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Original Scientific paper

MONOHYBRID RECESSIVE INHERITACE OF THE LENGTH OF GROWING PERIOD IN SOME ORIENTAL TOBACCO VARIETIES

Miroslav Dimitrieski,Gordana Miceska,

Un. "St. Kliment Ohridski" Bitola, Scientific tobacco institute, Prilep, Republic of Macedonia

e-mail:miroslavdimitrieski@yahoo.com, miceska.gordana@yahoo.com

ABSTRACT

Inheritance of length of growing period (days from transplanting to the beginning of flowering) was investigated by crossing Basma tobacco variety with shorter growing period and Veliki Hercegovac variety with longer growing period.

It could be stated that all plants in F1 generation began their flowering in the same time with parental variety with shorter growing period. From the self-pollinated hybrid individuals of F_1 generation, 3/4 of plants in F_2 generation had short growing period (under the influence of dominant allele) and 1/4 of plants had long growing period under the influence of recessive allele. Split ratio was 3:1 and frequencies distibution was assessed by X2 (chi-square) test.

Knowing that breeding work often imposes the use of characters inherited in a monohybrid recessive mode, we made efforts to use this type of inheritance in creation of new, more productive tobacco genotypes with longer growing period compared to the early maturing variety, preserving in the same time its quality features.

Keywords: production, monohybrid,growing period, tobacco, oriental

МОНОХИБРИДНО РЕЦЕСИВНО НАСЛЕДУВАЊЕ НА ДОЛЖИНАТА НА ВЕГЕТАЦИОНИОТ ПЕРИОД КАЈ НЕКОИ ОРИЕНТАЛСКИ СОРТИ ТУТУН

Со овие истражувања е проучувано наследувањето на должината на вегетациониот пери-од (денови од расадување до почеток на цветање), при вкрстување на една сорта тутун од типот басма (со кратка вегетација) и сортата Велики Херцеговац(со долга вегетација). Во F_1генерацијата е констатирано дека сите растенија започнуваат да цветаат истовремено со родителската сорта со кратка вегетација.

Од самоопрашените хибридни индивидуи од F_1 генерација, во следната F_2 генерација се добиени околу 3/4 од растенијата со кратка вегетација (условени од влијанието на доминантниот алел),а 1/4 од растенијата се со долга вегетација (условени од рецесивниот алел). Цепењето е во сооднос 3:1, а соодветноста на фреквенциите е тестирана по критериумот на X^2 (хиквадрат) тестот.Имајки го предвид фактот дека во селекционата работа често пати се налага потребата од искористување на својствата кои се наследуваат монофакторијално рецесивно, си поставиовме за цел овој начин на наследување да го примениме при создавањето на нови попродуктивни генотипови тутун со подолг вегетационен период од родителската раностасна сорта, а притоа задржувајки ги нејзините квалитетни својства.

Клучни зборови: производство, монохибридно,вегетационен период,тутун, ориенталски.

INTRODUCTION

In hybridization process, genetically stable parental components, according to Mendel, always have allelomorphic, i.e. allelic characters, which are homozygous and have AA or aa alleles (Borojevic S., Borojevic K., 1976; Stojkovski C., Ivanoska S., 2002, etc). These organisms produce one type of gametes. By merging the gametes with different alleles from both parents, the first hybrid generation (F_1) is obtained, the progeny of which is heterozygous (Aa). Hybrid units of this progeny are equal with regard to the character investigated, in accordance with the First Mendel's Law, i.e. the principle of uniformity of F_1 individuals. F_1 hybrid self-pollinate to create F_2 generation, in which segregation of characters in certain phenotypic and genotypic ratio is made, in accordance to the Second Mendel's Law on segregation in F_2 generation (Genchev 1980, Marinkovic 1982, Gershenson 1983, Ayala F. J., Kiger J.A.1984 etc.).

In modern selection, hybridization is the most appropriate method for creation of the necessary diversity of initial breeding material from which new varieties of plants can be created and stabilized.

Inselection work and hybridization, sometimes it is necessary to preserve some properties that have a recessive character. The goal of our investigations was to show practically the model of monohhybrid recessive inheritance and stabilizationof the character length of growing period in hybridization of the oriental variety Basma (with short growing period) and the variety Veliki Herzegovac (with long growing period).

MATERIAL AND METHODS

Trials were set upon the Experimental field of Tobacco Institute, Prilep, starting from2008.

Two tobacco varieties were used as starting material for hybridization: Basma (with short growing period) and Veliki Hercegovac (with long growing period). The length of the growing period from planting to the beginning of flowering ranged from 55 daysin Basma variety to over 120 days in Veliki Herzegovac. The length of the growing period from planting to the end of maturation of top leaves ranged from 95 - 110 days in Basma to over 180 days in Veliki Herzegovac (according Uzunoski, 1985).The choice of parental varieties was made on the basis of previous investigations. The crossing was completed in 2008, with Basmavariety as a mother, and Veliki Herzegovacas a father. F_1 progeny was obtained iIn 2009, F1 and F_2in 2010 and F_3 in 2011. The breeding process was carried outafter the method of intraspecies hybridization and Mendel's basic laws on inheritance, applying the pattern of monohybrid recessive inheritance of the investigated character (Scheme 1). Hybrid progenies of F_1, F_2 and F3 generations were grown on suitable area with sufficient number of individuals, complying with the needs of the proposed selection program. The F2 generation was monitored for cleavage (segregation) of the character and the goodness of fit for the frequencies was determined using the chi-squared ($x2$) test (Ayala F.J., Kiger J.A., 1984). Statistical analysis of cleavage in F_2 generation after this test wasobtained from the ratio between the actual (empirical) and theoretical values, according to which the probability P is determines in case when deviation is regular, using the Fisher's table. In agricultural sciences, the p-valueis conditionally defined to be equal to the value at 0.05.

RESULTS AND DISCUSSION

The process of hybridization started bycrossing the oriental shorter-growing variety Basma (AA) as maternal component and semioriental longer-growing variety Veliki Herzegovac (aa) as paternal component. In hybrid progeny of F1 generation heterozygous, phenotypically uniform individuals (Aa) with respect to the investigated character were obtained.

All hybrid individuals had a short short growing period, i.e. theyflowered almost simultaneously with the parent with shorter growing period, which indicates that this alternative character (allele) is dominant, and the longer growing period is recessive. Gornik (1973) reported thatthe period required for flowering sometimes appears as a dominant and sometimes as recessive character, depending on the varieties taken for hybridization.

Such mode of inheritance of the length of growing period was also stated by other authors. Thus, Chinchev (1979), usingthe top-cross method in his analysis of F1 hybrids, reported the lowest GCA valuefor the character days to flowering in oriental varieties Krumovgrad 988 and Plovdiv 7, as well as in Virginia varieties NC 2326 and Mc Nair 20, i.e. they had a shorter growing period, while longer growing period was observed in Virginia varieties 1349 and Coker 254. Stankev (1987) reported the highest GCA value in varieties Krumovgrad 90, Rila 544 and line 202-1a, i.e. those varieties showed the best GCA, i.e. longer growing period, regardless of the change of varieties that served as a tester. He also suggested that varieties Sandanski 321, No 888, Rila 9, Plovdiv 7 and Struma 75 can be used in cases where shorter growing period is required.

Bogdanceski (1984), in his three-year investigations of various hybrid combinations, reported differences in the inheritance of the character length of the growing period until 50% of flowering. Thus, Prilep x Nevrokop 261 and Prilep x Pazardzik 17 and their reciprocal crossings showed dominant inheritance of this character, i.e. all plants from F_1 flowered simultaneously with the variety Prilep (with shorter growing period), and other hybrid combinations showed intermediate inheritance.

From self-pollinated hybrid individuals of F1 generation, phenotypically different plants were derived in a ratio 3: 1, i.e. 3 plants (75%) are with shorter growing period and 1 plant (25%) is with longer growing period.

The results of statistical analysis (Table 1) of plants with short / long growing period ratio in F_2 progeny showed that theoretically expected segregation (3:1) was obtained.

Sinceinour casethe calculated value of $x2$=1.92 does not exceed the Table value for P = 0,05 (3,84), it means that actually obtained segregation is in compliance with theoretically expected 3:1 ratio.

Table 1. Ratio between the plants with short and long growing period in hybrid combination Basma x VelikiHerzegovac

Variants	Number of plants in F^2 generation		χ^2	P
	with short growing period	with long growing period		
Investigated individualsexperimental figures(**e**)	219	87	$\chi^2=\Sigma \mathbf{d}^2/\mathbf{t}$ χ^2=1,92	0,05=3,84
Expected individualstheoretical figures(**t**)	229,5	76,5		
Deviation (**d**)	10,5	10,5		
$\mathbf{d}^2$	110,25	110,25		
$\mathbf{d}^2/\mathbf{t}$	0,4803	1,4411		

Accordingly, it may be concluded that division (segregation) of the investigated character was observed in F_2 generation, where the theoretical ratio of the genotypes (presented on Scheme 1) was: 1AA : 2Aa: 1aa, i.e. 75% of the plants in this hybrid progenyhad short growing period (Aa and AA) and 25% were homozygous, with long growing period (aa).

Most of the plants (75%) of Aa and AA genotype with short growing periodwere not grouped by their time of flowering, because the subject of our investigations was the recessive character long growing period (aa).

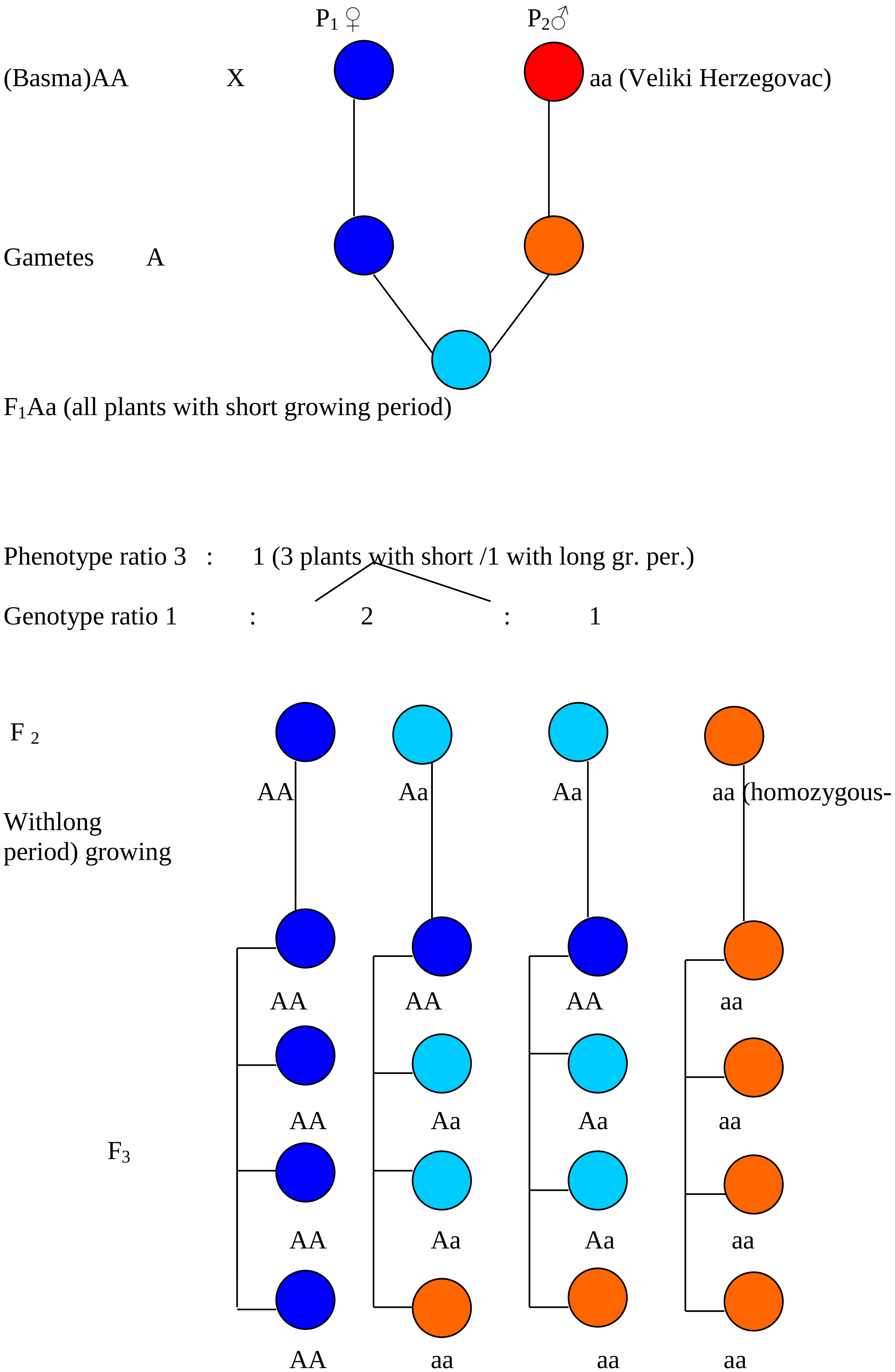
The plants with long growing period obtainedin F_2 (Figure1) progenyun doubtedly indicate that even in this generation we derived homozygous plants in relation to this recessive character. In order to confirm with certainty the resulting genotype with long growing periodin the hybrid population, 6 of the plants were selected and isolated (for self-pollination) in accordance with the intended aim of investigations.

These plants were used to create specific progenies in F_3 (Figure 2) generation and after inspection it was determined that all hybrid individuals of the investigated progenies had a long growing period, which indicates that they are homozygous with respect to this character.

Hence, it can be concluded that this character was permanently incorporated into their genome. Our next goal in selection will be to consolidate these progenies with other desired morphological, productional and qualitative characters.

Figure 1 - (Plot 169)

Figure 2 – (Plot 170)



Scheme N° 1 Monohybrid recessive inheritance of the character long growing period in F_1, F_2 and F_3 generation

CONCLUSION

Based on the results on monofactorial inheritance of the character length of growing period, in hybridization performed between the varieties Basma (with short growing period) and Veliki Herzegovac (with long growing period), the following conclusions can be drawn:

The character long growing period has a monohybrid recessive pattern of inheritance.

The recessive nature of the investigated character makes the selection process more complicated because it doesn't appear in F_1 generation. Therefore, it is necessary to transplant more plants (over 150) in F_2 generation, to achieve higher probability for determination of plants-carriers of this character.

In there is a need for creation of new line and varieties with long growing period, the monofactorial recessive inheritance of this character from the parent with long growing period will allow to obtain homozygous plants (aa) as early as F_2 generation, and monitoring of the character is easily controllable because flowering is a visible biological characteristic.

The applied selection scheme is simple and,beside monitoring of the monohybrid recessive character, it allows parallel selection of hybrid individuals with respect to other quantitative and qualitative characters desired.

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GRAPHIC ANALYSIS OF THE INHERITANCE OF LEAF SIZE IN SOME TOBACCO VARIETIES AND THEIR DIALLEL F1 AND F2 HYBRIDS

Ana Korubin-Aleksoska[1], Jane Aleksoski
[1]*Scientific Tobacco Institute – Prilep, Republic of Macedonia*
e-mail: anakorubin@yahoo.com

ABSTRACT

The paper presents investigations on the inheritance of length, width and area of middle belt leaf in oriental tobacco varieties Prilep (P0 10-3/2), Djebel (Pobeda P-2) and Yaka (YK 48), and the semi-oriental Forchheimer Ogrodowny - FO, including their six F1 and six F2 hybrids. Investigations and crosses were made in 2006 and 2007, and the final trial with parents and hybrids was set up in 2008, in the field of Scientific Tobacco Institute-Prilep, in randomized block system with four replications.
The aim of investigations was to present a comprehensive picture on the genetic system of inheritance of these characters by the use of graphic analysis.
Values of the major genetic components required for graphic presentation were calculated from the average values of the investigated characters, using the methods of Mather and Jinks (1974).
The regression graphs reveal partially dominant type of inheritance of the characters and absence of interallelic interaction. The position of points along the regression line reveals higher number of dominant genes in P 10-3/2 for inheritance of length, width and area of the middle belt leaves. P-2 variety has higher number of recessive genes. In YK 48 both dominant and additive genes are present, with small prevalence of ones over the others. In FO variety dominant genes prevail for inheritance of leaf length and width, and recssive genes for inheritance of middle belt leaf area.

Key words: tobacco (*Nicotiana tabacum L.*), inheritance, quantitative characters, graphic analysis

ГРАФИЧКА АНАЛИЗА ЗА НАСЛЕДУВАЊЕТО НА ДИМЕНЗИИТЕ НА ЛИСТОВИТЕ КАЈ ТУТУНСКИ СОРТИ И НИВНИТЕ ДИЈАЛЕЛНИ F1 И F2 КРСТОСКИ

Трудот опфаќа истражувања за наследување на должината, ширината и површината на листовите од средниот појас, кај сорти од ориенталските генотипови: прилеп (П 10-3/2), џебел (Победа П-2) и јака (ЈК-48), и Forchheimer Ogrodowny – FO од полуориенталски тип, како и нивните шест F1 и шест F2 крстоски. Вкрстувањата беа направени во 2006 и 2007 година, а финалниот опит со родителите и крстоските беше поставен во 2008 година на опитното поле во Научниот институт за тутун – Прилеп по случаен блок – систем во четири повторувања.

Целта на нашите истражувања е преку графичката анализа да се даде комплетна слика на генетскиот систем за наследување на наведените својства.

Вредностите на главните генетски компоненти потребни за графиконите беа добиени врз база на просечните вредности од мерењата на својствата, со примена на методите на Mather и Jinks (1974).

Од графиконите на регресија се открива парцијално доминантен начин на наследување на својствата и непостоење на интералелна интеракција. Од позицијата на точките на дисперзија по должината на линијата на регресија дознаваме дека сортата П 10-3/2 има повеќе доминантни гени за наследување на должината, ширината и површината на листовите од средниот појас. Сортата П-2 има повеќе рецесивни гени. ЈК 48 поседува доминантни и адитивни гени, со мали предности на едните или на другите. FO има претежно доминантни гени за наследување на должината и ширината на листовите, а рецесивни за наследување на нивната површина.

Клучни зборови: тутун (*Nicotiana tabacum L.*), наследување, квантитативни својства, графичка анализа.

INTRODUCTION AND AIM OF INVESTIGATIONS

Plant selection is a creative process of obtaining new and superior genotypes. For improvement of the genetic potential of wanted traits it is necessary to get a good knowledge on the mode of their inheritance, genetic regulations and selection methods. By application of the method of graphical analysis, a complete picture of the quantitative traits inheritance system can be obtained.

Many authors used the graphical analysis in their genetic investigations. Ibrahim and Avratovscukova (1982) in a diallel of five flue-cured tobacco varieties and ten F1 crosses showed overdominant type of inheritance for leaf length and partial dominant type for leaf width. Jung & co. (1982) in six oriental varieties and fifteen diallel F1 crosses revealed non-allelic gene interaction for leaf number, partial dominance for stalk height and overdominance for inheritance of yield. Lee & Chang (1984), in Korean local and oriental varieties and 28 F1 hybrids showed partial dominance for leaf number and leaf size. Dobhal (1988) revealed overdominant type of inheritance for leaf size and green/dry leaf mass yield in a diallel of ten parental genotypes of Nicotiana rustica. During the 4-years investigation of four Burley varieties and six F1 crosses, Butorac & co. (1999) revealed partial dominance in1992/1993 and total dominance in 1994/1995 for leaf number and overdominance for inheritance of yield.

The aim of our investigations was to present and interpret the regression graphs for inheritance of middle belt leaf size in tobacco varieties of different genotypes and their diallel F1 and F2 progenies. The methods of creation and interpretation of graphs can be used in genetic investigations of quantitative traits in many other crops.

MATERIALS AND METHODS

Based on previous investigations of the varieties available in Scientific Tobacco Institute-Prilep, four varieties were selected, of which three oriental (P 10-3/2, P-2 and YK 48) and one semi-oriental (Forchheimer Ogrodowny FO). After two-years diallel crossing and measurin, six F1 and six F2 crosses were obtained, and they served as a basic material for this paper. By application of genetic analyses, we developed a scheme applicable to any other diallel.

In 2006, in flowering stage, a seed of six diallel combinations for F1 generation was obtained by manual castration and pollination. In 2007, in a trial with selected homozygous parental genotypes and their F1 progeny, a seed for F2 generation was collected and additional diallel crossings were made to obtain seed for F1 generation. In 2008, a randomized trial was set up with four replications in the experimental field of Scientific Tobacco Institute-Prilep. Beside parents, it included six diallel F1 and six F2 crosses: P 10-3/2 x P-2, P 10-3/2 x JK 48, P 10-3/2 x FO, P-2 x JK 48, P-2 x FO and YK 48 x FO.

Parents and their crosses were sown in polyethylene covered seedbeds on 11.04.2008 and seedlings were transplanted on 2.06.2008. The trial was set up on diluvial-colluvial soil.

Space between rows was 45 cm, with protective raw at the beginning and end of each replication. Space within the row differed depending on the type of the parent, i.e. cross. For oriental parents it was 15 cm, for FO 25 cm, for combinations with only oriental varieties 15 cm and for those which included FO it was 20 cm.

The area of each replication was about 235 m^2. The total useful area was 940 m^2, and together with paths it approximated 1100 m^2.

All suitable cultural practices were applied during the growing period of tobacco.

Manifestation of quantitative characters greatly depends on the effect of environmental factors. Thus, in 2008, during tobacco growth in field (May - September), the mean monthly temperature was 19.91^{0}C and the total amount of precipitation 235.44 mm.

The following morphological characters were subject of analyses: length, width and area of the middle belt leaf. The length and width were measured during the tobacco flower stage (end July - August). In parental genotypes and F1 generation 100 leaves were measured from each replication, i.e. 400 leaves from middle primings, and in F2 200 leaves were measured from each replication, i.e. 800 leaves in the whole trial.

