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DIALLEL CROSSES TRIAL – THE BASIS FOR DETECTION OF RESISTANCE TO DISEASES IN TOBACCO

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ABSTRACT

Investigations were made with ten varieties of tobacco types : Prilep (P- 23 , P- 76 , P- 66-9/7 , P-84), Yaka (YK 10-7/1), Djebel (Xanthe, XDj-M), Basmak (MB-3), Samsun (SM-1), Sirdili (SM-LL) and Virginia (MV-1) and their 45 diallel crosses for resistance to diseases, with an emphasis on black shank - *Phytophthora parasitica var. nicotianae*. The trial with parental genotypes and their hybrids was set up in 2011, 2012 and 2013 at the Experimental field of the Scientific Tobacco Institute - Prilep in randomized block design with three replications. Traditional agricultural practices were applied during the growing season. The resistance/susceptibility degree was estimated according to a scale recommended by FAO.

The aim of this paper is the detection of resistance to black shank and creation of resistant lines, using diallel analysis to obtain a knowledge on the genetics of this disease.

The highest resistance to the disease was recorded in YK 10-7/1 and SM-LL, while the varieties MV-1 and P-76 showed to be the most susceptible. The highest resistance in the diallel was recorded in the crosses where one of the parents was YK 10-7/1, which indicates a possession of dominant gene of resistance. In the process of breeding, the method of Back-cross hybridization was used in order to increase the varieties resistance to the black shank disease.

Keywords: tobacco (*Nicotiana tabacum L.*), diallel crosses, Back-cross hybridization, resistance, black shank (*Phytophthora parasitica var. nicotianae*).

ОПИТ СО ДИЈАЛЕЛНИ КРСТОСКИ – БАЗА ЗА ОТКРИВАЊЕ ОТПОРНОСТ НА БОЛЕСТИ КАЈ ТУТУНОТ

Испитувани се десет сорти од типовите: Прилеп (П-23, П-76, П-66-9/7, П-84), Јака (ЈК 10-7/1), Џебел (Ксанти XDj-M), Басмак (МБ-3), Самсун (SM-1), Sirdili (SM-LL) и Вирџинија (МВ-1) и нивните 45 дијалелни крстоски за отпорност на болестите на тутунот со посебен акцент на црнилката - *Phytophthora parasitica var. nicotianae*. Опитот со родителските генотипови и нивните хибриди беше поставен на опитното поле при Научниот институт за тутун – Прилеп по случаен блок-систем во три повторувања во 2011, 2012 и 2013 година. Во текот на вегетацијата беа применети вообичаени агротехнички мерки. За проценката на степенот на резистентност односно сензибилност користевме скала пропишана од ФАО.

Целта на овој труд е откривање на отпорност кон црнилката, како и добивање на отпорни линии, а со дијалелната анализа и добивање сознанија за генетиката на болеста.

Прворангирани сорти отпорни на болеста се ЈК 10-7/1 и SM-LL, додека најголема осетливост покажаа МВ-1 и П-76. Највисока резистентност во дијалелот покажаа крстоските каде еден од родителите е ЈК 10-

7/1 што укажува поседување на доминантен ген за отпорност. За облагородување на сортите во насока на зголемување на отпорноста кон црнилката го користевме методот Повратно вкрстување (ВС).

Клучни зборови: тутун (*Nicotiana tabacum L.*), дијалелни крстоски, повратно вкрстување, отпорност, црнилка (*Phytophthora parasitica var. nicotianae*).

INTRODUCTION

Tobacco, like all other crops, is attacked by many diseases, parasites and pests. The lack of their control can lead to reduction in yield and quality and even to destruction of the entire crop. Nowadays there is a range of products for successful treatment of many diseases and control of pests, parasites and weeds. The most important law in nature, however, is the law of survival - all organisms tend to stay alive. This is performed through occurrence of new races of the pathogen - the causing agent of the disease or new individuals immune to the products for protection on one side and though creation of new resistant crops on the other.

Beside tobacco, which is dangerous to smokers health, the residues of the pesticides further increase the risk of diseases that threaten and destroy smokers life. Therefore, chemical products should be used carefully and properly as a precaution in the production and release of resistant varieties (Dimitrieski et al., 2012).

The purpose of this paper is to detect the resistance to economically important diseases in a trial with tobacco varieties and diallel crosses and to create new resistant lines. The same scheme can be applied in many other crops for various diseases.

