

# Pathogenic variation of *Phoma sorghina* on sorghum bicolor in Burkina Faso

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## ABSTRACT

*Phoma sorghina* is the most frequent fungus identified on sorghum seed in Burkina Faso. The isolates from different cereals and weed species in all the agro-climatic zone of the country indicated that morphological characters were so different and the statistical analysis have shown that isolates can be group in three distinct class. The objective of this study was to analysis pathogenic variation among *P. sorghina* isolates. To reach this objective thirteen isolates of *P. sorghina* were chosen in morphological class, according to plant species and climate zone. The method of seedling symptom test on sand was used. The effect of *P. sorghina* inocula on sorghum seed germination shown that isolates 1Dh08-01(3), 474So07-40 and 181.80 significantly reduced sorghum seed germination when compared with the control. The isolate 474So07-40 was the most virulent in the experimental conditions. By measuring weight and height of sorghum plant obtained from infected seeds, we have noticed that the infection by *P. sorghina* significantly diminishes infected plants synthesis capacity in comparison with the control. All the tested isolates significantly reduced the weight of the vegetative aerial organ of sorghum. Excepted isolates 1670So11-78, 1671So11-79 and 1672So11-80, the other *P. sorghina* tested isolates diminish the height of sorghum plant obtained from infected seed. The isolates Spf08-02 and 1341So07-65 were those which induced most mortality after emergence. The effects of *P. sorghina* isolates on sorghum seed germination, plant mortality and growth were variable. The isolates from weed were very aggressive on sorghum. This study clearly indicated the selectivity pressure of weed on the population of the pathogen.

**Keywords:** Pathogenicity, *Phoma sorghina*, germination, sorghum growth.

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## INTRODUCTION

In Burkina Faso sorghum is the main cereal grown. However, its production encounters many difficulties including fungal diseases. *Phoma sorghina* is the most frequent seed fungus identified in sorghum seed samples in Burkina Faso (Bonzi et al., 2012). According to the result of Boiron (2006), this fungus also secrete toxin harmful to human and animal. In Burkina Faso sorghum is the main cereal consumed. The high level of infection rate by *P. sorghina* can constitute in future a problem of public health. To prevent this, a method of control of *P. sorghina* must be found. Bonzi et al. (2012) shown that seed treatment with plant aqueous extract control the fungus efficiently. Plant resistance is also a best way to control *P. sorghina* infection on grain intended to human

consumption. For that, varietal selection for disease resistance requires a good knowledge of the pathogen. In this context, the selection must take into account the genetic diversity of the pathogen, its adaptability, in order to anticipate its evolution across time and space.

*Phoma sorghina* is one of the pathogens responsible of the development of mold on sorghum grain (Zida et al., 2010). This fungus has been identified on several plant species, which could imply the existence of pathogenic variability among the fungus isolates (Zainun and Parbery, 1974, Venkatasubbaiah et al., 1992; Porello and Moreno, 2005). Bonzi (2013) has shown a morphological diversity between isolates of *P. sorghina* on (malt) agar culture medium *in vitro*. Pazoutova (2009) also noticed

the existence of morphological variability in the isolates of *P. sorghina*. De Souza et al. (1988) indicated pathogenic variability among *P. sorghina* isolates in the same rice variety. In Burkina Faso, there were not enough research conducted on pathogenic variation of *P. sorghina* on sorghum. The objective of this study was to analyse pathogenic variability of *Phoma sorghina* isolates on sorghum in Burkina Faso

## MATERIALS AND METHODS

### Isolates of *Phoma sorghina*

Thirteen isolates of *Phoma sorghina* are selected according to their morphological characteristics defined in Table 1.

### Sorghum variety used

Pathogenicity tests were conducted under laboratory conditions using a local variety 1617So10 collected from the Southwest Region (Gaoua) of Burkina Faso. This variety was selected after a preliminary experiment conducted in laboratory with IRAT204 (suggested by breeder from Agricultural and Environmental Researches Institute) and 1617So10. This study reveals that the local variety 1617So10 was more sensitive to *Phoma sorghina* than IRAT 204. Moreover, the evaluation of mycoflora shows that local variety 1617So10 is less infected with *P. sorghina* which is an

advantage because the low presence of *P. sorghina* make grains disinfection easier.

