

## Evaluation of local common bean (*Phaseolus vulgaris* L.) genotypes for resistance to fungal diseases in the bimodal-rainfall forest zone of Cameroon

### Abstract

The general objective of the present study was to evaluate the susceptibility to fungal diseases of some bean collections in the bimodal rainfall forest zone of Cameroon. To this end, 12 bean collections from Foubot were grown at the University of Yaoundé I in a completely randomized block design without phytosanitary treatments. Symptoms identified on the leaves of diseased plants were used to characterize the fungal agents in the laboratory using an identification key. Incidence and severity were assessed, as well as the number of pods and seeds in plants under fungal pressure. Phenotypic observations of symptoms in the field led to the suspicion of 8 fungal diseases. Following the evolution of symptoms, all collections showed fungal disease attacks at different rates. Macroscopic and microscopic laboratory observations identified *Sclerotinia sclerotiorum*, *Uromyces appendiculatus* and *Fusarium solani* as the fungal agents responsible for these symptoms. The incidence of disease caused by *F. solani* was lower in the NJBPRNV (16.67%) and NJBV (22.22%) collections, as was severity (3.33% and 4.44%). On the other hand, the LGRTE (47.06%) and GGRBE (44.44%) LGRV (43.75%) collections showed high incidences of disease caused by *S. sclerotiorum*, *U. appendiculatus* and *F. solani* respectively. Considering the number of seeds, the KPGPV (403.2 seeds) and KPGPTV (350.4 seeds) collections recorded the highest number of seeds, compared with the GGRBE (15.93 seeds); LGRTE (23.87 seeds); GGRBTE (25.53 seeds); LGRV (42.4 seeds) and GGRTE (52.2 seeds) collections. However, the NJBPRNV collection, which had a late flowering and fruiting period of 129 and 131 days respectively, requires special attention in varietal creation, given its greater resistance to the fungal diseases identified.

**Key words:** *Phaseolus vulgaris*, fungal agents, sensitivity, incidence, severity.

### 1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important food legume widely grown in the temperate, tropical and sub-tropical areas of the world **Error! Reference source not**

**found..** According to the Agenda 2030, significant progress has been announced in several development sectors. The Sustainable Development Goals (SDGs), number 2, were entitled: "Eradicate hunger, ensure food security, improve nutrition and promote sustainable agriculture". In addition to food supply alone, four "pillar" dimensions were identified. These are availability (in quantity and quality, including production, distribution and trade), access to food, the establishment of diets that are nutritionally adequate, but also socially and health-wise, and stability over time, of both prices and foodstuffs[1]. To achieve these objectives, man, the pioneer of the ecosystem, must protect his environment by considering the ills that undermine the agricultural sector, particularly in highly anthropized ecosystems, and especially in agro-ecosystems. Certain agricultural practices deregulate the natural mechanisms that ensure system stability. Epidemics are often the result of this deregulation [3], as was the case in the 1970s for pine fusiform rust (*Cronartiumfusiforme*) and corn helminthosporiosis (*Helminthosporium maydis*). Plant diseases are an integral part of natural ecosystems, generally developing endemically. In Cameroon, the main common bean production basin includes the West and North West regions (with over 90%) of national production [4]; yields of local and exotic varieties are not clearly defined. All rural populations in the Western Highlands of Cameroon grow several varieties of common bean for food and commercial purposes. National, sub-regional and international demand for common beans has grown steadily over the years. However, national and African production remains low due to losses caused by pests and diseases. Among production constraints, angular spot disease (ASD), caused by *Phaeoisariopsisgriseola* (Sacc.) Ferraris, is the major pathological constraint [4]. According to our knowledge no study has been carried out on the susceptibility of local bean varieties to fungal diseases, hence the interest in studying their epidemiology in rainforest zones with bimodal rainfall. The aim of the present study is to assess the epidemiology of fungal diseases in bean collections in the bimodal rainforest zone of Cameroon.

