrid 719 (RG 8 x C 358) | -0,19 | 8,95 | 5,611 | -15,44 | 0,60 | 21,44 |
| Hybrid 725 (C 340 x C 358) | -0,42 | 11,54 | 8,778 | -26,08 | 0,59 | 11,93 |
| Hybrid 726 (C 358 x NC 729) | 1,78 | 10,33 | 11,212 | -36,16 | 0,76 | 36,41 |
| Hybrid 727 (C 358 x C 254) | 0,37 | 9,80 | 7,030 | -20,54 | 0,52 | 8,91 |

**Table 4.Genetic characteristic of width of the 13-14 leaf**

| Crosses | Tr | N | D | E | $h^2$ | Pp |
|---|---|---|---|---|---|---|
| Hybrid 714 (C 730 x C 254) | 2,19 | 3,46 | 28,36 | 0,22 | 0,47 | 6,26 |
| Hybrid 715 (C 730 x C 358) | 2,81 | 4,04 | 37,57 | 1,23 | 0,53 | 8,13 |
| Hybrid 719 (RG 8 x C 358) | 1,68 | 2,92 | 57,43 | -0,66 | 0,41 | 5,51 |
| Hybrid 725 (C 340 x C 358) | 0,52 | 4,17 | 64,62 | 1,54 | 0,55 | 7,34 |
| Hybrid 726 (C 358 x NC 729) | 1,76 | 3,22 | 43,78 | -0,85 | 0,44 | 6,03 |
| Hybrid 727 (C 358 x C 254) | 1,90 | 2,87 | 81,19 | 1,02 | 0,46 | 5,79 |

## CONCLUSIONS

In our investigations of Virginia tobacco samples, inheritance of the length and width of the leaves of the middle harvesting zone is overdominant and always in direction of the parent with the higher values.

Heterosis and transgression appear reliable means of increasing the width of the leaves from the middle harvesting zone in Virginia tobacco.

There are very few genes influencing the determination of the trait width of leaves. The phenotypic manifestation of leaf length is strongly influenced by the negative epistacy interactions.

There are significant values for the heritability coefficient, which is why the investigated traits will be effective in the early generations of the studied crosses of Virginia tobacco.

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Original Scientific paper

# INVESTIGATIONS OF THE VARIABILITY OF QUANTITATIVE CHARACTERS IN TOBACCO VARIETIES AND THEIR F1 AND F2 HYBRIDS

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## ABSTRACT

Six parental genotypes (P-23, MB-3, SM-1, YV 125/3, FL-5 and O-87) and their 15 diallel hybrids in F1 and F2 generations were investigated for two characters, stalk height without inflorescence and leaf number per stalk. Field trials were set up in Tobacco Institute-Prilep in 2008 and 2009 in a randomized block design with four replications. Standard agrotechnics was applied in tobacco growing.
The aim of investigations was to estimate the variability of characters using the basic statistical parameters standard deviation and degree of variability.
Parents and F1 generation showed a very low variability, which indicates a high level of uniformity. In F2 progeny high variability was estimated, indicating the existence of differences among individuals and higher possibility of choice for the breeders. The highest variability in F2 for these two characters in both investigating years was estimated in the hybrids P-23 x O-87 and P-23 x FL-5.

**Keywords:**Tobacco (*Nicotiana tanacumL.*), heredity, variability, standard deviation (σ), degree of variability (V).

## ИСТРАЖУВАЊА ЗА ВАРИЈАБИЛНОСТА НА КВАНТИТАТИВНИТЕ СВОЈСТВА КАЈ ТУТУНСКИ СОРТИ И НИВНИТЕ F1 И F2 КРСТОСКИ

Проучувани се шест родителски генотипови тутун (П-23, МБ-3, SM-1, JV 125/3, FL-5 и O-87) и нивните 15 дијалелни хибриди во F1 и F2 генерациите за својствата: висина на стракот без соцветие и број на листови по страк. Опитот беше поставен во текот на 2008 и 2009 година на опитното поле при Научниот институт за тутун-Прилеп, по случаен блок-систем во четири повторувања. При одгледувањето на тутунот се користеа стандардни агротехнички мерки.
Целта на истражувањата беше да направиме проценка на варијабилноста на својствата со помош на основните статистички параметри: стандардна девијација и степен на варијабилност.
Родителите и потомството на F1 генерацијата покажаа многу ниска варијабилност, што значи дека се одликуваат со висок степен на униформност. Кај потомството на F2 генерацијата е пресметана висока варијабилност, што укажува на постоење на различни индивидуи и можност за избор спрема желбата населекционерот. Највисока варијабилност за двете својства во двете години на истражување кај F2 генерацијата покажаа крстоските: П-23 x O-87 и П-23 x FL-5.

**Клучни зборови:**тутун (*Nicotiana tanacumL.*), наследност, варијабилност, стандардна девијација (σ), степен на варијабилност (V).

## INTRODUCTION

Quantitative characters, just as all other characters of the living organisms, are inheritable and changeable to a certain limit. Changeability of the characters is called variability. Carriers of the characters are the genomes and the reason for their variability are changes of the environment. The breeding activity is based on previous measurements of characters and determination of the mean values and variability.

The aim of the two-year investigations was to study the inheritance of stalk height and leaf number per stalk in six parental genotypes and their diallel progeny in F1 and F2 generations and to estimate the variability using the basic statistical parameters - standard deviation (σ) and variability coefficient (V)

## MATERIAL AND METHODS

Investigations included six tobacco genotypes - four oriental (Prilep P-23, Basma MB-3, Samsun SM-1, Yaka YV 125/3) and two semi-oriental (Floria FL-5, Otlia O-87).

15 diallel crosses for F1 were made, from which seed material for F2 generation was obtained. The trial was carried out in 2008 and 2009 in the field of Tobacco Institute-Prilep in a randomized block design with four replications. During the vegetation period, adequate cultural practices were applied on tobacco.

During tobacco vegetation in field (May - September) in 2008, mean monthly temperature was 19.91$^0$C, number of rainy days was 39 and total precipitation amount was 235.44 mm. In the same period in 2009, mean monthly temperature was 19.89$^0$C, number of rainy days 42 and total precipitation amount 240.6 mm.

Subject of the investigations were the characters stalk height without inflorescence and leaf number per stalk.

Determination of the mode of inheritance was based on test-significance of the mean values in F1 progeny compared to the parental average (Borojevic, 1981).

Standard deviation (σ) is an indicator of the variability of quantitative characters. It indicates the mean square deviation from the arithmetic mean and is a result obtained from the square root of the variance. It is calculated by the following formula:

$$\sigma = \pm \sqrt{\frac{\Sigma (x - \bar{x})^2}{n}} \quad \sigma = \pm \sqrt{\sigma^2}$$

If the representative sample consists of lower number of individuals, the following formula is used:

$$\sigma = \pm \sqrt{\frac{\Sigma (x - \bar{x})^2}{n - 1}}$$

Standard deviation is expressed with the same measurement with which the investigated character is measured.

The degree of variability of characters is calculated from the standard deviation by the following formula:

$$V\ (\%) = \frac{\sigma \cdot 100}{\bar{x}}$$

The above formulas for calculation of standard deviation and variability coefficient were used by Najceska (2002).

## RESULTS AND DISCUSSION

The two-year biometric investigations of parental genotypes for the characters stalk height without inflorescence and leaf number per stalk showed low standard deviation and low degree of variability, which is an indication of stability and uniformity as a result of their homozygotness. In both years of investigation, the lowest values for stalk height were recorded in YV 125/3, while for leaf number per stalk in SM-1 followed by O-87 in 2008 and O-87 followed by SM-1 in 2009 (Table 1).

**Table 1. Mean value and variability of the characters stalk height and leaf number**

| Parentales | 2008 | | | | | | 2009 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stalk height (cm) | | | Leaf number per stalk | | | Stalk height (cm) | | | Leaf number per stalk | | |
| | $\bar{x} \pm s\bar{x}$ | σ | V (%) | $\bar{x} \pm s\bar{x}$ | σ | V (%) | $\bar{x} \pm s\bar{x}$ | σ | V (%) | $\bar{x} \pm s\bar{x}$ | σ | V (%) |
| P1 | 78 ± 0,2 | 3,32 | 4,25 | 50 ± 0,1 | 2,10 | 4,19 | 79 ± 0,2 | 3,48 | 4,40 | 51 ± 0,1 | 2,15 | 4,22 |
| P2 | 88 ± 0,3 | 4,00 | 4,54 | 32 ± 0,1 | 1,73 | 5,41 | 89 ± 0,3 | 4,34 | 4,88 | 32 ± 0,1 | 1,82 | 5,69 |
| P3 | 91 ± 0,2 | 4,36 | 4,79 | 30 ± 0,1 | 1,02 | 3,40 | 90 ± 0,3 | 4,21 | 4,68 | 30 ± 0,1 | 1,28 | 4,27 |
| P4 | 120 ± 0,3 | 3,16 | 2,63 | 42 ± 0,1 | 1,58 | 3,76 | 122 ± 0,3 | 3,27 | 2,68 | 44 ± 0,1 | 1,90 | 4,32 |
| P5 | 125 ± 0,1 | 4,47 | 3,58 | 31 ± 0,1 | 1,48 | 4,78 | 126 ± 0,2 | 4,30 | 3,41 | 31 ± 0,1 | 1,54 | 4,97 |
| P6 | 138 ± 0,2 | 4,00 | 2,90 | 33 ± 0,1 | 1,23 | 3,73 | 140 ± 0,2 | 3,95 | 2,82 | 32 ± 0,1 | 1,25 | 3,91 |

Legend:
$\bar{x}$ - Mean value
$s\bar{x}$ - Error of mean value (±)
σ - Standard deviation
V - Coefficient of variability (%).
P1 - Prilep P-23
P2 - Basma MB-3
P3 - Samsun SM-1
P4 - Yaka YV 125/3
P5 - Floria FL - 5
P6 - Otlia O-87

In F1 progeny obtained from homozygous parents, variability parameters of stalk height without inflorescence are very low. The most frequent way of inheritance of this character in both investigating years was partial dominance. Occurence of positive heterosis was observed in hybrids MB-3 x FL-5, YV 125/3 x FL-5 and FL-5 x O-87.

In F2 progenies segregation of characters appears, as a result of which there are differences among the individuals. Therefore, their standard deviation and degree of variability are higher as compared with F1. For breeders, higher variability also means higher possibility of choice. The highest variability for this character was observed in the hybrid P-23 x O-87. In F2 progeny, inheritance of this character was most frequently intermediate or partially dominant, which indicates its secure and fast stabilization in further selection (Tables 2 and 3).

**Table 2. Mode of inheritance and variability of the character stalk height in F1 and F2 progenies in 2008**

| Hybrids | F1 | | | | F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) |
| 1. P-1 x P2 | 88,00 ± 1,02 | +d | 4,58 | 5,21 | 84,97 ± 1,14 | pd | 5,12 | 6,03 |
| 2. P-1 x P3 | 85,00 ± 0,87 | i | 3,87 | 4,56 | 85,55 ± 1,69 | i | 7,57 | 8,85 |
| 3. P-1 x P4 | 81,00 ± 0,91 | -d | 4,06 | 5,01 | 90,66 ± 1,55 | pd | 6,92 | 7,63 |
| 4. P-1 x P5 | 93,50 ± 1,12 | pd | 5,02 | 5,37 | 98,83 ± 3,48 | i | 15,57 | 15,76 |
| 5. P-1 x P6 | 85,50 ± 0,78 | pd | 3,50 | 4,09 | 96,45 ± 4,39 | pd | 19,62 | 20,34 |
| 6. P-2 x P3 | 90,25 ± 0,90 | pd | 4,02 | 4,46 | 89,35 ± 1,17 | i | 5,26 | 5,88 |
| 7. P-2 x P4 | 106,50 ± 0,94 | i | 4,21 | 3,96 | 105,05 ± 3,00 | i | 13,44 | 12,79 |
| 8. P-2 x P5 | 138,25 ± 0,89 | +h | 3,96 | 2,86 | 122,82 ± 1,86 | +d | 8,30 | 6,76 |
| 9. P-2 x P6 | 122,25 ± 0,90 | pd | 4,02 | 3,29 | 117,11 ± 3,50 | i | 15,67 | 13,38 |
| 10. P-3 x P4 | 100,00 ± 0,87 | pd | 3,87 | 3,87 | 103,64 ± 2,69 | i | 12,05 | 11,63 |
| 11. P-3 x P5 | 123,75 ± 0,48 | +d | 2,16 | 1,75 | 117,23 ± 3,11 | pd | 13,91 | 11,87 |
| 12. P-3 x P6 | 105,25 ± 0,90 | pd | 4,02 | 3,82 | 111,29 ± 3,06 | i | 13,68 | 12,29 |
| 13. P-4 x P5 | 130,75 ± 0,81 | +h | 3,63 | 2,78 | 128,35 ± 1,38 | +h | 6,19 | 4,82 |
| 14. P-4 x P6 | 125,50 ± 0,78 | pd | 3,50 | 2,79 | 127,41 ± 1,57 | i | 7,01 | 5,50 |
| 15. P-5 x P6 | 139,99 ± 0,91 | +h | 4,06 | 2,90 | 135,78 ± 1,81 | pd | 8,11 | 5,97 |

**Table 3. Mode of inheritance and variability of the character stalk height in F1 and F2 progenies in 2009**

| Hybrids | F1 | | | | F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) |
| 1. P-1 x P2 | 89,14 ± 1,03 | +d | 4,42 | 4,96 | 85,82 ± 1,17 | pd | 6,95 | 8,10 |
| 2. P-1 x P3 | 85,27 ± 0,82 | i | 3,83 | 4,49 | 85,77 ± 1,57 | i | 7,84 | 9,14 |
| 3. P-1 x P4 | 81,68 ± 0,94 | -d | 4,26 | 5,22 | 92,43 ± 1,59 | pd | 7,46 | 8,07 |
| 4. P-1 x P5 | 94,36 ± 1,10 | pd | 5,00 | 5,30 | 97,99 ± 3,56 | i | 17,15 | 17,50 |
| 5. P-1 x P6 | 87,02 ± 0,75 | pd | 3,97 | 4,56 | 98,25 ± 4,47 | pd | 19,94 | 20,30 |
| 6. P-2 x P3 | 89,72 ± 0,92 | pd | 4,18 | 4,66 | 89,87 ± 1,22 | pd | 5,91 | 6,58 |
| 7. P-2 x P4 | 107,33 ± 0,91 | i | 4,25 | 3,96 | 107,14 ± 3,05 | i | 14,62 | 13,65 |
| 8. P-2 x P5 | 139,45 ± 0,83 | +h | 4,85 | 3,48 | 123,51 ± 1,93 | +d | 8,59 | 6,95 |
| 9. P-2 x P6 | 123,51 ± 0,90 | pd | 4,19 | 3,39 | 118,35 ± 3,41 | i | 15,87 | 13,41 |
| 10. P-3 x P4 | 99,97 ± 0,85 | pd | 3,93 | 3,93 | 104,52 ± 2,72 | i | 12,73 | 12,18 |
| 11. P-3 x P5 | 123,92 ± 0,45 | +d | 2,45 | 1,98 | 116,73 ± 3,28 | pd | 14,36 | 12,30 |
| 12. P-3 x P6 | 106,08 ± 0,94 | pd | 4,19 | 3,95 | 112,05 ± 3,15 | i | 14,52 | 12,96 |
| 13. P-4 x P5 | 133,48 ± 0,87 | +h | 3,84 | 2,88 | 126,08 ± 1,68 | +d | 6,99 | 5,54 |
| 14. P-4 x P6 | 126,21 ± 0,75 | pd | 3,48 | 2,76 | 127,57 ± 1,59 | pd | 7,28 | 5,71 |
| 15. P-5 x P6 | 142,55 ± 0,90 | +h | 3,22 | 2,26 | 136,86 ± 1,88 | pd | 8,03 | 6,87 |

Low variability of the character leaf number per stalk in F1 progeny was observed during the two-year investigations. Modes of inheritance differred, but the most frequently represented was partial dominance. Positive heterozis was observed in MB-3 x SM-1 and negative heterosis in P-23 x MB-3, MB-3 x O-87 and SM-1 x O-87. As a result of previously presented reasons, the progeny of this generation is uniform.

Variability parameters in F2 generation were higher, which indicaes inequality of individuals in relation to this character. Higher variability also denotes higher possibilities for selection. In both years of investigation, the highest values were achieved in the hybrids P-23 x SM-1, P-23 x YV 125/3, P-23 x FL-5 and P-23 x O-87. The most represented inheritance is partial dominance, followed by the intermediate mode (Tables 4 and 5).

Data presented in the tables show that mean values of the investigated characters with their statistical errors in 2008 are approximately the same with those of 2009. Variability parameters are almost identical in both investigating years. Meteorological reports also reveal that 2008 and 2009 were very similar in relation to their mean monthly temperatures and precipitation amounts from May to September. This points out to precise estimations and good performance of the trial.

**Table 4. Mode of inheritance and variability of the character leaf number per stalk in F1 and F2 progenies in 2008**

| Hybrids | F1 | | | | F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) |
| 1. P-1 x P2 | 27,60 ± 0,19 | -h | 0,86 | 3,12 | 33,25 ± 0,79 | -d | 3,53 | 10,63 |
| 2. P-1 x P3 | 33,50 ± 0,29 | pd | 1,28 | 3,83 | 37,54 ± 1,23 | i | 5,50 | 14,65 |
| 3. P-1 x P4 | 41,75 ± 0,34 | -d | 1,51 | 3,62 | 43,87 ± 1,40 | pd | 6,27 | 14,29 |
| 4. P-1 x P5 | 36,70 ± 0,22 | pd | 1,00 | 2,74 | 39,62 ± 1,24 | i | 5,55 | 14,02 |
| 5. P-1 x P6 | 36,95 ± 0,22 | pd | 0,97 | 2,63 | 40,11 ± 1,21 | i | 5,43 | 13,54 |
| 6. P-2 x P3 | 32,30 ± 0,26 | +h | 1,19 | 3,68 | 30,40 ± 0,40 | pd | 1,79 | 5,90 |
| 7. P-2 x P4 | 32,70 ± 0,28 | -d | 1,27 | 3,88 | 34,79 ± 0,40 | pd | 1,80 | 5,19 |
| 8. P-2 x P5 | 31,10 ± 0,31 | -d | 1,41 | 4,54 | 30,96 ± 0,36 | -d | 1,63 | 5,26 |
| 9. P-2 x P6 | 29,50 ± 0,33 | -h | 1,50 | 5,08 | 31,93 ± 0,37 | -d | 1,65 | 5,17 |
| 10. P-3 x P4 | 34,85 ± 0,21 | i | 0,96 | 2,76 | 35,17 ± 0,60 | i | 2,67 | 7,59 |
| 11. P-3 x P5 | 30,20 ± 0,36 | pd | 1,63 | 5,40 | 30,18 ± 0,80 | pd | 3,59 | 11,89 |
| 12. P-3 x P6 | 29,45 ± 0,30 | -h | 1,32 | 4,49 | 31,21 ± 0,95 | i | 4,25 | 13,61 |
| 13. P-4 x P5 | 33,15 ± 0,26 | pd | 1,15 | 3,48 | 34,00 ± 0,94 | pd | 4,22 | 12,41 |
| 14. P-4 x P6 | 35,05 ± 0,23 | pd | 1,02 | 2,92 | 36,78 ± 0,89 | i | 3,96 | 10,78 |
| 15. P-5 x P6 | 31,55 ± 0,22 | pd | 0,97 | 3,08 | 31,30 ± 0,55 | pd | 2,45 | 7,83 |

