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Characterization and pathogenicity of soil borne pathogens inducing root rot symptoms in common bean in Rwanda

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Abstract:
Though Rwanda is among the highest common bean producers and consumers, this crop is still challenged by different factors including root rot diseases resulting in low yield potential. Root rot diseases can cause up to 100% of yield loss. Despite different soil borne pathogens causing bean root rot diseases; there is no recent literature that clearly documents the most aggressive ones affecting common beans in Rwanda. This study aimed at identifying the root rot pathogens affecting bean crop in Rwanda. A survey was conducted to collect bean samples showing root rot symptoms in 12 agro -ecological zones where bean crop is mostly grown. The selection of samples was based on the level of contamination and the purity of cultures. A total number of 132 samples were therefore selected and considered for further analysis. The pathogen isolation was done in Rubona at Rwanda Agriculture and Animal Resources Development Board (RAB) Biotechnology and Pathology laboratory. Structural characterization of soil borne pathogens was also carried out along with pathogenicity evaluation. The growth rate of identified pathogens were significantly different ($P<0.001$). Significant differences were observed among isolates inoculated on both plantlets and seeds ($P<001$). Four root rot diseases were identified and characterized including *Rhizoctonia spp, Fusarium spp,Sclerotium spp* and several *Pythium spp.* The four pathogens were mostly observed in Northern and Southern parts of Rwanda than East and Western parts of Rwanda. Morphological characterization showed that *Fusarium spp, Rhizoctonia spp and Sclerotium spp* were predominant than *Pythium species. Fusarium spp* was identified as the most predominant fungal pathogen while *Sclerotium spp* was the most virulent pathogen. The present study suggests bean root rot disease control program to reduce losses associated with these diseases especially the use of resistant varieties since the fungi are widespread and survive in soils for a long period of time**.**

Key words: *Rhizoctonia spp, Fusarium spp, Sclerotium spp, Pythium spp*

I. Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption in the world. Bean consumption is estimated to more than 300 million people's daily diet [5]. Rwanda is among the highest bean producers and consumers in Sub-Saharan Africa with consumption estimated at200g per capita per day [17]. In some cases, beans are called the meat of the poor due to its high concentration in proteins and its affordability [11],[3] The production in Rwanda is mainly secured by small farmers with average land holdings of less than one hectare and using limited technologies such as limited access to irrigation schemes, limited use of fertilizers and pesticides. Biotic and abiotic factors are the main constraints limiting common bean productivity in Sub-Saharan African countries [2] [17] [17]. Root rot disease caused by soil borne pathogens is among the biotic constraints affecting bean productivity. A number of these soil borne pathogens has been identified in Latin America and Africa such as *Fusarium solani f.*sp. *Phaseoli, Pythium spp, Rhizoctonia solani, Macrophomina phaseoli and Sclerotium rolfsii [6]*

Root rot diseases are most damaging when farming conditions are poor; including water logging, poor drainage capacity, intensification of the cultivation of beans, decline in soil fertility with absence of rotation and use of susceptible varieties [14]. The bean production system in Rwanda is characterized by series of different specific conditions where common bean varieties are grown under high demographic pressure, poor soils with very limited use of crop rotation[10] [18].

Symptoms and severity of root rot diseases depend on the environment conditions including rainfall, moisture, temperature, soil type, drainage, cropping system, and the interaction between responsible pathogens. The objective of this study was to identify and characterize root rot diseases caused by soil borne pathogens of common bean (*Phaseolus vulgaris*) in Rwanda.

II. Materials and methods

2.1. The study location and sampling methodology

The study was conducted in all 12agro-ecological zones of Rwanda where bean is grown**.** Samples were collected in common bean fields showing above ground root rot symptoms such as low plant vigor, yellowing and stunting. A total of 450 bean fields were visited. Three sectors were selected in each district and five bean fields in each sector were surveyed, considering the distance of 2km from one field to another. In each bean field, five quadrants each measuring $1m^2$ were randomly designed and bean plants showing root rot symptoms were collected from them. A total of 2118 bean samples were collected and samples that did not show any growth of mycelia were discarded along with samples that showed visual contamination with non-targeted pathogens. The disease plant was completely uprooted and put in labeled paper bags. In each sampled field, administrative and geographical information (district, sector, cell, village, altitude, and longitude, latitude, cropping system and soil type) were recorded. One hundred and thirty two samples were then brought to the laboratory for pathogen isolation.

2.2. Preparation of the media

Different types of culture media such as Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Tap Water Agar (TWA) were used for fungal isolation and growth.