## **2. Materials and methods**

### **2.1.Experimental site**

The present work was carried out on the site of the Genetics and Plant Breeding, Department of Plant Biology, Faculty of Science, University of Yaounde I, Yaounde, Cameroon, located in the southern zone of the Centre-Cameroon region between 3°58'-5°00'N longitude and 10°27'-10°38' E latitude (Fig. 1).

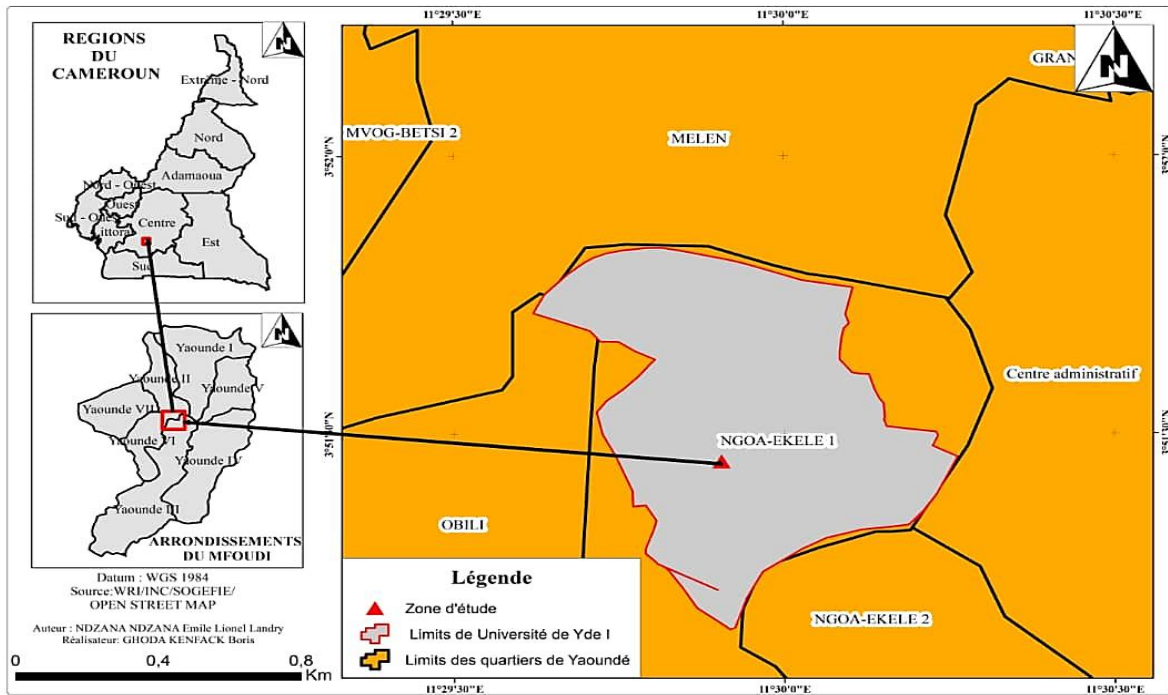


Fig. 1: Location map of experimental site.

### 2.2.2.2 Plant material

The trial used twelve (12) local collections of common bean (*Phaseolus vulgaris* L.) supplied by Foubot growers, including 4 upright and 8 voluble varieties.



Fig.2. Physical appearance of each of the 12 local common bean (*Phaseolus vulgaris* L.) Collections used. a: GGRFE, b: GGRBE, c: GGRBTE, d: KPGPTV, e: NJTRBV, f: NJBV, g: KPGPV, h: LGRTE, i: LGRV, j : PGRV, k: PGBV, l: NJBPRNV.

The acronyms of 12 local common bean (*P. vulgaris* L.) collections are listed in Table I.

Table I. Acronyms of bean collections.