**Table 5. Mode of inheritance and variability of the character leaf number per stalk in F1 and F2 progenies in 2009**

| Hybrids | F1 | | | | F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) | $\bar{x} \pm s\bar{x}$ (cm) | | σ | V (%) |
| 1. P-1 x P2 | 28,54 ± 0,21 | -h | 0,95 | 3,33 | 33,35 ± 1,09 | -d | 3,93 | 11,78 |
| 2. P-1 x P3 | 34,36 ± 0,31 | pd | 1,28 | 3,72 | 38,87 ± 1,13 | i | 5,42 | 13,94 |
| 3. P-1 x P4 | 43,58 ± 0,35 | -d | 1,44 | 3,30 | 44,91 ± 1,54 | pd | 6,76 | 15,05 |
| 4. P-1 x P5 | 37,75 ± 0,38 | pd | 1,27 | 3,36 | 40,89 ± 1,15 | i | 6,04 | 14,77 |
| 5. P-1 x P6 | 37,15 ± 0,33 | pd | 1,07 | 2,88 | 40,25 ± 1,37 | i | 5,92 | 14,71 |
| 6. P-2 x P3 | 34,84 ± 0,18 | +h | 1,26 | 3,62 | 31,24 ± 0,58 | i | 2,08 | 6,66 |
| 7. P-2 x P4 | 31,98 ± 0,19 | -d | 1,35 | 4,22 | 35,57 ± 0,67 | pd | 2,14 | 6,02 |
| 8. P-2 x P5 | 31,49 ± 0,27 | i | 1,54 | 4,89 | 31,86 ± 0,35 | pd | 2,18 | 6,84 |
| 9. P-2 x P6 | 30,05 ± 0,29 | -h | 1,68 | 5,59 | 31,32 ± 0,45 | -h | 1,99 | 6,35 |
| 10. P-3 x P4 | 35,65 ± 0,21 | i | 1,09 | 3,06 | 34,97 ± 0,86 | pd | 2,85 | 8,15 |
| 11. P-3 x P5 | 31,01 ± 0,36 | -d | 1,54 | 4,97 | 29,87 ± 0,92 | -d | 3,95 | 13,22 |
| 12. P-3 x P6 | 28,93 ± 0,30 | -h | 1,42 | 4,91 | 31,32 ± 1,00 | pd | 4,06 | 12,96 |
| 13. P-4 x P5 | 33,58 ± 0,25 | pd | 1,27 | 3,78 | 35,84 ± 0,82 | pd | 4,58 | 12,78 |
| 14. P-4 x P6 | 35,62 ± 0,25 | pd | 1,19 | 3,34 | 36,24 ± 0,79 | pd | 3,81 | 10,51 |
| 15. P-5 x P6 | 31,42 ± 0,20 | i | 1,08 | 3,44 | 31,83 ± 0,78 | pd | 2,62 | 8,23 |

## CONCLUSIONS

Based on the results of investigation, the following statements can be drawn:

- Inheritance of the characters stalk height without inflorescence and leaf number per stalk in six parental genotypes with their 15 F1 and 15 F2 hybrids is performed in different ways, but partial dominance and intermediate mode are dominating. Positive heterosis in F1 progeny for stalk height appears in MB-3 x FL-5, YV 125/3 x FL-5 and FL-5 x O-87, and for leaf number per stalk in MB-3 x SM-1. Negative heterotic effect appears only for leaf number per stalk in P-23 x MB-3, MB-3 x O-87 and SM-1 x O-87.
- Parental genotypes have a low standard deviation and variability of the investigated characters, which indicates high genetic homogeneity.
- Low variability of the characters in F1 progeny indicates high uniformity.
- Standard deviation and degree of variance for F2 progeny are significantly higher as compared with F1, which denotes that this generation offers a possibility for selection of individuals and improving the characters in further selection work. The highest variability for the two characters in F2 generation in both investigating years was estimated in the hybrids : P-23 x O-87 and P-23 x FL-5.

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Original Scientific paper

# *LASIODERMA SERRICORNE* F. - PEST OF TOBACCO AND TOBACCO PRODUCTS

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## ABSTRACT

Cigarette beetle *Lasioderma serricorne* F. is a well known pest of dried tobacco and tobacco products (cigarettes and cigars).
It can be found in warehouses for tobacco manipulation and fermentation and also in some purchase checkpoints where dried tobacco is stored.
Investigations carried out in 2011-2013 in laboratory conditions included tobacco types Prilep and Yaka from 2010 crop.
Standard methodology was applied during investigation.
Adults of this pest are reddish brown and their body is covered with fine and tiny ash grey hairs. The adults have an elongated oval body, approximately 2.5 mm long.
The egg is oval, milky white, less than 1 mm, and because of its small size it is hard to be determined in tobacco bales, especially in cut tobacco.
In our investigation, larvae are 4 mm long, dirty white in color and with numerous yellow brown hairs, densely distributed throughout their bodies. Insect droppings, tobacco dust and other debris adhere to their surface.
In laboratory conditions larval development lasts 20 to 40 days and in that time, feeding on tobacco, it makes serious economic damage.
The pupa is about 3 mm long. It is whitish in the beginning, but immediately after pupation its color is like that of the adult insect (reddish brown). Pupal stage lasts 7 to 9 days.
*L. serricorne* attacks dried tobacco and cut tobacco in warehouses, causing damage and contamination with its feces and remains of molting and metamorphosis. Initially it damages tobacco making small holes in it. In later development larvae bite larger parts of dried leaves and pile them one upon another.
If tobacco raw material attacked by cigarette beetle is stored for a longer period without any protection, only dust and debris will be seen when it is opened.
Larvae eat the wrapping paper of cigarettes, cigars and cigarillos, making round openings. It feeds on their content and contaminates it with feces, cast skin and remains of metamorphosis, making it unpleasnat in smoking.

**Keywords:** tobacco, cigarette beetle, *Lasioderma serricorne* F., harmfulness.

## *LASIODERMA SERRICORNE* F. ШТЕТНИК НА ТУТУНОТ И ТУТУНСКИТЕ ПРЕРАБОТКИ

Тутунската буба *Lasioderma serricorne* F. е познат штетник на исушениот тутун и производите од тутун (цигарите, цигаретите и пурите).
Се среќава во тутунските магацини за манипулација, ферментација и цигари, а се среќава и намножува и во пунктовите за откуп каде се чува сувиот тутун.
Проучувањата беа изведени во текот на 2011-2013 година во лабораториски услови. За материјал беа користени тутуни од тип Прилеп и тип Јака од реколта 2010 година.
За истражување на *L. serricorne* во лабораториски услови, за биолошки и други испитувања, беше применета стандардна методологија.
Имагото по боја е црвенкасто кафено, а телото е покриено со фини ситни влакненца со пепелавосива боја. Според нашите истражувања, имагата се со издолжено овална форма на телото и просечно се долги 2,5 mm.
Јајцето е овално, помало од 1 mm, со млечно бела боја, и заради неговата мала големина тешко се утврдува во тутунските бали, а посебно во режаниот тутун.
При нашите истражувања ларвите се со големина од 4 mm, имаат валканобела боја на телото и многубројни густи жолто кафени влакненца, распоредени по целото тело на ларвата. На нив се лепат изметот на инсектите, тутунска прашина и други остатоци.
Развојот на ларвите во лабораториски услови трае од 20 до 40 дена, при што исхранувајќи се со тутунот таа предизвикува економски значајни штети.
Куклата е долга околу 3 mm. Таа е во почетокот беличеста, меѓутоа веднаш по куклењето постепено се менува, така да подоцна добива боја на возрасен инсект (црвенокафена). Стадиумот кукла трае 7-9 дена.
*L. serricorne* го напаѓа сувиот тутун и режаниот тутун во магацините, го оштетува и загадува со измет, остатоците од преслекувањето и метаморфозата. Во почеток го оштетува тутунот правејќи мали дупчиња. Со текот на развитокот ларвите нагризуваат големи делови од сувите лисја наредени еден врз друг.
Кога суровината нападната од тутунската буба подолго време се чува, без да се заштитува, при отворањето останува само прашина и отпадоци.
На цигарите, цигаретите и пурите ларвите ја нагризуваат хартијата, омотниот лист, правејќи тркалезни отвори. Таа се храни со нивната содржина, а воедно ги загадува со изметот, остатоците од преслекувањето и метаморфозата, заради што тие стануваат непогодни за пушење.

**Клучни зборови:** тутун, тутунска буба , *Lasioderma serricorne* F., штетност.

## INTRODUCTION

*Lasioderma serricorne*, commonly known as cigarette beetle, cigar beetle or tobacco beetle, originates from tropical and subtropical regions of America, but it can be found in all parts of the world. According to other literature data, this pest comes from Africa.

Areal distribution of cigarette beetle is very wide and it can be said that it is a cosmopolitan species. It is found much more in areas with warm and moderate climate than in countries with sharp and cold winters.

*L. serricorne* was described as a harmful insect since long ago. According to Radovanovic (1961), the species was found 3000 years ago in Egypt, in an old tomb of Tutankamon, in a vase containing a substance similar to a resin. In Europe it was described for the first time in France, in 1972, according to some samples from the United States (21).

The oldest data which define it as tobacco pest originate from the U.S.A, 1886 (20).

Cigarette beetle is a polifagous pest that attacks various other products kept in the home.

In the Republic of Macedonia it is a permanent resident in the warmer southern parts of the country. It is often found in tobacco factories and warehouses, but its presence is still less than that of tobacco moth.

The larvae of cigarette beetle are poliphagous and beside tobacco products, bales and seeds they attack other stored products and food including rice, nuts, peanuts, beans, cocoa, coffee, chocolate, flours, dried fruits (dates, figs), dried fish, Chinesse tea, cashews, black pepper, red pepper and many other spices and seeds, dry dog food, etc. It also attacks many products that are stored in containers in kitchens and other places in the home.

It is also harmful to non-food products such as dried plants and herbarium specimens, dry floral arrangements, potpourri, decorative vine garlands, drugs and pills, insectariums, etc.

## MATERIAL AND METHODS

Two year investigations (2011-2013) were carried out in laboratory conditions with tobacco types Prilep and Yaka from the 2010 crop.

Standard methodology was applied for investigations on their biological characteristics and other analysis.

A part of tobacco samples was placed in cages to monitor the development cycle of cigarette beetle.

Also, larvae of *L. serricorne* were placed with tobacco leaves in Petri dishes until eclosion of adults.

Other samples were left as a whole into bags to monitor the damage caused by the pest.

The material was analysed on binocular in the laboratories of Scientific Tobacco Institute - Prilep.

## RESULTS AND DISCUSSION

*Lasioderma serricorne* Fabricius, 1792, belongs to the order Coleoptera- beetles, suborder Polyphaga, family Anobiidae.

Synonyms of this species (according to Targioni Tozzetti, cit. Tirelli, 1953) are: *Ptinus serricornis* F., *P. flavescens* Dahl, *P. ligniperda* Waltl., *Ptilinus testaceus* Duft, *Xyletinus testaceus* Sturm, *X. serricornis* Guer.

The species development undergoes complete metamorphosis (holometabolia).

Adults are reddish brown in color and their body is covered with fine ash grey hairs. The color may vary from dark red-brown to reddish yellow. The insect looks like in armour, because its head, chest, especially the pronotum and front wings are heavily chitinized (Fig. 1 , 2 , 3 , 4).

The adults of cigarette beetles are quite small, measuring about 2 to 4 mm (12, 13, 14, 15). They have an elongated oval body, approximately 2.5 mm long.

The head is completely concealed under the first thoracic segment and can not be seen from above, which gives the insect a hunchback appearance. The neck shield is wide in its base, like the elytrae, rounded on its margins and quite narrowed in the front side, with a small depression for the head. The head and mouthparts are bent downward at right angle to the body. The oral apparatus is adapted to biting, with very strong serrated mandibules. It has compound large black and oval eyes, in anterior lateral position. The antennae are long, with 11 saw-like segments.

Figure 1 Imago- dorzal view

Figure 2 Imago- lateral view

Figure 3 Imago- ventral view

Figure 4 Adults of tobacco beetle

The elytrae are arched and spread along the middle of the back, embossed, with many spots through the whole surface. The second pair of wings is membraneous, bent under the elytrae.

The abdomen consists of 5 visible soft segments, protected by elytra. The antenna is telescopic. Legs are short, capable to walk, the formula of the foot is 5-5-5.

Imagos are active at night. They fly in the cages, whereas at daytime they hide in various places, between cage edges, among dry tobacco leaves etc. The life cycle of adults usually takes 3-4 weeks.

Cigarette beetles “play dead” for a few seconds when they are disturbed.

The type is oviparous. Imagos copulate and the females lay the eggs on the substrate in which the tobacco beetle lives and feeds.

Eggs are laid without any order, individually or in a group. In tobacco samples and tobacco packs, the females attach the eggs on tobacco leaves along the leave nerves or along their edges.

In case of tobacco processed products, the female lays the eggs on open edges or on covering leaves of cigarettes, packaging boxes for tobacco products etc.

According to literature data, one female can lay 20-100 eggs (1, 5, 12, 13, 14, 17, 18).

The egg has an oval shape, smaller than 1 mm, with milky white color, and prior the hatching, it turns dull yellow.

Due to its small size, it is difficult to identify it in tobacco packs, especially in cut tobacco.

Larva is fat, crimped, with soft body, curled in the middle as "c", which is typical for this type of insects.

It is eucephalous, its head is chitinized, with brown color. Its jaws are darker, almost black, with triangular shape, with a slightly convex boundary externally, whereas the internal edge is serrated.

According to literature data, an adult larva can be 4 to 5 mm long (12, 13, 14, 15).

In our researches, larvae are 4 mm long, their body has opaque white color and numerous thick yellowish to brown hairs, distributed all over their body. The fecal material from insects, tobacco dust and other remains (figures 5, 6, 7, 8) are stuck on them.

The larva is oligopod, with three pairs of short legs with light brown color.

Figure 5 Larva after hatching

Figure 6 Larva

According to Vukasovic (1962), larvae hatch after 6-7 days in optimum conditions, whereas at temperature at 12 °C even after 8-12 days.
They immediately feed from the material where they hatched.

Figure 7 Larvae

Figure 8 Larvae and damage

The development of larvae is affected not only by the temperature and humidity, but also by the type of food.

The development of larvae in laboratory conditions lasts from 20 to 40 days, and during this time period, if reared on tobacco they cause significant economic damages.

According to Ilic (1961), if the larva is reared on cigars, the development lasts 42 days, if it is reared on cigarettes, the development lasts 36 days, and if it is reared on cut tobacco, only 30 days.

Radovanovic (1961), registered development of larva from 47-70 days, whereas according to Vukasovic (1962), the development of larva can take from 30 days to 70 days in winter conditions.

When the larva is fully grown, it is transformed into a cocoon, located into a semi-closed chamber. The larva builds the cocoon from secretion products, small food particles, tobacco or tobacco dust and fecal material in the substrate it rears on and it spends there the cocoon stadium (figure 9).

According to Krsteska et al. (2011), larvae sometimes make a pathway through card boxes and other packaging in search of spot for building a cocoon.

Figure 9 Cocoon

The cocoon is free cocoon type (pupa libera). The body parts are free and close to the body, whereas the jaws are immovable.

At first, the cocoon is white; later on the color gradually changes and assumes reddish brown color, similar to an adult insect. The cocoon is 3 mm long.

According to Ilic (1961), the length of cocoons is 3,5 mm, according to Radovanovic (1961), it is 2-3 mm, and according to Tirelli (1953), the length ranges from 2,5 to 3,75 mm.

The cocoon stadium lasts 4-10 days, sometimes up to 13 days (12, 15).

In laboratory conditions, this stadium lasted 7-9 days.

The life cycle of this harmful insect is variable. The length of life cycle is affected by climate conditions and the substrate it is reared on.

In warehouses where there are warmer locations, as well as in warehouses which are heated throughout the whole year, one can find *L. serricorne* in all stadiums of development. In the course of the summer, the development of this pest is faster.

In our regions, the tobacco beetle spends the winter in larva stadium. In spring, the larva forms a chamber in which it transforms itself into a cocoon.

One week later, the imago appears. During the first three days, the new imago stays in the cocoon chamber until its wings become stronger.

Usually, the adult insects appear during May and June.

According to a large number of authors, adult insects do not feed, but use the reserves in their body, stored while being in the larva stadium. Actually, adults can cause direct damage only when they eclode, because they make holes through which they go outside.

According to Krsteska et. al. (2011) and Radovanovic (1961), when a cocoon was in a cigarillo, cigarette or cigar, the adult imago makes a hole in order to go out. They can cause similar damages in tobacco packs.

For full development in laboratory conditions, this pest needs 35-55 days.

In our country, it is considered that the tobacco beetle produces 2-3 generations per year.

According to Dimitrov (2003), in Bulgaria, in years with late spring and early autumn, there were two generations, whereas in more favorable conditions there were 2 complete generations and the third generation developing during two calendar years.

## Harmfulness

*L. serricorne* attacks the dry tobacco in warehouses, causes damages to it by rearing on it and polluting it with fecal material, remains from changing and metamorphosis.

The unprocessed tobacco, upon fermentation in a mass or pack, becomes a very favorable environment for reproduction of this pest.

At first, it causes damage to the tobacco by making small holes. As it grows, larvae bite larger parts of dry leaves placed in layers one over another (figure 10, 11).

Figure 10 Damaged tobacco leaves

When the raw material attacked by tobacco beetle is stored for a longer period without any protection, during the opening of samples only dust and waste appear.

Figure 11 Damaged tobacco leaves

The type can cause significant economic damages in tobacco warehouses.

Most often, it attacks the best tobacco classes, in tonga or yarma packs, i.e. tobacco leaves with larger percentage of soluble carbohydrates and more matured, that is, older tobacco leaves.

According to Krsteska et. al. (2011) and Ilic (1961), the attack of tobacco beetles is particularly strong on quality and aromatic varieties of tobacco and causes greater damages in higher classes.

It avoids and more rarely attacks the fresh, non-fermented and lower class tobacco.

The type is important not only as a pest in dry tobacco, but one could say it is even more important as a pest in tobacco products such as cigarettes, cigarillos, cigars, chewing tobacco, pipe tobacco and snuff.

By rearing on cut tobacco, they damage and infest it with fecal materials, dead larvae, remains from changing and metamorphosis.

Figure 12 Damaged cigarettes

Larvae bite the paper and covering leaves on cigars, cigarettes and cigarillos and make round holes (figure 12, 13, 14). It rears on their contents, at the same time infesting them with its fecal materials, remains from changing and metamorphosis, which makes them inappropriate for smoking. When smoking such tobacco products, infested by tobacco beetle, one can sense a typical bad smell and taste.

Figure 13 Damaged cigarettes

Figure14 Damaged cigarettes

According to Ilic (1961), this type was also identified as a pest in tobacco seeds. It rears on it, infests it and even attaches pieces of the seed on its body.

## CONCLUSIONS

*L. serricorne* can cause significant economic damages when it reproduces in dry tobacco in warehouses, in tobacco manufacturing facilities and warehouses for manufactured tobacco products.

The development of larvae in laboratory conditions lasts from 20 to 40 days, and during this time period, if reared on tobacco they cause significant economic damages.

At first, it causes damage to the tobacco by making small holes. As it grows, larvae bite larger parts of dry leaves placed in layers one over another.

When the raw material attacked by tobacco beetle is stored for a longer period without any protection, during the opening of samples only dust and waste appear.

Besides the primary damages caused by dietary they done secondary damage by infest tobacco with fecal materials, dead larvae, remains from changing and metamorphosis.

Larvae bite the paper and covering leaves on cigars, cigarettes and cigarillos and make round holes. It rears on their contents, at the same time infesting them with its fecal materials, remains from changing and metamorphosis, which makes them inappropriate for smoking. When smoking such tobacco products, infested by tobacco beetle, one can sense a typical bad smell and taste.

Studies of the tobacco beetle will enable us timely and effective protection of tobacco and tobacco processing of this economically important pest.

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Original Scientific paper

# INVESTIGATIONS ON RESISTANCE OF SOME TOBACCO VARIETIES TO *PERONOSPORA TABACINA* ADAM IN THE CORESTA BLUE MOLD COLLABORATIVE EXPERIMENT

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## ABSTRACT

Blue Mold Collaborative Experiment includes tobacco varieties from various countries and climate conditions. The aim of investigation was to monitor the intensity of disease attack in a three-years period (2009 - 2011) and to estimate the degree of resistance to the pathogenic fungus *Peronospora tabacina* Adam.

Field trial was set up in randomized blocs with 4 replications. Cultural practices applied in the experiment were in compliance with CORESTA and AERET recommendations for large leaf tobaccos.

The most susceptible variety during the investigation period was Jupiter, while the variety BC1-09-VC1 showed to be tolerant to the disease. The most resistant varieties were Bel 61-10, Chemical Mutant and line V53 CMS F1. The other investigated varieties, among which B2/93 CMS F1, could also be considered as resistant.

