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Ergot Response for the Sorghum Genotype IS8525

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Abstract: Experiments were conducted during the 2002 and 2003 growing season at Texas A and M Agricultural Research Farm near College Station, TX to determine the response of two germplasm lines derived from IS8525 (juicy midrib = IS8525J and dry midrib = IS8525D) to ergot infection. Ergot inoculation was conducted at different flowering stages with bagging versus non-bagging of inoculated panicles to determine the disease response of IS8525. On IS8525J, bagged panicles had mean ergot severity of 21.3% compared to 1.8% for non-bagged panicles. Mean ergot severity for bagged panicles for IS8525D was 3.9% and non-bagged was 1.0%. The stage of anthesis at the time of inoculation also affected the severity of the disease with the highest level of infection occurring when panicles were inoculated at 25% flowering. Ergot severity decreased at later flowering stages. Bagging of the inoculated panicles at the beginning of anthesis generally increased ergot severity on both lines. In both years, IS8525J was more susceptible to ergot than IS8525D. Male-sterile hybrids derived from crosses between the two IS8525 lines with A_2T×623 were evaluated in 2004 and found to be highly susceptible to ergot. These results suggest that the tolerance of IS8525 is due to floral morphological characteristics and the flowering pattern.

Key words: *Claviceps africana*, sorghum ergot, sugary disease

INTRODUCTION

Globally, three *Claviceps* species cause ergot in sorghum. *Claviceps africana* Frederickson, Mantle and de Milliano is the most prevalent occurring throughout the Americas, Australia, Asia and Africa, while *C. sorghi* P. Kulkarni *et al.* is limited to Asia and *C. sorghicola* Tsukiboshi, Shimanuki and Uematsu is confined to Japan (Bandyopadhyay *et al.*, 1998; Pažoutová *et al.*, 2000; Tooley *et al.*, 2000). The species *C. africana* and *C. sorghi* share the same anamorph *Sphacelia sorghi*, which is different from that of *C. sorghicola* (Mantle and Bogo, 2002). The different species also are distinguished by several morphological features such as color, size and texture of the sclerotia and stromata and in their nucleotide sequences of transcribed spacer 1 and 5.8S rDNA (Frederickson *et al.*, 1991; Pažoutová *et al.*, 2000; Tsukiboshi *et al.*, 2001).

Sorghum ergot or sugary disease was confined to Asia and Africa until 1995 when it was diagnosed in Brazil (Casela *et al.*, 1999). Subsequently, the disease was found in sorghum in Australia in 1996 (Ryley and Henzell, 1999) and in the US in 1997 (Isakeit *et al.*, 1998). Presently, ergot is found in major sorghum growing regions in the US. Because the pathogen infects unfertilized ovaries, it poses a serious threat to the hybrid seed production industry. Male-sterile sorghum lines used in hybrid seed production are highly susceptible when pollination is delayed due to environmental or genetic factors (Futrell and Webster, 1965; Bandyopadhyay *et al.*, 1998). Yield losses due to ergot in hybrid seed production fields ranged from 10-80% in India and between 12-25% in Zimbabwe (Sangitrao *et al.*, 1999; Bandyopadhyay *et al.*, 1998). In addition to the reduction in yield, the sugary exudates produced by the pathogen reduces seed quality and makes harvesting and seed processing much more difficult (Bandyopadhyay *et al.*, 1998).

The presence of ergot in major sorghum growing regions of the world has increased the interest in developing control strategies. Chemical control through the use of fungicides has been inconsistent and it is not economically feasible for low input agricultural production (de Almeida *et al.*, 1999; Prom and Isakeit, 2003). Pollen management strategies in hybrid seed production have been helpful but cannot eliminate the problem due to environmental variation (Bandyopadhyay *et al.*, 1998). Ultimately, host plant resistance would be the best approach for controlling the disease.

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Ergot resistance sources have been reported by a number of researchers. Reed *et al.* (2002a) reported that IS14131 and IS14257 may possess genes for resistance based on evaluation of the resulting progeny when these lines were crossed with male-sterile cytoplasm. McLaren (1992) noted that SD1/91, RTAM428 and 28 other sorghum lines exhibited varying levels of resistance to ergot at two locations in South Africa. Six Ethiopian sorghum accessions ETS 1446, 2448, 2465, 3135, 4457 and 4927 were reported as resistant to ergot (Tegegne *et al.*, 1994). Musabyimana *et al.* (1995) identified 12 ergot resistant accessions, including IS 25533, 25576 and 25583.

