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Целосна Адреса - Full address
Kicevska bb-7500 Prilep – Republic of North Macedonia

www.tip.edu.mk, e-mail: tobacco_institute_prilep@yahoo.com

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CHEMICAL PROPERTIES OF SOME DIHAPLOID VARIETIES AND LINES FROM TYPE PRILEP

[1]Gordana Miceska, [1]Miroslav Dimitrieski , [1]Biljana Gveroska , [2]Jordanco Zografoski
[1.] "St. Kliment Ohridski" University- Bitola, Scientific tobacco institute- Prilep, Kicevska bb, Prilep,
[2]Tututnski Kombinat AD Prilep
Prilepski braniteli 85, Republic of North Macedonia
e- mail: gordana.miceska@uklo.edu.mk

ABSTRACT

Oriental tobacco in the Republic of Macedonia is selected by classic methods, which is quite a long process. Today, one of the most commonly used biotechnological methods in plants is the double haploid method, whose main purpose is to shorten selection processes in field conditions, while obtaining homozygous lines in the first generation. Tobacco is an ideal plant for the production of haploid plants. Tobacco crops produce an explosion of haploid plants used today in the process of producing dihaploid plants, which are then used in the hybridization process of tobacco. Dihaploidization was performed by direct androgenesis of haploid tobacco plants obtained in the laboratory in vitro at the Scientific tobacco Institute - Prilep, using the tissue culture method. Four dihaploid lines of tobacco P 146-7 / 1 DH, Jk.l.. 301/23 DH, Hybrid 301 / n DH, Jk.l.75-301 DH and their analogs (P 146-7/1 Ø, Jk.l. 301/23 Ø, Hybrid 301/n Ø and Jk.l.75-301 Ø) was tested. The nicotine content of the tested lines and varieties of tobacco ranges from 0.23% at Jk.l 75-301 DH to 0.60% at P 146-7 / 1 Ø. The nicotine content at dihaploid lines compared to their analogues are smaller and it can be concluded that all diaploid lines have lower nicotine content than their analogues.

Keywords: tobacco, dihaplod lines, hybridization, materiality,

ХЕМИСКИ СВОЈСТВА НА НЕКОИ ДИХАПЛОИДНИ СОРТИ И ЛИНИИ ТУТУН ОД ТИПОТ ПРИЛЕП

Ориенталските тутуни во Македонија се секционирани по класичните методи, што е доста долготраен процес. Денес, една од често употребуваните биотехнолошки методи кај растенијата е методот на двојни хаплоиди, чија главна цел е скратување на селекционите процеси во полски услови, при добивање на хомозиготни линии во првите генерации. Тутунот е идеално растение за добивање на хаплоидни растенија. Тутунските култури продуцираат експлозија на хаплоиди кои денес се користат при процесот на хибридизација на тутунот. Дихаплоидизацијата беше извршена со директна андрогенеза на хаплоидни тутунски растенија добиениво лабораторијата in vitro во Научниот институт за тутун - Прилеп, користејќи го метод култура на ткива Во испитувањата беа вклучени четири дихаплоидни линии тутун П 146-7 / 1 ДХ, Јк.л .301/23ДХ, Хибрид 30 /н ДХ, Јк.л.75-301 ДХ и нивните аналози (П 146-7 / 1 Ø, Јк.л.301/23 Ø, Хибрид 301/н Ø и Јк.л.75 . Содржината на никотин кај испитуваните линии и сорти тутун се движи од 0,23% кај Јк.л.75-301 ДХ до 0,60% П 146-7/1 Ø. Содржината на никотин кај дихаплоидните линни е помала во споредба сонивните аналози.

Клучни зборови: тутун, дихаплоидни линии, хибридизација, хемиски својства

INTRODUCTION

In the Republic of Macedonia, tobacco has been cultivated continuously for almost four centuries. Oriental, semi-oriental, and large-leaf tobacco types (Virginia and Burley) are grown during this period.

Higher tobacco production in the Republic of Macedonia was observed after World War II when tobacco production reached 36 221 tonnes in 1982. Today the production of oriental tobacco in the

Republic of Macedonia is organized on 15 881 ha (2009-2018), with an average production of 24 716 tons of tobacco.

Macedonia as a producer of oriental aromatic tobacco occupies a significant place in the world and is in the top eight countries of oriental tobacco producing 3% of the world production. Of the total arable land in the country, tobacco accounts for 3.4%, and in the total area under industrial crops around 81.1% of the total area.

In the economy of the Republic of Macedonia, tobacco occupies an important position due to the total value of the production itself and its economic - sociological significance.

Oriental tobacco in the Republic of Macedonia is selected by classic methods, which is quite a long process. Because the ontogenetic development of tobacco depends primarily on its biological properties, and heredity as a characteristic is one of the fundamental manifestations of plant organisms, which manifests itself differently depending on environmental conditions, in recent time there is a need for better knowledge of these processes.

Namely, man's efforts to refine plants in order to obtain a higher yield are probably as old as agriculture itself.

Today, in many institutions around the world as well as in our country, various methods of obtaining new plant varieties are applied, which in their genetic composition contain combinations of the best parental traits. In this way, varieties that produce higher yields are more resistant to disease and adverse climatic conditions.

One of the traits of plants is their ability for vegetative propagation. It means getting plants from different parts of the plant i.e. from buds, roots, stem, leaf, etc. This gives rise to many new plants belonging to the same sex generation as the mother.

There are several ways of vegetative in vitro propagation: micropropagation, organogenesis, somatic embryogenesis, development of haploid and dihaploid plants.

Today, one of the most commonly used biotechnological methods in plants is the double haploid method, whose main purpose is to shorten selection processes in field conditions, obtaining homozygous lines in the first generation, based on the different combinations that occur at gametes level, consolidation. of lines and the creation of new varieties (Morison, Evans 1988).

Tobacco is an ideal plant for the production of haploid plants, tobacco crops produce an explosion of haploid plants used today in the process of producing diaploid plants, which are then used in the hybridization process of tobacco.

The double haploid method is one of the most implemented biotechnological methods today. Seitz (Burchet, 2004) stated: "If the induction of hybrids was the greatest discovery in selection, and the use of biological laboratories second in size, the technology of double haploids would be the third in a row."

The main objective of this method is to shorten the process of obtaining new varieties in fields conditions where self-pollination and selection processes continue until uniform varieties are achieved and 100% of them become homozygous. It takes nine to eleven years to standardize some varieties or obtain new varieties (Patrascu, Ioan, 1984).

Dihaploid lines show a high level of uniformity (Devereux, Lameri, 1974), compared to standard varieties, and in other cases (Legg, Colins, 1968), some variations in morphological properties are observed between lines obtained from a single plant. Raymond (1987), in his studies, reveals significant differences in all indicators, except for the number of leaves among the dihaploid regenerators of the high hybrid variety NC 95.

There are some controversial points about the advantages and disadvantages of haploid methods, that is, the method of induced androgenesis in obtaining dihaploid homozygous plant lines. In order to overcome or clarify some of these doubts, in this paper we set out to investigate the quality properties of some dihaploids tobacco lines in comparison with their analogues, and to confirm the application of induced androgenesis, depending on the

objectives. of selection in obtaining homozygous dihaploid tobacco lines.
We hoped that the investigations and results obtained in this paper will form the basis of the selection processes and that biochemical methods (tissue culture and androgenesis) will be successfully applied in obtaining new superior homozygous lines and tobacco varieties.

MATERIAL AND METHODS

The tests were carried out in the field of study at the Tobacco Institute - Prilep in 2014. Four dihaploid lines of tobacco P 146-7 / 1 DH, Jk.l. 301/23 DH, Hybrid 301 /n DH, Jk.l. 75-301 DH and their analogs (P 146-7/1Ø, Jk.l. 301/23 Ø, Hybrid 301/ n Ø, Jk.l 75-301 Ø) Photo1-7.

Dihaploidization was performed by direct androgenesis of haploid tobacco plants obtained in vitro laboratory in Scientific Tobacco Institute - Prilep, using the tissue culture method (meristems, 0.5 cm leaf sections) on Murashige & Skoog, nutrition medium (Murashige T., Skoog F., 1962), modified for direct organogenesis and optimized with the following chemicals: casein - hydrolyzate - 1 mg / l; L - glutamine - 250 mg / l; glycine - 200 mg / l; IAA - 0.2 mg / l; BAP - 0.5 mg / l; adenine - 20 mg / l; kinetine - 3 mg / l and and myoinosite - 100 mg / l. The first dihaploid plants were grown in the biological laboratory in soil-pots: soil: perlite - (3: 1) until seed material was obtained from them.

The seedlings from the examined lines and varieties of tobacco were produced in the usual way, in the field of the Scientific Tobacco Institute – Prilep, all necessary agro-technical and protective measures were applied during the cultivation of the seed in order to obtain healthy and normally developed seedlings.The experiments were performed using the random block system (Randoum block system) in 4 repetitions. Planting was done at a distance of 40 cm between the rows, 15 cm in the row. The tobacco harvest was done manually, in the technical maturity of the leaves.

The usual agrotechnical and protective measures of tobacco against diseases and pests were applied during the vegetation of tobacco on the field. The qualitative assessment of dried tobacco was carried out according to the applicable Rulebook on Uniform Measures for Tobacco Purchase. Chemical properties were analyzes in the accredited laboratories of the STIP in accordance with the standard MKS EN ISO / IEC 17025: 2006, by standard methods. Chemical properties were analyzed for nicotine content, protein content, soluble sugar content, mineral content and quality of tobacco expressed by the Shmuk`s index.

Photo1.P 146-7/1 Ø and P146-7/1DH

Photo 2. Jk.l. 301/23 Ø

Photo 3. Jk.l. 301/23 DH

Photo 4. Hybrid 301/n Ø

Photo 5. Hybrid 301/nDH

Photo 6. Jк.л.75-301 Ø

Photo 7. Jк.л.75-301DH

AGROECOLOGICAL CONDITIONS

One of the important factors for the development, yield and especially the quality of the tobacco plant (chemical and physical properties) are the climatic conditions during the tobacco vegetation period. For this purpose, the following meteorological data were analyzed during our survey: mid-December and monthly air temperature, decadal and monthly precipitation amounts, and mid-decade and monthly relative humidity.

From the data presented in Table 1 it can be seen that the average average monthly air temperature in 2014 is 18,3 ° C. The average monthly minimum air temperature is 11.3 ° C and the average monthly maximum temperature is 27.0 ° C.

Table 1. Average decade and monthly air temperature from may – September 2014

Meteorological factors	**Decade**	**Months**					**Σ/X ºC**
		May	June,	July	August	September	
Average Decade Air Temperature ° C	I	12.4	16.8	21.5	20.6	17.4	
	II	13.6	18.7	19.8	22.5	17.2	
	III	16.3	20.5	21.3	22.0	13.6	
Average monthly air temperature ° C		14.1	18.7	20.9	21.7	16.0	18.3
Average monthly maximum air temperature ° C		21.8	27.7	30.2	31.6	23.9	27.0
Average monthly minimum air temperature ° C		7.7	11.4	12.9	13.6	11.1	11.3
Meteorological factors							**Σ/X l/m²**
Precipitation mm	I	15.0	1.0	0.0	16.0	76.0	
	II	8.0	10.0	43.0	0.0	16.0	
	III	14.0	0.0	8.0	1.0	15.0	
Total precipitation mm		37.0	11.0	51.0	17.0	107.0	223.0

According to Uzunoski (1985), the optimum air temperature for normal growth and development of tobacco plants ranges between 20 and 30 ° C, and the maximum temperature is between 40 and 50 ° C.

According to Atanasov (1962), temperature deficiency limits are below 18 ° C and excessive above 30 ° C.

From the average data in Table 1 it can be concluded that the temperatures during the vegetation are within the permissible temperature limits which ensure normal growth and development of tobacco.

According to the sum of precipitation (Table 1), in 2014 during vegetation a total of 223.0 mm of water sediment fell. According to the literature, this quantity corresponds to get fine-grained aromatic tobacco but still, the distribution of precipitation by decades and months is quite uneven.

The soil of the experiment field at the Scientific Tobacco Institute , Prilep, where the experiment wasset up in the year of the test, is deluvial (colluvial) type (Table 2). This type of soil is most prevalent in the Prilep production area, where tobacco is mainly grown as monoculture.

Table 2. Agrochemical properties of soil (location STI - Prilep)

Humus %	**Total nitrogen%**	**pH**		**CaCO3%**	**mg / 100g soil**		**Physical clay <0.02 mm%**
		H_2O	**KCl**		**P_2O_5**	**K_2O**	
0.57	0.019	6.09	5.00	-	24.2	19.2	24.5

According to the chemical properties (Table 2), it can be seen that the soil is very low in humus content, very low total nitrogen content, is carbon-free, with low acidic reaction, high content of available P2O5, and well supplied with potassium.

RESULTS AND DISCUSION

Chemical composition of tobacco

Chemical composition is one of the main indicators for determining the quality of tobacco raw material. The presences of certain chemical components and their interrelationship have a specific meaning to the quality of the tobacco, ie they are important to the quality value of the tobacco raw material.

The chemical composition as a material component of tobacco leaves is manifested both on their external appearance and on their smoking properties.

Uzunoski (1985), stated that the chemical composition to a certain extent is a typical and varietal characteristic and is highly variable depending on the conditions of cultivation during field tobacco vegetation, tillage and other factors.

Table 3 shows the results of the expeiment for the chemical composition of diahaploids lines and their analogues.

Байлов (1965) points out that the quality of tobacco depends not only on the chemical components contained in it but also on their interconnection, as well as on their relationship, changes resulting from the conditions of cultivation and the method of drying.

Table 3. Chemical composition of tobacco

Varieties/lines	**Nicotine (%)**	**Proteins (%)**	**Mineral supstances (%)**	**Redusing sugars (%)**	**Shmuks` index**
P 146-7/1 Ø	0.60	5.10	9.87	27.06	5.30
P 146-7/1DH	0.55	5.39	9.24	28.21	5.23
Jk.l.. 301/23 Ø	0.32	5.07	7.90	27.48	5.42
Jk.l. 301/23 DH	0.30	4.79	7.97	30.31	6.29
Hybrid 301/n Ø	0.39	5.67	14.92	24.00	4.23
Hybrid 301/n DH	0.36	5.81	11.90	21.67	3.73
Jk.l..75-301 Ø	0.48	5.23	8.58	26.68	5.10
Jk.l.75-301 DH	0.23	5.29	7.92	27.30	5.16

1. Nicotine content

The tobacco plant contains more alkaloids, but nicotine is one of the most important because of tobacco leaf wich is used for smoking. Nicotine is found in all parts of the tobacco plant, except in mature seeds. According to studies by a number of authors, nicotine content increases from lower to upper insertions.

The use of tobacco leaves is conditioned by the specificity of this chemical component, and is related to the physiological-narcotic effect of tobacco smoke on the smoker's nervous system.

According to Shmuk (1948) the optimum amount of nicotine in oriental tobacco is 1 to 1.5%.

Wolf (1962) points out that nicotine is certainly the most interesting and important ingredient of tobacco.

Nicotine content is a varietal characteristic, but its variations depending on environmental conditions and agrotechnical measures applied.

Dimitrieski et al. (1992) examining the varieties of type Yaka in the region of Prilep found that nicotine content was optimal in 1982 in the examined varieties of type Yaka is several times larger than in 1983 where the amount of rainfall was greater.

Dimitrova (1991) examining the chemical composition of newly acquired diaploid lines compared to their analogues of oriental type, concluded that dihaploid lines have a lower % of nicotine than their analogues 1,01% to 1,94% .

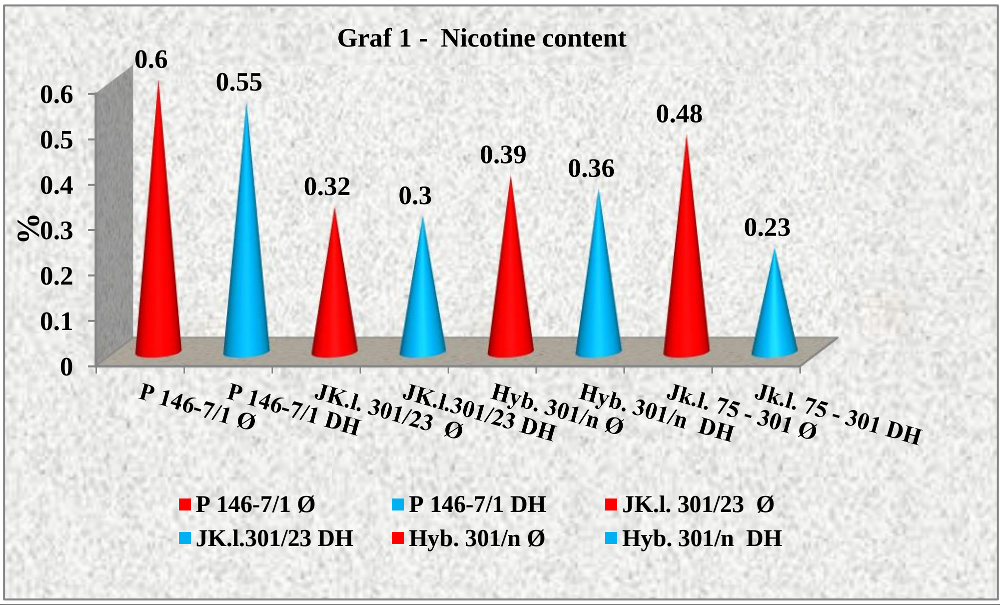
Analyzing the results for the nicotine content of the studied lines and varieties of tobacco, we can conclude that it (Table 3, Graf. 1) ranges from at Jk.l. 75-301 DH 0.23% to 0.60% at P 146-7 / 1 Ø.

Regarding the nicotine content of dihaploid lines compared to their analogues, it can be concluded that all dihaploid lines have lower nicotine content than their analogues.

Nicotine content in all tested lines and varieties has low values. It is due to the large amount of rainfall during the tobacco vegetation period and the irregular rainfall schedule. Namely, the precipitation content in the second and third decades of July is (51 mm), the first decade of August (16 mm), as well as the first decade of September (76 mm) in the period when these insertions were formed.

Lazaroski (1984), based on many years of Prilep-type tobacco research in the Prilep region, found that irrigation as an agrotechnical measure has a significant impact on reducing nicotine content in tobacco. Depending on the irrigation variant, the nicotine ranged from 0.80% to 1.14%.

Байлов(1965) points out that the same variety of tobacco in dry conditions can produce twice as much nicotine as in wet conditions. Increasing nicotine content is also affected by flowering violations.



2. *Protein content*

In the nitrogen complex, proteins are organic compounds that play the most important role in the tasting properties of tobacco raw materials.

Timov et al. (1974), point out that the favorable effect of proteins occurs in quantitatively optimal ranges between 5 and 10%. With less than 5% protein, unsatisfactory taste sensations of fullness and fullness of smoking are felt, and over 10% the taste of tobacco deteriorates and smells like bitterness, irritation and bad smell.

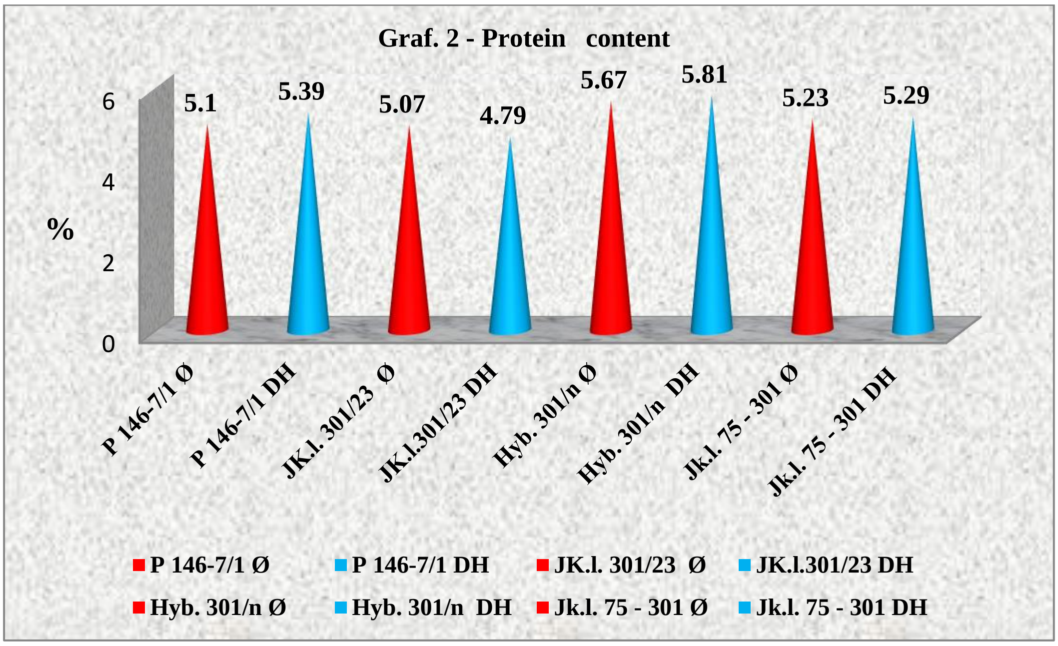
Table 3, Graf. 2 presents the data on the protein content of the dihaploid lines examined and their analogues. From the presented values it can be seen that the protein content ranges from 4.79% to 5.81%. The lowest protein content have dihaploid line Jk.l.301/23 DH (4.79%), and the highest protein content of 5.81% is in the Hybrid 301 / n DH.