Macedonian Virginia and Burley varieties (V53 CMS F1 and B2/93 CMS F1) showed considerably good results in all three years of investigation. These results are of particular importance not only for protection of tobacco from the disease but also for obtaining high yield and good quality of the raw material.

**Keywords:** Blue Mold, V53 CMS F1, B2/93 CMS F1, degree of infestation, degree of resistance.

# ИСПИТУВАЊЕ НА ОТПОРНОСТА НА НЕКОИ СОРТИ ТУТУН СПРЕМА *Peronospora tabacina* Adam ВО ЗАЕДНИЧКИОТ ОПИТ НА CORESTA

Заедничкиот оит на CORESTA за испитување на пламеницата опфаќа тутунски сорти со потекло од разни земји, со разни климатски услови. Целта на ова истражување беше да се следи интензитетот на напад од болеста во текот на тригодишниот период (2009-2011) и да се утврди реакцијата на сортата, односно, да се определи степенот на отпорноста спрема патогенот *Peronospora tabacina* Adam.

Поставен беше полски опит со испитуваните сорти во рандомизиран блок систем, во 4 повторувања. Применувани беа агротехнички операции во склад со насоките на CORESTA и AERET, како и производството на крупнолисни тутуни.

Во тригодишниот период на истражување како осетлива сорта се истакна Jupiter, а како толерантна BC1-09-VC1. На врвот на отпорните сорти се Bel 61-10, Chemical Mutant и линијата V53 ЦМС F1. Како отпорни може да се сметаат и другите испитувани сорти, меѓу кои и B2/93 ЦМС F1.

Македонските сорти од типовите вирџинија (V53 ЦМС F1) и берлеј (B2/93 ЦМС F1) покажаа доста добри резултати во текот на тригодишните истражувања.

Добиените резултати се од особен интерес како за заштитата на тутунот од оваа болест, но и за добивање на тутунска суровина со добар принос, а пред се, квалитет.

**Клучни зборови:** Blue Mold, V53 ЦМС F1, B2/93 ЦМС F1, интензитет на напад, степен на отпорност.

## INTRODUCTION

Blue mold, caused by pathogenic fungus *Peronospora tabacina* Adam, is one of the most harmful diseases on tobacco.

The disease was noticed for the first time in 1891 on cultivated and wild tobacco species in Australia. Its massive attack in 1961 caused serious loss in many tobacco producing countries, including Macedonia. Soon after its outbreak in Europe, it has become economically important disease, primarily because of the importance of tobacco culture and the damages it causes to tobacco yield and quality.

According to Mickovski (1984), climate is one of the major factors for occurrence of blue mold. High amplitudes between day and night temperatures (warm days and cold nights) are especially favorable for disease development. The optimum mean daily temperature for blue mold growth is 20$^0$C (24$^0$ /16$^0$ C day / night).

Blue mold intensity increases with the increase of relative humidity. Prolonged humid periods are suitable for formation of higher number of spores and for their germination. Another factor that favors occurrence of the disease and spreading of spores is the retention of water drops on leaf surface for at least 1 -3 hours. Therefore, the most suitable for occurrence and development of this disease is the period after heavy rainfalls - when the weather is calm and colder. Vice versa, in conditions of permanent light and aeration, possibilities for disease occurrence and growth are much lower.

The parasite is spread by strong winds, through mass conidia dissemination at wide distances. Therefore, monitoring of the disease in many countries, especially in the neighboring ones, is of particular importance and is considered as one of the preventive measures of protection.

Variety reaction to the pathogen is another important factor for occurrence of the disease. For this reason, investigation on various degrees of susceptibility and resistance in tobacco varieties is made by CORESTA (Center for coordination of scientific research on tobacco). Collaborative experiment on PTA monitoring has been successfully conducted since 2005, under the auspices of AERET (European Association for monitoring and research of tobacco). Scientific Tobacco Institute -Prilep is participating in this project with line V53 CMS F1 (hybrid) and variety B - 2/93 CMS F1, created in its Genetics and selection Department.

Line V53 CMS F1 was tested in 2002, in Tobacco Institute – Scafati, Italy and based on the results obtained it was included in the project. In 2003 Burley variety B-2/93 CMS F1 was tested and due to the satisfactory results it was also included in the experiment for PTA monitoring. Since then, the above two large-leaf tobacco varieties have continuously been a constituent part of the selection of tobaccos from all over the world in the scope of this international experiment.

During the whole period of investigation, these varieties showed satisfactory resistance to PTA (especially line V53 CMS F1).

The aim of this paper is to present the results of investigations to PTA resistant varieties in 26 countries-participants in this project, which belong to various climatic zones with different environmental conditions, e.g. Macedonia, Germany, USA, Guatemala and others.

## MATERIAL AND METHODS

Virginia and Burley varieties and lines originating from France, Germany, Switzerland, USA, etc. were taken as material for investigation. The complete list of tobacco varieties and seed materials included in the experiment can be obtained from the project coordinator. Planting takes place in different time periods, depending on climate conditions of the country where the experiment is carried out.

In R. Macedonia, the experiment was conducted in the field of Scientific Tobacco Institute–Prilep and sowing starts in March. During seedbed production, usual cultural practices were applied (use of herbicides, fertilization, irrigation, regulation of temperature regime, weeding). If conditions were favorable for disease occurrence, preventive protection with contact fungicide and insecticide was made. Soil processing was done in autumn, by plowing to a depth of 30-40 cm. Fertilization was made with NPK fertilizer (8:22:20), followed by two spring plowings. Prior to transplanting, harrowing was performed for herbicide incorporation in soil.

Transplanting was performed during May or in early June, on alluvial-coluvial soils. The experiment was set up in randomized blocks with four replications. Each replication consisted of 2 rows with 11 plants and planting density was 50x50 cm. During the growing period, tobacco was hoed twice, fertilized with 26% KAN and irrigated when necessary. From protective chemicals, only insecticides were allowed.

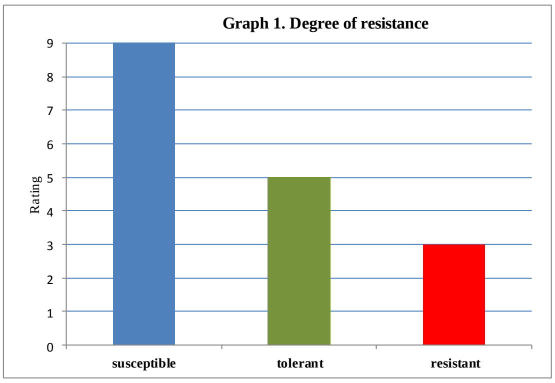
Monitoring (especially on blue mold occurrence) was done on daily basis, from planting to the end of growing season. The intensity of PTA attack is estimated on bottom, middle and top belts, according to CORESTA recommendations, using LTZ Augustenberg scale (Table 1). The degree of resistance is presented in Fig. 1 (Billenkamp and Dongmei, 2011).

**Table 1. Scale for assessment of the degree of infestation with *Peronospora tabacina* Adam**

| Scale | % leaf area damaged* |
|---|---|
| 1 | 0 - 0,6 % |
| 2 | 0,6 - 3 % |
| 2 | 3 - 6 % |
| 3 | 6 - 12 % |
| 4 | 12 - 25 % |
| 5 | 25 - 50 % |
| 6 | 50 - 75 % |
| 7 | 75 - 87 % |
| 8 | 87 - 93 % |
| 9 | 93 - 97 % |
| 9 | 97 - 100 % |
| 9 | 100% |

*****all PTA symptoms (sporulation, systemic)

Degree of resistance was calculated by the mean value of intensity of PTA attack in the three belts. The varieties were classified into 3 categories: resistant (1 - 2.99), tolerant (3 – 4.99) and susceptible (5-9) (Graph 1).




Growth stage of tobacco varieties was determined by certain morphological characters (leaf number, flowering percentage) and indicated by numerical code, according to the instructions given by CORESTA Guide No 7.

The list of investigated varieties is presented together with the results of investigation. The Jupiter variety was marked as susceptible to PTA and Bel 61-10 and Chemical Mutant were marked as resistant.

The annual results of the experiment obtained from each country – participant were sent to the project coordinator after the end of the growing season. After processing of data, a report was made with average results for PTA resistance for each variety.

## RESULTS AND DISCUSSION

The blue mold disease attacks tobacco in all stages of its growth, from seed to the final harvest (Fig. 1).

Disease symptoms in transplanted tobacco are recognized by the appearance of yellow spots on the leaf. They are localized in the beginning but later their number increases (Fig 2, 3). They often coalesce and occupy most of the leaf area, including leaf veins. Such symptoms can be observed in conditions of high intensity of the attack.

In favorable conditions for disease development, a greyish-blue downy mold appears on lower surfaces of leaves. Actually, it is made of conidiophores with conidia of the parasite fungus. Hence the name of the disease: blue mold (Fig. 4).

All symptoms of the disease are included in the above mentioned scale.

The average results of Blue Mold Collaborative Experiment in the period 2009-2012 are presented in Table 2.

Fig. 1 PTA in seedbeds

Fig. 2 Occurrence of disease

Fig.3 More severe attack of Blue mold

Fig. 4 Blue mold on tobacco leaves

In 2009, the lowest intensity of attack and the best rating was observed in line V53 CMS F1 (Table2)

The highest intensity of attack (5.66) was observed in Jupiter (susceptible check). No results are presented for the varieties Chemical Mutant and HYV 27. In other varieties, the intensity of attack ranged from 1.72 in Bel 61-10 (resistant check) to 3.13 in B-2/93 CMS F1.

According to the scale of resistance (Graph 1), most of the varieties in 2009 were marked as resistant, and the variety B-2/93 CMS F1 as tolerant.

In 2010, intensity of attack was higher in the most varieties. The lowest intensity was observed in Chemical Mutant (1.68) and the highest in Jupiter (6.26).

Varieties BCE/09/VC1, HYV 27, B911, ITB 583, Stella and B2/93 CMS F1 were rated as tolerant, whereas line V53 CMS F1 and varieties ITB 569 and ITB 420 were rated as resistant.

In 2011 the intensity of PTA attack was lower in almost all varieties. Similar to previous year, the highest susceptibility was observed in Jupiter, which achieved the highest intensity - 6.24. The most tolerant variety was BCE-09-VC1, with an intensity of 3.33.

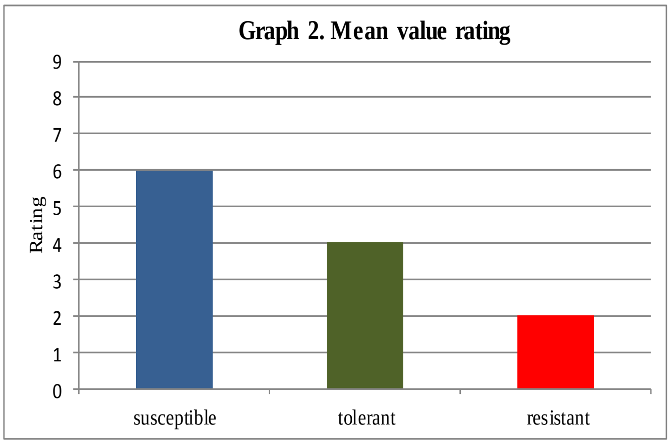
In addition to Bel 61-10 and Chemical Mutant (resistant checks), other varieties that were rated as resistant were ITB 569 (2.12), HYV 27 (2.29), B2/93 CMS F1 (2.31), V53 CMS F1 (2.44), Stella (2.64) and B911 (2.69).

Mean value of the intensity of attack in the varieties of corresponding category and their relation to the degree of resistance is presented in Graph 2. The average intensity of attack ranges from 5.68 in susceptible varieties, 3.05 in tolerant and 2 in resistant ones.

**Table 2. Assessment of the varieties reaction to *Peronospora tabacina* Adam**

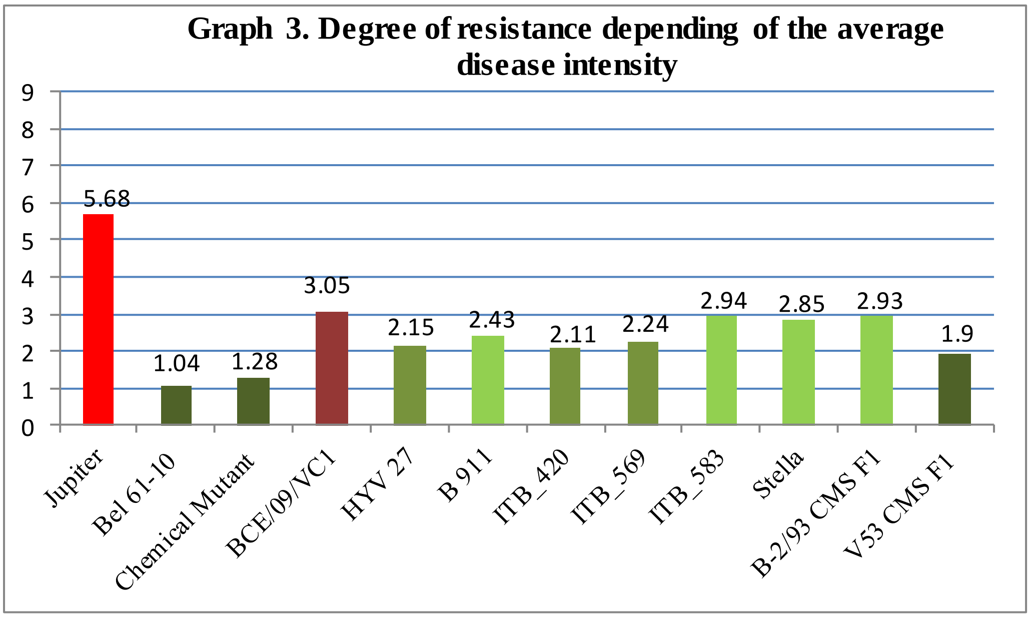
| N⁰. | Variety | Assessment | | | | | | Average | Rating |
|---|---|---|---|---|---|---|---|---|---|
| | | Year | | | | | | | |
| | | 2009 | Rating | 2010 | Rating | 2011 | Rating | | |
| 1 | Jupiter | 5,66 | 10 | 6,26 | 12 | 6,24 | 10 | 5,68 | 12 |
| 2 | Bel 61-10 | 1,72 | 2 | 2,21 | 4 | 1,09 | 1 | 1,04 | 1 |
| 3 | Chemical Mutant | | | 1,68 | 1 | 1,49 | 2 | 1,28 | 2 |
| 4 | BCE/09/VC1 | 2,92 | 8 | 3,77 | 11 | 3,33 | 9 | 3,05 | 11 |
| 5 | HYV 27*Germany | | | 3,06 | 6 | 2,29 | 4 | 2,15 | 5 |
| 6 | B911*Germany | 2,53 | 6 | 3,10 | 7 | 2,69 | 8 | 2,43 | 7 |
| 7 | ITB_420*France | 2,07 | 4 | 2,15 | 3 | | | 2,11 | 4 |
| 8 | ITB_569*France | 2,00 | 3 | 2,60 | 5 | 2,12 | 3 | 2,24 | 6 |
| 9 | ITB_583*France | 2,74 | 7 | 3,14 | 8 | | | 2,94 | 10 |
| 10 | Stella *Switzerland | 2,44 | 5 | 3,48 | 10 | 2,64 | 7 | 2,85 | 8 |
| 11 | B-2/93*FYROM | 3,13 | 9 | 3,35 | 9 | 2,31 | 5 | 2,93 | 9 |
| 12 | V53*FYROM | 1,20 | 1 | 2,06 | 2 | 2,44 | 6 | 1,90 | 3 |

*Country of origin of tobacco varieties/lines




The average results reveal the degree of resistance of investigated varieties in the period 2009-2011. The lowest intensity of PTA attack, i.e. the highest degree of resistance was observed in Bel 61-10 (1.04), Chemical Mutant (1.20) and V53 (1.90) (Table 2, Graph 3).

Other varieties that can be considered as resistant are ITB 420 (2.11), HYV 27 (2.15), ITB 569 (2.24), B911 (2.43), Stella (2.85), B2/93 ( 2.93) and ITB 583 (2.94).




The results of our susceptibility/ resistance investigations under conditions of natural infestation are in compliance with those of Marani et al. (1972). They made a crossing between two resistant and one susceptible local variety. F1 and F2 generations and their parents were investigated in a series of experiments in which tobacco seedlings were exposed to severe natural infection with *Peronospora tabacina* Adam.

Gilham et al. (1987) reported that monitoring of blue mold resistance, as well as other traits in tobacco lines obtained by crossing, will allow their further investigations as potentially commercial varieties.

The results of our investigations are in compliance with those of Pejcinovski and Mitrev (2007), who reported that lower disease index indicates higher degree of resistance, and vice versa. Accordingly, varieties Bel 61-10, Chemical Mutant and V53 CMS F1, which showed the lowest disease intensity, showed the highest degree of resistance to the pathogen *Peronospora tabacina* Adam. The highest intensity of attack was observed in Jupiter, due to which it was used as susceptible check.

It can be seen from the ratings that Bel 61-10 and Chemical Mutant (resistant check varieties), are immediately followed by the line V53 CMS F - creation of Tobacco Institute-Prilep. The lowest ranked variety is Jupiter. Variety B2/93 – another creation of Tobacco Institute-Prilep also belongs to the category of resistant varieties (Graph 2).

Considering the fact that Burley tobacco is genetically more susceptible compared to Virginia, it can be concluded that the above rating of B2/93 is quite a good result.

Selection of hybrids with resistance to *Peronospora tabacina* Adam can provide a significant reduction in pesticide use and improvement of safety and quality of tobacco (Tso, 1990). This claim is confirmed by our selection of large-leaf tobaccos with significant resistance to this pathogen.

The above data indicate that Macedonian tobaccos of the types Virginia (V53 CMS F1) and Burley (B2/93 CMS F1) successfully cope with other varieties originating from countries with much longer tradition in selection of large-leaf tobacco.

## CONCLUSIONS

-The most severe attack of PTA disease was observed in 2010.

-The most susceptible variety in the three year investigation period was Jupiter. -In 2009, the lowest intensity of attack was observed in Macedonian line V53 CMS F1 according to which it is considered as PTA resistant variety.

-In 2010, the lowest intensity of attack was observed in Chemical Mutant.

-In 2011, the lowest intensity of the disease was found in varieties Bel 61-10 and Chemical Mutant (resistant checks).

-According to the average results of investigations on severity of PTA infestation, line V53 has the second best rating after the resistant check varieties.

-With regard to PTA resistance, Macedonian large-leaf tobaccos successfully keep pace with countries which have much longer tradition in their production.

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Original Scientific paper

# ANTAGONISM OF *TRICHODERMA ASPERELLUM* TO *PHYTOPHTHORA PARASITICA VAR. NICOTIANAE*

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## ABSTRACT

Antagonistic effect of the fungus *Trichoderma asperellum* to the pathogenic fungus *Phytophthora parasitica var. nicotianae in vitro* and *in vivo* was investigated in this paper. *Trichoderma asperellum,* grown on nutrient medium in dual culture with pathogenic fungus *Phytophthora parasitica var. nicotianae* inhibits the growth of the pathogen and continues to develop on its colony. Development of the fungus grown in presence of the antagonist was reduced by 32.14%, and the percentage of inhibition was 67.86%. *T. asperellum* - biological agent for control of the soil-borne pathogen *P. parasitica var. nicotianae,* was investigated on tobacco seedlings in a protected area. Seedlings were inoculated both with pure culture of the pathogen and with dual culture grown in presence of the antagonist. In the seedlings treated with culture of the pathogen high percentage of infection was observed, while the seedlings treated with dual culture developed healthier and better.

**Keywords:** tobacco, *Trichoderma asperellum, Phytophthora parasitica var. nicotianae,* antagonism

## АНТАГОНИЗАМ НА *TRICHODERMA ASPERELLUM* ВРЗ *PHYTOPHTHORA PARASITICA VAR. NICOTIANAE*

Во овој труд е испитувано антагонистичкото дејство на габата *Trichoderma asperellum* врз патогената габа *Phytophthora parasitica var. nicotianae* во in vitro и in vivo услови. Габата *T. asperellum* одгледувана на хранлива подлога со патогенот *P. parasitica var. nicotianae* во двојна култура, го инхибира порастот на патогената габа и продолжува да се развива врз неговата колонија. Порастот на патогената габа одгледувана во присуство на антагонистот е намален во просек за 32,14%, додека процентот на инхибирање изнесува 67,86%. Биолошкиот агенс *T. asperellum* за контрола на почвениот патоген *P. parasitica var. nicotianae*, беше испитуван на тутунски расад во заштитен простор. Расадот беше инокулиран со чиста култура од патогенот и со двојна култура одгледувана во присуство на антагонистот. Кај расадот третиран со култура од патогенот имаше висок процент на зараза, додека расадот третиран со двојна култура беше здрав и со подобар развој.