### Sorghum grain disinfection

One hundred (100) grains of local variety 1617So10 were soaked in 1% sodium hypochlorite for 4 minutes. During the soaking period, the mixture is stirred from time to time with a spatula to allow the immersion of sorghum grains. At the end of the disinfection time, the seeds were rinsed three times with sterile water and dried under aseptic conditions for 24 hours.

### Inoculum preparation

To optimize *P. sorghina* conidial production, ten (10) disinfected seeds from local variety 1617So10 are introduced separately into test tubes containing 10 ml of 1% water agar. Then, a mycelial explant from each tested isolate was removed from the active growth zone of 5 -days old colony and placed near each grain with a curved needle. The test tubes were incubated for 10 days at 22°C by alternating cycle of 12 hours of artificial light and 12 hours of darkness.

The pycnidia grown in the tubes were shredded with a spatula and transferred into a test tube containing 3 ml of sterile water. The mixture was homogenized with a vortex mixer. After this phase of dispersal of conidia, the mixture was filtered with filter paper and the conidial concentration was adjusted to  $10^6$  conidia/ml using a Malassez cell.

**Table 1.** Characteristics of tested *Phoma sorghina* isolates.

Isolates Code	Plant organs used for isolation	Isolates Origin	Isolates class
1341So07-65	Sorghum red seed	South west region	(3)
1666So11-74	Sorghum white seed	North center region	(3)
1Dh08-01(3)	<i>Digitaria horizontalis</i> seed	High pond region	3
2Spf08-02	<i>Setaria pallide-fusca</i> seed	High pond region	3
803Ri06-03	Rice seed	South West region	1
181.80	Reference isolate	Netherlands	1
180.80	Reference isolate	Netherlands	1
591Mi06-04	Millet seed	High pond region	3
474So07-40	Sorghum red seed	East region	4
1Hs08-1	Sorrel leaf	High pond region	2
1670So11-78	Sorghum red seed	High pond region	-
1672So11-80	Sorghum white seed	High pond region	-
1671So11-79	Sorghum white seed	High pond region	-

- : These isolates have not been classified. Classes indicated in brackets are obtained from morphological classification done by Bonzi (2013).

### Preparation of the substrate

The substrate used was fine white sand taken from the sand quarry of Kôrô located at 13 km on the main road Bobo-Dioulasso Ouagadougou. The sand was sterilized using steam sterilization at 90°C for 3 to 4 hours. After cooling, 800 ml of sand were distributed by plate. The surface of each plate was well leveled and 1 cm depth pockets/holes were made in the sand previously moistened. In total, 25 holes were made per plate. These holes were organized in

concentric circles.

### Experimental design

The experimental setup was a simple randomization with 13 treatments which were constituted by the different isolates in Table 1. For each treatment, 100 grains of sorghum variety 1617So10 were used and each treatment was repeated 4 times.

### Seeding and inoculation

A disinfected seed was introduced into each seed hole and inoculated with 20 µl of conidial suspension using a micropipette. Seed holes were closed, and the pots were lid with plastic bags, in order to limit evaporation. The plastic bag was removed after the beginning of the emergence. The plants were watered after 24 hours as necessary. Incubation lasts 10 days under laboratory conditions at temperatures ranged between 28 and 33°C.

### Assessment

The assessment consisted in counting sorghum live and dead plants, ten (10) days after seeding (DAS). The percentage of live or dead plants was then calculated. The height (cm) and weight (cg) of five (5) well-developed plants per replicate were also evaluated at 10 DAS for height and 15 DAS for weight.

### Data analysis

The obtained data have been saved in Microsoft Excel software and then an analysis of variance has been done using SPSS 11.0

software. When the analysis of variance reveals significant differences between treatments, the averages were ranked at 5% threshold/limit according to Student-Newman Keuls' multiple classification method.

## RESULTS

### Effects of *Phoma sorghina* on sorghum seedling emergence

ANOVA Analysis shows highly significant differences among treatments. Isolates 1Dh08-01 (3) from weed and 474So07-40 collected on sorghum significantly reduced sorghum emergence compared with the control. They were more aggressive in our experimental conditions. The other tested isolates of *P. sorghina* have no repressive effects on seedling emergence. Some isolates like 1672So11-80, 1671So11-79 and 591Mi06-04 would even improve the emergence rate of sorghum plants (Figure 1).

