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Screening of local Italian maize varieties for resistance to *Fusarium verticillioides*

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Abstract

In order to find maize genetic sources of resistance to Fusarium attack and to fumonisin accumulation, 27 Italian local varieties collected at the CREA (Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di ricerca per la maiscoltura) maize gene bank, were evaluated in artificial inoculation field experiments during 2011 and 2012 seasons.

Primary ears were inoculated with a mixture of two *Fusarium verticillioides* toxigenic strains through kernel inoculation; non-inoculated and sterile water-inoculated ears were included as controls. Disease severity was estimated by counting the kernels with visible symptoms of infection at the inoculation point. The percentage of internally contaminated kernels was detected and fumonisin accumulation was recorded. Results showed that, under artificial inoculation, genotypes responded differentially to the Fusarium attack. Five out of 27 genotypes (VA56, VA70, VA74, VA114, and VA121) showed a low fumonisin content both in 2011 and 2012, seasons characterized by very different climatic conditions.

Keywords: maize, local varieties, artificial inoculation, *Fusarium verticillioides*, resistance

Introduction

Maize (*Zea mays* L) is constantly attacked by fungal pathogens throughout its entire life cycle. Toxigenic fungi produce mycotoxins, secondary metabolites, which may be toxic or have other debilitating effects on living organisms (CAST, 2003). Fusarium is one of the most common fungal genus associated with maize worldwide. Its infection can occur asymptomatically or cause rots in several tissues of the plant such as stalk and ear. The major consequence in kernel contamination is the accumulation of fumonisin that can cause several disorders in humans and animals (Voss et al, 2007). These mycotoxins are classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2002) and maximum levels for fumonisins, mainly B1 and B2, in food and feed have fixed by the European Union (Commission Regulation 1126/2007; Commission Recommendation 2006/576/EC).

The principal species of Fusarium causing ear rot in most maize-growing areas of Southern Europe is *Fusarium verticillioides*, a toxigenic fungus (Battilani et al, 2008; Covarelli et al, 2011; 2012) able to cause losses in grain yield, quality, and safety (De Curtis et al, 2011; Parsons and Munkvold, 2012). *F. verticillioides* can survive in crop residues, such as senescent roots and leaves in the soil, starting a subsequent infection (Munkvold and Caltron, 1997). Climatic conditions during the growing season and insect damage are determinant factors for *F. verticillioides* infection and for fumonisin accumulation in maize (Cao et al, 2014). Insects have been associated with fumonisin contamination, as their activity disperses the fungus and provides routes of entry into the ear and kernels (Alma et al, 2005).

Several factors can prevent Fusarium from entering the ear, such as adherent and tight husks and reduced open apical parts of the ear and thicker pericarp (Hoenish and Davis, 1994; Burton et al, 2006; Lanubile et al, 2012). Therefore, the development of plants able to overcome damages and restrict mycotoxin accumulation caused by fungal pathogens, either through transgene-mediated modification or plant breeding, is an important purpose for maize breeders. (Munkvold, 2003a; 2003b).

Maize is a major crop in Italy, playing an important role in animal feed, direct human consumption and commercial products. Around 10 million tons of maize per year are produced, prevalently in the Po river valley (Northern Italy), hence finding new genotypes resistant to Fusarium infection, to be included in breeding programs, could be an important objective for the improvement of maize cultivation (Balconi et al, 2010).

In recent decades it has been highlighted the importance of conservation and enhancement of local varieties as genetic source that could be important for agriculture and industry improvement both in supermarkets and in little local areas. Many of these varieties are distinguished by some chemical properties, such as antioxidants and carotenoids (Tafuri et al, 2014). Considering the recent attention to the consumption of foods with high nutritional value and

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low allergenic potential, the selection of interesting characters in these traditional genotypes could be a good goal. The largest collection of maize germplasm in Italy is kept at CREA-Unità di ricerca per la maiscoltura. This collection includes more than 1,200 varieties, of which nearly 700 local of Italian origin, gathered in different regions in the '50s, when the cultivation of hybrids was to replace traditional crops (Redaelli et al, 2013). So, it represents an interesting starting point for searching genotypes resistant to fungal pathogens in addition to good nutritional and safety characteristics (Berardo et al, 2009; Alfieri et al, 2012).