N°	Acronymes	Definition of the acronym	Habit	Notations
1.	GGRFE	Gros Grain Dark Red	Erected	a
2.	GGRBE	Gros Grain Red Brown	Erected	b
3.	GGRBTE	Large Grain Red Brown Spotted	Erected	c
4.	KPGPTV	Koki Small Grain Purple Spotted	Voluble	d
5.	NJTRBV	Njiembekouyou Land Red Brown	Voluble	e
6.	NJBV	Njiembekouyou	Voluble	f
7.	KPGPV	Koki Small Purple Grain	Voluble	g
8.	LGRTE	Long Grain Red Spotted	Erected	h
9.	LGRV	Long Grain Red	Voluble	i
10.	PGRV	Small Grain Red	Voluble	j
11.	PGBV	Small White Grain	Voluble	k
12.	NJBPRNV	Njoumbière Perennial Black King	Voluble	l

## 2.3.Methods

### 2.3.1. Experimental design, sowing and maintenance

The experimental set-up consisted of Completely Randomized Blocks (CRB) with 3 replications and no phytosanitary treatment was administered. Each block consisted of 12 experimental units, each measuring 2.70 m x 1.60 m. The distances between experimental units were 1 m and 1.20 m between blocks, i.e. a surface area of 36.75 m x 9.15 m (336.3 m<sup>2</sup>).

Three seeds were planted in each pokey, spaced 0.50 m apart between rows and 0.30 m between rows. Average density was 1,371 plants/36.26m<sup>2</sup>, or around 40,772 plants/ha. Maintenance consisted of weeding, manual weeding of weeds, followed by hoeing, and was carried out 3, 32 and 60 weeks after sowing.

### 2.3.2. Characterization of fungal pathogens

#### 2.3.2.1.Preparation of culture media

The use of PDA (Potato Dextrose Agar) culture medium enabled a good assessment of macroscopic criteria. Preparation of one liter of PDA required 200 g potatoes, 15 g dextrose, 15 g agar powder and distilled water. Peeled and cubed potatoes were macerated in water for

about half an hour, then cooked for 30 minutes. The resulting slurry is filtered through filter paper. Agar and dextrose are added to the collected juice in a 1000 ml beaker, and the whole is stirred and homogenized using a magnetic stirrer. The volume is then made up to the mark with distilled water, and the pH adjusted to 6.0. The resulting mixture is autoclaved at 120°C for 20 minutes and stored in the refrigerator [5]. Once the test tube had been removed from the autoclave, pouring was carried out by pouring the prepared liquid into the Petri dish. Once cooled and solidified, the culture medium is ready to receive the cultures of microorganisms (Fig. 3).



Fig.3. Preparation of culture media.

### **Organ sampling in the field**

In order to isolate and identify these various pathogens, samples of bean organs (leaves, fruits) showing symptoms of disease were taken during the field trip using the descriptor described by [6]. These samples were transported to the Plant Pathology Laboratory of the University of Yaoundé 1 for isolation and identification of the pathogen.

To ensure proper isolation of the endophytic fungi, diseased plants and fresh plant material were selected. Several mature and infected plants were selected and samples were randomly collected from various locations on the plants, placed in sterile plastic bags and transported to the laboratory. The collected samples were macerated for 30 min in distilled water, then spread out and stored in a fume hood until ready for use (Fig. 4).

