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RECENT DEVELOPMENTS IN CONTROL OF MAIZE DISEASES THROUGH BREEDING

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ABSTRACT

Within the maize breeding program, 63 inbreds and 62 maize hybrids were tested for resistance to leaf, ear and stalk diseases. Leaves were infected with now dominant race 2 of *Exserohilum turcicum*, which is a result of a wide and continued usage of the Ht1 gene. Line resistance to *E. turcicum* ranged between 0,5 and 4,5. Early hybrids from maturity group 100 and 200 were more resistant in comparison with those from groups 300 and 400, in which a wide range of estimates, from 1,3 to 4,4, was obtained.

Although *Gibberella zeae*, causing ear rot can appear sporadically in the world, it deserves attention because of the mycotoxins it produces. A large number of lines and hybrids was tested using for the first time a new Canadian method of artificial ear infection into the silk channel. Estimates obtained by a 1-7 scale varied from 1,0 to 5,1 for the lines and from 1,4 to 4,4 for the hybrids. Early hybrids proved to be resistant, wherease in groups 300, 400, 600, and 700 distinct differences in resistance were obtained.

Because of the growing maize production in monoculture, *Colletotrichum graminicola* is spreading as a cause of anthracnose stalk rot. Resistance of inbred lines was estimated based on outer stalk discoloration (1-9), and it ranged between 1,3 and 8,0. The most susceptible hybrids were those in group 100 and 200 with 15,8 to 83,4 % of rotted plants.

As a result of breeding for resistance, new versions of the early lines, F2 and Bc 252, resistant to anthracnose stalk rot have been obtained.

Key words: anthracnose stalk rot, breeding for resistance, gibberella ear rot, hybrids, lines, maize, methods of artificial infection

IZVLEČEK

SEDANJI RAZVOJ PRI ZATIRANJU KORUZNIH BOLEZNI Z ŽLAHTNJENJEM

V okviru programa žlahtnjenja koruze so testirali 63 inbridnih linij in 62 koruznih hibridov za odpornost proti boleznim listja, stebla in storža. Liste so okužili z zdaj dominantno raso 2 povzročiteljice koruzne progavosti (*Exserohilum turcicum*), ki je posledica obsežne in neprekinjene uporabe gena Ht 1. Odpornost linij proti *E. turcicum* je nihala med 0,5 do 4,5. Zgodnji hibridi iz zrelostnih razredov 100 in 200 so bili bolj odporni v primerjavi z zrelostnima razredoma 300 in 400, v katerih je bilo doseženih veliko ocen med 1,3 do 4,4.

Čeprav gliva *Gibberella zeae* le sporadično povzroča trohnobo koruznega storža po svetu, se ji vseeno namenja pozornost zaradi mikotoksinov, ki jih tvori. Prvič so testirali veliko število linij in hibridov z novo kanadsko metodo za umetno okuževanje v svilnati kanal. Po skali od 1 do 7 so ocene nihale od 1,0 do 5,1 pri linijah in od 1,4 do 4,4 pri hibridih. Zgodnji hibridi so bili odporni, medtem ko so bile v razredih 300, 400, 600 in 700 ugotovljene izrazite razlike v odpornosti.

Zaradi pridelovanja koruze v monokulturi, se gliva *Colletotrichum graminicola* širi kot povzročiteljica trohnobe in s tem poleganja stebla. Odpornost inbridnih linij so ocenili po zunanjem razbarvanju

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stebel po skali od 1 do 9 in je nihala med 1,3 do 8,0. Najbolj občutljivi hibridi so bili v razredih 100 in 200 z 15,8 do 83,4 strohnelih rastlin.
Kot rezultat žlahtnjenja na odpornost so dobili nove verzije zgodnjih linij, F2 in Bc 252, ki so odporne proti trohnobi stebla.