The aim of this research was to find new genetic sources for resistance to pathogens in order to improve maize safety and quality. For this purpose, during 2011 and 2012, a set of 27 maize local varieties were evaluated for *F. verticillioides* ear rot susceptibility and fumonisin accumulation, through kernel inoculation in field trials in Bergamo, Northern Italy.

Materials and Methods

Maize Genotypes

A set of 27 maize local varieties, (partially described by Regione Lombardia, 2002) stored at the CREA-Unità di ricerca per la maiscoltura gene bank, was tested. The main parameters of these varieties, such as origin, weight, harvest site and earliness of flowering (female and male expressed in GDU «Growing Degree Unit», high values are connected to late genotypes) are summarized in Table 1. The type of kernel colour (scale from white to purple), kernel type (ranging from flint to dent) and 1,000 kernels weight are also shown.

Field management and weather monitoring

The maize genotypes were grown in 2011 and 2012 at CREA, Bergamo Italy (45°68'N;9°64'E). This screening was conducted in a randomized complete block design with four replicates. The experimental unit consisted of a two row plot with a length of 5,1 m spaced 0,75 m; the plots were thinned to 20 plants per row. Irrigations during the growing season were applied to limit drought stress. Fertilization was implemented as follows kg ha^{-1}: N = 280, P_2O_5 = 115 and K_2O = 120. Environmental conditions, such as temperature, rainfall and humidity, were also recorded at the CREA-Unità di ricerca per la maiscoltura weather station.

Fusarium verticillioides artificial inoculation

A mixture of two of *F. verticillioides* strains (#289 and #294), chosen for their capacity to produce fumonisins (personal communication, Prof. Paola Battilani, Università Cattolica del Sacro Cuore di Piacenza), was used for artificial inoculation. The fungus was maintained on PDA (Potato Dextrose Agar) plates; spores were harvested after 15 days of incubation in a growing chamber (26°C, 62% humidity and 16 h light photoperiod) by washing the plates with 12 ml of sterile distilled water (SDW). The concentration of spore suspension was calculated in a Bürker haemocytometer and adjusted with SDW to the desired concentration (10^6 spores ml^{-1}).

Ten primary open-pollinated ears, at 15 days after mid-silking, were chosen for each genotype to be inoculated through Kernel Inoculation Assay (KIA) with a fresh spore suspension. This method involved the wounding, through the husks, of three kernels, placed in the lower middle of the ear, using a stainless fork, which was dipped in the spore suspension (Reid et al,1996; Ferrari and Balconi, 2008; Balconi et al, 2014). As controls, ears inoculated with SDW (internal method control) and non-inoculated ears, were included.

Ear and kernel evaluations

At physiological maturity (around 20% moisture) ears were manually harvested and de-husked. The severity of the *F. verticillioides* attack was evaluated as follows: i) for non-inoculated ears using a Disease Severity Rating (DSR) scale which includes a score based on percentage of kernels with visible symptoms of fungal infection (i.e. rot and mycelium) (DSR: 1 = 0%, 2 = 1-3%, 3 = 4-10%, 4 = 11-25%, 5 = 26-50%, 6 = 51-75%, 7 = 76-100%) (Reid et al, 1996; Balconi et al, 2014); ii) for Fusarium and water-inoculated ears, the Number of Infected Kernels (NIK), namely kernels showing visible symptoms of infection at the inoculation point, were counted. Non-inoculated ears after visual evaluation were shelled and percentage of moisture and hectolitric weight kg^{-1} hl^{-1}) were estimated using a grain analysis computer (Dickey-John GAC 2100).

After visual evaluation, ears of each plot and treatment were dried at 40°C for 7 days; then ears were shelled and kernels stored.

The kernels from each sample (10 ears) were accurately mixed before sub-sampling to ensure homogeneity. Samples were sorted by applying standard procedures (Berardo et al, 2005).

