International Scholars Journals

International Journal of Agroforestry and Silviculture ISSN 2375-1096 Vol. 7 (12), pp. 001-009, December, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

An improved method for screening *Fusarium* stalk rot resistance in grain sorghum (*Sorghum bicolor* [L.] Moench.)

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Accepted 12 September, 2019

Screening for resistance to *Fusarium* stalk rot under natural infection has been difficult because of irregularities in disease development. The toothpick inoculation technique was introduced to overcome this problem, but it lacked a mechanism to monitor inoculum dose. In this study, we introduce an improved procedure where inoculum doses are monitored and disease incubation periods are optimized. Five inoculum doses of *Fusarium verticillioides* (0, 1 × 10^3, 1 × 10^4, 1 × 10^5 and 1 × 10^6 conidia ml^{-1}) and five disease incubation periods (14, 21, 28, 35 and 42 days) were studied using sorghum genotypes of variable stalk rot reaction. Plants were inoculated on day 14 after flowering. Disease reaction was scored as length (cm) of necrotic lesion and number of nodes crossed. The effects of both inoculum dose and incubation period were significant. Inoculum doses of 1 × 10^4 conidia ml^{-1} and greater and incubation periods of 21 to 42 days clearly resolved differences among genotypes. Noting the burden of producing a highly concentrated inoculum and the lack of need to keep plants after physiological maturity, incubation for 28 days following inoculation with 1 × 10^4 to 1 × 10^5 conidia ml^{-1} is recommended for germplasm screening.

Key words: Stalk rot, *Fusarium*, *Sorghum bicolor*, inoculum dose (ID), incubation period (IP).

INTRODUCTION

Fusarium stalk rot is one of the major diseases of grain sorghum worldwide (Tarr, 1962), including Africa (Frowd, 1980; Zummo, 1980; Omar et al., 1985), India (Khune et al., 1984), Australia (Henzell et al., 1984) and the United States (Edmunds and Zummo, 1975; Duncan, 1983; Leslie et al., 1990; Jardine and Lesile, 1992). Damage to yield and quality becomes severe when disease development coincides with environmental stresses (Fredericksen, 1984; Khune et al., 1984). The disease is particularly aggravated by wet conditions that follow a prolonged dry period (Dodd, 1980; Hassan et al., 1996). The pathogens first colonize and destroy the root system and eventually advance above ground and infect the stalk causing red, pinkish or brownish coloration in the infected tissues, a characteristic symptom of the disease (Zummo 1980; Reed et al., 1983). Infected plants often have damaged vascular and cortical tissues in both the stalk and root system that may reduce water and nutrient absorption and translocation (Hundekar and Anahosur, 1994). Under severe conditions, the disease may result in complete disintegration of the root and stalk tissue, leading to lodging (Zummo, 1984).

Lack of consistent disease development under natural conditions and absence of sound inoculation protocols to initiate uniform infection (Zummo, 1984) have limited progresses towards deployment of resistance genes. The toothpick inoculation procedure originally developed for *Macrophomina phaseolina* (Rao et al., 1980; Seetharama et al., 1987) has been used to screen a limited number of sorghum genotypes against *Fusarium* (Bramel-Cox et al., 1988; Bramel-Cox and Claflin, 1989). Nonetheless, this technique lacks a mechanism to determine the amount of inoculum administered to each plant and thus may result in substantial bias. Inoculation with liquid *Fusarium* inoculum in corn has been shown to have improved the

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precision of scoring *Fusarium* ear rot than the traditional tooth pick inoculation (Clements et al., 2003). And because of its reliability and ease of use, this technique has been increasingly used for screening corn against ear rots caused by *Fusarium* and *Aspergilus* (Windham and Williams, 2002; Williams 2006; Henry et al., 2009). This study focuses on the development of simple and low cost screening protocol for stalk rot in sorghum. The technique allows manipulation of inoculum concentration and use of a modified syringe to deliver the desired volume of inoculum suspension to the target plant.

MATERIALS AND METHODS

Genetic materials and experimental design

The study was conducted at the Kansas State University agronomy research farm near Manhattan, KS over three years (2001, 2002 and 2008). In 2001, two sorghum hybrids with variable stalk rot reaction; Tx3042 × SC35 (susceptible) and Tx378 × SC599 (resistant) were studied. Test entries were increased to 10 hybrids in 2002 to include genotypes with a broader range of disease reaction. The entries were developed by crossing five pollinator parents of variable stalk rot resistance, SC1154, SC1039, SC134, SC35 and SC701, with two susceptible seed parents, Tx399 and Tx378. Previous studies have grouped six of the hybrids as resistant, two as moderately susceptible and the remaining two as susceptible to the disease (Tesso et al., 2004; 2005). The genotypes were evaluated against five levels of inoculum concentrations, 0 (control), 1×10^3, 1×10^4, 1×10^5 and 1×10^6 conidia ml^{-1} and five disease incubation periods, 14, 21, 28, 35 and 42 days, that were achieved by varying disease scoring dates.