Ključne besede: antraknozna trohnoba stebla, žlahtnjenje na odpornost, fuzarijska trohnoba storža, hibridi, linije, koruza, metode umetnega okuževanja

1 INTRODUCTION

Breeding for resistance is the most effective and ecologically the most justifiable way of maize diseases control. It involves several parts. Disease monitoring is used to observe the incidence and severity of infection, as well as possible occurrence of new diseases or pathotypes. Effective methods of artificial infection and disease severity rating scales are being developed. By testing a large number of maize inbreds resistant germplasm is identified, which is then incorporated into the adapted elite lines through breeding process. The knowledge of disease resistance inheritance plays an important role in it. The changes taking place in practice, such as assortment, plant density, fertilizer rates, cultural practices or irrigation can contribute to disease development. Therefore, breeding for resistance presents a continuing process.

Northern leaf blight in maize caused by *Exserohilum turcicum* (Pass) Leonard D. Suggs. Syn. *Helminthosporium turcicum* Pass is potentially the most important leaf disease in maize in more humid areas of growing. Following the discovery of monogenic resistance (Hooker, 1961), Ht 1gene was widely used, however, fifteen years later a new pathotype (race 2) was spread in the US, to which this gene was not effective (Turner, Johnson, 1980). Race 2 was found in Croatia in 1994 (Palaveršić, Lendler, 1996) and has become prevalent today, which is a results of a wide and continued exploitation of the Ht 1 gene (Palaveršić *et al.*, 1998).

In the world, gibberella ear rot, caused by *Fusarium graminearum*, the asexual state of *Gibberella zeae* (Schw.) can occur sporadically and only locally from year to year, without reducing yield. However, because of lower grain quality due to mycotoxins zearalenone and deoxynivalenol, produced by this pathogen, it is of considerable concern to livestock producers, particularly pig producers (Mirocha and Christensen, 1974, Prelusky *et al.*, 1994).

A new technique of artificial ear infection has recently been developed in Canada. It consists of injecting a *Fusarium graminearum* macroconidial spore suspension into the silk channel of primary ears (Reid *et al.*, 1992). The fungi of the genus *Fusarium* are the most common cause of maize stalk rot. Because of the increasing maize production in monoculture, higher incidence of anthracnose stalk rot has been reported. The cause of stalk anthracnose, *Colletotrichum graminicola* (Ces.) G. W. Wils., is a stronger parasite in comparison with *Fusarium graminearum*. However, overwintering of the fungus in the soil is limited and anthracnose therefore occurs in continuous cropping to maize (Vizvary, Warren 1982).

Artificial stalk infection with *C. graminicola* can considerably increase number of rotted and broken plants in susceptible genotypes (Milatović *et al.*, 1983, Brekalo 1991). Successfulness in anthracnose stalk rot resistance improvement of two (susceptible) early lines was also evaluated. The aim of this study was to test 63 maize lines and 62 hybrids of different maturity for resistance to artificial leaf, ear, and stalk infection. Resistance degree of the lines and hybrids were determined, which would allow to choose resistant lines for new breeding programs, as well as those susceptible which need improvement. At the same time, it was an opportunity to check the used methods of artificial infection and to see to what extent are resistant hybrids available to maize growers in control of these diseases.

2 MATERIALS AND METHODS

Testing lines and hybrids for resistance

Mostly Bc lines and hybrids were tested. Public lines and hybrids developed by other seed companies were used as standards:

	Trial		Entries
1.	Early lines		13
2.	Mid-late lines		29
3.	Late lines		21
4.	Hybrids FAO 100		6
5.		FAO 200	10
6.		FAO 300	10
7.		FAO 400	16
8.		FAO 500	10
9.		FAO 600-700	10

Trials with lines and hybrids were planted in three nurseries with artificial infection applied:

Et. R2 - *Exserohilum turcicum* - race 2 - nursery

Each genotype was planted in one row and two replications. Artificial infection with spore suspension was used, obtained from pure isolate culture 6/97. At 7-8 leaves, first infection was made, followed by the second one after one week into the leaf whorl of the very susceptible hybrid Minnesota 706, which was used as a spreader (Špehar, Palaveršić 1969). Ratings were taken 3 and 4 weeks after flowering using a 0,5-5 scale by Elliot, 1946.