**Клучни зборови:** Тутун, *Trichoderma asperellum, Phytophthora parasitica var. nicotianae,* антагонизам.

## INTRODUCTION

The soil pathogen *Phytophthora parasitica var. nicotianae* – the causing agent of black shank is a serious disease on tobacco. It attacks tobacco crop in all stages of its development. The disease appears in seedbeds and after transplanting in field, during the whole growing season. The first infections appear on root, root neck and bottom part of the stalk. The root of infested plants becomes necrotic, the stalks are dark brown and the leaves turn yellow and dry. The first occurrence of the disease was reported in Java, Indonesia in 1896 (Tashkoski, 1994), by the Dutch scientist J. van Breda de Haan. Presently it occurs in all continents in which tobacco is grown, causing severe losses to tobacco industry. In our country, losses caused by this pathogen in some plots of the regions Prilep and Strumitza approached 50-80 % (Tashkoski, 1999). According to literature data, in some tobacco producing areas the damage was 85-100 % (Fengli et al., 2011, Gallup et al., 2006), which brought into question the production of tobacco in those areas. Since the pathogen can infest tobacco in all stages of growth, protection of tobacco is of crucial importance. Application of some chemicals can reduce the percentage of infection, but they can not ensure complete and effective protection of tobacco. Chemical control of the disease can be effective only when used in combination with other measures. Therefore, tobacco protection from this pathogen requires an integral approach that includes modern agrotechniques, application of chemicals and breeding of resistant varieties. The best method for *Phytophthora* control is planting of resistant tobacco varieties (Gallup et al., 2006). Varieties with one gene of resistance as well as those with high level of partial resistance (Sullivan et al., 2005) provide a high degree of protection from the disease.

In the recent period, efforts have been made to reduce the application of chemicals in plant protection and higher attention was paid to biological control, i.e. to the use of bioproducts. Application of bioproducts in the control of harmful pathogens in agriculture is a subject of great interest for consumers and ecologists.

Among the most popular antagonistic fungi which are used in biological control of plant pathogens are the species of the genus *Trichoderma*, the antibiotic and antifungal properties of which have been known since the 1930-ies (Mudri & Sušinjak, 2000). These species act as parasites on plant pathogens (Anonymous, 2011) and as their competitors for food. They have an antagonistic effect and cause induced resistance in the host plant (Grahovac et al. 2009).

*Trichoderma* species showed high antagonistic effect against pathogenic fungus *P. parasitica var. nicotianae.* In investigations of Chen et al., (2009), isolates of the antagonistic fungus *Trichoderma viride* grown in dual culture inhibited the growth of the pathogenic fungus for 29.12 %. The same isolates, used in the control of black shank disease, showed 56.53 % higher effectiveness compared to metalaxyl. Application of the antagonistic fungus *T. harzianum* (Fernandes et al., 2002) largely decreases the inoculi of the pathogenic fungus in the soil, thereby reducing the intensity of black shank infection. High inhibitory activity against *P. parasitica var. nicotianae* was also observed in some antagonistic bacteria isolated from tobacco rhizosphere (Fengli et al., 2011). Trichoderma species, due to their nematicidal effect (Imran et al., 2001), are used in the control of plant nematodes. This is of particular importance for the spread of black shank disease in tobacco, because the nematodes, through the injuries they made in the root system, enable the pathogen to enter into the plant and to infect it. The nematodes control will reduce the possibilities for infection of tobacco by this pathogen. Today, a number of biofungicides are available for commercial use, obtained from *T. harzianum, T. viride, T. asperellum* and *T. polysporum* (Anonymous, 2011). In investigations of Tashkoski & Čifligaroski (2011), biofungicide Trifender WP, based on *T. asperellum,* showed high effectiveness in the control of soil-borne pathogens *Rhizoctonia solani* and *Pythium debaryanum* on tobacco seedlings grown in protected area.

The main goal of this investigation is to estimate the antagonistic effect of *T. asperellum* against the pathogenic fungus *P. parasitica var. nicotianae* in dual culture and biological control of the pathogen in tobacco seedlings.

## MATERIAL AND METHODS

Investigations were made in laboratory conditions on culture obtained from antagonistic and pathogenic fungi and on tobacco seedlings in protected area. Antagonistic effect of the fungus *Trichoderma asperellum* against pathogenic fungus *P. parasitica var. nicotianae* was estimated. Pure culture of the pathogenic fungus was obtained from infected tobacco plants and grown on potato dextrose agar, while the pure culture of the fungus *T. asperellum* on a nutritive surface potato-dextrose agar is derived from the biofungicide Trifender WP, on the basis of this fungus.

Antagonistic ability of the fungus *T. asperellum* against the pathogen *P. parasitica var. nicotianae* was tested by dual culture technique Dennis and Webster, (1971), (loc cit. Shalini and Kotasthane, 2007).

3 mm fragments with mycelia taken from the pathogenic fungus and from antagonist were placed 3 cm apart in 10-cm Petri dishes, on potato dextrose agar, in four experiments with three replications. Petri dishes were incubated in a thermostat at $25^0$C for a period of 10 days. Radial growth was measured for seven days on mycelial colony of the pathogen grown in presence of the antagonist and as a pure culture, which served as a check. The growth of mycelial colony of pathogenic fungus grown as pure culture was calculated by the equation of Siameto et al., (2010):

$$\% = \frac{\text{radial growth in the presence of the antagonist}}{\text{radial growth in the check}} \text{ x } 100$$

Percentage of inhibition of the pathogen by *T. asperellum* was calculated by the equation of Mudri & Sušinjak, (2000) and Siameto et al. (2010):

$$\text{inhibition \%} = (\text{a - b/a}) \text{ x } 100,$$

where:

a = radial growth of the pathogen in the check
b = radial growth of the pathogen in presence of the antagonist

According to Zivkovic et al., (2010), inhibition of pathogen's colony can be presented in the following scale:
0 = no inhibition, 1 = 1-25% inhibition, 2 = 26-50% inhibition, 3 = 51-75% inhibition, 4 = 76 -100 % inhibition.

Biological control of *P. parasitica var. nicotianae* was investigated on tobacco seedlings in protected area. Two experiments were conducted in three replications. Seedlings of the variety P-66 were sown in pots in an area of 380 cm$^2$, and in later phases it was inoculated with suspensions prepared from mycelium of the fungus in the following three variants:

- Seedlings treated with pure culture of the pathogen *P. parasitica var. nicotianae*

- Seedlings treated with dual culture of the pathogen and *T. asperellum*, and

- Check – untreated seedlings

Fungal culture was grown in nutrient medium potato dextrose agar, in thermostat at 25$^0$C for a period of 10 days. A culture of the pathogenic fungus was grown separately, while in other Petri dishes, pathogenic fungus was grown in dual culture with the antagonistic fungus.

Seedlings from a 380 cm$^2$ pot were inoculated in Petri dish with inoculum prepared from the mycelia. Mycelial colony was mixed in 200 ml distilled water and the obtained suspension was used for spraying tobacco seedlings. Inoculations of tobacco seedlings were performed on 22.6.2011 in the first variant and on 25.7.2011 in the second variant.

The seedlings that were used as a check were treated with pure water. The health condition of seedlings was evaluated according to the presence of infected plants, i.e. to the percentage of infected area.

## RESULTS AND DISCUSSION

*P. parasitica var. nicotianae* is a soil-borne pathogen which can be easily isolated as a pure culture from infected plants or from the soil. When grown on potato dextrose agar it forms white substrate mycelium (Fig. 1) in which a great number of conidia and chlamydospores are made. The antagonistic fungus *T. asperellum* in the beginning is similar to the pathogenic fungus and forms white mycelia which after a few days becomes green, as a result of conidiophores and conidia formation (Fig. 2 ).

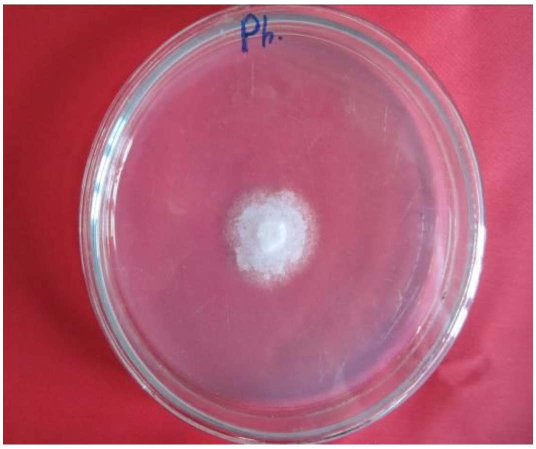


Fig. 1. Pure culture of *P. parasitica var. nicotianae*

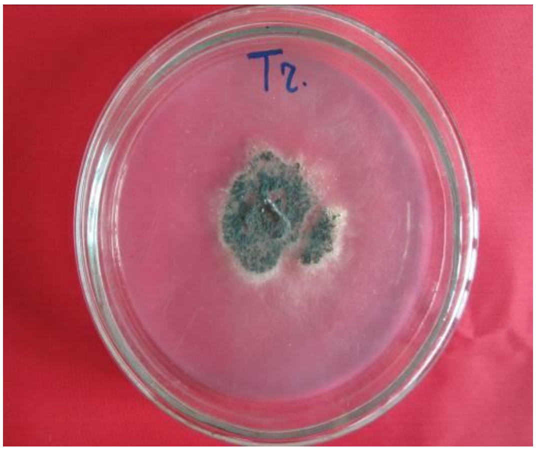


Fig. 2. Pure culture of *T. asperellum*

Percentage growth of pathogen's colony and percentage of its inhibition by the antagonist were calculated according to the results of measurements of colony's growth. Daily growth of fungi both in pure culture and in dual culture is presented in Table 1 and Fig. 3, in which mean values from the three replications in four trials are given.

**Table 1. Fungal growth (mm) during the incubation period by days**

| Variants | Days of incubation | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| ***P. parasitica var. nicotianae*** | 2,50 | 7,50 | 10,70 | 15,00 | 18,70 | 25,00 | **31,20** |
| ***P. parasitica var. nicotianae +T. asperellum*** | 2,50 | 6,00 | 9,00 | 10,00 | 10,00 | 10,00 | **10,00** |
| ***Trichoderma asperellum*** | **3,50** | **14,00** | **19,00** | **26,00** | **38,00** | **46,00** | **52,50** |

24 hours after sowing, radial growth of the pathogenic fungus *P. parasitica var. nicotianae* grown in pure culture was 2.50 mm, while seven days after, at the end of observations, it increased to 31.20 mm. Growth of the antagonistic fungus *T. asperellum* in pure culture was similar to that of the pathogenic fungus. Colony radius on the first day of observation was 3.50 mm and on the seventh day it increased to 52.50 mm. When pathogen was grown in dual culture with the antagonist, radial growth of the colony was 2.50 mm on the first day, increasing gradually up to the fourth day, when it amounted to 10 mm and stayed unchanged to the end of observation. Poor growth of mycelial colony is due to the presence of *T. asperellum* and its antagonistic effect on pathogenic fungus.

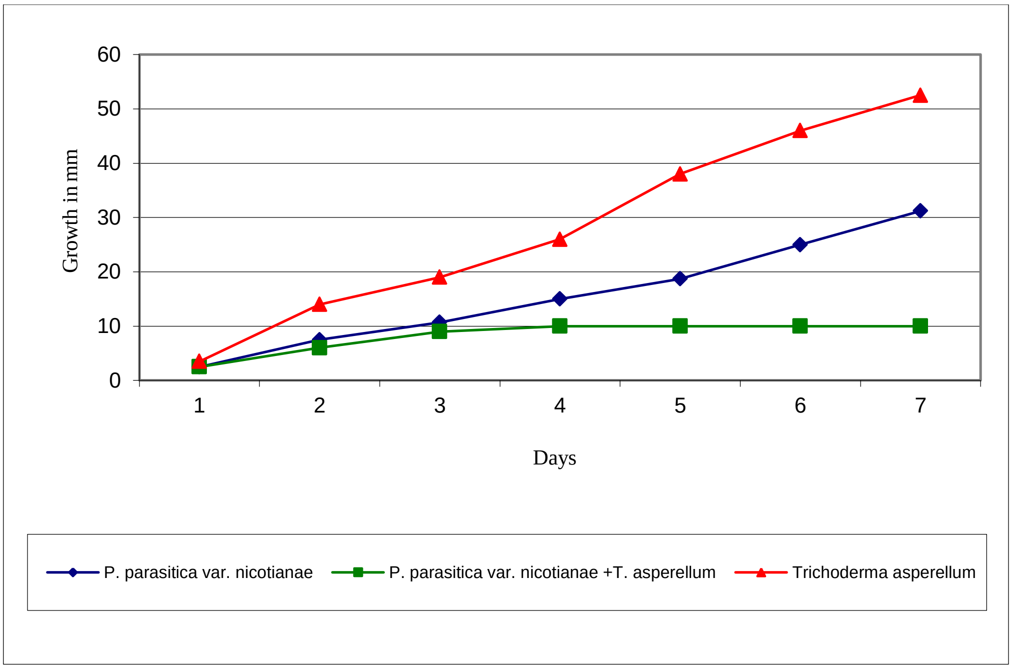


Fig.3 Daily growth of the colony

Similar results were obtained in all four trials on the seventh day of observation. No major differences were observed in growth of the fungal colony (Table 2). In the pathogen *P. parasitica var. nicotianae*, radial colony growth ranged 30.00 mm - 35.00 mm in the third trial, and in the fungus *T. asperellum* from 50.00 mm in the first and fourth trial to 55.00 mm in the second and third trial.

**Table 2. Colony growth of the fungus on the 7th day of incubation**

| Variants | Radial growth of the colony (mm) by trials | | | | Average, in mm |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| ***P. parasitica var. nicotianae*** | 30,00 | 30,00 | 35,00 | 30,00 | **31,25** |
| ***P. parasitica var. nicotianae +T. asperellum*** | 10,00 | 10,00 | 10,00 | 10,00 | **10,00** |
| ***Trichoderma asperellum*** | **50,00** | **55,00** | **55,00** | **50,00** | **52,50** |

Unlike this, on the seventh observation day, pathogenic fungus grown in dual culture with the antagonistic fungus had an increase of only 10 mm in all trials.

According to the results, pathogenic fungus *P. parasitica var. nicotianae* grown in pure culture had 32.14 % higher mycelial growth than when it was grown in presence of the antagonist (Table 3). Inhibition of its growth by the antagonist reached 67.86 % (Table 4). Chen et al., (2009) also reported that the isolate TG050609 of *T. viride* showed 29.12% inhibitory effect on the colony of *P. parasitica var. nicotianae* grown in dual culture.

**Table 3. Percentage growth of the colony of pathogenic fungus *P. parasitica var. nicotianae***

| Variants | Radial growth of colony in the check, mm | Radial growth of colony in presence of the antagonist, mm | Colony growth % |
|---|---|---|---|
| **Trial I** | 30,00 | 10,00 | **33,33** |
| **Trial II** | 30,00 | 10,00 | **33,33** |
| **Trial III** | 35,00 | 10,00 | **28,57** |
| **Trial IV** | 30,00 | 10,00 | **33,33** |
| **Average** | | | **32,14** |

**Table 4. Inhibitory effect of *T. asperellum* upon *P. parasitica var. nicotianae***

| Variant | Radial growth of colony in the check, mm | Radial growth of colony in presence of the antagonist, mm | Inhibition % | Index |
|---|---|---|---|---|
| **Trial I** | 30,00 | 10,00 | 66,67 | **3** |
| **Trial II** | 30,00 | 10,00 | 66,67 | **3** |
| **Trial III** | 35,00 | 10,00 | 71,43 | **3** |
| **Trial IV** | 30,00 | 10,00 | 66,67 | **3** |
| **Average** | | | **67,86** | |

The results obtained during investigation confirmed the high antagonistic effect of the fungus *T. asperellum* to *P. parasitica var. nicotianae*. The antagonist not only inhibits the growth of this pathogenic fungus, but successfully develops on its colony, suppressing its further growth (Fig. 4 and Fig. 5).

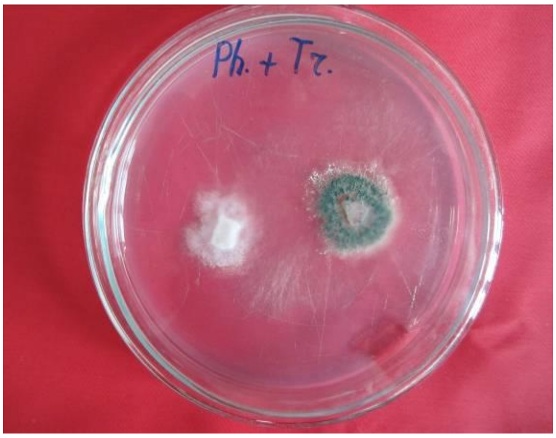

Fig. 4. Dual culture of *P. parasitica var. nicotianae* and *T. asperellum*

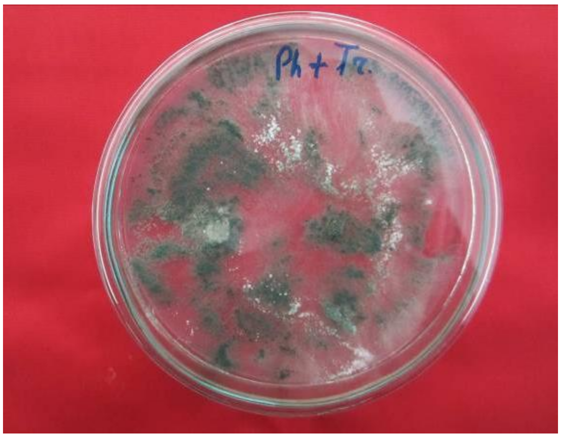

Fig. 5. Growth of *T. asperellum* on the colony of *P. parasitica var. nicotianae*

Good results were obtained with investigations of *T. asperellum* on tobacco seedlings in protected area for biological control of the phytopathogenic fungus *P. parasitica var. nicotianae*. Seedlings were inoculated with inoculi made from pure culture of the pathogen and from dual culture of the pathogen and the antagonistic fungus.

The first symptoms of infection in seedlings treated with pure culture of the pathogen in both trials occurred 2-3 days after inoculation. The infection spread very rapidly and in only a few days more than half of the seedlings were destroyed. In seedlings treated with inoculum obtained from the dual culture very small percentage of infected plants could be observed. Unlike this, low ocurrence of disease from natural infection was observed in the untreated check. 10-15 days after inoculation, seedlings treated with inoculum from pure culture were completely destroyed, while in seedlings treated with dual culture not only infection did not spread but they had better growth and development compared to the check ( Fig. 6 and Fig. 7).

Fig. 6. Inoculated seedlings (left - in pure culture , right – in dual culture ), I trial

Fig. 7. Inoculated seedlings (left - in pure culture , right – in dual culture ), II trial

High effectiveness of *Trichoderma* species in the control of *P. parasitica var. nicotianae* on tobacco was confirmed in investigations of Chen et al., (2009). *T. viride* isolate TG050609 showed 56.53 % higher effectiveness than metalaxyl.

In this investigation, made in laboratory conditions on pathogen culture and tobacco seedlings in protected area, *T. asperellum* proved to be good antagonist and true mycoparasite which inhibits the growth of pathogenic fungus *P. parasitica var. nicotianae*. Through its mechanisms – mycoparasitism and antagonism, it protects tobacco seedlings from infection by this soil-borne pathogen.

## CONCLUSIONS

The soil-borne pathogen *P. parasitica var. nicotianae* - the causing agent of black shank disease on tobacco, grown in pure culture on nutrient medium potato-dextrose agar has 32.14 % higher growth compared to that grown in dual culture with antagonistic fungus.

Inhibitory effect of the fungus *T. asperellum* on the growth of mycelial colony of the pathogenic fungus was 67.86 %.

