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Reaction of Some Sorghum Varieties Against Grain Mold and Fumonisin Accumulation

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Abstract
Grain mold caused by *Fusarium verticillioides* is one of the most dangerous food and feed safety challenges in sorghum production. The most efficient solution for reducing the hazards of the disease is breeding resistant varieties. In order to find the resistant sorghum varieties, nine sorghum varieties were evaluated for their reaction to *F. verticillioides* and fumonisin accumulation in their kernels under field conditions in Ardabil Agricultural Research Station, Moghan region, Iran (39° 41' N 47° 32' E, with 40-50 m above sea level), using a randomized complete block design with three replications. For artificial inoculation, sorghum panicles were sprayed with the mixture of spore suspension at 50% flowering stage. Two months after inoculation, disease severity was evaluated based on disease progress in each panicle. In addition, total fumonisin produced on sorghum kernels was measured using ELISA kits. The results showed that among studied varieties, KGS12 and KGS15 were moderately resistant and the other eight varieties were highly susceptible or susceptible to grain mold. The mean grain yield under artificial infection showed a reduction of 46.2% in comparison to the natural infection condition (control). The fumonisin accumulation in grains varied from 0.35 ppm (in KGS11 variety) to 1.83 ppm (in Sepideh variety) at the artificial infection condition, while the range was from 0 ppm (in the Kimia variety) to 0.16 (in the KGS15 variety) at the natural infection condition. The mean fumonisin accumulation under natural and artificial infection conditions was 0.07 and 1.00 ppm, respectively, which showed an increase of 1328% in comparison to the control treatment. In general, KGS12 and KGS15 were identified as superior varieties due to their lower disease severity and fumonisin accumulation and acceptable grain yield as compared with other varieties.

Keywords: Grain mold; Fumonisin; *Fusarium verticillioides*; Resistance; Sorghum

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in world after wheat, rice, maize and barely. It is cultivated annually over approximately 45 million ha, producing approximately 60 million tons of grain (FAO 2014). Sorghum grain is a major food in most areas of Africa, South Asia and Central America, and an important animal feed in USA, Australia, South America and Iran. In addition to these uses of the grain, sorghum crop residues and green plant also provide sources of animal feed and fuel (Reddy *et al.* 2010). In fact, the yield potential of sorghum is quite high; grain yields can exceed 10 t/ha under favorable conditions. However, there are some problems including drought, low soil fertility, pests and diseases that they cause this crop unable to take advantage of its potential (Singh *et al.* 2005).

Sorghum is the host of many diseases that are caused by fungi, bacteria, viruses and nematodes. Among over 50 diseases reported and described, only a few are economically important globally. However, there are several diseases that are regionally and locally important in specific ecosystems (Thakur *et al.* 2006). Grain mold (GM) is the most important diseases that affects sorghum crop in Iran. Major fungi involved in grain mold complex are *Fusarium verticillioides*, *F. thapsinum*, *Curvularia lunata*, *Phoma sorghina*, *Colletotrichum graminicola* and *Alternaria alternata*. Among these, *F. verticillioides* is more pathogenic than others (Thakur *et al.* 2003).

Damage resulting from grain mold infection includes reduced kernel development, discoloration of grain, colonization and degradation of endosperm and germ, decreased grain density, decreased germination, decreased seedling vigor and possible mycotoxin contamination particularly with *Fusarium* species (Navi *et al.* 2005). Production losses due to sorghum grain mold range from 30% to 100% depending on cultivar, time of flowering and prevailing weather conditions during flowering to harvesting (Singh and Bandyopadhyay 2000). Fumonisins are mycotoxins produced mainly by *F. verticillioides* in several crops, especially sorghum. This toxin causes leukoencephalomalacia in equines and rabbits, pulmonary edema in swine, and it has been reported as a probable cause of human esophageal cancer (Josefa *et al.* 2004).

Although cereals are important substrates, moisture level and temperature are the critical abiotic factors regulating the growth of *F. verticillioides* and production of fumonisins. The best temperature range is 20−28 °C for fumonisin production but low kernel moisture content (less than 22%) should reduce or prevent toxin production during storage (Fodor *et al.* 2006).

