UDC 633.71 ISSN 0494-3244

ТУТУН

TOBACCO

Vol. 68	Nº 1-6

BULLETIN OF TOBACCO SCIENCE AND PROFESSION

TUTUN TOBACCO	Vol. 68	Nº 1-6	pp:1-90	PRILEP REPUBLIC OF MACEDONIA	JANUARY JUNE	2018

ISSN 0494 – 3244

Тутун/Tobacco, Vol. 68, N^0 1-6, 3-12, 2018

UDC: 633.71-152:575.22(497.7)"2016/2017"

Original Scientific paper

INVESTIGATIONS OF SAME MORPHOLOGOCAL AND AGRONOMY TRAITS IN ORIENTAL TOBACCO GENOTYPES

Ana Korubin – Aleksoska

.“St. Kliment Ohridski” University – Bitola, Scientific Tobacco Institute – Prilep, str. "Kicevska" bb. Prilep, Republic of Macedonia
E-mail: anakorubin@yahoo.com, ana.korubin@uklo.edu.mk

ABSTRACT

Two oriental tobacco varieties and eight newly created lines were investigated for some major morphological and agronomic traits: height of the plant with inflorescence, leaf number per plant, length of the middle belt leaves and dry mass yield per plant. The trial was set up in the Experimental field of Scientific Tobacco Institute – Prilep in 2016 and 2017, in randomized block design with four replications, using traditional agricultural practices. Measurements of the first three traits were made in the stage of the beginning of flowering, while the dry mass yield was recorded during the treatment of cured tobacco. The results were statistically processed.
The aim of the research is to investigated the morphological traits and yield of Oriental genotypes, to determine the stability of the genotypes through their variability and to stand out the best among the selected material.
From the kit of genotypes we selected three superior lines with a high degree of homogeneity (one of the type Prilep and two of the type Yaka). The coefficient of variation of quantitative traits in these lines was lower than 10%, which is a sign of their stability. After their acceptance by the Commission for recognition and approval of varieties within the Ministry of Agriculture, Forestry and Water Economy of Republic of Macedonia, these genotypes will be released into circulation.

Keywords: tobacco, oriental genotypes, quantitative traits, variability.

ПРОУЧУВАЊА ЗА НЕКОИ МОРФОЛОШКИ И АГРОНОМСКИ СВОЈСТВА КАЈ ОРИЕНТАЛСКИ ГЕНОТИПОВИ ТУТУН

Испитувани беа две сорти и осум новокреирани линии тутун, за поважните морфолошки и агрономски својства: висина на растението со соцветие, број на листови по растение, должина на листовите од средниот појас и принос на сува маса по растение. Истражувањата беа направени на опит по случаен блок-систем во четири повторувања, поставен на опитното поле при Научниот институт за тутун – Прилеп во текот на 2016 и 2017 година. Опитот беше одгледуван со примена на вообичаени агротехнички мерки. Мерењата на првите три својства беа направени во периодот на почеток на цветање, а приносот на сува маса беше евидентиран при манипулацијата на сувиот тутун. Резултатите беа статистички обработени.

Целта на овој труд е да се проучат морфолошките својства и приносот на ориенталските генотипови, преку варијабилноста да се одреди стабилноста на популацијата и да се истакне најдоброто меѓу селектираниот материјал.

Од новосоздадените генотипови издвоивме три посупериорни линии кои покажаа висок степен на хомогеност (една од типот прилеп и две од типот јака). Коефициентот на варијабилност на квантитативните својства им е понизок од 10 % што е знак за нивната стабилност. По нивното признавање од страна на Комисијата за признавање и одобрување на сорти при Министерството за замјоделство, шумарство и водостопанство на Република Македонија, овие генотипови ќе може да се пуштат во промет.

Клучни зборови: тутун, ориенталски генотипови, квантитативни својства, варијабилност.

INTRODUCTION

Selection is a creative activity in which the breeder directs his creativity towards creating varieties more superior than the existing ones. At the Scientific Tobacco Institute - Prilep there is a centuries-old tradition of continuous successive selection work of tobacco varieties of different types, but generally of types Prilep, Yaka, Dzebel and Basmak - more recently. By 2014, the institute owns 10 varieties of Prilep type, 4 varieties of Yaka type, 4 varieties of Dzebel, 3 varieties of the type Otlya, 1 of the type Virginia and 3 of the type Burley (Kotubin - Aleksoska, 2004). Today, the variety fund is drastically increased and exceed the number of about 60 varieties.

The breeders from the Institute have published a number of papers on the topic of tobacco breeding, So for example Aleksoski and Korubin-Aleksoska (2011), studied the mode and level of inheritance of green and dry mass yield per stalk in four parental genotypes (Burley - B 2/93, Suchum - S1, Suchum - S2 and Prilep - P-84) and in their six diallel F1 hybrids, and found positive and negative heterosis. The higher heritability index of both types was recorded for dry mass yield. Mitreski (2012), studied height of the stalk with inflorescence in six oriental tobacco varieties of the type Prilep: P-23, P 12-2/1, NS-72, P 66-9 /7, P-79-94 and Prilep Basma 82. The average values for the trait ranged from 59,3 cm in Prilep P 12-2/1 to 148,1 cm in Prilep Basma 82. The same author in co-authorship with Korubin-Aleksoska (2014), made tasting of the cigarettes composed from the same varieties and informed that they have good degustational properties that are typical for oriental tobaccos. The authors (2015) of the same varieties examined some morphological traits and announce that Prilep Basma 82 had the highest average leaf number, and the lowest length and width of the middle belt leaves. Korubin-Aleksoska and Aleksoski (2013), presented investigations on the inheritance of length, width and area of middle belt leaves in oriental tobacco varieties Prilep (P0 10-3/2), Djebel (Pobeda P-2) and Yaka (YK 48), and the semi-oriental Forchheimer Ogrodowny - FO, including their six F1 and six F2 hybrids. The regression graphs reveal partially dominant type of inheritance of the characters and absence of interallelic interaction. Dimitrieski and Miceska (2015), offer information about new prospective variety of the oriental Yaka tobacco, and as the most perspective point out Yaka b 65 - 82/1. Korubin – Aleksoska (2016), explores heredity of the more important quantitative traits of four parental genotypes (Prilep P 10-3/2, Djebel A 42/3, Yaka YV 125/3, Floria FL-1) and their dialectic F1, F2 and BC1 generations, through genetic components of variance. The heritability is very high, indicating that the studied traits are highly heritable. Korubin – Aleksoska and Ayaz (2016), investigated height of the stalk with inflorescence, leaf number, length of the middle belt leaves and dry mass yield per stalk in five autochthonous tobacco varieties of types: Prilep (P 10-3/2 and P 12-2/1), Djebel (Dj No 1) and Yaka tobaccos (YK 7-4/2 and KY), and five commercial oriental varieties of Prilep tobacco (P-23, P-84, NS-72, P-66-9/7 and P-79-94). Differences between the genotypes in the investigation period were highly significant, which is a sign of their mutual genotypic and phenotypic diversity. The same authors (2016), studied ten oriental tobaccos of the types: Basmak (MK-1, MK-2, MB-2, MB-3, MS-8/1, MS-9/3 and YZ-7), Prilep (Prilep Basma-82), Djebel (Xanthi Djebel-1) and Yaka (YV 125/3), for some more important quantitative traits, and gave precise knowledge of the new type Basmak in Macedonia and the Balkans from genetic, morphological and agronomic aspects. All Basmak varieties are characterized by stability and uniformity as a result of their homozygotness. Dimitrieski, Gveroska (2017), studied some morphological traits, length of the growing season and resistance to powdery mildew disease in some oriental tobacco varieties and lines of the type Prilep. Miceska (2017), investigated some morphological, productional and quality characteristics in four new lines of the type Prilep obtained by generative hybridization (P .l. 14-65/1,P.l.14-66/7, P.l. 123-82/2,P.l. 14-67/7) and the variety P12-2/1 as a standard. Regarding the morphological properties (plant height, leaf number, largest leaf size), all lines showed very low variability, which is an indication of morphological uniformity and stability. Korubin – Aleksoska (2017), studied the oriental varieties in order to obtain data on their tolerance to drought. The highest degree of tolerance to drought was observed in genotypes P - 84 (type Prilep) and P - 2 (type Dzebel). These genotypes can be included in the programs for improvement of the investigated trait.

The aim of this paper is to show the way of assessment the stability of newly created lines of the Prilep and Yaka types, and then selecting the best for placing them in a comparative trial for varietal confirmation by the Commission for recognition and approval of varieties at the Ministry of Agriculture Forestry and Water Economy of R. Macedonia. Tha recognized varieties can be put into circulation.

MATERIAL AND METHODS

As a material for work, two oriental varieties were taken from which one of the type Prilep (P-66-9/7 – Fig. 1) and another of the type Yaka (YK-48 – Fig. 2), as well as eight newly created genotypes, of which four are of the type Yaka (L1 - Fig. 3, L2 - Fig. 4, L3 - Fig. 5 and L4 - Fig. 6), and four of the type Prilep (L5 - Fig. 7, L6 - Fig. 8, L7 - Fig. 9 and L8 - Fig. 10). The variety P-66-9/7 due to its mass application in tobacco production in Macedonia is taken as a control for comparison with line of the type Prilep. YK-48 is a control for lines of the type Yaka.

The experiment was set up in 2016 and 2017 on the Experimental Field of the Scientific Tobacco Institute - Prilep after a random block system in four repetitions. From the complete measurements, for this paper are separated: the height of plants with inflorescence, the number of leaves per plant, the length of the middle-belt leaves, and dry mass yield per plant. The obtained data are statistically processed through parameters of variability of traits and variance analysis (Najceska, 2002).

Figure 1. Prilep, P-66-9/7

Figure 2. Yaka, YK-48

Figure 3. L1 – type Yaka

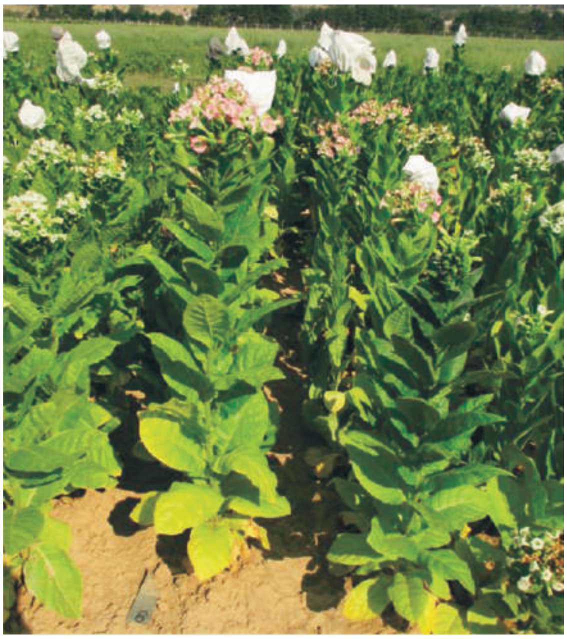

Figure 4. L2 – type Yaka

Figure 5. L3 – type Yaka

Figure 6. L4 – type Yaka

Figure 7. L5 – type Prilep

Figure 8. L6 – type Prilep

Figure 9. L7 – type Prilep

Figure 10. L8 – type Prilep

RESULTS AND DISCUSSION

In order to get acquainted with the genetic stability of the newly created lines, measurements of the quantitative traits were made and on the basis of the obtained values an analysis and their ranking was performed.

From the results shown in Table 1, the highest height of the plant with inflorescence was observed at the orienteal line of the type Yaka L3 (137.5 cm - 2016; 135.7 cm - 2017). This line is higher than the control variety YK-48 (127 cm - 2016; 126.8 cm - 2017), and the difference between them is significant fo 5%. The line L2 had a significantly lower value. Differences between the other variants have no significance. The smallest height is distinguished in oriental line L6 from type Prilep (67 cm - 2017). The newly created genotypes L5 and L7 are higher, and the differences are highly significant. All average values in 2017 are lower compared to those in 2016, due to the fact that 2017 was extremely dry during the vegetation.

The investigations on the variability of the trait in varieties and lines showed low values. The coefficient of variability (CV) ranges from 2.32% (2017) in P-66-9/7 to 7.40% (2016) in L4. The higher values of lines L4 and L5 point to the fact that they need additional successive selection which will enable their homogenization and stabilization. Lower values in 2017 are a sign of proper selection, directed to the stabilization of new genotypes. There is an exception in the populations of L4 and L7, but the difference is minimal and due to undefined environmental factors.

Table 1. Height of the plant with inflorescence (cm)

Genotypes	n	2016			2017		
		$\bar{x} \pm S\bar{x}$ (cm)	σ	CV (%)	$\bar{x} \pm S\bar{x}$ (cm)	σ	CV (%)
1. P-66-9/7 Ø	20	79.60 ± 0.53	2.37	2.98	71.88 ± 0.37	1.66	2.32
2. JK-48 Ø	20	127.00 ± 1.46	6.55	5.16	126.80 ± 0.73	3.27	2.58
3. L1	20	126.30 ± 1.11	4.87	3.94	122.50 ± 0.97	4.33	3.53
4. L2	20	122.00 ± 0.56	6.96	5.71	118.00 ± 0.74	3.32	2.81
5. L3	20	137.50 ± 1.09	4.87	3.54	135.70 ± 0.16	5.18	3.82

6. L4	20	132.50 ± 2.19	9.81	7.40	127.50 ± 2.11	9.42	7.39
7. L5	20	92.25 ± 1.25	5.58	6.04	89.10 ± 1.19	5.31	5.96
8. L6	20	76.20 ± 0.72	3.22	4.22	67.00 ± 0.73	3.26	4.86
9. L7	20	102.80 ± 1.10	4.91	4.78	95.65 ± 1.19	5.31	5.55
10. L8	20	72.90 ± 0.74	3.30	4.53	69.25 ± 0.51	2.30	3.32
		LSD $_{0.05}$ = 9.10 $_{0.01}$ = 16.39			LSD $_{0.05}$ = 7.84 $_{0.01}$ = 14.11		

The highest number of leaves per plant has L3 from type Yaka (59.35 - 2016; 57.85 – 2017), which can be seen from Table 2. The least leaves have the standard variety YK-48 (42.30 – 2016; 42 – 2017). Also, the higher number of leaves in the lines L4 from the Yaka type is highly significant. From the analysis of the number of leaves per plant in the two years of investigation, small differences can be observed, which points to the fact that it is a high-hereditary trait. The greatest difference occurs in P-66-9/7, from which can be conclude that this variety is sensitive to drought stress, and for its successful cultivation is necessary timely watering.

The values of the variability of the traits in the investigated genotypes are very low. The highest coefficient of variability has line L4 (6.16% - 2016; 6.11% - 2017), and the lowest L8 (2.40% - 2016) and YK-48 (2.61% - 2017). In nearly everyone newly created variants, the variability in the number of leaves per plant in 2017 is lower than in 2016, which is another confirmation of the proper selection aimed at stabilizing them.

Table 2. Number of leaves per stalk

Genotypes	n	2016			2017		
		x̄ ± Sx̄ (cm)	σ	CV (%)	x̄ ± Sx̄ (cm)	σ	CV (%)
1. P-66-9/7 Ø	20	57.25 ± 0.40	1.78	3.12	54.00 ± 0.56	2.51	4.65
2. JK-48 Ø	20	42.30 ± 0.35	1.58	3.74	42.00 ± 0.24	1.09	2.61
3. L1	20	45.20 ± 0.27	2.11	4.67	44.10 ± 0.33	1.48	3.36
4. L2	20	44.20 ± 0.40	1.78	4.02	42.65 ± 0.36	1.62	3.80
5. L3	20	59.35 ± 0.69	3.07	5.17	57.85 ± 0.50	2.24	3.88
6. L4	20	54.75 ± 0.75	3.37	6.16	53.85 ± 0.74	3.29	6.11
7. L5	20	51.20 ± 0.56	2.50	4.89	50.15 ± 0.48	2.15	4.29
8. L6	20	48.60 ± 0.47	2.08	4.29	47.35 ± 0.49	2.20	4.64
9. L7	20	46.90 ± 0.33	1.48	3.15	44.50 ± 0.39	1.75	3.92
10. L8	20	42.40 ± 0.23	1.02	2.40	44.50 ± 0.39	1.75	3.92
		LSD $_{0.05}$ = 1.76 $_{0.01}$ = 3.17			LSD $_{0.05}$ = 1.52 $_{0.01}$ = 2.73		

With the longest length of middlebelt leaves, in both years of investigation, the line L7 is characterized (Table 3). The average value of the trait in 2016 is 31.50 cm, and in 2017 it is 30.90 cm, and the difference in comparison with the control variety P-66-9/7 is highly significant. The difference between the genotypes from type Yaka is very small. From the results in the two years of investigations can seen that the differences are minimal, which is a sign of the high inheritance of the trait.

Low variability of the length of middlebelt leaves is an indicator of the stability of the trait, ie the low impact of environmental factors on its magnitude. The highest coefficient of variability has P-66-9/7 (9.61% - 2016; 7.07% - 2017), and the lowest JK-48 (4.97% - 2017). In nearly everyone newly created lines in 2017 have lower variability than those in 2016, which is an indicator of improving their stability.

Table 3. Length of the leaves from the middle belt (cm)

Genotypes	n	2016			2017		
		x̄ ± Sx̄ (cm)	σ	CV (%)	x̄ ± Sx̄ (cm)	σ	CV (%)
1. P-66-9/7 Ø	20	20.40 ± 0.44	1.96	9.61	20.00 ± 0.32	1.41	7.07
2. JK-48 Ø	20	21.65 ± 0.40	1.80	8.30	21.35 ± 0.24	1.06	4.97
3. L1	20	21.55 ± 0.33	1.50	6.96	21.10 ± 0.29	1.30	6.16
4. L2	20	20.50 ± 0.30	1.36	6.63	20.00 ± 0.28	1.26	6.32
5. L3	20	19.80 ± 0.23	1.03	5.20	19.50 ± 0.28	1.24	6.38
6. L4	20	20.70 ± 0.32	1.45	7.02	20.15 ± 0.28	1.24	6.13
7. L5	20	21.60 ± 0.30	1.36	6.28	21.05 ± 0.25	1.12	5.31
8. L6	20	21.55 ± 0.37	1.66	7.69	21.25 ± 0.28	1.26	5.93
9. L7	20	31.50 ± 0.45	2.01	6.39	30.90 ± 0.40	1.79	5.78
10. L8	20	21.15 ± 0.28	1.24	5.84	20.00 ± 0.33	1.48	7.42
		$LSD_{0.05}$ = 0.85 $_{0.01}$ = 1.52			$LSD_{0.05}$ = 1.21 $_{0.01}$ = 2.18		

The highest yield on the dry mass per plant among the parental genotypes has P-66-9/7 (20.15 g - 2016; 19.25 g - 2017), while between the newly created genotypes L7 (22.70 g - 2016; 21.48 g - 2017). The difference in values between the control of Prilep type and the line L7 is high-significant in both years of investigation. Between the lines of Yaka type, significantly higher yield have L1, L3 and L4. The yield of the whole genotype set in 2017 is lower than that in 2016. The data of the dry mass yield in the two years of investigations are shown in Table 5.

Table 4. Dry mass yield per plant (g)

Years	Genotypes									
	P1 Ø	P2 Ø	L1	L2	L3	L4	L5	L6	L7	L8
2016	20.15	17.74	20.28	17.52	21.12	19.75	19.80	19.01	22.70	17.80
2017	19.25	17.49	19.83	17.24	21.10	19.40	19.54	18.92	21.48	17.70
	2016: $LSD_{0.05}$ = 0.51 $_{0.01}$ = 0.91									
	2017: $LSD_{0.05}$ = 0.41 $_{0.01}$ = 0.75									

CONCLUSIONS

- ❖ The highest average height in the two years of investigation has the newly created line L3 (137.5 cm - 2016; 135.7 cm - 2017). In comparison with YK-48, only the lines L3 are significantly higher. The minimum average height has L6 in 2017 (67 cm). The YK-48 variety and Yaka-type lines have a greater height than P-66-9/7 and lines of the type Prilep. Differences in values between the two years in variants are minimal, which is a sign of a high degree of ecological stability.
- ❖ Highest number of leaves per stalk has L3 (59.35 - 2016; 57.85 - 2017). All lines from Yaka type have significantly higher number of leaves than the standard variety YK-48. The lines from Prilep type have significantly lower number of leaves than the control P-66-9/7.

- ❖ The highest length of leaves from the middle belt, in the control variants has YK-48 (21.65 cm - 2016; 21.35 cm - 2017), and in the lines has L7 (31.5 cm - 2016; 30.9 cm - 2017). Dimensions for the length of the leaves in the control and the lines of Yaka type are very similar. The newly created L7 line of the Prilep type has significantly longer leaves than the two control varieties.
- ❖ With highest yield on dry mass per plant among the standard varieties is P-66-9/7 (20.15 g - 2016; 19.25 g - 2017), while among the lines L7 (22.7 g - 2016; 21.48 g - 2017).
- ❖ Two-year investigations for variability of the traits in varieties and lines shows low values. The coefficient of variability (CV) for the height of the plant with inflorescence ranges from 2.32% (P-66-9/7 - 2017) to 7.4% (L4 - 2016), for the number of leaves per plant from 2.61% (YK-48 - 2017) to 6.16% (L4 - 2016), for the length of the leaves from the middle belt from 4.97% (JK-48 - 2017) to 9.61% (P-66-9/7 - 2016). The results indicate high genetic homogeneity, i.e. stability and uniformity of the newly created lines.
- ❖ The line L7 from the type Prilep and L1 and L3 from the type Yaka can be entered in the comparative labors for varietal recognition by the Ministry of Agriculture, Forestry and Water Economy of R. Macedonia.

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ISSN 0494 – 3244

Тутун/Tobacco, Vol. 68, N⁰ 1-6, 13-18, 2018

UDC : 663.97.051(497.7)"2009/2011"
633.71-152.61(497.7)"2009/2011"

Original Scientific paper

TASTING PROPERTIES OF SOME BASMAK TOBACCO VARIETIES

Karolina Kochoska [1], Milan Mitreski [1], Romina Kabranova [2], Ilija Risteski [1]

1.“St. Kliment Ohridski” University – Bitola, Scientific Tobacco Institute – Prilep, Repubilic of Macedonia
2. Faculty of Agricultural Sciences and Food Ss.”Cyril and Methodius”, Skopje, Repubilic of Macedonia

karolina_kocoska@yahoo.com

ABSTRACT

Tasting 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, 2010 and 2011 with one variety of Yaka tobacco (YK 7-4/2 Ø) and three varieties of the oriental type Basmak (MK-1, MB-2, MB-3). Tasting 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 standards and the "Key for taste evaluation of oriental aromatic tobacco”. The aim of this paper was to make comparative investigation on tasting properties of some varieties of Basmak tobacco grown under same conditions and to mark all the differences among them. The best average results regarding the investigated properties were obtained in the variety MK-1 (68.39 points). The Taste panel agreed that all investigated varieties show good tasting properties typical of oriental tobacco, but MB-2 and MB-3 are the most prominent among them. The investigations show that Basmak varieties, due to their good tasting characteristics, can be successfully grown in the Republic of Macedonia.

Keywords: Oriental, Nicotiana Tabacum L., testing, type.

ДЕГУСТАТИВНИ СВОЈСТВА НА НЕКОИ СОРТИ ТУТУН ОД ТИПОТ БАСМАК

Дегустационите својства на тутунот се еден од најважните параметри кои го одредуваат квалитетот на тутунот во пушењето. Испитувањата на овие својства се извршени во 2009, 2010 и 2011 година на една сорта од типот Јака (YK 7-4/2 Ø) и три сорти од ориенталскиот тип Басмак: МК-1, МБ-2, МБ-3. Дегустативните својства на испитуваните сорти тутун беа оценети од страна на Дегустативна комисија од Институтот за тутун - Прилеп, составен од седум члена, со методот на "анонимна дегустација" според одреден стандард и "Клуч за оценка на вкус на ориентален ароматичен тутун", овој труд требаше да направи компаративна истрага за дегустационите својства кај некои сорти тутун Басмак, одгледувани под исти услови и да ги обележат сите разлики меѓу нив. Најдобрите просечни резултати во однос на испитуваните својства беа добиени во сортата МК-1 (68,39 поени). Заедничкото мислење за Дегустацијата е дека сите испитувани сорти покажуваат добри дегустациони својства кои се типични за ориенталниот тутун, меѓутоа МБ-2 и МБ-3 се најистакнати меѓу нив. Истражувањата покажаа дека сортиte од типот Басмак, поради нивните добри дегустативни карактеристики, може успешно да се одгледува во Република Македонија.

