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Original Scientific paper

COMPARATIVE INVESTIGATIONS OF NEW VARIETIES OF THE TYPE BASMAK WITH POPULAR VARIETIES OF THE TYPES PRILEP, YAKA AND DJEBEL

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ABSTRACT

Investigations were carried out with ten oriental tobaccos of the types: Basmak (MB-2, MB-3, MK-1, MS-8/1, MS-9/3, YZ-7 and Dj-B-1), Prilep (P-23), Yaka (YV 125/3) and Djebel (Dj № 1), to study: height of the stalk with inflorescence, number of the leaves per stalk, length of the middle belt leaves and dry mass yield per stalk. The trial was set up in the Experimental field of Tobacco Institute–Prilep in 2013 and 2014, in randomized block design with three replications, using traditional agricultural practices.

The aim of the investigation is to study some more important quantitative traits of tobacco vatieties from type Basmak and to mace comparison with the popular varieties of Prilep, Yaka and Djebel types. This investigations will give precise knowledge of the new type Basmak in Macedonia from morphological and agronomic aspects.

The highest stalk among the investigated genotypes was recorded in the standard variety YV 125/3, while the highest number of leaves, length of the middle belt leaves and yield were measured in P-23. The highest stalk among the Basmak varieties was recorded in YZ-7, while leaf number, length of the middle belt leaves and yield were the highest in MS-9/3. All Basmak varieties are significantly higher than P-23 and Dj № 1 and lower than YV 125/3. Compared to P-23, all Basmak varieties are characterized by significantly lower leaf number, while highly significant differences in comparison with YV 125/3 were found in MB-2, MB-3, MK-1 and Dj-B-1. The leaf of Basmak varieties is significantly shorter compared to that of P-23 and significantly longer compared to Dj № 1. Basmak varieties have lower yield compared to P-23 and YV 125/3, but significantly higher compared to the variety Dj № 1.

Keywords: tobacco (Nicotiana tabacum L.); types: Basmak, Prilep, Yaka and Djebel; quantitative traits.

КОМПАРАТИВНИ ИСПИТУВАЊА НА НОВИТЕ СОРТИ ОД ТИПОТ БАСМАК СО ПОПУЛАРНИ СОРТИ ОД ТИПОВИТЕ ПРИЛЕП, ЈАКА И ЏЕБЕЛ

Испитувани се десет ориенталски сорти од типовите: басмак (МБ-2, МБ-3, МК-1, МС-8/1, МС-9/3, ЈЗ-7 и Џ-Б-1), прилеп (П-23), јака (ЈВ 125/3) и џебел (Џ № 1), за висина на стракот со соцветие, бројот на листови по страк, должина на листовите од средниот појас и приносот на сува маса по страк. Опитот беше поставен на

експерименталното поле при Научниот институт за тутун–Прилеп во 2013 и 2014 година, по случаен блок–систем во три повторувања, со примена на вообичаени агротехнички мерки.
Целта на истражувањата е да се проучат поважните квантитативни својства на тутунските сорти од типот басмак и да се направи споредба со популарните сорти од типовите прилеп, јака и џебел. Испитувањата ќе дадат точни сознанија за новиот тип басмак во Македонија од морфолошки и агрономски аспект.
Со најголема висина меѓу испитуваните генотипови се истакна стандардната сорта ЈВ 125/3, додека со најголем број на листови, најголема должина на листовите од средниот појас и највисок принос се издвои П-23. Со најголема висина меѓу сортите од типот басмак се окарактеризира ЈЗ-7, додека со најголем број на листови, најголема должина на листовите од средниот појас и највисок принос се истакна МС-9/3. Сите сорти од типот басмак се сигнификантно повисоки од П-23 и Џ № 1, и пониски од ЈВ 125/3. Споредбено со П-23 сите сорти од типот басмак имаат сигнификантно помал број на листови, додека споредбено со ЈВ 125/3 повеќе листови имаат МБ-2, МБ-3, МК-1 и Џ-Б-1, а разликите се високосигнификантни. Сортите од типот басмак имаат сигнификантно пократки листови споредбено со оние на П-23, а сигнификантно подолги листови од Џ № 1. Во однос на П-23 и ЈВ 125/3 сортите од типот басмак имаат понизок принос, а во однос на Џ № 1 имаат сигнификантно повисок принос.

Клучни зборови: тутун (Nicotiana tabacum L.); типови: басмак, прилеп, јака и џебел; квантитативни својства.

INTRODUCTION

Tobacco production in the Republic of Macedonia has a long tradition. It dates back to the Ottoman Empire. The introduced varieties had a long period of time for their adaptation, due to which they are stabilized and adjusted to the local conditions. Selection of new varieties started in 1924, with founding of the Tobacco Institute - Prilep. The newly created varieties of the oriental tobacco types Prilep, Yaka and Djebel and later of the semi-oriental type Otlia were released in production in 1930 (Korubin-Aleksoska, 2004). The above tobacco varietal structure remained unchanged until the large-leaf tobaccos Virginia and Burley were introduced in the mass production in 1975 and 1981, respectively. However, in 2002 the production of Virginia, Burley and Otlia tobaccos ceased, while the production of oriental tobacco varieties has continued. Later, in 2005, several new varieties of the oriental Basmak tobacco were included in regular production, at a request of tobacco purchasing companies founded with foreign capital. Basmak tobacco was created from the type Yaka, previously grown in large quantities in Greece. The total world production of oriental tobacco in 2006 reached 270,000 t, the greatest share of which belonged to Turkey -130,000 t, Bulgaria - 24720 t, Greece - 22 900 t (14,000 t of which is Basma tobacco) and Macedonia - 19640 t (mostly of the types Prilep - 12912 t and Yaka - 5054 t), (FAO, 2011). Today, the Tobacco Institute in collaboration with other companies - participants in the project for creation of new tobacco varieties has released seven varieties such as Basmak, two of which are already commercialized.

There are many studies related to Basma tobacco. The raw material of this type is highly estimated by the purchasers. For this reason, efforts have been made to expand and increase its production. Nikolova, Drachev and Nikolov (2005), in her studies of technological properties of some Basma tobacco varieties grown in different regions of Bulgaria concluded that the varieties grown in the regions where they are traditionally produced have a significantly better quality than the same varieties grown in other regions. Drachev, Nikolova and Popova (2006) made comparative trial with Bulgarian varieties of the ecotype Basma and the Greek variety Zihna, located in Djebelian tobacco producing region. In their investigation on the most important chemical parameters and degustational indicators of tobacco raw quality they observed significant differences that are the basis for a complex assessment of the average quality and rank of the studied varieties. Drachev, Nikolova and Nikolov (2007) made a comparative trial in the Yaka tobacco producing region to study the technological properties of some domestic varieties of the

oriental type Basma and Greek variety Zihna, in accordance with the emerging needs for changes in the varietal structure and placement of tobacco raw in the world market. Analyses were made using a qualitative index to estimate the quality of oriental tobacco, by which it was proved that the introduced varieties can be produce in the above regions. Masheva, Todorova and Dimanov (2009) studied the gene effect in the inheritance of plant height, leaf number per plant, leaf length and width and the period from planting to flowering in two hybrids obtained through interspecific hybridization: Krumovgrad 988 x Basma Xanthi 101 and Harmanly 134 x Basma Xanthi 101. The authors found additive and non-additive genetic effect and gave directions for further selection to obtain stable and uniform lines of Basma tobacco.

Nikolov (2009) in the research conducted in 2006 compared the chemical and technological traits of tobacco varieties Zihna and Komotini of Greek origin introduced in Bulgaria and the domestic variety Djebel K 81. He found that the quality of Zihna was lower than that of Djebel K 81 and the raw material obtained from The Djebelian tobacco producing region had a higher rank. Komotini and Zihna tobaccos grown in the region of Nevrokop were characterized by higher uniformity. Gixhari and Sulovari (2010) made three-years investigation (2007-2009) in two different environmental areas in Albania to study the inheritance of some major morphological and biological quantitative traits of eight parental genotypes and their hybrids. They found that the Greek variety Basma Xanthi had the highest stalk of 123,2 cm, with 36.4 leaves per stalk, 19,7 cm length and 12,1 cm width of the middle belt leaves, 91.9 days from planting to flowering and a yield of 259.6 kg/da. Dimanov and Masheva (2011) studied the new varieties of Basma tobacco selected in the Institute of tobacco and tobacco products – Markovo: Sekirka,, Djebel Basma 1, Djebel Basma 2, Basma 13, Plovdiv 380, Ustina 54, Kozarsko 339 and Srednogorska Yaka) in order to present the newly selected material. Darvishzadeha and Hatami Maleki (2011) made analysis on the genetic variance of 100 Iranian oriental and semi-oriental tobacco genotypes of local and foreign origin for the quantitative traits: days to 50% flowering, dry mass yield and leaf number per plant. The studied genotypes included varieties of tobacco types Samsoun, Izmir, Basma, Krumovgrad and Nevrokop, which can be grown in tobacco producing regions of Iran and in accordance with the needs of the world market. Kichukov (2012) reported that over 300 000 decares in Bulgaria are grown with oriental tobacco and that Basma varieties are grown in the most poorly productive regions. These are also the lowest-yielding varieties with about 120 kg/da and with the highest purchase price. The most suitable regions for growing Basma tobaccos are Goce Delchev, Haskovo and Kardzhali.

The subject of our investigation were some major morphological and agronomic traits of Basmak tobacco varieties selected in Tobacco Institute-Prilep and their comparison with known varieties of Prilep, Yaka and Djebel tobacco.

MATERIAL AND METHODS

These investigations include two-year study (2013 and 2014) on height of the stalk with inflorescence, number of the leaves per stalk, length of the middle belt leaves and dry mass yield per stalk. in seven varieties of the type Basmak: Basmak (MB-2 – Fig.1, MB-3 – Fig.2, MK-1 – Fig.3, MS-8 / 1 – Fig.4, MS-9 / 3 – Fig.5, YZ-7 – Fig.6 and Dj-B-1 – Fig.7), and one variety of the types: Prilep (P-23 – Fig.8), Yaka (YV 125/3 – Fig.9 and Djebel (Dj № 1 – Fig.10) for comparison. The trial was set up in the Experimental field of Tobacco Institute–Prilep, in randomized block design with three replications, using traditional agricultural practices. Investigations were performed at the stage of full flowering of tobacco, by standard methods.

Fig. 1. MB-2

Fig. 2. MB-3

Fig. 3. MK-1

Fig. 4. MS-8/1

Fig. 5. MS-9/3

Fig. 6. YZ-7

Fig. 7. Dj-B-1

Fig. 8. P-23

Fig. 9. JV 125/3

Fig. 10. Dj № 1

During tobacco vegetation in field (May - September) in 2013, mean monthly temperature was 19,40C, number of rainy days 34 and total precipitation amount 153 mm. In the same period in 2014 mean monthly temperature was 18,30C, number of rainy days 33 and total precipitation amount 223 mm.

RESULTS AND DISCUSSION

Popular varieties of the type Basma are ranked in the high-quality group of oriental aromatic tobaccos. Introduction and spread of Basma varieties in the Balkans require thorough quantitative and qualitative analysis of their adaptation to the conditions of the new areas of breeding. The name of this tobacco in Macedonia is Basmak and it consists of the newly created varieties MB-2, MB-3, MK-1, MK-2, MS-8/1, MS-9/3 and YZ-7, owned by Tobacco Institute-Prilep and tobacco companies – participants in the selection project. Since the variety MK-2 was no longer uniform, Bulgarian variety Dj-B-1

was included in the trial. Results of investigation on the new varieties of Basmak tobacco are presented in this paper and comparison is made with standard varieties of other tobacco types traditionally grown in R. Macedonia.

The highest stalk with inflorescence was observed in the standard variety of Yaka tobacco YV 125/3 ($\bar{x}$=123,5 cm). Among the Basmak varieties, YZ-7 is the highest ($\bar{x}$=118 cm) and MK-1 is the lowest ($\bar{x}$ = 89 cm). In relation to this trait, all Basmak varieties are significantly higher than P-23 and Dj № 1 and significantly lower than YV 125/3 (Table 1).

The highest number of leaves per stalk in the investigated genotypes was recorded in P-23 ($\bar{x}$=54), while in Basmak varieties the highest leaf number was found in MS-9/3 ($\bar{x}$=43.6). The lowest leaf number was recorded in MK-1 ($\bar{x}$=28.1) and Dj-B-1 ($\bar{x}$=28.25). Compared to P-23, all varieties of Basmak tobacco have significantly lower leaf number. Compared to YV 125/3, highly significant differences were observed in MB-2, MB-3, MK-1 and Dj-B-1, i.e. these varieties have significantly higher number of leaves per stalk (Table 1).

The longest middle belt leaf among the investigated genotypes was recorded in P-23 ($\bar{x}$=24,33 cm) and the shortest in Dj № 1 ($\bar{x}$ =18,52 cm). The longest leaf among Basmak tobaccos was measured in MS-9/3 ($\bar{x}$=22,65 cm) and the shortest in MB-2 ($\bar{x}$=20,35 cm). The length of the middle belt leaf in Basmak varieties is significantly lower compared to P-23 and significantly higher compared to Dj № 1. Compared to YV 125/3 no highly significant differences were observed, but in MB-2, MK-1 and MS-9/3 a difference at 5% level of significance was estimated (Table 1).

The highest yield among the investigated genotypes was achieved in P-23 ($\bar{x}$= 2558,5 kg/ha) and the lowest in Dj № 1 ($\bar{x}$=999,5 kg/ha). The highest yield among Basmak tobaccos was recorded in MS-9/3 ($\bar{x}$=2397,5 kg/ha) and the lowest in Dj-B-1 ($\bar{x}$=1345,5 kg/ha). Compared to P-23, all varieties of Basmak tobacco have significantly lower yields. Compared to YV 125/3 lower yield was observed in all the varieties except for MS-9/3, the yield of which was similar to that of the standard variety. All of the investigated varieties showed significantly higher yields compared to Dj № 1 (Table 1).

Table 1. Quantitative traits in tobacco varieties from types Basmak, Prilep, Yaka and Djebel

Tobacco varieties	Quantitative traits							
	Height of the stalk with inflorescence (cm)		Number of leaves per stalk		Length of the middle belt leaves (cm)		Dry mass yield per stalk (g)	
	2013	2014	2013	2014	2013	2014	2013	2014
1. Basmak – MB-2	93	94	29,5	30	19,95	20,76	1486	1507
2. Basmak – MB-3	98	97	33	34	21,22	21,35	1533	1652
3. Basmak – MK-1	88	90	27,7	28,5	20,73	20,56	1528	1581
4. Basmak – MS-8/1	105	110	41,5	42,8	21,95	22,35	2184	2282
5. Basmak – MS-9/3	111	115	43	44,2	22.24	23,06	2360	2435
6. Basmak – UZ-7	116	120	41	40,4	21,88	22,30	2105	2124
7. Djebel-Basma-1 – DJ-B-1	95	97	28,5	28	21,13	21,02	1299	1392
8. Prilep – P-23	68	68	53	55	24,59	24,07	2550	2567
9. Yaka – YV 125/3	122	125	41,7	42,6	21,57	21,77	2382	2465
10. Djebel – Dj № 1	79	80	28	29,8	18,45	18,59	994	1005
LSD 0,05	4,37	4,17	1,82	1,55	1,08	0,93	147,90	136,32
0,01	7,72	7,51	3,28	2,80	1,95	1,68	266,21	245,39

CONCLUSIONS

The highest stalk among the Basmak tobacco varieties was recorded in YZ-7, while the highest leaf number, leaf length and yield were measured in MS-9/3. The lowest stalk height was recorded in MK-1 and the lowest leaf number in MK-1 and Dj № 1. Variety MB-2 was characterized by the lowest length of the middle belt leaf and Dj-B-1 variety by the lowest yield.

All varieties of Basmak tobacco were significantly higher compared to P-23 and Dj № 1, but significantly shorter than YV 125/3. Significantly higher leaf number compared to YV 125/3 was counted in MB-2, MB-3, MK-1 and Dj-B-1, but the number of leaves in all varieties was significantly lower compared to P-23. All the varieties had significantly shorter leaves compared to P-23 and significantly longer leaves compared to Dj № 1. The yield of the investigated varieties was significantly lower compared to P-23 and YV 125/3 and significantly higher compared to Dj № 1.

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Original Scientific paper

INFLUENCE OF PARENTAL PHENOTYPIC DIVERGENCE ON HETEROSIS APPEARANCE IN TOBACCO

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ABSTRACT

Investigations were made on the influence of phenotypic divergence among genotypes of Burley tobacco on the heterosis appearance for yield in the F1 generation. Four varieties originate from Austria, three from Serbia and three from the USA. Simple crossing gave thirteen hybrid combinations, among which six showed significant higher yields. Average yield of F1 hybrids was higher from parental mean value for 34,2%. The effect of heterosis for yield was expressed in four F1 hybrids. The heterosis values compared to the parental mean value, ranged from 50,5% to 79,5% and in comparison to the better parent from 47,1% to 72,7%. The highest heterosis values comapred to the parental mena value (79,5%) and to the better parent (72,7%) were in the combination Bols 347 x TN 90. Results of the conducted studies showed that Burley varieties that were selected in different periods (using different parents), for various growing conditions, expressed divergence which contributed to the manifestation of the heterosis in some F1 hybrids.

Key words: Burley, phenotypic divergence, heterosis, yield, tobacco.

ВЛИЈАНИЕ НА ФЕНОТИПСКАТА ДИВЕРГЕНТНОСТ НА РОДИТЕЛИТЕ ВРЗ ПОЈАВАТА НА ХЕТЕРОЗИС КАЈ ТУТУНОТ

Испитувано е влијанието на фенотипската дивергентност на некои генотипови тутун од типот берлеј врз појавата на хетерозис во F1 генерацијата. Четири сорти беа со потекло од Австрија, три од Србија и три од САД. Со просто вкрстување добиени се тринаесет хибридни комбинации, од кои шест се истакнаа со повисок принос. Просечниот принос на F1 хибридите е за 34,2% повисок од просекот на родителите. Хетеротичен ефект за приносот е забележан кај F1 хибридите. Вредностите на хетерозисот во однос на родителскиот просек се движат од 50.5 до 79.5%, а во однос на подобриот родител 47,1 до 72,7%. Најголеми вредности на хетерозисот во однос на родителскиот просек (79,5%) и подобриот родител (72,7%) се забележани кај комбинацијата Bols 347 x TN 90. Резултатите од испитувањата покажаа дека берлејските сорти, што се

селекционирани во различни периоди (со користење на различни родители) и одгледувани во различни услови, покажаа дивергентност којашто придонесе за појавата на хетерозис кај некои F1 хибриди.

Клучни зборови: берлеј, тутун, фенотипска дивергентност, хетерозис, принос

INTRODUCTION

The term heterosis was introduced by Shull in 1914, while interpreting the benefits of F1 generation in increasing vigor at crossing genetically divergent parental gametes (Shull,1952.). The maximum parental divergence does not always produce the highest heterosis, but it increases to a certain degree in proportion to parental divergence, and afterwards decreases along with further divergence increasement (Moll et al.,1965). Numerous hypothesis on heterosis have been developed. However, the genetic mechanisms of heterosis occurance are mostly unknown. The problem of heterosis manifestation in tobacco have been studied by many researchers (Kostoff, 1941., Bolsunov, 1954., 1971., Kosmodemjanski, 1966., Chaplin,1966., Matzinger and Wernsman,1968., Povilaitis,1971., Mather and Jinks, 1982., Naumoski, 1982., Bogdančeski, 1984., Dražić, 1987., 1997., Korubin - Aleksoska Ana, 2008., Patel et al. 2012., and others). It was concluded that heterosis can be expressed in different properties, such as resistance, early maturity or some other, that are hardly transmitted in a homozygous state, which can be an effective way for their improvement. Also, it is noted that hybrids are more tolerant to climate changes (Dražić, 1997). Starting point in our studies is the hypothesis that in the hybrids obtained by crossing, the divergent parents will be superior.

MATERIAL AND METHODS

Previous studies of phenotypic divergence in Burley tobacco genotypes were used in selection of parental pairs for crossing. Earlier comparisons of variety distribution on dendogram showed that Bols 334, Poseidon, TN 86, TN 90 and SA 130 have relatively higher yield in relation to the other studied varieties (Dražić and Prodanović, 2001). Four varieties (Poseidon, Bols 334, Bols 347, Hy 71-442) originate from Austria, three from Serbia - Berlej 6, Berlej MR-1, Berlej T and three from the USA - SA 130, TN 90, TN 86 (Table 1). Obtained dendograms were also used for planning hybridization. Crossing was carried out among several divergent genotypes. This procedure eliminated the number of unnecessary crossings among similar varieties, e.g. domestic and Austrian varieties (Dražić and Prodanović 2001., Dražić and Prvulović, 2008). Simple crossing gave thirteen hybrid combinations. The two-year study analyzed ten parents and thirteen F1 hybrids. Studies were performed on chernozem soil in Stara Pazova, located about 35 km northwest of Belgrade. Planting was done in the third week of May at 80x50 cm spacing (25000 plants/ha). The main plot size was 12 m^2. During the study, we observed the values of parents and F1 generation for yield of dry leaves (g/plant). The following biometric parameters were calculated: mean value, variance and coefficient of variation. Results were analyzed using the method of variance analysis. Assessment of signifiance of yield changes was done by LSD test. Heterotic effect was calculated according to Jinks (1954). Heterosis (H1) in relation to parental mean value (MP) was calculated using the following formula:

$$H_1 = F_1 - MP, \quad MP = \frac{P_1 + P_2}{2};$$

$$SE_{(H_1)} = \sqrt{\text{variance } H_1}; \text{ t-test: } t = \frac{F_1 - MP}{SE_{(H_1)}}$$

Heterosis (H2) in relation to the better parent (BP) was calculated according to the formula:

$$H_2 = F_1 - BP, \quad SE_{(H_2)} = \sqrt{\text{variance } H_2}\,;$$

$$\text{t-test} = \frac{F_1 - BP}{SE_{(H_2)}}$$

RESULTS AND DISCUSSION

Mean value of parental yield was 91,7g, and that of hybrids 123,1 g/plant. In absolute amount, this difference was 31,4 g, which represents 34,2 % increase in favor of F1 hybrids. In their study of the heterosis occurence in oriental tobacco varieties, Marani and Sachs (1966) noted that F1 hybrids had yield increase of 21% compared to parental average.

