International Journal of Agriculture Innovations and Research
Volume 3, Issue4, ISSN (Online) 2319-1473

Detection of Resistance to Black Shank (*Phytophthora Parasitica Var. Nicotianae*) in a Trial with Diallel Crosses of Tobacco

Ana Korubin–Aleksoska
Department of Genetics, Selection and Seed control,
Scientific Tobacco Institute Prilep, University of St. Kliment Ohridski, Bitola, Republic of Macedonia
email: anakorubin@yahoo.com

Jane Aleksoski
Bispromet - Bitola,Republic of Macedonia
email: janealeksoski@yahoo.com

Abstract: **Investigations were made with nine varieties of tobacco types: Prilep (P-26, P-76, P-65, P-84), Yaka (YK 10-7/1), Djebel (Xanthe, XDj-M), Basmak (MB-3), Samsun (SM-1) and Virginia (MV-1) and their 36 one-way diallel crosses for resistance to Black shank - *Phytophthora parasitica var. nicotianae*. The trial with parental genotypes and their hybrids was set up in 2011 and 2012 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, 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: **Back-cross Hybridization, Black Shank (*Phytophthora Parasitica Var. Nicotianae)*, Diallel Crosses, Resistance, Tobacco (*Nicotiana Tabacum L.*)**

I. 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. The purpose of this paper is to detect the resistance to Black shank (*Phytophthora parasitica var. nicotianae)* - economically important diseas 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.

Testing the resistance of Black shank was performed using various techniques of inoculation to whole tobacco plant, root, stem or leaves. Check resistance with inoculation to root and entire leaves with a fragment of the fungus of mycelium performed Tedford [11] and found large differences in the development of necrosis on the surface of the leaf between sensitive and resistant varieties of tobacco (in sensitive varieties necrosis develops much faster than in leaves of resistant varieties), while resistant varieties inoculated by root showed comsplete resistance. In Macedonia, researcher Taskoski [9], [10] performed tests of resistance in some domestic and foreign varieties of tobacco using methods of inoculation to root with suspension of pure culture of the pathogen and inoculation in stem of tobacco plants with the fragment of mycelium of the fungus. Mitreski et al. [7], examined the resistance to Black shank in the crosses of four domestic and introduced varieties, and obtained highest resistance in the F1 generation of YK-48 x Florida-301. Dimitrieski et al. [2], investigated ten oriental genotypes (eight lines and two standard varieties) and obtained two lines resistant to this disease (P 65-54/90 and YK 1. 20-23/10).

Diallel crossing is applied in selection primarily for creation of hybrids and varieties with better yield and quality than the existing ones [5], 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.

II. MATERIAL AND METHODS

Investigation material included nine varieties representing six types of tobacco: Prilep (P-26, P-76 , P-65, P-84), Yaka (YK 10-7/1), Djebel (Xanthe, XDj-M), Basmak (MB-3), Samsun (SM-1) and Virginia (MV-1). In July and August 2010 we made diallel crossing and obtained seed from 36 F1 hybrids. The trial with parental genotypes and their F1 hybrids was set up in 2011 and

2012 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 pathogen of 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, 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 genotipes (Order – according one–way diallel):

Samsun, SM I – 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.

Virginia, VM I (authors: D. Cavkaroski and 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. 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 [6].

Yaka, YK 10–7/1 (author: A. Korubin–Aleksoska, 2009) – 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.

Prilep, P–26 (authors: K. Nikoloski and M. Mitreski, 2007) – variety of the type Prilep (sun-cured, oriental tobacco). The plant has a conical (fir tree-shaped) habitus, with average stalk height of 75 cm and 50 sessile leaves (21 cm x 10,6 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. Dry mass yield ranges 2200-2700 kg/ha.

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. Growth period from planting to flowering is 85-95 days (late maturing variety). Dry mass yield ranges 3500-4000 kg/ha [6].

Basmak,MB-3 (authors: group of breeders from Tobacco Institute – Prilep and Faculty of Agricultural Science and Food – Skopje, 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 1800-2200 kg/ha.

Prilep, P–65 (authors: M. Bogdanceski et al., 2001) – variety of the type Prilep (sun-cured, oriental tobacco). The plant has elliptic habitus, with average stalk height of 65 cm and 55 sessile leaves (24 cm x 12 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 2500-3500 kg/ha [6].

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 and are characterized by pleasant specific aroma. Dry mass yield ranges 500-700 kg/ha.

Prilep, P–84 (authors: K. Naumovski and 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 [6].

