

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

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### ABSTRACT

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

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

### ПРОИЗВОДНИ КАРАКТЕРИСТИКИ НА НЕКОИ ОРИЕНТАЛСКИ ЛИНИИ ТУТУН ОТПОРНИ НА ЦРНИЛКАТА (*Phytophthora parasitica* var. *nicotianae*)

Со изведените истражувања си поставивме за цел врз основа на добиените експериментални резултати да се утврдат морфолошко-производните и квалитетните својства, како и отпорноста спрема болеста црнилката (*Phytophthora parasitica* var. *nicotiana*) на 4 новосоздадени фертилни инбрид линии тутун во споредба со стандардната сорта ЈВ 125/3 (Ø неотпорна). Проучуваните линии се добиени по пат навнатревидова (меѓусортова) хибридизација. Селекциониот процес започна со вкрстување на странски ориенталски сорти, како и наши ориенталски стабилни линии тутун – отпорни на црнилката (AA), кои се користеа како мајчина компонента, а како таткова беа користени наши неотпорни ориенталски сорти и линии тутун (aa). Одбирањето од хибридниот потомство е вршено по методот “Педигре”. Испитуваните линии се консолидирани и по однос на висината на растенија, бројот, формата и големината на листовите. Според добиените резултати од полските опити новосоздадените отпорни линии се одликуваат со поголем број на листови по растение (45- 53 лист./растение) во однос на ЈВ 125/3(Ø) (37 лист./растение), повисок принос kg/ha (од 33,66 до 68,80%), повисока откупна цена ( од 14,3 до 27,7%) и поголем

економски ефект (41,01 до 81,29%). Отпорноста на испитуваните сорти и линии тутун спрема причинителот на болеста црнилка (*Phytophthoraparasitica* var. *nicotianae*) беше проучувана во текот на 2010 година во услови на вештачка инокулација во заштитен простор-стакленик (биолошката лабораторија) на Научниот институт за тутун- Прилеп. Од проучуваните 4 линии тутун, три се оценети како високоотпорни линии на црнилката, овие линии освен што ќе можат да најдат примена во производството, ќе можат да се користат и како извори на отпорност во селекционите програми.

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

## INTRODUCTION

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

chemicals in control of this disease. Current world tendency is to reduce the use of chemicals by introducing resistant varieties in tobacco production (Palakarcheva 1986, cited by Trancheva 2000).

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

## MATERIAL AND METHOD

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

The newly created resistant lines were obtained by intraspecies hybridization, using foreign resistant varieties and domestic non-resistant oriental varieties and lines as its components. They are

genetically stable and consolidated in terms of plant height and leaf number, shape and size.

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

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

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

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

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

- 0 - highly resistant- **no visible infection**
- 1 – resistant- **10 % infected plants**
- 2 - – moderately resistant - **40 % infected plants**
- 3 - susceptible- **50% infected plants**
- 4 – highly susceptible- **50% - 100% infected plants**

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

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

## RESULTS AND DISCUSSION

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

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

23/10 and Yaka l. 22-82/10 (53) and the lowest in the standard Yv. 125/3 (37).

From the data presented in Table 1 it can be seen that standard variety Yv 125/3 has the lowest values for the biggest leaf, with an average size of 19,7 cm in length and 9.5 cm

in width. Line Yk. 1. 301/23 has the highest leaf length (25.1 cm) and Line Yaka 1. 22-82 the highest width (11.8 cm).

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

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

Varieties Lines	Plant with inflorescence height cm	Leaf number per plant	Largest leaf size	
			Length	Width
Yv. 125/3Ø	98	37	19,7	9,5
Yk . 1.123-82	103	45	20,9	10,3
Yaka 1.20-23/10	118	53	20,9	9,6
Yaka 1.22-82/10	112	53	21,4	11,8
Yk. 1. 301 /23	103	47	25,1	11,7