Клучни зборови: ориенталски, (Nicotiana Tabacum L.), тестирање, тип

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 declines, but in developing countries it increases. All products that are consumed by humans are in solid or 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 tasting properties of tobacco are the physiological-tasting quality, aroma, strength and flavor of the smoke and they all have a great impact on quality evaluation. According to Sozonovic (1960), the quality of tobacco depends on interrelations among complex chemical matters in tobacco leaf and capability 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 picture of tobacco quality, because the properties. of tobacco and tobacco products are finally completed during 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 evaluated through sensory analysis, 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, methods of smoking, etc. The term degustation (Lat. degustatio – taste and aroma evaluation) denotes systematic investigation of human's response to physical and chemical properties of tobacco smoke. Characteristics of tobacco manifested while smoking are called tasting 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 tasting properties of some varieties of Basmak 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 manifested during smoking (irritation, taste, aroma and physiological strength). The material used for comparative investigation of tasting properties consisted of one variety of Yaka tobacco (YK 7-4/2 Ø) and three varieties of the oriental type Basmak (MK-1, MB-2, MB-3). Raw tobacco from the 2009, 2010 and 2011 crop was used for investigation purposes. The trial was set up at the experimental field of Tobacco Institute - Prilep. Tasting properties of tobacco varieties were evaluated by the Taste panel of Tobacco Institute – Prilep, composed of seven members, by the method of "anonymous sensory evaluation" according to the standard rules and the "Key for taste evaluation of oriental aromatic tobacco". The above evaluation also included investigation on cigarette combustibility. Although it is a characteristic of the raw material rather than smoke, it still needs to be monitored because of its interactive impact on tobacco smoke. Cigarettes made from tobacco of the above four varieties were coded (sampled by belts). The sensory evaluation of the twelve codes was done separately for each year of research. The following tasting properties were analyzed: irritation, taste, aroma, strength, combustion and ash compactness. At the end, the total number of points for the analysed properties was calculated.

RESULTS AND DISCUSSION

Irritation is a sensation felt when tobacco smoke passes through the mouth, throat and nose. Scoring was based on the following sensations: astringency, smoothness, burning, pricking, and harshness of the smoke. Some authors note this feature as a harshness of taste.

- Tasting properties by varieties and belts are presented in Table 1. The points for irritation ranged from 15.02 in the lower zone of YK 7-4/2 to 16.97 in the middle belt of MB-2. According to the scoring, varieties MK-1, MB-2 and MB-3 in the middle and upper belt were characterized by the following sensations: smoothness, no irritation while smoking, no burning, no scratching and no coating of the oral cavity. The points for irritation of the three belts ranged from 15.56 in YK 7-4/2 to 16.29 in MB-2 variety, which is 4.69% more than the check. Varieties that do not irritate during smoking are MB-2 with 16.29 and MK-1 with 16.17 points.

- The taste of tobacco smoke can be mild, bitter and sour, but by combining these sensations it can vary from very pleasant, pleasant to unpleasant.

In investigations by belts, the number of points received for the taste of tobacco smoke ranged from 14.43 in the lowest belt of MB-3 to 16.68 in the middle belt of MK-1. In average, the lowest number of points for this character was given to the check variety YK 7-4/2 (15.16 points) and the highest in variety MK-1 (15.77), which is 4.02% more than the check.

- The aroma of tobacco smoke is primarily a result of combustion of the aromatic complex in tobacco, i.e. resins, essential oils, polyphenols and other constituents. The lowest number of points received for this character was recorded in the lower belt of the check variety YK 7-4/2 (13.12) and the highest number of points was given to the upper belt of the MK-1 (16.34) and MB-2 (16.33).

The average values for this character ranged from 14.58 points in YK 7-4/2 to 15.45 points in MK-1, which is 6.70% more than the check. Upper belts of the varieties MK-1 and MB-2 are distinguished by a very fine, pleasant, penetrating and strongly expressed aroma (bouquet). According to the average values for the three belts, the raw material of these varieties gives a pleasant aroma and a clean refreshing smoke.

- With regard to the strength of cmoke, the lowest score for this character (12.02) was obtained in the upper belt of the check variety YK 7-4/2, and the highest (13.53) in the middle belt of MB-2 variety. Also, the average value for this character ranged from 12.77 in YK 7-4/2 to 13.35 in MB-2. According to the points obtained, these varieties give a medium-strength raw material.

- The average number of points for cigarette combustion was the highest in MB-2 variety (4.02), which is a sign of good combustibility.

For compactness of ashes, MK-1 and MB-2 received equal points (3.92), according to which they are close to tobaccos with light gray and compact ashes.

Table 1. Tasting properties of tobacco by belts (points)

VARIETY	Belts	Average values 2009-2011					
		Irritation	Taste	Aroma	Strength	Combustibility	Compactness of ashes
JK 7-4/2 ∅	lower	15.02	14.67	13.12	13.15	3.73	3.75
	middle	15.88	15.27	15.16	13.15	4.00	4.00
	upper	15.77	15.54	15.46	12.02	3.80	3.73
	Average	**15.56**	**15.16**	**14.58**	**12.77**	**3.84**	**3.83**
	Index	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
MK-1	lower	15.52	14.73	14.25	13.07	3.60	3.77
	middle	16.66	16.68	15.76	13.28	4.00	4.00
	upper	16.33	15.90	16.34	13.13	4.00	4.00
	Average	**16.17**	**15.77**	**15.45**	**13.16**	**3.87**	**3.92**
	Index	**103.92**	**104.02**	**105.97**	**103.05**	**100.78**	**102.35**
MB-2	lower	15.50	14.57	13.53	13.30	3.90	3.83
	middle	16.97	16.10	15.83	13.53	4.10	4.07
	upper	16.40	15.87	16.33	13.23	4.07	3.87
	Average	**16.29**	**15.51**	**15.23**	**13.35**	**4.02**	**3.92**
	Index	**104.69**	**102.31**	**104.46**	**104.54**	**104.69**	**102.35**
MB-3	lower	15.40	14.43	13.25	13.27	3.87	3.63
	middle	16.23	15.73	15.70	13.30	4.03	4.00
	upper	16.23	15.47	15.77	13.03	3.83	3.88
	Average	**15.95**	**15.21**	**14.91**	**13.20**	**3.91**	**3.84**
	Index	**102.51**	**100.33**	**102.26**	**103.37**	**101.82**	**100.26**

Data in Table 2 and Fig. 1 show that total number of points ranges from 62.80 in the lower belt of the check variety YK 7-4/2 in 2009 to 72.20 in the middle belt of MB-2 variety, also in 2009. The average number of points in the three belts the ranges from 63.43 in the lower belt of YK 7-4/2 to 70.60 in the middle belt of MB-2 variety. These data lead to the conclusion that the best tasting properties were recorded in the raw material from the middle belt, then in the upper and finally in the lower belt.

The average number of points from the three belts in the check variant was 66, while in other varieties it ranged from 67 to 68 points (Figura 2).

According to the total number of points, the first three in the rank were raw materials of the varieties MK-1, MB-2 and MB-3, while the check variety YK 7-4/2 ranked fourth.

Table 2. Tasting properties of tobacco varieties by belts (average)

No.	Variety	Belt	Year of investigation			Average Points Rank	Rank	Average points in the three belts	Rank
			2009	2010	2011				
1		lower	62.80	64.50	63.00	**63.43**	11		
2	YK 7-4/2 Ø	middle	69.80	68.01	66.20	**68.00**	6	**66**	**4**
3		upper	67.20	66.86	64.90	**66.32**	7		
4		lower	63.80	65.70	65.30	**64.93**	8		
5	MK-1	middle	71.00	70.14	70.20	**70.45**	2	**68**	**1**
6		upper	70.30	69.51	69.60	**69.80**	3		
7		lower	64.20	64.90	64.80	**64.63**	9		
8	MB-2	middle	72.20	70.20	69.40	**70.60**	1	**68**	**2**
9		upper	71.80	69.20	68.30	**69.77**	4		
10		lower	63.30	64.55	64.00	**63.95**	10		
11	MB-3	middle	70.00	69.60	67.40	**69.00**	5	**67**	**3**
12		upper	69.20	68.30	66.50	**68.00**	6		

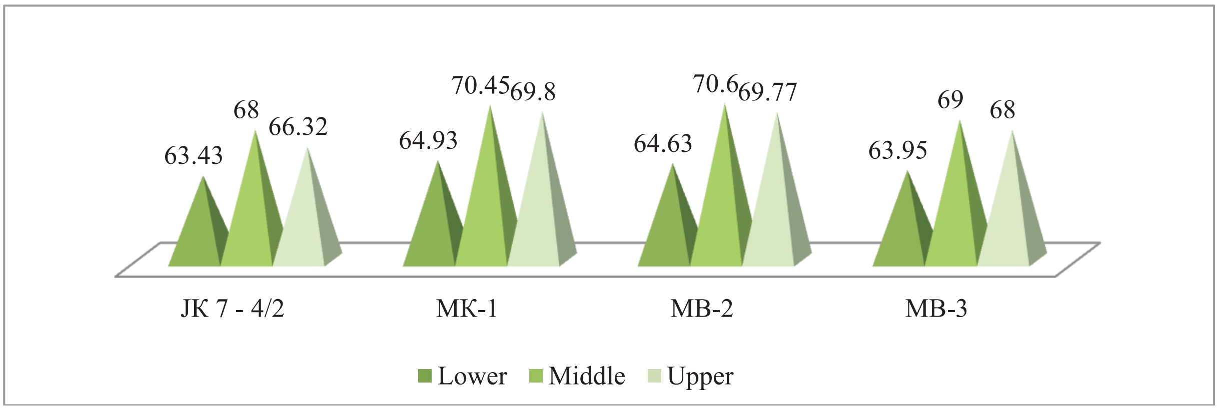

Fig.1 - Tasting properties of tobacco raw by belts (average 2009-2011)

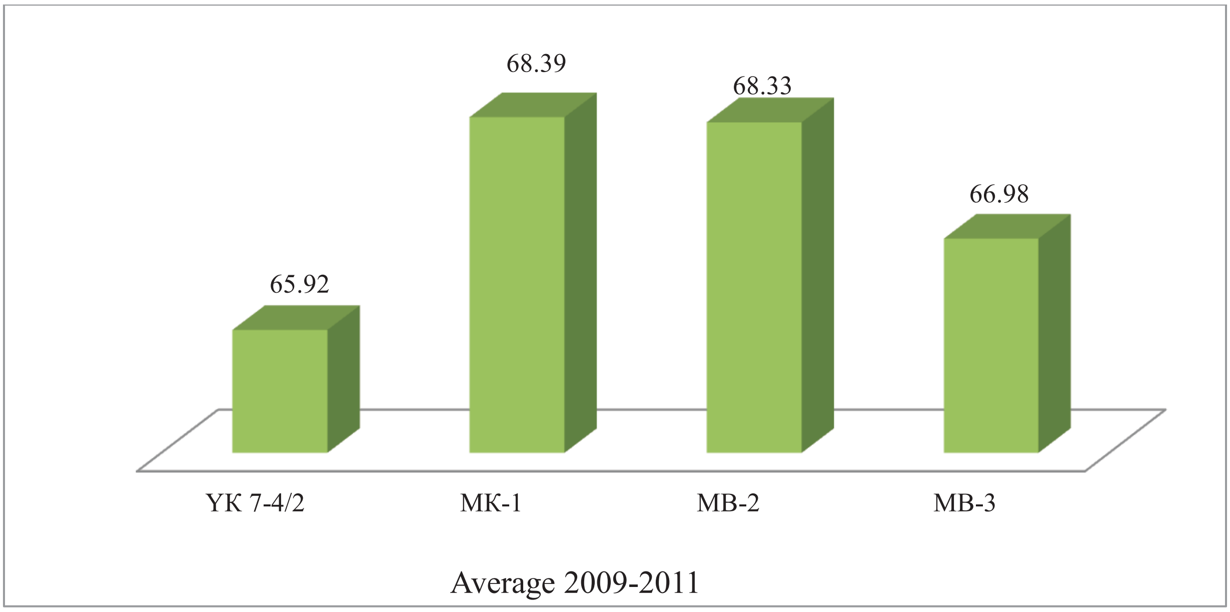

Fig. 2. Average number of points for the three belts

CONCLUSIONS

Based on the three-year investigations of tasting properties with three varieties of tobacco type Basmak and the check variety YK 7-4/2, the following conclusions can be drawn:

- The best variety with regard to tasting properties, which obtained the highest number of points was the check MK-1 (68.39), while the lowest number of points was given to MB-2 variety (68.33).

- General statement of the Taste panel is that all tobacco varieties included in the research show good tasting properties, typical for tobacco of oriental origin, but the varieties MK-1 and MK-2 deserve special emphasis.

- The investigated varieties of Basmak tobacco can be successfully included in blends for production of the highest quality cigarette brands in the world.

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ISSN 0494- 3244

Тутун/Tobacco, Vol. 68, N⁰ 1-6, 19-25, 2018

UDC: 633.71-152.61(497.775)"2016"
Original Scientific paper

MORPHOLOGICAL TRAITS AND VARIABILITY IN SOME FLUE-CURED GENOTYPES

Milan Mitreski[1], Jane Aleksoski[2], Ana Korubin-Aleksoska[1]

[1]. "St. Kliment Ohridski" University – Bitola, Scientific Tobacco Institute - Prilep, str.''Kicevska'' bb. Prilep, R. Macedonia
[2]Bis Promet Agrocentar - Bitola, str.''Kravarski pat'' bb. Bitola, R. Macedonia
Email: anakorubin@yahoo.com

ABSTRACT

The most important morphological traits for flue - cured tobacco (height of the plant with inflorescence, number of leaves per plant and length and width of leaves from the middle harvesting belt) were studied. The investigations are done in 2016 on experimental field in the Scientific Tobacco Institute in Prilep, with four repetitions with following genotypes: MV-1 (CMS) – control, Virginia K-326 and two newly created perspective lines MV-9-7/1 (CMS) and MV-1/14 (CMS).

The aim of this investigations is to show the variability of the most important flue-cured morphological traits, as well as to determine the uniformity and stability of the newly created lines of Virginia tobacco.

The results from the investigations are processed statistically by these parameters: average value ($\bar{x}$), average value error ($S\bar{x}$), standard deviation (S) and coefficient of variability (CV). From the research, we have noted that all genotypes are stable enough, the variability is very low because the coefficient of variability is lower than 10% everywhere. However, the newly created lines are with the slightest variation in the tested traits. Among other things, they are on average with the largest leaves, which is a positive characteristic in the creation of tobacco of this type.

Keywords: tobacco, flue-cured, morphological traits, variability.

МОРФОЛОШКИ ОСОБИНИ И ВАРИЈАБИЛНОСТ КАЈ НЕКОИ FLUE-CURED ГЕНОТИПОВИ

Во трудот се прикажани проучувања за најважните морфолошки својства кај flue - cured тутуните (висина на растението со соцветие, број на листови по растение, должина на листовите од средниот појаси и широчина на листовите од средниот појас). Испитувањата се извршени во 2016 година во Научниот институт за тутун – Прилеп, на полски опит во четири повторувања, со следниве сорти: Вирџинија МВ-1 (ЦМС) – контрола, Virginia К-326 и две новокреирани линии: МВ-9-7/1 (ЦМС) и МВ-1/14 (ЦМС).

Целта на овој труд е да се прикажеме варирањето на поважните морфолошки својства кај flue-cured тутуните, како и да се одреди униформноста и стабилноста на новосоздадените линии од типот вирџинија.

Резултатите од испитувањето се обработени варијационо-статистички преку параметрите: средна вредност ($\bar{x}$), грешка на средната вредност ($S\bar{x}$), стандардна девијација (S), варијационен коефициент (CV - %)и варијациона ширина (WV). Од истражувањата потврдивме дека испитуваните сорти се многу стабилни, односно варирањето на морфолошките својства е многу мало, бидејќи варијациониот коефициент секаде покажа вредност помала од 10 %. Истакнуваме дека новосоздадената линија МВ-1/14 – ЦМС има најмало варирање, истовремено има и најголеми листови, што е позитивно својство кај крупнолисниот тутун од овој тип.

Клучни зборови: тутун, flue-cured, морфолошки својства, варијабилност.

INTRODUCTION

In the agricultural economy of the Republic of Macedonia, tobacco belongs to the group of strategic cultures. Tobacco cultivation ranges between 12,000 and 15,000 hectares with an annual output of 20 to 25 million kilograms of quality tobacco raw material. From the aspect of the type of representation, over 95% of these areas belong to the oriental types of Prilep and Jaka (type Prilep is most present in our country), while the large (Virginia and Burley), in recent years, are almost encountered in the fields. By the end of the nineties of the last century, although to a lesser extent, they were growing in our country (an average of 1500 tons per year by the Virginia type only), which reduced the import of these tobacco raw materials - the main components in the harmanes for making the more popular "American bland" cigarettes. The type of Virginia in the composition of these cigarettes accounts for 45 to 65% (Mickovski, 2004). According to the author, the largest producers of tobacco of this type in the world are: China, USA, Brazil, Argentina, Italy, Spain, Zimbabwe and Oceania. It is important to point out that in the Republic of Macedonia there are regions with excellent conditions for growing quality tobacco of the type Virginia which is a challenge for its return to production. Having in mind the aforementioned, recent scientists from the Scientific Tobacco Institute - Prilep, create and select varieties that will meet the requirements and needs of the cigarette factories. Therefore, the object and purpose of this study is the variation of the most important morphological properties of Virgina tobacco type varieties that have good combination skills, which is a condition for creating new, more productive and better quality than existing ones. This would bring back the interest of the producers for this large type of tobacco, which is constantly requested in the world market, after which the financial effects would be positive and guaranteed to the satisfaction of everyone in the tobacco industry of the Republic of Macedonia.

MATERIAL AND METHOD OF WORK

We test were conducted on two varieties (MV-1 CMS – Fig. 1. as a control variety and Virginia К-326 – Fig. 2.) and two newly created Virginia-type line (MV-9-7/1, CMS – Fig. 3. and MV-1/14, CMS – Fig. 4.). The control variety MV-1 CMS (male-sterile) was created at the Tobacco Institute - Prilep. The Federal Classification Committee of the former Yugoslavia was recognized in 1987. Since then, by the end of the nineties of the last century, it was the only variety of Virginia-type tobacco that was produced in Macedonia and in certain regions in Serbia and Montenegro. The K-326 varietie is fertile and originate in the United States. In the past, it was fairly represented in Virginia production around the world, and to a lesser extent it is now produced in the home country and some countries in South America. This varietie as well as the control MV-1 CMS are still relevant due to their quality and other positive properties, and are used in the selection for the outward processing of tobacco of this type. The new lines MV-9-7/1 - CMS and MV-1/14 – CMS were created at the Scientific Tobacco Institute - Prilep by crossing and selecting Virginia tobacco varieties. The experiment was set in the experimental field of the Scientific Tobacco Institute- Prilep in 2016 on diluvial-colluvial soil in four repetitions. The tobacco is seeded manually at 80x50 cm. For basic fertilization, NPK fertilizer with combination 8:22:20 is used in quantity of 350 kg/ha. During the vegetation, the necessary agrotechnical operations are performed for ensuring normal growth and development of the plants (feeding with nitrogen fertilizer, trapping and treating tobacco according to the program of the Scientific Tobacco Institute - Prilep for protection from diseases, pests and weeds). The tobacco in the experiment is sprinkled 3 times with an average level of 400 m^3 / ha water. We note that the 2016 production was assessed as a good year for tobacco production. The studies on morphological properties (height of the plant with inflorescence, number of leaves and length and width of the largest leaf from the middle belt of the plant) were carried out in the field in the "full blossoming" phase of the tobacco by standard methods in the selection, is the mean value ($\overline{x}$), for each property is determined based on 15 randomly selected plants of each variety in the experiment. The obtained data from the measurements are statistically processed through parameters of property variability (Najcevska, 2002), and the results are shown in tables.

Fig.1. MV-1 (CMS)

Fig. 2. K-326

Fig. 3. MV-9-7/1 (CMS)

Fig. 4. MV-1/14 (CMS)

RESULTS AND DISCUSSION

The examined morphological features of the Virginia-type tobacco varieties belong to the group of quantitative properties. They are of great importance in the genetics and selection of tobacco because they determine (identify), the type and the varieties they belong to. In addition, the number and size of the leaves determine the yield and quality of tobacco. They are dictated by their own genotype but are also dependent on soil-climatic conditions in the region where it is grown, as well as from applied agrotechnical operation during vegetation. We have mentioned above that we present the results of the researches in tables, especially for each property for better visibility, comparison between the investigated varieties and the new line and drawing appropriate conclusions.

Height of the plant with inflorescence

The height of tobacco plants is a great feature. Uzunoski (1985), according to this characteristic, divides the tobacco varieties into three groups: 1. Varieties with low growth, the height of which is up to 70 cm (Prilep, low spot); 2. Medium growth varieties with height of plants with inflorescence, which varies from 70 to 130 cm (Jaka, Dzebel); 3. Varieties of high growth, with a height above 130 cm (Virginia and Burley). Risteski and Kochoska (2004), in their research on 6 varieties of tobacco of the Virginia type, point out that the height of the plant with the inflorescence ranged from 159 cm in the variety V-27/01 to 192 cm in the MV-1 CMS, which was and the highest. The results of our trials for this morphological feature are shown in Table 1.

Table 1. Height of the plant with its inflorescence (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV%	WV
MV-1 (CMS) Ø	15	190	1.76	6.81	3.59	180-200
Virginia K-326	15	155	0.97	4.32	2.79	150-165
MV-9-7/1 (CMS)	15	150	1.46	6.52	4.35	140-160
MV-1/14 (CMS)	15	180	1.24	4.81	2.66	170-185
	LSD 0.05 = 5.47 0.01 = 7.68					

The table shows that the average height of 190 cm is the highest control class MV-1 CMS, and with 150 cm the lowest is the MV-9-7/1. In terms of variation, it can be noted that the varieties tested are stable in this capacity, since the value of the variation coefficient (CV%) is low (everywhere is below 10%) and ranges from 2.66% to the new line MV-1/14 CMS up to 4.35% in MV-9-7/1 (CMS).

Number of leaves on the plant

The number of leaves of the plant depends on the genetic structure of the variety and the conditions of cultivation. It is thought that the number of tobacco leaves is one of the most stable quantitative properties. The number of leaves is a variegated feature and represents a high-yielding quantitative property (Atanasov, 1972). In their research on the number of leaves of 7 Virginia tobacco varieties Risteski and Kochoska (2014), found that the highest number of leaves of one plant is distinguished by the variety V-88/09 CMS, which on average for the two years of examination had 33.3 leaves, while the least leaves has the K-326 variety (28.8 leaves). Since the multi-year research of 5 Virginia tobacco varieties in the Republic of Croatia, it has been determined that the average number of leaves in the three newly created varieties is as follows: Kutjevo (H 30), 23 leaves, Drava (H 31), 22 leaves and Bilogora (H 32), has 22 leaves (Devcic and Triplat, 1982). In our research with the highest number of leaves (Table 2), is the variety MV-1 CMS, where on average we counted 28 leaves of the plant and with at least K-326 with 25 leaves.

Table 2. Number of leaves

Varieties	n	x̄	Sx̄	S	CV%	WV
MV-1 (CMS) Ø	15	28	0.32	1.25	4.48	26-30
Virginia K-326	15	25	0.29	1.31	5.23	22-27
MV-9-7/1 (CMS)	15	26	0.27	1.21	4.61	24-28
MV-1/14 (CMS)	15	27	0.23	0.88	3.26	25-28
	LSD 0.05 = 0.89 0.01 = 1.25					

Regarding the variability of this feature, the statistical parameters have shown that it is very small. The coefficient of variation is from 3.26% on the line MV-1/14 CMS to 5.23% in the K-326 variety. The standard deviation (S) is also small and ranges from 0.88 to 1.31 leaves.

Length of the leaves from the middle harvesting belt of the plant

The length of the leaves in all types of tobacco is an important feature because it is closely related to the quality of the tobacco raw material. In the type Virginia, the larger the leaves the higher the yield and the better the quality is. Boceski (2003), points out that the length and width, and therefore the surface of the leaves during curing, are reduced by 20 to 30%, which is very important in the technology of processing and processing of tobacco. Risteski and Kochoska (2014), examining the length of the leaves in 7 domestic and foreign varieties of the Virginia type, state that with the largest 10 leaves (the 10th leaf is the largest of the plant) is the variety V-79/09 CMS, with the length of the leaf of 62.4 cm, and with the smallest leaves, with a length of 44.3 cm is the Delcrist variety. The K-394 variety is on the fifth place with an average length of the 10th leaf of 52.5 cm. The results of our measurements and the variability of this property are shown in Table 3.