Diallel crosses (obtained from M.Sc. J. Aleksoski):
SM-1 x MV-1, SM-1 x YK 10-7/1, SM-1 x P-26, SM-1 x P-76, SM-1 x MB-3, SM-1 x P-65, SM-1 x XDJ-M, SM-1 x P-84, MV-1 x YK 10-7/1, MV-1 x P-26, MV-1 x P-76, MV-1 x MB-3, MV-1 x P-65, MV-1 x XDJ-M, MV-1 x P-84, YK 10-7/1 x P-26, YK 10-7/1 x P-76, YK 10-7/1 x MB-3, YK 10-7/1 x P-65, YK 10-7/1 x XDJ-M, YK 10-7/1 x P-84, P-26 x P-76, P-26 x MB-3, P-26 x P-65, P-26 x XDJ-M, P-26 x P-84, P-76 x MB-3, P-76 x P-65, P-76 x XDJ-M, P-76 x P-84, MB-3 x P-65, MB-3 x XDJ-M, MB-3 x P-84, P-65 x XDJ-M, P-65 x P-84 and XDJ-M x P-84.

III. RESULTS

Table 1: Assessment of the resistance level in parental genotypes

Parental genotypes	Percentual representation of the disease	Grade (FAO scale)	Rank
1. Prilep, P-26	69,5	7	7
2. Prilep, P-76	85.3	9	8
3. Prilep, P-65	21,2	1	2
4. Prilep, P-84	45.5	4 - 6	4
5. Yaka, YK 10-7/1	0	0	1
6. Xanthe-Djebel, XDJ-M	35,5	3 - 4	3
7. Basmak, MB-3	58,5	4 - 6	6
8. Samsun, SM-1	48, 5	4 - 6	5
9. Virginia, MV-1	99,5	9	9

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

F1 crosses	Disease (%)	Grade (FAO	Rank
1. SM-1 x MV-1	64,9	4 - 6	26
2. SM-1 x YK 10-7/1	1	0	2
3. SM-1 x P-26	61,5	4 - 6	24
4. SM-1 x P-76	72,6	7	30
5. SM-1 x MB-3	78,8	8	33
6. SM-1 x P-65	22,5	2 - 4	11
7. SM-1 x XDJ-M	40,1	2 - 4	16
8. SM-1 x P-84	45.3	4 - 6	18
9. MV-1 x YK 10-7/1	1,4	0	3
10. MV-1 x P-26	79,2	8	34
11. MV-1 x P-76	89,5	9	36
12. MV-1 x MB-3	71,3	7	29
13. MV-1 x P-65	24	2 - 4	13
14. MV-1 x XDJ-M	57	4 - 6	21
15. MV-1 x P-84	75,5	7	32
16. YK 10-7/1 x P-26	2	0	6
17. YK 10-7/1 x P-76	0,5	0	1
18. YK 10-7/1 x MB-3	3,5	0	7
19. YK 10-7/1 x P-65	1,5	0	4
20. YK 10-7/1 x XDJ-M	1,9	0	5
21. YK 10-7/1 x P-84	4,9	0	8
22. P-26 x P-76	86,5	8	35
23. P-26 x MB-3	70,5	7	28
24. P-26 x P-65	23,3	2 - 4	12
25. P-26 x XDJ-M	69,8	7	27
26. P-26 x P-84	57,5	4 - 6	22
27. P-76 x MB-3	73,4	7	31
28. P-76 x P-65	25,2	2 - 4	14
29. P-76 x XDJ-M	64,3	4 - 6	25
30. P-76 x P-84	60,7	4 - 6	23
31. MB-3 x P-65	25,5	2 - 4	15
32. MB-3 x XDJ-M	48,3	4 - 6	19
33. MB-3 x P-84	50,5	4 - 6	20
34. P-65 x XDJ-M	16,5	1	10
35. P-65 x P-84	12,4	1	9
36. XDJ-M x P-84	41,5	2 - 4	17

IV. DISCUSSION

Black shank is a very serious tobacco disease, first identified in 1893 on the islands Java and Sumatra. 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 [4].

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 [8], [1]. 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).

We followed the development of tobacco into trial in the field, counted diseased plants in each replication and on base of percentage assessment and application of the scale for resistance/susceptibility (FAO), made a ranking of all varieties (parents and hybrids). From parental genotype shown in Table1 the first-ranged is the variety YK 10-7/1 in which there were no diseased plants. In second ranked variety P-65 was about 21% diseased plants. In other varieties the percentage of diseased was substantially larger. The highest degree of sensitivity shown large-leaf variety MV-1 (99.5%), followed by late maturing oriental variety P-76 (85.3%). Table 2 shows the ranking of diallel crosses. The highest degree of resistance shown hybrids where one of the parents is oriental variety YK 10-7/1 (YK 10-7/1 x P-76, SM-1 x YK 10-7/1, MV-1 x YK 10-7/1, YK 10-7/1 x P-65, YK 10-7/1 x XDJ-M, YK 10-7/1 x P-26, YK 10-7/1 x MB-3 and YK 10-7/1 x P-84). Also showed high resistance the hybrids where one of the parents is oriental variety P-65 (P-65 x P-84, P-65 x XDJ-M, SM-1 x P-65, P-26 x P-65, MV-1 x P-65, P-76 x P-65 and MB-3 x P-65). The range of these crosses is from 1 to15.

