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SCREENING OF MUNG BEAN GENOTYPES [VIGNA RADIATA (L.) WILCZEK] FOR RESISTANCE TO MACROPHOMINA PHASEOLINA (TASSI) GOID

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ABSTRACT

Mung bean is a legume widely cultivated in subtropical and tropical regions of the world. Its introduction in Burkina Faso is of great interest for the food and nutritional security of the population. However, the development of its cultivation could be hampered by a number of biotic constraints, including fungal diseases caused by *Macrophomina phaseolina* (Tassi) Goid. To this end, 23 mung bean genotypes were screened in greenhouse with the most pathogenic isolate of the *M. phaseolina* fungus. Classification of genotypes according to recorded severity scores revealed three categories namely moderately resistant, susceptible and highly susceptible genotypes. No genotype proved resistant to the Sahelian isolate of *M. phaseolina*. However, two genotypes, M14 and lM2 showed moderate resistance to the Sahelian isolate of *M. phaseolina*. The results of this study could help in mung bean productivity program in Burkina Faso et in in drought-prone regions in the world.

Keywords: Fungal diseases, resistance, *Vigna radiata*

INTRODUCTION

Legumes represent an important component of human diet in many parts of the world, particularly in developing countries, where they supplement the lack of protein in cereals, roots and tubers[1]. In Burkina Faso, several legumes are grown and consumed. Moreover, their economic importance makes them crops of choice to meet the food security needs of the populations[2]. With high population growth and increased vulnerability to climate change, legumes are an alternative to alleviate the problem of food and nutritional deficits in Burkina Faso. Thus, the introduction of mung bean [*Vigna radiata* (L.) Wilczek] in addition to the other legumes already well-known in Burkina Faso, such as cowpea, soybean, groundnut and bambara groundnut, is of great interest[3]. It is much appreciated for its resilience to the effects of climate change, its high nutritional value and its potential in reducing chronic non-communicable diseases [4]. A tropical plant of the fabaceae family, originally from Asia, mung bean is widely consumed in Pakistani households[5], where it serves as both a food crop and a source of income. Indeed, it is rich in nutrients, notably protein (23-25%) and micronutrients (iron,calcium and zinc) [6,7,8].

Despite its growing potential in West Africa, mung bean production in Burkina Faso is threatened by root rot caused by *Macrophomina phaseolina*, a fungus increasingly prevalent in arid and semi-arid regions. Indeed, several biotic constraints limit legume production in Burkina Faso, including fungal diseases caused by *M. phaseolina* Tassi) Goid. It is one of the major constraints on legumes production and productivity due to dry root rot. This pathogen has been identified as one of the most pathogenic and widespread fungi in Burkina Faso [9]. It is a cosmopolitan pathogen that decimates numerous crops including bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.) Walp.) ,sorghum (*Sorghum bicolor* (L.) Moench), bambara groundnut (*Vigna subterranea* (L.) Verdcourt) and mung bean (*Vigna radiata* (L.) Wilczek.) [10,11,12,9]. It is transmitted through both soil and seed and causes yield losses up to 100% in epidemic event [13]. Strategies relying on cultural, chemical, or biological control are ineffective against the pathogen due to its biological characteristics. Using resistant varieties appears to be the most promising approach to limit damage and yield losses caused by *M. phaseolina* [10,14]. Yet, to date, little is known about the interaction between mung bean and local isolates of *M. phaseolina*. This study aims to fill this gap by identifying potential resistance sources to inform future breeding programs in order to support global efforts to develop climate-resilient legumes which could enhance food security in drought-prone regions in the world.

MATERIEL AND METHODS

STUDY PLACE

The study was carried out at Environmental and agricultural Researches and Training Center of Environment and Agricultural Research Institute (INERA), Burkina Faso. The center's geographical coordinates are 12° 28 latitude north, 1°32 longitude and an altitude of 296 m. Two tests were the subject of this study. These were the pathogenicity test and the screening test.

