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Original Scientific paper

INVESTIGATION OF SOME QUANTITATIVE TRAITS IN AUTOCHTHONOUS TOBACCO VARIETIES IN REPABLIC OF MACEDONIA

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ABSTRACT

Investigations were carried out with five autochthonous tobacco varieties of the types Prilep (P 10-3/2 and P 12-2/1), Djebel (Dj **№ 1**) and Yaka (YK 7-4/2 and KY), to study their quantitative traits: height of the stalk with inflorescence, number of leaves and dry mass yield per stalk. The trial was set up in the Experimental field of Tobacco Institute-Prilep in 2011 and 2012, in randomized block design with four replications. Traditional cultural practices were applied for realization of the experiment.

The aim of investigations was to evaluate the variability of the above quantitative traits typical for the autochthonous varieties by the use of biometric analysis and to give directions for their maintenance in future.

The significant differences observed among the traits of investigated varieties indicate that they are different cultivars. No significant differences were observed between the two years of investigation, which is an indication of highly heritable traits. Statistical parameters of variability are low, which is an indication of stable and homozygous genotypes, adapted to agro-ecological conditions of the region. Results on the standard deviation and variability coefficient were lower in 2012, because the seed sawn in this crop was obtained from one stalk for each variant isolated in 2011. The lowest statistical data on variability of stalk height and leaf number in both years were recorded in the varieties of Prilep tobacco, and for dry mass yield in the Yaka variety KY.

Keywords: tobacco (Nicotiana tabacum L.), autochthonous varieties, quantitative traits, standard deviation, variability coefficient

ПРОУЧУВАЊА НА ПОВАЖНИТЕ КВАНТИТАТИВНИ СВОЈСТВА КАЈ АВТОХТОНИ СОРТИ ТУТУН ВО РЕПУБЛИКА МАКЕДОНИЈА

Проучувани беа пет автохтони сорти тутун од типовите прилеп (П 10-3/2 и П 12-2/1), џебел (Џ бр.1) и јака (ЈК 7-4/2 и КЈ), за поважните квантитативни својства: висина на стракот со соцветие, број на листови по страк и принос на сува маса по страк. Опитот беше поставен на опитното поле при Научниот институт за тутун – Прилеп во текот на 2011 и 2012 година, по случаен блок-систем во четири повторувања. За време на вегетацијата беа реализирани вообичаени агротехнички мерки. Целта на истражувањата е преку биометричка анализа на наведените квантитативни својства карактеристични за автохтоните сорти да направиме евалуација на нивната варијабилност, како и да дадеме насоки за нивно понатамошно одржување. Својствата помеѓу варијантите сигнификантно се разликуваат што значи дека се работи за различни сорти. Не постојат сигнификантни разлики кај вредностите меѓу двете години на истражување, а тоа е знак за високо наследни својства. Статистичките параметри на варијабилност се ниски, што значи дека анализиравме стабилни, хомозиготни генотипови, адаптирани во агроеколошките услови на овие простори. Резултатите за стандардната девијација и коефициентот на варијабилност се пониски во 2012 година, бидејќи семето посеано за оваа реколта потекнува од по еден страк за секоја варијанта изолиран во 2011 година. Најниски статистички показатели за варијабилноста за првото и второто својство во двегодишните истражувања покажаа сортите од типот прилеп, а за третото својство сортата КЈ од типот јака.

Клучни зборови: тутун, автохтони сорти, квантитативни својства, стандардна девијација, коефициент на варијабилност.

INTRODUCTION

Autochthonous varieties are old local varieties which are no longer grown because their production has no economic justification. Still, they are the first ring in the chain of newly created superior varieties and present valuable genetic resources that must be preserved. Nowadays, the European Parliament issues laws by which farmers from the EU countries are obliged to grow only the crops from the List of approved varieties. The use of old and rare varieties will be punishable, thus leading to prevention of genetic diversity of the species and to a risk of extinction of the native (indigenous) plant species. In order to prevent this from happening, a number of projects have been activated for preservation of plant resources through establishment of regional network for cooperation and formation of working groups for various cultivated crops, including tobacco.

The aim of this paper is to study the autochthonous tobacco varieties and, through a biometric analysis of quantitative traits (stalk height with inflorescence, leaf number per stalk and dry mass yield per stalk), to make evaluation on their variability, as well as to give directions for their maintenance in the future.

MATERIAL AND METHOD

Investigations included studies of five oriental autochthonous tobacco varieties of the types Prilep (P 10-3/2 and P 12-2/1–Ph. 1), Djebel (Dj № 1) and Yaka (YK 7-4/2 – Ph.2 and KY - Kishinska Yaka). The trial was carried out in 2011 and 2012 in the field of Scientific 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 2011, mean monthly temperature was 19,04^{0}C, number of rainy days was 32 and total precipitation amount was 180 mm. In the same period in 2012, mean monthly temperature was 20,3^{0}C, number of rainy days 26 and total precipitation amount 180 mm.

Subject of the investigations were the quantitative trait: stalk height with inflorescence, leaf number per stalk and dry mass yield per stalk.

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:

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

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 Najčevska (2002).

RESULTS AND DISCUSSION

The two-year biometric investigations of autochthonous tobacco varieties in the Republic of Macedonia for the quantitative traits: stalk height with inflorescence, leaf number per stalk and dry mass yield 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 P 10-3/2 and P 12-2/1, while for leaf number per stalk and dry mass yield per stalk in Dj № 1 and YK 7-4/2. The highest values for the three studied traits showed KY (Table 1).

Table 1. Mean value and variability of the quantitative traits of the autochthonous tobacco varieties

Autochthonous tobacco varieties	Quantitative traits								
	Stalk height with inflorescence			Leaf number per stalk			Dry mass yield per stalk		
	$\bar{x} \pm s\bar{x}$ cm	δ	V (%)	$\bar{x} \pm s\bar{x}$	δ	V (%)	$\bar{x} \pm s\bar{x}$ g	δ	V (%)
2011									
1. P 10-3/2	52 ± 0,44	4,14	7,95	34 ± 0,39	1,76	5,15	11,3 ± 0,16	0,72	6,37
2. P 12-2/1	58 ± 0,35	3,32	5,72	37 ± 0,30	1,35	3,64	12,3 ± 0,17	0,75	6,12
3. Dj № 1	89 ± 0,62	5,92	6,66	30 ± 0,43	1,91	6,38	7,8 ± 0,12	0,54	6,92
4. YK 7-4/2	101 ± 0,67	6,33	6,27	29 ± 0,38	1,71	5,99	8,7 ± 0,12	0,54	6,23
5. KY	119 ± 0,85	8,10	6,81	42 ± 0,42	1,87	4,45	14,8 ± 0,12	0,52	3,53
2012									
1. P 10-3/2	54 ± 0,28	2,71	5,01	36 ± 0,32	1,41	3,93	10,2 ± 0,13	0,59	5,81
2. P 12-2/1	56 ± 0,33	3,09	5,52	38 ± 0,24	1,09	2,86	13,0 ± 0,15	0,66	5,06
3. Dj № 1	87 ± 0,46	4,34	4,98	28 ± 0,36	1,63	5,80	6,7 ± 0,10	0,47	6,93
4. YK 7-4/2	103 ± 0,66	6,23	6,05	31 ± 0,37	1,67	5,40	10,4 ± 0,11	0,51	4,96
5. KY	124 ± 0,53	5,07	4,09	41 ± 0,36	1,61	3,93	15,9 ± 0,12	0,52	3,26

Data presented in the table show that mean values of the investigated traits in 2011 with their statistical errors are approximately the same with those of 2012. Variability parameters are almost identical in both years of investigation. Meteorological reports also reveal that 2011 and 2012 were very similar with regard to their mean monthly temperatures and precipitation amounts from May to September. This points out to precise estimations and good performance of the trial.

Ph. 1. P12-2/1

Ph. 2. Yk 7-4/2

CONCLUSIONS

The autochthonous tobacco varieties Prilep P 10-3/2, P 12-2/1, Djebel Dj № 1, Yaka YK 7-4/2 and KY - Kishinian Yaka bear the following characteristics:

- They are homozygous, due to which their population is very uniform, with high genetic stability.

- They have a low standard deviation and variability of the investigated characters, which indicates high genetic homogeneity.

- They present a sound basis in selection of tobacco for obtaining superior varieties.

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PLANT BREEDING FOR CREATION OF LATE – MATURING ORIENTAL TOBACCO GENOTYPES

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ABSTRACT

Investigations were made with six oriental varieties of tobacco types Prilep, Djebel and Yaka (P-23, P-84, P10-3/2, P-76, Xanthi Djebel 1 and YV 125/3) and fifteen F1 hybrids for the characters flowering time and length of growing season from tobacco transplanting in field to the end of harvest. The field trial was set up in 2010 and 2011 in Tobacco Institute – Prilep in a randomized block design with four replications. All appropriate cultural practices were applied during the growing season. Statistical processing of data was performed by using the analysis of variance (ANOVA)

The aim of this work was to study the mode of inheritance and to detect possible heterotic effects for the above biological characters, which will allow a selection of lines with longer growing season, higher productivity and some other positive characters inherited from the early-maturing parent. They would be promising genotypes intended for arid regions with poorer soils and longer growing season.

The period from tobacco transplanting to 50% flowering in parental varieties ranged from 45 to 95 days in Xanthi Djebel-1 (XDj-1) and in P-76, respectively, while the period from transplanting to the end of leaf harvesting in these two genotypes ranged from 70 to 145 days. There were differences in inheritance of the two stages among hybrids. The most common types of inheritance were partial dominance and dominance. The early-maturing parent dominated in the first period, while the late-maturing parent dominated in the second period and there was also an appearance of heterosis.

Keywords: tobacco, diallel crosses, mode of inheritance, biological stages

СЕЛЕКЦИЈА ЗА КРЕИРАЊЕ НА ПОКАСНОСТАСНИ ГЕНОТИПОВИ ТУТУН

Испитувани беа шест ориенталски сорти од типовите прилеп, џебел и јака (П-23, П-84, П10-3/2, П-76, Ксанти Џебел-1 и ЈВ 125/3) и нивните 15 дијалелни F_1 хибриди за својствата време на цветање и должина на вегетациониот период од расадување на тутунот на нива до крајот на бербата. Опитот беше поставен во 2010 и 2011 година на опитното поле при Научниот институт за тутун - Прилеп по случаен блок - систем во четири повторувања. Во текот на вегетацијата беа применети соодветни агротехнички мерки. Податоците беа статистички обработени со анализа на варијансата (Anova).

Целта на овој труд беше да се проучи начинот на наследување и да се открие можен хетеротичен ефект за наведените биолошки својства, со што ќе се овозможи избор на линии со подолг вегетационен период, повисока продуктивност, но и со повеќе други позитивни особини наследени од раностасниот родител. Тоа би биле перспективни генотипови наменети за аридни подрачја, посиромашни почви и долга вегетација.

Периодот од расадување на тутунот до цветање на 50% од насадот кај родителските сорти се движеше од 45 (Ксанти Џебел-1) до 95 дена (П-76), додека периодот од расадување до завршување со бербата на листовите се движеше од 70 до 145 дена кај истите генотипови. Наследувањето на двете етапи кај крстоските беше различно. Најчест начин на наследување бече парцијално-доминантниот и доминантниот. Во првиот период доминираше пораностасниот родител, додека во вториот период доминираше покасностасниот родител, а имаше и појава на хетерозис.

Клучни зборови: тутун, дијалелни крстоски, начин на наследување, биолошки фази

INTRODUCTION

The activity of breeders is directed toward increasing the yield, quality and resistance, but they also monitor the length of growth period in various genotypes and provide directives for their cultivation in different localities. Tobacco is a crop that can be grown at different altitudes and in areas with different soil and climate conditions, but the variety is the key factor which will give the maximum genetic potential in particular environment.

The aim of this paper comes from the necessity that varietal regionalization in the Republic of Macedonia should be followed by creation of tobacco genotypes with longer growth period, higher yield and better quality, suitable for arid areas, poor soils and longer vegetation.

MATERIAL AND METHOD

To study the mode of inheritance of the time of flowering and length of growth period from planting in field to the end of harvest, one-way diallel crossings were made between six oriental varieties of the types: Prilep (P-23, P-84, P 10-3/2, P 76/86), Djebel (Xanthi Djebel XDj-1) and Yaka (YV 125/3). The choice of parents was made on the basis of previous studies on tobacco varieties produced in R. Macedonia.

Crossings were made in 2009 and 2010, with manual castration and pollination. In 2010 and 2011, a trial was set up on the Experimental field of Tobacco Institute - Prilep with 6 parental genotypes and their 15 F1 hybrids, in randomized block design with four replications. The inter-row spacing was 45 cm, and plant spacing 15 cm. Variants in each replication were planted in four rows, i.e. 16 rows in the whole trial. Cultural practices applied during the growing period were as usual for production of oriental aromatic tobaccos.

General characteristics of parental genotypes

Prilep P-23 is characterized by ellipticalal - conical (fir-tree shaped) habitus, approximately 65 cm in height and with 45-50 sessile leaves (20 cm x 10.5 cm), densely distributed on stalk (Korubin - Aleksoska, 2004-a). Leaves are small, very gentle, with poorly defined nervation and intensive pleasant aroma. The dry mass yield is 2000-2500kg/ha (Ph. 1).

Prilep P-84 is characterized by cylindrical to elongated elliptical habitus, approximately 65 cm in height, with 38-42 sessile leaves (20 cm x 10 cm), evenly distributed on stalk (Korubin - Aleksoska, 2004-a). The leaves are gentle, with marked nervation and pleasant aroma. The dry mass yield is 2500-3200kg/ha (Ph. 2).

Prilep P 10-3/2 is characterized by cup-like habitus, approximately 50 cm high, with 30-36 sessile leaves (21.5 cm x 10 cm), densely distributed (Korubin - Aleksoska, 2004-a). The leaves are gentle, with marked nervation and intensive aroma. The dry mass yield is 1100-1300kg/ha (Ph. 3).

Prilep P 76/86 is characterized by elliptical - conical habitus, approximately 90 cm high, with 60 densely distributed sessile leaves (23 cm x 11.5 cm) and with pleasant aroma (Korubin - Aleksoska, 2004-a). The leaves are with strongly expressed nervation and dark green color. The dry mass yield is 3500-4000 kg / ha (Ph. 4).

Xanthi Djebel XDj - 1 is characterized by elliptical habitus (Korubin - Aleksoska & Aleksoski, 2011). The average stalk height is 65 cm, with approximately 17 sessile leaves (17cm x 8.4 cm), with pleasant aroma, oval shape and slightly curved tip. The dry mass yield is 500-700 kg/ha (Ph. 5).

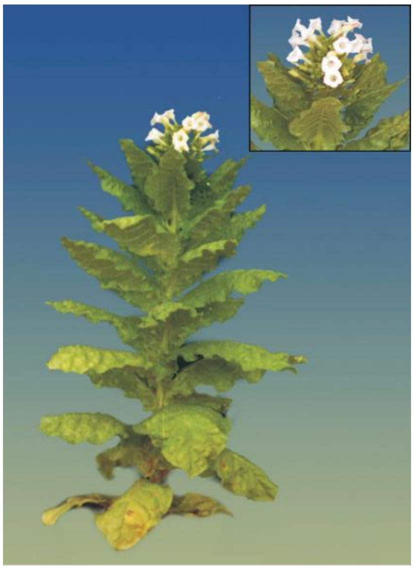
Yaka YV 125/3 is characterized by elliptical to cylindrical habitus . The average stalk height is 120 cm (Korubin - Aleksoska, 2004-b). It has approximately 40 sessile leaves (21.6 cm x 11.2 cm), with very pleasant aroma. The dry mass yield is 1500-1800 kg/ha (Ph. 6).

Processing of results

Data obtained for the traits time of flowering (from transplanting to beginning of flowering and to the stage when half of the plants are in blossom) and length of the growth period from transplanting tobacco in field to the end of harvest are processed statistically by the method of analysis of variance.

Mode of inheritance of investigated biological traits is evaluated on the basis of test - significance of the mean values of F1 progeny compared to parental average (Boroevic, 1981).

Ph. 1. Prilep P23



Ph. 2. Prilep P-84

Ph. 3. Prilep P 10-3/2

Ph. 4. Prilep P 76/86

Ph. 5. Xanthi Djebel XDj-1

Ph. 6. Yaka YV 125/3

Meteorological data

From May to September 2010, during the growth period of tobacco, the mean monthly temperature was 18.9 ^{0} C, mean monthly relative humidity was 56 % and rainfall amount in 35 days was 298 l/m².

In the same period in 2011, the mean monthly temperature was 19.04 ^{0} C, mean monthly relative humidity was 48.6 % and rainfall amount in 32 days was 180 l/m².

The above data on weather parameters were obtained from the Meteorological station located in the Experimental field of Tobacco Institute – Prilep.

RESULTS AND DISCUSSION

Parents and their F1 hybrids were transplanted on June 10 and June 15 in 2010 and 2011, respectively, but their further development was different (Table 1).

XDj - 1 variety is characterized by the shortest growth period. It begins to flower 43 days after planting and 50% of plants in plots blossom in four days. The period from planting to the end of harvest is 70 days. Variety P 76/86 has the longest growth period. Beginning of flowering stage in this variety occurs 90 days after planting and 50% of plants blossom in five days. Number of days from planting to the end of harvest is 145.

Hybrids with the shortest period to the beginning of flowering are: P-23 x XDj-1 (2010-45 days, 2011 - 46 days), P 10-3/2 x XDj-1 (2010 and 2011-45 days), XDj-1 x YV 125/3 (2010-45 days, 2011 - 44 days) and P-84 x XDj-1 (2010-46 days, 2011 – 43 days). In all of them, one parent is the early maturing variety XDj - 1. The longest period to the beginning of flowering was recorded in P 76/86 x YV 125/3 (2010-70 days, 2011-68 days).

The shortest period from planting to 50% flowering was observed in P-23 x XDj-1 (2010-48 days, 2011-47 days), in P 10-3/2 x XDj - 1 (2010 - 49 days, 2011 - 48 days) and XDj-1 x YV 125/3 (2010 and 2011-49 days). This period was the longest in P 76/86 x YV 125/3 (2010-75 days, 2011 - 73 days).

Period from planting to the end of harvest was the shortest in P-23 x XDj - 1 (2010 and 2011-100 days) and longest in P 76/86 x YV 125/3 (2010-150 days, 2011-148 days).

The most common mode of inheritance of biological traits in both years of investigations is partial dominance. Inheritance of the time to flowering (from planting to the beginning of flowering and from planting to 50 % flowering) does not coincide with inheritance of the time from planting to the end of harvest. For the time of flowering, dominant parent is the one with shorter vegetation, while for the time from planting to the end of harvest it is the late maturing parent. Negative heterosis with poor heterotic effect for the time of flowering was observed in P-84 x P 10-3/2, while for the time from planting to the end of harvest positive heterosis with poor heterotic effect occurs in P-23 x YV 125/3, P-84 x YV 125/3 and P 76/86 x YV 125/3 in both years of investigation. In all three hybrids where heterosis is present, variety YV 125/3 is one of the parents.

Numerous authors point out that the use of heterosis for yield increase in hybrids obtained from oriental tobacco is economically unjustified. Our investigations are related to the negative (for early maturation) and positive heterosis (for late maturation) which, due to their poor heterotic effect, supports the above statement.

The aim of our investigation was to create lines with longer growth period than the early-maturing parent, higher yield and better quality. For realization of this aim, the following hybrids should be pointed out: P-23 x XDj-1, P-84 x XDj-1, P 10-3/2 x XDj-1, P 76/86 x XDj-1 and XDj-1 x JV 125/3. In all these combinations, one of the parents is XDj-1, distinguished by a very pleasant aroma, low yield and shortest growth period (70 days from planting to the end of harvest). The inheritance of the length of growth period is partially dominant and positively dominant, and is dominated by the parent with longer growth period. This mode of inheritance ensures fast stabilization of the trait in future successive selection, aiming at the same time at yield and quality improvement. Such breeding activity will finally result in creation of prospective tobacco genotypes, suitable for arid area, poor soils and longer growth period.

Table 1. Inheritance of the time from transplanting to flowering and the length of growth period from transplanting to the end of harvest in some tobacco varieties and their diallel F1 hybrids

Parents and diallel F1 hybrids	Transplanting date: 10.06.2010			Transplanting date: 15.06.2011		
	Days to the beginning of flowering	Days to 50% flowering	Days to the end of flowering	Days to the beginning of flowering	Days to 50% flowering	Days to the end of flowering
P-23	50	53	115	48	51	110
P-84	59	64	135	60	64	135
P 10-3/2	54	58	110	50	55	105
P 76/86	90	95	145	87	92	143
XDj-1	43	47	70	40	45	70
YV 125/3	57	62	120	55	60	117
P-23 x P-84	50 -d	55 pd	133 +d	48 -d	52 -d	132 +d
P-23 x P 10-3/2	50 -d	53 -d	115 +d	48 -d	51 -d	110 +d
P-23 x P 76/86	60 pd	63 pd	135 pd	58 pd	62 pd	135 pd
P-23 x XDj-1	45 pd	48 pd	100 pd	46 pd	47 pd	100 pd
P-23 x YV 125/3	51 pd	55 pd	125 +h	50 pd	55 i	123 +h
P-84 x P 10-3/2	53 -h	57 -h	125 i	48 -h	56 -d	122 i
P-84 x P 76/86	60 -d	65 -d	143 pd	58 -d	63 -d	140 i
P-84 x XDj-1	46 pd	50 pd	120 pd	43 pd	49 pd	120 pd
P-84 x YV 125/3	57 -d	61 -d	140 +h	55 -d	60 -d	138 +h
P 10-3/2 x P 76/86	60 pd	65 pd	141 +d	60 pd	64 pd	139 +d
P 10-3/2 x XDj-1	45 pd	49 pd	105 +d	45 i	48 pd	105 +d
P 10-3/2 x YV 125/3	54 -d	59 pd	118 pd	54 pd	58 i	115 pd
P 76/86 x XDj-1	60 pd	65 i	120 pd	58 i	62 pd	118 pd
P 76/86 x YV 125/3	70 i	75 i	150 +h	68 i	73 i	148 +h
XDj-1 x YV 125/3	45 pd	49 pd	125 +d	44 pd	49 pd	122 +d

CONCLUSIONS

Based on the results of two-year investigations on the mode of inheritance of the time from planting to flowering of tobacco (beginning stage and 50 % flowering) and length of the growth period from planting to the end of harvest in six oriental varieties and their fifteen F1 diallel hybrids, the following conclusions can be drown:

- Parental genotypes P-23, P-84, P 10-3/2, P 76/86, XDj-1 and YV 125/3 are characterized by genetic homogeneity and significant differences among them, while in their diallel F1 progeny a high degree of uniformity was observed.