Tobacco seedlings inoculated with pure culture of the pathogenic fungus were infected and completely destroyed, while the seedlings inoculated with dual culture of the pathogen and the antagonist showed a very low percentage of infected plants. Seedlings grown in presence of the antagonistic fungus had faster growth and better development compared to the check variant.

*T. asperellum* proved to be a true antagonistic fungus and mycoparasite to the fungus *P. parasitica var. nicotianae* and it can be used for biological control of this phytopathogen in tobacco production.

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Original Scientific paper

# INVESTIGATIONS OF HERITABILITY AS AN INDICATOR OF THE INHERITANCE OF QUANTITATIVE CHARACTERS IN TOBACCO

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## ABSTRACT

Mode and level of inheritance of some major quantitative characters (stalk height, leaf number per stalk, middle belt leaf area, green and dry mass yield per stalk) was investigated in four parental genotypes (Burley - B 2/93, Suchum - S1, Suchum - S2 and Prilep - P-84) and their six diallel F1 hybrids. The trial was set up in 2007, 2008 and 2009 in the field of Tobacco Institute-Prilep in a randomized block design with four replications.
The aim of these investigations is to study the mode of inheritance and to determine heritability, i.e. degree of inheritance of major quantitative characters, which will allow us to give recommendations regarding the choice of parental genotypes and directions in creation of new cultivars, as our contribution to genetics of tobacco.
Mode of inheritance was estimated according to the test- significance of the mean value of F1 progeny compared to the parental average. Narrow-sense heritability was estimated after Allard (1960), while broad-sense heritability and genetic components after Mather and Jinks (1974).
The mode of inheritance in the hybrids was different. Positive heterosis for stalk height was recorded in S1 x S2 and S2 x P - 84, and for green and dry mass yields per stalk in S1 x S2. Negative heterosis occurs for leaf number and dry mass yield in S1 x P - 84 and S2 x P - 84 , while for dry mass yield in S1 x P - 84. Inheritance of the characters during the three years of investigation was identical. The highest narrow- and broad-sense heritability index during the three years of investigation was recorded for stalk height in 2007, and for dry mass yield in 2008 and 2009. The lowest values for both types of heritability were recorded for the character leaf number per stalk. In all investigated charcters, the values of broad-sense heritability were higher than those of narrow-sense heritability.

**Keywords:** tobacco (*Nicotiana tanacum* L.), heredity, intermediate, partial dominance, dominance, heterosis, narrow-sense and broad-sense heritability ($h^2$).

## ПРОУЧУВАЊА ЗА ХЕРИТАБИЛНОСТА КАКО ПОКАЗАТЕЛ ЗА НАСЛЕДНОСТА НА КВАНТИТАТИВНИТЕ СВОЈСТВА КАЈ ТУТУНОТ

Испитувањата ги опфаќаат начинот и степенот на наследување на поважните квантитативни својства: висина на страк, број на листови по страк, површина на листовите од средниот појас, принос на зелена и принос на сува маса по страк, кај четири родителски генотипови ( Берлеј – Б 2/93, Suhum – S1, , Suhum – S2 и Прилеп – П-84) и нивните шест дијалелни F1 хибриди. Опитот беше поставен во 2007, 2008 и 2009 година на опитното поле при Научниот институт за тутун – Прилеп по случаен блок – систем во четири повторувања.

Целта на овие истражувања е да го откриеме начинот на наследување и да ја одредиме херитабилноста т.е. степенот на наследување на поважните квантитативни својства, со што ќе дадеме напатствија за изборот на родителски генотипови и насоки во селекцијата за создавање на нови сорти, како и допринос во генетиката на тутунот.

Начинот на наследување се одреди врз база на тест сигнификантноста на средните вредности кај F1 потомството во однос на родителските просеци. Херитабилноста во потесна смисла се пресмета спрема Allard (1960), а херитабилноста во поширока смисла, како и генетските компоненти спрема Mather и Jinks (1974).

Наследувањето на својствата во трите години на истражување е идентично. Начинот на наследување кај крстоските е различен. Позитивен хетерозис се јавува за висина на стракот кај S1 x S2 и S2 x П-84, а за приносот на зелена маса и приносот на сува маса по страк кај S1 x S2. Негативен хетерозис се јави за бројот на листови и приносот на зелена маса по страк кај S1 x П-84 и S2 x П-84, додека за приносот на сува маса по страк кај S1 x П-84. Највисока процентуална вредност за херитабилноста во потесна и поширока смисла во 2007 година има својството висина на стракот, а во 2008 и 2009 година принос на сува маса по страк. Најниска процентуална вредност за двата типа на херитабилност во трите години на истражување има својството број на листови по страк. Кај сите проучувани својства вредностите за херитабилноста во поширока смисла се повисоки од оние за херитабилноста во потесна смисла.

**Клучни зборови:** тутун (Nicotiana tanacum L.), наследување, интермедијарност, парцијална доминантност, доминантност, хетерозис, херитабилност ($h^2$) во потесна и поширока смисла.

## INTRODUCTION

Heritability is genetic index for prognosis of the selection results. Many authors made investigations on the inheritance of characters in various cultures, including tobacco. Povilaitis (1966) reported low heritability in a diallel set of eight flue-cured varieties for leaf number and yield per stalk, while for the character leaf area the highest heritability was recorded in top leaves. Espino and Capote (1976), in diallel of seven dark tobacco varieties, reported medium heritability for stalk height and leaf number and low heritability for yield per stalk. Ibrahim and Avratovscukova (1984), in five flue-cured varieties and ten diallel F1 hybrids, recorded high to moderate broad-sense heritability for stalk height and yield, and moderate heritability for leaf number per stalk. Dobhal (1987) reported high heritability for leaf number in 25 genotypes of cigar wrapper tobacco. Naumovski (1987) reported high heritability in a diallel of oriental tobacco for leaf number. In 55 genotypes of Nicotiana rustica (hookah and chewing tobacco) Dobhal and Nageswara Rao (1988) reported moderate heritability for stalk height and yield. Legg (1989), in seven homozygous genotypes of dark and flue-cured tobacco and 21 F1 hybrids, found heritability high enough to justify the process of selection. Chaubey et al. (1990) obtained high heritability for leaf number and yield per stalk in 72 genotypes of Nicotiana rustica. Butorac (1999) investigated four parental varieties of Burly tobacco and their diallel F1, F2, BC1 and BC2 progenies and reported that their broad-sense heritability was higher than the narrow-sense heritability. The highest heritability was estimated in inheritance of the leaf area and the sixth leaf weight. Korubin-Aleksoska and Aleksoski (2009) in three oriental and one semi-oriental variety and their F1 and F2 progenies obtained very high index for both types, with predominance of the broad-sense heritability.

The aim of the three-years investigation was to determine the mode and grade of inheritance of some major quantitative characters of F1 progeny in four varieties in order to improve our knowledge on the genetic nature and to give further directions in selection of tobacco.

## MATERIAL AND METHODS

Four tobacco varieties were included in investigations: one large-leaf - Burley B2/93 ( Photo 1) and three oriental - Suhum S1 and S2 (Photo 2 and Photo 3) and Prilep P-84 (Photo 4), together with their six diallel F1 hybrids (Aleksoski, 2009). The trial with parental genotypes and their F1 progenies was set up in 2007, 2008 and 2009 in the field of Scientific Tobacco Institute-Prilep in randomized blocks with four replications. The area of each replication was 150 m$^2$ and the area of the total trial was 600 m$^2$. All suitable cultural practices were applied during the growth period of tobacco.

In 2007, during tobacco growth in field (May-September), mean monthly temperature was 20.88 $^0$C and the number of rainy days was 40, with 229.9 mm total amount of precipitation. In the same period in 2008, mean monthly temperature was 19.91$^0$ C, the number of rainy days 39 and the total amount of precipitations 235.4 mm. In 2009, mean monthly temperature was 19.89$^0$ C, the number of rainy days 42 and the total amount of precipitations 240.6 mm.

The following characters were investigated in this paper: stalk height without inflorescence, leaf number per stalk, middle belt leaf area, green mass yield per stalk and dry mass yield per stalk. Data obtained for each character were analysed using the analysis of variance.

Mode of inheritance of quantitative characters was determined by test-significance of the mean values in F1 progeny and their comparison with parental averages (Borojeviċ, 1981).

Heritability ($h^2$) is the ratio between the components of genetic and phenotypic variance. It can be presented in two ways and is expressed in percentages.

Photo 1. Burley B 2/93

Photo 2. Suchum S1

Photo 3. Suchum S2

Photo 4. Prilep P - 84

Narrow-sense heritability in some combinations is the ratio of the additive component of genetic variance over the phenotypic variance and is calculated using the Allard's formula (1960) :

$$h^2_{NS} = VA / VP \quad \text{or} \quad h^2 = \frac{\sigma^2 F_2 - \frac{\sigma^2 P_1 + \sigma^2 P_2 + \sigma^2 F_1}{3}}{\sigma^2 F_2} \cdot 100$$

Broad-sense heritability for all combinations of F1 progeny is the ratio of the total genetic variance (additive and dominant) over the phenotypic variance, and is calculated using the formula of Mather and Jinks (1974):

$$h^2_{BS} = (VA + VH) / VP \quad \text{or} \quad h^2 = \frac{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{4}H_2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{4}H_2 - \frac{1}{2}F + E}$$

Estimation of the genetic copmponents D, H1, H2 and F was made after Mather and Jinks (1974).

## RESULTS AND DISCUSSION

Our three-year investigations revealed different modes of inheritance of the character stalk height without inflorescence (Table 1). In hybrids where one of the parents is the large-leaf B 2/93 this character is inherited intermediary, except for the hybrid B 2/93 x FO in 2009, where the mode of inheritance was partial dominance. Positive heterosis was observed in S1 x S2 and S2 x FO. No occurrence of negative heterotic effect was observed in the diallel.

**Table 1. Mode of inheritance of the character stalk height without inflorescence in F1 progeny (cm)**

| Parents and F1 hybrids | 2007 $\bar{x} \pm s\bar{x}$ | | 2008 $\bar{x} \pm s\bar{x}$ | | 2009 $\bar{x} \pm s\bar{x}$ | | $\bar{x}$ | |
|---|---|---|---|---|---|---|---|---|
| 1. P1 | 147,09 ± 0,37 | | 141,85 ± 0,36 | | 149,88 ± 0,35 | | **146,27** | |
| 2. P2 | 69,97 ± 0,29 | | 67,83 ± 0,27 | | 70,09 ± 0,26 | | **69,30** | |
| 3. P3 | 69,53 ± 0,30 | | 66,79 ± 0,28 | | 70,01 ± 0,28 | | **68,78** | |
| 4. P4 | 57,75 ± 0,25 | | 57,29 ± 0,23 | | 57,82 ± 0,21 | | **57,62** | |
| 5. P1 x P2 | 104,85 ± 0,16 | i | 103,35 ± 0,18 | i | 108,20 ± 0,18 | i | **105,47** | i |
| 6. P1 x P3 | 102,50 ± 0,19 | i | 101,91 ± 0,19 | i | 102,65 ± 0,18 | i | **102,35** | i |
| 7. P1 x P4 | 100,50 ± 0,18 | i | 91,15 ± 0,19 | i | 99,89 ± 0,17 | pd | **97,18** | i |
| 8. P2x P3 | **70,19 ± 0,9** | **+h** | **68,85 ± 0,10** | **+h** | **71,48 ± 0,10** | **+h** | **70,17** | **+h** |
| 9. P2 x P4 | 70,98 ± 0,12 | +d | 68,97 ± 0,13 | +d | 71,08 ± 0,12 | +d | **70,34** | +d |
| 10. P3 x P4 | **71,82 ±0,14** | **+h** | **69.86 ± 0,12** | **+h** | **72.53 ± 0,14** | **+h** | **71,40** | **+h** |

Legend:

- P1 - Burley B - 2/93
- P2 - Suchum S1
- P3 - Suchum S2
- P4 - Prilep P - 84

- i - Intermediate
- pd - Partial dominance
- d - Dominance (positive and negative)
- h - Heterosis (positive and negative)

The inheritance of leaf number per stalk in hybrids where B 2/93 is one of the parents was negatively dominant in all three years of investigation. This indicates the dominance of the parent with lower number of leaves. In S1 x S2 partial and positive dominance were recorded, while in S1 x P-84 and S2 x P-84 there was occurrence of negative heterosis (Table 2).

**Table 2. Mode of inheritance of the character number of leaves per stalk in F1 progeny**

| Parents and F1 hybrids | 2007 $\bar{x} \pm s\bar{x}$ | | 2008 $\bar{x} \pm s\bar{x}$ | | 2009 $\bar{x} \pm s\bar{x}$ | | $\bar{x}$ | |
|---|---|---|---|---|---|---|---|---|
| 1. P1 | 35,61 ± 0,12 | | 34,19 ± 0,10 | | 35,45 ± 0,11 | | **35.08** | |
| 2. P2 | 46,70 ± 0,10 | | 44,66 ± 0,10 | | 46,81 ± 0,09 | | **46.06** | |
| 3. P3 | 47,09 ± 0,10 | | 44,87 ± 0,10 | | 46,92 ± 0,10 | | **46.29** | |
| 4. P4 | 53,47 ± 0,09 | | 52,37 ± 0,11 | | 53,33 ± 0,10 | | **53.06** | |
| 5. P1 x P2 | 35,24 ± 0,07 | -d | 34,54 ± 0,06 | -d | 35,47 ± 0,06 | -d | **35.08** | -d |
| 6. P1 x P3 | 35,85 ± 0,06 | -d | 34,41 ± 0,05 | -d | 35,58 ± 0,06 | -d | **35.28** | -d |
| 7. P1 x P4 | 36.88 ± 0,07 | -d | 35,22 ± 0,05 | -d | 36,79 ± 0,07 | -d | **36.30** | -d |
| 8. P2x P3 | 46,95 ± 0,04 | pd | 44,90 ± 0,03 | +d | 46,93 ± 0,04 | +d | **46.26** | +d |
| 9. P2 x P4 | 43,26 ± 0,05 | -h | 42,25 ± 0,04 | -h | 42,07 ± 0,05 | -h | **42.53** | -h |
| 10. P3 x P4 | 44,65 ± 0,05 | -h | 42,88 ± 0,04 | -h | 43,26 ± 0,04 | -h | **43.60** | -h |

The character middle belt leaf area was inherited with partial dominance, except for S2 x P-84 where the inheritance was intermediate. In selection, this indicates fast stabilization of the character in future (Table 3).

**Table 3. Mode of inheritance of the character middle belt leaf area in F1 progeny (cm²)**

| Parents and F1 hybrids | 2007 $\bar{x} \pm s\bar{x}$ | | 2008 $\bar{x} \pm s\bar{x}$ | | 2009 $\bar{x} \pm s\bar{x}$ | | $\bar{x}$ | |
|---|---|---|---|---|---|---|---|---|
| 1. P1 | 1263,88 ± 9,55 | | 1138,31 ± 9,43 | | 1304,21 ± 0,35 | | **1235.47** | |
| 2. P2 | 230,22 ± 1,73 | | 204,60 ± 1,77 | | 234,74 ± 0,26 | | **223.19** | |
| 3. P3 | 239,24 ± 1,71 | | 220,46 ± 1,62 | | 239,87 ± 0,28 | | **233.19** | |
| 4. P4 | 146,24 ± 1,87 | | 137,56 ± 1,75 | | 148,63 ± 0,21 | | **144.14** | |
| 5. P1 x P2 | 1063,44 ± 2,23 | pd | 1015,70 ± 2,53 | pd | 1063,17± 2,33 | pd | **1047.44** | pd |
| 6. P1 x P3 | 1074,49 ± 2,18 | pd | 988,24 ± 2,62 | pd | 1018,29 ± 2,25 | pd | **1027.01** | pd |
| 7. P1 x P4 | 903,39 ± 2,99 | pd | 832,39 ± 2,38 | pd | 942,43 ± 2,44 | pd | **892.74** | pd |
| 8. P2x P3 | 233,34 ± 1,73 | pd | 209,59 ± 1,44 | pd | 236,21 ± 1,53 | pd | **226.11** | pd |
| 9. P2 x P4 | 185,26 ± 1,73 | i | 173,40 ± 1,79 | i | 187,93 ±1,70 | i | **182.20** | i |
| 10. P3 x P4 | 174,53 ± 1,86 | pd | 158,22 ± 1,53 | pd | 176,28 ±1,67 | pd | **169.68** | pd |

The inheritance of green mass yield per stalk in progenies with B 2/93 as one of the parents was partially dominant. Positive heterosis ocurred in S1 x S2 and negative heterotic effect was recorded in the hybrids S1 x P-84 and S2 x P-84 (Table 4).

**Table 4. Mode of inheritance of the character green mass yield per stalk in F1 progeny (g)**

| Parents and F1 hybrids | 2007 $\bar{x}$ | | 2008 $\bar{x}$ | | 2009 $\bar{x}$ | | $\bar{x}$ | |
|---|---|---|---|---|---|---|---|---|
| 1. P1 | 1098,63 | | 1016,90 | | 1100,24 | | **1071,92** | |
| 2. P2 | 267,27 | | 209,64 | | 283,18 | | **253,36** | |
| 3. P3 | 260,45 | | 207,65 | | 275,41 | | **247,84** | |
| 4. P4 | 159,73 | | 157,88 | | 169,35 | | **162,32** | |
| 5. P1 x P2 | 812,77 | pd | 800,19 | pd | 812,93 | pd | **808,63** | pd |
| 6. P1 x P3 | 811,58 | pd | 808,36 | pd | 824,11 | pd | **814,68** | pd |
| 7. P1 x P4 | 795,45 | pd | 790,55 | pd | 832,39 | pd | **806,13** | pd |
| 8. P2x P3 | **269,44** | **+h** | **210,50** | **+h** | **294,27** | **+h** | **258,07** | **+h** |
| 9. P2 x P4 | 133,24 | -h | 130,26 | -h | 146,32 | -h | **136,61** | -h |
| 10. P3 x P4 | 135,11 | -h | 133,27 | -h | 148,24 | -h | **138,87** | -h |

Modes of inheritance and average values for the character dry mass yield per stalk in the investigating period are presented in Table 5. Positive heterosis was observed in S1 x S2 and negative heterosis in S1 x P-84.

**Table 5. Mode of inheritance of the character dry mass yield per stalk in F1 progeny (g)**

| Parents and F1 hybrids | 2007 $\bar{x}$ | | 2008 $\bar{x}$ | | 2009 $\bar{x}$ | | $\bar{x}$ | |
|---|---|---|---|---|---|---|---|---|
| 1. P1 | 185,43 | | 177,85 | | 190,55 | | **184.61** | |
| 2. P2 | 25,99 | | 24,73 | | 26,04 | | **25.59** | |
| 3. P3 | 26,03 | | 25,03 | | 26,17 | | **25.74** | |
| 4. P4 | 24,01 | | 23,68 | | 24,49 | | **24.06** | |
| 5. P1 x P2 | 132,15 | pd | 129,88 | pd | 130,42 | pd | **130,82** | pd |
| 6. P1 x P3 | 133,24 | pd | 130,04 | pd | 145,95 | pd | **136,41** | pd |
| 7. P1 x P4 | 122,15 | i | 117,33 | i | 116,31 | i | **118,60** | i |
| 8. P2x P3 | **26,40** | **+h** | **25,29** | **+h** | **26,88** | **+h** | **26,19** | **+h** |
| 9. P2 x P4 | 23,14 | -h | 22,74 | -h | 23,79 | -h | **23,22** | -h |
| 10. P3 x P4 | 24,05 | -d | 23,75 | -d | 24,29 | -d | **24,03** | -d |

Our investigations on the major quantitative characters of tobacco showed high values for both types of heritability, which indicates the presence of a very high genetic variability and insignificant environmental variability. For these reasons, the investigated characters appeared to be highly heritable, i.e. their manifestation can be easily predicted and their faster stabilization can be obtained.