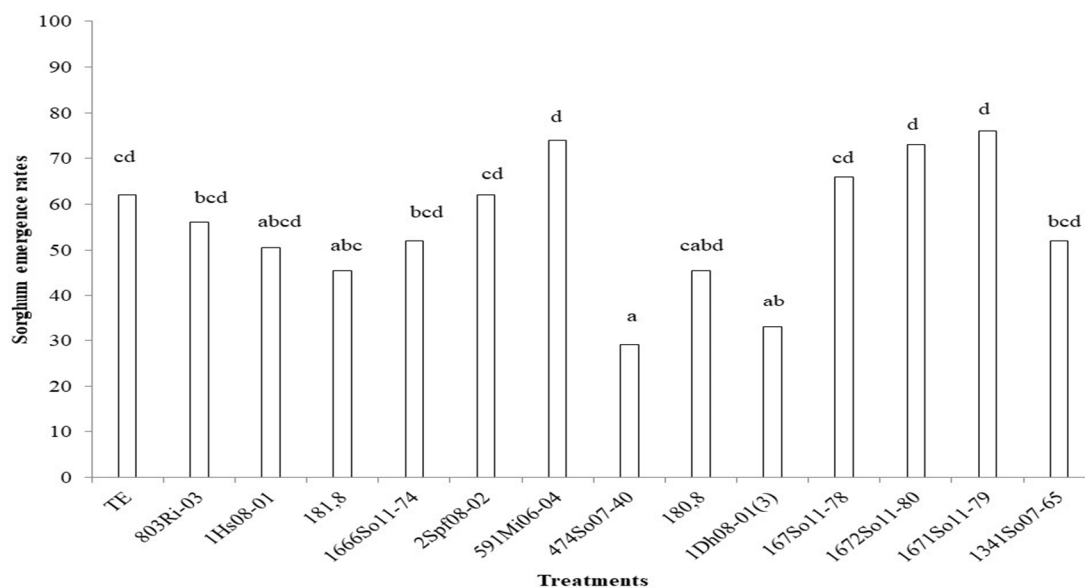


Figure 1. Effects of *Phoma sorghina* isolates on sorghum seedling emergence.

### Effect of *Phoma sorghina* on seedling mortality of sorghum

ANOVA analysis using number of sorghum dead plants after the emergence period shows differences between treatments that were highly significant. In general, the mortality of plants obtained from seeds inoculated with *P. sorghina* was relatively low. Isolates 2Spf08-02 collected on weed and 1341So07-65 significantly increased the number of dead plants compared to the control and the other tested isolates (Figure 2). Most of the isolates

collected on sorghum 1670So11-78, 1671So11-79, 1672So11-80 and 474So07-40 didn't cause seedling mortality when inoculated on sorghum plants, but isolates from others species in majority caused sorghum seedling mortality.

