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Responses to selection of S_5 inbreds for broad-based resistance to ear rots and grain mycotoxin contamination caused by *Fusarium* spp. in maize

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Abstract Random S_5 inbreds derived from three F_2 maize (*Zea mays* L.) populations (L1934 × LP918, LP915 × LP2541 and L7310 × L7266) were selected for ear rot resistance after inoculation with a low-fumonisin producing isolate belonging to *F. proliferatum.* The four less susceptible and the four most susceptible inbreds from each population were crossed and F_1 seeds were pooled. Resistant and susceptible pools from each population were evaluated for disease severity (percentage of the ear visibly diseased) after inoculation with the isolate used for selection, and high toxigenic isolates belonging to *F. verticillioides* and *F. graminearum*. Grain mycotoxin concentration was assessed by ELISA. Differences in disease resistance to each fungus were observed between resistant and susceptible pools in most populations and environments indicating that selection after inoculation with a single species might be effective to develop broad-based resistance to *Fusarium*. Resistant pools exhibited, after inoculation with *F. verticillioides*, low grain fumonisin concentrations in most populations and years. Positive genotypic correlations between disease severity and fumonisin concentration ($0.89 < r_g < 0.98$, depending on fungal species and year) indicate that selection for disease severity accounted for most of the variability for field fumonisin accumulation. Selection seemed to be also effective to reduce grain deoxynivalenol and zearalenone concentrations after inoculation with *F. graminearum*. Ratios between grain deoxynivalenol concentration and disease severity were lower in L7310 × L7266 than those observed in the other populations suggesting that mechanisms affecting mycotoxin accumulation might exist in this population and additional responses should be feasible if including deoxynivalenol concentration as another selection parameter.

Keywords Maize · Disease resistance · *Fusarium* · Fumonisins · Deoxynivalenol · Zearalenone

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Introduction

Fusarium verticillioides, (Saccardo) Nirenberg [(= *F. moniliforme* (Sheldon), teleomorph *Gibberella moniliformis* (Wineland) (= *G. fujikuroi* (Sawada Ito in Ito

& Kimura, mating population A)], *F. proliferatum* (Matsushima) Nirenberg (= *G. fujikuroi* mating population D; teleomorph *G. intermedia* (Kuhlman)] and *F. graminearum* (Schwabe) [teleomorph *G. zeae* (Schwein.) Petch] are among the most important pathogens causing ear rots in maize (*Zea mays* L.). *Fusarium* infections cause yield losses (Presello et al. 2008) and grain contamination with several mycotoxins, including fumonisins (FBs) produced by *F. verticillioides* and *F. proliferatum*, deoxynivalenol (DON), and zearalenone (ZEA) produced by *F. graminearum*. All of these mycotoxins are toxic to both humans and animals. *Fusarium* species coexist in field crops and prevalence of one species on another depends of environmental conditions during the growing season (Stewart et al. 2002). Since prevalence of ear rotting fungi can not be forecasted at planting time to be used as a criterion for choosing the hybrid on the basis of specific resistance, the development of new hybrids expressing broad resistance across prevalent ear rotting pathogens is currently needed.

Selection for disease resistance after silk or kernel inoculation seems to be effective for several *Fusarium* spp., such as *F. verticillioides* (De León and Pandey 1989) and *F. graminearum* (Presello et al. 2005). Previous reports also suggested that broad-based resistance across several *Fusarium* species exist in maize (Presello et al. 2006; Löffler et al. 2010), but there is scarce information on selection responses (Löffler et al. 2010) to develop maize germplasm expressing disease resistance to the most prevalent toxigenic fungi. Isolate variation for aggressiveness and mycotoxin production was observed for *F. graminearum* and ear rotting fungi belonging to Section *Liseola* (Leslie et al. 1992; Miedaner et al. 2010; Jardine and Leslie 1999; Iglesias et al. 2010). Since some mycotoxins, such as DON, affect isolate aggressiveness (Harris et al. 1999, Gardiner et al. 2009), using toxigenic fungal isolates as source of inoculum for selection is advisable. On the other hand, there is scarce evidence of FBs affecting isolate aggressiveness for ear rots in maize (Iglesias et al. 2010). Using low mycotoxin producer isolates as source of inoculum in breeding programs might be desirable to reduce exposure of breeding staff to these toxic compounds and there is scarce information on ability of these isolates to develop hybrids with low field mycotoxin accumulation.