Investigated parental genotypes differed in the amount of leaf yield per plant. Variety Berlej T (71,7 g) had the lowest yield, while variety TN 86 (124,3 g) showed the highest variation. Even though it had the highest yield, this variety did not give significant heterosis in any crossing combination. Beside TN 86 (Miller, 1987), other varieties that showed higher yields were: Berlej MR-1, Hy 71-442, Berlej 6 and Bols 347 (Table 1).

The hybrids had higher yields in comparison to the parents. These values ranged from 83,0 g (Berlej MR-1 x SA 130) to 166,1g (Bols 334 x TN 86). Beside combination Bols 334 x TN 86, several other F1 hybrids expressed significant yield increase: Bols 347 x TN 90, Berlej 6 x SA 130, Berlej 6 x TN 90, Poseidon x TN 90 and Poseidon x TN 86. Values for variance and yield variations in F1 hybrids varied and were generally higher in relation to the parents. Yield variations in parents and F1 hybrids are mainly caused by the environmental factors (Table 2).

In this paper we used results from earlier studies of Burley varieties (SA 130, TN 86, TN 90) that showed significant heredity, which indicates that yield was highly under genetic control. The obtained values had an impact on their involvement in this study (Dražić, 1999). Identification of favorable alleles' donor lines for improving the number of leaves, leaf area and yield also confirmed that genotypes SA 130, Bols 334 and TN 90 may be used as a potential donors for these properties (Dražić et. al., 2010).

In F1 generation four hybrid combinations expressed positive heterosis which was higher by 50% compared to the parental mean value (Poseidon x TN 90, Berlej 6 x SA 130, Berlej 6 x TN 90, Bols 347 x TN 90). In comparison to the better parent, the heterosis effect in these hybrids also showed positive values. Crossing combination Bols 347 x TN 90 had the highest positive value. Yield increase was very significant, both in relation to parental mean value (79,5%) and to the better parent (72,7%), (Table 3.).

Matzinger and Wernsman (1968) stated that yield of hybrids in F1 generation between flue-cured and oriental tobacco variety was 55-22% higher than parental mean value. However, in several cases, hybrids did not have higher yield than the better parent.

Table 1. Mean values and variability indicators for yield per plant (g)

No	Parents	x	S2	CV	Rank
1.	Poseidon	84,3	104,45	12,1	6
2.	Bols 334	81,3	4,71	2,7	8
3.	Bols 347	87,1*	37,10	7,0	5

4.	Hy 71-442	93,7**	106,10	11,0	3
5.	Berlej 6	88,5*	113,43	12,0	4
6.	Berlej MR-1	104,3**	81,72	8,7	2
7.	Berlej T	71,7	18,50	6,0	10
8.	SA 130	83,0	113,42	12,8	7
9.	TN 90	80,6	20,90	4,6	9
10.	TN 86	124,3**	396,00	15,4	1
	Average :	91,7	-	-	-

*, **significant at 0.05 and 0.01 probability level

Table 2. Mean values and variability hybrids for yield per plant (g)

No	F1 hybrids	x	S2	CV	Rank
1.	Poseidon x SA 130	114,0	548,60	19,0	8
2.	Poseidon x TN 90	124,1*	85,38	7,5	5
3.	Poseidon x TN 86	120,0*	778,40	23,3	6
4.	Bols 334 x TN 86	166,1**	804,30	17,1	1
5.	Bols 334 x TN 90	109,6	618,50	22,7	10
6.	Bols 347 x TN 90	150,4**	56,55	5,0	2
7.	Bols 347 x Berlej T	99,8	121,00	11,0	11
8.	Hy 71-442 x TN 90	95,7	44,90	7,0	12
9.	Berlej 6 x SA 130	143,0**	226,20	10,5	3
10.	Berlej 6 x TN 90	142,6**	478,30	15,3	4
11.	Berlej 6 x TN 86	114,8	147,14	10,6	7
12.	Berlej MR-1 x SA 130	83,0	76,91	11,0	13
13.	Berlej MR-1 x TN 90	113,2	81,72	8,0	9
	Average:	123,1	-	-	-

*, **significant at 0.05 and 0.01 probability level

Table 3. Percentage of heterosis for yield per plant

No	F1 hybrids	Heterosis	
		MP	BP
1.	Poseidon x SA 130	36,3	-
2.	Poseidon x TN 90	50,5**	47,1*
3.	Poseidon x TN 86	24,0	-
4.	Bols 334 x TN 86	29,0	-
5.	Bols 334 x TN 90	23,5	-
6.	Bols 347 x TN 90	79,5**	72,7**
7.	Bols 347 x Berlej T	17,0	-
8.	Hy 71-442 x TN 90	9,8	-
9.	Berlej 6 x SA 130	67,0**	62,0*
10.	Berlej 6 x TN 90	68,7*	61,1*
11.	Berlej 6 x TN 86	56,1	-
12.	Berlej MR-1 x SA 130	17,0	-
13.	Berlej MR-1 x TN 90	22,4	-

*, **significant at 0.05 and 0.01 probability level

CONCLUSION

The average yield of F1 hybrids was higher from parental mean value for 31,4 grams or 34,2%. Six of the thirteen studied hybrids had significantly higher yields. The analysis of heterosis based on the yield amount in parents and their hybrids showed occurence of heterosis in four hybrids. Combination Bols 347 x TN90 expressed highly significant heterosis in relation to both parental mean value (79,5%) and the better parent (72,7%), while other three hybrids also showed statistically significant variable values. Results of the conducted studies showed that Burley varieties that were selected in different periods (using different parents), for various growing conditions, expressed divergence which contributed to the manifestation of heterosis in some F1 hybrids.

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Original scientific paper

MONITORING OF HEALTH CONDITION OF THE BASMAK ORIENTAL TOBACCO

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ABSTRACT

Three-year investigations (2009 – 2011) were conducted with four oriental tobacco varieties, including the Yaka variety YK-7/4 as a check and three varieties of Basmak tobacco - MK-1, MB-2 and MB-3. The investigated varieties are characterized by a short growing season. This characteristic is in good correlation with climate changes in recent years and it allows timely harvesting and curing of tobacco. In the world and in our country, one of the priority issues for obtaining higher yields is to produce healthy tobacco. The most common field diseases in our country are Tobacco mosaic virus (TMV), Potato virus Y (PVY) and fungal disease Phytophthora parasitica var. nicotianae (black shank).
Taking into account that tobacco diseases cause major economic damage to tobacco, analyses were made on health status of the tested varieties during the three years of investigation. The obtained results show higher resistance of the varieties MK-1 and MB-2 to Tobacco mosaic virus (TMV), MK-1 and MB-3 to Potato virus Y (PVY) and MB-2 and MB-3 to Phytophthora parasitica var. nicotianae. These results can contribute to the variety selection in breeding this type of tobacco.

Keywords: tobacco, variety, Basmak, viruses, diseases

ПОВЕЌЕГОДИШНО НАБЉУДУВАЊЕ НА ЗДРАВСТВЕНАТА СОСТОЈБА НА ОРИЕНТАЛСКИ СОРТИ ОД ТИПОТ БАСМАК

Во тригодишните испитувања (2009 - 2011 година) беа вклучени четири ориенталски сорти од кои како стандард беше земена сортата ЈК-7/4 од типот Јака, а останатите три сорти беа од типот Басмак (МК-1, МБ-2 и МБ-3). Наведените сорти се со кратка вегетација, што е поволна карактеристика со оглед на настанатите климатски промени последниве години и истата им овозможува на производителите навремено берење и сушење на тутунот. Во цел свет, па и кај нас, еден од приоритетните проблеми за добивање на поголем принос е да имаме здрав тутун. Кај нас меѓу најприсутните болести во поле се јавуваат Tobacco mosaic virus (TMV), Potato virus Y (PVY) и габното заболување Phytophthora parasitica var. nicotianae (црнилка).
Имајќи го предвид тоа што болестите на тутунот нанесуваат големи економски штети во тутунопроизводството, си поставивме за цел да ја проучиме здравствената состојба на испитуваните сорти во текот на трите реколти. Добиените резултати од испитувањата покажуваат поголема отпорност на сортите МК-1 и МБ-2 кон Tobacco mosaic virus (TMV), поголема отпорност на МК-1 и МБ-3 кон Potato virus Y (PVY) и на МБ-2 и МБ-3 кон Phytophthora parasitica var. nicotianae над контролната сорта во опитот. Овие резултати можат многу да придонесат при изборот на сорта од страна на производителот, бидејќи влеваат извесна гаранција и сигурност за успешен производен циклус на овој тип тутун.

Клучни зборови: Тутун, сорта, басмак, вируси, болести.

INTRODUCTION

During its life cycle, tobacco is often attacked by different pathogenic fungi, bacteria and viral infections. In the world and in our country, high attention is paid to creation of varieties that will be more or less resistant to various diseases. The efforts to reduce the occurrence of diseases, besides the use of chemical products, include various measures such as crop rotation, adequate soil treatment, advanced agro-techniques, proper fertilization and irrigation.

Mickovski (1984), reported that Allard first isolated TMV in 1914 and described it as easily transmited and harmful virus which can reduce the yield up to 30%. Leaves infected with TMV are greener, often with visible deformities, hardly to moisten, easy to break, with higher protein content. They have a negative effect on the taste of oriental tobaccos.

Mickovski (1984), reported that the infection of tobacco plants caused by PVY occurs in two forms: simple and necrotic. This virus is spread throughout the world. The infection occurs in early spring, significantly reducing the quality and yield of tobacco. Symptoms vary depending on the plant host and strains of the virus. It begins with mild mosaic on leaf surface, leading to discoloration between the veins and leaf deformation, which eventually ends in death of the plant.

According to Mickovski (1984), black shank is widely spread disease, especially in the US. It attacks the stem and root of the plant. The causing agent of the disease is the pathogen P. parasitica var. nicotianae (Van Breda de Haan, 1896 and Tucker, 1931). Symptoms vary depending on climatic conditions and the stage of plant development. The infected plants are wilting and, later, the above-ground part of the stem turns dark brown.

MATERIAL AND METHODS

Three-year field trials were performed in Tobacco Institute - Prilep (2009, 2010, 2011) to study the health status of the variety YK 7-4/2 (standard) and three Basmak varieties - (MK-1, MB-2 and MB-3).

Seedling was produced in traditional way, with polyethylene covered seedbeds in Tobacco Institute - Prilep. 5 g/10 m^2 first-class seed material was used in the trial and all necessary agro-technical measures were applied (watering, fertilization and protection) in order to obtain healthy and well-developed seedlings.

Soil preparation before tobacco transplanting consisted of one autumn and two spring ploughings. Fertilization was done with 300 kg/ha NPK (8:22:20) and tobacco was transplanted on 2.6.2009, 7.6.2010 and 11.6.2011. Investigations were performed in randomized block design with 5 replications at 45 × 12 cm spacing between plants on previously prepared soil. The area of the main plot was 9 m^2 and the useful area was 6,16 m^2.

Each plot consisted of 5 rows, 3 of which were used for observation and 2 as protective zone. The number of plants in the row was 42 (38 stalks were harvested and 4 served as protective zone). Number of plants in the plot (3 rows) was 126 and number of plants for calculation was 114 (3 rows). Monitoring of the health status of plants was done at 100% flowering stage.

RESULTS AND DISCUSSION

During the three-year trials, appropriate protective measures were applied in seedbeds and field. Health inspection in the stage of 100% flowering revealed the existence of

the viral diseases TMV and PVY and fungal disease black shank. The infection of tobacco stalks differed depending on the resistance of the variety, distribution and amount of precipitation, air temperature and other factors. Data in Table 1 and Figure 1 show that the average percentage of TMV ranged from 1.12% (2009) in the check variety YK 7-4/2 to 0.18% (2009) in varieties MK-1 and MB-2. The average percentage of infection in the investigated period of three years ranged from 0.77% in the check YK 7-4/2 to 0.26 in variety MK-1.

According to Miceska et al. (2003), viral infection with TMV slows down the growth of tobacco plant regardless of the stage of development. It reduces the height of the plant with inflorescence by 1 – 6 cm and leaf number by 1-5 leaves. The conclusion of other researchers and our own is that attention should be paid to the creation of resistant varieties and to the use of appropriate preventive agro-technical measures. Acording to Dimitrieski et al. (2012), chemicals should be used carefully and properly as a preventive measure in production of resistant varieties. In the control of TMV, Taskoski et al. (2006), recommended application of phytosanitary measures, use of the same seed without plant residues, use of healthy seedlings for transplanting, crop rotation, harvest of healthy plants first and then of the infected ones, by which secondary infections would be reduced.

Table 1. Percentage of TMV infected plants in the period 2009-2011

Variety	Year	Number of observed plants	TMV infected plants %	Average 2009 /2011	Difference	
					Absolute	Relative
YK 7-4/2 Æ	2009	535	1.12	0.77	/	100.00
	2010	522	0.19			
	2011	505	0.99			
MK-1	2009	550	0.18	0.26	- 0.51	33.77
	2010	536	0.19			
	2011	500	0.40			
MB-2	2009	543	0.18	0.44	- 0.33	57.14
	2010	543	0.74			
	2011	505	0.39			
MB-3	2009	525	0.57	0.64	-1.13	83.11
	2010	524	0.94			
	2011	495	0.40			

Percentage of PVY infected plants (Table 2, Figure 1) is significantly lower than that of TMV and ranges from 0.40% in the check variety YK 7-4/2 (2011) to 0.18% in varieties MK-1 (2009) and MB-2 (2010). The average percentage of PVY infection ranges from 0.21% in the check variety YK 7-4/2 to 0.07% in variety MK-3. Therefrom it can be stated that the highest tolerance to PVY was recorded in the variety MB-3 and the lowest in the check variety YK 7-4/2. According to the registered percentage of infection, varieties MK-1, MB-2 and MB-3 have higher tolerance to TMV and PVY compared to the standard variety.

Mickoski (1984), stated that PVY can be easily transmitted mechanically by inoculation with infected juice, but natural infection is most often carried out by aphids. Therefore, attention should be paid to remove weeds that host the vector and to avoid tobacco producing in the vicinity of other plants such as potatoes, peppers and tomatoes.

Table 2. Percentage of PVY infected plants in the period 2009-2011

Variety	Year	Number of observed plants	PVY infected plants %	Average 2009 /2011	Difference	
					Absolute	Relative
YK 7-4/2 Æ	2009	535	/	0.21	/	100.00
	2010	522	/			
	2011	505	0.40			
MK-1	2009	550	0.18	0.13	- 0.08	61.90
	2010	536	/			
	2011	500	0.20			
MB-2	2009	543	/	0.19	-0.02	90.48
	2010	543	0.18			
	2011	505	0.39			
MB-3	2009	525	/	0.07	-0.14	33.33
	2010	524	/			
	2011	495	0.20			

Table 3. Percentage of plants infected with Phytophthora parasitica var. nicotianae (black shank) in the period 2009 -2011

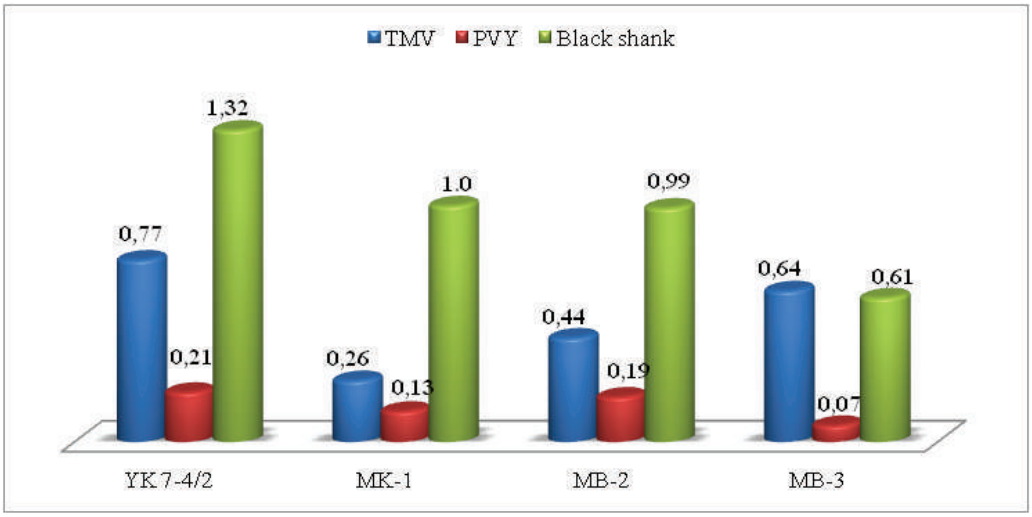
Variety	Year	Number of observed plants	Plants infected with black shank %	Average 2009 /2011	Difference	
					Absolute	Relative
YK 7-4/2 Æ	2009	535	/	1.32	/	100.00
	2010	522	/			
	2011	505	3.96			
MK-1	2009	550	/	1.00	-0.32	75.75
	2010	536	/			
	2011	500	3.00			
MB-2	2009	543	/	0.99	-9.52	75.00
	2010	543	/			
	2011	505	2.97			
MB-3	2009	525	/	0.61	-0.71	46.21
	2010	524	/			
	2011	495	1.82			

Table 3 shows that P. parasitica var. nicotianae was registered only in 2011. The presence of the disease can be associated with the occurrence of heavy precipitations at the end of seedlings production and the beginning of field production. The highest percentage of black shank was observed in the standard variety YK 7-4/2 (3.96%) and the lowest percentage was recorded in MB-3 variety (1.82%), which indicates that it has higher resistance to the disease. The three-year average ranged from 1.32% infection in the check variety YK 7-4/2 to 0.61% in MB-3 (Figure 1). General con-

clusion is that the newly created varieties, especially MB -3, show higher tolerance to P. parasitica var. nicotianae, but it can be also stated that the percentage of infection in other varieties of the trial did not achieve alarming proportions.

Taskoski (2003) reported that the application of chemicals cannot provide complete control of P. parasitica var. nicotianae in tobacco because it is a soil pathogen. For this reason, attention was turned to creation of resistant varieties. The same author noted that the first variety resistant to black shank disease was created by Tisdale in Florida, 1922 and it was named Florida 301. The next forty years this variety was used as a resistant parent to create a number of varieties resistant to black shank. In tobacco producing regions of R. Macedonia, black shank disease was recorded in 1986 (Taskoski et al., 2001). Due to the favorable climate conditions and intensive way of farming, it occurs in some tobacco regions almost every year, with various intensity.

Figure 1 – Average percentage of disease infection



CONCLUSIONS

- The three-year average infection of Tobacco Mosaic Virus (TMV) ranged from 0.77% in the standard variety YK 7-4/2 to 0.26% in MK-1, which is 66.23% less than the check variety. According to the obtained results, the newly created variety MK-1 has higher tolerance to TMV.
- The average infection of Potato virus Y (PVY) ranges from 0.21% in the standard variety YK 7-4/2 to 0.07% in variety MK-3. The highest tolerance to PVY was recorded in MB-3 variety and the lowest in the check variety YK 7-4/2.
- Phytophthora parasitica var. nicotianae (black shank) is present in small percentage, ranging from 1.32% in the standard variety YK 7-4/2 to 0.61% in MB-3. General conclusion is that the newly created varieties, especially MB -3, show higher tolerance to black shank, but it can be also stated that the percentage of infection in other varieties of the trial did not achieve alarming proportions.
- The obtained data on TMV and PVY infection show that varieties MK-1, MB-2 and MB-3 have higher tolerance than the standard variety.

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Original Scientific paper

LEAF NUMBER AND SIZE IN SOME VARIETIES OF PRILEP TOBACCO

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ABSTRACT

Number and size of leaves are morphological properties that have a major role in determination of the type or variety of tobacco. They are varietal characteristics and also indicators of tobacco yield. The aim of this paper is to present data on these two properties in some varieties of Prilep tobacco currently produced in the Republic of Macedonia. Field trials were set in 2009 and 2010 at Tobacco Institute - Prilep, in four replications, with the following varieties: P-23 (check), P 12-2/1, NS-72, P-66-9/7, P-79-94 and Prilep Basma 82. The highest average leaf number was recorded in Prilep Basma 82 (92 leaves) and the lowest in P 12-2/1 (39 leaves).
Referring to leaf size (length and width of the middle belt leaves), the highest leaf length was observed in P 12-2/1 (27,6 cm), and the lowest in Prilep Basma 82 (20,8 cm); the highest leaf width was recorded in NS-72 (13,4 cm) and the lowest in Prilep Basma 82 (10,7 cm).

Keywords: Prilep tobacco, type, varieties, leaf number, leaf size

БРОЈОТ И ГОЛЕМИНАТА НА ЛИСТОВИТЕ КАЈ СОРТИ ТУТУН ОД ТИПОТ ПРИЛЕП

Од морфолошките својства што го детерминираат типот на тутунот или сортата во еден тип, важно место имаат бројот и големината на листовите. Тие се сортни карактеристики, воедно и показатели на приносот кај тутунот.
Целта на овој труд е да ги прикажеме двете својства кај сорти од типот прилеп што се актуелни во производството во Република Македонија. Затоа, во 2009 и 2010 година во Научниот институт за тутун - Прилеп, поставивме полски опит во четири повторувања со следниве сорти: П-23 (контрола), П 12-2/1, НС-72, П-66-9/7, П-79-94 и прилеп басма 82.
Од испитувањата, утврдивме дека просечно со најголем број листови се карактеризира прилеп басма 82 (92 листа), а со најмал П 12-2/1 (39 листови).
Што се однесува до големината (должина и широчина на листовите од средниот појас), установивме дека со најдолги листови е П 12-2/1 (27,6 cm), а со најкуси е прилеп басма 82 (20,8 cm), односно најшироки листови има НС-72 (13,4 cm), а најтесни прилеп басма 82 (10,7 cm).

Клучни зборови: тип прилеп, сорти, број на листови, големина на листовите

INTRODUCTION

Tobacco is a strategic crop in the agricultural sector of the Republic of Macedonia. It also has a major social importance, as a main economic activity of around 35000 families.

Tobacco is an industrial plant that is grown because of the leaves. In R. Macedonia, oriental aromatic types (Prilep, Yaka, Basmak and Djebel) are grown on over 95% of the area under tobacco, but the leading position belongs to Prilep tobacco.

