Evaluation of the resistance of sorghum varieties against *Phoma sorghina* in field conditions in Burkina Faso

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Abstract

Phoma sorghina, one of mold pathogen agents, is commonly and abundantly found on sorghum seed. In order to contribute to the improvement of grain quality, we undertook to evaluate the resistance of sorghum varieties in field. *P. sorghina* was assessed on vegetative organs and on the harvested grains using Blotter method according to ISTA 1999. The results revealed that *P. sorghina* is present at variable frequencies depending on the organ and the variety of sorghum tested. On harvested grains, low infection rates have been noted from protected panicles in comparison with unprotected ones with relatively high rates infection. With regards to *P. sorghina* infection, we have identified that Gnonfing is susceptible and in opposite Sariaso 08 is resistant to infection by *P. sorghina*. The results of this activity can be used by sorghum breeders in order to improve the resistance of the most commonly varieties of sorghum against *P. sorghina*.

Key Word: Sorghum varieties, *Phoma sorghina*, sensitive, resistant, Burkina Faso.

Introduction

Phoma sorghina is one of the pathogens responsible of sorghum seeds mold [1, 2]. In Burkina Faso, the evaluation of sorghum seeds mycolfora revealed that *P. sorghina* was the most common fungus and the most abundant in seed samples collected in different agro-ecological zones [3, 4]. This fungus can also cause a loss of sorghum grain viability and post-emergence mortality of sorghum plants in the field [5, 6, 7]. In addition, it also produces mycotoxins that are very harmful to humans and animals [8, 9]. As sorghum is being the main cereal produced in Burkina Faso and consumed by about 80% of the population in rural areas, *P. sorghina* constitutes a real risk for public health [1].

In order to control effectively this fungus, we have developed a seed treatment method based on aqueous extracts of *Cymbopogon citratus* [4, 10]. This treatment makes it possible to obtain healthy and vigorous plants [1]. The use of chemicals products for seeds treatment has shown that chemicals treatments were also a good way to control *P. sorghina* on seeds. However, it turns out that infection by *P. sorghina* takes place in the field during the flowering and grain formation period. As a result, our effort to get healthy plants through

seeds treatment will only serve to reduce post-emergence mortalities and improve grain yield at harvest. But the harvested grains still had fairly high infection levels and the exposure risk of the population to mycotoxins produced by *P. sorghina* was still significant. Exploring other control methods, such as the use of resistant varieties, is necessary (is a must). So, we started to evaluate the resistance of 07 popularized varieties in Burkina Faso, against *P. sorghina* in the field. The overall objective of this study is to contribute to the development of an efficient method to control *P. sorghina*.

Materials
The study focused on eight (07) varieties of sorghum:
-Sariaso 05, Sariaso 08, Framida, ICSV 1049, Gnonfing and Grinkan which are long duration varieties of 120 days;
-Kapelga variety with a duration of 95days.
These varieties have been recommended to us by the Environmental Institute for Agricultural Research because they are most cultivated by producers and are resistant to mold.

Experimental site
The experimental design was set up on the station of the Environmental Institute for Agricultural Research (INERA) located at Farako-Bâ. The station of Farako-Bâ was located at 15 km in the southwest of Bobo-Dioulasso on the way leading to Banfora, 4°20 W longitude, 11°06 N latitude and at 405 meters altitude. According to [11] Guinko (1984), the southwestern zone of Burkina Faso in which Farako-Bâ is located belongs to the Sudano-Guinean climate. This climate is characterized by the alternation of a rainy season (May to October) with a dry season (November to April). The cumulative rainfall during the year was 4585.2 mm on a total of 80 days of rain. The wettest months are those of June and August.
The soil is a ferruginous tropical one with an argilo-sandy or sablo-muddy texture on the surface, low in organic matter (0.7%) and having a pH ranging between 5 to 5.5.

Methods
Experimental design
The experimental design was a Fisher block including seven (07) treatments repeated three times which gives a total of 21 elementary plots. The blocks are separated from each other by paths of 1.2 meter. Each elementary plot contains 4 lines and 11 seed holes by line, making a total of 44 seed holes.

Seed treatment
Two hundred (200) grains of each variety were separately introduced into 80 ml of sterile plastic tubes containing 30 ml of sterile water. The tubes were then placed in a water bath at 50°C for 40 min to disinfect the seeds. The disinfected seeds a were dried in aseptic conditions on sterile blotting paper for 12 h.

