Plant Breeding

Short communication

Gibberella stalk rot (*Fusarium graminearum* Schwabe) resistance of maize inbreds and their F₁ hybrids and their potential for use in resistance breeding programs.

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Abstract

Fusarium graminearum Schwabe is one of the predominant fungal species responsible for stalk rot of maize in Canada and many other countries. Adapted sources of resistance to this disease are scarce but recently a few have been identified. To evaluate the usefulness of these sources in a breeding program to improve stalk rot resistance, a complete diallel cross of five inbreds of varying susceptibility to stalk rot was conducted. Artificial inoculations of stalks were accomplished by injecting a conidial suspension into the first internode above the uppermost aerial root node. Stalk rot ratings, yield, and lodging in the parents, crosses and reciprocals were evaluated over two field seasons. There were significant differences between inbred lines and hybrids for stalk rot ratings. Inbreds CM174 and CO325 were highly susceptible, whereas inbreds 73405 and 73353 were classified as resistant and both had good general combining ability. Those inbreds could be used as donors of favourable alleles in breeding programs for *Gibberella* stalk rot resistance.

Keywords: *Zea mays*, *Fusarium graminearum*, stalk rot resistance, yield, diallel cross combining ability.

Introduction

The most frequently reported causative agents of stalk rot of maize (*Zea mays* L.) are fungi of the genus *Fusarium* (Kommendahl and Windels, 1981). *Fusarium graminearum* Schwabe [sexual stage: *Gibberella zae* (Schwein.) Petch] is only one of many species responsible for stalk rot, but it is considered to possess the highest pathogenicity (the ability to cause disease) and aggressiveness (the amount of disease caused) (Gilberton et al. 1985; Snijders, 1994).

Resistance to the spread of *F. graminearum* within the host has been identified after artificial inoculation of ears and stalks (Reid et al. 1996; Reid and Zhu 2005). Resistance to infection in the ears is polygenic and inherited in a mainly additive fashion (Hart et al. 1984; Gendloff et al. 1986; Chiang et al. 1987; Reid et al. 1992). Among the non-additive components, dominant gene action was noticeable (Reid et al. 1992). For infection in the stalk, most of the studies were focused on *Fusarium* stalk rot (*F. verticillioides*), and suggested the same inheritance pattern as that found in the ears: few genes involved mainly showing additive effects, although there was some evidence for dominance (Lal and Singh, 1984; Barrière, 1985). There is also evidence in some genotypes of a single dominant gene, *Rfg1*, responsible for *F. graminearum* stalk rot resistance (Yang et al., 2004).

Due to the scarcity of adapted sources of resistance to stalk rot, it is important to continue the search for new sources. Recently, we identified two inbreds, 73405 and 73353, with high levels of resistance to *F. graminearum* stalk rot under inoculated field conditions. The objectives of the current study was to determine the usefulness of these inbreds in future breeding programs by: (i) estimating the general combining ability (GCA), specific combining ability (SCA), and reciprocal effects for stalk rot resistance in

a complete diallel analysis of these two inbreds plus three others; and (ii), to evaluate the effects of stalk rot infection on yield and lodging in the five parents and their F₁ hybrids.

Materials and methods

Three maize inbred lines (CM174, CO325, and CO354) from Agriculture and Agri-Food Canada (AAFC) and two inbreds (73353 and 73405) from Cornell University were crossed in a complete 5 x 5 diallel in 2004. Lines 73353 and 73405 were chosen to represent the most resistant genotypes currently available to public corn breeders in this region. All possible F₁ single crosses, including reciprocals, and inbred parents were evaluated for resistance to stalk rot, yield and lodging in a randomized complete block design with three replicates in 2005 and 2006 at the Central Experimental Farm (AAFC). The main plot was a 3.5 m long row separated by 0.7 m and sown with 15 seeds per row.

Inoculum production followed the protocol developed by Reid et al. (1996) using three local DON-producing isolates, DAOM 180378, DAOM 194276 and DAOM 212678 obtained from the National AAFC Culture Collection. Inoculations were conducted 4-7 days after silk emergence following the protocol developed by Reid and Zhu, 2005. The central 10 plants of each 15-plant row were inoculated.

At harvest in mid-October, the ears were hand-harvested and used to calculate the yield in tones per hectare (t ha⁻¹). The number of broken or lodged plants was also recorded for each plot. The stalk of each inoculated plant was then split by hand and the severity of stalk rot infection was recorded using a rating scale where: 1 = no visible spread of the pathogen from inoculation point; 2 = 1- 25 %; 3 = 26- 50 %; 4 = 51- 75 %; 5 = > 75% of inoculated internode symptomatic; 6 = symptoms have spread to one adjacent internode; and, 7 = symptoms have spread to two or more adjacent internodes (Reid and Zhu, 2005). Plants were rated individually for stalk rot severity, and a mean rating was calculated for each plot.

