

(*Ph.parasitica*, var. *nicotiane*, Dast)

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**INHERITANCE OF RESISTANCE TO BLACK SHANK
 (*Ph. parasitica* var. *nicotiana*e, Dast) IN ORIENTAL TOBACCO**

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SUMMARY

Experiments were conducted in 2013-2014 at Tobacco and Tobacco Products Institute, Rila trial station. The aim of study was to establish the inheritance of resistance to black shank (*Ph. Parasitica* var. *nicotiana*e, Dast), in varieties and hybrids oriental tobacco. Seven oriental tobacco varieties with different sources of resistance and four hybrids were used as basic selection material. All investigated hybrids in F₁ generation revealed dominant and incomplete dominance which determined by the level of resistance in basic variety and susceptible components used in hybridization. Inheritance of resistance to black shank in tested samples is dominant and incomplete dominance. Genetic analysis F₂ hybrids showed monogenic inheritance source *N.debneyi* and polygenic in varieties with sources of resistance *N.tabacum* and *N.goodspeedii*.

Key words: resistance, black shank, inheritance, oriental tobacco

2013-2014 .
 (*Ph.parasitica*, var.*nicotiana*e, Dast)
 7
 F₁ 4
 F₂
N. debneyi.
N. tabacum
N.goodspeedii.
 : ,

INTRODUCTION

Black shank is among the most destructive and economically important tobacco diseases in Bulgaria. Most serious damage it inflicted to tobacco grown in areas along the Struma river – in Sandanski-Petrich tobacco area and Rila Valley. Black shank first appeared in these regions in 1927 (Dimitrov, 2003). Monoculture tobacco cultivation and often irrigations during the summer caused mass distribution and high density of infection in these areas. Black shank is caused by the fungus *Phytophthora parasitica* var. *nicotianae*. Black shank attacks mainly the base of the stems and the root system of the tobacco, turning them dark brown to black. The disease attacks the tobacco plant throughout the period of development (Dimitrov, 2003).

A dark brown to black, somewhat sunken, lesion usually appears on the stalk at or near the ground level. This lesion often extends up the stalk or shank of the plant causing it to turn black. Stalks, when split, (Fig.1) usually reveal the blackened pith separated into discrete disks (Bozukov, 2002).

Infection of the roots, stems and leaves may occur at any stage of plant growth, which results in root necrosis, wilting, chlorosis, stem lesion growth arrest and

(Csinos and Melton, 1983; Lucas, 1975).

death of the plant (Csinos and Melton, 1983; Lucas, 1975).



. 1.

. 1. Longitudinal section of a stem of a tobacco plant infected with black shank

(, 1984).
Phytophthora parasitica var. *nicotianae*
 4

Usually parasitic fungi are poorly differentiated and their phenotype genetic variability is judged by their physiological action (Enchev Chilikov 1984). There are four morphologically similar but genetically different forms – races of the *Phytophthora parasitica* var. *nicotianae*

0
 (Lucas, 1975). Litton et. al. (1970),
 : *N. longiflora*, *N. plumbaginifolia*, *N. stocktonii*, *N. nudicaulis*.

Race 0 is the predominant strain in most tobacco-growing regions around the world (Lucas, 1975). According to Litton et.al.(1970), resistant to rase 0 are species: *N. longiflora*, *N. plumbaginifolia*, *N. stocktonii*, *N. nudicaulis*.

Php a

Introduction of *Php* gene in

1 (Apple, 1967; Csinos, 2005; Sullivan, Melton and Shew, 2005).

Litton et. al. (1970),

N. nesophila, *N. repanda*, *N. rustica var. asiatica*, *N. longiflora*, , *N. stocktonii*.

Ph. parasitica var. nicotianae *N. longiflora* Cav. *N. plumbaginifolia* (Apple 1967; Chaplin, 1962).

N. plumbaginifolia *N. longiflora*

0,

1 *Ph. parasitica var. nicotianae* (Apple 1967).

2

Appel, 1967; Gallup, Shew, 2010; van Jaarsveld, Wingfield and Drenth, 2002).

3 *Ph. parasitica var. nicotianae* –

(McIntyre, Taylor, 1978). 3

Phl
Nicotiana longiflora,
Php *N. plumbaginifolia*.

