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Full Length Article

Phenotyping Bambara Groundnut Landraces for Resistance to *Macrophomina phaseolina* (Tassi) Goidnich

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Abstract

Bambara groundnut [*Vigna subterranea* (L.) Verdcourt] is one of the most important subsistance legumes cultivated in Burkina Faso, due to the high nutrient content of its seeds. However, bambara groundnut production can be seriously constrained by fungal diseases that reduce yields. *Macrophomina phaseolina* is one of fungal pathogen that causes important damage in bambara groundnut fields in Burkina Faso. The objective of this study was to identify sources of resistance to *M. phaseolina* among bambara groundnut landraces. Thus, a set of 20 genotypes was assessed under greenhouse conditions for their resistance to three strains of *M. phaseolina*. Comparison of the three strains of pathogen showed that Sahelian and Sudano-sahelian strains have the highest index of disease severity (7.13) on a scale of 1 to 9, respectively on landraces E86 and E62. The lowest index of disease severity (2.33) was recorded with Sahel strain of pathogen on landrace KVS 235_100GY. The genotypes were grouped in three classes such as resistant, moderately susceptible and susceptible genotypes. Each group consisted of genotypes with specific reaction to each strain. Six genotypes were resistant to all strains of *M. phaseolina*, three moderately susceptible and eleven specifically susceptible or resistant to each strain of the pathogen. The genotypes with stable resistance to all strains could be recommended to producers in all the three climatic zones and those with partial resistance according climatic zone. © 2019 Friends Science Publishers

Keywords: *Vigna subterranea*; Disease severity; Resistance; *Macrophomina phaseolina*; Burkina faso

Introduction

Bambara groundnut [*Vigna subterranea* (L.) Verdcourt] is the second major food legume after cowpea, for many populations in Burkina Faso. Its nutritious seeds have 15 to 21% protein content, 7 to 9% fat content, 50 to 65% of carbohydrate and 2 to 4% fiber (Nacoulma, 1996; Diallo *et al.*, 2015). The crop contributes to improve the quality of populations diet and soil nitrogen fertility through symbiotic nitrogen fixation (Nieuwenhuis and Nieuwelink, 2005).

Long considered as a healthy crop compared with cowpea, bambara groundnut production is, however, strongly handicapped by several biotic factors including viral and fungal diseases. In Burkina Faso, fungal diseases constitute the main constraint that significantly limits its production and productivity (Kiwallo, 1991; Sérémé *et al.*, 1991; Sérémé, 1992).

Macrophomina phaseolina (Tassi) Goidnicha has been identified as one of the most pathogenic and prevalent fungi species in Burkina Faso (Drabo *et al.*, 1997; Ouoba, 2017). It is a cosmopolitan pathogen that causes considerable damage to many crops including common bean (*Phaseolus vulgaris* L.), mung bean (*Vigna radiata* L. Wiltczek.), cowpea (Olaya *et al.*, 1996; Oladimeji *et al.*, 2012; Atiq *et al.*, 2014) and sorghum (*Sorghum bicolor* (L.) Moench) (Sharma *et al.*, 2014). Transmitted by both soil and seed, this fungus causes yield losses up to 100% in epidemic event (Iqbal *et al.*, 2010). Due to its virulence and highly efficient mode of transmissibility, cultural, chemical or biological strategies for the pathogen management are not adequate to control it efficiently or economically (Oladimeji *et al.*, 2012).

The use of resistant varieties to the pathogen appears promising for reducing damage and yield losses caused by the pathogen. The present study was therefore aimed to identify potential sources of resistance to *M. phaseolina* among 20 bambara groundnut genotypes in Burkina Faso.

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Materials and Methods

Experimental Details and Treatments

Plant material: Plant material provided by Gene Bank of Environment and Agricultural Research Institute (INERA, Ouagadougou, Burkina Faso) were composed of twenty (20) genotypes including nineteen (19) from Burkina Faso and one (1) from Togo (Table 1).

