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HETEROSIS AND COMBINING ABILITY FOR QUANTITATIVE BLUE MOULD (*PERONOSPORA TABACINA ADAM*) RESISTANCE IN TOBACCO

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Key words*: combining ability, quantitative resistance, Peronospora tabacina Adam, heterosis*

INTRODUCTION

Blue mould, caused by *Peronospora tabacina Adam*, is one of the most important fungus diseases that exist and cause serious damages to tobacco crops. The fungus has been a serious tobacco production problem in Albania since 1960. It is now present in all tobacco-growing regions.

Blue mould is a disease of seedbeds and field and can be exceedingly destructive in both, although, weather conditions largely confine it to being a field problem in Albania. It can be seen that the relatively mild and moist Albanian summer provides an excellent environment for blue mould.

Much of the oriental tobacco crop will escape serious damage in normal season because little rains are expected once the crop is planted out. Blue mould is difficult to control, particularly when environmental conditions are in its favour. On its control, cultural practices, fungicides and ***resistant cultivars*** are valuable aids to sound farming.

Resistance is graded in variety specifications and needs relating to particular disease and cropping situations. It is known that, in most types of tobacco, hybrids have been recommended for temporary situation or specific uses such as disease resistance.

Genes conditioning qualitative resistance have been intensively used in breeding of tobacco and other plants.

This has often resulted in development of virulent isolates (2, 3, 4, 7, 11, 13). Quantitative resistance introduced into cultivars with good agronomic performance offers a chance to reduce the selection pressure for virulence and to stabilize the host-pathogen system, where the level of quantitative resistance remains durable over a long period of time (2, 3, 4, 7, 9, 11, 13). This is more difficult than working with qualitative resistance. Thus, for better understanding of the genetic basis of quantitative resistance, heterosis and combining abilities were estimated and divided into their components by analyzing a diallel cross of tobacco, following Gardner and Eberhart (5).

MATERIAL AND METHODS

The experimental plants material is represented from eight selected tobacco lines with different relative levels of resistance to blue mould (*P. tabacina A*.).

The selected genotypes used as parental lines were: *Bel 61-9* (resistant), *Floria* (resistant), *Nevrokop* and *Krumovgrad* (susceptible), *Hicks-Resistant* (resistant), F_t*2-5* (resistant) and *Basma* (susceptible) (table 1). These 8 parental lines were crossed with each other giving a diallel series of crosses (28 crosses), without reciprocal crosses.

The experiment, containing 28 F_1 crosses and 8 parental lines, was arranged in a randomized block design with four replications. Experiments were conducted, for three years (2007-2009) at the experimental field of Tobacco Station of Cerrik, Albania. Plants were grown in two rows with 20 plants per plots. No fungicide effective against blue mould was applied in the seedbeds and in the field. The other cultural and curing practices used were the current ones applied in the area.

Symptoms of natural infestation of disease were observed and evaluated. Ratings were carried out upon first appearance of the pest, and further ratings were calculated at 15 days intervals. Ratings for upper, middle, and lower leaves were made separately. The scale of damage ratings was defined according to CORESTA rules defined by P. Schiltz (11).

Table1. Provenence, reaction against blue mould and tobacco varieties crossed in a diallel design

Genotype	Provenience	Reaction against blue mould
Bel 61-9	USA	resistent
Floria	Austria	resistent
Nevrokop	Bulgaria	susceptible
Krumovgrad	Bulgaria	susceptible
Samsun	Turkey	susceptible
Hicks-Rezistent	France	resistent
Ft2-5	Greece	resistent
Basma	Greece	susceptible

Data analysis

For each experiment, rating corresponding to the maximum of intensity for susceptible genotypes was taken into account in the following synthesis (Table 2, 3, 4, and 5).

The *general combining ability* (GCA) effects; the *specific combining ability* (SCA) effects and *heterosis* were the calculated parameters. The GCA effect of each line ($\mathbf{g_j}$) was calculated on the deviation of F_1s means of this variety ($\mathbf{y_j}$) from the overall mean of F_1s ($\mathbf{y_c}$), i.e: $\mathbf{g_j} = (\mathbf{p} - 1)/(\mathbf{p}-2)(\mathbf{y_j} - \mathbf{y_c})$; where, **p** is the number of homozygous lines/parents. These parameters were computed following Gardner and Eberhard (5) method II and Griffing (6).

