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Research Article
Identification of Resistant Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes against Charcoal under Artificial Inoculation

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Abstract

Background and Objective: Charcoal rot caused by *Macrophomina phaseolina* is known to be a serious threat to cowpea in Burkina Faso. Nowadays, control strategies other than host resistance are not much effective and economical. Therefore, the present study aimed to identify among cowpea germplasm, the genotypes endowed with stable resistance to *M. phaseolina*. **Materials and Methods:** Eighty cowpea (*Vigna unguiculata* (L.) Walp.) genotypes including wild, landrace and inbreeding lines were screened for their resistance to *Macrophomina phaseolina*, the charcoal rot fungus, in greenhouse experiments in Burkina Faso. The test was performed at Kamboinse research station of the Institute of Environment and Agricultural Research (INERA), using two pathogenic strains of *M. phaseolina* (I2 and I4) selected from a preliminary pathogenicity test involving four isolates. **Results:** Eight genotypes including 58-57, Bambey-21, CB27, CB46, Gourgou, KN-1, KVx404-8-1 and TVU 14 676 inoculated with the two isolates of *M. phaseolina* presented high emergence rates (80-100%). After emergence, five genotypes including B05-5a, B27 07a, CB27, SP369 A Profil-39B and SP88 Profil-13A stayed free of disease during the ten-days period of the study, four genotypes including Komsare, Kaya local, 58-57 and Gaoua local-2 showed low severity ($S \leq 10\%$) and 11 other genotypes including KVx 295-2-124-51, Pa local-2, Boalga local, TVU 14 676, Pouytenga-3, Apagbaala, N°91 profil-4, IT82D-849, B301, TV286b profil-12 and IT 98K-317-2 showed moderate disease severity indexes ($S \leq 20\%$) to both isolates of *M. phaseolina*. **Conclusion:** The present study gave the opportunity to identify under artificial inoculation, two cowpea genotypes including Kaya local and SP 369A profil-39B, having high and stable resistance to *M. phaseolina*, among 80 tested genotypes.

Key words: Cowpea, *Vigna unguiculata*, *Macrophomina phaseolina*, screening, resistance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cowpea is one of the most leguminous crops in the world with an estimated 14.5 M ha of land planted annually[1]. Global production of dried cowpeas in 2016 was 6.5 M metric tons. In Burkina Faso, the crop is the first leguminous used as food and the fourth economical crop. Cowpea production is a significant economic activity in many African countries[1,2], but the crop is increasingly damaged by charcoal rot caused by *Macrophomina phaseolina*. The fungal pathogen belongs to Coelomycetes class and is worldwide distributed on crop and non-crop plant species. It causes seedling blight, stem and pod rots and has more than 500 plant species as host range. Recognized as one of the most important diseases of legumes, including cowpea, this fungus causes yield losses from 10-50%[3].

Characteristic disease symptoms include presence of black sclerotia on the lower part of the stem and wilting and drying of the leaves and subsequently the whole plant at the flowering and fruiting stages. The fungus can also infect roots which show necrotic lesions, leading to pre- or post-emergence seedling damping-off or low plant growth.

In Burkina Faso, the crop faces many abiotic and biotic constraints such as striga and virus attacks that reduce the yield in field[4,5]. In addition, *M. phaseolina* is known to be a serious threat of cowpea[3]. No chemical control currently exists for charcoal rot and resistance has been hard to identify. Due to the soil-borne nature of the pathogen, control strategies other than host resistance are not much effective and economical. Varietal screenings carried out against this disease in cowpea have been reported[3,6,7]. However, in Burkina Faso, very few screening has been performed to identify resistant varieties to *M. phaseolina*.

The purpose of this study was to identify among cowpea germplasm, the genotypes endowed with stable resistance to *M. phaseolina*.

MATERIALS AND METHODS

Study area: The study was carried out from September, 2014 to December, 2015. It started with the collection of the isolates of *M. phaseolina* and ended with the screening, under artificial inoculation in the greenhouse, of 80 cowpea genotypes for resistance to *M. phaseolina*.

Cowpea varieties and fungal isolates: Eighty cowpea genotypes including 21 inbreed lines, 32 landraces and 27 wild genotypes were used. Four isolates of *M. phaseolina* isolated from *Arachis hypogaea*, *Vigna unguiculata*, *Sesamum indicum* and *Vigna subterranea* were used in the study as shown in Table 1.

Table 1: Host crops and sites of collection of the isolates of *M. phaseolina*

Isolate number	Host crop	Site
I1	*Arachis hypogaea*	Nobere
I2	*Vigna unguiculata*	Kamboinse
I3	*Sesamum indicum*	Kamboinse
I4	*Vigna subterranea*	Sabce

Collection of isolates of *M. phaseolina*: Plant tissues (seeds, roots, stems or leaves) of groundnut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), sesame (*Sesamum indicum*) and Bambara groundnut (*Vigna subterranea*) infected by *M. phaseolina* were collected from different crop producing areas in the country, Sabce, Kamboinse and Nobere (Table 1). The plant tissues were washed with tap water, cut into small pieces, surface-sterilized in 2% NaOCl for 1 min, rinsed three times with sterile distilled water and dried on paper bags in an oven at 37°C.