Table 3. Length of the leaves from the middle harvesting belt (cm)

Varieties	n	x̄	Sx̄	S	CV%	WV
MV-1 (CMS) Ø	15	60	0.32	1.22	2.04	58-62
Virginia K-326	15	56	0.27	1.21	2.15	54-58
MV-9-7/1 (CMS)	15	58	0.26	1.19	2.05	56-60
MV-1/14 (CMS)	15	62	0.25	0.96	1.55	60-63
	LSD 0.05 = 2.98 0.01 = 4.18					

The subject varieties have long leaves, which is characteristic for large-scale tobacco, including the type Virginia. The longest leaves of the plant have the new line MV-1/14 CMS ($\bar{x}$=62 cm), with the smallest is K-326 ($\bar{x}$=56 cm). The standard deviation is from 0.96 in the new line MV-1/14 (CMS), to 1.22 in the control, with a CV% within 1.55 to 2.15%, so it can be said that the variability is meaningless.

Width of the leaves from the middle harvesting belt of the plant

The width of the leaves and the length depends on the soil and climate conditions and technical measures during the cultivation of tobacco. Drazic et al. (2012), examining the morphological properties and yield of 12 newly created genotypes (7 in the Republic of Serbia and 5 in Republic of Macedonia), concluded that with the widest leaves the variety Hevesi 9 ($\bar{x}$=36 cm) was standard in the experiment. Of the new genotypes, with an average width of the largest leaf of 34 cm, the V-814 took the second place, while with a width of 24 cm, the genotype V-30/09 was in the last place. From our examinations we obtained the results we present in Table 4.

Table 4. Width of the leaves from the middle harvesting belt (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV%	WV
MV-1 (CMS) Ø	15	31	0.31	1.19	3.81	30-34
Virginia K-326	15	28	0.26	1.18	4.21	26-30
MV-9-7/1 (CMS)	15	30	0.26	1.16	3.85	28-32
MV-1/14 (CMS)	15	32	0.33	1.28	3.99	30-34
	LSD 0.05 = 1.26					
	0.01 = 1.77					

The table shows that on the average with the widest leaves is the line MV-1/14 CMS ($\bar{x}$=32 cm) and it is followed by the control with 31 cm, MV-9-7/1 with 30 cm, while with the narrowest leaves is K-326 with width of the largest leaf of 28 cm. The standard deviation ranges from 1.16 to 1.28, while CV% of 3.81 in control to 4.21 at K-326.

CONCLUSIONS

From the research we have got the following conclusions:

- The highest altitude is the control type MV-1 CMS ($\bar{x}$=190 cm), and the lowest is MV-9-7/1 CMS ($\bar{x}$=150 cm). The variation coefficient showed that with the slightest variation in this property is the line MV-1/14 CMS (CV=2.66%).
- At least leaves has Virginia K-326 (an average of 25), while with 28 leaves the control is in the first place. The newly created line MV-9-7/1 (CMS) has on average 26 leaves, and MV-1/14 CMS has 27 leaves. The variation coefficient yielded values that show the stability of this feature in the tobacco varieties concerned.
- Variation of the length of the largest plant leaf is the lowest in the line MV-1/14 (CMS) (CV=1.55%).
- From the measurements we found that with the widest leaves is the new line MV-1/14 CMS ($\bar{x}$=32 cm), and with the narrowest is Virginia K-326 ($\bar{x}$=28 cm).
- The tested Virginia type tobacco types are genetically stable, and the variation of the morphological properties is insignificant (CV is everywhere below 10%), which means they are a good material for the improvement and creation of new varieties. The new line MV-1/14 (CMS) is stable, with more positive properties, so it is expected to be included in the National Variety List of the Republic of Macedonia.

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ISSN 0494-3244

Тутун/Tobacco, Vol. 68, N⁰ 1-6, 26-34, 2018

UDC: 633.71-152.61(497.775)"2016/2017"

Original Scientific paper

COMPARATIVE ANALYSIS OF QUANTITATIVE TRAITS IN SOME VIRGINIA TOBACCO VARIETIES AND LINES

Ilija Risteski, Karolina Kocoska

"St. Kliment Ohridski" University – Bitola, Scientific Tobacco Institute - Prilep, Kicevski pat bb Republic of Macedonia
e-mail: ilija.r@t-home.mk

ABSTRACT

The investigations carried out in 2016 and 2017 in Scientific Tobacco Institute - Prilep included 3 introduced fertile varieties and 3 male-sterile hybrid lines created in the Institute. The following traits were subject of analysis during the vegetation period: time of flowering, length/width of the 5th, 10th and 15th leaf, stalk height and leaf number. The earliest beginning (62 days) and end of flowering (71 days) was recorded in the check variety Coker - 348, and the last to complete these stages was the hybrid line V-112/15 CMS F1. The highest values for leaf size, leaf number and stalk height were measured in the male-sterile hybrid lines. Complete data analysis shows that the male-sterile hybrid lines V-79/09 CMS F_1 and V-112/15 CMS F_1 in almost all investigated traits dominate over other varieties and lines included in the trial. The purpose of this study is to help the potential growers in their decision which genotype to choose in the start of the production cycle and to make wider presentation of the research work of Tobacco Institute-Prilep.

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

КОМПАРАТИВНА АНАЛИЗА НА НЕКОИ КВАНТИТАТИВНИ СВОЈСТВА КАЈ СОРТИ И ЛИНИИ ТУТУН ОД ТИПОТ ВИРЏИНИЈА

Испитувањата беа извршени во 2016 и 2017 во кругот на Научниот институт за тутун – Прилеп. Во истражувањата беа вклучени 3 странски фертилни сорти и 3 машкостерилни хибридни линии креации на Нучниот институт за тутун – Прилеп. Во текот на вегетацијата беа извршени следниве анализи: времето на цветање, должина и ширина на 5 ^{от} и 10 ^{от} и 15 ^{от} лист, висината на стакот и бројот на листовите. Најбргу почнува со цветање (62 дена) и најрано завршува (71 ден) контролната сорта Coker - 348, а сите овие фази последна ги завршува хибридната линија V-112/15 ЦМС F_1. Со најголеми димензии на анализираните листови, вклучувајќи го и број на листовите и висината на страковите, се одликуваа машкостерилните хибридни линии. При комплетната анализа на податоците дојдовме до заклучок дека машкостерилните хибридни линии V–79/09 ЦМС F_1 и V – 112/15 ЦМС F_1 во скоро сите испитувани својства доминираат над другите сорти и линии застапени во опитот. Целта на овие истражувања е преку добиените резултати да се даде придонес при одлуката на потенцијалните производители со кој генотип би стартувале во производниот циклус. Исто така имаме за цел и да ја запознаеме и пошироката научна јавност со достигнувањата на Научниот институт за тутун – Прилеп во оваа област.

Клучни зборови : тутун, вирџинија, цветање, листови,стракови , димензии

INTRODUCTION

Virginia tobacco is the most common type of tobacco in the world. Raw material of this tobacco participates with 60-70% in the composition of modern cigarette blends.
According to Beljo (1996) and Uzunoski (1985), Virginia tobacco belongs to the group of large-leaf and high tobaccos (about 200 cm high), which require precisely determined agro-ecological conditions and cultural practices for their growth and development. In the Republic of Macedonia, Virginia tobacco was grown until 2002 to satisfy the needs of domestic production. According to Risteski (2000), the annual production in the period 1976-1988 was 1633 tons and in 1989-1997 the production dropped to 1475 tons, with a tendency of further decline. After 2002, Virginia tobacco production was ceased, resulting in huge foreign currency losses and complete dependence on the import of this raw material. Exit from this situation or partial improvement can be seen in restarting the production with high-yield varieties, proper regionalization, modern agrotechniques, use of cheaper energy source for drying, etc. In the production chain of this tobacco, variety is highly important segment which determines the yield and quality of the raw material. Tobacco Institute – Prilep has created a number of hybrid lines in CMS form. In comparative trials they showed better results than the introduced varieties, which is a guarantee to the potential producers that this tobacco can be successfully grown in our agro-ecological conditions.

MATERIAL AND METHODS

Two-year trials (2016 and 2017) were conducted with 6 varieties and lines in fertile and CMS form, including the American fertile variety Coker 348 as a check, the fertile varieties Ky-51 (Zimbabwe) and L-16 (Australia) and three male sterile hybrid lines V-120/15 CMS F_1, V-112/15 CMS F_1 and V-79/09 CMS F_1 created in Scientific Tobacco Institute - Prilep. Autumn ploughing was carried out at 40 cm depth and in spring the soil was fertilized with 300 kg/ha 8:22:20 NPK, followed by two more ploughings. Prior to planting, the soil was treated with herbicide and then incorporated into the soil with a harrow. Healthy seedlings were planted manually by random selection of varieties, with 4 replications at 90 × 50 cm spacing. Before the second inter-row hoeing, the plants were nourished with 3-4 g 26% KAN. In the periods of insufficient water supply, several interventions with water were carried out. Plant protection from diseases and pests was performed with approved chemicals and preparations. During the growing season, time of flowering was recorded and morphological measurements were made on 5 stalks of each variety and line represented in the trial, from which the average values were estimated. The analysis includes only the leaves from the middle belt position (5th, 10th, 15th leaf), which account for 60-70% of the total leaf mass of the stalk. The analyses were made on leaf length and width, stalk height in cm and total leaf number of each variety and line.
The obtained data (mean values) were statistically processed by years, using the method analysis of variance and LSD test.

RESULTS AND DISCUSSION

The length of the vegetation period (flowering)

The beginning of flowering denotes the end of the vegetative stage of tobacco growth and the start of the reproductive stage, when generative organs, flowers and seeds are formed. According

to Uzunoski (1985), tobacco flowering begins 10 days after butonization of terminal bud. Naumoski et al. (1977) reported that tobacco plants already get 80% of their mass by the end of flowering. Hawks and Colins (1978) reported the presence of some tobaccos with mammoth traits that cause late flowering and give higher number of leaves per stalk.
According to the results for this trait presented in Table 1, the check variety Coker-348 is the first to start the flowering (62 days) and 50% flowering (66 days) and the first to end the flowering (71 days). The male sterile variety V-112/15 CMS F1 has the latest beginning of flowering (72 days), 50% flowering (77 days) and end of flowering (82 days, i.e.11 days later than the check).

Table 1. The length of the vegetation period (flowering)

Variety	Year	Beginning of flowering, in days after transplanting	Average 2016/17	Absolute differences from the average	50 % flowering, in days	Average 2016/17	Absolute differences from the average	End of flowering, in days	Average 2016/17	Absolute differences from the average
Coker-348 Ø	2016	63	62	/	67	66	/	72	71	/
	2017	61			65			70		
Ky-51	2016	63	63	+1	68	67	+1	73	72	+1
	2017	63			66			71		
L-16 Australia	2016	67	65	+3	71	70	+4	75	74	+3
	2017	63			69			73		
V-120/15 CMS F_1	2016	69	68	+6	74	73	+7	78	77	+6
	2017	67			72			76		
V-112/15 CMS F_1	2016	73	72	+10	79	77	+11	84	82	+11
	2017	71			75			80		
V-79/09 CMS F_1	2016	70	69	+7	74	74	+8	79	79	+8
	2017	68			74			79		

In other varieties and lines, flowering stage ranges from 63 days (beginning), 67 days (50% flowering) and 72 days (50% end of flowering) in the variety Ky -51, up to 69 days (beginning), 74 days (50 %) and 79 days (end of flowering) in line V-79/09 CMS F1.
Analyzing these data we can conclude that male-sterile hybrid lines require a longer period of time to pass the flowering stages compared to fertile varieties.

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

Leaf size is one of the basic criteria for valuation of tobacco. The longest leaves of Virginia tobacco are those of from the middle belt (5th – 15th) and they are the main carriers of its yield and quality. Although leaf size is genetically controlled trait, it also depends on the variety, agro-ecological conditions and applied agrotechniques. Only the middle belt leaves exceeding 35 cm length can be classified as the first class tobacco. Beljo (1996) reported that the length of tobacco leaves generally ranges from below 10 cm to above 80 cm. According to Risteski (1999), the talks of MV-1 variety grown on larger nutrient area have longer leaves. Devcic and Triplat (1982), describing some properties of the newly created Croatian varieties of Virginia tobacco, found the average length of hybrid varieties ranging from 45 cm in H-30, 46 cm in H-31 and 44 cm in H-32.

Data on the 5th, 10th, and 15th leaf length in varieties included in this study are presented in Table 2.

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

Variety	Year	5th leaf Length cm	5th leaf Average 2016/17	5th leaf Difference in cm	10th leaf Length cm	10th leaf Average 2016/17	10th leaf Difference in cm	15th leaf Length cm	15th leaf Average 2016/17	15th leaf Difference in cm
Coker-348 Ø	2016	51.7	50.5	/	59.5	56.2	/	57.4	56.8	/
	2017	49.3			52.8			56.2		
Ky-51	2016	52.0	50.9	+0.4	60.9	59.3	+3.1	57.9	56.3	-0.5
	2017	49.9			57.8^{++}			54.7		
L-16	2016	53.9	52.4	+1.9	63.5^{++}	62.8	+6.6	61.7^{++}	60.6	+3.8
	2017	50.9			62.1^{++}			59.5^{++}		
V-120/15 CMS F_1	2016	54.1^{+}	54.0	+3.5	63.4^{++}	63.6	+7.4	61.4^{++}	60.9	+4.1
	2017	54.0^{++}			63.8^{++}			60.4^{+}		
V-112/15 CMS F_1	2016	56.5^{++}	56.6	+6.1	64.4^{++}	64.3	+8.1	65.7^{++}	65.3	+8.5
	2017	56.8^{++}			64.2^{++}			65.0^{++}		
V-79/09 CMS F_1	2016	58.3^{++}	58.2	+7.7	71.7^{++}	70.8	+14.6	68.7^{++}	68.5	+11.7
	2017	58.1^{++}			70.0^{++}			68.4^{++}		

	2016 5th leaf	2016 10th leaf	2016 15th leaf	2017 5th leaf	2017 10th leaf	2017 15th leaf
LSD 5% $^{+}$	2.23 cm	2.13 cm	2.64 cm	2.82 cm	3.06 cm	3.24 cm
1%$^{++}$	3.08 cm	2.95 cm	3.66 cm	3.91 cm	4.23 cm	4.48 cm

Data in Table 2 show that the longest 5th leaf was recorded in variety V-79/09 CMS F_1 (58.2 cm), which is 7.7 cm more than the check variety Coker-348 (50.5 cm). In other varieties, this trait ranges from 50.9 cm in Ky-51 to 56.6 cm in line V-112/15 CMS F_1. In 2016, statistical significance of 5% was recorded only in line V120/15 CMS F1. Statistical significance of 1% was recorded in lines V-79/09 CMS F_1 and V-112/15 CMS F_1 in 2016 and 2017, and in line V-120/15 CMS F_1 such significance was estimated only in 2017.

The longest 10th leaf was measured in line V-79/09 CMS F_1 (70.8 cm) and it was 14.6 cm longer than the check variety Coker-348 (56.2 cm). In other varieties, the length of the 10th leaf ranged from 59.3 cm in variety Ky-51 to 64.3 cm in line V-112/15 CMS F_1. In both years, statistically significant differences of 1% was reached in variety L-16 and lines V-120/15 CMS F_1, V-112/15 CMS F_1 and V-79/09 CMS F_1 and in variety Ky-51 it was reached only in 2017. The highest average length on the 15th leaf (68.5 cm) was measured in line V-79/09 CMS F_1 and the smallest length was recorded in variety Ky-51 (56.3 cm), while in the check variety it measured 56.8 cm. In other varieties and lines, the 10th leaf length ranged from 60.6 cm in variety L-16 to 65.3 cm in line V-112/15 CMS F_1. Significant difference of 1% was obtained in variety L-16 and lines V-120/15 CMS F_1, V-112/15 CMS F_1 and V-79/09 CMS F_1 in both years of investigation.

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

Leaf width, just as length, is important morphological trait, genetically controlled and closely related to the leaf position on the stalk, environmental conditions and applied agrotechniques. The leaves of Virginia tobacco should have a larger length and width for better cigarette fabrication and the best length : width ratio is 2:1. Kocoska and Risteski (2008) studied six Virginia varieties in the Prilep region in 2003 and 2004 and reported that the width of the 5th leaf ranged 21 cm - 28 cm, of the 10th leaf 27 cm - 36 cm and of the 15th leaf 22 cm - 31 cm. Risteski et al. (2009), in their trials performed in the region of Prilep during 2005-2007 with 6 domestic and introduced varieties and lines found that the width of the 5th leaf ranged 26.8 cm - 30.3 cm, of the 10th leaf 32.0 cm - 35.7 cm and of the 15th leaf 22.1 cm - 27.9 cm.

Kalamanda (2009), in her trials conducted in the Republic of Srpska with varieties DH-17 and Hewissi-17 reported that the width of the middle belt leaves ranged from 19.4 cm in Hewissi-17 in 2004 to 25.4 cm in DH-17 in 2006. Data presented in Table 3 show that the largest average width of the 5th leaf (39.7 cm) was measured in line V-79/09 CMS F_1 and the smallest width was measured in variety Ky-51(33.0 cm). In the check variety, the width was 33.1 cm and in other varieties and lines it varied from 35.2 cm in L-16 to 39.0 cm in line V-120/15 CMS F_1. In 2016, statistical significance of 5% was estimated in lines V-120/15 CMS F_1 and V-112/15 CMS F_1, and significance of 1% was found only in line V-79/09 CMS F_1. In 2017, statistically significant difference of 1% was estimated in L-16 variety and in lines V-120/15 CMS F_1, V-112/15 CMS F_1 and V-79/09 CMS F_1. The largest average width of the 10 th leaf (41.5 cm) was observed in line V-120/15 CMS F_1, and the smallest width (37.3 cm) in variety Ky-51. 37.7 cm width was measured in the check variety Coker 348. In other varieties and lines, this trait ranged from 38.9 cm in line V-112/15 CMS F_1 to 41.5 cm in line V-120/15 CMS F_1.

Table 3. Width of the analyzed leaves

Variety	Year	Width of the 5th leaf			Width of the 10th leaf			Width of the 15th leaf		
		cm	Average 2016/17	Difference in cm	cm	Average 2016/17	Difference in cm	cm	Average 2016/17	Difference in cm
Coker-348 Ø	2016	36.3	33.1	/	39.3	37.7	/	30.7	29.9	/
	2017	29.9			36.2			29.1		
Ky-51	2016	34.3	33.0	-0.1	38.9	37.3	-0.4	29.3	29.1	-0.8
	2017	31.8			35.8			28.9		
L-16	2016	36.3	35.2	+2.1	41.7^{+}	41.2	+3.5	35.3^{+}	34.2	+4.3
	2017	34.2^{++}			40.7^{+}			33.2^{+}		
V-120/15 CMS $F_{1,}$	2016	39.2^{++}	39.0	+5.9	41.8^{+}	41.5	+3.8	33.8	33.2	+3.3
	2017	38.8^{++}			41.2^{++}			32.6		
V-112/15 CMS F_1	2016	38.8^{++}	38.6	+5.5	39.5	38.9	+1.2	34.4	33.7	+3.8
	2017	38.4^{++}			38.4			33.0^{+}		
V-79/09 CMS F_1	2016	40.1^{++}	39.7	+6.6	41.7^{+}	40.9	+3.2	36.4^{++}	35.9	+6.0
	2017	39.3^{++}			40.2^{+}			35.5^{++}		

	2016 5th leaf	2016 10th leaf	2016 15th leaf		2017 5th leaf	2017 10th leaf	2017 15th leaf
LSD 5% $^{+}$	= 1.79 cm	1.87 cm	3.83 cm	LSD 5% $^{+}$	= 2.48cm	3.39 cm	3.87 cm
1% $^{++}$	= 3.00 cm	2.59 cm	5.31 cm	1% $^{++}$	= 3.44 cm	4.69 cm	5.36 cm

Statistical significance of 5% in 2016 and 2017 was obtained in variety L-16 and line V-79/09 CMS F_1. Line V-120/15 CMS F_1 reached 5% statistical significance in 2016 and 1% in 2017. The largest width of the 15th leaf was measured in line V-79/09 CMS F_1 (35.9 cm) and the smallest width in variety Ky-51 (29.1 cm), while the width in the check variety was 29.9 cm. In other varieties and lines it ranged from 32.2 cm in line V-120/15 CMS F_1 to 34.2 cm in variety L-16. In both years of investigation, only the line V-79/09 CMS F_1 reached statistically significant difference of 1% compared to the check. Statistical significance of 5% was estimated in variety L-16 in 2016 and 2017 and in line V-112/15 CMS F1 in 2017.

Height of the stalk with inflorescence and Leaf number

The height of tobacco stalk is genetically controlled trait and varietal distinction. However, it is a variable category which depends on the environmental conditions and applied cultural practices. According to Uzunoski (1985), the height of tobacco stalk may vary from 25 cm to over 300 cm. The same author reported that the stalk of the introduced heterotic variety S-7 in certain conditions could reach a height of 300 cm. Devcic and Triplat (1982) reported that the average stalk height in Croatian hybrid varieties H-10, H-30 and H-31 was 170 cm. According to Beljo (1996), Virginia tobacco belongs to the group of high-stalk tobaccos. Data presented in Table 4 show that the highest stalk was measured in the line V-120/15 CMS F_1 - 207 cm, i.e. 31 cm more compared to the check variety Coker-348 (176 cm).

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

Variety	Year	Stalk height with inflorescence, cm	Average 2016/17	Height, cm	Rank	Leaf number	Average	Difference	Rank
Coker-348 Ø	2016	185	176	/	6	28.4	28.3	/	6
	2017	167				28.2			
Ky-51	2016	194	188	+12	5	30.2^{+}	29.8	+1.5	5
	2017	182^{+}				29.4			
L-16	2016	206^{+}	202	+26	4	31.2^{++}	31.1	+2.8	4
	2017	199^{++}				31.0^{++}			
V-120/15 CMS F_1	2016	212^{++}	207	+31	1	32.0^{++}	31.4	+3.1	3
	2017	202^{++}				30.8^{++}			
V-112/15 CMS F_1	2016	208^{+}	206	+30	2	34.4^{++}	34.1	+5.8	1
	2017	204^{++}				33.8^{++}			
V-79/09 CMS $F_{1,}$	2016	206^{+}	205	+29	3	33.6^{++}	33.5	+5.2	2
	2017	204^{++}				33.4^{++}			

	Stalk height 2016	Stalk height 2017		Leaf number 2016	Leaf number 2017
LSD 5% $^{+}$ =	18.61 cm	12.19 cm	LSD 5% $^{+}$ =	1.60	1.78
1% $^{++}$ =	25.77cm	16.89 cm	1% $^{++}$ =	2.22	2.48

In other varieties and lines, the average height of the stalk ranged from 188 cm in variety Ky-51 to 206 cm in line V-112/15 CMS F_1. In 2016, statistical significance of 5% was estimated in variety L-16 and lines V-112/15 CMS F_1 and V-79/09 CMS F_1, while 1% significance was obtained only in line V-120/15 CMS F_1. In 2017, statistically significant difference was estimated in variety L-16 and lines V-79/09 CMS F_1, V-112/15 CMS F_1 and V-120/15 CMS F_1. From economic point of view, leaf is the most important part of tobacco. According to Beljo (1996), leaf number ranges from 9 to 36, depending on the type, variety and other factors. Hawks (1978) noted that in most of the cases the varieties with higher stalks also have a higher leaf number. This was particularly evident in "non-flowering" mammoth varieties. In addition to leaf size, tobacco yield is also strongly influenced by their number. This trait is genetically controlled, but it also depends on agroecological conditions and applied agrotechniques during vegetation. Dražič et al. (2011) reported that the number of leaves in 13 varieties and lines in the locality of Nova Pazova (Serbia) ranged from 22 to 26. Kocoska and Risteski (2008) found that the number of leaves in some varieties and lines of Virginia tobacco ranged from 22 to 33. The results of our investigation presented in Table 4 show that the largest number of leaves (34.1) was counted in line V-112/15 CMS F_1, which is 5.8 leaves more compared to the check variety Coker – 348 (28.3 leaves). In other varieties and lines, leaf number ranged from 29.8 in variety Ky-51 to 33.5 in line V-79/09 CMS F_1. Statistically significant difference of 1% compared to the check in both years of investigation was achieved in variety L-16 and lines V-120/15 CMS F_1, V-112/15

CMS F_1 and V-79/09 CMS F_1. In Ky-51, statistically significant difference of 5% was achieved only in 2016.