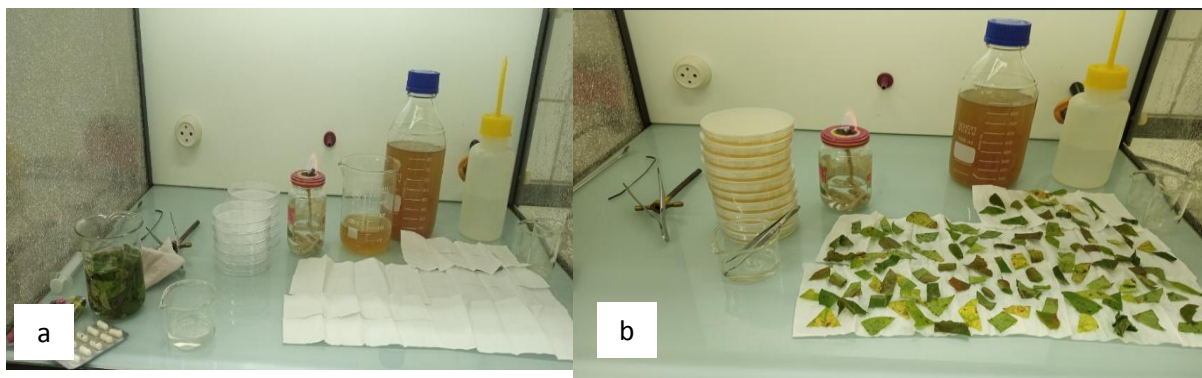


Fig.4. Sterilization and preservation of samples collected in a fume hood (a and b).

### 2.3.3. Laboratory identification of fungal pathogens

Identification consisted of observations of the morphological (macroscopic and microscopic) characteristics of emerging mycelia and an identification key.

### 2.3.4. Evaluation of epidemiological parameters of fungal diseases

#### 2.3.4.1. Fungal disease incidence

Disease incidence was determined by adopting the standard plant pathology formula used in the work of [7]:  $I(\%) = (n/N) \times 100$

Where: N represents the total number of plants per experimental unit; n the number of diseased plants on the same experimental unit and I (%) represents the incidence or frequency of the disease in the experimental unit.

#### 2.3.4.2. Evaluation of fungal disease severity

Disease severity was determined using the following formula  $S(\%) = (\sum(ab))/N \times 100$

Where:  $\sum(ab)$  is the sum of multiplications of the number of diseased plants (a) by the corresponding degree of infection (b) given in %; and N is the total number of plants. This same formula was used by [7]. The scale used for the degree of infection (b) is that proposed by [8]. Where 1 corresponds to 0% plant infection; 2: infection covering between 1 - 15% of the plant; 3: infection covering between 16 - 40% of the plant; 4: infection covering between 41 - 75%; 5: infection covering 76% - 100% of the plant [9].

### 2.3.5. Productivity of genotypes under fungal pressure

Pods and seeds were quantified using a simple counting method on five plants per ridge from twelve collections, with three replications, i.e. fifteen plants for each collection on day 74

after sowing. The NJBPRNV collection was not represented due to its late flowering and fruiting on days 129 and 131 after sowing respectively.

### **2.3.6. Statistical analysis**

The data collected on disease incidence and severity, as well as the number of seeds and pods in the different collections, were organized using EXCEL 2019 (Microsoft office) spreadsheet software and imported into XLSTAT 2023 for an Analysis of Variances (ANOVA). The Kruskal-Wallis multiple comparison test at the 5% probability threshold was used to highlight significant differences between means, using R software. Graphical representations of means were produced using Microsoft Excel 2019.

## **3. RESULTS AND DISCUSSION**

### **3.1. Results**

#### **3.1.1. Morphological characteristics of pathogens**

##### **3.1.1.1. Macroscopic observations**

The results of microscopic observation show white filaments with a cottony appearance (Fig. 5a), abundant white to brown downy mycelial filaments (Fig. 5b) and light-white to transparent mycelial filaments (Fig. 5c).



Fig.5. Pure strain obtained three days after transplanting.

##### **3.1.1.2. Macroscopic observations**

Microscopic observations of mycelial filaments under the light microscope shows isolated microconidia with zero to one oval to cylindrical septum and macroconidia with three to four medium to falciform or elongated septa. These characteristics are typical of *Fusarium solani* (Fig.6a), with small, irregularly shaped, non-septate spores or sclerotia (Fig.6b). The different

shapes observed show that this is *Sclerotiasclerotium*. Microscopic observations revealed uredia and uredospore which are typical of *Uromycesappendiculatus* (Fig. 6c).

