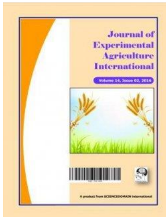

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Evaluation of French Bean (*Phaseolus vulgaris* L.) Varieties for Resistance to Anthracnose

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Authors' contributions

This work was carried out in collaboration between all authors. Author GJK designed the study, wrote the protocol and wrote the first draft of the manuscript. Author EEA reviewed the experimental design and all drafts of the manuscript. Author SKK performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

French bean (*Phaseolus vulgaris* L.) is a very important protein component of the diets of the majority of the population globally and in Kenya. It is a major vegetable export crop (48%) and income earner to the smallholder farmers who constitute more than 80% of producers in Kenya. However commercial varieties presently grown have the potential to produce more yields of above 1800 $kgha^{-1}$ but are limited by pests and disease. Among the diseases is anthracnose incited by *Colletotrichum lindemuthianum* (sacc.et. magn.) Lams. Scrip which causes a severe rapidly developing disease that can bring about complete plant defoliation and extensive yield and or quality loss. Studies were conducted to identify commercial varieties of French beans which are resistant to anthracnose disease. The objective of this study was to screen commercial varieties of French beans in controlled environmental conditions (glasshouse) for resistance to anthracnose disease. This was achieved through evaluation of incidence and phenotypic variation in anthracnose virulence among the bean varieties in relation to yields. Ten bean varieties were used during the study, among them were two controls; Julia, Andate, Amy, Organdia, Mara, Serengeti (resistant

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control), Venda (susceptible control), Conza, Strada and M_{uH13}. The experimental design used was Randomized Complete Block Design (RCBD) with four replications. A scale of 1-9 score was used to rate the disease reaction, which was subdivided into; 1-2 (resistant), 3-4(moderate), 5-9(susceptible). Data collected were subjected to ANOVA using SAS program version 9.1. Results exhibited three bean varieties (Julia, Mu_{H13} and Organdia) to have high significant resistance to anthracnose and one variety (Strada) to be tolerant. The study therefore suggests the use of desirable resistant varieties as the best way of increasing yields.

Keywords: French beans varieties; bean anthracnose; Colletotrichum lindemuthianum; susceptible; resistant; incidence; phenotypic variation.

1. INTRODUCTION

French bean is a very important protein component of the diets of the majority of the population in Kenya and other regions of the world. It is a major export crop in Kenya and it is popularly grown by both large and smallholder farmers [1].

Disease pressure is a very important biotic factor that affects the success of the French bean genotypes released in most breeding programs. High degree of susceptibility often results in a decreased yield and lowers the quality of the crop [2]. Anthracnose (*Colletotrichum lindemuthianum*) in French beans (*Phaseolus vulgaris* L.,) has been cited as the most severe disease that causes complete leaf loss (defoliation), poor quality and lower yields [3].

The use of own saved seeds is a problem because *Colletotrichum lindemuthianum* pathogen which causes Anthracnose disease is transmitted through infected seeds. Being mainly a seed borne disease, anthracnose is easily spread as farmers depend highly on farm saved seed and exchange of seed is common, and as such disease severity on farmer fields is perceived to be quite high [4]. With the sharing of farm saved seed between farmers, there is an increase in the chances of disease transmission between farmer fields, and between different agro-ecological zones where beans are grown [5].

Disease resistance in French beans is a very important component since a high degree of susceptibility may well result in decreased yield and quality of the crop [6].

Since other protein sources are too expensive to be consumed in nutritionally adequate quantities by resource-poor families, increase in French bean production has a major bearing on the health of most Kenyans [7].

French beans are immature green pods which are referred to as snap or green beans. The crop is cultivated for both fresh consumption and processing mainly canning and freezing. The optimum temperature range for growing French beans is 20-25ºC and an altitude range of 1,000-2,1000M. Rainfed cultivation is possible in areas with well distributed medium to high rainfall of 900-1,200 per year, but supplementary irrigation is required to maintain continuous production during off season. French beans performs best on well drained silty loams to heavy clay soil high in organic matter contents with slightly acidic to slightly alkaline PH of 6.5-7.5 [8].

Planting of French beans is carried out in single rows at 30×15cm or double rows at 60×30×10cm single seed per hole. The crop takes 45-50 days from planting to first picking [9]. Farm yard manure is recommended in poor soils at a rate of 10tons/ha applied in planting furrows. 200kg per ha of DAP fertilizer is applied in the furrows and mixed well before planting. At three leaf stage topdressing is carried out with 100kg of CAN and a second application follows at the onset of flowering. Foliar feed should be applied fortnightly from the fourth week to mid podding stage to promote high yields [10].