2.2.1. Preparation of tap water agar

Sixteen grams of agar extra-pure were added in 1000ml of tap water, shaken until the solution reaches the homogeneity stage and autoclaved at 121^oC for 20 minutes. The autoclaved media was allowed to cool down to 40^oCunder laminar flow then dispensed in 9 mm wide petri dishes in the rate of 20 ml per petri dish. The collected samples were prepared into piece of roots, isolated on tap water agar and incubated at 23^oC for 24 hours. Pathogen isolation was performed using a laboratory protocol as described in the International Center for Tropical Agriculture (CIAT) training manual (Mukamuhirwa *et al.,* 2017). All emerging fungi from the roots cultured on tap water agar were sub cultured on PDA and CMA.

2.2.2. Preparation of Potato dextrose Agar and Corn Meal Agar

Sixteen grams of PDA or CMA were added to 1000 ml of distilled sterilized water. The mixtures were shaken until the solutions reach the homogeneity stage and autoclaved at 121^oC for 20 minutes. The autoclaved media was allowed to cool down to 40^oC under laminar flow then dispensed in 9 mm wide petri dishes in the rate of 20 ml per petri dish. A bock agar from tap water media was transferred to the petri dishes with PDA to identify *Rhizoctonia spp, Sclerotium spp and Fusarium spp* while CMA was used to identify *Pithium spp* from other root rot pathogens. The petri dishes were incubated at 23^oC [14].

2.3. Pathogens isolation

The pathogen isolation was carried out in the Rwanda Agriculture and Animal Resources Development Board (RAB), Biotechnology and Pathology Laboratories. A single hyphal isolation was conducted under microscope on mycelium tips. All isolates were transferred on fresh medium of PDA and CMA with sterilized filter paper (Waltman paper n^o) for root rot pathogens long term conservation. Filter paper was cut into small pieces of about 4cm squared. The pieces of paper were then folded into aluminium paper and sterilized at 121^0C for 20 minutes. The pieces of paper were cooled down to room temperature. Using sterilized forceps under laminar flow, about 20 sterilized pieces of filter papers were placed on PDA and/ or CMA containing colonized agar block to allow the pathogens to grow and colonize the filter papers. Colonized filter papers were then transferred to new sterilized empty petri dishes. These petri dishes were

placed into sterilized desiccators for two to three days to allow the filter papers to dry. The dried colonized filter papers were removed and wrapped in sterile Aluminum foil, clearly labeled and stored under – 20^{o}C in freezer for further use.

2.4. Pathogens isolation

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2.5. Structural characterization of soil borne pathogens

Cultures were initiated for the growth rate measurements and phenotypic description of different pathogens. The description of the different root rot pathogens was done using their colony texture, mycelia color, presence or absence of sclerotia, color of sclerotia, colony margin and number of sclerotia, clear zonation and mycelia floral patterns. Microscopic identification of the pathogens was also done basing on the presence of micro and macro conidia (*Fusarium spp*.), and the hyphal branching at the perpendicular angles or T-shaped branching (*Rhizoctonia spp*.). Colony diameters for all cultures initiated for growth rate variations were measured after 24 hours of incubation. This was done by drawing two perpendicular lines at the base of the petri-dish and colony margins measured using a ruler along the lines. Measurements were carried on every day until when the plates were completely covered.

2.5. Pathogenicity evaluation

The pathogenicity test of isolated pathogens was performed using 132 isolates. The sorghum grain media was used for fungal inoculum production. Approximately 400ml of distilled water were added to every 300g of sorghum grains, loaded into a sterilized glass bottle, the autoclaved twice at 121^{o}C for 20min and allowed to cool for 12 hours. The sterilized sorghum was then inoculated with the liquid suspension of 100 µl scraped *pathogen* mycelia dissolved in sterile distilled water. The media substrate bottles were hand shaken daily to homogenize the fungal distribution and growth. The inoculum was incubated in the bottles over the sterilized sorghum grains in a sterile environment at room temperature for 14 days to allow uniform growth[4] [7]. Pathogenicity evaluation was done through 3 different experiments while the seed rot experiment was conducted in petri dishes following the MSU protocol where the colonized agar block was placed in the middle of fresh media and allowed to grow and infect the 10 sterilized healthy seed in the petri dish. The incubation was done for 7 days and the disease severity was assessed using 1-5 scale where 1: Germinated, 2: Delayed germination; 3: Germination and some Lesions; 4: Germination with coalesced lesions; 5: Seed colonized. The second experiment was conducted on laboratory bench where plastic boxes where used to plant sterilized healthy seed on sterilized paper towel. Bean seed were allowed to grow for 2 weeks, taking care of removal of any plant showing the symptoms of the disease. This was followed by inoculation of roots of plantlets with colonized sorghum grain, incubate for 7 days as explained above and evaluate the severity of the disease using 1-5CIAT scale. The third experiment was set in pots of 5liters in screen house following CIAT Training manual.