Gene–for–gene relationship:

The results in Table 1 and Table 2 indicates 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. This situation was defined by Flor [3] 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. Palikarcheva and D. Bajlov [6]. Pobeda 2 is originating from the wild species *Nicotiana debneyi* and a variety of Basma tobacco – Basma 239 (Fig. 1). *Nicotiana debneyi* (Fig. 2) 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.

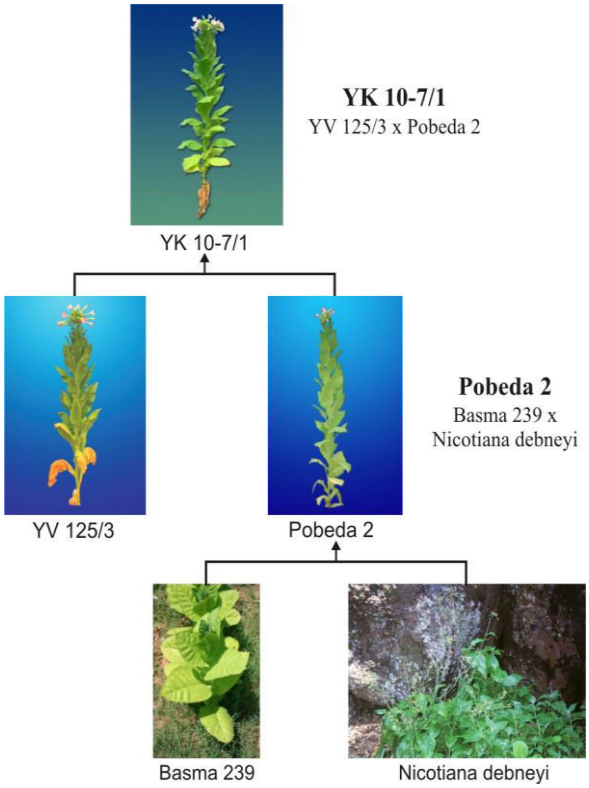

Fig. 1. Pedigree of Yaka YK 10–7/1

Fig. 2. Nicotiana debneyi

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 [8]. 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) will be eliminated, the heterozygous (Aa) will be avoided and selection will be made with the homozygous resistant (AA) individuals, which phenotypically resemble MV-1 and carry dominant genes for resistance to the disease.

The method for obtaining resistance to *Phytophthora parasitica var. nicotianae* can be applied in many other crops for various diseases, when it refers to ”vertical (specific) resistance”.

V. Conclusion

- A trial with parents and diallel 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. The highest susceptibility to the disease was recorded in MV-1 (large-leaf) and in P-76 (late maturing oriental variety).
- The highest resistance to *P. parasitica var. nicotianae* 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.

References

[1] Bonnet P., 1985. Differential reactions of tobacco to nine species of *Phytophthora* (original: Reactions differentielles du tabac a 9 especes de *Phytophtora*). Agronomie, 5: 54–60.

[2] Dimitrieski M., G. Miceska & P. Taskoski, 2012. Investigation of the resistance to Blue mold (*Perenospora tabacina Adam*) and Black shank (*Phytophthora parasitica var. nicotianae*) in some oriental tobacco cultivars and lines. Tutun/Tobacco, 1–12: 30–36.

[3] Flor H. H., 1971. Current status of the gene-for-gene concept. Annu Rev Phytopathol, 9: 275–296.

[4] Korubin–Aleksoska A., 1989. Resistance to *Phytophthora parasitica var. nicotianae* in tobacco (*Nicotiana tabacum L.*) [original: Resistencia a *Phytophthora parasitica var. nicotianae* en tabaco (*Nicotiana tabacum L.*)]. Specialization, IAMZ–Mediterranean Agronomic Institute of Zaragoza.

[5] Korubin–Aleksoska A., 2003. The effect of Back–cross hybridization on improving the characters of tobacco. Tutun/Tobacco, 1–2: 3–11.

[6] Korubin–Aleksoska A.,2004. Tobacco varieties from Tobacco Institute – Prilep. ITP, Prilep.