- The period from transplanting the seedlings in the field to the beginning of flowering in parents ranged from 40 days (2011) in the early-maturing variety XDj-1 to 90 days (2010) in the late-maturing P 76/86. In hybrids this period ranges from 43 days (2011) in P-84 x XDj-1 to 70 days (2010) in P 76/86 x YV 125/3. The period from planting to 50% flowering in parents ranges from 45 days (2011) in XDj-1 to 95 days (2010) in P 76/86 and in hybrids it is 47 days (2011) in P-23 x XDj-1 to 75 days (2010) in P 76/86 x YV 125/3. The period from planting the seedlings in the field to the end of harvest in parents ranges from 70 days (2010 and 2011) in XDj-1 to 145 days (2010) in P 76/86, and in hybrids it is 100 days (2010 and 2011) in P-23 x XDj-1 to 150 days (2010) in P 76/86 x YV 125/3.
- The mode of inheritance of biological stages in F1 progeny differs, but partial dominance is the most common. Inheritance of the time of flowering is dominated by the early-maturing parent, and the period from transplanting to the end of harvest is dominated by the late-maturing parent. Negative heterosis with poor heterotic effect on the time of flowering was recorded in P-84 x P 10-3/2. Positive heterosis with weak heterotic effect for the time from planting to the end of harvest in both years of investigation was recorded in hybrids where one of the parent was YV 125/3 (P-23 x YV 125/3, P-84 x YV 125/3 and P 76/86 x YV 125/3). The use of heterosis is economically unjustified, due to the poor heterotic effect and to the fact that our subject of investigation were oriental, small-leaf tobacco hybrids.
- For realization of the aim of our investigation we would point out to the hybrids where one of the parents is XDj-1, which has the shortest growth period, very pleasant aroma and low yield. Their F1 progeny has much longer growth period than the early-maturing parent. The inheritance is partial-dominant and dominant (dominated by the late-maturing parent), and it ensures rapid stabilization of the trait in future successive selection, aiming at the same time at increasing the yield and quality.

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Original Scientific paper

ANALYSIS OF GENE EFFECTS AND INHERITANCE OF SOME QUANTATIVE PARAMETERS IN ORIENTAL TOBACCO VARIETIES

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ABSTRACT

The generation, P_1, P_2, F_1, F_2, BCP_1 and BCP_2 of each of the crosses (Plovdiv 50 x basma Xanti 101) and (Krumovgrad 90 x Basma Xanti 101) were used for estimation of gene effects, for plant height, number of leaves per plant, and flowering time. The trial was set up in a randomizet block design with four replications in the experimental field of TTPI in 2009-2011. The observations were recorded on 80 plants from the P_1, P_2 and F_1 generations and 160 plants from F_2, BCP_1 and BCP_2 generations. The varieties used were divergent for all the characters.

In cross (Plovdiv 50 x Basma Xanthi 101) the presence of additive and non additive gene effects (plant height and flowering period) suggested the feasibility to evolve homozygous elite genotypes by cyclic method and inter se crossing among the desirable recombinants keeping adequate population size. Additive effects were predominant in the expression of leaves number and suggests that it be easier to isolate desired tobacco genotypes from this population by Pedigree method of selection.

In cross (Krumovgrad 90 Basma Xanthi 101) Involvement additive as well as epistatic (additive x additive and additive x dominance) effects of plant height, number of leaves and flowering period to conserve additive as well as nonadditive gene effects - cyclic method of breeding would be more effective.

Keywords: tobacco, gene effects, plant height, number of leave, flowering time

АНАЛИЗ НА ГЕННИТЕ ЕФЕКТИ И НАСЛЕДЯВАНЕ НА НЯКОИ КОЛИЧЕСТВЕНИ ПРИЗНАЦИ ПРИ СОРТОВЕ ОРИЕНТАЛСКИ

За оценка на генни ефекти по признаците височината на растенията, брой листа и вегетационен период са изследвани родителските сортове P_1, P_2 и F_1, F_2, BCP_1 и BCP_2 на хибридните комбинации (Пловдив 50 х Басма Ксанти 101) и (Крумовград 90 х Басма Ксанти 101). Опитът бе изведен в четири повторения в опитното поле на ИТТИ през периода 2009-2011. Броят на биометричните измервания е 80 растения за родителските сортове и F_1 и 160 растения за F_2, BCP_1 и BCP_2. Използваните сортовете ясно се различават по изследваните признаци.

В хибридната комбинация (Пловдив 50 х Басма Ксанти 101) установените адитивни и неалелни взаимодействия за признаците (височината на растенията и вегетационен период) предполагат възможност за отбор на хомозиготни елитни генотипове чрез периодичен метод на отбор сред желаните рекомбинанти като се запазва адекватен размера на популацията.

В експресията на признака брой листа адитивните генни ефекти са определящи, което позволява селекционната работа да се провежда чрез метода Pedigree.

В кръстоската (Крумовград 90 х Басма Ксанти 101) при наследяване на изследваните признаци участие вземат адитивни и епистатни генни ефекти (доминантни х доминантни и адитивни х доминатни). За запазване на алелните, както и неалелните генни ефекти – реципрочния периодичен отбор ще бъде по-ефективен.

Ключови думи: тютюн, генни ефекти, височина на растенията, брой листа, вегетационен период

INTRODUCTION

Optimization of the selection process to create new varieties of oriental tobacco is done by applying various models to describe processes that occur in inheritance in the hybrid generation (Yankulov, 1996; Manolov, 1985). Quantitative traits in tobacco are controlled by both alleles and by not alleles interactions that occur between genes. (Kurteva 1996, Petrova 1996).

The additive interaction among the alleles plays an essential role in nature as it provides flexibility environment (Petrova 1996, Stankev 1988, Metha et al., 1985). The degree of trait variation depends both on plant genotype and environment as well as on the interaction between them. In the selection of oriental tobacco, there are a number of quantitative indicators playing a role in the evaluation of new varieties.

Productivity, cigarette yield and homogeneity of the tobacco stuff are traits of great economic importance for tobacco varieties, but the results of genetic analysis are not always unidirectional, due to the large number of genes that are affected by each of them (Petrova1996, Kurteva 1996). The genotype plays a decisive great role on the expression of most important economic traits of Burley and oriental tobacco (Dyulgerski et al., 2013, Taskova et al., 2005).

Moreover, some of the genes controlling quantitative signs are stable with respect to their phenotypic expression, while others exhibit a large degree of variability. Since most tobacco traits important for successful breeding are of quantitative nature, the estimation of the mode of inheritance contributes to their better understanding (Boturac at al., 2004).

The objective of this study was to estimate mode of inheritance and gene effects and some quantitative traits of Oriental tobacco.

MATERIAL AND METHOD

The material consisted of four oriental varieties Plovdiv 50, Krumovgrad 90 and BasmaXanti 101. Six genetic population P_1, P_2, F_1, F_2, BCP_1 and BCP_2 were grown for each of the crosses (Plovdiv 50 x Basma Xanti 101) and (Krumovgrad 90 × Basma Xanti) in a randomized block design with four replication under each cross at the experimental field, Tobacco and Tobacco Products Institute, Plovdiv during 2009-2011. The three varieties were divergent in their morphological characters.

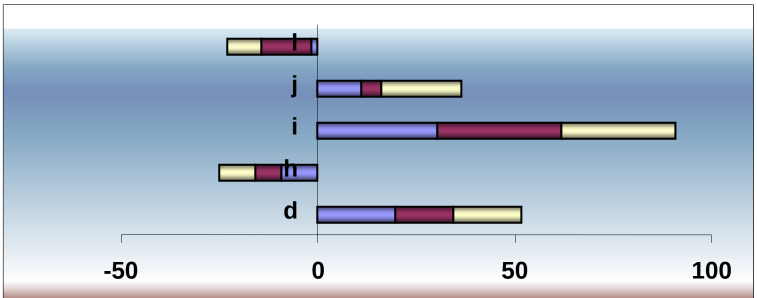
The plants were grown in accordance with the recommended practices for oriental tobacco cultivation. The observations were recorded on ten plants selected at random per plot from the P_1, P_2, F_1 generations and twenty from BC_1, BC_2 and F_2 generations.

The data were statistically analyzed (Genchev et al., 1975). The gene effects for all the characters was made after Cavalli L. test (Mather and Jinks, 1985).

RESULTS AND DISCUSSION

Plant height

The estimates of gene effects for plant height in cross (Plovdiv 50 x Basma Xanti101), are presented in fig.1.

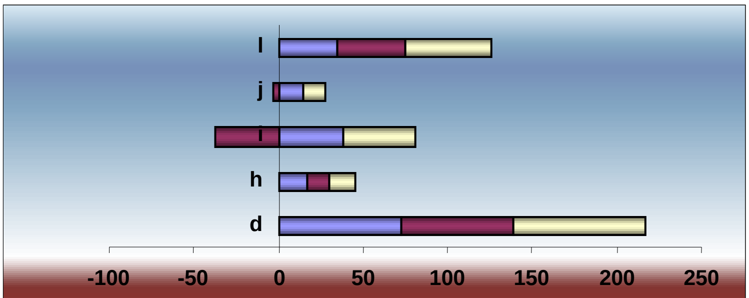


	d	h	i	j	l
2011	17.32**	-8.95	29.28**	20.2	-8.9
2010	14.65*	-6.87	31.18***	4.84	-12.59
2009	19.86***	-9.13	30.45***	11.2	-1.75

Figure1. Gene effects for plant height in cross (Plovdiv 50 x Basma Xanti 101)

Analysis of parameters describing the inheritance of plant height indicated that the additive as well as its higher order interaction (additive × additive) effects, governed the expression of this trait. For the entire period of examinations performed additive and (additive × additive) gene effects were relatively highest density in the formation of height of plants. During the whole period of their values were established at the highest level of probability.

In cross (Krumovgrad 90 x Basma Xanti 101) barring additive and dominance x dominance effects, all kinds of gene effects were nonsignificant.



	d	h	i	j	l
2011	78.45**	15.47	42.21	12.99	50.81**
2010	65.8	12.69	-37.36	-3.18	40.4*
2009	72.82*	17.33	38.55	14.63	34.79

Figure 2.Gene effects for plant height in cross (Krumovgrad 90 x Basma Xanti 101)

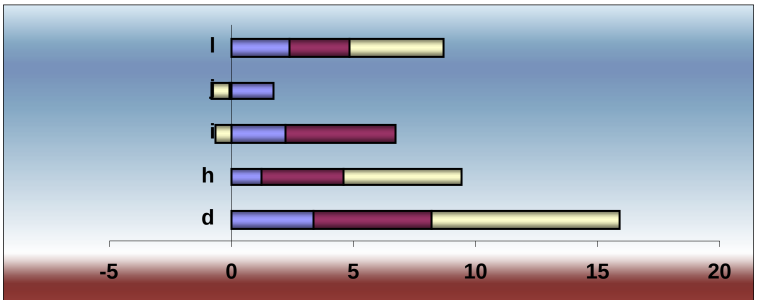
According to the previous investigations, the inheritance of plant height (Shoai Daylami and Honarneja 1996, Butorac et al. 1999) is more influenced by nonadditive variance. Our present results also point to the same conclusion (Masheva, V., 2008).

Number of leaves per plant

Number of leaves per plant is important characters closely related to the yield of tobacco crop. Leaf number is one of the strongly genetically conditioned traits. According to most studies this trait is inherited additively (Petrova 1996, Daylami and Honarneja 1996, Shamsuddin et al. 1980).

The assessment results in this trait indicate that both additive as well as epistatic (dominance x dominance), governed the number of leaves per plant in cross (Plovdiv 50 x Basma Xanti101) (fig.3).

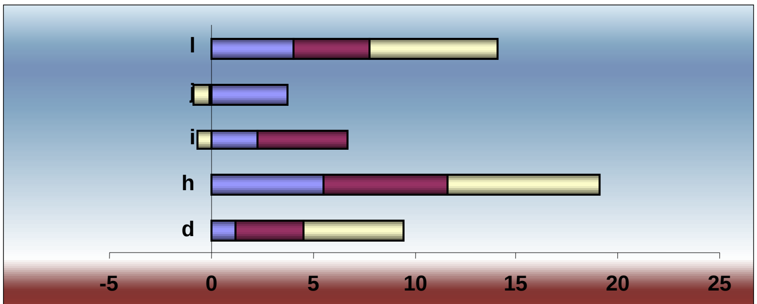
Leaf potential is a direct attribute of yield. The involvement of non additivity (epistasy) along with additive effects for number of leaves suggested that cyclic method of breeding would be more effective for improving this trait in the population of this cross.



	d	h	i	j	l
2011	7.72**	4.91	-0.68	-0.71	3.79
2010	4.81*	3.34	4.5	-0.12	2.52
2009	3.36*	1.21	2.24	1.71	2.35

Figure 3.Gene effects number of leaves in cross (Plovdiv 50 x Basma Xanti 101)

In cross (Krumovgrad 90 x basma Xanti 101) dominance and dominance x dominance (j) type of epistatic were important. Similar type of observations were recorded by Menta (1985) have reported the presence of both additive as well as nonadditive effects in the expression of leaf number.



	d	h	i	j	l
2011	4.91	7.53**	-0.68	-0.71	6.24**
2010	3.34	6.02*	4.5	-0.12	3.78*
2009	1.21	5.56*	2.24	3.72	4.02*

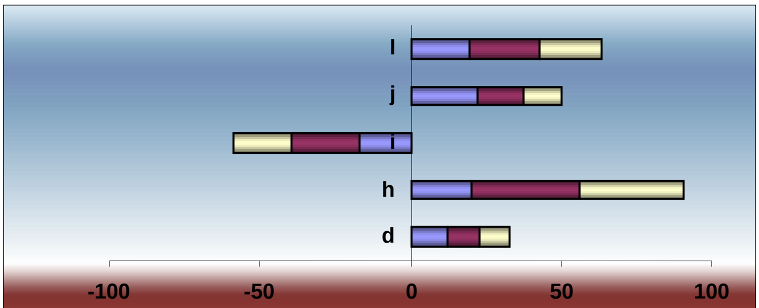
Figure 4.Gene effects number of leaves in cross (Krumovgrad 90 x Basma Xanti 101)

The variety Krumovgrad 90 possessed more number of leaves. When such type of varieties are to be used as donor parents, selection of plants are desirable for increasing the yields. Dominance (h) and interaction component (dominance x dominance) are associate with positive signs. This indicates an increase effect due to their gene action.

Flowering period

The estimates of gene effects for flowering period indicated that in the expression of this trait were involved all kind of gene effects. All parameters studied are presented and demonstrated high values and were significant over the entire period of study.

In fig. 5 are presented results of gene effects for flowering period in cross (Plovdiv 50 x Basma Xanti 101).

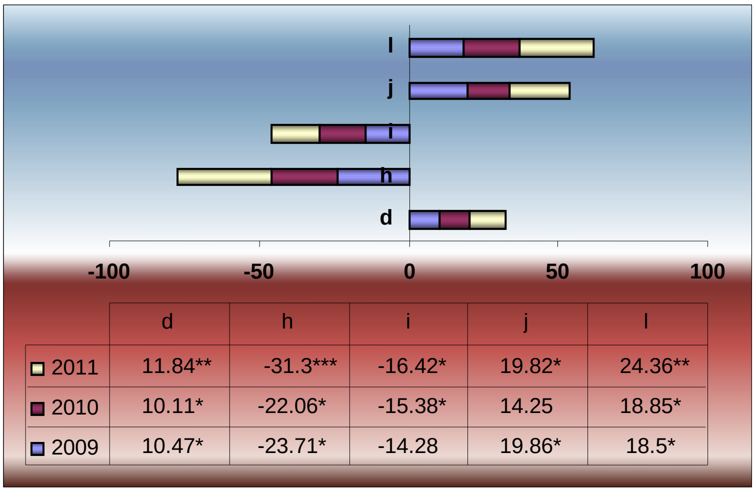


	d	h	i	j	l
2011	9.75*	34.35***	-19.82**	12.44	20.34*
2010	10.84*	35.7***	-22.3*	15.53*	23.48**
2009	12.11**	20.44*	-16.92*	22.2**	19.56*

Figure 5.Gene effects flowering period (Plovdiv 50 x Basma Xanti 101)

All kinds of gene effects significant in the expression of this trait in cross. Dominance and (dominance x dominance) variance were greater in magnitude, next in order being (additive x additive) and (additive x dominance).

In cross (Krumovgrad 90 x Basma Xanti 101) all kinds of gene effects were significant (fig. 6).



	d	h	i	j	l
2011	11.84**	-31.3***	-16.42*	19.82*	24.36**
2010	10.11*	-22.06*	-15.38*	14.25	18.85*
2009	10.47*	-23.71*	-14.28	19.86*	18.5*

Figure 6.Gene effects flowering period (Krumovgrad 90 x Basma Xanti 101)

The dominant (h) effect was significant and the most values. Negative signs of dominant effect indicate that the expression of the trait would be in the direction of a parent with a small length of flowering period. In this event that is very desirable, since a variety Krumovgrad 90 has a relatively long growing season and in years has created problems in the maturation, harvesting and drying of raw material.

Our previous results also point to the same conclusion (Masheva et al., 2009). Many workers like Butorac et al. (1999), Petrova (1996), Mehta et al. (1985) have reported the presence of both additive as well as nonadditive gene effects for this trait.

CONCLUSIONS

The estimation of gene effects of plant height, number of leaves and flowering period showed complex determination of these traits and define effective methods of selection:

- In cross (Plovdiv 50 x Basma Xanthi 101) the presence of additive and non additive gene effects (plant height and flowering period) suggested the feasibility to evolve homozygous elite genotypes by cyclic method and inter se crossing among the desirable recombinants keeping adequate population size. Additive effects were predominant in the expression of leaves number and suggests that it be easier to isolate desired tobacco genotypes from this population by Pedigree method of selection.

- In cross (Krumovgrad 90 Basma Xanthi 101) Involvement additive as well as epistatic (additive x additive and additive x dominance) effects of plant height, number of leaves and flowering period to conserve additive as well as nonadditive gene effects - cyclic method of breeding would be more effective.

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Original Scientific paper

DEGUSTATIONAL PROPERTIES OF SOME PRILEP TOBACCO VARIETIES

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ABSTRACT

Degustational properties of tobacco are one of the most important parameters that determine the quality of tobacco in smoking. Investigations of these properties were performed in 2009 and 2010 on six varieties of the oriental type Prilep: P-23 Ø, P 12-2/1, NS-72, P-66-9/7, P-79-94 and Prilep Basma 82. The best average results regarding the investigated properties were obtained in the check variety P-23 (73.34 points). Common opinion of the Taste panel is that all investigated varieties show good degustational properties that are typical for oriental tobacco, but P-23 and P 12-2/1 are the most prominent among them.

Keywords: tobacco (*Nicotiana Tabacum L.*), taste evaluation, type Prilep.

ДЕГУСТАТИВНИ СВОЈСТВА КАЈ НЕКОИ СОРТИ ОД ТИПОТ ПРИЛЕП

Дегустативните својства на тутунската суровина се меѓу најважните параметри кои го определуваат квалитетот на тутунот за пушење. Истражувањата за овие својства ги вршевме на шест ориенталски сорти тутун од типот прилеп: П-23 Ø, П 12-2-1, НС-72, П-66-9/7, П-79-94 и Прилеп басма 82 во 2009 и 2010 година. Просечните резултати од двегодишните испитувања покажаа дека најголем број бодови доби контролната сорта тутун П-23 (73,34 бода) и таа е најдобра во поглед на овие особини. Општо мислење на дегустативната комисија е дека сите испитувани сорти тутун имаат добри дегустативни својства, карактеристични за тутунската суровина од ориенталско потекло. Сепак, посебно треба да се истакнат сортите П-23 и П 12-2/1.

Клучни зборови: тутун (*Nicotiana Tabacum L.*), дегустација, тип прилеп.

INTRODUCTION

Tobacco is one of the most important industrial crops in the world. Due to the strong anti-smoking campaign, its consumption in developed countries has fallen, but increases in developing countries it is increasing.

All products that are used by man are in solid and liquid state, only tobacco is used in a form of smoke which is produced in combustion during the transition from solid to gaseous state.

Tobacco is commonly used in a form of processed products: cigarettes, cigarillos, cigars and pipe tobacco, and very little for chewing and snuffing. The most important degustational properties of tobacco are the physiological-tasting quality, aroma, strength and flavor of the smoke and they have a great influence on evaluation of its quality.

According to Sozonovic (1960), the quality of tobacco depends on interrelations of complex chemical matters in tobacco leaf, related to the properties of the products for combustion of these matters. Therefore, it is not possible to determine the quality of tobacco by technical measures. Chemical analysis can not provide complete estimation of quality according to the content of certain components. Also, the organoleptic assessment does not give objective assessment of tobacco quality, because the properties of tobacco and tobacco products are finally completed during the combustion of tobacco in the process of smoking, through the smoke effect on senses. Therefore, the final estimation of tobacco quality can be made only by experimental smoking, i.e. degustation.