2.6. Statistical analysis

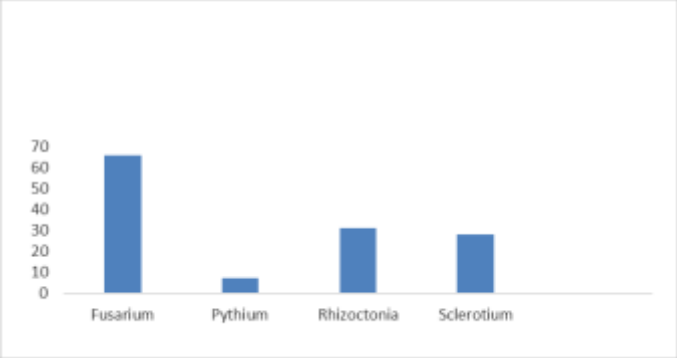
The slope between the first and last day of measurement was subjected to Analysis of Variance (ANOVA). The Disease Index (DI) scored on seed rot experiment was calculated as: DI = Σ(severity X n) / N [8] and the means were alsosubjected to ANOVA. Disease severity for plantlet inoculation in laboratory bench and screen house data in pots were recorded following CIAT scale and subjected to ANOVA.All the analysis were performed using Gen-stat Statistical Package.

III. Results

3.1. Morphological characteristics and distribution of root rot diseases in Rwanda

The colony type of identified isolates was 69 % cotton and 31% flat. The regular and or round colony margin was observed at 43 % while the irregular colony margin was observed at 57%. The mycelia of the identified pathogens were raised at 70% and thin at 30%. From the identified pathogens the mycelia color was 36% purple, 14% red, 25% cream and 25 % white. The growth rate of identified pathogens were significantly different ($P<0.001$) with the range of slope varying between 0.1 to 5.3 mm (Table 1). How many isolates were classified as fast growing, slow or medium?Among the 132 isolates, 68%, 9%, 32%, and 30% were*Fusarium spp, Pythium spp, Rhizoctonia spp, and Sclerotium spp* respectively. Morphological characterization showed that *Fusarium, Rhizoctonia* and *Sclerotium* isolates were more predominant than *Pythium* species (Figure 1).

Figure 1: Bean root rot pathogens identified in Rwanda.



Change	DF	SS	MS	v.r.	F pr.
Replication	2	0.322	0.2	0.2	0.9
Sample ID	131	505.3	3.8	2.7	1.0E-17
Residual	788	1108.2	1.4		
%CV	23.7				
SEM	0.3				

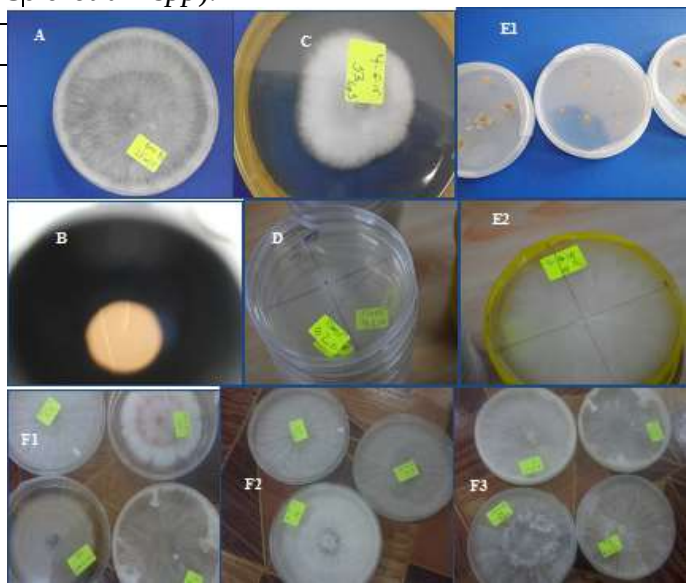
Table 1**.** Analysis of variance for slope of bean root rot diseases identified in different sites

3. 2. Pathogenic characterization

The most virulent isolates were selected for screening varieties for resistance to root rot pathogens. Selection of virulent isolate was done in 3 experiments which showed the same results. The study identified a high level of diversity in bean root rot diseases between (Figure 3:F1) and within species (Figure 3:F2 and F3). The four diseased were observed especially in Figure 1. The virulence study showed that isolates from southern and western locations were more virulent than the rest. Three most virulent and aggressive isolates were selected including *Sclerotium* 63 isolate collected from the District of Kamonyi in Runda sector, 84 *Rhizoctonia* 84 collected from Ruhango District, Byimana sector and *Fusarium* 196 collected from Southern Province of Rwanda (Table 2 and Figure 2). The Greenhouse experiment confirmed the results of seed rot assay and plantlet rot experiments (Table 3). The most aggressive isolates selected based on all experiments.