Five to six pieces of plant tissues were placed on Potato Dextrose Agar (PDA) medium (20 g of glucose, 20 g of agar and 1000 mL of water) in Petri dishes. The Petri dishes were then incubated at 25±1°C under alternating cycles of 12 hrs near UV light and 12 hrs darkness for 5 days. After incubation, each plant tissue was examined for the presence of fungi under a stereomicroscope and identification of *M. phaseolina* was confirmed by examining conidia under a compound microscope based on the description reported by Huda-Shakirah *et al.*[8]. Pure culture was obtained after consecutive sub culturing of the fungus on PDA.

Determination of the most pathogenic isolates of *M. phaseolina*

Pathogenicity test: A pathogenicity test was performed on *M. phaseolina* isolated from the different host plants mentioned above, in order to identify the most pathogenic isolates that used for the greenhouse screening of the 80 cowpea varieties. For this purpose, 3 cowpea varieties were selected on the basis of their resistance or susceptibility to *M. phaseolina*, Bambey-21 and CB46 as susceptible and IT93K-503-1 as resistant varieties[9]. Plastic pots (12×12×11 cm) containing a sandy clay soil (2:1) taken at about 10 cm deep from the rhizosphere at Kamboinse and sterilized at 121°C for 30 min in an autoclave were used for sowing. For each variety, 90 seeds were surface disinfected with a 1% sodium hypochlorite solution for 2 min. These seeds were then rinsed thoroughly with 3 successive baths of sterile distilled water in a 200 mL beaker and dried on sterile filter paper for 48 hrs.

Before sowing, two artificial methods of contamination were used:

- **Seed contamination:** The seed contamination method was used[10], consists to place 15 seeds of each variety in a Petri dish fully colonized by 5 day-old *M. phaseolina* mycelium for 5 hrs. Seeds placed in Petri dishes containing PDA free of *M. phaseolina* culture were used as control
- **Soil contamination:** According to Afouda *et al.*[7], the pots (12×12×11 cm) were first filled with 2000 g of sterile soil. The inoculum was prepared by suspending 4 day-old micro sclerotial/mycelial mat of *M. phaseolina* grown on potato, in tap water. The 400 mL of suspension were then thoroughly mixed with 3800 g of sterilized soil and each pot was supplemented with 380 g of the contaminated soil. Pots supplemented with 380 g of soil mixed with sterile distilled water (non-contaminated soil) were used as control

A split-plot design with 3 replications was used for each method of contamination. The cowpea varieties were assigned to the main plots and the isolates of *M. phaseolina* to the sub-plots. For each treatment, five seeds were sown per pot and per replication. Just after sowing, each pot was irrigated with 0.5 L of tap water.

Observations: Ten Days After Sowing (DAS), the number of emerged seedlings and the number of dead seedlings were counted. The percentages of emerged seedlings and dead seedlings were calculated as follow[10]:

$$\text{Emerged seedlings (\%)} = \frac{\text{Number of emerged seedlings}}{\text{Number of sown seeds}} \times 100$$

$$\text{Dead seedlings (\%)} = \frac{\text{Number of dead seedlings}}{\text{Number of emerged seedlings}} \times 100$$

Screening of cowpea genotypes against *M. phaseolina* in greenhouse: The experiment was carried out at Kamboinse Research Station. A total of 80 cowpea genotypes were screened against *M. phaseolina*. Seeds were sown in plastic alveolate trays. The trays were filled with fine white sand previously washed with tap water to remove the excess of clay and then, dried and sterilized at 121°C for 30 min in an autoclave.

The seeds of wild genotypes were scarified in order to facilitate water absorption and seed germination[11].

For each variety, 45 seeds were then disinfected with a sodium hypochlorite solution as described above.

Seed contamination and sowing: The artificial method of seed contamination described above was used for seed contamination with the two most pathogenic isolates of *M. phaseolina* selected from the pathogenicity test. For each genotype and per isolate, 15 seeds were sown in 3 replications of five each. Non-contaminated seeds were used as control.

The experimental design used was a split-plot design where the isolates of *M. phaseolina* were assigned to the main plots and the cowpea genotypes assigned to the sub-plots. Each treatment was repeated three times. The trays were watered twice a day.

Observations: The observations consisted to count the number of emerged, diseased and dead seedlings at 10 DAS. The severity of the disease was evaluated by giving a note to each seedling using the following scale used by Latunde-Dada[12]: 1: Healthy seedling, 2: Slightly attacked seedling, 3: Diseased seedling with symptoms on the leaves or on the stem, 4: Diseased seedling with severe symptoms but seedling still alive and 5: Completely dead seedling.

A severity index was calculated using the formula of Williams and Singh[13] and the results are expressed in percentage:

$$S\ (\%) = \frac{\Sigma\,[(xi-1)\,x\,ni]}{[E\,(x)-1]\times N} \times 100$$

Where:

xi = Disease note for each plant from the class i
ni = Number of plants from the class i
E (x) = Scale range (5)
N = Total number of seedlings observed
S = Severity index or ability of the fungus to invade the seedling (%)

These severity indexes were used to determine the susceptibility or resistance level of a genotype to a given isolate of *M. phaseolina* as follow:

- S = 0%: Immune genotype
- 0%<S≤5%: Very resistant genotype
- 5%<S≤10%: Resistant genotype
- 10%<S≤20%: Moderately resistant or moderately susceptible genotype
- 20%<S≤50%: Susceptible genotype
- S>50%: Very susceptible genotype

Statistical analysis: Analyses of variance (ANOVA) were performed on all the data recorded on pathogenicity test, seedling emergence, disease incidence, seedling mortality and disease severity, using the Statistical Analysis System, version 8. The comparison between treatment means was realized based on the Duncan's Multiple Range (DMR) or the Student-Newman-Keuls tests at 5% level of significance.