Soil preparation, sowing and plants management
Soil preparation consisted of plowing followed by harrowing in order to have a good seedbed. The sowing was carried out on July 25 and four (04) seeds were sown by seed hole according to the spacing of 80 cm between lines and 40 cm between seed holes.
Plants weeding by seed hole was done 14 days after sowing (DAS), to control weeds. The mineral fertilizer (NPK 15-15-15-6S-1B) was also applied the same day after weeding at the rate of 150 kg/ha, i.e. 210 g by elementary plot or 4.78 g by seed hole. Urea was added at 45 DAS at the rate of 50 kg/ha or 70.4 g per plot unit or 1.6 g by seed hole, followed by manually ridging to prevent plant fall, to control weeds and promotes infiltration. At the

swelling stage, one panicle was covered in some seed hole with craft paper envelopes to protect it against *P. sorghina* inoculum in the air. A total of three (03) panicles are protected per elementary plot. The harvest took place in November. For each variety, protected (P) and unprotected (NP) panicles were harvested separately in each elementary parcel.

Sampling and evaluation of mycoflora on vegetative organs and seeds.
At twenty-four (24) DAS, five (05) seedlings plants were collected on middle lines of each elementary plot and arranged separately. In laboratory, plants were washed with tap water to remove soil particles on roots. Some fragments were collected from each vegetative organ using a disinfected scalpel. Five fragments of each vegetative organ obtained were placed in Petri dishes containing three discs of moistened blotter papers The Petri dishes were incubated at 22°C under 12 hours alternating cycles of near ultraviolet (NUV) light and darkness. The leaves were evaluated after five (05) days of incubation while the stems and roots were evaluated after a 7-day-incubation period.
The harvested grains were incubated using the Blotter method described by [12, 13] ISTA, (1999) and Mathur and Kongsdal, (2003). Twenty-five (25) grains were placed in Petri dishes containing three (03) discs of blotting papers moistened with sterile water. The Petri dishes were incubated at 22°C under 12 hours alternating cycles of near ultraviolet (NUV) light and darkness for seven (07) days. A total of two hundred (200) grains per sample were evaluated.

Evaluation
a. Evaluation of the mycoflora of vegetative organs
At the end of the incubation period, vegetative organs were examined individually with stereoscopic material to detect the presence or absence of different fungi and particularly *P. sorghina*.

b. Evaluation of mycoflora of harvested grains
At maturity, the protected and unprotected panicles were harvested separately per elementary plot, dried and threshed separately. Sampling was carried out per elementary plot using a conical divider while taking into account (or considering) protected and unprotected panicles. Blotter method described above was used for mycoflora evaluation.

Data analysis and results presentation
Data collected on vegetative organs and harvested grains were analyzed using the software MINITAB version 16. ANOVA was calculated for all the parameters. The means were compared using Student Newman and Keuls multiple classification test at 5% when the analysis showed significant differences between treatments. The results are presented in tables.

Results
Evaluation of **Phoma sorghina** *transmission from seeds to vegetative organs of sorghum*
P. sorghina was detected on all the varieties tested. The ANOVA analysis did not show any difference between sorghum varieties (Table 1). Low infection level was observed on roots of Sariaso 05 (0.5%) and Sariaso 08 (0.2%) while high infection rate was obtained on roots of Framida, (0.67%) ICSV 1001 (0.67%) and Gnonfing (0.60%). Stem low infection rate was observed on Kapelga and ICSV (0.33%) and high on Sariaso 08 (0.60%). *P. sorghina* was less transmitted to leaves of Sariaso 08 (0.33%) and highly transmitted to Framida, Sariaso 05 and Kapelga (Table 1).

Table 1: Transmission of *Phoma sorghina* to vegetative organs of sorghum plants in field

Varieties	Roots	Stem	Leaves
Framida	0.67	0.53	0.67
Sariaso 05	0.13	0.47	0.67
ICSV 1049	0.67	0.33	0.47
Grinkan	0.47	0.53	0.47
Kapelga	0.40	0.33	0.67
Gnonfing	0.60	0.53	0.47
Sariaso 08	0.20	0.60	0.33
F value	**2.60**	**0.50**	**0.52**
Probability	**0.054**	**0.820**	**0.808**
Signification	**NS**	**NS**	**NS**

NS : Not significant

Evaluation of *Phoma sorghina* transmission to sorghum harvested grains

The analysis of variance showed highly significant differences between grain infection of both protected and unprotected panicles. *P. sorghina* was transmitted to grains from protected or unprotected panicles. However, variety Gnonfing differed from other varieties for its susceptibility to *P. sorghina* (74.33%) on grains from protected panicles. For other varieties, the infection rate varied from 9.17% for Sariaso 05 to 29.5% for Kapelga (Table 2). Compared to other varieties, lower infection rate (43.64 %) was obtained on unprotected panicles of Sariaso 08. It was even statistically different from Gnonfing which had high infection rate of 81.5% (Table 2).