F.g. - *Fusarium graminearum* nursery

Inoculum of *F. graminearum* was produced using a modified Bilay's liquid medium consisting of the following: 2 g KH_2OP_4, 2 g KNO_3,1 g $MgSO_4$, 1g KCl, 0,2 ppm $FeCl_3$, 0,2 ppm $MnSO_4$, 0,2 ppm $ZnSO_4$ and 1 g dextrose in 1 l of water. The fungus was grown in 250 ml erlenmayer flaks, containing 50 ml of the medium. After sterilization in an autoclave, a piece of agar was added containing pure culture of one of the four isolates of *F. graminearum* (74-2/97, 94/97, 99/97, 117/97), isolated from the infected ears collected from the previous vegetation. The culture was shaken for 1 hr at 4 hr intervals for 8 days under natural light supplemented with flourescent light. After the four isolates were mixed together, the mixture was diluted to a concentration of 5×10^5 conidia/ml.

Twenty plants were grown in each plot in three replications. Ten primary ears from center plants were infected by injecting 2 ml of condia suspension into the silk channel using a pistol-grip syringe (an 18-gauge Luer-lock - stainless steel hypodermic). Inoculations are best if done approximately 6 days after silk emergence, i.e. when slight browning is observed. Infection severities were rated at harvest (in October) using a scale consisting of 7 classes: 1 = no infection, 2 = 1-3 %, 3= 4-10 %, 4 = 11-25 %, 5 = 26-50 %, 6 = 51-75 % and 7 = > 75 % of infected kernels (*Reid et al.*, 1966).

Cg - *Colletotrichum graminicola* nursery

A mixture of five isolates (46/97, 68/97, 70/97, 72/97, 73/97) of the fungus isolated from leaves or stalks was used as inoculum. Oatmeal agar (Tuite, 1969) allowed to use conidia suspension of 1-2 x 10^6 conidia/ml. Inoculation of stalks was made with a pistol-grip syringe delivering 1 ml of the inoculum into the first elongated internode 7 ± 1 day after 50 % silking. A stainless steel needle with 3 mm in diameter and two side openings was used. All plants in the row were infected, in three replications. In trials with lines, outer stalk discoloration was rated (1-9), and in those with hybrids, percentage of rotted plants was determined by squeezing the first elongated internode between the thumb and forefinger (Brekalo 1991).

Evaluation of successfulness in breeding for resistance to anthracnose

Trial 1. Two maize hybrids, FAO 200 (an old version and an improved version) were tested for resistance to anthracnose stalk rot under artificial stalk infection with *C. graminicola*.

Trial 2. Two improved and two original maize hybrids were also tested for grain yield under natural conditions.

3 RESULTS AND DISCUSSION

Considerable differences were obtained in resistance levels of maize lines and hybrids to *E. turcicum* race 2, with the exception of the hybrids from FAO 100 (Table 1). Line resistance ranges from 0,5 to 4,5. The early hybrids from FAO 100 and 200 expressed higher resistance in comparison with those from groups 300 and 400, in which the range of estimated varied between 1,3 and 4,4. Resistant type of reaction was not found in the lines and hybrids tested, which is an indication that stable polygenic resistance is used by the breeders. Most of the tested lines possess medium resistance to race 2 of *E. turcicum*. There are more resistant lines than susceptible (Fig. 1).

Table 1: Average, highest and lowest resistance estimates for maize lines and hybrids to race 2 of *Exserohilum turcicum* in field trials - Rugvica 1998

Trial	n	*E. turcicum* (0,5-5) R2			range	LSD 5 %
		x	min.	max.		
LINES						
1. Early	13	2,5	0,9	4,5	3,6	0,6
2. Medium	29	2,4	1,1	3,8	2,7	0,7
3. Late	21	2,0	0,5	4,5	4,0	0,5
HYBRIDS						
4. FAO 100	6	2,1	1,0	2,9	1,9	NS
5. FAO 200	10	2,2	1,3	3,0	1,7	0,7
6. FAO 300	10	2,8	1,3	4,1	2,8	1,0
7. FAO 400	16	3,0	1,3	4,4	3,1	0,7
8. FAO 500	10	2,0	0,8	3,6	2,8	0,7
9. FAO 600-700	10	2,1	0,6	3,4	2,8	0,7