The properties of tobacco estimated by tasting, especially the physiological effect, strength, flavor and aroma, depend not only on the properties and composition of tobacco blend, but also on some technological factors, technical solutions, method of smoking, etc. The term *degustation* (Lat. degustatio – tasting, taste and aroma evaluation) denotes systematic investigation of human's response to physical and chemical properties of tobacco smoke.

Properties of tobacco manifested while smoking are called degustational properties (Uzunoski, 1985). According to Boceski (2003), the smoker receives "emotional satisfaction and pleasure". Alic-Dzemidjic et al. (1999) reported that chemical composition of tobacco and conditions of burning have a strong impact on smoke properties. Nuneski I. and Nuneski R. (2009) stated that all products of smoking are intended to give the smoker pleasant aroma and taste, as well as physiological pleasure.

The aim of this paper was to make comparative investigation on degustational properties in some varieties of Prilep tobacco grown under same conditions and to mark all the differences among them.

MATERIAL AND METHOD

Degustation as a method for quality assessment of tobacco and tobacco products is based on the properties that are manifested in smoking (irritation, taste, aroma and physiological strength).

The material used for comparative investigation of degustational properties consisted of the following six varieties of tobacco type Prilep: P -23 Ø, P 12-2- , NS -72, P -66-97/7, P-79-94 and Prilep Basma 82.

These varieties were subject of our investigations because for some period they have marked the production of Prilep tobacco in Republic of Macedonia and wider, except for the variety Prilep Basma 82 which was recognized in 2010, while our investigations were not finished yet.

Raw tobacco from the 2009 and 2010 crop was used for investigation purposes. The trial was set up at the experimental field of Tobacco Institute - Prilep.

Degustational properties of tobacco varieties were evaluated by the Taste panel of Tobacco Institute – Prilep, composed of seven members, by the method of "anonymous tasting" according to the standard and the "Key for taste evaluation of oriental aromatic tobacco”.

The above taste evaluation also included investigation on cigarette combustibility which, although not being smoking property but characteristic of the raw, still needs to be monitored because it has interactive impact on the smoking properties of tobacco smoke.

Samples of the investigated varieties were selected for making anonymously coded cigarettes (from 1 to 6) and the Taste panel evaluated the quality of each variety separately for both investigation years.

RESULTS AND DISCUSSION

Tobacco quality was estimated according to the total number of points for each degustational property and each variety. According to data presented in Table 1, the highest score in 2009 was observed in the check variety P -23 (72.70) and the lowest in NS -72 (67.90). With regard to their strength, all varieties were assessed as medium strong tobacco. In evaluation of other properties, the best results were recorded for the check variety P-23, followed by P -12- 2/1, P - 66-9 / 7, P - 79-94, Prilep Basma - 82 and NS - 72.

Similar results were obtained in 2010 crop (Table 2), when the highest total number of points (74) was also given to the check P - 23 and the lowest number to NS-72 (69.51). The variety P - 79-94 was the second best (72.01 points), followed by P 12-2/1, Prilep Basma - 82 and P- 66-9/7.

The average values for degustational properties of the varieties in both years of investigation are presented in Table 3. Here again, the best results were observed in P -23 and the worst in NS-72. The least irritation in smoking was obtained in the check variety, slightly poorer taste and aroma were observed in NS-72, all six varieties showed considerably good combustibility and ash compactness. According to the strength, all of them belong to the group of medium strong tobaccos. Degustational properties of tobacco (total number of points and the average in both years of investigation) are presented in Figure 1.

Besides the above presented values, the Taste panel also gave a descriptive grade for each variety separately:

Code 1 (P-23) - characterized by full, satisfying smoking. There is a slight irritation on draw, without any scratching or harshness. It leaves no coating sensation in oral cavity. The smoke is smoothly transmitted to the chest without negative sensation. The taste is sweetish, without bitterness, and pleasant on smoking. It has intensive aroma, typical for oriental type of tobacco. In terms of strength, it was evaluated as medium strong, still somewhat stronger than the other codes. Ash compactness is very good, with slight flaking. Combustibility of cigarette is good to very good, with small burning ring. The color of the ash is whitish gray to white. Typical for this variety is that its chemical components are composed so well that it gives the cigarette a harmonic, full and satisfying smoking.

Code 2 (P 12-2/1) – from the aspect of irritation, the raw is good, without in terms of irritation and pricking sensation perceived by the smoker on tongue. The taste is slightly weaker than that in Code 1. No bitterness, burning and coating of the oral cavity is felt during smoking. Compared to Code 1, it feels slightly emptier. The aroma is intensive, penetrating and slightly more noble. According to strength, it can be assessed as medium strong raw material. Ash compactness is good to very good, with slight flaking. Combustibility is good, with somewhat bigger burning ring. The color is whitish gray to white. In the opinion of the members of the panel, the taste on this variety slightly deviates from the other degustational properties.

Code 3 (NS-72) – the amount of irritation in this raw is more prominent, the taste is slightly sweetish, with no bitterness, pricking or coating sensation on oral cavity. The aroma is less intensive and incompatible with the above two degustational properties. It belongs to the group of medium strong tobaccos. Combustibility is good to very good, with occurrence of slight flaking. Ash compactness is good and the color of the ash is whitish gray.

Code 4 (P-66-9/7) - raw material with insignificant irritation. Pricking on the tongue and scratching of the throat is more pronounced than in Codes 1 and 2. The taste is mild, but slightly less pleasant. The aroma is defined as oriental, but less pronounced in comparison to Codes 1 and 2. It belongs to the group of medium strong tobaccos, with good compactness of ashes, where slight flaking is noticed. The color is grayish to white. Combustibility is good, with a burning ring slightly more pronounced than in Codes 1 and 2.

Code 5 (P - 79-94) –insignificant irritation, without scratching or pricking. The taste is pleasant and sweetish, without coating sensation on the oral cavity. It has pronounced aroma, typical for oriental tobaccos. The strength of the raw is medium. Ash compactness and combustability is good, with slightly wider burning ring. The color of the ashes is grayish to white.

Code 6 (Prilep Basma -82) - negligible irritation, no scratcing or pricking. The raw is pleasant for smoking. The aroma is less pronounced, but still typical for tobacco with oriental origin. It belongs to the group of medium strong tobaccos, with more pronounced strength. No resistance to draw is felt during smoking. Combustibility and compactness of ashes are good. Slight flaking can be observed.The color of the ashes is whitish gray to white.

General statement of the Taste panel is that all of the investigated codes (varieties) show good degustational properties, typical for Oriental tobacco. Somewhat better results, however, were obtained with Codes 1 and 2, i.e. the Prilep tobacco varieties P - 23 and P -12 -2/1, while poorer smoking characteristics were recorded in the Prilep variety NS-72.

CONCLUSIONS

Based on two-year investigations of degustational properties with six varieties of tobacco type Prilep, the following conclusions can be drawn:

• The highest number of points were given to the check P -23 (73.34), i.e. it was evaluated as the best variety with regard to degustational properties, and the lowest number of points were given to variety NS-72 (68,69).

• A general statement of the Taste panel is that all tobacco varieties included in the investigation have good degustational properties, typical for tobacco of oriental origin, but varieties Prilep P-23 and P 12-2/1 should be especially emphasized.

• The investigated varieties of tobacco type Prilep can be successfully used in mixtures for production of the highest quality cigarette brands in the world.

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Original Scientific paper

RESULTS OF INVESTIGATIONS OF SOME BIO -MORPHOLOGICAL TRAITS OF VIRGINIA TOBACCO VARIETIES AND LINES IN THE PRODUCING REGION OF PRILEP IN 2010 AND 2011

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ABSTRACT

Investigation was carried out in 2010 and 2011 with 7 Virginia varieties and male sterile hybrid varieties and lines in the region of Prilep. American fertile variety K-326 was used as a check. The following traits were analyzed during the growing season: time of flowering, length and width of the 5th , 10th and 15th leaf, stalk height and number of leaves. In most of these traits the male sterile hybrids proved to be superior to the fertile genotypes, with small advantage of the hybrid V-88/09 CMS F_1. The obtained results can help the producers to make decision which genotype to use in the start of the production cycle.

Keywords: tobacco, variety, Virginia tobacco, flowering, leaves, stalks, dimensions

РЕЗУЛТАТИ ОД ИСТРАЖУВАЊАТА НА НЕКОИ БИО-МОРФОЛОШКИ СВОЈСТВА КАЈ ВИРЏИНИСКИ СОРТИ И ЛИНИИ ТУТУНИ ВО ПРИЛЕПСКИОТ ПРОИЗВОДЕН РЕОН ВО 2010 И 2011 ГОДИНА

Во трудот презентирани се резултати од истражувањата со 7 вирџиниски сорти и машкостерилни хибридни линии во реонот на Прилеп, во 2010 и во 2011 година. Како контролна сорта беше користена американската фертилна сорта К-326. Во текот на вегетацијата беа анализирани: времето на цветање, должината и ширината на 5 ^{от}, 10 ^{от} и 15 ^{от} лист, висината на стракот и бројот на листовите. Во повеќето од овие својства машкостерилните хибриди се покажале како посупериорни од фертилните генотипови, со извесна предност на хибридот V-88/09 ЦМС F_1. Овие резултати можат да придонесат при одлуката на производителот со кој генотип ќе стартува во производниот циклус.

Клучни зборови: Тутун, сорта, вирџинија, цветање, листови, стракови, димензии.

INTRODUCTION

Presently, Virginia tobacco raw is inevitable component of modern cigarette types and it participates with different percentages in cigarette mixtures. To obtain a good quality raw material, two basic requirements should be met: good variety and strictly controlled production typical of the type. Macedonian fabrication has a need for Virginia tobacco, which is 100% imported. Up to 2002, some of the needs were satisfied by domestic production. According to (Risteski, 2000), Virginia tobacco production in R. Macedonia reached 1633 tons in the period 1976 - 1988 and 1475 tons in 1989-1997. In 2002 the production of this type ceased and this situation has remained unchanged until today. For successful restart, in our opinion, it would be necessary to use cheaper energy sources for curing (gas, gas-solar energy combination, cheap solid fuels), to introduce new, highly productive varieties and to distribute them most properly by the regions. All this will lower the price of the raw material, making it more competitive on the market.

Scientific Tobacco Institute in Prilep has been working successfully on creation of Virginia varieties with high economic value, which would be a guarantee for a successful restart of production of this tobacco. This statement is based on the results of comparative investigations with varieties from other countries known for production of this tobacco type. A part of these investigations will be presented in this paper.

MATERIAL AND METHOD

The two-years investigation (2010 2011) was carried out with 7 varieties, three of which were fertile (K - 326 from USA, Virginia SKR from Zimbabwe and V- 972 from Germany) and four male sterile hybrids (V-88/09 CMS F_1, V-63/04 CMS F_1, V-78/07 CMS F_1, V-82/07 CMS F_1), all of them created in Tobacco Institute – Prilep.

The American variety K - 326 was used as a check. The trial was set up in the field of Tobacco Institute on colluvial soil. The first ploughing was done at a depth of 40 cm. In spring, the plots were fertilized with 300 kg/ha NPK (8:22:20) and then ploughed two times more. Before transplanting, the plots were treated with selective herbicide and healthy seedlings were planted in randomized block design with 4 replications at 90 × 50 cm planting density.

Prior to second hoeing, 3 g / 26 % KAN was applied for nutrition of the plants. In the period of extended drought and in development stages when tobacco requirements for water were higher, additional irrigations were made. The plants were also treated with chemicals for their protection from pests and diseases. During the growing season, time of flowering was recorded and morphological measurements were made on 5 stalks of each variety. Analysis was made on the 5th, 10th and 15th leaf on the stalk, i.e. the belt which accounts for 60-70 % of the total leaf mass. According to (Uzunoski, 1983) these leaves are the largest in size (over 35 cm) and they are ranked in the I class. These leaves were also measured for their width. Each variety was analyzed for height of the stalk with inflorescence and total number of leaves per stalk. The obtained results were statistically processed using the method analysis of variance and tested with LSD test.

RESULTS AND DISCUSSION

- Length of the growing season (flowering)

This biological trait is considered as varietal characteristic, lasting in the period from planting to the end of flowering. (Rubin, 1971) reported that the first flower is the central (top) flower and the other bloom continuously within 10 to 15 days. According to (Hawks et al.,1994), tobacco varieties which bloom later usually have a higher number of leaves. Results on investigation of this trait are presented in Table 1.

Table 1. Length of the growing season (flowering)

Variety	Year	Beginning of flowering, in days	2010/ 2011 Average	Absolute differences from the average	50% flowering, in days	2010/ 2011 Average	Absolute differences from the average	End of flowering, in days	2010/ 2011 Average	Absolute differences from the average
K-326	2010	58	60.0	/	63	65.5	/	69	71.5	/
	2011	62			68			74		
Virginia SKR	2010	59	61.5	+1.5	65	67.0	+1.5	71	73.0	+1.5
	2011	64			69			75		
V-972	2010	60	62.0	+2.0	64	67.5	+2.0	70	73.0	+1.5
	2011	64			71			76		
V-88/09 CMS F_1	2010	63	66.5	+6.5	71	73.5	+8.0	77	79.0	+7.5
	2011	70			76			81		
V-63/04 CMS F_1	2010	61	62.5	+2.5	67	68.5	+3.0	73	74.5	+3.0
	2011	64			70			76		
V-78/07 CMS F_1	2010	62	65.5	+5.5	68	71.0	+5.5	73	77.0	+5.5
	2011	69			74			81		
V-82/07 CMS F_1	2010	64	66.5	+6.5	69	72.5	+7.0	75	79.0	+7.5
	2011	69			76			83		

From data in Table 1 it can be seen that earliest flowering was observed in varieties K - 326 (check) and Virginia SKR (60 and 61.5 days, respectively). Latest flowering was recorded in hybrids V-88/09 CMS F_1 and V-82/07 CMS F_1 (66.5 days, or 6.5 days longer compared to the check variety. In other varieties and lines, this parameter ranged from 62 days in variety V- 972 to 65.5 days in hybrid V-78/07 CMS F_1. Also, 50 % flowering was achieved first by varieties K - 326 and Virginia SKR (in about 65.5 and 67 days, respectively). Longest period to achieve this stage was observed in hybrid V-88/09 CMS F_1 (73.5 days, or 8 days longer than the check). In other varieties and hybrids, this parameter ranges between 67 days in variety Virginia SKR and 72.5 days in hybrid V-82/07 CMS F_1. According to (Naumoski et al., 1977), by the end of flowering almost 90 % of the total leaf mass has already been formed on the stalk. This period is shortest in the check variety K - 326 (71.5 days) and longest in hybrids V-88/09 CMS F_1 and V-82/07 CMS F_1 (79 days, or 7.5 days longer than the check variety). In other varieties and hybrids included in this investigation, the end of flowering stage ranges from 73 days in varieties V-972 and Virginia SKR to 77 days in hybrid V-78/07 CMS F_1.

General conclusion for all varieties and lines included in the trial is that male sterile hybrids need a longer period to complete all stages of flowering compared to the fertile varieties.

Length of the 5th, 10th and 15th leaf

Typical representative of leaves that carry the yield and quality of Virginia tobacco is the zone that includes the leaves (from the 5^{th} up to the 15th leaf). Their morphological characteristics are genetically controlled, but they are also affected by the soil and climate conditions and application of various agro-technical measures. In order to be ranked in the I class, these leaves must be longer than 35 cm. According to (Beljo, 1996), the length of tobacco leaves ranges from 10 cm to 80 cm. (Risteski, 1999) reported that leaves of the variety MB-1 from stalks grown at larger nutrition area are longer.

The size of the 5^{th}, 10^{th} and 15thy leaf are presented in Table 2.

Data from the above table reveal that the largest 5th leaf length of 54.6 cm was observed in hybrid V-88/09 CMS F_1, which is 5.9 cm more compared to the check variety K – 326 (48.7 cm). The lowest average length of the 5th leaf (40.7 cm) was observed in variety V- 972. In other varieties and hybrids this parameter ranged from 45.5 cm in hybrid V-63/04 CMS F_1 to 51.8 in hybrid V-82/07 CMS F_1. In 2010, statistically significant differences at 5 % level were recorded only in hybrid V-88/09 CMS F_1. In 2011, statistically significant differences at 5 % level were recorded in the variety Virginia SKR and at a level of 1 % in hybrids V-88/09 CMS F_1 and V-82/07 CMS F_1.

The largest 10th leaf length of 65.5 cm was found in hybrid V-88/09 CMS F_1, which is 10.1 cm more compared to the check variety K-326 (55.4 cm). The length of the 10th leaf was the lowest in variety V- 972 (52.0 cm).

In other varieties included in the trial, this parameter ranged from 60.3 cm in Virginia SKR to 63.0 cm in hybrid V-78/07 CMS F_1.

Statistically significant differences at 5 % level were recorded only in 2010 in the variety Virginia SKR. Significance level of 1% was observed in all male sterile varieties in both years of investigation and only in 2011 such level was found in the variety Virginia SKR.

The largest 15^{th} leaf length of 59.7 cm was recorded in the hybrid V -88/09 CMS F1, which is 6.2 cm more compared to the check variety K-326 (53.5 cm). In other varieties, the average length of the 15^{th} leaf ranges from 48.0 cm in V- 972 to 59.5 cm in hybrid V-78/07 CMS F1.

Statistically significant difference of 5% was recorded only in 2010 in variety Virginia SKR, hybrids V-82/07 CMS F1 and V 88/09 CMS F1. Significant difference at 1% level in both years of investigation was recorded in hybrid

V 78/07 CMS F1 and only in 2011 it was found in hybrids V-82/07 CMS F1, V-63/04 CMS F1 and V-88/09 CMS F1.

Table 2. Length of the 5th, 10th and 15th leaf

Variety	Year	Length of the 5th leaf in cm	average 2010/2011	difference in cm	Length of the 10th leaf in cm	average 2010/2011	difference in cm	Length of the 15th leaf in cm	average 2010/2011	difference in cm
K-326	2010	49.0	48.7	/	57.7	55.4	/	55.5	53.5	/
	2011	48.4			53.2			51.6		
Virginia SKR	2010	52.8	51.5	+2.8	61.5^{+}	60.3	+4.9	59.2^{+}	56.5	+3.0
	2011	50.2^{+}			59.2^{++}			53.8		
V-972	2010	41.4	40.7	-8.0	53.2	52.0	-3.4	48.6	48.0	-5.5
	2011	40.0			50.8			47.4		
V-88/09 CMS F_1	2010	54.8^{+}	54.6	+5.9	67.0^{++}	65.5	+10.1	60.0^{++}	59.7	+6.2
	2011	54.4^{++}			64.0^{++}			59.4^{++}		
V-63/04 CMS F_1	2010	46.0	45.5	-3.2	62.8^{++}	61.1	+5.7	58.2	56.8	+3.3
	2011	45.0			59.4^{++}			55.4^{++}		
V-78/07 CMS F_1	2010	50.4	50.4	+1.7	63.6^{++}	63.0	+7.6	60.8^{++}	59.5	+6.0
	2011	50.4			62.4^{++}			58.2^{++}		
V-82/07 CMS F_1	2010	51.6	51.8	+3.1	63.8^{++}	62.4	+7.0	59.2^{+}	57.7	+4.2
	2011	52.0^{++}			61.0^{++}			56.2^{++}		

	Length of the 5th leaf 2010	Length of the 5th leaf 2011	Length of the 10th leaf 2010	Length of the 10th leaf 2011	Length of the 15th leaf 2010	Length of the 15th leaf 2011
LSD	5% $^{+}$ = 4.57 cm	2.34 cm	5% $^{+}$ = 3.43 cm	2.86 cm	5% $^{+}$ = 3.67 cm	2.23 cm
	1% $^{++}$ = 6.77 cm	3.21 cm	1% $^{++}$ = 4.70 cm	3.93 cm	1% $^{++}$ = 5.03 cm	3.06 cm

From the above data it can be concluded that the 10th leaf has the largest length in all varieties and hybrids included in the trial.

Width of the 5th , 10th and 15th leaf

Width of the leaf, just as its length, is genetically controlled trait and varietal characteristic, but it is also influenced by soil and climate condition s and the applied agrotechniques. The best length and width ratio is 2:1.

Kalamanda (2009), in her investigations in Republika Srpska with varieties DH- 17 and Hewessi - 17, found that width of the leaves from the middle insertion ranged from 19.40 cm in Hewessi - 17 in 2004 to 25.40 cm in DH- 17 in 2006. (Devcic et al.,1982) reported an average leaf width ranging from 21 cm in varieties H- 30 and

H- 31 to 20 cm in H- 32. The data obtained for this parameter are presented in Table 3.

Table 3. Width of the 5th , 10th and 15th leaf

Variety	Year	Width of the 5th leaf			Width of the 10th leaf			Width of the 15th leaf		
		in cm	Average 2010/2011	Difference in cm	in cm	Average 2010/2011	Difference in cm	in cm	Average 2010/2011	Difference in cm
K-326	2010	29.7	30.1	/	33.3	32.5	/	30.5	29.4	/
	2011	30.6			31.8			28.4		
Virginia SKR	2010	32.5	31.8	+1.7	31.6	30.9	-1.6	30.5	29.9	+0.5
	2011	31.2			30.2			29.4		
V-972	2010	28.2	27.9	-2.2	39.4^{++}	38.0	+5.5	26.2	26.5	-2.9
	2011	27.6			36.6^{++}			26.8		
V-88/09 CMS F_1	2010	33.8^{+}	33.6	+3.5	41.8^{++}	41.4	+8.9	31.4	30.2	+0.8
	2011	33.4^{+}			41.0^{++}			29.0		
V-63/04 CMS F_1	2010	31.6	31.1	+1.0	37.2^{+}	35.9	+3.4	32.2	30.8	+1.4
	2011	30.6			34.6^{+}			29.4		
V-78/07 CMS F_1	2010	33.2	32.2	+2.0	39.2^{++}	38.6	+6.1	30.6	29.4	/
	2011	31.0			38.0^{++}			28.2		
V-82/07 CMS F_1	2010	34.6^{+}	34.2	+4.1	38.6^{++}	37.5	+5.0	30.0	29.3	-0.1
	2011	33.8^{++}			36.4^{++}			28.6		

	Width of the 5th leaf		Width of the 10th leaf		Width of the 15th lea	
	2010	2011	2010	2011	2010	2011
LSD	$5\%^{+}$ = 3.75 cm	2.34 cm	$5\%^{+}$ = 3.59 cm	2.33 cm	$5\%^{+}$ = 2.53 cm	1.89 cm N.S.
	$1\%^{++}$ = 5.19 cm	3.21 cm	$1\%^{++}$ = 4.92 cm	3.20 cm	$1\%^{++}$ = 3.47 cm	2.59 cm N.S.

