Field Crops Research

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 *F. 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.

KEYWORDS: *Zea mays*, heterotic group, breeding, *Fusarium verticillioides,* fumonisin accumulation.

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 5000-50000 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 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 (42º24´ N, 8º38´ 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 ºC in 2010 and 18 ºC 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; Velasco et al., 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 genotype was harvested according to its particular inoculation date. After rating, ears were dried at 35 ºC for one week, shelled and a representative kernel sample of approximately 200 g was ground and stored at 4 ºC until further chemical analyses. Kernels were ground through a 0.75 mm screen in a Pulverisette 14 rotor mill (Fritsch GmbH, Oberstein, Germany).

Ground samples were sent to the Food Technology Department of the University of Lleida, Spain, for determination of total fumonisin content (fumonisins B_1, B_2, and B_3) using a commercial ELISA kit (R-Biopharm Rhône Ltd, Glasgow, Scotland, UK). This kit is a competitive enzyme immunoassay for quantification of fumonisin residues in corn. The recovery rate of the test was approximately 60% with a mean coefficient of variation of approximately 8%; specifities for B_1, B_2, and B_3 were 100%, approximately 40%, and approximately 100%, respectively, and the detection limit was 0.025 g kg^{-1}. Extraction and preparation of samples, as well as test performance, were carried out as described in the kits.

2.3. Statistical analysis

Individual analyses of variance were performed for each year as described by Petersen (2005) and Scott and Milliken (1993) with genotypes as fixed effects and blocks as random effects. The inbred check PB130 was removed from the data analysis due to its extremely high levels of fumonisins and a large standard error. The two-way analysis of random year effects and fixed genotype effects was performed with the least square mean estimates of treatments obtained for each year (Federer et al., 2001). Mean comparisons among genotypes were computed using Fisher's protected LSD.

In order to get a representation of the fumonisin data across years we performed a standardization using the z-score scaling: variables recalculated as (X - mean of X)/s, where "s" is the standard deviation. As a result, all variables in the data set have equal means (0) and standard deviations (1) but different ranges.

Genotypes were grouped according to their kernel color and type, mutation, use and heterotic group. Analyses of variance and mean comparisons were performed for studying genetic variability for fumonisin content and Fusarium ear rot among these groups. Spearman correlations coefficients between Fusarium ear rot and fumonisin accumulation were computed with the adjusted means for each genotype. All statistical analyses were performed with SAS (SAS, 2008).

3. Results and discussion

The combined analyses of variance across years detected significant differences among genotypes for Fusarium ear rot and fumonisin content (Table 1). The year x genotype interaction was significant for fumonisin content and was due to changes in genotype ranking rather than magnitude changes across years. For this reason, mean comparison among genotypes for fumonisin content was kept separate for each year, but comparisons for ear rot symptoms were combined across years. Since fumonisin quantification requires expensive chemical analyses and this data indicated that these analyses should be performed in several environments due to the large environment x genotype interaction, it would be advantageous if a simpler less costly trait that was genetically correlated to fumonisin content could be used as an indirect selection criterion for increasing resistance to fumonisin accumulation.

Adjusted means for block differences of each genotype evaluated in both years plus the genotype characteristics with respect to use, kernel color, kernel type, heterotic group of field corn inbreds, and endosperm mutation are summarized in Table 2. Fifty eight inbreds from the Misión Biológica de Galicia Bank (A188, A509, A556, A619, A619*o2*, A630, A635, A637, A638, A641, A652, A654, A662, A666, A670, A680, A681, A682, B93, B98, BP1, BPM2, C68, DS69, EP16, EP17, EP28, EP31, EP32, EP39, EP45, EP47, EP55, EP56, EP57, EP65, EP69, EP72, EP73, EP76, EP77, EP78, F557, F575, F7, G, H95, H99, Mo20W, Oh43, PB57, PB97, PB98, V642, W153R, W572, W64A*o2* and Z77016) plus 3 private inbreds (Private 2,3,and 5) exhibited the lowest values for fumonisin content in 2010 and 2011 and the lowest averaged values across years for Fusarium ear rot severity (Table 2, Figure 1). Groups were made according to the least significant difference obtained for each trait; resistant inbreds do not differ significantly from the best. Inbreds had to be included in the resistant group for the three traits (FB10, FB11 and ear rot) in order to be considered as resistant or susceptible. Two of the more resistant inbreds, A188 and A635, have also been evaluated in crosses with FR1064 (a susceptible inbred) along with another 1587 inbreds under silk inoculation with *F. verticillioides* and were among the most resistant to fumonisin accumulation and Fusarium ear rot (Clements and White, 2004; Clements et al., 2004). We also confirmed the susceptibility to fumonisin accumulation of the inbred Va35 that was previously evaluated in the southern United States under kernel inoculation with *F. verticillioides* (Henry et al., 2009). These 61 inbreds included genotypes differing for kernel color, use, kernel type and heterotic group. When analyses of variance was performed to check for differences among these characteristics with respect to *Fusarium* resistance, the difference between white and yellow kernels was significant at 0.10 probability level for fumonisin content, differences between dent and flint kernels were significant at 0.10 probability level for fumonisin content and Fusarium ear rot and the interaction of year x use was significant at 0.05 probability level for both fumonisin accumulation and Fusarium ear rot (Table 3a). White corn had ($p < 0.10$) higher levels of fumonisin than yellow corn (Table 4), but it was still possible to find white inbreds, such as A188, EP65, F557, F575, Mo20W and PB98, 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 (just for 2011), there were some, such as V642 (parentage Sugar & Gold ♂) and C68 [derived from the population Whipple Yellow (Harris)], which were grouped with the most resistant 61 inbreds (Table 3b). Other authors have already observed variability for resistance to infection by *F. verticillioides* among sweet corn genotypes (Headrick and Pataky, 1991; Nankam and Pataky, 1996). In contrast to results by Löffler et al. (2010), the flint inbreds evaluated in our study had significantly ($p < 0.10$) less fumonisin content and Fusarium ear rot than the dent genotypes. These differences between studies could be due to the different genotypes evaluated; Löffler et al. (2009), (2010) used elite breeding materials of the KWS SAAT AG, Einbeck, Germany, while we used mostly public inbreds that have been released by universities or other public institutions and may represent a greater diversity of germplasm. Among the flint inbreds in our study, those related to the corn landraces from southern Europe, such as EP31, EP32, EP39, EP45, EP65, PB57, PB98, F7, F557, and F575, seemed to be the most promising sources of resistance to fumonisin accumulation.