The experiment was laid in a split plot design with randomized complete blocks. Genotypes were treated as the whole plot unit and the factorial combination of inoculum dose and disease incubation period was the subplot unit. Eight replications were used in the first year experiment, because of the large number of genotypes and relatively small variability between blocks observed in 2001, only two replications were used in 2002. Each plot consisted of two 5 m long rows spaced 0.7 m apart with 0.2 m between plants. The plots were managed using standard sorghum crop management recommendation. At flowering, a total of 75 plants in each whole plot unit (15 plants for each dose) were randomly selected and tagged with five distinct tapes.

Inoculum preparation and field inoculation

Inoculum suspension was initiated in potato dextrose broth (PDB-DIFCO) from the *Fusarium verticillioides* strain KSLM isolated from sorghum in Kansas. The suspension was incubated at room temperature on a rotary shaker until microconidia were produced and then strained through four layers of cheesecloth to separate the mycelial mass from the microconidia. Concentration of the suspension was determined by counting the number of conidia using a microscope and hemacytometer. The concentration was first adjusted to the required highest dose (1×10^6 conidia ml^{-1}) by diluting with phosphate buffered saline (PBS) solution and lower concentrations were obtained by serial dilution. On day 14 after flowering, plants tagged with distinct tapes were inoculated with a corresponding inoculum dose. Inoculations were performed with an Idico filler-plug gun (Forestry Suppliers, Inc., Jackson, MS) equipped with a stainless steel needle similar to that described by Toman and White (1993). This modified syringe can hold up to 1 L suspension and was calibrated to deliver 1.0 ml of the suspension at a time. Control plants were inoculated with a similar volume of PBS buffer. Beginning on day 14 after inoculation, 15 inoculated plants (three for each inoculum dose) in each whole plot unit were harvested every week and scored for disease severity. The rating was conducted by longitudinally splitting the stalks, measuring the length of visible necrotic lesion (cm) and counting the number of nodes contained within the necrotic region.

Germplasm screening using the improved procedure

In the year 2008, a validation exercise was carried out using the optimum inoculum dose (1×10^5 conidia ml^{-1}) and disease incubation period (35 days) identified in this study. A subset of a panel of sorghum accessions (59 genotypes) assembled from different geographical regions of the world was grown at the Kansas State University agronomy research farm. On day 14 after flowering, five plants were randomly selected from each genotype and inoculated with a liquid inoculum suspension of *F. thapsinum* (1×10^5 conidia ml^{-1}). On day 35 after inoculation, the plants were harvested and rated for disease severity. Management of the nursery, inoculum preparation, inoculation and data collection procedures were performed as previously done.

Statistical analysis

Data were subjected to statistical analysis with the General Linear Model in SAS (SAS, 1998). Independent genotype mean squares were computed for each level of inoculum dose and duration of incubation, and used as an index for selecting the level or levels that best explained variation among genotypes.

RESULTS

The effect of inoculum dose on disease severity

The analysis of variance for the 2001 and 2002 season experiments is presented in Table 1. Disease severity increased with increased levels of inoculum dose in both years (Tables 2 and 3). In 2001, mean lesion length across genotypes was shortest (4.03 cm) for the control treatment and longest (18.2 cm) for the highest inoculum dose. The trend was similar in the 2002 season; mean lesion length was still shortest (4.10 cm) in the control and longest (20.34 cm) for the highest inoculum dose. The intermediate levels produced a corresponding level of infection, resulting in a linear pattern of response to inoculum dose (Figure 1a). Genotypic response to inoculum dose was consistent in both years; disease severity was lowest among resistant hybrids and highest in moderate and susceptible hybrids. Hybrids involving resistant parents SC1039, SC1154, SC599 and SC134 had significantly less infection than hybrids produced using the susceptible parents SC701 (Tables 2 and 3).

A similar trend was observed for the number of nodes crossed (Tables 2 and 3) . In 2001, the average number of nodes contained within the necrotic region was 0.7 for the control treatment and 3.3 for the highest dosage treatment. This pattern was consistent in the year 2002;

Table 1. Mean squares from the analysis of variance for lesion length and number of nodes crossed following stalk rot inoculation.