Phl

(Gallup and Shew, 2010).

the *N. tabacum* genome causes the appearance of a new race of the disease - Race 1 (Apple, 1967; Csinos, 2005; Sullivan, Melton and Shew, 2005).

According to Litton et. al. (1970), highly resistant to race 1 are: *N. nesophila*, *N. repanda*, *N. rustica var. asiatica*, *N. longiflora*, *N. stocktonii*.

Sources of single resistance gene *Ph. parasitica var. nicotianae* are *N. longiflora* Cav. and *N. plumbaginifolia* (Apple, 1967; Chaplin, 1962

Resistance from *N. longiflora* and *N. plumbaginifolia* involve single, dominant genes that are highly effective against race 0 of the black shank pathogen but provide no protection against race 1 (Apple 1967).

Race 2 was found only in South Africa and poses no threat to tobacco from other countries (Apple, 1967; Gallup, Shew, 2010; van Jaarsveld, Wingfield and Drenth, 2002).

Race 3 *Ph. parasitica var. nicotianae* – it has been reported from Connecticut (McIntyre, Taylor, 1978). Race 3 is defined as overcoming *Phl* gene from *Nicotiana longiflora*, but not *Php* gene from *N. plumbaginifolia*. It is likely that the sensitivity of tobacco plants with a gene *Phl* be due to differences in gene expression in different plant organs (Gallup and Shew, 2010).

1949 . Kincaid -
N. debneyi *N.*
tabacum. Apple (1967) Chaplin
(1962)
N. plumbaginifolia
N. tabacum.

In 1949 Kincaid incorporated resistance to wild-type *N. debneyi* of *N. tabacum*. Apple (1967) and Chaplin (1962) reported to resistance transfer from *N. plumbaginifolia* in *N. tabacum*.

- By the methods of the inter-species hybridization are created a large number of breeding materials with resistance to black shank.

- The results confirm that one way the management of black shank is selection of resistant varieties as a continuous dynamic process.

- In Bulgaria were selected several oriental tobacco varieties resistant to black shank: Krumovgrad 90 (Manolov et al., 1977); Rila 82 (Stankev and Trancheva, 1984); Nevrokop 1146 (Gelemrov, 1987); Rila 89 (Stankev and Trancheva, 1993); Dupnitsa 126 Dupnitsa 160 (Stankev, 2010) – with different sources.

- The only effective prevention program for black shank is an integrated approach including cultivation practices, fungicides and resistant varieties (Melton and Broadwell, 2003).

Aim

- Study of the inheritance of resistance to black shank (*Ph. Parasitica* var. *nicotianae*, Dast) in Oriental tobacco hybrids with different sources of resistance.

90 (, 1977);
82 (, 1984);
1146 (,
1987); 89 (,
, 1993); 126,
160 (, 2010)
(Melton and Broadwell, 2003).

2013-2014

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321,
160,

7

()

O

10 m².

-

(1981)

F₂

-

-

χ^2

(

.,1975).

MATERIAL AND METHODS

Field experiment was conducted in 2013-2014 at Tobacco and Tobacco Products Institute, Rila trial station.

Basic selection material used tobacco varieties with different sources of resistance – Sandanski 321, Dupnitsa 126, Dupnitsa 160, Struma 75, not resistant Rila 202-1A, Prilep 7 and Sandanski 144 (control) and four hybrid combinations.

The field experiment by a completely randomized block design in two replications with size of 10 m². Twenty plants of each tobacco varieties and four hybrids were inoculated.

Plant inoculation – methodology Kutova (1981) through wounds in the stems of older plants.

Inheritance of black shank resistance in F₂ generation was statistically analyzed by χ^2 test (Genchev et al., 1975).

RESULTS AND DISCUSSION

The results of black shank resistance for the parental varieties are presented in Table. 1.

1.

1.

Table 1. Resistance to black shank of parental varieties

Variety	Total number studied plants	Resistant		Susceptible	
			%		%
321	20	16	80	4	20
160	20	18	90	2	10
126	20	20	100	0	0
7	20	5	25	15	75
75 *	20	20	100	0	0
202-1 *	20	2	10	18	90
144	20	1	5	19	95

The resistance of parental varieties in the study derived from sources: Dupnitsa 126 and Dupnitsa 160 is based on *N. debneyi*.

Variety resistance Sandanski 321 derived from *N. tabacum*, variety is produced by the method of individual breeding from local population (Chobanova, 1977).