CONCLUSIONS

- The check variety Coker - 348 is the first to begin the flowering stage (62 days) and also the first to complete 50% of flowering (66 days) and end of flowering (71 days). The last to begin the flowering was the variety V-112/15 CMS F_1 (72 days) and it was also the last to complete 50% of flowering (77 days) and end of flowering (82 days).
- The largest length of the 5th, 10th and 15th leaf was measured in variety V-79/09 CMS F_1 (58.2 cm, 70.8 cm and 68.5 cm, respectively). The smallest length of the 5th and 10th leaf was measured in the check variety Coker – 348 (50.5 cm and 56.2 cm, respectively). The smallest length of the 15th leaf was recorded in variety Ky-51 (56.3 cm).
- The largest width of the 5th and 15th leaf was measured in line V-79/09 CMS F_1(39.7 cm) and the largest width of the 10th leaf was recorded in line V-120/15 CMS F_1 (41.5 cm). The smallest width of the 5th, 10th and 15th leaf was recorded in variety Ky-51 (33.0 cm, 37.3 cm and 29.1 cm, respectively).
- The highest stalk was measured in line V-120/15 CMS F_1 (207 cm) and the shortest stalk in the check variety Coker - 348 (176 cm).
- The highest leaf number was counted in line V-112/15 CMS F_1 (34.1) and the smallest in the check variety Coker – 348 (28.3).
- In most of the analyzed traits, male-sterile hybrid lines were dominant over the other varieties investigated.

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Тутун/Tobacco, Vol. 68, N⁰ 1-6, 35-45, 2018

ISSN 0494-3244
UDC : 632.937.1
Original Scientific paper

BIOFUNGICIDES –NEEDS AND PERSPECTIVES IN SUSTAINABLE AGRICULTURAL PRODUCTION

Biljana Gveroska

"St. Kliment Ohridski" University , Bitola
Scientific Tobacco Institute-Prilep, Kicevska bb
e-mail: bgveros@yahoo.com

ABSTRACT

The environmental pollution and the consequences for human health coupled with the development of resistance to pathogens due to excessive use of agrochemicals, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture.

Biofungicides are potential alternatives to synthetic fungicides. Sources of biofungicides are readily available, easily biodegradable, exhibit various modes of action, are less expensive and have low toxicity to humans and non-target organisms. Acting by its base i.e active ingredients, they compete with chemicals with their effectiveness and their use promotes sustainable management and hence contribute towards sustainable agriculture.

This review discusses the current status of knowledge on biofungicides including their sources, production, formulation, commercialization, role in sustainable agriculture and their limitations. It also highlights future directions for strengthening sustainability and the perspectives of their application.

Keywords: biofungicides, ecological, non-toxic, sustainable agriclture

БИОФУНГИЦИДИ - ПОТРЕБИ И ПЕРСПЕКТИВИ ВО ОДРЖЛИВОТО ЗЕМЈОДЕЛСКО ПРОИЗВОДСТВО

Загадувањето на животната средина и последиците по здравјето на луѓето заедно со развојот на отпорност кон патогените поради прекумерната употреба на агрохемикалии, доведе до значителни промени во ставовите на луѓето кон употребата на пестициди во земјоделството.

Биофунгицидите се потенцијални алтернативи на синтетичките фунгициди. Изворите на биофунгицидите се лесно достапни, лесно биоразградливи, покажуваат различни начини на дејство, се помалку скапи и имаат ниска токсичност за луѓето и за нецелните организми. Делувајќи врз основа на својата активна материја тие се натпреваруваат со хемиските со нивната ефикасност. Нивната употреба промовира одржливо управување, а со тоа и придонесува кон одржливо земјоделско производство.

Овој труд го разгледува моменталниот статус на знаења за биофунгицидите, вклучувајќи ги и нивните извори, производство, формулација, комерцијализација, улога во одржливо земјоделство и нивните ограничувања. Исто така ги истакнува идните насоки за зајакнување на одржливоста и перспективите на нивната примена.

Клучни зборови: биофунгициди, еколошки, нетоксични, одржливо земјоделско производсто,

INTRODUCTION

Hazards and challenges in the use of conventional pesticides

Plant protection is based mainly on the use of chemical measures i.e. -pesticides. The use of synthetic chemicals is highly accepted due to their efficacy, reliability and quick knock down effect. But, like synthesized compounds, pesticides are of a different chemical composition, toxicological properties, persistence, so that they are polluters of the environment.
They have a health hazards for humans and environment due to their toxicity and pollution (Damallas and Koutrobuas, 2016). Synthetic pesticides also lead to environmental pollution due to the unbiodegradable nature of their constituent compounds. Therefore, Methyl bromide has been banned from agricultural use due to its negative impact on the environment. It is associated with depletion of ozone layer which contributes significantly to climate change. The constituent compounds of chemical pesticides, especially active ingredients contaminate soils (Kumari et al., 2014). They also pollute surface and ground water, killing aqua life after inhalation and consumption.
Continuous use of synthetic pesticides leads to development of resistant plant pathogen strains leading to their resurgence. Farmers apply more chemicals in control of the causing agents. In the process of managing target pests, synthetic pesticides kill non-target beneficial organisms and thereby disrupting biodiversity. After application, the active compounds of the synthetic pesticides are taken up and retained by crops.
Intermediate product of degradation are often more persistent than the starting compound, they remain for a long time in soil or water (groundwater),which can have consequences for subsequent plants in the crop rotation (Djordjevic, 2008, loc cit Grahovac et al., 2009).
Exposure to pesticides adversely affects the human population, directly or indirectly. Consumption of such crops poses chronic health problems to humans due to the accumulated toxic chemical residues. Also, continuous exposure to pesticides cause gene mutations, genetic damages, repro- ductive health problems and chronic diseases such as asthma, hypertension and cancer. (Kumari et al., 2014). They also reduce resistance of animals to disease-causing pathogen infections (Maksymiv, 2015).
The strong negative effect of pesticides on humans and the environment strengthened the first - people's awareness and then, interest and activity of the dominant and the most competent world organizations. Various other organizations are formed whose work is in the direction of human health and sustainable agricultural production. The World Healt Organization starts the strong fight against pesticides in the direction of protecting human health and the environment. The European Union (EU) set out strict regulations regarding registartion of active ingredients (especially for the toxicology requirements). There is strengthened application of Good Agriculture Practise.
Tobacco purchuasing companies also take the strong care abouth safety of the raw material exported to their markets (lowered MRLs in residue level). Therefore, they use the active ingredient from the more safety classes acording to WHO classification (STIP, 2019). There are lot of a.i banned by EU. There are efforts to withdrawal the another and supstitute by the other (MUCF, 2019).

NEEDS FOR THE USE OF THE BIOFUNGICIDES

Unknowledge in the application of some pesticides by operator and consumer leads to failure in solving some diseases and rapid emergence of resistant individuals in the population of some harmful agents. Also, the ban on the use of synthetic pesticides at the time of ripening and harvesting (especially in protected space), led to increased interest and publicity for introducing alternative measures in plant protection, where the deserved place finds biological preparations so and other non-pesticide measures (Damallas and Koutrobuas, 2016).

The development of integral protection system will include all methods and means to control the harmful agents with minimum effect on the environment and with no economic consequences. Creating unfavorable conditions for the development of the disease by application agro-technical measures, the application of biological products and antagonistic organisms, individually, or integrated with less risky pesticides are technologies the protection they are tending today (Gold, 2009). Strengthening the induced resistance is also one of the strategic goals of the plant protection.

Application of biopreparates is a good way to manage the environment in the process of achieving sustainable agriculture production (as well as tobacco). Environmental protection and sustainability are in harmony and sustainability can be achieved only with the protection of natural resources (Malik et al., 2012).

Trends in tobacco protection are in accordance with the basic requirements for sustainable agricultural production and in line with modern standards. Bioproducts are important part of the complex of measures for integrated pest management. Efforts for the wide application of biopreparates is promoting of their beneficial effect and the greater utilization of resources. Hence, scientific research in the field of tobacco production will continue to develop procedures and instructions for the most appropriate use of biopreparate (Gveroska, 2014).

Biofungicides are potential alternatives to synthetic pesticides. Sources of biofungicides are readily available, easily biodegradable, exhibit various modes of action, are less expensive and have low toxicity to humans and non-target organisms.

Back ground of biofungicides

The first bacterium called *Agrobacterium radiobacter* strain K 84 was registered with the United States Environmental Protection agency (EPA) for the control of crown gall in 1979. Ten years later the first fungus *Trichoderma harzianum* ATCC 20476 was registered with the EPA for the control of plant disesaes. Currently a total of 14 bacteria and 12 fungi have been registered with the EPA for the control of plant diseases. Most of these are sold commercially as one or more products. The technology of commercialization is still in its initial phase. 65% of the EPA registered organisms have been registered within the past 10 years while the remaining 36% regestered over the past 5 years. Many technological problems were overcome and shifts in tinking occurred for these products to reach the shelves. There are a number of biological plant protection agents that are available on the market.

In the world, a large number of countries and experts are involved in this field of research, both in education and in commercialization biopesticides. Today in the world are registered 185 biopesticidal preparations, of which 72 are active substances of bacteria, 47 mushrooms, 40 entomopathogenic nematodes, 24 viruses and two protozoa. They are applied to different plant species - vegetables, fruits, cereals, fodder plants and others cultivated species. The distribution of biopesticide was carried out according to the type of organisms it suppresses, on: bioinsecticides, biofungicides, bioherbicides, and others, or can be classified according to groups of living organisms (mushrooms, viruses, bacteria, nematodes) that are in the function of the active substance biopreparates (Djordjevic, loc.cit Grahovac et al., 2009).

In our country, there is the biofungicide Trianum P, based on alive spores of *T. harzianum* T22 Gveroska, 2017).

The ability of biofungicides to protect hosts from pathogens, take place on different plants in different conditions are the basis of their commercial success (Harman, 1996).

BIOFUNGICIDES - NATURALLY RESURSES BASED

Biofungicides are products and by-products of naturally occurring substances. There are several categories such biocontrol agents especially anatagonists, products of metabolism, botanicals, growth promoters etc. Among that, plants and microorganisms are the major sources of biopesticides due to the high components of bioactive compounds and antimicrobial agents.

Biofungicides include the use of useful microorganisms or their structures, products of their metabolism, the use of herbal extracts and essential oils in protection plants. Products metabolism mentioned above microorganisms are toxins, crystals, spores and antibiotics, which protect the plants by acting antagonistic to the causative agents diseases, harmful insects, nematodes and weeds, where abouts are harmless to humans and environmentally safe. Also, useful microorganisms produce both vitamins, enzymes, and plant hormones that can act on the immune system of plants, increasing their resistance (Grahovac et al., 2009).

The active compounds in plants include phenols, quinones, alkaloids, steroids, terpenes, alcohols and saponins Different plant families have varied antimicrobial bioactive compounds which include oil components such as α- and β-phillandrene, limonene, camphor, lina- lool, β-caryophyellene and linalyl acetate depending on the plant family.

Compost teas are filtrates of compost extracts and are similarly used as biopesticides.

Microbial biofungicides include bacteria species such as *Pseudomonas*, *Bacillus*, *Xanthomonas* and *Serratia* or fungi such as *Trichoderma*, *Verticillium* and *Beauveria* species. Biopesticides exhibit different modes of action against pathogens such as hyperparasitism, competition, lysis, induced resistance etc.

Plant growth promoting rhizobacteria protect plants from biotic and abiotic stresses and they also enhance plant growth and enhance formation of root hairs . The most common species of plant growth promoting rhizobacteria include *Bacillus, Agrobacterium*, *Microbacterium*, *Rhizobium*, *Pseudomonas*, *Chryseobacterion* and *Rhodococcus*. They colonize the environment around the plant roots, fix nitrogen, increase phosphate solubilisation and result in general increase in plant yield. Species of *Pseudomonas* and *Bacillus* have been used as biofertilizers with reports showing increase in plant growth. They also convert unreachible forms of certain chemical elements (P, Fe), turning them into readily available, therefore enlarge the yield of crops.

MICROORGANISMS AS SOURCES OF BIOPESTICIDES

Microorganism-based biocontrol agents form the bulk of commercialized bio-pesticides and they include bacteria, viruses, fungi, nematodes and protozoa (Koil, 2011). There are up to 175 reported microbial based biopesticide active agents and they have been used in management of pathogens, weeds, insects and ne- matodes (Singh, 2014). Majority of the microbial biofungicides are used to manage soil borne pathogens. The mechanisms of action exhibited by microorganisms against plant pathogens include hyperparasitism, competition, secretion of volatile compounds, antibiosis and parasitism (Howell, 2003; Suprpta, 2012). The most known biofungicides are based on the fungi of genus *Trichoderma* (Harman, 1996, 2004, 2006, Gveroska, 2013).

The major sources of microorganisms with pesticidal activity are agricultural fields where they coexist with other microorganisms including pathogens and beneficial species. The rhizosphere is usually concentrated with various classes of important microorganisms. According to Alizadeh et al. (2013), rhizobacteria are ideal biocontrol agents since they inhabit the rhizosphere and provide front-line defense against attack by pathogens.

Pathogens encounter antagonism from rhizobacteria before and during infection of the root. Formulation of the microbial fungicdes has a great contribution to the effectiveness of the resultant product and it is usually dependent on the substrate used. The activity was attributed to the high number of spores produced by the fungus. Gveroska (2016 a) estimated the greather biocontrol activity by the bigger cfu/g soil depending of the used manure.

Various strain of Bacillus has the biocontrol activity - there are reports for late blight (*Phytophthora infestans*) on tomato. There is a report for Bacillus subtillis Ch13 to damping off in tobacco (Gveroska, 2016 b). Also, B. subtillis QST 713 has the big opportunities to became the widely used in tobacco.

EM (effective microorganisms) technology is one of the main modes of environmental management aimed at establishing a sustainable production. The basic principle of this technology is the application and increase of the population of effective and beneficial microorganisms in soil which eliminate degenerative microorganisms, especially soil pathogens, thus creating a healthy environment for plants. There are data on the use of probiotics not only to improve the soil but to prevent the attack from diseases. Effective microorganisms continue to coexist in the rhizosphere and plants grow well, free of pathogens.

These studies indicate that microbial biopesticides can be incorporated in integrated pest management for sustainable agriculture (Gveroska 2014).

Mechanisms of action of biofungicides

Biofungicides act by:

- Direct Competition;
- Antibiosys;
- Hyperparasitism;
- Induced resistance of the host plant;
- Plant Growth Promotion

- Direct competence implies that the root of the plant (the host rhizosphere) must inhabit the organism that is used for the biological suppression of the causing agent disease or Biological Control Agent (BCA) has to be before the infection by pathogen. The rhizosphere is the source nutrients. In general, the organism that is being applied in biological control must be present in large number for competing with a pathogen.
- BCA should have antibiotic and antagonistic properties. They produce the low molecular weight compounds or an antibiotic by microorganisms that have a direct effect on the growth of plant pathogen *In situ* production of antibiotics by several different biocontrol agents has been approved despite the effective quantities are difficult to estimate because of the small quantities produced relative to the other, less toxic, organic compounds present in the phytosphere. An efficient bio control agent is one that produces sufficient quantities of antibiotics in the vicinity of the plant pathogen (Junaid et al., 2013).
- Parasitism implies that the organism that is being applied for biological control attacks pathogenic organism and it is fed. With such a mechanism of action, the biological agent must be present before the pathogen attack.

  Production of enzymes, toxins or antibiotics (antibiosis) are involved in this process, as well as contribute for capturing the territory
- Induced resistance occurs when attacked plants activate the defense system and do not immune, but an internal fight to slow down the infection. Host plants are deliberately

inoculated to induce this type resistance. A biological fungicide is a trigger for this phenomenon type of resistance.

- Antibiosis: The biological activity of biofungicides can be designed on the production of numerous metabolites that act antifungal and antibacterial. Bio agents are known to produce different types of antibiotics which act in different ways to supress the diseases or plant pathogens. Bio agents are known to produce three types of antibiotics viz., nonpolar/volatile, polar/ non-volatile and water soluble. Several bio-control strains are known to produce multiple antibiotics which can suppress one or more pathogens (Junaid et al., 2013). For example, *B. subtilis* is in the world used to prepare many preparations. Produces antibiotics (bacilysin and fengymycin, dithididine and oxydificidine, bacitracin, bacillin and balomycin b and iturin) that act on many types of aerobic and anaerobic bacteria.

  The products of metabolism, lipopeptides, act on different cell wall components, preventing adhesion of the pathogen on plant organs, and the enzyme subtilin blocks development pathogen (Klokočar-Šmit et al., 2006). Bacillus products for disease control have the mode of action of biopesticides: fungicidal lipopeptides production (and mimic cell membrane lipid, insert deply into membrane and disrupt packing of membrane lipids) and plant defence induction. There is the good shelf life, also (Bayer, 2017).
- Plant Growth Promotion: Bioagents can reduce the disease incidence of crops by increasing their growth at least during the early stages of the life cycle by the way of disease escape. The best example of this is the resistance of damping off of *Solanaceous* crops with advance of age. Bioagents both fungal and bacterial help in managing the plant diseases by promoting the growth of plants through increased solubilisation of nutrients, increased nutrient uptake with enhanced root growth and sequestration of nutrients.

USE OF THE BIOFUNGICIDES

Biofungicides are used: for the treatment of seeds, tubers potatoes before planting or before storage, foliar, for sinking or spraying seedlings before planting, watering plants after planting, planting plants, immersion of seedlings etc.

The application of this and similar biofungicides must be very common in order to provide a satisfactory fungal biomass antagonists and protect the seeedlings or plants. Gveroska (2013, 2016 a, 2017) highlights the best way of application of *T. harzianm* in tobacco seedlings. She recomended the sinking of seed 24-48h in the culture of the biocontrol agent or in suspension of the curent bioproduct.

For the full beneffit and sustainable usage, we have to understand mode of action; use as protecant: good coverage, protectant intervals; use under low to moderate disease pressure for best results; use in an integrated program, mixing or rotating with different modes of action; consider additional benefits in addition to efficacy (Bayer, 2017).

Possible combinations are three successive measures: thermal seed treatment, then application of growth stimulator (Azospirillum brasilense) and treatment with streptomycin sulphate for which increased efficiency is achieved, for example Better suppression of the cause of bacterial parasites (*Pseudomonas syringae* pv. tomato) (Bashan and Bashan, 2002).

Maximum allowed number of treatments for biofungicides is standard for every plant species. Number of cultivated plants which are protected by biofungicides is quite large, from vegetable, vegetable, fruit, decorative, medicinal and spice herbs (Grahovac et al., 2009).

Despite the huge perspective, ecological and sustainable approach, biofungicides are however faced with challenges of formulation, registration, commercialization, acceptance and adoption. Therefore, these are some aspects of biopesticide development, including their sources, production, formulation, commercialization, efficacy and role in sustainable agriculture.

COMMERCIALIZATION OF BIO CONTROL PRODUCTS

Although the number of biocontrol products in plant disease management is increasing, these products still represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture (Fravel, 2005). In recent years there are enlarged commercial production of bio control agents resulting into the entry of various biocontrol products into the world market.

Commercialization of biocontrol products is a multi-step process involving a wide range of activities:

a) Isolation of micro- organism from the natural ecosystem;
b) Evaluation of bio-agent both in vitro and under glass house conditions
c) Testing of the best isolate under field conditions
d) Mass production
e) Formulation
f) Delivery
g) Compatibility
h) Registration and release

The ultimate success of commercialization depends firstable on the base – isolation and the searching and screening process. Also, it depends from the target pathogen, the crop and the cropping system, presence or absence of the right isolate - antagonistic to the pathogen, real formulation, its stability etc.

FORMULATIONS OF BIOPREPARATES AND APPLICATION POSSIBILITIES

The formulation of biological preparations requires very good knowledge of the interaction of microorganisms and objects suppression. It is essential that the preparations can be produced on a liquid or semi-solid substrate and in sufficient quantities, then staying vital during storage and afterwards.

Application of the preparation, under selective pressure in the laboratory conditions do not lose the importance of biological importance suppression (vitality, variability, selectivity), as well as compatibility with application technology. This is it exceeds the use of avoided, nutritious and connective tissue matter or sticker and carrier as a major part in the formulation.

They are applied in the form of a polunative culture, or formulated in the form of a powder, a wettable powder, water-soluble granules, granules, pellets, microcapsules, gels and emulsifiers liquids. The short term storage of biopreparates exceeds by encapsulation of microorganisms, or their products, into the matrix of organic polymers. Biopreparaes may contain one or more microorganisms as active ingredients. Preparation Polyversum is based on Pythium oligandrum, and Trichoshield on the basis of Trichoderma harzianum + T. lignorum + Gliocladium virens + Bacillus subtilis (Klokočar-Šmit et al., 2006).

Unlike chemical, the content of active substance in the preparation of biofungicide is expressed in millions or billions of living cells and spore / g (B. subtilis isolates), or 2.5-5x10^{10} cells / ml (Pseudomonas fluorescence isolates), or 5 billion spores/g Penicillium vermiculatum) (Grahovac et al., 2009).

Existing formulation technologies suitable for biopesticides

Formulation needs to take into account not only that effcacy will be particularly dependent on environmental conditions, but that it will also be necessary to ensure stability of living organisms in distribution and storage.
Among formulation objectives for biopesticides, Dr Knowles lists: maintaining stability of the a.i. during processing and on storage and application; protecting the ai against degradation or loss of effectiveness on exposure to UV light; maintaining viability and effectiveness of the a.i. in the presence of other formulation components, especially for living organisms; and improving effectiveness of the a.i. under low humidity conditions, especially for living systems (Agrow, 2013).
Commercial biopesticides should be economical to produce, have persistent storage stability, high residual activity, be easy to handle, mix and apply, and provide consistently effective control of target pests. Different formulations of biopesticides should be introduced to overcome problems relating to their efficacy and their degradation and to be convenient during handling and application (Gašić and Tanović, 2013).

WHY BIOFUNGICIDES IN SUSTAINABLE AGRICULTURAL PRODUCTION ADVANTAGES VS. DISADVANTAGES OF BIOFUNGICIDE

Disadvantages of biological pesticides:

- They are more difficult to implement;
- Have a narrower spectrum of action;
- They act more slowly than chemical agents; act preventively, never eradicatively;
- Biopreparates have a shorter shelf life and therefore seem they are more expensive;
- They may be incompatible with other fungicides or bactericides
- Require repeated use and lowering the thresholds

Advantages of biological pesticides:

- The most powerful characteristic is that biopesticides are safety products both for the producer (applicant), the consumer and the environment. They have very short reentry intervals which guarantee safety for the applicant. They have no toxicity (Damalas and Koutroubas, 2015)
- Althought it is believed that the application of biopesticides in plant disease control requires more human labor and is less effective of chemical pesticides.
- Biofungicides are natural products. Biocontol agent are natural, resident of the curent ecosystem. They are non-toxic.
- They reduce the need for chemicals;
- Biofungicides do not cause resistanc in pathogens. So, they are extremely important in the preparation of an anti-persistent strategy and controlling the susceptibility of suppressed species to chemical pesticides; they are safer for use from chemicals;
- They are eco-friendly since they are easily biodegradable and therefore do not pollute the environment (Leng et al., 2011). They have more specific action on the target organism from chemical preparations;are and hence do not affect the beneficial organisms.
- They can take participation in decontamination of agricultural soils through introduction of beneficial microbial species and some products; reduced environmental impact; increased biodiversity (Anonymous 14).

- Have shorter working withdrawals and no quarantine and can be applied in various types of plant production (organic, integral, etc.); Consumer tastes and preferences fluctuate over time and following the demand for organically produced food, this makes biopesticides suitable alternatives to synthetic pesticides. Biopesticides have very short pre-harvest intervals and therefore are safe to use on fresh fruits and vegetables. Some of them are used for other purposes like food and feed. Aditionally, they can take a higher premiums for the production and sale of organic product (excluding pesticide residues);
- Availability of their source materials makes them inexpensive to attain since they are found within the natural environment.
- They compete with pesticides with their effectiveness and their use promotes sustainable management.
- Application of biological agents in programs of integral protection of plants allows the development of sustainable agricultural production;

Development and implementation of the new plant protection program it will take and will require significant investments. Key the factor is the education of workers abouth handling with a new "tool" – biological preparations and presentation of the beneffits. (Anonymous 14).