PATHOGENICITY TEST

For the pathogenicity test, three isolates of *M. phaseolina* from three climatic zones of Burkina Faso, namely the Sahelian zone (Dori), the Sudanian zone (Bobo Dioulasso) and the Sudano-Sahelian zone (Boromo), used (figure 1). Two mung bean accessions were used, namely the Ganga variety from Australia and a local BengTigré variety from Burkina Faso.

Inoculum was prepared from these three isolates using the method described in literature [15]. A 5 mm explant from a seven-day culture of each isolate was transferred to 250 ml Erlenmeyer flasks containing 100 ml PDB liquid medium (Potato- Dextrose-Broth) and incubated under laboratory conditions (27±3°C). After seven days' incubation, the conidial mass of isolates in the Erlenmeyer flasks was filtered through filter paper and transferred to 50 ml sterile distilled water. The conidial solution was then macerated in a mortar with a pestle for 1 min, then filtered through muslin cloth. The inoculum of each isolate, made up of the resulting suspension, was transferred to a beaker.

To inoculate the isolates into the two varieties, the seeds were first disinfected by soaking in a water bath. Seeds of each variety were soaked in a 1% sodium hypochlorite solution for 3 minutes, followed by two thorough rinses with sterile distilled water. The seeds were then drained and dried overnight in a laminar flow hood. Sterilized seeds of each variety were then germinated using the blotting paper method [16]. The disinfected seeds were placed equidistantly on a sheet of blotting paper soaked in sterile distilled water , then covered by another sheet also soaked in sterile distilled water. The roll of two layers of paper containing the seeds was lightly tied at the ends with elastic bands, then placed in a transparent plastic bag and incubated under laboratory conditions.

After seven days of incubation, normal seedlings (well-developed, with stem, roots and leaves) were selected and rinsed sterile distilled water. The roots of the drained seedlings were then immersed in the inoculum for one minute, then rearranged between two layers of blotting paper soaked in sterile distilled water as before, and incubated for 10 days in a four-replicate Fisher block design. The three treatments correspond to the three isolates plus one control. For each isolate and variety, 25 mung bean seedlings were used per replicate.

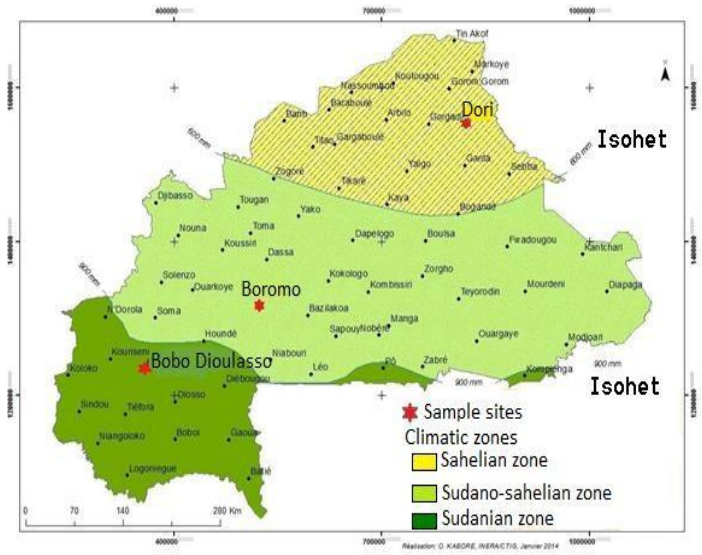

Figure 1. Site of origin of *M. phaseolina* isolates

SCREENING MUNG BEAN GENOTYPES FOR RESISTANCE TO *M. PHASEOLINA* SAHELIAN ISOLATE

To carry out the mung bean genotypes screening trial, the *M. phaseolina* isolate from the Sahelian zone was chosen on the basis of its apparent virulence. In addition, previous work [17] had also identified this isolate as the most virulent. The screening involved a total of 23 mung bean accessions from India and a local variety from Burkina Faso serving as a control.