Fungal material: The fungal material consists of three strains of *M. phaseolina* (Table 2) isolated from bambara groundnut diseased plants from three climatic zones (Sahelian, Sudano-sahelian and Sudanian) of Burkina Faso and identified after DNA sequencing (Ouoba, 2017).

Experimental set up: The inoculum was prepared with seven days old culture of *M. phaseolina* strains according to Abawi and Pastor-Corrales (1990) with some minor modifications. Thus, 60 g of rice seeds were soaked for 12 to 13 h and air dried under room temperature and placed in 250 mL conical flasks. The flask was then covered with cotton wool and wrapped in aluminum (foil). The prepared flask, was autoclaved at 121°C for 20 min. After cooling, the rice seeds (90 to 100 grains/dish) were directly deposited on *M. phaseolina* strains and incubated at room temperature in the dark for fifteen days. Genotypes were evaluated for each strain, in a split plot design (Fig. 1) with three replications. The experiment was set up in greenhouse in two-liter pots containing sterilized soil in the proportions of 50% sand, 33% clay and 17% organic matter. The seeds of bambara groundnut lines were sterilized with hot water after soaking in cold water for one hour followed by addition of the same amount of boiling water. The sterilized seeds were then sown after two hours of cooling, with three to four rice seeds contaminated with each strain of *M. phaseolina* mycelia. Temperature in greenhouse globally varied from 24.28°C ± 2.04 to 33.36°C ± 2.64 during the experiment.

Data Collection

Data collection included emergence at 14 days after sowing and infection severity. The emergence is the percentage of plants emerged at that date and calculated according the formula: $\text{Emergence} = \frac{Nt}{N} X\ 100$ where: Nt is the number of plants emerged at the date t and N is the total number of seeds sown. Severity of infection was evaluated basing on scale proposed by Abawi and Pastor-Corrales (1990). The severity index was recorded every week after emergence until the index of epidemic peak in the eighth week after sowing. The grades awarded are 1, 3, 5, 7 and 9 depending on the degree of plants infection. Index 1 is synonymous with the absence of symptoms and 9 for un-germinated seeds and fully attacked, withered or dead plants. Mean of severity index (MSI) was calculated from the formula: $\text{MSI} = \frac{Ng+Ndp+Nhp}{N}$ where: MSI is mean of severity index, Ng sum of severity index of un-germinated seeds, Ndp sum of severity index of diseased plants, Nhp sum of severity index of healthy plants and N number of seeds sown per landrace and per replication (N = 10). The correspondence of severity index according to the sensibility is followed as: 1: very resistant, 3: resistant, 5: tolerant, 7: susceptible, 9: very susceptible. This scale has been slightly modified to take into account the intermediate severity index obtained after calculating the mean of severity indexes. Thus, a landrace was considered:

- highly susceptible to a strain when its severity index (SI) is: 8≤ SI≤9;

Table 1: List of bambara groundnut landraces used in this study and their origin

Entry code	Landraces	Description	Country of origin
In-01	KVS246-1	Obtained by massal selection	Burkina Faso
In-02	KVS 235-100GY	Mutant obtained by gamma rays irradiation	Burkina Faso
In-03	KVS 235	Obtained by massal selection	Burkina Faso
In-04	KVS246-3	Obtained by massal selection	Burkina Faso
In-05	KVS246-2	Obtained by massal selection	Burkina Faso
In-06	Life16 141-1	Obtained by massal selection	Togo
Zs-12	E125	Collected to farmers	Burkina Faso
Zs-17	E119	Collected to farmers	Burkina Faso
Zs-18	E117	Collected to farmers	Burkina Faso
Zs-21	E105a	Collected to farmers	Burkina Faso
Zss-55	E56A	Collected to farmers	Burkina Faso
Zss-56	E130	Collected to farmers	Burkina Faso
Zss-59	E53a	Collected to farmers	Burkina Faso
Zss-71	Nob-Loc	Collected to farmers	Burkina Faso
Zss-72	E97	Collected to farmers	Burkina Faso
Zss-74	E63	Collected to farmers	Burkina Faso
Zss-76	E86	Collected to farmers	Burkina Faso
Zss-78	E62	Collected to farmers	Burkina Faso
Zss-84	E105b	Collected to farmers	Burkina Faso
Zss-89	E103	Collected to farmers	Burkina Faso