Diallel crossing is applied in selection primarily for creation of hybrids and varieties with better yield and quality than the existing ones (Korubin-Aleksoska, 2003), but one replication in the trial with parents and F1 hybrids can be set up on infected area or infestation can be made with pathogen of the disease, which will help to detect resistance among some parents and their hybrids. Diallel crossing provides maximum number of combinations to be made for each parent, by which accurate information on the inheritance of resistance can be obtained.

MATERIAL AND METHODS

Investigation material included 10 varieties representing different types of tobacco: Prilep (P-23 , P-76 , P-66-9/7, P-84), Yaka (YK 10-7/1), Djebel (Xanthe, XDj-M), Basmak (MB-3), Samsun (SM-1), Sirdili (SM-LL) and Virginia (MV-1). In July and August 2010 were made diallel crossing and obtained seed from 45 F1 hybrids (J. Aleksoski). The trial with parental genotypes and their F1 hybrids was set up at an area of 1471,5 m² in the Experimental field of Scientific Tobacco Institute - Prilep, using a randomized block design with three replications. The oriental parents and their hybrids were arranged in three rows per replication, with 34 plants in a row (spacing: 15 cm x 45 cm). The large-leaf parent and its hybrids were arranged in four rows per replication, the parent with nine plants (spacing : 60 cm x 90 cm) and hybrids with 15 plants in a row (spacing: 35 cm x 90 cm). The third replication was set up in a plot infected with black shank disease. Infestation was done with pathogens of powdery mildew (*Erysiphe cichoracearum*), blue mold (*Peronospora tabacina*) and wildfire (*Pseudomonas tabaci*). The results presented in this paper, however, are focused only on black shank disease - *Phytophthora parasitica var. nicotianae*.

For assessment of the resistance/ susceptibility degree of plants we used

a scale recommended by FAO: 0 - no information, 1 – immune, 2 - highly resistant, 3 to 4 – resistant, 4 to 6 – semi susceptible, 7 – moderately susceptible, 8 – susceptible and 9 - highly susceptible. This scale can be changed depending on the disease and crop and it is quite applicable in breeding, because each of its variants is adapted and internationally accepted.

Parental genotypes

(Order - according one-way diallel)

Samsun SM-1 – sun-cured, oriental, aromatic tobacco, brought in Tobacco Institute – Prilep from Turkey. The plant has cylindrical-elliptic habitus, with average stalk height of 85 cm and 25-30 sessile leaves (16 cm x 9 cm). Floral bud is semispherical, with light pink flowers. Cured leaves are gentle and elastic, golden yellow and orange in color, characterized by intensive and specific aroma. Dry mass yield ranges 1000 kg/ha (Fig. 1).

Virginia MV-1 (authors: D. Cavkaroski, M. Uzunoski – 1987) - variety of the type Virginia (flue-cured, large-leaf tobacco). The plant has conical (haystach-shaped) habitus, with average stalk height of 195 cm and 26-29 sessile leaves (55 cm x 35 cm). Floral bud is brushing, loose, cup-shaped, with pale pink flowers. Found both in male-sterile and fertile form (Korubin-Aleksoska, 2004). The middle belt dry leaves are golden-yellow in color. They are characterized by good elasticity, water retention and filling capacity, pleasant taste and aroma. Dry mass yield ranges 2500-3500 kg/ha (Fig. 2).

Yaka YK 10-7/1 (author: A. Korubin – Aleksoska – 2010) – variety of the type Yaka (sun-cured, oriental tobacco).The plant has cylindrical habitus, with average stalk height of 105 cm and 50-60 sessile leaves (17,5 cm x 9 cm). Floral bud is semispherical, with pale pink flowers. Cured leaves are with golden yellow color, characterized by pleasant sweetish taste and intensive specific aroma. Dry mass yield ranges 2500 kg/ha (Fig. 3).

Prilep P-23 (authors: K. Nikoloski, M. Mitreski, 2001) – variety of the type Prilep (sun-cured, oriental tobacco). The plant has a conical (fir tree-shaped) habitus, with average stalk height of 65 cm and 45-50 sessile leaves (20 cm x 10,5 cm). Floral buds are relatively small, dense and semispherical, with pale pink flowers. Cured leaves are golden yellow and the upper ones are light orange, elastic, rich in substance, with poorly defined nervation. They are characterized by an intense and specific aroma (Korubin-Aleksoska, 2004). Dry mass yield ranges 2000-2500 kg/ha (Fig. 4).