According to the data in the table, the largest average width of the 5th leaf (34.2 cm) was found in hybrid V-82/07 CMS F_1 and it is 4.1 cm more compared to the check variety K – 326 (30.1 cm). The lowest average width (27.9 cm) was recorded in the variety V- 972. In other varieties and hybrids, the average width ranges between 31.1 cm in hybrid V-63/04 CMS F_1 and 33.6 cm in hybrid V-88/09 CMS F_1. Statistically significant differences at 5 % level in relation to the check were recorded in hybrid V-88/09 CMS F_1 in 2010 and 2011, and in hybrid V-82/07 CMS F_1 in 2010. In the latter, statistically significant difference at 1 % level was recorded in 2011.

The largest average width of the 10th leaf (41.4 cm) was recorded in hybrid V-88/09 CMS F_1 and it is 8.9 cm more compared to the check variety K – 326 (32.5 cm). The largest average width of the leaf measured in variety Virginia SKR was 30.9 cm. In other varieties included in the experiment the average width ranged from

35.9 cm in hybrid V-63/04 CMS F_1 to 38.6 cm in hybrid V-78/07 CMS F_1. Statistically significant differences at 5 % level in both years of investigation were recorded in hybrid V-63/04 CMS F1. Significant difference at 1 % level was recorded in variety V- 972 and hybrids V-88/09 CMS F1, V-78/07 CMS F1 and V-82/07 CMS F_1. The largest average width of the 15th leaf (30.8 cm) was recorded in hybrid V-63/04 CMS F_1, which is 1.4 cm more than that of the check K - 326 (29.4 cm). The lowest average width of 26.5 cm was found in variety V- 972. In other varieties, the average width ranged between 29.3 cm and 30.2 cm. No statistically significant differences among varieties and hybrids in both years of investigation were recorded for this trait.

Height of the stalk with inflorescence and Number of leaves

According to Uzunoski (1985), morphological properties of tobacco are highly variable under the influence of environmental conditions and agrotechnical practices. Thus, height of the stalk varies from 25cm to 300 cm and over. These variations are also genetically controlled, i.e. they depend on the type or variety of tobacco. The same author (1983), in his investigations on Virginia tobacco in the region of Kicevo, reported that stalk height in the Italian variety S – 7, depending on the locality, ranged between 215 and 300 cm. According to (Devcic et al.,1982), the average stalk height in Croatian varieties H-10, H-31 and H-32 is 170 cm. (Risteski, 1999) reported that height of the stalks in variety MV - 1 is bigger in varieties which are grown on smaller nutrition area. Data on stalk height and leaf number are presented in Table 4.

According to these data, the lowest average height of 197 cm was observed in variety Virginia SKR and it is 27 cm higher than the check variety K - 326 (172 cm). The minimum height of 168 cm was obtained in variety V- 972. In other hybrids, the stalk height varies in the range from 182 to 186 cm. The difference between the highest and the lowest variety is 31 cm. Statistically significant differences compared to the control at 5 % level were observed in hybrid V-78/07 CMS F_1 in 2010 and 2011 and hybrid V-82/07 CMS F_1 in 2011. Significance level of 1 % in the two years of investigation were recorded in variety Virginia SKR and hybrids V-88/09 CMS F_1 and V-63/04 CMS F_1. In hybrid V-82/07 CMS F_1 such difference was observed only in 2010.

Number of leaves per stalk is also genetically controlled trait that is closely related to agroecological conditions, tobacco type, cultural practices etc. Hawks et al. (1994) came to a conclusion that in most of the cases the varieties with higher stalks also have a higher leaf number. This was particularly evident in varieties with mammoth traits which "do not blossom".

(Drazic et al., 2011) reported that the number of leaves in 13 varieties and lines investigated in locality Nova Pazova (Serbia) ranged from 22 to 26.

The results of our investigations (Table 4) show that the highest leaf number was obtained in hybrid V-88/09 CMS F_1 (33.3) and the lowest in check variety (28.8). In other varieties and hybrids in the trial the leaf number ranged from 29.0 to 30.4. The difference between the highest and the lowest leaf number (33.3 vs. 28.8) is 4.5 and it can significantly affect the total tobacco yield. Statistically significant differences compared to the check variety at 5 % level were obtained in Virginia SKR in 2010 and in hybrids V-63/04 CMS F_1 and V-78/07 CMS F_1 in 2011. Significant differences at 1 % level in both years of investigation were observed only in hybrid V-88/09 CMS F_1, while in 2011 such difference was registered in variety Virginia SKR and hybrid V-82/07 CMS F_1.

Table 4. Height of the stalk with inflorescence and Leaf number

Variety	Year	Stalk height with inflorescence	Average 2010/2011	Differences in cm	Rank	Leaf number	Average 2010/2011	Differences in the average	Rank
K-326	2010	173	172	/	6	29.8	28.8	/	7
	2011	172				27.8			
Virginia SKR	2010	201^{++}	197	+27	1	31.0^{+}	30.4	+1.6	2
	2011	193^{++}				29.8^{++}			
V-972	2010	168	168	-4	7	28.8	29.0	+0.2	6
	2011	168				29.2			
V-88/09 CMSF $_1$	2010	188^{++}	186	+14	2	33.6^{++}	33.3	+4.5	1
	2011	184^{++}				33.0^{++}			
V-63/04 CMS F $_1$	2010	186^{++}	185	+13	3	30.2	29.9	+1.1	5
	2011	184^{++}				29.6^{++}			
V-78/07 CMS F $_1$	2010	183^{+}	182	+10	5	30.6	30.1	+1.3	4
	2011	181^{+}				29.6^{++}			
V-82/07 CMS F $_1$	2010	187^{++}	183	+11	4	30.0	30.3	+1.5	3
	2011	179^{+}				30.6^{++}			

	Stalk height 2010	2011	Leaf number 2010	2011
LSD $5\%^{+}$ =	7.80	6.80	1.11	1.44
$1\%^{++}$ =	10.70	9.28	1.52	1.99

CONCLUSIONS

- The check variety K - 326 is the first one to begin with flowering (in 60 days) and the first to end (71.5 days). Hybrids V-88/09 CMS F_1 and V-82/09 CMS were the last to begin to flower (66.5 days) and the last to end (79.0 days).

- The largest length of the 5th, 10th and 15th leaf (54.6 cm, 65.5 cm and 59.7 cm, respectively) was observed in hybrid V-88/09 CMS F_1. The smallest length of the 5th, 10th and 15th leaf (40.7 cm, 52.0 cm and 48.0 cm) was recorded in variety V-972.

- The largest width of the 5th leaf (34.2 cm) was measured in hybrid V-82/07 CMS F_1 and the lowest (27.9 cm) in variety V- 972.

- The largest width of the 10th leaf was recorded in hybrid V-88/09 CMS F_1 (41.4 cm) and the lowest in Virginia SKR (30.9 cm).

- The largest width of the 15th leaf (30.8 cm) was observed in hybrid V-63/04 CMS F_1 and the lowest (26.5 cm) in variety V- 972.

- Stalk height with inflorescence is the largest in variety Virginia SKR (197 cm) and the lowest (168 cm) in variety V- 972.

- The highest number of leaves (33.3) was obtained in hybrid V-88/09 CMS F_1 and the lowest (28.8) in the check variety K – 326.

- Male sterile hybrids are dominant in most of the analyzed traits, with some advantage of hybrid V-88/09 CMS F_1.

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Original Scientific paper

THE EFFECT OF PLANT HORMONE KINETIN ON REDUCING THE INTENSITY OF BROWN SPOT DISEASE IN TOBACCO

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ABSTRACT

Brown spot disease is economically important disease which has a particular impact on the reduction of tobacco leaves quality and, hence, on the total economic effect. The main factors for its occurrence are the climate and irrational use of agrotechnical practices. Ontogenetic age of leaves has also a big influence on disease attack, i.e. its intensity increases with aging of the leaves. A number of fungicides are applied in the control of this disease.

The aim of integral protection, however, is to include preventive measures and to reduce the number of treatments. Also, the biointensive model of integral protection aims to replace them by natural resources. Our objective was to study the effect of plant hormone kinetin on the intensity of attack of brown spot disease.

Two concentrations of kinetin were applied (30 mg/l and 60 mg/l), with one and two treatments of tobacco plants. They were inoculated with a suspension of pure culture of the pathogenic fungus *Alternaria alternata* - the causing agent of the disease.

It was concluded that kinetin treatment has a positive effect on reducing the disease intensity. The lowest intensity in the two-year investigation was recorded in plants with a single treatment of kinetin -60 mg/l. Among treatments with 30 mg/l, lower intensity was recorded when two treatments were applied. Histological investigations of tobacco leaves confirmed the effect of kinetin in reduction of the possibilities for infection.

The investigations point out to the possibility for application of the plant hormone kinetin in tobacco as a biological and preventive measure in the control of brown spot disease.

Keywords: brown spot disease, intensity of attack, kinetin, reduction, preventive measure

ВЛИЈАНИЕ НА РАСТИТЕЛНИОТ ХОРМОН КИНТЕТИН ВРЗ НАМАЛУВАЊЕТО НА ИНТЕНЗИТЕТОТ НА БОЛЕСТА КАФЕНА ДАМКАВОСТ КАЈ ТУТУНОТ

Болеста кафена дамкавост е една од економските значајни болести, со особено влијание врз намалување на квалитетот на тутунските листови, а оттука, на вкупниот економски ефект. Како главни предиспонирачки фактори за нејзината појава се климатските услови и нерационалната примена на агротехничките мерки. Исто така, онтогенетската возраст на листовите има големо влијание врз интензитетот на напад, односно, истиот се зголемува со стареење на листовите.

За заштита од оваа болест се применуваат повеќе фунгициди. Но, една од целите на интегралната заштита на тутунот е најпрвин вклучување на превентивни мерки и намалување на бројот на третирања.

Исто така, биоинтензивниот модел на интегрална заштита се повеќе се насочува кон намалување на употребата на хемиски средства за заштита и користење на природните ресурси.

Оттука, нашата цел беше да го проучиме дејството на растителниот хормон кинетин врз интензитетот на напад од болеста кафена дамкавост.

Беа применети две концентрации на кинетин (30 mg / *l* и 60 mg / *l*)со едно и две третирања на тутунските растенија. Истите беа инокулирани со суспензија од чиста култура на предизвикувачот на болеста-патогената габа *Alternaria alternata*.

Беше констатирано дека третирањето со кинетин има позитивно дејство врз намалувањето на интензитетот на болеста. Во двете години на истражување, најмал интензитет беше утврден кај растенијата со едно третирање со кинетин -60 mg / *l*. Помеѓу третирањата со 30 mg / *l*, помал интензитет е утврден при две третирања.

Хистолошките испитувања на тутунските листови го потврдуваат влијанието на кинетинот во намалувањето на можноста за инфекција.

Овие истражувања укажуваат на можноста за примена на растителниот хормон кинетин кај тутунот како биолошка и превентивна мерка за заштита од болеста кафена дамкавост.

Клучни зборови: кафена дамкавост, интензитет на напад, кинетин, редукција, превентивна мерка

INTRODUCTION

Brown spot is one of the economically important diseases on tobacco. It attacks all tobacco types but achieves the highest intensity in large-leaf tobaccos. Its occurrence largely depends on climate conditions, variety and modes of tobacco production. Its intensity is increased in areas with warm climate and higher relative humidity or heavy rainfalls (Gveroska, 2006 a). Occurrence of the disease is greatly affected by the undue harvesting of tobacco leaves. In recent years, the intensive mode of production and efforts to obtain higher yields resulted in application of some cultural practices (excessive irrigation, low planting density, over-fertilization, etc.) which indirectly increase the disease incidence.

Recommended techniques for protection from the disease include application of preventive measures, resistant tobacco varieties and use of chemicals. The biointensive model for integral protection, however, is inclined towards reduced number of treatments and the use of natural resources.

The long-term use of chemicals increases both resistance to the pathogen and the costs. It also causes harmful effects to human health and the environment. Therefore, the scientific approach is focused o finding alternative modes for control of the disease.

The influence of ontogenetic age of tobacco on the intensity of disease (also known as "disease of weakness and old age") imposed investigations in another field - physiological basis of reducing the intensity of the attack.

Physiologically active substances are regularly used in agriculture. Plant hormones or phytohormones are substances produced by plants that are used for regulation of growth and development. As in many other cultures, they can cause physiological changes in tobacco (Miceska et al., 1999). They find practical application in increase of productivity and improvement of technological properties of plants (Petrova and Iliev, 1990), but they also have their role in the adaptive response of plants to various stressful situations (Atanasova et al., 1996).

According to Seilaniantz et al. (2007), plant hormones play an important role in growth regulation and in creation of a network of signals involved in plant response to a wide range of biotic and abiotic stresses.

Cytokinins promote a wide range of diverse biological activities that appear in the regulation of growth, formation of organs, aging process and dormation period. These substances, especially kinetin, have an influence on cell division, elongation and differentiation of cells, growth of the root tip and its initiation, initiation and growth of vegetative cone and on suspension of dormancy. Among many other functions, they increase plant tolerance to low and high temperatures and to fungal infection (Sarić et al., 1987).

Cytokinins affect the susceptiblity of leaves and other organs and increases the resistance to pathogens and extreme temperatures (Annonimus, 2011). It was stated that plants which have an ability to survive in suboptimal conditions contain increased amount of cytokinin. Such is the case with the beans exposed to AL toxin (Massos et al., 1994 , loc cit Atanassova et al., 1996).

No data have been found on the influence of plant hormones on tobacco reaction to certain diseases, but according to Haberlach et al. (1978), the resistance of tobacco in tissue culture can be controlled by regulation of the hormone regimen.

With reference to the effect of phytohormones on *Alternaria alternata*, Perez et al. (1995) report that the brown spot disease on fruits treated with giberelin acid is manifested with much lower severity.

Data on the effect of phytohormones on plant reaction to pathogens has raised the interest for investigation of their impact on tobacco and its reaction to brown spot disease. The aim of this paper was to study the effect of the plant hormone kinetin on the intensity of brown spot disease caused by the pathogenic fungus.

MATERIAL AND METHOD

Two-year investigations (2009 and 2010) were carried out in the biological laboratory of the Scientific Tobacco Institute - Prilep. Pots filled with 2:1 ratio of soil and sterilized manure were planted with 50 tobacco plants of the large-leaf variety B 2/93 for each variant. They were grown in traditional way, with the following variants:

1	30 mg /l kinetin	1 treatment
2		2 treatments
3	60 mg /l kinetin	1 treatment
4		2 treatments
5	Ø (control)	untreated plants

The first treatment of plants with prepared solutions containing appropriate concentrations of kinetin was made about a month after planting. The second treatment was made on half of the plants, a month after the first treatment.

Plants were inoculated with a suspension of fresh material, i.e. brown spot from infected leaves. The material was blended in a ratio 10 spots per 100 ml of distilled water and then filtered through a three-layer gauze. The leaves were wounded with karborundum, sprayed with 1 % solution of glucose, and then whole plants were sprayed with the prepared suspension. Inoculated tobacco plants were covered with polyethylene and stored in biological laboratory for 10 days.

Small deviation was made in 2010, when the period of humidification was 13 days, because of the very low temperatures that followed after inoculation.

Intensity of attack was assessed using a six-grade scale (0-5). The leaves were classified in appropriate category :0) healthy leaves ; 1) 1 spot on a leaf 2) 2-5 spots on a leaf 3) 6-10 spots on a leaf 4) 11-25 spots on a leaf 5) more than 1/2 of leafy area infected. Disease index was calculated according to the formula of Mc-Kiney:

$$i = \frac{\sum n \cdot k}{N \cdot K} x100$$

where:

n - number of leaves in the appropriate category
k - category
N - total number of analyzed leaves
K-Total number of categories

Before inoculation, leaf samples were taken from the investigated variants and they were used for making permanent preparations.

RESULTS AND DISCUSSION

Brown spot disease is manifested by the appearance of small brown spots which number is gradually increasing. At the same time, the spots enlarge and coalesce, spreading over the largest part of the leaf (Fig. 1). They become necrotic, with concentric rings surrounded by chlorotic area. In case of severe attack, irregular angular areas are formed and the infected tissue becomes stiff and falls off. These symptoms indicate that the tissue has undergone biochemical changes which deteriorate the quality of raw tobacco.

Fig 1. Symptoms of brown spot disease in large-leaf tobacco

The causing agent of this tobacco disease in R. Macedonia is the pathogenic fungus *A. alternata* (Fig. 2), subdivision *Deuteromycotina* (Fungi Imperfecti) - imperfect fungi, class *Hyphomycetes*, order *Hyphomycetales*, family *Dematiaceae* (Ivanović, 1992).

A. alternata – pure culture

This pathogen has a wide range of hosts. It attacks almost all types of tobacco and all the varieties are, more or less, susceptible. Still, the large-leaf varieties are subject to greater intensity of attack than the oriental and semi-oriental varieties. Among the large-leaf varieties, higher intensity of attack was observed in B 2/93 than in MV 1 (Gveroska, 2006b).

The symptoms of brown spot in this variety in conditions of natural and artificial inoculation are presented in Fig. 3 and 4.

Fig. 3 Brown spot in B2/93 -natural infestation

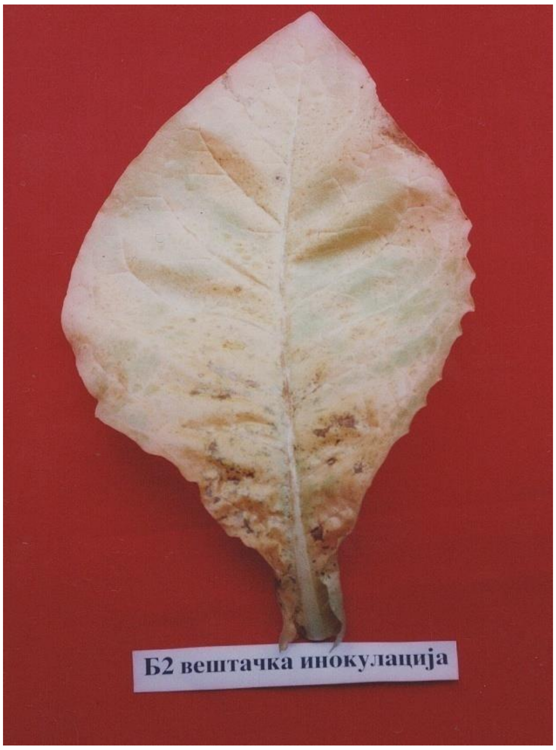


Fig.4 Brown spot in B2/93 – artificial infestation

In 2009, the intensity of disease attack ranged from 14.39% in plants treated with 60 mg/l kinetin (1 treatment) to 26.73% in plants treated with 30 mg/l kinetin (1 treatment). Between the two variants of treatment with 60 mg/l kinetin, higher intensity of attack was observed in those where two treatments were applied (20.85%). The highest intensity of 40.13% was observed in the check variant.

Table 1. The intensity of brown spot disease in 2009

Variant	Total number of analyzed leaves	Percentage of infected leaves	Intensity of attack (%)
30 mg / *l* kinetin 1 treatment	270	70,00	26,73
30 mg / *l* kinetin 2 treatments	165	61,82	26,67
60 mg / *l* kinetin 1 treatment	161	50,31	14,39
60 mg / *l* kinetin 2 treatments	227	51,10	20,85
Ø Check	179	89,38	40,13

In 2010, the intensity of attack in the check variant was much higher compared to 2009. The same situation was observed in the other variants investigated. It was reflected in the percentage of infected leaves (Table 2). Such a high percentage of infected leaves was due to the longer period of moistening. The temperature decrease in this case had no negative influence, because the infection was possible even at very low temperatures, which are not typical for tobacco growing season.

Table 2. The intensity of brown spot disease in 2010

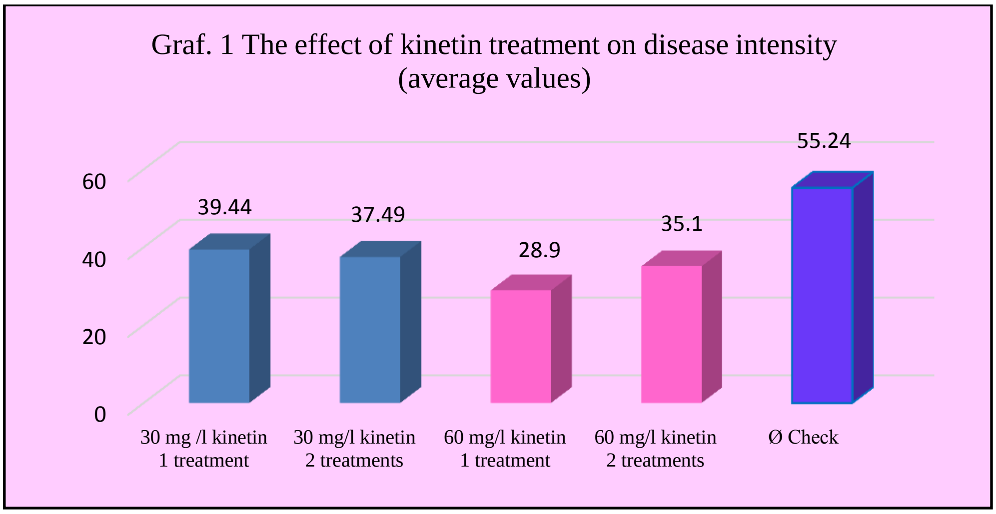
Variant	Total number of analyzed leaves	Percentage of infected leaves	Intensity of attack (%)
30 mg / *l* kinetin 1 treatment	132	94,69	52,15
30 mg / *l* kinetin 2 treatments	138	96,38	48,31
60 mg / *l* kinetin 1 treatment	138	96,37	43,42
60 mg / *l* kinetin 2 treatments	128	93,87	49,35
Ø Check	223	95,54	70,25

Percentage of infected leaves in the check variant is almost equal to that in the investigated variants. However, the number of analyzed leaves is higher and the infected leaves belong to higher categories in the six-grade scale, which has surely influenced the value of disease index in the check variants (Table 2).