For each combination the SCA effect was obtained by calculating the deviation between expected F_1 values (on the basis of GCA effects only) and observed F_1 performance, i.e: $\mathbf{S_{ij}} = \mathbf{y_{ij}} - \mathbf{y_c} - \mathbf{g_i} - \mathbf{g_j}$ where $\mathbf{y_{ij}}$ is the observed value of the F_1 between lines **i** and **j**.

Taking into account the values of the parental lines ($\mathbf{y_{jj}}$), heterosis was calculated and divided into *average heterosis* ($\mathbf{h_m} = \mathbf{y_c} - \mathbf{y_p}$); *variety heterosis* ($\mathbf{h_j} = \mathbf{g_j} - ½(\mathbf{y_{jj}} - \mathbf{y_p})$; and *specific heterosis* (corresponds to SCA) as proposed by Gardner and Eberhard. $\mathbf{y_p}$ is the mean of the parents. The difference between $\mathbf{y_{jj}}$ and $\mathbf{y_p}$ is the *variety effect* ($\mathbf{v_j}$) of cultivar **j**. For the analysis of variance, the fixed effects model was applied.

RESULTS AND DISCUSSION

Compatible host reaction of parents and F_1s occurred and leaf symptoms of disease were formed on all genotypes. ANOVA analysis revealed the presence of an important variability in the experimental materials, and significant quantitative differences of resistance between all genotypes were found. Mean squares for parents and hybrids were highly significant (at the P_{001} level of the probability) (table 2). In addition, the contribution of genotypes on total variance is very high ($\mathbf{R^2}$ = 0.9705). In figure 1 is given the distribution of the expected and observed values (mid parent/F_1 resistance) around the regression line.

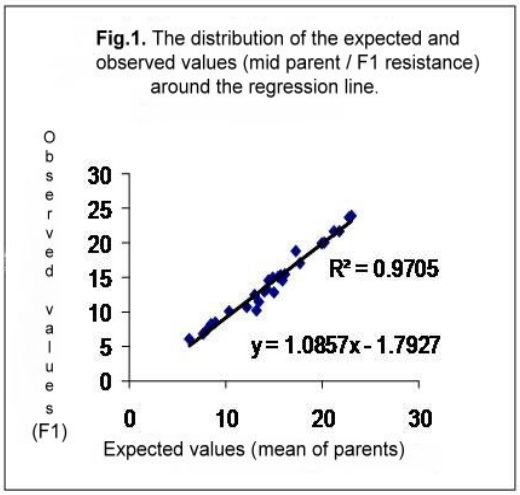

Fig.1. The distribution of the expected and observed values (mid parent / F1 resistance) around the regression line.

The distribution of the values (midparent/F_1 resistance) around the regression line ($\mathbf{y}$ = 1.0857x – 1.7927) proved that the observed quantitative resistance are heritable as shown in figure 1. The position of the values influenced by *Bel 61-9*, (the values ranged in low on the left of the regression line), proved that dominance for resistance occurred in crosses of this variety, whereas dominance for susceptibility occurred in crosses of *Samsoun* variety (the values ranged in upper position on the right of regression line).

In other crosses, expected heterosis was less expressed. The regression of F_1 on midparent for all crosses was 0.88721 (standard error). In our study, significant GCA effects ($\mathbf{g_j}$) were found whereas SCA effects were significant only in some individual crosses (Table 5).

Significant GCA effects ($\mathbf{g_j}$) and large values of variance ratio of additive and non-additive variances (GCA/SCA) proved that additive genetic variance is a more important component in the inheritance of *''quantitative resistance''* character (Tables 3, 4). Our results were similar to those reported by other authors (1,3,4,7,8,11,13) that have in other host-pathogen system found high values for additive gene action and where the most gene action among loci was additive (9, 11, 12, 1,2).