Leaf area was calculated by multiplication of length with the width and with the coefficient k=0.6354 (relative area).

Data obtained for each character by combinations for F1 and F2 generations were subject of variational-statistical analysis.

Type of inheritance was estimated according to the test-significance of the mean values of F1 and F2 in relation to the parental average (Borojevic , 1981).

Genetic components needed to compose the graphs for F1 were estimated by the methods of Jinks(1954) and Hayman (1963), and for F2 by the methods of MatherandJinks (1977).

Graphical analysis (regression analysis) was made by the methods of Mather and Jinks (1974). It is based on interpretation of $\overline{V_R}\ \overline{W_R}$ - graphs, where $\overline{V_R}$ is the variance of all progenies from all parents, and $\overline{W_R}$ is covariance of the parental progeny. Here, cross-section of the regression line with Wr-ordinate is very important, as well as the position of dispersion points on the diagram along the regression line. Points of the dispersion diagram are always within the limiting parabola ($\overline{W_R}^2 = \overline{V_R} \cdot Vp$), and the interallelic interaction depends on the quadrant in which they are placed. In case of absence of interallelic interaction, the regression line always equals to 1 (b=1). The cases where the regression coefficient "b" is significantly different from 1 and insignificantly different from 0, indicate the presence of interallelic interaction or epistatsis.

Cross-section of the expected regression line with Wr-ordinate in the origin is marked with "a" in the equation which denotes the direction of the line (y = a + bx) and it is an indication of the level of dominance. When D is higher than H1, and "a" has a positive value, the expected regression line intersects the Wr-ordinate above the origin, which denotes partial dominance. If D and H1 have an equal value, "a" equals 0 and the expected regression line cuts the origin, it is an indication of full dominance. When D is lower than H1, "a" has negative value and the expected regression line intersects the

WR-ordinate below the origin, it denotes a presence of overdominance.

Dispersion points on the diagram along the regression line shows the distribution of dominant and recessive genes in the parents. Points that are closer to the origin present the parents with a higher number of dominant genes and vice-versa, points on the opposite side of the origin present the parents with higher number of recessive genes. The completely dominant parents are positioned on the lowest part of the expected regression line, closest to the origin where it intersects the lower part of the limiting parabola. Parents in which recessive genes are predominant are positioned on the upper part of the expected regression line, where it intersects the limiting parabola at the most distant point from the origin.

Estimation of the regression coefficient $\beta = 1$ is made through the regression coefficient (b) and the standard error (Sb), according to Stell and Torrie (1960).

RESULTS AND DISCUSSIONS

Measuring of the characters and estimations of their average values in quantitative genetics are the basic ground for investigations of their inheritance.

Average values obtained for the characters length, width and area of the middle belt leaves and the mode of their inheritance are presented in Table 1. There are significant differences among parental genotypes. The smallest leaves were recorded in P-2 and the largest in FO. All modes of inheritance are present, but the intermediate is prevailing. Positive heterotic effect in F1 was recorded in P 10-3/2 x P-2 and P-2 x JK 48.

In further genetic invetigations, data presented in Table 1 will give innitial material for realization of biometrical methods and creation of regression graphs from which a more complete picture in the genetic system of inheritance will be obtained.

Table 1. Type of inheritance of leaf size in F1 and F2 generations

Parents and hybrids	Middle belt leaf length (cm)		Middle belt leaf width (cm)		Middle belt leaf area (cm²)	
	F1	F2	F1	F2	F1	F2
P 10-3/2	26,5	26,5	12,7	12,7	213,84	213,84
P-2	19,5	19,5	11,0	11,0	136,29	136,29
YK 48	21,8	21,8	11,9	11,9	164,83	164,83
FO	38,3	38,3	23,6	23,6	574,32	574,32
P10-3/2 x P-2	**29,4 +h**	25,2 pd	**15,5 +h**	11,4 pd	**289.55 +h**	182,54 i
P10-3/2 x YK 48	24,5 i	23,3 pd	12,3 i	11,8 -d	191,48 i	174,70 pd
P10-3/2 x FO	33,2 i	32,8 i	19,2 i	15,3 pd	405,03 i	318,87 pd
P-2 x YK 48	**25,7 +h**	21,9 +d	**14,8 +h**	11,8 +d	**241,68 +h**	164,20 +d
P-2 x FO	34,1 pd	29,8 i	20,4 pd	17.7 i	442,01 pd	335,15 i
YK 48 x FO	32,0 i	31.6 i	18,3 i	16.9 i	372,09 i	339,33 i

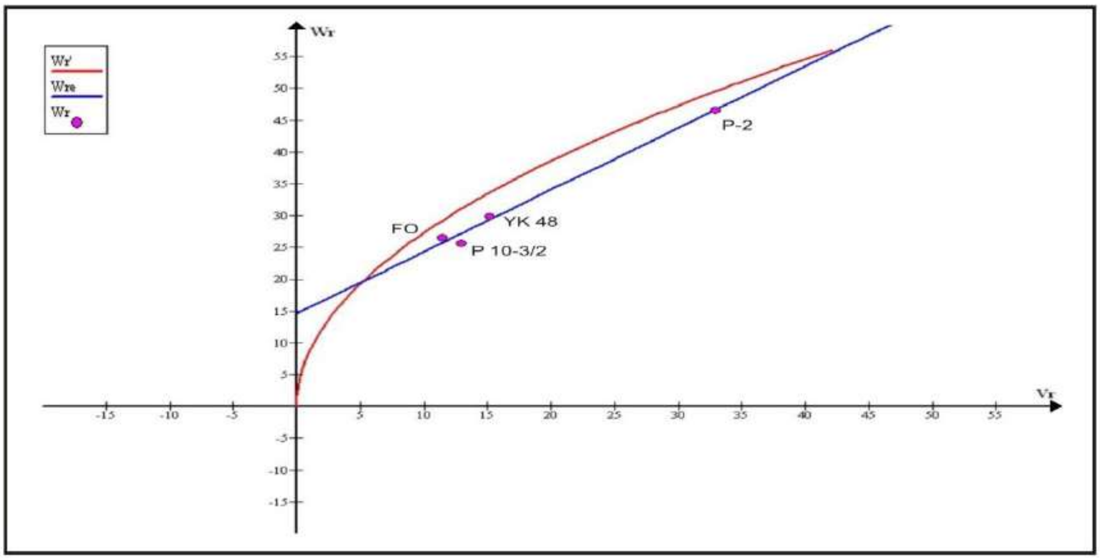
The graph of regression (VR, WR) for middle belt leaf length in F1 and F2 almost equals to 1 and differs significantly from 0, which indicates absence of interallelic interaction. The expected regression line is close to the limiting parabola (especially for F2 generation), which indicates predominance of additive genes in inheritance of this character. Cross-section of the expected regression line with Wr-ordinate is on the origin, which is an indication of partial dominant type of inheritance. Position of dispersion points on the diagram confirms the divergence of parental genotypes for the investigated character.

The position of the varieties FO, P 10-3/2 and YK 48 in the coordinate system shows significantly higher number of dominant than recessive genes in F1 generation (Fig.1). Their points are closest to the cross section of the parabola and the regression line on the side of the origin, according to which they possess mostly dominant genes in inheritance of this character. P-2 is positioned on the opposite side of the origin, which indicates predominance of additive gene effect.

From the graphic analysis of F2 generation (Graph 2), the position of FO and P 10-3/2 indicates that dominant genes prevail, whereas in YK 48 and P-2 additive genes prevail in inheritance of this character.

Values obtained in Graphs 1 and 2 are also presented in Table 2.

Graph 1- Graphic presentation of the inheritance of leaf length in F1 generation



Graph 2- Graphic presentation of the inheritance of leaf length in F2 generation

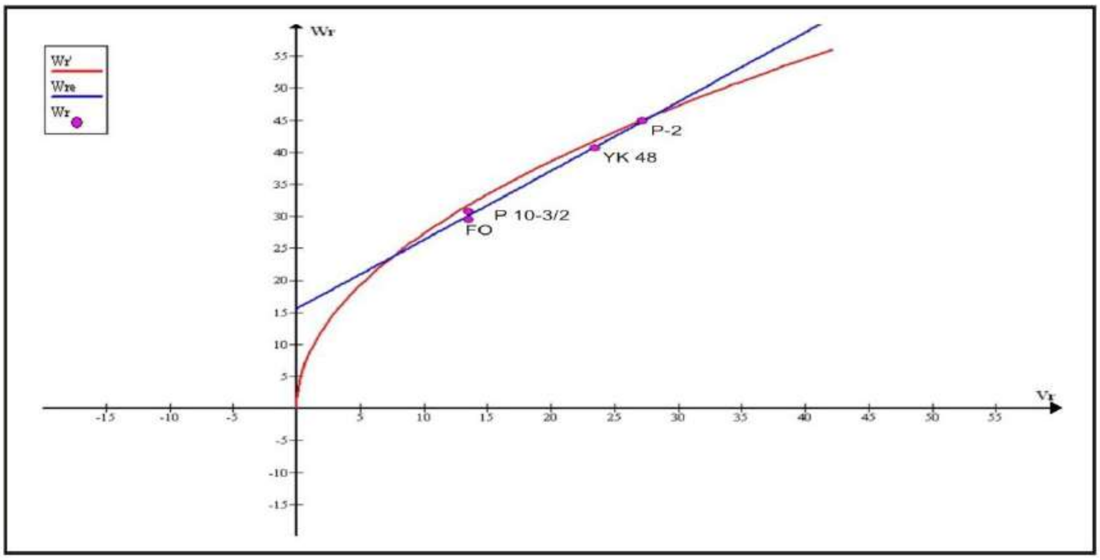


Table 2. The values for graphic presentation of inheritance for leaf length in F1 and F2 generations (Graph 1 and 2)

Parents	Generations	Coordinate System					
		Limiting parabola		Expected regression line		Distribuition points in the diagram	
		VR (x)	WR' (y)	VR (x)	WRE (y)	VR (x)	WR (y)
1. P 10-3/2	F1	13.01	31.03	13.01	27.12	13.01	25.65
2. P-2		32.93	49.55	32.93	46.62	32.93	46.61
3. YK 48		14.92	33.52	14.92	29.14	14.92	29.89
4. FO		11.43	29.19	11.43	25.72	11.43	26.56
Vp=74.56	Vm=14.57	a=1.726	b=0.972				
1. P 10-3/2	F2	13.49	31.77	13.49	30.10	13.49	30.78
2. P-2		27.14	44.99	27.14	44.82	27.14	44.91
3. YK 48		23.42	41.81	23.42	40.83	23.42	40.72
4. FO		13.52	31.75	13.52	30.13	13.52	29.49
Vp=74.56	Vm=18.22	a=1.824	b=1.078				

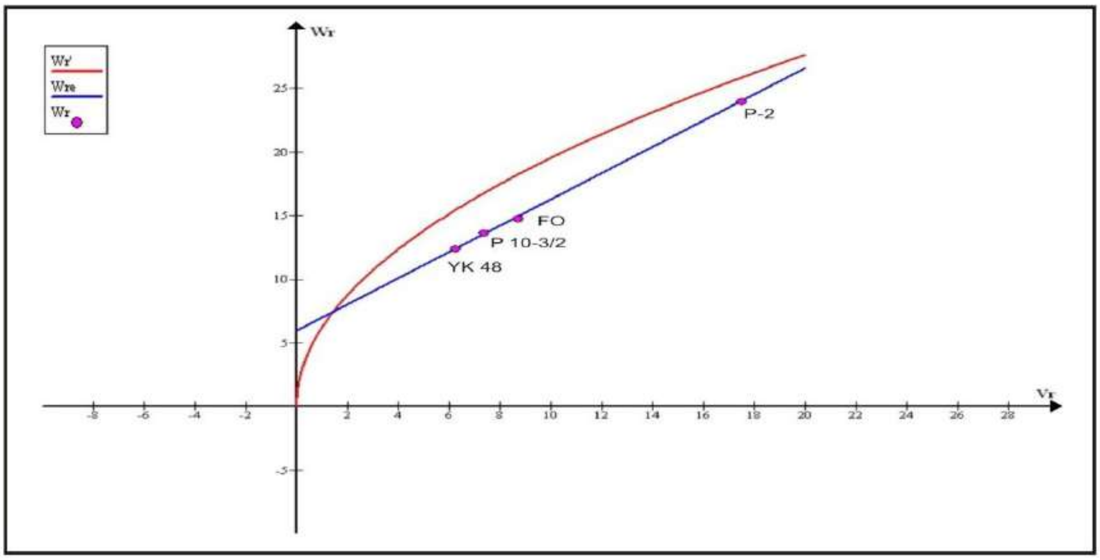
The regression graphs for the caharacter **middlebeltleaf width** in both generations has coefficient of regresionalmost equal to 1, and the position of regression line is very close to the limiting parabola (especially that of F2 generation). It denotes absence of interallelic interaction and dominance of the additive gene effect, which is specificity in inheritance of the quantitative characters. The expected regression line intersects the Wr-ordinate above the origin, which indicates partial dominance in inheritance of this character.

Distribution of points for F1 generation (Graph 3) shows that P 10-3/2 is closest to the point where parabola intersects the regression line on the side of ordinate. Accordingly, this variety has mostly dominant genes and neglectable number of recessive genes in inheritance of this character. YK 48, as well as P 10-3/2 and FO are positioned on the opposite side, which reveals presence of higher number of additive then dominant genes, indicating that additive gene effect prevails in inheritance of this character.

Distribution of points along the expected regression line for F2 (Graph 4) shows the distance of parents in relation to this character. The sequence of varieties is not identical to that of F1. Varieties YK 48, P 10-3/2 and FO are closer to the point of intersection of the parabola and the regression line on the side of ordinate, which denotes that dominant gene effect prevails in inheritance of this character. In P-2 variety recessive genes are prevailing, as can be seen from its position on the regression line.

Values of Graphs 3 and 4 are presented in Table 3.

Graph 3- Graphic presentation of the inheritance of leaf width in F1 generation



Graph 4- Graphic presentation of the inheritance of leaf width in F2 generation

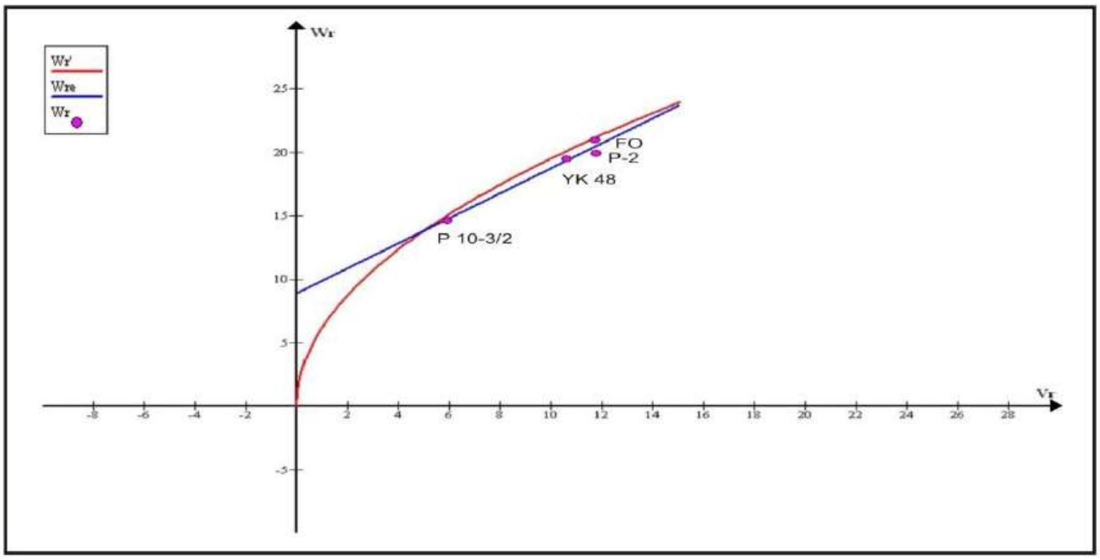


Table 3. The values for graphic presentation of inheritance for leaf width in F1 and F2 generations (Graph 3 and 4)

Parents	Generations	Coordinate System					
		Limiting parabola		Expected regression line		Distribuition points in the diagram	
		VR (x)	WR' (y)	VR (x)	WRE (y)	VR (x)	WR (y)
1. P10-3/2	F1	7.33	16.81	7.33	13.48	7.33	13.62
2. P-2		17.47	25.81	17.47	23.97	17.47	23.99
3. YK 48		6.19	15.41	6.19	12.39	6.19	12.41
4. FO		8.7	18.21	8.7	14.91	8.7	14.76
Vp=38.14	Vm=7.54	a=1.525	b=1.032				
1. P 10-3/2	F2	6.01	14.97	6.01	14.68	6.01	14.70
2. P-2		11.74	21.16	11.74	20.49	11.74	20.97
3. YK 48		10.59	20.11	10.59	19.37	10.59	19.51
4. FO		11.78	21.20	11.78	20.53	11.78	19.95
Vp=38.14	Vm=8.49	a=1.776	b=0.993				

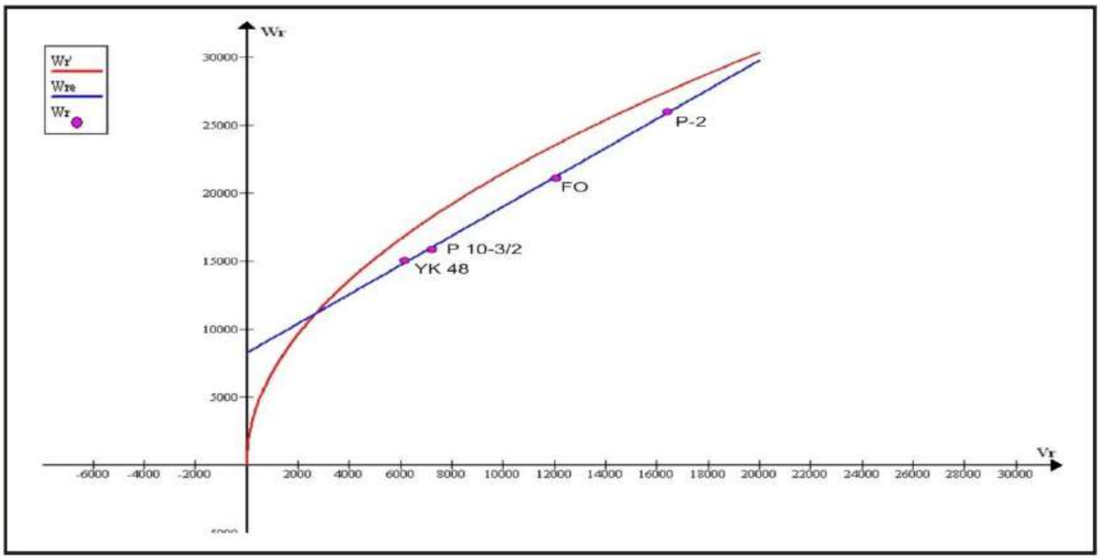
The coefficient of the regression graph for **middle belt leaf area** in F1 and F2 is almost equal to 1 and significantly different from 0, which indicates the absence of interallelic interaction. The expected regression line is close to the limiting parabola (especially the one for F2 generation) which denotes that additive genes have the leading role in inheritance of this character. The expected regression line intersects the Wr-ordinate above the origin, which points to partially dominant type of inheritance. Distribution of points in the diagram gives genetic picture of parental genotypes for the character investigated.

The position of YK 48 and P10-3/2 in the coordinate system for F1 generation shows the presence of significantly higher number of dominant genes over the recessive ones. Their points are closest to the cross-section of the parabola and regression line on the side of the origin, which shows that dominant gene effect prevails in inheritance of this character. The location of FO indicates approximately equal number of dominant and recessive genes, and the location of P-2 on the opposite side of origin shows dominance of the additive genes. Regression analysis for this character is presented in Graph 5.

From the graphic analysis of F2 generation presented in Graph 6 it can be concluded that in P 10-3/2 dominant gene effect prevails, in YK 48 and P-2 the number of dominant and recessive genes is almost identical and in FO additive genes prevail in inheritance of this character.

Values obtained in Graph 5 and 6 are presented in Table 4.