This research was conducted to investigate the feasibility to develop maize hybrids expressing broad-based disease resistance and low field mycotoxin accumulation by selecting parental inbreds on the basis of disease resistance to a low toxigenic *Fusarium* isolate.

Materials and methods

Selection and evaluation experiments were conducted in Pergamino, Province of Buenos Aires, Argentina, at 33°54′ south latitude and 60°35′ west longitude. Two hundred ninety-five random S_5 inbreds were developed by selfing three maize populations (Table 1). In 2005, single rows of each inbred consisting of 26 plants were sown at a rate of 5 plants/m. All plants were inoculated by injecting 2 ml of conidial suspensions into the silk channel four days after silking (Reid et al. 1996). P319, a low-fumonisin producer isolate belonging to *F. proliferatum* (Iglesias et al. 2010) was used to produce the inoculum. P319 was grown in a liquid medium following Reid et al. (1996). After 2 weeks, the cultures were filtered through cheesecloth to remove mycelium and conidial concentrations were adjusted to 1×10^6 conidia ml^{-1} with sterile water. Suspensions were stored at 4°C for a maximum

Table 1 Population, origin, number of inbreds and means of disease severity of the four less susceptible (Resistant) and the four most susceptible (Susceptible) S_5 maize inbreds selected after inoculation with *Fusarium proliferatum*

Population	Origin	Inbreds	Disease severity mean of selected inbreds[a]	
			Resistant	Susceptible
L1934 × LP918	Commercial hybrid Ax888	83	7.6	82.1
L7310 × L7266	Commercial hybrid C280	137	6.0	44.6
LP915 × LP2541	Stiff stalk synthetic	75	1.4	69.1

[a] Percentage of the ear visibly covered with mold

of 3 days prior to inoculation. When reaching about 18–20% grain moisture, all ears were harvested and area visibly affected by mold (%) was visually assessed.

In 2006, the four most symptomatic inbreds within each population were manually crossed to create six F_1 hybrids. After harvest, equal seed amounts from each F_1 hybrid were pooled to create the pool of susceptible hybrids. The same protocol was used to create the pool of resistant hybrids from the four less susceptible inbreds within population.

In 2007 and 2008, all six pools were tested after inoculation with P364, a high fumonisin producing isolate belonging to *F. verticillioides* (Iglesias et al. 2010); IV-II-3, a DON and ZEA producing isolate belonging to *F. graminearum* (G.A. Lori, personal communication) and P319, the isolate used for selection. P319 and P364 were isolated from maize collected in Pergamino and IV-II-3 from diseased wheat grains collected in La Plata, Province of Buenos Aires. A factorial experiment was conducted with the plant genotype and the fungal species as factors. Levels in the plant genotype factor consisted of the six pools and the susceptible check hybrid L4673 × L4671. A non-inoculated control treatment of L4673 × L4671 was also included. Treatments were randomized in a complete block design with four blocks. Each experimental unit consisted of two 5-m rows separated 0.7 m each other and sown at a rate of 5 seeds/m. Protocols for inoculum production, inoculum concentration adjustment, inoculation, and disease severity assessment were the same as those used for inbred selection. Disease severity was assessed in all plants and plot means were used for statistical analysis. Percentages of disease severity were transformed to arcsin (proportion of the ear affected by the fungus)$^{1/2}$ to normalize errors.