In treated plants, the highest intensity of attack was observed with 30 mg/l kinetin - 52,15 %. In plants where two treatments with the same concentration of kinetin were applied, the intensity was slightly lower - 48.31%.

The lowest intensity of disease attack (43.42 %) was recorded in plants with a single treatment of 60 mg /l kinetin. In variants treated twice with the same concentration of kinetin, the intensity of attack in this year was higher than the previously mentioned variant (49.35 %) (Table 2).

According to the average results on influence of the plant hormone kinetin on intensity of brown spot disease in the two-year investigation (Graph 1), the highest intensity of attack was recorded in plants treated with 30 mg /l kinetin (one treatment) and the lowest intensity of in plants treated with 60 mg / l kinetin (two treatments) (Graph 1).



Such an effect was determined in tobacco variety that was the most susceptible to this disease. Accordingly, the treatment with kinetin has a positive effect on reducing the intensity of brown spot attack in tobacco. Little is known about the interaction between plant hormones and the response to various pathogens.

Seilaniantz et al. (2007) investigated the interaction between the virulence of the pathogen and signaling networks involved in the response to various pathogens. The authors reported that the hormones can influence disease outcomes through their effect on SA (salicylic acid) or JA (jasmonic acid) signaling.

Similar statements were reported by Perez et al. (1995) in the treatment with giberellic acid. They claim that there is no direct effect on the pathogen, but rather physiological one, delaying senescence or healing wounded tissue.

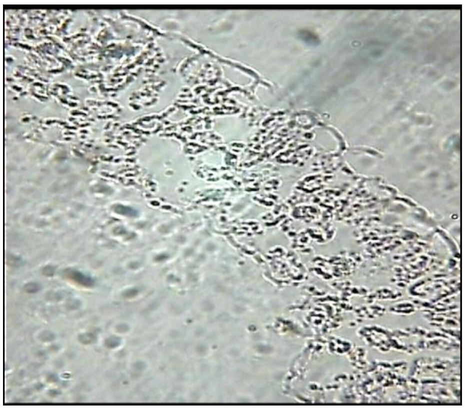


Fig. 5 Tobacco leaf – untreated plants

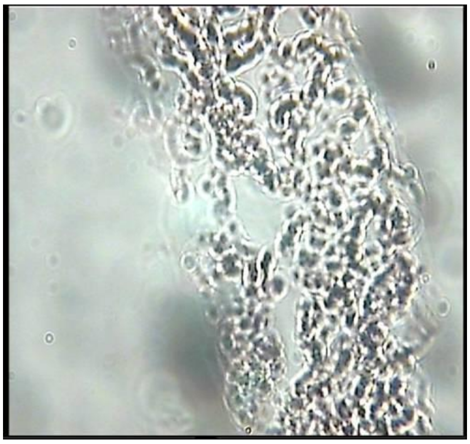


Fig. 6 Treatment with 30 mg/l kinetin

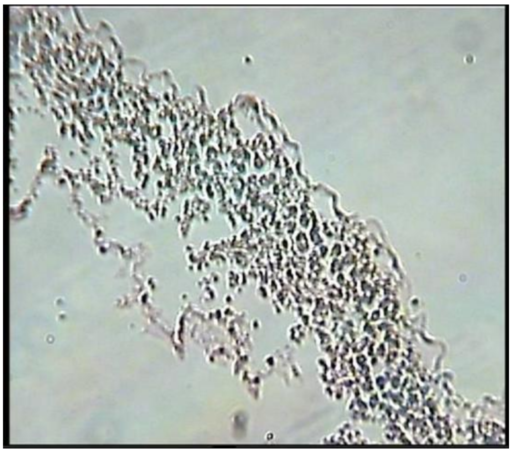


Fig. 7 Treatment with 60 mg/l kinetin

According to Geraats (2003), ethylene can induce resistance to plant diseases. In his experiments, tobacco plants that do not response to ethylene become susceptible to diseases. Ethylene plays important role in maturation of plants and ripening of fruits (Sarić et al., 1987). Although it is a hormone with completely different role, such information can contribute to explaining the role of cytokinins. The pathogen usually attacks the plant in a stage of turgor and uses its nutrients. This plant disease, however, is known as a disease of oldness and weakness and it attacks the old, overmature leaves. In this case cytokinins increase the activity of plants (slowing down the aging process) and thereby reduce the intensity of the disease.

In our investigation, higher intensity of disease was observed in the variant with two treatments of 60 mg/l kinetin, compared to the variant with one treatment. Petrova and Iliev (1990) reported that the increased concentration of cytokinins leads to reduced content of sugars. Brown spot is a disease that attacks the tissues with low content of sugars (Rotem, 1994). Hence, such treatment can lead to decrease of sugar content in tobacco leaves, which on the other hand will increase the susceptibility of the tissue to the pathogen. In support of this statement is the fact that the investigated tobacco variety is characterized by a low content of sugars and highest susceptibility to the disease causing agent.

Haberlach et al. (1978) reported that the increased concentration of cytokinin in tissue culture can have an influence on tobacco resistance. Hypersensitive reaction of the resistant varieties to *Phytophtora parasitica var. nicotianae* is lost and the tissue is poorly colonized with zoospores. However, in that case, too, when kinetin concentration was increased over 2 μм, the resistance was lost.

According to Turner (1986, loc cit. Atanassova et al., 1996), plants have the ability to respond to the pathogen through morphological and physiological changes, by which they can adapt to the changed conditions. In our study, the treatment with kinetin resulted in increase of length of the parenchymal tissue compared to that of untreated plants (Figure 5-7), which certainly reduced the possibility of infection and development of the disease.

Due to the role of cytokinins in plant response to diseases, their application may be useful in inducing resistance to pathogens and extreme temperatures (Atanassova, 1996; Annonimus, 2011). Therefore, the positive effect of treatment with kinetin enables its application in protection from brown spot disease.

CONCLUSIONS

1. The plant hormone kinetin affects the intensity of attack of brown spot disease in tobacco.
2. The highest intensity of attack was observed in plants treated with 30 mg/lkinetin (one treatment). In variant with two treaments with the same concentration of kinetin, the intensity of disease attack was lower.
3. Brown spot disease achieved the lowest intensity of attack in plants with a single treatment of 60 mg/l kinetin. However, higher intensity of attack was observed in plants where two treatments with 60 mg/l kinetin were applied.
4. In both years of investigation, the highest intensity of disease was recorded in plants treated with 30 mg/l kinetin (one treatment), and the lowest intensity was observed in plants treated with 60 mg/l kinetin (one treatment).
5. Treatment with kinetin induces histological changes in tobacco leaves, which leads to reducing the possibility

of infection and development of disease.

6. The positive effect of treatment with kinetin is a good reason for its use in protection from brown spot disease on tobacco.
7. The application of biochemical - physiological products in reducing the intensity of disease attack is a modern and environmentally acceptable measure in protection of tobacco crop.

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Original Scientific paper

CORRELATION BETWEEN THE CONTENT OF HEAVY METALS IN SOIL AND TOBACCO FROM SOME MUNICIPALITIES OF SKOPJE PRODUCTION REGION

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ABSTRACT

The purpose of this research was to determine the correlation between content of Pb, Cd, Zn, Cu, Mn and Fe in soil and tobacco leaves from production regions from Skopje. 39 soil samples and 117 leaves were taken for carrying out this research. Some tested soils have Mn and Fe content above the permitted limits that is probably result of the secondary pollution (traffic and industry). Lead, cadmium, manganese and iron content in tobacco raw from several smaller production sites are above maximum allowable concentration, but are close to the obtained results by several researchers. According to correlation analyses, clay and humus have prominent influence on the content of the Pb, Cd, Cu and Zn in the soil, and weak correlation only with the lead and copper in the tobacco leaves. There is no significant correlation between the content of heavy metals in the soil and in tobacco leaves.

Keywords: soil, tobacco, heavy metals, lead, cadmium, copper, zinc, manganese, iron.

КОРЕЛАЦИЈА МЕЃУ СОДРЖИНАТА НА ТЕШКИ МЕТАЛИ ВО ПОЧВАТА И ТУТУНОТ ОД НЕКОИ ОПШТИНИ ОД СКОПСКИОТ ПРОИЗВОДЕН РЕГИОН

Целта на истражувањето беше да се утврди корелацијата помеѓу содржината на Pb, Cd, Zn, Cu, Mn и Fe во почвата и тутунските лисја од производствените региони од Скопје. 39 почвени примероци и 117 листови тутун беа земени за извршување на ова истражување. Неколку примероци од анализираните почви имаат содржина на Mn и Fe над дозволените граници што е веројатно резултат на секундарно загадување (сообраќај и индустрија). Содржината на олово, кадмиум, манган и железо во тутунската суровина од неколку помали производствени места се над МДК, но се блиску до добиените резултати од страна на неколку истражувачи. Според корелационата анализа, содржината на глина и хумус имаат влијание врз содржината на Pb, Cd, Cu и Zn во почвата и слаба корелација само со оловото и бакарот во тутунските лисја. Не постои значајна корелација помеѓу содржината на тешки метали во почвата и во лисјата тутун.

Клучни зборови: soil, tobacco, heavy metals, lead, cadmium, copper, zinc, manganese, iron.

INTRODUCTION

Today, as a result of modern civilization, the world is facing a serious problem of pollution by harmful substances which certainly include heavy metals. Pollution is particularly critical near: power stations, mines, along the roads, soil fed with fertilizers and pesticides, landfills with industrial and organic wastes, etc. (Pelivanoska, 2007). The intensive use of mineral fertilizers and plant protection products containing Cu and Zn in agricultural production distort the balance of heavy metals in soil, allowing it to penetrate through the chain of animal and human nutrition through plants, through drinking water or through nutrition of farm animals that feed on surfaces contaminated with heavy metals. Heavy metals in form of fine particles of dust can be found in the atmosphere from where they are deposited in soils and water as insoluble compounds of carbonates, sulfates or sulfides. Soil pollution by heavy metals is a serious problem for agricultural production as basis of food production. Heavy metals accumulate in the biomass of successive food consumers. At the end of the food chain using this biomass are people. Heavy metals toxicity for living organisms and humans depends on their quantity for each item that is different and vary in wide range. Tobacco is widespread crop that is produced on every continent of the globe. Because of the specificity of its use, tobacco is treated as food product. Therefore, tobacco is subjected to stringent global criteria in terms of maximum concentration of certain heavy metals such as: Pb, Cd, Cu, Zn, Mn, Fe and others. There are 16 hot spots of contamination identified in Macedonia. Skopje is the capital and largest city in the country, and also an administrative political, economic, cultural, scientific and educational center. The industrial part of the city of Skopje is located on the southeast and east part where most of the tobacco production is placed.

Given that there are several industrial facilities in the Skopje production region that may pollute the soil, water and agricultural plants, our main goal was to determine the content of heavy metals in the tobacco raw produced in this region. Based on the obtained results we can conclude whether oriental tobacco grown in this region, despite its high quality and aroma represents environmentally safe raw material for both domestic and foreign market.

MATERIAL AND METHOD

Sampling locations

Studies were performed in 2008 in Skopje tobacco producing area. Soil and raw tobacco samples were taken from 11 settlements (Table 1). In most of the sampled places, oriental tobacco type Prilep and Yaka are produced. Only small areas produce semi- oriental tobacco type Otlia. Studies were performed of tobacco type Prilep which in this area represents over 97 %. There are 9 municipalities in Skopje production area: Studeničani, Butel, Zelenikovo, Petrovec, Gazi Baba, Aračinovo, Kisela Voda, Aerodrom and Čučer Sandevo. In the industrial part of the municipality Kisela Voda are located several large facilities in the chemical and metal industry (such as „Ohis“, „Tulana“ previous „Tipo“, newly opened factory for steel constructions „Prototip Stil“), glass factory, services and workshops etc. In the vicinity of the organic-chemical industry "Ohis" as one of the biggest polluters is located the cement factory "Usje" now "Titan", that performs excavation in municipalities of Dračevo and Studenicani. Also in this area, the textile factory “Hemteks” was located. The functioning of these industries is a hotspot for potentially environmental pollution in this tobacco producing area.

Soil analysis

39 composite soil samples were collected from the upper layer (0-30 cm) of each field with two replicates. At the same sites where soils were sampled, tobacco leaves with two replicates from three picking belt were collected. First picking belt included lower leaves, second middle and third picking belt, upper leaves. Samples were taken from Skopje municipalities given in Table 1. Preparation of soil samples was performed in accordance with ISO 11464:2006. Soil samples first were air-dried, after that crushed and sieved through a 2-mm sieve. Physical properties such as clay content (Resulovič H. 1971), pH (ISO 10390:2005), total nitrogen (ISO 11361:1995), humus (Tjurin, 1931, validated at the Scientific Tobacco Institute - Prilep, Macedonia), available phosphorus and potassium (*Džamić*, 1996, AL method, validated at the Scientific Tobacco Institute - Prilep, Macedonia) were measured. Concentration of metals was determined using the Aqua Regia (HCl-HNO_3, 3:1) extraction method (ISO 11466:1995) after digestion at 180ºC for 2 h. All reagents were of analytical grade (Merck, Germany). Heavy metals were determined by flame atomic-absorption spectrophotometry (FAAS, ISO 11047:1998). Cadmium and lead were measured by graphite furnace atomic-absorption spectrophotometry (GFAAS).

Plant analysis

Oriental tobacco leaves were washed to remove any adhering soil particles and rinsed with distilled water. After that, leaf samples were placed in paper bags, dried at 75 ^oC for 12 hours and ground using a mortar and pestle. Appropriate blanks were included in all extractions. Samples are mixture from three priming belts from each sample point. Heavy metals were determined by flame atomic-absorption spectrophotometry (FAAS, ISO 11047:1998). Cadmium and lead were measured by graphite furnace atomic-absorption spectrophotometry (GFAAS).

Statistical analysis

The data were statistically analyzed using correlation analysis (Pearson correlation, two-tailed). Results from two replicates were averaged prior to statistical analyses. Correlation analysis was used to establish relationships between physical and chemical characteristics of the soil samples, and between such characteristics and the heavy metal content of oriental tobacco leaf samples from three parts of the plant. Statistical analyses were performed with the aid of SPSS 9.0.

RESULTS AND DISCUSSION

Soil analyses

Agrochemical analyses of soils samples are given in Table 1. According to obtained data from agrochemical analysis, soil samples contain larger quantity of physical clay. Clayey soils are represented with 64.10 %, and loamy with 35.90 %. According to classification these soils are cambisols, very suitable for tobacco production. Only two samples had physical clay of over 80 % and it is advisable that they are omitted for production of the tobacco. High quality oriental type tobacco usually grows in soils with lower organic matter content. It is believed that the optimum content of humus in the soil for production of oriental tobacco is 1-1.5 % (Patće and Uzunoski 1966).

Soils that contain small amounts of nitrogen are also suitable for production of the oriental tobacco types. According to Georgieski (1957), the most adequate soils are the ones where total nitrogen content is from 0.08 % to 0.09 %. From our results, most of the samples had low to moderate content of nitrogen, and are very suitable for tobacco production.

Table 1. Agrochemical parameters of soils from the sampling locations from Skopje production region

Sampling location	Humus %	Total nitrogen %	pH		$CaCO_3$ %	mg/100 g soil		Clay <0,02 mm %	Texture soil classification according to Vigner
			H_2O	KCl		P_2O_5	K_2O		
Studeničani	1.02	0.054	8.01	7.23	2.35	35.85	16.81	29.6	Light loam
Studeničani	0.88	0.046	7.95	7.15	2.69	22.12	20.84	39.8	Medium loam
Studeničani	1.07	0.076	7.51	6.76	0.67	7.88	24.87	49.4	Heavy loam
Studeničani	1.81	0.112	7.46	6.46	9.58	3.05	23.53	70.8	Medium clay
Studeničani	0.87	0.048	7.82	7.08	1.68	62.55	19.83	22.9	Light loam
Studeničani	1.10	0.058	7.79	7.08	1.26	70.94	17.81	23.2	Light loam
Studeničani	1.41	0.067	7.58	6.76	3.19	78.31	29.91	44.5	Heavy loam
Studeničani	0.77	0.039	7.66	6.83	1.01	72.21	22.18	31.5	Medium loam
Morani	0.82	0.043	7.49	6.74	0.0	69.41	19.16	29.9	Light loam
Morani	2.03	0.109	7.51	6.70	20.07	16.53	29.58	77.6	Heavy clay
Morani	1.90	0.062	7.49	6.68	4.79	23.14	24.87	61.5	Medium clay
Morani	1.74	0.086	7.52	6.68	12.03	12.46	20.84	59.8	Light clay
Orešani	1.72	0.092	5.36	4.52	0.0	9.15	30.92	64.1	Medium clay
Orešani	2.10	0.105	4.79	3.83	0.0	7.88	28.90	69.1	Medium clay
Vražale	1.75	0.102	7.14	6.30	19.65	10.68	17.48	67.6	Medium clay
Vražale	2.00	0.086	7.21	6.42	1.51	15.76	18.82	56.3	Light clay
Vražale	1.92	0.086	5.15	4.09	0.0	3.97	20.84	59.7	Light clay
Vražale	1.38	0.084	7.18	6.30	0.0	3.81	17.48	67.5	Medium clay
Vražale	2.51	0.104	5.42	4.46	0.0	6.87	26.22	62.5	Medium clay
Zelenikovo	1.68	0.074	5.34	4.32	0.0	7.37	25.54	63.6	Medium clay
Zelenikovo	2.22	0.094	7.23	6.45	0.0	68.40	62.51	71.3	Medium clay
Pakoševo	2.17	0.137	7.41	6.54	2.52	63.57	67.80	71.1	Medium clay
Strahojadica	1.26	0.049	6.50	5.48	0.0	6.61	24.00	37.0	Medium loam
Strahojadica	1.33	0.069	6.46	5.46	0.0	7.88	29.20	53.7	Light clay
Gumajlevo	1.72	0.101	7.41	6.50	9.24	13.48	33.80	61.9	Medium clay
Gumajlevo	1.73	0.070	7.54	6.78	6.22	63.06	24.60	39.4	Medium loam
Gumajlevo	1.68	0.101	7.26	6.49	2.52	38.65	68.40	70.9	Medium clay
Gumajlevo	3.66	0.187	7.22	6.48	10.59	200.36	107.40	73.4	Medium clay
Gumajlevo	1.05	0.065	7.29	6.45	6.47	20.09	31.90	57.2	Light clay
Smesnica	1.60	0.069	5.95	4.99	0.0	3.71	25.00	54.7	Light clay
Smesnica	1.71	0.092	5.69	4.71	0.0	5.34	30.60	42.1	Heavy loam
Smesnica	1.62	0.099	6.28	5.18	0.0	9.31	34.50	61.5	Medium clay
Smesnica	1.21	0.078	6.27	5.12	0.0	4.73	32.70	60.1	Medium clay
Smesnica	1.83	0.079	6.52	5.54	0.0	39.41	71.50	61.4	Medium clay
Smesnica	1.18	0.068	6.21	5.13	0.0	3.56	18.50	47.4	Heavy loam
Smesnica	1.49	0.087	7.49	6.63	0.76	10.17	30.60	49.3	Heavy loam
Dračevo	2.37	0.145	7.64	6.70	4.12	12.20	30.60	84.6	Heavy clay
Dračevo	1.65	0.108	7.83	6.83	5.38	23.90	35.20	85.1	Heavy clay
Dolno konjare	2.24	0.102	7.75	6.78	2.69	21.61	33.80	49.2	Heavy loam

Based on the results, most of the samples (69.23%) have low organic matter content, and with medium content are 28.20 % of the soil samples. According to these results, except one sample, all sampled soils are adequate for production of high quality oriental tobacco.