According to Shmuk (1948), qyality tobacco does not contain more than 8% protein, while in less qyqlity tobacco they reach 20%.

Lazaroski (1976), examining the protein content in raw materi al of tobacco type prilep from 1973 and 1974 in Bitola region, found that it had an average of 8.01 – 8.85% protein. The same author (1983), in his three-year studies, found that the protein content in prilep tobacco ranges from 6.18% to 7.39%.

It also found that irrigation reduced the protein content by 16.73%.

Uzunoski (1985) states that the protein content increases from lower to upper insertions and from mature to immature tobacco leaves. Also, greater intermediate distance, more abundant nitrogen fertilization and flowering breakage increase the protein content.



Nuneski (2008), in their studies, concluded that the average protein content of all insects of basma tobacco in 2004 was 7,18% and in 2005 (7,65%), or for both harvests 7,41%.

Arsov et al. (2011) point out that the protein content below 5,5% of the dry matter of tobacco is a sign of one-sided taste, and a content higher than 7% is a sign of deteriorated taste of tobacco and the quality of tobacco.

Mitreski (2012), examining the chemical composition of several type prilep tobacco varieties, concluded that the protein content of some Prilep-type varieties in 2009 ranged from 6.34% at P 66-9 / 7 to 7.22% at P 79-94, and in 2010 it ranges from 6.35% in Prilep 79-94 to 6.75% in P-23.

Average results obtained from our tests are correlated with the cited literature data, i.e. the raw material from the examined dihaploid lines and their analogues is with protein content within the bounds of the corresponding commodity type.

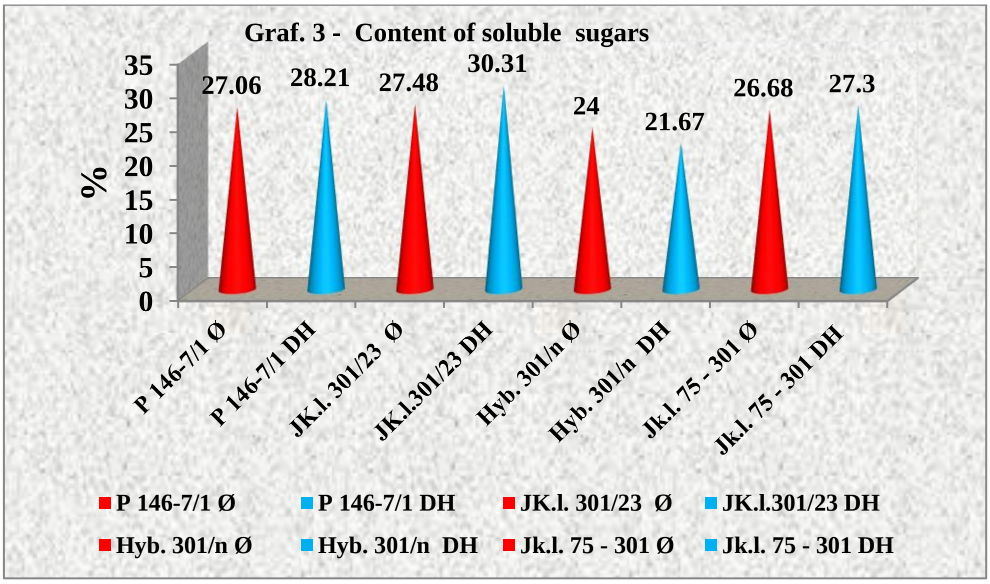
3. Content of redusing sugars

Redusing sugars are the most important non-toxic substances in the tobacco leaf, as they represent almost half of the total organic matter in the tobacco.

Redusing sugars are considered to be the primary building blocks of other organic compounds in photosynthesis. They have a positive impact on the quality of tobacco. The positive impact is that redusing sugars form a smoke with an acidic reaction, neutralizing the alkaline reaction produced by the proteins and other compounds of the leaf composition.

Oriental and virgin types of tobacco are characterized by a higher percentage of redusing sugars, as opposed to black tobacco and the burley type, where the percentage is quite low. Except for the type of tobacco and the way of drying, the influence of the soluble sugars has started to have a great impact - the climate conditions that prevailed during the vegetation period, the applied agrotechnics (fertilization and irrigation), etc.

Timov et al. (1974) concluded from their studies that the redusing sugar content of oriental tobacco in Bulgaria ranged from 8 to 15%.



According to the results from Table 3, Graf. 3 we can conclude that the lowest content of redusing sugars has dihaploid lines Hybrid 301 / n DH (21.67%), and the highest redusing sugar JK.l. 301/23 DH (30.31%) . All varieties tested and their dihaploids have a relatively higher dusing sugar content as a result of rains falling in the second and third decades of July (51 mm), in the first decade of August (16 mm), and in the first decade of September (76). mm), the period when the inserts were formed from the true middle leaf to the top.

According to Гюзелов et al. (1965), oriental tobacco containing less than 5% soluble sugars is of poor quality, with 10 - 11% being of medium quality, and tobacco with 11 - 16% being of high quality.

Lazaroski (1976) states that in Bitola region, tobacco type Prilep redusing sugars range from 18.42% in the lower, to 22.26% in the upper inserts. The author noted that the content of soluble sugars increased from lower to upper inserts.

According to Nuneski (1986), the percentage of soluble sugars in the Prilep type is about 18.41% for the lower middle leaf, 19.11% for the true middle leaf and 20.69% for the upper middle leaf.

4. *The quality of tobacco expressed by the Shmuk`s index*

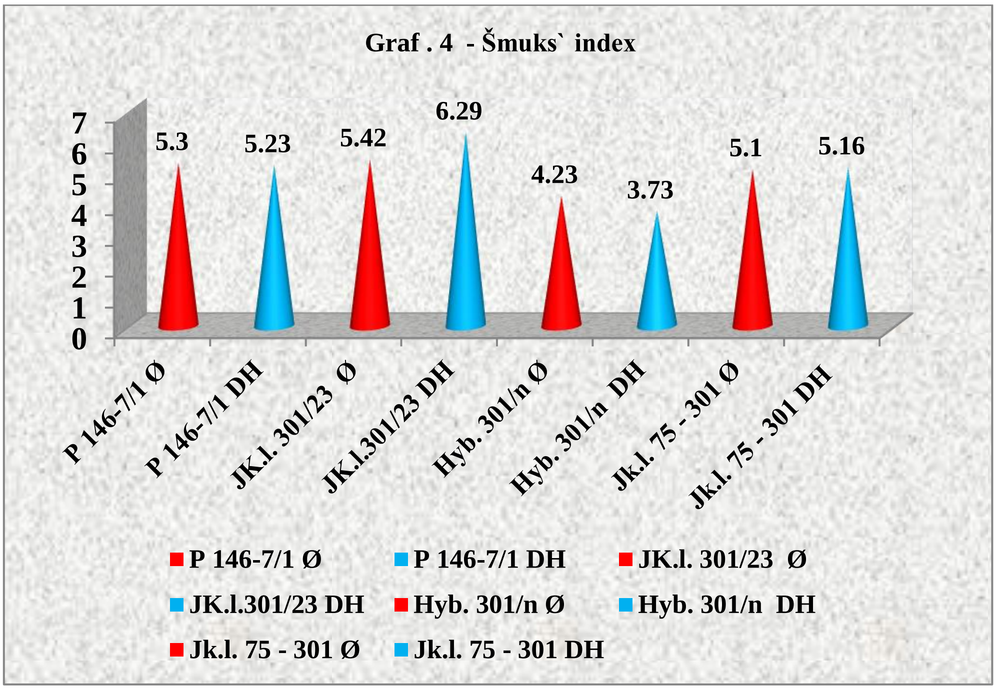
For the objective evaluation of the quality and value of use, in addition to the content of the components of the chemical composition of tobacco, their interrelationship is important. Shmuk's (1948) took the ratio of soluble sugars and proteins as the basis for assessing the quality of cigarette tobacco.

This ratio between soluble sugars and proteins is known as the Shmuk's index. The higher the Shmuk`s index, the better the quality of the tobacco. The data obtained from our tests for the Shmuk index are shown in Table 3 Graf. 4. The lowest index has a Hyb. 301/n DH (3,73), and the highest index per Shmuk`s has a Jk.l.301/23 DH (6,29).

All investigated varieties / lines have a relatively high Shmuk`s index , due to the redusing sugar content that results from the rains falling in the second and third decades of July (51 mm), the first decade of August (16 mm), and in the first decade. Decade in September (76 mm), in the period when the insertions were formed from the true middle leaf to the top

Analyzing the results for the Shmuks` index of diaploid lines compared to their analogues, the two lines (Jk.l.. 75-301 DH, Jk.l 301/23 Ø) have higher values of the Shmuks` index than their analogs for 0. 06 (Jk.l. 75-301 DH).

Mitreski (2012), in his researches stated that the Shmuks` index ranges from 1.74 in the variety Prilep P-79-94 to 3.06 in the variety P 66-9 / 7 in 2009 and from 2.69 in the variety NS 72 to 4.5 at the 2010, Prilep variety(P 79-94).



CONCLUSIONS

Having in mind the literature data as well as the data from our research we can come to the following conclusions:

• The nicotine content of the tested lines and varieties of tobacco ranges from 0.23% at Jk.l 75-301 DH to 0.60% at P 146-7 / 1 Ø, ie the nicotine content at dihaploid lines compared to their analogues are smaller and it can be concluded that all dihaploid lines have lower nicotine content than their analogues.

• The protein content of the examined dihaploid lines and their analogs ranges from 4.79% to Jk.l. 301/23 DH up to 5.81% with Hybrid 301 /n DH.

• The soluble sugar content of the dihaploid lines tested and their analogs ranges from 21.67% at the dihaploid line Hybrid 301 / n DH, to 30.31% at the diaploid line Jk.l 301/23 DH.

• The ***Šmuks` index*** of the investigated dihaploid lines and their analogs ranges from 3.73 Hybrid 301/n DH to 6.29 (Jk.l. 301/23 DH).

• Dihaploid lines according to their chemical properties successfully may be the starting material for further selection studies in obtaining new varieties of tobacco within the examined tobacco commodity type.

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Original Scientific paper

MONOHYBRID DOMINANT INHERITANCE OF TMV RESISTANCE IN SOME ORIENTAL VARIETIES OF YAKA TOBACCO

Miroslav Dimitrieski, Gordana Miceska
"St. Kliment Ohridski" University- Bitola, Scientific tobacco institute- Prilep,
Kicevska bb, Prilep, Republic of North Macedonia

A B S T R A C T

Investigations were made on the inheritance of resistance to tobacco mosaic virus (TMV) in some oriental tobacco varieties by the method of interspecies hybridization.

The selection process started with crossing of TMV resistant varieties (AA) with non-resistant aromatic varieties of the type Yaka. In F_1, TMV resistance was inherited dominantly, or the obtained plants were heterozigous (Aa). From self-pollinated hybrids of F_1 generation, the plants obtained in F_2 were varying in their genotypes in 3 : 1 ratio (3 resistant : 1 non-resistant plants). Genetic formula of the resistant plants is (AA), (Aa) and (Aa). When inoculated with infective sap, plants show necrotic reactions, which indicates that the investigated trait was dominant. In F_2 generation a number of resistant plants were selected to create separate progenies in F_3. Inoculation was carried out with infective sap from diseased plants, using the method of Ternovsky (1965, cit. by Trančeva, 1995). The aim of the paper was to study the mode of inheritance of TMV resistance in creation of new varieties of Yaka tobacco.

Key words : tobacco, TMV, resistant genotypes

МОНОХИБРИДНО ДОМИНАНТНО НАСЛЕДУВАЊЕ НА ОТПОРНОСТА НА ТМВ КАЈ НЕКОИ ОРИЕНТАЛСКИ СОРТИ ОД ТИПОТ ЈАКА

Беа направени истражувања за наследувањето на отпорноста кон обичниот мозаик вирус на тутунот (ТМВ), кај некои ориенталски сорти тутун по методот на меѓусортова хибридизација.

Селекциониот процес започна со вкрстување на отпорни сорти тутун на ТМВ (АА), со неотпорни ароматични сорти од типот Јака (аа). Во Ф1, отпорноста на ТМВ беше наследена доминантно, односно беа добиени отпорни хетерозивни растенија (Аа). Од самоопрашените хибридни растенија од Ф1 генерација, во Ф2 се добиени различни по генотип растенија во соодносс 3: 1 (3 отпорни: 1 неотпорно растение). Генетската формула на трите отпорни растенија е (АА), (Аа) и (аА). По инокулацијата со инфективен сок, растенијата покажуваат некротична реакција, што укажува на фактот дека проучуваното својство е доминантно. Во Ф2 генерацијата, беа избрани поголем број на отпорни растенија за создавање на посебни потомства во Ф3. Инокулацијата беше извршена со инфективен сок од заболени растенија, користејќи го методот на Терновски (1965 г., цит. Транчева, 1995).

Целта на трудот беше да се испита начинот на наследување на отпорноста на ТМВ вирусот, при создавање нови сорти тутун од типот Јака.

Клучни зборови: тутун, ТМВ, отпорни генотипови

INTRODUCTION

In hybridization process, monohybrid inheritance denotes hybridization between individuals of two varieties, when inheritance of only one character is observed, i.e. its two alternatives (pair of alleles) by which the parents are differed. A number of authors (S.Borojević, K.Borojevć, 1976, Genčev 1980, Marinković1981, Gershenzon 1983, Avala F.J. Kiger J.A. 1984, etc), emphasize the importance of Mendel's laws, according to which in the inheritance of two pure lines differing by a pair of alternative genes, one of them is represented in F1 hybrids (in full dominance) and all hybrid individuals are uniform in relation to the observed character.

The fact that the character resistance to TMV is inherited in monohybrid dominant way (according to many authors), offers a possibility for deliberately intended and fully controlled selection in breeding new TMV resistant oriental lines and varieties of the type Yaka.

MATERIALS AND METHODS

Investigations began at the experimental field and glasshouse of Tobacco Institute-Prilep in 1997, as a part of the project "Breeding of new oriental varieties resistant to TMV," with financial support of Macedonian Ministry of education and science (Dimitrieski M., Miceska G., et.all.2000).

Bulgarian TMV resistant varieties Rila 89 and Nevrokop 1146 were used as initial material for hybridization and crossed with local varieties of the type Yaka (Yaka 23, Yk. l. 65 and Yk. l. 123). Selection of parental varieties was based upon previous investigations. The method of intraspecies hybridization was used in the process of breeding, in accordance with Mendel's laws for character inheritance, applying the scheme of monohybrid dominant inheritance (Scheme 1). Hybrid progenies from F_1, F_2 and F_3 were tested on adequate surface and with sufficient number of individuals for each generation. Thus, 1-2 plants were taken from F_1 generation, 8-10 resistant individuals were selected and isolated from F_2 (special progenies of which were created in F_3), whereas in F3 generation resistant homozygous progenies were determined.

Juice from mosaic-infected tobacco plants was used for inoculation, according to the method of Ternovsky (1965, cit. by Trančeva, 1995). The juice from infected leaves was heated for 12 minutes in water bath at 80^0C, in order to inactivate all other viruses, e.g. cucumber mosaic virus (CMV) and Potato Virus Y (PVY). Observations were made between the 7th and 10th day of inoculation, after the appearance of local necrotic spots on resistant plants and mosaic patterns on the leaves of susceptible plants.

RESULTS AND DISCUSSION

The selection process started with crossing among resistant varieties of foreign origin (AA), used as maternal component, and domestic non-resistant varieties and lines (aa) of Yaka tobacco used as paternal component. In F_1 generation, heterozygous plants were obtained (Aa), i.e. all hybrids showed necrotic reaction after infestation, which indicates that resistant alleles are dominant and non-resistant alleles are recessive.

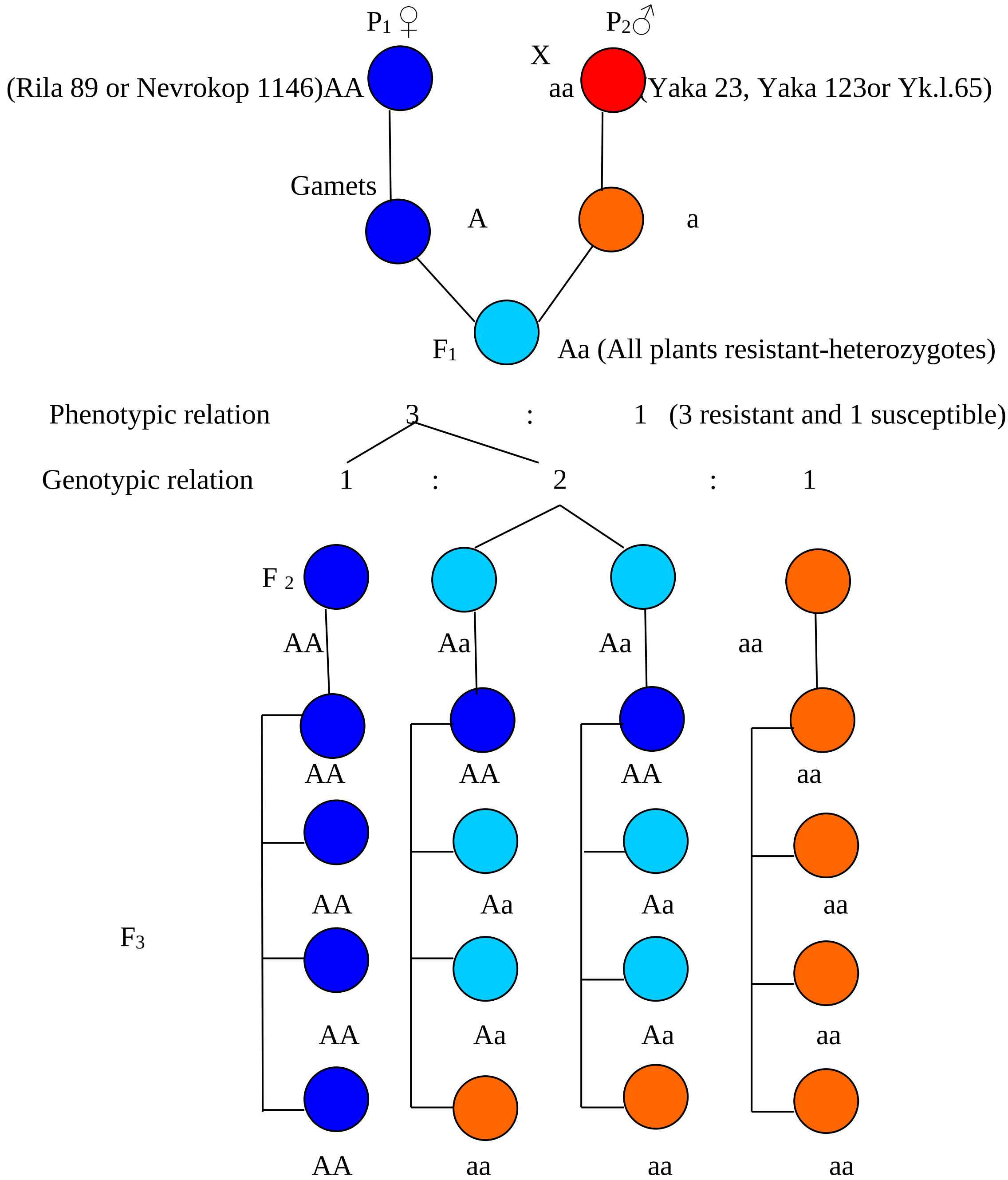
The dominant inheritance of this trait was also confirmed by other authors. Holmes, Kostov and Ternovskiy obtained homozygous forms of tobacco (N. tabacum) which succeeded to localize TMV. Holmes was the first to report from genetic aspect that one gene is responsible for localization of the virus, which usually appears as dominant (cited by Kostov, 1941-

1943). According to S. Stoyanov Gelemerov (2005), Kostov crossed the resistant form N. tabacum var. viri (N. tabacum x N. glutinosa) with Basma 36 and noted dominant inheritance of this trait (existence of local necrotic reaction). Ternovskiy used TMV resistant gene from N. glutinosa to create the varieies Đubek 7, Đubek 566, Trapezond 161 etc. He reported that Shabanov, Lulov and Manolov also worked on TMV resistant varieties. Petkova (2008) investigated three TMV resistant tobacco varieties which transmitted this trait in F1 with dominant inheritance. She reported that the same statement was confirmed by Manun (1981).
From self-pollinated hybrid individuals in F_1, genotypically diverse plants were obtained in F_2 generation, with ratio 3:1, i.e. 3 resistant plants : 1 non-resistant, or 75% of the individuals were resistant to TMV and 25% were non-resistant. Thus, it could be stated that segregation of the character resistance to TMV appeared in F_2 generation, according to the following pattern : 1AA : 2Aa : 1aa (AA, Aa, aA, aa), i.e. 25% of the hybrids were homozygous TMV resistant individuals, 50% resistant heterozygous individuals and 25% homozygous non-resistant individuals. However, in hybrids showing necrotic reaction, it is not clear whether resistant varieties are of AA genotype (homozygous) or Aa (heterozygous). In order to determine the genotype of resistant plants from F2 generation, 7-10 plants were isolated for F_3 generation. The higher number of randomly selected plants from F_2 progeny increased the possibility for determination of homozygous progenies (AA) totally resistant to TMV.
For realization of our goals, infestation was made of all individuals from each F3 progeny.
In hybrids where all hybrids showed necrotic reaction, complete resistance to TMV was achieved. It indicates that in relation to this character they are homozygous (AA), i.e. the character is permanently in their genome, which can be confirmed in F_4.
The resistant progenies should be further consolidated in relation to other morphological - productional and qualitative characters desired in the process of breeding.
In other progenies, which showed to be heterozygous (Aa) in F_2, as well as accidentally missed breeding materials up to F_3, F_4 or other generations, the monohybrid scheme of inheritance can be applied as soon as TMV resistant plants are determined.
Based on the monohybrid scheme of hybridization among above mentioned varieties, three homozygotus lines with resistance to TMV were created: Yk l.301/23, Yk l.65-82/1 and Yk l. 123/7. They are distinguished by their good productional and qualitative properties and present a good potential for the new tobacco varieties that should be included in tobacco production in the future(Dimitrieski M., Miceska G., et all.2005), as and three new homozygotus lines with resistance to TMV were created of prilep tobacco (Miceska G., Dimitrieski M. at all. 2005).