Internal Kernel Infection (IKI)

From each sample, 25 kernels were randomly chosen and surface-sterilized with 1% sodium hypochlorite for 2 min, with 90 % ethanol solution for 2 min, and finally rinsed four times in SDW. After drying under a sterile hood, five kernels were plated on a Fusarium selective medium (DRBC - Dichloran-Rose Bengal Agar; King et al, 1979) and incubated for seven days in a growing chamber. Infected kernels identified by visible Fusarium mycelium, according to the key of Nelson et al (1983), were recorded. At the same time, the percentage of germinated kernels was registered. Data were processed over five replications.

Fumonisin content analysis

Fumonisins were extracted from 5 g of flour sample after grinding kernels with a laboratory mill

Table 1 - List of maize local varieties: their origin, harvest site, kernel weights, type, colour, earliness of flowering (female and male expressed in GDU -Growing Degree Unit-, high values are connected to late genotypes) and 1,000 kernel weight (Regione Lombardia, 2002).,

Genotype	Name	Origin	harvest site	kernel type	kernel colour	earliness (GDU) FEM	earliness (GDU) MAL	1000 kernel weight (g)
VA56	Marano vicentino	Milano	Vimercate	Flint	Orange	553.3	526.1	155.0
VA62	Nostrano dell'isola	Sondrio	Pala Delebio	Semiflint	Orange	645.6	625.0	210.0
VA63	Nostrano locale	Sondrio	Pala Delebio	Dent	Yellow	655.6	616.1	223.0
VA65	Locale	Sondrio	Verceia	Dent	Yellow-orange	655.6	625.0	235.0
VA66	Locale	Sondrio	Verceia	Semiflint	Yellow-orange	671.7	645.6	270.0
VA67	Locale	Sondrio	Barbone	Flint	Orange	671.7	635.0	240.0
VA68	Nostrale	Sondrio	Madonna del Piano	Semiflint	Orange	663.3	625.0	215.0
VA69	Locale	Sondrio	Forte	Semiflint	Yellow-orange	663.3	625.0	255.0
VA70	Locale	Sondrio	Somaggia	Semiflint	Orange	645.6	606.1	355.0
VA74	Fiorentino	Belluno	Castion di Belluno	Semiflint	Yellow-orange	584.4	545.0	250.0
VA83	Bianco perla	Padova	Abano	Semiflint	White	645.6	606.1	321.0
VA89	Scagliolo Frassine	Padova	Montagnana	Dent	Yellow	663.3	635.0	203.0
VA90	Polenta rossa	Padova	Camposanpiero	Semiflint	Red	645.6	606.1	329.0
VA108	Ostesa (tipica)	Verona	Verona	Semident	Yellow	562.8	535.6	238.0
VA109	Ostesa	Verona	Verona	Semident	Yellow	593.9	535.6	254.0
VA111	Nostrano	Verona	Zimella	Dent	Orange	635.0	606.1	256.0
VA112	Pignolino nostrano	Verona	Veronella	Flint	Orange	625.0	571.7	202.0
VA113	Nostrano del Garda	Verona	Garda	Semiflint	Orange	593.9	526.1	216.0
VA114	Cinquantino Bianchi	Verona	Sorgà	Flint	Orange	571.7	509.4	170.0
VA121	Pinoletto d'oro	Vicenza	Bolzano vicentino	Flint	Orange	625.0	593.9	230.0
VA553	Scagliolo Marne	Bergamo	Marne	Flint	Yellow	730.0	716.0	290.0
VA572	Nostrano dell'isola	Bergamo	Madone	Flint	Orange	706.0	687.0	220.0
VA904	Cinquantino 2°raccolto	Milano	Alto Milanese	Flint	Orange	613.0	586.0	175.0
VA1196	Rostrato della Valchiavenna	Sondrio	Chiavenna	Semident	Orange-red	786.0	754.0	230.0
VA1269	Rostrato Esine	Brescia	Esine	Semident	Purple	780.0	755.0	213.0
VA1304	Spinato di Gandino	Bergamo	Gandino	Semiflint	Orange	783.0	752.0	271.0
VA1306	Rostrato rosso di Rovetta	Bergamo	Rovetta	Semident	Red	795.0	776.0	318.0

(Retsch ZM200) with a 1 mm sieve and stored at 4°C. The grain was treated with 25 ml of 70% methanol, and the obtained mixture was shaken for 3 min and then filtered through Whatman no.1 filter paper (Berardo et al, 2011). The fumonisins were quantified with a Ridascreen FBs ELISA test kit (R-Biopharm, Darmstadt, Germany) able to detect total Fumonisins at a minimum concentration of 0.025 mg kg^{-1}. Data were processed over two replicates.