Table 2. Trace elements content in soil samples

Sampling place	Pb mg/kg	Cd mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg
Studeničani	14.11	0.367	12.40	45.05	588.7	7684.8
Studeničani	14.33	0.267	14.74	54.34	979.7	7533.2
Studeničani	20.78	0.367	17.38	70.77	1056.9	7776.0
Studeničani	20.11	0.400	20.54	75.95	690.8	7830.7
Studeničani	13.00	0.367	12.00	49.15	470.1	6906.3
Studeničani	14.11	0.367	11.88	73.73	705.2	6790.2
Studeničani	16.33	0.367	20.47	75.38	756.1	7657.3
Studeničani	13.78	0.333	13.38	52.64	505.7	7332.6
Morani	14.00	0.300	12.61	49.50	539.3	7083.0
Morani	15.44	0.367	23.70	74.07	1056.8	7758.8
Morani	17.78	0.400	21.77	75.78	1009.3	7791.3
Morani	15.67	0.333	19.94	68.24	896.5	7737.7
Orešani	22.67	0.333	27.74	83.61	1142.3	7785.3
Orešani	24.44	0.433	38.70	90.45	1072.5	7788.0
Vražale	16.00	0.333	22.90	81.31	978.2	7757.0
Vražale	20.67	0.367	20.83	81.89	1443.0	7799.2
Vražale	18.11	0.267	14.02	78.88	593.9	7828.1
Vražale	18.78	0.367	24.27	84.73	1744.4	7836.6
Vražale	19.11	0.300	16.33	72.65	382.2	7818.0
Zelenikovo	21.33	0.333	34.08	75.34	963.8	7715.0
Zelenikovo	21.11	0.433	37.38	102.03	919.7	7826.7
Pakoševo	22.78	0.333	31.57	92.55	1315.9	7748.1
Strahojadica	15.44	0.367	15.08	72.11	883.9	7780.1
Strahojadica	18.33	0.233	17.80	80.49	827.1	7793.5
Gumajlevo	17.67	0.367	24.00	94.53	931.1	7823.7
Gumajlevo	13.11	0.433	16.20	62.28	1354.4	7658.2
Gumajlevo	14.67	0.367	22.10	92.52	724.2	7828.0
Gumajlevo	22.00	0.367	28.90	111.70	946.1	7791.2
Gumajlevo	12.11	0.367	16.98	72.91	775.3	7752.3
Smesnica	17.00	0.200	17.10	76.01	664.4	7779.9
Smesnica	19.67	0.233	25.34	113.87	783.7	7851.1
Smesnica	20.33	0.333	20.13	92.88	691.4	7788.5
Smesnica	16.67	0.367	18.40	74.78	659.6	7801.7
Smesnica	19.44	0.300	17.77	82.25	631.5	7813.0
Smesnica	15.00	0.300	16.34	76.25	658.5	7801.7
Smesnica	18.33	0.267	17.28	73.65	763.0	7757.8
Dračevo	21.67	0.300	26.77	86.65	1080.3	7807.2
Dračevo	20.11	0.367	25.61	85.02	1027.9	7793.0
Dolno konjare	16.33	0.367	21.58	75.03	1080.9	7790.9

Soil pH is from 5.15 to 8.01 that is also favorable condition for production of high quality tobacco. Most of the samples are non-carbonate soils, (41.02%). 10.26% have high carbonate content of over 10 %. Most soil samples are well supplied with available phosphorus. Very high content of this parameter is found in 11 soil samples. In these areas it is good to avoid tobacco cultivation or to avoid phosphorus fertilization for many years.

Heavy metal content of soil samples is given in Table 2. Based on the results we can conclude that Pb, Zn, Cu and Cd content are bellow the allowable limits (Sluzbeni list RH NN 1/97), therefore they are suitable for tobacco and agriculture production. Part of the sampled soils has Mn and Fe content that is above allowable limits. This is probably due the secondary pollution from the industry and traffic. These sampling spots are mostly in the settlements Oreshani, Smesnica and Gumajlevo that are very small production areas. According to Jekić (1985) Mn content in soil is 200-400 mg/kg (0.02-0.04 %) as total manganese, usually in the form of inorganic reserves. The content of manganese in soil and its accumulation in soil horizons depends on subsoil, soil pH, redox potential, moisture, humus and microbial activity (Stojkoska, 1987). Pelivanoska (2011) in soils for tobacco production found Mn content from 56.03 to 3 143 mg/kg. Our data are consistent with presented literature data.

Iron as one of the most widespread elements of the earth's crust in the tested soils ranges between 6790.2 to 7851.1 mg/kg. Average value of Fe is 7697.32 mg/kg, which is similar as soils from Prilep and Kumanovo area (Mitrikeski, 2000).

Plant analyses

Descriptive statistics of metals content from the oriental tobacco leaves from Skopje producing region in given in Table 3. As we can see average Pb content is from 22.06 mg/kg in middle leaves, 22.01 mg/kg in upper leaves to 22.75 mg/kg in the lower leaves. Variation coefficients for the lower and middle leaves are similar and are around 14.37 %, and in upper leaves it is 20.26 %. Data from these studies are close to the literature data from Pelivanoska (2007) and Golia et al., (2001) but significantly higher than the maximum permissible concentrations (Zakon za bezbednost na hrana, Sl. Vesnik 54, 2004). Average Cd content of the examined tobacco leaves is 2.32 mg/kg. Minimum content of Cd is 0.55 mg/kg in the lower leaves, and maximum 7.6 mg/kg in the upper leaves. According to descriptive statistics and coefficient of variation, cadmium content of tobacco leaves has large variation. This indicates inhomogeneity, and only in small number of localities tobacco has higher than tolerant content cadmium of 3.0 mg/kg (Tso, 1990). Nadkarni (1974) points out that in tobacco we can found 1-2 mg/kg cadmium. Based on our findings, Cd content above permissible limits is detected in some samples from Oreshani, Vrazhale, Zelenikovo, Starhojadica and Smesnica (Tables 1, 2). The survey results show a local increase in the cadmium content that can be explained by the existence of a large number of sources of pollution in the vicinity of the studied sites of the Skopje production region.

Zinc and copper are important trace elements and play an important role in enzyme activity and are involved in the chlorophyll formation (Foy et al, 1978). In plants Zn content is 20-100 mg/kg (Pelivanoska, 2007), and Cu content is to 20 mg/kg (Kastori 1993). Zink deficiency in plants is detected in contents less than 20-25 mg/kg, and Zn toxicity is detected at 400 mg/kg (Jakovlević et al., 1991). Jekić (1985) points out that zinc toxicity occurs very rarely. Average Zn content in the tobacco leaves is from 15.79 to 66.34 mg/kg. Highest content is found in the lower leaves from 6.73 до 68.01 mg/kg Zn, with average of 39.39 mg/kg. Middle leaves have 11.77 to 76.97 mg/kg Zn, or average 39.04 mg/kg. Upper leaves have the smallest content of Zn (17.23-74.73 mg/kg), with average measured values of 38.15 mg/kg. Our data is close to the data of Grabuloski (1985) and Pelivanoska (2010).

The average copper content depending on its location on the tobacco plant is highest in the upper harvests (7.54 mg/kg), lower in lower harvests (7.08 mg/kg) and lowest in the middle harvests (6.88 mg/kg). Variation coefficient is 35.87%-37.8%. According to Pashoski (1980) plant tissue has 1.5-2.0 mg/kg Cu. If the content is less, signs of deficiency appear, and if copper content of dry matter of plants is 20-30 mg/kg, it can adversely affect the yield and quality of the tobacco. Tso (1990) points out Cu content in tobacco of 15-21 mg/kg. The content of zinc and copper in tobacco leaves is lower than permissible limits in all tested samples and there is no danger of their detrimental effect on the quality of tobacco raw.

Table 3. Descriptive statistics of metals content from the Oriental tobacco leaves from Skopje producing region in mg/kg

		Tobacco sampling belt			
Heavy metal	Statistical index	Lower leaves	Middle leaves	Upper leaves	Average
Pb, mg/kg	Average mg/kg	22.74	22.06	22.01	22.18
	Minimum, mg/kg	12.00	11.50	11.50	11.60
	Maximum, mg/kg	28.50	28.50	27.00	28.20
Cd, mg/kg	Average, mg/kg	2.32	2.06	1.94	2.09
	Minimum, mg/kg	0.55	0.70	0.70	0.67
	Maximum, mg/kg	6.70	4.95	7.60	5.83
	CV, %	71.12	64.08	86.60	69.99
Cu, mg/kg	Average, mg/kg	7.08	6.88	7.54	7.52
	Minimum, mg/kg	3.40	2.90	3.25	3.07
	Maximum, mg/kg	12.60	11.90	12.65	12.19
	CV, %	35.87	35.90	37.80	36.27
Zn, mg/kg	Average, mg/kg	39.39	39.04	38.15	38.86
	Minimum, mg/kg	6.73	11.77	17.23	11.91
	Maximum, mg/kg	68.01	76.97	74.73	73.23
	CV, %	40.77	40.96	45.90	36.49
Mn, mg/kg	Average, mg/kg	147.48	105.13	122.02	116.98
	Minimum, mg/kg	30.40	44.50	32.45	39.27
	Maximum, mg/kg	670.70	300.55	578.35	430.14
	CV, %	72.75	45.42	85.11	58.82
Fe, mg/kg	Average, mg/kg	1050.1	821.63	659.83	843.85
	Minimum, mg/kg	302.80	327.15	225.00	284.98
	Maximum, mg/kg	2232	2108.15	2096.55	2145.57
	CV, %	47.19	56.54	59.93	49.05

Referent values for Mn content in tobacco leaves are in broad boundaries from 140 to 700 mg/kg (Pelivanoska, 2007). The survey results showed that the content of manganese is within range of 30.40 mg/kg in Smesnica to 670.70 mg/kg in Vrazhale. Lower harvest leaves have highest manganese content. These values are similar to those obtained by Grabuloski et al. (1985). These authors found that Mn content is higher in the lower leaves, while equally distributed in middle and upper leaves. Toshev (1969) detected the same distribution. According to these authors, Mn content in lower leaves is 145 mg/kg and 100 mg/kg in upper leaves.

Average Fe content of the tobacco raw from Skopje production area is from 284.98 to 2145.57 mg/kg. The highest average content of iron is in a leaves from lower harvest (1050.10 mg/kg), and lowest in the upper leaves (659.83 mg/kg). There is high coefficient of variation from all harvest zones. There are high fluctuations from 47.19% in lower leaves, to 59.93% in upper leaves. Our results are similar to ones from Grabuloski et al. (1985) where Fe content is to 1314 mg/kg. Same authors present data from many researches (Nadkarni, 1974; Jones et al., 1991, Campbell, 2000) that found Fe content in tobacco raw that is from 292 to 572 mg/kg. Pelivanoska (2010) detected values from 22.45 to 438.30 mg/kg. Miceska (2005) also found 0,37 до 0,64 mg/g Fe dry matter of tobacco type Prilep.

Correlation analysis

The content of heavy metals in tobacco leaves is a variable and depends on the conditions in which tobacco is grown, and mostly of the soil composition and its properties (Zaprijanova, 2010). One of the main soil parameters that affect the heavy metals content in tobacco is soil pH (Xian and Shokonifard, 1989, King and Hajjar 1990, Khan et al., 1992, Bell et al., 1992). Golia et al. (2007) found significant negative correlation among soil pH and heavy metal content in oriental tobacco. These authors found no relationship between total content of metals in the soil and in the leaves of oriental tobacco.

Table 4. Correlation among soil parameters and heavy metal concentration in both, tobacco leaves and corresponsive soil samples

Parameter	Pb-S	Cd-S	Cu-S	Zn-S	Mn-S	Fe-S	Pb-L	Cd-L	Cu-L	Zn-L	Mn-L	Fe-L
Humus	**0.612**	0.143	**0.604**	**0.670**	0.276	**0.492**	*0.366*	0.121	*0.356*	0.229	0.176	0.044
pH	**-0.447**	0.301	-0.290	*-0.345*	0.141	*-0.352**	*-0.411*	**-0.720**	**-0.609**	**-0.459**	-0.195	-0.285
Clay	**0.636**	0.134	**0.697**	**0.633**	*0.384*	**0.688**	*0.382*	0.164	*0.406*	0.388	0.164	0.125
Pb-S	1.000	0.025	**0.750**	**0.663**	*0.369*	**0.496**	0.292	*0.381**	**0.564**	**0.450**	*0.347*	0.135
Cd-S		1.000	0.310	*0.011*	0.332	0.038	**0.080**	0.303	0.080	-1.134	0.152	-0.022
Cu-S			1.000	**0.695**	**0.497**	**0.460**	*0.380*	*0.360**	**0.567**	**0.410**	0.312	0.096
Zn-S				1.000	0.303	**0.545**	*0.376*	*0.366**	**0.417**	**0.377**	0.211	0.202
Mn-S					1.000	*0.332**	0.190	-0.115	0.304	0.160	*0.394*	0.036
Fe-S						1.000	0.106	0.311	**0.438**	**0.555**	0.196	**0.465**
Pb-L							1.000	0.279	**0.462**	0.170	0.265	0.174
Cd-L								1.000	**0.744**	**0.657**	0.248	0.224
Cu-L									1.000	**0.768**	**0.462**	0.246
Zn-L										1.000	0.150	**0.469**
Mn-L											1.000	**0.536**
Fe-L												1.000

Pb- S is for soil samples; Pb- L is for tobacco leaves; Bold coefficients represent that correlation is significant at $\alpha = 0.01$; Italic coefficients represent that correlation is significant at $\alpha = 0.05$.

Correlation coefficients of soil parameters and the content of heavy metals in soil and tobacco leaves from Skopje production area are presented in Table 4. Based on the results it can be seen that the content of organic matter (or humus) in soil has a strong correlation with the content of Pb, Cu, Zn and Fe in the soil, and no correlation with the content of Cd and Mn. The content of humus in the soil has no significant relation to the contents of Pb and Cu in raw tobacco. Soil pH has significant correlation with Pb, Zn and Fe content from soil, while in tobacco raw this soil parameter has a strong correlation at the level of 0.01 with Pb, Cd, Cu and Zn, and no correlation with Mn and Fe. This research confirms the findings of the previously mentioned literature data. Pelivanoska et al., (2011) did not find correlation among soil pH and heavy metals in tobacco leaves. Cu content in soil has strong correlation with other soil parameters such as organic matter and clay, with soil Zn, Mn and Fe content and Cu and Zn content in the tobacco. Weak correlation is found among Cu soil content and Pb and Cd content from the tobacco raw. The results of the analysis indicate that the copper content in soil affects the copper content in tobacco leaves. Overall we can conclude that from all examined soil parameters as independent variables, soil pH showed the greatest influence on the content of the Pb, Cd, Cu and Zn, while it shows no influence on the content of Mn and Fe.

The clay and humus have significant influence on the content of the studied elements in the soil, and only weak correlations with lead and copper in tobacco raw.

CONCLUSIONS

According to agrochemical analysis of soils from Skopje production area, we can conclude that they are suitable for tobacco production. Only few soils samples make exception and are less convenient and these are soils where it is difficult to ensure the production of quality tobacco raw.

Pb, Cd, Cu and Zn content in soils are in allowable limits and these soils are suitable for production of tobacco as well as other crops. Part of the sampled soils have Mn and Fe content above reference values that might be result of natural background or pollution of air, industry and traffic. These samples are from Oreshani, Smesnica and Gumajlevo where tobacco production is low.

Results show geographical trends in lead and cadmium content with values above reference limits that can be explained by many sources of pollution in Skopje production area. Zn and Cu content in tobacco leaves are above the reference values, Mn is in the allowable limits and Fe content is above reference values only in few samples.

Soil pH has the most significant influence on the content of Pb, Cd, Cu and Zn, while no influence on the content of Mn and Fe. Clay and humus has most prominent influence on the content of heavy metals in soil samples, and less significant correlation only with lead and copper content in tobacco raw.

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Original Scientific paper

STATISTICAL MODELLING AND ANALYSIS OF NATURAL FERMENTATION PROCESS OF BULGARIAN ORIENTAL TOBACCO VARIETIES BASMI

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ABSTRACT

The tobacco production has a significant impact on economic, demographic and social problems in Bulgaria and other Balkan countries. The quality of the produced fine, light and aromatic oriental tobacco requires studying of the natural fermentation process. The current article presents regression model of the natural fermentation process in seven varieties of oriental tobacco Basmi in Bulgaria, first and second class. This model shows the relationship between tobacco temperature, relative humidity of the environment and time. The three stages of the natural fermentation process: pre-fermentation, active fermentation and "attenuation of the fermentation" are analyzed.

Keywords: oriental tobacco, natural fermentation, correlation, regression model

СТАТИСТИЧКО МОДЕЛИРАЊЕ И АНАЛИЗА НА ПРОЦЕСОТ НА ПРИРОДНА ФЕРМЕНТАЦИЈА НА БУГАРСКИТЕ ОРИЕНТАЛСКИ СОРТИ ТУТУН ОД ТИПОТ БАСМА

Производството на тутун има значајно влијание врз економскиот, демографскиот и социјалниот развој на Бугарија и другите држави на Балканот. Квалитетот на произведените нежни, светли и ароматични ориенталски тутуни бара проучување на процесот на природна ферментација. Во овој труд е прикажан регресиониот модел на процесот на природна ферментација кај седум сорти ориенталски тутун од типот басма во Бугарија, прва и втора класа. Овој модел укажува на врската меѓу температурата на тутунот, релативната влажност на надворешната околина и времето. Анализирани са трите фази на процесот на природна фермантација: предферментација, активна ферментација и „слабеење“ на ферментацијата.

Клучни зборови: ориенталски тутун, природна ферментација, корелација, регресионен модел

INTRODUCTION

Despite of decreasing production quantity in the last 10-15 years tobacco represents more than 12 % from the agrarian export of Bulgaria (Agrarian Academy-Sofia, 2011). In addition, the tobacco production has significant impact on demographic and social problems in some compact regions in the country. Bulgarian oriental tobacco is exported in EC and USA whose markets are very strict to quality issues. This requires scientific research on the technologies for processing oriental tobacco. Natural fermentation generally gives tobacco a more uniform color and a milder taste (Geiss al., 2007). The natural fermentation is being used more frequently for light, fine and aromatic oriental tobacco in order to achieve the balance between its aromatic and taste qualities (Гюзелев, 1978; Мохначев al., 1983).

The current article presents regression model building and analysis of the natural fermentation process of seven Bulgarian varieties of oriental tobacco Basmi.

MATERIAL AND METHOD

Experimental studies are carried out on the natural fermentation process of seven varieties of oriental tobacco Basmi in Bulgaria: Perustitsa-Ustina, Krumovgrad 90, Nevrokop 1146, Nevrokop 261, Melnik 812, Melnik 294 and Djebel 576. The temperature of tobacco y (^{o}C) and relative humidity of the environment φ (%) are measured in time t (days). Table 1 presents the obtained experimental data on the natural fermentation of oriental tobacco Nevrokop 1146, first class.

Table 1. Experimental data on the natural fermentation of tobacco Nevrokop 1146, first class

y (^{o}C)	φ (%)	t (days)	y (^{o}C)	φ (%)	t (days)
6.0	73.0	0	24.0	67.2	101
10.0	76.9	16	25.8	66.6	108
13.2	72.2	31	26.6	65.8	115
14.5	77.5	38	25.3	61.2	122
19.3	68.3	43	26.8	66.2	129
17.2	76.0	52	23.6	73.6	136
17.5	76.5	60	27.2	71.6	143
18.4	80.2	69	26.0	69.2	150
22.8	68.7	73	24.0	62.8	157
22.6	67.0	80	22.6	63.8	164
24.0	70.0	87	21.8	65.0	171
21.6	69.0	94	22.0	63.5	182

Correlation coefficients between temperature of tobacco **y** and time **t** as well as temperature of tobacco **y** and relative humidity of the environment **φ** are estimated (table 2).

Table 2. Correlation coefficients

Variety	Class	Correlation (y, t)	Correlation (y, φ)
Perustitsa-Ustina	I	0,699	-0,540
	II	0,670	-0,523
Krumovgrad 90	I	0,775	-0,610
	II	0,734	-0,582
Nevrokop-1146	I	0,851	-0,633
	II	0,791	-0,616
Nevrokop-261	I	0,768	-0,629
	II	0,723	-0,641
Melnik-812	I	0,762	-0,623
	II	0,796	-0,597
Melnik-294	I	0,782	-0,559
	II	0,784	-0,608
Djebel 576	I	0,675	-0,482
	II	0,683	-0,516

From the obtained results it is evident that:

- There are not significant differences in the values of correlation coefficient regarding varieties and classes as well;
- The effect of time **t** and relative humidity of the environment **φ** on the temperature of tobacco **y** is significant with an opposite sign.

The aim of statistical data processing is to establish the adequate model $y = F(t,\varphi)$ in order to learn more about the relationship between independent variables **(t, φ)** and dependent variable **y**. Besides, the model will give opportunity to study precisely the dynamic of natural fermentation process of the Bulgarian oriental tobacco varieties. The general stepwise regression procedure (Fowler et al., 2000) includes studying **t**, **φ, t^2**, **t^3**, **φ, t.φ, φ^2**. The regression model (1) is the best fit for all seven varieties of oriental tobacco Basmi. The software system SPSS is used for experimental data processing (Field, 2003).

$$y = a_0 + a_1 t + a_2 t^2 + b\varphi \, , \qquad \text{Model (1)}$$

where a_0, a_1, a_2 and b are model coefficients.

The adequacy of the regression model (1) is proved by using F-test at level of significance α = 0.05. This model explains the dynamic of the studied natural fermentation process in all seven varieties of Basmi tobacco in Bulgaria. Table 3 presents obtained R^2 values corresponding to the model (1) for varieties and classes of Basmi tobacco in Bulgaria. The R^2 values range from 0.912 for Melnik 812, II class to 0.962 for Nevrokop 1146, I class.

Table 3. R^2 values and coefficients of the the regression model

Variety	Class	R^2	a_0	a_1	a_2	b
Perustitsa-Ustina	I	0,940	21,9276	0,3650	-0,0017	-0,2255
	II	0,960	21,9895	0,4070	-0,0019	-0,2431
Krumovgrad 90	I	0,936	26,0865	0,2527	-0,0009	-0,2567
	II	0,928	26,3675	0,2643	-0,0011	-0,2628
Nevrokop 1146	I	0,962	19,8991	0,2300	-0,0008	-0,1837
	II	0,962	24,7199	0,2651	-0,0010	-0,2417
Nevrokop 261	I	0,917	30,2143	0,1325	-0,0003	-0,2520
	II	0,923	30,7023	0,1304	-0,0003	-0,2580
Melnik 812	I	0,923	25,7559	0,1746	-0,0007	-0,1787
	II	0,912	20,9945	0,1594	-0,0006	-0,1053
Melnik 294	I	0,932	17,9208	0,1684	-0,0006	-0,060
	II	0,919	25,1069	0,1461	-0,0004	-0,1603
Djebel 576	I	0,948	15,4784	0,4510	-0,0025	-0,1376
	II	0,948	20,5287	0,4170	-0,0023	-0,2020

The model coefficients a_0, a_1, a_2 *and* b as well as R^2 are different for the varieties and classes because of their specific biological features and the following technological factors: initial humidity of the tobacco, bale density and homogeneity of the tobacco class for natural fermentation. It is important that the structure of the regression model is the same for entire varieties and classes.