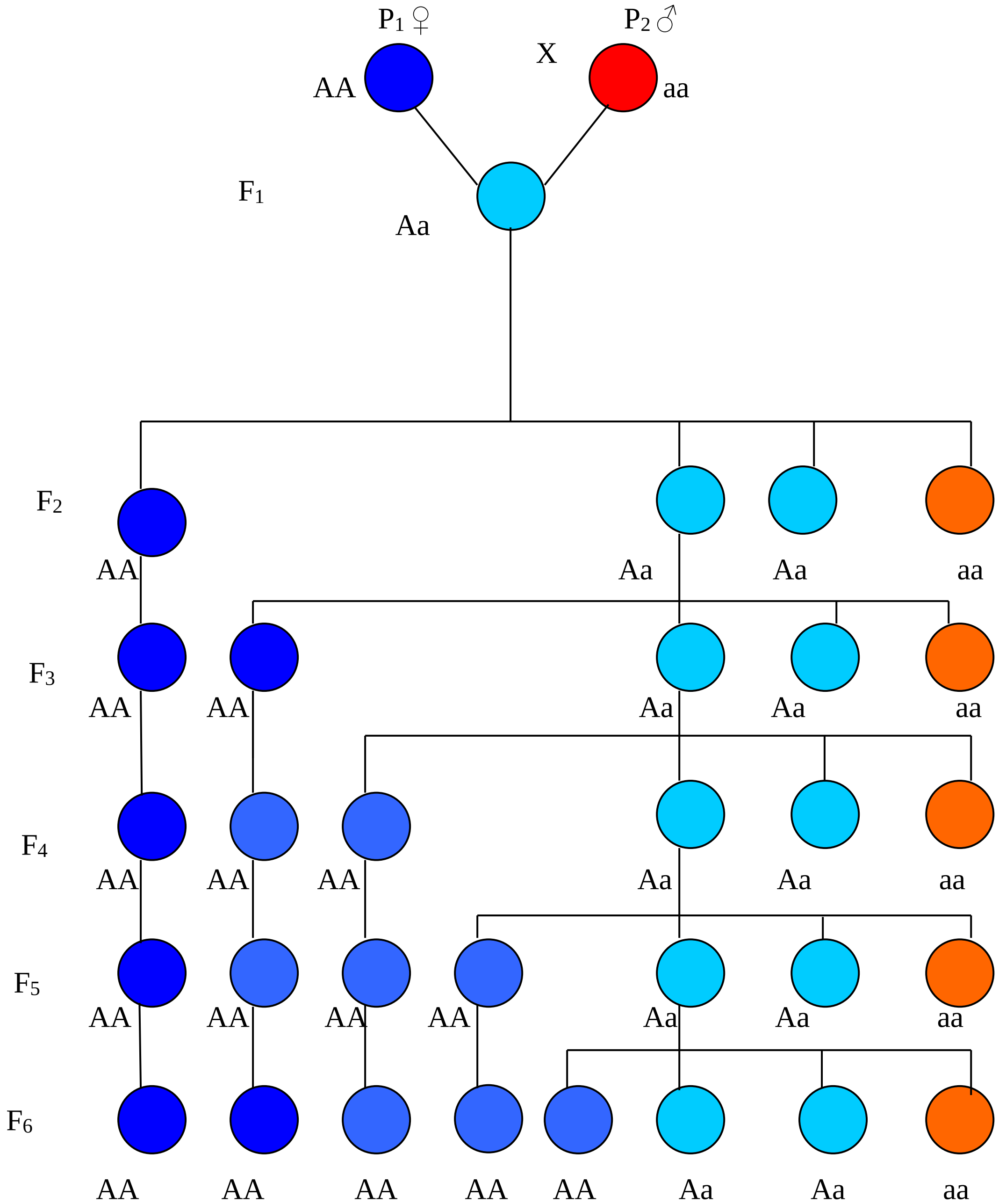
CONCLUSION

Investigations on monohybrid dominant inheritance of TMV resistance, in hybridization between introduced resistant oriental tobacco varieties and the susceptible varieties of the type Yaka, led to the following conclusions:
- A simple breeding scheme can be applied in creation of new tobacco varieties resistant to TMV, because the character inherited in a monohybrid dominant way is easy to control and also the method of inoculation is easily applicable.
- Monohybrid dominant inheritance of the character offers a possibility to isolate only one or two self-fertilized plants in F_1 and 8-10 plants in F_2, which show resistance to TMV. From these plants, special progenies will be created in F_3, and testing of all plants (60-80) will enable determination of homozygous F_3 progenies.

- The presented monohybrid scheme appeared to be highly rational and practical. As a result, three homozygous lines resistant to TMV have been obtained.(Yk.l.301/23, Yk.l.65-89/1 and Yk.l.123/7), which present the future potential and perspective of tobacco production.
- Beside selection of the character resistance to TMV, the monohybrid scheme allows simultaneous selection of hybrid individuals in relation to other quantitative and qualitative characters as well.



Scheme br. 1. Monohybrid dominant inheritance in F_1, F_2 and F_3 generation for the character resistance to TMV



Scheme 2 . Monohybrid inheritance of tobacco mosaic virus (TMV) with possibility for selection up to the F_6 generation

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EFFECTS OF CLIMATE CONDITIONS ON SOME QUANTITATIVE TRAITS OF BURLEY TOBACCO

Karolina Kocoska

Uneversity "St. Kliment Ohridski " – Bitola
Scientific Tobacco Institute - Prilep,
Kicevski pat bb 7500 Prilep, I. Macedonia
e-mail: karolina_kocoska@yahoo.com

ABSTRACT

Investigations were carried out in the Experimental field of the Scientific Tobacco Institute - Prilep during 2014 and 2015 with four foreign varieties of Burley tobacco (Hy-71Ø, B-963, SA-130, B-197/13) and two domestic male-sterile hybrid lines (B-209/13 CMS F1 and B-225/13 CMS F1). The trial was designed in randomized blocks with four replicates. Meteorological conditions during the growing season (June - September) were recorded in the Meteorological Station located in the field of the Institute. The aim of investigations was to study the influence of climate conditions on some quantitative traits of raw material obtained from Burley tobacco varieties and lines. The climate conditions were relatively good for production of Burley tobacco, but for normal development of plants during the driest months July and August, several irrigations of tobacco were applied. Tobacco was assessed according to the Uniform criteria for evaluation of raw tobacco quality. Data for each year of investigation were statistically processed using the method of analysis of variance and LSD test.

All varieties and lines grown in the same soil and climate conditions showed absolute dominance of the new lines B-225/13 CMS F1 and B-209/13 CMS F1 created in the Scientific Tobacco Institute - Prilep over the other varieties, which was statistically confirmed. From a practical point of view, these results can be a good guideline to tobacco growers in selection of tobacco variety.

Keywords: tobacco, variety, Burley, yield, line

ВЛИЈАНИЕ НА КЛИМАТСКИТЕ УСЛОВИ ВРЗ ОДРЕДЕНИ КВАНТИТАТИВНИ СВОЈСТВА ОД ТИПОТ БЕРЛЕЈ

Испитувањата беа направени во опитното поле во Научниот институт за тутун – Прилеп во текот на 2014 и 2015 година каде беа вклучени 4 берлејски фертилни сорти од странство (Ну-71Ø, Б-963, SA-130, Б-197/13), и две домашни машкостерилни хибридни линии (Б-209/13 ЦМС F_1 и Б-225/13 ЦМС F_1). Опитот беше поставен по методот Случаен блок ситем во 4 повторувања. Метеоролошките услови што владееа за време на вегетацијата (VI-IX месец) беа регистрирани од Метеоролошката станица лоцирана во кругот на Институтот. Цел на испитувањата беше да се испита влијанието на климатските услови врз одредени квантитативни својства на добиената суровина од берлејските сорти и линии. Климатските услови покажаа дека истите беа релативно добри за производство на типот берлеј, но потребата за вода за нормален развој на растенијата од овој тип на тутун во најсушниот период од месеците јули и август, тутунот беше неколки пати дополнително наводнуван. Проценката на тутунот се изврши по соодветниот - Правилник за единствени мерила за проценка на квалитет на суров тутун во лист од типот берлеј. Податоците од секоја година на истражувањето посебно беа и статистички обработени со помош на методот анализа на варијанса и LSD тестот.

Добиените резултати каде сите испитувани сорти и линии беа одгледувани во исти почвено климатски услови, покажаа апсолутна доминација на новодобиените линии креации на Научниот институт за тутун

– Прилеп, и тоа Б-225/13 ЦМС F_1 и Б-209/13 ЦМС F_1 над другите сорти чии резултати статистички беа потврдени. Од практичен аспект овие резултати во иднина можат да бидат добра насока при изборот на сорта од страна на примарните производители.

Клучни зборови: тутун, сорта, берлеј, принос, линија.

INTRODUCTION

Burley tobacco raw accounts for 30% - 40% in the composition of blend cigarettes. For this reason, production of large-leaf tobacco was introduced in R. Macedonia, in addition to the traditional oriental tobaccos. The first steps towards introducing Burley tobacco in R. Macedonia were made by Rudolf Gornik, who suggested (1953) that this tobacco can be successfully cultivated only in rich soils and humid climate with frequent rainfalls. In early 70-ies, efforts were made to create a variety that will be superior in most of the traits (primarily yield and quality). Burley tobacco is dried indoors under shade and is an integral part of blend cigarettes, but it is also consumed as pipe tobacco and chewing tobacco. Light, spongy tissue with high ability to absorb liquids is characteristic of Burley tobacco. It has high nicotine and protein content and only trace amount of sugar, as a result of long drying. This tobacco has a sharp taste (pungent ammonia) and is used in production of American blend-cigarettes, with up to 30% share (Stankovic, 2002, Georgiev, 2002, Radojčić, 2011). Several male-sterile varieties of Burley tobacco were created in Tobacco Institute - Prilep (B-96/85, Burley 1, B-2/93 and Pelagonec), each of them bearing specific characteristics, typical for Burley tobacco. Knowing that there is no ideal variety created once and for all, but some variety at a given moment is better than the others, the Department of genetics, selection and seed control in Tobacco Institute continued with creation of new varieties with improved yield and quality characteristics. Since these characters are governed by the genetic structure, parents in which these characters are predominant are used in the process of hybridization. This process is exclusively intervarietal and is conducted with the aim to obtain male sterile hybrid varieties. The best of them are tested in field, in comparative trials with other standard varieties (domestic and foreign) for a period of at least two years. If they show better results compared to the standard, they are submitted to the State Variety Commission for recognition. This paper presents the climate conditions in both years of investigation and their impact on yield (g/stalk and kg/ha) and quality of tobacco varieties and lines included in the trial. The yield of tobacco, as of many other crops, is affected by the genotype and genotype - environment interaction. It is a quantitative trait which is in close relation with number, size and thickness of the leaf. Different varieties of the same type of tobacco may have different yields, but still within the range typical for the type. Budim (1988) reported that the average yield of Burley tobacco in Zimbabwe in the period 1980-1985 ranged from 1202 to 1760 kg/ha. The development of selection of this tobacco in the world resulted in creation of new genotypes with significantly higher yields, without negative impact on quality. Stoyanov and Apostolova (1999) reported that the yields of B-1317 variety in some parts of Bulgaria can reach up to 3380 kg/ha. According to Djulgerski (2009), the yield of Burley tobacco should not fall below 3500 kg/ha. Risteski and Kocoska (2012) reported that Burley varieties created in Tobacco Institute- Prilep gave a yield of 3500-4500 kg/ha.

The yield of Burley type of tobacco is strongly affected by the applied agrotechnical measures. Pelivanoska (2001) reported that by the use of different variants of fertilization and irrigation, the yield of B-2/93 CMS F1 variety in the Ohrid-Struga region can reach up to 6000 kg/ha.

MATERIAL AND METHODS

The trial was set up in the Experimental field of Tobacco Institute – Prilep on colluvial-alluvial soil during 2014 and 2015 (Foto.1, Foto2). Investigations included four foreign Burley varieties in fertile form (Hy-71Ø, B-963, SA-130, B-197/13) and male-sterile hybrid lines (B-209/13 CMS F_1, B-225/13 CMS F_1), using Hy-71Ø as a check variety. Autumn ploughing was carried out at 40 cm depth and before spring ploughings the soil was fertilized with 300 kg/ha NPK 8:22:20. Prior to planting, the soil was treated with herbicide and then incorporated into the soil with a harrow. The trial was set up in 4 replicates using the randomized block design at 90 × 50 cm spacing. Two hoeings were performed and the plants were nourished with 5g 26% of KAN. During the growing season, several additional irrigations were made. Tobacco leaves were then stringed, matured and cured in special barns for Burley tobacco (air-curing). The qualitative assessment of cured tobacco was made according to the Rules for unique measurement of quality of Burley tobacco leaf. The adjusted yield per stalk and hectare were calculated according to the method of Rimker and the gross income (denars/ha) was obtained by multiplying the yield per hectare with the average price per 1 kg of raw tobacco. Variational-statistical processing of data was performed using the analysis of variance method (ANOVA). According to the records of the Weather Station located in the field of the Institute, meteorological conditions during the growing season (May-September) were relatively favorable for production of Burley tobacco. For better growth and development in the driest period (July/August), several additional irrigations were applied. The data for each harvest were statistically processed.

Foto 1. Experimental field of Tobacco Institute – Prilep 2014

Foto 2. Experimental field of Tobacco Institute – Prilep 2015

RESULTS AND DISCUSSION

- Meteorological data during the growing season in the years 2014 and 2015

Temperature and precipitations are major climate factors that have a decisive influence on yield and quality of tobacco and other crops. Data on the basic climate factors - temperature and precipitation in 2014/15 in the region of Prilep are presented in Table 1 and Fig. 1.

According to the meteorological data obtained from the Weather Station of Tobacco Institute – Prilep, the average mean monthly air temperature during the growing season in 2014 was 18.3^{0}C, while in 2015 it was 1.5^0 higher and reached 19.8^0 C. The maximum monthly average air temperature was 27.1^{0}C in 2014 and 28.9^{0}C in 2015. The minimum monthly average air temperature was 11.31^{0}C in 2014 and 12.41^{0}C in 2015, which indicates that there are no major differences between the two harvests. The mean monthly relative humidity of air was 53% in 2014 and 58% in 2015. Total amount of precipitation during the growing season May - September, was 223.0 mm in 2014 and 214.4 mm in 2015. According to the obtained data, the distribution of precipitation was uneven (Fig. 1), especially in June 2015, July 2014 and August 2015, but there was no significant difference with regard to the total amount of precipitation. For better growth, several irrigation interventions were applied during the growing season, depending on plant requirements.

Meteorological factors	Year	Months					Mean values by years
		May	June	July	August	September	
Mean monthly air temperature C^0	2014	14.1	18.7	20.9	21.7	16.0	18.3 C^0
	2015	16.5	18.7	23.8	22.1	18.0	19.8 C^0
Mean monthly maximum air temperature C^0	2014	21.8	27.7	30.2	31.6	23.9	27.1 C^0
	2015	25.4	27.2	33.7	31.7	26.5	28.9 C^0
Mean monthly minimum air temperature C^0	2014	7.7	11.4	12.9	13.6	11.1	11.3 C^0
	2015	9.1	11.4	14.7	14.2	12.5	12.4 C^0
Mean monthly relative air humidity %	2014	48	50	52	50	63	53%
	2015	59	61	48	56	64	68%
Total rainfall, mm	2014	21.0	26.0	12.0	48.0	107.0	214.4 mm
	2015	37.0	11.0	51.0	17.0	107.0	223.0 mm
Total number of rainy days	2014	6	4	5	6	12	33 days
	2015	6	7	4	6	10	33 days

Table 1. - Meteorological data 2014/15 (May – September)

Source: Report on project tasks Tobacco Institute -Prilep 2014
Report on project tasks Tobacco Institute -Prilep 2015

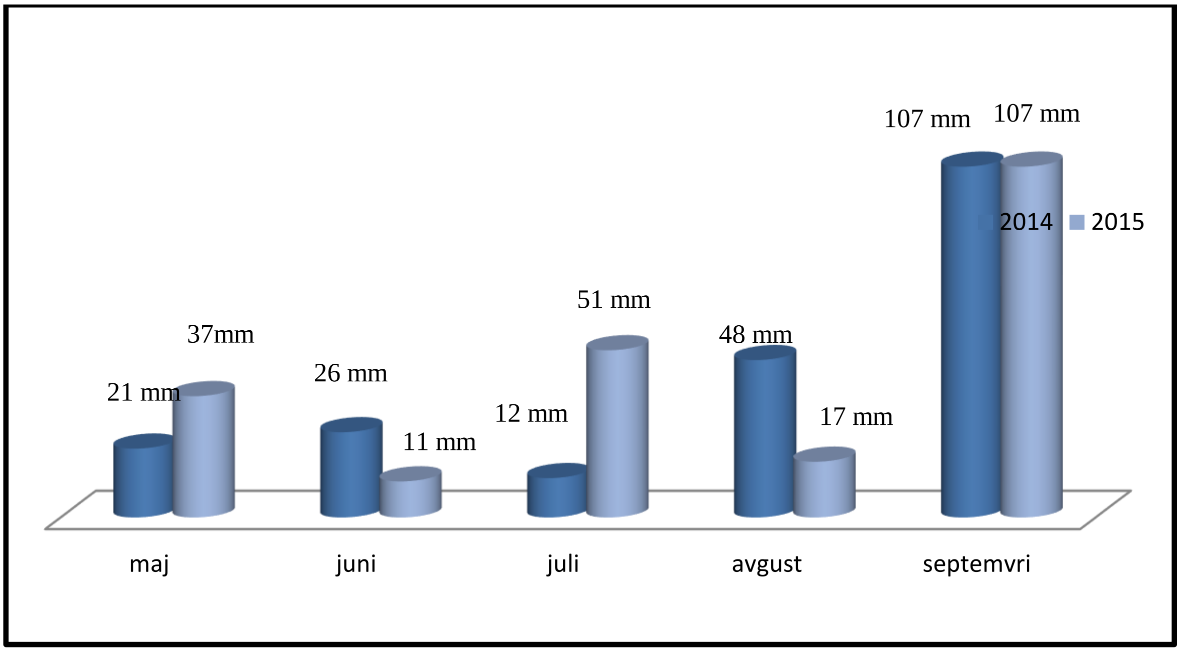

Fig. 1. Total precipitation in 2014 and 2015, in mm

Yield per stalk, g/stalk

According to the data in Table 2, the highest average yield of 163.7 g/stalk was obtained in line B-225/13 CMS F_1 and that is 80.0 g (95.58%) more compared to the check Hy-71, which has the lowest average yield of 83.7 g/stalk and is sixth-ranked variety. In other varieties and lines, the average yield ranged from 153.0 g/stalk in line B-209/13 CMS F1 to 111.6 g/stalk in variety B-963. In both years of investigation, statistically significant difference at 1% in relation to the check was recorded in lines B-225/13 CMS F_1, B-209/13 CMS F_1 and B-197/13, while the varieties SA-130 and B-963 reached 1% significance only in 2015. Statistical significance at 5% was recorded in variety SA-130 in 2014.