Biopesticides have sufficient efficacy to be able to be launched separately as products

Agrow (2013) highlights important data, which in some way are the directions of the chemical companies -magnates in the production of pesticides in terms of biofungicides:
Biologicals will have a meaningful role to play in helping growers to protect and enlarge their yields. It points out the challenges, such as limited shelf-life (dealing with living organisms), but it sees a high potential for future innovation in these areas. “We expect to see biologicals in the market with sufficient efficacy as stand-alone products as well as biologicals tailored for applications in combination with classic chemistries,” the company adds (BASF, 2013).
The use of biopesticides in a variety of scenarios offering varying degrees of efficacy. Some are designed for speciality production models. Some of them can be used alongside chemical crop protection products (in tank mixes or alternation programmes) to deliver comparable performance to standard and compete very effectively against chemical crop protection products in terms of disease control and yield benefits (Bayer , 2013).
Performance of biopesticides has improved over the years and they can be launched as individual products. When used in combination with chemicals, highquality seeds and established good agronomic practices, their performance can be signifficantly enhanced. Thus, the role of biopesticides in plant protection is ensured and soldified (Syngenta, 2013).

CONCLUSIONS

Use of synthetic chemicals has raised numerous concerns due to their negative effects on the environmental, human health, natural enemies and ecosystem balance. Global requirements for the need to reduce the use of chemicals pesticides enforced the use of harmless, new, sustainable strategies in plant protection.
Biofungicides are alternative to synthetic fungicides due to their low toxicity, biodegradability and low persistence in the environment. The base materials for biopesticides are readily available and inexpensive. They are real alternative to chemicals, despite the many challenges facing their adoption.
Formulation needs to be effcient, no dependent on environmental conditions, but that it will also be necessary to ensure stability of living organisms in distribution and storage.
Application modes need the knowledge of biocontrol agent which will enable the achievement of the best expression of biocontrol mechanisms.

Globally, researchers have conducted studies on effectiveness of natural plant protection products with significant results, in field conditions. Further research is recommended dedicate to close the gaps in formulation of biofungicides. They should work together with the industry as well as farmers to provide stable, durable formulations of biopesticides. Development and implementation of the new plant protection program it will take significant investments and education manufacturers, workers of forecasting and reporting services.
Any research is recommended to dedicate to all points of sustainability. This will strengthen and emphasize their role in sustainable agricultural production.

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ISSN 0494- 3244

Тутун/Tobacco, Vol. 68, N0 1-6, 46-55, 2018

UDC: 633.71-238(497.2)"2010/2011"

Original Scientific paper

TOBACCO VEIN MOTTLING VIRUS (TVMV) - NEW VIRUS OF TOBACCO IN BULGARIA

Yonko Yonchev*, Elisaveta Stoimenova**, Gancho Pasev***, Hristo Bozukov*

**Tobacco and Tobacco Products Institute (TTPI), Markovo, Bulgaria.*
***Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences.*
****Institute of Vegetable Crops, Plovdiv*
(e-mail:ionkogi@abv.bg)

ABSTRACT

Immunological studies have been carried out in the period 2010-2011 to prove the new tobacco-related viral disease in Bulgaria caused by TVMV. For this purpose, fresh material - leaves - were collected from tobacco plants in the experimental field of TTPI (Tobacco and Tobacco Products Institute) with different symptoms caused by potyviruses. Samples (isolates) were taken from 112 tobacco plants - 40 from Virginia tobacco, 51 from Burley and 21 from oriental tobacco. In Virginia and Burley tobaccos, TVMV was serologically detected, as the virus has been identified for the first time in Bulgaria. TVMV is most common in mixed infections with PVY in both Virginia and Burley tobacco. In Basma tobacco isolates, the presence of TVMV was not detected. In tobacco, the two potyviruses cannot be distinguished on the basis of symptoms alone. Accurate diagnosis is possible through the use of ELISA or PCR.
Key words: Virginia tobacco, Burley tobacco, Basma tobacco, TVMV, PVY

TOBACCO VEIN MOTTLING VIRUS (TVMV) – НОВ ВИРУС НА ТУТУНОТ ВО БУГАРИЈА

Во периодот 2010-2011 година спроведени се имунолошки проучувања за да се докаже новата вирусна болест на тутунот во Бугарија предизвикана од TVMV. За таа цел, свеж материјал - листови - беа собрани од тутунските растенија од Експерименталното поле на ИТТП (Институт за тутун и тутунски ппреработки) со различни симптоми предизвикани од потивируси. Примероците (изолати) се земени од 112 тутунски растенија - 40 од типот вирџинија, 51 од берлеј и 21 од ориенталски тутун. Во вирџиниските и берлејските сорти, TVMV беше откриен серолошки, бидејќи вирусот е идентификуван за првпат во Бугарија. TVMV е најчест кај мешаните инфекции со PVY и кај вирџинијата и кај берлејот. Во тутунските изолати од типот басма не беше откриено присуство на TVMV. Кај тутунот, двата потивируса не можат да се разликуваат само врз основа на симптомите. Точна дијагноза е можна преку примена на ELISA или PCR.

Клучни зборови: тутун тип вирџинија, тип берлеј, тип басма, TVMV, PVY

INTRODUCTION

Tobacco viral diseases pose a serious threat to tobacco production and cause considerable damage to this crop. Often, the losses they cause make tobacco growing unprofitable and sometimes impossible. The application of chemical means to combat viral vectors poses a risk to human health and the environment. In order to minimize these risks, it is necessary to use alternative methods, such as biological control. The establishment and deplopment of sustainable varieties, as well as the improvement of variety characteristics are still the cheapest and most tried method for reducing losses in tobacco and other crops, which produce environmentally friendly production, without polluting the environment. (Stoimenova, 2009; Drumeva - Yoncheva, 2007; Djulgersky and Yonchev, 2018; Keranova, 2018; Mihaylova, 2016).

Tobacco is natural host to over 20 viruses, among which the most significant and economically important are TMV; TSWV; CMV; AMV; TRSV; PVY; TEV and TVMV (Dukić, et al. 2006).

Potyviruses are the most common viruses that cause serious economic losses to tobacco. The basic vector responsible for the spread of the viruses from this group in tobacco are the aphids of the species Myzus persicae Sulz (Lukas, 1975; Gooding, 1985; Kovachevski, 1999; Vaneva - Gancheva, 2018). The three potyviruses PVY (Potato virus Y), TEV (Tobacco etch virus) and TVMV (Tobacco vein mottling virus) are often considered by some authors as a viral complex (Dietrich et al., 2007; Dietrich and Maiss 2003; Yonchev, 2014). In the literature, they are described as viruses of major economic importance for tobacco production in the United States, especially in regions such as Tennessee, Kentucky, Virginia and North Carolina (Pirone, 1989).

PVY has been identified in a number of countries but it presents the most serious problem for tobacco production in Chile, Hungary, Spain, South Africa and last but not least in the countries of the Balkan Peninsula (Gooding, 1985, Kovachevski et al. Dukić et al. 2006; Duduk et al., 2006; Dekić et al., 2007). Engraving is a disease caused by TEV. The virus was first reported in 1928 in Kentucky, United States, by Valleau and Johnson (1928). It was also identified in Canada, Venezuela, Nicaragua, India and Japan. According to Мицковски (1984), engraving is not found on tobacco grown in the Balkan Peninsula. Kovachevski and others (1999) reported that the disease was found in tomato and tattoo. The presence of TEV in Bulgaria was reported by Dimitrov and Bozukov (2004), on the basis of symptomatic diagnostics. In 2010, TEV virus was diagnosed serologically by ELISA immunoassay test in Virginia tobacco in the region of Plovdiv (Yonchev, et al., 2010). TVMV has not been described until 1970, when Gooding and Sun (1972) and Pirone et al. (1973) found a new strain in Burley tobacco in regions such as Tennessee, Kentucky, Virginia and North Carolina. Katisha and Makulu (2001) reported that strains of TVMV serologically associated with European strains of PVY have been identified on tobacco in Zambia. There is evidence of virus spreading in North America, Canada, Mexico, USA, Alaska, Italy, Portugal, Colombia and China (Kenedy, 2011; Horowitz and Ishaaya, 2004).

Symptoms induced by TVMV are barely noticeable. Burley tobacco varieties develop lightening of the veins on the developing leaves. On the mature leaves, parallel to the main veins, irregular green scratching spots are formed, followed by pronounced necrotic interruptions and staining on the veins, which differ from the continuous brown necrotic lines characteristic of PVY (Kovachevsky, 1999; Pirone et al., 1973). Necrotic stains resembling weather fleck may appear on the surface, and their number increases as vegetation progresses (Spetz et al., 2003).

Authors such as Gooding and Sun (1972), Chi et al. (2011), Kennedy (2011) believe that, under field conditions, all three potyviruses have characteristic symptoms that are generally not sufficient to identify viruses. Therefore, visual identification of viruses can be misleading and for correct diagnosis it is necessary to apply infestation of differentiator plants, ELISA test, RT-PCR and other methods.

MATERIAL AND METHODS

In the period 2010-2011, immunological studies were carried out to identify the new tobacco-related viral disease in Bulgaria caused by TVMV.

For this purpose, fresh material - leaves - has been collected from tobacco plants of the TPPI experimental fields, with different symptoms caused by potyviruses . Samples (isolates) were taken from 112 tobacco plants - 40 from Virginia tobacco, 51 from Burley and 21 from oriental tobacco. The collected samples were subjected to an ELISA test to detect the presence of TVMV and PVY.

For serological identification of the two potyviruses, kits from the French company SEDIAG S.A.S were used. The DAS-ELISA kit, identifying PVY, contains IgG against the PVY^o, PVY^c, and PVY^n serotypes and is administered at 1:100 dilution. DAS-ELISA was also used to detect TVMV, with IgG dilution of 1:200. The test to prove the two viruses was conducted according to the manufacturer's methodology .

The optical density (OD) reading in the ELISA test was performed using a Biotek Elx 808 spectrophotometer at 405 nm wavelengt, 60 minutes after dropping the substrate. For positive control, signals with OD values at least twice as high as those of the negative control are received.

The serological evidence of the two viruses has been carried out at the Laboratory for immune viral diseases of the Maritza Institute –Plovdiv.

For the bioassay test of TVMV, Rumex sp., a differentiating species for PVY and TVMV is C. frutescence, the first virus systematically infects the species, and the second virus is immune.

RESULTS AND DISCUSSION

According to literature data, the three potyviruses spread on tobacco (TVMV, TEV and PVY) cause similar symptoms (Shew and Lucas, 1991, Kennedy, 2011; Uzest, 2007). On the basis of these data, samples from plants with different symptoms of Sipaniza disease (caused by the three potyviruses) were collected and subjected to an ELISA test, to detect the presence of PVY and the new potivirus TVMV in Bulgaria. The experiment was conducted during 2010-2011 and the results are presented in Figures 1, 2 and 3 and Graphs 1 and 2.

The first pictures are plants with only PVY detected (Figure 1). The initial symptoms are vein lightening, which spreads across the leaf. As the symptoms develop, dark green strips appear parallel to the veins. Later, a slight mosaic grazing develops on young leaves due to the appearance of pale greenish or yellowish-green small chlorotic spots. The mature leaves of the diseased plants turn yellow and give a yellowish appearance to the whole plant.

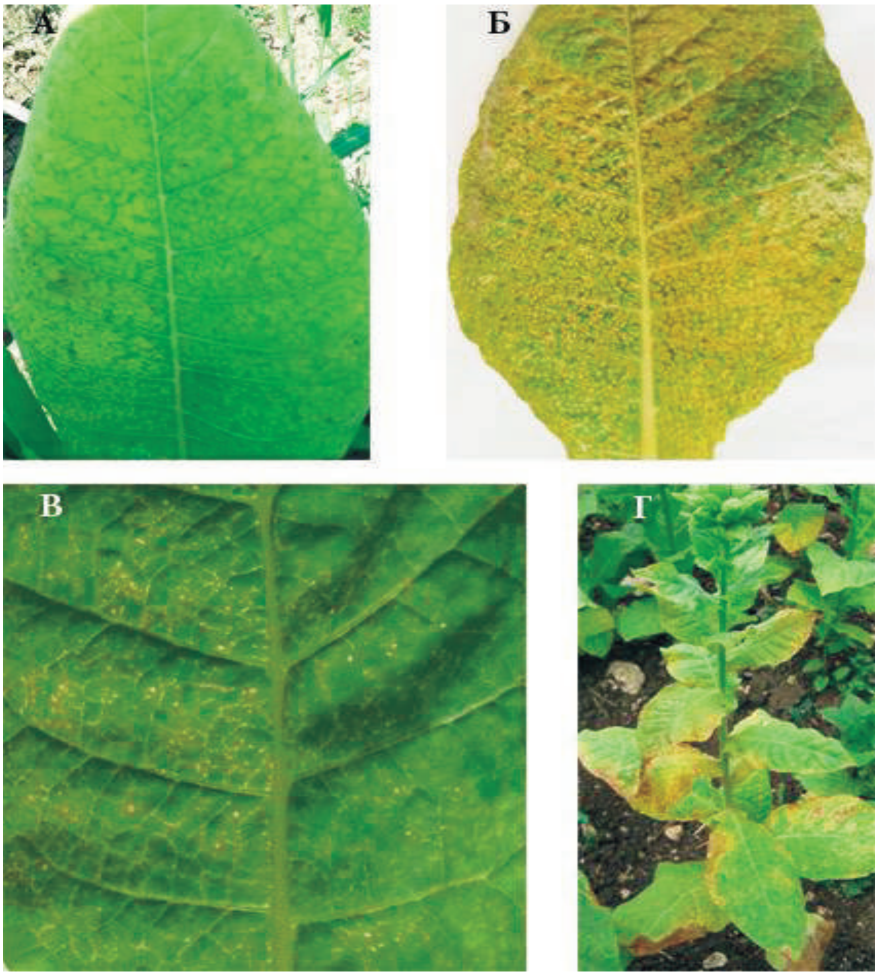


Fig.1 Symptoms caused by PVY. A) initial phase of the disease development - the usual form of pelargonium B) a number of chlorotic yellow spots characteristic of the disease together with vein necrosis on the upper side of leaves characteristic of the necrotic form B) necrotic small lines giving mesh appearance of the leaves, d) tobacco plant infected by Sipaniza.

The symptoms induced by TVMV begin with lightening of the veins on young leaves and almost all the mature leaves turn yellow, preserving the irregular green spots (Figure 2). Necrotic strips are observed on the upper side of the veins, and necrotic spots develop on the yellow areas. A characteristic symptom of the virus is the formation of pronounced necrotic discontinuous lines along the lower and upper surface of the veins, which differ from the continuous necrotic lines characteristic of PVY.

Fig. 2. Symptoms of disease caused by TVMV. A) necrosis of small veins on the upper side of the leaf B) necrotic broken lines on the underside of the main veins B) chlorosis of leaves with irregular green areas, necrotic spots and strips on the upper side of the veins.

In field conditions, under the influence of various factors, as a rule, there are no symptoms identical to those described for the virus. This is even more true for mixed infections.

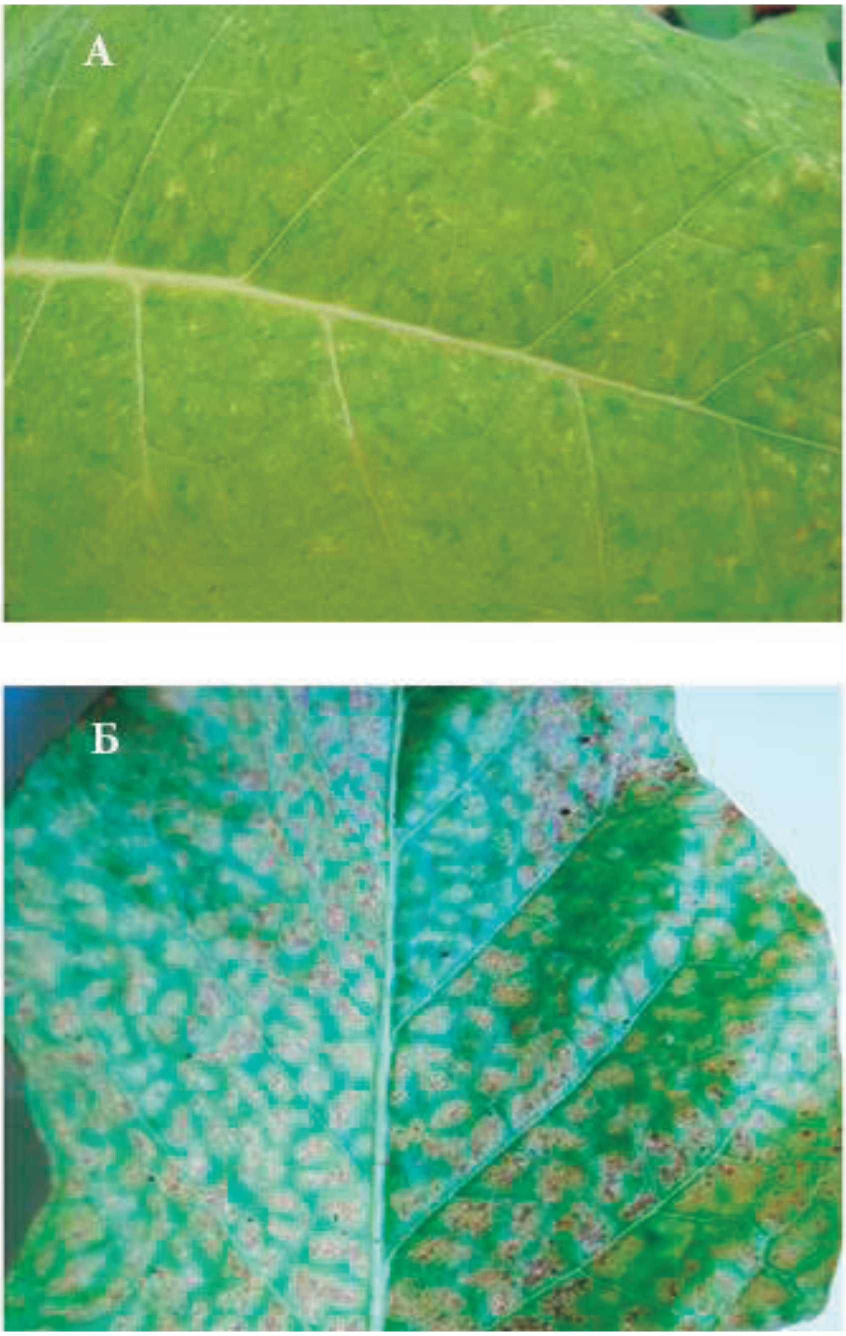
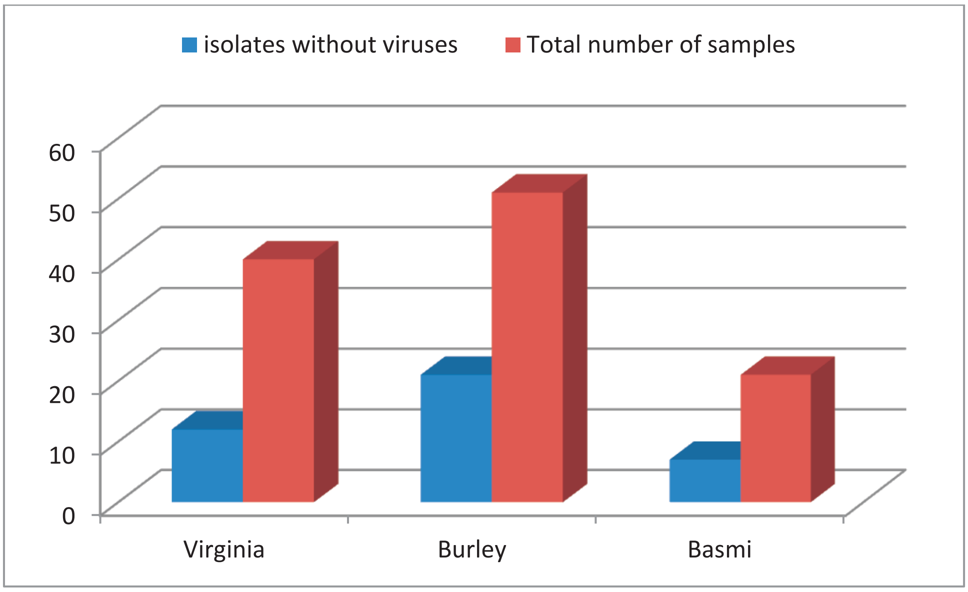


Fig. 3. Symptoms of tobacco leaves infected with mixed infection by PVY, TVMV. Fig. 3A and B show plant leaves with a mixed viral infection of PVY and TVMV, the first figure presenting the symptoms of soft mosaic, initial phase of disease development, and in Fig. 3B yellow and necrotic patches of irregular shape are observed.

Our results clearly show that the two potyviruses PVY and TVMV on tobacco cannot be distinguished on the basis of symptoms alone. Similar findings are made by many other authors (Gooding and Lapp, 1980; Kennedy, 2011; Chi et al., 2011; Gooding and Sun, 1972). The exact diagnosis is possible by the use of ELISA or PCR (Kennedy, 2011).

Graphics 1 and 2 present the results of ELISA testing of all 112 samples.

The two potyviruses were not found in 40 samples (38.4%) of the total number of tested models: 12 samples (40%) of Virginia tobacco, 21 samples (33.3%) of Burley tobacco and 7 samples (41.2%) of Basma tobacco (Figure 1).

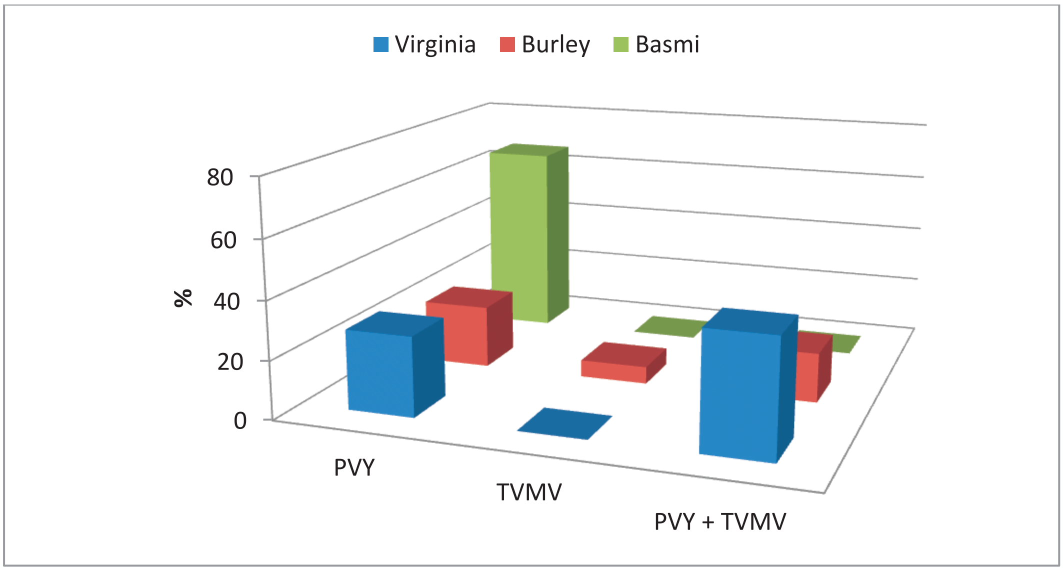


Graph 1 . Number of isolates in which no infection of the two potyviruses has been recorded

The relatively high percentage of samples in which no potyvirus was detected is due to the fact that they are taken not only from plants with typical symptoms of each of the potyviruses but also from those that differ significantly. We assumed that mixed infections could develop symptoms that are significantly different from typical symptoms.

PVY was independently identified in 11 (27.5%) isolates of Virginia tobacco, 11 (21.6%) isolates of Burley and 14 (66.7%) of Basma tobacco. TVMV was found in a single infection in 3 counts (5.9%) of Burley tobacco.

In mixed infections, the two potyviruses were identified in 16 (40%) and 17 (33.3%) isolates of Virginia and Burley tobacco, respectively. In all isolates of Basma tobacco, only a single infection of PVY was determined (Graph 2). Percentages show the ratio of single and mixed infections of both potyviruses by tobacco type.



Graph 2. Percentage distribution of single and mixed infections of the two potyviruses by tobacco types

CONCLUSION

1. PVY is the most common tobacco virus in the area under investigation, the virus was not found only in 3 (4.2%) of the positive isolates.
2. In Virginia and Burley tobacco varieties, TVMV was serologically diagnosed, as the virus has been identified for the first time in Bulgaria.
3. TVMV is most common in mixed infections with PVY in both the Virginia and Burley varieties of tobacco.
4. In isolates of the Basma tobacco varieties, the presence of TVMV was not detected.
5. In tobacco, the two potyviruses cannot be distinguished on the basis of symptoms alone. Accurate diagnosis is possible through the use of ELISA or PCR.