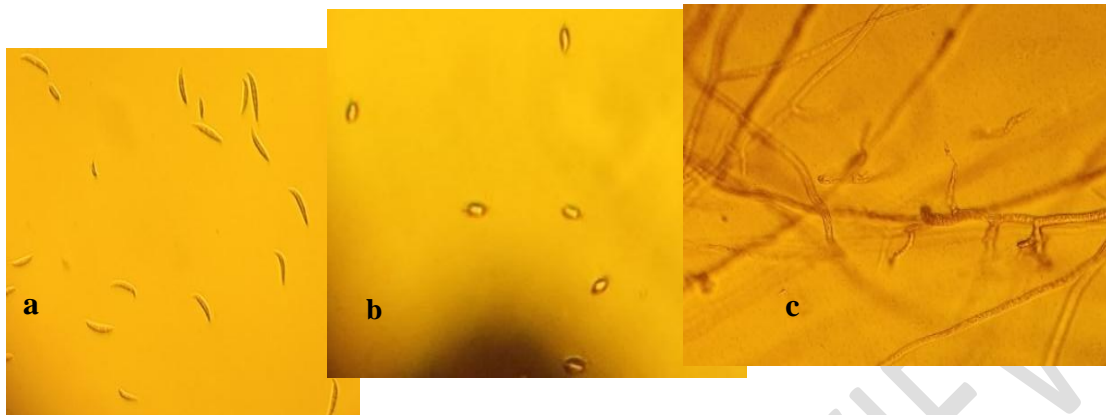


Fig.6. Microscopic identification of the three pathogens responsible for fungal diseases: a: *F.solani*; b: *S. sclerotiorum*; c: *U. appendiculatus*.

### 3.1.2. Pathogenicity of fungal diseases

#### 3.1.2.1. Sensitivity of collections to *Fusarium solani* pressure

The results show that the incidence of fusariosis varied from 16.67% to 43.75%. However, all collections were subject to *F. solani* attack. The GGRBTE and GGRBE, NJBPRNV and NJBV collections were less affected by the disease. Severities of 2.65, 2.92, 3.33 and 4.44% respectively were observed. On the other hand, the LGRV and LGRTE collections were the most attacked by disease, with severities of 16.41 and 14.70% respectively (Table II).

#### 3.1.2.2. Susceptibility of collections to *Sclerotiasclerotium* pressure

The results show that the NJBPRNV collection was the most resistant to the disease, as it was not attacked at all (0%). The NJBV, PGRV and PGRBV collections also showed fairly high tolerance to the disease caused by *S. sclerotiorum*, with incidences of 16.67%, 16.67% and 17.65% respectively, compared with the LGRTE and GGRBTE collections, which proved more susceptible to *S. sclerotiorum*, with incidences of 47.06% and 41.18% respectively (Table II).

The NJBPRNV collection showed no attack of the disease, and consequently no severity (0%). The NJBV, PGRV, PGBV, NJTRBV and GGRFE collections also showed relatively low levels of disease attack, with severity values of 3.33; 3.33 and 3.53, 4.29 and 4.71% respectively. However, high levels of disease attack were observed in the LGRTE and GGRBTE collections, which proved susceptible to the disease, with severity values of 16.71 and 14.70% respectively (Table II).

#### 3.1.2.2. Sensitivity of collections to *Uromycesappendiculatus* pressure

The results show that the NJBV and KPGPTV collections proved more resistant to the fungal disease caused by *U. appendiculatus*, with incidences of 16.67% and 19.05% respectively, compared with the GGRBE, GGRFE and GGRBTE collections, which proved more susceptible to *U. appendiculatus*, with incidences of 44.44%, 41.18% and 41.18% respectively (Table II).