Table 2. Corrected yield g/stalk

Variety	Year	Yield g/stalk	Average 2014/2015	Difference		Rank
				Absolute	Relative	
Hy-71Ø	2014	78.44	83.7	/	100.00	6
	2015	89.01				
Б-963	2014	97.36	111.6	+27.9	133.33	5
	2015	125.93^{++}				
SA-130	2014	101.90^{+}	111.8	+28.1	133.57	4
	2015	121.64^{++}				
B-197/13	2014	111.71^{++}	120.5	+36.8	143.96	3
	2015	129.29^{++}				
B-209/13 CMS F_1	2014	143.75^{++}	153.0	+69.3	182.79	2
	2015	162.36^{++}				

B-225/13 CMS F_1	2014	149.47^{++}	163.7	+80.0	195.58	1
	2015	178.05^{++}				

2014 LSD 5% = 19.49 g/stalk + 1% = 27.00 g/stalk ++
2015 LSD 5% = 9.23 g/stalk + 1% = 12.78 g/stalk ++

Yield per hectare, kg/ha

The yield of tobacco, as in many other crops, is affected by the genotype and by the genotype : environment interaction. Tobacco yield is a quantitative trait which is in close correlation with leaf number, size and thickness. Different tobacco varieties within the same type achieve different yields, but they still must bear characteristics of the given type. Yield per hectare is closely related with quality per stalk. The combination of these two traits is a good indicator for assessing the economic value of the genotype. Apart from the impact of the variety, this trait is also affected by the applied cultural practices. During the harvesting of the stalks, constant removal of suckers was carried out. Berenji and Nikolić (1996) reported that topping of the inflorescence in Burley tobacco, combined with sucker control, can result in 28% yield increase per hectare.

Data on yield per hectare in the varieties and lines included in the investigation are presented in Table 3 and Fig 2.

Table 3. Corrected yield per hectare, kg/ha

Variety	Years	Yield kg/ha	Average 2014/2015	Difference		Rank
				Absolute	Relative	
Hy-71Ø	2014	1748	1863	/	100.00	6
	2015	1978				
Б-963	2014	2164	2481	+618	133.17	4
	2015	2798++				
SA-130	2014	2264+	2447	+584	131.34	5
	2015	2631++				
B-197/13	2014	2482++	2677	+814	143.69	3
	2015	2873++				
B-209/13 CMS F_1	2014	3194++	3401	+1538	182.55	2
	2015	3608++				
B-225/13 CMS F_1	2014	3325++	3641	+1778	195.43	1
	2015	3957++				

2014 LSD 5% = 434.27 kg/ha+ 1% = 601.45 kg/ha++
2015 LSD 5% = 209.56 kg/ha + 1% = 290.23 kg/ha ++

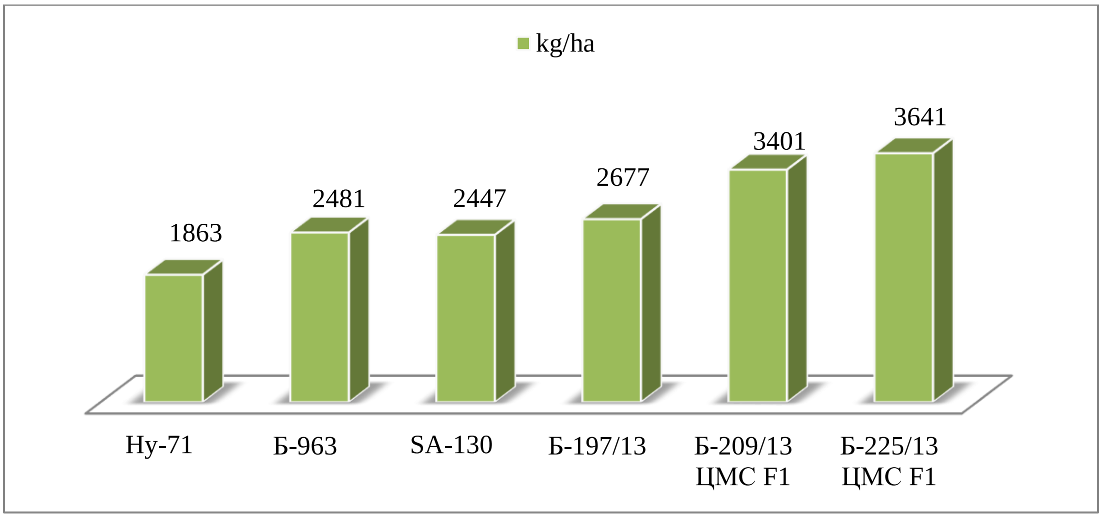

Fig. 2. Average yield, kg / ha

According to the data in the Table, the highest average yield per hectare of 3641 kg was recorded in line B-225/13 CMS F_1 and it was 1778 kg (95.45%) higher compared to the check variety Hy-71, which gave an average yield of 1863 kg/ha. In other varieties and lines, the average yield per hectare ranged from 3401 kg in line B-209/13 CMS F_1 to 2447 kg in variety SA-130 in both years of investigation (2014 and 2015).

CONCLUSIONS

-During the field trials, all varieties and lines developed and grew under the same conditions, but in the end they showed different results due to various reactions of the varieties dictated by their genetic structure.

- According to the obtained data, no big differences were recorded in the two years of investigation of maximum and minimum temperature, but the distribution of rainfalls during the growing season was very uneven, especially in June, July and August, so that additional irrigation interventions were applied depending on requirements.

- The highest average yield per stalk and hectare was obtained in B-225/13 CMS F_1 (163.7g/stalk), and the lowest in the check variety Hy-71Ø (83.7 g/stalk).

- The average yield per hectare was the highest in variety B-225/13 CMS F_1 (3641 kg/ha) and the lowest in the check variety Hy-71Ø (1863 kg/ha).

- The obtained results point to the conclusion that the variety has a crucial influence on some productional traits, but climate conditions during the growing season are also important. Therefore, when deciding which variety should be selected, it is highly important to have a good knowledge of the characteristics of variety, but also of the climate conditions and appropriate agrotechnical measures for its production.

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Original Scientific paper

A STUDY FOR INHERITANCE OF CHEMICAL COMPONENTS IN DRIED LEAVES OF TOBACCO VARIETIES OF DIFFERENT TYPES AND THEIR F_1 PROGENIES

Ana Korubin – Alesoska[1], Sime Dojcinov[2]

[1]University – St. Kliment Ohridski – Bitola, Scientific Tobacco Institute – Prilep
[2]Alliance One Macedonia - Kavadarci
e-mail: anakorubin@yahoo.com

ABSTRACT

Investigations were made with five tobacco varieties (Prilep P-23, Prilep P 8-9/80, Floria FL-7, Samsun S-1 and Virginia MV-1) and their four F_1 hybrids (P-23 x MV-1, P 8-9/80 x MV-1, FL-7 x MV-1 and S-1 x MV-1), for mode of inheritance for chemical components in dry leaves. The crossings were made in 2017, and the experiment with the parent genotypes and their hybrids was set up in 2018, on field trial in Scientific Tobacco Institute – Prilep in a randomized block design with four replications. All appropriate cultural practices were applied during the growing season.

The aim of this work was to study the mode of inheritance of the chemical components and to detect possible heterotic effects, which will allow a selection of lines with the most favorable chemical composition, and not to reduce yields and other quality features.

The results of the investigations indicate the fact that we got hybrids with reduced nicotine content, and favorable content of other chemical components. At the same time, we received valuable material for further selection activities. From the hybrids we singled it out S-1 x MV-1 as a cross with the best harmony of the chemical composition.

Keywords: tobacco, hybrids, inheritance, chemical components.

СТУДИЈА ЗА НАСЛЕДУВАЊЕТО НА ХЕМИСКИТЕ КОМПОНЕНТИ ВО СУВИТЕ ЛИСТОВИ НА ТУТУНСКИ СОРТИ ОД РАЗЛИЧНИ ТИПОВИ И НИВНОТО F_1 ПОТОМСТВО

Трудот опфаќа истражувања на пет тутунски сорти (Прилеп П-23, Прилеп П 8-9/80, Floria FL-7, Samsun S-1 и Вирџинија МВ-1) и нивните четири F_1 хибриди (П-23 x МВ-1, П 8-9/80 x МВ-1, FL-7 x МВ-1 and S-1 x МВ-1), за начинот на наследување на хемиските компоненти во сувите листови. Вкрстувањата беа направени во 2017 год., а полскиот опит со родителските генотипови и нивните крстоски беше поставен во 2018 год. во Научниот институт за тутун - Прилеп, по рондомизиран блок-систем во четири повторувања. За време на вегетацијата беа применети сите вообичаени агротехнички мерки.

Предмет на овој труд беше да се проучи начинот на наследување на хемиските компоненти и да се открие можен хетеротичен ефект, што ќе овозможи избор на линии со најповолен хемиски состав, а да не се намалат приносот и другите квалитетни карактеристики.

Резултатите од проучувањата укажуваат на фактот дека добивме хибриди со намалена содржина на никотин и поволна содржина на другите хемиски компоненти. Во исто време, добивме вреден материјал за понатамошна селекциона дејност. Од хибридите ги издвоивме S-1 x МВ-1 како крстоска со најдобра хармонија на хемискиот состав.

Клучни зборови: тутун, хибриди, наследност, хемиски компоненти.

INTRODUCTION

Chemical components and mutual harmony, especially nicotine content, make tobacco a natural means of enjoyment. There have been positives and anti-propaganda for centuries, but the importance of the ancient use of this plant, to this day, has not diminished its intensity.
Many authors have published their papers in which the main purpose are investigations of chemical components in the dried tobacco leaf, for example: Shah et al. (2009), in four barley varieties and one adapted barley variety as a control, and noted highly significant differences in genotypes for reduction of sugar levels and significant differences in nicotine content. Shah et al. (2017), realized two-year studies on the variability of 10 flue-cured hybrids, for more morphological and production properties, including the content of some chemical components. The studied hybrids showed insignificant differences in nicotine content and soluble sugars. Ahmed & Mohammad (2014), conducted two-year comparative trials of seven parent flue-cured varieties and their 42 diallel two-way F1 hybrids. at Tobacco Research Station - Mansehra (Pakistan), for more traits, including nicotine and soluble sugars. The analysis of variance reveals highly significant differences between genotypes and their interaction with the environment. The variety Spt G 28 had the highest percentage of nicotine and soluble sugars. Korubin – Aleksoska (2014), performed two-year studies in Scientific Tobacco Institute – Prilep on four tobacco varieties (three oriental and one semi-oriental), and their six F1 and the same number of F2 diallel crosses, for nicotine content, in order to investigate the genetics of inheritance, and revealed the predominance of partial dominance and intermediate, as well as the occurrence of heterotic effect. Ramachandra et al. (2015), made a genetic analysis of 62 genotypes (six lines of different types of tobacco, eight testers and their crosses) at the Agriculture research Station Nipaniat Belgaum – Karnataka (India), for more important quantitative traits and discovered two hybrids with optimal nicotine content. Kinay & Yilmaz (2016), in hybrids obtained by one-way crosses in the Turkish province of Tokat, they detected an average heterosis of 28.4%. Hybrids had about 16.6% less sugar and 10% more nicotine in dried leaves. Qaizar et al. (2019), at seven flue-cured genotypes and their two-way diallel crosses, in two locations (Mardan and Mansehra, Pakistan), for the inheritance of agronomic and biochemical properties and discovered positive and negative heterosis in the inheritance of chemical components in the dry leaf mass.
The aim of this paper is to study the mode of inheritance of chemical components, to detect a possible heterotic effect in F1 offspring and to determine guidelines for further selection activity.

MATERIAL AND METHODS

As a material of investigations, from the available assortment in Scientific Tobacco Institute – Prilep, we separated the following five varieties as parental genotypes: Prilep P-23 (Photo 1), Prilep P 8-9/80 (Photo 2), Floria FL-7 (Photo 3), Samsun S-1 (Photo 4) and Virginia MV-1 (Photo 5).
We used the large-leaf flue-cured variety Virginia MV-1 as a paternal parent, so with its pollen in 2017 we made the following four F1 hybrids: P-23 x MV-1 (Photo 6), P 8-9/80 x MV-1 (Photo 7), FL-7 x MV-1 (Photo 8) and S-1 x MV-1 (Photo 9).
They were tested the parent varieties and F1 hybrids by random block-system in four repetitions, in the experimental field at STI-Prilep, in 2018, on a working area of about 291.6 m^2, or on the total area of 655.2 m^2 (work area and paths). Large-leaf variety and F1 hybrids were planted at a planting distance of 90 cm (between rows) x 50 cm (between plants in a row), while the oriental varieties and the breeding line with a planting distance of 45 cm (between

rows) x 15 cm (between plants in the row).

The chemical components (percentage content of: nicotine, total nitrogen, proteins, soluble sugars and ash), in the dried tobacco leaves were determined in the accredited Laboratory for quality control of tobacco and tobacco products - L03 at STI-Prilep.

Mode of inheritance of the components was determined on the basis of test-significance of F1 generation in relation to the average of both parents, according to Borojevic (1981). Intermediate mode of inheritance (i) occurs when the mean value of one trait in the hybrid is equal to the parental average. Partial-dominant mode (pd), has when the mean value of hybrid offspring is approaching то one of the parent varieties. Dominance in inheritance (d), positive or negative, occurs when the mean value of the hybrid coincides with the mean value of one of the parents (+d - when a parent with a higher mean value dominates, -d - when a parent with a lower mean value dominates). Positive heterosis (+h) has a hybrid with a significantly higher value than that of the parent with a higher mean, while negative heterosis (-h) occurs in a hybrid with a significantly lower value than that of a parent with a lower mean value.

– Brief description of parental genotypes

Prilep P-23 is created by Kosta Nikoloski and Milan Mitreski. The variety belongs to the group of oriental tobaccos of the type Prilep. It is characterized by fir-habitus, stem 65 cm high, 45-50 seated leaves (20 cm x 10.5 cm). and semi-spherical compacted inflorescence with pink flowers (Korubin – Aleksoska, 2004). Belongs to the group of sun-cured tobaccos. The dried leaves have a golden yellow to light orange color, a delicate, elastic and compact leaf plate and a pronounced aroma. The dry leaf mass yield is 2000-2500 kg/ha (Photo 1.).

Prilep P 8-9/80 was created by Ana Korubin – Aleksoska. The variety belongs to the group of oriental tobacco of the type Prilep. It is characterized by a cylindrical to elongated elliptical habitus, the height of the stem with the inflorescence is 85 - 90 cm, there are about 45 sitting leaves light green with curly edges. The length of the largest leaf is 20 - 23, and the width 11 - 12.5 cm. The inflorescence is spherical with bright pink flowers. It belongs to the group of sun-cured tobaccos. The dried leaves are golden yellow to orange, with a moderately pronounced mainly rib and a thin weakly expressed secondary leaf veins. The yield of dry leaf mass is 2700-2900 kg/ha (Photo 2.). .

Floria FL-7 is a breeding linee, creation by Igor Bolsunov. The variety belongs to the group of semi-oriental tobaccos. It has a cylindrical elliptical habitus, the height of the stem with the inflorescence is 120 - 125 cm, анд about 30 sitting leaves. The length of the largest leaf is about 30 cm and the width is 17 cm. The leaves have a very delicate leaf plate. The inflorescence is spherical with bright pink flowers. It belongs to the group of sun-cured tobaccoc. The dried leaves are golden yellow to orange. This line has genes for resistance to blue mould (Peronospora tabacina Adam.). The yield of dry leaf mass is 2700-2900 kg/ha (Photo 3.).

Samsun, S-1 belongs to the group of Oriental Turkish tobacco of the type Samsun. The height of the stem is 80 - 120 cm, it has 28-36 heart-shaped leaves and a short handle, light green with curly edges. The length of the largest leaf is 20 – 23 cm, and the width 11 - 12.5 cm). The inflorescence is spherical with bright pink flowers. It belongs to the group of sun-cured tobaccos. Dried leaves have a yellow-reddish, orange or reddish-copper color, delicate, with a nice and pleasant aroma. The yield of dry mass is 700-1000 kg/ha (Photo 4.).

Virginia MV-1 is a large-leaf variety. Its authors are Dimche Cavkaroski and Mile Uzunoski. It is characterized by conical habitus, tall stem (195 cm), 26-29 sedentary leaves (55 cm x 35 cm) and a broom inflorescence with pale pink flowers. There is in fertile and male-sterile form (Korubin – Aleksoska, 2004). It belongs to the group of flue-cured tobacco. The dried leaves had a golden yellow color, pleasant taste and aroma. The yield of dry leaf mass is 3500 kg/ha (Photo 5.).

– Climatic and soil conditions in the area of investigations

The Earth's entire flora grows, develops and multiplies in favorable climatic and soil conditions and each change affectsто to the phenotype of the population. So with the tobacco plant, environmental factors make changes in a number of quantitative and qualitative traits, but those changes are differently limited, depending on the type of trait and the degree of its heredity. Therefore, in scientific research in terms of selection and genetics, it is necessary to take into account the environmental conditions in which the studies were performed. This is of particular importance in the analysis of chemical components, because their accumulation in the plant is directly dependent on changes in environmental factors.

During the tobacco vegetation in 2018, from May to September, the average monthly air temperature was 20,14^0 C, average monthly maximum air temperature 25,82^0 C, and the average monthly minimum air temperature 13,4^0 C. The average monthly relative humidity was 78.8 %. A total of 180.7 mm of precipitation fell during this five-month period.

Our research was conducted in the experimental field in the Scientific Tobacco Institute in Prilep on a deluvial (colluvial) soil type, without carbonates, characterized by low humus and total nitrogen content, moderately acidic to neutral reaction, low to extremely low security with easily accessible phosphorus and medium to good potassium supply.


Photo 1. P-23


Photo 2. P-8-9/80


Photo 3. FL-7


Photo 4. S-1


Photo 5. MV-1


Photo 6. P-23 x MV-1


Photo 7. P-8-9/80 x MV-1


Photo 8. FL-7 x MV-1


Photo 9. S-1 x MV-1

RESULTS AND DISCUSSION

The amount of chemical components in dried tobacco leaves is hereditary, but limited depending on environmental factors. For example, in dry conditions, or on nitrogen-rich soils, tobacco contains higher amounts of nicotine, while smaller amounts of soluble sugars. There are limits that are an indicator of quality oriental tobacco raw materials. It is known that nicotine in oriental varieties ranges from 0.8 to 2%. There are low-nicotine varieties in which the nicotine content does not exceed 0.7%. For proteins, a content of 4 to 9 % is considered an indicator of quality tobacco raw material. The content of soluble sugars is interpreted differently, but in oriental tobacco it should range from 15 to 25 %.

Our investigations was focused on the percentage of nicotine, total nitrogen, proteins, soluble sugars and ash in the dried leaves of the parent varieties and their four F1 hybrids.

The FL-7 variety has the lowest nicotine content among parents (0,37 %), and the highest P-23 (1.41%), while the lowest nicotine content among F1 hybrids has FL-7 x MV-1 (0,36%), and the highest P-23 x MV-1 (0,98 %). In inheritance of this component has the appearance of negative heterosis, that can be applied in tobacco production.

Negative heterosis in nicotine inheritance found: *Matsinger et al.* (1960), in the diallel crosses of flue-cured varieties, *Kara & Esendal* (1995), in the one-way diallel hybrids of the six Oriental varieties, *Butorac* (2000), in hybrids of four Burley varieties, *Korubin – Aleksoska* (2014), in the diallel crosses of 3 oriental and 1 semi-oriental varieties, *Qaizar et al.* (2019), in complete diallel of seven flue-cured genotypes.

The variety FL-7 is characterized with the lowest content of total nitrogen between parents (1,5 %), and with the highest MV-1 (2.78 %). While with the lowest content among F1 hybrids is characterized P 8-9/80 x MV-1 (1,57 %), and with the highest P-23 x MV-1 (3,27 %). Inheritance of total nitrogen is dominant in both directions, and there is a positive and negative heterosis.

The P 8-9/80 variety has the lowest protein content between parents (6,1 %), and the highest MV-1 (9,75 %), while with the lowest content among hybrids FL-7 x MV-1 (5,23 %), and with highest P-23 x MV-1 (8,43 %). Protein inheritance is intermediate and dominant with the predominance of the parent with lower content, and has a negative heterotic effect.

Different modes of inheriting of protein content in tobacco raw material have received and: *Butorac* (2000), in the hybrids of four Burley varieties, *Qaizar et al.* (2019), in a complete dial of seven flue-cured genotypes, etc.

The smallest content of soluble sugars has a large-leaf variety MV-1 (19,53 %), and the biggest FL-7 (28,68 %), while with the lowest content between hybrids P-23 x MV-1 (15,85 %), and

with the highest FL-7 x MV-1 (32 %). In the hybrids there is a positive heterosis (with the exception of P-23 x MV-1 where there is negative heterosis).
Heterosis in the inheritance of soluble sugars found: *Butorac* (2000), in hybrids of Burley tobacco varieties, *Ahmed & Mohammad* (2014), in a complete diallel of flue-cured varieties, *Kinay & Yilmaz* (2016), in Turkish tobacco hybrids, *Qaizar et al.* (2019), in two-way diallel hybrids of flue-cured genotypes, etc.
The ash content in the variants ranges from 8.64% in P 8-9/80 x MV-1, to 12.66 % in P-23 x MV-1. Inheritance of this component is dominant, and there is heterosis in both directions.,
Table 1. shows the percentage values for the chemical components in the dried tobacco leaves of the parents and the F_1 hybrids and the mode of inheritance, and Figure 1 shows the percentage size of the chemical components in the variants.