RESULTS AND DISCUSSION

Pathogenicity of the isolates of *M. phaseolina*: Effect of the isolates on seedling emergence and seedling mortality under the two methods of contamination: For seedling emergence, under both seed and soil contamination methods, the computed F-values of 1.62 and 0.35 for variety (Table 2) were smaller than their corresponding tabular F value (with f1 = 2 and f2 = 4 degrees of freedom) of 6.94 at the 5% level of significance, given by Gommez and Gomez[14], indicating that there is no significant difference between the emergence rates of the cowpea varieties. Similarly, the computed F values for inocula of 0.31 and 0.44 with seed and soil contamination methods respectively (Table 2), were smaller than their corresponding tabular F-value (with f1 = 4 and f2 = 24 degrees of freedom) of 2.78, indicating a non significant effect of the inocula of *M. phaseolina* on seedling emergence.

On the other hand, for plant mortality, the computed F-values of 2.77 and 2.93 for inocula (Table 3) were slightly equal to or greater than the corresponding tabular F-value of 2.78, indicating that the isolates of *M. phaseolina* had variable impacts under both methods of inoculation at the 5% level of significance. The results showed a nonsignificant interaction between varieties and inocula, indicating that the difference between the inocula was not depending to the tested variety and that the varietal effect did not differ significantly with the inoculum applied.

When contaminating the seeds and when contaminating the soil with *M. phaseolina*, the seedling emergence rates varied from 93.33-97.77 and from 86.66-95.55%, respectively and the different isolates showed no significant effect on seedling emergence (Table 4). However, under both contamination methods, all the isolates of *M. phaseolina* were pathogenic and induced significantly high seedling mortality rates (>6%) compared to the control (sterile distilled water or PDA) that caused no seedling mortality (Table 4). Under both contamination methods, isolate I2 from *Vigna unguiculata* and isolate I4 from *Vigna subterranean*, with respectively 18.33-20.37 and 13.88-14.81% of seedling mortality rates (Table 4) were the most pathogenic isolates.

Table 2: ANOVA results of the effect of the isolates of *M. phaseolina* on seedling emergence using two contamination methods

		Seeds contamination		Soil contamination	
Source of variation	DF	SS	F	SS	F
Variety	2	231.11	1.62^{NS}	160.00	0.35^{NS}
Inocula	4	88.88	0.31^{NS}	408.88	0.44^{NS}
Variety*Inocula	8	657.77	1162^{NS}	1617.77	0.88^{NS}

DF: Degree of freedom, SS: Some of squares, F: Value for testing the treatment effect, ^{NS}: Non significant at 5% level

Table 3: Effect of *M. phaseolina* isolates on seedling mortality of cowpea, using two contamination methods

		Seeds contamination		Soil contamination	
Source of variation	DF	SS	F	SS	F
Variety	2	401.11	1.26^{NS}	312.34	0.88^{NS}
Inocula	4	1763.3	2.77^{S}	2154.1	2.93^{S}
Variety*Inocula	8	1810	1.42^{NS}	2214.1	1.50^{NS}

DF: Degree of freedom, SS: Some of squares, F: Value for testing the treatment effect, ^{S}: Significant at 5% level, ^{NS}: Non significant

Table 4: Seedling emergence and plants mortality rates caused by 4 isolates of *M. phaseolina* using two contamination methods

	Seeds inoculation		Soil contamination	
Inocula	Seedling emergence (%)	Seedling mortality (%)	Seedling emergence (%)	Seedling mortality (%)
I0	95.55^{a}	0.00^{b}	93.33^{a}	0.00^{b}
I1	95.55^{a}	6.66^{ab}	91.11^{a}	7.22^{ab}
I2	95.55^{a}	18.33^{a}	86.66^{a}	20.37^{a}
I3	97.77^{a}	8.88^{ab}	95.55^{a}	8.88^{ab}
I4	93.33^{a}	13.88^{a}	93.33^{a}	14.81^{a}
P (5%)	0.8673	0.0450	0.7770	0.0372
Mean	95.55	9.55	92.00	10.25

Means under the same column with the same alphabetical letter are not significantly different at 5% level. I0: Control (PDA or sterile distilled water), I1: Isolate from *Arachis hypogaea*, I2: Isolate from *Vigna unguiculata*, I3: Isolate from *Sesamum indicum*, I4: Isolate from *Vigna subterranea*

Table 5: ANOVA results of the effects of two isolates of *M. phaseolina* on seedling emergence, seedling infection, seedling mortality and disease severity of 80 cowpea genotypes, under greenhouse conditions

		Emergence (%)		Incidence (%)		Mortality (%)		Severity (%)	
Sources of variation	DF	SS	F	SS	F	SS	F	SS	F
Isolates	2	96 534.44	93.82^{HS}	105 243.46	74.30^{HS}	66 427.42	53.08^{HS}	85 970.17	64.65^{HS}
Genotypes	79	226 113.33	5.56^{HS}	99 140.17	1.77^{HS}	80 385.85	1.63^{HS}	89 390.53	1.70^{HS}
Isolates × genotypes	158	131 376.66	1.62^{HS}	166 132.89	1.51^{HS}	154 195.86	1.50^{HS}	146 118.52	1.42^{HS}

DF: Degree of freedom, SS: Some of squares, F: Value for testing the treatment effect, ^{HS}: Highly significant