The only practical and economical method for the control of GM in sorghums are avoidance and/or sowing GM-resistant cultivars (Milliano *et al.* 1992). Breeding for GM-resistance began many years ago but the progress has been rather slow because of the association of several plant traits with resistance. Modern cultivars, especially F1 hybrids that have been deliberately bred for early and medium maturity, shorter plant height and higher grain yield with compact panicle in white-grain background often succumb to grain mold compared to late maturing, tall local land races with colored grain (Reddy *et al.* 2000; Thakur *et al.* 2006; Thakur *et al.* 2008). Several breeding methods, including pedigree selection, backcrossing and population breeding have been tried to generate GM-resistant inbred lines, varieties and populations. Analysis of the efficiency of various crossing methods indicated the superiority of biparental mating followed by pedigree selection in breaking linkages between undesirable plant traits and grain mold resistance (Reddy *et al.* 2000).

Considering that grain mold infection and development are greatly influenced by weather variables, such as temperature, relative humidity and rainfall quantity and distribution (Navi *et al.* 2005; Thakur *et al.* 2009), it is important to assess sorghum varieties for grain mold resistance in the target production areas. Therefore, the present study was designed to evaluate (1) resistance of some new advanced and commercial sorghum varieties to grain mold and (2) fumonisin accumulation rates in the studied sorghum varieties.

Materials and Methods

Nine sorghum varieties (Kimia, Sepideh, KGS11, KGS12, KGS15, KGS17, KGS23, KGS27, KGS32) were used in the present study. These varieties were evaluated for their reaction to *F. verticillioides* under field conditions. The experiment was carried out in the Moghan region

(39° 41' N 47° 32' E, with 40-50 m above sea level), Ardabil, Iran, using a randomized complete block design with three replications. A plot was made of four rows of 3 m length with a plant-to-plant spacing of 15 cm and row-to-row spacing of 75 cm. For artificial inoculation, sorghum panicles were sprayed by the mixture of spore suspension on one of the two middle rows in each plot at 50% flowering stage. Then, they were covered with paper bags for two weeks. To evaluate the development of the disease, 10 plants from artificial inoculation and natural infection (control) were randomly selected in each plot, two months after inoculation. In addition, total fumonisin produced on sorghum kernels was evaluated by ELISA kits (AgraQuant Fumonisin Kit; Romer Labs, Austria). In order to evaluate the disease development, 10 plants per plot were randomly selected from each of two middle rows of the plots at harvesting time and grain yield (g plant^{-1}) and infection severity (%) were determined. The grain mold severity was rated as the percentage of visibly infected kernels on each panicle. Then percentage of visibly infected kernels on each panicle surface transformed to a 9-class rating scale in which 1= no molded grain in a panicle, 2= 1 to 5% infection, 3= 6 to 10% infection, 4= 11 to 20% infection, 5= 21 to 30% infection, 6= 31 to 40% infection, 7= 41 to 50% infection, 8= 51 to 75% infection and 9= > 75% infection on a panicle (Thakur *et al*. 2006). Finally, according to Thakur *et al*. (2006), the studied varieties were classified into HR (highly resistant), R (resistant), MR (moderately resistant), S (susceptible) and HS (highly susceptible) categories based on disease severity scale (Table 1).

Collected data were initially subjected to analyses of variance (ANOVA) using MSTAT-C computer package. Infection severity values were transformed to log10 (1 + incidence of discolored kernels) to normalize the residuals. It was not possible to normalize the data with converting of the data in case of disease severity scale (1-9), therefore, Friedman test (nonparametric statistical method) was used to determine the differences between the varieties in terms of this trait. Friedman test was performed with SPSS software (version 15).

Table 1. The classification of studied varieties to highly resistant, resistant, moderately resistant, susceptible and highly susceptible based on 1 to 9 severity rating scale (Thakur *et al*. 2006)

Disease reaction class	Disease severity scale (1-9)
HR (highly resistant)	1(no symptoms)
R (resistant)	2-3 (up to 10% mold infection)
MR (moderately resistant)	4-5 (11-30% mold infection)
S (susceptible)	6-7 (31-50% mold infection)
HS (highly susceptible)	8-9 (> 50% mold infection)

Results and Discussion

The result of ANOVA showed significant difference among studied sorghum varieties in terms of disease severity at the artificial infection condition, however, the difference was not significant in the natural condition (Tables 2 and Table 3).