Table 2: Strains of *Macrophomina phaseolina* used in the study

Climatic zones	Strains
Sahel	*M. phaseolina* strain CPC 21498
Sudan-sahel	*M. phaseolina* strain CPC 21519
Sudan	*M. phaseolina* isolate 171

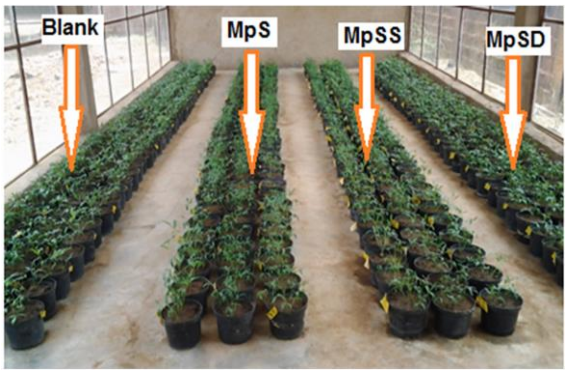

Fig. 1: Experimental layout
Blank: Non- inoculation conditions; MpS: inoculation conditions with sahel strain of *M. phaseolina*; MpSS: Inoculation conditions with sudan-sahel strain of *M. phaseolina*; MpSD: Inoculation conditions with sudan strain of *M. phaseolina*

Table 3: Results of variance analysis for genotypes emergence

Source of variation	df	Sum of square	Mean square	F value	P > F	
Genotype	19	15933.75	838.61	3.69	<.0001	***
Strains	3	404.93	134.97	0.59	0.6194	*
Genotype × Strain	57	17297.91	303.47	1.34	0.0820	*

df: degree of freedom ; ***$P < 0.05$, *$P > 0.05$

Table 4: Results of variance analysis for infection severity

Source of variation	df	Sum of Square	Mean Square	F value	P > F	
Genotype	19	17648.20	928.85	4.97	<.0001	**
Strains	3	75898.92	25 29 9.64	135.36	<0.0001	**
Genotype × Strain	57	16802.73	294.78	1.58	0.0143	**