The NJBV KPGPTV, KPGPV and PGRV collections proved less susceptible to the disease, with respective severities of 3.33; 3.81 and 4.44, 4.76%. In the GGRBE, GGRBTE and GGRFE collections, disease attacks were high, with severities of 15.87, 14.70 and 14.62% respectively (Table II).

**Table II. Sensitivity of *P. vulgaris* collections to fungal pressure.**

Collections	<i>Fusarium solani</i>		<i>Sclerotiasclerotiorum</i>		<i>Uromiceappendiculatus</i>	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
GGRFE	41,18	8,24	23,53	4,71	41,18	14,62
GGRBE	38,89	2,92	33,33	6,67	44,44	15,87
GGRBTE	35,29	2,65	41,18	14,70	41,18	14,70
NJTRBV	42,86	8,57	21,43	4,29	35,71	7,14
NJBV	22,22	4,44	16,67	3,33	16,67	3,33
KPGPV	33,33	6,67	38,10	7,62	23,81	4,76
KPGPTV	33,33	6,67	28,57	5,71	19,05	3,81
LGRTE	41,18	14,70	47,06	16,71	29,41	5,88
LGRV	43,75	16,41	25,00	5,00	25,00	5,00
PGRV	27,78	5,56	16,67	3,33	22,22	4,44
PGRBV	23,53	4,71	17,65	3,53	29,41	5,88
NJBPRNV	16,67	3,33	0,00	0,00	33,33	6,67

The twelve bean collections showed different severities and incidences of the three identified diseases. This may be due to the involvement of resistance genes against the latter. This difference in susceptibility may also be explained by the establishment of resistance mechanisms by local bean collections.

### 3.1.3. Production of common bean collections under fungal pressure

#### 3.1.3.1. Number of pods in common bean collections under fungal pressure

Considering the number of pods, 11 collections out of 12 were determined, with the KPGPTV and KPGPV collections showing the best results with an average number of pods of 149 and 148.9 respectively. There was no significant difference between the number of pods in these two collections. On the other hand, the GGRBE and LGRTE collections had the lowest number of pods, with averages of 11.8 and 31.63 respectively (Fig.7).

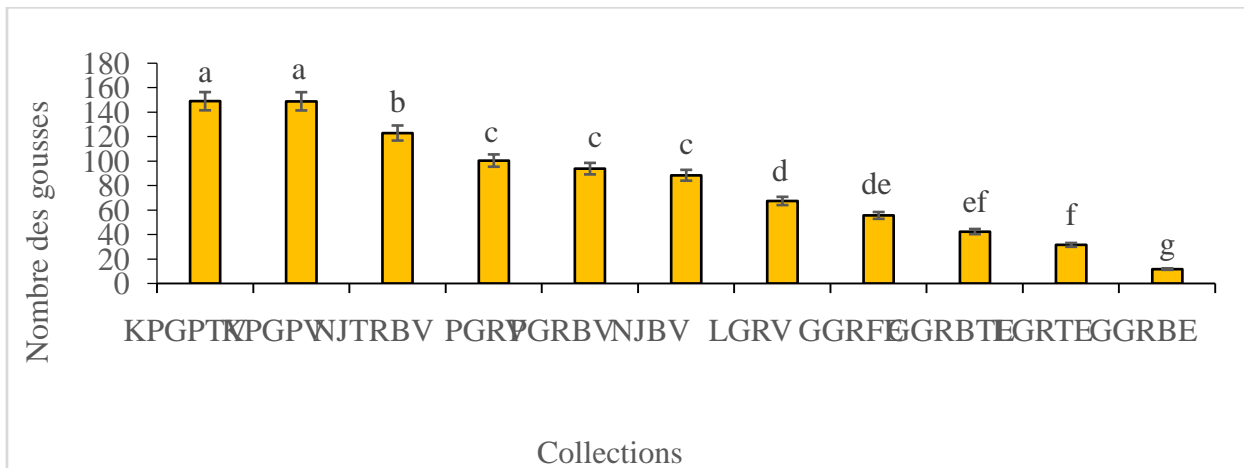


Fig.7. Number of pods in the different collections. Values followed by the same letter are not significantly different according to the Kruskal-Wallis test ( $p < 0.05$ ).