	2001			2002		
Source	**df**	**Lesion length**	**Nodes crossed**	**df**	**Lesion length**	**Nodes crossed**
Block (B)	7	47.27	1.79	1	48.81	4.11
Genotype (G)	1	7435.58**	150.51**	9	3050.27**	17.17**
G × B	7	35.07**	2.76**	9	58.36**	2.90**
Inoculum dose (ID)	4	2201.19**	69.17**	4	3520.75**	118.61**
Incubation period (IP)	4	571.13**	42.82**	4	783.54**	32.90**
ID×IP	16	18.5	0.56	16	45.44	1.12
G×ID	4	203.75**	4.65**	36	140.55**	0.85
G×IP	4	16.51	0.52	36	52.33*	0.83
G × ID x IP	16	20.76*	0.89	143	17.21	0.44
Error	314	9.03	0.58	210	19.69	0.65

**, * Statistically significant at P 0.01 and P 0.05, respectively.

Table 2. Variation in mean disease score between genotypes with contrasting stalk rot reaction as influenced by inoculum concentration and disease incubation period during the 2001 season.

	Inoculum concentration (conidia ml^{-1})									
	Lesion length					**Number of nodes crossed**				
	0	1×10^3	1×10^4	1×10^5	1×10^6	0	1×10^3	1×10^4	1×10^5	1×10^6
Tx378 × SC599	2.3	8.8	9.1	9.6	13.3	0.4	1.5	1.7	1.6	2.7
Tx3042 × SC35	5.8	17.5	20.0	21.2	23.5	0.9	2.6	3.1	3.4	4.0
Mean‡	4.1D	13.1C	14.5B	15.4B	18.4A	0.7d	2.1c	2.4b	2.5b	3.3a
Mean squares	400	1325	2335	2381	1592	5.1	22.8	38.8	57.2	31.7
LSD (0.05)	1.93	2.19	2.06	2.07	1.93	0.37	0.51	0.49	0.48	0.52
	Incubation period (days after inoculation)									
Genotypes	**14**	**21**	**28**	**35**	**42**	**14**	**21**	**28**	**35**	**42**
Tx378 × SC599	4.3	7.2	9.0	10.5	12.3	0.5	1.1	1.7	1.8	2.5
Tx3042 × SC35	14.3	15.8	18.9	19.3	20.7	1.7	2.3	3.0	3.3	3.6
Mean‡	9.3D	11.5C	14B	14.9B	16.5A	1.1d	1.7c	2.4b	2.6b	3.0a
Mean squares	1286	1336	1778	1415	1237	24.3	23.9	29.8	44.6	21.2
LSD (0.05)	2.37	2.58	2.57	2.61	2.63	0.41	0.49	0.6	0.61	0.64

‡ Means within rows followed by the same uppercase letters are not significantly different at P 0.05; Means within rows followed by the same lowercase letters are not significantly different at P 0.05.

the disease score ranged from 0.4 nodes in the control to 3.4 in the highest dose treatment. Similar to the lesion length, the difference between genotypes with respect to nodes crossed was evident for each dosage level (Figure 1a).

Variation in disease score steadily increased with increased dosage until the fourth level, after which not much increase or a slight decrease was observed (Table 2, Figure 1c and 1d). The trend was similar for both lesion length and nodes crossed in both years except that the variation for the highest inoculum dose was not high-er than the second highest level in both years (Tables 2 and 3). Variance for lesion length ranged from 400 in the control to 2381 in the 4th level (1 × 10^5 conidia ml^{-1}) in the year 2001 and from 192 in the control to 1059 in the 5th level (1 ×10^6 conidia ml^{-1}) in the year 2002. Similarly, variance for nodes crossed was lowest (5.1) in the control and highest (57.2) in the 4th level in 2001 and lowest (1.4) in the control and highest (6.7) in the 5th level in 2002 (Figure 2a).

Effect of incubation period

Similar to inoculum dose, disease severity was significantly different for the different disease incubation periods in both years. In the year 2001, the shortest (4.26 cm) lesion length was recorded in the resistant hybrid

Table 3. Variation in mean disease score as affected by inoculum dose and disease incubation period during the 2002 season.

Genotypes	Inoculum concentration (conidia/ml)									
	Lesion length (cm)					Number of nodes crossed				
	0	1×10^3	1×10^4	1×10^5	1×10^6	0	1×10^3	1×10^4	1×10^5	1×10^6
Tx378 × SC1039	2.7	4.7	9.4	13.2	15.5	0	0.9	1.6	2.4	2.8
Tx378 × SC1154	3.4	6.3	9.8	12.1	15.3	0.6	0.7	1.7	2.2	3.7
Tx378 × SC134	2.6	6	8.5	13.5	16.4	0.4	0.9	1.2	2.3	3.2
Tx378 × SC35	4.2	9.3	11.7	17	20.2	0.2	1.8	2.3	3.6	4.6
Tx378 × SC701	8.8	24.9	30.4	37.5	44.1	0.6	1.8	2.8	3.6	4.3
Tx399 × SC1039	1.8	5.4	7.9	11.1	12.4	0.1	0.7	1.6	2.3	2.8
Tx399 × SC1154	2	4.6	6	8	9.6	0	0.6	1.5	1.5	2.2
Tx399 × SC134	3.1	6.8	6.9	12.5	12.1	0.1	1.1	1.2	2.1	2.6
Tx399 × SC35	3.7	8.7	13.3	12.3	16.1	0.9	2	2.6	3.1	3.7
Tx399 × SC701	7.9	21.3	31.8	36.2	39.2	1.1	1.9	3.1	4.1	4.3
Mean‡	4.0E	9.8D	13.6C	17.3B	20.1A	0.4e	1.2d	2.0c	2.7b	3.4a
Mean squares	192	375	756	1020	1059	1.4	2.9	4.5	6	6.7
LSD (0.05)	2.87	4.23	9.47	6.02	6.39	0.64	0.82	0.99	1.05	1.1

