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NEWLY CREATED LINES OF ORIENTAL YAKA TOBACCO RESISTANT TO THE COMMON MOSAIC VIRUS (TMV) AND BLACK SHANK DISEASE (*Phytophthora Parasitica* var. *Nicotianae*)

Miroslav Dimitrieski, Gordana Miceska

Scientific Tobacco Institute - Prilep

E-mail: miroslavdimitrieski@yahoo.com

Abstract: *The common tobacco mosaic virus and black shank disease can cause severe economic loss to tobacco production in some microregions of R. of Macedonia and in other tobacco producing countries worldwide. Considering the actuality of the problem, by means of generative hybridization a number of oriental lines with high resistance to the above mentioned diseases have been created in Tobacco Institute-Prilep. Five of these lines and varieties were included in investigations carried out in the Experimental field and in Bio-laboratory of the Institute. Three of them had high resistance to TMV and black shank, one had high resistance to TMV and medium resistance to black shank, while the standard variety YV125/3 was susceptible to both diseases. The resistant lines can be used as a starting material in hybridization, for creation of new tobacco genotypes resistant to the economically important diseases.*

Key words: *tobacco, disease, common mosaic virus, black shank, oriental varieties, lines*

Introduction

Each year a large number of diseases of different origin (viral, bacterial, pathogenic, etc.) cause severe damages to tobacco yield and quality. Tobacco mosaic is the most widely spread viral disease in the world. If the infection occurs immediately after transplanting, 50 to 100 % of tobacco plants may be infected, the yield may decrease by 30% and the total value of tobacco by 50 % (Mickovski, 1984; Dimitrov, 2003). The virus is difficult to be controlled because of its adaptability, heat resistance, unusual ability for modification and rapid replication in leaf tissue (Trancheva, 2008). The danger becomes even greater knowing that no chemical product can prevent the occurrence of disease and that the only possibility left is to take preventive measures. Black shank is another economically important disease on oriental tobacco. In years with favorable conditions for its occurrence it can cause severe damage to the mass tobacco production. A number of authors reported epiphytotic occurrence of the disease in many regions, with enormous damage caused to tobacco industry (Mickovski 1984, 1988; Trancheva-Petkova, 2001; Gelemerov 2005; Taškoski et al. 2008; Dimitrieski et al. 2011, 2012; etc.). The pathogen persists in the soil for a long period of time, which makes more difficult the application of chemicals in disease control. Bearing in mind the importance of the problem, our investigations were directed towards creation of new resistant varieties and lines of oriental tobacco. A number of oriental lines with high resistance to TMV and black shank have been obtained, but subject of this study are 4 lines of Yaka tobacco, compared with the standard variety YV 125/3.

Material and methods

Investigation was carried out in Biological laboratory of Tobacco Institute - Prilep in 2010, with the following varieties and lines of oriental Yaka tobacco: Yk 1 20-23/10, Yk 1 22-82/10, Yk 1 301/23 and Yk 1 123 -82. The newly created resistant lines included in investigation were obtained by intrapecies

hybridization, using introduced resistant varieties and local non-resistant oriental varieties and lines. Resistance of the plants from hybrid progenies was determined by artificial inoculation. For assessment of TMV resistance, 25 plants of each line and variety were planted in pots and inoculated.

Juice from mosaic-infected tobacco plants was used for inoculation, according to the method of Ternovsky 1965, (cit. by Tranceva, 15). The juice from infected leaves was heated for 12 minutes in water bath at 80 °C, 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.

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 (Tashkoski, Pejcinovski 2002). 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, 2000), 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

According to the results of investigations presented in Table 1, the four newly created lines (Yk 1 123-82, Yk 1 20-23/10, Yk-1 22-82/10 and Yk 1 301/23) are resistant to TMV. After inoculation with infective juice, 100 % resistance to TMV was determined in these plants, while in the standard variety YV 125/3 all of the plants were infected and showed systemic reaction to TMV.

Dimitrieski, Miceska (2010) made two assessments on the occurrence and spread of the disease in top leaves and suckers during the growing season of tobacco in field. They reported that no symptoms of TMV virus were observed in lines Yk 1 123-82 and Yk 1 301/23, neither in harvested nor in non-harvested plants, while the standard variety YV 125/3 was determined as susceptible to the virus.

Table 1 –TMV resistance in lines and varieties of Yaka tobacco

Varieties-lines	Number of transplanted plants	Number of investigated plants	Number of inoculated plants	Resistant plants, %
YV 125/3 Ø	25	25	25	0
Yk. 1. 123-82	25	24	24	100
Yk. 1. 20-23/10	25	25	25	100
Yk. 1. 22-82/10	25	23	23	100
Yk. 1. 301/23	25	25	25	100

According to the results on the resistance to black shank in conditions of artificial inoculation (Table 2), 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). 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 was rated as highly susceptible (index 4) to the pathogen (*Phytophthora parasitica* 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 2. Tobacco cultivars inoculated with a culture of *Pytophthora parasitica* var. *nicotianae*- greenhouse 2010

Varieties -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

Conclusion

From the investigations on resistance to common mosaic virus and black shank disease in Yaka tobacco varieties and lines, the following conclusions can be drawn:

Three of the investigated lines (Yk l 20-23/10, Yk l 22-82/10 and Yk l 301/23) showed high resistance to TMV and black shank, while the line Yk l 123-82 showed high resistance to TMV and medium resistance (index 2) to black shank.

Standard variety YV 125/3 showed susceptibility to TMV and high susceptibility to black shank disease.

The lines with high resistance to TMV and black shank will not only find their place in mass tobacco production, but they can be used in hybridization as sources of resistance for creation of new resistant varieties.

References

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