The properties by which different varieties are recognizable and which determine their yield and quality are divided into three groups: morphological, biological and qualitative. Most important morphological properties are leaf number and size (length and width). These properties in some varieties of Prilep tobacco were the subject of our two-years investigation. Atanasov (1972) reported that the number of leaves in a selected variety is a constant parameter. Uzunoski (1985) reported that leaf number varies depending on agro-ecological conditions and is important characteristic which largely determines the tobacco yield. According to the author, leaf number in different varieties varies from 10 to 70.

Leaf size (length and width) is another important characteristic in determination of tobacco quality. In assessment of dry tobacco, leaves which length exceeds 20 cm are classified as additional tobacco because they have very little or no aroma. According to Tomić (1973), the length of middle belt leaves in Prilep tobacco during the drying process is reduced by 10.73% and the width by 20.99%. The author points out that tobacco plants that are fertilized (especially with nitrogen fertilizers) and irrigated, show greater reduction (contraction) of leaves after drying.

MATERIAL AND METHODS

Investigations included the following six varieties of Prilep tobacco: P-23 Ø, P 12-2/1, NS-72, P-66-9/7, P-79-94 and Prilep Basma 82 (Fig. 1 - 6). They are listed on the National List of Varieties in the group of aromatic oriental type Prilep, which is highly esteemed on the world tobacco market. For a longer period they have been grown in a mass production in the country, some of them are grown on small areas even today and are still valued because of their quality and are used in the selection and breeding. In recent years, the most common variety of Prilep tobacco in the regions where oriental tobacco is produced is P-66-9/7 (over 90%).

The trial was set up in the field of Scientific Tobacco Institute - Prilep in 2009 and 2010, on deluvial-colluvial soil with four replications. Tobacco was planted manually, at a spacing of 40 x 12 cm and fertilized with 250 kg/ha of NPK fertilizer (8:22:20), using traditional cultural practices to ensure normal growth and development of plants. Tobacco was irrigated three times in n 2009 and twice in 2010 (due to heavy rainfall), with irrigation rate of 250 m^3 water per hectare. Investigations were performed at the stage of full flowering of tobacco, by standard methods, i.e. the average leaf number per stalk was determined on fifteen randomly selected plants of each variety from each replication, while leaf length and width were measured on the middle belt (the belt with the largest leaves). The length was measured from the base to the top and the width on the second third of the leaf because in this part it is the widest.

Photo 1. Prilep P-23

Photo 2. Prilep P 12-2/1

Photo 3. Prilep NS-72

Photo 4. Prilep P 66-9/7

Photo 5. Prilep P 79-94

Photo 6. Prilep Basma 82

RESULTS AND DISCUSSION

The investigation results are presented in tables, separately for each trait.

Leaf number per stalk

The number of leaves on a plant varies from stalk to stalk, but not significantly, so that this is the most stable trait and it presents a variety characteristic.

Korubin-Aleksoska (2004) reported that the number of leaves in different varieties of Prilep tobacco ranges as follows: from 34 to 38 in P 12-2 /1, 38 to 42 in P-84, 45 to 50 in P-23 and 50 to 55 in P-79-94.

Dimitrieski and Miceska (2011) noted that the average number of leaves per stalk in variety P-66-9/7 was 52.

In our investigations both in 2009 and 2010, all varieties had almost the same number of leaves. The obtained results are presented in Table 1.

Table 1. Leaf number per stalk

Variety	Year	Average leaf number	Difference Abs.	Difference Rel.	Average 2009/2010	Difference Abs.	Difference Rel.
P-23 Ø	2009	56	/	100	55,5	/	100
	2010	55	/	100			
P 12-2/1	2009	39	-17	69,64	39,5	-16	71,17
	2010	40	-15	72,73			
NS-72	2009	43	-13	76,79	43,5	-12	78,38
	2010	44	-11	80,00			
P-66-9/7	2009	55	-1	98,21	55,0	-0,5	99,10
	2010	55	/	/			
P-79-94	2009	57	+1	101,79	58,0	+2,5	104,50
	2010	59++	+4	107,27			
P. Basma 82	2009	93+++	+37	166,07	92,5	+37	166,67
	2010	92+++	+37	167,27			

	2009		2010
LSD	0,05=3,6 leaves +	LSD	0,05=2,7 leaves+
	0,01=4,9 leaves ++		0,01=3,7 leaves ++
	0,001=6,8 leaves +++		0,001=5,2 leaves +++

The table shows that each variety has its characteristic number of leaves which is almost the same in both years. It means that these are genotypically different varieties, i.e. that they have their own genetic code. The average number of leaves in the period of investigation ranged from 55.5 in the check, 55.0 in P-66-9/7, 58 in P-79-94 to 92.5 in Prilep Basma 82, while lower leaf number compared to the check (P-23) was obtained in varieties NS-72 (43.5) and P 12-2/1 (39.5). Statistical analysis of the results showed highly significant differences in Prilep Basma 82 in both years of investigation, whereas P-79-94 showed highly significant differences compared to the check only in 2010.

Length of the largest leaf on the stalk

The length of the leaves in oriental aromatic types of tobacco is an important parameter in assessment of quality of the raw tobacco. Measurement of size was performed on fresh (green) tobacco leaves. In the process of drying, the size of tobacco leaf (especially in irrigated and fertilized varieties) , reduces up to 30%. Leaf length primarily depends on the type and variety of tobacco, but it is highly affected by soil and climate conditions and applied cultural practices during production.

Korubin-Aleksoska (2004) found that the average length of the middle belt leaf in Prilep tobacco varieties ranged from 20 cm in P-23 and P-84, 22,5 cm in P 12- 2 / 1 and 23 cm in P-79-94.

Mitreski (2005) reported the following average lengths of the largest leaf: 25,8 cm in P-10-3/2, 25,0 cm in P-23 and 24,7 cm in P-84.

The results obtained in our investigations on this trait are presented in Table 2.

Table 2. Length of the largest leaf on the stalk in cm

Variety	Year	Average leaf number	Difference		Average	Difference	
			Abs.	Rel.	2009/2010	Abs.	Rel.
P 12-2/1	2009	26,6	+2,9	112,24	27,7	+3,1	112,60
	2010	28,7+++	+3,3	112,99			
NS-72	2009	27,0	+3,3	113,92	26,1	+1,5	106,10
	2010	25,1	-0,3	98,82			
P-66-9/7	2009	25,1	+1,4	105,91	24,3	-0,3	98,78
	2010	23,4	-2,0	92,13			
P-79-94	2009	23,5	-0,2	99,16	22,8	-1,8	92,68
	2010	22,1	-3,3	87,00			
P. Basma 82	2009	20,8	-2,9	87,76	20,8	-3,8	84,55
	2010	20,8	-4,6	81,89			

	2009		2010
LSD	0,05=n.s.	LSD	0,05=1,4 cm
	0,01=n.s.		0,01=2,0 cm
	0,001=n.s.		0,001=2,7 cm

According to Table 2, the length of the largest leaf in the check (P-23) was 24,6 cm (average of two years). Higher length of the middle belt leaves was obtained in NS-72 (26,1 cm) and P 12-2/1 (27,7 cm), which in this case could affect the assessment of tobacco during purchase. Shorter leaves compared to the check were measured in varieties P-66-9/7 (24,3) and P-79-94 (22,8 cm), while the lowest length of the middle belt leaves was obtained in Prilep Basma 82 (20,8 cm). Statistically processed values for this trait in 2009 showed no significant differences, and in 2010 highly significant difference compared to the check was observed in P 12-2/1.

Width of the largest leaf on the stalk

The width and length of the leaves depends on soil and climatic conditions and agrotechnical practices applied in tobacco cultivation. In oriental aromatic tobaccos, too wide leaves are not desirable because they increase the percentage of stalk. It is considered that the length-width ratio in the middle belt leaves should be in the range from 1,8 to 2,3:1.

Korubin-Aleksoska (2004) reported that the width of the middle belt leaf in Prilep tobacco varieties ranged from 10 cm in P-84, 10.5 in P-23, 11 cm in P 12-2/1 to 12 cm in P-79-94.

Dimitrieski and Miceska (2005), in their investigations of Prilep tobacco lines and varieties, found that the width of the largest leaf in P-23 was 11,7 cm and in P 12-2/1 it was 12,4 cm. The average values obtained in our investigations are presented in Table 3.

Table 3. Width of the largest leaf on the stalk in cm

Variety	Year	Average leaf number	Difference		Average	Difference	
			Abs.	Rel.	2009/2010	Abs.	Rel.
P 12-2/1	2009	11,3	+0,2	101,80	11,9	+0,4	103,48
	2010	12,5	+0,6	105,04			
NS-72	2009	13,7	+2,6	123,42	13,4	+1,9	116,52
	2010	13,1+	+1,2	110,08			
P-66-9/7	2009	11,4	+0,3	102,70	11,5	/	/
	2010	11,6	-0,3	97,48			
P-79-94	2009	11,3	+0,2	101,80	11,1	-0,4	96,52
	2010	10,9	-1,0	91,60			
P. Basma 82	2009	11,0	-0,1	99,10	10,7	-0,8	93,04
	2010	10,4	-1,5	87,39			

	2009		2010
LSD	0,05=n.s. 0,01=n.s. 0,001=n.s.	LSD	0,05=1,0 cm 0,01=1,3 cm 0,001=1,8 cm

The table shows that an average width of the largest leaf on the stalk (2009/2010) was 11,5 cm both in the check variety and in P-66-9/7. Wider leaves were measured in P 12-2/1 (11,9 cm) and NS-72 (13,4 cm), and narrower leaves compared to the check were measured in Prilep Basma 82 (10,7 cm) and P-79 -94 (11,1 cm).

No statistically significant differences for this trait were observed in 2009, while in 2010 significant differences compared to the check were determined in variety NS-72.

CONCLUSIONS

The two-year investigations lead to the following conclusions:

The number and size of leaves in the oriental aromatic tobacco type Prilep are major morphological (quantitative) traits which determine the yield and quality.

The average leaf number in the investigated varieties ranged from 39 in P 12-2/1 and 55 in the check variety P 23 to 92 in Prilep Basma 82.

The length of the largest leaf on the stalk ranged from 20,8 cm in Prilep Basma 82 to 27,7 cm in P 12-2/1. Considering the contraction of leaves during the drying process (20 to 30%), we believe that varieties P 12-2/1 and NS-72 (with larger middle belt leaves) will be classified as oriental aromatic tobaccos.

The lowest leaf width was measured in Prilep Basma 82 (10,7 cm) and the highest width in NS-72 (13,4 cm). In the check variety, the width of the largest leaf was 11,5 cm, the same as in P-66-9/7; in P 12-2/1 the largest leaf width reached 11,9 cm and in P- 79-94 it was 11,1 cm.

Tobacco is a flexible plant which can, to some extent, modify its quantitative traits under the influence of soil and climate conditions and applied cultural practices, but nonetheless, it can be stated that the number and size of leaves are varietal characteristics. The investigated varieties are stable, different, with their own genetic constitution which makes them recognizable in the mass production, as representatives of Prilep tobacco.

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Original scientific paper

NEW FUNGICIDE OPPORTUNITIES IN THE CONTROL OF Rhizoctonia solani IN TOBACCO SEEDLINGS

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ABSTRACT

Damping off is the most destructive disease on tobacco seedlings. Effectiveness of some fungicides against this disease was tested in seedbeds and in biological laboratory.
The aim of investigations was to test the fungicide already used in seedlings protection along with some other fungicides - active ingredients and to determine their effectiveness when applied in higher and lower concentrations. The obtained results may lead to expanding the list of products used in protection of tobacco seedlings from damping off disease.
Signum 33 WG and Quadris 25 SC fungicides showed higher effectiveness in the control of pathogenic fungus Rhizoctonia solani compared to the standard product Top M (0.1%). Fungicides Signum 33 WG at concentrations of 0.1% and 0.15% and Quadris 25 SC at concentrations of 0.15% and 0.2% offer new opportunities in protection of tobacco seedlings from damping off disease.

Keywords: Rhizoctonia solani, fungicide, active ingredient, effectiveness

НОВИ ФУНГИЦИДНИ МОЖНОСТИ ЗА СУЗБИВАЊЕ НА Rhizoctonia solani КАЈ ТУТУНСКИОТ РАСАД

Болеста сечење е најдеструктивна болест на тутунскиот расад. Испитувањата за ефикасноста на некои фунгициди за заштита од оваа болест беа извршени во леи и вобиолошка лабораторија.
Тие имаа цел да се испита ефикасноста на фунгицидот кој веќе се употребува во производството на тутунски расад, но и на некои други фунгициди, односно активни материи, и да се утврди концентрацијата во која новите препарати покажуваат најголема ефикасност, односно каква е таа во случај на намалена концентрација. Резултатите би придонесле за можно проширување на листата на препарати за заштита на тутунскиот расад од сечењето.
Фунгицидите Signum 33 WG и Quadris 25 SC покажаа повисока ефикасност во сузбивањето на патогената габа Rhizoctonia solani од стандардниот препарат Top M (0,1%). Signum 33 WG во концентрации 0,1% и 0,15% и Quadris 25 SC во концентрации 0,15% и 0,2% претставуваат нови фунгицидни можности за заштита на тутунскиот расад од болеста сечење.

Клучни зборови: Rhizoctonia solani, фунгицид, концентација, ефикасност

INTRODUCTION

Production of healthy and good quality tobacco largely depends on successful seedling production and on providing enough quantities of healthy planting material to enable timely and complete seeding of the areas. However, the main problem in production of tobacco seedling is the outbreak of damping off disease. In most cases, pathogenic fungus Rhizoctonia solani is the causing agent of the disease.

According to Gutierez et al. (2001), the fungus can cause two types of diseases: target spot and stem rot (sore shin or damping off). The latter is usually seen in the early stages of seedlings development. The author described the symptoms of damping off disease.

According to Ceresini (1999), some diseases caused by Rhizoctonia in beans, sugar beet and tobacco occur as a result of infection from basidiospores. However, sclerotia and/or mycelium present in the soil or in host-plant tissue are primary inoculum. R. solani persists for many years as mycelium in organic matter or as sclerotia in soils, in variable environmental conditions. The sclerotia can also survive in water (Pataky, 1988; Grosh, 2003).

R. solani is a widespread pathogen which causes serious damage to many agricultural and horticultural plants (Grosh, 2003). The reduction of yield is proportional to agricultural areas and can reach up to 50% (Wallwork, 2000, loc cit. Hollaway, 2008).

Destructive effects caused by this fungus are the reason why the control of this pathogen is so important (SMBSC, 2012).

Protection programmess should be primarily based on preventing pathogen invasion (Pataky, 1988). Uchida (2012) emphasized the importance of such measures, and similar statement was made by Miller and Miller (2009), with special emphasis on seed treatment with fungicide.

Saprophytic ability, however, and wide range of host plants, along with the limited opportunity for crop rotation greatly obstruct the disease control (Hollaway, 2008). Still, efforts are made to maintain the loss at minimum level. The application of chemicals is still the most acceptable protective measure.

Azoxystrobin, trifoxystrobin, and tebuconazole are effective active ingredients against Rhizoctonia (Mocioni et al., 2003). According to Koenning (2007), fungicides containing PCNB (Terrachlor), Iprodione (Rovral) or Azoxystrobin (Quadris) are effective in the control of R.solani. Recommended products for control of this pathogen in potatoes are fludioxinil, maneb, penthiopyrad, thiophanate-methyl, PCNB and azoxystrobin, with their trade names and modes of application (Schwartz and Gent, 2012).

Azoxystrobin (Quadris) is recommended against root rot caused by R.solani (Bredehoert, 2012). Compared to penthiopyrad, Azoxystrobin applied in protection of sugar beet proved efficient in all variants of treatment. Even in severe attacks of Rhizoctonia, the mortality of plants in treatments with azoxystrobin was significantly lower (Poindexter and Wenzel, 2013).

Out of the wide range of products with various trade names and active ingredients for control of R. solani in soybean seedlings, the most recommended fungicides are strobilurins pyraclostrobin and trifloxystrobin (Mueller, 2014). In protection of beans, the recomended fungicide (besides Quadris) is Signum, in which pyraclostrobin is combined with boscalid (Annonimus, 2015). This combination enables a wide range of activities and reduced risk of resistance to different target pathogens (Hauke et al. , 2004).

Signum is a fungicide with preventive and systemic action against many diseases in a variety of crops (BASF, 2008).

Despite the great number of active ingredients, the number of fungicides for control

of this pathogenic fungus in our conditions is limited. The most commonly used fungicide in protection from the disease is Top M is (Gveroska, 2012).

Some active ingredients are effective in tobacco protection from the pathogen. Csinos and Stephenson (1999) recommend flutolanil, iprodione, fluazinam and tebuconazole to prevent the spread of infection in seedbeds.

Bertrand (2012) recommends azoxystrobin (Quadris F) in the control of target spot on tobacco caused by R.solani AG-3. Application of Quadris against this disease is also recommended by the manufacturer (Syngenta, 2006).

According to LaMondia (2012), seedlings treatment with azoxystrobin (Quadris) may be the key measure in protection from sore shin caused by R.solani. The author made in vitro and in vivo tests and concluded that the effectiveness of the product at in vitro conditions is not as good as in field and in biological laboratory. These data focused the investigations on the use of fungicides in conditions of natural infection by the pathogen.

The aim of our investigations was to study the effectiveness of some new fungicides and the concentrations in which they will be effective in various conditions of infection. This can create new fungicide opportunities in protection of tobacco seedlings from damping off disease.

MATERIALS AND METHODS

Investigations were conducted in Scientific Tobacco Institute - Prilep in seedbeds and in biological laboratory.

- Tobacco seedbeds

Sowing was carried out on 21.4.2015, in 10 m^2 seedbeds, using a rate 0,67 g/m^2, in traditional way. Seedlings were fertilized once with 10 g/m^2 ammonium saltpeter, with no other fungcicide treatments.

The trial was set up with three replicates, in randomized block system. The area of each variant was 2,5 m^2. First treatment was made on 15.5.2015 and the second one in 27.5.2015. The assessment of the infected area was made on 8.6.2015, after which the the effectiveness of tested fungicides was evaluated.

- Biological laboratory

The trial was set up two times with 3 replicates at 0,1 m^2. Tobacco seed was sown at a rate of 0,5 g/m^2 and seedlings were grown in traditional way.

The first sowing was carried out on 2.6.2015. Treatment of seedlings was made on 23.6.2015 and assessment of disease infection (% of infested area) on 6.7.2015.

The second sowing was carried out on 11.9.2015, seedbeds were treated on 28.09.2015 and assessment of disease infection was made on 9.10.2015.

The fungicides were applied in appropriate concentrations, using 200 ml water.

Fungicides for both investigations were chosen according to the target pathogen, literature data and data from the investigations and experience in production and protection of tobacco seedlings in the Scientific Tobacco Institute-Prilep. In most cases, the causing agent of damping off disease was the pathogenic fungus Rhizoctonia solani.

After estimation of the disease infection, artificial isolation of the pathogen was made on nutrient medium potato dextrose agar and it was found that damping off was caused by this pathogen.

After assessment of the effectiveness of above fungicides in seedbeds, we tested their effectiveness in higher and lower concentrations. Therefore, two concentrations of the products, along with the standard fungicide Top M, were tested in biological laboratory,

Results on tested fungicides and concentrations in both investigations are given in Table 1.

Table 1. Investigated fungicides

Fungicide	Active ingredient	A.i. content	Seedbeds	Biolaboratory
			Concentration (%)	
Top - M 70% WP	Thiophanate- methyl	70%	0,1	0,1
Signum® 33 WG	Boscalid + pyraclostrobin	26,7% +6,7%	0,1	0,1 0,15
Quadris 25 SC	Azoksistrobin	250 g/l	0,2	0,15 0,2

Seedlings development and disease severity were monitored daily. The observed difference in growth between the two trials was due to the period of sowing. The fungicides were applied before the stage of rapid growth. Assessment of disease was made 7-12 days after infection. Fungicides effectiveness was estimated by the method of Abbott, based on the mean value of the percentage of infected area in the three replicates and the check. Figure 1 is a graphic representation of the mean value of fungicides effectiveness in both trials.

RESULTS AND DISCUSSION

Specific symptom of the disease is that seedlings immediately rot and fall over the soil surface.

The first symptom is the appearance of small watery lesions on the stalk close to the ground, which quickly become brown and hollow. In favorable temperature and humidity conditions the lesions expand, turn brown and inhibit further growth of the plant. Symptoms like these were also described by Gutierrez et al. (2001).

Sometimes, when moisture in the seedbed is higher, a whitish mold can be observed on seedlings. The infected area increases, making patches which coalesce to form large necrotic areas, destroying most of the seedbed. Such symptoms on tobacco seedlings were also observed in the biological laboratory (Ph. 1 and 2).

Ph. 1. Symptoms of damping off disease in tobacco seedbeds

Ph. 2. Symptoms of damping off disease in pots

Although it is not possible to achieve complete control of Rhizoctonia, severity of the disease can be reduced in the early stages of vegetation (Miller and Miller, 2009). By treatment with the investigated fungicides, the infected area in tobacco seedbeds is reduced, i.e. it is significantly smaller compared to the check and to the area treated with the standard fungicide (Table 2). It can be noted that the infected area in all

variants is lower in the second replicate. The lowest effectiveness was achieved with the standard fungicide Top M (0,1%) and the highest with Quadris 25 SC (0,2%), but high effectiveness in protection of tobacco seedlings from damping off disease was also obtained with the fungicide Signum® 33 WG (0,1%) (Table 2).

The manufacturer recommends application of Signum in protection of beans immediately after the first signs of disease. For optimal control, spraying must be repeated at 3-4 week intervals, depending on the disease development (BASF, 2008). Hence, our results on the effectiveness of this product in seedbeds, where two treatments of seedlings were applied, are in accordance with these recommendations.

Post-planting application of Signum in lettuce also provides good protection against three pathogens, including R. solani (AHDB, 2011).