The inoculum was prepared using sorghum grains as a carrier according to the method described in literature [18] with a slight modification. The method involved soaking sorghum grains in batches of 100 g in 500 ml Erlenmeyer flasks containing 300 ml water for 18 h, then draining and autoclaving at 121°C for 30 min.

After cooling, each seed lot was aseptically inoculated with two explants (5 mm Ø) of each 5-day- old *M. phaseolina* isolate culture. The flasks were then incubated at 25°C in the dark. From the second day onwards, the flasks were shaken daily by hand to ensure homogeneous fungal colonization of the grains and avoid the formation of aggregates. After seven days of incubation, the sorghum seeds were completely colonized by the fungus. The colonized grains were then air-dried for two days. The control treatment used sorghum grains that had been sterilized but not inoculated. Soil preparation consisted in sterilizing the potting soil under the autoclave, cooling it and filling the seed tray cells. For inoculation, the cells filled with sterilized potting soil were first watered, then covered with black plastic film to prevent contamination. Contamination was then carried out with contaminated

sorghum grains and incubated for five days before sowing. The screening test was then carried out in the greenhouse using a split plot design with five replicates, with *M. phaseolina* isolate as the main factor and the varieties as the second factor. Sowing was carried out with three seeds and de-sowing with one plant per plot. Each variety was represented by four plants. Plates were then placed on tables in the greenhouse and watered appropriately until germination and development of the plants. Maintenance work consisted of de-matting, watering and regular monitoring of the plants.

DATA COLLECTION

For the pathogenicity , data were collected 10 days incubation of the inoculated seedlings. The number of contaminated seedlings was counted per treatment and per replicate. Severity was assessed 10 days after inoculation. This involved recording the degree of contamination of 40 plants per isolate, divided into four, i.e. 10 per replicate, according to the following scale: 0= Healthy seedlings; 1= Slight symptoms only on roots; 2= Symptoms on roots; 3= Symptoms on roots and part of stem; 4= Symptoms on roots and whole stem; 5= Seedling completely invaded and dead.

For the screening test, data were collected on emergence at 14 days after sowing and severity noted each week after emergence up to the maximum epidemic score at the sixth week after sowing. Emer- gence at 14 days after sowing is the percentage of plants emerging at this date calculated from the formula: Emergence= (Nt/N) x100 where : Nt=number of plants emerged at date t and N=total number of seeds sown. The disease rating scale described in literature [19] (table 1) was used. Average severity indices were calculated with the scores following the formula used by autors [20] and the result expressed as a percentage: ASI (%)= {Σ (xi - 1) / [E (xi) - 1] x N} x 100 where: xi= Score assigned to each seedling of class i, ni= Number of seedlings belonging to class i, E(xi) = Range of rating scale,N = Total number of seedlings observed, IMS = Severity of infection or ability of the fungus to invade the plant (%).

DATA ANALYSIS

The data collected were subjected to analyses of variance (ANOVA) at the 5% threshold to discriminate between isolates and varieties using XLSTAT 2019 software. A classification of the entries according to their reaction to the pathogen was then carried out on the basis of the average severity indexes obtained for each them, using the scale in table 1.

Table 1. Scale for assessing genotype susceptibility to the pathogen

Scale	Class (%)	Sensitivity
1	1 -10	Resistant
3	11 - 20	Moderately resistant
5	21 - 30	Moderately sensitive
7	31 - 50	Sensitive
9	51 - 100	Highly sensitive

RESULTS AND DISCUSSION

PATHOGENICITY OF *M. PHASEOLINA* ISOLATES AND ASSOCIATED SYMPTOMS ON MUNG BEAN

The analysis of variance for pathogenicity (Table 2) revealed highly significant differences among *M. phaseolina* isolates ($P < 0.0001$), whereas differences among mung bean genotypes were not statistically significant ($P=0.477$). Likewise, the interaction between genotypes and isolates was non-significant ($P = 0.471$), indicating a uniform susceptibility of the tested genotypes to the pathogen. This suggests that the pathogenic effect of the isolates was not dependent on varietal differences.