The effectiveness of the factor "time", based on the regression model of the fermentation process for Nevrokop 1146, I class can be computed as follows:

$$\frac{y}{t} = \frac{19{,}899 + 0{,}23 * t - 0{,}0008 * t^2 - 0{,}184 * \varphi}{t}$$

This estimation shows the effectiveness of the factor “time” during the natural fermentation process. It is calculated for constant value of **φ.** Figure 1 shows that the effectiveness of this factor is declining during the studying period of time. The results of estimations of other varieties and classes of the studied oriental tobacco varieties are similar.

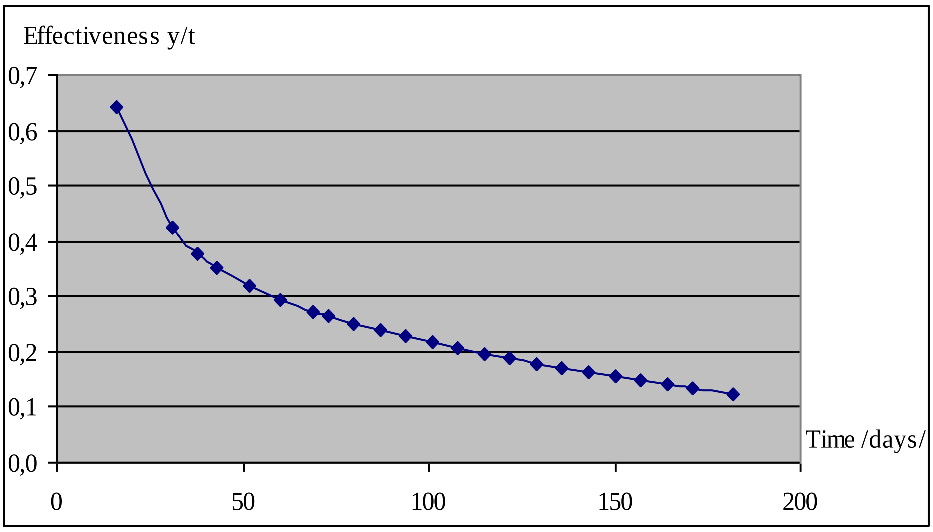

Figure 1. Effectiveness of the factor “time” during natural fermentation of tobacco Nevrokop-1146, I class

Fig. 2 shows the tobacco temperature y (^{o}C) during natural fermentation of tobacco Nevrokop-1146, I class under the regression model. It describes the three stages of the natural fermentation process: pre-fermentation (60-70 days), active fermentation (70-80 days) and “attenuation of the fermentation” (20-30 days). It should be noted that:

- The specific characteristics of all varieties of oriental tobacco affect the duration of the fermentation stages. The studied process is natural, therefore the real climate conditions also affect the duration of fermentation;

- The expert activities continue after the stage “attenuation of the fermentation” with the stage “watching the tobacco”. It is necessary because of two main reasons: a risk of pests and the probability of deformation of the bales. The stage “watching the tobacco” is important in order to save the tobacco quality.

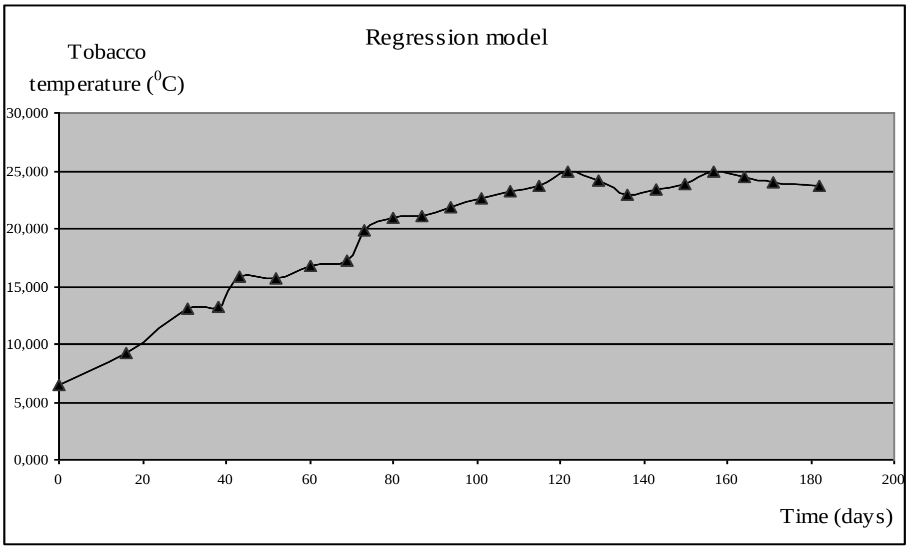

Figure 2. Tobacco temperature (^{0}C) during natural fermentation of tobacco Nevrokop-1146, I class

CONCLUSIONS

The developed regression model explains the relationship between tobacco temperature, time and relative humidity of the environment for the process of natural fermentation of seven oriental varieties Basmi of tobacco in Bulgaria. It describes the three stages of the natural fermentation process: pre-fermentation, active fermentation and "attenuation of the fermentation". The current tendency for applying natural fermentation of oriental tobacco has a positive impact on the development of small and medium tobacco enterprises in Bulgaria and other Balkan countries. The aim of the future scientific work is to assess precisely the effect of natural fermentation on the quality of the cigarettes and last but not least on the health of large group of people.

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Original Scientific paper

DYNAMIC PRESENTATION OF THE ORIENTAL AND SEMI-ORIENTAL TOBACCO PRODUCTION IN THE BALKAN COUNTRIES AND IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Dynamic presentation of oriental and semi-oriental tobacco production in some Balkan countries and data on the share of some tobacco types in the world and in R. Macedonia are presented in this paper.

Tobacco production is traditional activity in some Balkan countries, which also play important role in world trade with this crop.

In some periods the production of oriental and semi-oriental tobaccos showed a downward trend. The average production in the countries that produce and export these tobaccos in the period 2008 - 2012 was 143.360 tons, which is 57.91% of the world production.

In the Republic of Macedonia, however, tobacco production shows an upward tendency, as a result of the reduced subsidies in Greece and the shift of Greek farmers to other crops.

Republic of Macedonia is one of the five leading countries - producers and exporters of oriental and semi-oriental tobaccos. For that reason, the government should continue to subsidize tobacco production in the country, which plays an important role in world trade with these tobaccos.

Keywords: production, oriental, semi-oriental, types, world, structure

ДИНАМИЧКИ ПРИКАЗ НА ПРОИЗВОДСТВОТО НА ОРИЕНТАЛСКИ И ПОЛУОРИЕНТАЛСКИ ТУТУНИ ВО БАЛКАНСКИТЕ ЗЕМЈИ И РЕПУБЛИКА МАКЕДОНИЈА

Во содржината на трудот презентирани се податоци за динамичкиот приказ на производството на ориенталски и полуориенталски тутуни во некои покарактеристични балкански земји, податоци за застапеноста на одредени типови тутун како во светот ,така и воРепублика Македонија..

Имено, производството на горенаведените тутуни е традиционално во некои покарактеристични балкански земји, кои истовремено се и извозници на овие тутуни

Во одреден временски период забележана е тенденција на намалување на нивното производство. Просечното производство кај земјите , производители и извозници на овие тутуни, за периодот 2008-2012 година изнесува 143.360 тони тутун, или тоа во проценти изнесува 57,91%.од светското производство .

Што се однесува до производството во Република Македонија , напротив постои тенденција на зголемување кое се должи како резултат на намалување на субвенциите во Република Грција и премин кон производство на други култури.

Република Македонија им припаѓа на првите пет земји производители и извозници на овие тутуни.За да го задржи производството на овие тутуни на едно задоволително ниво неопходен е факторот субвенционирањето од страна на државата која завзема значајно место во светската трговија на овие тутуни.

Клучни зборови: производство, ориенталски, полуориенталски, типови свет структура

INTRODUCTION

In recent years, the development of world production has been subject to important changes in type structure, depending on the changes of usable value of certain tobacco products and also on taste preferences of the smokers.

The best quality oriental and semi-oriental tobacco is traditionally produced in the Balkan countries, which are also exporters of these tobaccos. In some periods, however, there was a tendency of reduction of these tobaccos.

The average production in these countries for the period 2008-2012 was 143 360 tons, and that is 57.93 % of the world production (247.440 tons).

The Republic of Macedonia is one of the five leading countries - traditional producers and exporters of these tobaccos used in cigarette manufacture.

The upward trend in Macedonian production comes as a result of reduction in world production of oriental tobacco, but the reason for their decline is the abolition of subsidies in Greece and the shift towards the production of olives and development of tourism in Turkey.

Republic of Macedonia can keep tobacco production at a satisfactory level only if the government continues its subsidies for the producers.

The aim of the paper is to show the dynamics of the oriental and semi-oriental tobacco production in some Balkan countries that produce and export tobacco, for the period 2008-2012. In the scope of the study, data will be presented on the structure of tobacco production in Macedonia and in the world, by types.

MATERIAL AND METHOD

The paper presents data on the production of oriental and semi-oriental tobaccos in some Balkan countries and their share by types and varieties, both in the Republic of Macedonia and in the world, for the period 2008-2012.

Several methods were used during the investigation, among which the analytical method was the most commonly used one.

Internal secondary data sources and data from domestic and foreign literature were also used in the investigation.

RESULTS AND DISCUSSION

Production of oriental and semi-oriental tobaccos in some Balkan countries

Tobacco is a very important crop with great economic importance both for the Balkan countries and for the world.

Prior to making any statement on the production of oriental and semi-oriental tobacco, it is necessary to have adequate knowledge about the dynamics of the world production.

Semi-oriental and oriental tobaccos with a relatively high quality have been traditionally produced in some Balkan countries as a product intended for export. These tobaccos are also produced in some Asian countries as well as in former Soviet republics. In this study data will be presented on the production of some more typical Balkan countries.

Production of oriental and semi-oriental tobaccos in the countries that are traditional manufacturers and exporters of these tobaccos are presented in Table 1, Fig. 1.

Table 1. Traditional oriental and semi-oriental tobacco producing and exporting countries

Country	Year 2008	2009	2010	2011	2012 *	Average 2008/2012
Bulgaria	21.900	28.800	29.100	17.000	18.300	23.020
Greece	20.200	20.500	17.500	17.700	18.700	18.920
India	4.400	6.400	9.200	2.700	2.000	5.140
Macedonia	17.000	23.200	26.400	21.000	24.400	22.400
Thailand	10.000	10.000	10.000	7.900	9.700	9.520
Turkey	92.400	80.100	50.300	43.600	55.300	64.340
Total Exporters	**165.900**	**169.100**	**142.500**	**110.900**	**128.400**	**143.360**
Other producers						
Albania	2.500	2.500	2.500	2.500	2.500	2.500
CIS	49.500	47.300	48.400	45.000	42.700	46.580
Iran	2.100	2.500	2.800	1.100	1.300	1.960
Lebanon	7.200	7.200	5.500	5.200	5.200	6.060
Pakistan	15.000	15.000	14.000	15.000	15.000	14.800
P.R.China	12.000	18.000	18.000	22.500	22.500	18.600
Syria	11.000	11.000	10.800	10.800	10.800	10.800
Others	1.900	2.400	3.200	3.100	3.300	2.780
Total other producers	101.200	105.900	105.100	105.100	103.200	104.160
World Total Orient. – Semi-orient.	**267.100**	**274.900**	**247.600**	**216.000**	**231.600**	**247.440**

Source :Universal Corporation, 2013, Master thesis by Klime Damcheski

*Predicted production

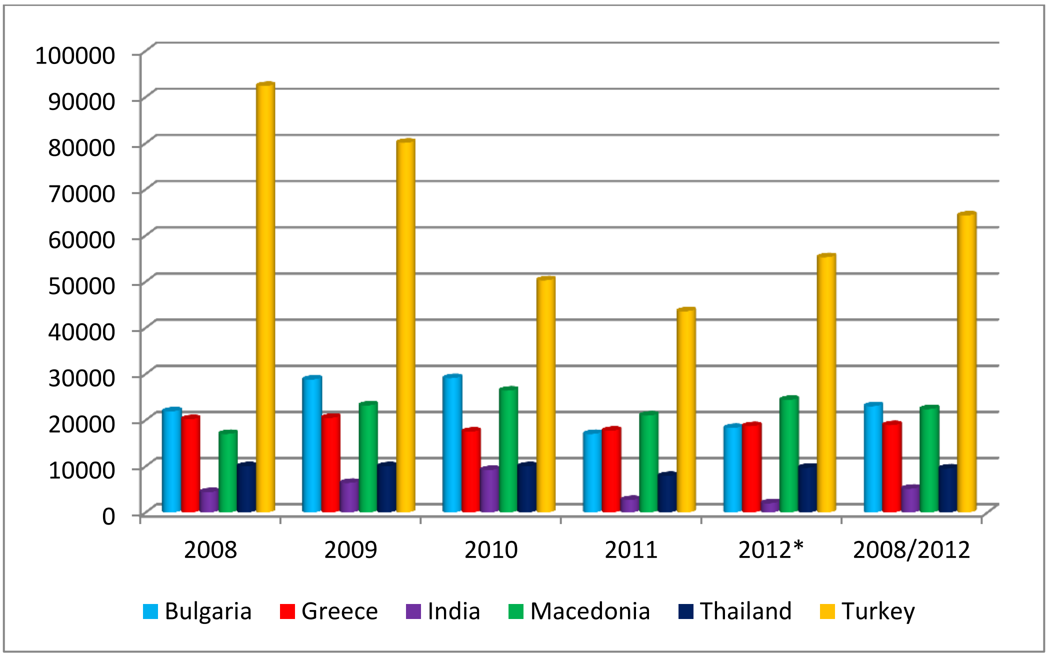

Fig. 1. Traditional oriental and semi-oriental tobacco producing and exporting countries

Data in Table 1, Fig. 1 present the dynamics of production of oriental and semi-oriental tobacco production in some traditional Balkan countries in the period 2008-2012. The average five-year production in these countries was 140.360 tons, or 57.93 % of the world production, while in the other countries it was 104.260 tons or 42.12 %. Turkey is the largest producer with an average production of 64.340 tons, and it is followed by Bulgaria – 23.020 t, Macedonia – 22.400 t, Greece 18.920 t, Thailand -9.520 t and India - 5140 t. From the other countries, higher average production was recorded in CIS, People's Republic of China, Pakistan, Syria and also Albania, with a production of only 2500 tons oriental and semi-oriental tobaccos.

The trend of increasing Macedonian production comes as a result of decreased production of oriental and semi-oriental tobacco in the world. R. Macedonia plays an important role in world trade with this tobaccos and a major exporter. It has two modern factories for cigarette production (in Skopje and Prilep) with a capacity and skilled staff for production of 35.000 t of oriental and semi-oriental tobacco.

The structure of Macedonian and world production of tobacco by type, in tons

In recent years, there were dramatic changes in the structure of tobacco production in the world, mainly as a result of changes in the usable value of certain tobacco products.

Basically, the structure of the world production consists of many types and varieties of tobacco , but the most significant are four commercial types: Virginia , Burley , dark air-cured and fire-cured tobaccos, as well as sun-cured oriental and semi-oriental tobaccos.

Data on the structure of tobacco production in the world and in Macedonia by types are presented in Table 2 and Figure 2.

Table 2. World production of oriental tobacco by types, in tons

Tobacco types	**Year**					**Average**
	2008	2009	2010	2011	2012	
Virginia	4.172.900	4.319.800	4.540.400	4.492.400	4.578.600	4.420,82
Burley	735.000	835.300	749.800	774.900	583.600	735.720
Dark air-cured	134.800	128.900	130.200	134.100	122.000	130.000
Dark fire-cured	54.100	52.800	54.500	54.300	50.700	53.280
Oriental sun-cured	267.100	274.900	247.600	216.000	231.600	247.440
World Total	**5.363.900**	**5.611.700**	**5.722.500**	**5.671.700**	**5.566.500**	**5.587.260**
% Oriental./World **	**4.98**	**4.90**	**4.33**	**3.81**	**4.16**	
% Oriental Maced./World* *	**6,36**	**8,44**	**10,66**	**9,72**	**10,53**	
% Macedonia/World **	**4,98**	**4,90**	**4,33**	**3,81**	**4,16**	

Source: Universal corporation, 2013,

* Predicted production

** Calculations based on data from Universal corporation, 2013

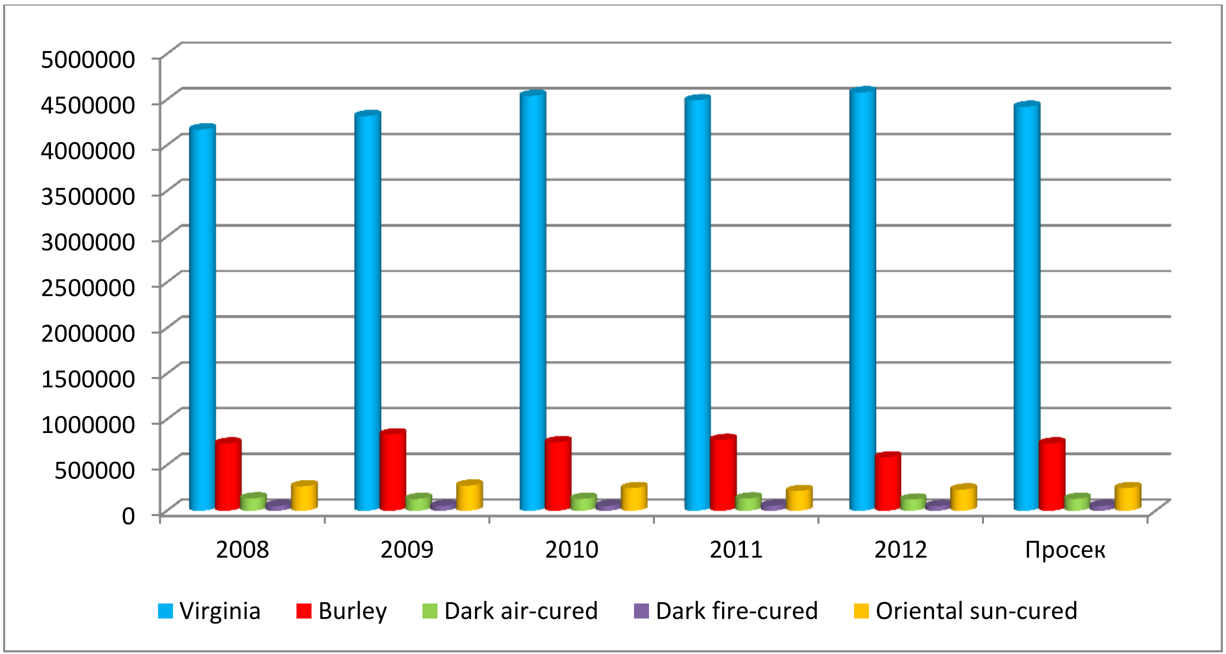

Fig. 2 World production of oriental tobacco by types, in tons

The highest average production of Virginia tobacco in the world was 4.420,820,00 tons, and that of Burley - 735,720 tons, dark air-cured tobaccos - 135,000 tons, dark fire-cured tobaccos - 53,280 tons and oriental sun-cured tobaccos - 246 240 tons. The share of oriental tobacco in the world production ranges from 3.81 % in 2011 to 4.98 % in 2008. Macedonian production, compared to the world production of oriental and semi-oriental tobacco ranges from 6.36 % in 2008 to 10.66 % in 2010 and compared to the total world production it ranges from 0.32 % in 2008 to 0.46 % in 2010 .

Macedonia is traditional producer and exporter of oriental tobacco which is used in manufacture of major cigarette brands in the world and it also plays important role in the global trade of tobacco. It has favorable soil and climate conditions which create conditions for rapid and synchronous growth of different types of tobacco. High quality oriental tobaccos are obtained in areas with nutrient poor soils, with low amount of moisture during the growing season.

With regard to the tobacco production in R. Macedonia in 2008-2011 by types, the total average production of oriental tobacco was 21,265 tons, of which 13,918 tons (65 %) belongs to the type Prilep, 5.272 (25 %) to Yaka and 1.900 tons (9 %) to Basmak. The poorest yield was obtained from the type Dzebel - only 1.75 tons (0.82%) (Table 3 , Fig.3).