Dahlberg *et al.* (1998) identified IS8525 as a potential source of ergot resistance and indicated that the resistance was non-pollen based. Reed *et al.* (2002b) evaluated IS8525 at several locations and found it resistant to ergot. Greenhouse and field trials in Texas and Puerto Rico have shown that the ergot response of IS8525 was highly variable (Prom and Erpelding, unpublished data). Thus, the aim of this study was to determine the effect of flowering stage, bagging and nonbagging after inoculation on ergot severity of IS8525 lines and on the male-sterile F_1 hybrids derived from crosses between IS8525J and IS8525D with A_2T×623.

MATERIALS AND METHODS

Sorghum line: IS8525 (PI563092) a sorghum landrace collected from Ethiopia and is considered heterogeneous. Two morphological phenotypes based on midrib juiciness were selected from IS8525 as described by Dahlberg *et al.* (1998). They were subsequently designated as IS8525J (juicy midrib) and IS8525D (dry midrib). These two lines differ in height, maturity and several other morphological characteristics. Since the accession is heterogeneous, the relationship between these two lines is unknown. For the purposes of this study, both lines were obtained from the sorghum breeding program at Texas A and M University in College Station, TX.

Field trials: Experiments were conducted during the 2002 and 2003 growing season at the Texas A and M Agricultural Research Farm located in Burleson County, TX near College Station, TX. Seeds of IS8525J and IS8525D were planted in 6 m rows at 0.31 m spacing between rows. Field preparation included fall plowing and incorporation of the compound fertilizer 60-40-40 (N-P_2O_5-K_2O) at 175 kg N/ha and 116.5 kg/ha P Q and K_2O before planting. An additional 175 kg N/ha was applied as top dressing at the seedling stage (5 weeks after planting). A pre-emergent insecticide 'Counter 20 CR' (BASF Group, Southfield, MI) and herbicide 'Atrazine' (Syngenta Crop Protection Inc. Greenboro, NC) were applied before planting to control weeds and protect against seedling insects. All plots were also kept free of weeds by hand weeding during the growing season.

Experimental design: The experiment was a split-split plot design with cultivar as whole plot, incubation (bagged or nonbagged) as sub-plot and treatment (flowering stage: before anthesis and at 25, 50, 75 and 100% flowering) as sub-sub-plot. Three panicles from each cultivar per incubation/treatment combination were used in 2002 and six panicles were used in 2003. Each treated panicle was considered a replicate.

Inoculation protocol: Conidia of *C. africana* (Texas isolate) were harvested from ergot-infected panicles of greenhouse-grown sorghum plants. Infected panicles were soaked in plastic pans containing a 0.1% solution of Tween 20 in sterile distilled water and vigorously agitated to dislodge the conidia. The suspension was filtered through four layers of sterile cheese cloth into clean plastic pans and diluted to a final concentration of 1×10^6 conidia mL^{-1} using a hemacytometer.

Panicles of IS8525J and IS8525D were tagged before anthesis and at 25, 50, 75 and 100% flowering stages and spray-inoculated with the *C. africana* spore suspension until run-off using a hand-held spray bottle. After inoculation, panicles at each developmental stage of flowering were either bagged with paper bags for 7 days or non-bagged. Control panicles at the same flowering stages were sprayed with sterile distilled water and either bagged or non-bagged. The number of panicles used for the control treatment was the same number used for the inoculated treatments in each year. Due to the differences in the rate of maturity between the IS8525J and IS8525D genotypes, panicles from each of the lines were inoculated on different dates.

Disease assessment: Disease evaluation on each inoculated or control panicle was conducted at 10, 15 and 20 days after inoculation. Percent ergot severity was based on the number of infected florets in each panicle divided by the total number of florets multiplied by 100.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software (version 8.1, SAS Institute, Cary, NC). Means separation was by Tukey's Studentized Range test at the 5% probability level. Due to the heterogeneity of error, data for the two years were analyzed separately.

Male-sterile hybrids: IS8525 lines were used to pollinate the male sterile line, A_2Tx623 to obtain male-sterile F_1 plants. Hybrid seed was planted in single rows at three locations. Approximately 60 plants from each cross were inoculated with ergot and evaluated in College Station, TX in 2004. In Celaya, Mexico, 100 plants of each cross were inoculated and evaluated for ergot in 2004. Ergot evaluation in Isabela, Puerto Rico was conducted in 2003 without inoculation.

RESULTS

Year 2002: There were significant differences in ergot severity due to sorghum line, treatment (flowering stage), sorghum line x treatment and sorghum line×incubation interactions. The main effect of date of disease assessment was not significant. The significant cultivar by treatment interaction indicates that IS8525J and IS8525D sorghum genotypes did not react similarly to ergot infection at the different flowering stages. Ergot infection on the control panicles ranged from 0 to 0.3%, which represents the level of infection from naturally occurring inoculum.

