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IDENTIFICATION OF POTENTIAL SOURCES OF ROOT ROT RESISTANCE AMONG RWANDAN BEAN LINES

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ABSTRACT

A study was conducted in 2016 at RAB Rubona in Rwanda to identify root rot resistance among 114 lines of beans. The inoculum was prepared from the most virulent isolates including Sclerotium sp 63 collected from the District of Kamonyi in Runda sector, Rhizoctonia sp 84 collected from Ruhango District, in Byimana/Kirengeri and Fusarium sp 196 collected from western part of Rwanda. Treatments consisting of bean lines were inoculated with the same quantity of inoculum under greenhouse conditions in Rubona research centre of Rwanda Agriculture and Animal Resources Development Board. The inoculum was prepared by inoculating one petri in 300g net sterilized sorghum which were then mixed with 13.5kg of loam sandy soil previously sterilized by steaming on firewood. Disease assessment was carried out twenty-one days after planting. Data were analyzed using analysis of variance with GenStat. This study showed that bean lines such as NUA 566, BOA5-1/8, CAL 96, EQUADOR 299 x G 122164 TU, EQUADOR x ACC 714, G 122164 TU x EQUADOR 299, G 12727 AB 136 x G 122164 TU, IGISUBIZO, NUA 377, RWIBARURA, RWR 1668, RWR 2154, RWV 3006 x G 122164 TU, RWV 3317, SCR 16, USCR9 x RWR 2074 x G2333 X RWR 719 F2-1-6 and RWR 3228 had the lowest disease mean scores than the local resistant check

Keywords: Beans, Fusarium, Rhizoctonia, Sclerotium. Root rot resistance

1. INTRODUCTION

Common bean (Phaseolus vulgaris L.) is the third most important food legume crop worldwide after soybean and peanut in production (Popelka et al. 2004; FAOSTAT 2014). Common bean forms a significant part of the diet in Africa and provides more than 45% of the total proteins consumed (CIAT 2001). It is the second most important source of proteins for over 100 million people in the rural and poor communities (Wachenje 2002). In Rwanda, the annual per capital consumption is estimated at 55 kg (Rubyogo et al. 2010). According to Petry et. al. (2015), 200-300g of beans are consumed per capita per day. It is consumed in a variety of dishes as green or dry grains, leafy or snap vegetables and as processed or blended products (Musoni A., 2011). Common bean provides quality proteins, energy, fiber and micronutrients, including vitamin A, folic acid, iron, and zinc (Blair et al. 2009, CGIAR Independent Science and Partnership Council (ISPC), 2014). Due to its high nutrition value, it was described as a near-complete food; meat for the poor and green meat for the rich (Musoni A, 2016). Bean production is affected by root rot

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diseases, which is exacerbated by low soil fertility, acidity and high intensification of bean production. Bean root rot diseases are caused by species of pathogenic fungi including Fusarium phaseoli, Rhizoctonia solani, Sclerotium spp and Pythium spp., and by lesion of nematodes (Pratylenchus Spp.). Root rot severity and incidence varies according to environmental and soil conditions as well as the number and type of root rot pathogens present under given conditions. Moreover, it is often occurred in a complex of two or more pathogens. Root rot diseases indirectly affect the uptake and efficiency use of nutrients (Abawi, 1989), and make plants more susceptible to stress factors such as drought, temperature extremes and other biological stresses. Infections of these soil borne pathogens may result in different symptoms and their severity will depend not only on the presence of the appropriate environmental conditions but also in the synergistic interaction among these pathogens (Singh, 2009). Depending on which stage of these pathogens is active, they may cause different symptoms and diseases. The anamorph stage is considered to be the cause of damping off, root rot, crown blights and fruit rots. However, it may cause leaf blights such as Web Blight when it is in its teleomorph stage and when it is in the right environmental conditions.