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ISSN 0494- 3244

Тутун/Tobacco, Vol. 68, N⁰ 1-6, 56-65, 2018

UDC: 633.71-276.81(497.775)"2016/2017"

Original Scientific paper

SPECIES OF THE FAMILY PENTATOMIDAE-HEMIPTERA - PESTS ON TOBACCO PLANTS

Vesna Krsteska

St. "Kliment Ohridski" University-Bitola, Scientific Tobacco Institute- Prilep, Kicevska, bb, 700 Prilep, Republic of Macedonia
e-mail: vkrsteska@yahoo.com

ABSTRACT

Nezara viridula, Dolycoris baccarum and *Eurydema ornata* are polyphagous feeders attacking important major horticultural and field crops. Hosts include variety of weeds. These pests have cosmopolitan distribution and they are resistant to many pesticides.

During 2016/2017, a sweep net method was used around the tobacco plots (ten replications/10-day intervals) in the region of Prilep. The occurrence of *D. baccarum* and *E. ornata* was investigated on 100 tobacco plants/decade, in 4 replications. Samples of *N. viridula* were collected directly from tobacco leaves, and bred in the laboratories of Scientific Tobacco Institute.

Dry warm weather 2016/2017 allowed development of phytophagous bugs (Pentatomidae, Hemiptera) on tobacco. The nymphs and adults fed on the upper or lower surface of the tobacco leaves. They injected saliva into the leaf tissue and suck up nutrients from plant juices. Tobacco leaves were pierced and deformed.

N. viridula and *D. baccurum* were established in all development stadiums on tobacco and *E. ornata* only as an adult. The quantitative presence of investigated pentatomids was bigger in 2017. In August 2017 on surrounding weeds was identified great attack, 201 samples of *N. viridula*, 96 samples of *D. baccarum* and 58 samples of *E. ornata.* In September 2017, on top tobacco leaves we found 628 samples of *D. baccarum* and 243 samples of *E. ornata.* Individal samples of *N. viridula* fed on the margins of the tobacco plots.

Investigated species are an economically -important pests in most of the areas in which they occurs. On tobacco plants they cause injury to plant tissues, reduce quality of tobacco and therefore, cause economic loss. Effective integrated control measures will reduce pest damage in tobacco production.

Keywords: *Nezara viridula, Dolycoris baccurum, Eurydema ornata, tobacco.*

ВИДОВИ ОД ФАМИЛИЈАТА PENTATOMIDAE-HEMIPTERA - ШТЕТНИЦИ НА ТУТУНСКИТЕ РАСТЕНИЈА

Nezara viridula, Dolycoris baccarum и *Eurydema ornata* се полифагни видови, напаѓаат економски значајни градинарски и поледелски култури, а опсегот на домаќини вклучува и различни видови на плевели. Овие штетници имаат космополитска дистрибуција и се резистентни на многу пестициди.

Во текот на 2016/2017 година, во околината на Прилеп се користеше методот косење со кечер околу тутунските парцели (десет повторувања/ 10-дневни интервали). Појавата на *D. baccarum* и *E. ornata* била испитувана на 100 тутунски растенија/ по декади, во 4 повторувања. Примероците на *N. viridula* беа собрани директно од листовите од тутун и се одгледуваа во лабораториите на Научниот институт за тутун.

Сувото и топло време во текот на 2016/2017 овозможи развој на фитофагни стеници (Pentatomidae, Hemiptera) на тутун. Нимфите и возрасните се хранат на горната или долната површина од тутунските листови. Тие инјектираат плунка во лисното ткиво и ги цицаат хранливите сокови од растенијата. Тутунските листови беа прободени и деформирани.

N. viridula и D. baccurum беа застапени во сите развојни стадиуми на тутунот, а *E. ornata* само како возрасни. Квантитативното присуство на испитувани стеници беше поголемо во 2017 година. Во август

2017 година на околните плевели беше утврден голем напад, 201 примерок од *N. viridula*, 96 примероци од *D. baccarum* и 58 примероци на *E. ornata*. Во септември 2017 година, на врвните тутунски листови беа утврдени 628 примероци на *D. baccarum* и 243 примероци на *E. ornata*. Поединечни примероци на *N. viridula* се хранат на маргините на тутунските парцели.
Проучуваните видови се економски-значајни штетници во повеќето области во кои се појавуваат. На тутунските растенија тие предизвикуваат повреда на растителните ткива, го намалуваат квалитетот на тутунот и притоа предизвикуваат економска загуба. Ефективните интегрирани мерки за контрола ќе го намалат оштетувањето од штетниците при производството на тутунот.

Keywords: *Nezara viridula, Dolycoris baccurum, Eurydema ornata,* тутун

INTRODUCTION

Nezara viridula is cosmopolitan and it is known as southern green stink bug or green vegetable bug. It is believed to have originated in Ethiopia. Its distribution now includes the tropical and subtropical regions of Europe, Asia, Africa and the Americas. The pest feed on wide variety of plants, and hosts include native species, weeds, garden flowers, vegetables, maize, fruit crops and macadamia nuts.
Dolycoris baccarum is one of the most widely distributed Pentatomide. It is found on a variety of plants, herbs and etc., as wheat, barley, maize, cabbage, carrots, peas, onions, tobacco and other crops. The pest is known as Hairy shieldbug, Sloe Bug or Stinking Bug.
Eurydema ornata, known as red cabbage bugs occurs in Europe, North Africa, South and East Asia. It prefers open areas with low vegetation. This shield bug sucks the sap of plants, especially crucifers (Brassicaceae family), such as cabbage, cress and radish.

MATERIAL AND METHODS

We observed *N. viridula, D. baccarum* and *E. ornata* as pests in commercial tobacco fields in Prilep (2016-2017).
Samples of *N. viridula* are collected directly from tobacco leaves, and a sweep net method was used around the tobacco plots (ten replications/10-day intervals). Collected material was analyzed, nourished and bred in the laboratories of Scientific Tobacco Institute.
The occurrence of *D. baccarum* and *E. ornata* was investigated on 100 tobacco plants/decade, in 4 replications. Annually (are analyzed 3600 tobacco stalks. Also they are collected using a sweep net method.

RESULTS AND DISCUSSION

Dry warm weather 2016/2017 allowed development of three species of Pentatomidae (Hemiptera) on tobacco: *N. viridula, D. baccarum* and *E. ornata*. They are hemimetabolous insects. The nymphs and adults fed on the upper or lower surface of the tobacco leaves. They injected saliva into the leaf tissue and suck up nutrients from plant juices. Tobacco leaves were pierced and deformed. Young strongly attacked plants can die.
The species N. *viridula* is a new pest on tobacco fields in Macedonia (Figure 1).
Backlay, 2004, state that the pest attacks on other species of the Solanaceae family such as tomato.
According to Tirelli, 1953, this species is not found in northern and central Europe, and is common in the south.
In August 2016 we saw groups of eggs on tobacco leaves. The number of eggs on tobacco was almost identical with the number of *D. bacarum*, but they differ in color. The eggs of *D. baccarum* have orange color, and those eggs had whitish yellow-pink color. Some days later, eclode black spherical larvae and they looked like a small spiders.

Fig. 1. Adult of *N.viridula* after eclossion 8/2017 (photo: V. Krsteska)

During 2016 we have identified individual samples of *N. viridula* on tobacco.
According to Finke, Oonincx, 2014, *N. viridula* mainly feeds at night.
The quantitative presence of green vegetables is bigger in 2017 and the pest feeds on the margins of the tobacco plots. At the end of August we register greatest attack on surrounding weeds (Table 1 and Figure 2).

Fig. 2. *N. viridula* attack on surrounding weeds (photo: V. Krsteska)

The adult has green body with yellow brown antennas and legs. The antennae are with five segments and the tarsi are 3-segmented. It has sucking mouthparts, in which the modified mandibles and maxillae form a "stylet" which is sheathed within a modified labium. The stylet is capable of piercing tissues and sucking liquids, while the labium supports it. The body is trapezoidal in shape (shield-shaped), and medium-sized. At the base of the scutellum, it has three white spots, arranged in line. The front lateral margin of the scutellum also has two black specks.
Adult's body length in laboratory conditions is 10-11 mm. According to Squitier, 1997, the males are in average 12.1 mm in length and females 13.15 mm.
The wings completely cover the abdomen. The forewings -hemelytra, with the basal half thickened while the apex is membranous (as are the hindwings).
The female lays yellow-pink eggs in groups (14-30) on the upper or underside of tobacco leaves (Figure 3).

According to Squitier, 1997, the eggs are deposited in masses that range from 30 to 130 eggs per mass.
Adults lay their eggs on the lower page of leaves or other parts of the plants, in groups in the form of covering crusts from 40 to just over 100 eggs (Tirelli, 1953).

Fig. 3. Eggs of *N.viridula* on tobacco (photo: V. Krsteska)

The eggs are about 1 mm to 1.3 mm in size. In the beginning the eggs are yellowish then they turn in pink before hatching. The eggs were firmly glued to each other and to the substrate. According Tirelli, 1953, the egg is sub-cylindrical, flesh-colored, with an areolated surface with short, uniform projections around the operculum, 1.3-1.4 mm long, 1.1-1.2 mm wide..
One of the measurements of IPM control of this species is collecting the leaves on which the eggs were laid and destroying them.
There are five nymphal instars, and every instar has specific color, patterns and differs by the length (Figure 4-6). Each instar resembles the adult more than the previous one. In L_1 and L_2 the larvae are almost black and spherical. The third and fourth instar is also black, but has more white and yellow spots on upper side of the abdomen. The fifth instar nymphs are green with a pink, yellow or white abdominal spots

Fig. 4. L_1 and L_2 of *N.viridula* on tobacco (photo: V. Krsteska)

Fig. 5. L_3 of *N. viridula*
(photo: V. Krsteska)

Fig. 6. L_5 of *N. viridula*
(photo: V. Krsteska)

The young larvae aggregate together on the host plant. In the last two instars they split up, spread through plant crops and cause damages. The adults are active fliers and are spreading across the fields. During the late autumn adult color became dark green (Figure 7).

Fig. 7. Color variation of adults
(photo: V. Krsteska)

The green stink bug *N. viridula* was established in all development stadiums on tobacco. According to Kogan et al., 1999, it had become a severe pest due to its polyphagous habit that enables *N. viridula* to damage many vegetable and field crops.

The adults of *N. viridula* winter in various shelters and in spring they come out and feed on herbaceous and arboreal plants of many families: Cruciferae, Solanaceae, Malvaceae, Fabaceae, Poaceae (Tirelli, 1953).

D. baccarum is one of our commonest bugs and can occur in a wide variety of habitats. On tobacco it was established in all development stadiums.

Popular name of these species in our country is “Stinking Martin” because it produces a pungent unpleasant odor released from pores in the thorax.

Adult is medium-sized, oval bug and can reach a length of about 10–12 mm (Figure 8).

According to Tanasijevic, Simova-Tosic, 1985, body length is in average 10 mm.

The basic color of the body is reddish-brown or grayish brown, with yellowish markings. The margins of the abdomen are alternately mottled with yellowish and black spots. The whole body is covered with hairs. During the winter adult color became dull brown (Figure 9).

Fig. 8. Adult of *D. baccarum* 7/2017
(photo: V. Krsteska)

Fig. 9. Adult of *D. baccurum* 9/2017
(photo: V. Krsteska)

Eggs are cylindrical and have pale yellow-orange color. They are neatly arranged into groups (Figure 10). On the tobacco leaves were established from 13 to 20 eggs laid in groups.

According to Tanasievic, Ilic, 1969, the eggs are deposited in masses, 40 eggs per mass.

Fig. 10. Eggs and L_1 of *D. baccarum*
(photo: V. Krsteska)

The nymphs moult several times as they grow. Larvae are dorsal covered with fine small hairs and have different spots during the larval development (Figure 11).

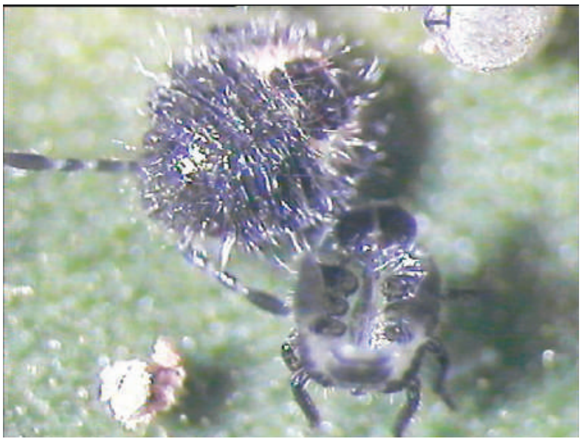

Fig.11. L_2 of *D. baccarum*
(photo: V. Krsteska)

In April/May, on young seedlings in tobacco beds we determine individual adults of *D. baccarum*. Also in June/July we established individual samples of bugs on tobacco plants.
In sunny hours the pests are climbed to the top of the tobacco plant, where they feed, mate and lay eggs. When the weather is hot adults are often lurking in the lower part of the tobacco plant.
The eggs are deposited on the upper side of the tobacco leaves from mid July (frst observation 12.07.2016; 14.07. 2017) till the end of August (last observation 20.08.2016; 26.08. 2017).
Adults frequent fly from one to another tobacco plants. When it is disturbed, it leaves behind an awful stinking substance to protect it selves and discourages predators.
From the end of August and during September on tobacco leaves and seed capsules were found great attack by *D. baccarum.* Up to 5 shield-bugs per plant occur on top leaves, and leaves developed light-colored spots at the punctured loci, resulting wilt and desiccate of the plants.
On tobacco was determined *E. ornata* (Hemiptera Pentatomidae) in adult stadium. Adult has black head and general reddish color of the body covered with small black spots (Figure 12). It can reach a length of 7-10 mm.

Fig.12. Adult of E. ornata 9/2017
(photo: V. Krsteska)

The species *E. ornata* can be found on the top of tobacco plants on sunny days. Adult suck up plant juices from top leaves and flower buds of tobacco, cause punctures and leaves brown spots (Figure 13).

Fig.13. Adult of *E. ornata* 7/2017
(photo: V. Krsteska)

According to Postolovski, Lazarevska, 2014, representatives may cause damage to plant parts (flowers, flower stalks, seeds), and in case of mass appearance can cause a reduction of up to 20% of the yield.

Table 1. Quantitative representation of investigated pentatomids on weeds (method: insect's sweep net)

Species	Year of investigations	Junu	July	August	September	Total
N. viridula	2016	-	-	38	7	45
D.baccarum		17	23	73	25	138
E. ornata		13	5	36	11	65
N.viridula	2017	-	10	201	37	248
D.baccarum		25	21	96	51	193
E. ornata		29	11	58	14	112

At the end of August are register greatest quantitative representation of investigated pentatomids on weeds around the tobacco plots (Tab. 1). One of IPM measurements is frequent control of weeds within and around the crops it is necessary.
According to Table 2 great quantitative representation of investigated species are register in August and September. In September 2017, on top leaves we found 628 samples of *D. baccarum* and 243 samples of *E. ornata.*

Table 2. Number of tobacco stalks attacked by *D. baccarum* and *E. ornata*
(method: survey of 100 randomly selected tobacco plants)

Species	Year of investigations	July	August	September	Total
D. baccarum	2016	97	209	321	627
E. ornata		60	95	127	282
D. baccarum	2017	139	277	628	1044
E. ornata		72	174	243	489

In autumn adults (influenced by low temperature in October 2016/ November 2017) stop their activities, and look for place for wintering. They spend the winter as an adult in the ground-based soil cracks or under plant debris, hides in the bark of trees or walls or other locations to obtain protection from the weather.
In spring pentatomid bugs move out of the winter cover and begin feeding on leaves and flower buds of the plants and cause damage on plants especially on seed pods.
Investigated pests cause direct damage on tobacco plants, but they also may transmit plant pathogens which increase their damage potential.
A strong linear relationship exists between stink bug density, seed damage, and yield loss (Daugherty et al., 1964; McPherson et al., 1974; Yeargan, 1977 cit. Rupe, Luttrell, 2008).

CONCLUSION

- Dry warm weather 2016/2017 allowed development of three species of Pentatomidae (Hemiptera) on tobacco: *N. viridula, D. baccarum* and *E. ornata*.
- *N. viridula* is a new pest on tobacco fields in Macedonia and on tobacco it was established in all development stadiums. Adult's body length in laboratory conditions is 10-11 mm. The female lays yellow-pink eggs in groups (14-30) on the upper or underside of tobacco leaves. The quantitative presence of green vegetables is bigger in 2017 and the pest feeds on the margins of the tobacco plots. At the end of August we register greatest attack on surrounding weeds.
- The species *D. baccarum* was found in all development stadiums on tobacco. On the tobacco leaves were laid from 13 to 20 eggs in groups. According quantitative representation it attacked 627 tobacco stalks in 2016 and 1044 in 2017.
- On tobacco was determined *E. ornata* only in adult stadium. In 2016, *E. ornata* attacked 282 tobacco stalks, and 489 tobacco stalks in 2017.
- Investigated pests cause injury to plant tissues, reduce quality of tobacco and therefore, cause economic loss.

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ISSN 0494 – 3244

Тутун/Tobacco, Vol. 68, N^0 1-6, 66-74, 2018

UDC: 633.71:581.192]:543.423.1(497.7)
631.416:543.423.1]:633.71(497.7)

Original Scientific paper

COMPARATIVE ASSESSMENT AND DIFFERENTIATION BY MULTIELEMENT COMPOSITION OF MACEDONIAN ORIENTAL TOBACCO AND CORRESPONDING SOIL

Biljana Jordanoska Šiškoska[1], Valentina Pelivanoska[1], Trajče Stafilov[2]

[1] "St. Kliment Ohridski" University , Bitola , Scientific Tobacco Institute, – Prilep, Kičevska bb, Republic of Macedonia;
[2] Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss Cyril and Methodius University, Arhimedova 5, Skopje, Republic of Macedonia
e-mail: bi.jordanoska@gmail.com

ABSTRACT

Data of the elemental composition of the tobacco and corresponding soil samples from 19 municipalities from three tobacco growing regions in the Republic of Macedonia were analyzed using multivariate statistical technique (factor and cluster analyses). The main aim was to determine the correlation between the content of elements in tobacco samples and to investigate the possibility of differentiating production areas on the basis of element associations. In the group of 18 elements (Ag, Al, Ba, Cd, Cr, Cu, Li, Fe, Mn, Na, Ni, Pb, V, Sr, N, P, K and Zn) analyzed by atomic emission spectrometry with inductively coupled plasma (ICP-AES), only Fe and V content in soil has a statistically significant dependence with corresponding content in plant material. Both soil and plant samples are well clustered according to their locality. Multivariate analyses indicated that given data may be differentiated according to the association of elements of the soil and elements derived from human activity as well as and by producing areas.

Key words: tobacco, soil, multivariate analysis, differentiation

КОМПАРАТИВНА ПРОЦЕНКА И ДИФЕРЕНЦИЈАЦИЈА СО МНОГУЕЛЕМЕНТЕН СОСТАВ НА МАКЕДОНСКИОТ ОРИЕНТАЛЕН ТУТУН И ПОЧВИ

Елементниот состав на примероци од ориентален тутун и соодветни почвени екстрати од 19 општини од три тутуно-производни реони во Република Македонија се анализирани со помош на мултиваријатна статистичка техника (факторна и кластерна анализа). Главната цел е да се одреди корелацијата помеѓу елементниот состав на примероците тутун и да се одреди можноста за диференцијација на производните области врз база на асоцијацијата на елементи. Во групата од 18 елементи (Ag, Al, Ba, Cd, Cr, Cu, Li, Fe, Mn, Na, Ni, Pb, V, Sr, N, P, K and Zn) анализирани со помош на атомска емисиона спектрометрија со индуктивно спрегната плазма (ICP-AES), само содржината на Fe и V во почвените примероци има статистички значајна зависност со составот на соодветниот растителен материјал. Почвените и растителните примероци добро се групираат согласно нивното потекло. Мултиваријатната анализа покажува дека дадените податоци можат да се диференцираат согласно групации на елементи од антропогено и литогено потекло, а со тоа и по производните области.

Клучни зборови: тутун, почва, мултиваријатна анализа, диференцијација

INTRODUCTION

Tobacco as a culture has high availability to adapt on soil and climate conditions, but high-quality tobacco is produced only on soil with favorable conditions. Republic of Macedonia is one of the countries where production of oriental Tobacco (*Nicotiana tabacum* L.) is main lucrative crop. Same as all plants, the element content in species has two major sources: lithogenic and anthropogenic. Natural sources depend from the parent material of the soil. On the other side anthropogenic sources include various origins such as: application of organic and mineral fertilizers, pollution of the environment and other chemicals used in the process of growth and protection of the tobacco raw. The aim of this study was to provide statistical comparison of tobacco major and trace element composition with the composition of the corresponding soil samples.

Chemical content in both, plant and soil samples have been assessed for many reasons and due to the significance of this culture it is used as indicator for providing information on possible trace element contamination. There are three main growing regions in the Republic of Macedonia: Pelagonia, Vardar Valley and South-eastern region. In most areas the tradition extends from XVI century (Mickoski, 2004). Pelagonia is known as the largest tobacco production area with three mines and the region rich in lignite (Yossifova et al., 2009; Barandovski et al., 2012; Jordanoska, 2014). The second largest region is the South-eastern region where recent studies are focused on biomonitoring correlated with copper mine and flotation plant (Balabanova et al., 2010; Balabanova et al., 2011; Stafilov et al., 2010a). The region of tobacco production in the Vardar Valley is also associated with the lead and zinc and nickel industries in the recent past with the possible impact of soil, vegetable and fruit contamination (Stafilov et al., 2010b, 2010c; Bačeva et al., 2011, 2012; Pančevski et al., 2016; Stafilov and Šajn, 2016).

In this work data of the elemental composition of the tobacco and corresponding soil samples from 19 municipalities from these three tobacco growing regions were analyzed using factor and cluster analyses. Cluster analyses were performed to discover the similarity of the samples from studied localities. Initially according to the analyses, the nearness of the objects defined by their variables, reflect the similarity of the properties. Correlation analyses were carried out to reveal the relationship with ambient soil properties and chemical composition of tobacco plant. Statistical multivariate analysis was done to obtain these correlations and to investigate the possibility of differentiating production areas on the basis of element associations. The goal was to obtain distinction and to determine the origin of the chemical composition of tobacco raw and corresponding soil samples by statistical comparison.

MATERIALS AND METHODS

Analyses of plant and soil material

Hundred and fifty oriental tobacco samples and 150 soil samples were taken from 19 municipalities in the three tobacco growing areas: Pelagonia, Vardar Valley and South-eastern region. All terrains are with deluvial and alluvial soils. Tobacco leaves from three harvestings were collected and mixed for 150 representative samples. Leaves were washed carefully to remove any adhering soil particles and rinsed with redistilled water. The plant material was dried to a constant weight at 75^{o}C for 12 hours and homogenized. Soil composites were sampled at fixed depth (0-30 cm) in each field with two replicates (Hawks and Collins, 1987). Summary of the selected methods and instrumentation are given in Table 1. The reference standard materials JSAC 0401 (soil) as well moss samples M-2 and M-3 (Steinnes et al., 1997) were used for quality control.

Table 1. **Summary of the selected methods and instrumentation**

SOIL SAMPLES	*METHOD*
Total nitrogen	ISO 11261 - Micro-Kjeldaal method, modified
Organic matters (OM)	Wet oxidation method (Pelivanoska, 2012)
pH	ISO 10390 - potentiometric with pH meter
Available phosphorus and Potassium	Ammonium lactate method (Pelivanoska, 2012)
Cation exchange capacity (CEC)	Sumner and Miller (1996)
Total content of the elements	For total digestion, soil samples (0.2500 g) were placed in a Teflon digestion vessel and digested on a hot plate (ISO 14869-1) measured by ICP-AES (Varian, 715-ES)
PLANT SAMPLES	*METHOD*
Total content of the elements	Digested in Teflon vessels with HNO_3 and H_2O_2 using the Mars microwave system (CEM, USA) measured by ICP-AES with ultrasonic nebulizer CETAC (ICP/U-5000 AT)
Nicotine content	ISO 2881:2010 - Determination of alkaloid content by Spectrometric method
Total nitrogen	Modified Kjeldahl method (Srbinoska, 2012)
Sugars content	Schmuk and Bertrand (Srbinoska, 2012)

Statistical analyses

The data were treated statistically with the program IMB Statistics SPSS v. 19. The correlation between macronutrient elements and other selected soil properties are expressed by Pearson two tailed correlation coefficients. All outlying values of the analyzed parameters were excluded. For the purpose of multivariate analyses, each of the 150 tobacco samples was considered to be a vector with 24 variables (soil properties and element content). Data matrix with dimensions 150x24 was basis for cluster, correlation, principal component and factor analyses. All variables were transformed to values of normal distribution. Cluster analyses were done in order to discover the similarity of the samples according to studied localities. Principal component analyses was done to reduce the number of original variables according to their linear combination and to discover their further dependencies. Factor analyses were used to establish basic data structure of the variables in tobacco leaves by varimax rotation technique.

