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A NEW METHOD FOR THE EVALUATION OF THE RESISTANCE OF MAIZE HYBRIDS TO FUSARIAL EAR ROT – THE TOXIN-MOULD-INDEX (TMI)

Árpád BATA* – Pál RAFAI** – Gábor KOVÁCS*** – Anna HALÁSZ****

*Department of Biochemistry and Food Technology,
Technical University of Budapest
H–1502 Budapest, Pf. 91
**Department of Animal Hygiene,
University of Veterinary Sciences, Budapest
***Plant Selection Institute,
Szentes, Hungary
****Central Research Institute of Food Industry,
Budapest, Hungary

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Abstract

30 maize hybrids grown in Hungary representing the groups FAO 200 – 299, FAO 300 – 399, FAO 400 – 499, and FAO 500 – were studied in order to gain information about genotypic resistance to *Fusarium* moulds. The plants grown on an experimental farm were artificially infected using the tooth pick method with *F. graminearum* and *F. oxysporum* resp. In addition maize grain meals were also inoculated with isolates of moulds. The surface area of ears covered by moulds 9 weeks after inoculation and the toxin content (zearalenone and T-2 toxin) of infected maize grain meals were measured.

Big differences were observed in the mould- covered area of the ear surface (2.00 to 38.88%) and also in the zearalenone content (2.73 to 80.33 mg per kg) and T-2 toxin content (5.37 to 52.33 mg per kg) between genotypes. Poor correlation was found between the mould-covered area and the toxin content measured in the maize grain meal of the same genotypes.

For characterization of resistance resp. susceptibility of different genotypes, a new method was introduced using the *toxin-mould-index (TMI)* taking in to consideration both the growth rate of moulds and their toxin production. In every FAO group resistant genotypes were found confirming the view that not only the duration of the vegetation period influences the resistance or susceptibility.

Keywords: maize, ear rot, Fusaria, resistance, toxin-mould-index.

Introduction

In regions of moderate climate the infection of maize by *Fusarium* moulds occurs relatively often, particularly in rainy autumns. Generally *F. graminearum, F. culmorum* and *F. sporotrichoides* are present in the infected maize plants. Both *Fusarium* ear rot and *Fusarium* stalk rot occur under Hungarian conditions, but in the experiments described in this paper only the ear rot was studied in detail. Due to the fact that the contamination of maize with mycotoxins (first at all with zearalenone and T-2 toxin) produced by *Fusarium* moulds may cause significant losses in animal husbandry, the possible ways of prevention of mycotoxicoses are intensively studied in many countries including Hungary. One of the possible ways of prevention of mycotoxicoses may be the growing of resistant feed grains.

Experiments carried out so far (MESTERHÁZY – KOVÁCS 1986, CULLEN et al. 1983, CHIANG et al. 1987, KING – SCOTT 1981, SCOTT – KING 1984) have shown differences in the response of maize hybrids to*Fusarium* infection, suggesting that some of the hybrids may have resistance against fusarial stalk and ear rot. It was also suggested that the possible resistance is connected both with genetic and agrotechnical factors.

Although abundant data have been published owing to differences in evaluation and different pathogenicity even of the isolates of the same strain, further investigations are needed to improve prevention of the mycotoxicoses by better breeding and selection methods as well as with changes in agrotechnique. The aim of the experiments to be reported in this paper was to study the genetically determined resistance of maize hybrids grown in Hungary to most common *Fusarium* species.

Materials and Methods

Materials

30 hybrids grown in Hungary were studied in 1993 and 1994. The hybrids provided by IKR Company (Bábolna, Hungary) represented four vegetation periods (FAO 200 – 299, FAO 300 – 399, FAO 400 – 499, FAO 500 –). The field experiments were carried out at the Experimental Farm of the AGROSELECT Company for Plant Breeding and Trade in Szarvas (Hungary). In addition, laboratory experiments were also performed with maize meals.

Pairs of isolates of *F. graminearum* and *F. culmorum* of different pathogenicities were used in experiments for artificial inoculation of maize plants and maize grain meals, respectively.

Methods

Random block design with three replications for every maize hybrid was used for the growing of maize hybrids and the generally known agrotechnology was applied. For artificial infection the tooth pick method was used. The inoculum of *Fusarium* strains was transferred into the middle of ears 10 – 12 days after female flowering of the plants (under Hungarian conditions July 26 – August 6). The evaluation of the fungal damage of the ears was performed 9 weeks after artificial infection. The basis of the evaluation was the proportion of ear surface covered by moulds.