### 3.1.3.2. Number of seeds in collections under fungal pressure

With regard to the number of seeds in the eleven remaining collections, the KPGPV and KPGPTV collections showed the best results in terms of number of seeds, with mean values of 403.20 and 350.4 seeds respectively. On the other hand, the GGRBE, LGRTE and GGRBTE collections had the lowest seed count values, at 15.93, 23.87 and 25.53 respectively (Fig.8).

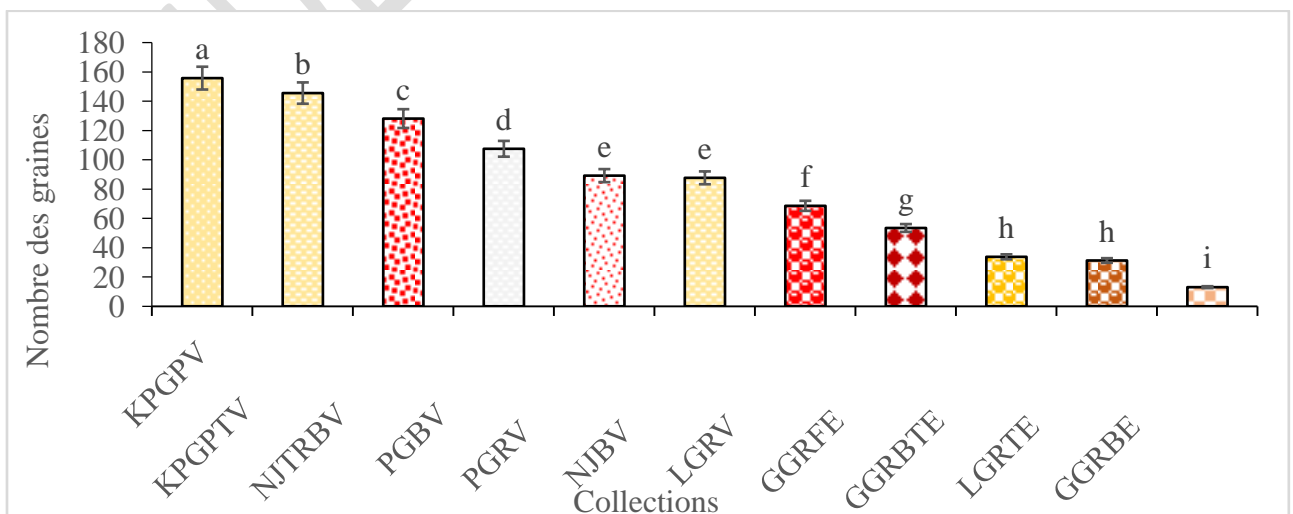


Fig.8. Number of seeds in pods from different collections. Values followed by the same letter are not significantly different according to the Kruskal-Wallis test ( $p < 0.05$ ).

### 3.2.DISCUSSION

Analysis of the 8 symptoms observed on the leaves of common bean plants in the field enabled the laboratory to identify *Fusarium solani*, *Sclerotinia sclerotiorum* and *Uromyces appendiculatus* as being responsible for said symptoms. With the exception of *Fusarium*, these fungal agents are different from those observed by [10] in the Western region of Cameroon precisely in Menouaon bean seeds in storage. This difference may be due to the sampling period. Indeed, these authors worked on bean seeds in storage, unlike this work carried out on samples of plant leaves in the field.