Table 6: Percentages of seedling emergence, seedling infection, seedling mortality and disease severity induced by two isolates of *M. phaseolina* on 80 cowpea genotypes, under greenhouse conditions

Isolates of *M. phaseolina*	Emergence (%)	Incidence (%)	Mortality (%)	Severity (%)
Control (I0)	75.66^{a}	4.61^{c}	4.32^{c}	4.48^{c}
Isolate 2 (I2)	48.25^{c}	34.92^{a}	28.69^{a}	32.02^{a}
Isolate 4 (I4)	68.25^{b}	24.45^{b}	20.09^{b}	22.31^{b}
Average	64.05	20.95	17.39	19.26
p-value	<0.0001	<0.0001	<0.0001	<0.0001

Means under the same column with the same alphabetical letter are not significantly different at 5% level

Reaction of cowpea genotypes to two isolates of *M. phaseolina*, under semi-controlled conditions: Table 5 presented the ANOVA results of the effects of two isolates of *M. phaseolina* on seedling emergence, seedling mortality, disease incidence and disease severity of 80 cowpea genotypes, under greenhouse conditions. For seedling emergence, disease incidence, plant mortality and disease severity, the computed F-values for isolates of 93.82, 74.30, 53.08 and 64.65, respectively (Table 5), were greater than the corresponding tabular F-value (with f1 = 2 and f2 = 4 degrees of freedom) of 18.00 at the 1% level of significance, indicating that inoculation of seeds with *M. phaseolina* isolats had widely varying effects on seedling emergence, disease incidence, seedling mortality and severity of attack. Similarly, the computed F-values for genotypes (5.56, 1.77, 1.63 and 1.70, respectively) were greater than the corresponding tabular F value (with f1 = 79 and f2 = 474 degrees of freedom) of 1.47 at the 1% level of significance, indicating that cowpea genotypes exhibited very different reactions to *M. phaseolina* isolates. The results in Table 5 also showed that the computed F-values for (isolates × genotypes) of 1.42-1.62 were greater than the corresponding tabular F-value of 1.32. These results revealed a significant interaction between isolates and genotypes, indicating that the varietal difference was significantly affected by the isolat used for inoculation and the isolat effect significantly varied with the tested genotype.

Effect of the isolates of *M. phaseolina* on seedling emergence, disease incidence, seedling mortality and disease severity of cowpea: Here, I2 isolated from *Vigna unguiculata* and I4 isolated from *Vigna subterranean* were the most pathogenic isolates selected from the pathogenicity test and used for the greenhouse screening.

Seeds contaminated with isolates I2 and I4 showed emergence rates significantly lower than that recorded on non-contaminated seeds (control), the lowest rate (48.25%) being obtained with seeds inoculated with isolate I2 (Table 6). The two isolates also induced seedling infection (24.45-34.92% of infected seedlings), seedling mortality (20.09-28.69% of dead seedlings) and disease severity (22.31-32.02% of disease severity index) rates significantly higher than those observed on the control (4.61, 4.32 and 4.48%, respectively), however, the isolate I2 showed the highest rates (Table 6).

Seedling emergence and mortality rates, disease incidence and severity indexes of 80 cowpea genotypes contaminated or not by *M. phaseolina* isolates were presented in Table 7.

Seedling emergence rates significantly varied between the non-contaminated genotypes (control) ($p<0.0001$) as well as between those inoculated with *M. phaseolina* isolate I2 ($p<0.0001$) and with isolate I4 ($p = 0.0001$). The average rates observed with the isolates I0 (control), I2 and I4 were respectively 75.66, 48.25 and 68.25%. The 47, 13 and 33 genotypes inoculated with I0, the isolate I2 and the isolate I4 respectively, showed high emergence rates ($\geq$80%). Eight of these genotypes including 58-57, Bambey-21, CB27, CB46, Gourgou, KN-1, KVx 404-8-1 and TVU 14 676 presented 80-100% emergence rate whatever the isolate of *M. phaseolina* used for inoculation.

For disease incidence and disease severity, significant differences were seen between the genotypes inoculated with the isolate I2 ($p = 0.0056$, $p = 0.0166$) and moderate significant differences were seen between the genotypes inoculated with the isolate I4 ($p = 0.0564$, $p = 0.0720$) while no difference was seen between the control genotypes (inoculated with I0) ($p = 0.1439$, $p = 0.1128$) (Table 7). The average disease incidence rates recorded were 4.61, 34.91 and 24.45% for I0,

Table 7: Effect of *M. phaseolina* on seedling emergence, disease incidence, plants mortality and disease severity of cowpea genotypes in greenhouse conditions in Burkina Faso

