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Original Scientific paper

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

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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 22^0C 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 25^0C 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 25^0C.
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 25^0C 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.

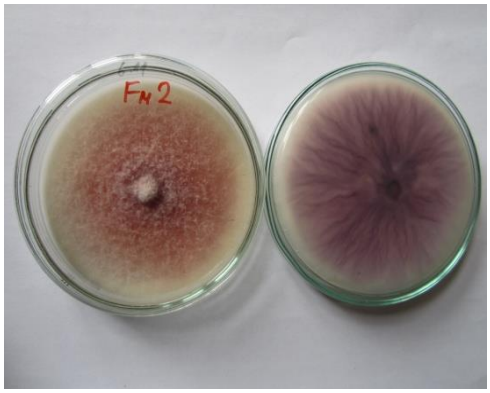

Fig. 3 *Fusarium* spp. Pure culture-isolate FM2 Fig.

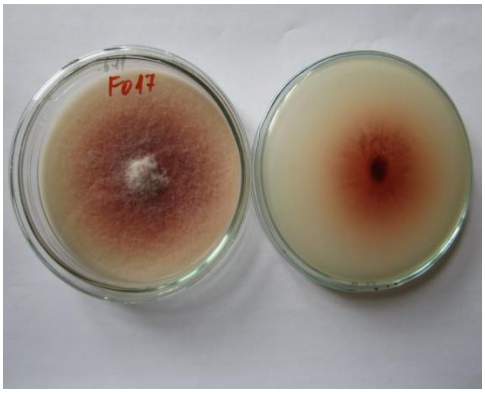

Fig. 4 *Fusarium* spp. Pure culture-isolate FO17

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

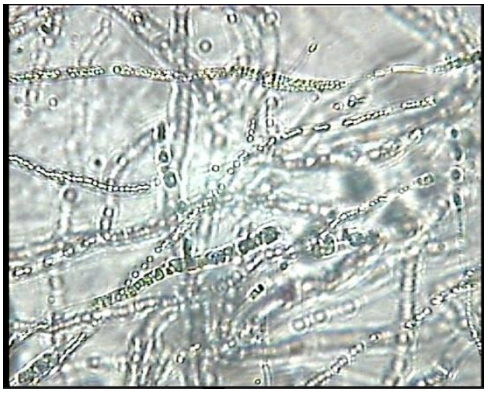

Fig.6 *Fusarium* spp. Fungal mycelia - 400x

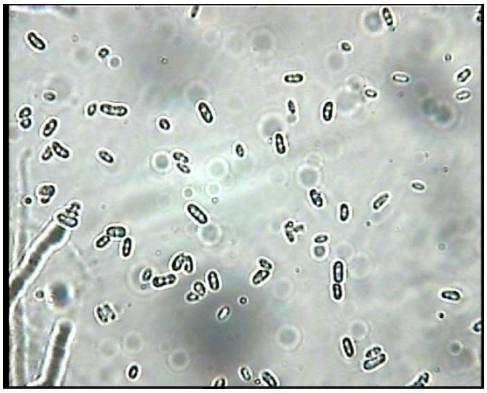

Fig.7. *Fusarium* spp. Microconidia-400x

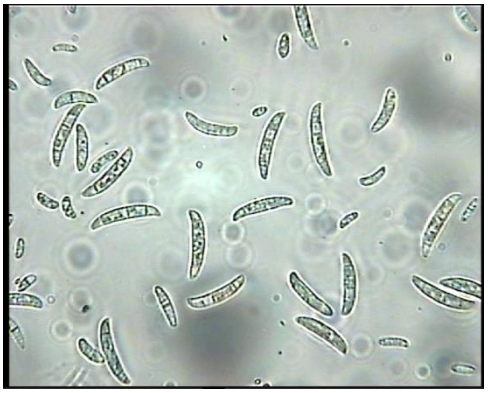

Fig.8. *Fusarium* spp. Macroconidia - 400x

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	-

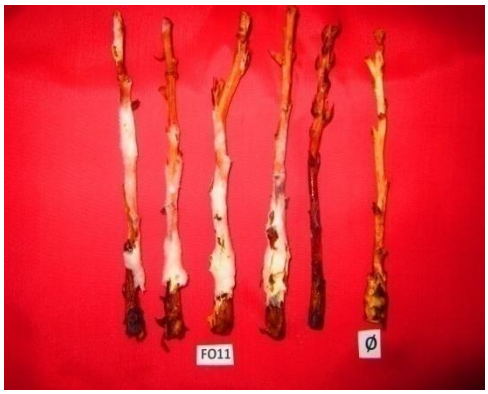

Fig. 9. *O. ramosa* - Inoculated stalks

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).

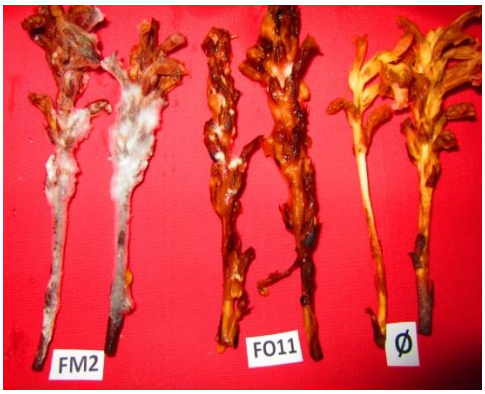

Fig. 11. *O.ramosa* - Inoculated inflorescences

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

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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 сличаен 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 пострак 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:

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

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 (cm^2)			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 II - 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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