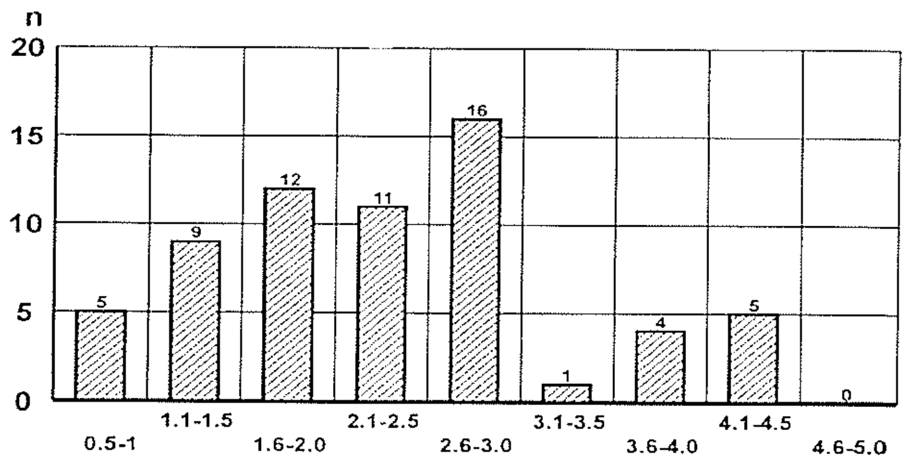

Figure 1: Frequency distribution for *E. turcicum* (race 2) ratings (0.5-5), for 63 maize inbreds, Rugvica 1998

First application of the Canadian technique of ear infection with *F. graminearum* into the silk channel gave good results, although dry weather did not favour infection and the irrigation, which is recommended, was not used (Table 2). Efforts were made to follow the method closely (Reid *et al.*, 1996).

Table 2: Temperatures and rainfalls for July and August, Rugvica 1998

10 day period	Mean temperatures (°C)	Total rainfall (mm)
July		
I	19,2	65,3
II	20,9	17,3
III	23,6	31,3
August		
I	23,8	0,8
II	22,4	0,0
III	17,1	69,6

In trials with lines, considerable differences in resistance degree among the tested lines were obtained (Table 3). Significant differences in resistance to ear rot were found among the tested hybrids from FAO 300, 400, 500, and 600-700. However, in groups 100 and 200, where least ranges in resistance were found, the differences were not significant (Table 3). The ranges of average estimates for the lines were between 1,0 and 5,1, and for hybrids between 1,4 and 4,4.

Table 3: Average, highest and lowest resistance estimates for maize lines and hybrids to gibberella ear rot in field trials - Rugvica 1998

Trial	n	*F. graminearum* (1-7)			range	LSD 5%
		x	min.	max.		
LINES						
1. Early	13	1,8	1,0	3,2	2,2	1,2
2. Medium	29	2,6	1,2	4,9	3,7	1,3
3. Late	21	3,2	1,3	5,1	3,8	1,1
HYBRIDS						
4. FAO 100	6	1,6	1,4	1,9	0,6	NS
5. FAO 200	10	2,0	1,4	2,7	1,3	NS
6. FAO 300	10	2,7	1,8	3,9	2,1	0,7
7. FAO 400	16	2,8	2,0	4,4	2,4	0,9
8. FAO 500	10	2,6	1,9	3,2	1,3	0,9
9. FAO 600-700	10	2,9	1,8	4,2	2,4	1,1

Early hybrids proved as resistant, and in groups 300, 400 and 600-700, clear difference in resistance degree were found. During and after the inoculation of early hybrids, there was a period of dry weather, whereas in the third decade of August, there was abundant rainfall, which is probably why the inoculation was more sucessful in later hybrids (Table 2). This deficiency can be eliminated with irrigation or by many-year testing. Resistant lines to ear rot were prevailing, and only several were very susceptible (Fig. 2). The advantage of the inoculation with conidial suspension into the silk channel over the method with toothsticks overgrown with the fungus (Sutton, Baliko, 1981), which had been in use until then, is that no toothpicks are required and there is no risk that they will be displaced as silks elongate. The inoculation failed only in one line because it had short husks and no silk channel.