In variety Struma 75 resistance was acquired by Australian variety S-394-5 whose genome involved *N. goodspeedii*.

During the study with the highest percentage of resistance were parental varieties Dupnitsa 126 and Struma 75 (100%). In other samples resistance ranges from 10% (variety Sandanski 144) to 80% (variety Sandanski 321).

Results confirmed the literature data obtained by others authors and show the possibility of using such varieties as components in

F₁

hybridization in selection program to generate resistant hybrids.

Inheritance of resistance to black shank in F₁ hybrids

The results for four hybrid combinations are presented in Table 2.

2. F₁
Table 2. Resistance to black shank in the F₁ generation

Hybrid	Total number plants	Resistant		Susceptible	
			%		%
(Prilep 7 x Sandanski 321)	20	16	80	4	20
(Prilep 7 x Dupnitsa 160)	20	20	100	0	0
(Prilep 7 x Sandanski 126)	20	16	80	4	20
(Struma 75 x Rila 202-1)*	20	19	95	1	5

7

(Prilep 7 x Dupnitsa 160) 100%
 (Prilep 7 x Sandanski 321) 80%
 (Struma 75 x Rila 202-1) - 90% -
 (1973)

The table shows that inheritance of resistance to black shank in four hybrids was dominantly. Hybrids F₁ (Prilep 7 x Dupnitsa 160) were 100% resistant.

In hybrids (Prilep 7 x Sandanski 321) and (Prilep 7 x Dupnitsa 126) 80% of infected plants have no symptoms, while (Struma 75 x Rila 202-1A) - 90% - incomplete dominance.

According to Genchev (1973) resistance of higher plants against parasitic fungi inherited most often dominantly, while the property of virulence of the parasite -

(1977), 1987; (1987).
 F₁
 F₁ *plumbaginifolia* (1966) *goodspeedii*,
 F₂

recessive.

Similar results were obtained by other authors (Trancheva, 1987; Chobanova, 1977) as the dominance depends on the level of resistance of parental variety and the influence of modifying factors (Trancheva, 1987).

Incomplete dominance of resistance in F₁ is due to the action of genes modifiers existent in the sensitive parental variety acting inhibition of expression of the main gene.

That explanation for incomplete dominance in F₁ hybrids source *N. plumbaginifolia* reported Chaplin (1966) and source *N. goodspeedii*, Chobanova (1977).

Inheritance of resistance to black shank in F₂ hybrids

In studies on the genetic structure plant immunity, it has been found that even for the same parasite and the same plant results by genetic analysis sometimes vary.

This is due to the genetic characteristics of different varieties, the physiological differentiation of the parasite and the conditions under which it was carried out.

Given that the response of the host to the parasite can be

modified by external conditions widely, it is clear how complicated is breeding work aimed at creating immune or resistant varieties in varying degrees.

The results of the genetic analysis of inheritance of resistance to black shank are presented in Table. 3.

3.

3.

F₂

Table 3. Ratio between resistant and susceptible plants to the black shank in F₂ hybrids

Hybrid		/ Ratio				χ^2_{ex}	χ^2_{st} P _{0,05}
		Obtained		Expected			
		R	S	R	S		
7	321	4	16	15	5	32,27	3,84
7	160	16	4	15	5	0,27	3,84
7	126	12	8	15	5	2,40	3,84
(75 x 202-1)*		19	1	15	5	4,27	3,84

χ^2

(7

(7

/

126

160
N.debneyi.
(1987)

F₂

126)

160)

3:1.

Data was analyzed by χ^2 test. In hybrids F₂ (Prilep 7 x Dupnitsa 126) and (Prilep 7 x Dupnitsa 160) resistance was controlled by a simple dominant gene and correlates with Mendelian distribution – resistant/susceptible 3:1. Statistical analysis of the data shows monogenic control of black shank resistance in varieties Dupnitsa 126 and Dupnitsa160 source of resistance *N.debneyi*.

Trancheva (1987) also found monogenic control in some

hybrid's varieties Nevrokop B-12,
 Petrich 84 Imuniy 580 (*N.debneyi*).
 -12, 84,
 580
 (*N.debneyi*).
 (7 x 321)
 (75 x 202-1)
 χ^2 χ^2_{st} . 32,27
 7,20,
 ,
 .
N. tabacum N.
goodspeedii.
 321 (*N. tabacum*)
 75 (*N.goodspeedii*),
 (1987)

hybrid's varieties Nevrokop B-12,
 Petrich 84 Imuniy 580 (*N.debneyi*).