Overall mean ergot severity was significantly greater for IS8525J compared to IS8525D (Table 1). Bagging of the inoculated panicles for 7 days significantly increased ergot severity compared to nonbagged panicles in both lines (Table 2). IS8525J panicles that were inoculated and bagged exhibited a significantly higher ergot severity across flowering stages compared to IS8525D and non-bagged panicles. Bagged panicles of the IS8525D line had twice as much disease as non-bagged. Mean disease severity was not significant between IS8525J and IS8525D for the non-bagged.

Year 2003: The level of ergot severity was affected by sorghum line, date of disease assessment, incubation and sorghum line×incubation interaction. The main effect of treatment (flowering stage) and cultivar×treatment interaction were non-significant. The non-significant cultivar by treatment interaction indicates that IS8525J and IS8525D sorghum genotypes reacted similarly to ergot infection at the different flowering stages. No infection was observed on the control panicles sprayed with sterile distilled water.

IS8525J also was found to exhibit the highest levels of ergot severity in 2003 (Table 1). Bagging of the inoculated panicles significantly increased ergot

Table 1: Percent ergot severity of bagged and nonbagged juicy and dry midrib sister-lines of the IS8525 sorghum inoculated with *Claviceps africana* averaged over five different flowering stages in 2002 and 2003

	Mean ergot severity (%)[a]	
Sorghum line	Year 2002	Year 2003
IS8525J (juicy midrib)	16.4a[b]	6.7a
IS8525D (dry midrib)	2.5b	2.3b

[a]Mean percent ergot severity based on the number of infected florets per panicle divided by the total number of florets multiplied by 100. [b]Mean comparisons by Tukey's Studentized Range test. Means within a column with the same letter are not significantly different at the 5% probability level

Table 2: Mean percent ergot severity in bagged and non-bagged panicles of IS8525J and IS8525D inoculated with *Claviceps africana* averaged over five different flowering stages in 2002 and 2003

	Mean percent ergot severity[a]			
	Year 2002		Year 2003	
Sorghum line	Bagged	Nonbagged	Bagged	Nonbagged
IS8525J (juicy midrib)	29.9a[b]	2.8c	12.7a	0.7c
IS8525D (dry midrib)	3.3b	1.6c	4.4b	0.3c

[a]Mean percent ergot severity based on the number of infected florets per panicle divided by the total number of florets multiplied by 100.[b]Mean comparisons by Tukey's Studentized Range test. Means within year and across columns (bagged vs nonbagged) and within a column (juicy vs dry) with the same letter are not significantly different at the 5% probability level

Table 3: Effect of flowering stage on mean percent ergot severity of IS8525J and IS8525D sorghum genotypes inoculated with *Claviceps africana* in 2002 and 2003

	Mean ergot severity (%)[a]	
Flowering stage[b]	Year 2002	Year 2003
0	6.1b[c]	4.7[d]
25	12.4a	7.0
50	10.6ab	4.3
75	10.6ab	4.2
100	6.1b	2.4

[a]Mean percent ergot severity based on the number of infected florets per panicle divided by the total number of florets multiplied by 100.[b]Flowering stages: 0 = before anthesis; 25 = 25% of panicles had completed flowering; 50 = 50% of panicles had completed flowering; 75 = 75% of panicles had completed flowering and 100 = 100% of panicles had completed flowering. [c]Mean comparison by Tukey's Studentized Range test. Means within a column with the same letters are not significantly different at the 5% probability level. [d]The treatment effect was non-significant at the 5% probability level

Table 4: Mean ergot severity on IS8525D (dry midrib), IS8525J (juicy midrib), male-sterile line A_2T×623 and male-sterile F_1 hybrids obtained from crosses between the two IS8525 lines and A_2T×623 and planted at three different locations

	Location[a]		
	Celaya	College station	Isabela
Sorghum line	Ergot severity (%)	Ergot severity (%)	Ergot severity (%)
IS8525D	0	1	5[d]
IS8525J	5	3	4
A_2T×623	90	50	90
IS8525D×A_2Tx623[b]	90	30	90
IS8525J×A_2T×623[c]	90	36	90

[a]Locations: Celaya, Mexico; College Station, Texas and Isabela, Puerto Rico. [b]IS8525D×A_2T×623 = Male-sterile F1 hybrids obtained from a cross between IS8525D and A_2T×623.[c]IS8525J×A_2T×623 = Male-sterile F_1 hybrids obtained from a cross between IS8525J and A_2Tx623. [d]Means for the IS8525D and IS8525J were averaged over experiments conducted over several growing seasons in Isabela, Puerto Rico during 2001, 2002 and 2003

severities in the lines with IS8525J having the highest level of infection (Table 2). The effect of flowering stage was non-significant in 2003, although panicles inoculated at 100% flowering had the least amount of ergot infection (Table 3).