Table 2. Effectiveness of fungicides in the seedbeds

Fungicide (concentration)	Infected area %			Average value	Effectiveness %
	Replicates				
	I	II	III		
Top M (0,1%)	2,23	0,91	2,51	1,88	65,82
Signum® 33 WG (0,1%)	1,27	0,22	1,77	1,09	80,18
Quadris 25 SC (0,2%)	0,56	0,06	0,89	0,50	90,91
Check Ø	6,53	1,38	8,59	5,50	-

Tests conducted in biological laboratory revealed that the intensity of damping off attack was higher compared to that in seedbeds, which is certainly a result of the time period and the conditions in the lab. In the first trial, damping off attacked the untreated seedlings (check variant) with an average percentage of 41.66% of planted area. In seedlings treated with fungicides, damping off disease was observed only in variant with Top M 0,1% (Ph. 3). Seedlings treated with Signum 33 WG and Quadris 25 SC showed no symptoms of disease. Such condition was observed with both concentrations. In the variant with Quadris, for example, no outbreak of damping off was observed even in lower concentration (Table 3). Thus, reducing the concentration of this product seems justified. Brantner and Windels (2011) confirmed that in conditions of less severe attack of damping off disease, the benefit from the use of Quadris in any concentration is more than enough to pay off its application.

Top M (01%), achieved 78.00% effectiveness, compared to Signum (0,1 and 0,15%) and Quadris (0,15 and 0,2%) which achieved an effectiveness of 100% (Ph. 4, 5 and 6).

Table 3. The effectiveness of fungicides in biolaboratory (I trial)

Fungicide (concentration)	Infected area %			Average value	Effectiveness %
	Replicates				
	I	II	III		
Top M (0,1%)	10,00	10,00	8,33	9,44	78,00
Signum® 33 WG (0,1%)	-	-	-	-	100,00
Signum® 33 WG (0,15)	-	-	-	-	100,00
Quadris 25 SC (0,15%)	-	-	-	-	100,00
Quadris 25 SC (0,2%)	-	-	-	-	100,00
Check Ø	43,33	25,00	56,66	41,66	-

- no symptoms of disease

Ph. 3 Infected area in the check variant - Fungicides Top M and Signum 33WG

Ph.4 Effectiveness of investigated fungicides

Ph. 5. Infected area - fungicide Signum (0,1 and 0,15%)

Ph. 6. Infected area – fungicide Quadris (0,15 and 0,2%)

In the second trial, the intensity of disease attack in the check (mean value 10.33%) was lower compared to the first trial. However, symptoms of disease were found in almost all variants and replicates.

The calculated effectiveness of the fungicides is in compliance with the infected area. It was the highest (3.15%) in Top M (0,1%), i.e. the lowest effectiveness was achieved with this preparate. Similar value (79.00%) was achieved with Signum (0,1%), while the higher concentration of this fungicide increased the effectiveness (Table 4, Ph 7).

Table 4. The effectiveness of fungicides in biolaboratory (II trial)

Fungicide (concentration)	Infected area %			Average value	Effectiveness %
	Replicates				
	I	II	III		
Top M (0,1%)	3,59	3,06	2,82	3,15	70,00
Signum® 33 WG (0,1%)	-	4,35	2,23	2,19	79,00
Signum® 33 WG (0,15)	2,47	1,29	0,53	1,43	87,00
Quadris 25 SC (0,15%)	-	-	1,53	0,51	96,00
Quadris 25 SC (0,2%)	-	0,26	0,11	0,12	99,00
Check Ø	5,00	17,66	8,33	10,33	-

- no symptoms of diseas

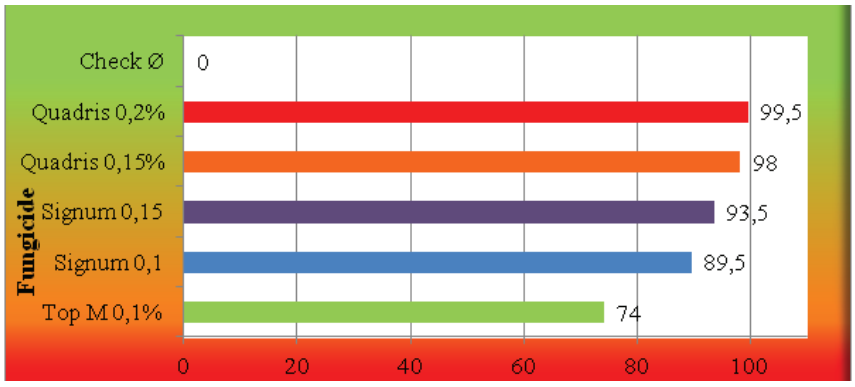
Ph.7. Infected area in variants with Top M and Signum 33 WG (0,1%)

Ph. 8. Infected area in the variant with Quadris 25 Sc (0,15%)

Ph. 9. Infected area in the variant with Quadris 25 SC (0,2%)

The lowest percentage of infected area (0.12%) was obtained with Quadris (0,2%). However, even with reduced concentration of this product (0.15%), the percentage of infected area was considerably low (Table 4, Ph 8). The highest effectiveness was achieved in the variants with Quadris 25 SC (0,2%) - 99,00% and Quadris (0,15%) - 96,00% (Ph. 8 and 9).

Fig. 1. The effectiveness of fungicides (average value of two trials)



The mean value of fungicide effectiveness in both trials confirmed the factual situation (Figure 1), i.e. the lowest effectiveness in control of the pathogen R. solani was obtained with the fungicide Top M (0,1%). Similar results were reported in investigations of Gveroska (2012).

The highest effectiveness was obtained with Quadris 25 SC (0,2%) - 99,5%, but it should be noted that even with lower concentration (0.15%), the fungicide achieved 98.00% effectiveness. Hence, the treatment of tobacco seedlings with azoxystrobin (Quadris) could be a key measure in the control of R.solani (LaMondia, 2012). The high effectiveness of Quadris is due to the direct effect of azoxystrobin in the soil inoculum and the mutual effective combination with the plant tissue. The obtained results are certainly due to the timely treatment of tobacco seedlings, monitoring the conditions for disease outbreak and the optimum amount of water. This was confirmed by Khan (2015), who reported that Quadris application should begin prior to disease development and not as a curative treatment, preferably in the stage of cotyledone (4-6 leaf stage), especially when conditions for infection are favorable. It should be applied in enough water for thorough coverage (Syngenta, 2006).

Signum® 33 WG fungicide in concentrations of 0,1% and 0,15% also provided high effectiveness against damping off disesase - 89.50% and 93.50%. This effectiveness may be due to the fact that both active ingredients are an excellent combination of two different biochemical modes of action in the fungal cell respiration (Hauke et al., 2004).

According to AHDB (2011), fungicides application at less than full label rate, in the case of lower risk of infection, should be justified by well-grounded facts. In this sense, our results on the effectiveness of investigated fungicides in concentration that differs from the recommended, confirms the possibility of their application from both practical and economic aspects.

CONCLUSIONS

Standard fungicide in the control of the pathogen R. solani Top M (0.1%) showed the lowest effectiveness both in seedbeds and in biological laboratory.

- In seedbed trials, 80.18% effectiveness was achieved with Signum 33 WG (0,1%) and 90,91% effectiveness with Quadris 25 SC (0,2%).
- The effectiveness of investigated fungicides in biological laboratory was lower in the second trial.
- The effectiveness of the fungicide Signum 33 WG (0,1%) was 100% in the first trial and 79% in the second. With increased concentration (0.15%), effectiveness of 100% was achieved in the first and 87.00% in the second trial.
- Quadris 25 SC achieved very high effectiveness in the control of this pathogen, reaching 100% and 99.00% at 0.2% concentration in the first and second trial, respectively. Even at reduced concentration of 0.15%, the fungicide had 100.00% and 96.00% effectiveness in the first and second trial, respectively.
- The fungicides Signum 33 WG and Quadris 25 SC showed higher effectiveness in the control of pathogenic fungus R. solani compared to the standard fungicide Top M.

- Signum 33 WG at concentrations of 0.1 and 0.15% and Quadris 25 SC at concentrations of 0.15% and 0.2% offer new fungicide opportunities in protection of tobacco seedlings from damping off disease.

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Original scientific paper

APPLICATION OF THE SIGNUM FUNGICIDE IN CONTROL OF *PYTHIUM DEBARYANUM* HESSE ON TOBACCO SEEDLINGS

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ABSTRACT

Favorable microclimate conditions in seedbeds contribute to the outbreak of damping off disease on tobacco seedlings. The aim of this paper was to estimate the effectiveness of the fungicide Signum in control of *Pythium debaryanum*, the disease causing agent. Investigations were made during 2013 and 2014 in the biological laboratory of the Scientific Tobacco Institute -Prilep. Seedlings of the variety NS 72 were sown in 0,3 m^2 plastic trays. Standard fungicide Proplant 722 SL 0,25% (722 g/l propamocarb-hydrochlorid) and the preparations Orvego 0,1% (ametoctradin 300 + dimethomorph 225), Enervin 0,2% (ametoctradin 120 + metiram 440) and Signum 0,1% (boscalid 267 + pyraklostrobin 67) were used in the investigations. Two waterings were applied on seedlings with 1 l/m^2 suspension prepared from the fungicides. The first watering was applied prior to the 4th-leaf stage and the second one 15 days after. The coefficient of fungicide effectiveness was calculated by applying the formula of Abbott (1925). In both years of investigation, Signum 0,1% showed the highest effectiveness in protection of the seedlings. No infected plants were observed during the growing season in variants where this fungicide was applied.

Keywords: tobacco seedlings, disease, intensity, fungicides, effectiveness

ПРИМЕНА НА ПРЕПАРАТОТ SIGNUM ВО ЗАШТИТАТА НА ТУТУНСКИОТ РАСАД ОД ПАТОГЕНОТ *PYTHIUM DEBARYANUM* HESSE

Поволните микроклиматски услови во леите допринесуваат за честа појава на болеста сечење односно "топење" на расадот. Целта на испитувањето беше да се провери ефикасноста на фунгицидот Signum за сузбивање на патогенот *Pythium debaryanum* причинител на ова заболување. Испитувањата се изведени во текот на 2013 и 2014 година во биолошката лабораторија на Научниот институт за тутун. Тутунскиот расад од сортата НС72 беше одгледуван во пластични корита со површина од 0,3 m^2. За испитување беа користени стандардниот препарат Proplant 722 SL 0,25% (722 g/l propamocarb-hydrochlorid) и препаратите Orvego 0,1% (ametoctradin 300 g/l+dimethomorph 225 g/l), Enervin 0,2% (ametoctradin 120 g/kg+metiram 440 g/kg) и Signum 0,1% (boscalid 267 g/kg+pyraklostrobin 67 g/kg). Расадот е третиран со полевање со суспензија подготвена од препаратите. Направени се две полевања на расадот со по 1 l раствор на m^2. Првото полевање е направено во фаза вкрстување на расадот, додека второто полевање е извршено после 15 дена од првото. Коефициентот на ефикасност на фунгицидите е пресметан според формулата на Abbott

(1925). Во двете испитувани години препаратот Signum 0,1% покажа највисока ефикасност во заштитата на тутунскиот расад. Во сите варијанти каде што беше применет овој препарат, во текот на вегетацијата немаше забележано појава на заразени растенија.

Клучни зборови: тутунски расад, болест, интензитет, фунгициди, ефикасност

INTRODUCTION

In the Republic of Macedonia, tobacco seedlings are traditionally grown in cold seedbeds covered with polyethylene, while in most of the large-leaf tobacco producing countries they are grown in hydroponic nutrient solution - float system (Boić et al., 1999). The period of seedling production consists of the following stages: seed germination, emergence, 4th leaf stage, root formation and the period for transplanting, when seedlings are highly susceptible to diseases. For successful production of tobacco it is necessary to produce healthy and high quality seedlings and to provide suitable preventive measures.

Tobacco is a host to many plant parasites. The diseases caused by these parasites can deteriorate the whole tobacco production, if appropriate measures are not taken. Protection of tobacco, particularly of seedlings, is very important but delicate question. Some of the seedling diseases are the following: black root rot (caused by *Thielaviopsis basicola*), root burn (*Olpidium brasicae*), root rot (*Rhizoctonia solani*), damping off (*Pythium debaryanum*), etc. According to literature data (Boić et al., 1999), the most common diseases on tobacco seedlings in America are root rot (*Rhizoctonia solani*), sclerotinia (*Sclerotinia sclerotiorum*) and black leg (*Erwinia carotovora*). Until recently it was considered that disease of tobacco seedlings in seedbeds was caused by the phytopathogenic fungus *R. solani*. Last few years, however, it has been determined in several tobacco producing regions that the causing agent of damping off disease is *P. debaryanum*. Common characteristic of fungi diseases in seedlings is that they occur in densely planted beds, with excessive moisture in the seedbed, insufficient aeration and unfavorable climate conditions (cloudy and rainy weather).

The genus Pythium consists of more than 100 species (Ćosić et al., 2006), the most important of which are *P. debaryanum*, *P. irregulare*, *P. ultimum*, *P. splendens*, *P. aphanidermatum* etc. It is optional parasite which lives in soil rich in organic matter and infects a large range of hosts, including vegetables, ornamental plants, fruit and forest species (Pejcinovski et al., 2009), but it especially attacks sugar beet and tobacco (Ćosić et al., 2006). The most susceptible are young plants at the stage of germination and emergence while the older plants are not endangered (Ćosić et al., 2006). Species of the genus Pythium regularly appear all around the world, making a huge damage. They often cause complete destruction of germ while in emerged plants they necrotise the root and the stem base, as a result of which the plants collapse and are covered with mycelium (Jurković et al., 2009). The plants die quickly, and at higher humidity and temperature they are "melting", due to which the disease is also known as melting of seedlings. The disease spreads in the seedbed in a form of circles, forming smaller or larger empty patches, depending on the intensity of attack. Similar symptoms, however, can be caused by other fungal species, including *Fusarium spp*., *R. solani*, *Phytophthora parasitica var. nicotianae,* etc.

All external factors that negatively affect the seedlings germination favor the development of disease causing agents: heavy and poorly drained soils, acidic soils, low temperatures after germination, high hu-

midity and higher temperatures in the rapid growth stage, poor aeration, etc. One of the common errors in the production of tobacco seedlings is excessive irrigation (Radunović, 7). In such conditions, plants have poorly developed shallow-rooted system and, due to favorable conditions, the seedlings are infected by pathogenic fungi from the surface layer of the soil. Similar problems in the production of seedlings in the vegetable crops, in addition to recommended preventive measures and cultural practices (Balaž, 1), led to the use of chemicals, i.e. fungicides Previcur 607 SL and Previcur Energy (Radunović, 7). High effectiveness in the control of this pathogen in tobacco was also achieved by formulations with active ingredient etridiazol (Seebold, 2008; Kenneth, 2011).

Signum is a new fungicide developed by BASF, applied in recent years against fungal diseases. According to Hauke (2004), it showed high effectiveness in the control of *Botrytis cinerea* in strawberries and gave good protection against some species of powdery mildew and Phytophthora. Good results with application of this fungicide against *Botrytis cinerea* were also observed in lettuce Callens (2005).

The aim of this study was to estimate the effectiveness of some new fungicides, including Signum, in protection of tobacco seedlings from the phytopathogenic fungus *P. debaryanum*, the causing agent of damping off disease.

MATERIAL AND METHODS

Investigations were conducted in the biological laboratory of the Scientific Tobacco Institute-Prilep in 2013 and 2014. Seedlings of the variety NS72 were planted in 0.3 m^2 polystyrene trays on 7.5.2013 and 5.5.2014, in two trials with three replications. Seedlings in the first trial were grown on soil inoculated with pure culture of the fungus and in the second trial they were grown in naturally infected soil without additional artificial inoculation. Fungal culture was grown on potato dextrose agar in a thermostat at 25^{0}C for 10 days.

The inoculum for the 0.3 m^2 tray was prepared from mycelial colony in two Petri dishes and mixed in 200 ml distilled water. The suspension was added in the tray before sowing the seedlings. Two treatments with fungicides were made during seedlings growth, the first one in the stage of 4th leaf on 20.5.2013 and 19.5.2014, and the second one in the stage of rapid growth on 4.6.2013 and 3.6.2014. The seedlings were treated with 1 l fungicide solution/m^2 and the check variants were poured only with pure water.

Table 1. Investigated fungicides

Fungicide	Active ingredient	Concentration %
Orvego	300 g/l ametoctradin + 225 g/l dimethomorph	0,1
Enervin WG	120 g/kg ametoctradin + 440 g/kg metiram	0,2
Signum	267 g/kg boscalid + 67 g/kg pyraklostrobin	0,1
Proplant 722 SL	722 g/l propamocarb hydrochlorid	0,25

Two assessments were made during the growing season, the first one on 12.6.2013 and the second on 18.6.2013, i.e. on 16.6.2014 and 23.6.2014. After the inspection of seedlings health condition, the infected area was measured in the sites where infection occurred. The measurement data were used to evaluate the percentage of empty area, i.e. the disease intensity. According to the disease intensity measured in the second assessment, the coefficient of fungicide effectiveness was calculated by the Abbott's (1925) formula.

RESULTS AND DISCUSSION

Results of the investigation on fungicides effectiveness in the control of *P. debaryanum* on tobacco seedlings are presented in tables. Data obtained in 2013, from the trials with seedlings grown in soil inoculated with culture of the pathogenic fungus, are presented in Table 2. In the check variant, the infection ranged from 73.00% in the first assessment to 100.00% in the second assessment (Fig. 1). The infection in seedlings treated with Proplant 0,25% reached 94.00% and in treatment with Orvego 0,1% it ranged from 57.00% in the first assessment to 80.00% in the second assessment (Fig. 2). The lowest rate of infection (3.00%) was evaluated in the variant treated with Enervin 0,2% (Fig. 3). In the variant treated with Signum 0,1%, alone or in combination with other fungicides, no incidence of infection was observed (Fig. 4). This fungicide showed high effectiveness in the control of *P. debaryanum* on tobacco seedlings.

Table 2. Disease intensity in seedlings grown in soil inoculated with culture of the fungus P. debaryanum in 2013

Variant	Infected area %	
	I Assessment	II Assessment
Check	73,00	100,00
Orvego 0,1%	57,00	80,00
Enervin 0,2%	3,00	3,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	94,00	94,00

Fig. 1. P. debaryanum – Infected seedlings in the check variant

Fig. 2. Seedlings treated with Orvego 0,1%

Fig. 3. Seedlings treated with Enervin 0,2%

Fig. 4. Seedlings treated with Signum 0,1%

Similar results were obtained with seedlings grown on naturally infected soil (Table 3). In the first assessment, the infected area in the check variant was 51.00%. In the second assessment the disease affected larger area and the infection increased to 86.00%.

As in the previous case, treatment with Orvego 0,1% did not give satisfactory results and the disease intensity ranged from 47.00% in the first assessment to 60.00% in the second.

Table 3. Intensity of the disease in seedlings grown on naturally infected soil in 2013

Variant	Infected area %	
	I Assessment	II Assessment
Check	51,00	86,00
Orvego 0,1%	47,00	60,00
Enervin 0,2%	1,00	1,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	20,00	53,00

Somewhat better results were obtained with Proplant fungicide used at a concentration of 0.25%, with disease intensity ranging from 20.00% in the first assessment to 53.00% in the second. Once again, the best results were obtained with Enervin 0,2%, with only 1.00% infected area, and Signum 0,1% in all variants, with no outbreak of disease.

Data on the occurrence and intensity of the disease caused by *P. debaryanum* received in the second assessment were used to calculate the effectiveness of the applied fungicides. According to the results given in Table 4, the highest effectiveness (100.00%) in the control of this pathogen both in seedlings grown in soil inoculated with pure culture of the fungus and in seedlings grown in naturally infected soil was obtained with Signum 0 1%, where no occurrence of infection in tobacco seedling was recorded. With Enervin 0,2%, the percentage of infected plants was insignificant and its effectiveness ranged 97.00% and 98.83%, respectively. The lowest effectiveness was obtained with Orvego 0,1% (20,00%, i.e. 30.23%) and Proplant 0,25% (6.00% in seedlings grown in inoculated soil and 38.37% in naturally infected soil).

Table 4. The effectiveness of fungicides tested in 2013

Variant	Effectiveness %	
	Seedlings grown on inoculated soil	Seedlings grown on naturally infected soil
Check	-	-
Orvego 0,1%	20,00	30,23
Enervin 0,2%	97,00	98,83
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,25%+Signum 0,1%	100,00	100,00
Proplant 0,25%	6,00	38,37

In the investigations of 2014, seedlings grown in soil inoculated with fungal culture showed high disease infection in the check variant, with intensity ranging from 80.00 to 90.00% (Table 5). No major difference in seedlings damage was observed in the treatment with Orvego 0,1%, where the intensity of disease was 65.00% and 75.00%, respectively. Similar results were obtained with Enervin 0,2% and Proplant 0,25%. In the first assessment the percentage of infected area was 19.70% and 15.50%, while in the second assessment the infection was more severe, with disease intensity reaching 40,00% in Enervin 0,2% and 34.67% in Proplant 0,25%.

Table 5. Disease intensity in seedlings grown in soil inoculated with culture of the fungus *P. debaryanum* in 2014

Variant	Infected area %	
	I Assessment	II Assessment
Check	80,00	90,00
Orvego 0,1%	65,00	75,00
Enervin 0,2%	19,70	40,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	15,50	34,67

On the other side, no outbreak of disease was observed in variants treated with Signum 0,1 alone or in combination with other agents.
In the trial where seedlings were grown in naturally infected soil, no major differences in infection rate were observed compared to previously obtained results. The intensity of disease in the check ranged from 60.00% in the first assessment to 85.00% in the second (Table 6).

Table 6. Disease intensity in seedlings grown on naturally infected soil in 2014

Variant	Infected area %	
	I Assessment	II Assessment
Check	60,00	85,00
Orvego 0,1%	55,00	68,00
Enervin 0,2%	20,00	20,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	5,00	18,00

In the variant treated with Orvego 0,1%, 55.00% infected area was measured in the first assessment and 68.00% in the second. In this case, too, good results were obtained in treatments with Enervin 0,2% and Proplant 0,25%, with disease intensity of 20.00% and 18.00%, respectively. Only the seedlings treated with Signum 0,1% in all variants showed no symptoms of disease.

Table 7 shows the results on product effectiveness in seedling protection from the pathogen *P. debaryanum* during 2014. In variants where seedlings were grown in soil inoculated with fungus culture, the poorest attack (16.66%) was recorded with Orvego 0,1%. Good results were obtained with preparations Enervin 0,2% (55,55%) and Proplant 0,25%, which achieved an effectiveness of 61.47%. The highest effectiveness of 100.00% was obtained with Signum 0,1% in all three combinations, with no symptoms of disease on treated seedlings.