Analysis of variance (ANOVA) was performed with PROC GLM (SAS Institute, 2004) considering replication, years and corresponding interactions as random effects and the remaining variables as fixed effects. In the combined analysis of variance for each trait, means squares of genotypes were partitioned into components due to inbred lines, hybrids, and inbreds vs hybrids. Variation among hybrids of the diallel was further partitioned into GCA, SCA, and reciprocal effects. Griffing's Method 3, Model I (fixed effects) (Griffing, 1956) was used to determine GCA, SCA, and reciprocal effects for stalk rot ratings, yield, and lodging. The diallel was analyzed using a program developed by Zhang and Kang (1997). The standard errors for GCA, SCA, reciprocal effects, and differences among GCA and SCA effects were calculated according to Griffing (1956). For each trait, simple correlations were calculated between GCA effects for each inbred and its performance *per se*.

Results and discussion

Inbreds and hybrids exhibited significant differences for stalk rot ratings when inoculated with the macroconidial suspension of *F. graminearum*. The inbreds 73405 and 73353 were resistant inbreds, followed by CO354 as intermediate and two highly susceptible genotypes, CO325 and CM174 (Table 1). The results were as expected according to previous evaluations.

Hybrid combinations of any parental line with the 73405 exhibited higher resistance, while most of hybrid combinations with the inbred line CM174 exhibited higher susceptibility (Table 1). There were one highly resistant hybrid, 73353 x CO325, with a mean stalk rot rating (3.33) lower than the mean of any of the resistant inbreds. The hybrid x year interaction was significant suggesting that differences among hybrids varied across years; however, these differences, mainly focused in the GCA, were due to a wider spread of disease severity ratings recorded in 2006 than in 2005 rather than changes in genotype rankings between years (data not shown).

The hybrid effects for the mean stalk rot ratings were subdivided into SCA and GCA effects, both were significant. SCA effects explained 12% of the sum of squares due to hybrids, while GCA effects explained 69%, showing that for this set of hybrid most of variation for stalk rot was controlled by genes with additive gene action as opposed to other types of gene action (dominance, epistasis, overdominance, pseudo-overdominance, etc), making this trait highly heritable. Similar results were found for Gibberella ear rot and Fusarium stalk rot (Reid et al. 1992; Donahue, 1989). Heritability could be estimate in the current study considering the parents as a sample of genotypes from a reference population (random effects), in this sense we get a medium heritability $h=0.55$ that could be manage by diverse breeding methods (Hallauer and Miranda, 1981).

There were neither significant reciprocal effects nor significant year x reciprocal interaction for stalk rot ratings. This means that for the genotypes studied, the maternal effects were not important in the inheritance of resistance to stalk rot under these inoculated conditions although maternal effects could have importance in the emergence and stand counts as it has been pointed out for *F. moniliforme* by Lunsford et al. (1975).

GCA and SCA negative effects are associated with resistance (lower stalk rot ratings) and positive effects are associated with susceptibility (higher stalk rot ratings). As expected, the two most resistant inbreds, 73353 and 73405, showed significant negative GCA effects for stalk rot with scores (Table 1). The correlation between the GCA effects and the inbred lines disease ratings was $r = 0.87$, which was significant at the 0.05 level. This indicates that these inbred lines, especially 73353, would make good parents in a maize improvement program that stresses stalk rot resistance. In contrast, the inbred CM174 showed the highest positive values for GCA (associated with susceptibility) in agreement with the high susceptibility of its hybrids (Table 1) indicating that this inbred when crossed to other inbreds results in the worst combinations in relation to severity ratings. Some hybrids obtained from the five inbreds lines performed significantly better or worse than expected based on the GCA of their parents. For example, 73353 x 73405, CM174 x CO325 and CO354 x 73353 showed higher severity ratings than those expected, while CO325 x CO354 and CO325 x 73353 had less disease than expected.

No significant differences were found among hybrids for yield in the combined analyses (2 years) due to a significant genotype x year interaction, thus individual analyses were computed. There were significant differences among GCA and SCA effects for yield under inoculation with *F. graminearum* in 2005 (Table 1). As the

191 contribution of SCA effects to hybrid differences for yield was greater than that of GCA
192 effects, there was not any inbred line that performed significantly better than any other in
193 hybrid combination.

194 Percentage of lodging was important in the inbred line CM174 (60%), and
195 noticeable in the inbred CO325 (3%), and this unfavorable characteristic was inherited by
196 their hybrids (data not shown). The lodged plants cannot be harvested by the combines
197 resulting in a large negative impact on yield; however, in the present study, we could not
198 determine the contribution of lodging to yield loss because ears were hand harvested.
199 Precise yield loss data for most stalk rots is difficult to ascertain. Nevertheless, lodging,
200 which results from stalk rot, can have such an impact on harvest losses that many plant
201 pathologists consider stalk rots to be the most significant yield limiting disease of maize
202 (Thomison and Paul, 2006).