Graph 5- Graphic presentation of the inheritance of leaf area in F1 generation



Graph 6- Graphic presentation of the inheritance of leaf area in F2 generation

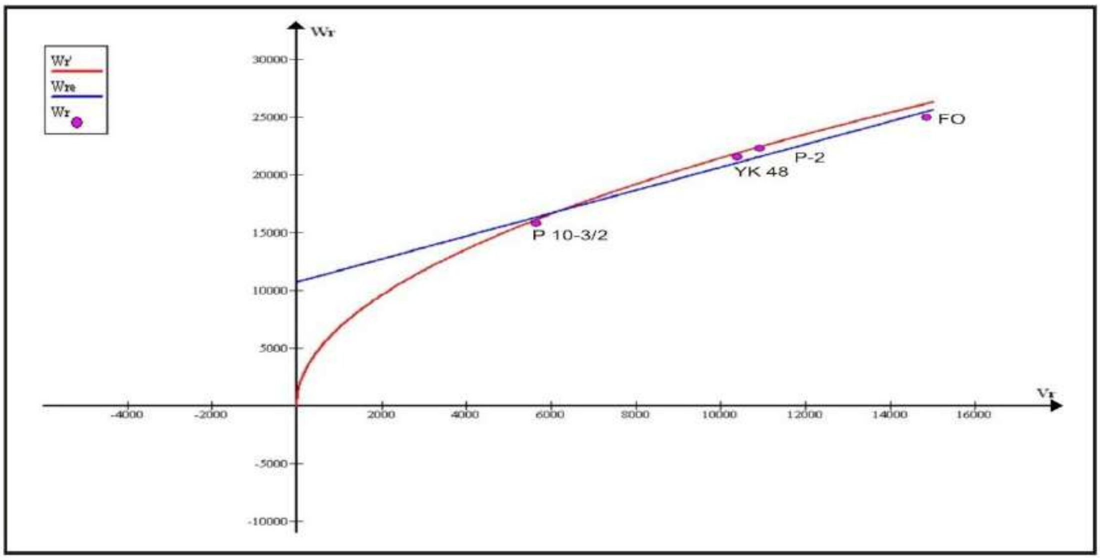


Table 4. The values for graphic presentation of inheritance of leaf area in F1 and F2 generations (Graph 5 and 6)

Parents	Generations	Coordinate System					
		Limiting parabola		Expected regression line		Distribuition points in the diagram	
		VR (x)	WR' (y)	VR (x)	WRE (y)	VR (x)	WR (y)
1. P10-3/2	F1	7236.05	18253.98	7236.05	16032.87	7236.05	15859.12
2. P-2		16389.31	27472.19	16389.31	25884.21	16389.31	25978.63
3. YK 48		6143.14	16819.32	6143.14	14857.05	6143.14	15068.09
4. FO		12068.57	23574.41	12068.57	21234.21	12068.57	21103.11
Vp=46049.60	Vm=8718.11	a=1.864	b=1.076				
1. P10-3/2	F2	5638.07	16113.20	5638.07	16322.13	5638.07	15802.45
2. P-2		10899.94	22403.97	10899.94	21627.10	10899.94	22340.25
3. YK 48		10390.05	21873.90	10390.05	21113.00	10390.05	21553.19
4. FO		14859.20	26158.37	14859.20	25619.26	14859.20	24985.34
Vp=46049.60	Vm=9862.29	a=2.026	b=1.008				

CONCLUSIONS

- Parental genotypes are homogenous, with significant differences among them.
- The inheritance of leaf size in F1 and F2 progenies differs, but the most represented type is intermediate inheritance. Positive heterosis in F1 progeny was observed in P 10-3/2 x P-2 and in P-2 x YK 48.
- All distribution points on the regression graph are located on the interior side of limiting parabola. The expected regression line intersects the Wr (y) - axis above the origin, which indicates partial dominant type of inheritance. The regression line differs significantly from 1, which denotes absence of interallelic interaction. The patterns of distribution points along the regrssion line of the diagram reveal prevalence of dominant gene effect in P 10-3/2 for inheritance of length, width and area of the middle belt leaves. In P-2 recesive genes prevail in inheritance of the above characters. In YK 48 has both dominant and additive genes, with small prevalence of one over the other, depending on the character or on generation. The semi-oriental FO is governed mostly by dominant genes in inheritance of length and width and recesive genes in inheritance of area of the middle belt leaves.

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Original Scientific paper

QUALITY RESEARCH OF THE *KRUMOVGRAD 90* ORIENTAL TOBACCO CULTIVAR, GROWN IN THE *MESTA* TOBACCO REGION UNDER VARIED FERTILIZING CONDITIONS

NurettinTahsin, Tatyana Ortomarova, ShteliyanaKalinova

Agricultural University-Plovdiv, Bulgaria
E-mail: s_kalinova@yahoo.com

ABSTRACT

The quest for quality-specific raw tobacco aimed at obtaining homogenous lots preferred on the international market has led to the replacement of the typical *Nevrokop* tobacco cultivars by the *Krumovgrad* tobacco ecotypes and their growing in untypical regions such as the *Mesta* tobacco region. The mass entering of the*Krumovgrad* cultivars into almost all tobacco growing regions in Bulgaria has to a certain extent changed also the quality characteristics of the raw material. That fact posed the need of changing the traditional agro techniques applied for those types of tobacco in line with the peculiarities of the region and with a view to obtaining high quality raw tobacco. The traditional agro techniques for the *Nevrokop* tobaccos, used for the growing of the *Krumovgrad 90* in the *Mesta* tobacco region is most likely one of the reasons for obtaining raw tobacco inferior in its consumer and technological characteristics to that produced in its typical regions.
The present research proves the assertion that the use of the combined soil fertilizer – *Hydro* 4-21-21+ammonium nitrate and the combined soil fertilizer – *Cropcare* 12-22-8 + foliar spray – *Ferticare* 6-14-31 influence positively the quality of the *Krumovgrad 90* cultivar.

Key words: oriental tobacco, agro techniques, fertilizing, expert evaluation, quality

ИСТРАЖУВАЊЕ НА КВАЛИТЕТОТ НА ОРИЕНТАЛСКАТА СОРТА КРУМОВГРАД 90 ПРОИЗВЕДЕНА ВО ТУТУНОПРОИЗВОДНИОТ РЕОН МЕСТА ВО РАЗЛИЧНИ УСЛОВИ НА ЃУБРЕЊЕ

Потрагата по тутунска суровина со специфичен квалитет со цел да се добијат хомогенисерии какви што бара меѓународниот пазар доведе до замена на типичните тутунски сорти неврокоп со екотиповите од тутунот крумовград и до нивно одгледување во нетипични региони како што е тутунопроизводниот реон Места. Масовното навлегување на крумоврадските сорти во скоро сите тутунопроизводни реони во Бугарија до извесна мера ги измени и квалитативните својства на новиот материјал. Овој факт ја наметна потребата од промена на традиционалните агротехнички мерки кои се применуваат кај овие типови, за да одговараат на особеностите на реонот, со цел да се добие висококвалитетна тутунска суровина.

Примената на агротехничките мерки што се вообичаени за сортите неврокоп и во производството на Крумовград 90 во реонот на Места е, најверојатно, една од причините што добиената тутунска суовина по своите потрошувачки и технолошки својства е полоша во споредба со онаа што се произведува во својот типичен реон.

Со ова истражување се потврди дека примената на комбинираното почвено ѓубре Hydro 4 -21-21 + амониум нитрат и комбинираното почвено ѓубре Cropcare 12-22-8 + folijарно прскање со Ferticare 6-14-31 има позитивно влијание врз квалитетот на сортата Крумовград 90.

Клучни зборови: ориенталски тутун, агротехнички мерки, ѓубрење, стручна проценка, квалитет

INTRODUCTION

It is mainly oriental tobacco that is grown in Bulgaria. Around 80% of its production is to be found in the Rila-and-Pirin and the Rhodopi-and-Trakia districts (Slavova, Drachev, 2004). The tobacco ecotypes, marked by their specific technological features, are set up in differentiated regions under the impact of mainly two basic facts – the local soil and climatic conditions. Tobacco grown on unsuitable soils, loses to some extent its type (Georgiev, 2005), apart from its quality. The Krumovgrad 90 cultivar, grown in the Mesta tobacco region does not realize its biological potential and is inferior in biological and economic traits to the same cultivar, grown in the typical Bashibalijski region. In the latter region the Krumovgrad 90 cultivar forms thicker, larger and richer leaves of dominant red hues featuring a raw material of a pleasant flavour, specific aroma and medium to above- medium strength (Nikolova, Nikolov, Drachev, 2007; Drachev, Nicolova, 2006; Nicolova, 2007). Asto thequality of the obtained raw leaves expressed in grade percentages in the Mesta tobacco region, the first grade percentage is 34% whereas in the Bashibalijski region it exeeds45% (Georgiev, 2005; Mladenov, 2006). The technological investigations on the quality of the cured tobacco of the Krumovgrad 90 cultivar, grown in the Mesta tobacco region and on the tobacco typical of the Bashibalijski region, showed that the crude tobacco obtained from Krumovgrad 90 grown in the regions and sub-regions of the Nevrokop ecotype is inferior to the raw material from the typical Bashibalijski region in relation to all quality features. The detected differences are in the exterior quality traits, chemical content and smoking properties (Drachev, Nicolova, 2006).

Suitable agro ecological conditions are a necessary prerequisite for the full and adequate unfolding of the genotype potential. Under intensive production terms tobacco realizes its maximum biological potential only when applying the right and up-to-date agro techniques (Zdraveska, 2006; Zdraveska et all., 2007; Pelivanoska 2008; Trajкosкi, Pelivanoska, 2003). Fertilization and irrigation are the most important of the complex agro technical measures, exerting a direct influence on the biological and economic features of raw tobacco (Hristoskiet all., 2007). Only when matching the appropriate cultivars, soils and technology with the producers’ experience is it possible to obtain high-quality tobacco and profitable production (Georgiev, 2005).

The aim of the present research was to study the exterior features of the Krumovgrad 90 cultivar, grown in the untypical Mestatobacco region with different variants of fertilizer application.

MATERIAL AND METHODS

The object of investigation was the *Krumovgrad 90* oriental tobacco cultivar grown in the *Mesta* tobacco region. The trials were carried out in 2007 and 2008 in two sub-regions, representative of the *Mesta* tobacco region: the *Polski* sub-region /valley-type/ and the *Yaka* sub-region. The *Polski* sub-region /valley-type/ comprised the micro-regions of *Borovo* and *Banichan* while the *Yaka* sub-region included the micro-regions of *Lazhnitsa*, *Kornitsa* and *Breznitsa*. The fertilizer rates in kg/da of active substance (Table 1) were fixed on the basis of preliminary soil analysis. Both single and combined fertilizers were used for the purpose of the research in the following variants: variant 1 /control/– non-fertilized; variant 2 – applying ammonium nitrate, triple superphosphate, potassium sulphate; variant 3 – using the combined soil fertilizer – *Hydro* 4-21-21+ammonium nitrate; variant 4 – applying the combined soil fertilizer – *Cropcare* 12-22-8 + foliar spray – *Ferticare* 6-14-31 in a concentration of 0.4%. Thefertilizers were incorporated in the rows at the stage of transplanting. The foliar spray was applied three times at an interval of 10 days, the first application done at the beginning of the intensive growth stage. The tobacco growing was implemented in compliance with the technology, well-recognized at the time of the research,for oriental tobacco cultivation consistent with the agro technical recommendations of the author of the cultivar – *Manolov* (1979). The deadline for transplanting was within the agro technical period for Southern Bulgaria (30th April –20th May). The quality rating of the cured tobacco was done by using the *expertise* method directly comparing the samples.

Table 1. Fertilizer rates in kg/da active substance

Sub-region*Polski* /valley-type/		Sub-region *Yaka*		
Micro-region*Borovo*	Micro-region *Banichan*	Micro-region *Kornitsa*	Micro-region *Lazhnitsa*	Micro-region *Breznitsa*
N – 3.0	N – 3.8	N – 3.5	N – 3.5	N – 2.0
P – 6.0	P – 8.0	P – 7.0	P – 7.0	P – 6.0
K – 6.0	K – 7.0	K – 7.0	K – 7.0	K – 5.0

RESULTS AND DISCUSSION

The expert evaluation plays a significant role in the quality grading of oriental tobacco taking into consideration its peculiarities as both a consumer product and commodity meant mostly for export. It is done on the basis of the exterior quality traits, their peculiarities and degrees of manifestation in the varied leaf categories.

The expert evaluation of tobacco is a must at all stages, from its purchase to its inclusion in separate trading batches andpresence in the cigarette blends.

In the present research the method of *profile description* was used in carrying out the expert evaluation with a view to achieving a more definitive characterization of the exterior quality traits of the separate samples. The degree of manifestation of each quality trait was determined by accrediting a certain number of points.The final evaluation was the sum total of the pointsand the listingaccording to the sum. The sample with the fewest pointswas the bestand was given rank 1.

The results of the expert evaluation of the tobaccos grown in the *Polski* sub-region /valley-type/ and the *Yaka* sub-region during the 2007 yield year are given in Table 4. The data show that when grading the tobaccos of the 4 variants of fertilizer application in the 5 micro-regions, the highest quality of the exterior traits was observed in the control (the non-fertilized variant), followed by variant 3.

The evaluation for the separate sub-regionswas as follows:

Sub-region *Polski* /valley-type/ (Table 2): No significant differences were detected in the compared samplesin the *Banichan*micro-region. The control ranked first, followed by variant 3, variants 4 and 2 coming last.

In the *Borovo* micro-region the control showed the best exterior quality traits. There was no credible difference between variants 4 and 3, ranking second and third respectively, since the differences in the sums of the pointsof the two samples were insignificant. The variant 2 sample was inferior to both the control and the rest of the tested variants regarding the exterior quality traits.

Sub-region *Yaka*(Table 2)**:**In reference tothe *Breznitsa* micro-region: The obtained results showed variant 3 to possess the best exterior quality traits, followed by the control and variant 4 ranking in the last place.

In the *Kornitsa* micro-region the grading of the tobaccos grown with different fertilizer application variants was as follows: the control demonstrated the best exterior quality traits, variants 2 and 4 coming next and variant 3 in the last place.

In the *Lazhnitsa* micro-region the control sample had the best exterior quality traits, followed by variants 3 and 4, variant 2 coming in the last place. It should be noted, however, that the differences between variants 3 and 4 were insignificant.

The results of the expert evaluation of the tobaccos grown in the *Polski* sub-region /valley-type/ and the *Yaka* sub-region during the 2008-yield year are given in Table 3.

The obtained outcomes show that for the 4 variants of fertilizer application in the 5 micro-regions during 2008, the highest quality of the exterior traits was observed in variant 3 from the *Polski* sub-region /valley-type/ and variant 4 from the *Yaka* sub-region.

The expert evaluation for the micro-regionsand fertilizer variants was as follows:

Sub-region *Polski* /valley-type/ (Table 3):

The *Banichan* micro-region demonstrated the best tobacco quality in the control. No significant difference was observed among the rest of the variants. The sums of the pointswere quite close, especially in variants 2 and 3, standing in third and fourth place respectively.

In micro-region *Borovo* variant 3 manifested the best traits, variant 2 having the lowest values.

Sub-region *Yaka* (Table3)

In the *Breznitsa* micro-region the control showed the best quality traits, followed by variant 4. The differences in quality among the variants were most sharply expressed in that specific micro-region.

In the *Kornitsa* micro-region variant 4 was of the best traits. The control conceded considerably as to the number of points(31.2). The two other variants possessed traits similar to those of the control.

In the *Lazhnitsa* micro-region the positioning of the variants was as follows: variant 4 demonstrated the best quality traits in the expert evaluation, variant 2coming next. The quality traits values were lower, as compared with the control, only in variant 3. Variant 4 ranked either first or second, immediately after the control, in all micro-regions at the expert evaluation of tobaccos for the 2008-yield year.

Table 2.Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/and *Yaka* sub-region for the 2007 yield

Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/for the 2007 yield																		
		Indicators																
Variant	Micro-region	1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Banichan	1	1	2	1	1	2	1	1.5	2	3	3	1	1	20.50	1		
1*	Borovo	1.5	1.75	2	1	1	2	1	1	2	3.5	3	1	1	21.75	1	21.12	1
	Banichan	1	1	2	1	1	2	2	1.5	2	3	3	1	2.5	23.00	4		
2*	Borovo	1	1.75	2.5	1	2	2	2	3	2	3	3	1	1	25.25	4	24.12	4
	Banichan	1	1	2	1.5	1.5	2	1	1.5	2	3	3	1	1	21.50	2		
3*	Borovo	1.5	1.75	2	1	1	2	1	3	2	3.5	3	1	1	23.75	3	22.62	2
	Banichan	1	1	2.5	1.5	1	3	3	1.5	2	1	3	1	1	22.50	3		
4*	Borovo	1	1	2	1.5	2	2	2	1	2	3.5	3	1	1	23.00	2	22.75	3
Expert evaluation of the tobaccos from the *Yaka* sub-region for the 2007 yield																		
		Indicators																
Variant	Micro-region	1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Breznitsa	2	1	2.5	2	2	2	3	1	2	3	3	1	1	25.50	2		
	Kornitsa	1.5	1	1.5	1	1	2	2	1	2	2.5	3	1	1	20.50	1		
1*	Lazhnitsa	1	1	1.5	1.5	1	2	1.5	1.5	1	1.5	2	1	1	17.50	1	21.17	1
	Breznitsa	1.5	2	2.5	3.5	3	3	3	3.5	2	4	3	1	1	33.00	3		
	Kornitsa	1.5	1	2	1.5	2	2	2	2	1.5	3	3	1	1	23.50	2		
2*	Lazhnitsa	1	1.5	2.5	1.5	1	2	2.5	3.5	2.5	3	3	1	1	26.00	4	27.50	3
	Breznitsa	1	1.75	2.5	2	2	2	2.5	1	1.5	3	3	1	1	24.25	1		
	Kornitsa	2	2.5	2.5	2.5	3	3	3	4.5	4	4.5	3	1	1	36.50	4		
3*	Lazhnitsa	1	1.5	2	2	2	2	2	1.5	1	1.5	2	1	1	20.50	2	27.08	2
	Breznitsa	1.5	2.5	2.5	2	3	3	3	4	4	4.5	4	1	2.5	37.50	4		
	Kornitsa	1	1	2.5	2.5	2.5	3	3.5	3.5	2	3	3	1	1	29.50	3		
4*	Lazhnitsa	1	1	1.5	1.5	1.5	2	2	1	1	3.5	3	1	1	21.00	3	29.33	4

Table 3.Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/and *Yaka* sub-region for the 2008 yield

Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/for the 2008 yield																		
Variant	Micro-region	Indicators																
		1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Banichan	1.8	1	2	2	1	2.5	1.8	1	1.8	1	3	1	1	20.90	1		
1*	Borovo	2	1	2	1.5	1	2.5	2	1.5	2	3	3	1	1	23.50	3	22.20	2
	Banichan	1.5	1	2	1	1	3	2	1.5	2	3.5	3	1	1	23.50	3		
2*	Borovo	2	1	2.5	1.5	1.5	2.8	2.5	1	2	3	3	1	1	24.80	4	24.10	4
	Banichan	1.5	1	2	1	1	2.5	3	1.8	2	3	3	1	1	23.80	4		
3*	Borovo	1.7	1	17	1.3	1	2	1.2	1	1.5	1.5	2	1	1	17.90	1	20.80	1
	Banichan	1	1	2	1.5	1	2.5	2.5	1	2	3	3	1	1	22.50	2		
4*	Borovo	2	1	2.2	1.8	2	2	2	1	1.7	3	2.5	1	1	23.20	2	22.80	3
Expert evaluation of the tobaccos from the *Yaka* sub-region for the 2008 yield																		
Variant	Micro-region	Indicators																
		1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Breznitsa	1.5	1	2.5	2	2	3	3	1	2	3.5	3	1	1	26.50	1		
	Kornitsa	1.5	1.7	2.5	2	2	3	4	2	3	4.5	3	1	1	31.20	4		
1*	Lazhnitsa	1.5	1	2	2	2	3	2	1	2	4.5	3	1	1	26.00	3	27.90	3
	Breznitsa	2	1	3	3	3	3	4	2	2	4.5	3	1	1	32.50	3		
	Kornitsa	1.7	1	2	2	2	2.5	2.5	1	2	3.5	3	1	1	25.20	3		
2*	Lazhnitsa	2	1	2	2	2	3	2	1	2	3.5	3	1	1	25.50	2	27.70	2
	Breznitsa	2	1	3	4	3	3	4	4	3.7	3	3	1	1	35.70	4		
	Kornitsa	2	1	2	1.5	2	2.5	2	1	2	3.5	3	1	1	24.50	2		
3*	Lazhnitsa	1.5	1	2.2	2	2	3	3	1	2	4.5	3	1	1	27.20	4	29.10	4
	Breznitsa	2	1.7	2.2	2.5	2	3	2.5	2	2	3.5	3	1	1	28.40	2		
	Kornitsa	1.3	1	2	1.8	1	3	3	1	2	3	3	1	1	24.10	1		
4*	Lazhnitsa	1.5	1	2	2	2	2.5	2	1	2	3.5	3	1	1	24.50	1	25.70	1

1* - non-fertilized variant (control); 2* - application of ammonium nitrate, triple superphosphate and potassium sulphate; 3* - using a combined soil fertilizer and a supplement of ammonium nitrate; 4*- a combination of a soil fertilizer and foliar spray.
1-cropping (pulling) area of the stalk; 2 –ripeness; 3 –richness; 4 –leaf structure; 5 –elasticity; 6 –resin content;7 –aroma; 8 –basic colour; 9 – basic colourintensity; 10 – hues; 11 – gloss; 12 – injuries caused by diseases and pests; 13 – injuries caused in the curing process.

It is obvious from the mean data (Table 4) for the expert evaluation of the tested tobaccos that the control exhibited the best exterior quality traits in the Polski sub-region /valley-type/, followed by variant 3, the differences in the ranks being insignificant – 21.66 forvariant 1 and 21.71 for variant 3.
In the Yaka sub-region variant 4 demonstrated the best exterior quality traits out of the fertilized variants – 27.51.

Table 4.Expert evaluation of tobaccosin 2007 – 2008

Expert evaluation of tobaccosin 2007 – 2008								
	Variants of fertilizer application							
	1*		2*		3*		4*	
Sub-region	Average	Grading	Average	Grading	Average	Grading	Average	Grading
Polski/valley-type/	21.66	1	24.11	4	21.71	2	22.77	3
Yaka	24.53	1	27.60	3	28.09	4	27.51	2

CONCLUSIONS

Based on the data about the impact of the different kinds of fertilizers on the exterior quality traits of the tobaccos grown in the Polski sub-region /valley-type/ and the Yaka sub-region, the following conclusions could be drawn:
Under the conditions of the Polski sub-region /valley-type/, out of the variants with fertilizer application, variant 3 exhibited the best exterior quality traits /when using the combined soil fertilizer – Hydro 4-21-21+ammonium nitrate/.
Under the conditions of the Yaka sub-region, out of the variants with fertilizer application, variant 4 exhibited the best exterior quality traits /when applying the combined soil fertilizer – Cropcare 12-22-8 + foliar spray – Ferticare 6-14-31/.