Prilep P-76 (authors: D. Cavkaroski et al. - 1987) – variety of the type Prilep (sun-cured, oriental tobacco).The plant has elliptic-conical habitus, with average stalk height of 90 cm and 59 sessile leaves (23 cm x 11,5 cm). Floral bud is dense and semispherical, with white to pale pink flowers. Cured lower leaves are yellow, middle leaves are orange and the upper ones reddish orange, characterized by specific aroma (Korubin-Aleksoska, 2004). Growth period from planting to flowering is 85-95 days (late maturing variety). Dry mass yield ranges 3500-4000 kg/ha (Fig. 5).

Basmak – MB-3 (authors: group of breeders from Tobacco Institute – Prilep and Faculty of Agricultural Sciences and Food - 2010) – variety of the type Basmak (sun-cured, oriental, aromatic tobacco). The plant has cylindrical habitus, with average stalk height of 70 cm and 35-45 sessile leaves (19 cm x 9,7 cm). Floral bud is semispherical, with light pink flowers. Cured lower leaves are yellow-orange and the upper ones red-orange in color. They are characterized by intensive specific aroma. Dry mass yield

ranges 2000-2500 kg/ha (Fig. 6).

Prilep P-66-9/7 (authors: M. Dimitrieski, G. Miceska, A. Siskoski – 2001) – variety of the type Prilep (sun-cured, oriental tobacco).The plant has elliptic habitus, with average stalk height of 80 cm and 45-55 sessile leaves (18 cm x 9 cm). Floral bud is dense and semispherical, with pale pink flowers. Cured lower leaves are yellow and the upper ones are reddish to orange, characterized by intensive specific aroma. Dry mass yield ranges 3000-3500 kg/ha (Fig. 7).

Sirdili, SM-LL – oriental variety of tobacco. It has a cup-like habitus, with average stalk height of 55 cm and 33 elongated sessile leaves (27,5 cm x 6 cm). Floral bud is semi-spherical and sessile in apical leaves, with white-pink flowers. Cured leaves are characterized by pleasant specific aroma. Dry mass yield ranges 1200-1500 kg/ha (Fig. 8).

Xanthe-Djebel, XDJ-M - variety of the type Djebel (sun-cured, oriental, aromatic tobacco). The plant has elliptic habitus, with average stalk height of 65 cm and 17 sessile leaves (17 cm x 8,4 cm), with oval shape and slightly curved tip. Floral bud is loose, with pale pink flowers. Growth period from planting to flowering is 40-45 days (early maturing variety). Cured leaves are golden yellow to light red in color. They are characterized by pleasant specific aroma. Dry mass yield ranges 500-700 kg/ha (Fig. 9).

Prilep P-84 (authors: K. Naumovski, A. Korubin – Aleksoska - 1988) - variety of the type Prilep (sun-cured, oriental tobacco). The plant has cylindrical to oblong-elliptic habitus, with average stalk height 65 cm; 38-42 sessile leaves (20 cm x 10 cm). Floral bud is medium large, semispherical, with pale pink flowers. Cured lower leaves are yellow, middle leaves are orange and the upper ones are red orange in color. They are characterized by specific aroma. Dry mass yield ranges 2500-3200 kg/ha (Fig. 10).

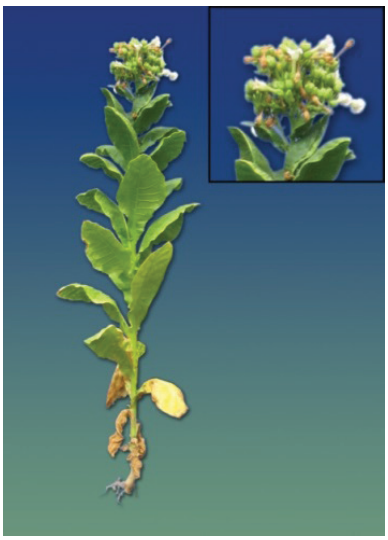

Fig. 1. Samsun, SM-1

Fig. 2. Virginia MV-1

Fig. 3. Yaka JK 10-7/1

Fig. 4. Prilep P-23

Fig. 5. Prilep P-76

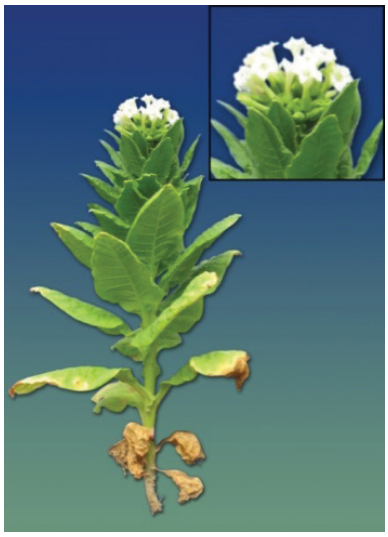

Fig. 6. Basmak MB-3

Fig. 7. Prilep P-66-9/7

Fig. 8. Sirdili, SM-LL

Fig.9. Xanthe-Djebel, XDJ-M

Fig. 10. Prilep P-84

Diallel crosses

(Obtained from J. Aleksoski)