Table 1. Mode of inheritance of chemical components in dry tobacco leaf in diallel F_1 hybrids (%)

S.Nº	Genotypes and F_1 hybrids		Chemical components (%)				
			Nicotine	Total nitrogen	Proteins	Soluble sugars	Ash
1.	P-23	P1 (♀)	1.41	1.78	6.58	26.74	9.69
2.	P 8-9/80	P1 (♀)	1.16	1.63	6.10	27.10	9.80
3.	FL-7	P1 (♀)	0.37	1.50	6.23	28.68	9.59
4.	S-1	P1 (♀)	1.18	2.19	7.67	24.22	10.75
5.	MV-1	P2 (♂)	1.07	2.78	9.75	19.53	11.21
6.	P-23 x MV-1	F_1	0.98 $^{-h}$	3.27 $^{+h}$	8.43 ^{i}	15,.5 $^{-h}$	12.66 $^{+h}$
7.	P 8-9/80 x MV-1	F_1	0.67 $^{-h}$	1,57 $^{-d}$	6.51 $^{-d}$	29.52 $^{+h}$	8.64 $^{-h}$
8.	FL-7 x MV-1	F_1	0.36 $^{-d}$	1.63 $^{-d}$	5.23 $^{-h}$	32.00 $^{+h}$	9.67 $^{-d}$
9.	S-1 x MV-1	F_1	0.57 $^{-h}$	1.70 $^{-h}$	5.70 $^{-h}$	28.71 $^{+h}$	11.27 $^{+d}$

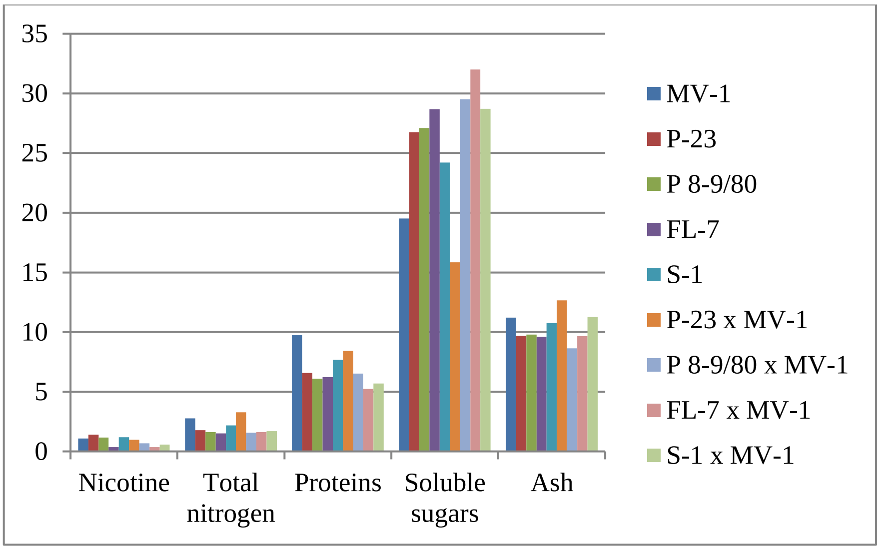

Figure 1. Display of the content of chemical components in dry tobacco leaves in parental genotypes and their F_1 hybrids (%)

CONCLUSIONS

From our investigations on the inheritance of chemical components in dry tobacco leaves from the varieties to their F_1 offspring, we have brought the following conclusions:

- The tobacco varieties that are the subject of this investigations differ significantly from each other and are characterized by a high degree of stability and equality, as a result of their homozygosity.
- There is a negative heterosis in inheritance of the nicotine content in dried leaves (only in FL-7 x MV-1 there is a negative dominance, ie dominance of the parent with lower alkaloid content).
- Inheritance of total nitrogen in dry leaves is dominant with a dominant effect in both directions, and there is a positive and negative heterosis
- Proteins are inherited intermediately and negatively dominant, and there is negative heterosis.
- Inheritance of soluble sugars has a positive heterotic effect, except in the hybrid P-23 x MV-1 in which there is negative heterosis, ie. dominance of the weaker parent for this character.
- In inheritance of ash content in the dry leaf mass occurs dominance and heterosis in both directions.
- With these investigations we obtained hybrids and provided material for further selection activity. From the hybrid variants, the cross S-1 x MV-1 can be distinguished (with the dominance of a more supportive parent), in which there is negative heterosis in nicotine inheritance and positive heterosis in the inheritance of soluble sugars.
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Original Scientific paper

EFFECTIVE CONTROL OF THE TOBACCO SEEDLINGS FROM DAMPING OFF DISEASE IN DUAL INFECTION IN BEDS

Biljana Gveroska

University "St. Kliment Ohridski"- Bitola,
Scientific Tobacco Institute –Prilep,
Republic of North Macedonia
e-mail:bgveros@yahoo.com

ABSTRACT

Damping off disease is caused by pathogenic fungi *Rhizoctonia solani* and *Pythium debarianum*. Its control is very difficult because of similarity of symptoms and use of non compatible fungicides for the target pathogens. The bigest problem is the dual infection which is very often, therefore the use of the proper control is necessary. According these facts, the aim of this study was to determine the most effective way of control.

The investigations were carried out in tobacco beds, with two applications of fungicides. They were chosen by instructions, results from artificial inoculation and the previous practice.

The most effective control is a fungicide who acts against two pathogens or combination who act separately to each pathogen or simultaneously.

The highest effectiveness is achieved by Quadris 25 SC (250g/l azoxystrobin) at 0.15% - 100% effectiveness and Signum 33 WG (267 g/kg boscalid + 67g/kg pyraclostrobin) at 0.1% - 85.05%.

Two combinations: Orvego (300g/l ametoctradin + 225g/l dimetomorph) at 0.1% + Manfil 80WP (800 g/kg mancozeb) at 0.25% - 88.64% and Orvego at 0.1% + Signum 33 WG at 0.1% - 84.23% effectiveness also achieved high results.

These fungicides are excellent chemical control for damping off disease in dual infection by two pathogens in tobacco seedbeds.

Keywords: damping off, fungicides, effectiveness, Quadris 25 SC, Signum 33 WG, Orvego

ЕФИКАСНА ЗАШТИТА НА ТУТУНСКИОТ РАСАД ОД БОЛЕСТА СЕЧЕЊЕ ПРИ ДВОЈНА ИНФЕКЦИЈА ВО ЛЕИ

Болеста сечење кај тутунскиот расад е предизвикана од патогените габи *Rhizoctonia solani* и *Pythium debarianum.* Нејзината заштита е доста тешка поради сличноста на симптомите и примена на некомпатибилни фунгициди за целните патогени. Најголем проблем е двојната инфекција која е доста честа, поради што примена на правилна заштита е неопходна.

Согласно овие факти, целта на ова истражување беше да се утврди најефикасната заштита.

Испитувањата беа изведени во тутунски леи. Направени беа две апликации на фунгициди. Тие беа одбрани врз основа на упатствата, резултатите од вештачката инокулација и практичните искуства.

Најефикасна заштита претставува комбинација од двата фунгицида кои делуваат одделно кон секој патоген или истовремено. Најголема ефикаспост беше постигната со Quadris 25 SC 0.15% (250g/l azoxystrobin) -100% и Signum 33 WG (267 g/kg boscalid + 67g/kg pyraclostrobin) 0.1% - 85.05%.

Двете комбинации: Orvego (300g/l ametoctradin + 225g/l dimetomorph) 0.1% + Manfil 80WP (800 g/kg mancozeb) 0.25% -88.64% и Orvego 0.1% + Signum 33 WG 0.1% - 84.23% исто така постигнаа добри резултати.
Овие фунгициди претставуваат одлична хемиска заштита од болеста сечење при двојна инфекција од двата патогени во тутунските леи.

Клучни зборови: сечење, фунгициди, ефикасност, Quadris 25 SC, Signum 33 WG, Orvego

INTRODUCTION

The significance of tobacco seedlings for tobacco production is well-known. The success of tobacco yield and quality depends on a healthy and quality tobacco seedlings. But tobacco seedlings production can be degraded by the occurrence of many diseases. The most destructive is damping off disease. It can occur in all stages of development of seedlings, starting from the germination of seeds to the full growth.

The causing agents of the disease are *Rhizoctonia solani* and *Pythium debarianum*. In many cases, there is the mutual infection. Therefore, control of the disease is very hard.

The soil pathogen *Rhizoctonia solani* is difficult to control because it is common in various soil types and has many hosts. The fungus causes serious damage to many important field and horticultural crops (Nunez, 2005).

Pythiace family (to which it belongs *Pythium debarianum*) are considered opportunistic fungi preying on weakness and taking advantage of conditions which are not ideal for the plant. They can reproduce sexually (in the form of oospores), as well as asexually. They are almost always found in the root system (Morel Diffusion, 2017).

The symptoms they caused are similar. The true determination is possible only with microscopy and isolation of the pathogen. But, in practice this is unachievable.

The chemical control is still the most effective method of protection from the pathogens. But, its control is very difficult because of similarity of symptoms and wrong determination which lead to the noneffective control i.e use of non compatible fungicides.

The biggest problem is the dual infection which is very often, therefore the use of the true control is necessary. Targeted chemical control strategies become limited when more than one pathogenic agent contributes to the disease as the application of the specific substance may not necessarily result in succesful disease management (Lamichhane and Venturi, 2015).

There are numerous data on the activity of certain active substances, respective for each pathogen.

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 fludioxonil, maneb, penthiopyrad, thiophanate-methyl, PCNB and azoxystrobin, with their trade names and modes of application (Schwartz and Gent, 2012). Azoxystrobin, trifloxystrobin, and tebuconazole are effective active ingredients against *Rhizoctonia* (Mocioni et al., 2003).

Azoxystrobin (Quadris) is recommended against root rot caused by *R.solani* (Bredehoert, 2012, Poindexter and Wenzel, 2013). For control of *R. solani* in soybean seedlings, the most recommended fungicides are strobilurins pyraclostrobin and trifloxystrobin (Mueller, 2014).

There are some reports for use strobilurins in tobacco. Azoxystrobin is used for control of *R.solani* in tobacco (Bertrand, 2012; LaMondia, 2012). Application of Quadris against this disease is also recommended by the manufacturer (Syngenta, 2006).

The most commonly used active ingredients in control of the disease is thiophanate-methyl. But, the fungicides Signum 33 WG and Quadris 25 SC acchieved higher effectiveness in the control of pathogenic fungus *R. solani* in artificial inoclation and natural infection in seedbeds (Gveroska, 2015).

Prasad et al. (2014), testing in vitro ten fungicides against *Rhizoctonia solani* and *Pythium debarianum*, estimated no equal results for two pathogens. Tebuconazole +Trifloxystrobin, Propiconazole, Captan+Hexaconazole and Carbendazim showed 100 % inhibition in control of *Rhizoctonia solani*. In inhibitin of *Pythium debarianum*, Metalaxyl, Propiconazole, Tebuconazole +Trifloxystrobin, Metiram+Pyraclostrobin, Captan+Hexaconazole amd Thiram showed the best results.
Mihajlović et al. (2013) have tested 5 fungicides and 1 biofungicide against *P. aphanidermatum* – *in vitro* and *in vivo*. They pointed toxicity of Quadris in vitro and 100% efficacy of Fosetyl-Al in greenhouse assays.
As solution of *Pythium* control Chase (2013) lists etridiazole, mefenoxam, fluopicolide, phosphonates, strobilurins and *Trichoderma* sp. In tobacco seedling control propamocarb is the mostly used active ingredients against *P. debarianum*. BAYER (2019) also offers propamocarb hydrochloride (Banol®).
Meadows et al. (2017) reported different active ingredients against *Rhizoctonia* (benomyl, azoxystrobin, thiophanate-methyl, iprodione) and *Pythium* (etridiazole and metalaxyl) as well as broad spectrum fungicides in chemical control of damping off in seedlings. They suggested combined application in treatment of unknown pathogen or mutual infection.

Despite the great number of active ingredients and fungicides, control of these pathogenic fungi in our conditions was limited. Recently, many new fungicides and active ingredients have appeared on the market. But, there were not any results for the use in tobacco. This was one reason that imposed these investigations.
Effective control has to reduce the damages and achieve healthy seedlings by the minimum costs for plant protection products. It has to be performed using the contemporary standards of Sustainable Tobacco Production, which refers to the use of chemicals, too.
According to the previous practice for the investigated fungicides as well as the results from artificial inoculation, the aim of this study was determined - to find the most effective control of damping off in dual infection on tobacco seedlings in seedbeds.

MATERIAL AND METHODS

Investigations were conducted in tobacco seedlings production in seedbeds, in Scientific Tobacco Institute-Prilep. The seedlings were cultivated in the usual way.
It was treated twice, in the stage before and the full development stage. Investigated fungicides –commercial names, active ingredients and used concentration are shown in Table 1.
They were applied in 3 replications of 10 m^2, with the use of 1L water for 10m^2.
They were chosen according to instructions, results from artifficial inoculation and the previous practice.
Isolation and determination of the pathogen/s were made from the infected plants in check plots, before the treatments i.e. before the each estimation.
Two assessments of the intensity of damping off disease - the percentage of infected area were made, 10-15 days after the each treatment. Effectiveness of the tested fungicides for the each assessment was calculated by the formula of Abbott.
Figure 1 shows the average value (from the two estimations) of the fungicide's efficacy in trials.

Table 1. Investigated fungicides

Fungicide (Commercial name)	Active ingredient / s	Concentration (%)
Top-M 70%WP	70% thiophanate –methyl	0.1%
Proplant 722 SL	722g/l propamocarb	0.15%
Enervin WG	120 g/kg ametoctradin + 440 g/kg metiram	0.2%
Signum 33 WG	267 g/kg boscalid + 67g/kg pyraclostrobin	0.1%
Orvego™	300 g/l ametoctradin + 225 g/l dimetomorph	0.1%
Quadris 25 SC	250 g/l azoxystrobin	0.15%
Top-M 70%WP + Proplant 722 SL	70% thiophanate –methyl + 722g/l propamocarb	0.1% + 0.15%
Signum 33 WG + Proplant 722 SL	267 g/kg boscalid + 67g/kg pyraclostrobin + 722g/l propamocarb	0.1% + 0.15%
Signum 33 WG + Orvego™	267 g/kg boscalid + 67g/kg pyraclostrobin + 300 g/l ametoctradin + 225 g/l dimetomorph	0.1% + 0.1%
Orvego ™ + Manfil 80WP	300 g/l ametoctradin + 225 g/l dimetomorph + 800 g/kg mancozeb	0.1% +0.25%

RESULTS AND DISSCUSION

Symptoms of the disease begins with the appearance of small watery spot at the base of the stalk which is starting to spread. The tissue becamec necrotic. So, the movement of water and nutrients is disabled, the plant falls on land as it is cuted - where the name comes from (Fig. 1a,b).

The tissue becomes disorganized, loses its firmness and take on a watery look. The young stalk is weakened and flops, bringing down the whole of the above-ground part of the plant. The disease may go so far as to make the plants literally dissolve and disappear from the surface, so it is also referred to as seedling melt (post-appartion melt) (Fig. 1c,d). Larger plants can get over thanks to their growth; the symptoms they exhibit are withering or yellowing of a leaf or a few leaves (Morel Diffusion, 2017).

The infection quickly spreads, infected fireplaces in its beds expand and converge, because much of the seedlings were destroyed (Fig 2 a,b,c). The danger is evident not only in seedlings production, but also in the whole tobacco production.

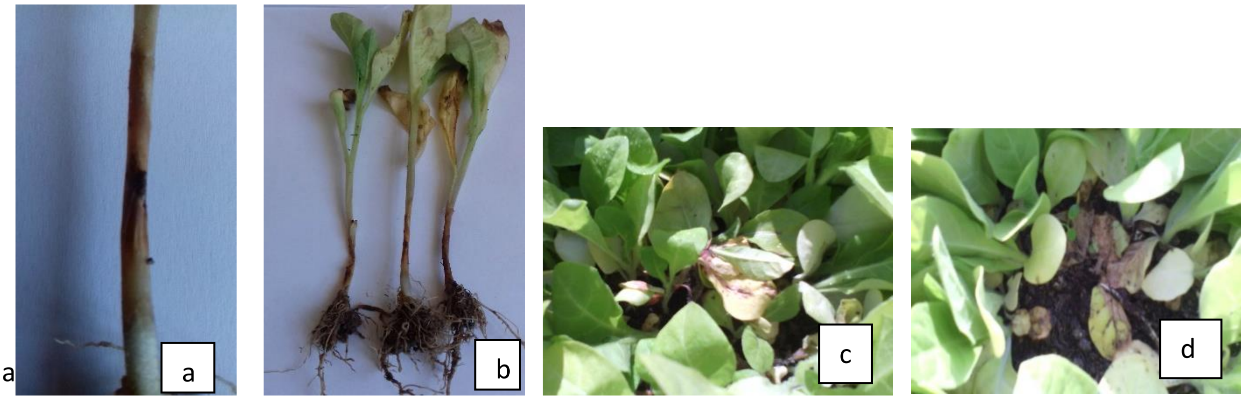


Figure 1 (a-d). Symptoms of the disease

Figure 2 (a-c). Expansion and merge of the infected sites by spreading the disease

The each test of the causing agent/s during investigations showed the presence of both pathogens *R. solani* and *P*. *debarianum*. Pure cultures of these fungi are presented in Figure 3 and 4.

They have different taxonomy i.e morphological, physiological, generic and reproductive characteristics (Ivanović, 1994). Both pathogenic fungi are well known damping off agents causing significant damage in nurseries (Blancard, 2012).

Symptoms they caused are similar and hardly to differentiating and their presence at the same time suggests that it is a double infection. According to Lamichhane and Venturi (2015) there are synergism between microbial pahtogens in plant disease complex. The universality of synergism need to better consider in the future that one pathogenic agent can team up with others rather than acting alone. Therefore, that might have important implications in plant disease epidemiology and consequent development of effective disease control strategies.

These efforts for successful damping off disease management in tobacco via these assays yielded the following results.

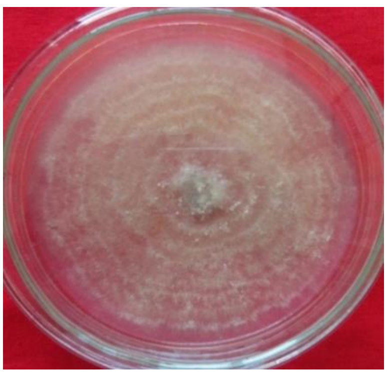


***Figure 3. Rhizoctonia solani* –pure culture**

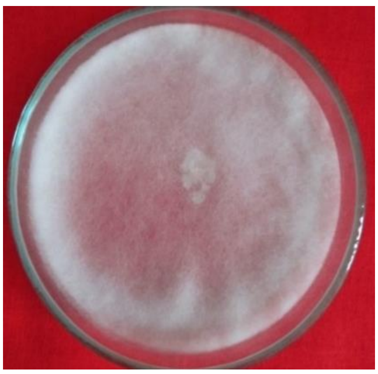


***Figure 4. Pythium debarianum* – pure culture**

The mean value (from individual replications) of disease intensity ranged from 0.06% in Proplant 722 SL to 0.19% in contact fungicide Enervin WG. The highest intensity was observed in the control, whereas in the seedlings treated with Quadris 25 SC no disease was present (Table 2).
According to intensity, the tested fungicides showed adequate efficacy. The lowest effectiveness was shown by the Enervin WG and the highest by Quadris 25 SC (100%).
In this rating Proplant 722 SL showed good efficiency – 82.35%. In contact fungicide Enervin WG it is only 44.12%. Other preparations and combinations showed about 70% efficacy, with the highest value in Orvego TM and its combination - Orvego TM + Manfil 80WP (79.41%).

Most of fungicides showed lower values at the second estimation. It ranges from 0.01% in Orvego TM + Manfil 80WP and Signum 33 EG + OrvegoTM to 0.26% in Enervin WG. However, treatment with Top-M 70% WP as well as the Signum 33 EG + Proplant 722 SL also showed a very low attack intensity – 0.02%. As the previous estimation, at Quadris 25 SC had no disease.
Calculated effectiveness has higher values than in the first estimation. It is the smallest in the Enervin WG - almost the same as in the first rating. Very high efficiency is achieved with Orvego TM + Manfil 80WP and Signum 33 EG + OrvegoTM – 97.87% as well as Top-M 70% WP and Signum 33 EG + Proplant 722 SL-95.74%. The effectiveness of Signum EG is also excellent -93.62%. Orvego TM has high value, too -82.98%. Application of Proplant 722 SL alone or in combination with Top-M had a lower efficacy -72.4% and 78.73%.

