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429

Incidence and Levels of Fumonisin Contamination in Maize Genotypes Grown in Europe and Africa

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The natural occurrence of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) has been investigated in 26 maize inbred lines grown in Italy and in 72 maize hybrids grown in Croatia (19), Poland (7), Portugal (9), Romania (6), Benin (9), and Zambia (20). The incidence and levels of fumonisin contamination resulted in two major groups of countries. The first with high contamination included Italy, Portugal, Zambia, and Benin, with incidence of 100, 100, 100, and 82%, and fumonisin (FB_1 + FB_2) levels up to 2850, 4450, 1710, and 3310 ng/g, respectively. The second group, including Croatia, Poland, and Romania, showed very low levels of contamination ($\leq$70 ng/g) with 50% incidence of positive samples. A general trend for higher contamination levels was observed in maize genotypes with higher FAO maturity class or dent-type endosperm. Although the environmental conditions of the specific area of cultivation seem to play a role in the formation of fumonisin in maize, further investigations are needed to thoroughly establish the genotype–area–season interaction.

Keywords: *Fumonisin; Fusarium; maize genotypes; Europe; Africa*

INTRODUCTION

Fumonisins are a group of recently discovered mycotoxins produced primarily by the fungus *Fusarium moniliforme* Sheldon (Gelderblom *et al.*, 1988; Bezuidenhout *et al.*, 1988), one of the most prevalent seed-borne fungi of maize (Booth, 1971). Fumonisins can contaminate maize destined for animal feeding or human consumption as a result of the fungal invasion before and after harvest. Of the six structurally related metabolites (FB_1, FB_2, FB_3, FB_4, FA_1, and FA_2) isolated from cultures of *F. moniliforme* (Bezuidenhout *et al.*, 1988; Cawood *et al.*, 1991), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 are the major compounds usually present both in maize fungal cultures and in naturally contaminated maize (Cawood *et al.*, 1991; Hopmans and Murphy, 1993; Visconti and Doko, 1994; Doko and Visconti, 1994).

Toxicological investigations of *F. moniliforme* cultures or naturally infected maize revealed that fumonisins, particularly FB_1 and in some extent FB_2, are the causal agents of several animal diseases. FB_1, in purified form or combined with FB_2 in fungal cultures and in naturally contaminated maize or maize-based feeds, has been shown to cause equine leukoencephalomalacia (ELEM), porcine pulmonary edema (PPE), and rat liver cancer, and it has been associated with several field outbreaks of mycotoxicoses (Marasas *et al.*, 1988; Harrison *et al.*, 1990; Kellerman *et al.*, 1990; Gelderblom *et al.*, 1992; Ross *et al.*, 1990, 1992; Thiel *et al.*, 1991b, 1992; Wilson *et al.*, 1990; Caramelli *et al.*, 1993). In particular, maize screenings have often been implicated in field cases of ELEM and PPE (Ross *et al.*, 1990, 1992; Wilson *et al.*, 1990; Caramelli *et al.*, 1993) and have been shown to contain some of the highest fumonisin levels

in animal feeds, reaching FB_1 levels as high as 360 µg/g (Plattner *et al.*, 1991; Stack and Eppley, 1992; Ross *et al.*, 1992; Murphy *et al.*, 1993). These high concentrations of fumonisins in naturally contaminated maize are certainly of concern, particularly if compared with the much lower levels of other mycotoxins, such as aflatoxin, zearalenone, or T-2 toxin, that can occur in feedstuffs (despite the relatively higher toxicity of these mycotoxins). The widespread infection of maize kernels by *F. moniliforme* and the subsequent fumonisin contamination of maize and maize-based products for human consumption has become a worldwide chronic phenomenon, which raises serious concerns for human health. Moldy home-grown maize intended for human consumption has been associated with the high incidence of esophageal cancer in southern Africa (Sydenham *et al.*, 1990; Thiel *et al.*, 1992). In northeastern Italy, where polenta (food derived from maize flour) is a major staple food, an increasing risk of developing human esophageal cancer with increasing consumption of maize has been reported (Franceschi *et al.*, 1990). Furthermore, the presence of fumonisins has recently been revealed in maize and maize-based foods marketed in several countries worldwide (Sydenham *et al.*, 1991; Pittet *et al.*, 1992; Stack and Eppley, 1992; Hopmans and Murphy, 1993; Doko and Visconti, 1994).