Table 7. The effectiveness of investigated fungicides in 2014

Variant	Effectiveness %	
	Seedlings grown in inoculated soil	Seedlings grown in naturally infected soil
Контрола	-	-
Orvego 0,1%	16,66	20,00
Enervin 0,2%	55,55	76,47
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,25%+Signum 0,1%	100,00	100,00
Proplant 0,25%	61,47	78,82

Similar results were obtained with seedlings grown in naturally infected soil: 20,00% effectiveness was obtained with Orvego 0,1%, and somewhat higher effectiveness was achieved with preparations Enervin 0,2% and Proplant 0,25% (76,47% and 78.82%, respectively). Only the seedlings treated with Signum 0,1% showed no symptoms of disease. High effectiveness with this fungicide was achieved in protection of tobacco seedlings in field conditions (Tashkoski et al., 2014), and according to literature data, high effectiveness against *Botrytis cinerea* was observed in strawberries and lettuce (Hauke, 2004; Callens, 2005). According to Gutierrez (2012), the best results in protection of tobacco seedlings from this pathogen were achieved by

application of the strobilurin fungicides, with active ingredient azoxystrobin.
This confirms the results obtained with the product Signum 0,1%, which active ingredient is pyraklostrobin, belonging to the group of strobilurins.

CONCLUSION

-The results obtained indicate that damping off disease occurs with almost the same intensity in both years of investigation. Thus, in 2013 the disease intensity in the check variant ranged from 86.00% in seedlings grown in naturally infected soil to 100.00% in soil inoculated with fungus culture. In 2014 similar results were obtained, with infection in the check ranging from 85.00% to 90.00%. Unlike this, in the varieties treated with fungicides in both years of investigation, infection of tobacco seedlings was much lower.
-The lowest effectiveness in 2013 in both variants was observed with Orvego 0.1%, with 20.00% effectiveness and 30.23%. Similar effectiveness was achieved with Proplant 0,25%, ranging from 6.00% in seedlings grown in inoculated soil to 38.37% in seedlings grown in naturally infected soil. Effectiveness of 97.00% and 98,83% was achieved with Enervin 0,2% in both variants.
-In 2014, the lowest effectiveness of about 20.00% was obtained with Orvego 0,1% in seedlings grown in naturally infected soil. The fungicides Proplant 0,25% and Enervin 0,2% gave approximately the same results and they did not provide complete protection of seedlings.
-The highest effectiveness in seedlings protection in both years and in both variants was achieved by the fungicide Signum 0,1%, applied separately or in combination with other fungicides. Wherever this preparation was applied during the growing season, no symptoms of disease were observed on seedlings, they were healthy and had a good development. The high effectiveness recorded during the investigation gives this fungicide an opportunity to find practical application in protection of tobacco seedlings from the pathogen *P. debaryanum*.

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Original scientific paper

CADMIUM UPTAKE BY DIFFERENT PARTS OF ORIENTAL TOBACCO GROWN IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Some plants have the ability to absorb and accumulate heavy metals that do not have important biological functions in their diet and development. Compared with other crops tobacco is well known accumulator of heavy metals even when grown on uncontaminated soil.The main aim of this investigation is to study cadmium uptake by oriental tobacco and its correlation between soil properties as well as the content of this element in different parts of tobacco. Samples were taken from 150 farms that grow oriental tobacco from three well known tobacco regions in Macedonia (Pelagonia, Southeastern Region and Vardar Valley). All analyzed samples are from the areas which are typically agricultural and with low anthropogenic pressure. It was found that the oriental tobacco has a high tendency for accumulation and transfer of Cd in top parts of the plant.

Keywords: cadmium, oriental tobacco, uptake, soil

ИЗВЛЕКУВАЊЕ НА КАДМИУМОТ ОД РАЗЛИЧНИ ДЕЛОВИ НА ОРИЕНТАЛЕН ТУТУН ОДГЛЕДУВАН ВО РЕПУБЛИКА МАКЕДОНИЈА

Одредени видови растенија имаат способност да апсорбираат и акумулираат тешки метали кои немаат значајни биолошки функции во нивната исхрана и развој. Во споредба со други земјоделски култури тутунот е познат акумулатор на тешки метали дури и кога се одгледува на незагадени почви. Главната цел на ова истражување е да се проучи извлекувањето на кадмиум кое го врши ориенталниот тутун, неговата корелација со почвените параметри, како и содржината на овој елемент во различните органи на тутунското растение. Примероци се земени од 150 локалитети од три познати реони во Македонија каде се одгледува ориентален тутун (Пелагонија, Југоисточниот и Вардарскиот тутуно-производен реон). Сите анализирани примероци се во граници кои се типични за земјоделски површини и ниски антропогени влијанија. Утврдено е дека ориенталениот тутун има голема тенденција за акумулација и пренос на Cd во антенските делови.

Клучни зборови: кадмиум, ориентален тутун, извлекување, почва

INTRODUCTION

Compared with other heavy metals, cadmium is characterized by considerable mobility in the system soil - plant and accumulates in the antenna parts of the plant to a much greater extent than other heavy metals (Symeonides & McRae, 1977). Cadmium is one of the most toxic and most harmful elements. Phytotoxicity of Cd is well known due to the high rate of transfer and the light accumulation in plants (Alloway & Ayres, 1994). It is well known that tobacco (*Nicotina tabacum*) easily accumulates Pb and Cd (Gondola & Kadar, 1995). According to some data (Golia et al., 2009) Cd content in tobacco variety Berlej is from 0.045 to 3.6 mg/kg, and variety Virginia has Cd content of 0.01–3.4 mg/kg. According to the results of Tso (1990), cadmium content of tobacco leafs exceeds 11.6 mg/kg. These values are much higher than the obtained values for most plant cultures. Usually its content is never above 0.05 mg/kg. Addition of nitrogen fertilizers, especially those containing chlorides, increases the availability of cadmium for plants (Alkorta et al., 2004). The absorption of macro and trace elements from plants and their accumulation in vegetative and reproductive organs has a very specific character both in terms of the types of plants, and in terms of the particular element.

The main aim in this investigation is to study cadmium uptake by oriental tobacco from the Republic of Macedonia and its correlation between soil properties as well as the content of this element in different organs of tobacco.

MATERIALS AND METHODS

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

The following soil properties were determined: mechanical composition (Đamić, 1996), pH (ISO 10390:2005), total nitrogen (ISO 11261:1995), organic matter (OM) by the wet oxidation method (Đamić, 1996), and calcium carbonate equivalent determined volumetrically (ISO 10693). Electroconductivity was measured in a saturation extract, the content of the extractable phosphorus and potassium according to the ammonium lactate method, while the cation exchange capacity (CEC) was measured by the method of Sumner and Miller (1996).

For the determination of total content of cadmium in soil the samples were digested with mixture of 4 acids (HNO_3, HF, $HClO_4$ and HCl) according to ISO 14869-1.

Plant available fraction of Cd was determined by the extraction method using buffered solution of diethylentetraaminepentaacetic acid (DTPA) at pH 7.3 (ISO 14870). Extracts were collected after filtration through Whatman No. 42 filter paper.

Tobacco samples (root, stems, leaves, blossoms and seeds) were selected from plants at the same sites where soils were sampled. Tobacco leaves from three primings were collected with the total of 450 representative samples from 150 sampling locations. Tobacco samples were washed carefully to remove any adhering soil particles and rinsed

with redistilled water. The plant material was dried and homogenized to a constant weight after drying at 75 °C for 12 hours. For the analysis of the total content of Cd, plant samples (0.5000 g) were digested in Teflon vessels with HNO_3 and H_2O_2 using the microwave digestion system (Mars, CEM, USA). The plant samples were digested at 180°C. After cooling the digested samples were quantitatively transferred into 25 mL calibrated flasks (Bačeva et al., 2012).

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

RESULTS AND DISCUSSION

Descriptive statistics of analyzed soil properties are given in Table 1. Soil samples vary from silt loam (18.8 % clay) to silt clay loams (77.6 % clay). According to soil texture, average OM content is generally low to moderate (Table 1). Available phosphorus and potassium contents varied differently. Soils had an adequate CEC for agricultural production. All analyzed parameters pointed out the levels which are typical for agriculture except few sampled points from Pelagonia production region that had high content of available phosphorus.

Table 1. Basic soil properties, descriptive statistics of three tobacco production areas (Jordanoska et al., 2013)

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

OM - organic matter, TN – total nitrogen, CEC - cation exchange capacity, EC – Electroconductivity, s_a - standard deviation, Min - minimum, Max - maximum

The total Cd content in soil samples was under the detection limits (<1 mg/kg) and therefore is not presented. Average values from different tobacco growing areas are given in Table 2. As it can be seen from data presented in Table 2, the mobile cadmium content varies from 0.02 mg/kg to 0.3 mg/kg. The content of Cd in tobacco leaves varies from 0.1-1.9 mg/kg, with an average value of 0.48 mg/kg. The highest content of Cd have samples from Vardar Valley.

Table 2. The content of Cd in tobacco leaves and the content of DTPA extractable Cd from different tobacco growing areas (given in mg/kg)

	LOD	Pelagonia Region			South-eastern Region			Vardar Valley		
	mg/kg	X_g	s_a	Min-Max	X_g	s_a	Min-Max	X_g	s_a	Min-Max
Cd – leaves	0.005	0.35	0.17	0.09-0.89	0.45	0.25	0.18-1.27	0.8	0.5	0.3-1.9
Mobile Cd	0.005	0.02	0.01	0.01-0.07	0.06	0.04	0.05-0.2	0.2	0.1	0.1-0.3

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

Average content of Cd in tobacco leaves distributed by municipalities is given in Figure 1. As it can be seen, the samples from the municipality of Caška have the highest average values. All samples taken from this municipality have content in the range of 0.8-1.9 mg/kg Cd. This high content of Cd in the tobacco from this area is due to the polluted soil from the metallurgical activities of the former lead and zinc smelter located near the city of Veles (Stafilov et al., 2010). According to Tso (1990) tolerable content of Cd in tobacco is 3.0 mg/kg. No sampled tobacco leaves contains more than 2.0 mg/kg.

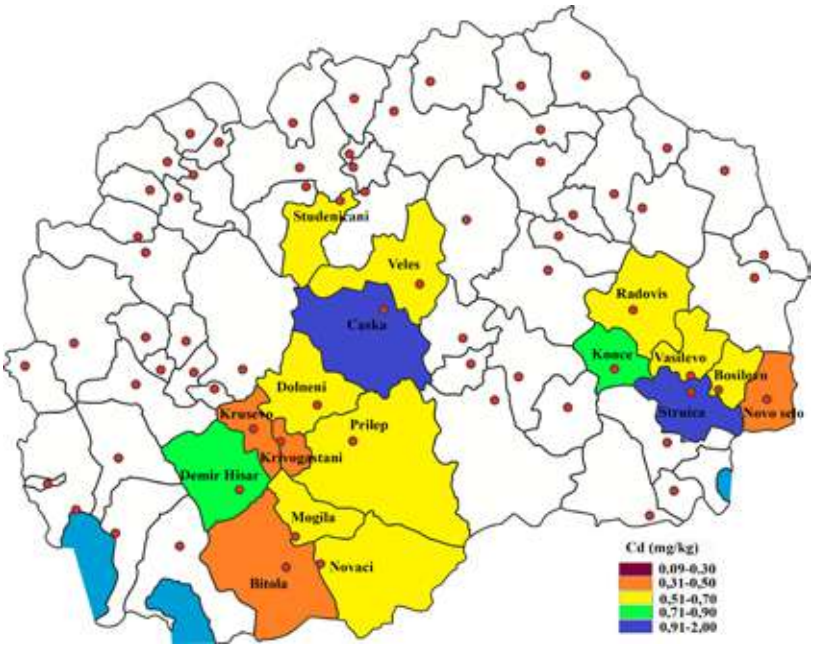

Figure 1. Ranges of content of Cd in tobacco leaves in samples from different studied municipalities in the Republic of Macedonia

The average content of Cd in different tobacco parts are given in Figure 2. The highest accumulation was recorded in leaves from the first harvesting zone (first priming). The content of Cd in the leaves is almost five times higher than the content in other vegetative organs. Compared to the other organs, stem and flower have almost equal distribution of 0.08 mg/kg. Although cadmium is a non-essential element for plants, it can easily be accumulated through the root system and by foliar feeding. Cadmium is easily transported from the roots to the aerial organs. Its transfer through

the whole plant may be limited because it can easily be kept in places where there are active components found in cells (Kabata-Pendias, 2011).

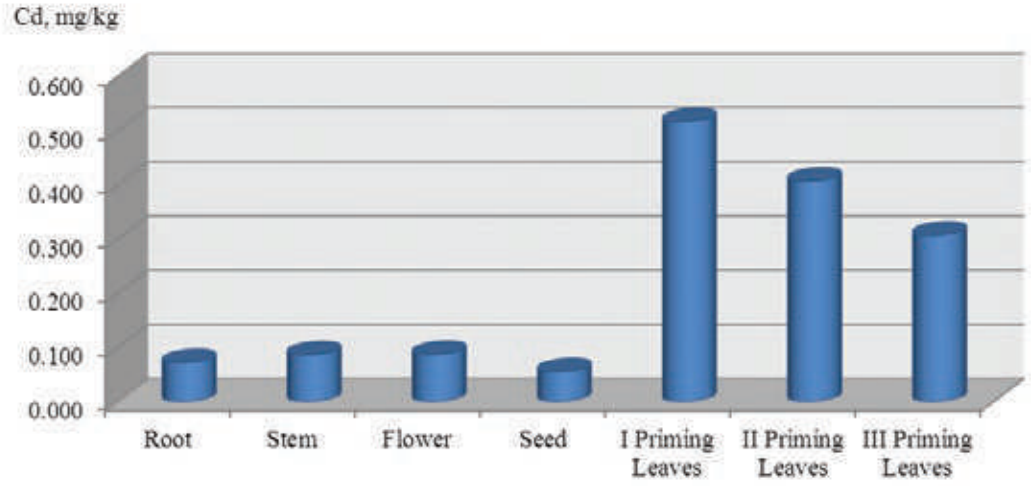

Figure 2. The average Cd content in different tobacco parts

Using determined contents in different parts (root, stem, flower, leaves and seed) of tobacco plant and total element content of corresponding soils the Biological Accumulation Factor (BAF) was calculated. This factor is defined as the ratio between total content of the elements in all parts of studied plant and in corresponding soil. The BAF for Cd is calculated taking the value which corresponds to the half of the limit of detection obtaining BAF of 2.58. Compared to other trace elements only Cu (BAF=3.2) has higher factor than Cd. This means that oriental tobacco accumulates high content of this observed element.

To assess the bioaccumulation ability of certain elements in the parts of the tobacco plant, despite biological accumulation factor, another parameter - Biological Transfer Factor (BTF) is also used. This factor represents the ratio of the content of the element in the aerial tissues of the plant and the root. This factor indicates the efficiency of transfer of the elements to the antenna parts of the plant. According to the values of this factor we found that Cd has the biggest BTF compared to all analyzed micro and macro elements. This means that the biggest transfer in the aerial organs of oriental tobacco has Cd. Also, this means that the oriental tobacco has a high tendency for accumulation of Cd in the antenna parts (BTF = 20.2), similar to that of Li (BTF = 20.0).

The correlation dependencies among the important parameters of soil and total quantities of Cd in tobacco leaves as well in the DTPA extract were processed using correlation analysis (Pearson correlation, two-tailed). The obtained results are given in Table 3 and 4. According to the data of the regression and correlation analysis, significant correlation was observed only among soil clay and Cd content in the leaves from the third priming (Table 3). Soil pH and Cd content of all vegetative organs of oriental tobacco have no significant correlations. This is not in accordance to literature data that study mainly the relation of pH and element content of the leaves (Gondola & Kadar, 1993; Adamu, 1989). There are no significant correlations between the organic matter and Cd in all parts of the tobacco plant, as well as its available fraction. Significant correlations were determined with some moving elements from the soil and Cd in leaves from the first and second harvest belt and are given in Table 3.

Flower and root, and leaves from second and third priming have significant correlation at level 0.01 of significance (Table 4). Cadmium content in flower has linear dependence with the amount of Cd in the root. From the determined coefficients we can conclude that around 80% of the Cd in the flower depends of its content in the root.

Table 3. Significant correlation coefficients of Cd content in tobacco leaves and soil properties

Parameter	Leaves			Root	Stem	Flower	Seed
	I priming	II priming	III priming				
Cd-DTPA	**0.309**	**0.265**	**0.462**	0.088	-0.050	0.084	0.051
Clay	**0.311**	0.081	0.190	1.000	0.000	0.081	**0.274**
P_2O_5	**-0.317**	-0.108	-0.118	0.035	-0.158	0.037	0.097
K_2O	**0.267**	0.015	0.054	-0.211	0.125	-0.207	-0.103

Bold numbers present significant correlation at 0.01 levels

Some weak correlations are also found between the total content of Al, Na, Ni and Sr in the soil and the content of Cd in the second harvest belt. Negative significant correlation coefficients are determined and between Cd content in the leaves of the first priming and total content of Ba, Ca, Na and Sr of the soil samples.

Table 4. Significant correlation coefficients of Cd content in tobacco parts and micro and macro elements in the soil samples

Parameter	Leaves			Root	Stem	Flower	Seed
	I priming	II priming	III priming				
Cd II priming	**0,469**	**0,561**	1,000	0,190	-0,022	0,185	-0,042
Cd stem	-0,162	0,081	0,190	1,000	0,000	**0,991**	0,034
Al	**-0,419**	-0,184	-0,275	-0,009	0,035	-0,012	-0,026
Ba	**-0,287**	-0,055	-0,129	-0,063	-0,046	-0,060	0,112
Ca	**-0,282**	0,002	-0,222	0,110	-0,112	0,117	0,040
Na	**-0,349**	**-0,271**	**-0,274**	0,085	0,137	0,080	-0,073
Ni	0,148	**0,314**	**0,258**	0,019	-0,153	0,027	0,200
P	**-0,317**	-0,108	-0,118	0,035	-0,158	0,037	0,097
Sr	**-0,326**	-0,172	**-0,232**	0,118	-0,003	0,127	0,067

Bold numbers present significant correlation at 0.01 levels

CONCLUSIONS

According to the determined soil properties, all analyzed soil samples are in good condition for the production of the high-quality oriental tobacco. Cadmium content of soil samples war under the detection limits (<1 mg/kg), so all analyzed samples pointed out levels which are typical of agricultural and low anthropogenic pressure areas. All plant samples had Cd content under limits considered critical. According to distribution of this element in tobacco vegetative organs, the highest accumulation was recorded in

leaves from the first harvesting zone (first priming). According to the value for BAF it can be concluded that oriental tobacco plant accumulates high content of P, K, Cu and Cd. From all analyzed samples and calculated BTF it can be concluded that oriental tobacco has a high tendency for transfer and accumulation of Cd in antenna parts. According to data of the regression and correlation analysis, significant correlation was observed only among the content of soil clay and the content of Cd in the leaves from the third priming. Regression dependencies of some significance are observed between the mobile cadmium in the soil and most of the soil parameters except with the available forms of phosphorus and potassium.

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Original scientific paper

MORPHOLOGICAL CHANGES IN *NICOTIANA TABACUM* TYPE PRILEP, VARIETY P 12-2/1, INFECTED WITH FRESH *AGROBACTERIUM RHIZOGENES* A4 CULTURE IN *IN VITRO* CONDITIONS

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ABSTRACT

The aim of this paper is to show that infection of tobacco (*Nicotiana tabacum*) with a fresh culture of *Agrobacterium rhizogenes* A4, under *in vitro* conditions, lead to genetic transformation. The interaction between the t-DNA sequence of Ri plasmid of *Agrobacterium rhizogenes* A4 and recipient cells of *Nicotiana tabacum* seed type Prilep variety P 12-2/1, induces the appearance of "Hairy Roots-HR" and positive morphological modifications are manifested by increased surface area, number of leaves and lateral roots, and increased length of root and offspring. The recent research assume that these transgenic plants with altered phenotype can be very usefull in future in industry for production of biofuel.

Keywords: *Agrobacterium rhizogenes*, tobacco (*Nicotiana tabacum L.*), morphological modifications, "Hairy Roots-HR"

МОРФОЛОШКИ ПРОМЕНИ КАЈ *NICOTIANA TABACUM* ТИП ПРИЛЕП, СОРТА П 12-2/1 ПРИ ИНФЕКЦИЈА СО СВЕЖА КУЛТУРА ОД *AGROBACTERIUM RHIZOGENES* А4 ВО *IN VITRO* УСЛОВИ

АПСТРАКТ

Овој труд имаше за цел да покаже дека при инфекција на тутун (*Nicotiana tabacum*) во *in vitro* услови со свежа култура од *Agrobacterium rhizogenes* А4, настанува генетска трансформација. Интеракцијата помеѓу t-DNA секвенцата од Ri плазмидот на *Agrobacterium rhizogenes* и клетката реципиент на семето од *Nicotiana tabacum* тип Прилеп сорта П 12-2/1 индуцира појава на "Hairy Roots-HR"-"влакнести корени" и позитивни морфолошки модификации кои се манифестираат со зголемена површина и број на листови и странични корени, како и зголемена должина на изданок и корен. Најновите истражувања претпоставуваат дека овие трансгенични растенија со променет фенотип во иднина можат да бидат од голема корист во индустријата за производство на биогориво.

Клучни зборови: *Agrobacterium rhizogenes*, тутун (*Nicotiana tabacum L.*), морфолошки модификации, "Hairy Roots-HR"-"влакнести корени".