Statistical analyses

The response of genotypes among treatments (non-inoculated, water-inoculated, and Fusarium-inoculated) were evaluated separately for each year, in a split-plot model with treatment and genotype as main plot and subplot, respectively. The analysis of variance at 95% (ANOVA) was calculated with AGROBASE software. These analyses were conducted on five replicates for visual evaluation and double analysis for fumonisin content quantification.

Results

Climatic conditions

Environmental data, such as rainfall and mean temperature during both years, recorded at the CREA-Unità di ricerca per la maiscoltura weather station, are reported in Figure 1. Mean temperature during the summer period (June - August) was higher in 2012 than in 2011, ranging from 22°C to 27°C, and from 20°C to 26°C, respectively. Rainfall was also higher in 2012 than in 2011 for the entire period of cultivation (831 mm in 2012 vs. 742 mm in 2011), but with an unfavorable distribution for the crop, with more than 90% concentrated during spring (March - May).

In addition, Growing Degree Days (GDD) reached 2,315 GDDs in 2012 and 2,275 GDDs in 2011 and the accumulation period (number of days from flowering to physiological maturity) in 2011 was higher than in 2012 (63 vs. 56 days; Mazzinelli et al, 2012; 2013).

Evaluation of non-inoculated ears

At physiological maturity, ears were harvested manually and shelled, and moisture and hectolitric weight were measured. Values of moisture in 2011 ranged from 10.7% to 17.2% (average 12.8%), and from 12.1% to 17.3% during 2012 (average 13.85%). This parameter was significantly different between the two seasons (LSD0,05 = 0,67).

Hectolitric weight ranged from 57.6 kg hl^{-1} to 78.5 kg hl^{-1} and from 58.1 kg hl^{-1} to 81.0 kg hl^{-1} in 2011 and 2012 seasons, respectively. Test weight was not significantly different across years, although the values resulted slightly higher in 2012 (average 72.12 kg hl^{-1}) with respect to 2011 (average 74.41 kg hl^{-1}).

The DSR average for non-inoculated primary ears, was quite similar during both years (Table 2), ranging from 1.4 (VA74) to 4 (VA68) in 2011, and from 1.44 (VA56 and VA112) to 4.14 (VA 68) in 2012, suggesting that less than 10% of kernels per ear showed visible symptoms such as rot and mycelium growth under natural environmental conditions. For this parameter no significant statistical difference between two years (analysis of variance conducted on 5 replicates), was observed.

Response to Fusarium inoculation

The average of NIK, during 2011 was higher than that recorded in 2012, both for the Fusarium-inocu-