Differences among *waxy* and wild versions of the same inbred were not significant for either ear rot severity or fumonisin accumulation; however, *opaque* and wild versions of the same inbred differed significantly at the 0.10 probability level for fumonisin content (Table 4a). Although *waxy* inbreds tended to have higher levels of fumonisin than their wild counterparts, differences were not significant due to the high standard deviation associated with each mean as a consequence of the low number (four) of inbreds evaluated (Table 4b). Blandino and Reyneri (2007) also observed higher concentrations of fumonisins in *waxy* than in wild genotypes. *Waxy* corn contains only amylopectin and no amylose starch molecules unlike wild corn genotypes that contain both. It has been shown that amylopectin induces FB1 production in *F. verticillioides* (Bluhm and Woloshuk, 2005). *Opaque* inbreds had significantly ($p < 0.10$) less fumonisin accumulation than their corresponding wild versions (Table 4b). This result does not agree with results reported previously in which *o2* mutants were significantly more susceptible to *F. verticillioides* than their wild isolines (Lanzanova et al., 2009). Therefore, the effect of the *o2* mutant on fumonisin accumulation should be studied in more detail as well as the effect of other mutations which affect the protein profile of the corn kernel.

The Spearman correlation coefficients between fumonisin content and Fusarium ear rot were moderate and significant (r = 0.64, $p < 0.0001$, across years, r = 0.56, $p < 0.0001$, 2010 and 2011 respectively) (Table 5) supporting previous results that showed a better correlation between both traits after wound inoculation than under silk inoculation with .*F. verticillioides* (Schaafsma et al., 2006). Fusarium ear rot could be useful for screening multiple inbred lines in a single environment because this trait seemed stable across environments (Henry et al., 2009). Selected inbreds exhibiting resistance could then be tested for fumonisin accumulation in different environments. This breeding procedure would work well when applied to white kernel genotypes or genotypes belonging to the miscellaneous heterotic group because Spearman correlation coefficients between fumonisin content and Fusarium ear rot were high in both years in both of these groups; while selection for reduced Fusarium ear rot would not be a good indirect criterion for diminishing fumonisin content in popcorn and sweet corn.

In summary, significant differences for resistance to Fusarium ear rot and fumonisin accumulation were found among the genotypes evaluated in this study. Several of the inbreds could be used to develop new populations and inbred lines with improved resistance. If a pedigree breeding program was used, evaluations for Fusarium ear rot in a single environment could be done on early generations as this trait appeared stable across environments. This could be followed by evaluations of the selected advanced progenies for disease severity as well as the more costly and time consuming quantification of kernel fumonisin content in several environments. This study also showed that it is not necessary to transfer resistance from a given kernel color, type or use to another since acceptable levels of resistance were found in all of the different groups we studied. Finally, to take advantage of the adaptation and heterosis of the heterotic patterns ‘Reid Dent’ x ‘European Flint’ and ‘Lancaster Dent’ x ‘European Flint’, we propose to initiate a pedigree breeding program for obtaining field corn inbreds with the following crosses: EP31 x EP39 and F575 x EP65 (European Flint), B93 x Oh43 and A670 x H95 (Lancaster Dent) and A630 x A635 and A654 x A666 (Reid Dent).

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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	222	4369**	221	17076**	236	37900**	218	1.48**	217	1.21**	234	1.81**
Year (Y)					1	3181					1	1.32
Y x G					207	22579**					201	0.72
Error	35	557	34	4009	69	2283	35	0.70	34	0.42	69	0.56

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Table 2. Adjusted means for block differences of each inbred evaluated for *Fusarium* ear rot severity and fumonisin accumulation (FB) in 2010 and 2011and each inbred characteristic as related to use, kernel color of field corn inbreds, type of kernel of field corn inbreds, endosperm mutation and heterotic group of field corn inbreds. *Sixty one resistant inbreds to fumonisins are highlighted.