Figure 2: Map of the study area

Figure 3**:** Pathogen isolation on Tap Water Agar (A), Subculture on Potato Dextrose Agar (B) Single hyphal tip under microscopic observation (C), pure culture (D) growth measurement (E1 at the first day &E2 at the last day) and colony features assessment and diversity (F1: the four pathogens identified, F2 diversity in *Rhizoctonia spp*, F3 diversity in *Sclerotium spp*).



Number	District	ID	Pathogen	Meanscore	Number	District	ID	Pathogen	Meanscore
1	Muhanga	33	*Fusarium*	2	67	Muhanga	67-1	*Fusarium*	2
2	Muhanga	124	*Fusarium*	4	68	Kayonza	45-2	*Fusarium*	2
3	Kamonyi	62-1	*Fusarium*	1	69	Huye	158	*Fusarium*	2
4	Kamonyi	144	*Fusarium*	4	70	Ruhango	10	*Fusarium*	3
5	Kamonyi	184	*Fusarium*	1	71	Ruhango	213	*Fusarium*	2
6	Kamonyi	*1-2	*Fusarium*	2	72	Ruhango	75	*Fusarium*	2
7	Kamonyi	*1-1	*Fusarium*	1	73	Bugesera	210	*Fusarium*	2
8	Gatsibo	220	*Fusarium*	2	74	Muhanga	41-1	*Fusarium*	1
9	Burera	141	*Fusarium*	1	75	Muhanga	125	*Fusarium*	2
10	Kamonyi	3	*Fusarium*	1	76	Muhanga	171	*Fusarium*	3
11	Kamonyi	129	*Fusarium*	2	77	Muhanga	221	*Fusarium*	2
12	Kamonyi	72	*Fusarium*	2	78	Ruhango	11	*Fusarium*	2
13	Rwamagana	47	*Fusarium*	4	79	Kamonyi	58	*Fusarium*	2
14	Rwamagana	47-1	*Fusarium*	3	80	Gatsibo	185	*Fusarium*	2
15	Kayonza	39	*Fusarium*	1	81	Kicukiro	182-2	*Fusarium*	2
16	Kamonyi	1	*Fusarium*	2	82	Gatsibo	185	*Fusarium*	3
17	Kamonyi	201	*Fusarium*	1	83	Gatsibo	218	*Fusarium*	3
18	Kamonyi	121	*Fusarium*	2	84	Gicumbi	203	*Fusarium*	3
19	Kicukiro	217	*Fusarium*	2	85	Rwamagana	188	*Fusarium*	3
20	Kicukiro	182	*Fusarium*	3	86	Rwamagana	inconue	*Fusarium*	2
21	BUGESERA	126	*Fusarium*	3	87	Kamonyi	63	*Sclerotium*	5
22	Nyagatare	187	*Fusarium*	3	88	Muhanga	51	*Sclerotium*	5
23	Nyamagabe	200	*Fusarium*	3	89	Kamonyi	32	*Sclerotium*	5
24	Kayonza	29	*Fusarium*	4	90	Huye	94	*Sclerotium*	5
25	Musanze	111-1	*Fusarium*	3	91	Kayonza	20	*Sclerotium*	4
26	Musanze	14	*Fusarium*	3	92	Kayonza	31	*Sclerotium*	4
27	Muhanga	6	*Fusarium*	4	93	Huye	90	*Sclerotium*	5
28	Muhanga	6	*Fusarium*	4	94	Huye	95	*Sclerotium*	5
29	Burera	146	*Fusarium*	3	95	Huye	89	*Sclerotium*	5
30	Huye	202-2	*Fusarium*	1	96	Kamonyi	4	*Sclerotium*	5
31	Kamonyi	24	*Fusarium*	2	97	Muhanga	71	*Sclerotium*	5
32	Muhanga	67-1	*Fusarium*	2	98	Kayonza	68	*Sclerotium*	5
33	Muhanga	67-1	*Fusarium*	2	99	Kayonza	76	*Sclerotium*	5
34	Burera	148-1	*Fusarium*	2	100	Kamonyi	19	*Sclerotium*	4
35	Rwamagana	191	*Fusarium*	2	101	Ruhango	14	*Sclerotium*	4
36	Ruhango	12	*Fusarium*	3	102	Huye	91	*Sclerotium*	4
37	Musanze	115-2	*Fusarium*	2	103	Muhanga	50	*Sclerotium*	5
38	Ruhango	166	*Fusarium*	1	104	Kamonyi	60	*Sclerotium*	4
39	Ngororero	139	*Fusarium*	1	105	Kamonyi	63	*Sclerotium*	5
40	Kamonyi	174	*Fusarium*	3	106	Nyamasheke	132	*Sclerotium*	4