In beans, Rhizoctonia root rot is one of the most economically important root and hypocotyl disease in the world (Sikora, 2004). Rhizoctonia root rot occurs mostly on young bean plants; causing partial or complete hypocotyl girdling during seedling germination and emergence. Lesions on hypocotyls and roots start as small, elongate, and produce sunken reddish brown areas that may increase with time. Severe infections cause plant stunting which eventually may lead to plant death. Infection occurs in two different ways, through penetration of individual hyphae on natural openings and wounds, or when infection pegs are produced from infection cushions penetrating the cuticle and epidermis. The pathogen survives between crop seasons as mycelia or sclerotia in plant debris or in the upper region of the soil (10 to 15 cm). The disease is more severe in moderately wet soils than dry or waterlogged soils. Most isolates can infect in a range of 15°C to 18°C. However, at temperatures below 9°C and above 21°C the lesions are substantially reduced. Nevertheless, some isolates can remain active at temperatures reaching 35°C. It was also reported that root rot diseases are associated with intensive and near-continuous bean production, and a subsequent build-up of inoculum in the soil together with low soil fertility which results in less plant tolerance to root rot infection. The ability of a bean crop to tolerate root rots is related to soil nutrient supply. With high soil fertility, the crop grows vigorously and tolerates root rots and the application of fertilizers or readily decomposed organic manures has been shown to improve tolerance to root rots (CIAT, 1992; Mutitu et al., 1985 and 1989).

Good Agronomic Practices (GAP) constitute an efficient and excellent tool for effective root rot disease management. The genetic improvement using diversity germplasm for root rot diseases resistance has been suggested to contribute to disease resistance (CIAT, 1992). Positive effects of combining more than one agricultural practice with resistance or tolerance varieties were reported by Abawi and Pastor-Corrales (1990), and CIAT (1992). Buruchara et al. (1993) and CIAT (1993) reported that the integration of organic amendments, use of raised beds and resistant bean varieties have shown to be advantageous over the use of single component in controlling the severity of root rot diseases.

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The sustainability of crop production and food security will depend on the availability of improved varieties that can tolerate various biotic and abiotic stresses. Genetic improvement using the available diversity found in the two common bean gene pools and their wild relatives could be explored to develop root rot resistance lines. The use of resistance is probably the cheapest and most cost effective control measure against Fusarium root rot in developing countries (Mukankunsi, et al. 2011). Plant breeders must be equipped with tools to adapt and improve crops to a wide range of pressure. The objective of this study was to identify root rot resistance among bean lines.

2. MATERIALS AND METHODS

Plant materials and root rot isolates

A set of 114 lines of Phaseolus vulgaris selected among Rwandan bean germplasm were used in this study (Table 1). It includes released varieties, landraces, and improved lines selected based on their good characteristics to breeders and consumers. They constitute different market classes, seed types and color including red mottle, purple, yellow, cranberry, white and kidney. Inoculum used in this evaluation was prepared from the most virulent isolates including Sclerotium 63 collected from the District of Kamonyi in Runda sector, Rhizoctonia 84 collected from Ruhango/Byimana/Kirengeri and Fusarium 196 collected from western part of Rwanda.

Table 1: Description of tested bean lines

No	Lines	Origin	Description		
			Type	Color	Size
1	665SI-4/1	CIAT	Climber	Cream	Small
2	ACC 714	CIAT	Bush	Black	Small
3	ACC 714 Temoin	CIAT	Bush	Black	Small
4	BOA5-1/16	CIAT	Bush	Pale red	Small
5	BOA5-1/8	CIAT	Bush	Red	Small
6	CAB 2	CIAT	Climber	White	Small
7	CAL 96	CIAT	Bush	Red mottle	Large
8	CAL 96 Temoin	CIAT	Bush	Red mottle	Large
9	CIM RM00321 L	CIAT	Bush	Red mottle	Large