All M. phaseolina isolates exhibited pathogenicity on mung bean, with the isolate originating from the Sahelian zone of Burkina Faso being the most virulent (Figure 2). Comparable observations were previously made on Bambara groundnut [17]. The high pathogenic potential of *M. phaseolina* in semi-arid and tropical regions has been widely documented, underscoring its economic importance in these agroecological zones [21]. In addition, isolates originating from Sahelian environments characterized by low precipitation levels have been shown to display increased aggressiveness [22].

The Sahelian isolate of *M. phaseolina* induced disease symptoms across the different mung bean genotypes, beginning with seed non-germination, followed by chlorosis at two weeks after sowing (WAS), and progressing to wilting and complete desiccation at six WAS (Figure 3). Disease severity increased from 19.56% at two WAS to a maximum severity rating at six WAS with a severity index of 76.23% (Figure 4). These symptoms, particularly chlorosis and desiccation, are similar to those previously described on common bean, cowpea, and bambara groundnut [17, 23, 24].

Other symptoms such as charcoal rot and dry root rot also caused by *M. phaseolina* have been reported from previous studies[23,10]. Chlorosis is generally followed by wilting, leading to plant death. According to literature this is because the *M. phaseolina* pathogen generally affects the fibrovascular system of the roots, clogging the xylem vessels with microsclerotia [25]. This reduces the transport of nutrients and water to the upper parts of the plant, causing wilting and premature death of the plant. Previous studies [26] , reported that *M. phaseolina* is responsible for damping off, which leads to poor seedling emergence. Contrary to the observations of these studies, the emergence of inoculated entries with the Sahelian isolate of *M. phaseolina* in the present study was not significantly affected comparatively to the non-inoculated control. According to several others studies preemergence mortality results from the ability of *M. phaseolina* race to infect the embryo[27]. Earlier studies [28] reported that races of the fungus that do not cause pre-emergence mortality move to the upper parts of the plant, and the pathogenicity of these races is therefore linked to the degree of nutritional compatibility with the host tissue.

Table 2. Analysis of variance results for pathogenicity of different isolates

Source	DDL	SS	MS	F	Pr> F
Vars	1	0.125	0.125	0.521	0.477Ns
Isolates	3	59.250	19.750	82.292	< 0.0001***
Genotypes*Isolates	3	0.625	0.208	0.868	0.471Ns

SS: Sum of squares ; MS: mean square ;Ns: Not significant, ***: Highly significant

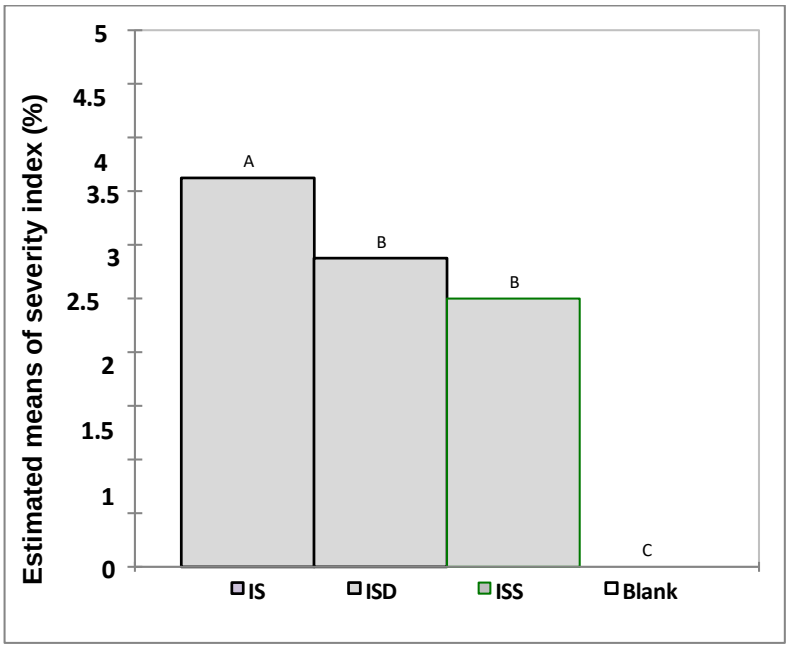

Figure 2. Severity of Isolates on genotypes
SI: Severity index; IS: Isolate from Sahelian zone , ISD: Isolate from sudanian zone, ISS: Isolate from Sudan- Sahelian zone