Inbred	**Color**	**Use**	**Type**	**Mutant**	**Heterotic group**	**FB(10) 2010**	**FB(11) 2011**	**Fusarium ear rot**
K305	White	Field corn	Dent	Wild	Lancaster	49.64	74.1	2.84
33-16	White	Field corn	Dent	Wild	Other Corn Belt	63.58	71.99	3.22
A188 *	White	Field corn	Dent	Wild	Other Corn Belt	-20.85	30.81	2.24
EP10	White	Field corn	Dent	Wild	Other Corn Belt	1677.66	59.92	4.89
EP81	White	Field corn	Dent	Wild	Other Corn Belt	113.22	204.86	4.57
H105W	White	Field corn	Dent	Wild	Reid	50.18	9.6	2.9
H106W	White	Field corn	Dent	Wild	Reid	1407.04	281.42	5.41
K301	White	Field corn	Dent	Wild	Reid	24.48	55.72	3.75
Mo20W *	White	Field corn	Dent	Wild	Unknown	25.49	29.61	2.33
EP64	White	Field corn	Flint	Wild	European	46.51	16.4	2.51
EP65 *	White	Field corn	Flint	Wild	European	6.67	8.69	2.15
EP66	White	Field corn	Flint	Wild	European	24.64	36.09	2.98
EP71	White	Field corn	Flint	Wild	European	18.28	201.63	3.49
F557 *	White	Field corn	Flint	Wild	European	20.05	32.74	2.41
F575	White	Field corn	Flint	Wild	European	7.86	6.89	2.23
PB40	White	Field corn	Flint	Wild	European	59.77	155.79	3.66
PB98 *	White	Field corn	Flint	Wild	European	-0.09	23.37	0.95
FP1	White	Field corn	nd*	Wild	Miscellaneous	42.5	64.03	2.54
EA3076	Yellow	Field corn	Dent	Wild	European	98.52	163.5	3.68
A295	Yellow	Field corn	Dent	Wild	Lancaster	10.12	54.59	2.94
A427	Yellow	Field corn	Dent	Wild	Lancaster	104.5	154.66	5
A619 *	Yellow	Field corn	Dent	Wild	Lancaster	24.57	28.5	2.41
A624	Yellow	Field corn	Dent	Wild	Lancaster	58.08	-19.63	2.71
A670 *	Yellow	Field corn	Dent	Wild	Lancaster	11.87	15.61	2
A671	Yellow	Field corn	Dent	Wild	Lancaster	70.12	41.64	2.21
A677	Yellow	Field corn	Dent	Wild	Lancaster	136.4	59.15	1.73
A682 *	Yellow	Field corn	Dent	Wild	Lancaster	28.14	38.87	2.26
B93 *	Yellow	Field corn	Dent	Wild	Lancaster	8.64	-51.02	1.57
C123	Yellow	Field corn	Dent	Wild	Lancaster	55.31	214.25	3.17
EP2	Yellow	Field corn	Dent	Wild	Lancaster	45.13	198.62	3.09
H95 *	Yellow	Field corn	Dent	Wild	Lancaster	10.77	27.5	2.07
H99 *	Yellow	Field corn	Dent	Wild	Lancaster	-0.98	44.06	0.77
Mo17	Yellow	Field corn	Dent	Wild	Lancaster	128.93	39.46	3.02
Oh43 *	Yellow	Field corn	Dent	Wild	Lancaster	11.18	33.38	1.47
Oh545	Yellow	Field corn	Dent	Wild	Lancaster	36.22	54.95	3.57
Va35	Yellow	Field corn	Dent	Wild	Lancaster	26.6	140.82	2.44

Private 3 *	Yellow	Field corn	Dent	Wild	Lancaster	-22.04	47.14	1.52
Private 4	Yellow	Field corn	Dent	Wild	Lancaster	586.82	196.31	4.84
Private 6	Yellow	Field corn	Dent	Wild	Lancaster	235.4	181	2.9
LH51	Yellow	Field corn	Dent	Wild	Lancaster	32.54	-8.49	2.54
A495	Yellow	Field corn	Dent	Wild	Minnesota n13	2.84	179.76	3.15
A509 *	Yellow	Field corn	Dent	Wild	Minnesota n13	2.94	20.55	1.89
W117	Yellow	Field corn	Dent	Wild	Minnesota n13	60.64	39.46	3.48
W153R *	Yellow	Field corn	Dent	Wild	Minnesota n13	28.15	40.42	1.6
W552	Yellow	Field corn	Dent	Wild	Minnesota n13	0.96	160.7	2.27
EP57*	Yellow	Field corn	Dent	Wild	Minnesota n13	33.87	5.37	1.85
A673	Yellow	Field corn	Dent	Wild	Miscellaneous	34.1	48.77	2.67
EP51	Yellow	Field corn	Dent	Wild	Miscellaneous	555.49	171.35	4.53
EP55 *	Yellow	Field corn	Dent	Wild	Miscellaneous	12.54	16.98	2.06
EP67	Yellow	Field corn	Dent	Wild	Miscellaneous	71.91	191.47	3.85
EP72 *	Yellow	Field corn	Dent	Wild	Miscellaneous	35.66	46.3	1.79
EP74	Yellow	Field corn	Dent	Wild	Miscellaneous	11.51	64.24	2.54
EP75	Yellow	Field corn	Dent	Wild	Miscellaneous	9.39	85.54	2.94
EP76 *	Yellow	Field corn	Dent	Wild	Miscellaneous	29.92	18.72	1.58
EP77 *	Yellow	Field corn	Dent	Wild	Miscellaneous	9.15	18.83	1.62
EP78 *	Yellow	Field corn	Dent	Wild	Miscellaneous	-17.24	51.06	2.38
Private 2 *	Yellow	Field corn	Dent	Wild	Miscellaneous	-19.03	12.4	2.19
A251	Yellow	Field corn	Dent	Wild	Other Corn Belt	32.57	15.67	2.84
A340	Yellow	Field corn	Dent	Wild	Other Corn Belt	14.41	40.83	3.61
A659	Yellow	Field corn	Dent	Wild	Other Corn Belt	102.78	31.57	3.11
A661	Yellow	Field corn	Dent	Wild	Other Corn Belt	5.01	38.09	2.72
A662 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	2.88	42.91	1.75
A73	Yellow	Field corn	Dent	Wild	Other Corn Belt	59.11	19.11	2.76
B87	Yellow	Field corn	Dent	Wild	Other Corn Belt	28.27	16.98	2.55
B97	Yellow	Field corn	Dent	Wild	Other Corn Belt	654.76	54.57	3.52
B98 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	8.79	-1.23	1.56
B9A	Yellow	Field corn	Dent	Wild	Other Corn Belt	0.9	56.31	2.75
CO106	Yellow	Field corn	Dent	Wild	Other Corn Belt	82.61	62.6	3.68
CO125	Yellow	Field corn	Dent	Wild	Other Corn Belt	18.14	153.84	3.09
CO328	Yellow	Field corn	Dent	Wild	Other Corn Belt	46.83	120.98	3.11
EP27	Yellow	Field corn	Dent	Wild	Other Corn Belt	3.99	144.13	3.03
EP28 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	12.45	58.05	1.93
EP29	Yellow	Field corn	Dent	Wild	Other Corn Belt	118.54	121.91	4.05
EP52	Yellow	Field corn	Dent	Wild	Other Corn Belt	26.92	45.81	2.97
EP56 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	9.35	10.71	1.86
G *	Yellow	Field corn	Dent	Wild	Other Corn Belt	44.51	37.6	2.17
W401	Yellow	Field corn	Dent	Wild	Other Corn Belt	2.13	19.55	2.61
A239	Yellow	Field corn	Dent	Wild	Reid	595.3	87.75	4.36
A554	Yellow	Field corn	Dent	Wild	Reid	14.04	47.14	2.67
A556 *	Yellow	Field corn	Dent	Wild	Reid	8.22	54.43	2.39
A630 *	Yellow	Field corn	Dent	Wild	Reid	23.34	-24.68	2.28