[7] Mitreski M., J. Aleksoski & A. Korubin–Aleksoska, 2006. Obtaining a resistance to Black shank (*Phytophthora parasitica var. nicotianae*) in tobacco. Tutun/Tobacco, 1–2: 3–10.

[8] Sanchez–Monge E., 1974. Fitogedetica. Instituto Nacional de Investigaciones Agrarias, Madrid.

[9] Taskoski P., 2003. Estimation of tobacco resistance to *Phytophthora parasitica var. nicotianae* by root inoculation. Tutun/Tobacco, 1–2: 53–61.

[10] Taskoski P., 2005. Reaction of some tobacco varieties to Black shank disease in inoculation of the stalk with races 0 and 1. Tutun/Tobacco, 7–8: 175–185.
[11] Tedford E. C., T. L. Miller & M. T. Nielsen, 1990. A detached – leaf technique for detectiong rrsistance to *Phytophthora parasitica var. nicotianae* in tobacco. Plant Disease, 4: 313–316.

AUTHOR'S PROFILE

Dr. Ana Korubin-Aleksoska was born in Prilep, Republic of Macedonia, on 1.10.1957. She graduated from the Faculty of Agriculture - Ss. Cyril and Methodius University - Skopje in 1983. Educational background: Doctorin agricultural science: Faculty of Agriculture, Ss. Cyril and Methodius University - Skopje, 1998 (doctoral dissertation: *Inheritance of some morphological and quality characters in hybrids obtained by diallel crossing of tobacco (Nicotiana tabacum L.)*; Master in agricultural science: Faculty of Agriculture, Ss. Cyril and Methodius University - Skopje, 1988 (MSc thesis**:** *Contribution to the knowledge of changes in tobacco raw in the process of production of reconstituted tobacco*); Graduated from the Faculty of Agriculture - Ss. Cyril and Methodius University - Skopje, 1983 (graduate thesis**:** *Technology of production of reconstituted tobacco in Tobacco Company-Prilep).* She completed the specialization at the International Centre for Advanced Mediterranean Agronomic Studies– Mediterranean Agronomic Institute of Zaragoza –Spain, 1989 (specialized thesis**:** Resistencia a *Phytophthora parasitica var. nicotianae* en tabaco (*Nicotiana tabacum L.*). She has been working in the Scientific Tobacco Institute – Prilep since 1985 and currently she has a tiltle of RESEARCH ADVISOR. She is also a FULL PROFESSOR at the postgraduate study programmes of the Tobacco Institute (University of St. Kliment Ohridski, Bitola, Macedonia). Presently, she is head of Department of Genetics, Selection and Seed control in Scientific Tobacco Institute – Prilep.

She has published 85 scientific papers in International and National reputable scientific Journals, a book and a handbook of genetics, breeding and seed control in tobacco. She has over 60 presentaciones at scientific meetings – congresses, symposia etc. She is co-author of two and author of four registered oriental tobacco varieties.

She likes most her 3 publications:

Korubin-Aleksoska Ana, "Tobacco varieties from Tobacco Institute–Prilep". Prilep, Republic of Macedonia, Tobacco Institute – Prilep, 2004.

Korubin-Aleksoska Ana, Nikova Violeta & Aleksoski Jane, "Regression analysis of the inheritance of leaf size in F1 and F2 progenies in various tobacco genotypes". Plovdiv, Republic of Bulgaria, BIOTECHNOL.& BIOTECHNOL, 24: 401 – 406, 2010.

Korubin-Aleksoska Ana & Aleksoski Jane, "Heritability of Quantitative Traits in F1 and F2 Progenies of some Tobacco Varieties". Zagreb, Croatia, Agriculturae Conspectus Scientificus (ACS), 4: 1-4, 2012.

Dr. Korubin-Aleksoska is a member of the Society of Arts and Sciences - Prilep, Commission for registration of plant varieties in the Ministry of Agriculture, Forestry and Water Management of R. Macedonia, Commission for approval and recognition of field and garden crops in the Ministry of Agriculture, Forestry and Water Management of R. Macedonia and various other commissions and bodies. She is a member of the Editorial board of the international publication "Tutun-Tobacco".

Ph. D. Ana Korubin - Aleksoska
Head of Department of Genetics, Selection and Seed control
Scientific Tobacco Institute-Prilep
University St. "Kliment Ohridski"-Bitola
st. "Kicevski pat" bb, 7500 Prilep
Republic of Macedonia
Tel.: + 389 48 412 760
fax: + 389 48 412 763
e -mail: anakorubin@yahoo.com
web: http://www.tip.edu.mk