INTRODUCTION

In the eighties of the last century first attempts were made to use *Agrobacterium rhizogenes* A4 as a vector for introduction of foreign genes of interest in the plant genome (Horch et al., 1985). Cultures of hairy roots (HR) are obtained by integration of the t-DNA fragment of Ri plasmid of *Agrobacterium rhizogenes* into the plant cell genome. Then, the place of infection of the plant tissue induced development of transgenic hairy roots (Chilton et al., 1982). During the transformation process with *Agrobacterium rhizogenes* one or two t-DNA sequences were transferred into the plant genome (Jouanin, 1984). With integration of T_L t-DNA sequence in the genome of the plant cell allows the expression of *rol* genes that have essential role in induction, development of the phenotype and HR (Binns and Costantino, 1998). On the other hand, the integration of T_R t-DNA sequence is enabling expression of genes responsible for the biosynthesis of auxin in the plant cell genome, with the increased content of auxin, and the need for exogenous phytohormones for development and growth transformed HR decreases. Also with transmission of T_R t-DNA segment of the Ri plasmid of *Agrobacterium rhizogenes* into the plant cell genome occurs expression of genes for the biosynthesis of specific opini (Rhodes et al., 1990). Cultures of the HR have the capability of spontaneous regeneration in transgenic plants that are characterized by expression of active t-DNA genes (Tepfer, 1984). The regenerated transgenic plants have modified morphological characteristics in comparison to non-transformed plants, a newly phenotype known as "hairy root phenotype" or "T - phenotype". This phenotype is characterized by curved leaves, reduced plate surface, the emergence of asymmetrical and colorful leaves, highly developed root system, reduced apical dominance, reduced length of internodes, etc (Tepfer, 1984; Hamamoto et al., 1990). Growing of crops of genetically transformed roots were characterized by two physiological parameters (Kuzovkina and Schneider, 2006): linear elongation of the root tip and exponentially formation of lateral roots. The curve growth is characterized by a short resting phase (lag phase), the following exponential increase of the biomass of the HR culture. The cell cycle of HR cultures is 2-7 days (Wilson et al., 1987). The exponential growth phase of HR ends by forming a dense combination of intertwined fibrous roots. During the growing HR limiting the necessary growth factors that restrict the space and reduced supply of oxygen and nutrients from the nutrient medium. Also cultures of HR have the ability to form idioblast. Idioblast does not contain chloroplasts and represent cells with thickened cell walls. The presence of lipophilic vesicles and vacuoles in idioblast are of great importance in the accumulation of secondary metabolites. The process of growing the HR is incomparably more intense than untransformed roots. HR have the ability to form new root meristems high degree of lateral branching (Oksman-Caldentey and Hilyunen, 1996).

MATERIAL AND METHODS

Determination of growth optimal conditions for *Agrobacterium rhizogenes* A4

A. rhizogenes A4 is part of Collection of Microorganisms, from Department of Microbiology and Microbial Biotechnology, Faculty of Natural Sciences and Mathematics in Skopje. The initial culture of *A. rhizogenes* A4 was inoculated on three media: Mannitol Broth, Nutrient Broth and Muller Hinton Broth, with incubation time

of 48 hours and a temperature of 28-30°C. Before and after the incubation period it was measured turbidity of bacteria in order to determine the most suitable medium for incubation. Then, in order to determine the optimum pH value of the medium, the initial culture was incubated in appropriate medium in five different pH values: 6, 6.5, 7, 7.5 and 8, during the incubation of 72 hours and a temperature of 28-30°C. After the incubation period, it was measured turbidity of bacteria in order to determine the optimum pH. Finally, *A. rhizogenes* A4 was incubated in an appropriate medium, appropriate optimal pH, at three different temperatures: 23/25◦C, 28◦C and 37◦C for 48 hours. Turbidity measurements before and after incubation were varied, whereby to determine the most appropriate temperature for incubation of bacteria.

Preparation of fresh bacterial suspension of *Agrobacterium rhizogenes A4*

Bacterial suspension of *A rhizogenes A4* was prepared in Muller Hinton broth with a pH adjusted to 6.5 with 0.1N NaOH. 3 ml of bacteria were incubated in 300 ml broth, on 28°C, during 48 h, on shaker. After two days it was obtained fresh culture for infection of seed tobacco *(Nicotiana tabacum)* type Prilep, variety P 12-2 /1.

Infection and micropropagation of *Nicotiana tabacum* type Prilep, variety P12-2 /1

One part of tobacco seed was infected with *A. rhizogenes* A4, and one part was uninfected, and utilized as control for the experiment. Infection was carried out for a period of 30 minutes. After that, seeds were placed in double-distilled water for imbibitions, than it was surface sterilized in 70% ethanol (1 minute) and 1% Izosan-G (10 minutes). After washing several times in distilled deionized water it was planted in small jars (40 ml) with ½ MS basal medium (Murashige and Skoog, 1962, Gamborg, 1968). The basal medium contains 30 g·L^{-1} sucrose, 7 g·L^{-1} agar, MS mineral solution (Murashige и Skoog, 1962, Gamborg, 1968) vitamin B5 solution, organic (Table 1) and inorganic components (Table 2). After 14 days of micropropagation, from the sterile sprouts seeds we were isolated apical buds in length of 1-3 mm, as initial explants. These explants were placed on MS medium.

Table 1. Organic components of the MS basal medium.

Organic components	Concentration (mg·L^{-1})
Thiamine (vitamin B1)	0,1
Pyridoxine (vitamin B6)	1,0
Nicotinic acid	0,1
Casein hirolizat	200,0
myo-inositol	100,0

Table 2. Inorganic components in the MS basal medium.

Inorganic components	Concentration (mg·L^{-1})
NH_4NO_3	1650
KNO_3	1900
$CaCl_2$x $2H_2O$	441

$MgSO_4$x $7H_2O$	370
KH_2PO4	170
H_3BO_3	6,2
$MnSO_4$ x H_2O	16,9
$ZnSO_4$ x $7H_2O$	8,6
KJ	0,83
Na_2MoO_4 x $2H_2O$	0,25
$CuSO_4$ x $5H_2O$	0,25
$CoCl_2$ x $6H_2O$	0,25
Na_2EDTA	37,26
$FeSO_4$ x $7H_2O$	27,8

The cultures were kept in a climate chamber under controlled aseptic conditions. The seeds sprout successfully for a period of 14 days under *in vitro* conditions. After the 15th day it was measured the length of the sprout and the root, number of lateral roots and leaves on sprout, the length and width of each leaf, and the values were used to calculate the surface of the leaves. Eventually the plant material of experimental and control groups was lyophilized to absolutely dry condition.

RESULTS AND DISCUSSION

Determination of growth optimal conditions for *Agrobacterium rhizogenes* A4

To select the most suitable medium for incubation of *Agrobacterium rhizogenes* A4, it was tested three different media: Mannitol Broth, Nutrient Broth and Muller Hinton Broth, with the incubation time of 48 hours and a temperature of 28-30◦C. Growth was monitored by turbidity change before and after the incubation period because the turbidity is proportional to the number of bacteria in the medium. Table 3 shows that the most appropriate medium is Muller Hinton broth (1 FAU before/ 280 FAU after).

Table 3. Media for culturing bacteria.

medium	**turbidity before incubation (FAU)**	**turbidity after incubation (FAU)**
Nutrient Broth	0	156
Mannitol Broth	0	267
Muller Hinton Broth	1	280

To select the most suitable pH value for the incubation of *Agrobacterium rhizogenes* A4, bacteria was inoculated on MH medium with five different pH values (6, 6.5, 7, 7.5 and 8). According to turbidity, the most suitable medium was the one with a pH 6.5 (Table 4).

Table 4. pH values for culturing bacteria.

pH	pH turbidity before incubation (FAU)	turbidity after of incubation (FAU)
6	4	372
6,5	14	555
7	3	418
7,5	0	235
8	4	529

To choose the optimal temperature for cultivation of *A. rhizogenes* A4, bacteria were exposed to three different temperatures, 23/25°C, 28°C and 37°C for a period of 48 hours. After incubation it was obtained the results shown on Table 5.

Table 5. Temperatures for culturing bacteria.

Temperature	turbidity before incubation (FAU)	turbidity after incubation (FAU)
23/25°C	12	233
28°C	4	371
37°C	1	179

Morphological changes in *Nicotiana tabacum* type Prilep, variety p 12-2/1 infected with fresh *Agrobacterium rhizogenes* A4 culture in *in vitro* conditions

Agrobacterium rhizogenes infects higher plants to produce so-called ''hairy roots'' with altered phenotype from the wound sites. Transformed root cultures possess some properties that make them very attractive in comparison with untransformed root cultures and undifferentiated cell suspension and tissue cultures (Kuzovkina and Schneider, 2006).

In our study, one half of tobacco seeds was infected with *A. rhizogenes* A4 and one half which was not infected with bacteria was used as a control for the experiment. Sown seeds were left to germinate for a period of 14 days. After the germination of the seed and growing of tobacco plant, it was analysed morphological features of infected tobacco and control. In the experiment we included morphological characteristics as the length of the sprout and the root, number of lateral roots and leaves on sprout, the length and width of each leaf. After lyophilization, dry weight of control was 0.019g, while the treated material 0.126g.

Hairy roots are fast growing and laterally highly branched, and are able to grow in hormone-free medium. Moreover, these organs are not susceptible to geotropism anymore (Sevon and Oksman-Caldentey, 2002).

Table 6. Length of the sprout and the root, number of leaves per sprout and number of lateral roots in five control samples.

	Length of the sprout (mm)	Root length (mm)	No. of leaves per sprout	No. of lateral roots
Control 1	19	38	7	11

Control 2	18	22	7	7
Control 3	14	19	6	7
Control 4	12	31	6	6
Control 5	9	7	7	3
Average	14,4	23,4	6	7
SD	4,159327	11,84483	0,547723	2,863564
Max	19	38	7	11
Min	9	7	6	3

* Control 1-5: random samples were taken from five uninfected material; Average: arithmetic average of all obtained values for each parameter; SD: standard deviation; Max: maximum value; Min: minimum value

Table 7. Length, width and area of the leaves in five control samples.

	Length of leaf (mm)	Width of leaf (mm)	**Area of leaf (mm^2)**
C1	11	9	32
	7	5	35
	11	9	33
	6	7	42
	7	4	28
	6	3	34
	7	3	77
	11	7	74
	13	6	82
A.v, C1	8,8	5,9	48,6
C2	6	5	30
	6	5	30
	3	2	6
	4	3	12
	4	3	12
	4	2	8
	4	3	12
A.v, C2	4,4	3,2	15,7
C3	5	3	15
	5	2	10
	4	2	8
	4	3	12
	7	6	42
	7	6	42

	4	3	12
A.v, C3	5,2	3,6	20,2
C4	5	3	15
	5	3	15
	6	4	24
	8	5	40
	7	4	28
	4	3	12
A.v, C4	5,8	3,7	22,4
C5	3	2	6
	4	3	12
	2	2	4
	4	1	4
	2	2	4
	6	5	30
	2	2	4
A.v, C5	3,3	2,4	9,1
Average value	5,5	3,7	12,9

* A.v, C1-C5. mean value of control leaves ** average value of Av, C1-C5

The transformed roots can be excised to establish axenic root cultures and indefinitely propagated in growth regulator free medium. The root exhibit fast, plagiotropic growth characterized by profuse lateral branching and rapid root tip elongation, just like in several earlier papers (Tepfer and Tempé, 1981; Chilton et al., 1982; Tepfer, 1984).

Table 8. Length of the sprout and the root, number of leaves per sprout and number of lateral roots in five samples treated with *A. rhizogenes* A4

	Length of the sprout (mm)	Root length (mm)	No. of leaves per sprout	No. of lateral roots
T1	27	35	7	9
T2	56	45	9	13
T3	65	32	5	11
T4	46	52	5	16
T5	42	49	7	9
Average	47,2	42,6	7	12
SD	14,4118007	8,73498712	1,67332005	2,96647939
Max	65	52	9	16
Min	27	32	5	9

*T1-5: random samples were taken from five infected material; Average: arithmetic average of all obtained values for each parameter; SD: standard deviation; Max: maximum value; Min: minimum value

Table 9. Length, width and area of the leaves in five samples treated with *Agrobacterium rhizogenes* A4.

	Length of leaf (mm)	Width of leaf (mm)	Area of leaf (mm^2)
T1	4	3	12
	4	3	12
	16	11	176
	11	7	77
	7	4	28
	7	3	21
	8	6	48
A.v,T1	8,2	5,3	54
T2	11	9	99
	7	5	35
	11	9	99
	11	7	42
	7	4	28
	18	13	234
	19	13	247
	22	17	374
	21	12	252
A.v, T2	13,6	9,9	156,7
T3	15	11	165
	9	8	72
	7	9	63
	10	11	110
	8	9	72
A.v,T3	9,8	5,3	96,4
T4	13	8	104
	11	8	88
	12	8	96
	9	7	63
	6	5	30
A.v,T4	10,2	7,2	76,2
T5	9	6	54
	8	7	56
	9	8	72
	9	6	54
	8	5	40
	8	6	48
	5	5	25
A.v, T5	8	6,2	49,8
Average value	9,9	6,7	86,5

* A.v, T1-T5. mean value of treated leaves ** average value of average value, T1-T5.

Morphological modifications in comparison to the length of the sprout, the length of the root, the number of lateral roots, the number of leaves per sprout (Table 8) and the surface area of the leaves (Table 9) were positive and they are manifested in in-

creased growth. According to these observations, treated sprouts and roots of infected samples had a length which is 3 times higher than in control samples (Table 6 and Table 7).

The experimental data in our study showed similar behavior as results published for *V. vinifera* cultivars (Martins et al., 2003; Peros et al., 1998).

Fig1. Germinated non-infected seeds of tobacco (*Nicotiana tabacum*), type Prilep, variety P12-2 /1.

Fig2. Germinated infected seeds of tobacco (*Nicotiana tabacum*), type Prilep, variety P12-2 /1.

The number of leaves and number of lateral roots is increased by about 2 times. There was also positive morphological modifications in length, width and surface of leaves. A. rhizogenes, like A. tumefaciens, invokes morphological changes in infected plant tissues and allows growth of transformed tissues in vitro in the absence of exogenous plant growth regulators. However, rather than undifferentiated tumors, highly branched, ageotropic roots emerge from sites of A. rhizogenes infection. Transformed roots can be regenerated into plants which, in many species, have a characteristic morphology (called the “hairy root” phenotype) that includes stunted growth, shortened internodes, reduced apical dominance, severely wrinkled leaves, atypical flower morphology and reduced fertility (Tepfer, 1984).

In infected tobacco samples appeared hairy root syndrome (Hairy root) as a result of the transfer of genetic material (t-DNA) of bacteria cells in the tobacco seed (Figure2), whereas in the control samples this phenomenon was absent (Figure1).

The promising developments and applications of hairy root cultures indicate that in the near future these cultures will provide researchers with powerful tools for further biotechnological research.

CONCLUSIONS

The results of investigations lead to the following conclusions:

- Optimal conditions for growth of *Agrobacterium rhizogenes* A4 are: Muller Hinton Broth – as a medium; 6,5 - pH value; 28°C - temperature.
- In *in vitro* conditions we successfully obtained multiplied sprouts from isolated buds from tobacco (*Nicotiana tabacum*), type Prilep, variety P12-2 /1 on MS mineral medium.

As a result of the contact between the t-DNA sequences of the Ri plasmid of the bacterium and the recipient cell from tobacco seeds, the treated material was characterized by the appearance of hairy roots, while in the control material this phenomenon is absent.

The control material was characterized normal phenotype, and the treated material was altered morphology. Morphological modifications of the treated material were positive, and values were increased two to three times compared to the control.

Overall, hairy roots seem to be becoming useful in the production of proteins, in environmental biotechnology for phytoremediation of pollutants from waste water, and for the regeneration of genetically altered plants

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Original scientific paper

PHYSICAL, TECHNOLOGICAL, CHEMICAL AND SMOKING PROPERTIES OF NEW LINES AND HYBRIDS BURLEY TOBACCO

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ABSTRACT

The aim of the research is a comparative analysis of the physical, technological, chemical and the smoking properties of our new lines Burley tobacco. Investigated are eight new lines of Burley tobacco varieties created at Tobacco and Tobacco Products Institute -TTPI in Plovdiv. Results of the research show that the best performance values differs Line 1458, followed by Line 1354 and Hybrid1470. In the smoking properties as near taste- aromatic complex is observed with Line 1458, Line 1472, Line 1416, followed Hybrid 1470. With the best utility value is Line 1458, followed by Hybrid 1470. There is connection between the smoking properties and chemical composition of investigated tobacco lines. Not found such as between physical and technological indicators and the smoking properties. Based on the results it can be concluded that it is possible in Republic of Bulgaria to create new, promising and competitive lines Burley tobacco comparable with imported ones.

Keywords: tobacco, Burley , physical, technological, chemical indicators, smoking properties

ФИЗИЧКИ, ТЕХНОЛОШКИ, ХЕМИСКИ И ПУШАЧКИ СВОЈСТВА НА НОВИТЕ ЛИНИИ И ХИБРИДИ ТУТУН ОД ТИПОТ БЕРЛЕЈ

Целта на ова истражување е компаративна анализа на физичките, технолошките, хемиските и пушачките својства на нашите нови линии тутун од типот Берлеј. Испитувани се осум нови линии тутун од сортите од типот Берлеј создадени во Институтот за тутун и тутунски производи од Пловдив. Резултатите од истражувањето покажуваат дека најдобри карактеристики покажуваат линијата 1458, проследено со линијата 1354 и хибридот 1470. Според пушачките својства со најароматични комплекс се одликуваат линијата 1458, линијата 1472, линијата 1416, проследено со хибридот 1470. Со најдобра употребна вредност е линијата 1458, проследено со хибридот 1470. Постои поврзаност помеѓу пушачките својства и хемискиот состав на испитуваните линии тутун. Не е пронајдена поврзаност меѓу физичките и технолошките индикатори и пушачките својства. Врз основа на резултатите може да се заклучи дека во Бугарија е можно да се создадат нови, ветувачки и перспективни линии тутун од типот Берлеј кои ќе бидат конкурентни на увезени тутуни.

Клучни зборови: тутун, Берлеј, физички, технолошки, хемиски индикатори, пушачки својства

INTRODUCTION

In recent years, firmly holds the trend of increasing consumption of American blend cigarettes, both globally and in our country (Bozukov, 2012). Burley tobacco is a major component of this type of cigarettes. In Bulgaria the production of Burley tobacco is a relatively „new“. Due to the increased share of production increases more attracted

to utility qualities (Resnik, 1974; Lewyn, 1979; Tso, 1988; Spears and Tones, 1981).
In a number of studies listed evidence of impaired typicality of the produced in our Burley tobacco (Tomov and Minev, 1996). With its high nicotine content and specific smoking and properties imported Burley tobaccos superior Bulgarian samples. Local tobacco could hardly be equivalent substitutes imported in cigarette blends. (Popova et al., 2003; Kirkova, 2005; Kirkova and Taskova, 2005; Kirkova et al., 2006; Nicolova et al., 2006; Popova et al., 2006).
The aim of our research is a comparative analysis of the physical, technological indicators, and chemical properties of smoking and our new lines Burley tobacco.

MATERIAL AND METHODS

The experimental work is carried out in Tobacco and Tobacco Products Institute –TTPI Markovo-Plovdiv. Studies covering the period from 2008 to 2010. Studied are eight lines Burley tobacco created in TTPI. The selection of lines is based on economic performance yield and percentage of first class. For all variants is applied uniform technology of cultivation. Harvesting of tobacco is performed whole plants and air dried plants.
Of each line is allocated a representative sample. Analysis and data processing are used standardized methods /ISO 15152, ISO 15154, ISO 2817, БДС 15836, БДС 16255, БДС 8389 and e.t..

RESULTS AND DISCUSSION

1. Physical and technological indicators

Regarding the number of leaves per kilogram of tobacco, the result meets the standards of Burley tobacco, the most favorable is the index in Hybrid 1470. The highest conditional cigarette yield differs Line 1458 followed by Line 1354. These options give 1800 tobacco cigarettes per kilogram . Overall, the results are within the limits of Burley tobacco and are visualized in Figure № 1.

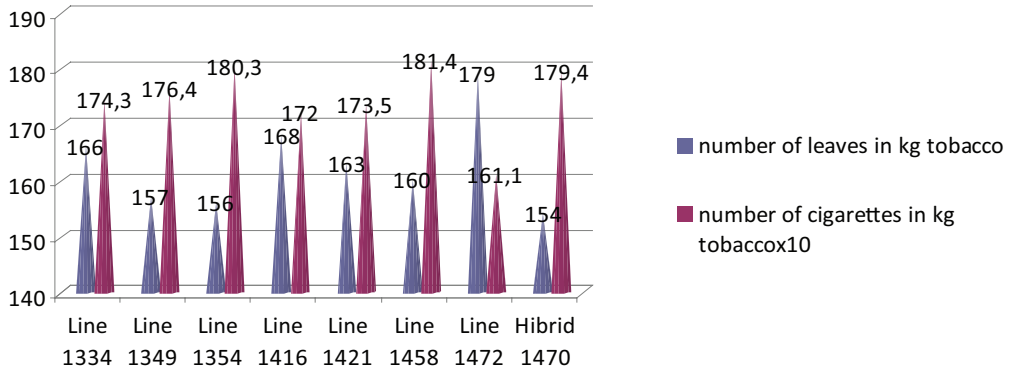

Figure 1. Number of leaves in kilogram tobacco and conditional yield

The data obtained for the width of the leaves are also within the requirements of the variety group in all variant. In this case the most favorable indicators are Line 1354, Line 1416, Line Hybrid 1458 and Hybrid 1470. With regard to the length of the leaves, the indicators in all variants are in norms for Burley tobacco. The highest values of the studies index are Line 1354, Line 1458 and Hybrid 1470. The percent of main steams is in standards of Burley tobacco in all researches tobacco samples. In the most favorable values is Hybrid 1470 - figure № 2.

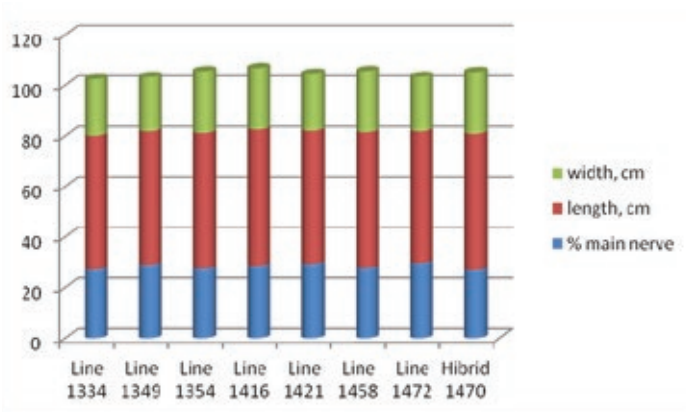

Figure 2. Characteristics of tobacco leaves

The results indicate that the largest size of the leaves, which is desirable in Burley tobacco are Hybrid 1470, Line 1354 and Line 1458. The proportion lengths to width in nearly all the variant is favorable, and is in the norms are varietal group.