Grain mold severity for all nine varieties ranged from 5 (i.e., 21 to 30% grain mold symptom) to 8 (i.e., 51 to 75% grain mold symptom) in the artificial infection condition. Variety KGS15 had the lowest disease severity level with 23.3% grain mold infection among the studied varieties, while Sepideh had the highest disease severity with 70% grain mold infection in artificial infection condition (Table 4). Overall, none of the studied varieties were resistant to the disease.

Table 2. Analysis of variance for grain yield per plant and infection severity percent in sorghum varieties under study

SOV	df	Grain yield per plant		Infection severity percent		Fumonisin at artificial infection
		Natural infection	Artificial infection	Natural infection	Artificial infection	
Replication	2	8.529	71.004	0.081	0.022	0.06
Variety	8	413.257**	551.357**	0.221^{ns}	0.080**	4.88**
Error	16	67.562	22.987	0.263	0.004	0.22

^{ns} and **: Non-significant and significant at 1% probability level, respectively.

In general, based on reactions of the studied varieties to the grain mold disease, varieties Kimia, Sepideh, KGS11, KGS17 and KGS23 were highly susceptible (HS) as evident by the disease incidence percent (> 50% mold infection). In the same way, the varieties KGS12 and KGS15 appeared as moderately resistant (MR) while KGS27 and KGS32 were recognized as susceptible (S) varieties (Table 4).

Table 3. Friedman test for comparing sorghum varieties in terms of disease severity scale (1-9)

Trait	Chi-square	df	P-value
Disease severity at artificial infection	20.500**	8	0.009
Disease severity at natural infection	7.124 ^{ns}	8	0.523

^{ns} and **: Non-significant and significant at 1% probability level, respectively.

Thakur *et al*. (2008) showed that among 156 studied accessions, 19 were highly resistant (1.0 score), 134 resistant (1.1 to 3.0 score) and 3 moderately resistant (3.1–3.5 score). Resistant materials were reported in Thakur *et al*. (2008), because of using landrace material. Most commercial varieties have moderately susceptible or susceptible reaction to the disease (Thakur *et al*. 2008). The presence of resistance genes in native populations can be useful in producing advanced sorghum varieties.

Table4. Mean of grain yield per plant, disease incidence (%) and disease severity for the studied sorghum varieties

Varieties	Grain yield per plant (g)			Fumonisin accumulation (mg kg^{-1})		Disease incidence (%)		Disease severity		Variety reaction category*
	Natural infection (control)	Artificial infection	Reduction %	Natural infection (control)	Artificial infection	Natural infection (control)	Artificial infection	Natural infection (control)	Artificial infection	
Kimia	85.1	34.1	60.0	0.00	1.14	1.7	63.3	2	8	HS
Sepideh	72.7	66.9	8.0	0.03	1.83	10.0	70.0	3	8	HS
KGS11	78.5	34.2	56.5	0.02	0.35	3.3	65.0	2	8	HS
KGS12	81.9	53.3	34.8	0.05	1.54	3.3	30.0	2	5	MR
KGS15	75.4	21.3	71.7	0.16	0.41	0.0	23.3	1	5	MR
KGS17	68.3	41.3	39.6	0.03	0.97	6.7	60.0	3	8	HS
KGS23	80.1	32.9	58.9	0.12	1.24	3.3	56.7	2	8	HS
KGS27	49.1	34.9	29.0	0.15	1.09	3.3	46.7	2	7	S
KGS32	58.8	30.7	47.8	0.07	0.44	0.0	38.3	1	6	S
Mean	72.2	38.8	46.2	0.07	1.00	-	-	-	-	-

*MR (moderately resistant), S (susceptible), HS (highly susceptible)

It is well known that variation exists for grain mold resistance in sorghum germplasm, but breeding to improve for resistance has had limited success. This is partly due to incomplete understanding of the genetics of resistance and the complex interaction of traits that influence grain mold resistance (Thakur *et al.* 2006).