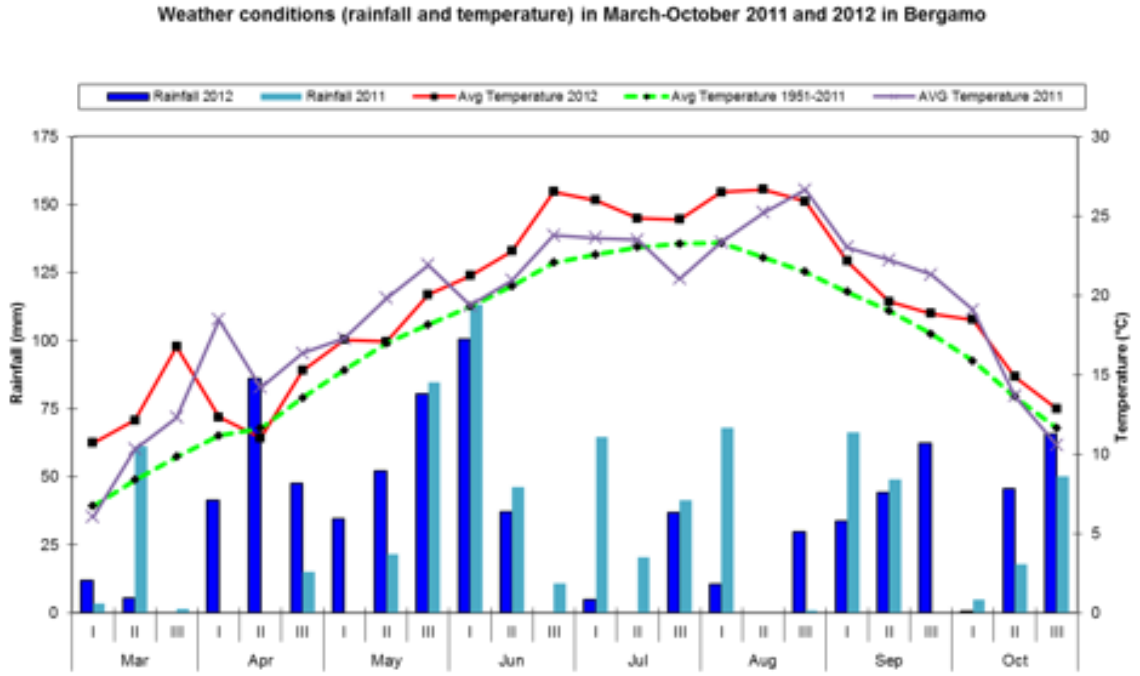

Figure 1 - Environmental data (rainfall and average temperature) recorded at the CREA-Unità di ricerca per la maiscoltura weather station during 2011 and 2012.

lated and internal controls materials; average NIK of water-inoculated samples was 17.77 and 13.92 in 2011 and 2012 respectively. Analysis of variance at 95% showed that there was statistically significant difference between inoculated samples and water-inoculated controls both in 2011 ($LSD_{0,05}$ = 8.08) and 2012 ($LSD_{0,05}$ = 6.21 -data not shown-). In 2011, the average of NIK for Fusarium-inoculated samples 33.05, while in 2012, was 30.16 (Table 2).

Comparing single genotypes, a large variability in the response to *F. verticillioides* inoculation during both years, was evident (Table 2). It is worth highlighting that some genotypes showed a DSR between 1 and 2 (VA56, VA74, VA112, and VA114), i.e., less than 3% of ear showed visible symptoms of Fusarium ear-rot. Values ranged from a minimum of 1.4 (VA111) in 2011 and 1.44 (VA56) in 2012, to a maximum of 4 (VA68) in 2011 and 4.14 (VA68) in 2012. This parameter was statistically different during both years among genotypes (2011: $LSD_{0,05}$ = 1.28 and 2012: $LSD_{0,05}$ = 1.26). Based on DSR-ranking, genotypes can be divided in three groups: low (from 1 to 9, green in Table 2), medium (from 10 to 18, yellow in Table 2) and high (from 19 to 27, red in Table 2). It can be noted that 11 out of 27 genotypes (40.74%) belong to the first group during both years, while three out of 27 (11.11%) belong to the second group and no genotype belongs to the third group, during both years. Several genotypes (13 out of 27 - 48,14%), belong to different groups through the years.

Observing NIK values for each genotype inoculated with *F. verticillioides*, a large variability can be noted. During 2011, NIK values ranged from 9.8 (VA62) to 99.8 (VA1269), and from 9 (VA 1306) to 102.2 (VA 111) in 2012 (Table 2). Among genotypes, there was no statistically significant difference in 2011, while differences were significant at 95% in 2012 (LSD0,05 = 40.95). Based on NIK rankings, genotypes can be divided into three groups: low (from 1 to 9, green in Table 2), medium (from 10 to 18, yellow in Table 2) and high (from 19 to 27, red in Table 2). During both years, four out of 27 genotypes (14.81%) belong to the first group, four out of 27 (14.81%) belong to the second group both, and two out of 27 genotypes (7.4%) belong to the third group. Many genotypes (16 out of 27 – 59.25%) belong to different groups during different seasons.