203 Some inferences can be made on the potential use of the evaluated inbreds for
204 developing new inbreds with resistance to *Gibberella* stalk rot. First, the use and
205 improvement of resistant genotypes for reducing stalk rot incidence is advisable. Second,
206 the most resistant lines 73353 and 73405 had the largest negative GCA and little SCA
207 effects, meaning that they will produce resistant F₁'s with most other parents. Finally,
208 those inbreds could be used as donors of favourable alleles in breeding programs for
209 *Gibberella* stalk rot resistance.

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Table 1. Means, general combining ability effects (GCA inbreds section) and specific combining ability effects (SCA hybrids section) for disease ratings and yield in the diallel cross with reciprocals evaluated in 2005 and 2006 [†].

Genotypes	Stalk rot ratings (1-7)		Yield (t ha ⁻¹)			
	2 years	2 years	2005	2005	2006	2 years
<i>Inbreds</i>	Means	GCA [‡]	Means	GCA ^{‡‡}	Means	Means
CM174	7.00a	0.69*	3.57a	0.23	6.08ab	4.71a
CO325	6.85a	0.15	2.88a	-1.00*	4.53bc	3.94a
CO354	5.41b	0.36	4.60a	0.87*	6.84a	5.61a
73353	4.18c	-0.66*	2.22a	0.30	1.82d	1.65b
73405	3.98c	-0.54*	††	-0.40	3.42cd	1.87b
LSD (P ≤ 0.05)	0.54				1.66	1.90
<i>Hybrids</i>	Means	SCA [‡]	Means	SCA ^{‡‡}	Means	Means
CM174 x CO325	6.47a	0.72*	8.79fg	0.02	12.15a	10.18a
CM174 x CO354	5.90ab	-0.11	11.99ab	-0.35*	13.20a	12.97a
CM174 x 73353	4.71d-g	-0.17	10.30b-f	-1.05*	10.07a	10.04a
CM174 x 73405	4.43e-g	-0.24	12.54a	1.38*	11.57a	10.86a
CO325 x CM174	6.38a	0.72*	9.54d-g	0.02	10.03a	10.50a
CO325 x CO354	5.37b-d	-0.62*	11.38a-d	1.16*	15.21a	12.87a
CO325 x 73353	4.14f-h	-0.44*	10.39b-f	1.04*	12.18a	11.15a

310	CO325 x 73405	4.87c-g	0.13	7.57g	-2.22*	11.49a	9.53a
311	CO354 x CM174	5.72a-c	-0.11	12.18ab	-0.35*	13.37a	12.56a
312	CO354 x CO325	4.92c-f	-0.62*	11.24a-d	1.16*	13.23a	11.70a
313	CO354 x 73353	5.12b-e	0.41*	9.86c-f	-0.81*	11.06a	10.24a
314	CO354 x 73405	4.49d-g	-0.06	11.99ab	0.01	11.61a	11.48a
315	73353 x CM174	4.74d-g	-0.17	11.51a-d	-1.05*	12.75a	10.89a
316	73353 x CO325	3.33h	-0.44*	10.39b-f	1.04*	12.77a	11.05a
317	73353 x CO354	4.85c-g	0.41*	11.63a-c	-0.81*	10.46a	11.57a
318	73353 x 73405	4.00gh	0.37*	11.63a-c	0.83*	11.16a	11.14a
319	73405 x CM174	4.72d-g	-0.24	12.19ab	1.38*	12.43a	11.71a
320	73405 x CO325	4.35e-g	0.13	9.04e-g	-2.22*	11.24a	10.52a
321	73405 x CO354	4.76d-g	-0.06	10.90a-e	0.01	14.51a	13.30a
322	73405 x 73353	4.07f-h	0.37*	10.31b-f	0.83*	11.97a	11.25a
323	LSD(P ≤ 0.05)	0.89		2.04			

324 Means within a column and group followed by the same letter are not significantly
325 different at 0.05 level of probability..

326 †For stalk rot ratings, GCA and SCA effects were computed with data across years; while
327 data from year 2005 were used for yield (significant differences for hybrids just in this
328 year).

329 * GCA (general combining ability) or SCA (specific combining ability) estimate differed
330 significantly from zero. Significance at 0.05 level of probability.

331 [‡]LSD (GCA) = 0.66; LSD (SCA_{ij}-SCA_{jk}) = 0.47; LSD (SCA_{ij}-SCA_{kl}) = 0.33

332 ^{‡‡}LSD (GCA) = 0.79; LSD (SCA_{ij}-SCA_{jk}) = 1.03; LSD (SCA_{ij}-SCA_{kl}) = 0.73

333 ^{††}Yield for the inbred 73405 could not be estimated due to the higher moisture in the ears
334 during the 2005 harvest time.