	M. phaseolina isolate I0 (Control)				*M. phaseolina* isolate I2				*M. phaseolina* isolate I4			
Genotypes	Emerg. (%)	Incid. (%)	Morta. (%)	Sever. (%)	Emerg. (%)	Incid. (%)	Morta. (%)	Sever. (%)	Emerg. (%)	Incid. (%)	Morta. (%)	Sever. (%)
524B	66.67	0.00	0.00	0.00	60.00	0.00	0.00	0.00	60.00	44.44	33.33	36.11
58-57	86.67	0.00	0.00	0.00	80.00	8.33	8.33	8.33	86.67	0.00	0.00	0.00
Apagbaala	66.67	36.11	36.11	36.11	40.00	0.00	0.00	0.00	40.00	16.67	16.67	16.67
B05 5a*	66.67	8.33	8.33	8.33	46.67	0.00	0.00	0.00	66.67	0.00	0.00	0.00
B12-07a*	60.00	0.00	0.00	0.00	40.00	0.00	0.00	0.00	73.33	24.44	24.44	24.44
B27 07a*	73.33	11.11	11.11	11.11	6.67	0.00	0.00	0.00	46.67	0.00	0.00	0.00
B30 01*	60.00	0.00	0.00	0.00	40.00	11.11	11.11	11.11	60.00	33.33	33.33	33.33
B301	73.33	0.00	0.00	0.00	73.33	16.67	8.33	8.33	93.33	15.00	15.00	15.00
Bambey-21	100.00	0.00	0.00	0.00	86.67	53.33	46.67	51.67	86.67	6.67	0.00	5.00
Bolga local	86.67	30.00	30.00	21.67	33.33	16.67	16.67	16.67	93.33	0.00	0.00	0.00
CB27	93.33	0.00	0.00	0.00	93.33	0.00	0.00	0.00	93.33	0.00	0.00	0.00
CB46	93.33	0.00	0.00	0.00	93.33	50.00	43.33	46.67	100.00	20.00	20.00	20.00
Djouroum local	73.33	13.33	13.33	13.33	73.33	33.33	25.00	27.08	80.00	6.67	6.67	6.67
Gaoua local-2	93.33	0.00	0.00	0.00	66.67	8.33	8.33	8.33	86.67	6.67	6.67	6.67
Goinkoro-2	73.33	0.00	0.00	0.00	46.67	44.44	44.44	44.44	93.33	13.33	13.33	13.33
Gorom local	80.00	0.00	0.00	0.00	66.67	16.67	16.67	16.67	46.67	38.89	38.89	38.89
Gourgou	86.67	6.67	6.67	6.67	80.00	25.00	25.00	25.00	86.67	46.67	30.00	38.33
HTR	100.00	0.00	0.00	0.00	80.00	34.44	27.78	32.78	60.00	12.50	12.50	12.50
IT82D-849	66.67	20.00	20.00	20.00	40.00	0.00	0.00	0.00	46.67	22.22	11.11	19.44
IT84S-2049	86.67	0.00	0.00	0.00	40.00	50.00	50.00	50.00	53.33	27.78	27.78	27.78
IT84S-2246	86.67	0.00	0.00	0.00	73.33	16.67	16.67	16.67	80.00	52.78	39.44	49.44
IT93K-503-1	93.33	0.00	0.00	0.00	53.33	8.33	0.00	8.33	86.67	26.67	20.00	25.00
IT93K-693-2	86.67	0.00	0.00	0.00	26.67	25.00	25.00	25.00	53.33	80.56	69.44	77.78
IT95K-14 79	86.67	16.67	0.00	10.42	60.00	30.00	30.00	30.00	93.33	44.44	36.67	41.67
IT95K-627-4	86.67	0.00	0.00	0.00	26.67	66.67	50.00	62.50	60.00	11.11	11.11	11.11
IT97K-207-15	0.00	-	-	-	46.67	38.89	38.89	38.89	60.00	8.33	0.00	2.08
IT97K-499-35	66.67	0.00	0.00	0.00	20.00	0.00	0.00	0.00	46.67	55.56	55.56	55.56
IT98K-317-2	100.00	0.00	0.00	0.00	73.33	13.33	0.00	10.00	93.33	15.00	15.00	15.00
Kaya local	93.33	0.00	0.00	0.00	73.33	8.33	0.00	6.25	80.00	6.67	0.00	5.00
KN-1	93.33	0.00	0.00	0.00	86.67	53.33	53.33	51.33	93.33	13.33	0.00	6.67
Koakin local	100.00	0.00	0.00	0.00	73.33	33.33	33.33	33.33	80.00	0.00	0.00	0.00
Kolondura local	86.67	0.00	0.00	0.00	66.67	33.33	33.33	33.33	86.67	20.00	0.00	13.33
Komkallé	66.67	0.00	0.00	0.00	66.67	11.11	11.11	11.11	60.00	11.11	11.11	11.11
Komsaré	60.00	11.11	11.11	11.11	26.67	0.00	0.00	0.00	40.00	16.67	0.00	4.17
KVx 295-2-124-51	33.33	16.67	16.67	16.67	93.33	23.33	8.33	16.25	33.33	0.00	0.00	0.00
KVx 396-4-5-2D	100.00	0.00	0.00	0.00	33.33	50.00	37.50	40.63	60.00	55.56	55.56	55.56
KVx 402-5-2	60.00	0.00	0.00	0.00	53.33	43.33	43.33	43.33	40.00	27.78	27.78	27.78
KVx 404-8-1	93.33	0.00	0.00	0.00	86.67	41.67	25.00	35.42	80.00	13.33	13.33	13.33
KVx 414-22-2	93.33	0.00	0.00	0.00	46.67	66.67	66.67	66.67	73.33	19.44	8.33	16.67
KVx 525	80.00	0.00	0.00	0.00	26.67	50.00	50.00	50.00	66.67	41.67	33.33	39.58
KVx 61-1	80.00	0.00	0.00	0.00	26.67	100.00	100.00	100.00	66.67	50.00	16.67	33.33
KVx 640	66.67	0.00	0.00	0.00	33.33	50.00	50.00	50.00	80.00	47.78	32.78	40.69
KVx 65-114	100.00	6.67	6.67	6.67	6.67	0.00	0.00	0.00	86.67	51.67	31.67	35.00
KVx 745-11P	93.33	0.00	0.00	0.00	46.67	50.00	50.00	50.00	66.67	0.00	0.00	0.00
KVx 780-1	40.00	0.00	0.00	0.00	13.33	100.00	100.00	100.00	53.33	33.33	33.33	33.33
KVx 780-3	86.67	0.00	0.00	0.00	20.00	25.00	0.00	18.75	80.00	28.33	28.33	28.33
KVx 780-4	93.33	0.00	0.00	0.00	20.00	66.67	33.33	50.00	93.33	35.00	13.33	20.83
KVx 780-6	93.33	13.33	0.00	10.00	46.67	66.67	58.33	64.58	80.00	13.33	13.33	13.33
KVx 780-9	80.00	0.00	0.00	0.00	66.67	86.67	40.00	80.00	73.33	27.78	27.78	27.78
MelaKH	100.00	6.67	6.67	6.67	73.33	33.33	8.33	27.08	80.00	33.33	33.33	33.33
Moussa local	86.67	0.00	0.00	0.00	40.00	66.67	66.67	66.67	33.33	44.44	44.44	44.44
N°3076 Profil 51b*	53.33	0.00	0.00	0.00	20.00	100.00	25.00	56.25	60.00	25.00	25.00	25.00
N°3076-Profil-22*	73.33	19.44	19.44	19.44	33.33	50.00	50.00	50.00	73.33	8.33	8.33	8.33
N°91 Profil 4*	13.33	50.00	50.00	50.00	33.33	0.00	0.00	0.00	26.67	16.67	16.67	16.67
Nafi	86.67	0.00	0.00	0.00	53.33	60.00	13.33	28.33	73.33	16.67	16.67	16.67