A632	Yellow	Field corn	Dent	Wild	Reid	100.07	73.36	2.38
A634	Yellow	Field corn	Dent	Wild	Reid	43.62	123.45	3.77
A635 *	Yellow	Field corn	Dent	Wild	Reid	11.76	15.35	2.33
A637 *	Yellow	Field corn	Dent	Wild	Reid	11.1	-51.01	2.03
A638 *	Yellow	Field corn	Dent	Wild	Reid	5.52	35.2	1.66
A639	Yellow	Field corn	Dent	Wild	Reid	22.61	215.31	3.38
A641 *	Yellow	Field corn	Dent	Wild	Reid	30.04	8.01	2.25
A652 *	Yellow	Field corn	Dent	Wild	Reid	26.32	97.96	2.16
A654 *	Yellow	Field corn	Dent	Wild	Reid	6.82	9.06	2.23
A657	Yellow	Field corn	Dent	Wild	Reid	550.79	163.18	3.12
A664	Yellow	Field corn	Dent	Wild	Reid	23.33	198.24	3.03
A665	Yellow	Field corn	Dent	Wild	Reid	10.57	63.26	2.49
A666 *	Yellow	Field corn	Dent	Wild	Reid	-23.2	27.25	1.96
A672	Yellow	Field corn	Dent	Wild	Reid	67.32	113.5	3.56
A675	Yellow	Field corn	Dent	Wild	Reid	4	16.62	3.52
A680 *	Yellow	Field corn	Dent	Wild	Reid	45.87	35.06	1.97
A681 *	Yellow	Field corn	Dent	Wild	Reid	34.84	55.2	1.68
B14A	Yellow	Field corn	Dent	Wild	Reid	286.53	101.78	2.95
B37	Yellow	Field corn	Dent	Wild	Reid	394.52	57.43	3.93
B73	Yellow	Field corn	Dent	Wild	Reid	492.8	112.88	2.64
B84	Yellow	Field corn	Dent	Wild	Reid	32.43	50.62	3.24
CM105	Yellow	Field corn	Dent	Wild	Reid	98.04	207.63	4.06
CM109	Yellow	Field corn	Dent	Wild	Reid	169.18	71.49	2.32
CM139	Yellow	Field corn	Dent	Wild	Reid	11.56	111.04	2.68
CM169	Yellow	Field corn	Dent	Wild	Reid	8.5	156.29	2.56
CM99	Yellow	Field corn	Dent	Wild	Reid	14.38	53.81	2.72
H84	Yellow	Field corn	Dent	Wild	Reid	66.75	-21.74	2.85
W182E	Yellow	Field corn	Dent	Wild	Reid	61.3	134.71	3.2
W22	Yellow	Field corn	Dent	Wild	Reid	313.56	104.67	3.8
W570	Yellow	Field corn	Dent	Wild	Reid	103.62	49.16	2.38
W572 *	Yellow	Field corn	Dent	Wild	Reid	29.38	116.49	1.93
W64A	Yellow	Field corn	Dent	Wild	Reid	29.72	191.52	2.45
Private 1	Yellow	Field corn	Dent	Wild	Reid	306.17	287.37	3.54
Private 7	Yellow	Field corn	Dent	Wild	Reid	93.22	50.57	2.86
Private 5 *	Yellow	Field corn	Dent	Wild	Reid	32.18	8.78	1.94
B52	Yellow	Field corn	Dent	Wild	Unknown	1520.65	95.72	4.78
GT114	Yellow	Field corn	Dent	Wild	Unknown	217.45	213.44	4.92
MS1334	Yellow	Field corn	Dent	Wild	Unknown	5.73	-2.6	2.58
EA2024	Yellow	Field corn	Flint	Wild	Argentinian Flint	30.91	54.15	2.71
EP31 *	Yellow	Field corn	Flint	Wild	European	6.17	-44.09	2.07
EP32 *	Yellow	Field corn	Flint	Wild	European	24.52	118.59	2.39
EP39 *	Yellow	Field corn	Flint	Wild	European	14.59	-14.83	1.97
EP42	Yellow	Field corn	Flint	Wild	European	40.24	73.13	3.8
EP43	Yellow	Field corn	Flint	Wild	European	229.26	108.3	3.56
EP45 *	Yellow	Field corn	Flint	Wild	European	23.74	73.06	1.98