SM-1 x MV-1, SM-1 x YK 10-7/1, SM-1 x P-23, SM-1 x P-76, SM-1 x MB-3, SM-1 x P-66-9/7, SM-1 x SM-LL, SM-1 x XDJ-M, SM-1 x P-84, MV-1 x YK 10-7/1, MV-1 x P-23, MV-1 x P-76, MV-1 x MB-3, MV-1 x P-66-9/7, MV-1 x SM-LL, MV-1 x XDJ-M, MV-1 x P-84, YK 10-7/1 x P-23, YK 10-7/1 x P-76, YK 10-7/1 x MB-3, YK 10-7/1 x P-66-9/7, YK 10-7/1 x SM-LL, YK 10-7/1 x XDJ-M, YK 10-7/1 x P-84, P-23 x P-76, P-23 x MB-3, P-23 x P-66-9/7, P-23 x SM-LL, P-23 x XDJ-M, P-23 x P-84, P-76 x MB-3, P-76 x P-66-9/7, P-76 x SM-LL, P-76 x XDJ-M, P-76 x P-84, MB-3 x P-66-9/7, MB-3 x SM-LL, MB-3 x XDJ-M, MB-3 x P-84, P-66-9/7 x SM-LL, P-66-9/7 x XDJ-M, P-66-9/7 x P-84, SM-LL x XDJ-M, SM-LL x P-84, XDJ-M x P-84

RESULTS AND DISCUSSION

Black shank is a very serious tobacco disease, first identified in 1893 on the islands Java and Sumatra (Fig.11). In the United States it occurred in 1915 on large-leaf tobacco. Later it was observed in some countries of Africa, South America and Europe. In Bulgaria it was first observed in 1928, in Greece in 1975, in Montenegro in 1982 and in Macedonia in 1983 (A. Korubin-Aleksoska, 1989).

Inoculation

A replication of the diallel trial with parental genotypes and F1 hybrids was set up in previously infected soil and additional inoculation with fungus culture was applied by irrigation of the stalk base (Sanches - Monge 1974, Bonnet 1985). This method seems to be the most acceptable because it is cheap, fast and reliable. The inoculum was prepared from stalks of diseased plants (a mixture of the pathogen and optimum amount of water).

Table 1. Assessment of the resistance level in parental genotypes

Parental genotypes	Percentual representation of the disease	Grade (FAO scale)	Rank
1. Samsun, SM-1	48, 5	4 - 6	6
2. Virginia, MV-1	99,5	9	10
3. Yaka, YK 10-7/1	0	0	1
4. Prilep, P-23	72,5	7	8
5. Prilep, P -76	85.3	9	9
6. Basmak, MB-3	59,7	4 - 6	7
7. Prilep, P-66-9/7	19,2	1	3
8. Sirdili, SM-LL	11,7	1	2
9. Xanthe-Djebel, XDJ-M	35,5	3 - 4	4
10. Prilep, P -84	46.8	4 - 6	5