IKI analysis indicated that, during both years and for all treatments analysed, most of genotypes were internally contaminated by *F. verticillioides* with values ranged from 98.81% for non-inoculated samples to 100% for inoculated materials during 2011 and from 88.44% for non-inoculated kernels to 97.63% for inoculated samples during the 2012 season. The germination percentage of kernels was evaluated both years, it ranged from 71.55% for inoculated samples to 85.18% for non-inoculated materials during 2011 and from 67.55% for inoculated samples to 82.07% for water-inoculated materials during 2012; this parameter was not affected by Fusarium inoculation.

Response to fumonisin accumulation

The average of fumonisin content in inoculated local varieties, was statistically higher than in controls

Table 2 - List of maize local varieties: their origin, harvest site, kernel weights, type, colour, earliness of flowering (female and male expressed in GDU -Growing Degree Unit-, high values are connected to late genotypes) and 1,000 kernel weight (Regione Lombardia, 2002).

	2011				2012			
	DSR		NIK		DSR		NIK	
Genotype	Rank	DSR	Rank	NIK	Rank	DSR	Rank	NIK
VA 56	2	1.60	23	49.40	1	1.44	9	20.40
VA 62	6	2.40	1	9.80	11	2.30	10	21.80
VA 63	11	3.60	3	16.60	15	2.80	8	18.80
VA 65	7	2.60	24	53.00	14	2.70	11	22.80
VA 66	8	2.80	17	30.33	16	2.88	23	54.80
VA 67	9	3.20	9	20.60	19	3.50	20	41.40
VA 68	12	4.00	13	22.80	21	4.14	9	20.40
VA 69	11	3.60	10	21.40	12	2.44	18	36.40
VA 70	10	3.40	22	49.00	13	2.67	7	17.20
VA 74	1	1.40	15	26.00	7	2.00	12	23.60
VA 83	9	3.20	8	20.20	17	3.00	3	12.60
VA 89	7	2.60	16	26.80	18	3.33	13	24.00
VA 90	6	2.40	20	38.00	10	2.29	15	29.00
VA 108	3	1.80	7	18.80	5	1.89	16	30.40
VA 109	4	2.00	18	31.60	4	1.80	17	35.20
VA 111	1	1.40	12	22.60	13	2.67	25	102.20
VA 112	4	2.00	21	40.40	1	1.44	22	45.60
VA 113	6	2.40	2	15.60	2	1.50	21	41.80
VA 114	2	1.60	11	21.80	6	1.90	19	41.00
VA 121	7	2.60	19	33.80	3	1.70	6	17.00
VA 553	11	3.60	26	89.60	6	1.90	24	68.00
VA 572	10	3.40	25	56.20	9	2.20	5	14.60
VA 904	2	1.60	14	24.60	15	2.80	2	12.25
VA 1196	6	2.40	5	18.20	20	3.83	5	14.60
VA 1269	5	2.20	27	99.80	8	2.10	4	14.00
VA 1304	9	3.20	4	17.00	9	2.20	14	25.60
VA 1306	4	2.00	6	18.40	4	1.80	1	9.00
Mean		2.56		33.05		2.42		30.16
$LSD_{0.05}$		1.28		n.s.		1.26		40.95

during both years; during 2011, fumonisin content resulted 20.08 mg kg^{-1} in non-inoculated materials and 33.33 mg kg^{-1} in water-inoculated materials. During 2012, a lower trend in fumonisin content was observed in comparison to the previous year with an average fumonisin content of 7.4 mg kg^{-1} in non-inoculated samples and 9.3 mg kg^{-1} in water-inoculated samples (data not shown).