Table 7: Continued

Genotypes	*M. phaseolina* isolate I0 (Control)				*M. phaseolina* isolate I2				*M. phaseolina* isolate I4			
	Emerg. (%)	Incid. (%)	Morta. (%)	Sever. (%)	Emerg. (%)	Incid. (%)	Morta. (%)	Sever. (%)	Emerg. (%)	Incid. (%)	Morta. (%)	Sever. (%)
Nafi HT-1	86.67	6.67	6.67	6.67	20.00	66.67	66.67	66.67	73.33	16.67	16.67	16.67
Nafi HT-2	86.67	8.33	8.33	8.33	60.00	41.67	30.56	38.89	100.00	33.33	26.67	28.33
Niizwè (IT98K-205-8)	80.00	0.00	0.00	0.00	33.33	66.67	66.67	66.67	73.33	16.67	16.67	16.67
NS-1 BF*	33.33	0.00	16.67	16.67	20.00	0.00	0.00	0.00	40.00	33.33	33.33	33.33
Pâ local-2	86.67	0.00	0.00	0.00	100.00	20.00	13.33	16.67	66.67	0.00	0.00	0.00
Pa local-GJ	86.67	13.33	13.33	13.33	20.00	100.00	100.00	100.00	66.67	90.00	80.00	87.50
Pobé local	100.00	13.33	6.67	13.33	53.33	33.33	33.33	33.33	66.67	70.00	58.89	67.22
Pouytenga-3	60.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	60.00	16.67	16.67	16.67
Sakoula local	93.33	15.00	6.67	15.00	13.33	100.00	50.00	87.50	40.00	58.33	41.67	54.17
SP118 Profil-24*	46.87	16.67	16.67	16.67	13.33	50.00	50.00	50.00	33.33	16.67	16.67	16.67
SP130 Profil-19*	86.67	0.00	0.00	0.00	33.33	50.00	16.67	41.67	80.00	28.33	28.33	28.33
SP17 Profil-30b*	46.67	0.00	0.00	0.00	26.67	75.00	50.00	50.00	40.00	16.67	16.67	16.67
SP180*	66.67	0.00	0.00	0.00	86.67	68.89	44.44	52.78	86.67	40.00	40.00	40
SP369A Profil-39B*	66.67	0.00	0.00	0.00	26.67	0.00	0.00	0.00	46.67	0.00	0.00	0.00
SP5 Profil-51b*	60.00	0.00	0.00	0.00	26.67	33.33	33.33	33.33	53.33	50.00	50.00	50.00
SP88 Profil-13A*	86.67	0.00	0.00	0.00	20.00	0.00	0.00	0.00	66.67	0.00	0.00	0.00
SP9 Profil-49a*	0.00	-	-	-	0.00	-	-	0.00	33.33	20.00	20.00	20.00
Tiligré	66.67	16.67	16.67	16.67	73.33	56.67	56.67	56.67	46.67	25.00	25.00	25.00
TN88-63	80.00	0.00	0.00	0.00	40.00	25.00	25.00	25.00	86.67	46.67	38.33	42.50
TV286b Profil-12*	73.33	0.00	0.00	0.00	66.67	6.67	0.00	5.00	86.67	31.67	23.33	19.17
TV359 Profil-34*	80.00	8.33	8.33	8.33	20.00	35.00	35.00	35.00	80.00	0.00	0.00	0.00
TV709 Profil-7*	26.67	0.00	0.00	0.00	20.00	75.00	75.00	75.00	53.33	8.33	8.33	8.33
TVU 14 676	100.00	0.00	0.00	0.00	80.00	26.67	17.78	19.86	93.33	28.33	15.00	18.33
Woango-1	86.67	0.00	0.00	0.00	46.67	66.67	66.67	66.67	60.00	11.11	0.00	8.33
Yiis-yandé (IT99K-573-2-1)	66.67	0.00	0.00	0.00	80.00	32.78	11.11	27.36	80.00	11.11	11.11	11.11
P (5%)	<0.0001	0.1439	0.0260	0.1128	<0.0001	0.0056	0.0093	0.0166	0.0001	0.0564	0.1164	0.0720
Mean	75.66	4.61	4.32	4.48	*48.25*	*34.91*	*28.69*	32.02	*68.25*	*24.45*	*20.09*	22.31