EP53	Yellow	Field corn	Flint	Wild	European	49.39	110.42	3.28
EP80	Yellow	Field corn	Flint	Wild	European	25.6	86.77	3.18
F2	Yellow	Field corn	Flint	Wild	European	-22.74	42.27	2.89
F7 *	Yellow	Field corn	Flint	Wild	European	-3.15	5.57	1.27
PB97 *	Yellow	Field corn	Flint	Wild	European	15.36	6.57	1.37
Z77016 *	Yellow	Field corn	Flint	Wild	European	3.56	48.86	2.02
Z78007	Yellow	Field corn	Flint	Wild	European	35.94	23.26	2.8
PB57 *	Yellow	Field corn	Flint	Wild	European	-2.74	9.24	1.48
EP86	Yellow	Field corn	Flint	Wild	European	20.58	56.54	3.33
EP17 *	Yellow	Field corn	Flint	Wild	Miscellaneous	-2.62	47.46	1.62
EP18	Yellow	Field corn	Flint	Wild	Miscellaneous	9.09	160.7	2.11
EP19	Yellow	Field corn	Flint	Wild	Miscellaneous	49.64	49.58	3.4
EP68	Yellow	Field corn	Flint	Wild	Miscellaneous	19.93	78.03	2.65
EP69 *	Yellow	Field corn	Flint	Wild	Miscellaneous	8.36	29.48	2.24
EP73 *	Yellow	Field corn	Flint	Wild	Miscellaneous	1.3	33.25	2.04
EP79	Yellow	Field corn	Flint	Wild	Miscellaneous	2.44	351.07	3.66
EP16 *	Yellow	Field corn	Flint	Wild	Northern Flint	2.47	48.61	2.39
EP3	Yellow	Field corn	Flint	Wild	Northern Flint	3.14	-9.62	2.45
EP4	Yellow	Field corn	Flint	Wild	Northern Flint	58.94	-50.79	2.66
EP46	Yellow	Field corn	Flint	Wild	Northern Flint	53.16	59.78	2.5
EP47 *	Yellow	Field corn	Flint	Wild	Northern Flint	18.23	-39.42	2.08
TD16	Yellow	Field corn	nd*	Wild	Unknown	193.92	143.77	4.94
TD25	Yellow	Field corn	nd*	Wild	Unknown	281.78	55.7	4.43
BP1	--------	Popcorn	-------	Wild	-----------------------	10.28	39.42	1.57
BP2	--------	Popcorn	-------	Wild	-----------------------	32.72	122.78	4.43
BP3	--------	Popcorn	-------	Wild	-----------------------	84.13	43.26	2.12
BPM2 *	--------	Popcorn	-------	Wild	-----------------------	34.15	29.35	2.29
DS28	--------	Popcorn	-------	Wild	-----------------------	46.23	187.53	2.85
DS69 *	--------	Popcorn	-------	Wild	-----------------------	17.31	-14.99	2.37
DS91	--------	Popcorn	-------	Wild	-----------------------	93.09	19.9	3.72
C23*sh2*	--------	Sweet corn	-------	*sh2*	-----------------------	96.8	352.8	6.04
C40*sh2*	--------	Sweet corn	-------	*sh2*	-----------------------	33.65	417	2.7
EP84	--------	Sweet corn	-------	*sh2*	-----------------------	73.36	-22.04	3.53
EP85	--------	Sweet corn	-------	*sh2*	-----------------------	56.71	2.11	2.93
IL101t	--------	Sweet corn	-------	*sh2*	-----------------------	23.93	87.75	4.96
C13	--------	Sweet corn	-------	*su1*	-----------------------	64.41	164.11	4.2
C6	--------	Sweet corn	-------	*su1*	-----------------------	0.77	29.95	2.46
C68 *	--------	Sweet corn	-------	*su1*	-----------------------	-7.94	20.18	2.33
CO108	--------	Sweet corn	-------	*su1*	-----------------------	22.89	33.97	3.86
EP58	--------	Sweet corn	-------	*su1*	-----------------------	71.09	65.64	4.16
EP59	--------	Sweet corn	-------	*su1*	-----------------------	68.12	76.01	3.07
EP60	--------	Sweet corn	-------	*su1*	-----------------------	130.72	142.38	2.94
EP61	--------	Sweet corn	-------	*su1*	-----------------------	42.11	73.68	2.82
EP62	--------	Sweet corn	-------	*su1*	-----------------------	66.16	45.57	2.92
EP82	--------	Sweet corn	-------	*su1*	-----------------------	440.78	1057.38	5.41