### Effect of *Phoma sorghina* isolates on seedling weight of sorghum

ANOVA analysis indicated significant differences among

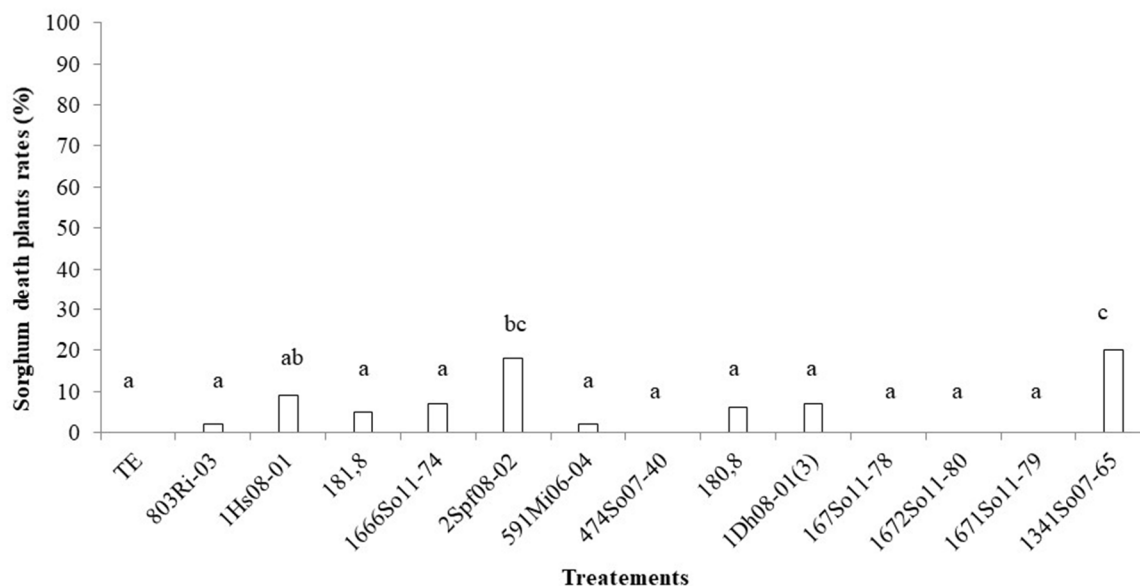


Figure 2. Effects of *Phoma sorghum* isolates on sorghum seedling mortality.

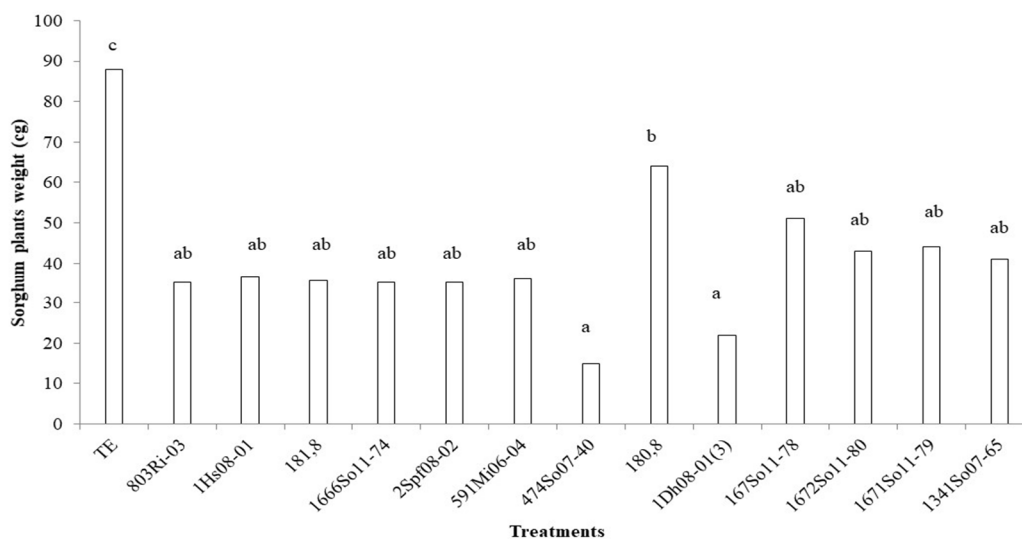


Figure 3. Effects of *Phoma sorghum* isolates on sorghum seedling weight.

treatments regarding seedling weight. In general, all the tested isolates meaningfully reduced the fresh weight of sorghum seedlings in comparison with the untreated control. Isolates 474So07-40 and 1Dh08-01 (3) from weed have negative effects on plants development. These isolates deeply reduced the weight of sorghum seedlings comparing with reference isolate 180.80 and the control (Figure 3).

