

**INVESTIGATION OF THE RESISTANCE TO BLUE MOLD  
(*Peronospora tabacina* Adam) AND BLACK SHANK (*Phytophthora parasitica*  
*var. nicotianae*) IN SOME ORIENTAL TOBACCO CULTIVARS AND LINES**

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**ABSTRACT**

Blue mold and black shank are among the most common and harmful diseases in R. Macedonia and other tobacco producing countries in the world. Considering the actuality of the problem and economic losses it produces, the aim of our paper was to investigate the resistance to these diseases in 8 newly created tobacco lines and two standard cultivars (P12-2/1 and YV 125/3). Investigations were carried out in Biological laboratory of Tobacco Institute-Prilep during 2010. Resistance of investigated cultivars and lines to the causing agents of the diseases was studied in conditions of artificial inoculation. Resistance was reported in three lines of the investigated tobacco, two of which were resistant to blue mold and one to black shank.

The new resistant lines will not only find application in practice, but they will be also used as sources of resistance in breeding programs.

**Key words:** tobacco, disease, blue mold, black shank, oriental tobacco, cultivars, line

**ПРОУЧУВАЊЕ НА ОТПОРНОСТА СПРЕМА ПЛАМЕНИЦА (*Peronospora*  
*tabacina* Adam) И ЦРНИЛКАТА (*Phytophthora parasitica var. nicotianae*) КАЈ НЕКОИ  
ОРИЕНТАЛСКИ СОРТИ И ЛИНИИ ТУТУН**

Пламеницата и црнилката на тутунот спаѓаат во редот на најраспространетите и најштетните болести во нашата република и останатите земји производители на тутун од целиот свет. Имајќи ја во предвид актуелноста на проблемот, како и големите економски штети кои ги причинуваат овие две болести во тутунопроизводството, си поставивме за цел да ја проучиме отпорноста спрема пламеницата и црнилката на 8 новосоздадени ориенталски линии и две стандардни сорти П12-2/1 и ЈВ 125/3. Испитувањата се извршени во биолошката лабораторија на Институтот за тутун во текот на 2010 година. Отпорноста на испитуваните сорти и линии спрема причинителот на овие болести е проучувана во услови на вештачка инокулација. Од проучуваните 10 ориенталски сорти и линии тутун се добиени податоци за отпорноста спрема двете болести (пламеница и црнилка) кај 3 линии, две линии покажуваат отпорност кон црнилката, а една линија е отпорна на пламеницата. Овие новосоздадени отпорни 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, пламеница, црнилка, ориенталски сорти, линии

## INTRODUCTION

In selection of oriental aromatic tobaccos, the main interest of breeders is always directed toward increasing the yield and improving the quality of tobacco. In our country, however, there is little or no work on creation of oriental tobacco cultivars resistant to economically important diseases. Such diseases are blue mold and black shank, which in some years and under favorable conditions for their appearance may cause serious damage to tobacco production (4, 5, 6, 9, 8). Gelemerov (1) reported that growing of oriental tobacco as a monoculture in the region of Nevrokop leads to frequent occurrence of diseases in epiphytotic form. According to him, the problem can be solved with creation and use of resistant cultivars. Currently, the world tendency in modern tobacco production is to limit the use of chemicals by introducing resistant cultivars (Palakarceva, 1986, cit. by Tranceva, 9). She reported that significant results on this subject were achieved in Bulgaria, with creation of cultivars Basma 15, Krumovgrad 90, Rila 82,

Jubilej 816, Nevrokop A-24, Djebel 169, Pobeda 3 etc., resistant to both diseases. Accordingly, the most effective control of these pathogens will be achieved only by creating and introducing new resistant lines and cultivars of oriental tobacco in primary production (2,3,10).

From the aspect of relevance of the problem, our recent investigations have been directed toward implementation of the achievements in intracultivar hybridization in creation of new oriental tobacco cultivars and lines, by which more oriental lines with high resistance to blue mold and black shank were obtained. Some of these lines and cultivars are subject of this study. Investigated tobacco cultivars and lines were analyzed in terms of their resistance to blue mold and black shank and the possibility of their utilization not only in production but also as a starting material for hybridization in creating new cultivars resistant to these diseases.

## MATERIAL AND METHODS

Investigations were made in Biological laboratory of Tobacco Institute-Prilep during 2010 and they included 10 cultivars and lines of oriental tobacco (P12-2/1, Hybrid 301/N, I. P 65-54/09, I. P 123-65/82, I. P 123-65/82, YV 125/3, Yk.l. 123-82, Yk.l. 20-23/10, Yk.l. 22-82/10, Yk.l. 301/23).

The newly created resistant lines included in investigations were obtained by intracultivar hybridization, using introduced resistant cultivars and domestic non-resistant cultivars and lines of oriental tobacco.

For investigation of resistance to blue mold, 24 plants of each cultivar were transplanted and monitored. In order to improve the conditions for occurrence and development of the disease, tobacco pots were placed on hemp canvas and covered with polyethylene. Canvases were regularly moistened in order to increase relative humidity under the polyethylene and thus to create favorable conditions for disease occurrence. The symptoms of the disease in certain tobacco cultivars and lines appeared as a result of natural infection by the pathogen.