The recent concern for naturally occurring fumonisins in maize and maize products and the possible consequences for human and animal health prompted the current study of screening for the fumonisin content in different maize genotypes that varied mainly in the geographic area of cultivation. Specific characteristics of the maize genotypes (inbred lines and hybrids), such as endosperm and FAO maturity class, were considered as possible factors influencing the accumulation of fumonisins in maize grains.

EXPERIMENTAL PROCEDURES

Maize Genotypes. A total of 98 samples of maize genotypes (26 maize inbred lines and 72 maize hybrids) grown in various European and African countries were screened for

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Table 1. Characteristics and Fumonisin (FB₁ and FB₂) Levels in Maize Inbred Lines Grown in Italy and in Maize Hybrids Grown in Portugal

maize genotype	crop year	endosperm characteristics	maturity class (FAO)	fumonisin concn (ng/g)[a]		
				FB₁	FB₂	FB₁ + FB₂
Italy						
W 153R	1989	dent	300	230	20	250
W 182R	1989	dent	300	10	nd	10
A 660	1990	dent	400	680	160	840
H 99	1989	dent	500	60	nd	60
OH 43	1990	dent	500	70	10	80
W 22	1991	dent	500	1250	200	1450
H 95	1990	dent	500–600	40	nd	40
Mo 17	1989	semident	500–600	40	nd	40
K 55	1990	dent	600	40	nd	40
Ky 226	1990	dent	600	1960	400	2360
LO 876	1990	dent	600	40	nd	40
R 805	1990	dent	600	690	180	870
33–16	1990	dent	600–700	30	nd	30
C 103	1990	semident	600–700	150	30	180
B 14	1990	dent	600–700	2330	520	2850
B 37	1990	dent	600–700	130	20	150
B 73	1990	dent	600–700	930	200	1130
B 84	1990	dent	600–700	120	20	140
B 87	1990	dent	600–700	50	nd	50
B 89	1990	dent	600–700	90	20	110
FR 27	1990	dent	600–700	30	nd	30
Pa 884	1989	dent	600–700	810	80	890
Va 60	1990	dent	600–700	30	nd	30
Va 85	1989	dent	600–700	30	nd	30
K 816	1990	dent	700	50	nd	50
TX 325	1990	dent	700	40	nd	40
Portugal[b]						
AIOA-Pioneer			500	2300	690	2990
CONSTANZA-Pioneer			600	2070	850	2920
ATRIX-Coop.do Pau			600	90	nd	90
AGENCIA-Coop.do Pau			600	1660	620	2280
CORKI-Coop.do Pau			700	670	250	920
G-4507-Sapec			700	860	320	1180
DRACMA-Sapec			660	180	840	
PRISMA-Sapec			3370	1080	4450	
XL-75-A-Dekalb			1300	370	1670	

[a] nd = not detected, <10 ng/g. [b] Maize hybrid samples of 1992 crop.

their FB₁ and FB₂ contents. None of the lines and hybrids from one country were represented in any other country. All cultivars from one country were grown in the same environment in the specified year. The 26 maize inbreds, cultivated in a breeding station of northern Italy during the years 1989, 1990, and 1991, were obtained from the Department of Genetics, University of Milan (Dr. E. Pé). The 72 maize hybrids all originated from the 1992 crop in the following countries: 9 from Portugal obtained from Agricultural High School of Coimbra; 7 from Poland obtained from the Department of Plant Pathology, Agricultural University, Warsaw (Prof. J. Chelkowski); 19 from Croatia obtained from the Institute for Breeding and Production of Field Crops, Zagreb (Dr. B. Palaversic); 6 from Romania obtained from the Research Institute for Cereals and Industrial Crops, Fundulea (Dr. M. Ittu); 11 from Benin, 9 of which obtained from the Agricultural Research Station of INA, Parakou, and 2 from a local commercial source; and 20 from Zambia supplied by Dr. L. D. Ristanovic, Golden Valley Regional Research Station, Chisamba. All of the above maize samples appeared healthy (no evident grain damage) and homogeneous. Specific characteristics relevant to endosperm (dent, flint–dent, flint, high lysine) and vegetative cycle (FAO maturity class) of most tested inbreds and hybrids are reported in Tables 1–3. Fumonisins (FB₁ and FB₂) analyses were performed as described below, based mainly on the method of Shephard et al. (1990).