41	Burera	145	*Fusarium*	3	107	Rutsiro	205	*Sclerotium*	3
42	Burera	131	*Fusarium*	3	108	Huye	85	*Sclerotium*	4
43	Burera	219	*Fusarium*	4	109	Kamonyi	69	*Rhizoctonia*	3
44	Kayonza	181	*Fusarium*	3	110	Kayonza	31	*Rhizoctonia*	1
45	Muhanga	127	*Fusarium*	1	111	Kayonza	31	*Rhizoctonia*	4
46	Kamonyi	117	*Fusarium*	2	112	Ruhango	84	*Rhizoctonia*	3
47	Nyamagabe	173	*Fusarium*	1	113	Ruhango	84	*Rhizoctonia*	4
48	Gasabo	156	*Fusarium*	3	114	Nyarugenge	124	*Rhizoctonia*	1
49	BURERA	140	*Fusarium*	3	115	Kayonza	21	*Rhizoctonia*	3
50	Burera	151	*Fusarium*	3	116	Ruhango	82	*Rhizoctonia*	4
51	Nyaruguru	196	*Fusarium*	4	117	Rwamagana	183	*Rhizoctonia*	3
52	Burera	147	*Fusarium*	2	118	Ruhango	17	*Rhizoctonia*	4
53	Huye	87	*Fusarium*	2	119	Ruhango	10	*Rhizoctonia*	4
54	Gatsibo	189	*Fusarium*	3	120	Kamonyi	62-2	*Rhizoctonia*	3
55	Huye	202-1	*Fusarium*	2	121	Musanze	97	*Rhizoctonia*	3
56	Ruhango	55	*Fusarium*	2	122	Musanze	106	*Rhizoctonia*	4
57	Musanze	113-1	*Fusarium*	2	123	Ruhango	16	*Rhizoctonia*	5
58	Burera	142-1	*Fusarium*	3	124	Musanze	102	*Rhizoctonia*	4
59	Gisagara	175	*Fusarium*	2	125	Kamonyi	48	*Rhizoctonia*	4
60	Huye	216	*Fusarium*	2	126	Ngoma	RG2-1	*Pythium*	3
61	Muhanga	74-2	*Fusarium*	2	127	Burera	RG3-4	*Pythium*	2
62	Huye	88	*Fusarium*	2	128	Kicukiro	RG4-3	*Pythium*	3
63	Rwamagana	191-1	*Fusarium*	1	129	Kicukiro	RG4-3	*Pythium*	2
64	Muhanga	38	*Fusarium*	1	130	Ngoma	RG5-6	*Pythium*	3
65	Ruhango	198	*Fusarium*	2	131	Musanze	RG6-8	*Pythium*	2
66	Rulinndo	199	*Fusarium*	2	132	Rwamagana	RG7-4	*Pythium*	3

Table 2:.**Identification of root rots pathogens inducing root rot symptoms**

Change	DF	SS	MS	v.r.	F pr.
Rep	2	0.27966	0.13983	1.214153	
Isolate	131	211.0758	1.611266	13.99072	<0.001
Residual	262	30.17368	0.115167		
Total	395	241.5292			
CV			9		
LSD			0.5012		

Analysis of variance of bench method

Change	DF	SS	MS	v.r.	F pr.
Rep	2	284	142	1444.051	
Isolate	131	360.5104	2.751988	27.986	<.001
Residual	262	25.76363	0.098334		
Total	395	670.274	1.696896		
% CV			11.58		
LSD			0.4451		

Analysis of Variance of screen house experiment

Change	DF	SS	MS	v.r.	F pr.
Rep	2	0.2797	0.1399	0.922795	0.3987
Isolate	131	201.5433	1.5385	10.15173	<.001