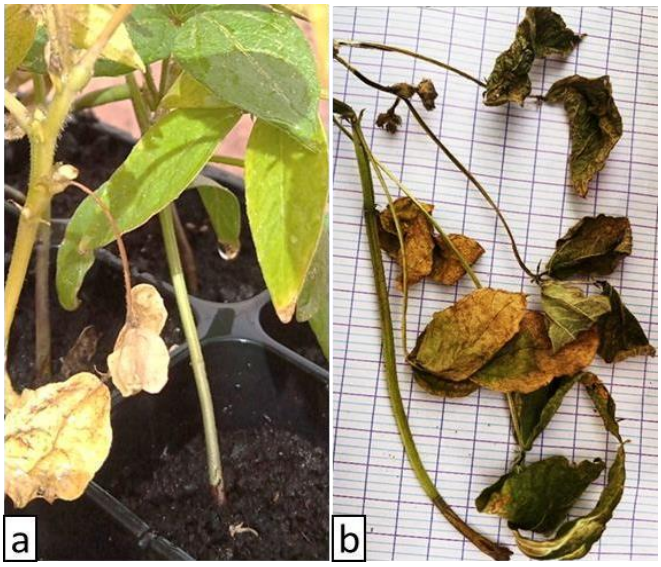

Figure 3. Symptoms of *M. phaseolina* on diseased mung bean plants
a: chlorotic plants, b: drying out of plants

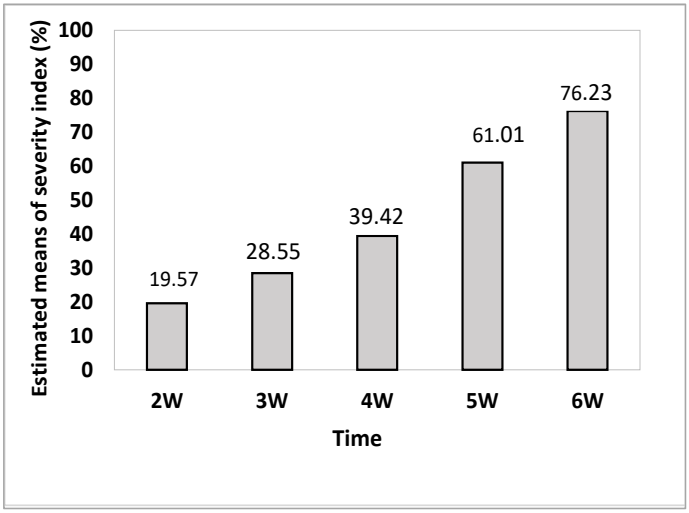

Figure 4. Evolution of disease severity
w: week after sowing

RESPONSE OF 23 MUNG BEAN GENOTYPES TO *M. PHASEOLINA* SAHELIAN ISOLATE

Identifying sources of resistance among mung bean genotypes in Burkina Faso is a crucial step in launching a breeding program to develop resistant varieties. Non-significant differences between genotypes and between the control and the Sahelian isolate of *M. phaseolina* were observed for plant emergence at 14 days after sowing. Thus, emergence showed a non-significant variation with emergence rate of 99.78% for plants inoculated with the Sahelian isolate and 100% for the non-inoculated control (figure 5). A non-significant interaction between genotypes and isolates was observed for the same parameter (table 3). However, in terms of severity of infection (table 4), highly significant differences were recorded between genotypes on the one hand, and between the control and plants inoculated with the Sahelian isolate of *M. phaseolina* on the other.