Extraction and Cleanup. Sample size varied from 0.5 to 2.0 kg (except samples from Poland, <200 g). Subsamples of about 200 g were finely ground in a Buehler laboratory mill and thoroughly mixed. Aliquots (25 g) of the ground subsamples were blended with 50 mL of methanol/water (3:1) for 3 min and filtered through Whatman no. 4. While maintaining the flow rate below 2 mL/min, 10 mL of the filtered extract was applied to a Bond-Elut strong anion exchange (SAX) cartridge (Varian, Harbor City, CA) fitted to a Supelco solid-phase extraction (SPE) manifold (Supelco, Bellefonte, PA), previously conditioned by the successive passage of methanol (5 mL) and methanol/water (3:1, 5 mL). The cartridge was then washed with methanol/water (3:1, 8 mL) followed by methanol (3 mL), and fumonisins were eluted with 0.5% acetic acid in methanol (14 mL). The eluate was evaporated to dryness at 40 °C, under a moderate stream of nitrogen, and stored dry at −18 °C until HPLC analysis.

HPLC Analysis. The residue after cleanup was redissolved in 200 μL of acetonitrile/water (1:1). An aliquot (50 μL) of this solution was derivatized with 200 μL of o-phthaldialdehyde (OPA) solution obtained by adding 5 mL of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol to 1 mL of methanol containing 40 mg of OPA. The fumonisin OPA derivatives (10 μL of solution) were analyzed using a reversed-phase HPLC/fluorescence detection system. The HPLC system consisted of a 2150 LKB pump (Bromma, Sweden) connected to a Perkin-Elmer MPF-44 B spectrofluorometric detector and a Perkin-Elmer LCI-100 integrator (Perkin-Elmer, Norwalk, CT). Chromatographic separations were performed on a stainless steel Spherisorb ODS 2, C-18 reverse-phase column (15 cm × 4.6 mm i.d., 5-μm particle size; Phase Preparations Ltd., Deeside Clwyd, U.K.) connected to a Nova-Pak C18 precolumn cartridge (Waters, Milford, MA). Methanol/0.1 M sodium dihydrogen phosphate (75:25) solution adjusted to pH 3.35 with orthophosphoric acid was used as mobile phase, at a flow rate of 1.5 mL/min. Fluorescence of the fumonisin OPA derivatives was recorded at excitation and emission wavelengths of 335 and 440 nm, respectively. Fumonisins quantification was performed by peak height measurements and comparing with a reference standard solution. The latter was

Fumonisin Contamination in Maize Genotypes

J. Agric. Food Chem., Vol. 43, No. 2, 1995 **431**

Table 2. Characteristics and Fumonisin (FB₁ and FB₂) Levels in Maize Hybrids Grown in Croatia, Poland, and Romania in 1992

maize genotype	endosperm characteristics	maturity class (FAO)	FB_1	FB_2	$FB_1 + FB_2$
		Croatia			
Bc 9201	flint−dent	100	10	nd	10
Bc 9202	dent	100	10	nd	10
Bc 9203	flint−dent	200	10	nd	10
Bc 9204	dent	200	nd	nd	nd
Bc 9205	dent	300	10	nd	10
Bc 9206	dent	300	10	nd	10
Bc 9207	dent	300	10	nd	10
Bc 9208	dent	300	nd	nd	nd
Bc 9209	dent	300	nd	nd	nd
Bc 9211	dent	400	20	10	30
Bc 9212	dent	400	nd	nd	nd
Bc 9213	dent	400	40	10	50
Bc 9215	dent	400	nd	nd	nd
Bc 9216	dent	400	nd	nd	nd
Bc 9218	dent	400	nd	nd	nd
Bc 9219	dent	400	60	10	70
Bc 9210	flint−dent	400	10	10	20
Bc 9214	dent	500	20	nd	20
Bc 9217	dent	600	nd	nd	nd
		Poland			
Zenit	flint−dent	210	nd	nd	nd
KLG 2210	flint	230	10	nd	10
Betulisa	flint (HL)[b]	240	nd	nd	nd
RAH BE 90102	flint (HL)[b]	240	nd	nd	nd
RAH BE 86101	flint (HL)[b]	240	nd	nd	nd
Smolimag	flint	240	nd	nd	nd
Ruten	flint−dent	240	20	10	30
		Romania			
Fundulea 2671-92	dent−flint	230	nd	nd	nd
Fundulea 2727-92	dent	260	20	10	30
Fundulea 2659-92	dent−flint	580	nd	nd	nd
Fundulea 2639-92	dent	630	10	nd	10
Fundulea 2643-92	dent	630	10	nd	10
Fundulea 2603-92	flint	630	nd	nd	nd