Table 2. Intensity of disease in the first estimation and effectiveness of fungicides

Variant (fungicide) Concentration (%)	Intensity Replication I	II	III	Average value	Effectiveness
Check Ø	0.36	0.42	0.24	0.34	-
Top-M 70%WP 0.1%	0.08	0.08	1.15	0.10	70.59
Proplant 722 SL 0.15%	0.09	0.07	0.02	0.06	**82.35**
Enervin WG 0.2%	0.23	0.13	0.21	0.19	44.12
Signum 33 WG 0.1%	0.04	0.11	0.09	0.08	76.47
Orvego™ 0.1%	0.04	0.09	0.08	0.07	79.41
Quadris 25 SC 0.15%	0	0	0	0	**100.00**
Top-M 70%WP +Proplant 722 SL 0.1% + 0.15%	0.11	0.04	0.09	0.08	76.47
Signum 33 WG + Proplant 722 SL 0.1% + 0.5%	0.08	0.12	0.1	0.10	70.59
Signum 33 WG +Orvego™ 0.1% + 0.1%	0.10	0,.1	0.09	0.10	70.59
Orvego ™ +Manfil 80WP 0.1% +0.25%	0	0.13	0.07	0.07	79.41

The average value of the fungicide effectiveness is presented in Graph. 1. The effect of fungicide's treatment is shown in Figure 5-7.

The least effective was contact fungicide Enervin WG, which is quite understandable. The standard Top-M 70% WP + Proplant 722 SL combination also had poor results.
Quadris 25 SC-100% achieved the best effectiveness during the tests. But also Signum 33 EG with 85.05%, as well as its combinations - Signum 33 EG + OrvegoTM (84.23%) and Signum 33 EG + Proplant 722 SL (83.17%).

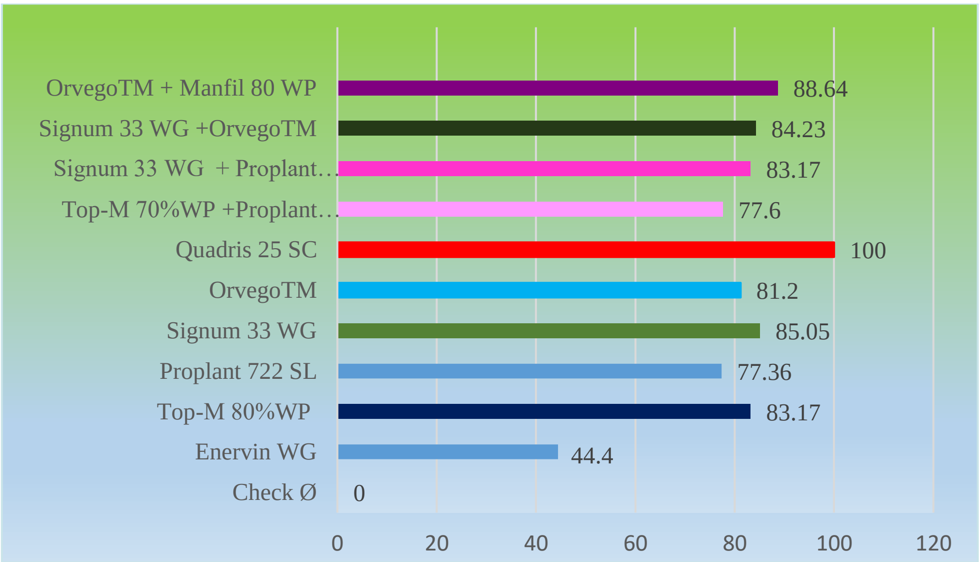
The high effectiveness (88.64%) was achieved with treatment with Orvego TM + Manfil 80WP.

Table 3. Intensity of disease in the second estimation and effectiveness of fungicides

Variant (fungicide) Concentration (%)	Intensity Replication I	II	III	Average value	Effectiveness
Check Ø	0.48	0.43	0.50	0.47	-
Top-M 70%WP 0.1%	0	0	0.07	0.02	**95.74**
Proplant 722 SL 0.15%	0.16	0.11	0.12	0.13	72.34
Enervin WG 0.2%	0.29	0.33	0.16	0.26	44.68
Signum 33 WG 0.1%	0.05	0	0.04	0.03	**93.62**
Orvego^{TM} 0.1%	0.12	0.13	0	0.08	82.98
Quadris 25 SC 0.15%	0	0	0	0	**100.00**
Top-M 70%WP +Proplant 722 SL 0.1% + 0.15%	0.11	0.12	0.08	0.10	78.73
Signum 33 WG + Proplant 722 SL 0.1% + 0.15%	0.01	0.06	0	0.02	**95.74**
Signum 33 WG +Orvego^{TM} 0.1% + 0.1%	0	0.04	0	0.01	**97.87**
Orvego ^{TM} +Manfil 80WP 0.1% +0.25%	0.04	0	0	0.01	**97.87**

Achieved effectiveness of strobilurins in these assays confirmed the findings of the others: (Bertrand (2012), LaMondia (2012), Syngenta (2006). Towards effective protection of many crops with Azoxystrobin against root diseases (Bredehoert, 2012; Poindexter and Wenzel, 2013; Schwartz and Gent, 2012; Koenning (2007) tobacco is also joining. This active ingredient showed the best effectiveness in the case of dual infection in the seedbeds. The efficacy of Quadris is due to the direct effect of azoxystrobin on the inoculum in the soil, as well as its effective combination with plant tissue (LaMondia, 2012).

According to BASF (2008), combination of pyraclostrobin and boscalid (Signum fungicide) - has preventive and systemic activity for use against certain diseases in various crops. This includes tobacco / tobacco seedling because this combination has shown high efficacy in control of the damping off disease. The efficacy of Signum may be due to the fact that the two active ingredients are an excellent combination of two different biochemical modes of action on cellular respiration of fungi (Hauke et al., 2004).



Graph 1. Effectiveness of the investigated fungicides (average value of two estimations)

Figure 5. Seedlings treated with Quadris 25 SC

Figure 6. Seedbed - Proplant 722 SL + Top M 70% WP

Figure 7. Seedbeds - Quadris 25 SC (left) and Orvego™ +Manfil (right)

Combination of many active ingredients with those of Signum with different mode of action achieved a big success. too. It has to be concluded that mixtures combine the effectiveness of different fungicides for broad-spectrum activity.

If the specific fungus causing damping-off is not known. Meadows et al (2017) suggested the broad spectrum fungicide or two specific fungicides (etridiazole + thiophanate methyl) either combination of fungicides (benomyl plus. etridiazole or metalaxyl). More precisely, each a.i. in combination should be acts against the specific pathogen in mutual control of *Rhizoctonia* and *Pythium.*

These statements are confirmed by these investigations, too. Combinations of fungicides (active ingredients), combination of a.i. with preventive and systemic action and use of fungicides with a wide range of activities had the best results in control of damping off in dual infection by two pathogens.

CONCLUSIONS

- Damping off disease in tobacco seedings production is a serious problem especially in the dual infection by two pathogens.
- This model of fungicide treatment offers a new opportunity for effective control disease.
- The most effective control is a fungicide who acts against two pathogens or combination who act separatelly to each pathogen or simultaneously.
- Fungicide Quadris 25 SC (250g/l azoxystrobin) 0.1% had the best effectiveness - 100%
- The highest effectiveness is achieved by two combinations: Orvego (300g/l ametoctradin + 225g/l dimetomorph) 0.1% + Manfil 80WP (800 g/kg mancozeb) 0.25% - 88.64% and Orvego 0.1% + Signum 33 WG 0.1% - 84.23% effectiveness.
- The common fungicides used even in their combination had a weaker results than new active ingredients.
- The pointed fungicides and combinations provide the excellent chemical control for damping off disease in dual infection by two pathogenic fungi in tobacco beds.
- These results contribute to enlarging the list of active ingredients for tobacco seedling protection.

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Original Scientific paper

EXAMINATION OF THE SIZES OF LEAVES OF PERSPEKTIVE LINES ECOTYPE „NEVROKOP" TOBACCO

Daniela Vitanova, Yovko Dyulgerski

Tobacco and Tobacco Products Institute (TTPI), Markovo, Bulgaria
(e-mail:yovk_dulg@abv.bg)

ABSTRACT

Eightt genotypes ecotype Nevrokop tobacco, of which six perspective lines and Nevrokop 1146 and Krumovgrad 90 varieties are studied for length and width of leaves, during the three year period (2010-2012) in the agro-ecological conditions of the Nevrokop region. The results of the study show that both the length and the width of the leaves in all the harvesting belts in first rank is Line 5, except for the width of the leaves of the highest harvesting belt, where the league is seated by Line 2. The latter also ranks second in the other surveyed indicators. This line is formed as the most typical in terms of the studied parameters. Created valuable selection material of oriental tobacco ecotype Nevrokop, and the newly-selected lines with the highest value are Line 5 and Line 2, which can successfully be included in a future breeding programs.

Key words: ecotype Nevrokop tobacco, new lines, length of leaves, width of leaves

ИСТРАЖУВАЊЕ НА ДИМЕНЗИИТЕ НА ЛИСТОВИТЕ НА ПРОСПЕКТИВНИ ЛИНИИ ТУТУН ОД ЕКОТИПОТ „НЕВЕРОКОП"

За време на тригодишен период (2010-2012 година) во агроеколошките услови на Неврокопскиот регион беа изучувани должината и ширината на лисјата на осум примероци од тутун од екотипот Неврокоп, од кои шест перспективни линии и сортите Неврокоп 1146 и Крумовград 90. Резултатите од истражувањата покажуваат дека и по должина и ширина збирот на лисјата во сите појаси, на прво место е Линијата 5, освен ширината на листовите од горниот појас, каде на прво место е Линијата 2. Втората, исто така, се наоѓа на второто место од другите проучени индикатори. Оваа линија е исто така создадена како најтипична во однос на испитуваните својства. Создаден е квалитетен материјал за селекција од ориентален тутунски екоотип Неврокоп. Од новоизбраните линии со најголема вредност се издвојуваат Линиите 5 и Линијата 2, кои можат успешно да се вклучат во следните програми за селекција.

Клучни зборови: тутунски екотип Неврокоп, нови линии, должина на лисја, ширина на лисја.

INTRODUCTION

Neurocope ecotype Oriental tobacco is grown in the area of Gotse Delchev and is of great importance for the livelihood of the local population (Dimanov, 2011; Dimanov and Masheva, 2011). This tobacco ecotype is one of our most valuable tobacco and its preservation is of great importance (Dimanov and Vitanova, 2011; Drachev et all, 2009; Nikolova et all, 2007, Nikolova, 2006, 2007). The breeding work with tNevrokop ecotype is at the heart of its further development (Dimanov et all., 2013).

One of the most important varietal characteristics of all types of tobacco is the leaf size (Masheva et al., 2004; Risteski et all., 2010; 2012). Leaf sizes are of great importance for both yield and quality (Tomov, 1985a, 1985b, 1989, 1991; Masheva, 2011). The length of the leaves from the lower belt and the middle belt determine the shape of the plant, which is an important variety trait (Dyulgerski, 2011). The length-to-width ratio, in turn, determines the shape of the leaves (Tomov, 1990).

The purpose of the present study is to characterize the leaf sizes of newly created tobacco lines of the Nevrokop ecotype for estimating their breeding value by these indicators.

MATERIAL AND METHODS

During the three-year period (2010-2012), biological indicators in the agro-environmental conditions of the Nevrokop region on eight tobacco samples from the Nevrokop ecotype are examined, including six perspective lines in the advanced generation, as well as the Nevrokop 1146 and Krumovgrad 90 varieties are used as controls. The first is the tobacco standard of this ecotype, and the second is used for control, as the most common variety in the area. The experience is derived from the Experimental Field - Gotse Delchev, Tobacco and Tobacco Products Institute, Markovo, Plovdiv,Bulgaria.

For field testing, a block method was used, with the area of the experimental plot being 20 m², each variant being tested in three replicates according to the method of Shanin and Barov, (1965). The conditions for plant cultivation are in accordance with the requirements of the Oriental tobacco varieties specific for the Nevrokop ecotype and are derived according to the accepted agricultural technology (Dimanov and Vitanova, 2011).

Biometric measurements of leaves were made. For this purpose, 50 plants of variants of length and width of 7th, 14th, 21st and 28th leaf were measured. The ratio of leaf length to width for each harvest belt was calculated. ANOVA and Duncan (1995) multivariate test is used to determine differences between the variants.

RESULTS AND DISCUSSION

1. Length of leaves

With the largest length of the 7th leaf in 2010, Line 2 and Line 5 are presented with almost the same indicators and Line 3 with the smallest indexes, and only it deviates by this index of the two standard varieties (Table 1). The greatest length of the 7th leaf in 2011 is characterized by Line 5, followed by Line 2 and Line 4 respectively, which are very slightly different from each other (Table 2). Again, Line 3 has the smallest length and is the only option that gives way to both standards. This year, all variants develop the smallest length of leaves compared to the other two years. With the largest length of the 7th leaf in 2012, again the first place is taken by Line 5, followed by Line 1, and again Line 3 is with the smallest length and is still the only variant which is inferior to both standard varieties.. (Table 3). This year, all variants, with the exception of the standard Krumovgrad 90 variety, develop longer leaf lengths than the other two years of study. Overall, there is a slight variation in this indicator over the years of the study.

Average for the period of the study with the longest length of the 7th leaf, Line 5 is formed, which exceeds with proven difference one standard Krumovgrad 90 variety (Table 4). Second place, but with still unproven difference of more than 2 cm from the first place is Line 2. The shortest length of the leaves from the lower harvest belt is represented by Line 3, which is inferior to both control varieties. With the exception of this line, however, all other newly selected lines outperform the two standard varieties - Nevrokop 1146 and Krumovgrad 90.

With the largest length of the 14th leaf in 2010 stands out Line 5, which is far superior to the other options in this respect. Second and third place with minimal difference from each other are taken by Line 2 and Line 3. All new lines exceed the readings of the standard varieties except Line 4, which is slightly inferior to Krumograd 90 variety (Table 1). The largest length of the 14th leaf in 2011 is characterized in Line 5 and the smallest in Line 3 (Table 2). This year, all variants develop the smallest length of this leaf compared to the other two years. With the largest length of the 14th leaf 2012, again the first place is taken by Line 5 and the last is Line 4 (Table 3). Overall, slight variation is observed over the years of study of this indicator, with the exception of Line 5, where variation is highly expressed.

Average for the study period with the longest leaf length of the 14th stands out Line 5, which is far superior to the other variants according to this indicator (Table 4). Second place with the same indicators are Line 2 and Line 6. Line 3 is aligned with the readings of the standard varieties, and Line 4, which is slightly inferior to them, has the smallest length of leaves from the middle harvest belt.

All variants have a shorter length of the 14th leaf compared to the 7th leaf, which is a prerequisite for the pyramidal shape of the plants.

With the largest length of the 21st leaf in 2010 stands out Line 5. Only Line 4 and Line 6 slightly are deviated to one standard - Krumovgrad 90 variety (Table 1). The largest length of the 21st leaf in 2011 is characterized by Line 5 and the smallest in Line 1, which is almost twice smaller from the length of the first one (Table 2). This year, all variants develop the smallest length of the 21st leaf compared to the other two years. The longest length of the 21st leaf in 2012 is again at the first place in Line 5, and the smallest length - the standard Nevrokop 1146 variety (Table 3). There is a little variation in this indicator over the years. All variants have a shorter leaf length on the 21st leaf compared to the 14th leaf.

On average over the study period with the longest leaf length of the 21st leaf - Line 5 stands out, which is far superior to the other variants of this indicator, although there is no proven difference to them (Table 5). Second in line with the same indicators are Line 1 and Line 6. The standard Nevrokop 1146 variety has the shortest length of leaves from the upper harvest belt.

The longest leaf size of the 28th leaf in 2010 is characterized in Line 5, and the smallest in the standard Nevrokop 1146 variety (Table 1). The same is true for the next 2011 (Table 2). The largest length of this leaf in 2012 is first Krumovgrad 90 variety, and the smallest one is in Line 1 (Table 3). Generally it is observed slight variation over the years of study of this indicator.

All variants have a smaller leaf length in each successive upper belt than the lower one, which determines the pyramidal shape of the plants.

Average for the period of study with the greatest length of the 28th leaf is presented Line 5 (Table 5). With a minimal difference, the second rank is Line 2. The standard Neurocope 1146 variety has the shortest leaf length of the uppermost harvest belt.

During all the years of study, Line 5 stands out strongly for all four long-leaf harvest belts.

2. Width of leaves

With the widest width of the 7th leaf in 2010, Line 2 and Line 5 are presented with almost identical indicators, and Line 3 is the smallest, with the only one falling behind this index of the two standard varieties (Table 1). The largest width of this leaf in 2011 is characterized Line 5, and again Line 3 has the smallest length and is the only variant that fits on one standard variety and is aligned with the other - in the case of Krumovgrad 90 variety (Table 2). This year, all variants have the smallest width of the 7th leaf compared to the other two years. With the largest width of the 7th leaf in 2012, again the first place is taken by Line 5, and in Line 3 is the smallest again and is still the only option that breaks the both standard varieties. (Table 3). There is almost no variation in this indicator over years of study.

During the study period, Line 5 is formed with the largest length of the 7th leaf, which exceeds the proven difference of both standard varieties (Table 4). Line 2 is placed in second place with a difference of less than 1 cm. from the first. Line 3, which is inferior to the standard varieties, is presented with the smallest length of the leaves from the lower harvest belt. With the exception of this line, however, all other newly selected lines outperform the two standard varieties - Nevrokop 1146 and Krumovgrad 90.

The greatest width of 14th leaf in 2010 stands Line 5, which strongly outperforms other options on this indicator. The second is Line 2. All new lines exceed the readings of the standard varieties (Table 1). Line 5 has the largest width of the 14th leaf in 2011, and all other new lines are inferior to this indicator by both standards (Table 2). This year, all variants develop the smallest width of this leaf compared to the other two years. The results obtained for the width of the 14th leaf in 2012 are similar to the results in 2011, except that the variants develop a wider width of this leaf this year (Table 3). Overall, slight variation is observed over the years of study of this indicator, with the exception of Line 5, where variation is strongly expressed.

Average for the study period with the largest width of the 14th leaf stands out Line 5, which is far superior to the other variants in this indicator (Table 4). On the second place with the same indicators are Line 2 and the standard Krumovgrad 90 variety. On the third place again with the same indicators is the other standard variety and Line 6, and Line 4 has the smallest leaf width of the middle harvest belt.

All variants have a smaller width of the 14th leaf compared to the 7th leaf.

The largest leaf width of the 21st leaf in 2010 is Line 2 followed by only 0.1 cm difference from Line 5. The smallest leaf width this year is Krumovgrad 90 vatiety (Table 1). The largest width of the 21st leaf in 2011 is characterized by Line 5 and the smallest Line 1, which is almost twice smaller compared to the width of the first one (Table 2). This year, all variants develop the smallest width of this leaf compared to the other two years. With the largest width of the 21st leaf in 2012, again the first place is Line 5, and the smallest - the standard Nevrokop 1146 variety (Table 3). There is a little variation in this indicator over the years.

All variants have a smaller leaf width of the 21st leaf compared to the 14th leaf.

Average for the study period with the largest width of the 21st leaf stands out Line 5, which is far superior to the other variants of this indicator, although there is no proven difference with them (Table 5). Second in line is Line 2. The standard Nevrokop 1146 variety has the shortest leaf length of above average harvest belt.

The largest leaf width of the 28th leaf in 2010 is characterized by Line 5, and the smallest with equal indicators are the standard Nevrokop 1146 variety and Line 1 (Table 1).

With the largest width on the 28th leaf in 2010, with the same indexes, the first rank is Line 5 and Line 2, and the smallest with the same indicators are the standard Nevrokop 1146 variety and Line 1. (Table 2). The largest leaf length in 2012 is Line 2, and second is Krumovgrad 90 variety. The smallest width of the 28th leaf is Line 1 (Table 3). Overall, there is a slight variation in this indicator over the years of the study.

On average Line 2 (Table 5) is presented by the largest 28th leaf during the study period. With a difference of 1 cm, second is Line 5. The standard Nevrokop 1146 variety, has the smallest width of leaves and the uppermost harvest belt. For the selection of the Nevrokop ecotype, it is important that the upper leaves are larger in size, and this is expressed in the tested lines.

All variants have smaller leaf width in each successive upper belt than the lower one. The ratio of leaf length is quite close both between the variants and the years and across the four harvest belts. In general, the leaf shape is typical for the Nevrokop ecotype tobacco.

In almost all variants in terms of all indicators, the worst results are obtained in 2011. While Line 5 exhibits expressed variation in leaf size in all harvesting belts, the other varieties have not been significantly identified, i.e. they are stable with respect to these indicators.

During all the years of study, in all four harvesting belts with the largest leaf sizes, Line 5 stands out strongly, and second is Line 2. This line also gives the leaves with the largest width in the upper harvest belt. Line 3 is with the smallest leaf.