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Original Scientific paper

THE INFLUENCE OF GENOTYPE ON YIELD, QUALITY AND ECONOMIC EFFECTS OF BURLEY TOBACCO

Ilija Risteski, Karolina Kočoska

University St. Climent Ohridski - Bitola - Scientific Tobacco Institute - Prilep, Republic of Macedonia
e-mail: ilija.r@t-home.mk

ABSTRACT

During 2010 and 2011 investigations with 6 Burley tobacco varieties and lines were made in order to study their influence on yield and quality of the obtained raw material, and to evaluate their economic effects. The results of investigations showed absolute dominance of variety Pelagonec CMS F_1 and line B-98/N CMS F_9 over the other varieties, which was statistically confirmed. From a practical point of view, these results can be a good guideline to tobacco growers in selection of tobacco variety.

Key words: tobacco, variety, Burley, yields, economic effects

ВЛИЈАНИЕТО НА ГЕНОТИПОТ ВРЗ ПРИНОСОТ, КВАЛИТЕТОТ И ЕКОНОМСКИТЕ ЕФЕКТИ КАЈ ТУТУНОТ ОД ТИПОТ БЕРЛЕЈ

Во текот на 2010 и 2011 година во испитувањата беа вклучени 6 берлејски сорти и линии тутун со цел да се испита нивното влијание врз приносот и квалитетот на добиената суровина и економскиот ефект што го даваат истите. Добиените резултати од испитуваните својства покажаа апсолутна доминација на сортата Пелагонец ЦМС F_1 и линијата Б-98/N ЦМС F_9 над другите сорти што и статистички беше потврдено. Од практичен аспект овие резултати во иднина можат да бидат добра смерница при изборот на сорта од страна на примарните производители.

Клучни зборови: тутун, сорта, берлеј, приноси, економски ефект.

INTRODUCTION

Raw material of Burley and Virginia tobaccos participate in the composition of blend cigarettes with about 80%. The first steps towards introducing the type Burley in the Republic of Macedonia were made by Rudolf Gornik, who reported (1953) that this tobacco can be successfully cultivated only in rich soils and humid climate with frequent rainfalls. In early 70ies efforts were made towards creating a variety which will prove to be the best in most of the properties, especially in yield and quality. In that period, the main representative of this type of tobacco in the Republic of Macedonia was the Croatian male sterile variety Chulinec. Later on, male sterile varieties Burley B-96/85 CMS F_1, Burley 1 CMS F_1, B-2/93 CMS F1 and

Pelagonec CMS F1 were created in Tobacco Institute - Prilep. These varieties were a satisfactory substitute for the variety Chulinec, and some of them found their way beyond the borders of Macedonia. The fact that there is no ideal variety created once and for all, but that some variety at a given moment is better than the others, motivated the breeders of Tobacco Institute - Prilep to create new varieties (genotypes) with improved characters, i.e. with higher yields and quality. Since these characters are governed by the genetic structure, parents in which these characters are predominant are used in the process of hybridization. This process is exclusively intervarietal and is conducted with the aim to obtain male sterile hybrid varieties. The best of them are tested in field, in comparative trials with other standard varieties (domestic and foreign) for a period of at least two years. If they show better results than the standard, they are submitted to the State Variety Commission for recognition. This paper will present the results of investigations on yield and quality of the raw material obtained from the varieties and lines represented in the research.

MATERIAL AND METHODS

The investigations were carried out in the Experimental field of Tobacco Institute - Prilep during 2010 and 2011, on coluvial-alluvial soil. It included three introduced fertile varieties of Burley tobacco (B-21 from USA, Banquet 21 from Zimbabwe and B- 1317 from Bulgaria), the male sterile hybrid variety Pelagonec CMS F_1 and lines B-98/N CMS F_9, B-136/07. The variety B-21 was used as a check. Autumn ploughing was carried out at about 40 cm depth and prior to spring ploughing, the soil was fertilized with 300 kg/ha NPK 8: 22: 20. Before transplanting, the soil was treated with herbicide and, immediately after, it was incorporated into the soil by harrowing. The trial was set up in randomized blocks with 4 replications, at 90 × 50 cm spacing. Two hoeings of tobacco were applied, followed by addition of 5g of 26% CAN. A few additional irrigations during the growing period were applied when necessary. After harvest and stringing, tobacco was yellowed and air-cured in special curing barns for Burley tobacco. Qualitative estimation of dried tobacco was made according to the Rules for standard measurements of quality of leaf tobacco of the type Burley. Corrected yield per stalk and per hectare was estimated by the method of Rimker and gross income (denars/ha) was assessed when the yield per hectare was multiplied with the average price per 1 kg of raw tobacco. Statistical processing of data was performed using the analysis of variance technique.

RESULTS AND DISCUSSION

The yield of tobacco, as in many other crops, is affected by the genotype, as well as genotype : environment interaction. Tobacco yield as quantitative character is in close correlation with leaf number, size and thickness. There are differences between the varieties of the same type, but it still must be typical for that type. Budim T. (1988) reports that the average yield of Burley tobacco in Zimbabwe in the period 1980-1985 ranged from 1202 to 1760 kg/ha. The development of selection of this tobacco in the world resulted in creation of new genotypes that produce significantly higher yields, without negative effects on quality. Stoyanov Boris and Apostolova

Elena (1999) reported that the yields of B-1317 variety in some parts of Bulgaria can reach up to 3380 kg / ha.
According to Djulgerski Yovko (2009), the yield of Burley tobacco should not be lower than 3500 kg/ha. Ilija Risteski and Karolina Kocoska (2012) reported that Burley varieties created in Tobacco Institute- Prilep gave a yield of 3500-4500 kg/ha. The yield of this tobacco type is strongly affected by agrotechnical measures applied. Pelivanoska V. (2001) reported that by different variants of fertilization and irrigation, the yields of B-2/93 CMS F1 varieties in the region Ohrid-Struga can reach up to 6000 kg/ha.

Yield per stalk (g/stalk)

Data on variations of yields per stalk in varieties and lines investigated in the trial are presented in Table 1.

Table 1 Corrected yield, g/stalk

Varieties	Years	Average yield, g/stalk	Average 2010/11	Differences from the average: Absolute	Differences from the average: Relative	Range
B-21	2010	123.5	122.3	/	100.00	3
	2011	121.1				
B-1317	2010	118.8	119.7	- 2.6	97.84	5
	2011	120.6				
Banquet 21	2010	122.3	118.9	- 3.4	97.22	6
	2011	115.9				
B-136/07	2010	121.4	121.6	- 0.7	99.42	4
	2011	121.9				
B-98/N CMS F_9	2010	158.3++	166.0	+ 43.7	135.73	2
	2011	173.7++				
Pelagonec CMS F_1	2010	168.3++	176.0	+ 53.7	143.91	1
	2011	183.8++				

2010	2011
LSD 5% = 21.11 g/stalk +	LSD 5% = 14.97 g/stalk +
1% = 29. 23 g/stalk ++	1% = 20.74 g/stalk ++

According to the above data, the highest average yield per stalk of 176.0 g was recorded in the variety Pelagonec CMS F_1. It is 53.7 g or 43.91% higher compared to the check variety B-21, which average yield was 122.3g/stalk. The lowest average yield of 118.9 g/stalk was achieved in the variety Banquet 21, and it is 3.4 grams or 2.78% less than that of the check variety. In the other varieties and lines, the average yield ranges from 119.7 g/stalk in variety B-1317 to 166.0 g/stalk in line B-98/N CMS F_9. In both years of investigations, statistically significant differences at a level of 1% compared to the check were estimated in the variety Pelagonec CMS F_1 and line B-98/N CMS F9.

Yield per hectare (kg / ha)

The yield per hectare is closely related with quality per stalk. The combination of these two characters is a more expressive indicator in assessing the economic value of the genotype. Beside the impact of the variety, this character is also affected by some agro-technical measures. Janos Berenji and Miroslava Nikolic (1996) found that topping of the inflorescence, combined with sucker control in Burley tobacco can result in 28% yield increase per hectare.

Table 2 Corrected yield per hectare (kg/ha)

Varieties	Years	Average yield, kg/ha	Average 2010/11	Differences from the average		
				Absolute	Relative	Range
B-21	2010	2744	2717	/	100.00	3
	2011	2691				
B-1317	2010	2641	2661	- 56	97.93	5
	2011	2681				
Banquet 21	2010	2641	2608	- 109	95.99	6
	2011	2575				
B-136/07	2010	2698	2704	- 13	99.52	4
	2011	2710				
B-98/N CMS F_9	2010	3520^{++}	3692	+ 975	135.88	2
	2011	3864^{++}				
Pelagonec CMS F_1	2010	3740^{++}	3912	+ 1195	143.98	1
	2011	4085^{++}				

	2010	2011
LSD 5% =	466.56 kg/ha+	LSD 5% = 332.72 kg/ha +
1% =	646.17 kg/ha++	1% = 460.82 kg/ha ++

According to the data presented in Table 2, the highest average yield per hectare of 3912 kg was recorded in the variety Pelagonec CMS F_1, which is 1195 kg (43.98%) higher compared to the check variety B-21, which average yield was 2717 kg/ha. The lowest average yield per hectare of 2608 kg was obtained in variety Banquet 21. In other varieties and lines, the average yield per hectare ranged from 2661 kg in the variety B-1317 to 3692 kg in line B-98 / N CMS F_9 in both years of investigations (2010 and 2011).

Average price, denars / kg

The average price is, in fact, an indicator of quality of the obtained tobacco raw expressed in monetary value. However, the quality of tobacco is a very complex concept, affected by many mutually dependent factors and influences. So this indicator is only the beginning of a series of procedures for estimation of tobacco quality (physical and chemical properties, degustation, etc.). The quality of tobacco raw and the average price depend on a number of adequately performed cultural practices in field, in the time of harvest, yellowing, curing, etc. Data on the average price per 1 kg of dry tobacco in investigated varieties and lines are presented in Table 3.

Table 3 Average price denars/ kg

Varieties	Years	Average price, denars/kg	Average 2010/11	Differences from the average: Absolute	Differences from the average: Relative	Range
B-21	2010	30.98	32.63	/	100.00	5
	2011	34.29				
B-1317	2010	36.27	34.57	+ 1.94	105.94	3
	2011	32.87				
Banquet 21	2010	28.10	28.77	- 3.86	88.17	6
	2011	29.44				
B-136/07	2010	36.27	32.74	+0.11	100.33	4
	2011	29.21				
B-98/N CMS F_9	2010	37.22^{++}	40.38	+7.75	123.75	2
	2011	43.55^{++}				
Pelagonec CMS F_1	2010	44.67^{++}	44.79	+12.16	137.26	1
	2011	44.92^{++}				

2010	2011
LSD 5% = 2.27 kg/ha+	LSD 5% = 3.15 kg/ha +
1% = 3.14 kg/ha++	1% = 4..36 kg/ha ++

Data from the table show that the highest average price of 44.79 denars / kg was obtained with variety Pelagonec CMS F_1, and that is 12.16 denars / kg or 37.26% higher compared to the check variety with an average price of 32.98 denars / kg.

The lowest quality and the lowest average price of only 28.77 denars / kg was recorded in the variety Banquet 21. In other varieties and lines, the average price ranges from 32.74 denars/kg in line B-136/07 to 40.38 day / kg in line B-98/N CMS F_9. Statistical differences at 1% significance level compared to the check in both years of investigation were obtained in the variety Pelagonec CMS F_1 and the line B-98 / N CMS F_9.

Gross income, denars/ha

The most important factors in the formation of this character are the average yield per hectare and the average price of 1 kg raw tobacco, i.e. it represents the yield and quality achieved by the varieties and lines investigated in the trial.

Table 4 Gross income (economic effect, denars /ha)

Varieties	Years	Gross income, denars/ha	Average 2010/11	Differences from the average: Absolute	Relative	Range
B-21	2010	85 568	88 588	/	100.00	5
	2011	91 609				
B-1317	2010	98 094	93 212	+ 4 624	105.22	3
	2011	88 330				
Banquet 21	2010	76 260	79 530	- 9 058	89.77	6
	2011	82 801				
B-136/07	2010	98 094	88 738	+150	100.17	4
	2011	79 383				
B-98/N CMS F_9	2010	131 596++	150 438	+61 850	169.91	2
	2011	169 281++				
Pelagonec CMS F_1	2010	167 613++	175 528	+86 940	198.0	1
	2011	183 443++				

2010 2011

LSD 5% = 20 843 kg/ha+ LSD 5% = 14 186 kg/ha +

1% = 28 867kg/ha++ 1% = 19 647 kg/ha ++

According to the above data, the highest average gross income of 175 528 denars/ha was recorded in the variety Pelagonec CMS F_1, which is 86 940 denars/ha, i.e. 98.13% higher than the check variety B-21, which achieved 88 588 denars/ha. This indicator has the lowest value in the variety Banquet 21 (79 530 denars / ha). In other varieties and lines, the gross income ranges from 88 738 denars/ha in line B-136/07 to 150 438 denars/ha in line B-98/N CMS F_9. Statistically significant differences at 1% level compared to the check variety were estimated in variety Pelagonec CMS F1 and line B-98/N CMS F_9 in both years of investigation (2010 and 2011).

CONCLUSIONS

Based on the data obtained during the investigation, the following conclusions can be drawn:

- All varieties and lines included in the field trial developed under the same conditions of growing, but in the end they showed different results, as a product of various reactions of the varieties dictated by their genetic structure.
- The yields per stalk and per hectare were the highest in the variety Pelagonec CMS F_1 (176.0 g/stalk and 3912 kg/ha), and the lowest in the variety Banquet 21 (118.9 g/stalk and 2608 kg/ha).
- The average price for 1 kg of raw tobacco was the highest in the variety Pelagonec CMS F_1 (44.79 denars/kg) and the lowest in Banquet 21 (28.77 denars /kg).

- The gross income was the highest in the variety Pelagonec CMS F_1 (175 528 denars/ha), and the lowest in the variety Banquet 21 (79 530 denars/ha).
- Data obtained from the investigations show absolute dominance of the variety Pelagonec CMS F_1 and line B-98/N CMS F_9 over the other varieties and lines, which has been confirmed statistically.
- The obtained results lead to a conclusion that the variety has a very big influence on some productional characters. For this reason, in selection of varieties it is very important to have a deep knowledge of their properties.

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Original Scientific paper

THE EFFECTIVENESS OF FUNGICIDES IN THE CONTROL OF ALTERNARIA ALTERNATA DEPENDING ON THEIR IMPACT ON PATHOGEN BIOLOGY

Biljana Gveroska

University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute-Prilep, Kicevski pat bb, 7500 Prilep
e-mail: gveroska@t-home.mk

ABSTRACT

Investigations were carried out to study the effectiveness of some fungicides in the control of pathogenic fungus Alternaria alternata in laboratory conditions. Their impact on conidial germination and growth in solid and in liquid media was studied in this paper.

The highest reducing effect on conidia germination was obtained by application of fungicides Folicur EW-250, Score 250-EC and Acrobat MZ – with only 2.97%, 11.60% and 15.19% germinated conidia. The poorest results were obtained with Dithane M-45 and Antracol WP-70.

Fungal growth in solid and liquid media was prevented by application of Folicur EW-250. With application of Score 250 EC it reached only 8.30 mm and with Baycor WP 25 -14.70 mm

However, high yield of dry biomass was obtained with Baycor WP 25 (140.00 mg), while with Dithane M-45 it was 20.00 mg.

According to the investigations, the effectiveness of fungicides varies in different environmental conditions, depending on its impact on the biology of the pathogen.

Fungicides Folicur EW-250 (0.1%), Score 250-EC (0.05%) and Acrobat MZ (0.25%) showed the best results in control of the pathogen.

Keywords: Alternaria alternata, fungicide, inhibition, biology

ЕФИКАНОСТ НА ФУНГИЦИДИТЕ ЗА СУЗБИВАЊЕ НА *ALTERNARIA ALTERNATA* ВО ЗАВИСНОСТ ОД НИВНОТО ВЛИЈАНИЕ ВРЗ БИОЛОГИЈАТА НА ПАТОГЕНОТ

Истражувањата беа извршени со цел да се испита ефикасноста на фунгицидите за сузбивање на патогената габа Alternaria alternata во лабораториски услови. Беше испитувано влијанието врз 'ртењето на конидиите како и развојот врз цврста и течна подлога.

Најголем редуцирачки ефект врз 'ртењето на конидиите покажаа фунгицидите Folicur EW-250, Score 250-EC и Acrobat MZ со само 2.97%, 11.60 % и 15.19% из'ртени конидии. Најслаби резултати имаа Dithane M-45 и Antracol WP-70.

Габата не се развиваше на цврста и течна подлога со препаратот Folicur EW-250. Кај Score 250-EC достигна само 8.30 mm а кај Baycor WP 25 -14.70 mm

Но, приносот на сувата биомаса кај Baycor WP 25 е голем (140.00 mg), а кај Dithane M-45 тој изнесува 20.00 mg.

Според истражувањата, ефикасноста на фунгицидите варира во разни еколошки услови, во зависност од влијанието врз биологијата на патогенот.

Фунгицидите Folicur EW-250 (0.1%), Score 250-EC (0.05%) и Acrobat MZ (0.25%) покажаа најдобри резултати во сузбивањето на патогенот.

Клучни зборови: Alternaria alternata, фунгицид, инхибиција, биологија.

INTRODUCTION

Brown spot is one of the fungal diseases in the Republic of Macedonia which appears each growing season in all tobacco types. Its presence in the oriental tobacco is particularly harmful because it occurs at the end of the season, on the top leaves which have the best quality. According to Rotem (1994), in diseases caused by fungi of the genus Alternaria, the yield may be reduced through: a) reduction of photosynthetic activity and leaf production, without direct infection, b) direct attack by the pathogen, c) reduction of tobacco quality, d) combination of all the activities.

The causing agent of brown spot disease on tobacco - Alternaria alternata reduces the total economic effect of tobacco production through a combination of all mechanisms that affect tobacco yield and quality. It also has an impact on the smoking properties of tobacco raw. As severity of the disease increases, the "good" taste is reduced in favor of the "bad" taste (Lucas, 1975, loc cit Rotem, 1994). Also, the creation of AT toxin by this fungus in tobacco and its persistence in the raw material affects the smokers' health.

The disease is becoming even more important because the intensive way of tobacco production disables the proper performance of agro-technical operations, i.e. the application of preventive measures for protection. The outbreak of the disease is especially affected by the undue harvest of tobacco leaves and excessive irrigation. Because of the above reasons, the application of chemical protection is unavoidable. Therefore, continuous investigations are made on the effectiveness of some active ingredients and fungicides in the control of this pathogenic fungus.

In investigations of Shaeik and Taha (1984), Dithane M 45 applied in concentration of 0.25% appeared to be effective in control of the disease. Nagarajan (2000) recommended the application of Mancozeb, Difenoconazol and Propiconazol for this purpose.

In investigations of Colturato et al. (2009) on the effect of some active ingredients and their combinations on disease intensity and yield increase, all fungicides included in investigation showed a positive effect on reducing the intensity, but trifloxystrobin + propiconazole appeared to be the most effective in increasing the yield.

Fungicides effectiveness in control of A. alternata has been investigated in vitro. Bozukov (2002) studied the biological effect of 15 fungicides in order to determine the most appropriate preparations for tobacco protection from the brown spot disease.

Survilience and Dambrauskiene (2006) reported that active ingredients showed significant inhibitory effect on the development of several species of Alternaria. Issiakhem and Bouznad (2010) found such an effect of Difenoconazole and Chlorthalonil on A. solani and A. alternata. Inhibitory effect on the growth of A. alternata was found in five contact and five systemic fungicides (Chandhary and Patel, 2010).

Mahatabi et al. (2001), in their investigations in vitro and in vivo, found several active ingredients that can be applied in protection of tobacco from brown spot disease. Sometimes, however, certain ambiguities arise between the results obtained in laboratory and in field conditions (Zellner et al., 2011).

Research of biological effect of fungicides in the control of certain pathogen begins with investigation of their impact on its biology as a basic principle for effective protection. Therefore, the aim of this study was to examine the impact of several fungicides on the biology of this pathogenic fungus.

MATERIAL AND METHOD

The selection of fungicides for this investigation was made in accordance with our intention to include higher number of active ingredients known for their protection of tobacco, as well as other substances which proved to be efficient in the control of Alternaria. Some ambiguities in fungicides investigations in field conditions also imposed the need for this research.

The list of systemic and contact fungicides applied on tobacco in recommended concentrations is presented in Table 1.

Table 1. Investigated fungicides

Fungicide	Active ingredient	a.i. content	Concentration, %
Dithane M-45 (WP)	Mancozeb	80 %	0.25
Acrobat MZ (WP)	Dimetomorf +Mancozeb	(9 + 60)%	0.25
Ridomil MZ 72 (WP)	Metalaksil +Mancozeb	(8 +64) %	0.3
Antracol WP –70	Propineb	70 %	0.2
Score 250 -EC	Difenokonazol	250 g/l	0.05
Euparen multi WP 50	Tolyfluanid	50%	0.25
Baycor WP 25	Bitertranol	25 %	0.25
Folicur EW- 250	Tebuconazol	250g/dm^3	0.1
Poliram DF (WG)	Metiram	80 %	0.2

Fungicides effect on conidia germination

Conidia from infected leaves, previously kept in Petri dish on moistened filter paper for 24 hours were used as material for investigation. Water solutions of the fungicides were prepared in appropriate concentrations. The percentage of germinated conidia was estimated by the method of Ko et al. (1975).