EP83	--------	Sweet corn	-------	*su1*	------------------------	24.91	52.51	2.81
H3	--------	Sweet corn	-------	*su1*	------------------------	13.73	258.37	3.94
H5	--------	Sweet corn	-------	*su1*	------------------------	67.47	92.55	3.9
H6	--------	Sweet corn	-------	*su1*	------------------------	65.43	24.76	3.14
H7	--------	Sweet corn	-------	*su1*	------------------------	43.69	45.98	2.79
I453	--------	Sweet corn	-------	*su1*	------------------------	22.1	-36.37	2.83
I5125	--------	Sweet corn	-------	*su1*	------------------------	193.92	92.54	4.54
I5177	--------	Sweet corn	-------	*su1*	------------------------	133.77	542.83	4.87
I5492	--------	Sweet corn	-------	*su1*	------------------------	-3.13	64.48	2.81
P39	--------	Sweet corn	-------	*su1*	------------------------	-2.93	14.49	3.1
T244su	--------	Sweet corn	-------	*su1*	------------------------	28.25	89.6	2.702
TA21	--------	Sweet corn	-------	*su1*	------------------------	38.61	23.17	4.23
TA22	--------	Sweet corn	-------	*su1*	------------------------	25.58	-11.46	4.03
TA25	--------	Sweet corn	-------	*su1*	------------------------	8.13	102.65	2.89
TA26	--------	Sweet corn	-------	*su1*	------------------------	2.6	281.22	1.92
TA29	--------	Sweet corn	-------	*su1*	------------------------	4.26	88.13	5.06
TA30	--------	Sweet corn	-------	*su1*	------------------------	54.21	92.4	4.65
TD101	--------	Sweet corn	-------	*su1*	------------------------	9.26	59.04	3.59
V576	--------	Sweet corn	-------	*su1*	------------------------	47.54	59.81	4.92
V642 *	--------	Sweet corn	-------	*su1*	------------------------	13.43	-0.94	1.2
V663	--------	Sweet corn	-------	*su1*	------------------------	37.68	302.13	3.6
V679	--------	Sweet corn	-------	*su1*	------------------------	196.51	69.97	3.98
IL767b	--------	Sweet corn	-------	*su1se1*	------------------------	71.08	59.04	3.87
P51	--------	Sweet corn	-------	*su1*	------------------------	35.88	85.59	3.97
A619*o2* *	--------	Specialty	-------	*opaque*	Lancaster	39.06	9.91	2.32
Mo17*o2*	--------	Specialty	-------	*opaque*	Lancaster	43.72	16.49	3.14
EP3*o2*	--------	Specialty	-------	*opaque*	Northern Flint	13.23	54.11	3.43
EP4*o2*	--------	Specialty	-------	*opaque*	Northern Flint	11.91	47.93	2.69
EP5*o2*	--------	Specialty	-------	*opaque*	Other Corn Belt	42.18	51.94	3.22
A632*o2*	--------	Specialty	-------	*opaque*	Reid	28.03	10.42	2.94
B37*o2*	--------	Specialty	-------	*opaque*	Reid	84.13	53.2	2.7
W22*o2*	--------	Specialty	-------	*opaque*	Reid	58.72	84.14	2.9
W64A*o2* *	--------	Specialty	-------	*opaque*	Reid	29.33	3.41	2.42
C103*wx*	--------	Specialty	-------	*waxy*	Lancaster	468.4	811.92	5.12
Oh43*wx*	--------	Specialty	-------	*waxy*	Lancaster	56.84	-10.47	2.11
A632*wx*	--------	Specialty	-------	*waxy*	Reid	160.9	154.86	3.5
B37*wx*	--------	Specialty	-------	*waxy*	Reid	55.38	163.5	3.27
LSD for checks (0.05)						21.43	57.54	0.5
LSD for new genotypes (0.05)						71.1	190.84	1.67
LSD check *vs* new genotypes (0.05)						55.05	147.83	1.3

*n.d.= not determined.

[1] recorded in 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).

Table 3a. Mean squares (MS) and degrees of freedom (df) of the analyses of variance of corn kernel colors (C: white and yellow), uses (U: field corn, popcorn and sweet corn), kernel types (T: dent and flint) and heterotic groups (G: European, Reid, Lancaster, Northern Flint, Minnesota No 13, other Corn Belt, and Miscellaneous) for fumonisin content and Fusarium ear rot severity recorded in years 2010 and 2011.

	Fumonisin		**Fusarium ear rot**			**Fumonisin**		**Fusarium ear rot**	
	df	***MS***	***df***	***MS***		***df***	***MS***	***df***	***MS***
Year (Y)	1	169921**	1	5.62**	**Year (Y)**	1	12037	1	0.58
Color (C)	1	94281*	1	2.51	**Use (U)**	2	13336	2	23.90
Y x C	1	88512	1	1.30	**Y x U**	2	105760**	2	5.07**
Error	303	32764	300	1.17	**Error**	399	31518	393	1.22
	df	***MS***	***df***	***MS***		***df***	***MS***	***df***	***MS***
Year (Y)	1	41705	1	4.15†	**Year (Y)**	1	3213	1	1.05
Type (T)	1	104785*	1	3.85 *	**Group (G)**	6	23797	6	1.04
Y x T	1	11089	1	0.01	**Y x G**	6	17076	6	0.75
Error	269	34835	266	1.11	**Error**	276	27976	273	1.12

*, **, Significant at 0.10 and 0.05 probability levels, respectively.

Table 3b. Least square means ± standard deviations of groups of corn inbreds representing different kernel colors (white and yellow), kernel types (dent and flint) and uses (sweet corn, field corn and popcorn) evaluated in 2010 and 2011 for fumonisin content and Fusarium ear rot severity.

	Year	**Fumonisin mg/kg**	**Fusarium ear rot (1-7)[1]**
Color			
White	**Across**	133± 29 a[2]	------
Yellow	**years**	81 ± 11 a	------
Type			
Dent	**Across**	96 ± 13 a[2]	2.8 ± 0.1a[2]
Flint	**years**	51 ± 22 a	2.5 ± 0.1 a
Use			
Sweet	**2010**	58 ± 28 b	3.4 ± 0.2 b
Field		103 ± 14 ab	2.9 ± 0.1 c
Popcorn		45 ± 67 ab	2.7 ± 0.4 bcd
Sweet	**2011**	139 ± 27 a	3.9 ± 0.2 a
Field		71 ± 14 b	2.6 ± 0.1 d
Popcorn		61 ± 67ab	2.9 ± 0.4 bcd

Within each column, means followed by the same letter did not significantly differ at 0.05 probability level.

[1] Recorded in 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.

[2] Within this group, means significantly differed at 0.10 probability level.

Table 4a. Mean squares (MS) and degrees of freedom (df) of the analyses of variance of *waxy vs* wild versions of the same inbreds and *opaque vs* wild versions of the same inbreds evaluated for fumonisin content and Fusarium ear rot severity in years 2010 and 2011.