Residual	262	39.7062	0.1516		
Total	395	234.248			
% CV	12.84				
LSD	0.5628				

Table 3: **Analysis of variance of seed rot assay**

Sample ID	Means Score	Group	Sample ID	Means Score	Group	Sample ID	Means Score	Group
11	5	A	71	5	ab	188	4	defghijklm
4	5	A	38	5	abc	189	4	defghijklm
13	5	A	81	4	bcd	203	4	defghijklm
16	5	A	133	4	cde	218	4	defghijklmn
19	5	A	183	4	cdef	111-1	4	defghijklmn
20	5	A	131-1	4	cdef	120	4	defghijklmno
48	5	A	141	4	cdefg	167	4	defghijklmno
50	5	A	216	4	cdefg	127	4	defghijklmnop
51	5	A	23	4	cdefgh	90	4	efghijklmnopq
60	5	A	128	4	cdefgh	166	4	efghijklmnopq
68	5	A	221	4	cdefgh	197	4	efghijklmnopq
69	5	A	182	4	cdefghi	220	4	efghijklmnopq
76	5	A	67-2	4	cdefghi	47-1	4	efghijklmnopq
84	5	A	2	4	defghij	74-2	4	efghijklmnopq
88	5	A	75	4	defghij	87	4	fghijklmnopqr
89	5	A	142-1	4	defghij	146	4	fghijklmnopqr
94	5	A	125	4	defghijk	176	4	fghijklmnopqr
95	5	A	147	4	defghijkl	202	4	fghijklmnopqr
63	5	A	182	4	defghijkl	41-1	4	fghijklmnopqr
8	5	A	217	4	defghijkl	35	4	ghijklmnopqrs

Sample ID	Means	Group	Sample ID	Means Score	Group
140	4	ghijklmnopqrs	158	4	lmnopqrstuvwxy
144	4	ghijklmnopqrs	74-1	4	lmnopqrstuvwxy
151	4	ghijklmnopqrs	12	4	mnopqrstuvwxy
175	4	ghijklmnopqrs	163	4	mnopqrstuvwxy
190	4	ghijklmnopqrs	124	4	nopqrstuvwxyz
115-1	4	ghijklmnopqrs	139	4	nopqrstuvwxyz
39	4	hijklmnopqrst	171	4	nopqrstuvwxyz
132	4	hijklmnopqrst	23-3	4	nopqrstuvwxyz
135	4	hijklmnopqrst	125	4	opqrstuvwxyzA
187	4	hijklmnopqrst	182	4	opqrstuvwxyzA
206	4	hijklmnopqrst	185	4	opqrstuvwxyzA
85	4	hijklmnopqrstu	191	4	opqrstuvwxyzA
191	4	hijklmnopqrstu	202	4	opqrstuvwxyzA
204	4	ijklmnopqrstuv			
118	4	jklmnopqrstuvw			
126	4	jklmnopqrstuvw			
189	4	jklmnopqrstuvw			
64	4	klmnopqrstuvwx			
174	4	klmnopqrstuvwx			
3	4	lmnopqrstuvwxy			

Table 4: Means scores of the selected virulent isolates under seed rot assay experiment

Sample ID	Disease	Average	Max	Minimum
51	*Sclerotium*	5	5	3
32	*Sclerotium*	5	5	4
20	*Sclerotium*	4	5	4
90	*Sclerotium*	5	5	3
95	*Sclerotium*	5	5	4
89	*Sclerotium*	5	5	4
4	*Sclerotium*	5	5	4
71	*Sclerotium*	5	5	4
68	*Sclerotium*	5	5	4
50	*Sclerotium*	5	5	4
63	*Sclerotium*	5	5	5
31	*Rhizoctonia*	4	5	2
84	*Rhizoctonia*	5	5	3
21	*Rhizoctonia*	3	5	1
82	*Rhizoctonia*	4	5	1
17	*Rhizoctonia*	4	5	1
17	*Rhizoctonia*	4	5	1
10	*Rhizoctonia*	4	5	1
97	*Rhizoctonia*	3	5	1

106	*Rhizoctonia*	4	5	2
16	*Rhizoctonia*	5	5	3
102	*Rhizoctonia*	4	5	2
33	*Fusarium*	2	4	1
196	*Fusarium*	4	5	3
14	*Fusarium*	4	5	2
124	*Fusarium*	4	5	2
219	*Fusarium*	4	5	2
47	*Fusarium*	4	5	2
6	*Fusarium*	4	5	2
141	*Fusarium*	4	5	2
174	*Fusarium*	3	5	1
*1-2	*Fusarium*	2	5	1
220	*Fusarium*	2	4	1
129	*Fusarium*	2	4	1
1	*Fusarium*	2	4	1
72	*Fusarium*	2	4	1

Table 5: Means scores of the selected virulent isolates under bench (Plantlet rot) Experiment