In laboratory experiments 50 grams of grain were ground. The meal was then mixed with 50 ml of distilled water and sterilized at 105°C for two hours. On the following day the sterilization process was repeated under the same conditions. The sterilized mixture, containing about 22 – 25% water was inoculated with mycelia of *F. graminearum* and *F. Oxysporum* respectively. The moulds had been grown on Czapek agar medium. The final inoculum contained 100.000 to 1000.000 microbes per gram.

The inoculated substrate was incubated for 7 days at 20 – 22°C, then for another 7 days at a temperature of 4°C and finally it was again incubated for 7 days at 20 – 22°C in a thermostat. After incubation, the material was sterilized at 150°C for 2 hours and this procedure was repeated 24 hours later under the same conditions.

The zearalenone and T-2 toxin content of the materials were determined by capillary GC as described by BATA et al. (1983).

Evaluation of the Tolerance

Comparing the results obtained by means of the foregoing two methods, poor correlation was found between the proportion of mould-covered surface of maize ears and the quantity of T-2 toxin and zearalenone in the infected maize grain meals. Therefore in addition to the

- mould-covered surface proportion of the maize ear,
- zearalenone content of the infected maize grain meal, and
- T-2 toxin contrent of the infected maize grain meal,

the so called *Toxin-Mould-Index (TMI)* was also calculated by multiplying the sum of mycotoxin contents, expressed in mg per kg, by the proportion of mould-covered surface of maize ear expressed as per cent of the total surface

$$\mathrm{TMI} = (\mathrm{Z} + \mathrm{T\text{-}2}) \cdot \mathrm{A}$$

where Z – zearalenone content of maize meal,
T-2 – toxin (T-2 toxin) content of the maize meal,
A – proportion of the mould covered area of ears in %-s of the total areas of ears.

Based on TMI values, the hybrids were classified as follows

- highly tolerant hybrids: TMI lower than 200,
- moderately tolerant hybrids: TMI 200 – 400,
- hybrids sensitive to *Fusarium* infection: TMI over 400.

Results and Discussion

Toxin Content of Infected Maize Grain Meals

The toxin ciontents of infected maize meals studied are summarized in *Table 1.* As it is shown, low T-2 toxin content (lower than 18 mg per kg) was found in hybrids No. 27, 19, 18, 4, 12, and 7. Only two hybrids were classified as moderately tolerant (19 to 28 mkg per kg), and all others as sensitive to infection by *Fusaria.*

Low zearalenone (under 9 mg per kg) is characteristic of hybrids No. 16, 4, 30, 23, 24, and 5. Moderate toxin content (10 to 30 mg per kg) was found in the meals of hybrids No. 2, 18, 3, 7, 12, 19, and 22. Other hybrids may be classified as sensitive to *Fusarium* infection based on the zearalenone content. If the sum of two toxins is considered, the tolerant group (under 28 mg per kg) is as follows: No. 1, 8, 9, 10, 21, 28, and 29. Hihhest values viz. high sensitivity was observed for hybrids No. 13, 14, 14, 23, 6, 24, 15, 26, 25, and 17.

Growth of Moulds on Infected Ears

The proportions surface area of ears covered by moulds 9 weeks after artificial infection of maize plants grown in experimental fields is shown in *Table 2.* Lowest values (2.0 – 3.1%) were found in the case of hybrids No. 2, 1, 3, all belonging to the FAO 200 – 299 group and the highest ones (26.5 – 38.8%) on the ears of hybrids No. 25, and 28, but also on those of No. 15 which is a relatively early ripening hybrid (FAO 300 – 399).

Toxin-mould-index

As it may be seen by comparing the data in *Tables 1* and *2,* the correlation between the results of field and laboratory experiments is relatively poor. To find a better evaluation of tolerance of the hybrids which is more suitable for practical purposes from the point of view of feeding, we introduced a

Table 1
Toxin content of mould-infected maize grain meals (mg/kg)