Genotypes	Incubation period (days after inoculation)[A]									
	14	21	28	35	42	14	21	28	35	42
Tx378 × SC1039	5.2	8.7	9	10.3	12.4	0.8	1.4	1.5	1.8	2.6
Tx378 × SC1154	7.7	9.2	9.3	9.9	11.2	1.1	1.1	1.9	2.2	2.4
Tx378 × SC134	5.9	7.3	10	11.9	11.9	0.6	0.9	2	2.4	2.3
Tx378 × SC35	7.8	10.7	12.3	15.6	16	1.5	2.7	2.5	3	3
Tx378 × SC701	17.8	28.2	32.1	34.3	33.3	1.3	2.6	2.9	3.1	3.4
Tx399 × SC1039	5.8	7.8	7.8	7.3	11.5	0.8	1.2	1.5	1.5	2.6
Tx399 × SC1154	3	5.8	6.6	6.8	7.9	0.5	1.2	1.4	1.3	1.7
Tx399 × SC134	5.6	7.9	9.6	9.6	9.8	0.5	1.2	1.6	1.9	1.8
Tx399 × SC35	8	10.3	9.5	10.7	15	0.8	2.3	2.1	3.1	3.9
Tx399 × SC701	18.1	24.9	26.1	31.2	42.3	1.5	3	2.9	3.4	4.1
Mean‡	8.5D	12.1C	13.2C	14.8B	17.1A	0.9c	1.8b	2.0b	2.4a	2.8a
Mean squares	285	677	720	906	903	1.5	3.9	5	5.3	4.9
LSD (0.05)	4.9	6.65	6.56	7.42	9.66	0.94	1.27	1.18	0.42	1.54

‡ Means in rows (lesion length) followed by the same upper case letters are not significantly different at P 0.05; means in rows (nodes crossed) followed by same lowercase letters are not significantly different at P 0.05.

with the shortest exposure (14 days) and the longest (20.70 cm) lesion length was in the susceptible hybrid incubated for 42 days (Table 2) . The overall mean lesion length due to incubation period ranged from 9.3 cm in the 14 day incubation to 16.1 cm in the 42 day incubation. Again the trend was similar in the year 2002; the mean lesion length ranged from 8.5 cm in the 14 day incubation period to 17.1 cm for the longest (42 day) incubation period (Table 3 and Figure 1b). Results for the number of nodes crossed followed a similar pattern. The mean number of nodes crossed ranged from 1.1 to 3.0 in 2001 (Table 2) and 0.9 and 2.8 in 2002; the highest and lowest scores correspond to the shortest and longest incubation periods in both years (Tables 2 and 3).

Variation in disease score among the different incubation periods was again remarkably high; variation consistently increased as the incubation period increased, but the rate of the increase in the year 2001 was much lower than in the year 2002 for both lesion length and nodes crossed. But unlike inoculum dose, the longest level for incubation period resulted in less variability than the preceding level for both lesion length and number of nodes crossed in both years (Tables 2 and 3). In 2001, lesion length mean square for lesion length and nodes crossed slightly increased with increased level of incubation period until the 4th level and was lower in the 5th level (Table 2). The pattern in the year 2002 was similar to that of year 2001, except the mean square was generally lower in 2002 than 2001 (Table 3, Figure 1c and 1d). The low mean square reading appears to be the result of the number of genotypes evaluated, which was reflected in the degree

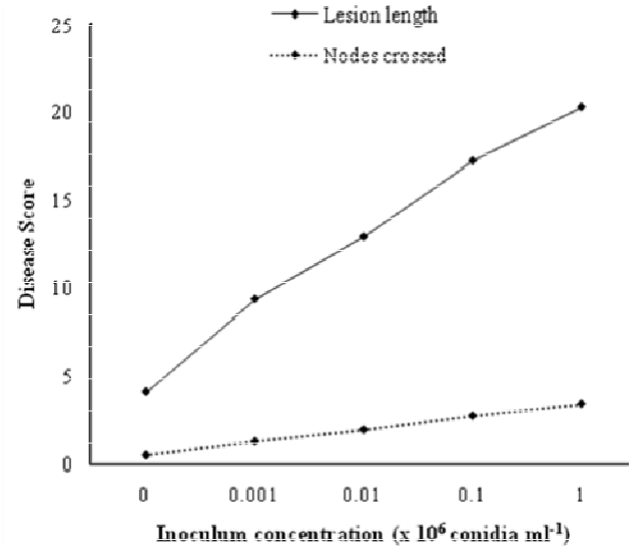

Figure 1a. Pattern of genotypic reaction to various doses of inoculum for the different levels of the two factors.