However excessive nitrogen promotes growth of leaves instead of pods. Regular watering is essential for promotion of high yields, uniformity and high quality. The crop is sensitive to water stress at flowering .Water logging should also be avoided. Application of 35mm of water per week at planting to 10 days after germination and 50mm thereafter to flowering stage is recommended for good growth of French beans. Timely weed control is absolutely essential [11].

Major production constraints of French beans are biotic and abiotic factors. Biotic factors are pests and diseases. Among the diseases is anthracnose which is known wherever French beans are cultivated [12]. It was first discovered in Germany in 1875 and appeared in England five years later. It is not very common in Britain but may be severe in cool, wet summer and is fairly widespread throughout Europe, America, Australia, New Zealand, Africa and India [13].

The fungus is capable of tolerating temperatures as low as -15°C to -20°C, for several days, but is sensitive to high temperatures; the optimum and maximum for growth lies between 22°C and 23°C and 30°C and 31°C respectively. The optimum temperature for sporulation is near 15°C with the minimum and maximum at 4°C and 38°C respectively. Germination of the spores takes place more rapidly at temperatures higher than the optimum for growth, but not normally above 27.5°C, the critical temperature occurring between 32°C and 35°C [14]. Cool moist weather during the summer affords the best conditions for the prevalence of anthracnose, and high atmospheric humidity (more than 95%) is essential, not only for the success of initial infections but also for the dissemination of the spores in the fields [15].

Pathogenicity of the fungus varies greatly over the world and affects the stability and effectiveness of varietal resistance [16]. The production of disease-resistant French beans is the most desirable method of achieving control of pathogens. However total immunity produced by breeding is rare and the durability of resistant varieties of narrow genetic base has often proved short-lived [17].

One of the most urgent needs in agriculture is to identify and develop French beans varieties that can resist anthracnose disease which infects French beans causing damage and finally yield losses [18]. Also the ideal way of controlling anthracnose is for farmers to use resistant varieties which is more economical than other control methods and thus lowers cost of production [19] and increased yields [20].

2. MATERIALS AND METHODS

2.1 Description of the Study Site

The study was conducted at the University of Eldoret, Department of Biotechnology (laboratory) in a glass house. The glass house had humidity of more than 90% and temperatures ranging from 22°C – 25°C. University of Eldoret is located between longitude 35°18' E and latitude 0°30'N and at an altitude of 2140 m above the sea level. Rainfall ranges from 900 to 1300 mm with an annual average of 1124 mm. The average annual temperature is 23°C with a minimum of 10°C. The type of soil is ferralsols [21].

2.2 Description of the Commercial French Bean Varieties

The study was conducted on ten French bean varieties: Julia, Amy, Organdia, Serengeti (resistant control), Conza, Venda (susceptible control), Mara, Strada, Andate and Mu_{H13} (Table 1).

Table 1. Seed colour and growth habit of the ten French bean varieties.

Varieties	Seed colour	Growth habit
Julia	Black	dwarf
Andate	Pink	dwarf
Mara	Pink	dwarf
Amy	White	dwarf
Serengeti	Pink	dwarf
Conza	Pink	dwarf
M_{uH13}	black	dwarf
Venda	Pink	dwarf
Strada	Pink	dwarf
Organdia	Black	dwarf

2.3 Description of the Planting Procedures

The beans were first pre-germinated in petri-dishes at room temperatures inside the laboratory and after germination; they were transferred and planted in plastic pots. The experiment was conducted using 40 – plastic pots, each variety was planted using 10 plastic pots. Manure and soil were mixed in ratio of (1:2) respectively and was applied to each pot before planting [22]. A maximum of five seeds were planted in each plastic pot. The experiment was arranged in a Randomized Complete Block Design (RCBD) with four replicates. Throughout the experimental period the pots were kept weed-free, watering was done regularly on each pot.

2.4 Fungus Isolation

The fungus was isolated from diseased leaves. Small pieces of infected tissue were surface sterilized and incubated on petri dishes containing a modification of Mathur's medium.

The media contained dextrose (8 g liter^{-1}), $MGSO_{4}.7\ H_2O$ (2.5 g liter^{-1}), neopeptone (2.4 g liter^{-1}), yeast extract (2.0 g liter^{-1}) and agar (16 g liter^{-1}). Conidia harvested from sporulating colonies were placed in a sterile solution of 10% peptone and 20% sucrose for medium-term storage. The spore suspension was impregnated on sterile pieces of filter paper that were desiccated for 8 days over sterilized silica gel prior to impregnation.