For hybrid combinations
 (Prilep 7 x Sandanski 321) and
 (Struma 75 x Rila 202-1A) null
 hypothesis is not confirmed.
 Values χ^2 χ^2_{st} are respectively
 32.27 and 7.20, which is reason to
 assume that the observed
 experimental results is not
 correspond to the theoretical
 prediction.

Sources of resistance in two
 varieties respectively *N. tabacum*
 and *N. goodspeedii*.

As a result of genetic studies
 of resistance to black shank in
 varieties Krumovgrad 90,
 Sandanski 321(*N.tabacum*) and
 Struma 75 (*N. goodspeedii*),
 Trancheva (1987) found that the
 resistance is inherited dominantly
 and is controlled by three
 dominant gene, which confirms
 our results.

CONCLUSIONS

1. -
 126 ,
 75 321.
 2. -

1. The highest percentage of
 resistance to black shank have
 varieties Dupnitsa 126, Struma 75
 and Sandanski 321.

2. Inheritance of resistance to
 black shank in tested samples is
 dominant and incomplete
 dominance.

3.	-	3. Genetic testing showed monogenic inheritance source <i>N.debneyi</i> and polygenic in varieties with sources of resistance <i>N.tabacum</i> and <i>N. goodspeedii</i> .
<i>N. Goodspeedii</i> .	-	

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<i>Parasitica var. nicotianae, Dast.</i>	-	

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ECONOMIC AND CHEMICAL INDICATORS OF INTRODUCED MALE STERILE LINES OF *BURLEY* TOBACCO

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SUMMARY

2010-2012 .

2002.

7%-28%
2002.

The study was conducted during the 2010-2012 period at the Khan Krum DP Experimental Station of Agriculture in the municipality of Shumen. The economic and chemical indicators of five male sterile lines of introduced *Burley* tobacco were studied. *Pliska 2002* variety was used as a standard. The resulting material was of better quality and gave a higher yield of cured tobacco by 7%-28% compared with the *Pliska 2002* standard. The lines were characterized by a balanced chemical composition and met the essential requirements for that tobacco type – a high nicotine content and low content of soluble carbohydrates.

Key words: tobacco, cytoplasmic male sterility, yield, quality

INTRODUCTION

The use of CMS in the heterosis selection of tobacco has been recognized as a potential opportunity for solving the

at the expense of changing one of the indicators – it depends on the enhancement of the overall system of indicators which are in a close interaction amongst themselves as well as with the factors of the environment (, 1974, 1987).

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-
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The use of tobacco varieties of cytoplasmic male sterility is still quite limited in Bulgarian production. The creation and use of new CMS lines and analogues in the future are bound to result in the yield increase and quality improvement of both the leaf and seed produce.

- The aim of the research was
- to study the economic and chemical traits of five introduced male sterile lines of Burley tobacco in order to estimate the chances for using them in the heterosis selection which would lead to
 - reducing hand labour in the process of hybrid seed production.

MATERIAL AND METHODS

2010-2012
 –
 24 m².
 100 40

The study was carried out during the 2010-2012 period in the Khan Krum DP experimental field. The experiments were performed by the block method in four replications, the size of the yield plot being 24 m².

The planting of the tobacco was done following the 100 40 cm pattern by the end of May. The basic cultivation and technical

cm

()

2002

- activities as well as the control of
 - pests and diseases were carried out in compliance with the adopted technology for growing *Burley* tobacco in the region. Five introduced male sterile lines and *Pliska 2002* (control) were involved in the research.

(2010-2012 .)

50-

The meteorological conditions during the period of the study (2010-2012) were characterized by a great variety during the years as well as when compared with the average figures over a 50-year period perceived as

normal for the region. The precipitation quantity was higher than the normal for May when the tobacco was planted. The years were characterized by an uneven rainfall distribution during the tobacco vegetation from May to September. The years 2011 and 2012 were averagely dry with high mean monthly temperatures and low relative air humidity. All those factors influenced the growth and development of the tobacco plants.

2011-2012

- The tobacco was harvested
 - when technically mature in four pickings and the mean yield and quality of the cured tobacco were estimated.