The resistance was estimated according to the EPPO scale:

- 0 – highly resistant- **no visible infection**
- 1 – resistant - **5 % infected plants**
- 2 – moderately resistant - **5 - 25 % infected plants**
- 3 - susceptible- **25 - 50% infected plants**
- 4 – highly susceptible- **50% - 100% infected plants**

Resistance to black shank disease of the investigated cultivars and lines was studied in conditions of artificial inoculation. 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 P 25, 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 100 ml distilled water.

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 (7). After that, 30 ml of the prepared suspension was added to each plant by watering, and 30 ml 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, 9), with minor corrections, all varieties are classified into 5 categories:

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**

## RESULTS AND DISCUSSION

In the investigations of black shank resistance which included 10 oriental tobacco cultivars and lines (Table 1), high resistance to the pathogen (index 0) was observed in 4 of the lines (I.P123-65/82, I.P301-11/46, Yk l. 22-82/10 and Yk l.301/23). During the observations and assessment of the occurrence and spread of the pathogen among the cultivars and lines tested in Biological Laboratory, there were no symptoms of disease in conditions of natural infection. Such symptoms appeared in other cultivars tested under the same conditions, as a result of natural infection by the pathogen. Among these, one tobacco line (Yk l. 20-23/10) showed to be

resistant (index 1), two standard cultivars (P12-2/1 and JV125/3) and the line Hybrid 301/H were moderately resistant (index 2), and two lines (I.P65-54/09 and JK l.123-82) were highly susceptible (index 4). The most susceptible line L.P65-54/09, in which 100% of the plants were infected, may serve as non-resistant check in future investigations of this disease.

The four highly resistant lines (I.P123-65/82, I.P301-11/46, Yk l. 22-82/10 and Yk l.301/23), beside their implementation in mass production, can be used as sources of resistance in creation of new cultivars.

Table 1 Tobacco cultivars naturally infected with black shank - greenhouse 2010-

Cultivars-lines	Number of observed plants	Number of infected plants	infected plants, %	Index <sup>(1)</sup>
P 12-2/1	20	4	20.0	2
Hybrid 301/N	24	5	20.83	2
I.P 65-54/09	24	24	100.0	4
I.P 123-65/82	20	0	0.0	0
I.P 301-11/46	24	0	0.0	0
YV125/3	24	3	12.5	2
Yk.l. 123-82	24	12	50.0	4
Yk.l. 20-23/10	20	1	5.0	1
Yk.l.22-82/10	24	0	0.0	0
Yk.l.301/23	20	0	0.0	0

- <sup>0</sup> – highly resistant- **no visible infection**  
 1 – resistant - **5 % infected plants**  
 2 – moderately resistant - **5 - 25 % infected plants**  
 3 - susceptible- **25 - 50% infected plants**  
 4 – highly susceptible- **50% - 100% infected plants**

According to the results on the resistance to black shank in conditions of artificial inoculation ( Table 2), out of the 10 cultivars and lines of oriental tobacco included in investigations, 5 lines were highly resistant (l. P 65-54/09, l.P 301-11/46, 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 l. P. 123 was estimated as resistant (index 1), and the lines Yaka l.123-82 and Hybrid 301/N as moderately resistant (index 0).

The other two standard cultivars, Prilep 12-2/1 (as non-resistant control) and YV 125/3 (Fig. no. 1) were rated as

highly susceptible (index 4) to the pathogen (*Phytophthora parasitica* var. *Nicotianae*). In these cultivars, the percentage of infected plants after inoculation was 100 and 87.5%, respectively.

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 Rila 82 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: l. Q 65-54/09, l.P 301-11/46, Yaka l. 20-23/10, Yaka l. 22-82/10 and Yk l.301/23.

Table 2 Tobacco cultivars inoculated with a culture of *Phytophthora parasitica* var. *nicotianae* - greenhouse 2010

Cultivars-lines	Inoculated plants	Total No. of infected plants	Infestation, %	Level of resistance	Index <sup>(1)</sup>
P 12-2/1	20	20	100.00	-	4
Hybid 301/N	24	8	33.33	66.67	2
l.P 65-54/09	24	0	0.00	100.00	0
l.P 123-65/82	20	1	5.00	40.00	1
l.P 301-11/46	24	0	0.00	100.00	0
YV 125/3	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

- <sup>1</sup>0 - highly resistant - **no visible infection**  
1 - resistant - **10 % infected plants**  
2 - moderately resistant - **up to 40% infected plants**



Photo 1 YV 125/3

- 3 - susceptible - **up to 50% infected plants**  
4 - highly susceptible - **over 50% infected plants**



Photo 2 Yk l. 301/23

## CONCLUSION

The following conclusions can be drawn from the investigations on resistance to blue mold and black shank disease in some oriental tobacco cultivars and lines:

- From 10 studied oriental tobacco cultivars and lines, three lines show high resistance to blue mold and black shank (l. P. 301-11/46, Yk.l.22-82/10, Yk.l.301/23), two lines show high resistance to black shank (l. P65-54/09 and Yk l. 20-23/10) and the line l. P 123-65/82 shows high resistance to blue mold.

- Two lines were reported as resistant

with index 1 (Yk. l. 20-23/10 to blue mold and l.P 123-65/82 to black shank).

- Standard cultivars P 12-2/1 and YV 125/3 proved to be highly susceptible to black shank and moderately resistant to blue mold, while lines l. P65-54/09 and Jk.l. 123-82 as highly susceptible to blue mold..

- Beside their use in commercial production, the lines with high resistance to blue mold and black shank can be used as sources of resistance in creation of new cultivars resistant to these diseases.

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