2.5 Preparation of Bean Pod Agar Media

This was prepared using; 500 grams of green pods, 16 grams of Agar and 1 liter distilled water. Two ends of the French bean pod were cut off, the pods were washed and autoclaved for 15-20 minutes, the water Agar was prepared (16 grams of Agar in 1 liter of distilled water) and autoclaved for 20 minutes, 3 millilitres of water Agar was poured into autoclaved clean glass tubes, the bean pod were put upright in each tube and closed using cotton wool, they were autoclaved for 20 minutes and left to solidify [8,13].

2.6 Inoculum Preparation

Since the fungus was already grown in Petri dish, but not sporulating on artificial media French bean pod agar provided nutrients needed for sporulation. Using a sterile needle small pieces of agar with the fungus were cut from the corner of the plates and agar plugs placed in the tubes with sterile French bean pods, the tubes were placed in the incubator at 25°C and were monitored for 3-4 days when the fungus had fully grown, the fungus was re-plated into new tubes containing media with pod and the fungus were ready for inoculation after two weeks [15,22].

2.7 Inoculation Procedure

Ten drops of Tween20 was added to 1 liter of distilled water, a spatula was used to remove the pods from the tubes which were placed in a beaker and 200 milliliter of distilled water was added. Magnetic stir plate was used to star until the suspension was formed. Cheesecloth was placed on the top of the funnel, the funnel with cheesecloth was placed on the flask and the spore suspension was poured into the flask. The spore suspension was counted and the concentration of the spores was adjusted to $1.2*10^6$ Spores/ml. The French beans were then inoculated using the inoculum prepared and the fungus was re-isolated from the inoculated plants.

2.8 Disease Screening in the Glasshouse

Inoculum for glasshouse screening was prepared by suspending in water the conidia collected from *C. lindemuthianum* cultures. A heamocytometer was used to adjust the concentration of the inoculum Inglis et al. CIAT researchers used a conidial concentration of 1.2×10^6 for glasshouse inoculations (Pastor-Corrales et al. Seven-day-old seedlings were inoculated and kept seven days in a chamber at 22°C at 95-100% relative humidity. Inoculum was applied to the seedlings until all the seedlings had a runoff on the stem and the surface of the unifoliate leaves. Plants were placed in a chamber with high humidity (> 95%) for 48 hours at 22-25°C and evaluated for disease reaction at 7th, 14th, 21st and 28th day after inoculation [12]. Eight plants of each variety were evaluated using a 1-9 scale to calculate a mean disease rating (Table 2).

Table 2. Evaluation scales for glasshouse screening for anthracnose reaction

1 – 9 Scale	Symptoms on 7- day- old seedlings
1-2	No symptoms (Resistant)
3-4	Very small lesions, mostly on primary leaves (Resistant)
5-9	Numerous enlarged lesions or sunken cancers on the lower sides of the leaves or hypocotyls (Susceptible)

Source: Balardin et al. [20]

Disease incidence, were recorded at four different plant growth stages (at 7th, 14th, 21st and 28th day after inoculation).

2.9 Statistical Data Analysis

All the data obtained were subjected to analysis of variance (ANOVA) using SAS program version 9.1. Mean values were separated at 5% level of significance and the two mean values were declared significantly different when the difference between them was greater than the least significant difference (LSD).

3. RESULTS

The results showed significant phenotypic variability among the commercial French bean varieties. Anthracnose incidence indicated significant differences between the French bean varieties ($p<.05$) (Table 3). French bean varieties; Julia, Organdia, and Mu_{H13} consistently had lower anthracnose incidence levels throughout the study. The varieties which showed average anthracnose incidence is Strada which can be considered to be tolerant while those which had high incidence levels were Amy, Conza, Mara, Andate.

The effects of the disease on the leaves and the stalk of plants were pronounced with immature leaves falling down and showing the whitish lesion and some with chlorosis and black spots on the leaves (Fig. 1). Being an aggressive foliar disease, anthracnose destroys plant photo-synthetic tissue causing premature defoliation and early maturation thus lowering yields (Fig. 2), this is in agreement with earlier researchers [21].

Fig. 1. Chlorotic effects of anthracnose disease on French bean variety (Amy)

From the results, the significant differences in anthracnose incidence and damage levels between varieties could be indicative of differences in susceptibility levels (Table 2). The French bean seeds with black colour (Table 1) showed some resistance to anthracnose.

The mean incidence of the bean varieties showed that there was significant difference at $P<.05$ among the varieties, therefore varietal resistance to anthracnose differed significantly ($P<.05$) (Table 3). The varieties which showed resistance to anthracnose were; Julia, Mu_{H13}, and Organdia, those which exhibited susceptibility to anthracnose were; Andate, Mara, Venda, Amy and Conza and Strada exhibited tolerance to anthracnose (Table 3).