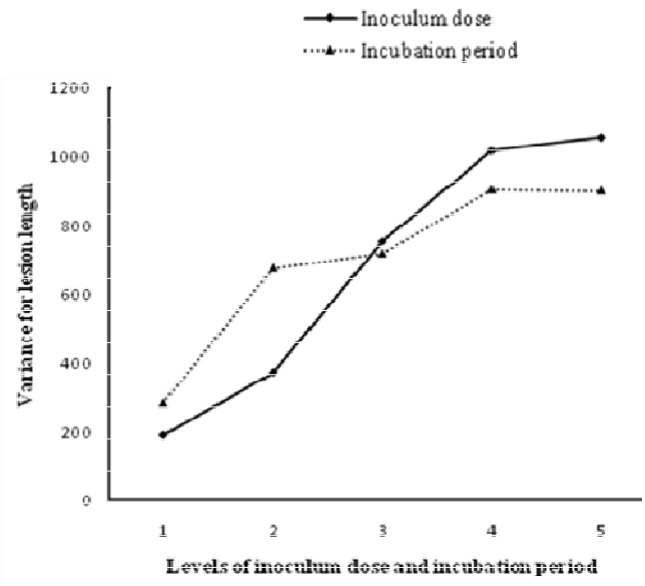

Figure 1c. Pattern of genotypic reaction to various doses of inoculum and the extent of genotypic variability in lesion length for the different levels of the two factors.

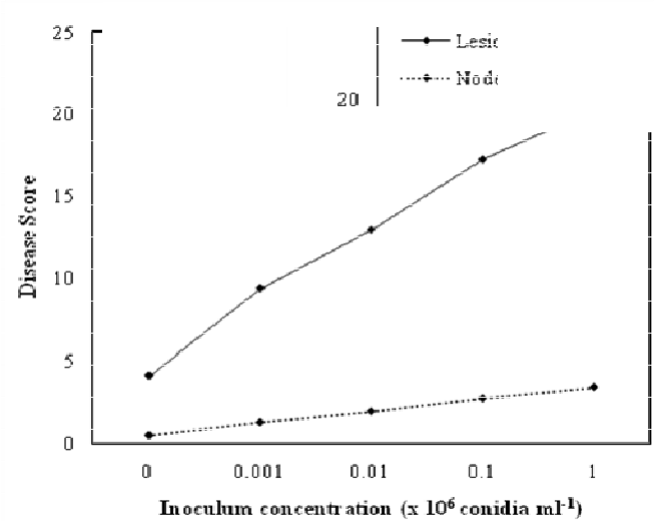

Figure 1b. Pattern of genotypic reaction to various doses of inoculum disease incubation period for the different levels of the two factors.

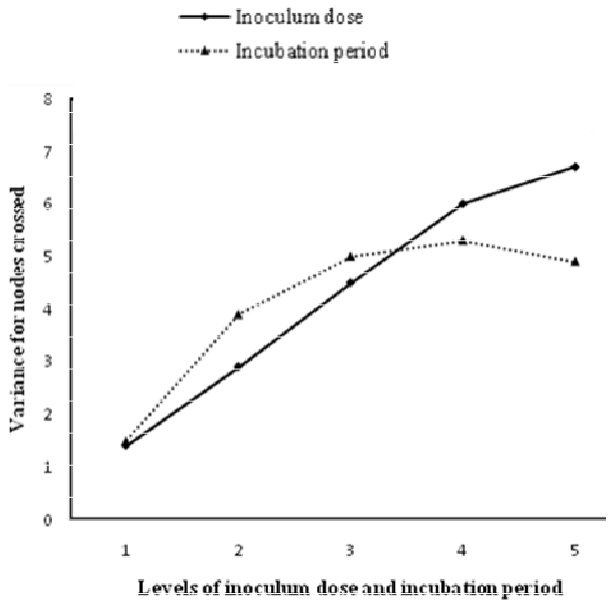

Figure 1d. Pattern of genotypic reaction to various doses of inoculum and nodes crossed for the different levels of the two factors.

of freedom and thus the amount of mean square for genotypes. Looking at both pattern of variability among levels of inoculum dose and disease incubation period and the actual disease scores for levels of the two factors, inoculum dose appears to have better resolved genetic differences among genotypes than incubation period.