After disease severity assessment, ears were let dry naturally and shelled. Grain was thoroughly mixed and a 1-kg sample was taken from each experimental unit for mycotoxin and/or ergosterol analyses. Grain FB concentration was assessed by ELISA (Ridascreen® Fast Fumonisin, R-Biopharm AG, Darmstadt, Germany) in samples from treatments inoculated with P319 and P364, and from natural infection control. Deoxynivalenol and ZEA were assessed by R-Biopharm ELISA kits (Ridascreen® Fast DON and Ridascreen® Fast Zearalenon, respectively) in samples from treatments inoculated with IV-II-3. For FB and DON assessments, the mycotoxins were extracted by blending 5 g of milled culture in 25 ml of 70% methanol. The mix was shaken by 2 min in a vortex Boeco V-1 (Boeckel & Co., Hamburg, Germany), filtered through filter Whatman No. 1 and diluted 1:14 with sterile distilled water. Diluted extracts and five standards, at mycotoxin concentrations of 0.000, 0.222, 0.667, 2.000 and 6.000 μg g^{-1} were subjected to ELISA. Protocols for ZEA assessments were similar to those used for the other two mycotoxins but the mycotoxin was extracted with water and standard concentrations were 0.00, 0.05, 0.10, 0.20 and 0.40 μg g^{-1}. For the three mycotoxins, well absorbance was measured at 450 nm with a microplate reader Biotek ELx 800 (Biotek Instruments Inc., Winooski, USA). Absorbance values of positive standards and samples were divided by the absorbance value of the first standard (standard zero). Mycotoxin concentration of the samples was estimated on the basis of a logit-log function between mycotoxin concentration and relative absorbance of the four positive standards [RIDA®SOFT Win software (R-Biopharm AG, Darmstadt, Germany)]. Grain mycotoxin concentrations were subjected to logarithmic transformations to normalize errors.

Grain ergosterol concentration was assessed by HPLC (Hewlett-Packard model 1050 system) in treatments inoculated with *F. graminearum,* following Seitz et al. (1977), with some modifications. 15 ml of methanol and 1 g of lyophilized milled sample were mixed for 2 min in a 125 ml Erlenmeyer flask. The blend was poured into a 50 ml capped polypropylene centrifuge tube. The remaining blend from the erlenmeyer flask was washed off with 15 ml of methanol and poured into the centrifuge tube. The final extract was then centrifuged 15 min at 3,000×*g*. The supernatant was poured off. The residue was re-suspended in 10 ml of methanol, shaken for 30 s, and centrifuged as before. Supernatant portions were combined, mixed with 8.5 g of KOH and 25 ml of ethanol, and refluxed for 30 min at 65°C. The cooled, saponified mixture was diluted with 5 ml of distilled water and extracted three times with 10 ml of hexane. Hexane extracts were combined and evaporated to dryness under nitrogen with heating (35°C) in a rotary evaporator. The resultant residue was dissolved in 5 ml methanol (HPLC grade). The solution was transferred to vials for HPLC analysis after filtration (0.22 μm). HPLC analysis was carried out with a Hewlett-Packard model

1050 system. Elution was performed at room temperature on a Hypersil ODS C18 microbore column (200 × 2.1 mm i.d. 5 μm) using an isocratic mobile phase consisting of methanol at a flow rate of 0.3 ml/min and detection at 282 nm. A volume of 10 μl was injected into the HPLC. The ergosterol peak was eluted at about 6.6 min. The quantification was made by external standardization (Ergosterol from Sigma, USA) with a calibration curve range from 1.0 to 150 μg ml^{-1}. Analyses were performed in duplicated and the results were obtained using the average.

Analyses of variance and mean comparisons (*t* test) were performed with INFOSTAT (2002). Genotypic variances were estimated with "Correlations" procedure in the "Experimental Statistics" module of GENES software (Cruz 2006). All statistical tests were conducted at a significance level of $P = 0.05$.

Results

Non-inoculated control hybrid showed ear rot symptoms and FB contamination which indicates that natural infections of *F. verticillioides* and/or *F. proliferatum* occurred (Table 2). Natural infections of *F. graminearum* were not detected since grain samples from the same hybrid exhibited non-detectable levels of DON and ZEA (Table 3). Resistant pools exhibited lower disease severity means than those of susceptible pools for most populations and isolates in both years (Tables 2, 3). Resistant pools exhibited lower grain FB concentrations, after inoculation with *F. verticillioides*, and lower grain ZEA concentrations, after inoculation with *F. graminearum*, than those observed in susceptible pools in most populations and environments (Tables 2, 3). Differences between resistant and susceptible pools for grain DON concentration were observed only in some populations in 2008 (Table 3). Resistant and susceptible pools exhibited similar grain FB concentrations after inoculation with the low toxigenic isolate P319 in most populations and environments.