The severity of infection depended on the isolate, hence the highly significant interaction between isolates and genotypes recorded. Infection severity varied very significantly from 0.37% for the control to 50.37% for the Sahelian isolate (figure 5). Within genotypes (figure 6) , disease severity varied very significantly from 6.67% for genotype M14 to 34.72% for genotype M24, while disease clearance did not vary significantly from one genotype to another (97.5% for genotype M3 to 100% for genotype M14).So the different genotypes tested showed different levels of susceptibility to Sahelian isolate of *M. phaseolina*. These differences in susceptibility of genotypes to Sahelian isolate of *M. phaseolina* had previously been reported by somes studies [30,9] on mung bean. Sources of resistance to this pathogen has been identified within the local bambara groundnut germplasm in Burkina Faso [17] .

The results of the classification of genotypes according to average severity index (figure7) show a breakdown of these entries into three groups: Moderately Resistant (MR) , Susceptible (S) and Highly Susceptible (HS). Group 1, which groups moderately resistant entries, contains two gen- otypes: M14 and M2. Group 2, comprising susceptible entries, contains seven genotypes (M7, M25, M16, M17, M8, M6 and M5), while group 3, comprising highly susceptible entries, con- tains 14 genotypes. No entry was found to be resistant to the Sahelian isolate of *M.phaseolina.*

Host plant resistance is the most feasible and cost-effective measure for reducing yield losses due to diseases caused by this fungus [29]. But no mention of mung bean resistance to Sahelian isolate of *M. phaseolina* in Burkina Faso has yet been reported in literature. More and more, biotechnological approaches have increasingly guided methods to identify resistance sources to this fungus [31,32].

Table 3. Results of analysis of variance on mung bean genotype emergence at 14 days after sowing

Source	DDL	SS	MC	F	Pr> F
Isolates	1	2.228	2.228	0.008	0.928Ns
Genotypes	22	157.244	7.147	0.026	1.000 Ns
Isolates*genotypes	22	170.120	7.733	0.028	1.000 Ns

DDL: Degree of freedom; Ns: not significant; SS: Sum of squares; MS: Mean of squares.

Table 4. Results of analysis of variance on disease severity on mung bean genotypes

Source	DDL	SC	MC	F	Pr> F
Isolates	1	145152.108		573.287	<
		145152.108			0.0001**
Genotypes	22	14105.594	641.163	2.532	0.000***
Isolates*genotypes	22	13975.205	635.237	2.509	0.000***

highly significant; * highly significant; SC: Sum of squares; MC: Mean of squares

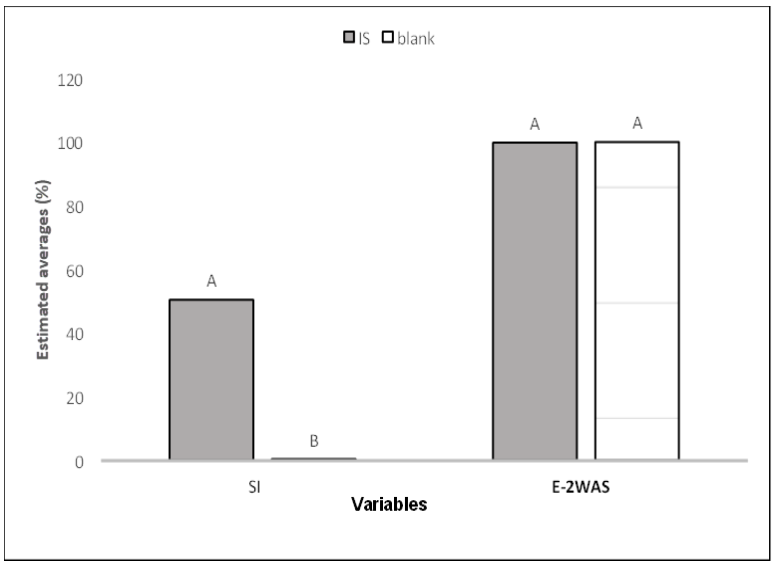

Figure 5. Disease severity and effect of Sahelian isolate on genotype emergence SI: Severity Index, E-2WS: Emergence two weeks after sowing