Conidia were transferred from lesions with sterile bacteriological needle into the drops of prepared fungicide solutions. They were placed in Van-Tieghem chambers and incubated in a thermostat at 28^0C. The percentage of germinated conidia was estimated after 4-5 hours. The moment when the length of initial hypha was equal to the width was taken as a criterion for germinated conidium.

Five microscopic preparations of each chemical were observed, with 10 visual fields. Conidia suspension in a drop of distilled water was used as a check. The trial was replicated three times and the results present the mean value of the investigations.

The percentage of inhibition of conidia germination was estimated by the Ogbebor and Adekunle formula (2005).

Fungicides effect on pathogen development in solid nutrient media with addition of fungicide

Pure culture of *A. alternata* was used in the trial, obtained by conidia transfer from fresh infected plant material on potato dextrose agar.

The same media was used for monitoring the fungus development in a presence of fungicide. It was prepared in a usual way and fungicides were added after sterilization and cooling to $40\text{-}50^0$C. The media with appropriate concentration of the fungicide was spread on 110 mm Petri dishes.

3-5 mm^2 fragments of the pure culture (7-10 days old, incubated at 25^oC) were transferred in media with fungicide. The Petri dishes were incubated at the same temperature. The trial was set up in three replications; with every replication five Petri dishes were sown for each variant. The development of the fungus was followed and colony diameter was measured each day, in two opposite directions at right angle. The results given for the 3rd, 5th, 10th and 15th day represent the mean value of the replications. A colony grown in solid media without addition of fungicide was used as a check.

Percentage of reduction of pathogen development in solid and liquid media was estimated according to the formula of Shovan et al. (2008).

Fungicides effect on pathogen development on liquid nutrient media with addition of fungicide

For these investigations, liquid potato dextrose agar was prepared and fungicides were added after sterilization and cooling. 20ml of the media with fungicide was dispensed in Petri dishes and then sowing was performed with fragments of the mycelium using a sterile needle. Five Petri dishes were sown for each variant, and the trial was set up in three replications. In this case, too, the media without fungicide was used as a check. Incubation lasted 15 days at 25^0 C, and it was followed by separation (filtration) of the fungus from the liquid media and drying of the filtrate at 25-28^{0}C up to 3 consecutive measurements with constant value, according to the method of Sarić (1986). The mean value of all replications shows the yield of dry biomass in mg.

RESULTS AND DISCUSSION

All investigated fungicides showed a reducing effect on conidial germination of *A. alternata*. Some fungicides make their impact by inhibiting the development of germ tube, appressorium formation and mycelial growth (Obanor et al., 2005).

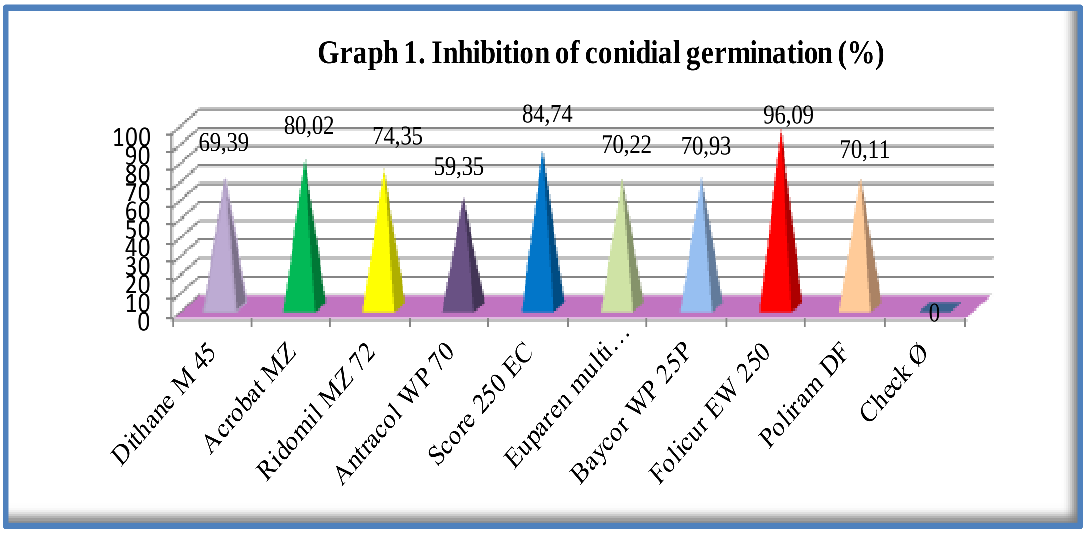
The lowest percentage of germinated conidia was determined with Folicur EW-250, only 2.97%, but also in fungicides Score 250 EC and Acrobat MZ - 11.60% and 15:19, respectively. The highest percentage of germinated conidia was determined with fungicides Dithane M-45 and Antracol WP-70 (Table 2). According to the above data, the lowest percentage of inhibition was obtained with the fungicide Antracol WP-70 - 59.35%. The highest percentage of inhibition of conidia germination was obtained with the fungicide Folicur EW-250 - 96.09%, but also with Score 250-EC and Acrobat MZ (Figure 1).

Table 2. The effect of fungicides on conidia germination

Fungicide	Dithane M-45	Acrobat MZ	Ridomil MZ 72	Antracol WP -70	Score 250 EC	Euparen multi WP 50	Baycor WP25	Folicur EW 250	Poliram DF	Check Ø
Concentration, %	0.25	0.25	0.3	0.2	0.05	0.25	0.25	0.1	0.2	-
Germinated conidia, %	23.27	15.19	19.50	**3.90**	11.60	22.64	22.10	**2.97**	22.72	76.02
Inhibition of conidia germination, %	69.39	80.02	74.35	**59.35**	84.74	70.22	70.93	**96.09**	70.11	-

Similar results were reported by Issiakhem and Bouznad (2010), who also noted that Difenoconazole is effective in conidial germination of *A. alternata* and *A. solani*.

Fungicides differentiated their effectiveness in specified concentration. Those with higher inhibitory activity on germination, even at lower concentrations when the effect on spore germination is slightly reduced, still have an influence which can be seen through the fact that the length of germ tube is significantly reduced (Obanor et al., 2005).



With respect to the development of pathogenic fungus in solid nutrient media with the addition of fungicide, it can be noted that in the initial days of incubation no colony growth was observed with Score 250 EC, as well as with Folicur EW 250, and with the latter it does not appear throughout the entire incubation period (Table 3).

The products start to differentiate with respect to their effect about the fifth day, but in Antracol WP-70, the good development that was observed at the beginning continues up to the end, when the colony size is the largest - 70.90mm. In Ridomil MZ 72, Dithane M-45 and Poliram DF, the colony diameters range from 41.73 to 54.98 mm. These results are similar to those reported by Zellner et al. (2011), in which Poliram WG appeared to have almost no effect on *A. alternata*.

According Chandhary and Patel (2010), Mancozeb shows good results in the inhibition of fungus growth, which is not the case in this type of investigations.

The largest diameter of the colony was observed in the media with Antracol WP-70, i.e. this chemical showed the lowest percentage of inhibition of colony growth - 33.92% (Table 3, Figure 2).

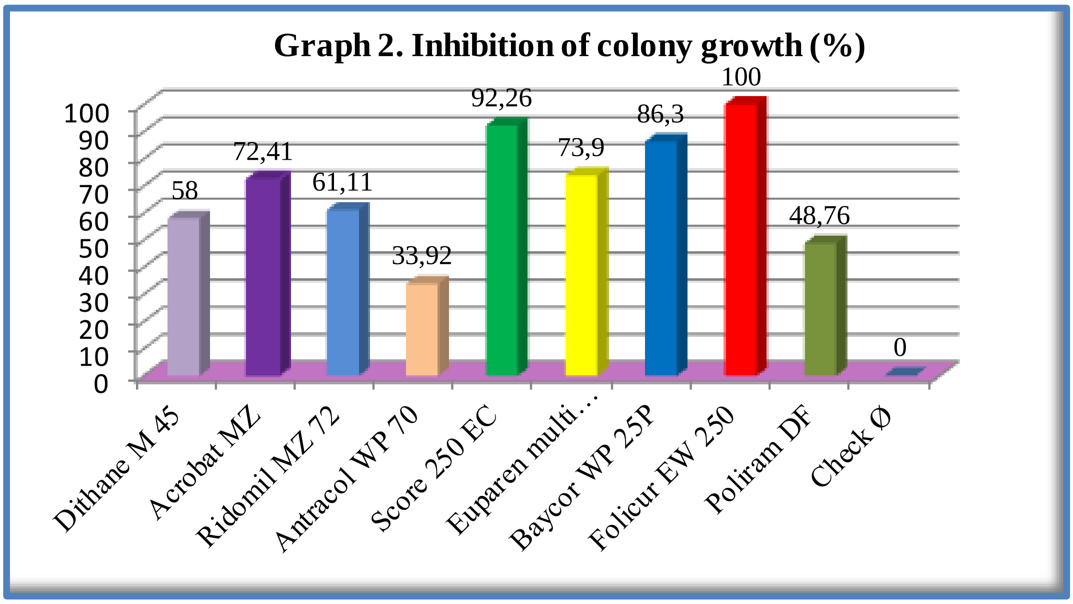
Table 3. The effect of fungicides on colony growth in solid nutrient media

Fungicide	Concentration%	Diameter of the colony (mm)				Inhibition of colony growth %
		3rd day	5th day	10 th day	15 th day	
Dithane M-45	0.25	6.16	8.33	26.50	45.07	58.00
Acrobat MZ	0.25	6.00	8.40	17.70	29.60	72.41
Ridomil MZ 72	0.3	6.00	8.75	24.15	41.73	61.11
Antracol WP-70	0.2	8.50	21.50	42.40	**70.90**	**33.92**
Score 250 EC	0.05	0.00	6.00	6.80	**8.30**	**92.26**
Euparen multi WP 50	0,25	9.30	12.50	21.40	28.00	73.90
BaycorWP25	0.25	6.00	6.00	10.40	14.70	86.30
Folicur EW 250	0.1	0.00	0.00	0.00	**0.00**	**100.00**
Poliram DF	0.2	6.60	10.60	32.23	54.98	48.76
Check Ø	-	27.20	44.02	85.59	107.30	-

Ridomil MZ 72 did not show a significant percentage of inhibition of colony growth (Graph 2, Figure 1). In investigations of Batta (2001), the formulation metalaxyl + mancozeb showed significant curative effect in the disease caused by *A. alternata*. This chemical is also used in the control of other pathogens in tobacco. However, *A. alternata* is less invasive parasite in the complex of fungi that attack tobacco and it is even regarded as secondary parasite (Rotem, 1994). Therefore, certain chemicals may affect other pathogens, which causes an error in determination of the intensity of attack of *A. alternata*. Such explanations are also given by Zellner et al. (2011), for differences in fungicides effect appearing in field and laboratory investigations.

Of the investigated chemicals, only Folicur EW 250 showed 100% inhibition of colony growth (Graph 2, Figure 1 and 2). These results are in accordance with investigations on biological effect of 15 fungicides made by Bozukov (2002), according to which the fungicide effect was recognized in only five chemicals, and the others showed fungistatic and inhibitory effect.

In vitro investigations made by Survilience and Dambrauskiene (2006) revealed that all investigated fungicides have shown satisfactory inhibition of *Alternaria spp*. colonies, reducing them in average 94 to 25% after the 21st day. Folicur EW 250 (Tebuconazol), however, differed by its inhibitory activity which persisted at 71% to 62% even after the 21st day.



Score 250 EC have also achieved high percentage in inhibition of colony growth - 92.26% (Graph 2, Figure 2). These results are in agreement with those of Issiakhem and Bouznad (2010), according to which Difenoconazole is efficient in inhibition of colonial growth of *A. solani* and *A. alternata* and has better effectiveness than the active substance Chlorthalonil.

According to Dahmen and Staub (1992), Difenoconazole shows high effectiveness against the the Ascomycetes, Basidiomycetes and Deuteromycetes classes of fungi.

According to Batta (2001), Difenoconazole together with Cyprodinil + Flodioxonil are the most efficient preventive fungicides in the control of this pathogen in fruits.

In liquid nutrient media with Folicur EW 250, no yield of dry biomass was observed, confirming 100% inhibitory effect of the fungicide in these trials, too. With Acrobat MZ and Score 250 EC it was only 10 mg, or both have shown

95.24% inhibition. These three products showed the best results in the inhibition of dry biomass yield (Table 4, Graph 3).

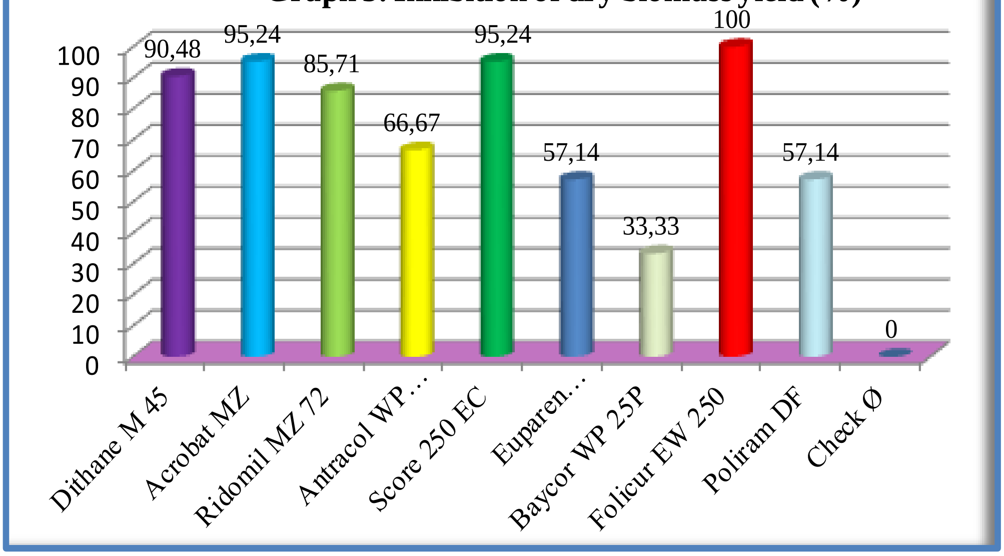
Baycor WP25 showed high efficiency in inhibition of pathogen growth. However, the inhibition of dry biomass yield in liquid nutrient media was only 33.33%, which is the lowest value (Table 3 and 4, Graph 3). Despite the modest results of this product (Table 2) and the small diameter, the colony had a specific look, i.e. it was very thick, in white color, with pronounced aerial growth (Figure 2).

The lowest effectiveness in these trials, besiden Baycor WP 25, were observed with Antracol WP 70, Euparen multi WP 50 and Poliram DF (Graph 3).

With Dithane M-45 fungicide, which showed low inhibitory effect on colony growth, the dry biomass yield was 20mg. It means that in this case the fungicide showed good inhibitory effect, i.e. 90.48% inhibition of dry biomass yield. Similar to this, Chandhary and Patel (2010) reported that percentage of inhibition with mancozeb was 97.80%.

Table 4. The effect of fungicides on dry biomass yield

Fungicide	Dithane M-45	Acrobat MZ	Ridomil MZ 72	Antracol WP -70	Score 250-EC	Euparen multi WP 50	Baycor WP 25	Folicur EW-250	Poliram DF	Check Ø
Concentration, %	0.25	0.25	0.3	0.2	0.05	0.25	0.25	0.1	0.2	
Dry biomass, mg	20.00	10.00	30.00	70.00	10.00	90.00	140.00	**0.00**	**90,00**	210.00
Inhibition of dry biomass yield %	90.48	95.24	85.71	66.67	95.24	57.14	33.33	**100.00**	**57.14**	



Graph 3. Inhibition of dry biomass yield (%)

During investigations, some of the fungicides showed better results in inhibition of colony growth than in conidial germination. The ineffectiveness of certain preparation in protection from conidial germination is due to the fact that it causes higher inhibitory effect on the development of hyphae than on spore germination (Obanor et al., 2005).

Some fungicides (e.g. Acrobat MZ and Dithane M-45), had higher inhibitory impact in liquid than in solid media. Our results are in accordance with findings of Ko et al. (1976), according to which the agar affects the diffusion of fungicides, i.e. it binds to a fungicide, thereby reducing its activity.

Among the investigated fungicides, the following were distinguished by their inhibitory effect on the biology of *A.alternata*: Folicur EW-250 (0.1%) (Tebuconazol), Score 250-EC (0.05%) (Difenoconazole) and Acrobat MZ (0.25%) (Dimetomorf +Mancozeb).

In vitro and in vivo investigations of several active ingredients have shown that Propiconazol, Tebuconazol and Mancozeb in concentration 0.1, 0.1 and 0.2%, respectively, can provide good protection from brown spot disease on tobacco (Mahtabi et al., 2001).

Figure 1. The effect of some fungicides on fungus growth in solid nutrient media

Figure 2. The effect of: (down, left to right) Baycor WP 25, Score 250-EC and Folicur EW 250

CONCLUSIONS

All fungicides investigated had an inhibitory effect on pathogenic fungus A. alternata.

• The best results in the inhibition of conidial germination were obtained with fungicide Folicur EW-250 (0.1%). Good results were also obtained with Score 250-EC (0.05%) and Acrobat MZ (0.25%).

• Folicur EW-250 (0.1%) showed the highest inhibitory effect on pathogen growth. The fungus does not grow in the presence of this product, neither in solid nor in liquid media.

• The lowest yield of dry biomass was obtained with the fungicide Acrobat MZ (0.25%), which showed also good results in solid media.

• Fungicides Baycor WP 25 (0.25%) and Dithane M-45 (0.25%) showed contradictory results in investigations in solid and in liquid media.

• Fuingicides Antracol WP-70 (0.2%) and Poliram EW-250 (0.2%) showed the poorest inhibitory results.
• Fungicides Folicur EW-250 (0.1%), Score 250-EC (0.05%) and Acrobat MZ (0.25%) had the best results in these trials.

They can be used in protection of tobacco from brown disease.
SAccording to the results, the effectiveness of fungicides to a particular pathogen varies in different environmental conditions, depending on their impact on biological properties.

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Original Scientific paper

FUSARIUM ROT ON *OROBANCHE RAMOSA* - BROOMRAPE ON TOBACCO IN THE REPUBLIC OF MACEDONIA

Petre Tashkoski

University "St. KlimentOhridski "- Bitola, Scientific Tobacco Institute, Kicevski pat, bb. 7500 Prilep, R. Macedonia, E-mail: taskoskip@yahoo.com

ABSTRACT

The first observations of withered tobacco stalks in R. Macedonia, caused by broomrape (*Orobancheramosa*),weremade in the summer 2012, onbroomrape infected fields intobacco producing region of Prilep. Drying was caused by Fusarium fungi, which are not pathogenic to tobacco plant. The causing agentwas isolated from field collected material. Based onmicroscopic analyzes and literature data,it was stated that all isolates obtained from the broomrape infected stalks belonged to species of the genus Fusarium. These isolates were used ininoculation of broomrape stalks and tobacco as well as broomrape inflorescences and tobacco leaves. Investigations confirmed that the isolates are highly pathogenic tobroomrape stalks and inflorescences, with a damage ranging up to 100%, but they have no negative effect on inoculated stalks and tobacco leaves.

Keywords:tobacco, broomrape, *Orobancheramosa*, Fusarium spp.

ФУЗАРИОЗНО ГНИЕЊЕ НА *OROBANCHE RAMOSA*- ЧУМА НА ТУТУНОТ ВО РЕПУБЛИКА МАКЕДОНИЈА

Кај тутунот одгледуван на површините во прилепскиот реон каде имаше појава на чума (*Orobancheramosa)*, во летото 2012 година за прв пат во Р. Македонија, забележавме појава на сушење на стебла од чумата. Сушењето беше причинето од габи на Fusarium, кои не се патогени за тутунското растение. Од собраниот материјал на терен беше изолиран причинителот на оваа појава, а по извршените микроскопски анализи и на основа на податоците добиени од литература, констатиравме дека сите изолати кои беа добиени од заразените стебла на чума припаѓаат на видови од родот Fusarium. Добиените изолати беа искористени за инокулирање на стебла од чума и тутун, како и соцветија од чума и тутунски листови.

Во сите испитувања се потврди дека изолатите имаат висока патогеност врз стеблата и соцветијата на чумата, каде оштетувањата изнесуваа до 100%, додека истите немаа негативно влијание врз инокулираните стебла и листови од тутун.

Клучни зборови: Тутун, чума, *Orobancheramosa*, Fusarium spp.

INTRODUCTION

During the summer 2012, symptoms of broomrape disease (*Orobancheramosa*) were recorded in the region of Prilep, at several sites where tobacco was grown as monoculture. It was also noticed that the broomrape stalks were withered. It was the first time that such phenomenon was registered in R. Macedonia. Necrosis was observed on the surface of infected stalks,whichwere dark brown to black in color and they were rotten and dry.

Drying was observed in stalks just emerging from the soil, as well as in the stalks which succeeded to bloom, but no seed was formed in their seed capsules. Necrosis caused high mortality on broomrape stalks, but tobacco plants were not affected. Such phenomenon was observed in 2002 on tobacco fieldsin southern Italy (Nanniet al., 2005), where large number of broomrape stalks were infected by necrosis caused by strains of the fungus *Fusariumoxysporum*. Necrosis caused high mortality on *Orobancheramosa*, but made no damage totobacco plants.

According to Boutitiet al., (2008), broomrape rottening is caused by species of the genus Fusarium, isolated from infected *O.crenata* and *O.foetida*on beans. From the isolates of *O.aegyptiaca*on tomatoes, the species *F. oxysporum* and *F.solani*were identified (Ghannam et al., 2007). High effectiveness of *F.solaniza*in the control of *O.aegyptiaca* broomrape in tomatoes was investigated by Reza and Hadi (2012), because of the high level of infection on broomrape areas which were untreated and deserted.