Table 2: *Phoma sorghina* transmission to protected and unprotected seeds of sorghum

Varieties	*Phoma sorghina* infection rate %)	
	Protected panicles	Unprotected panicles
Framida	12.33[b]	67.50[ab]
Sariaso 05	9.17[b]	60.67[ab]
ICSV 1049	15.33[b]	67.00[ab]
Grinkan	24.17[b]	67.17[ab]
Kapelga	29.50[b]	71.00[ab]
Gnonfing	74.33[a]	81.50[a]
Sariaso 08	10.17[b]	43.67[b]
F value	**28.39**	**4.10**
Probability	**0.000**	**0.014**
Signification	**HS**	**HS**

HS: Highly significant

Compared effect of panicles protection on the infection rate of *Phoma sorghina* on harvested grains

Protection of panicles before swelling period contributed to the reduction of *P. sorghina* infection rate. Comparative ANOVA showed highly significant differences between protected and unprotected panicles, with the exception of Gnonfing (Table 2). The Student Newman and Keuls multiple classification indicated a separation between infection rates of grains obtained from protected panicles and those from unprotected panicles of the other varieties of sorghum tested. Infection level of protected panicles of tested varieties excepted Gnonfing was between 9.17 to 29.5 % and that of unprotected panicles was between 43.67 to 81.5 % (Table 2). The comparative analysis between protected an unprotected panicle

showed that panicle protection before swelling had not significant effect on the reduction of infection rate of Gnonfing.

Tableau 3: Comparison of the effects of protection and unprotection of panicles on the infection rate of *Phoma sorghina*

Varieties	*Phoma sorghina* infection rate %
Framida P	12.33[d]
Sariaso 05 P	9.17[d]
ICSV 1049 P	15.33[d]
Grinkan P	24.17[cd]
Kapelga P	29.50[cd]
Gnonfing P	74.33[a]
Sariaso 08 P	10.17[d]
Framida NP	67.50[ab]
Sariaso 05 NP	60.67[ab]
ICSV 1049 NP	67.00[ab]
Grinkan NP	67.17[ab]
Kapelga NP	71.00[a]
Gnonfing NP	81.50[a]
Sariaso 08 NP	43.67[bc]
F value	**29.29**
Probability	**0.000**
Signification	**HS**

HS: Highly significant; P: Protected panicles et NP: Unprotected panicle

Discussion

Sorghum was the main cereal consumed in Burkina Faso. The evaluation of mycoflora showed that these grains were very infected by a mycotoxinogenic fungus *Phoma sorghina.* As part of the search for effective control methods against this fungus, varieties well adopted by producers were evaluated in field against this seed-borne fungus. Evaluation of mycoflora on vegetative organs of different sorghum varieties showed that *P. sorghina* infects all organs regardless the variety tested. Similar results were obtained by [14] Bonzi *et al.* (2013) who showed that *P. sorghina* was transmitted to leaves, stems and roots of sorghum plants obtained from infected kernels. The analysis of grain mycoflora of different varieties revealed the presence of *P. sorghina* in the grain samples of all the varieties tested. Infection rate however depends on the variety. Our work indicates that Gnonfing is susceptible to *P. sorghina* and Sariaso 08 was resistant to this fungus. This variety, which is declared vulnerable to mold agents, has a fairly high infection rate (71%) by *P. sorghina*. The protection of sorghum panicles before the swelling period allows a significant reduction in *P. sorghina* infection, except for Gnonfing, for which the protection did not induce a significant reduction. These results corroborate those obtained by [15] Bonzi *et al.* (2012) who showed that the protection of sorghum panicles before the heading period reduces *P. sorghina* infection in grains obtained from protected panicles. This work also confirms those of [1] Bonzi (2013) regarding the role of *P. sorghina* inoculum found in the air in increasing *P. sorghina* infection rate.

Conclusion

The most cultivated varieties in Burkina Faso were susceptible to infection by *Phoma sorghina*. But, the results of our study point out that Sariaso 08 has shown good resistance

against this fungus in field. In order to reduce the infection rate by *P. sorghina* in harvested grains we firstly sensitize producers to use this variety. In second point sorghum breeder must use Sariaso 08 as materiel to improve the resistance of the other sorghum varieties against *P sorghina*, the mold and mycotoxigenic fungus of sorghum in Burkina Faso.

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