Interaction effects were generally not significant, except for genotype × inoculum dose for both lesion length and nodes crossed in the year 2001 and genotype × inoculum dose and genotype × incubation period for lesion length in the year 2002 (Table 2). Interaction between concentration and incubation period and the three-way interaction between genotype, inoculum dose, and incubation period were generally not significant, except for lesion length in the year 2001. In general the interaction effects were much smaller than main effects.

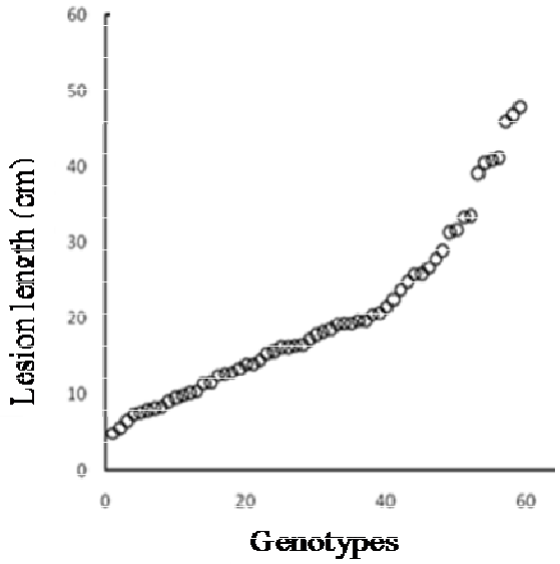

Figure 2a. Variation in mean lesion length among a subset of the sorghum diversity panel evaluated with the optimal procedure.

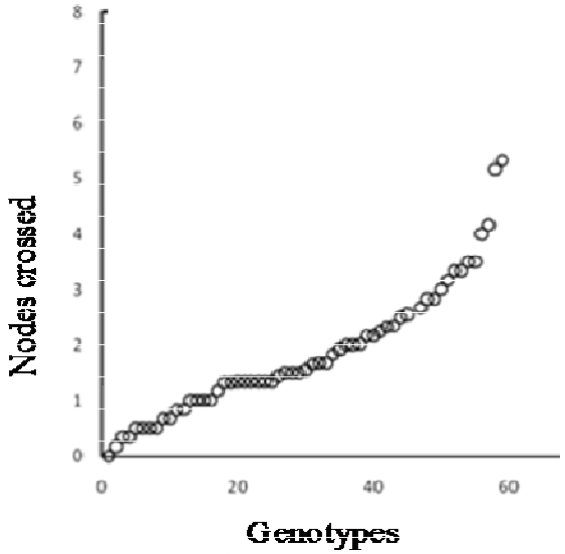

Figure 2b. Variation in nodes crossed among a subset of the sorghum diversity panel evaluated with the optimal procedure.

Validation of the bioassay procedure

The validation exercise was conducted on a subset of the 300 sorghum diversity panel grown at Manhattan during the 2008 season. The fourth inoculum dose (1×10^5 period were used to characterize stalk rot reaction of the genotypes. There was a wide range of variability among genotypes. Mean lesion length ranged from 4.9 cm in the known resistant genotype SC599 to 47.8 cm in SC284, a new susceptible line not characterized before. Similarly, mean number of nodes enclosed within the lesion ranged from 0 to more than 5 in genotypes Tx641 and SC405, respectively (Table 4). The variation between genotypes was continuous for both disease score parameters, with the known resistance (SC599) and susceptible (SC284) sources having scores near the extreme ends (Figure 2a and 2b) (which ones are known resistance or susceptible sources?). Many genotypes previously not characterized for stalk rot reaction exhibited comparable levels of reaction with the known resistance sources. These genotypes include P9517, Tx641, Tx642, and Tx2911. These are public inbred lines bred at the Texas and Purdue sorghum breeding programs and are known to have elements of drought tolerance and/or disease resistance.

DISCUSSION

Disease severity increased with increased inoculum dose and incubation period (Tables 2, 3 and Figure 1a, 1b). This trend was anticipated because plants inoculated with higher inoculum doses come in contact with large population of infective conidia that not only initiate quicker infection but also spread faster and colonize a larger area of tissue. Similarly, a longer incubation period would allow sufficient time for disease to develop. This result is in agreement with common knowledge on the effect of inoculum dose and duration of exposure on disease development and severity (Agrios, 1997). However, plants inoculated with the control solution (PBS) also appeared to have slight infection. It is not clear if this was the effect of opportunistic pathogens that may have used the wounds created by the needle to enter the plants, the result of natural plant response to mechanical wounds, or combination of the two. Attempts to isolate pathogens from the seemingly infected area did not yield any dominant pathogen group but rather produced a complex colony of fungi including the pathogen being used in the study. A similar attempt on the pathogen inoculated plants recovered large amount of the causal inoculum, but trace amounts of other groups of fungi were also recovered. In our earlier work (Tesso et al., 2005) we noted similar results in a series of genotypes inoculated with the control solution. But as in the present study, the amount of lesion observed in control plants was very small compared with the infection that developed as a result of inoculation with infective conidia.