Since no interaction between population and selection level was observed for ratios between grain mycotoxin concentration and disease severity, only population means were compared and discussed for these traits. P364 caused higher FB to disease severity ratios (FB/DS) than those caused by P319

Table 2 Ear rot severity and grain fumonisin concentration, after inoculation with *Fusarium proliferatum and F. verticillioides*, of F_1 hybrid pools attained by crossing the four less susceptible (Resistant) and the four most susceptible (Susceptible) S_5 inbreds from three maize populations

Population	Pool	Disease severity[a]		Grain fumonisin concentration[b]	
		2007	2008	2007	2008
Fusarium proliferatum					
L1934 × LP918	Resistant	13.9*	23.2*	10.1	16.3
L1934 × LP918	Susceptible	40.4	56.9	14.8	19.1
L7310 × L7266	Resistant	40.2*	41.3*	24.5	22.3*
L7310 × L7266	Susceptible	67.5	76.1	50.8	45.7
LP915 × LP2541	Resistant	13.8*	35.3*	6.6	18.5
LP915 × LP2541	Susceptible	42.0	49.4	18.3	8.2
Susceptible control hybrid[c]		58.6	52.5	17.9	16.3
Fusarium verticillioides					
L1934 × LP918	Resistant	19.4	31.3*	30.4	93.2*
L1934 × LP918	Susceptible	30.4	70.0	25.5	193.2
L7310 × L7266	Resistant	41.5*	66.5*	54.0*	121.2*
L7310 × L7266	Susceptible	65.5	80.7	184.3	253.7
LP915 × LP2541	Resistant	17.2*	34.9*	18.4*	86.3*
LP915 × LP2541	Susceptible	29.5	58.5	29.9	104.3
Susceptible control hybrid[c]		58.5	63.6	47.7	141.8
Non-inoculated control[c]		22.4	4.9	7.4	13.7

[a] Percentage of the ear visibly covered with mold

[b] mg kg^{-1}

[c] L4673 × L4671

* Differences between resistant and susceptible pools significant at a probability level of 0.05 (*t* test)

Table 3 Ear rot severity and grain mycotoxin concentration, after inoculation with *Fusarium graminearum*, of F_1 hybrid pools attained by crossing the four less susceptible (Resistant) and the four most susceptible (Susceptible) S_5 inbreds from three maize populations

Population	Pool	Disease severity [a]		Grain mycotoxin concentration[b]			
				Deoxynivalenol		Zearalenone	
		2007	2008	2007	2008	2007	2008
L1934 × LP918	Resistant	22.9*	26.6*	3.1	2.0*	7.7*	2.1*
L1934 × LP918	Susceptible	51.3	67.8	5.8	11.1	12.6	12.7
L7310 × L7266	Resistant	43.3*	43.3*	1.1	1.1	0.6*	1.3*
L7310 × L7266	Susceptible	62.3	80.7	2.8	3.6	4.7	8.4
LP915 × LP2541	Resistant	32.8*	27.5*	3.6	1.6*	9.2	2.0*
LP915 × LP2541	Susceptible	45.8	69.1	6.8	8.3	12.2	10.5
Susceptible control hybrid[c]		59.5	64.8	11.5	3.9	19.8	6.9
Non-inoculated control[c]		22.4	4.9	ND	ND	ND	ND

[a] Percentage of the ear visibly covered with mold after inoculation with *F. graminearum*

[b] mg kg^{-1}

[c] L4673 × L4671

* Differences between resistant and susceptible pools significant at a probability level of 0.05 (*t* test)

ND non-detectable

Table 4 Ratios between grain mycotoxin concentration (mg kg^{-1}) and visibly diseased ear area (percentage) in maize populations inoculated with *Fusarium* spp

Population	*Fusarium graminearum*				*F. proliferatum*		*F. verticicillioides*	
	Deoxynivalenol		Zearalenone		Fumonisins			
	2007	2008	2007	2008	2007	2008	2007	2008
L7310 × L7266	0.04a	0.05a	0.04a	0.06a	0.48a	0.36a	1.04a	2.13a
LP915 × LP2541	0.12b	0.27b	0.09ab	0.11a	0.55a	0.44a	1.31a	2.35a
L1934 × LP918	0.14b	0.33b	0.12b	0.13a	0.69a	0.54a	2.06b	3.02a

Means followed by the same letter are not different at a probability level of 0.05 (*t* test)

(Table 4). Within each fungal species, all populations exhibited similar FB/DS in most environments (Table 4). Genotypic correlation coefficients between disease severity and FB concentration were all significant and varied from 0.98 to 0.94 for P319 and from 0.94 to 0.89 for P364 in 2007 and 2008, respectively.