The collections studied showed different levels of susceptibility to the pathogens identified. The work carried out on the development of fungal diseases in the field showed that the NJBPRNV collection was the one that developed the lowest sensitivity to the fungal diseases identified. This sensitivity could be due to the collection's genetic heritage, which contains pathogen resistance genes in its genotype. The presence of resistance genes has also been shown to act as a barrier to infection in bean cultivars [11]. The disease tolerance of certain collections has also been reported in other work under different ecological conditions [12]. Indeed, studies carried out in Colombia have identified disease resistance genes using AFLP molecular markers in bean genotype G 10474 [16], RAPD markers [17] and on the Brazilian cultivar 'Ougo Negro' [14]. However, the existence of high variability in pathogenicity within disease populations suggests the need for a study of the genetic diversity of pathogen populations in Cameroon, in order to understand their biology and genetic structure, and to devise effective control strategies [18]. In addition, the strong influence of *Fusarium solani*, responsible for root rot, for example, has been reported as a fundamental cause of the absence of resistance genes [19]. The severity of the various fungal diseases recorded varies. The GGRBTE, NJBV and NJBPRNV collections showed severities of 3.33, 2.65; and 0% respectively for *Sclerotinia sclerotiorum*, *Fusarium solani*; and *Uromyces appendiculatus* were the least susceptible. This low level of susceptibility could be explained by the fact that these collections possess genes for resistance to the diseases studied. This variation in susceptibility between genotypes has also been observed within 25 bean genotypes [20] (Fekede et al.,

2022). Analyses of symptom severity values observed on leaves from different collections corroborated those of severity. Thus, symptom intensity, which is easier to determine, could be used as an indicator for assessing the varietal susceptibility of common bean to disease. This was confirmed by the work of [21], who assessed the resistance of certain common bean varieties to angular leaf spot disease.

The R8 pod-filling stage was the most susceptible in the different collections with voluble habit as well as those with erect habit. Indeed, it has been established that the flowering and pod-filling stage is the most susceptible to the fungal disease [4]. This sensitivity could be due partly to the approach of senescence in beans and partly to the abundance of inoculum. Indeed, the primary inoculum developed by the first symptoms would have multiplied (during stages R6 and R7) in the form of secondary and tertiary inoculum to infect plants at stage R8. The voluble collections produced a higher number of pods and seeds than the erect collections; this is justified by the fact that, on the one hand, these are long-cycle collections which are able to reach light quickly thanks to the supports and consequently increase their photosynthetic activity and, on the other, have a sufficiently large above-ground biomass (leaves) to supply the high number of flowers and pods formed with carbohydrates. Indeed, in common bean collections with a voluble habit, there is continuous flowering as the plant develops on the support, which increases the number of inflorescences, pods and seeds. Similar results were reported by [22], who showed that voluble collections produced a higher number of pods and seeds than dwarf collections.

#### 4. Conclusion

At the end of this study, the general objective of which was to assess the epidemiology of fungal diseases in bean collections in the southern zone of Cameroon, it was found that the eight voluble and four erect collections tested showed susceptibility to the fungal diseases studied. Of the 08 fungal diseases suspected in the field, 03 were isolated in the laboratory. These pathogens influenced the number of pods and seeds, as well as the variability of the incidence and severity of the collections over time. Erect collections were more susceptible to disease than voluble collections. Of all the collections studied, NJBPRNV (16.67%) and NJBV (22.22%) were the least susceptible to fungal disease caused by *Fusarium solani*, while LGRTE (47.06%) and GGRBE (44.44%) were more susceptible to fungal diseases caused by *Sclerotinia sclerotiorum* and *Uromyces appendiculatus*. Although the diseases significantly reduced the number of seeds in the various collections studied, the KPGPV (403.2 seeds) and

KPGPTV (350.4 seeds) collections performed well, presenting the highest number of seeds, while the lowest number of seeds was obtained in the GGRBE (15.93 seeds); LGRTE (23.87 seeds); GGRBTE (25.53 seeds); LGRV (42.4 seeds) and GGRTE (52.2 seeds). The KPGPV and KPGPTV collections can therefore be recommended to farmers in this locality.

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