Table 2. Analysis of variance for 8 tobacco cultivars and 28 F_1s infected by Blue mould (*P. tabacina Adam*) (Means of three years)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-values
Genotypes	4304,1236	35	122,9749	136,06**
Hybrids	2779,5058	27	103,1302	114,11**
Parents	1524,6178	7	217,8025	240,98**
Blocks	6,7703	3	2,2567	2,4969
Residual	94,9036	105	0,9038 = (Me)	
Total	4405,7975	143		

Table 3. Analysis of variance for GCA effects and SCA effects (specific heterosis), average Heterosis (h_m), variety heterosis ($\mathbf{h_j}$) and variety effects ($\mathbf{v_j}$)

Source of variation	Sum of squares	Degrees of freedom	Mean square (Ms)	F-values
GCA	1058,2748	7	151,1821	Ms/Mé = 668,95**
SCA(specific heterosis)	19,0807	28	0,6815	" " = 3,01**
Average heterosis ($\mathbf{h_m}$)	233,9335	1	233,9335	Ms/Me = 247,77**
Variety effect ($\mathbf{v_j}$)	1524,6144	7	217,8020	" " = 240,98**
Variety heterosis ($\mathbf{h_j}$)	20,1051	7	2,8721	" " = 3,18**
Residual	94,9036	105	0,9038 (Me)	

(Mé = Me/nb; where, **nb** → number of blocks = 4)

Table 4. *Quantitative Blue mould resistance* of eight tobacco cultivars ($\mathbf{y_{jj}}$), *variety effects* ($\mathbf{v_j}$), *variety heterosis* ($\mathbf{h_j}$) and F_1s means according varieties ($\mathbf{y_j}$) and GCA effects ($\mathbf{g_j}$)

No	**Varieties**	$\mathbf{y_{ii}}$	Range	$\mathbf{v_i}$	$\mathbf{h_i}$	$\mathbf{y_i}$	$\mathbf{g_i^*}$	$\mathbf{g_i^{**}}$
1	*Bel 61-9*	5,50	**a**	-9,45**	-1,145**	9,40	-5,88**	-5,41**
2	*Floria*	10,00	**c**	-4,95**	0,495*	12,74	-2,12**	-2,17**
3	*Nevrokop*	21,00	**e**	6,05**	-0,125	16,93	2,90**	2,95**
4	*Krumovgrad*	21,50	**e**	6,55**	0,145	17,37	3,42**	3,36**
5	*Samsun*	24,62	**f**	9,67**	0,565*	19,07	5,40**	5,17**
6	*Hicks-Rezistent*	7,12	**b**	-7,83**	-0,125	10,98	-4,04**	-3,99**
7	*F_t2-5*	10,87	**c**	-4,08**	-0,080	12,62	-1,98**	-2,08**
8	*Basma*	19,00	**d**	4,05**	0,245	16,39	2,27**	2,17**
	$LSD_{0,05}$			1,30	0,416		0.333	0,333
	$LSD_{0,01}$			1,76	0,566		0,492	0,492

Note 1: $\mathbf{g_j^*}$ is calculated following Gardner & Eberhart (5) and $\mathbf{g_j^{**}}$ according to Griffing (6).
Note 2: The variety values ($\mathbf{y_{jj}}$) followed by the same letter are not significantly different by Duncan's multiple range test (P=5%).

Significant of SCA effects ($\mathbf{S_{ij}}$) in some individual crosses proved that, in particular crosses, the *specific heterosis* plays an evident role in the inheritance of ''*resistance*'' character. Marani and Sachs (9), Jinks (8) and Matzinger et al (10) found high values for additive and dominance variance, and where dominance effects became greater in the adult plants stages (9). Several published results showed that dominance and epistatic effects occurred despite additive effects (1, 2, 3, 4, 8, 10, 11).