The dry tobacco was analyzed in order to determine the following indicators: nicotine – by the Coresta method (CORESTA RM 20, 1968),
 - soluble carbohydrates – by the School's method (BSS 9143 – 1988), total

15836-1988)

„BIOSTAT” (Penchev et al., 1989-1991).

(nitrogen – by the re method (BSS 15836-1988).

The statistical processing of the results was performed by using BIOSTAT (Penchev et al., 1989-1991).

RESULTS AND DISCUSSION

The average yields of dry tobacco from the tested male sterile lines are given in Table 1.

1.

1. Cured tobacco yield, kg/da
Table 1. Cured tobacco yield, kg/da

/ Variety	2010		2011		2012		/ Mean	
	kg/da	%	kg/da	%	kg/da	%	kg/da	%
2002st	279,25	100,00	354,63	100,00	316,39	100,00	316,76	100,00
Pliska 2002 st.								
- 01	366,71+++	131,32	443,75+++	125,13	388,38+++	122,47	399,61+	126,16
MS – 01								
- 02	416,96+++	149,31	416,67++	117,49	385,75+++	121,92	406,46++	128,32
MS – 02								
- 03	293,29	105,40	362,25	102,15	378,50+++	119,63	344,68	108,81
MS – 03								
- 04	324,59+++	116,24	488,79+++	137,83	341,00	107,78	384,79+	121,48
MS – 04								
- 05	315,50++	112,98	386,04	108,86	319,38	100,95	340,31	107,43
MS – 05								
GD 5%	22,43		38,80		24,66		62,81	
1%	31,01		53,66		34,10		89,34	
0,1%	42,86		74,17		47,13		129,32	

It is noteworthy that the tested materials produced the highest yields in the second year of the research – 362.25-488.79 g/da, the excess in MS-04 as compared with the standard *Pliska 2002* being 37.83% while in MS-01 and MS-02 it was within 17.49 – 25.13%, the differences being of a high credibility extent. On the average for the three-year test period, MS-02 occupied first position for cured tobacco yield

2. , %
Table 2. Cured tobacco quality by classes, %

/ Variety	2010			2011			2012			/ Mean		
	1	2	3	1	2	3	1	2	3	1	2	3
2002	11,36	43,25	45,39	18,99	60,03	20,98	11,31	66,13	22,56	13,88	56,48	29,64
Pliska 2002 st.												
- 01	12,67	62,43	24,60	27,32	52,39	20,29	30,62	49,00	20,38	23,54	54,71	21,75
MS - 01												
- 02	25,25	45,62	29,13	17,47	60,53	22,00	24,51	58,65	16,84	22,41	54,93	22,66
MS - 02												
- 03	9,41	54,41	36,18	13,79	64,68	21,53	17,93	62,87	19,20	13,71	60,65	25,64
MS - 03												
- 04	10,40	60,98	28,62	35,26	53,50	11,24	24,21	58,90	16,89	23,29	57,79	18,92
MS - 04												
- 05	5,90	57,58	36,52	15,17	50,00	34,83	19,56	63,25	17,19	13,54	56,94	29,52
MS - 05												
GD 5%										11,34	14,04	10,07
1%										16,14	19,97	14,33
0,1%										23,36	28,90	20,74

3.

- The results for nicotine
- content from our research over the
- years are presented in Table 3. It
is obvious that the meteorological
conditions during the years
influenced considerably the
percentage of nicotine content.

3. , %
Table 3. Content of nicotine, %

/ Variety	/ Year			/ Mean	%
	2010	2011	2012		
2002	1,23	1,36	1,07	1,22	100,00
Pliska 2002 st.					
- 01	1,75	2,09	2,56	2,13	174,59
MS - 01					
- 02	1,69	2,14	2,17	2,00	163,93
MS - 02					
- 03	2,43	2,89	2,92	2,75	225,41
MS - 03					
- 04	1,62	2,27	2,38	2,09	171,31
MS - 04					
- 05	1,39	2,24	2,35	1,99	163,11
MS - 05					
/ Mean	1,96	2,17	2,24	2,03	166,39

- 2011 2012
2,17%

- During the drier 2011 and
2012 the nicotine content
averagely for the group was 2.17%

2,24%,
2010

1,69%.

-03 2,43% 2,92%
(225,4 %)

63,1 74,6%.