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No	Lines	Origin	Description		
			Type	Color	Size
10	COLTA	Burundi	Bush	Yellow	Small
11	COLTA X EQUADOR 299	Rwanda	Climber	Black	Medium
12	COLTA X RWR 2245	Rwanda	Semi Climber	Yellow	Medium
13	COLTA XMAC 42	Rwanda	Climber	Pale-yellow	Medium
14	EQUADOR 299	CIAT	Bush	Black	Small
15	EQUADOR 299 X G 122164 TU	Rwanda	Bush	Black	Small
16	EQUADOR X ACC 714	Rwanda	Semi Climber	Black	Small
17	EQUADOR X RWR 2245	Rwanda	Bush	Black	Medium
18	G 122164 TU X EQUADOR 299	Rwanda	Bush	Black	Small
19	G 122164 TU X G 121 727 A B 136	Rwanda	Bush	Red	Small
20	G 122164 TU X MAC 42	Rwanda	Bush	Red	Medium
21	G 12727 AB 136 X ACC714	Rwanda	Bush	Black	Small
22	G 12727 AB 136 X COLTA	Rwanda	Climber	Yellow	Medium
23	G 12727 AB 136 X EQUADOR 299	Rwanda	Climber	Black	Small
24	G 12727 AB 136 X G 122164 TU	Rwanda	Climber	Black	Small
25	G 12727 AB 136 X MAC 42	Rwanda	Climber	Sugar	Medium
26	G 12727 AB 136 X RWR 2245	Rwanda	Bush	Red mottle	Medium
27	G 14619 X SEL 1461 F2-2-2-4-4-4/3	Rwanda	Bush	Black	Medium
28	G122164 TU	CIAT	Bush	Red	Small

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No	Lines	Origin	Description		
			Type	Color	Size
29	G2331	CIAT	Climber	Yellow	Small
30	GASIRIDA	Rwanda	Climber	Purple	Medium
31	IB ANGA 2	Rwanda	Climber	Pale pink	Small
32	IGISUBIZO	Rwanda	Climber	Pale Pink	Small
33	KAB06F2-8-27	CIAT	Bush	Red mottle	Medium
34	MAC 28	CIAT	Climber	Red mottle	Medium
35	MAC 42	CIAT	Climber	Sugar	Medium
36	MAC 42 X COLTA	Rwanda	Climber	Yellow	Medium
37	MAC 44	CIAT	Climber	Red mottle	Medium
38	MAC 49	CIAT	Climber	Red mottle	Medium
39	MAC 9	CIAT	Climber	Red mottle	Medium
40	MBC 23	CIAT	Climber	Red mottle	Medium
41	MBC 25	CIAT	Climber	Red mottle	Medium
42	MBC 64	CIAT	Climber	White	Large
43	MBC 71	CIAT	Climber	Red mottle	Large
44	MBC 71 X RWR 2245	Rwanda	Climber	Red mottle	Large
45	MEXICO 235	CIAT	Bush	Black	Small
46	MEXICO 235 X MBC 71	Rwanda	Climber	Black	Medium
47	MEXICO 235 X REDRANDSPIONNEER	Rwanda	Bush	Black	Small
48	MEXICO 235 X RWV 3006	Rwanda	Climber	Black	Medium

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No	Lines	Origin	Description		
			Type	Color	Size
49	MEXICO 54	CIAT	Bush	Black	Small
50	MEXICO 54 X MEXICO 235	Rwanda	Climber	Cream	Small
51	MWIRASI	Rwanda	Climber	Purple	Small
52	NYIRAGASAZI	Rwanda	Climber	White-black	Large
53	NR 1263-1/A	CIAT	Bush	Red mottle	Medium
54	NUA 377	CIAT	Bush	Red mottle	Large
55	NUA 566	CIAT	Bush	Red mottle	Small
56	NYIRAMAGOROLI	Rwanda	Climber	Dark red	Small
57	NYIRAMAGOROLI D	Rwanda	Climber	Dark red	Small
58	RED RANDISPIONNEER X G 1287227 AB 136	Rwanda	Bush	Red	Small
59	RED RANDISPIONNER	CIAT	Bush	Red	Small
60	RED RANDISPIONNER X EQUADOR 299	Rwanda	Bush	Black	Small
61	RED RANDISPIONNER X MAC 42	Rwanda	Climber	Red	Medium
62	RED RANDISPIONNER X RWV 3006	Rwanda	Climber	Red	Medium
63	RED RANDSPIONNEER X TU	Rwanda	Bush	Black	Small
64	REDRANDISPIONEER X MEXICO 235	Rwanda	Semi Climber	Black	Medium
65	RW R 1180	Rwanda	Bush	Red	Large
66	RWIBARURA	Rwanda	Climber	Kaki	Large