[a] nd = not detected, <10 ng/g. [b] HL = high lysine.

obtained by dissolving pure FB_1 and FB_2 (CSIR, Division of Food Science and Technology, Pretoria, South Africa) in acetonitrile/water (1:1), at concentrations of 100 and 50 μg/mL, respectively. Appropriate dilutions of standards and/or sample extracts were made with acetonitrile/water (1:1). The limit of detection of the analytical method was 10 ng/g for both toxins.

Statistics. The GraphPAD software InStat (Sigma, St. Louis, MO) was used to determine differences between maize genotypes from individual countries or groups of countries by the Kruskal−Wallis one-way analysis of variance (nonparametric ANOVA test), followed by the Dunn's multiple comparison test. Linear regression analysis was performed for correlation between FB_1 and FB_2 in contaminated samples, and between fumonisin content and FAO maturity class in maize inbreds and hybrids.

RESULTS AND DISCUSSION

Individual results of the FB_1 and FB_2 analysis in the examined maize inbreds and hybrids are reported in Tables 1−3, together with sum of fumonisins (FB_1 + FB_2), crop year, endosperm characteristics, and FAO maturity class. The overall data have been divided into three groups of geographic areas representing Western European countries, i.e. Italy and Portugal (Table 1), Central European countries, i.e. Croatia, Poland, and Romania (Table 2), and African countries, i.e. Benin and Zambia (Table 3).

All genotypes from Western Europe contained fumonisins (FB_1 + FB_2) at levels ranging from 10 to 2850 ng/g in maize inbred lines grown in Italy and from 90 to 4450 ng/g in maize hybrids grown in Portugal (Table

Table 3. Characteristics and Fumonisin (FB₁ and FB₂) Levels in Maize Hybrids Grown in Benin and Zambia in 1992

maize genotype	maturity class (FAO)	FB_1	FB_2	$FB_1 + FB_2$
	Benin			
TZ ESR W	400−500	30	nd	30
DMR ESR W	400−500	450	80	530
POOL 16 SR	400−500	160	30	190
ACR 87 POOL 16 SR	400−500	nd	nd	nd
EV 8443 SR	600−700	20	nd	20
EV 8422 SR	600−700	540	110	650
TZB SR	600−700	160	20	180
TZB SR SE	600−700	2630	680	3310
OKOMOSA	600−700	nd	nd	nd
unknown A[b]		280	60	340
unknown B[b]		280	50	330
	Zambia			
MM 501	−	1420	290	1710
MM 502		260	50	310
MM 504		590	150	740
MM 601		165	30	195
MM 603		190	50	240
MM 604		70	20	90
MM 612		120	20	140
MM 752		95	30	125
MM 501-4		80	20	100
MM 509		80	20	100
MM 505		40	nd	40
MM 605		130	30	160
MM 602-2		60	10	70
MM 601-4		80	20	100
MM 705		45	10	55
MM 609		20	nd	20
MM 701-1		40	10	50
MM 2519		40	nd	40
MM 608		40	nd	40
MM 701		50	nd	50

[a] nd = not detected, <10 ng/g. [b] From local commercial source.

1). The level of fumonisin contamination in Portugal was higher than in Italy, with mean concentrations determined at 1930 and 450 ng/g, and medians at 1670 and 70 ng/g, respectively (Table 4).