The data of F1s and parents were combined to perform Analysis II as proposed by Gardner and Eberhard (1966). Significance of variety heterosis ($\mathbf{h_j}$), variety effects ($\mathbf{v_j}$), GCA effects ($\mathbf{g_j}$) and parents were obtained too, and significant average heterosis ($\mathbf{h_m}$) was also obtained but its effect was small. Analysis of data for GCA components ($\mathbf{g_j = h_j + ½ v_j}$) show that, significant differences, among eight parental lines for $\mathbf{g_j}$, $\mathbf{h_j}$ and $\mathbf{v_j}$ were found (see tables 3, 4). In table 4, the relation between the quantitative resistances of varieties ($\mathbf{y_{jj}}$) and variety effects ($\mathbf{v_j}$), GCA effects ($\mathbf{g_j}$) and variety heterosis is given. No significant relation exists between $\mathbf{y_{jj}}$ and $\mathbf{h_j}$, and significance relation exists between $\mathbf{y_{jj}}$ and $\mathbf{g_j}$.

Our results, similar to those reported by Bulmer (1), proved that this correlation might also be negative.

This means that if parental value attempts is higher, the potential value of heterosis attempts is lower (1, 8, 9). The ranking of the varieties according to their GCA effects calculated according Gardner and Eberhard (5) and Griffing (6) was similar, and the ranking of hosts according to their pure line performance ($\mathbf{y_{jj}}$) corresponds to that resulting from GCA effects ($\mathbf{g_j}$) (see table 4). Nevertheless, it becomes evident that a great part of the observed variation in GCA ($\mathbf{g_j}$) was conditioned by varieties effects ($\mathbf{v_j}$). By using homozygous varieties (i.e. when $\mathbf{d_j}$

= 0) these variety effects (contain additive $\mathbf{a_j}$ gene action) are representing the contribution of homozygous loci to the $\mathbf{j^{th}}$ variety mean (6,8).

Such effects can be used by breeding pure lines and, since differences exist, selection for improved quantitative blue mould resistance may be effective (1, 6, 8, 12).

In our study, the differences between F_1 and parent means were significant in a great part of individual crosses. Expressed in percentage of heterosis, the *average heterosis* for all *Bel 61-9* crosses was -13.7%; for *Krumovgrad* crosses -0.83% and for *Samsoun* crosses it was -2.87%; but the observed difference $(\mathbf{y_c - y_p})$ calculated for all data combined was -0.513.

Table 5. Values of SCA effects ($\mathbf{S_{ij}}$)

	2	3	4	5	6	7	8
1	0.29	-1,21**	-0,48	-1,09*	1,58**	1,06*	2,08**
2		-0,24	-0,51	1,01*	-0,17	-0,22	2,05**
3			0,99*	1,01*	-0,30	-0,60	2,55**
4				0,74	-0,45	-0,24	2,13**
5					-0,68	-0,60	1,80**
6						0,22	1,99**
7							2,57**

($LSD_{0,05}$ = 0.88 and $LSD_{0,01}$ = 1.19)

Summarising the data presented and the published results (1, 2, 3, 4, 5, 7, 8, 9, 12), it becomes evident that the predominance of additive effects is very common in *host-pathogen* systems. Among the fixed set of parents analysed, *Bel 61-9* and *Hick-Resistant* are the best for further crosses and for improvement of quantitative blue mould (*P. tabacina*) resistance in tobacco.

ABSTRACT

In this study, the estimation of heterosis and the combining abilities for quantitative resistance against Blue Mould (*Peronospora tabacina Adam*) of eight oriental tobacco cultivars are presented. For this purpose, a half-diallel cross and its parents were arranged in four replications of a randomized block design. Symptoms of natural infestation of disease were observed and evaluated acording to CORESTA-methodology, during three years.

From the data presented on the combining ability and heterosis for quantitative Blue mould (Peronospora tabacina Adam) resistance in oriental tobacco, the following statements might be drawn:

- Significant general combining ability (GCA) was found, whereas the specific combining ability (SCA) was significant only in some individual crosses, and a great part of the general combining ability could be explained by *variety effects*. Significant *variety heterosis* was obtained too, and significant *average heterosis* was also obtained, but its effect was small.
- Among those selected for this study, "*Bel 61-9''* and ‘’*Hicks- Rezistent*" were the best for further crosses for tobacco resistance against *P. tabacina Adam*.

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