The species *F. oxysporumf. sp. Orthoceras* is a potential agent for biological control of the parasitic weed *O.cumana*, which significantly reduces the percent of broomrape seeds germination (Thomas et al., 1999). In the large number of *O.crenata* isolates, themajority of which belonged to Fusarium(about 66%), the most common were *F.solani*and *F.oxysporum*, which inhibited seed germination for more than 60% (Abouzeid, 2009, Abouzeid and El-Tarabily, 2010). Crops that are most affected by broomrape are tomatoes, cucumbers, tobacco, sunflowersand other dicotyledones (Hasanneiad et al, 2006, Saremi and Okhoyvat, 2008). BesidesFusarium, the genus Trichoderma (Abdel-Kader and El-Mougy, 2009) was also used as biological control agent. Its species *T. harzianum* and *T. viride*showed high effectiveness in the control of broomrape ongarden crops.

Parasitic weedsattack many cultivated garden, industrial and weed plants.The most important and most commonparasitic weeds in our country aredodder – *Cuscutaspp* and broomrape-*Orobanche spp*. They draw the water and nutrients from the plant host by theirhaustoria, making the plant weaker andslower in growth. Due to high temperatures during the day,it loses its turgor and gives lower yields and poor quality.

Cultural plants are attacked by about 130 species of the genus *Orobanche,* the most common of which are *O. ramosa, O.crenata, O.cumana, O. minor, O.aegiptiaca*, appearing on many vegetables and industrial crops (Jovičić, 2012, Ćosić et al., 2006).

They are obligate parasites that attack many dicotyledoneplant species of various families in warm and dry areas throughoutthe world (Europe, Australia, Russia, China India, Mongolia, Iran, Iraq, Egypt, Algeria) (Ćosić et al., 2006). They are especially common on sugar beet, sunflower, maize and tobacco, but they also parasitize tomatoes, peas, beans, hops andcannabis. The most important for sunflower is *O.cumana* (Ćosić et al., 2006). According to literature data (Boca, 2007), the yield of sunflower was significantly reduced due to broomrape attack (18-38%). Tobacco in Italy as well as in Macedonia is parasitized by the species *O. ramosa* (Мицковски, 1984, Nanni et al., 2005). It has been observed in the regions of Kavadarci, Prilep, Strumiza, Kumanovo, RadovisVinica, etc. The highest damage can be made on poor soils, in dry conditions and early appearance of the parasitic weed.

All broomrape species have atrophied root system and live as parasites on roots of other plants. They are chlorophyll-free and have unbranching stalkcovered with small scales that ends ingrape-like inflorescence with pink or light blue blossoms. A single plant can host many broomrape stalks, sometimes over 100 (Jovičić, 2012).

In favorable moisture and temperature conditions, root secretions simulate germination of broomrape seeds. Thread-like hypha of the germ tube adheres to the root system of the plant and forms a thickening (appresorium). On the bottom side of the appresorium appears a nail-likeprotrusion that penetrates through the root into the conducting vesselsof the host plant (phloem, xylem) to draw its water and nutrient elements. On the upper side of appresorium there are buds from which the stalks of the parasite develop. The broomrape seeds are retained in the soil for a long period (8-12 years) and are the main source of infection (Jovičić, 2012).

The aim of theinvestigation was to determine the cause for occurrence of necrosis and drying of broomrape stalks, to isolate the causingagentand to assess the infectivity and pathogenicity of the isolates of *Fusarium* species on broomrape and on tobacco plant.

MATERIAL AND METHODS

Healthy stalks of *O.ramosa*, stalks with symptoms of infection and dry stalks were selected from field. The plants were wrapped in plastic bags and kept in refrigerator until use. Healthy plants were collected for seed and for inoculation, in order to determine the infective ability and pathogenicity of the obtained isolates.

Infected stalks were collected for isolation of the causing agent, using fragmentswith disease symptoms, washed with distilled water, disinfected with 1% sodiumhypochlorite for 5 minutes and rinsed several times with sterile distilled water.

Fragments were placed in Petri dishes on potato dextrose agar (PDA) as nutrient medium and incubated at 22^{0}C until fungus development. Obtained isolates were used for inoculation of broomrape stalks and inflorescences and also for tobacco stalks and leaves. Inoculation was carried out by the method of Goussouset al. (2008).
Healthy and fresh broomrape stalks 15 cm in size and disinfected as described above were used for inoculation. The stalkswere placed upright, and then a 4 mm fragment of the fungus culture was taken and carefully placed with the upper sideon cross-sectionof the stalk.

In order to preserve the moisture, the inoculated part was covered with moistened cotton and wrapped in aluminum foil. Controlstalks were wrapped only in moistened cotton. Inoculated stalks were placed in a moist chamber and incubated at 25^{0}C for 7 days. The trial was set up in three replications, with 10 plants inoculated for each replication. The stalk infection levels were ranged on a 0-4 scale as follows: 0 = no symptoms; 1= symptoms localized at the point of inoculation (5% of stalk tissue infected); 2 =infection spread around the point of inoculation (30% infection); 3 = maceration tissue extends several cm above the point of inoculation (60-70% of the stalk infected); 4 = loss of consistency of the whole stalk for 7 days (100% infection). The same method was used for inoculation of stalks of tobacco plant, to check whether the isolates were pathogenic to tobacco.

Pathogenicity of isolates was also tested on broomrape inflorescences. Fresh inflorescences taken from healthy plants were disinfected as above and inoculated with a suspension prepared from thefungus culture. Fungal suspension was prepared by adding 25 ml of sterile distilled water in the culture of onePetri dish, after whichits surface was scratched and mixed. Inflorescences were soaked in the suspension and then placed in wet chambers. Control inflorescences were soaked in sterile distilled water. Inoculated inflorescence were incubated for 7 days at 25^{0}C.

The assessment is made according to the following scale: 0=the inflorescence is not infected; 1 = dark discoloration of surface tissues (50% of tissues infected); 2 =inflorescences are black, with maceration of internal tissues (100% infection). 10 plants of each example were inoculated and the trial was set up with three replications.

The same method was used for inoculation of tobacco stalks. Tobacco leaves were inoculated by putting the base of the leaf in the suspension and control leaveswere placed in sterile distilled water. Inoculated leaves were incubated in wet chambers at 25^{0}C for a period of 7 days, and the evaluation was made according to the percentage of infected leaf surface.

RESULTS AND DISCUSSION

Fungi coloniesisolated fromdryplantsof *O.ramosa* (Fig. 1, Fig. 2) had a well-developed substrate and aeria lmycelia of cotton-white color, with a tendency to be more or less colored in the central part. After colorization, the coloniesturned peach, pink or violet (Fig. 3, Fig. 4), and sometimes they remained white (Fig. 5). In few days (5-7), the colony reached maximum growth in 9cm diameter Petridish. The mycelia of all isolate swereseptated (Fig. 6) and produced many oval microconidia with 1-2 septa (Fig. 7), many falciform macroconidia with 3-5 septa (Fig. 8) andshort conidiophores.

Fig. 1. *O. ramosa*-Broomrape on tobacco

Fig. 2. *O. ramosa*-Dry broomrape

20 isolates were obtained for this study and microscopic examination confirmed that all of them belonged to the genus Fusarium. Boutitiet al. (2008) also confirmed that the isolates belonged to Fusarium, and according to Nanniet al., (2005), the obtained isolates were identified as *F.oxysporum*. The *F.oxysporum* isolates are colored peach or pale orange, dark pink to red and the colony of *F.solani*isolates is light pink, dark pink to red (Ghannam et al., 2007). The mycelium in all isolates is segmented, microconidia are oval with reduced basis, and in some isolates chlamydosporeswere found.

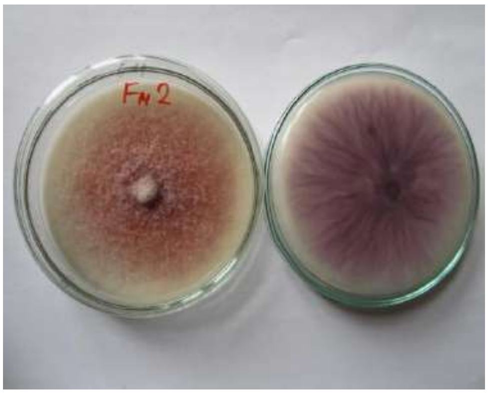

Fig. 3 *Fusarium* spp. Pure culture-isolate FM2 Fig.

Fig. 4 *Fusarium* spp. Pure culture-isolate FO17

Fig.5 *Fusarium* spp. Pure culture-isolate FM10

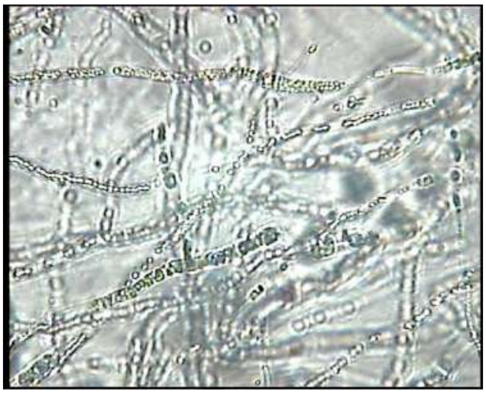
Fig.6 *Fusarium* spp. Fungal mycelia - 400x


Fig.7. *Fusarium* spp. Microconidia-400x

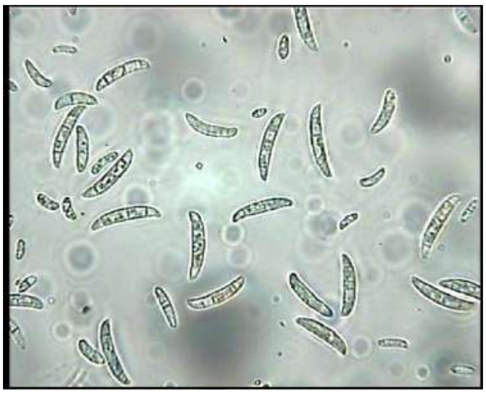
Fig.8. *Fusarium* spp. Macroconidia - 400x

For inoculation of broomrape stalks with fragments of the fungal colony, theisolates FM2, FO11 and FO14 were used.The first symptoms were observed on the third day of inoculation, by occurrence of web-like myceliumon stalk surface. All isolates investigated showedalmost identical level 4of symptoms and damage on inoculatedstalksof *O.ramosa*, i.e. their tissue was 100% damaged. These isolates showed high effectivenesson broomrape, reaching up to 100%damageof the stalk tissue (Table 1). The symptoms caused by these isolates were characterized by the occurrence of white cotton-like mycelia along stalk surface, necrosis, soft rot and loss of consistency in 7 days after inoculation (Fig. 9). Similarly, the isolates *F. oxysporum* and *F.solani*, reached an effectiveness of 80-92% in inoculation of *O. cernua* stalksbythis method (Goussous et al., 2008).

Table 1. Inoculated stalks of broomrape and tobacco

Variant	Inoculum	Inoculated stalks	Infected stalks	Index	Infection %
Broomrape Stalks	Fragment	30	30	4	100
Tobacco Stalks	Fragment	30	-	0	-
Broomrape Inflorescences	Suspension	30	30	2	100
Tobacco Stalks	Suspension	30	-	0	-
Tobacco Leaves	Suspension	30	-	0	-

Fig. 9. *O. ramosa* - Inoculated stalks

Fig. 10. Inoculated tobacco stalks

Tobacco plants inoculated with fragment hadno symptoms of infection, change of color or necrosis, the stalks were healthy and without damage (Fig.10). This confirmed thatisolates of Fusariumspecies are pathogenic to *O.ramosa*, but they are not harmful to tobacco. The high percentage of broomrape infection with Fusarium (over 90%) in tobacco regions was confirmed by Nanniet al.,(2005). Due to the fact that the fungus is not harmfulto tobacco, but is deadly to *O.ramosa*, it can be included in the program for biological control of this parasitic weed.

Fusariumcan be used as biological control agent against *O. crenata* and *O. foetida*in beans, because the isolates of this genus reduced the germinability of broomrapeseed for 27-93%, and some of them reduced the occurrence of broomrape stalks for even 98% (Boutiti et al., 2008). In tomatoes, percentage of dead broomrape was increased for 67.3 to 100% compared to the check, and none of the isolates had a negative impact on cultivated plants (Ghannam et al., 2007).

The suspension used for inoculation of broomrape inflorescences and tobacco stalks was prepared from the same isolates. On the third day of inoculation, white cotton-like mycelium appeared among floral buds and spread so rapidly that in a few days almost the entire surface of the inflorescence was covered (Fig. 11). In tobacco stalks, however, no symptoms of infection were present (Fig. 12).

The situation was similar with all investigated isolates. In evaluation made on the 7th day, all inoculated inflorescences were rated with index 2, i.e. the tissue was 100% damaged (Table 1).

Fig. 11. *O.ramosa* - Inoculated inflorescences

Fig. 12. Inoculated inflorescences and tobacco stalks

Fig. 13 Inoculated tobacco leaves

In tobacco leaves inoculated with fungal suspension there was no appearance of necrosis or change in color - all leaves were healthy, without any damage (Fig. 13). This confirmed that the isolates are highly pathogenic to the broomrape, but have no negative effect to tobacco plant. These results show that Fusarium isolates can be used as biological agents in the control of broomrape (*O. ramosa*) on tobacco.

CONCLUSION

Drying of the plants from broomrape is caused by fungi of the genus Fusarium, which were isolated from dry stalks on potato dextrose agar (PDA) medium.

All inoculated broomrape plants were 100% infected with isolates of the fungus *Fusarium spp*. They were entirely covered with the fungal mycelium, their stalks were wet and rotten and the tissue was in a stage of decomposition.

Inoculated stalks and leaves of tobacco were healthy and showed no symptoms of infection.

Some species of the genus Fusarium can be used in biological control of *Orobanche spp*. – the weed that attacks tobacco and many other plant species.

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Original Scientific paper

THE MODE OF INHERITANCE OF QUANTITATIVE CHARACTERS AND HETEROTIC EFFECT IN F1 HYBRIDS IN A DIALLEL OF DIFFERENT TOBACCO TYPES

Jane Aleksoski

Prilep, Republic of Macedonia

Correspondence to: Jane Aleksoski

E-mail: aleksoskijane@yahoo.com

ABSTRACT

The paper concerns the mode of inheritance and assessment of heterotic effect in six diallel F1 crosses of four parental genotipes (large – leaf variety Burley B – 2/93 and the oriental Suchum S1 with pink flowers, Suchum S2 with white flowers and Prilep P – 84), for the following characters: stalk without inflorescence, leaf number per stalk, middle belt leaf area, green mass yield and dry mass yield per stalk. The trial was set up in the field of Scientific tobacco institute – Prilep, in randomized blocks with four replications during 2007, 2008 and 2009 and traditional cultural practices were applied in tobacco growing.

The analysis of variance was used to determine statistically significant differences between parents and their hybrids for the characters investigated during the three – years period. Positive heterosis with poor heterotic effect was recorded in S1 x S2 hybrid for stalk height without inflorescence and for green/dry mass yields per stalk, while in S2 x P-84 only for height of the stalk without inflorescence. Negative heterosis with poor heterotic effect was recorded in hybrids S1 x P-84 for leaf number and for green/dry mass yields and S2 x P-84 for leaf number and green massyield per stalk. The low heterotic effect indicates that utilization of heterosis in investigated tobacco genotypes is without economical justification, but in the same time it points out to the eventual breeding activities for creation of new more superior varieties.

Keywords: tobacco NicotianatabacumL., diallel crosses, mode of inheritance, heterosis, heterotic effect.

НАЧИН НА НАСЛЕДУВАЊЕ НА КВАНТИТАТИВНИТЕ СВОЈСТВА И ПРОЦЕНКА НА ХЕТЕРОТИЧНИОТ ЕФЕКТ КАЈ F1 ХИБРИДИТЕ КАЈ ДИЈАЛЕЛ НА РАЗЛИЧНИ ТИПОВИ ТУТУН

Трудот опфаќа проучувања за начинот на наследување и проценка на хетеротичниот ефект кај шест еднонасочни дијалелни крстоски на четири родителски генотипови (крупнолисната сорта Берлеј Б - 2/93 и ориенталските сорти Сухум S1 со розови цветови, Сухум S2 со бели цветови и Прилеп П - 84), за својствата: висина на стракот без соцветие, бројот на листови по страк, површина на листовите од средниот појас, принос на зелена маса по страк и принос на сува маса по страк. Опитот беше поставен на опитното поле при Научниот институт за тутун – Прилеп по случаен блок – систем во четири повторувања во 2007, 2008 и 2009 година. Со анализа на варијансата беа утврдени статистички значајни разлики помеѓу родителите и нивните хибриди за својствата во трите години на истражувања.

Позитивен хетерозис со слаб хетеротичен ефект е утврден кај крстоската S1 x S2 за висината на стракот без соцветие и приносот на зелена и сува маса по страк и кај S2 x П-84само за висината на стракот без соцветие. Негативен хетерозис со слаб хетеротичен ефект е утврден кај крстоските S1 x П-84 за бројот на листови по страк и за приносот на зелена и сува маса постарк и S2 x П-84 за бројот на листови и принос на зелена маса по страк.

Нискиот хетеротичен ефект укажува на економската неоправданост на искористувањето на хетерозисот кај проучуваните генотипови тутун, но истовремено укажува на можни идни селекциони активности за создавање на нови сорти посупериорни од родителите.

Клучни зборови: тутун - *Nicotiana tabacum L.*, диалелни крстоски, начин на наследување, хетерозис, хетеротичен ефект.

INTRODUCTION

The phenomenon in F1 when progenies of genetically divergent lines are more vigorous and achieve higher yields thah parents is called heterosis. It is used in mass production especially of side-fertilizing cultures, in which it is difficult to obtain homogenous and stable varieties and where each subsequent reproduction differs from the previous ones due to the free fertilization.

Heterosis is not used in the production and breeding of oriental tobacco because it is considered as economically unjustified measure. However, genetic investigations on inheritance of characters in various crops have always been completed by determination of the heterotic effect in F1 hybrids. Genetic mechanism of the heterosis enables early prognosis of the breeding value of hybrid combinations. There is great probability that new lines with preferred characters can be obtained from the varieties with high heterotic effect.

Hybrid vigor of F1 hybrids in different tobacco varieties has been investigated in many papers, but we only present those in which oriental tobaccos are included. Marani and Sachs (1966) obtained positive heterosis for height and leaf number in hybrids of oriental tobacco. Matzinger and Wernsman (1968) recorded positive heterosis only for stalk height in flue-cured and oriental varieties. Tomov (1975) found strong positive heterotic effect for stalk height in domestic varieties of oriental tobacco. Jung et al. (1982), in diallel analysis of six orienral and fifteen F1 hybrids revealed positive heterosis for stalk height, leaf number and yield, with strong heterotic effects in hybrids Samsun x Izmir and Xanthi x Izmir). Terrill et al. (1982) revealed positive heterosis for stalk height and yield in 12 varieties of sun-cured, flue-cured, dark-fired, Burley, Maryland and cigar tobacco and their diallel F1 hybrids. Lee & Chang (1984) found positive heterosis for leaf length and width and for leaf mass yield in their analysis of local and oriental Korean varieties and 28 F1 hybrids. Kara &Esendal (1995) in six oriental varieties and their 15 F1 hybrids (excluding reciprocal crosses) revealed negative heterosis for leaf number and significant heterosis for yield (the average yield of the hybrids was 15.2% higher than the parents). Korubin-Aleksoska (2008) in analysis of three oriental varieties and one semi-oriental and their diallel F1 progenies found positive heterosis for stalk height (YV 125/3 x FO), for middle belt leaf area and dry mass yield (P 12-2/1 x P-2 and P-2 x YV 125/3) and for green mass yield (P 12-2/1 x P-2). The cross P-2 x YV 125/3 showed negative heterosis for leaf number per stalk. The authors reported that application of heterosis in tobacco production is economically unjustified, except for hybrids resistant to some disease.

The aim of the investigations was to reveal heterosis and to estimate its effect on major quantitative characters in F1 progenies of different tobacco types, in order to contribute to the genetics of this crop and to predict the perspectiveness of the new lines in the diallel.

MATERIALS AND METHODS

Investigations on major quantitative characters and heterotic effect in F1 progeny was performed with four tobacco varieties, one of which were large-leaf variety (Burley B- 2/93 in CMS form) and three were oriental (Suchum S1 with pink flowers, Suchum S2 with white flowers and Prilep P-84 with red flowers). The diallel crossings provided the maximum number of combinations that can be made between some parental genotypes.

Crossings were made in the Experimental field of Tobacco Institute-Prilep during 2006, 2007 and 2008. The seed from six combinations for F1 generation was obtained by hand castration and pollination. The trial was set up during 2007, 2008 and 2009 in randomized blocks with four replications. Investigations included parental genotypes and progenies of the following F1 hybrids:

1. Burley B-2/93 x Suchum S1
2. Burley B-2/93 x Suchum S2
3. Burley B-2/93 x Prilep P-84
4. Suchum S1 x Suchum S2
5. Suchum S1 x Prilep P-84
6. Suchum S2 x Prilep P-84

Each replication was performed at an area of 147.6 m². The whole trial was set up at of 590.4 m² usable area, i.e. at 838 m² total area, together with the paths.

All suitable cultural practices were applied during the growing season.

Analysis was made on the following quantitative traits: stalk height without inflorescence, leaf number per stalk, middle belt leaf area, green mass yield per stalk and dry mass yield per stalk.

The first two characters were investigated in the period of tobacco flowering, at the end of July and August. 50 stalks from each replication were measured, or a total of 200 stalks from the whole trial, with the same number of leaves from the middle primings.

Leaf area was calculated by multiplication of their length and width with the coefficient k=0,6354.