The fumonisin content of *F. verticillioides* inoculated materials, showed large variability among genotypes for both years (Table 3); fumonisin content ranged from 8.8 mg kg^{-1} (VA114) to 214.44 mg kg^{-1} (VA65) during 2011 and from 1.19 mg kg^{-1} (VA121) to 136.9 mg kg^{-1} (VA553) during 2012. During both years there were statistically significant differences among genotypes at 95% (2011: $LSD_{0,05}$ = 14.03; 2012: $LSD_{0,05}$ = 8.77). Fumonisin content in Fusarium-inoculated samples during 2011 (average 77.10 mg kg^{-1}) was statistically different from samples inoculated in 2012 (average 33.0 mg kg^{-1}) with $LSD_{0,05}$ = 2.19 (Table 3).

For each season, genotypes were grouped into three classes based on relative rankings, the classes were 1) low, for genotypes ranking 1-9 (green in Table 3) ; 2) medium, for genotypes ranking 10-18 (yellow in Table 3); 3) high for genotypes ranking 19-27 (red in Table 3). Results showed that five genotypes (18.51%) maintained a low ranking (from 1 to 9) during both years: VA56, VA70, VA74, VA114, and VA121, two genotypes (7.4%) maintained medium rankings for both years (VA90 and VA112), and three genotypes (11.11%), maintained a high rank over both years (VA66, VA111, and VA553). Many genotypes (17 out of 27; 62.96%) belonged to different groups in different years. Comparing genotypes with low fumonisin content separately in the two years, in 2011, VA56 and VA121 were statistically significantly different; on the other hand, in 2012, only VA121 was significantly different from others.

Discussion

Temperature, drought stress, insect damage, and other fungal diseases are the most influential risk factors with regard to maize Fusarium ear rot and fumonisin accumulation (Miller, 2001; Cao et al, 2014). The weather plays a fundamental role on these factors, as uncontrollable variable, so the use of resistant genotypes could be a good strategy to contrast the propagation of *F. verticillioides*, an endemic fungus in the Italian areal. Breeders usually rely

Table 3 - Average of fumonisin content (mg kg^{-1}) and relative rank for 27 maize local varieties inoculated with Fusarium spores in 2011 ($LSD_{0.05}$ = 14.03) and 2012 ($LSD_{0.05}$ = 8.77) at CREA-Unità di ricerca per la maiscoltura. Rank is highlighted with colours corresponding to the three different classes: low (green), medium (yellow), high (red).

	2011		2012	
Genotype	Rank	mg kg^{-1}	Rank	mg kg^{-1}
VA 56	9	43.30	4	10.70
VA 62	6	34.30	15	25.90
VA 63	19	90.60	11	19.40
VA 65	27	214.44	10	17.10
VA 66	22	102.90	20	41.40
VA 67	7	35.90	13	25.60
VA 68	20	92.90	9	16.20
VA 69	5	33.30	12	24.10
VA 70	3	21.60	2	5.90
VA 74	2	16.60	7	13.90
VA 83	18	81.90	23	59.00
VA 89	24	136.10	19	31.10
VA 90	16	71.30	14	25.70
VA 108	15	70.10	24	93.70
VA 109	10	43.40	22	51.30
VA 111	21	101.50	21	50.50
VA 112	12	49.60	18	29.30
VA 113	17	72.50	25	97.40
VA 114	1	8.80	6	12.80
VA 121	8	36.40	1	1.19
VA 553	25	201.25	26	136.90
VA 572	23	118.50	17	26.20
VA 904	4	27.20	16	26.10
VA 1196	11	44.20	5	11.10
VA 1269	26	205.68	9	16.20
VA 1304	13	59.50	8	15.90
VA 1306	14	68.00	3	6.50
Mean		77.10		33.00
$LSD_{0.05}$		14.03		8.77

on natural contamination to observe infection severity and then to select genotypes resistant to fungal disease. However, natural infection is not sufficiently uniform in most locations to make classical breeding efficient and successful (Mesterházy et al, 2012). Artificial infection is a reliable tool to test genotypes for resistance to ear rot and mycotoxin production (Reid et al, 1996; Miedaner et al, 2010; Balconi et al, 2014).