Male-sterile hybrids: Male-sterile F_1 plants obtained from crosses between the IS8525 lines with A_2T×623 were found to be highly susceptible to ergot in College Station, Texas, Isabela, Puerto Rico and Celaya, Mexico (Table 4).

DISCUSSION

The use of host resistance offers the best means for controlling plant diseases. The ergot pathogen primarily infects unfertilized ovaries and therefore male-sterile lines used in sorghum hybrid seed production are highly vulnerable to the disease. In order for ergot resistance sources to be useful in hybrid seed production, resistance will need to be expressed in male sterile lines. Dahlberg *et al.* (1998) noted that IS8525 resistance was non-pollen based, suggesting a physiological source of ergot resistance. Reed *et al.* (2002b) indicated that IS8525 was the best source of ergot resistance for use in male-sterile parental lines. This study has shown that IS8525J and IS8525D sorghum genotypes exhibited different levels of tolerance to ergot when inoculated with *C. africana*. Similar results have been observed in Isabela, Puerto Rico using inoculation and panicle bagging (Prom and Erpelding unpublished results). The two lines have been evaluated over several growing seasons and ergot infection has ranged from 5-20%. Reed *et al.* (2002b) reported ergot severities of 3.68% in Guayanilla, Puerto Rico, 2.64% in Mexico and 13.39% over three locations in South Africa for IS8525. Significantly higher ergot infection has been observed for the juicy midrib line (IS8525J) compare to the dry midrib line (IS8525D) suggesting genetic variation for ergot tolerance. Also, IS8525J flowers approximately 10 days later than IS8525D. The tolerance to ergot infection of IS8525 may provide suitable field resistance under natural infection or in non-epiphytotic years. However, the tolerance or field resistance of IS8525 appears to be due to floral morphological and phenological characteristics. Anthesis is rapid and occurs as the panicle is emerging from the flag leaf potential limiting infection.

Reed *et al.* (2002b) reported that ergot resistance in IS8525 was pollen based when male-sterile hybrids were produced using A_3-cytoplasm male-sterile testers and evaluated for ergot response. They reported mean ergot severity of 27.57% in Puerto Rico and 39.85% in Mexico for IS8525 crossed to five A_3-cytoplasm male-sterile testers. In this study, male-sterile F_1 hybrids derived from IS8525 lines were found to be highly susceptible when planted in the Texas, Mexico and Puerto Rico. The mean ergot severity for the male-sterile F_1 hybrids of IS8525 was 71% over the three locations. In addition, emasculated florets of IS8525 are completely susceptible when inoculated with *C. africana* spores suggesting no physiological mechanism of resistance. No sorghum genotype with a significant amount of resistance to ergot in the absence of pollen has yet been identified (Bandyopadhyay *et al.*, 1998; Frederickson and McLaren 2000; Mantle and Bogo, 2002). According to these authors, sorghum lines that were reported as resistant to ergot have the capacity to escape infection by rapid pollination and fertilization.

Ergot severity was markedly affected by bagging and the stage of flowering when panicles of IS8525 were inoculated in this study. Similar to results obtained by Tegegne *et al.* (1994), Musabyimana *et al.* (1995) and Prom *et al.* 2005), bagging of inoculated panicles significantly increased ergot severity. Bagging of inoculated panicle enhances the ability of the pathogen to cause infection, excludes external pollen (Musabyimana *et al.*, 1995) and increase the humidity around the panicles.

The flowering stage of the panicles at the time of inoculation also affected the level of ergot infection. Futrell and Webster (1965) and Musabyimana *et al.* (1995) noted that the unpollinated florets were susceptible to ergot infection, whereas florets became resistant once pollinated. Ergot inoculation of IS8525 at the start of anthesis resulted in a greater infection response with a decrease in ergot severity when inoculations were conducted at later flowering stages.

We have shown that both IS8525J and IS8525D exhibit tolerance to ergot when inoculated with *C. africana*. Variation in ergot reactions of IS8525 have been observed in different environments in this study. Reed *et al.* (2002b) also observed variation in disease response of IS8525 in different locations. A line of IS8525 obtained from R. G. Henzell (Queensland Department of Primary Industries, Warwick, Australia) exhibited 20-80% ergot severity under natural infection in Weslaco, TX during the fall of 2003 (data not shown). Environmental conditions can significantly influence ergot response; therefore, bagging and the flowering stage at inoculation are two important factors to consider when screening sorghum germplasm for resistance to ergot.

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