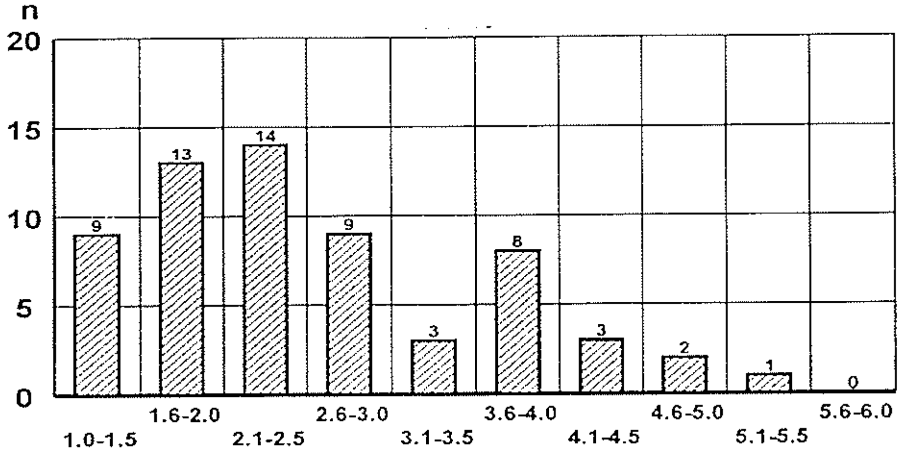

Figure 2: Frequency distribution for *Gibberella* ear rot ratings (1-7) for 62 maize inbreds, Rugvica 1998

When evaluating genotypes for resistance to gibberella ear rot, one needs to consider also kernel infection, i.e. adequate kernel inoculation technique (Reid, Hamilton, 1996). Maize producers and especially pig producers are recommended to observe the occurrence of Fusarium ear rot and avoid to use susceptible hybrids.

Significant differences in resistance levels to stalk anthracnose were obtained for lines and hybrids with the exception of the hybrids from FAO 100 and 500 (Table 4). The most susceptible were early lines and some early hybrids from FAO 100 and 200. The lowest average severities were found in hybrids from FAO 300. In later hybrids, average severities were higher, but the range was smaller. The line Bc 10 was used for improving resistance of the susceptible early lines, Bc 252 and F2. Table 5 gives the results of resistance to stalk anthracnose for the original hybrids and those with improved resistance. The hybrid (2), Bc 706-9 x Bc 10252 and (4), Bc 406/405 x Bc 102 appeared to be significantly more resistant to stalk anthracnose in comparison with the original hybrids 1 and 3. The hybrids with improved resistance also produced higher yields by 24,3 and 6,7 % respectively, but, unfortunately, with higher moisture by 2,6 % and 0,6 % (Table 6). What is especially important is that resistance to natural infection and Fusarium stalk rot, appears to be improved, i.e. percent of rotted plants dropped from 32,4 and 46,2 to only 0,8 and 2,2 % respectively.

Because breeding for resistance is a complex trait and largely influenced by the environment, it is recommended to use large seggregating populations in pedigree selection in order to achieve even slight improvements (Bauman, 1977). By inoculating stalks with *C. graminicola* (breeding for resistance to anthracnose), resistance to Fusarium stalk rot is also improved, which is its advantage. When growing maize in monoculture and with short rotation, it is important to choose the hybrids resistant to anthracnose.