In this study, 27 maize local varieties, representing a good source of genetic variability of traditional Italian varieties, were chosen and analyzed in Bergamo during two seasons (2011 and 2012). To avoid a possible difference in the fumonisin producer complex, as reported by Bottalico (1998), kernel inoculation was performed with spore suspensions obtained from a mix of two toxigenic *F. verticillioides* strains. This method (KIA) is a good technique to have an effective infection with consequent fumonisin accumulation, and represents a suitable tool to select resistant/susceptible genotypes (Reid et al, 1996; Miedaner et al, 2010; Balconi et al, 2014). As internal control, ears inoculated with sterile water, allow the evaluation of environmental fungal infection through ear injury, because fungal spores can use wounds to enter and infect the ear. Moreover, an evaluation of non-inoculated ears in order to detect environmental infection was performed. The two-year period of this study was characterized by different climatic conditions. In fact, in 2012 more rainfall was registered than in 2011, but only during spring (March-May), and temperatures were higher in the summer period (June-August).

Our results showed that natural infection is quite low in fact, less than 10% of the ear is covered by mycelium during each year, indicating that environmental contamination does not affect the evaluation of the artificial inoculations; in addiction, there was no significant difference on DSR between 2011 and 2012 so both years can be evaluated and compared. At the inoculation point, significant differences between water-inoculated (control) and Fusarium-inoculated materials were observed, underlining how the method of inoculation can be successful in discriminating genotypic responses to *F. verticillioides*.

Fumonisins are present in all treatments, but in controls the amount of these mycotoxins was significantly lower than in inoculated materials, indicating that toxigenic strains used for experimentation produced a higher amount of mycotoxins in field trials than natural ones. The internal infection analysis didn't show significant differences among treatments, because all of them highlighted a high similar internal contamination (over 88%); suggesting that symptomless kernels could be internally contaminated.

Visual screening results, evinced that there was a large variability among genotypes both for DSR (statistically different both years) and for NIK (significantly different only in 2012). Genotypes were divided into three classes based on increasing rank for the two above mentioned parameters. Most genotypes (11 out of 27) had a low DSR-ranking, so this parameter was not strictly discriminating for resistant/susceptible genotypes. On the contrary, observing NIK-rankings, four genotypes (VA63, VA83, VA1196, and VA1306) had a low NIK-score during both years. Hence, it can be assumed that NIK is a good parameter to identify resistant genotypes. During both years, it was also interesting to underline that VA1306 had a low rank both for DSR and NIK parameters, highlighting that this variety could be a good candidate for further studies.

With respect to fumonisin accumulation in single genotypes, a large, significant variability could be observed during both years. Five out of 27 genotypes (VA56, VA70, VA74, VA114, and VA121) had a low-rank for fumonisin content during both years.

The correlation coefficient of fumonisin content and NIK in Fusarium inoculated samples, was determined for both years, but no correlation could be found between these parameters (data not shown), similarly to research reported by Butron et al (2006).

In other studies, on Italian inbred lines, a correlation between these two factors was reported (Balconi et al, 2014). Maize varieties genotypes used in this study are characterized by a high genetic variability, and consequently by low homogeneity for many parameters, such as ear/kernel size.

A significant difference between two years was evident only for grain moisture content, likely due to the different climatic conditions. Hectolitric weight, a parameter connected to the type of kernel, with high values corresponding to a hard kernel, did not change across the two seasons. A possible correlation between pathological parameters (visible symptoms and fumonisin content) and moisture and/or test weight was also considered, but no correlation was found during both years considered.

In conclusion, this research showed that Italian germplasm offers some interesting sources of resistance to Fusarium ear rot. Five genotypes (VA56, VA70, VA74, VA114, and VA121) showed a low fumonisin accumulation, both in 2011 and 2012, two seasons with different climatic conditions. These genotypes, with their particular nutritional properties, such as carotenoids and high total antioxidant capacity (Tafuri et al, 2014) and with bioactive compounds useful both for industry and agriculture, are suitable for introduction into advanced breeding programs together with gene bank accessions, to enhance and improve biodiversity potential. In addition, these genotypes could represent starting materials for future research focused on the evaluation of the response to other toxigenic fungal pathogens, such as *Aspergillus flavus*, present in the maize growing area.

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