Ratios between grain DON concentration and disease severity (DON/DS) after inoculation with *F. graminearum* depended on population and environment. L7310 × L7266 exhibited in both years lower DON/DS than those observed in the other two populations (Table 4). L7310 × L7266 also tended to exhibit lower ZEA/DS ratios in comparison to those of the other populations but differences were significant only in 2008 (Table 4). Genotypic correlation coefficients between disease severity and grain concentration of the mycotoxins produced by *F. graminearum* were not significant for DON in both years and significant for ZEA ($r_g = 0.92$), in 2008 only. Since differences among ratios between grain mycotoxin concentrations and visibly diseased ear areas were observed for *F. graminearum*, grain ergosterol concentration was measured in grain samples from plots inoculated with this fungus to assess how accurately visible symptoms estimate fungal biomass.

Positive associations between grain ergosterol concentration and ear area visibly diseased were observed in both years (r = 0.87 and 0.94 in 2007 and 2008, respectively) indicating that visible symptoms estimated the amount of disease quite accurately. Thus, at the same level of fungal invasion *F. graminearum* produced different ELISA-detectable mycotoxin concentrations depending on host genotype.

Discussion

Selection of S_5 inbreds after *Fusarium* inoculation was effective to improve ear rot resistance in the three maize populations. These results are consistent with previous reports indicating that inbred selection for resistance to *Fusarium* is more effective in later generations when genotypic variability reaches maximum values (Presello et al. 2005). Since resistant and susceptible pools consisted in composite samples of hybrids, screening in S_5 generation seems to be a valuable approach to develop parental inbreds of ear rot resistant hybrids. Selection for disease resistance to *F. proliferatum* caused indirect responses in disease resistance to the ear rots caused by *F. verticillioides* and *F. graminearum* indicating, consistently with previous reports (Presello et al. 2007; Löffler et al. 2010), that broad-based resistance to *Fusarium* exists. Thus, selection after inoculation of a single species should be effective to develop maize genotypes with improved resistance to prevalent ear rotting *Fusarium* pathogens.

Selection after inoculation of a low fumonisin producer isolate was effective to develop maize genotypes expressing resistance to field FB accumulation in some environments. High association between disease severity and FB concentration after inoculation with low and high toxigenic isolates belonging to Section *Liseola*, which was observed here and in previous reports (Presello et al. 2008), indicates that selection for disease resistance accounts for most of the variability for FB accumulation. Thus, since maize selection projects usually involve large inbred samples and accurate mycotoxin assessment is quite cost and time demanding, discarding inbreds by disease severity only might be worthwhile in early stages of selection.

Selection for disease resistance also seemed to cause indirect responses for DON and ZEA accumulation after inoculation with *F. graminearum* but grain mycotoxin concentration was affected by host population. Considering the same amount of disease, ELISA detected lower grain DON concentrations in pools from L7310 × L7266 in comparison to those observed in pools from the other two populations. Intra-specific variability for DON/DS ratio had been reported in some other plant species hosting *F. graminearum*, such as winter rye (Perkowski et al. 1995) and wheat (Muthomi et al. 2002), and results from this research indicate that is also available in maize. Since genotypic correlations between disease severity and grain DON concentration were not significant, phenotypic differences observed among populations for DON/DS might arise from specific maize genes affecting ability of *F. graminearum* to produce DON. Thus, assuming that genotypic variability for DON/DS ratio is available within maize populations, additional genetic progress would be achieved by including grain DON concentration as a selection parameter. Nevertheless, maize cells are able to metabolize the mycotoxin into products that can not be detected by standard ELISA or HPLC techniques (Sewald et al. 1992) and further research is needed to elucidate the specific mechanisms affecting grain DON/DS ratio in L7310 × L7266.