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No	Lines	Origin	Description		
			Type	Color	Size
67	RWK 10	Rwanda	Bush	Sugar	Medium
68	RWK 10 X ACC 714	Rwanda	Bush	Black	Medium
69	RWR 1668	Rwanda	Bush	Purple	Large
70	RWR 2154 X SCAM3 F2-7-2	Rwanda	Bush	Sugar	Large
71	RWR 2154	Rwanda	Bush	Sugar	Medium
72	RWR 2245	Rwanda	Bush	Red mottle	Medium
73	RWR 2245 X G12727 AB136	Rwanda	Semi Climber	Black	Small
74	RWR 2245 X RED RANDSPIONER	Rwanda	Bush	Red	Medium
75	RWR 2245 X RWR 2245	Rwanda	Bush	Red mottle	Medium
76	RWR 2245 X RWV 3006	Rwanda	Bush	Red mottle	Medium
77	RWR 3194	Rwanda	Bush	Red mottle	Medium
78	RWR 3228	Rwanda	Bush	Red mottle	Medium
79	RWR 390	Rwanda	Bush	Red mottle	Medium
80	RWV 1129	Rwanda	Climber	Pink	Medium
81	RWV 1348	Rwanda	Climber	Red	Small
82	RWV 2070	Rwanda	Climber	Brown	Medium
83	RWV 2269	Rwanda	Climber	Yellow	Small
84	RWV 2350-2B	Rwanda	Climber	Red	Medium
85	RWV 2352- 1A	Rwanda	Climber	White	Small
86	RWV 2357-B-3	Rwanda	Climber	Red	Small

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No	Lines	Origin	Description		
			Type	Color	Size
87	RWV 2361	Rwanda	Climber	Sugar	Medium
88	RWV 2365-2	Rwanda	Climber	Red	Small
89	RWV 23794	Rwanda	Climber	Red	Small
90	RWV 2411A- 2	Rwanda	Climber	White	Small
91	RWV 2699-1	Rwanda	climber	Sugar	Large
92	RWV 2828-1	Rwanda	climber	Red	Small
93	RWV 2872	Rwanda	Climber	Sugar	Medium
94	RWV 2887	Rwanda	Climber	Dark red	Medium
95	RWV 3006	Rwanda	Climber	White	Large
96	RWV 3006 X G 122164 TU	Rwanda	Climber	Red	Medium
97	RWV 3316	Rwanda	Climber	Red	Small
98	RWV 3317	Rwanda	Climber	Sugar	Medium
99	RWV 3346	Rwanda	Climber	Purple	Medium
100	RWV 3347	Rwanda	Climber	Dark red	Medium
101	SAB 16	CIAT	Bush	Red mottle	Medium
102	SC B790	CIAT	Bush	Green	Small
103	SCR 16	CIAT	Bush	Red pale	Small
104	SER 13	CIAT	Bush	Dark red	Small
105	SER 16	CIAT	Bush	Dark red	Small
106	SER 30	CIAT	Bush	Dark red	Small

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No	Lines	Origin	Description		
			Type	Color	Size
107	TU	CIAT	Bush	Black	Small
108	TU X G 12727 AB 136	Rwanda	Bush	Black	Small
109	TU X MBC 71	Rwanda	Bush	Black	Small
110	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-2	Rwanda	Bush	Pink mottle	Medium
111	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-4	Rwanda	Bush	Pink mottle	Medium
112	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-6	Rwanda	Bush	Pink mottle	Medium
113	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-9	Rwanda	Bush	Pink mottle	Medium
114	VRA 17	CIAT	Climber	Red	Large

Identification of inoculum dose

Pathogens were reactivated by sub-culturing on a fresh potato dextrose agar (PDA) culture media where to 16g of agar extra-pure were added 1000ml of distilled water, and autoclaved at 121^{o}C for 20 minutes. After autoclaved and cooled down to 40oC in laminar flow, the media was dispensed in Petri dishes (90mm wide) in rate of 20ml per petri dish. An agar block colonized by the pathogen was transferred to a fresh medium, incubated at room temperature for 7 days and transferred to bottle filled with sorghum substrate. Sorghum grains were used as a medium for fungal pathogen growth. 400ml of distilled water and 300g of sorghum grains were put in bottles, sterilized and allowed to cool for 12 hours. Colonized agar bearing the pathogen culture in one petri dish was inoculated in each bottle containing 300g of sorghum and leave inoculated bottles in a sterile environment at a room temperature for 14 days to allow uniform growth. The identification of inoculum dose performed with four soil treatments, 100g of inoculum inoculated respectively to 1.5 kg, 3kg, 4.5 kg and 6 kg of soil. It was carried out under greenhouse located at Rubona research center of Rwanda Agriculture Board located in Huye District, Rusatira sector, Kiruhura cell of Southern province.