Mean disease scores were significantly different among genotypes for all levels of inoculum dose and incubation period, suggesting that any level or combination of levels of the two factors could be used for rating the reaction of sorghum accessions to stalk rot infection. Higher doses of inoculum and longer incubation periods appear to have better resolved the difference among genotypes than lower doses and short incubation periods. This was evident from

Table 4. Mean disease score of the diverse set of genotypes screened with the optimized protocol.

No.	Genotypes	Geographical origin	Botanical race	Disease score	
				Lesion length (cm)	Nodes crossed
1	(SN142)S	United States	Inbred line	29.00	3.50
2	00MN7645	United States	Inbred line	12.50	0.50
3	BOK11	United States	Inbred line	13.33	2.00
4	BQL41	Australia	Inbred line	7.59	0.50
5	BT × 2752	United States	Inbred line	12.67	1.92
6	BT × 3042	United States	Inbred line	14.50	1.83
7	BT × 3197	United States	Inbred line	8.34	1.67
8	BT × 378	United States	Inbred line	11.50	1.33
9	BT × 399	United States	Inbred line	8.17	1.33
10	BT ×615	United States	Inbred line	9.84	1.34
11	BT × 623	United States	Inbred line	18.17	2.83
12	BT × 641	United States	Inbred line	7.33	0.00
13	BT × 642	United States	Inbred line	9.00	0.34
14	BT × 643	United States	Inbred line	15.34	2.25
15	BT × ARG-1	United States	Inbred line	14.00	2.55
16	Dorado	Niger	Cultivar	20.67	0.84
17	El Mota	Niger	Cultivar	41.17	2.02
18	Feterita	Sudan	Cultivar	31.67	3.34
19	HEGARI	-	Cultivar	20.50	2.83
20	Macia	South Africa	Cultivar	16.17	2.33
21	P9517	United States	Inbred line	6.50	0.17
22	RT × 2783	United States	Inbred line	19.33	1.00
23	RT × 430	United States	Inbred line	14.00	1.00
24	SA5330/M	United States	Inbred line	16.50	1.34
25	SC132	Ethiopia	Dura Bicolor	19.34	1.50
26	SC134	Ethiopia	Dura	7.90	0.50
27	SC146	Ethiopia	Dura	46.00	0.67
28	SC15	Ethiopia	Guinea Bicolor	11.34	1.17
29	SC202	India	Dura	33.33	3.17
30	SC241	-	-	24.83	2.34
31	SC243	-	-	17.17	0.34
32	SC283	Tanzania	Guinea	22.50	1.34
33	SC284	-	-	47.84	2.50
34	SC317	India	Guinea Bicolor	40.83	2.17
35	SC328	Uganda	Caudatum	19.67	1.00
36	SC33	Ethiopia	Dura	19.67	2.34
37	SC348	Nigeria	Caudatum	33.50	2.67
38	SC35	Ethiopia	Dura	16.34	2.00
39	SC405	-	-	25.83	5.17
40	SC418	Tanzania	Kafir Caudatum	27.84	4.00
41	SC424	Japan	Caudatum	18.50	1.34
42	SC452	-	-	9.50	1.67
43	SC504	-	-	46.83	3.00
44	SC520	-	-	17.84	0.83
45	SC56	Sudan	Caudatum	21.50	1.50
46	SC564	Uganda	Caudatum	10.17	1.44
47	SC575	-	-	19.17	1.65
48	SC599	United States	Caudatum	4.85	0.50
49	SC60	Sudan	Caudatum	16.17	1.50
50	SC62	Sudan	Caudatum	25.75	1.34

Table 4. Contd.

No.	Genotypes	Geographical origin	Botanical race	Disease score	
				Lesion length (cm)	Lesion length(cm)
51	SC627	South Africa	Kafir	39.17	5.33
52	SC663	United States	Guinea Kafir	23.75	1.55
53	SC702	Sudan	Caudatum	26.67	3.34
54	SRN39	Sudan	Cultivar	12.84	2.17
55	SU629	Sudan	-	15.67	1.34
56	SURENO	Central America	Cultivar	10.34	1.00
57	San Chi	China	Cultivar	31.34	4.17
58	Segeolane	-	Cultivar	40.50	3.50
59	RT × 2911	United States	Inbred line	5.50	0.67
	Mean			20.19	1.89
	LSD (0.05)			16.58	1.90