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References

Cruz CD (2006) Programa Genes: Análise multivariada e simulação. Editora UFV, Viçosa (MG) Brazil, p 175

De León C, Pandey S (1989) Improvement of resistance to ear and stalk rots and agronomic traits in tropical maize gene pools. Crop Sci 29:12–17

Gardiner DM, Kazan K, Manners JM (2009) Novel genes of *Fusarium graminearum* that negatively regulate deoxynivalenol production and virulence. Mol Plant Microbe Interact 22:1588–1600

Harris LJ, Desjardins AE, Plattner RD, Nicholson P, Butler G, Young JC, Weston G, Proctor RH, Hohn TM (1999) Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Dis 83:954–960

Iglesias J, Presello DA, Botta G, Lori GA, Fauguel CM (2010) Aggressiveness of Fusarium section *Liseola* isolates

causing maize ear rot in Argentina. J Plant Pathol 92: 205–211

Infostat (2002) Infostat Version 1.1. Grupo Infostat. F.C.A. Universidad Nacional de Córdoba, Argentina

Jardine DJ, Leslie JF (1999) Aggressiveness to mature maize plants of *Fusarium* strains differing in ability to produce fumonisin. Plant Dis 83:690–693

Leslie JF, Plattner RD, Desjardins AE, Klittich CJR (1992) Fumonisin B1 production by strains from different mating population of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 82:341–345

Löffler M, Kessel B, Ouzunova M, Miedaner T (2010) Population parameters for resistance to *Fusarium graminearum* and *Fusarium verticillioides* ear rot among large sets of early, mid-late and late maturing European maize (*Zea mays* L.) inbred lines. Theor Appl Genet 120:1053–1062

Miedaner T, Bolduan C, Melchinger AE (2010) Aggressiveness and mycotoxin production of eight isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for ear rot on susceptible and resistant early maize inbred lines. Eur J Plant Pathol 127:113–123

Muthomi JW, Oerke EC, Dehne HW, Mutitu EW (2002) Susceptibility of Kenyan wheat varieties to head blight, fungal invasion and deoxynivalenol accumulation inoculated with *Fusarium graminearum*. J Phytopathol 150:30–36

Perkowski J, Miedamer T, Geiger HH, Müller HM, Chelkowski J (1995) Occurrence of deoxynivalenol (DON), 3-Acetyl-DON, zearalenone, and ergosterol in winter rye inoculated with *Fusarium culmorum*. Cereal Chem 72: 205–209

Presello DA, Reid LM, Butler G, Mather DE (2005) Pedigree selection for *Gibberella* ear rot resistance in maize populations. Euphytica 143:1–8

Presello DA, Iglesias J, Botta G, Lori GA, Eyherabide GH (2006) Stability of maize resistance to the ear rots caused by *Fusarium graminearum* and *F. verticillioides* in Argentinean and Canadian environments. Euphytica 147: 403–407

Presello DA, Iglesias J, Botta G, Eyherabide GH (2007) Severity of Fusarium ear rot and concentration of fumonisins in Argentinian maize hybrids. Crop Prot 26:852–855

Presello DA, Botta G, Iglesias J, Eyherabide GH (2008) Effect of disease severity on yield and grain fumonisin concentration of maize hybrids inoculated with *Fusarium verticillioides*. Crop Prot 27:572–576

Reid LM, Hamilton RI, Mather DE (1996) Screening maize for resistance to gibberella ear rot. In: Tech Bull Publ 1996–5E. Agriculture and Agri-Food Canada, ON

Seitz LM, Mohr HE, Burroughs R, Sauer B (1977) Ergosterol as an indicator of fungal invasion in grains. Cereal Chem 54:1207–1217

Sewald N, Lepschy von Gleissenthall J, Schuster M, Müeller G, Aplin RT (1992) Structure elucidation of a plant metabolite of 4-desoxynivalenol. Tetrahedron Asymmetry 3:953–960

Stewart DW, Reid LM, Nicol RW, Schaafsma AW (2002) A mathematical simulation of growth of *Fusarium* in maize ears after artificial inoculation. Phytopathology 92:534–541