The screening of maize hybrids from the three countries of Central Europe, i.e. Croatia, Poland, and Romania, indicated definitely lower incidence of contamination (50%) and toxin levels (Table 2). The latter varied from 10 to 70 ng/g fumonisins (FB_1 + FB_2), with no samples from Romania and Poland exceeding 30 ng/g. The median of fumonisin concentration in maize samples from these three countries was calculated at 5 ng/g, which is below the detection limit.

In maize hybrids from Benin and Zambia (Table 3), the fumonisin levels ranged from 0 to 3310 ng/g and from 20 to 1710 ng/g, with mean fumonisin content of positive samples at 700 and 220 ng/g, and medians at 190 and 100 ng/g, respectively (Table 4). The incidence of toxin contamination in these two countries was 82% and 100%, respectively.

The Kruskal−Wallis analysis of variance indicated a significant variation among countries and groups of countries. The multiple comparison test showed extremely significant difference ($P < 0.001$) in fumonisin contents between genotypes from Central Europe (Croatia, Poland, and Romania) and Western Europe (Italy and Portugal) and between Central Europe and Africa (Benin and Zambia), whereas no significant difference ($P > 0.05$) was observed between samples from Western Europe and Africa.

The occurrence levels and the distribution pattern of fumonisins (FB_1 + FB_2) in the overall maize genotypes

Table 4. Occurrence and Distribution of Fumonisins (FB_1 + FB_2) in Maize Genotypes Grown in Europe and Africa

country	occurrence of fumonisin positives[a]	fumonisins (FB_1 + FB_2) (ng/g)			no. of positive samples		
		range	median	mean of positives	<100	100−1000	>1000 (ng/g)
		High-Occurrence Areas					
Benin	9/11 (82)	0−3310	190	700	2	6	1
Italy	26/26 (100)	10−2850	70	450	14	8	4
Portugal	9/9 (100)	90−4450	1670	1930	1	2	6
Zambia	20/20 (100)	20−1710	100	220	9	10	1
total	**64/66 (97)**	**0−4450**	**130**	**610**	**26**	**26**	**12**
		Low-Occurrence Areas					
Croatia	11/19 (58)	0−70	10	20	11	0	0
Poland	2/7 (29)	0−30	0	20	2	0	0
Romania	3/6 (50)	0−30	5	20	3	0	0
total	**16/32 (50)**	**0−70**	**5**	**20**	**16**	**0**	**0**

[a] Percent incidence is reported in parentheses.

considered herein are reported in Table 4, from which two clear-cut groups of areas can be inferred. The first with high toxin incidence and contamination levels, including Benin, Italy, Portugal, and Zambia, and the second with low toxin incidence and contamination levels, including Croatia, Poland, and Romania (the P value for populations of these two groups of countries was <0.001, extremely significant). The distribution of positive samples from high-occurrence areas among groups with different contamination levels, resulted in the following: 41% (26/64), 41% (26/64), and 18% (12/64) at fumonisins (FB_1 + FB_2) levels lower than 100 ng/g, from 100 to 1000 ng/g, and above 1000 ng/g, respectively. None of the fumonisin-positive maize samples from low-occurrence areas (16/32) contained more than 100 ng/g fumonisins.

In spite of the absence of fumonisins in high lysine genotypes, the available data (only three samples from Poland, see Table 2) were not enough to conclude that high lysine endosperm prevents the formation of fumonisin.

Almost all samples from Italy (24/26) derive from dent-type endosperm, and the great variability of fumonisins content among them seemed to indicate a lack of influence by this characteristics in respect to the formation of fumonisins. However, when considering the overall samples from Italy, Croatia, Poland, and Romania (for which endosperm characteristics were known) a trend could be observed indicating low fumonisin contamination in flint-type endosperm (only 1/6 positive at very low level, 10 ng/g), compared to intermediate contamination in flint−dent or semident endosperm (6/9 positive, mean-positive concentration 50 ng/g), and high contamination in dent endosperm (34/43 positive, mean-positive concentration 290 ng/g).

No correlation was observed between fumonisin content and genotype's vegetative cycle within each country. However, when considering the overall data, a trend could be observed leading to higher contamination by increasing the length of the vegetative cycle. In particular, the linear regression analysis of fumonisin content vs FAO maturity class showed a slope significantly different from zero (P < 0.01), with a correlation coefficient of 0.3146. This finding suggests a positive correlation between fumonisin contamination and time of exposure of the maize kernels to fungal infection.