Emerg.: Emergence, Incid.: Incidence, Morta.: Mortality, Sever.: Severity, *Wild genotypes, - : Non evaluate

I2 and I4, respectively and the average disease severity indexes (S) were 4.48, 32.02 and 22.31%, respectively. Five genotypes including B05-5a, B27 07a, CB27, SP369 A Profil-39B and SP88 Profil-13A were free of disease (S = 0%) to both isolates I2 and I4; four genotypes including Komsare, Kaya local, 58-57 and Gaoua local-2 showed low severity (S≤10%) and 11 other genotypes including KVx 295-2-124-51, Pa local-2, Boalga local, TVU 14 676, Pouytenga-3, Apagbaala, N°91 profil-4, IT82D-849, B301, TV286b profil-12 and IT 98K-317-2 showed moderate disease severity indexes (S≤20%) to both isolates of *M. phaseolina*. In the other hand, several genotypes such as KVx 396-4-5-2D, KVx 525, KVx 61-1, KVx 640, KVx 780-1, Moussa local and SP180 were susceptible to both isolates of *M. phaseolina* and showed high disease incidence (33.33-100%), high severity (33.33-100%) and high seedling mortality (16.67-55.56%) rates compared to their corresponding control treatments (I0) which were free of disease. The following genotypes were singularly susceptible to the isolate I2 (severity index>20%) and indemne to the isolate I4: Kaokin local, KVx 745-11P and TV359 Profil-34. At the opposite, 524B, B12-07a, IT97K-499-35, KVx 65-114 and NS-1 BF were susceptible to the isolate I4 (severity index>20%) and indemne to the isolate I2.

Regarding seedling mortality, the average rates recorded were 4.32% for the control genotypes, 28.69% for those inoculated with the isolate I2 and 20.09% for those inoculated with the isolate I4 (Table 7). The results revealed significant differences between the genotypes inoculated with I0 (p = 0.0260), and between the genotypes inoculated with the isolate I2 (p = 0.0093). In addition to the five genotypes immune to both of the isolates listed above, two other genotypes including Kaya local and Komsare showed no dead seedling despite the contamination of their seeds with both *M. phaseolina* isolates. Four genotypes including Gaoua local-2, IT82D-849, Komcalle and KVx 295-2-124-51, presented low mortality rates (0-11%) to both isolates. Strangely, nine (9) genotypes (Apagbaala, Boalga local, IT82D-849, KVx 295-2-124-51, N°076 Profil-22, N°91 Profil-4, NS-1 BF, SP118 Profil-24 and Tiligre showed significant high mortality rates (16.66-50%) although their seeds have not been contaminated with *M. phaseolina* (control).

Results on pathogenicity test showed that independently to the original host of the isolate, all the isolates of *M. phaseolina* used were pathogenic and able to cause seedling mortality on cowpea. Additionally, in the present study, the significant difference between the isolates of *M. phaseolina* indicated the presence of sufficient variability between the tested isolates of *M. phaseolina*, which is valuable for the screening for identification of stable resistant genotypes to the fungus. Despite the low number of isolates included in the present study, current results are in agreement with those of several other authors15, 16, 17, 18, 19,20 who worked on the genetic diversity in *M. phaseolina* and found that this fungus had no host-specificity and was highly variable for virulence or aggressiveness. The four isolates used in this study were originated from three different locations of Burkina Faso and were found pathogenically different. Recently, Kumar *et al*.[21] also demonstrated that *M. phaseolina* from soybean was pathogenic on different crops as chickpea, mungbean, urd bean and cowpea and advised to avoid crop rotations of soybean with any pulse crop in the future. Among 84 isolates of *M. phaseolina* from different geographical regions of Mexico, Mayek-Prez *et al*.[22] identified 43 distinct pathotypes. Khan *et al*.[23], later also demonstrated variation in morphology, cultural characters and pathogenicity among isolates of *M. phaseolina* recovered from various hosts and geographical regions. In the present study, the most pathogenic isolates identified were those recovered from *Vigna unguiculata* and *Vigna subterranea*, originated from Kamboinse and Nobere, respectively.

In greenhouse experiment, seedling emergence and mortality, disease incidence and severity were significantly different for the different isolates of *M. phaseolina* ($p<0.0001$ for all parameters) and for the different genotypes of cowpea ($p<0.0001$, $p = 0.0013$, $p = 0.0002$, $p = 0.0005$, respectively). In addition, significant interactions were found between the isolates and the genotypes ($p<0.0001$, $p = 0.0008$, $p = 0.0006$, $p = 0.0032$, respectively). These parameters placed the different isolates and the different genotypes under different pathogenicity and different resistance classes, respectively, with variable reactions of the genotypes depending to the isolate used for the inoculation. In their study, Kumar *et al*.[21] confirmed current results by showing wide variation among 16 isolates of *M. phaseolina* causing charcoal rot of soybean, on seedling root damage, seedling mortality and disease incidence of chickpea, mungbean, urd bean and cowpea.