Data about productional characteristics of the newly created tobacco lines compared to the standard Yv. 125/3 are presented in Table 2. The lowest average yield was obtained in the standard variety (1628 kg/ha), and the highest in Yaka 1. 20-23/10 (2748 kg/ha), which is 68.80% higher than the standard. The yields of the other three lines were 33.66% - 60.50% higher compared to the standard. With regard to yield per hectare the newly created tobacco lines showed statistically significant differences at 1% level, compared to the standard variety YV-125/3. The highest purchase price was recorded for Yaka 1. 22-82/10 (2,08 €/kg), which is 11,82% higher than the standard Yv. 125/3 (1,86 €/kg). The other lines, showed equal or somewhat higher purchase price compared to the standard. In relation to the economic effect, relatively high differences were observed between the investigated lines and the standard (Table 2). Thus, the

lowest gross income was achieved in the standard Yv.125/3 (3.028,08 €/ha), and the highest in Yaka 1. 20-23/10 (5.496,00 €/ha), which is 81.50% higher compared to the standard. Economic effects of the other three lines, too, were 40.85% to 70,86% higher compared to the standard. Regarding the gross income three of the newly created lines showed statistical significance at 1% while in line YK 1.123-82 the significance level was 5%, compared to the standard variety YV-125/3.

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

**Table 2 Productional characteristics of Investigated lines and the standard variety**

Varieties Lines	Yield		Average purchase price		Average economic effect	
	kg/ha	%	€ /kg	%	€ /ha	%
Yv. 125/3Ø	1628	100,00	1,86	100,00	3.028,08	100,00
Yk. L. 123-82	2176	133,66	1,96	105,38	4.264,96	140,85
Yaka l.20-23/10	2748	168,80	2,00	107,52	5.496,00	181,50
Yaka l.22-82/10	2259	138,76	2,08	111,82	4.698,72	155,17
Yk. l.301/23	2613	160,50	1,98	106,44	5.173,74	170,86
LSD p=0,05=143kg/ha P=0,01=203kg/ha			LSD p=0.05=1015€ /ha p=0.01=1425€ /ha			

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

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

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

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

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

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

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

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

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

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

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

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



**Photo 1.** YV 125/3



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

## CONCLUSIONS

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

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

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

- The purchase price (den/kg) and economic effect (den/ha) in newly created

perspective lines was 41.01% - 81.29% higher compared to the standard variety YV 125/3.

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

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

## REFERENCES

1. Горник Р., 1973. Облагородување на тутунот. Прилеп
2. Димитриески М., Мицеска Г. Ташкоски П. 2011. Отпорност кон црнилката (*Phytophthora parasitica* var. *nicotianae*) кај некои сорти и линии ориенталски тутун. Заштита на растенијата , I Конгрес за заштита на растенијата, Година XXII, Vol.XXII, Скопје .23- 27.
3. Dimitrieski M., Miceska G., Taskoski P.2012. Investigation of the resistance to blue mold(*Peronosporatabacina* Adam)and black shank (*Phytophthoraparasitica* var. *nicotianae*) in some oriental tobacco cultivars and lines.Тутун /Tobacco Vol. 62 N°стр. 30-35.
4. Димитров А., 2003. Нарбчник по заштита на тютюна от болести, непријатели и плевели. Институт по тютюна и тютюневите изделия, Пловдив.
5. Мицковски Ј., 1984. Болести на тутунот. Стопански Весник, Скопје.
6. Мицковски Ј. 1988. Фитофтората (црнилката) важен проблем во тутунопроизводството. Тутун/ Tobacco, Vol. 38, No 9-10, 317-327, Прилеп.
7. Ташкоски П., Пејчиновски Ф. 2002. Проверување на вирулентноста на изолатите и идентификација на физиолошките раси од габата *Phytophthora parasitica* var. *nicotianae*. Тутун/ Tobacco, Vol. 52, No 3-4, 89-100, Прилеп.
8. Ташкоски П., Димитриески М., Мицеска Г. 2008. Проучување на отпорноста спрема *Phytophthora parasitica* var. *nicotianae* кај некои ориенталски сорти тутун. Заштита на растенија, Год. XIX, Vol. XIX, 103-107, Скопје.
9. Гранчева Р. 2000. Проучување на отпорноста спрема ТМВ (*Tobacco mosaic virus*), пламеница (*Peronospora tabacina* Adam) и црnilка (*Phytophthora parasitica* var. *nicotianae*) на бугарски и интродуцирани тутунски сорти. Тутун/ Tobacco, Vol. 50, No 7-8, 123-127, Прилеп.
10. Гранчева - Петкова Р. 2001. Проучување на отпорноста кон црнилката (*Phytophthora parasitica* /Dastur/ var. *nicotianae* /Breda de Naan/Tucker) на сортите и линиите тутун за создавање на селекционен материјал отпорен на причинителот на болеста. Тутун/ Tobacco, Vol. 51, No 3-4, 75-84, Прилеп.