RESULTS AND DISCUSSION

Chemical composition of tobacco leaves and corresponding soil samples are given in Table 2. Content of the studied elements in tobacco samples have greater scatter than those in soil samples. Wide range of physical and chemical properties based on their geochemical origin and long cultivation period are given in (Jordanoska et al. 2014, 2018). Element contents of the tobacco samples corresponds to the literature for the quality oriental tobacco leaves and is all below the contamination limit for plants (Tso, 1990, Gondola and Kadar, 1994; Metsi et al., 2002; Golia et al., 2009; Rodgman and Perfetti, 2009; Pelivanoska et al., 2011, Kabata-Pendias, 2011).

Total chemical content of soil samples (Table 2) are comparable to those obtained from the study on agricultural soils of Europe (VROM 2000, Salminen et al. 2005; Soriano-Disla et al. 2012). In most soil samples from Vardar Valley nickel content is higher as described by Stafilov and Šajn (2016), due the parent rock material and anthropogenic influence. Content of barium in all three production regions was above the optimum value of 200 mg/kg (VROM, 2000) that is related to the lithogenic origin (Stafilov and Šajn, 2016). Content of Fe, above 5% higher than the Fe content in European topsoil average of 2.2 % (Salminen et al. 2005), was observed at Bitola and Mogila sampling areas as influenced from the open coal deposit in the Pelagonia area (Stafilov et al. 2018).

Table 2. Descriptive statistics of total chemical composition of tobacco leaves and soil samples (n=150). The values for Al, Ca, Fe, K, Mg and Na in soil samples and TN, SS, nicotine in tobacco samples are given in %, for the rest of the elements in mg/kg, X_g – geometrical mean, Md - median, *SD*– standard deviation, TN- total nitrogen, SS- soluble sugars, SN- Shmuk number

	Tobacco leaves					Soil samples				
	X_g	Md	SD	Min	Max	X_g	Md	SD	Min	Max
Al	1472	1305	822	158	4527	4.4	4.4	1.0	2.3	6.8
Ba	45	44	16	13	103	318	296	116	133	764
Ca	14920	14603	3398	7707	28065	9.9	9.3	6.2	1.5	41
Cd	0.41	0.3	0.3	0.1	1.9	-	-	-	-	-
Cr	2.7	1.8	4	0.3	33	49	44	32	13	292
Cu	12	11	4	4	35	18	15	9.6	5	53
Fe	871	732	484	147	2679	2.3	2.2	0.8	0.9	4.9
K	7610	7653	2225	2575	13292	1.0	0.9	0.3	0.4	2.0
Li	13.9	11	10	2	49	14	13	7.0	3.0	53.0
Mg	1305	1311	231	744	1883	0.5	0.5	0.2	0.1	2.0
Mn	68	57	62	27	749	527	496	163	218	1268
Na	64	59	20	38	150	0.8	0.8	0.3	0.1	1.7
Ni	2.5	2.1	2	0.6	16	25	20	18.9	5.0	124
P	834	807	239	439	1991	453	377	295	73	2098
Pb	1.0	0.9	1	0.5	4.0	14	12	4.9	10	30

Sr	48	48	16	12	109	122	107	83.0	23	537
V	2.6	2.1	2	0.5	11.1	63	56	28.6	20	217
Zn	22	21	7	8	52	65	50	127	21	1534
TN	2.4	2.4	0.4	1.4	3.6					
SS	14	13.9	4	5.8	25					
SN	1.9	1.88	1	0.5	4.6					
Nicotine	1.7	1.7	0.5	0.7	3.1					

A cluster analysis was used to find the similarity of the tobacco and soil samples using all determined parameters. In the Fig. 1a) tobacco samples from Pelagonia Region and South-eastern Region are each clustered together indicating well distinguish from the others. Tobacco samples are better clustered than soils from studied areas (Fig. 1b).

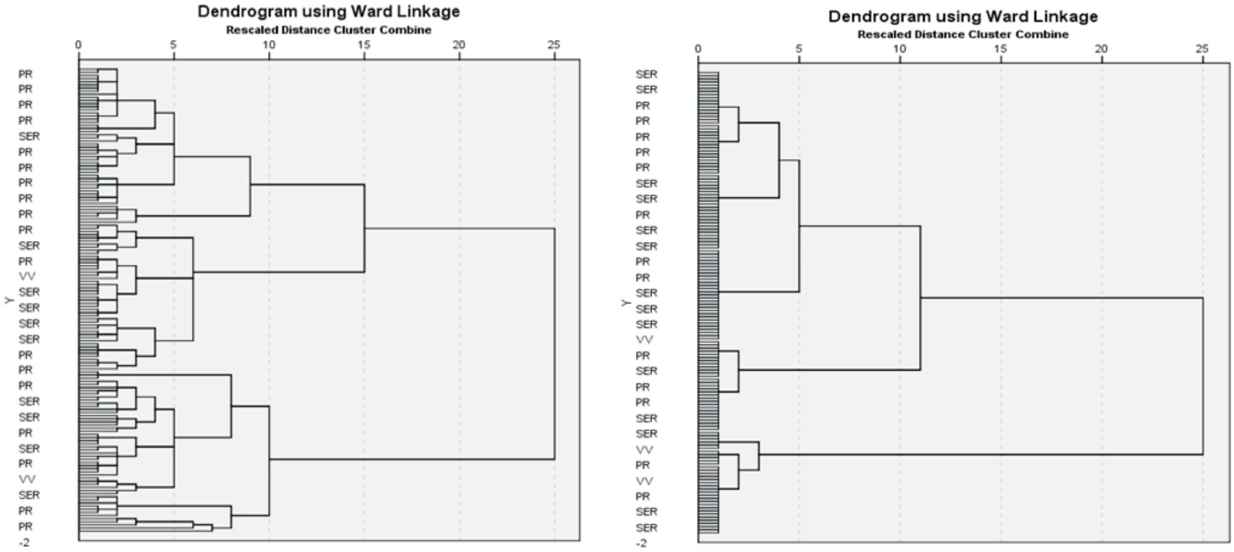

Fig. 1 Dendrograms of the cluster analysis of tobacco (a) and soil samples
(PR- Pelagonia region, SER-South Eastern region, VV-Vardar valley)

It was found that only Fe and V content in soil has a statistically significant dependence with corresponding content in plant material (correlation coefficients >0.600). This means that content of both elements in plant material is dependent on their content in soil samples. The content of all other determined elements between the soil and plant material are generally independent. This can be explained with various uptakes and transport of the ions (Fijałkowski et al. 2012). Bivariate correlation between soil parameters and chemical content of soil and plant material was also investigated. Correlations among total element content in soil and soil parameters are generally not significant. Correlation coefficients above 0.5 and significance at the 0.01 level are further discussed. Besides inter element correlation, only clay exhibits significant negative correlation with Na content of tobacco (r = -0.559). Most lithophile elements are positively mutually correlated (Mg with Cr, and Sr with Ba, Ca, Na). Significant positive correlation is found among Fe and V (r = 0.908), Cu (r =0.625), Mn (r =0.681) and Cu and Zn (r =0.656). Significant dependence between total nitrogen content and nicotine (r = 0.625), and nitrogen content and soluble sugars (r = -0.707) of the tobacco samples was observed.

PCA was performed to reduce the number of original variables and to determine the relationship between the element content in tobacco samples. The first six principal components explained 76.54 % of the total variability among 22 variables. First component (PC1) contributes with 24.03% of the total variance and the second component (PC2) contributed with 14.03 %. The results of the loading plots of the variables presented on PC1-PC2 plot is given in Fig. 2a). It can be seen that most of the analyzed elements dominate in the positive values of PC1. On the other hand, chemical parameters that determine the tobacco quality such as Shmuk number and soluble sugars (SS) show considerably negative values in the in the same component PC1.

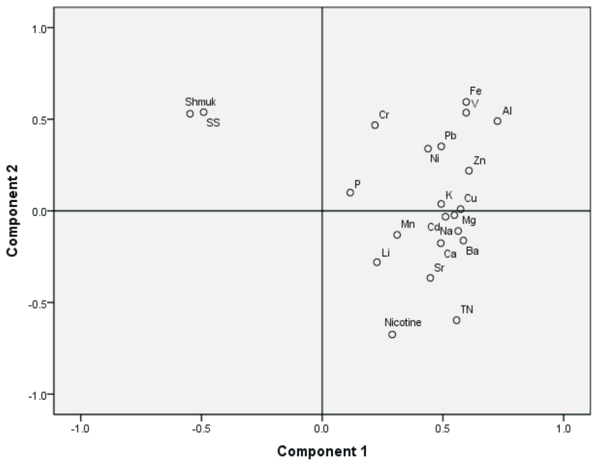

Fig. 2a. Loading plots for principal component analyses in tobacco samples

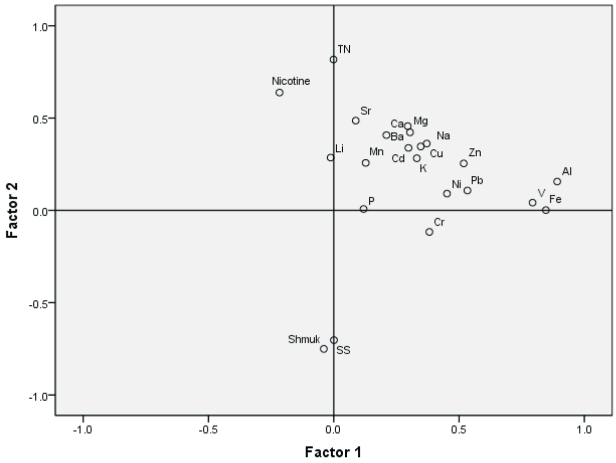

Fig. 2b. Loading plots for first two factors in tobacco samples after varimax rotation

Associations between the variables in tobacco samples were determined and by using factor analysis (FA). The first six extracted components explain 74.95 % of the total variance. In the Fig. 3b are given factor loading plots of the two factors after varimax rotation. Factor 1 represents 27.30% of the total variance and the second factor 14.90%. Similar as PCA, there is domination in association of the variables in the factor 1 (Fig. 2b, Table 3).

Table 3. Rotate component matrix (n=150) for 18 selected elements in tobacco leaves, Com- Communality (%), Var- Variance (%)

Element	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*Com*
Fe	**0.96**	0.03	0.05	-0.02	0.15	0.02	94.91
V	**0.92**	0.13	0.03	-0.06	0.11	0.04	88.94
Al	**0.92**	0.19	0.16	0.05	0.08	0.06	92.84
Pb	**0.65**	0.00	0.17	0.22	0.04	-0.08	50.34
Sr	-0.05	**0.91**	-0.04	0.08	-0.04	0.15	85.87
Mg	0.13	**0.77**	0.30	-0.11	0.15	-0.19	76.93
Na	0.22	**0.64**	0.13	0.34	-0.07	0.01	60.03
Ba	0.16	**0.64**	0.16	0.09	0.02	0.43	66.07
Zn	0.24	0.11	**0.80**	0.26	0.09	0.01	78.56
Cd	0.01	0.03	**0.77**	0.11	0.13	0.33	72.26
Cu	0.16	0.25	**0.75**	-0.10	0.08	0.04	67.09
P	0.00	-0.02	-0.14	**0.86**	-0.01	-0.02	76.03
K	0.16	0.04	0.26	**0.74**	0.01	0.22	69.60
Ca	0.01	0.37	0.19	**0.66**	0.01	0.02	60.75
Cr	0.18	-0.03	-0.01	-0.02	**0.93**	-0.09	90.76
Ni	0.13	0.06	0.31	0.04	**0.88**	0.09	90.75
Mn	0.12	0.08	0.02	-0.02	0.05	**0.81**	67.65
Li	-0.20	0.04	0.27	0.19	-0.09	**0.66**	59.65
Var	27.34	14.90	9.95	9.40	7.43	5.94	74.95

Analyzed trace elements further can be grouped in two geogenic, two mixed (geogenic-anthropogenic) and two anthropogenic associations (Table 3). Factors connected to the nature of the elements are factor 2 (Sr, Mg, Na and Ba) and factor 6 (Mn and Li). Compared to other production regions, the highest levels of both associations are measured in the samples from Pelagonia region where three mines rich in lignite are located (Yossifova et al. 2009; Barandovski et al. 2012; Jordanoska et. al. 2014). Mixed factors are presented by first factor (Fe, V, Al and Pb) and fourth factor (P-K-Ca). According to our study (Jordanoska et al. 2018), all mentioned elements of this factors are most absorbed by tobacco leaves rather than the other oriental tobacco organs. Associations of elements affected by human activity are given in factor 3 (Zn, Cd, Cu) and fifth factor (Cr and Ni). Both associations are correlated with the lead and zinc industry in Vardar Valley region, as well as copper mine in South Eastern region. This is consistent with the previous findings of Balabanova et al. (2012) and Barandovski et al. (2008, 2012). Content of the group of elements connected to the anthropogenic influence in tobacco samples corresponds with elevated levels in the corresponding soil samples.

CONCLUSIONS

Statistically significant dependence between the content of the tobacco samples and corresponding soil sample is exhibited only for Fe and V. It was also observed that the nicotine and soluble sugars content is affected by nitrogen content of the tobacco samples. Soil clustering did not follow tobacco clustering, indicating the individuality practices of the producers. Generally, samples are well clustered according to their locality. Most of the lithophile elements have a predominant positive correlation. Factor analyses and principal component analyses indicated that given data may be differentiated according to elements from the soil and elements derived from human activity. It has been shown that there is possible differentiation for the producing areas and that multivariate analyses application is suitable for analyzing such extensive data set.

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ISSN 0494 – 3244

Тутун/Tobacco, Vol. 68, N⁰ 1-6, 75-82, 2018

UDC: 633.71-152.61:631.84(497.774)"2010/2013"
Original Scientific paper

EFFECTS OF DIFFERENT NITROGEN RATES ON THE YIELD AND QUALITY OF ORIENTAL TOBACCO PRODUCED IN PELAGONIA REGION

Valentina Pelivanoska, Biljana Jordanoska Šiškoska

"St. Kliment Ohridski" University, Bitola, Scientific Tobacco Institute, Prilep, Kičevska bb, Republic of Macedonia
e-mail: vpelivanoska@yahoo.com

ABSTRACT

Field trials were carried out during 2010 - 2013 in Dobrushevo, Pelagonija Region, to investigate the effects of different nitrogen rates upon yield and quality of tobacco varieties: Prilep P-23, Prilep P-79-94 (domestic varieties), and Basma1 and Elenski 817 (introduced varieties). Investigation included four varieties, two nitrogen rates (20 and 30 kg/ha) and constant amount of phosphorus (60 kg/ha) and potassium (40 kg/ha). According to the results, the best effect on the yield had fertilized varieties with the 30 kg N/ha, where yield increases of 24.14%, 26.75%, 23.57% and 24.29 % respectively, as opposed to the non fertilized variant. The average purchase price of tobacco, expressed in % of quality classes, has a slight decrease with increasing nitrogen rates in all investigated varieties. In all investigated varieties was observed increasing content of nicotine, total nitrogen and mineral matter and decreasing content of the soluble sugar with increasing nitrogen quantities.

Keywords: oriental tobacco, nutrients, yield, quality, chemical composition

ЕФЕКТИ ОД РАЗЛИЧНИ КОЛИЧИНИ НА АЗОТ НА ПРИНОСОТ И КВАЛИТЕТОТ НА ОРИЕНТАЛСКИ ТУТУН ПРОИЗВОДЕН ВО РЕГИОНОТ ПЕЛАГОНИЈА

Полски опити се спроведени во периодот од 2010-2013 година во Добрушево, Пелагониски регион, за да се испитаат ефектите од различните количини азот врз приносот и квалитетот на тутунските сорти: Прилеп П-23, Прилеп Р-79-94 (домашни сорти) и Басма1 и Еленски 817 (интродуцирани сорти). Истражувањето вклучува четири сорти, две дози азот (20 и 30 kg/ha) и постојано количество фосфор (60 kg/ha) и калиум (40 kg/ha). Според резултатите, најдобар ефект врз приносот има ѓубрењето со 30 kg N/ha, каде приносот се зголемува од 24,14%, 26,75%, 23,57% и 24,29%, соодветно, наспроти не ѓубрената варијанта. Просечната откупна цена на тутунот, изразена преку учеството на квалитетни класи во %, има мало намалување со зголемување на количините на азот кај сите испитани сорти. Кај сите тестирани сорти забележано е зголемување на содржината на никотин, вкупен азот, минерални материи и намалување на содржината на растворливиот шеќер со зголемување на количините на азот.

Клучни зборови: ориентален тутун, нутриенти, принос, квалитет, хемиски состав

INTRODUCTION

Pelagonija is one of the larger regions for small leaf aromatic tobacco production, in the Republic of Macedonia. Yield and quality of oriental tobacco are closely associated with its availability to absorb nutrients from soil. Tobacco is particularly sensitive to the quantities of nitrogen in soil. There are many

models that have been developed to predict the release of nitrogen from applied fertilizers (Cheng-Wei et al. 2014, Fan and Li 2010, Lobell 2007). Nitrogen fertilizers are absorbed directly by plants or converted into various other forms were excess of nitrogen is lost through denitrification, leaching or volatilization (Cheng-Wei et al. 2014, Tamme et al, 2009, Brady et al. 2008). This very important nutrient has a positive impact on yield and quality of tobacco to a certain limit. After that, the yield can be increased but the quality of produced tobacco substantially declines (Akehurst 1981; Kozumplik & Cavlek 1986, Pelivanoska 1999). In conditions of strong fertilization and higher nitrogen availability tobacco forms larger leaves with prominent nervature, rougher and thicker tissue, difficult to dry and with poor color, with higher percentage of nitrogen and protein, reduced percentage of sugar and bad smoke properties.
Uncontrolled use of fertilizers is an important issue for sustainable agriculture and has a negative effect not only on tobacco quality but it also increases the production costs and has negative impact on the surrounding environment Zebarth et al. (2009). Every agricultural system should include yield and environmental quality during management. In this respect, during four year period, the influence of increasing rates of nitrogen in fertilization in agro ecological condition in Pelagonija region, village Dobrushevo on yield and quality was investigated.
In recent years, the tobacco market has shown the tendency to buy raw materials from a limited number of varieties. The aim of the study is to determine the impact of agro-ecological conditions on the productivity and quality of domestic and introduced varieties of aromatic tobacco. Considering the importance of the mineral nutrition on the growth and development of tobacco during 2010, 2011, 2012 and 2013, we examined the impact of different doses of nitrogen in the mineral fertilizers on the yield and quality of domestic and introduced varieties of aromatic tobacco.

MATERIAL AND METHODS

Four-year trials were carried out in the Pelagonia region, village Dobrushevo, with oriental tobacco varieties Prilep P-23, Prilep P-79-94 (domestic varieties), and Basma1 and Elenski 817 (introduced varieties). Experiment was carried out on fluvisol soil, in randomized complete block design with three replications, two nitrogen rates (20 and 30 kg/ha) with constant amount of phosphorus (60 kg/ha) and potassium (40 kg/ha).
Soil preparation was performed with one autumn (30 cm depth) and two spring plowings (8-20 cm depth). Before the trial was set up, the soil was tested to determine its agrochemical and physical properties. Fertilization was done using 200 kg/ha inorganic mineral fertilizer NPK 10:30:20, and 27% KAN for top up. Fertilizer was applied in the last plowing, prior to planting, together with phoshorus and potassium. The rest of the nitrogen amount for the third variant is applied on the first hoeing.
Each elementary plot has 4 rows with 41 plants in a row, or a total of 164 plants in the plot. The total number of plants in the trial was 6642, with 40 x 12 cm spacing. All necessary agro-technical and plant protection practices were applied during the vegetation period of tobacco. During the vegetation, three irrigations were made, depending on the needs of the plants. Harvesting was done manualy in 5 harvests. After the processes of yellowing and sun-curing, tobacco was graded and weighed. Agrochemical parameters of soil and chemical components of tobacco raw were determined by standard methods in accredited laboratories of the Scientific Tobacco Institute - Prilep. The obtained results were statistically processed with ANOVA – LSD test.

Soil and climate conditions

The experiment is set on the soil type fluvisol. According to the mechanical composition the tasted soil is light loamy. The fraction of physical clay (powder + clay) is fractionally represented by 25.70% in the first layer and 27.30% in the second layer, while the remaining 75% constitutes the fraction of coarse and fine sand. According to agrochemical parameters, the soil has an average humus content, low content of easily accessible phosphorus, optimal availability with easily available potassium and neutral to poorly acidic pH reaction and is a suitable environment for growing oriental tobacco. For production of quality small leaf oriental tobacco, soil must contain 1-1.5 % humus (Mitreski, 2012), 0.5-2.8 % humus and 0.05-0.15% nitrogen Domitrov et al. (2005), moderate phosphorus content, and well potassium level, Naumoski et al. (1977). According to Bailov (1965), good quality conditions give soils with light mechanical composition that have weak acidity or are acid, scarce humus content, where most of the soils have favorable potassium regime.

Table 1 Agrohemical propertis of the soil

Depth (cm)	pH		Humus	mg/100 g soil		Physical clay
	H_2O	KCl	%	P_2O_5	K_2O	%
0 - 30	7.15	6.18	1.77	6.53	21.72	25.70
30 - 60	7.00	6.03	1.51	6.11	20.32	27.30
Classification	Neutral	Slightly acid	Medium	Low	Optimum	Light loamy

Climatic factors have great influence on yield and tobacco quality,(Shmuk, 1938, Nunski 1986). Given data presents average air temperature that varies in tight range of 2 ^{0}C. During the testing period, monthly average temperature is 20.71 ^{0}C that meets the requirement of the tobacco plant. According to the metrological data (Table 2), during the vegetation period are noted precipitation differences of about 90 mm among year 2012 (233.9 mm) and year 2013 (154.8 mm).

Table 2 Meteorological data during the growing season -Bitola

Month	Year	Average air temperature (^{o}C)			Precipitations	Days with
		Daily	Min	Max	mm	precipitations
May	2010	16.9	10.2	23.2	82.4	9
	2011	14.7	8.6	21.3	82.7	14
	2012	16	9.8	22.3	101.5	14
	2013	17.8	10.8	24.2	61.3	6
June	2010	20.2	13.1	26.8	43.2	9
	2011	19.9	12.7	26.8	31.9	7
	2012	22.6	13.4	30	4.7	1
	2013	20.4	12.7	27.7	50.8	6
July	2010	22.5	15.2	29.2	26.5	6
	2011	23.7	14.2	31.3	9.6	5
	2012	25.9	16.1	34.1	5.6	2
	2013	22.6	14.4	30.2	17.2	5
August	2010	24.1	15.1	32.3	-	4
	2011	23	14.2	32	13.7	4
	2012	23.7	14.5	32.3	48.1	5
	2013	23.8	14.8	31.9	6.3	4
September	2010	17.7	11	24.9	46.3	7
	2011	20.3	12.4	29	44	2
	2012	19.4	11.6	27.5	74	4
	2013	18.3	9.9	26.2	18.7	7
Average/Total (V - IX)	2010	20.28	12.92	27.28	198.4	31
	2011	20.32	12.14	27.26	184.2	37
	2012	21.52	13.08	29.24	233.9	24
	2013	21.50	13.1	29.2	154.3	28
Annual average	2010-2013	20.91	12.81	28.245	192.7	
	1999 -2009	**20.0**	**12.2**	**27.1**	**207.7**	

There is no significant difference between the years 2010 and 2011, 198.4 and 184.2 mm, respectively. Considering the literature that small leaf oriental tobacco can be successfully breed in the precipitation range of 100-250 mm, where optimal amount is 120-150 mm (Atanasov 1972), precipitation is in the permitted range during the years of the given research. Although the quantities of precipitation are favorable (Table 2), it is seen that the rainfall distribution is very uneven, which strongly reflects on the yield and the gross profit per unit area. The impact of climate factors on yield and gross income over years is evident, especially in drought 2013, compared to other years.