The significant variations observed in seedling emergence and seedling mortality for the genotypes used as control could be related to the effects of seed-borne *Macrophomina* or other seed-borne pathogens. In fact, the use of *Macrophomina*-contaminated seed could partially explain this situation. The seed disinfection method used before sowing, consisting of a simple seed surface disinfection with sodium hypochlorite solution, could be insufficient to eliminate the whole seed-borne inoculum since the fungus was known to survive as sclerotia or dormant mycelium embedded in the seed coats.

The two isolates I2 and I4, by inducing low seedling emergence rates (48.25-68.25% on average) and high seedling mortality (20.09-28.69%) rates, compared to the control (75.66% for emergence and 4.32% for mortality), exhibited their abilities to cause severe pre emergence and post emergence damping-off on cowpea, as demonstrated by Mohanapriya *et al*.[24]. In contrast to the results obtained in the pathogenicity test, the results from greenhouse screening suggested the isolate I2 more pathogenic by inducing significantly lower seedling emergence rates and significantly higher disease incidence, seedling mortality and disease severity rates on the genotypes than the isolate I4. The observed inconstancy in these results could be due to the great amount of data recorded in the greenhouse (two isolates tested on 80 genotypes, using three repetitions) that allowed more robust statistical analyses than the amount of data recorded in the pathogenicity test where the isolates were tested on only three genotypes.

Among the screened genotypes, eight including 58-57, Bambey-21, CB27, CB46, Gourgou, KN-1, KVx404-8-1 and TVU 14 676 inoculated with the two isolates of *M. phaseolina* presented high emergence rates (80-100%), suggesting that these genotypes were resistant to *M. phaseolina* in seedling stage.

After emergence, five of the 80 tested genotypes including B05-5a, B27 07a, CB27, SP369 A Profil-39B and SP88 Profil-13A stayed free of disease during the ten-day period of the study, suggesting that they were resistant to *M. phaseolina* in vegetative growth stage. However, it was noted that comparatively to their corresponding control treatments (non-contaminated seeds), these genotypes presented low emergence rates (6.67-66.67%) when inoculated with the two isolates, except the genotype CB27 which showed 93.33% emergence rate in all cases. The genotype CB27 could be considered resistant to *M. phaseolina* in seedling and vegetative growth stages in greenhouse conditions. In the present study where two isolates of *M. phaseolina* were used for inoculation, the genotype CB27 was indemne to both isolates, in contrast to a previous study conducted by Muchero *et al*.[25] where this genotype was susceptible. In addition, the genotypes Bambey-21, 524B and IT93K-503-1 considered as resistant

were susceptible to one of the isolates used in curent study. In the other hand, current results demonstrating the susceptibility of CB46 and IT84S-2049 to the disease, were in agreement with those obtained by Muchero *et al.*[25].

The four genotypes (Komsaré, Kaya local, 58-57 and Gaoua local-2) exhibiting low disease severity (S≤10%) could be classed resistant and the 11 other genotypes (KVx 295-2-124-51, Pâ local-2, Boalga local, TVU 14 676, Pouytenga-3, Apagbaala, N°91 profil-4, IT82D-849, B301, TV286b profil-12 and IT 98K-317-2) with a moderate disease severity (S≤20%) to both isolates of *M. phaseolina* could be classed moderate resistant/moderate susceptible to *M. phaseolina*.

Several genotypes inoculated with the two isolates including Pa local-GJ, Melakh, Pobe local, Sakoula local, KVX 396-4-5-2D, KVx 525, KVx 61-1, KVx 640, KVx 780-1, Moussa local and SP180 showing high disease incidence (33.33-100%), high severity (33.33-100%) and high seedling mortality rates (16.67-55.56%) to both isolates were susceptible to very susceptible to *M. phaseolina*.

Despite the isolate I2 was more pathogenic than the isolate I4, some genotypes including 524B, B12-07a, IT97K-499-35, KVx 65-114 and NS-1 BF were strangely susceptible to the isolate I4 (severity index>20%) and indemne to the isolate I2.These genotypes showed more infected seedlings and more dead seedlings when inoculated with the isolate I4 than when they were inoculated with the isolate I2. Nevertheless, the isolate I2 induced high pre emergence damping-off rates (10-93%) on each of these genotypes.

CONCLUSION

Greenhouse screening studies gave the opportunity to investigate on the genetic variability of *M. phaseolina* and to evaluate 80 cowpea genotypes resistance against charcoal rot fungus, *M. phaseolina*. Variation in pathogenicity of the isolates of *M. phaseolina* was demonstrated. Two genotypes including Kaya local and SP 369A profil-39B, having high and stable resistance to *M. phaseolina*, under artificial inoculation were identified. In addition, Gourgou, Woango-1 and N°3076 profil-51b also revealed a good resistance under greenhouse. Several genotypes susceptible to *M. phaseolina* were also identified and included Pa local-GJ and Pobe local.

SIGNIFICANCE STATEMENT

This study discovered the existence of stable sources of resistance to charcoal rot of cowpea in Burkina Faso, that can be beneficial for breeding programs for improving the resistance of available cowpea varieties and preferred by farmers but that is susceptible to the disease. This study will help the researchers to uncover the critical areas of genetic resistance to charcoal rot of cowpea, caused by *M. phaseolina*, that many researchers were not able to explore. Thus a new theory on the control of *M. phaseolina* of cowpea may be arrived at.

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