Different agronomic traits contribute towards the yield of the bean varieties [2]. From the study, the days to maturity, pod length and yields were significant at $P<.001$, $P<.05$ and $P<.001$ respectively (Table 4).

The results showed that all the varieties yielded significantly different ($P<.001$) (Table 4). The varieties which exhibited high yields were; Julia, Mu_{H13} and Organdia and Strada (Fig. 2). These varieties show significant resistance and tolerance to anthracnose disease, therefore host plant resistance enhances high yields. This is in agreement with other earlier researchers [6,19] (Table 4). The potential yields for French beans are above 1800 kg/ha but due to biotic and abiotic factors they remain below the expected potential yields [1,3].

Table 3. Incidence of anthracnose disease on the ten French bean varieties

Variety	Days after inoculation				Means	1-9 scores
	7th	14th	21st	28th		
Julia	2.5	5.2	9.8	18.6	9.3^{cd}	3-4 (resistant)
Serengeti (control)	2.0	4.1	8.0	16.3	7.6^{d}	1-2 (resistant)
MuH13	2.2	3.8	7.4	14.2	6.9^{d}	1-2 (resistant)
Organdia	2.4	4.5	8.8	16.5	8.1^{d}	3-4 (resistant)
Andate	3.8	6.3	12.2	24.5	11.7^{c}	5-9 (susceptible)
Mara	4.5	7.8	14.3	27.8	13.6^{abc}	5-9 (susceptible)
Venda(control)	4.2	7.2	14.0	27.3	$13.2a^{bc}$	5-9 (susceptible)
Amy	5.5	9.6	16.7	32.4	16.1^{a}	5-9 (susceptible)
Strada	3.6	6.8	13.3	25.0	11.5^{c}	3-4 (tolerant)
Conza	4.0	8.3	15.8	30.8	14.7^{ab}	5-9 (susceptible)
Grand Mean					11.5	
LSD (0.05)					3.4	
CV (%)					20.6	

Means with the same letters within the column are not significantly different at (P<.05), Scale of 1-9 scores (Balardin, [20]) – (1-2 resistant), 3-4 (resistant/tolerant), 5-9 (susceptible).

Table 4. Agronomic traits of the French bean varieties

Varieties	Days to maturity	Pod width (mm)	Pod length (cm)	Yields Kg/ha
Julia	59.50^{a}	8.25^{a}	13.25^{ab}	1572.50^{b}
Serengeti	59.25^{a}	8.25^{a}	12.75^{abc}	1577.50^{b}
MuH13	59.00^{a}	8.00^{ab}	13.25^{ab}	1550.00^{b}
Organdia	58.75^{a}	7.50^{abc}	13.75^{a}	1637.50^{a}
Andate	54.50^{b}	7.25^{abc}	12.50^{bc}	1437.50^{c}
Mara	53.00^{c}	8.00^{ab}	13.00^{abc}	1420.00^{c}
Venda	50.25^{d}	6.25^{c}	12.25^{bc}	1190.00^{e}
Amy	49.25^{de}	6.75^{bc}	12.75^{abc}	972.50^{f}
Strada	49.00^{de}	7.00^{abc}	12.00^{c}	1327.50^{d}
Conza	48.50^{e}	6.75^{bc}	13.25^{ab}	1165.00^{e}
Means	54.10	7.40	12.88	1385.00
LSD	1.43	1.43	1.01	27.66
Varieties	***	ns	**	***
CV (%)	1.83	13.29	5.43	1.38

*Means with the same letters within the column are not significantly different at P<.05, * significance at P<.05, ** significance at P<.01, *** significance at P<.001, ^{ns} not significant.*

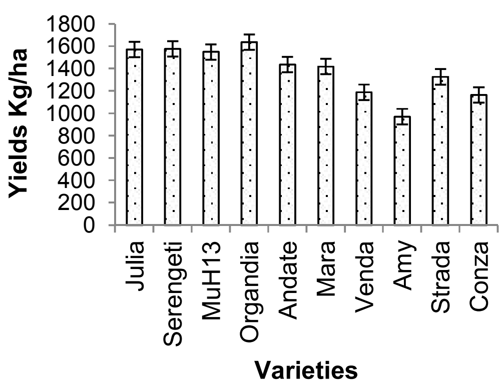

Fig. 2. Yields of the French bean varieties during the study

4. CONCLUSION AND RECOMMENDATION

The significant high yields observed for the resistant varieties justify the importance of using resistance to manage anthracnose. It could therefore be suggested from the study that the use of suitable anthracnose resistant or tolerant varieties would be the best option in the management of anthracnose.

It is recommended that a breeding program be established to help in the introgression of anthracnose resistant genes into the susceptible French bean varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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