The indicator weigh unit leaf area in g/sm2², in all variants is in the range of the standard on Burley tobacco. In the most favorable indicators are Line 1458 and Line 1354. At all variants observed values in the standards of Burley tobacco on the density of the leaves. The most favorable performance have Line 1354 and Line 1458. The results of the filling power of correspond to the results obtained in terms of the density of the leaves. In all variants they are within the standard of Burley tobacco. Best data differ Line 1354 and Line 1458. The results are displayed in Figure 3.

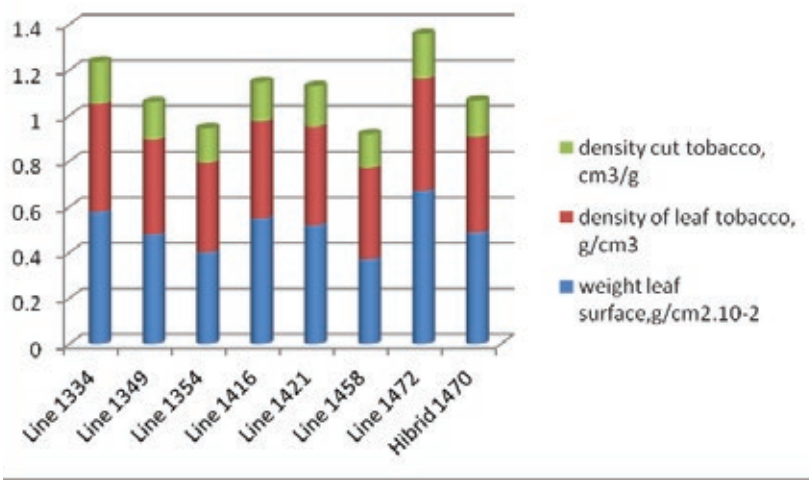

Figure 3. Technological characteristics

The indicator free burning in minutes is most favorable values in Line 1458 and Hybrid 1470. In Line 1472 the result is unsatisfactory for Burley tobacco - Figure 4.

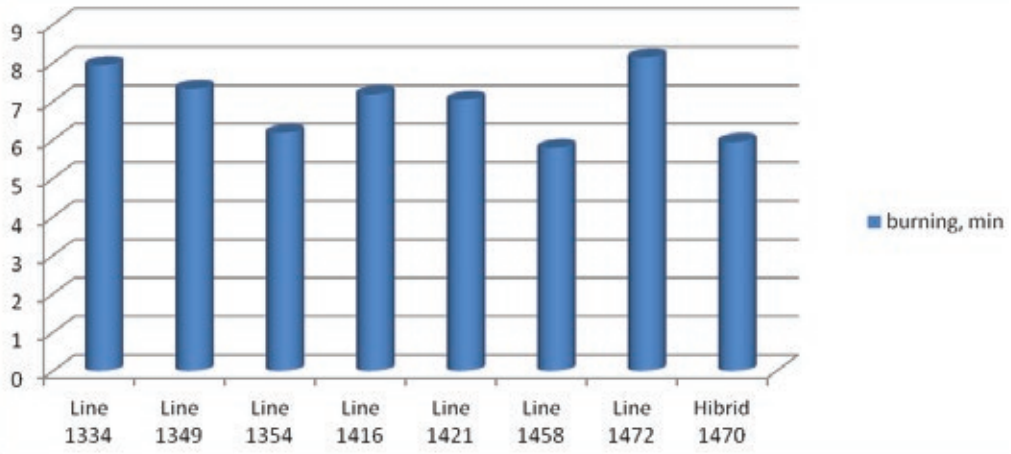

Figure 4. Free burning in minutes

From the results it is clear that all options are explored with performance corresponding to the accepted standard in Burley tobacco. Most of them are responsible for average quality.

In the most favorable data regarding the physical and technological parameters differs Line 1458. With very good results stand also Line 1354 and Hybrid 1470.

2. Chemical indicators

As regards the content of nicotine with the best results is Line 1458. Good results and Line 1472, Line 1354 and Hybrid 1470, which revealed more than 3% of the same content. Other options show satisfactory values. Values for sugars are lower in Line 1458. The results of the other options are satisfactory and in Line 1421 the sugar content is high standards in Burley tobacco. The content of total nitrogen for all variants is generally accepted standards for the type of tobacco. The highest values realized Line 1458. The ash content in all variant is optimal values for Burley tobacco. The lowest content of ammonia and chlorine, which are highly undesirable in Burley tobacco, is Line 1458. For Line 1472 values are good. All variant give protein content in the standards of Burley tobacco. The results of the chemical composition of the tested lines are presented in Figure 5.

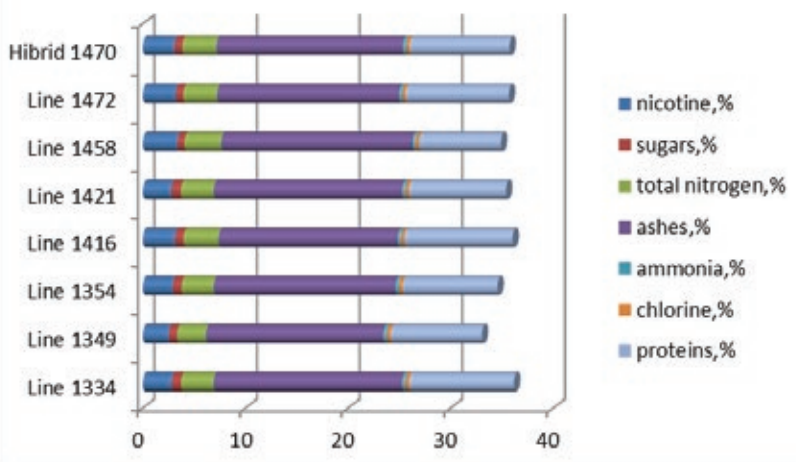

Figure 5. Chemical composition of the investigated variants Burley tobacco (%)

It is well known that the smoking properties of tobacco is determined by complex chemical indicators. In the case stands out Line 1458, followed by Line 1472. Hybrid 1470 also reveals a balanced chemical composition. The chemical composition of the distinguished options is displayed in Figure 6.

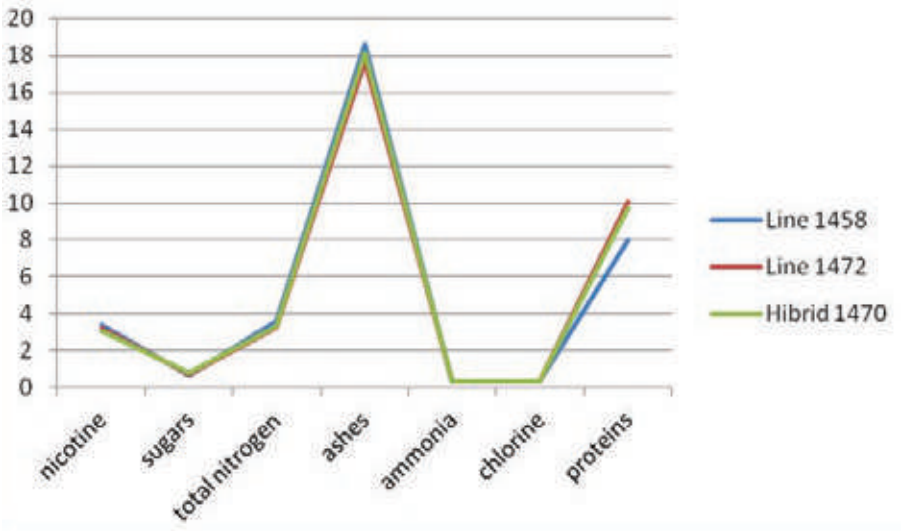

Figure 6. Chemical composition of the standout studied tobacco

3. Smoking properties

Tasting evaluation is performed on mono cigarettes the investigated lines under equal conditions, without a filter segment. Results in basic perceptions aroma, flavor and physiological force are displayed in Figure 7 and 8. With good smoking and properties distinguish Line 1458, Line 1472, Line 1416, followed by Hybrid 1470.

Can be reported link between the smoking properties and chemical composition of tobacco researches. Not found correlation between physical and technological indicators and the smoking properties.

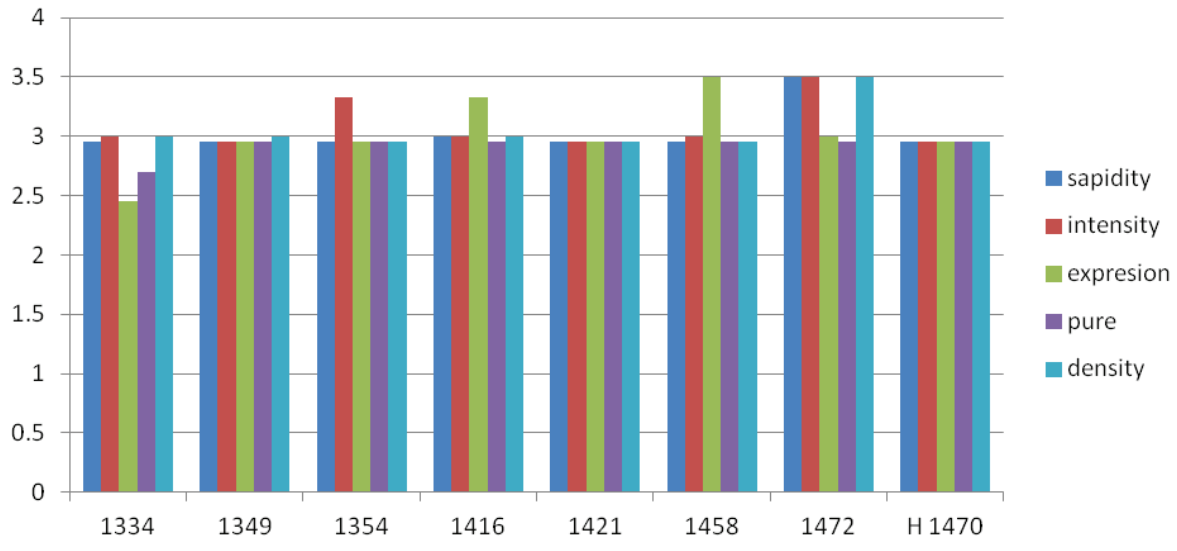

Figure 7. Aroma

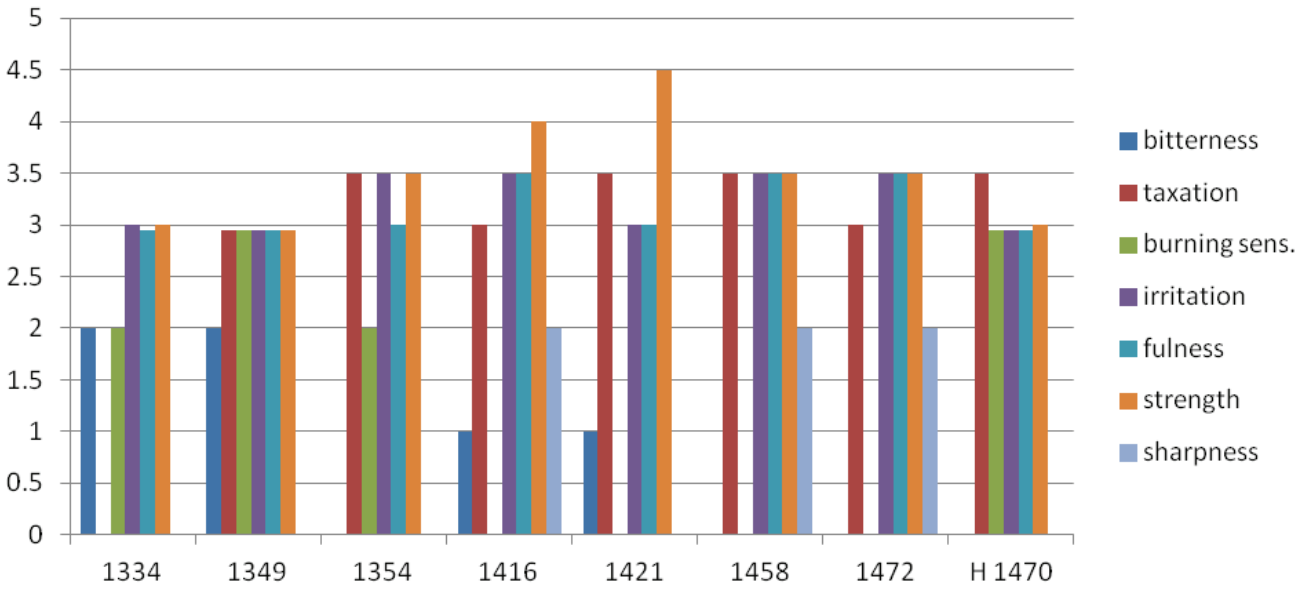

Figure 8. Taste and physiological strength

rom the results it can be generalized conclusion that the desired characteristics and properties of tobacco Burley type, in most approaches Line 1458. Hybrid 1470 also reveals good comprehensive indicators. These two options have markedly high utility value. Line 1458 may be used as genetic material for improvement of the production characteristics of the indigenous varieties of Burley tobacco.

The other options have results largely which satisfy the conditions of the varietal group. They are classified with moderate to good level of quality.

The newly created selection materials Burley tobacco favorable physical-technological, chemical indicators and smoking properties are success for selection work in Bulgaria.

CONCLUSION

With best values in physical and technological parameters differs Line 1458, followed by Line 1354 and Hybrid 1470. With best formed chemical complex also features Line 1458, followed by Line 1472. For Hybrid 1470 it can be argued that has a balanced chemical composition.

In the smoking properties are distinguished Line 1458, Line 1472 and Line 1416, followed by 1470 Hybrid.

Observe connection between the smoking properties and chemical composition of tobacco researches. Not found such as between physical and technological and the smoking properties.

It can be concluded that it is possible in Bulgaria to create new, promising and competitive lines Burley tobacco relentless imported ones.

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Original scientific paper

SOME ASPECTS OF SUSTAINABLE DEVELOPMENT IN AGRICULTURE WITH REFERENCE TO TOBACCO PRODUCTION AND ENVIRONMENTAL PROTECTION

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ABSTRACT

Sustainable development of agriculture cannot be imagined without economic capacity, environmental protection, social responsibility and ethical focusation.Economically capable agriculture implies application of new methods of economy, which also takes into account the aspect of environmental protection.Environmentally protected agriculture prefers rational use of natural resources, application of optimum-minimum amounts of fertilizers and agrochemical products for protection and special care for the environment, with genetic sustainability of plants and animals. Social responsibility is focused on engagement of agricultural workers, their motivation and dedication to ecologically clean agricultural production. Eco-ethics is a principle focused on many areas, including the mode of soil cultivation, crop breeding, treatment of plant and animal populations and care for the environment.Tobacco production is agricultural activity that engages about 10% of the population in the Republic of Macedonia. Its development must correspond to the intentions of the European Union and global world policy defined by various government and non-government organizations, which in practice means production of high quality tobacco, use of tobacco as a bioenergy crop, gradual decrease in tobacco consumption and its substitution with other crops, with continuous improvement in environmental protection.This paper determines the courses of the sustainable development of agricultural production with special reference to the dynamics of tobacco production development.

Keywords: sustainable development,tobacco,agriculture,areas,production

НЕКОИ АСПЕКТИ НА ОДРЖЛИВ РАЗВОЈ НА ЗЕМЈОДЕЛСТВОТО СО ОСВРТ НА ПРОИЗВОДСТВОТО НА ТУТУН И ЗАШТИТА НА ЖИВОТНАТА СРЕДИНА

Одржливиот развој на земјоделството не може да се замисли без неговата економска способност,еколошка заштитеност, социјална одговорност и етичка фокусираност.Економски способно земјоделство подразбира примена на нови начини на стопанисување, што во своите калкулации го зема предвид и аспектот на заштита на животната средина.Еколошки заштитеното земјоделство преферира рационално користење на природните ресурси, примена на оптимално-минимална количина на ѓубрива и агрохемиски заштитни средства и водење на посебна грижа за зачувување на животната средина,со генетска одржливост на растителниот и животинскиот свет.Социјалната одговорност е насочена кон обезбедување на ангажираност на земјоделските работници, нивна мотивираност и посветеност кон еколошко -земјоделско производство. Еко-етиката е морално начело кое се фокусира на многу подрачја,вклучувајќи го и начинот на кој луѓето ја обработуваат почвата,начинот на производство на земјоделски култури, начинот на постапување со разновидниот растителен и животински свет и грижата за животната средина.Производството на тутун, како земјоделска активност со која се занимаваат околу 10% од населението во Р.Македонија, мора да

се одвива во согласност со интенциите на Европската унија и глобалната позитивна светска политика дефинирана од владини и невладини организации, што всушност значи производство на квалитетен тутун,негово користење за биоенергија и негово постепено намалување и заменување со други култури, притоа водејќи посебна грижа за заштитата на животната средина.Во овој труд е направен краток преглед кон насоките за одржлив развој на земјоделското производство, со посебен осврт на динамиката на развој на тутунопроизводството.

Клучни зборови: одржлив развој,тутун,земјоделие,реони,производство

INTRODUCTION

Sustainable development is a globally accepted philosophy of development and progress and it summarizes the spiritual ideals of humanity into a single logical unit. Top advantages of sustainable development are equality, fairness, ethics and responsibility towards present and future generations. Fact is that mankind has always faced a number of choices related to the quality of life and environment. Each choice has an impact on the world we are leaving to future generations. Some of the choices will contribute to creating a world in which economic and social needs are balanced with the capacity of natural resources and ecosystems, others will lead to a world with devastated environment ruled by poverty and hunger.Through sustainable development collective responsibility is taken for promoting economic development, social equity and environmental protection at local, national and global level.

Sustainable development is defined as development that satisfies the needs of present generations without compromising the ability of future generations to satisfy theirs. The concept implies a new attitude towards the environment in which we act as if we have not inherited it from our ancestors, but we have borrowed it from our children. In other words, sustainable development is development which meets the needs of the present, without endangering the possibility of future generations to meet their own needs.

Unsustainable development

Unsustainable development is an increase of poverty, destruction of natural resources, pollution effects on health. Unsustainable development is when trees are cut down without being replaced by other trees, when fish are caught in amounts that threaten their survival, exploitation of waters, plants, stones, mineral resources without responsibility for the future. Unsustainable development is when people buy and drive cars which pollute the environment and release exhaust gas.Unsustainable development is also when too many pesticides are added in the soil on which crops used as a food are grown. Unsustainable trends in reflected in climate change, use of energy, poverty and social exclusion, demographic pressure and aging, threats to the public health, the use of natural resources and loss of biodiversity are processes that need urgent action.

THE CONCEPT OF SUSTAINABLE DEVELOPMENT

We live in a consumer society. Every day we buy and use a range of products, but what we rarely ask is how these products are produced and what impact they have on the environment. All manufactured products consume large amount of natural resources, various harmful chemicals that are released into water and soil together with thousands tons of carbon dioxide and other greenhouse gases emitted into the atmosphere.

In other words, people take the resources from nature, they use them to create products they need and after some period of time they throw these materials and then burn them in landfills. This principle has long been considered acceptable until people realized that nature and natural resources are not found in unlimited quantities and that at some point the available resources will be significantly reduced and some will be completely exhausted. Faced with this problem, people developed the concept of sustainable development.**Sustainable development is development that meets the needs of present generations without compromising the ability of future generations to meet their own needs**.Sustainable development assumes collective responsibility for advancing the economic development, social equity and environmental protection at local, national and global level. The concept implies a new attitude towards the environment in which we act as if we have not inherited it from our ancestors, but we have borrowed it from our children. In other words, sustainable development is development which meets the needs of the present, without endangering the possibility of future generations to meet their own needs.

Sustainable development, as a global concept, is a challenge for everyone. Its main goal is to meet current needs without compromising the needs of future generations. Realization of this goal means creation of economy with full employment and highly qualified employees, high quality health care, social and territorial cohesion and environmental protection, all this in conditions of peace and security throughout the world.Active participation of Europe in the global process of sustainable development discussed at the conference in Rio de Janeiro in 1992 resulted in adoption of the first Sustainable Development Strategy (SDS) of the EU in Gothenburg in 2001, revised in 2006 by the European Council.Present generation has a duty to preserve and support our planet's diversity and to ensure its sustainable development. The overall aim of the revised strategy is to establish a set of measures and actions that will allow continuous improvement of quality of life of present and future generations through the creation of sustainable communities able to manage and use resources efficiently, able to develop the ecological and social innovation potential of the economy and able to ensure prosperity, environmental protection and social cohesion.The renewed Sustainable Development Strategy (EU SDS) concerns the global nature of sustainable development and the need for global solidarity, inclusion of all countries, particularly those with rapid development which have a significant impact on global growth. This strategy complements the Lisbon Declaration and largely corresponds to the Millennium Development Goals (MDGs). The key objectives of EU SDS are related to environmental protection, social equity and cohesion, economic prosperity and fulfillment of international obligations.This concept implies a new attitude towards the environment in which we act as if we have not inherited it from our ancestors, but we have borrowed it from our children. In other words, sustainable development is development which meets the needs of the present, without endangering the possibility of future generations to meet their own needs. Sustainable development is a harmonious relationship between ecology and economy, natural resources and the idea of preserving our planet for future generations. It is a collection of methods to create and sustain development which seeks to reduce poverty, create equitable standards of living, satisfy the basic needs of all peoples and establish sustainable political practices, all while taking the steps necessary to avoid irreversible damages to natural capital. Sustainable development can be conceptually divided into four pillars: environmental, economic, social and political sustainability (Fig. 1).

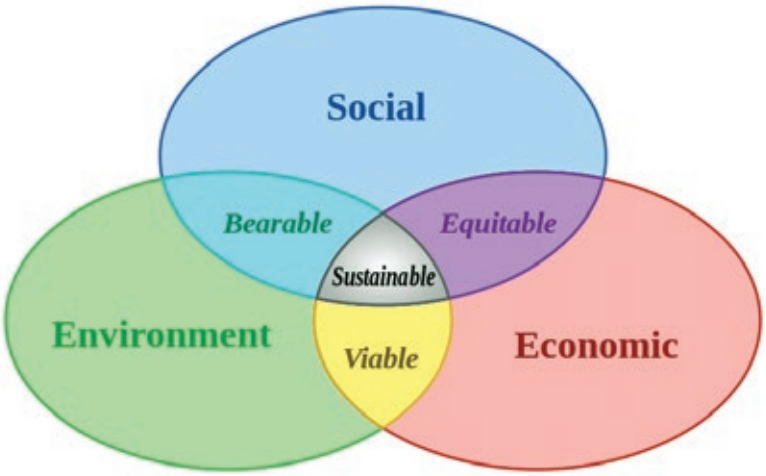


Figure 1. Pillars of sustainable development

Sustainable development is a harmonious relationship between ecology and economy, natural resources and preservation of our planet for future generations. Sustainable development is a set of methods to produce sustainable growth which aims to eradicate poverty and provide equal living standards, satisfying the basic needs of all people and establishing sustainable political practices, while taking all the steps necessary to avoid irreversible damages to natural capital.