The highest narrow- and broad- sense heritability in 2007 (Table 6) was recorded for the character stalk height without inflorescence ($h^2_{NS}$ = 0,9776, $h^2_{BS}$ = 0,9988), while in 2008 and 2009 for dry mass yield per stalk (2008 - $h^2_{NS}$ = 0,9687, $h^2_{BS}$ = 0,9988; 2009 - $h^2_{NS}$ = 0,9788, $h^2_{BS}$ = 0,9989). The lowest values for both heritability types in the three-year investigations was recorded for leaf number per stalk ((2007 - $h^2_{NS}$ = 0,7447, $h^2_{BS}$ = 0,9867; 2008 - $h^2_{NS}$ = 0,7577, $h^2_{BS}$ = 0,9879; 2009 - $h^2_{NS}$ = 0,7563, $h^2_{BS}$ = 0,9869).

High heritability values were also reported by the following authors: Ibrahim and Avratovscukova (1984) in five flue-cured varieties and ten F1 hybrids for stalk height and yield per stalk, Dobhal (1987) in 25 cigar wrapper genotypes, Naumovski (1987) in a diallel of oriental varieties for leaf number per stalk, Chaubey et al. (1990) in 72 genotypes of Nicotiana rustica for leaf number and yield per stalk and Korubin –Aleksoska анд Aleksoski (2009) in a diallel of three oriental and one semi-oriental variety for some more important quantitative characters in tobacco. Butorac (1990) reported higher broad-sense heritability compared to the narrow-sense heritability in a diallel of four Burley varieties, which coincides with the results of the above mentioned authors and with data presented in this paper.

**Table 6. Heritability of the quantitative characters in F1 progeny**

| Heritability (%) | Stalk height without inflorescence | Leaf number per stalk | Middle belt leaf area | Green mass yield per stalk | Dry mass yield per stalk |
|---|---|---|---|---|---|
| | | | 2007 | | |
| Narrow - sense | 0.9776 | 0.7447 | 0.9335 | 0.9578 | 0.9714 |
| Broad - sense | 0.9988 | 0.9867 | 0.9983 | 0.9965 | 0.9954 |
| | | | 2008 | | |
| Narrow - sense | 0.9676 | 0.7577 | 0.9184 | 0.9418 | 0.9687 |
| Broad - sense | 0.9973 | 0.9879 | 0.9948 | 0.9983 | 0.9988 |
| | | | 2009 | | |
| Narrow - sense | 0.9753 | 0.7563 | 0.9245 | 0.9492 | 0.9788 |
| Broad - sense | 0.9983 | 0.9869 | 0.9978 | 0.9972 | 0.9989 |

## CONCLUSIONS

On the basis of presented data and analysis, the following conclusions can be drawn:

- Parental genotypes B 2/93, S1, S2 and P-84 and their F1 progenies are uniform. Stalk height without inflorescence was most frequently inherited with intermediate mode, leaf number per stalk with negative dominance and middle belt leaf area, green mass yield and dry mass yield per stalk with partial dominance. Positive heterosis for stalk height was observed in S1 x S2 and S2 x P - 84, and for green mass yield in S1 x S2. Negative heterotic effect for leaf number and green mass yield was observed in S1 x P-84 and S2 x P-84, while for dry mass yield in S1 x P-84. The mode of inheritance of quantitative characters was identical in all three years of investigation (except for the characters stalk height in B 2/93 x P - 84 and leaf number per stalk in S1 x S2, in which insignificant differences provoked by non-genetic factors were observed).
- The highest value of narrow- and broad-sense heritability in 2007 was recorded for stalk height and in 2008/2009 for dry mass yield. The lowest value for both heritability types during the three years of investigation was observed in leaf number. In all characters investigated, the values for broad-sense heritability were higher than those for narrow-sense heritability.
- High genetic variance is manifested in investigated quantitative characters, which can be noted from the high percentual values for heritability. Therefore, it is about highly heritable characters which will be fixed and stabilized in a short period of time.

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Original Scientific paper

# INTRODUCTION OF ORIENTAL TOBACCO VARIETIES UNDER THE ENVIROMENT OF THE AREA NEVROKOP

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## ABSTRACT

In three years (2010-2012) studied the biological and economic characteristics of two varieties - Katerini M and Katerini 53 under the environment of the area Nevrokop. Experience is displayed in the experimental field of ETS (Experimental Tobacco Station) G. Delchev .

Variety Katerini 53 and Katerini M differ materially from cultivated tobacco varieties in the region . They are relatively low, with short and dense internodiyas smaller leaves then Bulgarian varieties traditionally grown, so keeping them is associated with high labor costs . The vegetation period is much shorter. Leaves are shortly ripes, which creates a problem for timely harvesting.

The study is detailed and shows that Greek varieties inferior in resistance to important indicator for the region diseases. This, and the above findings, making them inappropriate in our growing in Nevrokop region. Varieties Katerini 53 and Katerini M have good vegetative morphology and uniformity and can be included in breeding programs for new varieties with good smoking and qualities demanded by the firms.

**Keywords:** oriental varieties , varietal groups , agroecological conditions , introduction, region diseases

## ВНЕСУВАЊЕ НА ОРИЕНТАЛСКИ СОРТИ ТУТУН ВО ЕКОЛОШКИТЕ УСЛОВИ НА РЕОНОТ НА НЕВРОКОП

Извршени се тригодишни испитувања (2010-2012) на биолошките и економските својства на две сорти тутун – Катерини М и Катерини 53 во агроеколошките услови на неврокопскиот реон. Опитот се изведуваше на опитното поле од Експерименталната станица за тутун „Гоце Делчев“.

Сортите Катерини 53 и Катерини М се разликуваа од култивираните тутунски сорти во овој реон. Тие се релативно ниски, со кратки и густи интернодии и со помали листови во споредба со традиционално одгледуваните бугарски сорти, поради што нивното одржување е поврзано со високи трошоци на трудот. Нивниот вегетационен период е многу пократок. Листовите за брзо време зреат, што претставува проблем за навремената берба.

Проучувањето е детално и покажа дека грчките сорти се со послаба отпорност на поважните болести што се појавуваат во овој реон. Ова, како и горенаведените сознанија ги прават овие сорти неадекватни за неврокопскиот реон. Сортите Катерини 53 и Катерини М имаат добра вегетативна градба и униформност и можат да бидат вклучени во селекционите програми за создавање на нови сорти со добри пушачки и квалитетни својства какви што бараат компаниите.

**Клучни зборови:** ориенталски сорти, вариетени групи, агроеколошки услови, внесување, регионални болести.

## INTRODUCTION

Tobacco is the taste - flavoring product and as a commodity is sold mainly on the basis of the established specific dietary qualities. Under the influence of climate , topography and soils in limited geographic environments are formed territorial units in which the varieties constructed material with certain properties. The formation of the typical variety chemical, technological and tasting indicators need specific growing environment. Unlike other crops, tobacco varieties exhibit extreme precision to conditions to express their genetic potential. Each artificial, balanced plant population and especially tobacco varieties maintain their equilibrium only under specific growing conditions (Masheva, V., 2011)

Distribution of world tobacco markets and changing economic situation in the country necessary to obtain material from different varieties and regions allowing the formation of a specific batch typicality and character. Due to ever changing market conditions globally is the tendency for growing atypical for ecotype Nevrokop tobacco varieties - bulgarian and alien. In number of mass growers in the region are varieties of Bulgarian varietal group - Basma - ecotype Kroumovgrad and Greek varieties of Katerini variety group Samsun. These tobaccos are grown in Greece in Katerini area. The main commercial properties that have made them famous tobacco are the great nobility, a very pleasant aroma, mild flavor and excellent burn well as its quality is maintained during long term storage.

The aim of the study was to investigate the morphological characteristics and biological properties of Basma and Samsun groups varieties under atypical theirs conditions to Nevrokop area.

## MATERIAL AND METHOD

We studied Katerini 53 and Katerini M varieties nake stalk form - from the famous Turkish varietal group Samsun distributed southwest of Thessaloniki in Katerini and Kroumovgrad 90 variety from ecotype Kroumovgrad, Basma groups. Nevrokop 1146 variety was used as a control.

The study was conducted during the period 2010 - 2012 in the experimental field of ETS (Experimental Tobacco Station) G. Delchev .

Experience is set in triplicate with the experimental plot size 20 sq. Planting distances of 45 cm between rows and 15 cm within rows. Data were recorded on 10 plants of each repetition . The technology of cultivation and agrotechnical measures comply with the requirements of oriental tobacco.

Biometric measurements include:

- plant height
- number of leaves
- sizes 7$^{th}$ , 14$^{th}$ , 21$^{st}$ , 28$^{th}$ leaf
- length of the growing period in days - from transplanting to full bloom
- disease resistance in natural and artificial infection background

## RESULTS AND DISCUSSION

Katerini is located in the central part of Greece, in the area of Pieria Piraeus plane. It is situated between Mount Olympus and the Thessalonian Gulf. On the east it borders the beach The average monthly data based on observations of several decades are presented in Table 1 . (www.sec.bg / userfiles / life)

**Table 1.Katerini region meteorological characteristics**

| indices | Performance period | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | V | | VI | | VII | | VIII | | IX | |
| Temperature | min | max | min | max | min | max | min | max | min | max |
| ($C^0$) | 12,1 | 24,5 | 16,3 | 29,2 | 18,6 | 31,5 | 18,3 | 31,1 | 14,9 | 27,2 |
| Rainfall mm | 44,4 | | 29,6 | | 23,9 | | 20,4 | | 27,4 | |
| Relative humidity (%) | 64,3 | | 56,3 | | 53,6 | | 55,4 | | 62,5 | |
| Rainy days (number) | 10,7 | | 7,5 | | 5,9 | | 4,7 | | 5,9 | |

The values of these indicators highlight the fact that the area of Katerini features all the characteristics of a Mediterranean climate typical of the fluctuations from year to year are small (Kapouzos, DK et al., 2010) .

Nevrokop area is located in Gotse Delchev Hollow in widespread around periphery of hills and low and high places (Timov, A at all. 1974). In terms of climate, the region belongs to continental - Mediterranean climate region with a warm climate without manifest large variations in individual annual periods. Characterized by the following: (Table 2).

**Table 2. Nevrokop area meteorological characteristics**

| **indices** | **Performance period** | | | | |
|---|---|---|---|---|---|
| | **V** | **VI** | **VII** | **VIII** | **IX** |
| Temperature ($C^0$) | 15,7 | 19,3 | 21,2 | 20,5 | 17,1 |
| Rainfall mm | 67 | 66 | 63 | 62 | 67 |
| relative humidity (%) | 62 | 52 | 44 | 35 | 28 |

Comparing the data for the two regions is noteworthy that there is little difference in temperature between 3-4 $C^0$ , which for this period of development of the tobacco plant (V- IX month) is not essential. Temperature sum is optimal for the production of oriental tobacco. As regards the other two parameters - relative humidity (%) and the amount of Rainfall (mm), the difference is about 10 % lower for the atmospheric humidity and Nevrokop area by 20 mm lower Rainfall which in turn has a significant interest on transferred to the new varieties and depressive environment for them .

It is known that plant growth in new areas is first changed physiology - biochemical processes that affect major tobacco signs. Valuable are those varieties that have large adaptation abilities allowing the formation of stable yields. (Dimanov, D. at all. 2012).

Upon removal of the Polish experiment is noteworthy that the Greek varieties with good morphological and vegetative uniformity enabling correctness results Biometric data show that both form the Greek variety plant height of 87-89 cm, and were significantly lower compared to the control Nevrokop 1146 and variety Kroumovgrad 90. This symptom differences were demonstrated in the highest degree of probability (Table 3). Such values for attributes obtained in other studies with varieties of the same group (Shabanov, D., V. Pophristev and N. Tomov, 1969) .

The leaves of the tobacco plant are the main site for industrial use and consumption. Their number in different varieties and different is directly related to the production of tobacco crop. The number of leaves is one indicator, relatively less than the height of the plant is susceptible to modifying effect of external conditions on consolidation varieties. Throughout the test period both Greek variety and variety Kroumovgrad 90 formed fewer leaves (Table 3).

They are 28 to 30, an average of 29 pieces, with 32 pieces for Nevrokop 1146. Found differences with the control options have proven that confirms our previous observations regarding this feature. (Dimanov, D., D. Vitanova, 2011). Internodiyas are short 3.1 to 3.2 cm, so that the leaves are more closely spaced than two Bulgarian variety. Upon comparison of the parameter of the dynamic growth shows that controls Nevrokop 1146 and Kroumovgrad 90 have a pronounced growth after transplanting. Katerini varieties have slower growth, but faster leaf formation.

Size of the tobacco leaves are important morphological trait. On the one hand, they are an indicator of the type characterized tobacco, and the other appeared element in determining the yield and quality of oriental tobacco. Shaped leaves of Katerini 53 and Katerini M are heart-shaped, with a short handle naked. (Fig. 1) lamina is slightly wrinkled and the color is dark green.

**Table 3. Plants height and number of leaves**

| varieties | indices | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | height | | | | number of leaves | | | |
| | 2010 г. | 2011 г. | 2012 г | average | 2010 г. | 2011 г. | 2012 г | average |
| Katerini 53 | 89,33 | 85,70 | 88,00 | 89,33$^{c}$--- | 30,00 | 30,00 | 30,00 | 29,00$^{b-}$ |
| Katerini M | 89,33 | 85,70 | 88,00 | 87,33$^{c}$--- | 31,00 | 28,00 | 29,00 | 29,33$^{a-}$ |
| Kroumovgrad 90 | 121,33 | 117,00 | 121,00 | 121,33$^{np}$ | 32,00 | 28,00 | 28,00 | 29,33$^{a-}$ |
| Nevrokop 1146 | 133,30 | 128,00 | 132,00 | 133,33 | 33,00 | 32,00 | 32,00 | 32,33 |

Gd 5%(a)-2,080;

Gd 1%(b)-3,145;

Gd 0,1%(c)-5,064

np Gd 5%(a)-15,952;

Gd 1%(b)-24,126;

Gd 0,1%(c)-38,848

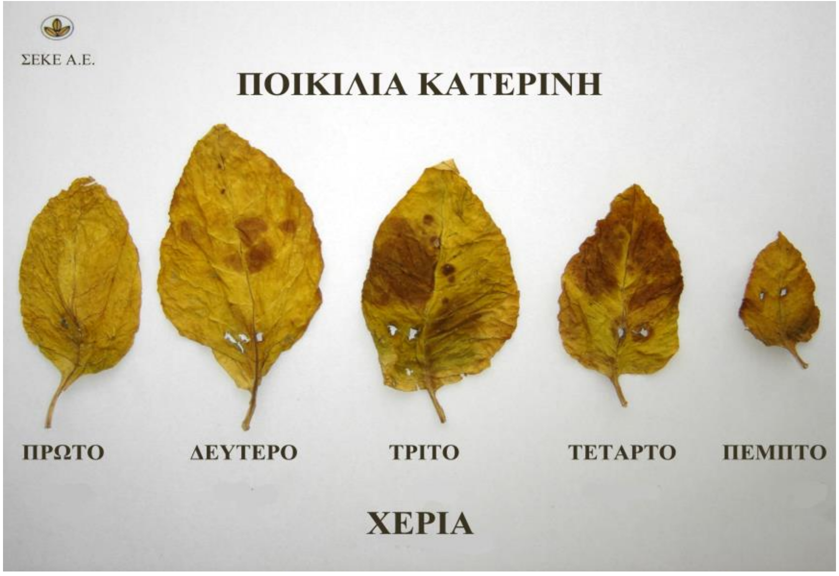


Fig. 1

Compared with the attribute number of leaves, the variation in the dimensions of the leaf is more pronounced and is influenced by the growing environment. Data attributes leaf size - length and width of the $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ leaf (Table 4,5,6,7) show that the three varieties studied form smaller leaves. This trend continued for all harvesting zones. Of two components - the length is highly variable characteristics.

**Table 4. Leaves sizes $7^{th}$ leaf**

| Varieties | Indices | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Length $7^{th}$ leaf | | | | Width $7^{th}$ leaf | | | |
| | 2010 г. | 2011 г. | 2012 г | average | 2010 г. | 2011 г. | 2012 г | average |
| Katerini 53 | 21,00 | 16,60 | 16,80 | 18,13⁻⁻ | 12,90 | 10,90 | 11,60 | 11,80⁻⁻⁻ |
| Katerini M | 19,70 | 15,80 | 16,00 | 17,17⁻⁻ | 12,10 | 11,20 | 11,20 | 11,50⁻⁻⁻ |
| Kroumovgrad 90 | 23,10 | 18,20 | 23,70 | $21,67^{np}$ | 13,80 | 11,80 | 14,40 | 13,33⁻⁻ |
| Nevrokop 1146 | 25,40 | 22,60 | 25,70 | 24,57 | 16,70 | 14,10 | 16,80 | 15,87 |

Gd 5%(a)-1,440;

Gd 1%(b)-2,178;

Gd 0,1%(c)-3,507

np Gd 5%(a)-3,038;

Gd 1%(b)-4,596;

Gd 0,1%(c)-7,400

**Table 5. Leaves sizes $14^{th}$ leaf**

| Varieties | Indices | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Length of $14^{th}$ leaf** | | | | **Width of $14^{th}$ leaf** | | | |
| | 2010 г. | 2011 г. | 2012 г | average | 2010 г. | 2011 г. | 2012 г | average |
| Katerini 53 | 18,20 | 15,20 | 14,30 | $15,90^{-}$ | 10,10 | 10,10 | 9,30 | $9,83^{np}$ |
| Katerini M | 16,10 | 16,70 | 15,10 | $15,97^{-}$ | 9,40 | 8,30 | 9,20 | $9,70^{np}$ |
| Kroumovgrad 90 | 18,70 | 14,10 | 18,20 | $17,00^{-}$ | 11,60 | 10,50 | 11,30 | $10,40^{np}$ |
| Nevrokop 1146 | 28,40 | 17,40 | 22,70 | 22,83 | 19,50 | 8,80 | 14,70 | 14,77 |

np Gd 5%(a)-5,266;

Gd 5%(a)-5,111;

Gd 1%(b)-7,965;

Gd 1%(b)-7,730;

d 0,1%(c)-12,823

Gd 0,1%(c)-12,447

**Table 6. Leaves Sizes $21^{st}$ leaf**

| Varieties | Indices | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Length of $21^{st}$ leaf** | | | | **Width of $21^{st}$ leaf** | | | |
| | 2010 г. | 2011 г. | 2012 г | average | 2010 г. | 2011 г. | 2012 г | average |
| Katerini 53 | 13,7 | 10,80 | 10,50 | $11,66^{-}$ | 7,80 | 6,60 | 6,40 | $6,93^{np}$ |
| Katerini M | 12,10 | 10,50 | 10,20 | $10,93^{--}$ | 6,80 | 6,79 | 6,78 | $6,79^{np}$ |
| Kroumovgrad 90 | 13,70 | 9,70 | 13,90 | $12,17^{-}$ | 7,30 | 5,40 | 8,20 | $6,97^{np}$ |
| Nevrokop 1146 | 17,60 | 12,00 | 18,40 | 16,00 | 10,30 | 6,20 | 11,70 | 9,40 |

np Gd 5%(a)-3,348;

Gd 5%(a)-2,777;

Gd 1%(b)-5,063;

Gd 1%(b)-4,199;

Gd 0,1%(c)-8,153

Gd 0,1%(c)-6,762

The results show proved negative differences at different levels of significance for signs in both Greek variety and not proven for a variety Kroumovgrad 90 seventh and $28^{th}$ leaf.

As mentioned, the width of the leaf is more constant characteristic. In it there is less variation in the values characteristic of the variety. Proven negative differences at different levels of significance were obtained for the width of the $7^{th}$ and $28^{th}$ leaf and unproven in the $14^{th}$ and $21^{st}$ leaf for all test options.

**Table 7. Leaves sizes 28th leaf**

| Varieties | Indices | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Length of 28th leaf** | | | | **Width of 28th leaf** | | | |
| | 2010 г. | 2011 г. | 2012 г | average | 2010 г. | 2011 г. | 2012 г | average |
| Katerini 53 | 9,40 | 8,00 | 7,80 | $8,40^{--}$ | 4,60 | 3,50 | 3,60 | $3,90^{---}$ |
| Katerini M | 8,40 | 7,60 | 7,60 | $7,87^{--}$ | 3,70 | 3,20 | 3,20 | $3,37^{---}$ |
| Kroumovgrad 90 | 11,40 | 7,70 | 10,80 | $9,67^{np}$ | 5,70 | 4,40 | 5,40 | $5,17^{--}$ |
| Nevrokop 1146 | 13,70 | 9,20 | 12,80 | 11,90 | 7,70 | 5,80 | 6,80 | 6,77 |

Gd 5%(a)-2,215; Gd 1%(b)-3,350; Gd 0,1%(c)-5,393 np Gd 5%(a)-0,704; Gd 1%(b)-1,065; Gd 0,1%(c)-1,714

Length of the growing season (planting - full flowering) is a varietal mark . Its duration has significance on the course of all phenophases tobacco plant. This is especially true for oriental tobacco in which the length of the growing season, although subject to the biology of individual species showed a clear dependence on the conditions of the external environment.