Table 4: Average, highest and lowest resistance estimates for maize lines and hybrids to anthracnose stalk rot in field trials - Rugvica 1998

Trial	n	*C. graminicola* (1-9)			range	LSD
		x	min.	max.		
LINES						
1. Early	13	6,1	3,9	8,0	4,1	1,3
2. Medium	29	4,7	1,8	7,5	5,7	1,3
3. Late	21	3,5	1,3	5,4	4,1	1,7
HYBRIDS		Rotted plants, %				
4. FAO 100	6	46,7	14,8	70,6	55,8	-
5. FAO 200	10	42,7	16,3	83,4	67,1	-
6. FAO 300	10	33,7	20,4	65,4	45,0	-
7. FAO 400	16	41,4	18,5	65,6	47,1	-
8. FAO 500	10	43,2	23,3	71,1	47,8	-
9. FAO 600-700	10	54,4	38,1	76,4	38,3	-
		Transformed data - arc.			sin x	
4. FAO 100	6	41,8	22,4	55,5	33,1	NS
5. FAO 200	10	39,9	19,8	66,6	46,8	16,1
6. FAO 300	10	35,0	25,5	54,6	29,1	14,9
7. FAO 400	16	39,6	24,9	54,7	29,8	13,2
8. FAO 500	10	41,1	25,8	58,3	32,5	NS
9. FAO 600-700	10	47,6	37,8	61,4	23,6	9,9

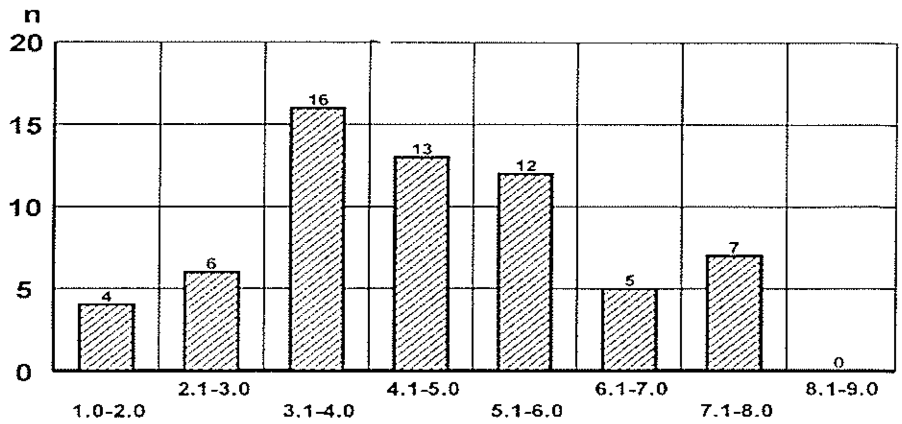

Figure 3: Frequency distribution for antracnose stalk rot ratings (1-9) for 63 maize inbreds, Rugvica 1998

Table 5: Results of testing maize hybrids for resistance to anthracnose stalk rot, Rugvica 1998

Hybrids	Stalk breakage %		Stalk rot %	
	C. graminicola infection	Check	*C. graminicola* infection	Check
1. Bc 706-9 x Bc 252 ST-1	47,6	30,8	61,5	13,5
2. Bc 706-9 x Bc 10252	17,7	24,2	16,3 **	0
3. Rx 406/405 x F2 ST-2	16,1	31,0	51,9	14,6
4. Rx 406/405 x Bc 102	31,2	13,7	14,0 **	8,7
x	28,2	24,9	35,9	9,2

** significantly more resistant to stalk rot in comparison with the standard

Table 6: Grain yield, grain moisture, percent of broken and rotted plants in a trial in Rugvica, 1998

Hybrid	Grain yield at 14 % moisture, dt/ha	Harvest grain moisture, %	Stalk breakage %	Stalk rot %
1. Bc 706-9 x Bc 252 ST-1	73,29	23,13	22,3	32,4
2. Bc 706-9 x Bc 10252	91,11	25,77	5,2	0,8 **
3. Rx 406/405 x F2 ST-2	77,00	24,67	5,3	46,2
4. Rx 406/405 x Bc 102	82,21	25,27	12,3	2,2 **

** significantly more resistant to stalk rot in comparison with the standard

4 CONCLUSIONS

The applied techniques of artificial infection significantly differentiated the tested lines and hybrids by their resistance degree to Northern Leaf Blight, gibberella ear rot and stalk anthracnose.

In control of the above three diseases, maize growers are offered a good selection of resistant hybrids.

Resistance of the early lines, Bc 252 and F2, to anthracnose stalk rot has been successfully improved.

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