Bean lines Screening for root rot disease resistance

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The experiment was set at Rubona research centre of Rwanda Agriculture and Animal Resources Development Board (RAB). Wooden flat trays of 20kg of capacity were used to evaluate different bean genotypes. After incubation, 300g of colonized sorghum inoculum were mixed with 13.5kg of loam sandy soil previously sterilized by steaming on firewood for four hours and left overnight to cool. These wooden flat trays were arranged in Randomized Complete Block Design (RCBD) with 5 replications, and in each tray it was planted 10 seed of test varieties. A susceptible check (RWR 2245) and a resistant check (ACC 714) were included in each tray.

Data collection and analysis

Germinated plants were counted, and disease assessment was carried out twenty-one days after planting. Seedlings of each bean line was carefully uprooted without damaging roots and hypocotyls, and washed with clean tap water. Number of germinated plants was recorded. The disease severity was assessed using CIAT scoring scale of 1-9 for Fusarium sp and Rhizoctonia sp, where 1 stand for no visible symptoms, 3: light discoloration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions, 5: approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system, 7: approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting and reduction of root system, and 9: approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with severe reduction in the root system. For Sclerotium sp, disease severity was assessed base on 1-5 scale, where 1 represent a healthy plant, 2: plant with disease symptoms without fungal growth, 3: plant with disease symptoms with fungal growth, 4: wilting plant, and 5: plant completely death (Abawi and Pastor-Corrales 1990). Data collected was subjected to analysis of variance using GenStat statistical package.

3.RESULTS AND DISCUSSION

Inoculums dose for screening dry bean for root rot diseases

All four treatments used in the study revealed differences in disease symptom expression at $P<0.05$. The identification of the inoculum dose for bean root rot disease screening showed that 4.5kg of soil inoculated with 100g of colonized sorghum produces visible symptoms on plants. Completely plant death was observed on 1.5kg of soil inoculated with 100g, while 6kg of soil inoculated with 100g produces non-assessable symptoms on plants. According to Anne Kadaari Kivisi (2015), root rot severity varied significantly at $P<0.05$ in green house experiment among seed treatments when 10g of the infected sorghum seed were spread at 1cm below soil in pots containing sterile soil. Therefore, production of root rot symptoms on plants grown on 4.5kg of soil inoculated with 100g of colonized sorghum was due to the virulence of the pathogen collected in Rwanda during the study period. These results are also supported by two other treatments where 1.5 kg of soil inoculated with 100g caused a completely plant death, while 6kg of soil inoculated with 100g produces invisible symptoms on plants. Similarities in management of inoculum and isolates may lead to similar conclusions but difference in quantity of inoculum producing symptoms indicates that different strains may be favorable in different environmental

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conditions (D.J Hagedorn and D.A.Inglis, 1986). High inoculum levels in soil is likely to results into severe disease infections leading to low yield.

Analysis of variance

The analysis of variance of root rot disease data (Sclerotium sp, Rhizotonia sp and Fusarium sp) revealed a high significant variations P< 0.01) among bean lines inoculated with each of the three pathogens (Table2).

Table 2: Analysis of variance of root rot diseases (Sclerotium , Rhizotonia and Fusarium) in common bean lines tested under greenhouse conditions

Source of variation	Plant germination		Disease score			
	d.f.	s.s.	d.f.	*Sclerotium*	*Rhizotonia*	*Fusarium*
Replication	4	0.65	4	5.4338	13.9046	33.4817
Bean line	113	2669.55**	113	36.1735**	544.7669**	694.2796**
Disease	2	13121.83**	-	-	-	-
Bean lines x Disease	226	3903.57**	-	-	-	-
Residual	1658	1869.47	550	73.2723	205.1589	546.5525
Total	2003	21566.17	667	114.8795	763.8304	1274.314