df: degree of freedom; **$P < 0.05$

Table 5: Impact of strains of *M. phaseolina* on bambara groundnut genoty

Genotypes	Blank		MpS		MpSS		MpSD	
	RE(%)	SI	RE (%)	SI	RE(%)	SI	RE(%)	SI
KVS246-1	53.33^{a}	0	56.66^{ab}	5.26^{ab}	73.33^{a}	4.53^{ab}	63.33^{a}	4.46^{a}
KVS 235-100GY	63.33^{a}	0	86.66^{a}	2.33^{b}	76.67^{a}	3.00^{b}	76.67^{a}	2.86^{a}
KVS 235	76.67^{a}	0	76.66^{ab}	3.13^{b}	53.33^{a}	4.96^{ab}	73.33^{a}	3.33^{a}
KVS246-3	70.00^{a}	0	83.33^{ab}	3.36^{b}	66.67^{a}	4.66^{ab}	63.33^{a}	4.70^{a}
KVS246-2	70.00^{a}	0	63.33^{ab}	3.93^{ab}	86.67^{a}	2.86^{b}	70.00^{a}	3.40^{a}
Life16 141-1	93.33^{a}	0	76.66^{ab}	3.33^{b}	90.00^{a}	3.23^{b}	86.67^{a}	2.60^{a}
E125	70.00^{a}	0	63.33^{ab}	4.46^{ab}	56.67^{a}	6.20^{ab}	60.00^{a}	4.46^{a}
E119	80.00^{a}	0	76.66^{ab}	3.40^{b}	73.33^{a}	3.40^{ab}	73.33^{a}	2.86^{a}
E117	80.00^{a}	0	70.00^{ab}	3.80^{ab}	66.67^{a}	4.16	80.00^{a}	3.13^{a}
E105a	76.67^{a}	0	76.66^{ab}	3.16^{b}	83.33^{a}	2.46^{b}	73.33^{a}	3.40^{a}
E56A	70.00^{a}	0	43.33^{ab}	5.26^{ab}	53.33^{a}	5.53^{ab}	83.33^{a}	3.66^{a}
E130	73.33^{a}	0	43.33^{ab}	5.83^{ab}	76.67^{a}	3.40^{ab}	63.33^{a}	4.20^{a}
E53a	70.00^{a}	0	70.00^{ab}	3.70^{ab}	70.00^{a}	4.73^{ab}	56.67^{a}	3.93^{a}
Nob-Loc	76.67^{a}	0	40.00^{ab}	5.80^{ab}	53.33^{a}	5.06^{ab}	56.67^{a}	4.93^{a}
E97	76.67^{a}	0	76.66^{ab}	4.600^{ab}	70.00^{a}	4.96^{ab}	56.67^{a}	4.93^{a}
E63	53.33^{a}	0	80.00^{ab}	4.90^{ab}	66.67^{a}	6.36^{ab}	70.00^{a}	4.93^{a}
E86	50.00^{a}	0	36.66^{b}	7.13^{a}	60.00^{a}	4.40^{ab}	53.33^{a}	5.26^{a}
E62	60.00^{a}	0	70.00^{ab}	5.60^{ab}	53.33^{a}	7.13^{a}	70.00^{a}	4.46^{a}
E105b	86.67^{a}	0	83.33^{ab}	3.23^{b}	80.00^{a}	2.53^{b}	66.67^{a}	3.93^{a}
E103	70.00^{a}	0	73.33^{ab}	3.40^{b}	66.67^{a}	4.76^{ab}	76.67^{a}	3.40^{a}
Mean	71.00	0	67.33	4.28	68.83	4.42	68.66	3.94

Blank: no- inoculation conditions; MpS: Inoculation conditions with sahel strain of *M. phaseolina;* MpSS: Inoculation conditions with sudan-sahel strain of *M. phaseolina;* MpSD: Inoculation conditions with sudan strain of *M. phaseolina;* RE: Rate of Emergence; SI: Severity Index; Same letters: non significant differences; Different letters: significant differences

- susceptible when its severity index (SI) is: 6≤ SI<8;
- moderately susceptible when its severity index is: 4≤ SI<6;
- resistant if its severity index (SI) is: 2≤ SI<4;
- highly resistant if its severity index (SI) is: 1≤ SI<2.

The fragments of organs of diseased plants were surface disinfected with alcohol (70°) and incubated on Potato Dextrose Agar medium for five days under room temperature to confirm or deny the presence of fungus. Un-germinated seed samples were directly subjected to sanitary tests for the presence or absence of fungus.

Statistical Analysis

The variance analysis was carried with SAS software 9.1 based on confidence interval of 95% in order to examine the effect of genotype, strains of *M. phaseolina* and their interaction on seeds emergence and infection severity. Newman-Keuls Student test was used for means separation.

Results

Symptoms Caused by *M. phaseolina*

The symptoms observed with different strains of *M. phaseolina* inoculated to different bambara groundnut landraces are mainly non-germinated seeds covered by the fungus, chlorotic plants observed at two weeks after sowing, evolved to wilting and complete death (Fig. 2) a week after the onset of symptoms. The analysis of diseased plant organ fragments and un-germinated seeds revealed the presence of *M. phaseolina.*

Reaction of Bambara Groundnut Genotypes to Strains of *M. phaseolina*

Significant and non-significant differences among tested genotypes, strains of *M. phaseolina* and interaction between genotypes and strains for plant emergence (Table 3) and infection severity (Table 4) were observed.