Sample ID	Disease	Average	Max	Minimum
51	*Scolerotium*	5	5	3
32	*Scolerotium*	5	5	4
20	*Sclerotium*	4	5	4
90	*Sclerotium*	5	5	3
95	*Sclerotium*	5	5	4
89	*Sclerotium*	5	5	4
71	*Sclerotium*	5	5	4
68	*Sclerotium*	5	5	4
50	*Sclerotium*	5	5	4
63	*Sclerotium*	5	5	5
84	*Rhizoctonia*	5	5	3
16	*Rhizoctonia*	5	5	3
102	*Rhizoctonia*	4	5	2
33	*Fusarium*	2	4	1
196	*Fusarium*	4	5	3
219	*Fusarium*	4	5	2
174	*Fusarium*	3	5	1
1	*Fusarium*	2	5	1
69	*Rhizoctonia*	3	4	2
94	*Scoleratium*	3	5	2
19	*Scoleratium*	4	5	3
218	*Rusarium*	3	4	3
131	*Fusarium*	3	5	1
151	*Fusarium*	3	4	2
121	*Fusarium*	2	5	1
60	*Scoleratium*	4	5	3
175	*Fusarium*	3	4	3

74/2	*Fusarium*	2	4	1
188	*Fusarium*	3	4	2

Table 6: Mean scores of the selected virulent isolates under green house experiment, plantlet rot on bench experiment and seed rot experiment in petri dishes

IV. Discussions

The isolation protocol used in this study allowed finding out different isolates of bean root rot

diseases. Using morphological characterization, our study showed that *Fusarium spp, Rhizoctonia spp and Sclerotium spp* were predominant than *pythium spp.*

CIAT reported that *Pythium* and *Fusarium* species are more important particularly under high soil moisture content and low temperatures which favor the root rot disease development [14] reported that the *Pythium* species identified in Rwanda were pathogenic on common bean. The present study revealed the four pathogens causing bean root rot diseases including *Fusarium spp, Pythium spp, Rhizoctonia spp and Sclerotium spp* where *Fusarium spp* is more predominant while *Sclerotium spp* is more aggressive than the rest. This is consistent with several studies where it was shown that bean root rot diseases are worldwide spread. *Fusarium* root rot was found to be present wherever dry beans are grown. In his study, [14] revealed the wide distribution of *Pythium s*pecies in Rwanda. He notified the presence of several *Pythium* species including *P. vexansPythium indigoferae, Pythium torulosum, Pythium, ultimum* and *Pythium rostratifingens, Pythiumcucurbitacearum*, *Pythium arrhenomanes*, *Pythium pachycaule* and *Pythium rostratum.*

In the present study, diseased locations were observed especially in northern and southern parts of Rwanda while east and western parts were less affected. Several studies showed that the distribution of bean root rot diseases depend on several factors including species type[14]. The most virulent pathogens were found within *Sclerotium* isolates and it was scored high compared to other pathogens. This fungus can infect any part of the plant and the variability among isolates is well documented. The aggressiveness depends on the environment interaction among other factors.

V. Conclusion and recommendations

Four root rot diseases were identified and characterized including *Rhizoctoniaspp, Fusariumspp, Sclerotiumspp* and several *Pythium spp.* Morphological characterization showed that *Fusarium spp, Rhizoctonia spp and Sclerotium spp* were predominant than *Pythium species.* The four diseased were observed especially in northern and southern parts of Rwanda than east and western parts of Rwanda. The collected and characterized isolates constitute an important resource for screening and improvement of dry bean germoplasm in Rwanda. *Fusarium* was identified as the most predominant fungal pathogen in Rwanda. It was observed that *Sclerotium* was the most virulent pathogens since most of its isolates scored high compared to other pathogens. The selected isolates are highly recommended to be used in selecting breeding materials in Rwanda. The total bean root rot disease control program is recommended to reduce losses associated with these diseases especially the use of resistant varieties since the fungi are widespread and survive in soils for a long time. It is highly recommended to promote the use of integrated technologies to reduce bean root rot causing pathogens especially *Sclerotium*. Further studies are recommended to characterize the identified pathogens.

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VII. References

[1] Asare-Marfo, D., Birol, Katsvairo,E.L., Manirere, J.A., Maniriho, F. and Roy D. (2004) "Farmer Choice of Bean Varieties in Rwanda: Lessons learnt for HarvestPlus Delivery and Marketing Strategies." HarvestPlus.CIAT (2004). Enhancing farmers' access to seed of improved bean varieties in Rwanda, CIAT, pp. 2.

[2] Bayuelo-Jimenez,J.S. Debouck, D.G. and Lynch J. P. (2003) Growth, gas exchange, water relations, and ion composition of Phaseolus species grown under saline conditions. Field Crop Research 80:207–222.doi:10.1016/S0378-4290(02)00179-X

[3] Beebe, S.; Gonzalez, A.V.; Rengifo, J. (2000). Research on trace minerals in common bean. Food. Nutr.Bull (21): 387-391.