In the natural infection condition, grain mold disease severity for all nine varieties ranged from 1 (no infection) to 3 (6 to 10% grain mold infection). Varieties KGS15 and KGS32 had the lowest disease severity level among the studied varieties (Table 4). Although, grain mold is present at low levels on most field-grown sorghums, but may spark to high levels depending on both the environment and genetics of the host plant.

Fumonisins produced by *F. verticillioides* have been associated with potentially serious toxicoses of animals and humans. Thus, varieties with low fumonisin accumulation in grain will be valuable for the production of sorghum-based human food and animal feed products (Kleinschmidt *et al.* 2005). Generally, high fumonisin accumulation in grain has been associated with environmental conditions, such as drought and high relative humidity (Clements *et al.* 2004), which frequently occur in Moghan region, Iran. In our study, the fumonisin accumulation varied from 0.00 (in the variety Kimia) to 0.16 ppm (in the variety KGS15) under natural infection condition and from 0.35 ppm (in the variety KGS11) to 1.83 ppm (in the variety Sepideh) under artificial infection condition. Our results were consistent with Rahjoo *et al.* (2011). They concluded that fumonisin levels in sorghum kernels ranged from 0.2 ppm to 4.8 ppm in the artificial infection condition. On the other hand, variety Sepideh (highly susceptible variety) had significantly higher levels of fumonisin in the grain and higher infection rate (70.0% grain mold infection) than the other genotypes used in this investigation. Rahjoo *et al.* (2011) found that more susceptible sorghum varieties to grain mold had a significantly greater infection rate than either the resistant or intermediate varieties.

Mean fumonisin accumulation under natural infection condition was 0.07 ppm while mean fumonisin accumulation under artificial infection condition was 1.00 ppm, which showed an increasing of 1328% in comparison to the natural infection condition (Table 4). This means that under favorable conditions and disease occurrence, fumonisin accumulation would increase significantly and this could be serious risk to human health. Therefore, more attention should be focused on this issue and will get more priority than the improvement of resistant varieties to this disease. To select varieties with low fumonisin contamination, the direct selection through fumonisin accumulation is more efficient than the indirect selection through grain mold. However, measuring the fumonisin accumulation is time consuming and expensive. Therefore, visually assessing the percentage of grain mold and scoring is often used for identifying sources of resistance (Shiri and Ebrahimi 2017).

There were significant differences among sorghum varieties for grain yield under *F. verticillioides* inoculation (Table 2). This result implies that sorghum varieties reacted differently when inoculated with *F. verticillioides*. It also indicates the presence or absence of host resistance genes in different sorghum genotypes against this fungus. In this study, the grain yield varied from 49.1 grams (in the variety KGS27) to 85.1 grams (in the variety Kimia) per plant under natural infection condition and from 21.3 grams (in the variety KGS15) to 66.9 grams (in the variety Sepideh) per plant under artificial infection condition. Mean grain yield under natural infection condition was 72.2 grams per plant, while it was 38.8 grams per plant under artificial infection condition, which showed a reduction of 46.2% in comparison to the natural infection condition. The highest reduction in yield per plant (71.7%) due to *Fusarium* infection occurred in the variety KGS15 (Table 4). Although maximum disease severity and fumonisin accumulation were observed in the Sepideh variety, however, this variety showed the minimum yield reduction per plants (8.0%) due to *Fusarium* infection. The disease severity was measured based on the observed symptoms not the grain rotting. On the other hand, fumonisin can even accumulate in grains without symptoms. Therefore, this variety was highly sensitive despite minimal grain yield reduction per plant (Table 4).

Conclusion

Management of grain mold using resistant cultivars is considered as an important strategy. In the current study, reaction of sorghum varieties to grain mold was assessed on the basis of disease severity and fumonisin accumulation in grains. Significant variations were noticed among the nine tested sorghum varieties for their response to grain mold. None of the varieties were found immune or highly resistant against grain mold, although moderate resistance and low fumonisin accumulation were observed in KGS12 and KGS15 varieties.

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