The effect of *M. phaseolina* strains under inoculation and non-inoculation conditions on genotypes emergence and infection severity showed significant differences between genotypes for emergence in inoculation conditions only with Sahelian strain of *M. phaseolina* (Table 5). On the other hand, significant differences between genotypes for infection severity were observed in inoculation conditions with Sahelian and Sudano-sahelian strain of *M. phaseolina*. The rate of emergence varied from 36.66% for genotype E86 in inoculation conditions with Sahelian strain of *M. phaseolina* to 93.33% for genotype life 16-141-1 in non-inoculation conditions. In general, the mean of emergence rate for all genotypes in non-inoculation conditions (71%) was higher than the mean of emergence rate for all genotypes in inoculation conditions with Sahelian (67.33%), Sudano-sahelian (68.83%) and Sudan (68.66%) strains of *M. phaseolina.* In inoculation conditions, severity index varied from 2.33 for genotype KVS 235-100GY with Sahelian strain to 7.33 for genotypes E86 and E62 respectively with Sahelian and Sudano-sahelian strains.

Classification of Genotypes According to their Sensibility to *M. phaseolina* strains

The classification carried out according to genotypes reaction to strains of *M. phaseolina*, revealed three groups of genotypes: moderately susceptible, susceptible and resistant genotypes (Table 6). However, the sensibility of genotypes varied between strains. Each group consisted of genotypes with specific sensibility to each strain. Nevertheless, six genotypes were resistant to all strains of *M. phaseolina* and three genotypes moderately susceptible to all strains. The eleven others genotypes were specifically

Table 6: Reaction of genotypes to strains of *M. phaseolina*

Genotypes	MpS			MpSS			MpSD		
	SI	Relative variation	Sensibility	SI	Relative variation	Sensibility	SI	Relative variation	Sensibility
KVS246-1	5.26	0.70	MS	4.53	0.56	MS	4.46	0.53	MS
KVS235-100GY	2.33	0.26	R	3.00	0.91	R	2.86	0.70	R
KVS 235	3.13	0.96	R	4.96	0.43	MS	3.33	0.73	R
KVS246-3	3.36	0.46	R	4.66	0.56	MS	4.70	1.13	MS
KVS246-2	3.93	1.41	R	2.86	1.48	R	3.40	0.46	R
Life16141-1	3.33	0.40	R	3.23	0.74	R	2.60	0.80	R
E125	4.46	0.53	MS	6.20	1.20	S	4.46	0.26	MS
E119	3.40	0.80	R	3.40	0.92	R	2.86	0.70	R
E117	3.80	0.23	R	4.16	0.81	MS	3.13	0.70	R
E105a	3.16	0.51	R	2.46	0.66	R	3.40	0.80	R
E56A	5.26	0.70	MS	5.53	0.26	MS	3.66	0.53	R
E130	5.83	0.03	MS	3.40	0.92	R	4.20	0.80	MS
E53a	3.70	0.50	R	4.73	0.70	MS	3.93	0.26	R
Nob-Loc	5.80	0.46	MS	5.06	0.52	MS	4.93	0.76	MS
E97	4.60	1.05	MS	4.96	0.46	MS	4.93	1.15	MS
E63	4.90	1.06	MS	6.36	0.28	S	4.93	0.86	MS
E86	7.13	0.67	S	4.40	0.30	MS	5.26	1.16	MS
E62	5.60	0.61	MS	7.13	0.70	S	4.46	0.53	MS
E105b	3.23	0.52	R	2.53	0.46	R	3.93	0.70	R
E103	3.40	0.92	R	4.76	0.71	MS	3.40	0.00	R

SI: Severity Index; S: susceptible (6≤ SI <8); MS: moderately susceptible (4≤ SI <6); R: resistant (2≤ SI <4)

Fig. 2: Symptoms of *M. phaseolina* on bambara groundnut diseased plants

a: ungerminated seed covered by the fungus, b: chlorotic plants, c: wilted plant, d: Dried plants

susceptible or resistant to each strain of the pathogen. The genotypes KVS235-100GY, KVS246-2, life16-141-1, E119, E105a and E105b were resistant to all strains of *M. phaseolina*. Only genotypes KVS246-1, Nob-Loc and E97 were moderately susceptible to all strains of *M. phaseolina.*