The period can be divided into two phases. During the vegetative phase of the tobacco plant are formed the number, size and thicknessof the foliar i.e. signs with a direct effect on the yield and create conditions largely on the quality of the tobacco. By entering the plant at reproductive stage, the process of removal of the plastic materials from the leaves to reproductive organs. For these reasons, a growing season in tobacco varieties to be lasting allowing for the optimal flow of the two phases (Masheva, V. 2007) Katerini varieties have a short growing season, 57 to 58 days (Table 8). Compared with the two Bulgarian varieties grown in the area observed by 5 days Nevrokop 1146 and 10 days with a variety Kroumovgrad 90. The short growing season requires quick handling of the first phase. Leaves with Greek varieties ripen quickly, which creates a problem for the timely collection.

**Table 8. Length of the growing period**

| Varieties | Indices | | | |
|---|---|---|---|---|
| | length of the growing period | | | |
| | 2010 г. | 2011 г. | 2012 г | average |
| Katerini 53 | 56 | 58 | 58 | $57,33^{---}$ |
| Katerini M | 58 | 60 | 58 | $58,67^{---}$ |
| Kroumovgrad 90 | 69 | 70 | 68 | $69^{+++}$ |
| Nevrokop 1146 | 62 | 62 | 62 | 62,67 |

np Gd 5%(a)-1,374 Gd 1%(b)-2,078; Gd 0,1%(c)-3,016

During the vegetation made observations on resistance to some important diseases of tobacco – rot, TMB, mildew and root rotting of natural and artificial infection background. An area Nevrokop are of particular importance rot and TMB. Sustainability was established by inoculation by the method of mechanical inoculation (Kutova I., 1982; Ternovski M., 1956) for TMB and rot and natural infective background of mildew.

In the seedling stage varieties Katerini M and Katerini 53 are highly susceptible to root roting The results of the readings are as follows: to rot Kroumovgrad 90 - 100% sustainability, Nevrokop 1146 to 90 % sustainability and 10% moderately susceptible, Katerini 53 and Katerini M - 0% resistance. For TMB - Kroumovgrad 90 - 60% of sustainability, Nevrokop 1146 to 80 % sustainability and 20% moderately sensitive and 0% resistance to both Greek variety. Observations of mildew made of natural infective background. 2010 all tested variants are susceptible to mildew, the other two years of testing mildew was observed.

## CONCLUSIONS

Variety Katerini 53 and Katerini M differ materially from cultivated tobacco varieties in the region. They are relatively low, with short and dense internodyas, smaller leaves Bulgarian varieties traditionally grown, so keeping them is associated with high labor costs.

The vegetation period is much shorter. Leaves are ripen quickly, which creates a problem for timely collection.

The study shows that Greek varieties inferior in resistance index of important disease area. This, and the above findings, making them inappropriate in our growing in region Nevrokop.

Variety Katerini 53 and Katerini M have good vegetative morphology and uniformity and can be included in breeding programs for new varieties with good smoking and qualities demanded by the firms.

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Original Scientific paper

# CREATING COMPARATIVE ADVANTAGE THROUGH MARKETING RESEARCH COMPANIES

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## ABSTRACT

Marketing researching is a systematic and objective approach to development and information supply, making decision in the process of marketing management. This process includes the system, objectivity, awareness and rationality in making decisions. In fact, the marketing researching includes systematic planning, analysis, data and findings reporting for the specific marketing situation facing a specific company.The marketing managers are responsible to make decisions on the base of the marketing researching information, whereby the managers and marketing researchers is necessary to be creative, good planners, implementers and auditors. In marketing researching that significantly affects competitive advantage of the companies especially should be observe the phases of the process starting with problem definition and objectives, researching project, data sources, forms and method for data collection, method of sample, processing and data analysis and research report. Such separation in of the phases in the researching process allowstonoticethe distinction between the problem and opportunity.

According the importance of marketing research there is no dilemma about its application. The time and resources spent on marketing research is a good investment which contributes for right decisions. The consequences of making inappropriate decisions based on intuition are larger and long-term than the costs made during the researching process. So entrepreneurs have to pay for the mistakes they have made, also for the marketing research for the market which can help to avoid such mistakes.

**Keywords:** marketing research, competition, competitive strategy, market follower, market orientation.

## СОЗДАВАЊЕ НА КОНКУРЕНТСКА ПРЕДНОСТ ВО КОМПАНИИТЕ ПРЕКУ МАРКЕТИНГ ИСТРАЖУВАЊЕ

Маркетинг истражувањето е систематски и објективен приод на развојот и обезбедување наинформации за донесување одлука во процесот на маркетинг менаџментот. Во овој процес вклучена е систематичноста, објективноста,информираноста и рационалноста во донесувањето на одлуките. Всушност, маркетинг истражувањето опфаќа систематско планирање, собирање, анализирање и известување за податоците и наодите значајни за специфична маркетиншка ситуација сокоја се соочува една компанија.Маркетинг менаџерите имаат одговорност да донесуваат одлуки врз база на информациите од маркетингистражувачите, пришто и менаџерите и истражувачите неопходно е да бидат креативни, добри планери ,имплементатори и контролори. При маркетинги стражувањето кое значително влијае на конкурентската предност на компаниите посебно треба да се почитуваат фазите на процесот кои започнуваат со дефинирање на проблемот и целите, истражувачки проект, податоци и извори, методи и форми за собирање податоци, метод на примерок, обработка и анализа на податоците и извештај од истражувањето.Со ваквото разграничување на фазите при истражувањето се овозможува да се направи разлика меѓу она што значи проблем и она што значи можност.

Имајќи го во предвид значењето на маркетингистражувањето, дилемата околу неговата примена не постои. Времето и средствата што се трошат за маркетингистражување се корисна инвестиција која придонесува за правилни одлуки. Последиците од донесувањето на погрешни одлуки за сновани на интуиција, се многу поголеми и подолгорочни од трошоците што би се направиле со истражувањето. Значи, претприемачите мораат да плаќаат-било за направените грешки,било за маркетиншко истражување на пазарот кое може да помогне тие грешки да се избегнат.

**Клучни зборови:** маркетинг истражување ,конкуренција, конкурентска стратегија, пазарен следбеник, пазарна ориентација

## INTRODUCTION

The competitive advantage is an important element in the formulation of the business and development policy and strategy of the companies. To plan an effective marketing strategy, a company must find out what is necessary for its competitors. The company must constantly compare its products, prices, distribution channels and promotions with those of the competitors. Thus the company can find branches with a potentialcompetitive advantage or disadvantage. Also the company can launch more effective marketing campaign against its competitors and prepare more effective defense of the activities that can be taken by the competitors.

Hence, first the competitors should be determinate, as well as their goals and strategy, advantages and disadvantages and make a variety of competitors to attack or avoid. At any time the marketing – managers of the company should have a full access to reliable information that might effect to their decisions on any of their competitors. To accomplish this requires a proper marketing strategy that depends of the position of the company, its objectives, opportunities and resources.

## IDENTIFICATION OF THE COMPANY

The competition is one of the most important elements of the market economy without which the market mechanism cannot function. It should provide an effective supply of the market with quality products and affordable prices, and other conditions associated with the purchase. The firm and the intensity of competition on the market largely depend on the exercise price of the company.

Many companies identify the competitors of industrial perspective (industry is a group of firms offering products and services that are close substitute to each other). But some companies identify the competitors from a market perspective rather than industrial. Here are considered the competitors that persist to supply a client or same group of clients. Generally, the market research to competition provides companies an extended aspect of real and potential competitors that leads to better long-term planning of the market.

The methodological approach to the study of the competition includes the following activities:

1. Identifying of the competition and establishing a database:
2. Analysis the basic parameters of the business and development policy of competitors at domestic and foreign market.

Within the first stage it is necessary to:

- Identify and analysis the competitors' offering system:
- Identify and analysis the competitors' post-sale services:
- Identify the strength and weakness of competitor:
- Analysis the usage of the instruments of marketing mix in supplying formation on different markets:
- Identifying the criteria on the base on which the costumers evaluate binding of different competitors.

❖ In the second stage the competitors are classified according a certain criteria especially on the effect of business policy of the competitive firm. It is made to determinate the basic parameters and relations toward the competition firms and develop an application on particular perspective for maintaining the required level of competitive ability of their own company.

❖ The identification of competition means creating an information base to all companies producing products that supply the same need (same or substitutes). Database should contains information regarding the number of competitors, location, market area activity, development strategy, technical and technological capability, marketing and management facility, sales potential, market implementation, range of product dimensions, quality of the product, additional activity, location of sales-network marketing, export, total export share production, main suppliers, development of public relations, cooperation with scientific institutions, etc.

❖ Important for the company are data products of competition, their characteristics, delivery terms, delivery method, etc. Also sales channels, price, discounts, the communication technique with costumers etc. should be analyzed.

❖ When the main competitors are identified marketing – management team have to find an answer to the requirement of each competition on the market and what arose its behavior?

❖ Initially may be assumed all competitors tend to maximize the profit and arrange the activities. But the companies differ according the emphasis put on a short term rather than long-term profits, some competitors are oriented towards satisfying rather than maximizing profits. They have profitable objectives that satisfy even though the strategies can increase the profit.

❖ Marketing – managers must look beyond the competitor's profit objective, because each competitor has mixed objectives, each objective is with a different importance. The company wants to know the relative importance that competitors put on the current profit of increment of market share that takes the cash flows, technological leadership, service delivery leadership and other objectives.

Through knowledge or identifying the objectives of the competitor, the company finds out whether it is satisfied with the current situation and what their further reactions will be. For example, a company that tends for a leadership that is not expensive will react very strongly to the breakthrough of competitive production that has decreased the expenses than the increased advertising of the same competitor.If the company determines that competition has discovered a new segment which can be consider as competition's opportunity. If the company discovers the competition plans new strokes on the current segments then the company will be prepared and disposed to react.

## ASSESSMENT OF THE COMPETITIVE REACTION SCHEMES

- The competitor's objectives, strategies, advantages and disadvantages explain the possible actions and reactions as reducing prices, increasing of promotion or presentation of a new product. Every management has its own working philosophy, specific internal culture and leading investments. Therefore, if marketing managers want to understand how the competitor will react, they need deeperunderstanding of competitor's mentality.Thus they receive indications how to react and defend their current position.
- When the target clients are determinate, the distribution channels and marketing mix strategy, the company has already determinate the main competitors, as well as the strategic group to which it belongs.
- Most companies prefer to direct their attacks against weaker competitors that it takes less time and resources. But the activities should be direct to the competitors whereby they sharpening the skills.
- Analysis of the costumer value is useful tool to determine competitor's strengths and weaknesses, establishing which benefits they value and how they appreciate the company compared to the competition. This analysis shows the vulnerable parts of company in terms of competing activities.
- In this context, it is important to keep in mind that a company really needs the competitors and their benefits. The existence of competitive outcomes results with the following benefits:
    - Helps to increase overall demand;
    - Provides larger differentiation of the products and services;
    - Stimulates the introduction and application of new technologies;
    - Stimulates faster and more dynamic development;
- However, despite the mentioned strategic objectives, it is important to keep in mind that there are two types of competition:
    1. A competition that acts according the rules at a certain branch, favors reasonable prices in costs relation, motivates other companies to decrease the costs or enhance the difference and accept a reasonable level of market share and profits:
    2. A competition that does not respect the rules, undertakes risks, invests in excessive capacity that lead to destabilization.
- If we consider the previous comprehension of creating competitive advantage of a company, we realize for leader in certain activityis necessary to act on 4 different segments:
    - the company must find a way to increase entire manufacture;
    - try to increase its market share even though the market is not changed;
    - reduce the costs;
    - protect its market share through good offensive and defensive tactics;

Competitive strategy

| | |
|---|---|
| New market demand<br>New users<br>Larger utilization | Gaining market share<br>Gaining new costumers<br>Gaining a loyalty |
| Improvement of productivity<br>Decreasing the costs<br>Improving the production mix<br>Improving the value | Defending the position<br>Static defense<br>Proactive defense<br>Reactive defense |

- The market leader often bares the enormous costs associated with development of new products and new markets, the cost of distributive channels and market information and education. Such work ensue a reward but also a risk that is result of the market leadership. On the other hand the market follower can use the experience of the market leader to improve the products, services and marketing program with lower investment. Although the follower cannot reach the leader he can also make a profit.
- Considering the fact that the market follower in significantly copies the leader realizes that the follower do not have to develop own marketing strategy. But in practice each market follower must have own strategy on which will strive to take characteristic advantages in its entire market (location, services, financing). This means that the following is not the same as the passive behavior because it must define its increasing path, have quality products and high level services and lower market costs.
- Firms "market followers" belong to one of the following type:
  1. **Cloners** – entirely copy the products, distribution, advertisement and other marketing movements of market leader. Because of this they cannot create anything and try to maintain on the leader investments.
  2. **Imitators** – copy some things of the leader, but keep certain differences in a manner of realization, packing, prices etc. Usually the leader does not mind the imitator's acting in avoiding the monopoly costs until it does not attack seriously.
  3. **Adjusters** – the leader's products are rewarded also the marketing programs are improved at the same time. Often the adjuster appears on different markets to avoid conflict with the market leader.
- To create competitive advantages through marketing research it is necessary to consider the strategy of the firms that appear as participants in a specialized part of the market. Their objectiveis not the entire market but market's separate segments or sub-segments. This particularly applies to small frills with limited resources and with a small share can highly be profitable through reasonable sub-segmentation.
- The main reason for the profitability of these firms is that the participant on the specialized market investigates the target group of costumers and contends their needs and may impose a significant amount of the costs on a certain value.

At the same time the idea of the participants in specialized market is the specialization within the market, the object, the product or the market mix. Thus the following specialist services are created on the market:

- End user specialist products;
- Specialist of vertical levels;
- Costumer range specialist;
- Specialist for specific costumers;
- Geographic specialist;
- Ratio specialists ( quality – price)

- When a company is a market leader the challenger or the follower company must find competitive marketing strategy that will positioned most effectively despite its competitors. The company must constantly adapt its strategy to the rapidly changing competitive environment. The positive is the company constantly searches for weaknesses in its position and reveals the weaknesses of the competitors, but the negative is insteadthe company focus to the clients its main occupation is directed to the competitors. If we look back in the history the companies have passed through four orientations or levels. First they were oriented to the products, without giving attention to the competitors and clients, second they have been oriented to the clients and even on the third level they have oriented to the competitors. Today the companies need to be market- oriented and equally dedicated influence and balance between the clients and competitors.

To prepare an effective market strategy a company must scan the competitors as well as the permanent and potential clients. The company must analyze the competitors and develop competitive market strategies which will make it effective in relation to its competitors, also will have the strongest possible competitive advantage.

## CONCLUSION

- At any time the marketing – managers of the company should have a full access to reliable information that might effect to their decisions on any of their competitors. The main alternative competitor positions are management costs, differentiation and focus. Many companies fail because they did not follow the above mentioned competitive positions so they remain stuck in the middle.
- Which of the competitive market strategy is the most significant depends on the company's position in a given industry and of its objectives, possibilities and resources. The company's competitive market strategy depends on whether the company is a market leader, market challenger, market follower or a participant in the specialized market. The market leader wants to find a way how to expand the market, the leader also looks for new costumers, new and grater usage.

The market challenger is a company that stridently tries to increase its market share and attacks the market leader, other smaller and associated companies in the industry.

- The challenger as an attack can choose one of the different strategies including the frontal attack, sideways attack, the rounding attack, the surrounding attack and the guerrilla attack.
- The participant in the specialized market is a small firm serving a market share that is unlikely to attract larger firms. The participants in the specialized market often become specialists for a certain products for definitive usage, for vertical levels, the greatness of the client, the geographic area or some other remarkable characteristic of the product or service.
- The competitive orientation is important for the market today, but the companies should not exceed the focusing on the competition. The companies that balance the importance between costumer and competitor practice the real market orientation.

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Original Scientific paper

# RATIO BETWEEN GREEN AND DRY MASS IN SOME VARIETIES OF YAKA AND BASMAK TOBACCO

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## ABSTRACT

Water content in tobacco plant is variable and is closely related to tobacco variety, stage of development, plant organs (root, stem and leaf), climate conditions during the growing season and other factors. In 2009, 2010 and 2011 investigations were carried out on green : dry mass ratio in four tobacco varieties - one of the type Yaka (YK 7-4/2), used as a check, and three of the type Basmak (MK-1 MB-2 and MB-3).

Varieties MK - 1 and MB- 3 participate with smaller amounts of green mass to yield 1 kg dry mass (MK - 1 with 4.983 kg and MB- 3 with 5.262 kg). Somewhat higher share of green mass to obtain 1 kg dry mass was recorded with the check variety YK 7-4/2 (5365 kg) and with MB-2 (5575 kg).

The aim of our investigations was to present qualitative characteristics of investigated varieties, since it is considered that varieties which contain lower amounts of water have a better quality.

**Keywords:** oriental tobacco, green and dray mass, varieties, Yaka, Basmak

## СООДНОС НА ЗЕЛЕНА СПРЕМА СУВА МАСА ТУТУН КАЈ НЕКОИ СОРТИ ОД ТИПОТ ЈАКА И БАСМАК

Содржината на вода во тутунското растение е различна и е во тесна врска со сортата тутун, фазата на развој, органите на растението (коренот, листот и стеблото), од климатските услови кои владеат за време на вегетацијата и други фактори. Во текот на 2009, 2010 и 2011 година беа извршени истражувања на соодносот на зелена спрема сува маса тутун кај четири сорти тутун и тоа : ЈК 7-4/2 како контрола која е од типот јака и три сорти од типот басмак МК-1, МБ-2 и МБ-3.

Сортите МК-1 и МБ-3 учествуваат со помала количина на зелена маса за да се добие 1 kg сув тутун, каде МК-1 учествуваше со 4.983 kg зелен : 1 kg сув тутун а МБ-3 со 5.262 kg зелен : 1 kg сув тутун. Додека контролната сорта ЈК 7-4/2 и МБ-2 учествуваа со нешто поголема количина на зелена маса за да се добие 1 kg сув тутун, каде контролната сорта ЈК 7-4/2 учествуваше со 5.365 kg зелен : 1 kg сув тутун и сортата МБ-2 со 5,575 kg зелен : 1 kg сув тутун.

Цел ни беше со овие истражувања да се прикажат и квалитативните карактеристики на испитуваните сорти, затоа што се смета дека сортите што содржат помала количина на вода во себе имаат и подобри квалитативни карактеристики.

**Клучни зборови:** ориенталски тутун, зелена и сува маса, сорти, јака, басмак

## INTRODUCTION

Water is an environment in which all physiological processes in living cell take place and a basic element in creation of organic matter in the process of photosynthesis. In the process of curing, water is separated from tobacco leaf, which causes loss not only of water but also of a part of dry matter. Water content in leaf usually decreases from the lower to the upper insertions and it differs in various types of tobacco. Karajankov (1995) in his three-year investigation concluded that technically mature leaves of Macedonian oriental varieties (P 10-3/2, YK 7-4/2 and Djebel No.1) contain approximately 77.43 % of water. Studying the water content in different organs of the plant, the author reported that 50.08 % of the water in YK 7-4/2 is found in the leaf, 25.15 % in the stalk, 12.87 % in the root and 10.90 % in the inflorescence.

According to Boceski (2003), the loss of dry matter is 16 to 20 % of its initial content. Dimitrov (1964) points out that the increase of nitrogen rates in green tobacco leaves leads to an increased amount of water. In cured tobacco, the higher amount of water indicates low quality of tobacco leaves and vice versa.

According to Uzunoski (1985), the water amount in technically mature leaves is considerably high (75 % - 90 %) and depends on the variety, growing conditions and insertion. The amount of water in cured leaves ranges 6 - 10 % of the total leaf weight.