The correlation between FB_1 and FB_2 was determined for individual countries and for all countries of the high-occurrence areas. Remarkedly, the correlation coefficient was very close to 1 for all tests, and the slope (FB_2/FB_1) varied from 0.21 for Italy and Zambia to 0.33 for Portugal (0.29 average for all countries). These data

are in agreement with previous reports relevant to both naturally contaminated maize-based feeds or foods and maize cultures of fumonisin-producing Fusarium isolates, indicating that FB_2 accounts for about 20−35% of the FB_1 content in the same sample (Murphy et al., 1993; Caramelli et al., 1993; Visconti and Doko, 1994; Doko and Visconti, 1994).

Surveys on Fusarium species and their mycotoxins carried out in western European countries, such as Italy, France, and Spain, have revealed F. moniliforme as the most frequently isolated fungal species from maize in the field as well as in commercial maize and maize-based commodities (Logrieco and Bottalico, 1988; Bottalico et al., 1989, Rapior et al., 1993, Cabanes et al., 1993). In addition, F. moniliforme isolated from maize and maize-based feeds in the same countries yielded (when cultured on sterilized maize) high amounts of fumonisins at levels reaching 4.1 mg/g of FB_1 and 0.8 mg/g of FB_2 (Visconti and Doko, 1994; Caramelli et al., 1993). In contrast with the high incidence recorded in the above countries, in Poland F. moniliforme comprised only 2−10% of Fusarium isolates from maize grains (Czaplinka et al., 1979; Chelkowski and Lew, 1992), although the production of fumonisins by fungal strains isolated in this country has reached levels as high as 2.9 mg/g of FB_1 and 1.0 mg/g of FB_2 (Chelkowski and Lew, 1992; Visconti and Doko, 1994).

Among African countries, where maize is a basic dietary staple of humans, the prevalence of F. moniliforme in maize has been reported in Transkei (South Africa) and Zambia (Marasas et al., 1988; Bache and Forthun, 1993), and fumonisin production on maize cultures by fungal strains isolated in these countries has reached levels up to 17.9 mg/g FB_1 and 3.0 mg/g FB_2 (Alberts et al., 1990; Thiel et al., 1991) and up to 3.1 mg/g FB_1 and 0.8 mg/g FB_2 (unpublished data), respectively. F. proliferatum isolated from maize in Sierra Leone and from sorghum in South Africa has also been shown to produce fumonisins (Thiel et al., 1991). Moreover, the presence of fumonisin-producing Fusarium species, with the prevalence of F. moniliforme, has been reported on millet grain and sorghum from Nigeria, Lesotho, and Zimbabwe (Onyike et al., 1991; Onyike and Nelson, 1993).

The finding of 100% contamination of maize inbreds grown in Italy supports earlier reports that revealed in Italy about 85−90% incidence of fumonisin contamination in corn-based feeds and corn-based foods, reaching FB_1 levels up to 8400 and 6100 ng/g, respectively (Minervini et al., 1992; Doko and Visconti, 1994). A significant percent of samples with fumonisin levels higher than 1 ppm, a level of concern, was observed in

Fumonisin Contamination in Maize Genotypes

J. Agric. Food Chem., Vol. 43, No. 2, 1995 **433**

healthy looking maize from some of the examined countries, indicating that fungal infection can also occur in symptomless maize kernels. On the basis of the results of the present investigation, it seems that kernel infection with fumonisin-producing *Fusarium* species and the subsequent natural occurrence of fumonisins in maize genotypes is a widespread phenomenon in some western European and African countries, whereas it is less pronounced and/or sporadic in central European countries, such as Croatia, Poland, and Romania. However, the low levels and incidence of toxin contamination in hybrids from central Europe should not be considered as a perfect guarantee against fungal invasion, since favorable environmental conditions both in the field and/or during storage of maize may result in mold spoilage, which decreases the quality of the grain and introduces potential hazards of fumonisins. In conclusion, the environmental conditions of the specific area of cultivation seem to play a role in the formation of fumonisin in maize. However, due to the fact that in the present study the same genotypes were not represented in different countries and/or in different seasons, further investigations are necessary to thoroughly establish the genotype—area—season interaction.

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