evident from the mean square of genotypes for different levels of the two factors (Tables 2, 3 and Figure 1c, 1d). With respect to inoculum dose, the greatest variation among genotypes was obtained for the 1×10^5 conidia ml^{-1} in the first year and 1×10^6 conidia ml^{-1} in the second year both for lesion length and number of nodes crossed (Figure 1c and 1d). Similarly, the largest variation for incubation period was noted for the 35 day incubation period in both years (Figure 1c and 1d). This result suggests that screening sorghum germplasm against *Fusarium* stalk rot can be performed with various inoculum doses and disease ratings can be made during the 14 to 42 days post-inoculation period. But, the highest inoculum dose (1×10^6 condia ml^{-1}) and longest incubation period (42 days) may be difficult to achieve in practical conditions. Use of the highest inoculum dose imposes the burden of producing highly concentrated inoculum suspension. Certain low sporulating strains do not produce enough conidia and this may pose a problem in administering higher inoculums doses, especially when a large number of genotypes are screened. In a related study in which we compared virulence between four *Fusarium* species and another four mating populations of *Fusarium monliforme*, we noticed marked variation in conidia production between the different species and mating populations (data not published). Likewise, an extended period of incubation beyond 35 days may not be advantageous. For genotypes with short grain fill duration, extended stay in the field may encourage invasion by and development of spontaneous saprophytic fungi that could complicate the scoring process. Tables 2, 3 and Figures 1c and 1d show that the highest levels of both factors (inoculum dose and incubation period) did not have much contribution toward further resolving differences among genotypes and that the potential benefit from using the highest levels of both factors is limited. Therefore, inoculation with 1×10^4 to 1×10^5 conidia ml^{-1} followed by 4 weeks of incubation is the optimal assay condition for field screening of sorghum germplasm for resistance to *Fusarium* stalk rot.

Furthermore, data showed that disease severity was generally not affected by interaction between inoculum dose and incubation period. In situations where the above recommendations are difficult to achieve, lower levels of one or both of these factors can be used. The significant interaction between genotype and inoculum dose did not involve change of ranks and hence would not affect the possibility of using combination of various levels of the two factors. Such interaction largely occurs because of the slow response of stalk rot resistant genotypes to various levels of inoculum doses. Crosses involving resistant males SC1154, SC1039 and SC134 showed only small differences as concentration of inoculum dose varied, whereas the susceptible entries showed marked variation, resulting in significant genotype x inoculums dose interaction (Table 3).

This new assay condition (1×10^5 conidia ml^{-1} dose and 35 day incubation period) was used to evaluate an array of sorghum genotypes for stalk rot resistance. The procedure revealed marked difference among genotypes. Known resistant and susceptible sources were clearly resolved with this procedure. Disease severity among susceptible genotypes was 3 to 4 times higher than resistant sources, indicating that the method can be applied in large-scale germplasm screening. A large number of genotypes with previously unknown stalk rot reaction were stacked between the susceptible and resistant sources, resulting in a continuous variation in the ratings (Figures 2a and 2b). Some of the new genotypes had remarkably low infection scores and were grouped with the resistant sources. Most of these genotypes are public inbred parents bred at Texas and Purdue and are tolerant to post flowering drought stress.

The greatest advantage of this technique over the tooth pick inoculation is that it allows monitoring of inoculum doses administered to each plant and as a result improve accuracy in the data collected. In this study we did not compare the accuracy of the results from the old (tooth pick) and the improved method, but the defect with the old method was well recognized both in corn and sorghum.

In corn, liquid inoculum injection was proven to provide more accurate estimation of ear rot infection than the traditional toothpick method (Celements et al., 2003). In addition, this technique is much simpler both in terms of inoculum preparation procedure as well in the actual inoculation. A single 250 ml beaker suspension culture can produce sufficient inoculum to inoculate 5000 plants and this can be produced in 48 h. It will take more than 20 petri-dishes and at least 7 days to produce enough tooth pick inoculums to inoculate this many plants.

Conclusion

Screening sorghum germplasm for resistance to *Fusarium* stalk rot can be effectively done with the liquid inoculation procedure. Although various inoculum doses and disease incubation periods can be used, inoculation with 1×10^4 to 1×10^6 conidia ml^{-1} followed by 4 to 6 weeks of incubation gives the best result. But the highest inoculum dose of 1×10^6 conidia ml^{-1} may be difficult to achieve for large-scale inoculation especially with low sporulating strains. Also, it may not be practical to keep inoculated plants for up to 6 weeks in the field or greenhouse. We recommend use of a liquid inoculum suspension containing 1×10^4 to 1×10^5 conidia ml^{-1} and incubation periods of 4 weeks as an optimum procedure for screening of wide range of sorghum genotypes.

ACKNOWLEDGEMENTS

The authors wish to thank the Kansas Sorghum Commission for financial support of this study and the Kansas State University Department of Agronomy for providing field and laboratory facilities for conducting the experiment.

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