Table 2. Assessment of the resistance level in diallel F1 crosses

A				B			
F1 crosses	Disease (%)	Grade (FAO)	Rank	F1 crosses	Disease (%)	Grade (FAO)	Rank
1. SM-1 x MV-1	64,9	4 - 6	35	24. YK 10-7/1 x P-84	4,9	0	9
2. SM-1 x YK 10-7/1	1	0	2	25. P-23 x P-76	86,5	8	44
3. SM-1 x P-23	61,5	4 - 6	33	26. P-23 x MB-3	70,5	7	37
4. SM-1 x P-76	72,6	7	39	27. P-23 x P-66-9/7	23,3	2 - 4	21
5. SM-1 x MB-3	78,8	8	42	28. P-23 x SM-LL	9,5	0	14
6. SM-1 x P-66-9/7	22,5	2 - 4	20	29. P-23 x XDJ-M	69,8	7	36
7. SM-1 x SM-LL	8,7	0	12	30. P-23 x P-84	57,5	4 - 6	31
8. SM-1 x XDJ-M	40,1	2 - 4	25	31. P-76 x MB-3	73,4	7	40
9. SM-1 x P-84	45.3	4 - 6	27	32. P-76 x P-66-9/7	25,2	2 - 4	23
10. MV-1 x YK 10-7/1	1,4	0	3	33. P-76 x SM-LL	5,9	0	10
11. MV-1 x P-23	79,2	8	43	34. P-76 x XDJ-M	64,3	4 - 6	34
12. MV-1 x P-76	89,5	9	45	35. P-76 x P-84	60,7	4 - 6	32
13. MV-1 x MB-3	71,3	7	38	36. MB-3 x P-66-9/7	25,5	2 - 4	24
14. MV-1 x P-66-9/7	24	2 - 4	22	37. MB-3 x SM-LL	12	1	15
15. MV-1 x SM-LL	9	0	13	38. MB-3 x XDJ-M	48,3	4 - 6	28
16. MV-1 x XDJ-M	57	4 - 6	30	39. MB-3 x P-84	50,5	4 - 6	29
17. MV-1 x P-84	75,5	7	41	40. P-66-9/7 x SM-LL	6,5	0	11
17. YK 10-7/1 x P-23	2	0	6	41. P-66-9/7 x XDJ-M	16,5	1	19
19. YK 10-7/1 x P-76	0,5	0	1	42. P-66-9/7 x P-84	12,4	1	16
20. YK 10-7/1 x MB-3	3,5	0	8	43. SM-LL x XDJ-M	15,2	1	18
21. YK 10-7/1 x P-66-9/7	1,5	0	4	44. SM-LL x P-84	12,8	1	17
22. YK 10-7/1 x SM-LL	2,5	0	7	45. XDJ-M x P-84	41,5	2 - 4	26
23. YK 10-7/1 x XDJ-M	1,9	0	5				

Fig. 11. Phytophthora parasitica Dast. var. nicotianae Breda de Haan - Black shank (crnilka)

Gene – for – gene relationship

The results in Table 4 indicate vertical resistance to Phytophthora parasitica var. Nicotianae in YK 10-7/1. This type of resistance may be determined by a single gene – monogene or several genes - oligogenes with a strong effect, so called major genes. This situation is present when the pathogen does not contain virulence genes. The disease occurs when the pathogen contains additional virulence genes and the plant does not have resistance genes. This is defined as gene - for - gene relationship, which results in specific resistance to certain races of the pathogen (Korubin - Aleksoska, 1989). This situation was defined by Flor (1971), and it denotes that for each pair of resistance or susceptibility specific genes in the host there is a corresponding pair of virulence or avirulence specific genes inside the pathogen.

Pedigree of tobacco variety Yaka YK 10-7/1

The Yaka variety YK 10-7/1 was created by crossing of Yaka YV 125/3 and the Djebelian variety Pobeda 2 (authors: M. Palakarcheva and D. Bajlov). Pobeda 2 is originating from the wild species *Nicotiana debneyi* and a variety of Basma tobacco. *Nicotiana debneyi* is a wild species originating from Australia that blooms throughout the growing season. It brings resistance to many diseases, one of which is the black shank.

Breeding for obtaining the resistance to black shank disease

The highest resistance in the diallel trial was observed in YK 10-7/1 and the lowest resistance, i.e. the highest susceptibility to black shank was observed in MV-1.

In order to increase the MV-1 resistance to black shank the Back-cross hybridization method was used (E. Sanchez-Monge, 1974). Cultivar YK 10-7/1 (resistant to the

pathogen) was used as a mother and MV-1 (susceptible to the pathogen) as a father. After seven successive back-crossings with MV-1 and one self-fertilization of heterozygous individuals (Aa), the susceptible ones (aa) were eliminated, the heterozygous (Aa) were avoided and selection was made with the homozygous resistant (AA) individuals, which phenotypically resemble MV-1 and carry dominant genes for resistance to the disease (Fig. 12).