	Fumonisin		**Fusarium ear rot**	
waxy	***df***	***MS***	***df***	***MS***
Year (Y)	1	2770	1	0.24
Mutant (M)	1	26036	1	1.24
Y x M	1	16665	1	0.07
Error	11	59803	11	2.21
opaque				
Year (Y)	1	13099	1	2.78 *
Mutant (M)	1	25326†	1	0.04
Y x M	1	10892	1	0.14
Error	28	6947	28	0.68

* Significant at 0.10 probability level.

Table 4b. Least square means ± standard deviations of corn mutants evaluated in 2010 and 2011 for fumonisin content and Fusarium ear rot severity.

Inbred version	**Fumonisin mg/kg**	**Fusarium ear rot (1-7)[1]**
waxy	233 ± 86 a	3.5 ± 0.5 a
Wild	149 ± 93 a	2.9 ± 0.6 a
opaque	37 ± 21 b	2.8 ± 0.2 a
Wild	93 ± 21 a	2.9 ± 0.2 a

Within each column, means followed by the same letter did not significantly differ at 0.10 probability level.

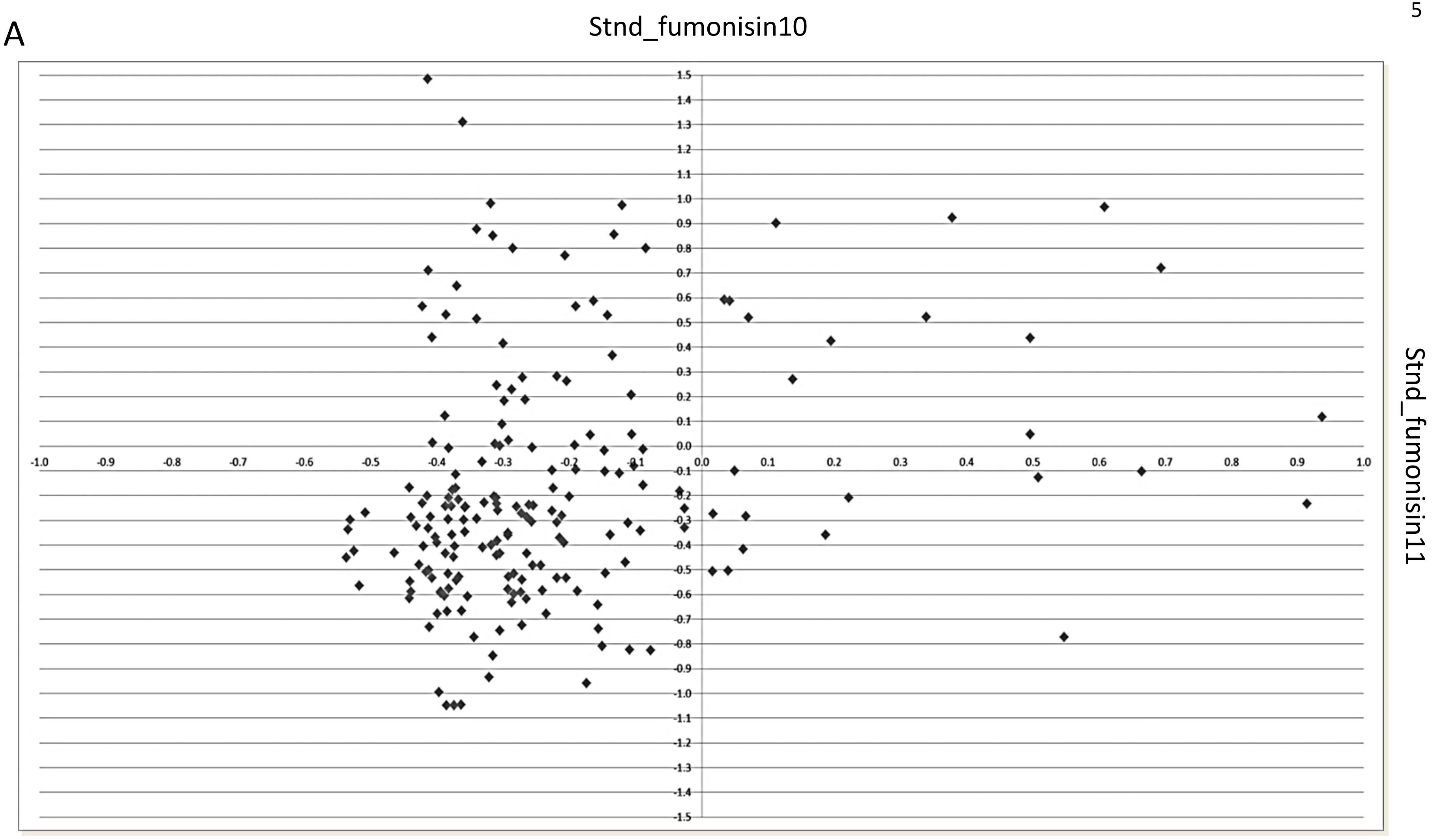
[1] Recorded in a seven-point scale 1= no visible disease symptoms, 2 =1-3 %, 3 = 4-10 %, 4 = 11-25 %, 5 = 26-50 %, 6 = 51-75 %, and 7 = 76-100 % of kernels exhibiting visual symptoms of infection, respectively.