SUSTAINABLE DEVELOPMENT– A CHALLENGE TO THE PRESENCE AND THE FUTURE

„We do not inherit the Earth from our ancestors,we borrow in from our children“
Lacota Indians

In the last decades, the concept of sustainable development has been accepted as a way of life in harmony with the environment. One of the definitions on sustainable development is that of the World Commission on Environment and Development (Brundtland Commission): “Sustainable development is development that meets the needs of the present, not compromising the ability of future generations to meet their own needs.”At the Earth Summit on Environment and Development (UNCED) held in Rio de Janeiro, 1992, world leaders established Agenda 21, which provides recommendations on sustainable management of land, water and forest resources in the 21st century.Sustainable development, as a global concept, is a challenge to every man and every country in the world. Its main goal is to secure continuous improvement of quality of life and prosperity of the country and to ensure sustainable development for present and for future generations. Unsustainable trends reflected in climate change, use of energy, poverty and social exclusion, demographic pressure and aging, threats to the public health, the use of natural resources and loss of biodiversity are processes that need urgent action.

Active participation of Europe in the global process of sustainable development discussed at the conference in Rio de Janeiro in 1992 resulted in adoption of the first Sustainable Development Strategy (SDS) of the EU in Gothenburg in 2001, revised in 2006 by the European Council.The renewed Sustainable Development Strategy (EU SDS) concerns the global nature of sustainable development and the need for global solidarity, inclusion of all countries,

particularly those with rapid development which have a significant impact on global growth. This strategy complements the Lisbon Declaration and largely corresponds to the Millennium Development Goals (MDGs). The key objectives of EU SDS are related to environmental protection, social equity and cohesion, economic prosperity and fulfillment of international obligations.

„The greatest threat to humanity comes not from its wrongs, but from its success“
Ernest Friedrich

How can humans continue their civilization on Earth without destroying the nature and themselves? Certainly not with ruthless development conceived as mere piling up of material wealth, followed by profligate use of natural resources through which all countries compete and take care only of their GDP (gross domestic product), as a measure of their success and development, blind to the price the nature pays for their progress. The answer to the question lies in implementation of the concept of sustainable development.Sustainable development means improving the quality of life within the capacity of existing systems. It is a development that provides basic environmental, social and economic services to all members of the community, without threatening the vitality of natural, artificial and social systems on which those services depend.The core principal of sustainable development is the belief that social and economic goals and goals related to environmental protection should be mutually complemented and interrelated throughout the whole process of development. Hence, sustainable development should be treated as an issue based on the integration of economic and social development in the context of high quality environmental management. In this respect, it is necessary to implement the strategy of sustainable development. The Sustainable Development Strategy is an important document which defines the courses for optimum development of the local community, taking into account the characteristics, advantages and limitations of the environment.

OBJECTIVE OF THE SUSTAINABLE DEVELOPMENT

„.. Through a jointed and timely action of morality and knowledge sustainable development is possible, because scientific truth is marvelous, but moral truth is divine...“
Horace Mann

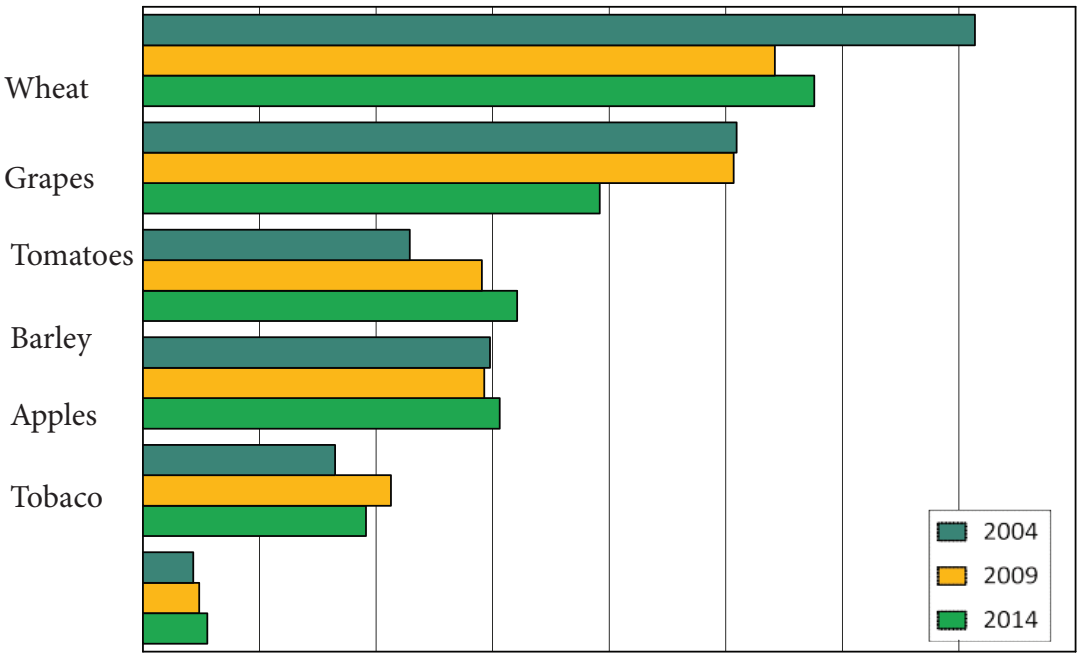
The main objectives of the EU Sustainable Development Strategy are to ensure, environmental protection, social equity and cohesion, economic prosperity and fulfillment of international obligations.The key challenges are:

- Climate change and clean energy - to limit climate change and its costs and negative effects to society and the environment;
- Sustainable transport – to meet the community needs for traffic whilst minimising its undesirable impacts on the economy, society and the environment
- Sustainable consumption and production- to divide the economic growth from degradation of the environment
- Conservation and management of natural resources - to avoid over-exploatation of natural resources

- Public Health – to secure equal access to a good-quality healthcare and to improve protection against health threats
- Social inclusion, demography and migration – to increase the quality of life of citizens as a precondition for lasting individual well-being
- Global poverty and sustainable development challenges – to reduce poverty, to promote sustainable development and to ensure that the internal and external policies are consistent with global sustainable development and its international commitments.

The progress in realization of the objectives of sustainable development is monitored by a set of indicators defined by the European Commission. The sum of indicators is organized into major themes following the economic, social, environmental and institutional dimension of sustainable development. The indicators are organized into three levels according to the subgroup to which they belong.

The fate of future generations lies in the hands of the present generation - we have a duty to preserve the Earth's capacity to support life in all its diversity and to ensure sustainable development. Agriculture in Republic of Macedonia is tending towards application of the principles of sustainable development. In context of sustainable model of production and consumption, statistical data on the production, area and yields of various crops, including tobacco, in a period of ten years (2004-2014) are presented in this paper (Graph 1, Table 1 and 2).



Graph 1.Realized production of agricultural crops

Table 1 shows some oscillations in the areas planted with barley, tobacco and tomatoes from 2004 to 2014, but there is still some stability in the use of these crops. Higher oscillations and significant reduction (24635 ha less) was recorded in the area planted with wheat - from 101321 down to 76686 ha.

The oscillations were more stable in the number of fruit-bearing apple trees and grape vines.

Table 1. Area and production of agricultural crops

	2004	2009	2014
Wheat	101321	88151	76686
Barley	44739	48622	41157
Tobacco	17716	17800	17756
Tomato	5972	5731	5720
Apples (No. of fruit-bearing trees in 000)	3625	4049	3776
Grapes (No. of fruit-bearing vines in 000)	88840	75228	84481

Data on the average yields of above crops are presented in Table 2. The table gives data on the average yield of crops over a 10-years period represented by 2004, 2009 and 2014.
It can be seen that there are also significant oscillations in the average yield, with a rise in 2014. It can be also noted that the average yield of tomatoes and tobacco showed an upward movement in 2009 and 2014. This is primarily due to the varietal structure of oriental tobaccos - primarily of the type Prilep, and to the climate conditions and applied cultural practices.

Table 2. Average yields of agricultural crops

	Unit	2004	2009	2014
Wheat	kg/ha	3522	3076	3755
Barley	kg/ha	3328	3010	3719
Tobacco	kg/ha	1221	1355	1553
Tomatoes	kg/ha	19171	25370	28065
Apples	kg/tree	23	26	25
Grapes	kg/vine	3	3	2

The concern for sustainable development

The fate of future generations is in the hands of current, which have a duty to preserve the planet's capacity to support life in its overall diversity and ensure sustainable development. Thus, care for sustainable development is in the hands of current and future generations and it should be encompassed by science and, with even greater magnitude, by practice. The present generations should understand that they have an obligation to use reasonably all that they inherited from previous generations, to spend it but also to renew it and pass on to future generations. Science through its research, debates and creativity should regularly refer to sustainable development in all areas in their countries and in the world, not endangering the stability of the cosmos. The practice through its practical actions should make a reasonable use of the limited resources, in order to secure the human and biological survival.

CONCLUSIONS

Sustainable development, as a global concept, is a challenge to every man and every country in the world. Its main goal is to secure continuous improvement of quality of life and prosperity of the country and to ensure sustainable development for present and for future generations. Unsustainable trends reflected in climate change, utilization of energy, poverty and social exclusion, demographic pressure and aging, threats to the public health and loss of biodiversity are processes that need urgent action. The fate of future generations lies in the hands of the present generation, which has a duty to preserve the Earth's capacity to support life in all its diversity and to ensure sus-

tainable development. Sustainable development offers a harmonious relationship between human and natural resources and a model of just and progressive world. It implies harmonization of economic, social and environmental aims, their integration whenever it is possible through creating mutually supportive policies and practices and making compromises when the integration between opposite aims is not possible. National Strategy for Demographic Development of the Republic of Macedonia is a basic document that identifies priority areas and tasks in formulating demographic policy. The aim of these priorities and tasks is to develop a trend of long-term stabilization of population growth, increasing birth rate and reducing mortality rate and the rapid aging of the population by enabling a high quality of the human capital, better quality of life for every Macedonian citizen and family and creating conditions for progress towards a socially cohesive community of citizens. Sustainable development is a development that satisfies the needs of present generations without compromising the ability of future generations to satisfy theirs. Realization of this goal means creation of economy with full employment and highly qualified employees, high quality health care, social and territorial cohesion and environmental protection, all this in conditions of peace and security throughout the world

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Professional papers

CHEMICAL PROPERTIES OF TOBACCO IN SOME ORIENTAL VARIETIES FROM THE TYPE PRILEP

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ABSTRACT

The chemical composition of tobacco, comprise about 3000 components, but we studied those that most affect its quality, and through him the smoking properties of tobacco: nicotine, total N, protein, soluble sugars and minerals. Investigations were carried out in the Scientific Tobacco Institute – Prilep and included four varieties of Prilep tobacco: Prilep P-23 (Ø), P-66-9/7, NS-72 and P-79-94. The trial was set up in randomized block design with four replicates. The analysis of chemical composition of cured tobacco was made by standard and accepted methods in the accredited laboratory L03 of the Scientific Tobacco Institute - Prilep. From the results we can conclude that all the varieties are distinguished by good chemical properties, or it comes to good quality of tobacco raw type prilep.

Keywords: type prilep, chemical properties, tobacco varieties

ХЕМИСКИ СВОЈСТВА НА ТУТУНОТ КАЈ НЕКОИ ОРИЕНТАЛСКИ СОРТИ ОД ТИПОТ ПРИЛЕП

Хемискиот состав на тутунот го сочинуваат околу 3 000 компоненти, но ние ги проучивме оние што најмногу влијаат на неговиот квалитет, а преку него и на пушачките својства на цигарите: никотинот, вкупниот азот, белковините, растворливите шеќери и минералните материи. Истражувањата се извршени во Научниот институт за тутун во Прилеп. Опитот беше поставен во четири повторувања по методот Рандомизирани блокови. Испитувани се четири сорти од типот прилеп: прилеп П-23 (контрола); П-66-9/7; НС-72 и П-79-94. Анализите за хемискиот состав на сувиот тутун се направени по стандардни и општоприфатени методи во акредитираната Лабораторија - L03 во Одделението за хемија на тутунот, тутунскиот чад и остатоци од пестициди при Научниот институт за тутун - Прилеп. Од добиените резултати можеме да констатираме дека предметните сорти се одликуваат со добри хемиски својства, односно станува збор за квалитетна тутунска суровина од типот прилеп.

Клучни зборови:тип прилеп, хемиски својства, сорти тутун

INTRODUCTION

The consumption of tobacco in the world is increasing steadily. In recent decades, with the introduction of filter cigarettes, the manufacture of cigarettes with low nicotine content and the use of appropriate materials, greatly contribute to increasing growth.
Tobacco production takes an important place in the economy of the Republic of Macedonia. Its importance is reflected by the fact that the raw material is primarily intended for export to world tobacco markets. Our country is known producer of high-quality aromatic oriental tobacco type, participating in the recipes for making the finest cigarette brands in the world.

Uzunoski (1985), divides elements of quality that characterized tobacco into three groups:
- Chemical indicators of quality
- Physical indicators of quality
- Tasting properties of tobacco

A number of research and practical findings suggest that in the formation of the chemical composition of tobacco, the biggest impact have the type, variety, ecological conditions in the region and applied agro-techniques.
From the connection of these factors depends the intern relationship of chemical components, which in turn reflect the quality of smoking tobacco.
Our purpose with this study was to investigate the chemical and display those properties that most affect the quality of tobacco: total nitrogen, nicotine, proteins, soluble sugars and minerals.

MATERIAL AND METHOD

The trial was set up in 2014 on dilluvial-colluvial soil in the field of Tobacco Institute-Prielp with four varieties of Prilep tobacco (P-23, P-66-9/7, NS-72 and P-79-94). Seedlings were produced in cold seedbeds. The second spring ploughing was followed by fertilization with NPK 10:30:20 in a rate of 250 kg/ha. The experiment was designed in randomized blocks with four replicates. Tobacco was transplanted manually at 40 cm spacing between the rows and 12 cm between the stalks. The area of each plot was 10 m^2. All necessary agro-technical measures for normal growth and development were applied in the field during the growing season. In July watering of the trial with wing sprinklers system was applied with 25 l/m^2 water. After harvesting, tobacco was sun-cured in barns specially designed for drying of oriental tobacco. Average samples for chemical analysis were taken from fermented tobacco by standard methods. Analyses of chemical composition were performed in accredited laboratory L03 in Tobacco Institute-Prielp, by recognized and generally accepted methods.
Subject of our investigations was the content of the following chemical compounds in dry tobacco leaves: nicotine, Total nitrogen, proteins, soluble sugars and mineral matters. Shmuk quality index was also determined.

SOIL CONDITIONS

Production of high quality tobacco is closely related to the soil type. Soil with its mechanical composition and nutrients content is a medium in which tobacco plants grow, develop and reach their genetic potential.

The trials were performed on delluvial–colluvial, the most represented soil type in the region of Prilep. Agrochemical characteristics of the soil on which the trial was conducted are presented in Table 1.

Table 1. Agrochemical characteristics of the soil

Horizon	Depth (cm)	Humus (%)	CaCo3 (%)	pH in		Available nutrients mg/100gr		N (%)	C:N
				H2O	KCl	P2O5	K2O		
I	0 - 30	0,53	-	6,00	4,96	7,25	13,3	0,055	5,59
II	30 - 57	0,43	-	5,94	4,78	1,75	19,2	0,055	4,53
III	57 - 83	0,39	-	6,15	4,95	1,9	18,5	0,055	4,11
IV	83 - 100	0,26	-	6,29	5,05	1,25	13,3	0,049	3,08
V	> 100	0,24	-	6,70	6,01	2,00	9,3	0,046	3,03

Data presented in Table 1 show that the soil on which the trial was conducted is characterized by a very low content of organic matter, low total nitrogen, moderately acidic, slightly acidic to neutral pH of the soil solution, low to extremely low supply of P2O5 and medium to good supply of physiologically active K2O.

RESULTS AND DISCUSSION

Chemical composition of tobacco is a complex of substances such as organic acids, organic bases, alkaloids, nitrogen compounds, proteins, carbohydrates, essential oils and resins. Tobacco is very adaptable plant to the external environment which highly affects its chemical composition, anatomic structure and physical properties. Any change of water, nutrient and light regime leads to modification of the basic properties of raw tobacco (Vesselinov, 1964). The results of our investigation on chemical composition of some varieties are presented in Table 2.

Table 2. Chemical composition of tobacco

No.	Variety	Nicotine %	Toatal N %	Proteins %	Soluble sugars %	Mineral matters %	Shmuk quality index
1.	P - 23 Ø	0.94	1.90	6.59	14.15	16.66	2.15
2.	P-66-9/7	0.87	2.16	6.63	17.99	14.29	2.71
3.	NS-72	0.88	2.30	6.98	14.67	15.78	2.10
4.	P-79-94	0.91	2.20	6.75	13.67	16.49	2.03

Nicotine

Nicotine as a chemical compound is one of the major representatives of the alkaloids contained in tobacco plant and an important indicator of quality of tobacco raw. In fresh condition it is oil-like liquid, without color and smell. When exposed to air it turns yellow and then dark (Uzunoski, 1985). The nicotine content is a variety characteristic that varies depending on the agri-environmental conditions and the cultural practices applied. Nicotine is synthetized in the roots and then through the stalk it comes to the leaves.

Turšić (1993), reports that tobacco fertilization with certain doses of nitrogen can increase the nicotine content from 20.7 to 34.9%.

In the tested varieties from type Prilep in 2014, the content of nicotine was slightly

lower and it is from 0.88% in the variety NS-72, to 0.94% in the control variety P-23. This reduction is due to the larger amount of rain during the vegetation.

Total N

A number of authors reported that higher amount of Total N in tobacco has a negative impact on its quality.
According to Uzunoski (1985), total nitrogen content increases from lower to the upper insertions and varies depending on the conditions of growing and the type of tobacco.
The average values show that maximum total N (2.30%) has the variety NS-72, and the minimum has the control variety (1.90%). In the other two varieties the total N content is nearly the same or is from 2.16 for P-66-9/7, to 2.20% in P-79-94.

Proteins

Proteins are complex organic compounds that have a special place in the chemical composition of tobacco, expressed through the smoking and taste properties of tobacco raw material.
According to Timov et co. (1974), the optimum content of Total N ranges between 5% and 10 %. The contents below 5% impairs the taste of tobacco and the smoker feels bitterness, irritation of throat and unpleasant smell.
According to Shmuk (1948), good quality tobacco should contain between 7 and 9% protein.
The results of our investigations have shown that the content of proteins for three varieties of the type Prilep are in optimal range as an expression of the quality of tobacco raw material, or it is from 6.59 for control variety, to 6.98% for NS-72.

Soluble sugars

Soluble sugars (monosaccharides and disaccharides) are the first products of the photosynthesis. The quality of tobacco is highly affected by the content of soluble sugars. The higher content of soluble sugars has a beneficial influence on tobacco taste and improves the aroma through neutralization of the negative impact of proteins, giving tobacco smoke an acidic reaction.
Veselinov (quoted by Uzunoski, 1985), reported that high quality oriental tobaccos should contain 14 to 18% of soluble sugars. Table 2 clearly shows that the variety P-66-9/7 contains the greatest amount of soluble sugars (17.99%), and lowest amount we have for the variety P-79-94 (13.67%). And for the check variety P-23, the value of this property is 14.15%.

Mineral matters

Mineral matters are important part of the chemical composition of tobacco and their share in the total dry mass content ranges from 9 to 30%. They are expressed through the ashes content that remains after the burning of tobacco. It is considered that higher content of mineral matters has a negative impact on tobacco quality.
Mitreski (2012) reported that the content of mineral matter in Prilep tobacco varieties ranges between 10.7% and 14.5%.
According to Alić - Đemidžić et al (1999), and Butorac (2009), the most important ingredients of the mineral complex of tobacco are potassium, calcium and magnesium.
In our research (Table 2), the average content of mineral substances is from 14.29 for P-66-9/7, to 16.66 for P-23 (Ø). The data presented in the table for this property, shows that it is a quality varieties tobacco from type Prilep.

Shmuk quality index

Shmuk index is the ratio between soluble sugars and proteins and it presents a coefficient for evaluation of tobacco quality. Its higher value denotes better quality of tobacco raw. In our investigations, the Shmuk index ranges from 2.03 in the variety P-79-94, 2.10 in NS-72, 2.15 in P-23 to 2.71 variety P-66-9/7.

Donev and Zlatev (1974), pointed out that the soluble sugars and proteins ratio is used as an objective criterion for estimation of tobacco quality. According to them, good quality tobaccos are those with Shmuk index 2 and over 2. Therefrom, it can be stated that the investigated varieties of Prilep tobacco are characterized by a very good quality.

CONCLUSION

Based on the results obtained from research on the chemical properties of the subject varieties, we made the following conclusions:
The content of total nitrogen is within the limits of oriental tobaccos from type Prilep. In all varieties, the total nitrogen is below 3%, which will positively reflect in the tobacco smoking material.
Nicotine content is from 0.87 for P-66-9/7, to 0.94% of P-23. Although the content of nicotine is a variety feature, it depends on soil and climatic conditions in the region of production.
The average protein content is within the limits of oriental tobacco. The same ranges from 6.63 in P-66-9/7, to 6.98% in NS-72, which is a sign of quality tobacco raw.
Soluble sugars are also in the optimal range. Their proportion of protein is a versatile, which indicates on good quality tobacco.
The average values of minerals are moving from 14.29 in P-66-9 / 7, to 16.66% in P-23. In the other two varieties the average content of minerals is at 15.78 NS-72, or 16.49% in the variety P-79-94.
Shmuk quality index has a positive value (greater than one), for all tested varieties from the type Prilep.
Studies on the chemical composition of the varieties have shown that it is a high quality oriental tobacco raw which is likely to have good tasting features.

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