Discussion

Macrophomina phaseolina, a soil and a seed-borne fungus, induces diseases in different crops including bambara groundnut and causes considerable damages. In this study, the 20 landraces of bambara groundnut assessed for resistance to three strains of *M. phaseolina* belonging to three climatic zones of Burkina Faso showed variations in disease severity in the different landraces due to their sensibility and strains pathogenecity. The symptoms of chlorosis, wilt and complete death of plants were observed with the different strains of *M. phaseolina*. These symptoms are similar to those reported in common bean by Abawi and Pastor-Corrales (1990) and in cowpea by Oladimeji *et al.* (2012). Other symptoms such as coal rot (dark lesions) and dry rot of root (Abawi and Pastor-Corrales, 1990; Oladimeji *et al.,* 2012) are also caused by *M. phaseolina*. However, even some studies cited *M. phaseolina* as a pathogen of bambara groundnut (Drabo *et al.,* 1997), no study has reported so far the manifestations of diseases caused by this fungus on bambara groundnut. Symptoms similar to those observed in this study with strains of *M. phaseolina* (chlorosis and wilt) may also be attributed to some pathogens such as *Fusarium solani* f. sp. Phaseoli (Abawi and Pastor-Corrales, 1990), but the analysis of samples from diseased plants confirmed the presence of *M. phaseolina* and allowed to attribute to it the symptoms observed. Chlorosis and wilt leading to plant death, can be explained by the fact that pathogen (*M. phaseolina*) generally affects the fibrovascular system of roots and obstructs xylem vessels with microsclerotia (Khan, 2007). This reduces the transport of nutrients and water to the upper parts of the plant causing progressive wilting and premature death of the plant.

Pre-emergence symptoms are characterized by poor emergence (Abawi and Pastor-Corrales, 1990) that can explain the lowest emergence rate obtained in inoculation conditions with the strains of pathogen. Previous studies

(Dhingra and Sinclair, 1978; Burney *et al.*, 1984; Francl *et al.*, 1988) explained pre-emergence mortality as the ability of *M. phaseolina* to infect the embryo. These fungus strains are responsible for pre-emergence mortality and can penetrate a thick membrane of 5 to 10 μ. According to Khan *et al.* (2000), strains of the fungus that do not cause pre-emergence mortality move to the upper parts of the plant and the pathogenicity of these strains is related to the degree of nutritional compatibility with the host tissue.

Resistance of the host plant is the most feasible and economical measure to reduce yield losses due to diseases caused by *M. phaseolina* (Iqbal *et al.*, 2010). The identification of sources of resistance among bambara groundnut landraces grown in Burkina Faso is an important pre-requisite for initiating an improvement program aimed at developing resistant varieties. This study revealed a significant variability within the germplasm of bambara for resistance to different strains of *M. phaseolina* under controlled conditions. The different landraces showed different levels of sensibility to three strains of *M. phaseolina*. These differences in the response of bambara groundnut landraces to strains of *M. phaseolina* were also observed by Khan and Shuaib (2007) and Atiq *et al.* (2014) on mung bean.

Methods of identification of resistance sources to *M. phaseolina* have evolved into biotechnology. Indeed, in the common bean, two RAPD markers related to resistance to this fungus were detected and mapped (Hernández-Delgado *et al.*, 2009). In sorghum, studies have revealed an important role of enzymes such as chitinase and stilbene in improving its resistance to *M. phaseolina* (Sharma *et al.*, 2014). Although, local bambara groundnut genotypes have been identified as resistant to fungal foliar diseases (Kiwallo, 1991), no mention of their resistance to *M. phaseolina* has yet been reported in the literature.

Conclusion

This study identified three groups of genotypes such as resistant, moderately susceptible and susceptible genotypes based on the sensibility of bambara groundnut landraces to three strains of *Macrophomina phaseolina.* Six genotypes showed resistance to all strains of *M. phaseolina*, three genotypes moderately susceptible to all strains and eleven genotypes with specific susceptibility or resistance to each strain of this pathogen. Sources of resistance to this pathogen could be used in a varietal improvement program of bambara groundnut. However, this study deserves to be deepened with modern biotechnology tools, in order to better appreciate the level of resistance of varieties to this fungus.

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