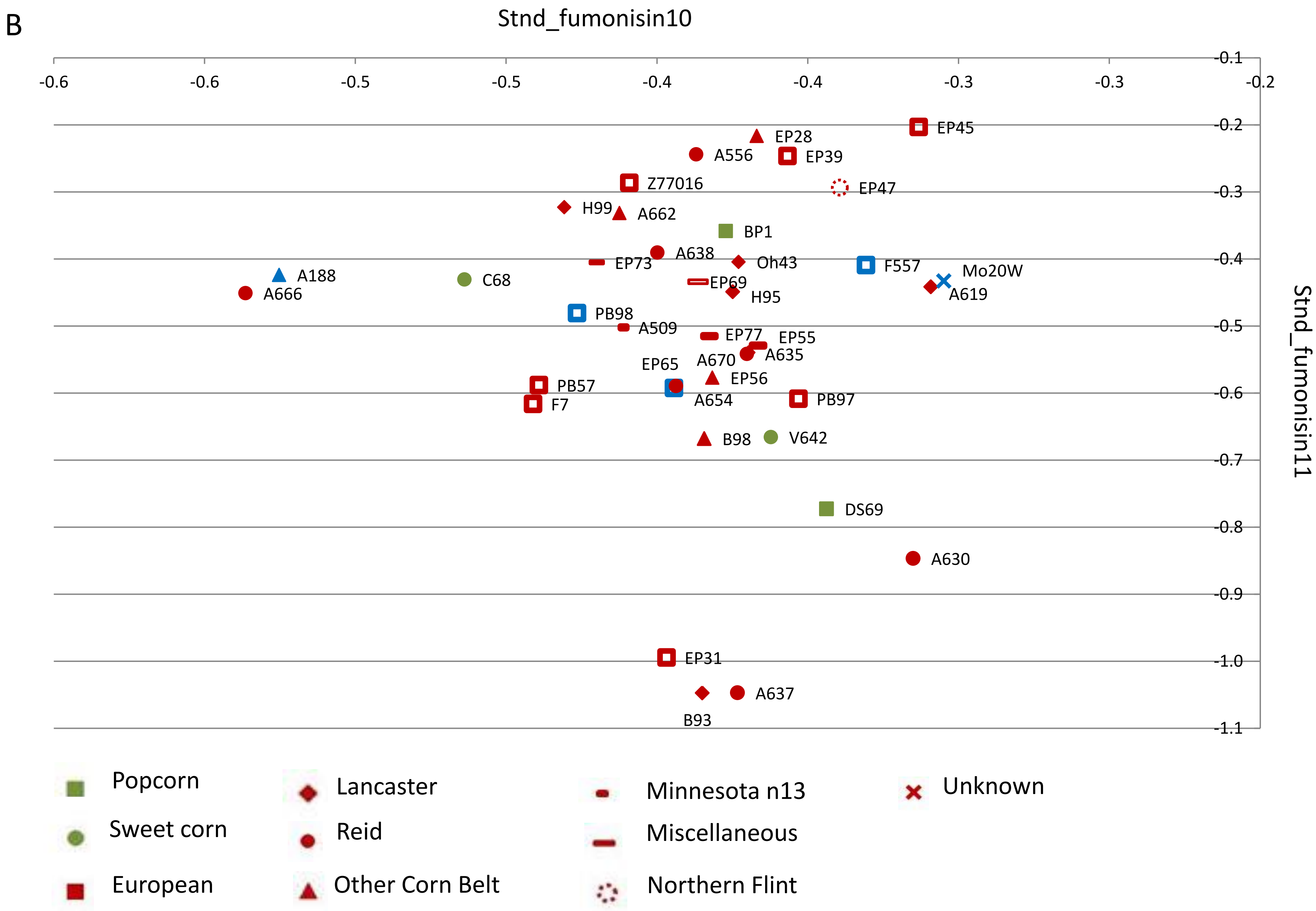
Table 5. Spearman correlation coefficients (r) between Fusarium ear rot severity and fumonisin content in corn inbreds evaluated in two years under inoculation with *Fusarium verticillioides* and belonging to different groups with respect to kernel color (white and yellow) and type (dent and flint), use (field corn, popcorn and sweet corn) and heterotic group (European, Reid, Lancaster, Other Corn Belt, and Miscellaneous). The number of inbreds (N) used for computing each correlation coefficient is indicated.

	All inbreds	**White kernel**	**Yellow kernel**	**Field corn**	**Popcorn**	**Sweet corn**	**Dent kernel**	**Flint kernel**	**Other Belt**	**European**	**Lancaster**	**Reid**	**Miscellaneous**
2010													
r	0.56**	0.73**	0.62**	0.62**	0.70	0.46**	0.63**	0.72**	0.64**	0.73**	0.61**	0.61**	0.79**
N	219	19	132	151	7	39	99	35	23	27	23	44	17
2011													
r	0.56**	0.78**	0.63**	0.65**	0.67	0.50**	0.67**	0.49**	0.56**	0.58**	0.79**	0.59**	0.76**
N	219	19	131	150	7	41	100	34	24	25	25	43	16

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Figure 1. Standardized distributions of the fumonisin contamination in 2010 and 2011. A) Representation of the whole array of inbred lines evaluated. B) Enlarged area representing the genotypes resistant and stable in both years (limited to the genotypes with negative standardized values in both years and included in the resistant group). Kernel color: white (in blue) and yellow (in red); kernel type: dent (solid fill) and flint (unfilled); special corn in green. Heterotic group and type of special corn in the figure B legend.




B
Stnd_fumonisin10
Stnd_fumonisin11
-0.6
-0.6
-0.5
-0.5
-0.4
-0.4
-0.3
-0.3
-0.2
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
EP45
EP28
A556
EP39
Z77016
EP47
H99
A662
BP1
A638
EP73
Oh43
F557
Mo20W
A188
C68
EP69
A666
H95
A619
PB98
A509
EP77
EP55
A635
A670
EP65
EP56
PB57
A654
PB97
F7
B98
V642
DS69
A630
EP31
A637
B93
Popcorn
Sweet corn
European
Lancaster
Reid
Other Corn Belt
Minnesota n13
Miscellaneous
Northern Flint
Unknown