The results of the study show that Line 5, followed by Line 2, has the highest economic value in terms of the studied indicators. The latter is presented as the most typical Oriental tobacco of the Nevrokop ecotype.

Table 1. Sizes of leaves (length and width) of the studied variants for 2010

Variety/Line	Length/ Width of 7th leaf	Proportion	Length/Width of 14th leaf	Proportion	Length/ Width of 21st leaf	Proportion	Length/ Width of 28th leaf	Proportion
Nevrokop 1146	20,7/13,0	1,6	16,0/9,5	1,7	10,7/6,4	1,7	6,8/4,0	1,7
Krumovgrad 90	21,2/13,0	1,6	17,2/10,3	1,7	14,8/8,6	1,7	13,1/7,2	1,8
Line 1	24,1/14,8	1,6	18,2/10,4	1,8	16,4/8,5	1,9	7,6/4,0	1,9
Line 2	25,5/16,8	1,5	19,8/12,8	1,5	15,6/10,4	1,5	12,2/8,7	1,4
Line 3	20,1/11,1	1,8	19,4/11,0	1,8	14,4/8,2	1,8	12,9/7,0	1,8
Line 4	22,0/15,4	1,4	16,5/10,8	1,5	12,8/7,8	1,6	12,7/7,0	1,8
Line 5	25,4/16,7	1,5	28,4/19,5	1,5	17,6/10,3	1,7	13,7/7,7	1,9
Line 6	23,1/13,8	1,7	18,7/11,6	1,6	13,4/7,3	1,8	11,4/6,0	1,9

Table 2. Sizes of leaves (length and width) of the studied variants for 2011

Variety/Lne	Length/ Width of 7th leaf	Proportion	Length/Width of 14th leaf	Proportion	Length/ Width of 21st leaf	Proportion	Length/ Width of 28th leaf	Proportion
Nevrokop 1146	18,5/12,2	1,5	15,6/9,4	1,7	7,9/4,9	1,6	5,8/3,6	1,6
Krumovgrad 90	17,4/10,7	1,6	14,3/8,8	1,6	7,2/4,5	1,6	6,8/4,2	1,6
Line 1	19,4/12,4	1,6	13,6/7,4	1,8	6,5/3,9	1,7	6,9/3,6	1,9

Line 2	18,5/12,6	1,5	13,2/8,1	1,6	8,9/5,9	1,5	8,2/5,4	1,5
Line 3	17,1/10,7	1,6	12,1/7,5	1,6	6,9/4,3	1,6	6,1/4,1	1,5
Line 4	19,2/12,6	1,5	13,0/8,2	1,6	6,8/4,2	1,6	5,9/4,0	1,5
Line 5	22,6/14,1	1,6	17,4/10,1	1,7	12,0/6,5	1,8	9,2/5,4	1,7
Line 6	18,2/11,8	1,5	14,1/8,3	1,7	9,7/5,4	1,8	7,7 4,4	1,8

Table 3. Sizes of leaves (length and width) of the studied variants for 2012

Variety/line	Length/ Width of 7th leaf	Proportion	Length/ Width of 14th leaf	Proportion	Length/ Width of 21st leaf	Proportion	Length/ Width of 28th leaf	Proportion
Nevrokop 1146	21,8/13,6	1,6	18,4/12,2	1,5	11,6/6,9	1,7	7,4/4,4	1,7
Krumovgrad 90	20,6/12,3	1,7	19,0/13,0	1,5	14,2/8,4	1,7	9,6/6,0	1,6
Line 1	24,9/14,4	1,7	18,5/11,6	1,6	13,9/7,6	1,8	6,1/4,0	1,5
Line 2	23,5/15,7	1,5	18,0/11,2	1,6	11,7/7,3	1,6	9,5/6,6	1,4
Line 3	20,3/11,4	1,8	18,6/11,8	1,6	12,4/7,4	1,7	8,0/4,4	1,8
Line 4	23,2/14,1	1,6	17,7/10,9	1,6	14,1/8,2	1,7	7,6/4,2	1,8
Line 5	25,7/16,8	1,5	22,7/14,7	1,5	18,4/11,7	1,6	8,3/4,6	1,8
Line 6	23,7/14,4	1,6	18,2/11,3	1,6	13,9/8,2	1,7	8,4/4,4	1,9

Table 4. Sizes of 7 and 14 leaf (length and width) of leaves of the studied variants average for the period of study

Variety/Line	7th leaf		14th leaf	
	Length	Width	Length/	Length/
Nevrokop 1146	20,3^{ab}	12,9^{bcd}	16,7^{ab}	10,4^{ab}
Krumovgrad 90	19,7^{b}	12,0^{cd}	16,8^{ab}	10,7^{ab}
Line 1	22,8^{ab}	13,9^{abc}	16,8^{ab}	9,8^{b}
Line 2	22,5^{ab}	15,0^{ab}	17,0^{ab}	10,7^{ab}
Line 3	19,2^{b}	11,1^{d}	16,7^{ab}	10,1^{ab}
Line 4	21,5^{ab}	14,0^{abc}	15,7^{b}	10,0^{ab}
Line 5	24,6^{a}	15,9^{a}	22,8^{a}	14,8^{a}
Line 6	21,7^{ab}	13,3^{abcd}	17,0^{ab}	10,4^{ab}
	SD = 0,73; GD 5% =1,161; GD1% = 1,609; GD0,1% =2,236 ;	SD = 0,54; GD 5% =1,570; GD1% =2,175; GD0,1% =3,022 ;	SD = 1,41; GD 5% =1,484; GD1%=2,056; GD0,1%=2,857 ;	SD = 1,26; GD 5% =3,032; GD1% =4,202; GD0,1% =5,837;

Table 5. Sizes of 21 and 28 leaf (length and width) of leaves of the studied variants average for the period of study

Variety/Line	21st leaf		28th leaf	
	Length	Width	Length	Width
Nevrokop 1146	10,1[a]	6,1[a]	6,7[a]	4,0[a]
Krumovgrad 90	12,1[a]	7,2[a]	9,8[a]	5,8[ab]
Line 1	12,3[a]	6,7[a]	6,9[a]	3,9[a]
Line 2	12,1[a]	7,9[a]	10,0[a]	6,9[a]
Line 3	11,2[a]	6,6[a]	9,0[a]	5,2[ab]
Line 4	11,2[a]	6,7[a]	8,7[a]	5,1[ab]
Line 5	16,0[a]	9,5[a]	10,4[a]	5,9[ab]
Line 6	12,3[a]	7,0[a]	9,2[a]	4,9[ab]
	SD =1,26; GD 5% =2,709; GD1% =3,755; GD0,1% =5,216 ;	SD = 1,15; GD 5% =2,473; GD1%=3,427; GD0,1% =4,761;	SD = 0,73; GD 5% =1,570; GD1%=2,175; GD0,1%=3,022;	SD = 0,58; GD 5% =1,247; GD1% =1,728; GD0,1% =2,401;

CONCLUSION

-Average over the study period Line 5 is represented by the largest length of the 7th leaf, the 14th leaf, the 21st leaf and the 28th leaf. This line also forms the largest width of the 7th leaf, the 14th leaf, the 21st leaf and the largest width of the 28th leaf is characterized by Line 2. Line 2 also ranks second on the studied other indicators.

-Line 5 and Line 2 are distinguished from the newly selected lines with the highest economic value.

-With the exception of Line 3, the other newly selected lines meet the requirements of the Nevrokop ecotype in terms of performance trials, and may be included in a future breeding programs.

-Line 5 can be used to increase leaf sizes as a donor for hybridization in breeding work. Therefor Line 2 can also be successfully used for the uppermost harvest belt.

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Original Scientific paper

WORLDWIDE STATES AND TENDENCIES IN CIGARETTE CONSUMPTION

Silvana Pašovska
"St.Kliment Ohridski" – University, Bitola, Scientific tobacco institute, Prilep,
"Kicevska" bb, Republic of Macedonia
e-mail: s_pasovska@yahoo.com

ABSTRACT

Consumption of cigarettes belongs to the group of conventional consumption of goods and as a relevant factor in personal consumption is characterized by the following:

- About 35% of the world's population, as consumers, participate in the cigarette market,
- The cigarette market is heterogeneous and smokers are differentiated from one another in many aspects: demographic, geographical, economic, etc. ,
- The way of buying and the motives for cigarette consumption are of an emotional nature-need-reason.

Cigarette consumption is influenced by a number of factors, including the tendency to smoke, the purchasing power of the smokers, the price of the cigarettes, the acceptance of cigarettes as a means of calming, relaxation, enjoyment, etc.

The World Health Organization (WHO), through the governments of many countries around the world, continuously influences the creation of various difficulties against the spread of smoking, thereby directly affecting the tobacco industry. The key measures to achieve this are the increase of excise taxes and taxes on cigarettes, the increase of their prices, as well as the prohibition of smoking in public places, offices, cabinets etc. This has the effect of creating an organized anti-smoking lobby against the tobacco industry. But such a WHO policy, in many countries in Eastern Europe and Asia where there is low purchasing power of the population, is leading to an increase in illegal cigarette trafficking, which reduces state budget revenues and directly damages the tobacco industry.

Despite such a WHO policy aimed at reducing cigarette consumption, however, with the globalization of the multinational companies, a greater capacity for cigarette production is being created, reaching a greater concentration on the production to a greater market control, and reduced production and trade costs. The effect of such movements is to increase the profits from the production of cigarettes and their sale. This will lead to a new cycle of investment in the tobacco industry and to all that it regenerates.

Keywords: cigarettes, consumption, smoking units, anti-smoking lobby, World Health Organization.

СОСТОЈБИ И ТЕНДЕНЦИИ ВО ПОТРОШУВАЧКАТА НА ЦИГАРИ ВО СВЕТОТ

Консумацијата-потрошувачката на цигари спаѓа во групата на конвенционална потрошувачка на стоки и таа како релевантен фактор во личната потрошувачка се карактеризира со следново:

- На пазарот на цигари учествуваат просечно околу 35% од населението во светот,како потрошувачи,
- Пазарот на цигари е хетероген и пушачите меѓусебно се издиференцирани од повеќе аспекти: демографски,географски,економски итн. ,
- Начинот на купување и мотивите за консумација на цигари се од емотивна природа-потреба-причина.

Врз консумацијата на цигари влијаат голем број фактори меѓу кои доминантни се:склоноста кон пушењето, куповната сила на пушачот, цената на цигарите, прифаќање на цигарите како средство за смирување,релаксирање,уживање итн.

Светската здравствена организација (СЗО), преку владите на многу држави во светот, континуирано влијае на создавање на разни потешкотии против ширењето на пушењето, а со тоа директно влијае на

тутунската индустрија.Како ударни мерки за тоа да се постигне се покачување на акцизите и таксите на цигарите, зголемување на нивната цена, како и забрана за пушење на јавни места, канцеларии, кабинети и др. Со тоа се влијае на организирано создавање на антипушачко лоби против тутунската индустрија. Но, ваквата политика на СЗО, во многу земји во Источна Европа и Азија каде постои ниска куповна моќ на населението, доведува до пораст на нелегалната трговија со цигари, која ги намалува приходите во државните буџети и нанесува директна штета на тутунската индустрија.Покрај ваквата политика на СЗО насочена кон намалување на консумацијата на цигари, сепак со глобализацијата на мултинационалните компании се создаваат поголеми капацитетни можности за производство на цигари, се доаѓа до голема концентрација на производството, до поголема контрола и владеење со пазарите, како и до намалување на производните и прометните трошоци.Ефектот од ваквите движења е зголемување на профитот од производството на цигари и нивната продажба. Тоа ќе доведе до нов циклус на инвестирање во тутунската индустрија и на се она што таа го регенерира.
Клучни зборови: цигари, потрошувачка, пушачки единици, антипушачко лоби, Светска здравствена организација.

INTRODUCTION

More than fifteen years have passed since the adoption of the 2003 Framework Convention on Tobacco Control (FCTC). During this period, the Convention has been continuously supplemented in terms of introducing additional measures for more efficient operation and restriction of smoking and reducing its impact on human health.

During this period, the President of the World Health Organization, who was appointed from 2008 to 2018, was replaced. WHO's president during this period was Margaret Chan, and how her engagement as WHO president succeeded in influencing the consumption of cigarettes worldwide through the FCTC measures, can be seen through the region's (continent) cigarette consumption that will be processed in the continuation of the paper.

PREDICTIONS IN EXPECTATIONS OF WORLDWIDE CIGARETTE CONSUMPTION

The World Health Organization analysis indicates that in 1997 there were 1,100,000,000 smokers in the world, and the prediction is that the number of smokers by the year 2050 to increase to 1,600,000,000, with a worldwide population of between 7,000,000,000 and 9,000,000,000. The movement of cigarette consumption worldwide in certain regions (continents) gives the state and the tendency of its movement. The data for the state of cigarette consumption for 2017 released by the World Bank where the same worldwide consumption per capita is shown, indicate the following facts:

Table 1. Worldwide cigarette consumption by region (continents) in thousands of cigarette units for 2017.

Continents	Number of countries	Population	Cigarette consumption	%
Europe	44	727.980.000	1.307.158.000	16.5
Asia	50	4.270.714.000	5.600.232.000	70.6
Africa	54	874.365.000	364.261.000	4,6
North and Central America	23	558.146.000	430.328.000	5.5
South America	12	407.040.000	183.344.000	2.3
Australia and Oceania	14	39.881.000	40.375.000	0.5
Total:		6.887.126.000	7.925.698.000	100

Source: World Bank

We can see from the table that the total consumption in the world for 2017 is 7.925.698.000 thousand smoking units or 7.925.698 conditional tons. To see how and how much cigarette consumption increased in 2017 compared to the cigarette production achieved in 2005 when 5,820,000 of conditional tones were achieved, which means, for the 12 years worldwide, the increase is 36%, ie an average annual increase of 3%.

Of the total cigarette consumption worldwide, 70.6% is in the Asian countries that are considered as developing countries. If we add to these Asian countries the growth of the African countries, then the consumption of cigarettes in developing countries will be 75.2%, while the developed countries of Europe and the countries of North and Central America will account for 22%. The rest of the 2.8% cigarette consumption belongs to the countries of Australia and Oceania and South America.

If we apply the growth rate of cigarette consumption worldwide, the production (consumption) of cigarettes would increase by 24%, ie 982,786,552,000 thousand smoking units by 2025.

The aforementioned indicators of world consumption movements for certain markets coincide with the predictions of the renowned tobacco analyst Lambert who analyzes the period from 2010-2015 for the movement of the worldwide cigarette consumption and concludes that the international cigarette market will reach 6,500,000 conditional tones. Of this amount, 70% will be realized in developing countries.

The total consumption of cigarettes in the world markets by regions is as follows:

Table 2. Cigarette consumption per capita worldwide in smoking units for 2017

Market Areas-Continents	Smoking units
Europe	1.800
Asia	1.540
North and Central America	800
Africa	420
South America	450
Australia and Oceania	1.000
Average units	1.000

Source: World Bank

The average cigarette consumption is 1000 smoking units worldwide. Europe has the highest per capita consumption, although it has the largest and most regulated cigarette consumption under the Framework Convention. This consumption of cigarettes per capita does not take into account the other tobacco products such as: electronic cigarettes, hand-rolled cigarettes, cigars, cigarillos, smokeless tobacco (chewing tobacco). If these tobacco products were also taken into account, then the consumption of cigarettes and other products would be higher.

The question arises whether the supply of tobacco raw material meets the needs of around 7,000,000 tones when there is a greater demand for tobacco products? This consumption of tobacco products would be a consequence of increasing tobacco production worldwide. It should be emphasized here that the actual tobacco consumption in cigarettes is actually 25% lower in a smoking unit depending on the commercial length of the cigarette. The average consumption per unit of cigarette ranges from 0.700 to 0.750 grams of tobacco. Thus, the world market for tobacco raw material meets the needs of the cigarette industry, and even more so as the multinational companies continue to seek to reduce the amount of raw tobacco in a cigarette beneath 700 grams.

In 2018, the Bulgarian Tobacco Magazine 1-2 / 2018 reported that in the European Union 80 million packages of A-20 cigarettes are consumed daily, and this amount is estimated at 29 billion packs of cigarettes annually. When these indicators are reduced to annual production (EU cigarette consumption), it would be 1,600,000,000 smoking units or 1,600,000 conditional tones of cigarettes spent. Based on data from 2017 according to the World Bank, in the EU, or in its 28 countries, 710.830.000 smoking units or 1380 smoking units per capita are spent or consumed. Based on this data on the total production of smoking units of 1.600.000.000 when we extract the consumption of 710.830.000 smoking units, the rest of the cigarettes produced in the EU member states is focused on the export outside the EU member states. The estimated cigarette consumption is 889.170.000 smoking units according to the World Bank. Annually, 35.540.000 packages of cigarettes are spent by the EU members. The EU's annual cigarette consumption according to the above estimated production is 1,700 smoking units per capita (2017). The difference between the estimation of cigarette consumption per capita and that from the year 2018 means that the difference between these two indicators is 0.320 smoking units or represents a stock of unsold smoking units.

Table 3. EU cigarette consumption in "000" smoking units

Country	Population in "000"	Production per capita	Total consumption in tones
Austria	8373	1,9	15908
Belgium	11827	2,4	28384
Bulgaria	7364	1,7	12518
Great Britain	63240	0,8	50592
Germany	81930	1,6	131090
Greece	11306	2,1	23742
Denmark	5544	1,3	7207
Ireland	4471	1,0	4471
Italy	60400	1,5	90600
Poland	38540	1,4	53956
Portugal	16640	1,1	18304
Romania	21360	1,2	25632
Finland	5577	1,1	6134
France	65447	1,1	71992
Netherlands	16620	1,5	24930
Croatia	4435	1,6	7096
Sweden	9394	0,7	6576
Spain	46073	1,5	69110
Czech Republic	10520	2,4	25248
Slovakia	5430	1,5	8145
Slovenia	2057	2,2	4525
Hungary	10013	2,1	21027
Lithuania	3229	1,3	4198
Latvia	2236	1,2	2683
Luxembourg	502	0,3	151
Malta	416	1,5	624
Estonia	1340	1,2	1608
Cyprus	800	2,0	1600
Total:	**514.814**		**718.051**

In the EU member states the cigarette consumption is 718,051,000 smoking units or 718,051 tones or 1,395 smoking units per capita.

Although the data are calculated from two different sources, they overlap in per capita consumption in the EU countries. Although EU member states most effectively implement the FCTC, the fact is that it has contributed very little in reducing smoking and implementing its measures to limit it.

Such behavior by the WHO and its authorities devalues the effectiveness and the impact of the Framework Convention on the basis of the results achieved in the production and consumption of cigarettes in the EU.

One of the measures most advocated by the WHO is to increase the excise tax on cigarettes, which has had a counter-effect, namely the hand-rolling of cigarettes by consumers or smokers has been expanded. In the EU, smokers are thought to use more than 50,000 tones of cut tobacco for hand-rolling cigarettes and this is on the rise. Besides the cigarettes that are essential in the production of tobacco products that are 90% or more, they also produce their own alternatives, such as electronic cigarettes, the smokeless cigarettes Snus (for chewing), and other types of cigarettes such as (Qas), the production of Philip Morris and its contents consists of 1/3 tobacco and 2/3 filter and this tobacco does not burn. Another alternative is the NEET cigarette. These cigarettes are packaged in 20 smoking units and have a limited number of breaths and they are with low flavor. All these phenomena and alternatives have little effect on the consumption of classic cigarettes.

The result of the measures taken by the WHO, according to the cigarette consumption data, was over 8,000,000,000 smoking units in 2017, without taking into account the hand-rolling of cigarettes that was spread around the world. Other tobacco products such as cigars, cigarillos, smokeless cigarettes, etc. are also not covered. It indicates that the WHO measures covered by the Framework Convention for these 10 years have had no particular effect, except that they have had little impact on the development of the dynamics of the tobacco industry. It is certain that with the change of the WHO leadership and the arrival of the Ethiopian Dr Tedros Adhanom Ghebreyesus as its head, it is expected that his policy and commitment to the tobacco industry will not be so aggressive and negatively oriented, but more tolerant and inclined. This finding is due to the real situation in cigarette consumption and the ineffectiveness of the Framework Convention's measures because of its universality and its weaknesses that it cannot take into account the different situations in countries around the world in terms of their particular unwritten market laws, the habits of the smokers, the religious and other characteristics, and above all the socio-economic needs that are different in the countries around the world.

The increase in excise taxes due to the emergence and the growth of the illegal markets has its impact and damages the markets of all countries where there is an illegal trade estimated at between 530,000,000-600,000,000 conditional tones of smoking units, that damages the countries' state budgets by over 40 billions of Euros (source-1/2017 Bulgarian Tobacco). From the first speech of the newly-appointed WHO President, it can be seen that tobacco and the issues of the tobacco industry are not his priority, or as he says he will pay more attention to five basic world problems such as: universal acceptable health, managing complex conditions in the area of health management, the welfare of women and children around the world, the consequences of climate change, and openness in decision-making and providing accountability for his work.