1%

(4)

1%,

and 2.24% while in the more humid 2010 it was 1.69%. The data showed that line MS-03 had the highest nicotine content during the test period – from 2.43% to 2.92% (surpassing the standard by 225.4%). The rest of the lines surpassed the standard *Pliska 2002* indicator by 63.1% - 74.6% over the years.

The basic characteristic trait of *Burley* tobacco distinguishing it from *Virginia* and *Oriental* is the low content of soluble carbohydrates, it being under 1% down to the so called traces.

The data from our investigation (Table 4) show that the content of soluble carbohydrates in the studied varieties over the years was under or around 1% which is typical of that tobacco type.

4.
Table 4. Soluble sugars,%

/ Variety	/ Year			/ Mean	%
	2010	2011	2012		
2002	0,57	0,52	0,68	0,59	100,00
Pliska 2002 st.					
- 01	0,43	0,27	0,54	0,41	69,49
MS – 01					
- 02	0,14	0,52	0,54	0,40	67,80
MS – 02					
- 03	0,29	0,39	0,68	0,45	76,27
MS – 03					
- 04	0,14	0,12	0,55	0,27	45,76
MS – 04					
- 05	0,57	0,21	0,81	0,53	89,83
MS – 05					
/ Mean	0,36	0,34	0,63	0,44	74,58

-
 -
 -
 0,34%
 (2011 .) 0,63% (2012 .).
 -
 0,27% (45,8%
 -04 -
 -
 67,8-89,8%
 2002.
 ,
 0,27-0,59%,
 -
 -
 (Popovic, 1985;
 , 1996; , 2002)
 .
 3,08 4,36%.
 5. 3-
 ,
 -
 3,80%
 2002.
 12,4
 16, 8% -
 .
 ,

Averagely for the group during the testing period the content of soluble carbohydrates was within the limits of 0.34% (2011) to 0,63% (2012). The lowest figures for that indicator averagely for the period had line MS-04 – 0.27% (45.8% compared with the standard). The rest of the lines had lower values of 67.8%-89.8% compared with the standard *Pliska 2002* variety.

The data analysis showed that on the average in that experiment the level of soluble carbohydrates was within the limits of 0.27-0.59% which had also been confirmed by the research of several authors (Popovic, 1985; Drachev, 1996; Pelivanovska, 2002).

The total nitrogen quantity was in a negative correlation with the tobacco quality. In the typical American *Burley* tobacco the total nitrogen varies from 3.08 to 4.36%.

The results of the chemical analysis for the total nitrogen content are given in Table 5. Averagely for the three-year testing period as well as for the years of the highest total nitrogen content 3.80% was the standard *Pliska 2002*. The studied male sterile lines had 12.4% to 16.8% lower total nitrogen content as compared with the control.

The influence of the protein substances on the consumer qualities of the tobacco was negative on the whole since when

disintegration of proteins under high temperature took place nitrogen substances of unpleasant smell and irritating taste resulted in the tobacco smoke.

5. Total nitrogen content, %

/ Variety	/ Year			/ Mean	%
	2010	2011	2012		
2002	3,54	3,90	3,95	3,80	100,00
Pliska 2002 st.					
- 01	3,20	3,04	3,24	3,16	83,15
MS - 01					
- 02	3,32	3,11	3,04	3,16	83,15
MS - 02					
- 03	3,91	2,96	3,11	3,33	87,63
MS - 03					
- 04	3,36	3,02	3,42	3,27	86,05
MS - 04					
- 05	3,45	3,05	3,25	3,25	85,53
MS - 05					
/ Mean	3,46	3,18	3,34	3,33	87,63

As a result of the cured tobacco chemical analysis of the indicators for the nicotine content, soluble carbohydrates and total nitrogen, lines MS-02 and MS-04 stood out clearly.

CONCLUSIONS

The introduced male sterile lines MS-01, MS-02 and MS-04 are high-yielding and of high quality. Averagely for the period they surpassed the standard *Pliska 2002* by 21.5 -28.3% for the cured tobacco yield.

For the nicotine content, soluble carbohydrates and total nitrogen indicators, the lines had better results than the *Pliska 2002* standard and met the major requirements for that type of tobacco – high nicotine content

-02 -04.

-01, -02

-04

2002 21,5 28,3%.

2002

and low content of soluble carbohydrates.

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