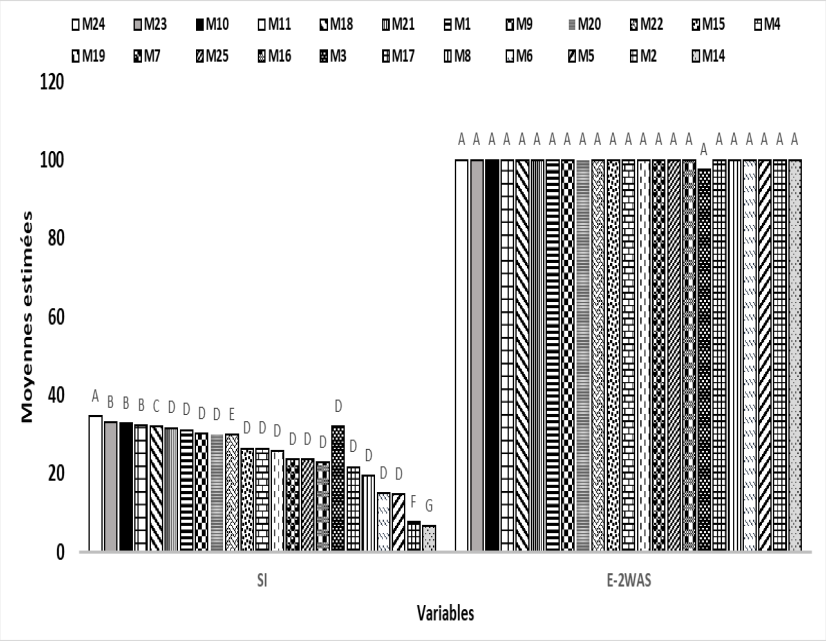

Figure 6. Disease severity and seedling emergence within genotypes
SI: Severity Index, E-2WS: Emergence two weeks after sowing

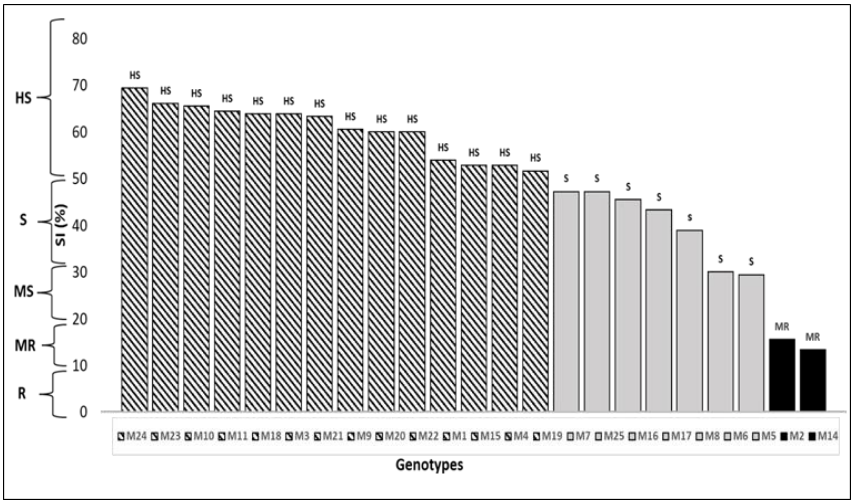

Figure 7. Distribution of genotypes according to SI of Sahelian isolate of *M. phaseolina*
SI: Severity Index , R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, HS: highly susceptible.

CONCLUSION

Among the three isolates of *M. phaseolina* tested, the Sahelian isolate was identified as the most virulent on mung bean genotypes. Screening of 23 accessions revealed significant variability in their responses to infection, although no genotype exhibited complete resistance. Two genotypes, M14 and M2, showed moderate resistance, while the remaining accessions ranged from susceptible to highly susceptible. These findings lay a foundation for the genetic improvement of mung bean in Burkina Faso and highlight the urgent need to incorporate resistant traits through breeding programs. Future studies should integrate molecular characterization and environmental interactions to better understand the genetic basis of resistance and support the development of resilient mung bean cultivars suited to semi-arid regions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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