Hybrid	T-2 toxin	Zearalenone	T-2 + zearalenone
1.	23.20	4.20	27.40
2.	34.70	11.70	46.40
3.	31.70	19.50	51.20
4.	8.60	6.80	15.40
5.	47.80	8.20	56.00
6.	50.10	41.30	91.40
7.	18.00	19.50	37.50
8.	48.20	31.15	79.35
9.	35.40	29.50	63.90
10.	30.30	53.17	83.47
11.	37.60	70.80	108.40
12.	11.50	20.70	32.20
13.	18.80	55.20	74.00
14.	31.70	44.40	76.10
15.	104.00	71.20	175.20
16.	41.60	4.20	45.80
17.	122.50	62.30	184.80
18.	11.40	13.50	24.90
19.	3.00	24.70	27.70
20.	27.60	31.45	59.05
21.	65.10	60.21	125.31
22.	30.90	26.30	57.20
23.	79.90	8.00	87.90
24.	90.80	8.20	99.00
25.	89.50	38.30	137.80
26.	72.40	42.20	114.60
27.	1.60	33.50	35.10
28.	66.20	44.13	100.33
29.	72.40	37.19	109.59
30.	21.40	7.40	28.80

new value, the *toxin-mould-index (TMI)* calculated as shown in section Materials and Methods. The TMI data are shown in *Table 3.*

If we look at the group with the lowest TMI (hybrids No. 4, 2, 18, 7, 19, and 3) it can be stated that the hybrids belong not only to the FAO 200 – 299 group, but also to the FAO 300 – 399 group. None of FAO 400 – 499 or FAO 500 – group hybrids is present in the low TMI group. The group of the highest TMI values (hybrids No. 16, 11, 23, 17, 22, 5, 26, and 25) concerning FAO groups is more heterogeneous (only FAO 200 – 299 hybrids are missing).

Table 2

Proportion of mould-covered surface area of the ears infected by Fusarium moulds

Hybrid	Area %	Hybrid	Area %
2.	2.00	8.	7.63
1.	2.18	23.	7.78
3.	3.10	21.	8.60
7.	3.15	12.	8.68
6.	3.28	10.	9.20
24.	3.88	27.	10.18
13.	4.20	20.	10.75
18.	4.53	16.	12.20
14.	4.83	29.	18.04
4.	5.15	22.	24.30
19.	5.25	5.	25.25
11.	5.63	26.	26.50
17.	6.08	25.	29.83
9.	6.58	28.	31.15
30.	7.53	15.	38.88

The results obtained in the experiments confirm the views of MESTERHÁZY (1974, 1978) and KOVÁCS et al. (1988) suggesting that although full resistance does not exist significant differences may be found between different hybrids.

The effect of duration of the vegetation period on the susceptibility is doubtful, nevertheless it is important to note that in every FAO group there are genotypes being much less susceptible than the average of the group, e. g. hybrid No. 4 in the FAO 300 – 399 group, No. 24 in the FAO 400 – 499 group, and No. 30 in the FAO 500 – group.

If, in addition, we consider the investigations by different authors (BOLING – GROGAN 1965, ODIEMAH – MANNINGER 1982, CHIANG et al. 1987) showing that the resistance to ear rot is inherited in a partially dominant way, there exists a real genetical basis for improving the resistance of maize.

Contradictory data were published concerning the methodology of the determination of resistance. ATLIN et al. (1983) are of the opinion that the study of only one *Fusarium* strain is enough for the determination of the susceptibility. Oppositely, NAIK et al. (1978), CULLEN et al. (1983), NAGY et al. (1988) have found differences in growth and toxin production of different strains after artificial infection of field-grown ears maize. Our experience shows that the two strains (*F. graminearum* and *F. culmorum*) most commmonly occurring in Hungary and other countries of moderate climate (TANAKA et al., 1988) are very similar in their action and the

Table 3
Toxin-mould-index (TMI) of maize hybrids

Hybrid	Index	FAO-group
1.	59.73	
2.	92.80	200 – 299
3.	158.72	
4.	79.31	
5.	1414.00	
6.	299.79	
7.	118.13	
8.	605.44	
9.	420.46	
10.	767.43	
11.	610.29	300 – 399
12.	279.50	
13.	310.80	
14.	367.56	
15.	6797.76	
16.	558.76	
17.	1123.58	
18.	112.80	
19.	145.43	
20.	1231.95	
21.	1077.66	
22.	1389.96	400 – 499
23.	683.86	
24.	384.12	
25.	3812.27	
26.	3036.90	
27.	357.32	500 –
28.	3146.35	
29.	1977.00	
30.	216.86	

differences are small and generally not significant. In every case use of more strains may improve the reliability of the conclusions.

Summarizing the results it could be stated that although very early ripening hybrids (FAO 200 – 299 group) generally seem to be more resistant to *Fusarium* infection, and late ripening hybrids seem to be more sensitive, in every group occur hybrids being more resistant than other members of the group. These differences between genotypes may probably be used in breeding practice to select less sensitive hybrids.

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