Green mass yield was measured after each harvest. Total weight of tobacco from each plot was added and the addition was divided with the number of stalks from which tobacco leaves were picked. The same method was used to calculate dry mass yield per stalk, i.e. tobacco was measured after manipulation and formulae for corrected yield were applied.

Processing of results

Data obtained from measurements of each character by combinations for parental genotypes and their F1 progeny were processed by the variational-statistical method.

Mode of inheritance was estimated according to the test-significance of the mean value of F1 progeny compared to the parental average Borojević (1981). Significantly higher mean value of the hybrid obtained from parent with higher average value denotes the appearance of positive heterosis (+h), whereas significantly lower mean value of the hybrid obtained from parent with lower average value denotes negative heterosis (-h).

Heterozis (h) is a phenomenon in which the progeny of the first generation possesses more strongly expressed characters, both positive and negative, compared to the parents. Its effect is estimated as follows:

$h = \overline{F1} - \overline{BP}$

where:

$\overline{F1}$- mean value of F1 generation

$\overline{BP}$ - mean value of the better parent

Standard error of heterosis in relation to $\overline{BP}$is estimated by the formula:

$Se(h) = \sqrt{h \text{ of variance}}$

The significance of $\overline{F1}$generation in relation to$\overline{BP}$is tested with t-test:

$t = \overline{F1} - \overline{BP}/SE(h)$

Meteorological data

Manifestation of quantitative characters greatly depends on the effect of environmental factors. In 2007, during tobacco growth in field (May-September), mean monthly temperature was 20.88ºC and the number of rainy days was 41, with total amount of precipitation 229.9 mm. In the same period in 2008, mean monthly temperature was 19.91ºC and the number of rainy days was 39, with total amount of precipitations 235.4 mm. In the same period in 2009, mean monthly temperature was 19.89ºC and the number of rainy days was 42, with total amount of precipitations 240,6 mm.

Values of the above parameters indicate optimum climate conditions for production of oriental tobaccos. They reveal approximately identical conditions in the three investigating years.

RESULTS AND DISCUSSION

The most common mode of inheritance in F1 hybrids, where one of the parents is the large leaf variety B-2/93, is partial dominance. In these crosses, inheritance of height is intermediary, and that of the number of leaves is negatively dominant. In crosses where both parents are of oriental type, all possible modes of inheritance are present, but heterosis is predominant.

Heterosis (h) is a consequence of heterozigosity of F1 progeny, in which some diallel and non-allelic genes in interaction affect certain character, exceeding the parents in positive or negative direction. Expression of the strength of this phenomenon is called heterotic effect. It is manifested only in F1 generation, while in the successive generations it disappears, due to impossibility of its fixation.

The reveal of heterosis is based on previous investigations on inheritance of the characters. In our three-year investigation, the highest among parents was the large-leaf variety B-2/93, and P-84 was the shortest. Among hybrids, B-2/93 x Suchum S1 was the highest, while S1 x P-84 and S2 x P-84 were the shortest. The highest leaf number among parental genotypes was observed in P-84 and the lowest in B-2/93, while among hybrids this character was highest in S1xS2 and lowest in crossings where B-2/93 was one of the parents. The largest leaves and highest yield of green and dry mass were observed in B-2/93, whereas the smallest leaf and lowest green and dry mass yields were found in P-84. Among hybrids, predominant for all three characters were those in which B-2/93 was one of the parents. The smallest leaf area and lowest green and dry mass yield was noticed in S1 x P-84 and S2 x P-84, in which negative heterosis appeared. Values for the quantitative characters in parents and F1 progeny are presented at Table 1.

Table 1.Inheritance of some quantitative characters from parents of the F1 progeny and appearance of heterozis

Parents and F1 hybrids	Quantitative characters														
	Height of the stalk without inflorescence (cm)			Number of leaves / stalk			Middle belt leaf area (cm²)			Green mass yield / stalk (g)			Dry mass yield / stalk (g)		
	2007	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**
1. Burley B-2/93	147	142	145	36	34	36	1264	1138	1282	1099	1017	1055	185	178	182
2. Sochoumi S1	70	68	69	47	45	45	230	205	220	267	210	253	26	25	25
3. Sochoumi S2	69	67	70	47	45	46	239	220	225	260	208	220	26	25	26
4. PrilepP-84	58	57	58	53	52	52	146	138	142	160	158	160	24	24	24
5. B - 2/93 x S1	105 i	103 i	104 i	35 -d	34 -d	35 -d	1063 pd	1016 pd	1050 pd	813 pd	800 pd	809 pd	132 pd	130 pd	131 pd
6. B - 2/93 x S2	102 i	102 i	103 i	36 -d	34 -d	36 -d	1074 pd	988 pd	1040 pd	811 pd	808 pd	810 pd	133 pd	130 pd	133 pd
7. B - 2/93 x П - 84	100 i	91 i	95 i	37 -d	35 -d	36 -d	903 pd	832 pd	888 pd	795 pd	790 pd	789 pd	122 i	117 i	120 i
8. S1 x S2	70 +h	69 +h	71+h	47 i	45 i	46 +d	233 pd	210 pd	225 +d	269 +h	211 +h	275 +h	26 +h	26 +h	27 +h
9. S1 x P - 84	71+d	69 +d	70 +d	43 -h	42 -h	42 -h	185 i	173 i	180 i	133 -h	130 -h	135 -h	23 -h	23 -h	23 -h
10. S2 x P - 84	72 +h	70 +h	73 +h	45 -h	43 -h	43 -h	174 pd	158 pd	170 pd	135 -h	133 -h	137 -h	24 -d	24 -d	25 i

Positive significant heterotic effect for stalk height without inflorescence appeared in S1 x S2 and in S2 x P-84. Positive heterotic effect for this character was also reported: Marani and Sachs (1966), Matzinger and Wernsman (1968), Tomov (1975), Jung, Hwang and Son(1982), Terrill, Aycock, Link and Conner (1982) and Korubin - Aleksoska (2008).

Negative heterotic effect for leaf number was observed in S1 x P-84 and S2 x P-84. The same effect for this character was also reported Karaand Esendal (1995) and Korubin - Aleksoska (2008).

Positive heterotic effect for green and dry mass yields in our investigations was observed in S1 x S2. The same effect for this character was also reported Jung, Hwang and Son(1982), Terrill, Aycock, Link and Conner (1982), Lee and Chang (1984), Kara and Esendal (1995) and Korubin - Aleksoska (2008). Negative heterotic effect for green and dry mass yields was found in S1 x P-84, and only for green mass yield in S2 x P-84.

Heterotic effect of the characters that were subject of our investigation is presented in Table 2.

Table 2.Heterotic effect of quantitative characters in F1 hybrids

Parents and F1 hybrids	Quantitative characters														
	Height of the stalk without inflorescence (cm)			Number of leaves / stalk			Middle belt leaf area (cm²)			Green mass yield / stalk (g)			Dry mass yield / stalk (g)		
	2007	2008	2009	2007	2008	2009	2007	2008	2009	2007	2008	2009	2007	2008	2009
5. B - 2/93 x S1															
6. B - 2/93 x S2															
7. B - 2/93 x П - 84															
8. S1 x S2	+ 0.22	+ 1.02	+1.12							+ 2.17	+ 0.86	+21.62	+ 0.37	+ 0.26	+1.22
9. S1 x P - 84				– 4.91	– 2.41	-3.26				– 26.49	– 27.62	-25.14	– 0.87	– 0.94	-0.97
10. S2 x P - 84	+ 2.26	+ 2.03	+2.94	– 4.91	– 1.99	-2.92				– 24.62	– 24.61	-23.03			

CONCLUSIONS

- Selected parents (Burley B-2/93, Suchum S1, Suchum S2 and P-84) are genetically homogeneous and significantly different;
- The modes of inheritance of investigated quantitative characters are different. In crosses where one of the parents is B-2/93 partial dominance is prevailing, whereas in crosses from oriental varieties heterosis is predominant, but other modes of inheritance are also present.
- Positive heterotic effects for stalk height without inflorescence, as well as for green and dry mass yields, were observed in hybrid S1 x S2, and only for the stalk without inflorescence in S2 x P-84. Negative heterotic effect for leaf number per stalk and for green and dry mass was found in hybrid S1 x P-84, whereas for leaf number and green mass yield it was observed in S2 x P-84. The positive heterotic effect is low and economically unjustified;
- F1 hybrids are the basis from which, through successive selection in future, perspective lines will be selected, with improved characters which will stabilized very soon.

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Original Scientific paper

ECONOMIC EFFECTS OF TOBACCO FARMING IN MACEDONIA - CASE STUDY OF 2011 CROP

Snezana Stojanoska[1], Lazar Stojanoski[2]

1"St. Kliment Ohridski" University - Bitola, Scientific Tobacco Institute - Prilep, Macedonia

2 "St. Kliment Ohridski" University - Bitola, , Faculty of economics-Prilep, Macedonia

e-mail: snezana.stojanoska @ yahoo.com

ABSTRACT

The main hypothesis of this investigation is the poll of 40 tobacco growers from the southwest and 20 from the southeast part of R. Macedonia, whose farms are larger than one hectare. The results obtained from the processing of questionnaire related to the basic data on tobacco production, cost structure and other factors which determine this production are presented and commented in this paper. Calculations are made for 2011 tobacco crop, which includes 16 typical tobacco growers. The average costs ranged from 19 300 den/kg to 4020 day/kg and the average yield from 100 kg/decare to 270 kg/decare. Some of the farmers engaged additional labor force which amounted 8640 hours, against the 2880 hours of the household members. Productivity of labor in tobacco production ranges from 36 to 200 minutes per kg. The planned revenues of the monitored group of producers ranges between 479,800 and 2,721,200 denars, in calculations based on average purchase price of 230 denars/kg and a premium of 80 denars/kg dry tobacco.

Keywords: production, yield, cost calculation, revenue

ЕКОНОМСКИ ЕФЕКТИ ОД ФАРМЕРСКОТО ПРОИЗВОДСТВО НА ТУТУН ВО РЕПУБЛИКА МАКЕДОНИЈА-СТУДИЈА НА СЛУЧАЈ ЗА РЕКОЛТА 2011 ГОДИНА

Основна хипотеза на истражувањето претставува спроведената анкета на 40 производители на тутун од југозападниот и 20 производители од југоисточниот реон кои што одгледуваат тутун на површина поголема од еден хектар. Во продолжение презентирани и коментирани се резултати добиени од обработениот анкетен прашалник, поврзан со прашањата што се однесуваат на основните податоци за производството на тутун, прашањата поврзани за структурата на трошоците и на крај прашањата што го детерминираат ова производство Во содржината на трудот презентирана е планска калкулација за производство на тутун за реколта 2011 година, и тоа за 16 покарактеристични производители. Просечните трошоци кај 16-те производители се движат во распон од 19 300 ден/kg до 4020 ден/kg., просечниот принос од 100 kg/декар до 270 kg/декар. Кај некои производители ангажирана е дополнителна работна сила која изнесува 8640 саати, наспроти 2880 саати од членовите на семејството.

Продуктивноста во производстото на тутун се движи во границите од 36 до 200 минути за еден килиграм тутун. Планираната добивка кај евидентираната група производители се движи во интервал 479.800 денари до 2.721.200 денари кога во пресметките беше користена средна откупна цена од 230 ден/kg и премија од 80 ден/kg сув тутун.

Клучни зборови: производство, принос ,трошоци ,калкулација, добивка

INTRODUCTION

Despite the aggressive antismoking campaign, tobacco still takes an important place in the agricultural and food sector of the Republic of Macedonia and is important financial source both for the households and for the state.

Global changes imposed a necessity to make strategic planning of tobacco production in the country, and as a result of that to design a conception for further development of this crop, with intentions to produce about 35 000 tons of dry tobacco in the future. Therefore, it is necessary to direct the strategic development of tobacco production toward its expansion and quality improvement and to raise it at a level of family business (farming), which will provide employment for members of the household and for other persons.

1. THE AIM OF INVESTIGATION

The main objective of the research is to create conception for strategic development of tobacco production in the Republic of Macedonia. These aim will be realized through organized research, analysis, forecasts and planning in order to prove the basic hypothesis of the paper, that R. Macedonia has suitable conditions to produce about 35 000 tons of dry tobacco, which will lead to appropriate economic effects both for the farmers and for the state.

2. MATERIAL AND METHODS

The basic material for realization of this aim are the official data published in statistical yearbooks of R. Macedonia, and data obtained in the poll of tobacco farmers conducted from 15 to 30 September 2011 using a structured questionnaire consisting of three sections with 27 questions.

The above sources were used to collect relevant data about area under tobacco and its average yields, quantity and value.

3. RESULTS AND DISCUSSION

With regard to the denoted issue and the basic hypothesis of the study, it was necessary to make a poll among tobacco growers. Respondents were 40 tobacco farmers from the southwest and 20 farmers from the southeast production area which grow tobacco on lands larger than one hectare. These regions were selected due to their highest contribution in the total tobacco production of R. Macedonia. Special attention in processing the collected data was paid to tobacco growers who use a farm model of production, with contemporary agro-techniques, and who engage additional force of labor besides that of their own household.

3. 1. The structure of tobacco production costs

Tobacco production is associated with costs and the following components of productional calculation will be presented here: rent for land, ploughing and number of ploughings, manure (artificial and organic), irrigation (number of irrigations, costs for using water from hydro-systems), threads for stringing tobacco leaves, curing barns, costs for insurance, other costs (for transportation, protection products, polyethylene, seedlings) and costs for labor force.

In order to protect the personal data, planning calculation of tobacco production for 2011 was made for 16 typical tobacco growers who will remain unnamed.

Table 1- Calculation of planning of dry tobacco production in 2011 crop

No. of tobacco grower	Planted area, decares	Rent	Ploughing	Fertilization	Irrigation	Transportation	Threads	Curing	Additional labor costs	Other costs	Total costs	Average costs per decare
1	55	38.500	176.000	51.000	60.000	20.000	14.000	55.000	600.000	47.000	1.061.500	19.300
2	40	20.800	96.000	30.000	42.000	15.000	8.000	20.000	360.000	35.000	626.800	15.670
3	22	44.000	52.800	30.000	23.000	15.000	4.000	20.000	147.000	35.000	370.800	16.854
4	27	16.200	64.800	26.000	-	9.000	12.000	18.000	120.000	15.000	281.000	10.407
5	60	36.000	144.000	72.000	-	150.000	7.000	12.000	432.000	30.000	883.000	14.716
6	40	120.000	96.000	30.000	-	130.000	20.000	30.000	216.000	12.000	654.000	16.350
7	15	45.000	36.000	28.000	-	20.000	4.000	7.000	-	5.000	145.000	9.666
8	12	24.000	33.600	18.000	-	30.000	2.500	4.000	-	22.000	134.100	11.175
9	20	20.000	42.000	13.000	4.000	20.000	10.000	10.000	40.000	5.000	164.000	8.200
10	18	24.300	43.200	12.500	2.000	18.000	7.000	7.000	-	2.100	116.100	6.450
11	14	16.800	29.400	9.100	-	10.000	7.000	6.000	-	3.000	81.300	5.807
12	20	10.000	36.000	-	6.000	15.000	3.000	12.000	25.000	6.000	113.000	5.650
13	20	10.000	36.000	7.000	7.000	15.000	3.500	12.000	24.000	6.000	120.500	6.025
14	20	12.000	36.000	7.500	-	5.000	4.000	8.000	-	9.000	81.500	4.075
15	25	15.000	45.000	10.500	-	5.000	5.000	10.000	-	10.000	100.500	4.020,
16	20	12.000	36.000	7.500	-	14.000	4.000	8.000	-	9.000	90.500	4.525
Average	26,75	29.038	62.675	22.006		30.688	7.188	14.938		15.694	313.975	9.931

According to the the above calculation, the total cost of tobacco production ranged between 1 061 500 denars for 55 decares to 81 500 denars for 20 decares. The average costs ranged from 19 300 denars/decare to 4020 denars/decare. The big difference in production costs is due to the fact that tobacco grower No 1 applies the most contemporary cultural practices and hires 15 workers as additional labor in a period of 100 days and 5 hours daily work, which makes a total of 7500 additional labor hours. The average cost of the first five tobacco growers was 16,578 denars/decare, while that of the growers who engage none or negligible additional labor approximated 5 250 denars/decare. The expected yields in the analyzed group of tobacco growers ranged from 2000 kg to 11 000 kg, which gives an average yield of 100 kg - 270 kg/decare.

3.1.1. Labor force engaged in tobacco production

In this investigation efforts were made to determine the engaged labor force in tobacco production among the above mentioned 16 producers. The processed data are concerning the total engaged labor (estimated in hours), as a sum of labor of the household members and additionally engaged labor force in 2011 crop. The participation of the above labor categories will be presented in Table 2.

Table 2 - Engaged labor force

No. of tobacco grower	Engaged labor force, in hours		Total hours
	Household members	Additional labor force	
1	3.400	7.500	10.900
2	1.920	4.500	6.420
3	2.400	1.840	4.240
4	6.000	1.500	7.500
5	2.880	8.640	11.520
6	9.000	4.320	13.320
7	7.200	-	7.200
8	5.175	-	5.175
9	2.880	4.000	6.880
10	5.040	-	5.040
11	3.900	-	3.900
12	4.000	250	4.250
13	4.800	240	5.040
14	4.410	-	4.410
15	5.880	-	5.880
16	4.410	-	4.410
Average	4.581	2.049	6.630

It is evident from the above data that some tobacco growers engage extra manpower, e.g. the tobacco grower No. 5 engaged 8640 hours additional labor, against 2880 hours labor of his household members. In general, it can be concluded that tobacco growers who accepted the farm production engage more additional labor in 2:1ratio. There is a clear tendency among the growers to increase the area planted with tobacco and to use additional labor for its processing. The average hourly wages in 2011 crop was around 90 denars.

3.1.2. Productivity of individual tobacco growers

Productivity in tobacco growing will be estimated by the following model, using the tobacco grower No. 1 as an example:

$$\text{Productivity (P)} = \frac{\text{Total labor force in hours}}{\text{Total production in kg}} = \frac{10\ 900}{11\ 000} = 0{,}99 \text{ hours}$$

$$P = 0{,}99 \text{ hours} = 59{,}45 \text{ minutes/kg}$$

The above estimation, expressed through the time required for production of 1 kilogram dry tobacco will be presented in Table 3:

Table 3 - Time required for production of 1 kg dry tobacco

No. of tobacco grower	Time required for production of 1 kg dry tobacco	
	hours	minutes
1	0,99	59
2	0,60	36
3	0,85	51
4	1,82	109
5	1,92	115
6	3,33	200
7	2,77	166
8	2,59	155
9	0,82	49
10	1,87	112
11	1,70	102
12	1,42	85
13	1,26	76
14	1,47	88
15	1,55	93
16	1,47	88
Average	1,65	99

According to the table, productivity of dry tobacco production in the investigated group of growers ranges from 36 to 200 minutes/kg. Big differences in productivity are determined by the mode of tobacco production. Tobacco growers who apply modern cultural practices and appropriate irrigation achieved high productivity ranging in an interval from 30 to 50 minutes for production of one kilogram dry tobacco. The productivity of the majority of growers ranged between 50 and 100 minutes, or in average it takes 70 minutes to produce 1 kg dry tobacco. Very few manufacturers achieved a productivity of over 100 minutes per kilogram tobacco, which is a result of the untimely irrigation and labor intensive mode of production.

3.1.3. Economic effects of tobacco farming

The economic effects of tobacco will be presented in Table 4.

Table 4-Planned revenue in denars

No. of tobacco grower	Total planned production, in kg	Total planned revenue, in denars	Planned expenditures	Planned profit	Hourly wage for all household members	Number of household members	Hourly wage per member of the household
1	11.000	3.410.000	1.061.500	2.348.500	691	4	172,82
2	10.800	3.348.000	626.800	2.721.200	1.417	4	354,24
3	5.000	1.550.000	370.800	1.179.200	492	5	98,40
4	4.100	1.255.500	281.000	974.500	162	4	40,59
5	6.000	1.860.000	883.000	977.000	339	2	169,74
6	4.000	1.240.000	654.000	586.000	66	5	12,92
7	2.600	790.500	145.000	645.500	90	5	17,84
8	2.000	613.800	134.000	479.800	93	3	31,37
9	4.000	1.240.000	164.000	1.076.000	374	3	124,85
10	2.700	837.000	116.200	720.800	143	6	23,99
11	2.300	694.400	81.300	613.100	158	5	31,37
12	3.000	930.000	113.000	817.000	204	4	51,05
13	4.000	1.000.240	120.500	879.740	233	5	47,37
14	3.000	930.000	81.500	848.500	192	3	63,96
15	3.800	1.162.500	100.500	1.062.000	181	4	44,90
16	3.000	930.000	90.500	839.500	190	3	63,35

The table shows variations in planned profits of tobacco growers in 2011 crop. Estimations were made with projected average price of 230 denars/kg and premium of 80 denars/kg dry tobacco. The projection was based on the data from the poll, but the factual situation still can not be determined because the purchase of tobacco from 2011 crop has not been finished yet. The planned profit ranges from 479 800 denars to 2 721 200 denars. In this case, too, high disproportions are resulting from the mode of tobacco production. The lowest profit was realized by the grower who applies labor intensive mode of production and to whom tobacco is additional source of income in the family budget, beside the incomes from other agricultural crops (cereals, garden crops, etc.).

Especially interesting is the data on planned hourly wage of the household members engaged in tobacco production. This wage is determined for tobacco grower No. 1 through the following algorithm:

Hourly wage of household members = $\frac{\text{planned profit}}{\text{totla number of hours of household members}} =$

$$= \frac{2\ 348500}{3\ 400} = \mathbf{691\ denar/hour}$$

or

691 : 4 = 172.82 denars/hour.