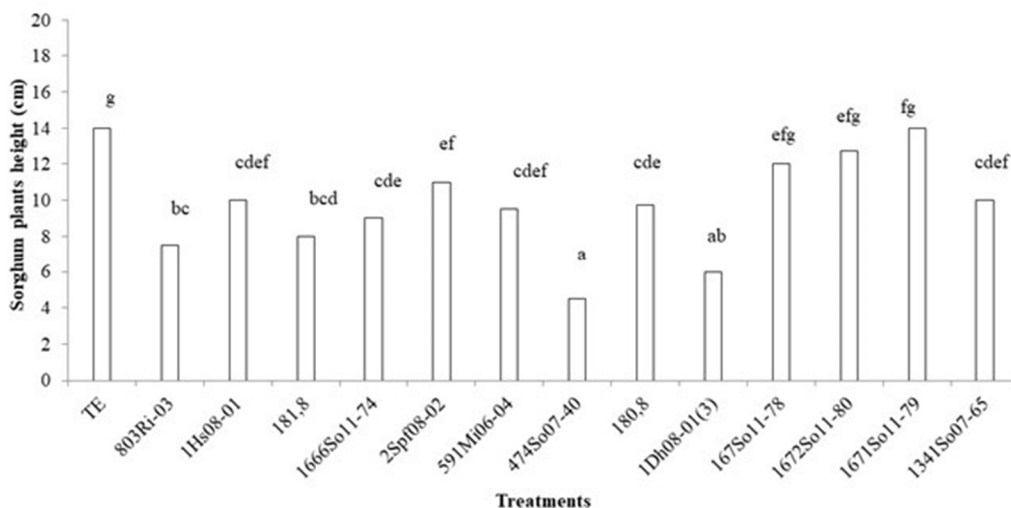
#### Effects of *P. sorghina* on sorghum plants height

ANOVA analysis on plants height reveals highly

significant differences among treatments. Except isolates 1670So11-78, 1672So11-80 and 1671So11-79, the other tested isolates significantly reduced plants height compared with the untreated control. Isolates 474So07-40 and 1Dh08-01 (3) significantly reduced plants height and appear as the most aggressive isolates (Figure 4).

#### DISCUSSION

*Phoma sorghina* is one of the sorghum grain mold pathogen. The pathogenic study of 13 well characterized isolates of *P. sorghina* on sorghum seedling emergence



**Figure 4.** Effects of *Phoma sorghum* isolates on sorghum seedling height

indicated that there was pathogenic variability among *P. sorghina* isolates. According to our experience the isolates 474So07-40 and 1Dh08-01 (3) were the most aggressive isolates tested. Similar, results were obtained by de Souza et al. (1988) in the study of rice glume blight, where they have detected variable levels on aggressiveness in *P. sorghina* isolates when inoculated on the same rice cultivar. On sorghum seedling emergence, isolates 474So07-40 and 1Dh08-01 caused a significant decrease in the rate of emerged plants compared with the control. The results of Castor and Frederiksen (1980), Gopinath and Shetty (1980), Williams and Rao (1984), Zida et al. (2010) also shown that *P. sorghina* was not only responsible of failure to emerge, it is also involved in post-emergence seedling mortality in sorghum and other plant species. The results of Punithalingam (1985) proved that *P. sorghina* is implicated in post emergence mortality of Sorghum, Macroptilium and Stylosanthes. In our experimental conditions, we have observed post-emergence mortality for all isolates except isolates 474So07-40, 1670So11-78, 1671So11-79 and 1672So11-80 collected on sorghum seed. The post emergence mortality was mostly caused by isolate from others plants species. The measuring of the weight on the 5 apparently healthy plants obtained from seeds artificially inoculated with a conidial suspension of *P. sorghina*, reveals that infection by *P. sorghina* reduced the synthesis ability of infected plants compared with no-inoculated ones. The consequences of low synthesis ability noticed on infected plants lead to weak plants with low weight. *P. sorghina* infection also affects sorghum plant height during the period of growth. The results of our experiment show for all the isolates employed, a significant decrease on the plant height resulting from seeds inoculated by *P. sorghina* compared with the control ; except isolates 1670So11-78, 1672So11-80 and 1679So11-79. At this

level also, isolate 474So07-40 was more aggressive than all *P. sorghina* isolates used in the experiment. Venkatasubbaiah et al. (1992) showed that phytotoxins such as epoxydon and diphenylether produced by *P. sorghina* inhibit sorghum plants roots growth at 1000 micrograms per Petri dish compared with the water control. Reduced roots development may affect the absorption capacity of the plant, thus its normal growth.

## CONCLUSION

The study of pathogenic variation of *P. sorghina* isolates clearly indicates the existence of pathogenic variability among isolates on sorghum in Burkina Faso. Some isolates from sorghum (474So07-40 and 1341So07-65) and the others from weed (1Dh08-01 (3) Spf08-02) are more aggressive. Most of *Phoma sorghina* isolates from others plant species caused post emergence mortality and have more negative effects on sorghum plant growth.

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