Source data on cigarette consumption and other tobacco products, as well as their alternative substitutes, show that the tobacco industry is an efficient and growing branch, and this is confirmed by the increase in tobacco raw material production worldwide. The tobacco industry is more concerned than the WHO because it has made great efforts to protect the consumer – smoker, by reducing tobacco raw material in a smoking unit by 30-40%, has also made efforts to offer the smoker different alternatives to nicotine and tar in cigarette participation, and it has succeeded in refining cigarette filters by increasing their absorptive power of retaining the harmful substances nicotine and tar. But unfortunately, despite all the facts, the opponents of the smoking lobby do not recognize these pledges and benefits.

CONCLUSION

From the previously presented observations and analyzes regarding the state and tendencies of cigarette consumption in the world, can be said that the measures taken by the World Health Organization in the direction of anti-smoking propaganda and reduction of cigarette consumption have a negative effect and efficiency. Namely, despite all the anti-smoking propaganda measures that have been taken, the results achieved are minimal and the statistical data obtained are contradictory. This means that the cigarette consumption worldwide is on the rise, with both men and women smoking, with an increasing tendency for women to smoke. With young smokers the smoking limit is rapidly decreasing, while with the older smokers the abstinence is within the limits of easy and voluntary renunciation or due to impaired health.

The tendency of the anti-smoking propaganda to reduce, or even to eliminate the tobacco use goes to the limits of forgetting the benefits that the tobacco industry gives to the producer countries, such as hiring a large workforce, gaining from foreign exchange, as well as huge state revenues that go directly into the state budget. In such a situation it is realistic to ask the question what would happen if this anti-smoking lobbyist's policy was implemented in practice? It will certainly be a serious blow to social instability and economic disintegration.

In this context it should be emphasized that the most popular measure to reduce cigarette consumption - increasing its price, has a short-term effect due to the fact that the cigarette consumption is inelastic consumption, where the increase of the price changes the structure of the consumption, but not its quantity.

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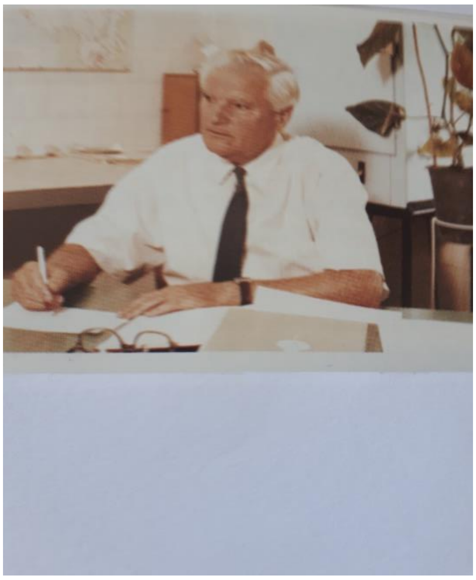
UDC: 929 BOGDANCESKI M.

IN MEMORIAM



Dr. Milan Bogdančeski
Scientific Advisor

On 18/09/2017, our dear colleague and associate, Dr. Milan Bogdančeski, suddenly passed away. Another eminent retired scientific counselor of the Department of Genetics, Selection and Seed Control of the Tobacco Institute – Prilep, has departed for good.

Dr. Milan Bogdančeski was born in 1931 in Varoš – Prilep, in a working-class family. He finished primary education and secondary tobacco school in Prilep and Faculty of Agricultural Science in Skopje, in 1958. Upon graduation, he worked as agronomy engineer in the Tobacco Combine – Prilep from 1958 to 1960, where he especially distinguishes himself. From 1960 to 1966, he worked in the Secondary Tobacco School – Prilep, as professor of some of the vocational courses in the field of tobacco processing. Here, Dr. Milan Bogdančeski especially rises to prominence with his expertise on the subject Tobacco Manipulation (domestic and industrial), where he significantly contributes to the theoretical and practical development of the middle-skill staff of tobacco technicians as per the requirements of the tobacco enterprises of the Republic of Macedonia and the region of former Yugoslavia. In September 1966, he began working in the Tobacco Institute in Prilep and stayed there until his retirement in 1996, in the Department of Genetics, Selection and Seed Control, which he heads for over 25 years. In 1967, he was promoted to the rank of assistant, then in 1972 he became scientific associate, in 1985 a senior scientific associate, and in 1990 – a scientific counselor. He completed postgraduate studies in the area of genetics and plant selection in 1971 at the Faculty of Agricultural Science in Skopje, where he also successfully defended his doctoral dissertation on the subject of "Examining the Heterotic Effect in the Macedonian Tobacco Varieties and Cultivars". In 1974, he had a brief study visit to the Tobacco and Tobacco Products Institute – Plovdiv, Bulgaria, and in 1981, he underwent specialization at the All-Union Tobacco and Makhorka Institute in Krasnodar, USSR. In the course of his work, he was involved as head researcher or associate in several scientific and research projects in the area of genetics and tobacco selection, submitted to the Ministry of Education and Science and Jugotutun AD – Skopje. Dr. Bogdančeski has published over 40 scientific papers both as writer and co-writer and he participated in multiple symposiums, congresses and councils in the country and abroad. Moreover, he was the first to author 6 tobacco varieties: 3 of the Prilep type, 2 of the Yaka type and 1 variety of the Otlja type.

In his thirty years of working experience in the Tobacco Institute, Dr. Milan Bogdančeski has provided significant contribution, both to the development of the scientific research and application in the area of tobacco genetics, selection and seed production, as well as of the primary tobacco production, for which we owe him immeasurable gratitude.

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UDC: 929 GRABULOSKI T.

IN MEMORIAM

M-r Tome Kiro Grabuloski

On March 15, 2019, the life of one of our esteemed colleagues, friends and parents ended. He was born on March 22, 1937 in Prilep. Primary and secondary education ends in Prilep, and the Faculty of Natural Sciences and Mathematics - group Chemistry ends in Skopje. where he graduated in 1962. After graduating, she works at the Borka Taleski Chemical and Food School in Prilep, as a professor of Organic and Analytical Chemistry.
Since 1964. works as an assistant in the Department of Tobacco Biochemistry at the Institute of Tobacco - Prilep. He completed his postgraduate studies at the Faculty of Chemistry-Skopje, in the group Organic Chemistry in 1978, and as head of the Department of Chemistry at the Institute was almost his entire working life.
He was the Deputy Director from January 1, 1986 to 1987, and the Director of the Tobacco Institute - Prilep from 1988-1993. He remained at the Institute until 2000, when he retired.
He has been repeatedly elected to the governing bodies of the Institute, and has been chairman of the Workers' and Scientific Council. He was a member of DNU-Prilep since its establishment. He actively participated in the creativity and development of the chemical profession of tobacco raw materials, cigarettes and raw materials related to the quality properties of tobacco and its processing. As the head of the Department, in the field of his profession he has studied and set, several methods for studying the biochemical components of tobacco and tobacco smoke, remnants of applied chemical preparations and others. He has presented his scientific and professional papers abroad and in the country at several symposia

and congresses. He has published 47 papers in the field of tobacco, which will contribute to many young people who will continue to work in this field of science.

Employees of the Tobacco Scientific Institute - Prilep, by emphasizing his life values, by emphasizing his professional and social engagement, pays great respect to him and let his name remain forever to be remembered.

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UDC: 929 LJUBEN VASILEV

IN MEMORIAM

Dr. Ljuben Vasilev
Scientific Advisor

The Scientific Tobacco Institute - Prilep is saying goodbye to Dr. Ljuben Vasilev, a retired scientific advisor, Head of the Department for Tobacco Protection from Diseases, Pests and Weeds, a prominent scientist, an expert, a social worker.

It is an enormous loss of a scientist who was always open to cooperate, with an optimistic attitude, a respected and a self-sacrificing colleague.

Dr. Ljuben Vasilev was born on November 27, 1933 in the village of Rajchilovci, Bosilegrad, Serbia.

He finished primary education in his hometown, and high school in Bosilegrad. He graduated from the Faculty of Agriculture and Forestry in Skopje, in 1957. After the graduation, from 1957 to 1960 he was working in the Agricultural Cooperative “11 Oktomvri” in the village Mazhuchiste, and after he finished his military service, he was working in the Regional Station for Plant Protection - Prilep.

In 1968 he was employed at the Tobacco Institute – Prilep. Initially, he was appointed assistant in the work unit Tobacco Protection at the time, when he stepped on the path of the scientific thought and he followed this path until his retirement - in 1997. Throughout his working life, he worked tirelessly on entomology, solving various problems in the field of tobacco protection.

He completed his master studies in 1971 at the Faculty of Agriculture in Skopje and in 1972 he was elected a scientific associate. In 1976 he defended his doctoral dissertation on the topic: “Biology and Ecology of the Tobacco Cotton Bollworm in the Socialist Republic of Macedonia.”

In 1977 he was elected Senior Scientific Associate, and in 1981 he was elected Scientific Advisor.

In 1980 he was appointed Head of the Department for Tobacco Protection, which he managed until his retirement.

During the accomplishment of his fruitful scientific activity, Dr. Ljuben Vasilev strived for and actively participated in the overall development of the Institute. Some of the project activities he coordinated are still being conducted.

In 1993 he was elected Vice-Rector of the University “St. Kliment Ohridski – Bitola”. He made a significant contribution to the commencement of the doctoral studies at the Public Scientific Institution Tobacco Institute - Prilep.

Dr. Ljuben Vasilev is a distinguished scientist and an expert in the field of entomology, a contemporary of the rise of plant protection, who made a great contribution to the development of this scientific field. He was a prominent member of the Plant Protection Association of the Socialist Republic of Macedonia and a member of the presidency of the Union of Plant Protection Associations of the SFRY.

He was also the holder of numerous social functions: President of the Republic Commission for Awarding Individual Tobacco Producers, Member of the Council for Scientific and Technical Creativity at the Assembly of the Socialist Republic of Macedonia, Vice President of the Association for Science and Art from Prilep, and for many years he was also the association’s Secretary, President of the Locust Destruction Staff, member of the Republic Trade Union Council for Innovations and Competitions, delegate of the self-governing interest-based community for scientific activities, President of the Agriculture Commission within the District Committee of the League of Communists - Prilep.

He specialized in Poland and in USSR at the time, and in June 1981 at the Federal Institute of Tobacco and Mahorka in Krasnodar (USSR).

He is distinguished for his fruitful scientific-research activity. He presented his achievements in the field of plant protection by participating in international symposia and congresses. With his speeches, he proved to be an experienced scientific worker in the field of plant protection. He attended a number of gatherings, symposia and congresses in our country in the field of tobacco.

He is the author of numerous scientific papers, as well as professional papers and publications.

Until his retirement, Dr. Ljuben Vasilev translated and conveyed his knowledge into a written word and passed it on to the younger generations. He was a good colleague, open to cooperate and help younger colleagues, who selflessly made efforts to solve specific problems. A dear scientist, an intellectual, who has left visible traces in the field of plant protection and the tobacco science and profession in general.

The staff of the Scientific Tobacco Institute - Prilep would like to express their gratitude and thank him for his contribution to the development of the scientific thought and the prosperity of the tobacco science and profession. His scientific achievements will be the impetus for future generations.

His character will be remembered in our hearts and memories.

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IN MEMORIAM

Dr. Josif Mickovski
Scientific Advisor

1925 - 2016

The Scientific Institute for Tobacco-Prilep has forever lost Dr. Josif Mickovski, a retired scientific advisor and a longtime Head of the Department for Tobacco Protection from Diseases, Pests and Weeds, as well as Director of the Institute.

The collective lost a respected director, a scientist who was always open to cooperate, and above all, a man who was adorned with wisdom, perseverance and empathy. His character will be remembered in our hearts and memories.

The dedicated space is so small to write everything about the scientific opus of Dr. Josif Mickovski. In fact, he left a rich archive of papers, articles, reports, etc. which testify to his scientific activity, to the rich creative opus, to the significance of the scientific results. This is only a small reminder of his character and work.

His loss is only a physical absence, because he is always present with his work. His book Tobacco Diseases is a kind of a Bible on the phytopathology of the tobacco culture. He laid the foundations of tobacco virology and worked on the most modern methods of his time. He vigorously led the chemical struggle with the blue mold, for which several active substances are still used as a solution today.

Dr. Josif Mickovski was born on July 7, 1925 in the village Golemo Crcko - Kichevo.

He finished primary education and high school in Bitola. He enrolled at the Faculty of Agriculture and Forestry in Skopje in 1946, and he graduated from this faculty in 1950.

During his studies he was an active member of the Committee of the League of Communists. After graduating, he was working in the regional united alliance in Kichevo. In 1953 he completed his specialization in plant protection in Belgrade and Zagreb. After completing his specialization, he returned to Prilep and established the scientific service for Tobacco Protection from diseases and pests at the Tobacco Institute in Prilep. He was engaged and participated in the organization of a series of actions for tobacco protection. During his working life, he tirelessly worked to solve problems in the field of tobacco protection.

With his scientific work, Dr. Josif Mickovski is differentiated as a prominent scientist and expert in the field of plant protection, who made a great contribution to the development of this scientific field.

In 1954 he was appointed Director of the Tobacco Institute. He held that position for 17 years, until 1960.

He received his PhD from the Faculty of Agriculture in Zemun in 1964 and after the review of his entire scientific activity, he was awarded the highest scientific title - scientific advisor.

He devoted his scientific thought to phytopathology and this bore fruit in the resolution of problems with tobacco diseases. He is distinguished for his fruitful scientific research activity. He is the author of approximately 95 scientific and professional papers that are important for science and practice. He presented his achievements by participating in numerous international congresses, symposia and conferences in Paris, Vienna, Rome, Lisbon, Athens, Hamburg, Montreux, Varna, Tokyo, Manila, etc.

His prolific scholar scientific-research work was internationally recognized and affirmed. He was one of the founders and a member of the scientific committee CORESTA (International Organization for the Study of Tobacco). He was also a member of the Yugoslav Commission for Cooperation with the United Nations Food and Agriculture Organization (FAO). As a United Nations consultant in the FAO for tobacco, he occasionally resided in Algeria, taking part in the overall issue of tobacco production in this country.

From 1960 to 1976 he was the editor-in-chief of the only Yugoslav magazine “Tutun (Tobacco)” and he was also appointed President of the scientific council of the Tobacco Institute in Prilep.

Being a longtime director, he showed outstanding managerial skills and succeeded to develop the Institute into a state-of-the-art, modernly equipped scientific institution that incorporates all scientific tobacco disciplines. He strengthened the image of the Institute as a recognized scientific institution and at the same time enabled its affirmation in the country and worldwide. He made a huge contribution to the promotion of the tobacco industry as a whole.

For special merits in the field of the tobacco science, he was awarded the award “3 Noemvri” of the town of Prilep, and he also received two gold plaques.

He was awarded the Order of Labor with gold wreath “9 Oktomvri” in 1965, and the Order of the Red Banner of Labour in July 1975.

Although he was retired, he lived with all the scientific findings in the field of phytopathology. He unselfishly shared his experiences, especially with the young staff, but also stimulated them to deepen their knowledge, to introduce new methods and solutions. Current and future generations are the indebted successors of his work. His scientific thought will continue to be the foundation for future generations, and his achievements will be a guiding star in the sky of modern science.

The Scientific Tobacco Institute - Prilep owes him immense gratitude for everything that Dr. Josif Mickovski did for the prosperity and the development of the Institute, for the science of tobacco and the tobacco economy.

INSTRUCTIONS TO AUTHORS

"Tutun/Tobacco" is published biannually (double issues).

Since the publication is of an international character, all manuscripts should be submitted in English. Authors whose native language is not English should have their papers checked by research workers from the related fields who have good proficiency in the English language. All manuscripts must be proofread prior to submission. Language and style of the manuscripts are responsibility of the author.

The publication presents:original scientific papers,review articles, short reports, professional papers and other works related to tobacco science and practice.

Original scientific papers-should contain original scientific research results, previously unpublished. They must be presented in a manner enabling the experiment, i.e. research method, to be repeated and accuracy of the analysis, results and conclusions confirmed.

Review articles-should contain critical surveys of the accomplishments in the fields encompassed in the Journal, papers by an individual researcher or a group of researchers with the purpose to undertake, analyze, evaluate or synthesize previously published information. They should present the latest ideas and theories or new scientific achievements.

Preliminary communications -should contain new scientific conclusions whose character suggests quick publishing. They do not have to enable repetition of the experiment and examination of the presented results and can be used as a basis for further research.

This part also contains Letters to the editor or Short notes.

Professional papers-should present useful contributions from the field of an applied science whose problematic is not related to the original research. The aim of these papers is not to present new findings but to use already acquired knowledge and implement it into practice.

Other articles published in this journal will not be categorized.

Manuscripts should be submitted to the Editorial Board in typescript and electronically, on CD and via E-mail (tobaccotip@yahoo.com). Papers must be written in a clear and concise manner using Times New Romanand 12 pt font size, with single spacing. The complete manuscript should be no longer than 10 pages, A4 format, with margins2.5cm for all sides. Text must be justified (no tabs), without hyphenation, avoiding excess white space between words. The Abstract should be translated in Macedonian, using Times New Roman font with Macedonian support.

Manuscripts should follow the format INTRODUCTION, MATERIAL AND METHODS, RESULTS AND DISCUSSION AND CONCLUSION, for experimental research where events are presented in chronological order.

Titles in the text (INTRODUCTION, MATERIAL AND METHODS, RESULTS...) should be centered, boldfaced, written with capital letters, font size 12; **Subtitles** should be written with initial capital letter, boldfaced, 12-point font size, aligned to the center;
Titles and subtitles must be separated with 1 empty row.

Arrangement of the paper:

Title-in capital letters, boldfaced, 12-point font size, aligned to the center;
Full name and surname of the first author-capital initial letter, other letters small, font size 12, centered;

Name of the institution-for multiple authors from different institutions, each author's surname should be followed by identifying superscript number associated with the appropriate institution.
Address of the institution–full postal address of the institution, as well as the e-mail of the principal author; italic,centered.

ABSTRACT-at the beginning of the paper, both in English and Macedonian, should not exceed150 words. It should mention the techniques used without going into methodological detailsand should summarize the most important results. Abstracts should not include citations toreferences.Font size 10, centered.

Keywords-up to 5essential words, in English and Macedonian.
For non–Macedonian authors,the Editorial board will provide translation of title, abstract and keywords in Macedonian.

INTRODUCTION should provide a brief statement of the subject, comprehensive survey of the relevant lietrature and objectives of the paper;

MATERIAL AND METHODS should be short and concise. Well-known techniques and methods should be indicated by a reference: only new methods or relevant modifications should be described in sufficient detail to allow reproduction of the investigation by others;

RESULTS AND DISCUSSION should be presented in tables, figures, diagrams and photographs, which must accurately describe the findings of the study, ordered sequentially as they appear in the text;

Tables should be numbered with Arabic numeralsaccording to their sequence in the text. The table title should be always above the table,centered, in 10 pt font, with one empty row between the title and the table and another one between the table and the text.Tables should be simple and should not duplicate the information given in figures.

Reference to the Table, example: It could be seen from Table 1...., or: The nicotine content in tobacco is 0.98% (Table 4).

Illustrations should be numbered consecutively in Arabic numerals, with centered titles below each of them.

All graphical presentations (including graphs, schemes, drawings, photographs etc) should be submitted on CD together with the text. Minimum resolution for images is 200 -300 dots per inch.

References in the text citations in the text should consist of the author's last name and the year of publication in parenthesis (Miceska, 2010) Dimeska et al. (2007), Tso et al. (1990), (Adamu1989, Campbell 2000). Each citation must correspond to the Reference list at the end of the paper.

Nomenclature of genera and species names must agree with the International Code of Zoological Nomenclature(ICZN, latest edition). Taxonomic affiliation, followed by author(s) and year of description, should be presented in complete form at least once in the main text (usually when first mentioned),and in subsequent appearances only the abbreviated form is presented (MetasyrphuscorollaFabricius, 1794 asM. corolla).

Units–measurements should be given in SI units.

CONCLUSIONS should provide a brief and clear summary of the study findings and their contribution in science and practice.

REFERENCE LIST -is arranged alphabetically, in the following order: surname and initial of author(s) first name, year of publication, title of the article, name of publication, volume number and page.
For books, author's name, complete title, publisher and date of publishing should be listed.

For journals:

Mickoski J., 1988. Ispituvanje na infektivnata sposobnost na peronosporata i pepelnicata na tutunot. Tutun/Tobacco 1-2, 21-40, Institut za tutun-Prilep.
Weybrew J.A., Wan Ismail W. A., Long R. C., 1983. The cultural management of flue-cured Tobacco quality.Tob. Sci. 27, 56-61.

For books: Russel E. W., 1973. Soil conditions and plant growth. 10th ed., Longman, London.

References are cited on the language of original papers. In literature references, use the International Serials Catalogue for abbreviation of journal names.

NOTE: Manuscripts that are not arranged and submitted according to the above instructions, will not be taken in consideration for reviewing and publishing.

Тутун/Tobacco
Tobacco Institute 7500 Prilep
Kicevska bb, Republic of Macedonia
E-mail: tobacco_institute_ prilep@yahoo.com