Since this grower has engaged four household members in production of tobacco, the hourly rate is 172.82 denars, or 2,81 EUR. per person. Considering the fact that household members are engaged 8 hours per day in production of tobacco, the daily wage per household member is

2.81 x 8 = 22.46 EUR, i.e. 1381 denars.

It can be seen in Table 4 that hourly rate per household member ranges between 12.92 and 354.24 denars. This disproportion appears as a result of the same reasons that were given for the planned profit.

CONCLUSIONS

It is undeniable fact that a great number of growers find their economic existence in production of tobacco and for others the production of this crop is additional source to supplement their family budget. Therefore, the importance of this branch of agriculture in the Republic of Macedonia will increase, which will be reflected in improvement of the social status of the poorest part of the population. Therefore, it is necessary to direct the strategic development of tobacco production toward its expansion and quality improvement and to raise it at a level of family business (farm production), which will provide employment not only for members of the household but for other persons too.

The findings from the poll lead to conclusion that tobacco production of the Republic of Macedonia is performed on small plots by individual tobacco growers or in private farms with an average area of 1.5 hectares per farmer. In the last period there was an increase of area planted with tobacco and its cultivation in a model of farm. It can be stated from the poll that 22,86% of the farmers grow tobacco on an area of about 3 hectares and four of the farmers grow this crop on an area larger than 5 hectares.

In accordance with the above data, it can be concluded that for development and establishment of tobacco production in the Republic of Macedonia, it is necessary to concentrate on varietal structure of tobacco, to create responsible partnership between tobacco growers and companies that purchase and process tobacco and to pay for purchased tobacco on time, at prices which should be economically justified and with positive reflection on producers profitability. From the above elaboration that is aimed towards increasing the production of tobacco, the need for government support to tobacco growers becomes more evident.

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Original Scientific paper

USE OF A CHLOROPHYLL METER AS A TOOL TO DIAGNOSE THE NITROGEN STATUS OF ORIENTAL TOBACCO

Radka Bozhinova[1], Hristo Filipov[2]
[1]Tobacco and Tobacco Products Institute, 4108 Markovo, Bulgaria,
e-mail: rbojinova@yahoo.com
[2]Institute of Plant Genetic Resources, Sadovo, Bulgaria

ABSTRACT

The aim of this work was to assess the usefulness of non-destructive and quick measurements of leaf chlorophyll content with a Hydro N-Tester as indicator of tobacco nitrogen status. For the purposes of the study four nitrogen rates were tested in a stationary field trial. During the vegetation period leaf chlorophyll content was measured with a portable instrument Hydro N-Tester.

Chlorophyll content in the leaves ranged from 283 to 559 HNT units and was affected by leaf measurement date and N fertilizing rates. Chlorophyll meter readings of youngest, fully formed leaves (third or fourth leaf from the top) could be used to accurately discriminate N-deficient from N-sufficient treatment. Chlorophyll meter values were highly correlated with concentration of total N in tobacco leaves and yield of cured leaves. After further testing Hydro-N-tester values can be used as an indirect method for assessing the nitrogen nutrition of tobacco at different stages of vegetation.

Key words: tobacco, chlorophyll content, Hydro N-Tester chlorophyll meter

УПОТРЕБА НА ХЛОРОФИЛМЕТАРОТ КАКО СРЕДСТВО ЗА ОДРЕДУВАЊЕ НА НИВОТО НА АЗОТ КАЈ ОРИЕНТАЛСКИОТ ТУТУН

Целта на овој труд е да се процени корисноста од недеструктивните и брзи мерења на содржината на хлорофил во листот со помош на Hydro N-Tester како индикатор на нивото на азот во тутунот. За целите на истражувањето испитувани се четири дози на азот во стациониран полски опит. За време на вегетациониот период содржината на лисниот хлорофил е мерена со подвижен рачен инструмент Hydro N-Tester.

Хлорофил во лисјата се движи од 283 до 559 HNT единици, а врз него влијаат датумот на мерење на листовите и конценрациите на азотнот ѓубре. Мерењата на најмладите, целосно оформени листови (третиот или четвртиот лист од врвот) со хлорофилметар можат да се користат за да се направи прецизна разлика помеѓу N-дефицитарните од N-суфицитарните третирања. Вредностите од хлорофилотметарот се во корелација со концентрацијата на вкупен N во тутунските листови и приносот на сушените листови. По натамошно тестирање, вредностите добиени со Hydro-N-tester можат да се користат како индиректен метод за проценка на азотното прихранување на тутунот во различните фази на вегетација.

Клучни зборови: тутун,содржина на хлорофил, Hydro N-Tester за мерење на хлорофил

INTRODUCTION

Nitrogen management, rate and time of nitrogen application are critical factors in optimizing oriental tobacco yield and quality. Although a high nitrogen supply generally increases yield, it is necessary to achieve a balance between maximum yield and optimal quality TsoT.C (1999). The value of soil tests prior to transplanting to evaluate fertilizer-N requirements for tobacco has been well established in our country. Various plant tests (assessment of leaf chlorophyll, tissue total N and NO_3-levels) during the growing season are also used to estimate tobacco N status and to make fertilizer N recommendations.

Leaf chlorophyll concentration is strongly correlated with plant N concentration, which is a good predictor of yield potential because of the association between photosynthetic activity and leaf N Evens J.R. (1983). The traditional method of measuring leaf chlorophyll concentration involves wet chemical procedures. Recently chlorophyll meters were introduced to nondestructively measure relative chlorophyll content. The chlorophyll meter offers a possible alternative technique to estimate dryland winter wheat N status and determine the need for additional N fertilizer Follet et.all.(1992). According to MacKown and Sutton and Lin et al. (2007) chlorophyll meters can be used for evaluating nitrogen nutrition of tobacco plants. Lin et al. (2007) observed that chlorophyll meter readings varied with year, location, N rate, leaf position on the stem and part of the leaf. Castelli and Contillo (2009) in pot experiment with flue-cured tobacco varieties found that SPAD values were well-correlated with both total chlorophyll and total N leaf concentration, and one leaf stalk position alone is able to monitor the N-status of the whole tobacco plant during the first six weeks after transplanting, without distinction of year and variety effects.

The aim of this work was to assess the usefulness of non-destructive and quick measurements of leaf chlorophyll content with a Hydro N-Tester (HNT) as indicator of oriental tobacco nitrogen status.

MATERIAL AND METODS

For the purposes of the study four nitrogen rates (0, 25, 50 and 100 kg ha^{-1}) were tested in a stationary field trial. A long-term fertilizer experiment with continuous tobacco cropping system was established at Tobacco and Tobacco Products Institute – Markovo, Bulgaria on rendzina soil in 1966. The experimental design was a randomized complete block replicated three times. In 2006 oriental tobacco plants (Nicotiana tabacum L. cv. Plovdiv 7) were grown in the stationary field. The plot area was 6.25 m^2 (2.5 X 2.5 m). Nitrogen was applied as urea before transplanting. Tobacco seedlings were transplanted at a 0.5 x 0.12 m distance (166 000 plants ha^{-1}). All cultural practices were in accordance with those used by the growers for oriental tobacco production. Leaves were harvested in three primings and were sun-cured.

At the beginning of the experiment, the air-dried soil had the following characteristics in the top layer: pH - 8.5, total humus - 3.01%, available P_2O_5 - 15 mg kg^{-1} soil and available K_2O - 400-500 mg kg^{-1} soil (10).

Leaf chlorophyll content was measured with a Hydro N-Tester (HNT), a portable instrument measures the light transmittances of the leaf at 650 nm and 960 nm wavelength, red and near infrared chlorophyll absorption. Measurements were carried out during the following stages: rosette (35 days after transplanting - DAT), ripeness of basal leaves (56 DAT) and ripeness of middle leaves (77 DAT) on thirty youngest fully formed leaves (third or fourth leaf from the top). At the same dates leaves were collected and tissue total N was analyzed using the Kjeldahl method.

The relationships between N rate, chlorophyll meter readings and selected dependent variables (yield of cured leaves, percentage of I^{st} grade, chlorophyll meter values and total N content) were studied by regression analysis.

RESULTS AND DISCUSSION

Yield of cured leaves was greatly affected by the rate of N application (Fig. 1). The average yield was 509 kg ha^{-1}, 733 kg ha^{-1}, 847 kg ha^{-1} and 905 kg ha^{-1} for the N_0, N_{25}, N_{50} and N_{100} treatment, respectively.

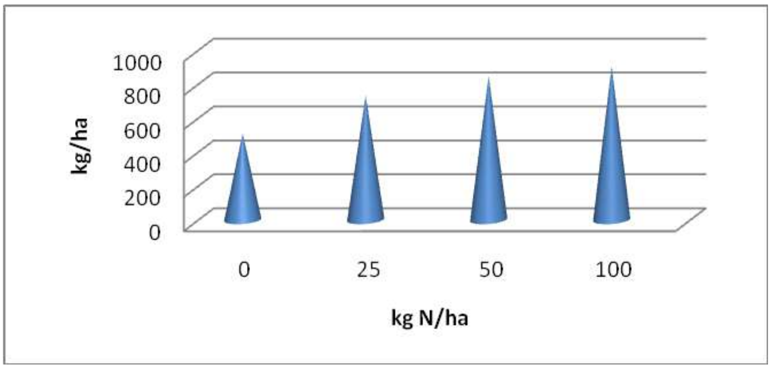

Figure 1. Effect of N rate on yield of cured leaves

The fertilizer N treatments resulted in diminishing rates of increase in leaf yield with increasing amounts of N fertilizer. The N rate of 25 kg N ha^{-1} increased cured-leaf yield by 44% compared to the unfertilized control. A further increase of nitrogen to 50 and 100 kg ha^{-1} resulted in an increase in leaf yield by 66 and 78%. The response in leaf yield (Y) to applied N (N) fitted quadratic model:

$$Y = 513.4 + 9.793N - 0.059N^2,\ R^2 = 0.997$$

Raw tobacco product quality was also influenced by N fertilizing rates (Fig. 2). The highest average percentage of I^{st} grade was obtained from the unfertilized plots. Leaf quality declined as nitrogen fertilization increased from 0 to 100 kg N ha^{-1}. The percentage of I^{st} grade was the lowest (6.3%) as a result of applying the largest nitrogen rate.

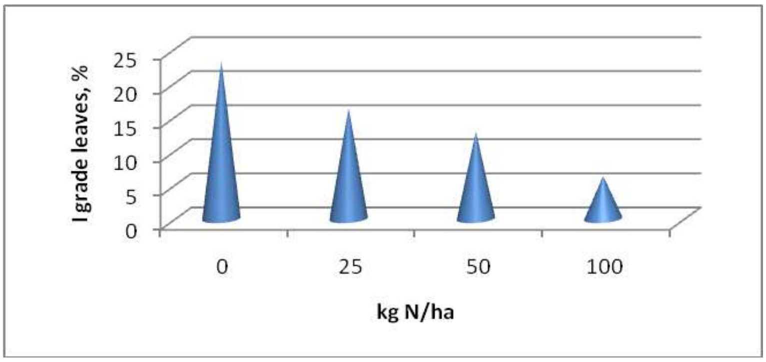

Figure 2. Effect of N rate on tobacco leaf quality

Model for linear relationships among nitrogen treatment rates (N) and changes in tobacco leaf quality (Y) was significant ($P < 0.05$):

$$Y = 21.74 - 0.16N,\ R^2 = 0.964$$

Given the above data we can point out that under our experimental climatic and soil conditions annual fertilizing with 50 kg N ha^{-1} led to obtaining relatively high yield and quality of the raw product of Oriental tobacco grown as monoculture.

Chlorophyll meter readings were in a highly significant positive correlation with total N of the tobacco leaves (Lin L. et.al. 2007). Kowalczyk-Juśko and Kościk (2002) also established a close correlation between nitrogen accumulation and chlorophyll meter readings for Virginia tobacco. Under the experimental conditions the correlation coefficients between chlorophyll meter readings and concentration of total N in tobacco leaves were 0.968, 0.818 and 0.927 for 35, 56 and 77 DAT, respectively. The correlation was statistically significant at the first date. At the subsequent stages the correlation was high but not significant.

Chlorophyll content in the tobacco leaves ranged from 283 to 559 HNT units (Table 1). Chlorophyll meter readings were the highest at the rosette stage (35 DAT). With the exception of the N_{25} treatment the lowest levels of chlorophyll meter readings were found in leaves measured at 56 DAT. Chlorophyll content was increased with an increase in nitrogen fertilizer level. Leaves from the unfertilized plots contained the least chlorophyll at all the measurement times. The average HNT values from the plots with an optimal nitrogen rate (50 kg N ha^{-1}) were from 385 to 534. Chlorophyll content in tobacco from 50 kg N ha^{-1} treatment was 534 HNT units during the rosette stage (35 DAT) when the last cultivation and application of sidedress N would be made. The values obtained from the unfertilized plots were much lower than the optimum N rate. The chlorophyll content at the 100 kg N ha^{-1} treatment ranged between 382 – 559 HNT units. Small differences in chlorophyll content were found between 25, 50 and 100 kg N ha^{-1} treatments during the first and second measurement. Greater differences between plots receiving supplemental N fertilizer were noted 77 days after transplanting.

Table 1. Impact of N fertilizing rate on chlorophyll content (in HNT units) and leaf total N concentration

Treatment kg N ha^{-1}	Chlorophyll content - HNT units			Total N, % dry weight		
	Days after transplanting			Days after transplanting		
	35	56	77	35	56	77
0	403	283	313	2.43	1.66	1.74
25	514	357	343	3.31	2.11	2.34
50	534	385	411	3.54	2.34	3.48
100	559	382	520	3.42	3.02	3.80

Regression equations describing the relationship between the N fertilizing rates and the chlorophyll meter values are presented in Table 2. Statistically significant linear dependency was noted between nitrogen treatments and chlorophyll content in the leaf tissues at 77 DAT.

Concentration of total N in tobacco leaves generally increased with increasing fertilizer N applied (Table 1). The total N concentration of the lamina tissue decreased between 35 and 56 DAT, and increased at 77 DAT. Therefore, patterns of nitrogen accumulation and chlorophyll synthesis during vegetation were similar. Kowalczyk-Juśko and Kościk (2002) reported that the highest N accumulation was observed during the first measurement and subsequent analyses showed lesser N concentration in the tobacco leaves. According to Mylonas (1984) nitrogen concentration in tobacco leaves decreased from the 4th to the 11th week after transplanting. The same author explained this pattern by the high leaf growth rate compared to that of roots for the period from the 4th to the 9th week and by diminishing available soil nitrogen in later growing season. The increased content of total nitrogen in leaves at the last measurement is likely to be related to favorable environmental condition (soil temperature and moisture) for a higher N mineralization in late growth period. There was significant linear interaction between N fertilizing rates and the concentration of N in the leaf tissues at 56 DAT (Table 2). Small differences in N concentration were found between 25, 50 and 100 kg N ha^{-1} treatments during the first measurement and the correlation coefficient was lower at this stage.

Table 2. Relationships between the N fertilizing rates and changes in the chlorophyll meter values and total N content

Dependent variables	DAT	Relationship	Correlation coefficient (r)
Chlorophyll content - HNT units	35	Y = 442.4 + 1.37x	0.852
	56	Y = 313.2 + 0.88x	0.792
	77	Y = 303.2 + 2.14x	0.994**
Total N, % dry weight	35	Y = 2.81 + 0.01x	0.712
	56	Y = 1.70 + 0.01x	0.996**
	77	Y = 1.92 + 0.02x	0.935

** Significant at 0.01 probability level

The yield response of cured leaves to HNT values measured with the chlorophyll meter was described by a linear model (Table 3). At 35 and 56 DAT there was strong significant correlation between chlorophyll meter readings and leaf yield, but there was no significant interaction between the chlorophyll meter values and leaf yield at 77 DAT. The increase by 1 HNT unit produced a rise of 1.64 to 3.60 kg ha^{-1} in yield of cured leaves. Borges et al. (1) found an increase of 11.4-20 kg ha^{-1} in wrapper yield with the rise of the SPAD value by 1 unit. Negative relationship between HNT values and raw tobacco product quality assessed on the basis of the percent of I^{st} grade was observed (Table 3).

Table 3. Relationships between chlorophyll meter readings and yield of cured leaves and tobacco leaf quality

Dependent variables	DAT	Relationship	Correlation coefficient (r)
Yield of cured leaves, kg ha^{-1}	35	Y = -509.7 + 2.50x	0.985*
	56	Y = -518.0 + 3.60x	0.978*
	77	Y = 96.3 + 1.64x	0.745
Tobacco leaf quality, percent of I^{st} grade	35	Y = 62.8 - 0.10x	0.936
	56	Y = 61.0 - 0.13x	0.889
	77	Y = 43.8 - 0.07x	0.958*

* Significant at the 0.05 probability level

CONCLUSIONS

Chlorophyll content in tobacco leaves ranged from 283 to 559 HNT units and was affected by leaf measurement date and N fertilizing rates. Chlorophyll meter readings were highly correlated with concentration of total N in tobacco leaves and yield of cured leaves. Chlorophyll meter readings of youngest, fully formed leaves (third or fourth leaf from the top) could be used to accurately separate N-deficient from N-sufficient treatment. Soil tests prior transplanting of oriental tobacco have been taken to make fertilizer N recommendations. The chlorophyll meter offers a possibility to estimate tobacco N status and determine the need for additional nitrogen fertilizer before onset of rapid growth.

Additional studies with different cultivars of tobacco, as well as in different soil and climate conditions are necessary. Consequently, after more testing, Hydro-N-tester values can be used as an indirect method for assessing the nitrogen nutrition of oriental tobacco at different stages of vegetation.

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In memoriam

Dr Iskra Hristovska

On 22.2.2013 we lost our dear colleague, senior research fellow in Department of tobacco protection from diseases, pests and weeds at Tobacco Institute-Prilep.

The news of her untimely death shook all the employees of Tobacco Institute-Prilep. We knew that she fights with a serious disease, but we hoped that she would win yet another victory in her life. Unfortunately, we lost forever a young person, full of energy.

Dr Iskra Hristovska was born on 5.3.1965 in Skopje, where she completed her elementary school and high school education. She graduated from the Faculty of Agriculture in Skopje in 1989. Throughout her education, she was a distinguished and successful student, with high average grades.

In 1989 she enrolled postgraduate studies in Genetics and selection of plants with seed production at The Faculty of Agriculture in Skopje and finished it with 9.20 average grade.

In 1990 she worked as teaching assistant at the Faculty of Agriculture – Skopje and received a scholarship from the Ministry of Science of the Republic of Macedonia.

In 1991 she took the course on genetic engineering in MANU (Macedonian Academy of Science and Arts). Together with a group of professors from the Faculty of Agriculture she was included in the project “Gene-Bank”. Her good proficiency in English allowed her to follow the contemporary foreign literature and to take participation in projects.

On 10.3.1995 she defended her master thesis entitled “Investigation of the variability of some producing and quality characteristics of soybean (*Glycine hispida Max*.) in the region of Skopje”. The same year she submitted a proposal for doctoral dissertation and in 1996 she started with the experimental part of the thesis.

In 1997 she took three months specialization on the methods for investigation of tobacco resistance to diseases at the Institute of Genetic Engineering in Kostinbrod- Bulgaria.

On 1.1.1998 she was employed at Tobacco Institute-Prilep, in Department for tobacco protection from diseases pests and weeds.

On 25.5. 1999 she defended the doctoral dissertation entitled “*Phytophthora parasitica var. nicotianae* and possible measures for its control” .

From 2004 to 2008 she was a member of the Commission on pesticides in the *Ministry of Agriculture, Forestry and Water Economy*.

She presented her research work at many scientific meetings, congresses and symposia and she is also an author of several research papers published in scientific journal *TUTUN/Tobacco*.

Dr Iskra Hristovska successfully combined her career and motherhood. She was a good colleague, open and honest person. She always had a positive attitude toward solving the working assignments. She remained optimistic until the last moments of her life.

In 1999 Dr Iskra Hristovska became our representative in Skopje, engaged on assignments related to the work of the Institute that have a big impact to tobacco economy.

With her scientific-research activity on diseases that attack tobacco, Iskra Hristovska gave a great contribution in solving the actual problems, and with her commitment she contributed to the development of tobacco science.

She became a modern researcher, but she stopped only a step before being promoted to the highest level in her profession. Death was an obstacle to her further creative work in the field of tobacco science. The most painful thing, however, is that her young life ended too early and separated her from her loved ones, from the joys of life.

Staff of the Scientific Tobacco Institute – Prilep express its deepest condolences to her family and gratitude to her contribution in the development of scientific thought and the prosperity of tobacco science.

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Since the publication is of an international character, all manuscripts should be submitted in English. Authorswhose native language is not English should have their paperschecked by research workers from the related fields who have good proficiency in the English language. All manuscripts must be proofread prior to submission. Language and style of the manuscripts are responsibility of the author.

The publication presents:original scientific papers,review articles, short reports, professional

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suggests quick publishing. They do not have to enable repetition of the experiment and examination of the presented results and can be used as a basis for further research.

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Tables should be numbered with Arabic numeralsaccording to their sequence in the text. The table title should be always above the table,centered, in 10 pt font, with one empty row between the title and the table and another one between the table and the text. Tables should be simple

and shouldnot duplicate the information given in figures.

Reference to the Table, example: It could be seen from Table 1...., or: The nicotine content in tobacco is 0.98% (Table 4).

Illustrations should be numbered consecutively in Arabic numerals, with centered titles below each of them.

All graphical presentations (including graphs, schemes, drawings, photographs etc) should be submitted on CD together with the text and saved as separate files (graphs should be prepared as Excel files -XLS extension, and schemes, drawings and images should be submitted as JPG or .TIF files). Minimum resolution for images is 200 -300 dots per inch.

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