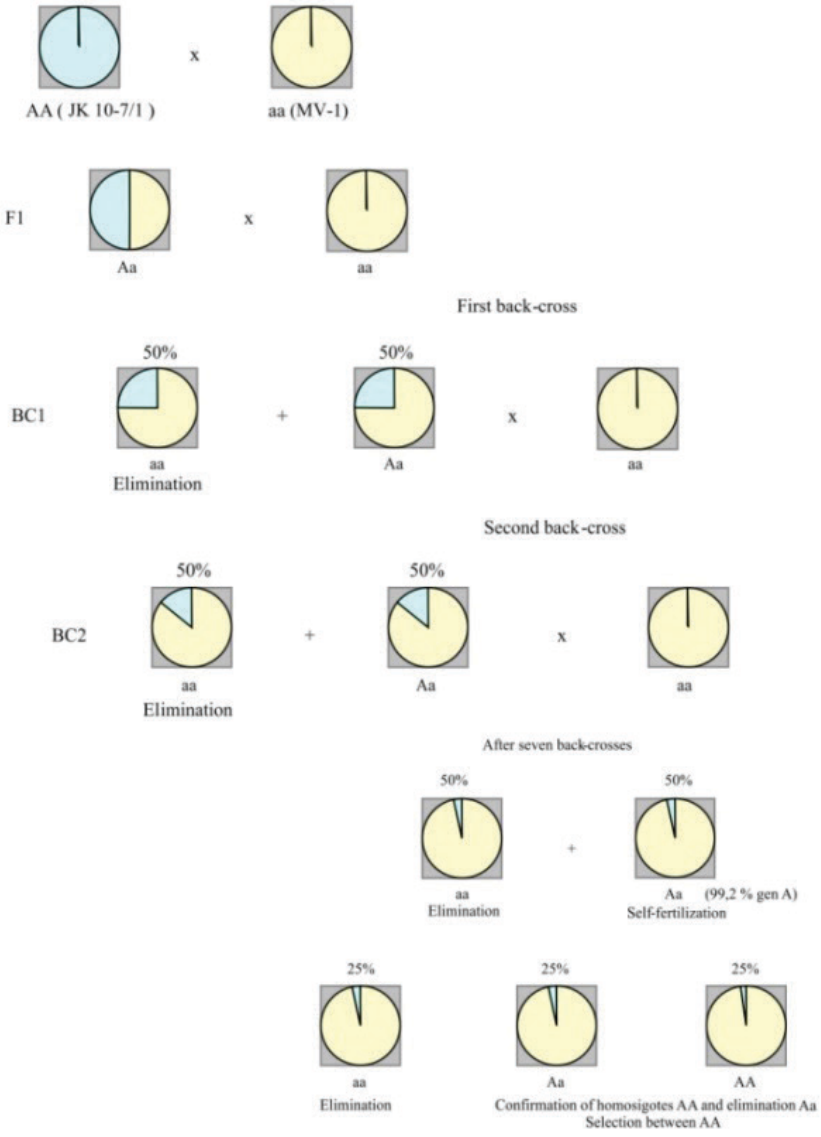

Fig. 12. Developing a resistance to Black shank disease (Phytophthora parasitica var. nicotianae) in tobacco by the use of Back-cross hybridization (E. Sanchez-Monge, 1974).

CONCLUSION

Based on the results of our investigations on detection and selection of disease-resistant tobacco cultivars and F1 hybrids from their diallel crosses, the following conclusions can be drawn:

- A trial with parents and diallel F1 crosses is used for obtaining hybrids

and creation of new superior cultivars; it also offers a possibility for detection of resistant genotypes. The diallel consists of maximum number of combinations which can be obtained among parental genotypes and the diallel analysis will give us the knowledge on the mode of inheritance of characters investigated.

- Among parental genotypes, the first ranked cultivar with resistance to *Phytophthora parasitica var. nicotianae* was YK 10-7/1, and it was followed by Sirdili SM-LL. The highest susceptibility to the disease was recorded in Virginia MV-1 (large-leaf) and in P-76 (oriental).
- The highest resistance to *P. parasitica* in the diallel was recorded in the crosses where one of the parents was YK 10-7/1, indicating a possession of dominant genome for resistance.
- The method of back-cross hybridization was applied to increase the cultivars resistance to black shank. Cultivar YK 10-7/1 (resistant to the pathogen) was used as a mother and MV-1 (susceptible to the pathogen) as a father. After seven successive back-crossings with MV-1 and one self-fertilization of heterozygous individuals (Aa), the susceptible ones (aa) were eliminated, the heterozygous (Aa) were avoided and selection was made with the homozygous resistant (AA) individuals, which phenotypically resemble MV-1 and carry dominant genes for resistance to the disease.
- The scheme for obtaining resistance to *Phytophthora parasitica var. nicotianae* can be applied in many other crops for various diseases, when it reffers to ”vertical (specific) resistance”.

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