Table 3. Production of oriental and semi-oriental tobacco in R. Macedonia, in tons

Year	Types				Total
	Prilep	Yaka	Dzebel	Basmak	
2008	9.065	4.465	46	2549	16.125
2009	13.393	6.294	212	3.321	23.220
2010	19.000	5.000	400	1.000	25.400
2011	14.212	5.327	44	730	20.313
Average 2008/11	**13.918**	**5.272**	**175**	**1900**	**21.265**
%	**65,45**	**24,79**	**0,82**	**8,93**	

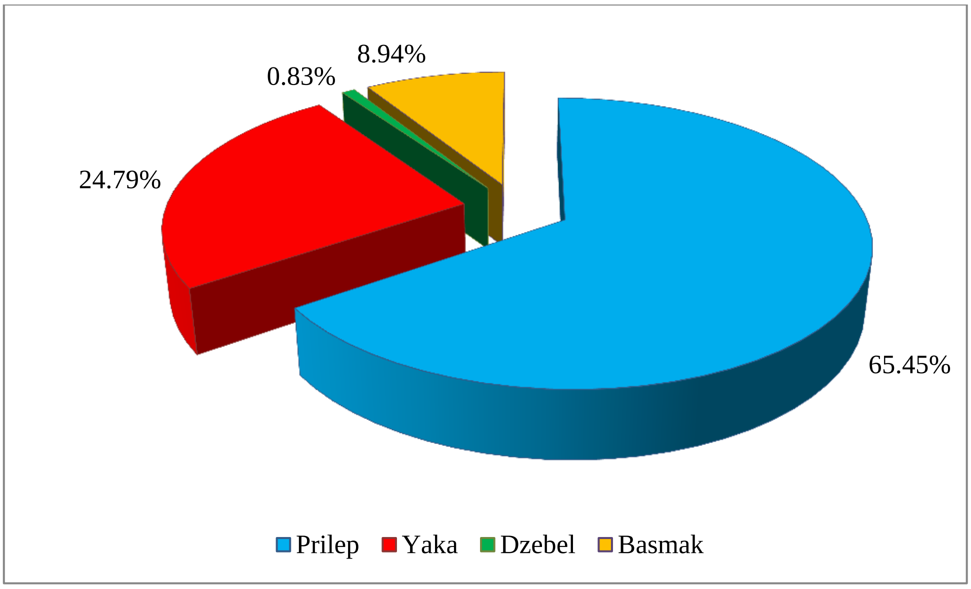

Fig. 3. Production of oriental and semi-oriental tobacco in R. Macedonia, in tons

CONCLUSIONS

Production of oriental and semi-oriental tobacco in the world and in the Republic of Macedonia varies from year to year, in proportions that are not only a result of fluctuations of natural factors. This paper observes the instruments of economic policy that determine the importance of tobacco industry in economic development of the country.

Based on the presented data, the following conclusions can be drawn:

1. High quality oriental and semi-oriental tobacco is traditionally produced in the Balkan countries that are mostly producers and exporters of these tobaccos.
2. The five-year average production of oriental and semi-oriental types in the Balkan countries is 140.360 tons, or 57 % of the world production of these tobaccos.
3. The upward trend in Macedonian production comes as a result of reduction in the world production of oriental and semi-oriental tobacco.
4. The Republic of Macedonia plays an important role in world trade of these tobaccos and it is also one of the major exporters .
5. In the structure of world tobacco production, the following commercial types play the most important role: Virginia, Burley, dark air-cured and fire-cured tobacco and sun- cured oriental and semi-oriental tobacco.
6. The highest average tobacco production in the world was obtained with the types Virginia, Burley, dark air-cured and fire-cured tobacco and sun-cured oriental tobacco.
7. Macedonia has favorable soil and climate conditions for rapid and synchronous development of many types and varieties of tobacco.
8. The total average production of oriental tobacco in R. Macedonia during the investigated period (2008/2011) was 22.265 tons .
9. The highest yield was obtained from the type Prilep. It was followed by the types Yaka and Basmak, and the lowest yield was obtained from Dzebel.
10. R. of Macedonia has two modern cigarette factories and professional staff, with ability to produce more than 35.000 tons of tobacco.
11. Republic of Macedonia can keep tobacco production at a satisfactory level only if the government continues its subsidies for the producers.
12. General conclusion would be that satisfactory amounts of high quality oriental and semi-oriental tobacco can be produced both in the world and in the Republic of Macedonia. After all, favorable natural conditions are a guarantee for further utilization of the available potentials. About 80 million annual net foreign exchange earnings can be realized from exports of the existing types of oriental tobacco.

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Original Scientific paper

EPHESTIA ELUTELLA HÜB. ON TOBACCO

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ABSTRACT

Ephestia elutella Hüb. is one of the economically most important warehouse moths in the world.

Investigations were carried out during 2011-2013 with Prilep and Yaka tobacco from the 2010 crop. Biological and other investigations of *E. elutella* were performed in laboratory conditions, using a standard methodology.

Imagos are small butterflies, with bodies and wings covered with tiny scales. During our investigations their color varied from light grey brown to dark gray brown. They were 6 to 9 mm long, with a 10-13 mm wingspan.

Eggs are elliptical and less than 1 mm long. During laying they are off-white in color and later, prior to hatching, they turn darker.

After hatching, the larvae are also off-white and as they grow older the caterpillars turn brown. The body of the caterpillar is covered with sparse hairs. The length of the adult larva in our investigation was 8 – 9 mm.

Tobacco moth causes damage to tobacco in larval stage. Larvae feed greedily on dry leaf tissue, skeletizing the leaf, and in case of stronger attack only the main nerve remains.

Caterpillars also cause indirect damage. They wrap the leaves with silky threads and inside the leaf they leave black grainy excrements and remains from molting and metamorphosis.

Larval stage lasts from 30 to 35 days and during this long period it causes significant damage.

The pupa is covered, mummy-formed pupa (pupa obtecta), about 7-8 mm in length, light brown in the beginning and prior to eclosion the butterfly turns darker, almost black.

The pupal stage lasts 6 – 7 days.

Direct and indirect damages caused by caterpillars strongly affect the tobacco value and make it unsuitable for further fabrication and export.

Keywords: tobacco, tobacco moth, *Ephestia elutella* Hüb., damage

EPHESTIA ELUTELLA HÜB. НА ТУТУНОТ

Ephestia elutella Hüb. е еден од економски најзначајните штетници во магацините за тутун во светот.

Проучувањата беа изведени во текот 2011-2013 година во лабораториски услови. За материјал беа користени тутуни од тип Прилеп и тип Јака од реколта 2010 година. За истражување на *E. elutella* во лабораториски услови, за биолошки и други испитувања, беше применета стандардна методологија.

Имагата се ситни пеперутки, а бојата на телото при испитувањата варираше од светлосивокафена до темносиво кафена боја. Целото тело и крилјата на пеперутките им се препокриени со лушпенца.

Должината на телото, при нашите истражувања во лабораториски услови, изнесува од 6 до 9 mm, а распонот на крилјата изнесува околу 10-13 mm.

Јајцата имаат елиптичен облик со должина помала од 1 mm. При полагањето се валканобели, а покасно, пред пилењето на ларвата добиваат потемна боја.

Исто така и ларвите по пилењето имаат валканобела боја, а кога ќе пораснат гасениците стануваат кафени. Телото на гасеницата е покриено со ретки влакненца. Должината на возрасната ларва, според нашите истражувања, изнесуваше од 8- 9 mm.

Тутунскиот молец предизвикува штети на тутунот во стадиумот ларва. Ларвите многу лакомо се хранат со ткаеницата на сувите листови тутун, го скелетираат листот, а при посилен напад останува само главниот нерв.

Гасениците предизвикуваат и индиректни штети. Нападнатите листови ги замотуваат со свилекасти конци, а во внатрешноста се наоѓа црн зрнест измет, екскременти, остатоци од преслекувањето и метаморфозата.

Стадиумот ларва при нашите истражувања се одвиваше од 30 до 35 дена и за тој долг период таа нанесува значајни ошетувања.

Куклата е покриена, кукла мумија (pupa obtecta). Должината и е околу 7-8 mm. Таа во почеток е светлокафена, а покасно се менува и пред еклозија на пеперутката станува потемна, скоро црна.

Стадиумот на кукла трае 6 -7 дена.

Директните и индиректните штети кои ги предизвикуваат гасениците, мошне го декласираат тутунот, а ваквиот тутун е непогоден за понатамошна фабрикација и извоз.

Клучни зборови: тутун, тутунскиот молец, *Ephestia elutella* Hüb., штетност

INTRODUCTION

The origin of *E. elutella* is probably Europe, wherefrom it has been transported to all parts of the world, even to Australia. Presently, it is a cosmopolitan species, distributed over a wide area, especially in regions with warm and moderate climate in warehouses, food factories and shops.

It is known under common names tobacco moth, mite, cocoa moth or warehouse moth.

E. elutella is a poliphagous species. Its larvae usually infest the food of vegetative origin. Beside tobacco and cocoa, the pest is also found in chocolate, different kinds of cereals, hop, sunflower, dried fruits and vegetables, peanuts, almonds, nuts, hazelnuts, coffee grains, candies, red pepper, flours and various products made of it (bisquits, bran, pasta), dairy products etc.

E. elutella was identified as a pest on cocoa and as a chocolate pest it was first described in France (Radovanovic, 1961). Presently, it is one of the most dangerous pests in chocolate factories, but it is frequently found in warehouses for flour and dried dough.

Less common tobacco moth food includes meat and carrions, insect collections and dry wood.

Tobacco moth has been defined as economically important pest on tobacco at the beginning of the last century. In Europe it was first described in tobacco warehouses by Mokrzecki in 1909. In the USA it was identified in 1897, but was defined as a pest on tobacco in 1930 (Radovanovic, 1961).

Tobacco moth is spread all over R. Macedonia, mainly in warehouses where small-leaf (oriental) tobacco is stored (Boceski, 1984, 2003; Vukasovic, 1962; Jovanovic, 2001).

Very often this pest can make damage even before tobacco manipulation by the farmers (Todoroski, 1969). The infected tobacco is then transported in bales and stored in warehouses (non-fermented tobacco, tobacco with seasonal fermentation, 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.

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 of biological characteristics and other analysis of *E. elutella*.

A part of tobacco samples was placed in cages to monitor the development cycle of tobacco moth. Also, larvae of *E. elutella* were placed with tobacco leaves in Petri dishes until eclosion of adults.

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

RESULTS AND DISCUSSION

Ephestia elutella Hübner (1796) belongs to the order Lepidoptera- butterflies, suborder Microlepidoptera small butterflies, family Pyralidae, subfamily Phycitinae.

The species is known under several synonyms: *Ephestia amarella* Dyar (1904), *E. icosiella* Ragonot (1888), *Ephestia infumatella* Ragonot (1887), *Ephestia roxburghii* Gregson (1873), *Ephestia uniformata* Dufrane (1942), *Homoeosoma affusella* Ragonot (1888), *Hyphantidium sericarium* Scott (1859), *Phycis angusta* Haworth (1811), *Phycis elutea* Haworth (1811); *Phycis rufa* Haworth (1811), *Phycis semirufa* Haworth (1811), *Tinea elutella* Hübner (1796).

The species development undergoes complete metamorphosis (holometabolia).

Imagos are tiny butterflies and the color of their bodies in our investigations varied from light gray brown to dark gray brown. The whole body and wings of the butterflies are covered with scales (Fig. 1).

According to literature data, the color of the wings is not constant and it can vary from dark gray brown, gray to gray yellowish or even brown and it probably depends on the food in which they develop.

In our investigations, the body length varied from 6 to 9 mm and the wingspan was 10-13 mm.

According to other authors, body length is 10 mm and the wingspan is 14- 20 mm (Radovanovic, 1961; Tanasievic, Simova-Tosic, 1985; Tanasievic, Ilic, 1969).

The fore wings are slightly darker and narrower than the hind wings, elongate-triangular, with characteristic transverse stripes.

Some individuals are striped more noticeably than others.

The hind wings are monochromatic, light gray, with long dark brown fringes in the margins.

Fig. 1 Imago

The head is relatively small and round, with well developed compound eyes and two eyes on the top of the head. The antennae are located between the compound eyes.

The body is elongated and spindle-shaped. The thorax segments are fused and carry two pairs of membranous wings with rich innervation. Legs are adapted for walking, with 5–segmented feet.

Ovipositors of the females are telescopic and feromones are secreted from the glands located in intersegmental membrane of the genital segments.

Butterflies are active and fly during the night. During the daytime they hide in various places between the edges of the cages, among dry tobacco leaves etc. They are very photophillic and move to the top of the cages, toward the light.

Butterflies do not feed. They use the food collected previously, at larval stage.

Fig. 2 Mating

Butterflies are oviparous. Egg-laying starts 1-2 days after mating. According to Radovanovic (1961), the females lay their eggs randomly, single or in small groups, in tobacco leaves, bales or in packing material, sticking them firmly to the base.

Fecundity of a single female is 130 to 200 or even 300 eggs (Radovanovic, 1961; Tanasievic, Simova-Tosic, 1985; Tanasievic, Ilic, 1969).

Shortly after laying their eggs, butterflies die.

The eggs are elliptical in shape, less than 1mm long. Immediately after hatching they are dirty- white and later become darker.

Embryonic development, according to various authors, significantly differs and vary from 3 to 17 days, depending on the temperature (Radovanovic, 1961).

The larvae are eucefalic, with well developed mouthparts adapted for biting. They have short antennae and 6 stemmata (larval eyes). The head and neck shield are brown and the jaws are darker (Fig. 3).

They are polypodous, with 16 legs, three pairs of which are thoracic and five abdominal.

During their lifetime, caterpillars shed their skin five times.

After hatching, they are off- white in color and as they grow up the caterpillars turn brown.

The body of the caterpillar is covered with sparse hairs.

Along dorsal and lateral side of the body they have four rows of black spots, with one tiny hair protruding from each spot.

In our investigations the adult larva was 8 – 9 mm long, while literature data report a length of 9 - 15 mm (Radovanovic, 1961, Tanasievic, Simova-Tosic, 1985; Tanasievic, Ilic, 1969).

Fig. 3 Larva

The larvae are phytophagous and they can cause significant economic damage in tobacco production.

Immediately after hatching the caterpillar feeds on dry tobacco leaves. Larvae feed on leaves from the surface layer of samples (Fig 4).

Caterpillars bite the tissue between leaf veins, and later they eat whole pieces of cured leaves, leaving only the leaf nervation (Fig 5).

According to Krsteska et al. (2011), if tobacco is packed in bales, caterpillars bore through them and make tunnels while feeding on tobacco. The tunnels are found mostly at the surface, not going deeper into the bale.

Unlike cigarette beetle, tobacco moth does not infest ready tobacco products.

Fig. 4 Damaged tobacco leaves, from the surface layer of samples

Caterpillars can also cause indirect damage. The affected leaves are wrapped with silky threads and inside the leaf they leave black grainy excrements and remains from molting and metamorphosis (Fig. 6).

Fig. 5 Damaged tobacco leaves (only the leaf nervation)

At higher humidity in warehouses, damaged leaves create good conditions for development of mold which makes serious damage to tobacco (Radovanovic, 1961; Todoroski 1960).

Damages caused by larvae negatively affect the quality and quantity of tobacco. The products made of such tobacco (cigarettes and cigars) have a bad taste and unpleasant aroma.

Caterpillars are especially greedy when they feed on high quality oriental tobacco, but they do not spare tobaccos of lower quality, too (Radovanovic, 1961; Todoroski 1960).

Larvae prefer Virginia and oriental tobaccos with a high sugar content and low nicotine (Alic-Dzemidzic et al. 1999; Radovanovic, 1961; Todoroski 1960).

Fig. 6 Damaged tobacco leaves, with silky threads and black grainy excrements

In our investigations, the larval stage lasted from 30 to 35 days and during that period it caused serious damage.

According to literature data, the caterpillar stage usually lasts 50-60 days. Depending on temperature conditions, duration of this stage varies significantly. The development can be completed in 25-40 days in favorable conditions, but it can be prolonged up to 240 days when the conditions are unfavorable (Radovanovic, 1961;, Todoroski 1960; Tanasievic, Simova-Tosic, 1985; Tanasievic, Ilic, 1969).

Before the caterpillar turns into a pupa, it spins a silky whitish cocoon (Fig 7).

Such cocoons can be found in tobacco samples or in the corners of the cages, under the filter paper or on dry leaves in Petri dishes.

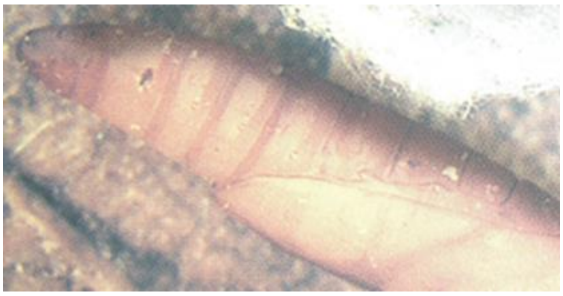

Fig. 7 Pupa

Pupae are also found at different sites in warehouses - in gunny sacks, inside or outside the bale, etc. According to Radovanovic (1961), in severely infected units, tobacco bales can be completely wrapped in a web spun by caterpillars before they turn into pupae.

Fig. 8 Ecloded pupa in a cobweb

The pupae are covered (pupa obtecta) and their appendages are closely bound to the body (Fig. 8). In our investigations they are 7-8 mm long, light brown in color at the beginning and later, before eclosion, the butterfly turns darker, almost black. Pupal stage lasts from 6-7 days.

According to literature data, the pupa can reach 10-12 mm in length (Radovnovic, 1961). Pupal stage lasts from 6 days in favorable weather conditions to10-12 days when conditions are unfavorable (Radovanovic, 1961, Todoroski, 1964).

Tobacco moth prefers warm environment but it successfully develops in areas with moderate climate.

Under suitable conditions, the moth develops and reproduces continuously, so that its eggs, larvae, pupae and adults can be observed simultaneously. In summer, the pest develops faster.

Adult larvae from the last generation are overwintering in tobacco bales, in packing material, hidden in window casings or in various cracks on the walls, floor and other places in warehouses.

Caterpillars turn into a pupa during April. Very often, the pest overwinters in this stage. The first butterflies appear in the warehouses in late April or early May, depending on the temperature conditions and humidity. In areas with warmer climate they appear earlier.

In unheated warehouses, imagos from different generations appear at warmer temperatures – from late April to October.

In different countries the number of generations varies depending on the climate conditions. According to Dimitrov (2003) and Meng et al. (1990) it is considered that the pest has 2-3 generations annually, and in years with early and warm spring and long autumn it can develop up to 4 generations.

Under climate conditions of the Republic of Macedonia, tobacco moth develops 2-3 generations per year.

CONCLUSIONS

The larvae are phytophagous and they cause significant economic loss in tobacco production.

These pests can cause severe losses in warehouses and they have significant economic importance to tobacco quality and quantity.

In our investigation, larval stage lasted 30 - 35 days and during this long period it caused serious damage.

Caterpillars bite the tissue between leaf veins, and later they eat whole pieces of the cured leaves, leaving only the leaf nervation. In the case of stronger infestation, tobacco is skeletonized and only the main nerve remains.

Caterpillars also cause indirect damage. They wrap the leaves with silky threads and inside the leaf they leave black grainy excrements and remains from molting and metamorphosis. Feeding on tobacco, the larvae decrease its market value.

The investigations will result in timely and successful protection of tobacco from this dangerous pest.

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IN MEMORIAM

Prof. Dr. Simeon Karajankov
(1943-2014)

Prof. Dr. Simeon Karajankov, our respected colleague and outstanding tobacco expert passed away on 24.1.2014. He was professor to many generations of students from the Faculty of Agricultural Sciences and Food at the Ss. Cyril and Methodius University in Skopje.

He was born on March 26, 1943 in Kochani, in a working-class family. After finishing the elementary school in his hometown and Secondary Tobacco School in Prilep, in 1962, he was employed as a regional instructor in Yugotutun - the enterprise for tobacco production and processing in Kochani, where he worked until September 1965. The same year he enrolled as a regular student at the Faculty of Agriculture and Forestry (program: Field crop production) and graduated in July 1969.

The further course of life brings him in Prilep, where he formed a family and was employed as a young agronomist in Tobacco Company-Prilep (TCP), first as a technologist in primary production and later in Development sector, due to his interest in research work. His tasks were directly related to development of the Company, particularly in the field of selection and primary production. In those years, TCP was in its golden period of financial, technological and professional expansion and it cooperated with renowned experts from former Yugoslavia and abroad. At that time, Dr. Karajankov, together with other young agronomists, had the honor to meet and work with the renowned tobacco breeders Rudolph Gornik and Igor Bolsunov, especially in the field of large-leaf tobaccos. In that time he began his professional co-operation with Prof. Dr. Dusko Boceski, which continued until the end of their lives. Their visionary view on the future of tobacco production in Macedonia is closely related to the creation of many new oriental tobacco varieties with higher productivity and better quality.

Their concept that no progress can be reached with low yielding varieties and poor mechanization of the production processes was not properly understood in that time. It took more than one decade and a good part of their life for their concept to be applied. Without adequate support from tobacco professionals in that workplace, he decided to move to the former Institute of horticulture and field crop production at the Faculty of Agriculture in Skopje and worked as assistant on a group of tobacco-related subjects, patiently building his career. In 1981 he obtained his M. Sci. degree and in 1995 became a doctor of agricultural sciences from the Faculty of Agriculture in Skopje. In 1996 Karajankov was appointed assistant professor (docent), in 2001 as associate professor and in 2006 as full professor on the subject Tobacco production. After the years of devoted work in scientific and educational processes, in 2008 he retired.

During his working age, Prof. Karajankov performed various functions at the University, such as: member of the Faculty administration, Head of the Department of special farming and Tobacco, member of the Council of Tobacco Institute - Prilep, member of the Approbation Commission for tobacco seed and head of the Tobacco section at postgraduate studies in the Institute, etc.

He was also a member of different government bodies and other organizations: working group for drafting the Law on tobacco and tobacco products in 2006, State Commission for identification of tobacco varieties in R. Macedonia, Commission for preparation of standards for assessment of tobacco quality in buy-out, coordinating body of the Ministry of Agriculture, Forestry and Water Management of R. Macedonia, president of the State commission for graduation exam in the Bureau for education development in the Ministry of Education and Science, etc.

As author or co-author of research papers, Dr. Simeon Karajankov participated in many symposia and meetings in Macedonia and abroad. He took active part in realization of four national and 2 international projects in the field of tobacco. As a result of his active scientific and applicative work in tobacco selection, Dr. Karajankov was a co-author of 10 newly created oriental varieties represented in the list of approved tobacco varieties of R. Macedonia. The quality of the approved varieties is confirmed by the fact that some of them have been included in mass production for years.

Dr. Karajankov has published more than 40 papers in national and international journals. As a mentor or commission member, he participated in the preparation and defense of two specialistic, six master and five doctoral thesis.

His death is a great loss to tobacco science and profession in the Republic of Macedonia. We express our gratitude for its generosity, support and commitment. He was a man of cheerful spirit and he will forever remain in our memory.

May his noble soul rest in eternal peace.

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Dr. Romina Kabranova,
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