RESULTS AND DISCUSSION

According to the average results (Tables 3), it can be noted that the yield is proportionally increased with the increasing quantities of nitrogen. Variants fertilized with the highest quantities of nitrogen has increased yield at all varieties, P-23 for 24.14%, P-79-94 for 26.75%, Basma 1 23.57% and Elenski 817 24.29%, compared to the control. Based on the statistical analysis of the achieved yield by years, in all fertilized variants there is statistically significant impact on all three levels of probability. This reflects the full justification of the applied fertilizing during the cultivation of the domestic and introduced tobacco varieties. The table also presents data where is given comparison between the yield in given years of the standard variety P-23, as a control (100%). Based on the data we can see that fertilized variants of domestic variety has higher yield from 11.36% to 24.15% and 8.23 to 20.67%, respectively, where introduced varieties have significantly lowest yield, compared with the control.

Table 3. Average tobacco yeld kg/ha (2010-2013)

Variant	Variety	Year				**Average kg/ha**	Index	Index
		2010	2011	2012	2013			
Ø	P-23	3071	3057	3065	2321	**2878**	100,00	100.00
$N_{20}P_{60}K_{40}$	P-23	3396	3315	3262	2848	**3205**	111,36	111.36
$N_{30}P_{60}K_{40}$	P-23	3693	3688	3508	3405	**3573**	124,14	124.15
Ø	P-79-94	2925	2869	2929	2236	**2740**	100,00	95.21
$N_{20}P_{60}K_{40}$	P-79-94	3299	3334	3132	2694	**3115**	113,68	108.23
$N_{30}P_{60}K_{40}$	P-79-94	3587	3651	3396	3259	**3473**	126,75	120.67
Ø	Basma 1	2137	2178	2060	2005	**2095**	100,00	72.79
$N_{20}P_{60}K_{40}$	Basma 1	2443	2398	2254	2367	**2365**	112,88	82.18
$N_{30}P_{60}K_{40}$	Basma 1	2681	2560	2455	2661	**2589**	123,57	89.96
Ø	Elenski 817	2199	2190	2122	2102	**2153**	100,00	74.81
$N_{20}P_{60}K_{40}$	Elenski 817	2483	2459	2373	2412	**2432**	112,95	84.50
$N_{30}P_{60}K_{40}$	Elenski 817	2681	2725	2542	2758	**2676**	124,29	92.98
LSD	0.5	0.1	0.01		LSD	0.5	0.1	0.01
P23	238.4	361.1	580.5		Basma	114.3	173.2	278.4
P-79-94	210.0	318.1	511.4		Elenski	91.8	139.0	223.4

The data of the average purchase price per kilogram dry tobacco is presented in Table 4. From the results we can point out that all variants in all studied varieties have lower average price compared to the control. Best results are achieved at the variety P-23, control non fertilized. In all fertilized variants, was noted a slight decrease in the average price of around 3-5%, compared to the control. Statistical processing of the results showed that the fertilization has no significant impact on the average price of tobacco, in all examined varieties and varieties.

Table 4. Average tobacco price, den/kg (2010-2013)

Variant	Variety	Year				Average den/kg	Index	Index
		2010	2011	2012	2013			
Ø	P-23	132.0	135.3	127.1	139.9	133.6	100.00	100.00
$N_{20}P_{60}K_{40}$	P-23	127.1	125.6	126.3	138.1	129.3	96.78	96.78
$N_{30}P_{60}K_{40}$	P-23	124.0	124.9	123.1	137.9	127.4	95.36	95.36
Ø	P-79-94	126.4	131.1	122.9	137.8	129.5	100.00	96.93
$N_{20}P_{60}K_{40}$	P-79-94	121.6	123.2	119.2	136.2	125.0	96.53	93.56
$N_{30}P_{60}K_{40}$	P-79-94	121.1	122.1	118.4	135.8	124.3	95.98	93.04
Ø	Basma 1	135.3	138.9	129.8	142.2	136.5	100.00	102.17
$N_{20}P_{60}K_{40}$	Basma 1	131.7	132.8	126.1	140.4	132.7	97.22	99.33
$N_{30}P_{60}K_{40}$	Basma 1	128.3	132.6	124.6	139.1	131.1	96.04	98.13
Ø	Elenski 817	133.6	134.2	127.8	138.7	133.6	100.00	100.00
$N_{20}P_{60}K_{40}$	Elenski 817	126.9	131.5	125.5	137.5	130.3	97.53	97.53
$N_{30}P_{60}K_{40}$	Elenski 817	124.1	130.0	122.0	134.8	127.7	95.58	95.58
LSD 0.5	P23	**NS**	P-79-94	**NS**	Basma	**NS**	Elenski	**NS**

Economical effect as gross income of tobacco per ha, (Table 5) represents the yield and quality of tobacco for each set of variants. Great yield per hectare and quality of raw tobacco (average price per kg of dry tobacco) means greater economic impact and vice versa. In the examined varieties lowest gross income have controls (378168 den/ha, 360539 den/ha, 275 405 den/ha, 276 788 den/ha), and the highest from variant 3 (464365den/ha, 450176den/ha, 328409den/ha, 348929den/ha), respectively. That is gross income of 22.79% at variety P-23, and 24.86% at variety P-79-94, 19.04% at variety Basma 1 and 26.06% at variety Elenski 817, more than unfertilized variants. The results clearly show that fertilization has a significantly impact on increasing the gross income of all varieties, compared to the control.

Table 5. Gross income of tobacco, den/ha (2010-2013)

Variant	Variety	Year				Average kg/ha	Index	Index
		2010	2011	2012	2013			
Ø	P-23	403642	402436	378485	328109	378168	100.00	100.00
$N_{20}P_{60}K_{40}$	P-23	445682	463232	422526	413681	436280	115.37	115.37
$N_{30}P_{60}K_{40}$	P-23	491366	457001	427585	481508	464365	122.79	122.79
Ø	P-79-94	378424	374940	365090	323703	360539	100.00	95.34
$N_{20}P_{60}K_{40}$	P-79-94	428872	447730	404021	401424	420512	116.34	111.20
$N_{30}P_{60}K_{40}$	P-79-94	460183	453547	413833	473141	450176	124.86	119.04
Ø	Basma 1	271728	288885	252442	288563	275405	100.00	72.83
$N_{20}P_{60}K_{40}$	Basma 1	305333	285832	278537	344655	303589	110.23	80.28
$N_{30}P_{60}K_{40}$	Basma 1	312767	309844	303724	387302	328409	119.25	86.84
Ø	Elenski 817	276605	269090	258038	303420	276788	100.00	73.19
$N_{20}P_{60}K_{40}$	Elenski 817	309212	299781	291611	355560	314041	113.46	83.04
$N_{30}P_{60}K_{40}$	Elenski 817	339315	342645	307869	405888	348929	126.06	92.27
LSD	0.5	0.1	0.01		LSD	0.5	0.1	0.01
P23	44132	66857	107472		Basma	30720	46539	74811
P-79-94	39073	59193	95152		Elenski	20311	30769	49461

According to the results during the years of the investigation, we can see that besides the influence of the nitrogen rates, there is influence and from the climate conditions to the yield, economical benefit and average price. During the year 2012 as the most relevant year, there is higher yield and gross income, but there is slight reduction of the average purchase price of one kilogram of dry tobacco in comparison with year 2013 in all tested varieties.

Chemical properties analyses were performed as very important indicators of the tobacco leaves quality. Chemical composition depends from genetic material, type, variety, breed conditions, time for harvesting, drying processes and fermentation (Mitreski 2012, Dimitrieski 1992). Pecijareski (1965) stated that chemical composition of the tobacco primary depends on climate and soil conditions, then on agrotechnical measures and normal performances of after harvesting processes.

From Table 6 and 7 it can be seen that the increase of nitrogen content (20 and 30 kg/ha) statistically increases the content of humus (from 4.0 to 10.0%), total nitrogen (13.0% to 27.54 %) and mineral matter (6.9% -12.59%), in all investigated varieties. The content of soluble sugars is the highest in an unfertilized variant, and as the rate of nitrogen increases, its average content decreases. The highest average content of humus has the variety P-23 (1.74%), and the lowest varieties Basma 1 (1.49%). Total nitrogen has the highest content in the variety P-79-94 (2.07%), and the lowest in the varieties Basma 1 (1.67%).

Table 6. Nicotine and total nitrogen content, in %

Variety	Nicotine content, in %					Total nitrogen, in %				
	Variant			Average	%	Variant			Average	%
	Ø	$N_{20}P_{60}K_{40}$	$N_{30}P_{60}K_{40}$			Ø	$N_{20}P_{60}K_{40}$	$N_{30}P_{60}K_{40}$		
P-23	1.70	1.73	1.80	1.74	100.0	1.78	1.94	2.19	1.97	100.0
P-79-94	1.47	1.52	1.61	1.53	87.93	1.80	2.03	2.39	2.07	105.7
Basma 1	1.43	1.50	1.63	1.52	87.35	1.46	1.65	1.90	1.67	79.69
Elenski817	1.42	1.49	1.57	1.49	85.63	1.66	1.94	2.05	1.88	95.43
Average	1.50	1.56	1.65	1.57	90.22	1.67	1.89	2.13	**1.90**	**84.77**
%	100.0	**104.0**	**110.0**			**100.0**	**113.17**	**127.54**		
LSD	0.05=0.037	0.01=0.056	0.001=0.090			LSD 0.05=0.10	0.01=0.16	0.001=0.25		

The quality of oriental tobacco largely depends on the content of soluble sugars. The higher content of soluble sugars gives better taste of the tobacco, because during the combustion tobacco gives substances that influence the flavor and taste of tobacco, neutralizing the negative impact of protein and forming an acidic reaction to tobacco smoke.

The examined, domestic and introduced varieties according to the content of soluble sugar are ranked in the group of high quality tobacco. The highest average sugar content has the variety P-23 (16.5%), followed by the variety Elenski 817 (15.57 %), Basma 1 (15.08 %) and the lowest content is the variety P-79-94 (14.75 %). Tobacco whose content of soluble sugars ranges from 11 to 16 % is high quality tobacco (Mitreski, 2012). Mineral substances in the total dry matter content account from 9 to 30 %.

Tobacco that contains over 15% of minerals is of poor quality. According to the obtained results, the investigated varieties fall into the group of medium-quality tobacco, where the content of ash ranges from 12.90 in the variety Elena 817 to 13.66% in the variety P-79-94.

From the obtained results for the chemical properties we can see that the fertilization with 20 and 30 kg of nitrogen/ha has a positive effect on the increase of the nicotine content, which is a positive property, but at the same time it affects a slight increase in the total nitrogen and mineral content and reduction the content of soluble sugar. This means that with the further increase in nitrogen quantities, the harmony of the chemical properties of tobacco can be significantly worsened.

Table 7. Content of soluble sugars and mineral matter, in %

Variety	Soluble sugars, in %					Mineral matter, in %				
	Variant			Average	%	Variant			Average	%
	Ø	$N_{20}P_{60}K_{40}$	$N_{30}P_{60}K_{40}$			Ø	$N_{20}P_{60}K_{40}$	$N_{30}P_{60}K_{40}$		
P-23	17.63	16.45	14.06	16.5	100.0	12.29	12.95	13.93	13.06	100.0
P-79-94	15.86	14.90	13.51	14.75	91.90	12.86	13.76	14.36	13.66	104.59
Basma 1	16.17	15.06	14.03	15.08	93.95	11.70	13.01	13.76	12.82	98.16
Elenski817	17.00	15.75	13.96	15.57	97.0	12.37	12.92	13.41	12.90	98.77
Average	16.66	15.54	13.89	15.36	95.7	12.31	13.16	13.86	13.11	100.38
%	**100.0**	**93.27**	**83.37**	**92.19**		100,0	**106.90**	**112.59**	106.46	
LSD	0.05=NS					0.05=0.41	0.01=0.62		0.001=0.99	

CONCLUSIONS

Studies have shown that the average yield and gross income per unit of production area increases with increasing of the nitrogen doses, and the average price per kilogram of tobacco decreases slightly in all investigated varieties. The introduced varieties Basma 1 and Elenski 81 at the same agrotechnology in the Pelagonia agro-climatic region, village Dobrushevo gave significantly lower results compared to the P-23 variety, as control. Various rates of nitrogen in fertilizers, has a certain influence on the chemical composition of tobacco. In all examined varieties, higher content of nicotine, protein and mineral matter was observed, as well as a reduction in the content of reduced sugars by increasing the nitrogen dose. Based on the obtained results it can be concluded that due to the lower yield and gross income per ha, the introduced varieties Basma 1 and Elenski 817 are not perspective varieties for wider production in the Pelagonija region.

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ISSN 0494 - 3244

Тутун/Tobacco, Vol. 68, N⁰ 1-6, 83-87, 2018

UDC: 633.71-116.642:338.516.47(497.7)

Original scientific paper

INTERNAL PARITY OF PRICES BETWEEN THE RAW TOBACCO GRADES

Silvana Pašovska

"St.Kliment Ohridski"- University, Bitola, Scientific tobacco institute,Prilep, "Kicevska" bb,Republic of Macedonia

e-mail:s_pasovska@yahoo.com

ABSTRACT

Tobacco, along with other agricultural crops, is important segment of the productional structure of individual producers. Depending on crop prices, the farmers plan and direct their activities toward one or several cultivars that will guarantee the highest income. This relationship is called parity ratio of the prices of agricultural crops.
According to this quality, farmers make decision to which agricultural structure they will direct their economy. In practice, it is about parity between two and more crops due to differences in their prices, also referred as disparity. Disparity is an indicator to which crop and to which extent will the farmers direct their activities. This is also valid for tobacco crop in terms of its market price compared to other cultures.
Beside this, there is also internal parity. In tobacco, it is the parity between different tobacco types and varieties as well as different grades within the variety. In determining and creating the prices of tobacco, internal parity should be carefully considered because it affects the need to increase or decrease the quality of tobacco depending on market demand. In the first case, parity between different types and varieties of tobacco can lead to increase of tobacco production, which can result in possible market surplus of one tobacco type and lack of another. In the second case - internal parity of grades within certain variety has a significant role in stimulating and maintaining the quality of tobacco, taking care not to cause high approximation (equalization) of quality between tobacco grades. Parity and internal parity will be discussed and analysed in this paper.

Keywords: crops, prices, grades, quality, parity

ИНТЕРЕН ПАРИТЕТ НА ЦЕНИТЕ ПОМЕЃУ КЛАСИТЕ НА СУРОВИОТ ТУТУН

Тутунот како земјоделска култура заедно со другите култури ја сочинуваат производната структура кај индивидуалните производители.Во зависност од цените на одредени земјоделски култури кои се застапени во производната структура на земјоделецот ,тие ја формираат и насочуваат неговата активност кон културата или неколку земјоделски култури кои гарантираат најголем доход.Во тој случај производителот ја одредува неговата активност кон која култура ќе ја насочи за да обезбеди најголем доход.Овој однос е тн.паритетен однос на цените помеѓу земјоделските култури. На оваа основа и паритет се донесува одлука на која земјоделска структура земјоделецот ќе го насочи неговото стопанство.Во пракса се зборува за паритет меѓу две и повеќе култури поради постоење на разлики во нивните цени односно т.н. диспаритет.Овој диспаритет е индикатор каде и колку ќе ја усмери и која култура ќе ја насочи во структурата на своето стопанство.Ова се одесува и на тутунската култура во однос на тоа каква е нејзината пазарна цена во однос на други култури.Покрај овој паритет постои и интерен паритет,конкретно кај тутунот тој паритет е паритет помеѓу одредени типови на тутун,сорти на тутуни и тутунски класи во сортата на тутунот.Во одредување и креирање на цените кај тутунот треба добро да се внимава на овој паритет зошто преку него се влијае на потребата и желбата за зголемување или намалување на квалитетот на тутунот во зависност од пазарната побарувачка.Во првиот случај кога се зборува за паритет меѓу одредени типови и сорти на тутун паритетот може да влијае на пораст на производството на тутун така да можи да се јави од една страна пазарен вишок на еден тип тутун и недостаток на друг тип тутун.Интерниот паритет на одредена сорта на тутун по класи има значајна улога и функција во стимулирање и одржување на квалитетот на тутунот,водејќи сметка да не дојде до големо

приближување(егализација) на квалитетот помеѓу класите на тутунот. Паритетот и интерниот паритет ќе бидат аргументирано разработени во содржината на трудот.

Клучни зборови:култури,цени,класи,квалитет,паритет.

INTRODUCTION

If particular agricultural product has a more favorable price compared to other crops, the interest and activities of the producers are directed towards those crops that provide higher income. In this context, the relation between the price of raw tobacco and the price of one or more agricultural crops is called parity. In practice and in theory, however, when talking about parity between agricultural crops and differences between the prices of two or more crops we actually talk about price disparity. The disparity is a good indicator of the changes occurring in the market relations and conditions among agricultural crops. In tobacco industry, particularly in the stage of primary production and maintenance of its stability, it is very important to maintain the parity of tobacco in relation to other agricultural crops. If it is not maintained, it can find itself in "price scissors". The parity of tobacco in relation to other crops is very important in terms of timely determination of prices agreed between the buyer and manufacturer. By the rule, the price should be determined immediately after the end of the harvest and its purchase and the period for determination should be as short as possible. Late determination of purchase prices affects the decision of tobacco grower on the structure of agricultural goods, i.e. which and how much of them will be accepted depending on prices and their profitability. Timely determination of prices will also help tobacco grower to estimate the size of agricultural area for production and to forecast the profitability of tobacco compared to other crops. Untimely determination of prices leads to oscillation of the number of growers involved in tobacco production.

INTERNAL PARITY OF THE PRICES OF RAW TOBACCO

Internal parity denotes the relationship among prices of purchase grades of raw tobacco and it enables the obtaining of a good quality tobacco material. Inadequate assessment and establishment of inadequate parity can lead to overproduction or lack of certain variety of tobacco, which is not in the interest of parity. The dynamics of the internal parity per kilogram of tobacco and the prices of tobacco grades are presented in Table 1.

Table 1. Purchase price of the Prilep tobacco raw material (MKD/kg), internal parity and ratio

	1993			1994		
Grade	Price	Index	Ratio	Price	Index	Ratio
1-aromat	85	100.00		85	100.00	
2	73	85.88	1:1,164	73	85.88	1:1,164
3a	55	64.70	1:1,545	55	64.70	1:1,545
3b	44	51.76	1:1,931	44	51.76	1:1,931
4	27	31.76	1:3,148	27	31.76	1:3,148
5	14	16.47	1:6,071	14	16.47	1:6,071
	1995			**1997**		
Grade	Price	Index	Ratio	Price	Index	Ratio
1-aromat	100	100.00		120	100.00	
2	84	84.00	1:1,190	97	88.83	1:1,123
3a	63	63.00	1:1,587	72	60.00	1:1,667
3b	49	49.00	1:2,040	54	45.00	1:2,222
4	30	30.00	1:3,333	33	27.50	1:3,636
5	15	15.00	1:6,667	17	14.17	1:7,059

Source: Analysis of the work of AD Yugotutun-Skopje

It can be noted from the table that the ratio between internal parity and the price of tobacco per kg is rarely changed and the proportions are not dramatically disturbed. However, in certain years of production they undergo some changes in terms of pricing policy set by the purchase companies depending on the quality of tobacco at the time of purchase, which is characteristic of the period 1995-1997 in relation to the period 1993-1994. Following the internal prices which are result of an agreement between tobacco buyers and growers, changes recorded in the harvests of 2010 and 2011 are presented in Table 2.

Table 2. Purchase price of the Prilep tobacco raw (MKD/kg), internal parity and ratio

	2010			2011		
Grade	Price	Index	Ratio	Price	Index	Ratio
1-aromat	208	100		208	100	
2	163	78	1:1,282	165	79	1:1,266
3a	125	60	1:1,667	112	53	1:1,887
3b	85	40	1:2,5			
4	49	24	1:4,167	50	24	1:4,167
5	23	11	1:9,091			

Source: Analysis of the work of AD Yugotutun-Skopje

According to the table, the parity from the 2nd to the 5th grade significantly differs compared to previous periods and shows a tendency of higher differentiation in quality. Based on the internal parity, the extent of oscillation of tobacco raw quality between the grades can be estimated. Insufficient assessment and establishment of inadequate parity in the internal grades can impair the quality of a particular variety, which may have implications on the intensity of its sale and on creation of inadequate tobacco stocks.

Particularly indicative in the internal parity is the amount of tobacco from consecutive grades that can be bought for the price of the first grade. For example, the price for the first grade tobacco was enough for buying 6,71 kg of the fifth grade in 1993 and 1994 and 6,66 kg in 1995. In 2010, the price for the first-grade tobacco was enough to buy 9 kg of the fifth grade, 4,2 kg of the fourth grade, 1.6kg of the third grade and 1.27 kg of the second grade. In 2011, the price of the first grade could buy 4.16 kg of the fourth grade, 1.88kg of the third grade and 1.26 of the second grade tobacco. The above ratios show that despite the agreed price by grades, there are conflicting situations between farmers and dealers in the time of purchase, due to the change of tobacco quality. Tobacco raw from different insertions (belts) has a different quality and thus the decrease or increase of grades and their impact on quality in domestic and industrial manipulation of tobacco is highly questionable.The presented data show that in reality the fourth and fifth grade exist and are present during the purchase, but in practice their reduction is obvious and they seem to disappear, especially in the last few years. However, the purchaser permits dislocation of these quality groups in the first, second and third grade, which means that the industrial manipulation accepts these changes. The table below shows the movement of internal parity in the period 2015-2017.

Table 3. Purchase price of the Prilep tobacco raw (2015-2017), internal parity and ratio

Purchase grades	2015			2016			2017		
	Price	Index	Ratio	Price	Index	Ratio	Price	Index	Ratio
1	249	100		270	100		280	100	
2	178	71	1:1,398	188	70	1:1,436	195	73	1:1,435
3	136	54	1:1,830	136	50	1:1,985	142	56	1:1,972
4	55	22	1:4,527	55	20	1:4,909	60	33	1:4,667

Source: Association of tobacco producers of R. Macedonia

The table shows the changes in internal parity of tobacco grades 1 – 4, as well as additional indicators for comparison between internal parity of grades during the purchase realized in the following crops:

- 1993 – with the price of the 1st grade it was possible to purchase 1.164 kg of the 2nd grade, 1.931 kg of 3b, 1,545 kg of 3a; 3,148 kg of the 4th and 6,071 kg of the 5th grade tobacco.

- 1994 – the price of the 1st grade tobacco was enough for 1,164 kg of the 2nd grade, 1,931 kg of 3b, 1,545 kg of 3a; 3,148 kg of the 4th and 6,071 kg of the 5th grade tobacco.

- 1995 - the price of the 1st grade tobacco was enough for 1,190 kg of the 2nd grade, 1,587 kg of 3a grade, 2,040 kg of 3b grade, 3,333 kg of the 4th grade and 6,667 kg of the 5 grade tobacco.

- 1997 - the price of the 1st grade was enough for 1,123 kg of the 2nd grade, 1,667 kg of 3a grade, 2,222 kg of 3b grade, 3,636 kg of the 4th grade and 7,059 kg of the 5th grade tobacco.

- 2010 - the price of the 1st grade tobacco was enough for 1,282 kg of the 2nd grade, 1,667 kg of 3a grade; 2.5kg of 3b grade; 4,167kg of the 4th grade and 9,091 of the 5th grade tobacco.

- 2011 - the price of the 1st grade was enough for 1,266 kg of the 2nd grade; 1,887kg of 3a grade and 4,167kg of the 4th grade tobacco.

- 2015 - the price of the 1st grade was enough for 1,398 kg of the 2nd grade; 1,830 kg of the 3rd grade and 4,527 kg of the 4th grade tobacco.

- 2016 - the price of the 1st grade was enough for 1,436 kg of the 2nd grade; 1,985 kg of the 3rd grade and 4,909 kg of the 4th grade tobacco.

- 2017- with the price of the 1st grade it was possible to purchase 1,435 kg of the 2nd; 1,972 kg of the 3rd grade and 4,667 kg of the 4th grade tobacco.

CONCLUSION

Based on previous analyses and research work presented in the tables, it can be concluded that there were five purchase grades of tobacco, with grade 3b in between. During the period of analyses, the number of grades was reduced from five to four, but recently it has been suggested to reduce them to three purchase grades. It should be taken into account, however, that there are five grades according to leaf position and belts, and they are of different quality. By reducing them to only three purchase grades, the fourth and fifth grade will be moved into the third, second and first grade, which will lead to degradation of quality of the above three grades.

It can be stated that the internal parity of prices of purchase grades is variable. The changes are usually small, although under different weather conditions they can reach extreme values and change the internal parity. It is characteristic that in 2014 the fourth grade accounted for 23.3% of the total rate of yield, while in the succeeding years it participated with only 2%. It gives grounds to exclude this grade from purchase grades, which is quite disputable.

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