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Assessment of corn resistance to fumonisin accumulation in a broad collection of inbred lines

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ABSTRACT

Genetic improvement is an effective and environmentally safe method to reduce the levels of fumonisin mycotoxins in corn kernels infected with Fusarium verticillioides. In order to find new sources of resistance, a wide collection of corn inbred lines were evaluated for Fusarium ear rot and fumonisin accumulation after inoculation of the kernels with *Fusarium verticillioides*. Augmented designs were used for testing 240 un-replicated inbreds and 6 inbred checks in 2010 and 2011. Sixty-one inbreds were found to have the highest levels of resistance to Fusarium ear rot and fumonisin accumulation across years. Inbreds differing in kernel color, use, kernel type and heterotic group were all represented in this group of 61 inbreds. White corn inbreds had higher levels of fumonisin than yellow corn inbreds, but it was still possible to find white inbreds with comparable resistance to fumonisin accumulation to that of the most resistant yellow inbreds. Similarly, although the sweet corn inbreds evaluated in this study were less resistant to infection by *F. verticillioides* than the field corn inbreds, there were some which were grouped in the most resistant 61 inbreds. Many of these inbreds can be used to improve resistance to *F. verticillioides* infection and fumonisin accumulation by crossing the most resistant inbreds of each subgroup.

1. Introduction

Fusarium verticillioides is the most prevalent fungus found on corn in Spain (Butrón et al., 2006) and poses a feed and food safety problem because most *F. verticillioides* isolates are capable of producing the fumonisin mycotoxins (Abarca et al., 2000; de Oliveira Rocha et al., 2011; Tancic et al., 2012). These toxins are accumulated in corn kernels. Fumonisin toxicity is related to a capacity to disrupt the biosynthesis of sphingolipids, the main components of the plasmatic membrane of the cell, resulting in apoptosis and disturbances of cellular processes such as cell growth, and cell differentiation and morphology (SCF, 2000; Voss et al., 2007). In humans, fumonisins are suspected risk factors for esophageal cancer and neural tube defects (Bennett and Klich., 2003). The International Agency for Research on Cancer has classified fumonisins as probable carcinogens (IARC, 1993). In livestock, these toxins cause leukoencephalomalacia in horses, pulmonary edema in pigs, reduced growth in poultry and hepatic and immune disorders in cattle (Logrieco et al., 2003; Voss et al., 2007). Legislation to limit the amount of fumonisins in foods and feedstuffs has been implemented in many parts of the world (FAO, 2004). The European Union established threshold fumonisin contents of 4000 μg/kg in non-processed corn, 1000 μg/kg in corn intended for direct human consumption, 800 μg/kg in corn-based breakfast cereals and snacks, 200 μg/kg in corn-based products for infants and young children, 1400 μg/kg in milling fractions with particle size >500 μm, 2000 μg/kg in milling fractions with particle size <500 μm (1126/2007/EC, 2007), and recommends levels below 5000–50,000 μg/kg, depending on the animal, for livestock feed (576/2006/EC, 2006).

As genetic improvement is emerging as an effective and environmentally safe method to reduce the levels of fumonisins in corn (Eller et al., 2008), the search for sources of resistance has been the focus of many studies (Pascale et al., 2002; Kleinschmidt et al., 2005; Afolabi et al., 2007; Presello et al., 2007; Henry et al., 2009; Löffler et al., 2010). Kernel and silk channel inoculations mimic kernel infection vectored by insects and by spores deposited on silks by rain or/and wind, respectively. Of these, Schaasfsma et al. (2006) found a consistent correlation between Fusarium ear rot and fumonisin accumulation after inoculation by kernel wounding with *F. verticillioides* but not with channel inoculation. This study suggests that kernel inoculation would be more suitable inoculation technique to use when screening genotypes for resistance to fumonisin accumulation by using Fusarium ear rot disease severity as an indirect selection criterion. Partial resistance to infection by *F. verticillioides* and to the accumulation of fumonisins have been

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identified in several different types of corn germplasm (Clements et al., 2004; Afolabi et al., 2007; Presello et al., 2007; Henry et al., 2009; Löffler et al., 2010); however, until now, an exhaustive evaluation of the germplasm adapted to the environmental conditions of the southern European Atlantic Coast has not been carried out. The inbred collection maintained at the Misión Biológica de Galicia (Spanish Council of Scientific Research) is well adapted to the southern European Atlantic Coast and includes materials developed from Spanish landraces which may supply more rare alleles than other European landraces since Spain was the main entrance of corn to Europe from the Americas (Revilla et al., 2003). The purpose of the current study was: (1) to evaluate a collection of inbred lines from the Misión Biológica de Galicia in order to study the relationship between fumonisin accumulation and Fusarium ear rot in this adapted germplasm base; (2) to investigate the influence of kernel characteristics, such as color (white and yellow), type (dent and flint), use (popcorn, sweet corn and field corn), mutation (waxy, opaque), and heterotic group (European, Reid, Lancaster, Northern Flint, Minnesota No 13, other Corn Belt, and miscellaneous) on Fusarium ear rot and fumonisin accumulation; and 3), to identify sources of resistance for incorporation into a breeding program to develop resistance to *F. verticillioides* in this geographical region.