According to Atanassov (1962), green : dry mass ratio of the oriental tobacco type Ustina ranged 5.5 : 1 to 6.7 : 1 in lower primings, 4.8 : 1 to 5.1 : 1 in middle primings and 3.9 : 1 to 5.0 1 in upper primings. The same author reported that in Burley tobacco this ratio is 6.7 : 1 to 8.3 : 1 and in Virginia 5.5 : 1 to 6.7 : 1.

Green : dry tobacco ratio in the investigated varieties is mainly a varietal characteristics. According to Mitreski (2011), the average ratio in some varieties of the type Prilep in 2009 and 2010 ranged from 6.11 : 1 in variety P 66-9/7 to 6.95 : 1 in P 12-2/1. According то Ristevski (2006), green: dry tobacco ratio in Burley varieties ranged from 6389 g : 1000 g in Croatian variety Chulinec to 6230 g: 1000 g in variety B -96/85. The green : dry tobacco ratio will be presented in our research.

## MATERIAL AND METHODS

Investigations were carried out in 2009, 2010 and 2011 with four tobacco varieties, one of which Yaka YK 7-4/2 as a check (ø) and three Basma varieties: MK-1, MB-2 and MB-3. Seedling from the investigated varieties was produced in traditional way, under polyethylene covered beds in Scientific Tobacco Institute - Prilep. Elite seed material of 5g/10 $m^2$ was used for investigation. During seedling production, all necessary cultural practices and protection measures were applied simultaneously. After preparation of the soil with one autumn and two spring ploughings, investigations were set up with 5 replications designed in randomized blocks, with planting density of 45 × 12 cm. The length of the trial was 5 m and the width of the basic plot was 1,80 m. The main plot area in field was 9 $m^2$ and the useful plot area was 6.16 $m^2$.

Each plot consisted of 5 rows, three of which were used for harvest and two served for protection. The number of plants in the row was 42 and 38 of them were stalks for harvesting. The number of plants in the plot (for 3 rows) was 126 and the number of plants for calculation (3 rows) was 114. Total number of stalks from each variety in the whole trial was

1050. Harvesting and stringing of the leaves were carried out manually, in 7 primings in the stage of technical maturity and curing was performed in the sun, on horizontal frames. Green and dry tobacco of each variety was measured separately during the three years of investigation and their ratio was obtained mathematically (Photos 1 and 2).

The obtained data were processed statistically by the method of analysis of variance (ANOVA) and tested with LSD test .


Photo 1. Green tobacco


Photo 2. Dry tobacco

## RESULTS AND DISCUSSION

Basma tobacco is represented with a very small percentage in varietal structure of R. Macedonia, which is not satisfactory, given the fact that, compared to some other oriental tobaccos (Prilep, Yaka and Djebel), it has a good quality (strong and pleasant aroma).

According to the results of investigations carried out in 2009 (Table 1), the average green : dry tobacco ratio ranges from 5.256 kg : 1 kg in Basmak MB- 3 to 5.805 kg: 1 kg in Basmak MB- 2, the index of which is 3.15 % higher than that of the check variety YK 7-4/2. In YK 7-4/2 this ratio was 5.617 kg green : 1 kg dry tobacco and in MK – 1 variety it ranged 5.359 kg green : 1 kg of dry tobacco. So, the investigated tobacco varieties showed no statistically significant differences in 2010 crop.

**Table 1. Green : dry tobacco ratio in 2009**

| Varieties | Replications | | | | | Average | Index |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | |
| YK 7-4/2 ∅ | 5.233 : 1 | 5.206 : 1 | 6.111 : 1 | 6.214 : 1 | 5.321 : 1 | 5.617: 1 | 100.00 |
| MK-1 | 5.500 : 1 | 4.580 : 1 | 5.428 : 1 | 5.918 : 1 | 5.367 : 1 | 5.359: 1 | 95.41 |
| MB-2 | 5.879 : 1 | 5.470 : 1 | 5.442 : 1 | 6.424 : 1 | 5.808 : 1 | 5.805: 1 | 103.35 |
| MB-3 | 5.229 : 1 | 5.347 : 1 | 5.040 : 1 | 5.675 : 1 | 4.991 : 1 | 5.256: 1 | 93.57 |

2009 crop
LSD 5% 0.460 g $^{+}$ n.s.
1% 0.647 g $^{++}$ n.s.

In 2010, the lowest green: dry tobacco ratio was recorded in variety Basmak MK-1 (4.837 kg : 1 kg) and the highest in the variety MB-2 ( 5.591 kg : 1 kg), which is 5.27% higher than the check (Table 2). In YK 7-4/2 this ratio averaged 5.311 kg : 1 kg and in MB-3 it reached 5.341 kg : 1 kg. Thus, no statistically significant differences were observed among investigated varieties.

**Table 2 Green : dry tobacco ratio in 2010**

| Varieties | Replications | | | | | Average | Index |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | |
| YK 7-4/2 ∅ | 5.128 : 1 | 5.438 : 1 | 5.377 : 1 | 5.885 : 1 | 4.729 : 1 | 5.311: 1 | 100.00 |
| MK-1 | 5.046 : 1 | 5.217 : 1 | 4.316 : 1 | 4.380 : 1 | 5.227 : 1 | 4.837: 1 | 91.08 |
| MB-2 | 5.364 : 1 | 4.937 : 1 | 6.185 : 1 | 5.602 : 1 | 5.864 : 1 | 5.591: 1 | 105.27 |
| MB-3 | 6.024 : 1 | 5.730 : 1 | 4.598 : 1 | 5.393 : 1 | 4.962 : 1 | 5.341: 1 | 100.56 |

2010 crop

LSD 5% 0.837 $g^{+}$ n.s.

1% 1.176 $g^{++}$ n.s.

The data collected in 2011 (Table 3) show the lowest green: dry tobacco ratio in variety Basmak MK-1 again (4.753kg : 1 kg), and the highest ratio was observed in variety Basmak MB-2 (5.330kg : for 1 kg), which is 3.15% higher than the check. The Basma varieties showed no statistical differences compared to the check.

**Table 3. Green : dry tobacco ratio in 2011**

| Varieties | Replications | | | | | Average | Index |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | |
| YK 7-4/2 ∅ | 5.290 : 1 | 5.294 : 1 | 5.000 : 1 | 5.000 : 1 | 5250 : 1 | 5.167: 1 | 100.00 |
| MK-1 | 4.720 : 1 | 4.760 : 1 | 5.219 : 1 | 4.446 : 1 | 4.622 : 1 | 4.753: 1 | 91.99 |
| MB-2 | 5.150 : 1 | 4.895 : 1 | 5.709 : 1 | 5.211 : 1 | 5.686 : 1 | 5.330: 1 | 103.15 |
| MB-3 | 5.385 : 1 | 5.352 : 1 | 4.364 : 1 | 4.970 : 1 | 5.896 : 1 | 5.193: 1 | 100.50 |

2011 crop

LSD 5% 0.592 $g^{+}$ n.s.

1 % 0.832 $g^{++}$ n.s.

According to Table 4, the average green : dry tobacco ratio ranged from 4.983 kg : 1 kg in MK-1 to 5.575 kg : 1 kg in MB- 2, with relative difference being lower for 7.12 % in MK - 1 and 1.92 % in MB - 3, compared to the check variety . It can be concluded from the results that MK - 1 and MB- 3 varieties participate with a lower amount of green mass to obtain 1 kg dry tobacco, but there are no statistically significant differences compared to the check. According to the data obtained, the green : dry tobacco ratio is primarily a varietal trait varieties feature, but it also depends on other factors as cultural practices, climate, soil etc.

**Table 4. The ratio between green and dry tobacco (in kg)**

| VARIETY | Year | green : dry tobacco | Average Period 2009 / 2011 | Difference | | Range |
|---|---|---|---|---|---|---|
| | | | | Absolute | Relative | |
| YK 7-4/2 ∅ | 2009 | 5.617 : 1000 | 5.365 :1 | | 100.00 | 2 |
| | 2010 | 5.311 : 1000 | | | | |
| | 2011 | 5.167 : 1000 | | | | |
| MK-1 | 2009 | 5.359 : 1000 | 4.983 :1 | - 382 | 92.88 | 4 |
| | 2010 | 4.837 : 1000 | | | | |
| | 2011 | 4.753 : 1000 | | | | |
| MB-2 | 2009 | 5.805 : 1000 | 5.575 :1 | +210 | 103.91 | 1 |
| | 2010 | 5.591 : 1000 | | | | |
| | 2011 | 5.330 : 1000 | | | | |
| MB-3 | 2009 | 5.256 : 1000 | 5.262 :1 | -103 | 98.08 | 3 |
| | 2010 | 5.341 : 1000 | | | | |
| | 2011 | 5.193 : 1000 | | | | |

The average ratio green : dry tobacco mass in investigated tobacco varieties is presented more vividly in Figure 1 and it ranges from 5.58 : 1 in MB-2 to 5.00 : 1 in variety MK-1.

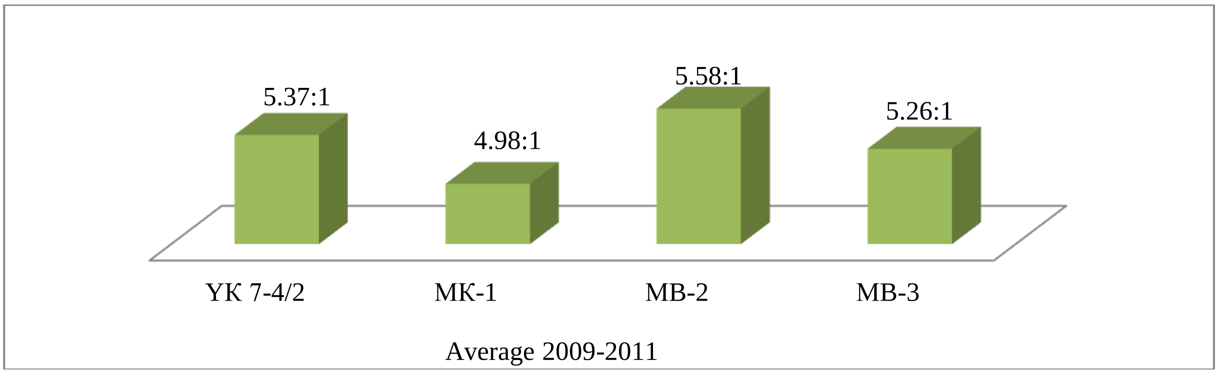


Figure 1. Green : dry tobacco mass in kg
(average values)

## CONCLUSIONS

As a result of the obtained data, the following conclusions can be drawn:

Green : dry tobacco ratio in 2009 ranged from 5.256 kg : 1 kg in variety Basmak MB- 3 to 5.805 kg : 1kg in variety Basmak MB -2.

In 2010, green : dry tobacco ratio was the lowest in variety Basmak MK - 1 (4.837 kg: 1 kg) and the highest in variety MB-2 (5.591 kg : 1 kg).

Similar data were obtained in 2011 crop, when the lowest green : dry tobacco ratio was recorded in variety Basmak MK - 1 ( 4.753 kg: 1 kg) and the highest in variety Basmak MB-2 (5.330 kg : 1 kg).

From the three-year results it can be concluded that varieties MK - 1 and MB - 3 participate with smaller amount of green mass to obtain 1 kg dry tobacco, i.e. the shares of green tobacco from MK - 1 and MB - 3 to obtain 1 kg dry tobacco are 4.983 kg and 5.262 kg, respectively.

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Original Scientific paper

# REGIONALIZATION OF TOBACCO PRODUCTION IN THE REPUBLIC OF MACEDONIA

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## ABSTRACT

The paper presents data on tobacco planted areas,average production and yields in various regions of R. Macedonia in the period 2007 – 2011. The analysis revealed that the biggest areas planted with tobacco are found in the southwest region of R. Macedonia (4604 hectares in average) and the smallest in the northeast and west regions. The highest average production was achieved also in the southwest region – 5426 tons, and the lowest in northeast region – 103 tons. The highest average yield per unit area was achieved in the central region (9505 kg/ha) and the lowest in the northeast region (2585 kg/ha). According to the obtained results, adequate and in-time regionalization is one of the most important conditions for improvement of tobacco production in R. Macedonia.

**Keywords:** regions, areas, production, yield, tobacco

## РЕГИОНАЛИЗАЦИЈА НА ТУТУНОПРОИЗВОДСТВОТО ВО РЕПУБЛИКА МАКЕДОНИЈА

Во содржината на трудот презентирани се податоци за просечите површините засадени со тутун во регионите (реоните) на Р. Македонија, оствареното просечно производство и приносот по единица површина, за периодот 2007-2011 година. Од извршената анализа доаѓаме до сознание дека во југозападниот производен регион на Р. Македонија засадени се најголеми површини со тутун кој во просек изнесуваат 4604 хектари, а најмали површини забележани се во североисточниот и западниот регион. Исто така и по однос на оствареното производтвото предност има југозападниот производен регион, со остварено просечно производство од 5426 тони, а најмало просечно производсво забележано е во североисточниот регион (103 тони). Просечниот принос по единица површина во регионите (реоните) на Р. Македонија за периодот 2007-2011 година најголем е во централноисточниот производен регион 9505 кг/ха и најмал во североисточниот производен регион (2585 кг/ха) . Неоспорен е фактот дека во Р. Македонија потребно е да се изврши правилна и навремена регионализација (реонизација) на производството на тутун бидејќи тоа е еден од условите за негово подобрување и унапредување.

**Клучни зборови:** региони, реони, површина, производство, принос, тутун.

## INTRODUCTION

Republic of Macedonia has favorable environmental conditions for production of high-quality oriental, semi-oriental and (from recently) Basma tobacco varieties. The complex of natural conditions (climate: soil: relief) is, however, very limited, i.e. in some regions the conditions for growing certain tobacco types are optimal and in others they are very poor. Care must be taken to choose those tobacco types that will be the most suitable for given soil type and climate conditions of the region in order to obtain highly valued raw product. Some of the regions offer favorable conditions for combined production of various types and subtypes, which will enable diversification of tobacco production.

It is undisputable that proper distribution of tobacco types and varieties by regions is essential for rational use of available areas suitable for tobacco production, which is one of the conditions for its development and improvement.

According to the decision of the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia, the following five tobacco producing regions have been differentiated: northeast, west, southwest, central east and southeast.

The starting point in defining the aim of our investigation was the data analysis on area planted with tobacco and average yields in certain regions of R. Macedonia for the period 2007-2011.

## MATERIAL AND METHODS

Basic material for realization of the research were the data published in the Statistical yearbook of R. Macedonia and Statistical Review on crop farming, fruit growing and viticulture, 2007 -2011.

Methods used in data processing and conclusion making were typical for this type of investigation, but the most usual one was the comparative-analytical method.

## RESULTS AND DISCUSSION

This paper presents data on the average area planted with tobacco, realized average production and average yield in the regions of R. Macedonia for the period 2007-2011.

Analysis of the above data shows that the average area planted with tobacco in all five regions amounted 1817 hectares. The largest average area of 4604 hectares was recorded in the southwest region, and it was followed by the southeast region with 3300 hectares and centraleast region with 1053. The smallest area planted with tobacco was recorded in the northeast and west producing regions, with only 85 i.e 43 hectares, respectively. According to this, the largest average area under tobacco for the period2007-2011 was planted in the years 2010 (2008 ha) and 2011 (1923 ha) .

**Table 1. Areas planted with tobacco in the regions of R. Macedonia for the period 2007 - 2011**

In hectares

| Regions | Years | | | | | |
|---|---|---|---|---|---|---|
| | 2007 | 2008 | 2009 | 2010 | 2011 | **Average** |
| **Northeast** | 81 | 83 | 80 | 88 | 91 | 85 |
| **West** | 37 | 41 | 46 | 46 | 44 | 43 |
| **Southwest** | 4044 | 4242 | 4602 | 5140 | 4992 | 4604 |
| **Central-east** | 1096 | 1027 | 1024 | 1081 | 1039 | 1053 |
| **Southeast** | 3114 | 3067 | 3179 | 3692 | 3448 | 3300 |
| **Average** | 1674 | 1692 | 1786 | 2008 | 1923 | 1817 |

Data on the total tobacco production and realized yields in the regions of R. Macedonia are presented in Tables 2 and 3.

**Table2. Tobacco production in the regions of R. Macedonia for the period 2007-2011**

In tons

| Regions | Years | | | | | |
|---|---|---|---|---|---|---|
| | 2007 | 2008 | 2009 | 2010 | 2011 | **Average** |
| **Northeast** | 79 | 87 | 88 | 138 | 123 | 103 |
| **West** | 26 | 56 | 82 | 36 | 36 | 47 |
| **Southwest** | 4870 | 4106 | 5607 | 6650 | 5898 | 5426 |
| **Central-east** | 1595 | 1003 | 1636 | 2009 | 1588 | 1566 |
| **Southeast** | 3999 | 2870 | 4367 | 5729 | 5141 | 4421 |
| **Average** | 2114 | 1624 | 2356 | 2912 | 2557 | 2313 |

Data presented in Table 2 show thatthe average tobacco production in R. Macedonia for all five regions was 2313 tons. The highest production was recorded in the southwest (5426 t) and southeast region (4421t), in the central east region it was lower (1566 t), andthe lowest values were recorded in the northeast (103 t) and west region (47 t). The highest average production was recorded in the years 2010 and 2011, amounting 2912 and 2557 tons, respectively.

The average yield achieved in all five regions was 5612 kg/ha. The highest average yield was recorded in the central eastregion-9505kg/ha, followed by the south east region with5629kg/ha and the west region with5238kg/ha. The lowest yield (2585 kg/ha) was obtained in the north east region. The highest average yields for the period 2007-2011were a chievedin 2009, 2010 and 2011 (6258,5963,5936 kg/ha ) (Table 3).

**Table 3. Tobacco yields obtained in the regions of R. Macedonia, 2007-2011 crop**

| Regions | Years | | | | | |
|---|---|---|---|---|---|---|
| | 2007 | 2008 | 2009 | 2010 | 2011 | **Average** |
| **Northeast** | 1944 | 2105 | 2151 | 3972 | 2755 | 2585 |
| **West** | 2780 | 6160 | 7700 | 2850 | 6700 | 5238 |
| **Southwest** | 5157 | 4279 | 5396 | 5583 | 5103 | 5104 |
| **Central-east** | 8983 | 7803 | 10610 | 10587 | 9541 | 9505 |
| **Southeast** | 6151 | 4154 | 5434 | 6823 | 5582 | 5629 |
| **Average** | 5003 | 4900 | 6258 | 5963 | 5936 | 5612 |

## CONCLUSIONS

Based on the datapresented in the paper, the following conclusions can be drawn that are of major interest to the tobacco industry of R. Macedonia:

1.Tobacco producing regions in R. Macedonia could hardly be shifted to other agricultural crops or economic activities, although tobacco industry is subjected to many fluctuations in the production and market and imposes huge risks to the farmers, due to which the regions remain under developed.

2. The Republic of Macedonia has exceptionally favorable agro-ecological conditions for production of the highest quality oriental aromatic types of tobacco, and some of the regions with their specific conditions enable a successful growing of semi-oriental and Basma tobacco varieties.

3. Tobacco production should be adequately developed in the regions of R. Macedonia in order to rationally exploit the available area for tobacco growing.

4. The need for regionalization is undisputable, because it is one of the conditions for improvement and development of tobacco production.

5. The average area planted with tobacco in all five regions in R. Macedonia was 1817 hectares. The largest area under tobacco wasrecorded in the southwest, and the smallest in the southeast and west region.

6. The average production of tobacco in all five regions in R. Macedonia was 2313 ton. The highest production was achieved in the southwest region, and the smallest in the northeast and the west.

7. The average yield per hectare achieved in all five regions was 5612 kg/ha. The highest averageyield (kg/ha) wasrecorded in the central eastregion, and the lowestinthe northeast region.

8. Tobacco industry of the Republic of Macedonia and its further development should be directed toward grouping of oriental tobaccosat one level and, to a lesser extent, introducing of semi-oriental and Basma types of tobacco.

9. Measures which should be taken in realization of tobacco production will result in obtaining a better and cheaper product. For this purpose it is necessary to make a detailed analysis of conditions in the tobacco producing regions,i.e. to predict which tobacco type would be the most appropriate for given region.

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