[4] Binagwa, P.H. , Bonsi,C.K., Msolla, S. N. and Ritte, I. I. (2015). Morphological and molecular identification of Pythium spp. isolated from common beans (Phaseolus vulgaris) infected with root rot disease. African Journal of Plant Science. Vol. 10(1), pp. 1-9, DOI: 10.5897/AJPS2015.1359 (http://www.academicjournals.org/AJPS)

[5] Blair, M., Gonzales,L.F., Kimani, P.M.; Butare, L. (2010) Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from central africa. *Theor. Appl. Genet.* 2010, *121*, 237–248.

[6] Buruchara, R., Estévez de Jensen, C., Godoy, G., Abawi, G., Pasche, J.and Lobo Junior, M., Mukankusi, C. (2015). A review of the root rot diseases of common bean with emphasis in LatinAmerica and AfricaCommon Bean Disease on AngularLeaf Spot and Root Rot oral paper presentation in the conference.Protea Hotel Kruger Gate Skukuza, South Africa July 20-23, 2015 (http://arsftfbean.uprm.edu/bean/wp-content/uploads/2015/09/BDW-Day2-Robin-Buruchara.pdf .

[7] Chikoti, M. (2016). "Morphological and Molecular Identification and Characterization of Dry Bean Fungal Root Rot Pathogens in Zambia" (2016).Theses, Dissertations, and Student Research in Agronomy and Horticulture.Paper 112. http://digitalcommons.unl.edu/agronhortdiss/112

[8] Chilvers, M. and Hanson, L.(2015). Soil Borne Diseases of Dry Bean, avalaible on line at http://www.fieldcroppathology.msu.edu, accessed in May 2015CIAT-AFrica. Highlights. *CIAT*, 1-2.

[9] CIAT, (2008). Farm-level impacts of improved bean varieties and agronomic technologies in Rwanda'*Highlights, CIAT in Africa, no.41,* Kampala: CIAT

[10] Graham, P.H., Rosas J.C., de Jensen, E.C., Peralta, E., B., lustyT., Acosta-Gallegos, J., Arraes Pereira, P. (2003). Addressing edaphic constraints to bean production:the bean/cowpea CRSP project in perspective. Field Crop Research 82, 179-192.

[11] Larochelle, C.,Alwang, J., Norton, G.W., Katungi,E. and Labarta,R.A. (2014) "Ex-Post Impact of Adopting Improved Bean Varieties on Poverty and Food Security in Uganda and Rwanda."Selected Paper prepared for presentation at the Agricultural & Applied Economics Association's2014 AAEA Annual Meeting, Minneapolis, MN, July 27-29, 2014.

[12] Larochelle, C. and Alwang J. (2014). Impacts of Improved Bean Varieties on Food Security in Rwanda.Selected Paper prepared for presentation at the Agricultural & Applied Economics Association's2014 AAEA Annual Meeting, Minneapolis, MN, July 27-29, 2014. http://www.Impacts%20of%20Improved%20Bean%20Varieties%20on%20Food%20Security%20in%20Rwanda_AAEA.pdf

[13] Mukamuhirwa F. Mukankusi M.C., Tusiime, G., Butar L., Musoni A., Ngaboyisonga C., Gahakwa D., Gibson P. and Kelly K. (2017). Resistance Levels to Root Rot and Angular Leaf Spot Diseases in Selected High Iron Bean Genotypes. Journal of Advances in Crop Science and Technology. Adv. Crop Sci. Tech 5: 274. doi: 10.4172/2329-8863.1000274

[14] Nzungize, J.,Gepts P.,Buruchara,R., S. Buah, P. Ragama, J. P. Busogoro andJ. P. Baudoin. (2011). Pathogenic and molecular characterization of *Pythium*species inducing root rot symptoms of common beanin Rwanda

[15] Petry, N., Boy, E., Wirth J.P. and Hurrell,R.F. (2015) Review: The Potential of the Common Bean (Phaseolus vulgaris) as a Vehicle for Iron Biofortification . Nutrients 2015, 7, 1144-1173; doi:10.3390/nu7021144 (ISSN 2072-6643 www.mdpi.com/journal/nutrients)

[16] Rainey, K.M. and Griffiths, P.D.(2005). Inheritance of heat tolerance during reproductive development in snap bean (Phaseolus vulgaris L.). J. Am. Soc Hort. Sci., 130(5): 700-706

[17] Sharma, K.K. and Lavanya M. (2002).Recent developments in trans-genics for abiotic stress in legumes of the semi-arid tropics. JIR-CAS Working Report: 61–73

[18] Wortmann,C.S, Kirkby,R.A., Eledu,C.A. and Allen,D.J., (1998).Atlas of common bean *(Phaseoulus vulgaris) production in Africa. International Center for Trocal Agriculture, cali, Colombia.*