2. Materials and methods

2.1. Plant material and experimental design

Three hundred entries were evaluated for resistance to *F. verticilioides* at Pontevedra, Spain (42°24′ N, 8°38′ W, 20 m above sea level) using an augmented design in 2010 and 2011. This experimental design is suitable for screening large numbers of new and untried treatments (Federer, 2002). Two hundred and forty un-replicated inbreds were randomly assigned to ten blocks, while six inbred checks (A509, CO125, EP42, EP77, EP80, and PB130) were replicated, being assigned at random to plots within each of the ten blocks; therefore, each block comprised 24 un-replicated inbreds to be screened plus the six inbred checks. The checks were chosen on the basis of their differential performance under inoculation with *F. verticillioides* shown in preliminary evaluations: resistant A509 and EP42; intermediate EP77; susceptible CO125 and PB130 (unpublished data). Genotypes evaluated were organized with respect to use (field corn, popcorn or sweet corn), kernel color (white or yellow), kernel type (dent or flint), heterotic group for the field corn inbreds (European, Reid, Lancaster, Northern Flint, Minnesota No 13, other Corn Belt, and miscellaneous), and endosperm mutation (*waxy*, *opaque*, *su1*, *su1se1*, *sh2* or wild). The heterotic group called 'other Corn Belt' included all germplasm from the USA that was neither Reid, nor Lancaster, nor Northern Flint nor Minnesota No13. In each trial, estimates of recorded traits for the un-replicated inbreds were adjusted for block differences which were measured by the inbred checks (Petersen, 1985). Each entry was assigned to a row of 11 plants with 0.18 m spacing between plants and 0.8 m between rows.

This study was conducted at the southern Atlantic European Coast which has a unique climate with summer temperatures averaging around 23 °C and relatively mild spring, fall, and winter seasons; the region also experiences high rainfall (from 900 to 2000 mm a year), particularly in the fall-winter-spring period. In 2010 and 2011, the mean rainfall in the growing season (May to October) was 66 mm and 57 mm, respectively, while the mean temperature was 19 °C in 2010 and 18 °C in 2011. Some differences in the distribution of the rainfall should be noted as there was considerably less rainfall in August 2010 (7.2 mm) than in 2011 (120 mm).

The main corn borer in the Mediterranean area, *Sesamia nonagrioides* (Lef.), preferentially damages the stem and the shank of the plant and has been described as an important vector for *F. verticillioides* infection (Velasco et al., 2002, 2007; Avantaggiato et al., 2003). The relationship between kernel damage by *S. nonagrioides* and fumonisin content has been established (Avantaggiato et al., 2003). However, it is important to note that for our study in these particular environmental conditions, kernel damage by borers tended to be low.

2.2. Artificial inoculations and fumonisin determinations

Since natural infection cannot guarantee sufficient and homogeneous inoculum levels to adequately differentiate genotypic variation in resistance to *F. verticillioides* (Mesterhazy et al., 2012), we inoculated the plants using a kernel inoculation technique. It has been previously shown that with kernel inoculations the probability of identifying hybrids as resistant when they are actually susceptible in environments that favor fumonisin accumulation is low; although kernel inoculation may overcome low levels of natural resistance, such as that provided by tight husks (Kleinschmidt-DeMasters, 2009). In each row (genotype), approximately seven to 14 days after silking, all primary ears were inoculated with 2 ml of a spore suspension of *F. verticillioides*. Each genotype was inoculated according to its singular flowering date. This fungal isolate is an aggressive toxigenic isolate adapted to the local environment and previously isolated from a maize ear; the isolate is deposited in the Culture Collection of the Misión Biológica de Galicia (CSIC), Spain. The spore suspension contained 10^6 spores per ml and was injected into the center of the ear using a four-needle vaccinator which perforated the husks and injured three to four kernels (Reid et al., 1996). Ears from each row (genotype) were collected two months after inoculation and were individually rated for Fusarium ear rot using a seven-point scale (1 = no visible disease symptoms, 2 = 1–3%, 3 = 4–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–75%, and 7 = 76–100% of kernels exhibiting visual symptoms of infection, respectively) devised by Reid and Zhu (Reid and Zhu, 2005). Each

Table 1
Mean squares (MS) and degrees of freedom (df) of the analysis of variance of 245 corn inbred lines (240 un-replicated inbreds and five inbred checks) evaluated for *Fusarium* resistance in two years using an augmented design.

	Fumonisin						Fusarium ear rot					
	2010		2011		Across years		2010		2011		Across years	
	df	*MS*	*df*	*MS*	*df*	*MS*	*df*	*MS*	*df*	*MS*	*df*	*MS*
Block	9	485	9	4179			9	1.52*	9	1.10*		
Genotype (G)	222	4369**	221	17,076**	236	37,900**	218	1.48**	217	1.21**	234	1.81**
Year (Y)					1	3181					1	1.32
Y × G					207	22,579**					201	0.72
Error	35	557	34	4009	69	2283	35	0.70	34	0.42	69	0.56

* Significant at 0.05 probability levels.
** Significant at 0.01 probability levels.