(** significant at P-value of 0.01)

From the table above, significant differences were observed among bean genotypes for resistance to both Sclerotium sp, Fusarium sp and Rhizoctonia sp at P<0.01. This implies that among tested genotypes, there were resistant genotypes for both diseases. The comparison of resistance of bean varieties to three different diseases showed weak correlation. Therefore, it was concluded that It is difficult to get a bean variety that is resistant to all three tested diseases. The tested genotypes have different genetic makeup, the Rhizoctonia and Sclerotium isolates collected from Rwanda were so aggressive than Fusariun collected in Uganda (Mukamuhirwa et al., 2017). Comparison of disease resistance data for Aphanomyces and Fusarium root rots in peas showed weak correlation (Grünwald et al, 2003). Therefore, understanding root rot pathogen biology and disease risks in an evolutionary context can support breeding for resistance programs and strategies for root rot management in common beans. The present study was conducted in screen house where conditions were under control. It is known that environmental conditions affect soil

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borne pathogens density (University of Sydney, 2003; Industry and Investment NSW 2009, Naseri B, 2014). Therefore, the disease occurrence and severity may depend on the weather. Understanding resilience of soil borne pathogens against stress can potentially guide disease management plans (Manici et al., 2014). Our results regarding bean root rot and Sclerotium sp virulence in particular should be of great importance in Rwanda breeding program. In their study on root rot pathogens in fields in relation to common bean disease and seed production, Bita Nasei and Seyyed Mousavi (2015) reported that Fusarium and Rhizoctonia root rots are major causes of bean losses. This study showed that Sclerotium was more important than Fusarium and Rhizoctonia root rots in beans. In Rwanda and neighbouring countries, Pythium spp are the fungal pathogens most frequently associated wih severe root rot epidemics (Rusuku et al., 1997, Nzungize, 2012). In the previous study by Nzungize et al 2012, except Sclerotiun rilfisii all isolated fungi were found in exprefectures investigated during 4 seasons where Pythium was the most frequently isolated fungi but the recent study by Mukamuhirwa et al 2018, Fusarium was the most predominant even if they did not identify pathogens at the species level. Pythium species collected in Rwanda and identified in the study by Mukamuhirwa et al., 2018, showed no agressivity to bean genotypes comparing to the three pathogens. In the study by Nzungize et al., 2011, bean varieties under their investigation showed differences in their reaction to inoculation with 16 Pythium species. They reported that the varieties CAL 96, RWR 617-97A, Urugezi and RWR 1668 were susceptible to all Pythium species while the G2331, AND6, MLB 40-89A, Vuninkingi, AND 1064 and RWR 719 showed a high level of resistance to all Pythiun species used in the study. The high level of resistance to Pythium root rot reported in diverse number of Common bean varieties grown in Rwanda, can be exploited as potential parents to improve resistance to pythium root rot disease in most popular varieties grown in Rwanda. The results of this study also revealed germplasm with resistance genes to consider in developing new bean lines with resistance to most root rot diseases.

Different reactions against isolates collected from Rwanda were observed on resistant and susceptible checks. The study showed that bean lines including NUA 566, BOA5-1/8, CAL 96, EQUADOR 299 x G 122164 TU, EQUADOR x ACC 714, G 122164 TU x EQUADOR 299, G 12727 AB 136 x G 122164 TU, IGISUBIZO, NUA 377, RWIBARURA, RWR 1668, RWR 2154, RWV 3006 x G 122164 TU, RWV 3317, SCR 16 and USCR9 x RWR 2074 x G2333 XRWR 719 F2-1-6 had the lowest disease mean scores than the local resistant checks (Table 3). RWR 3228 resisted Fusarium sp. and Rhizoctonia sp. Lines that showed a higher disease score compared to the local susceptible checks (Table 4) include highly susceptible lines CAB 2, Colta, RWV 1129, RWV 1348, RWV 2350-2B, RWV 2070, RWV 2269, RWV 2352- 1A, RWV 2357-B-3, RWV 2361, RWV 2365-2, RWV 23794, RWV 2411A- 2, RWV 2699-1, RWV 2828-1, RWV 2872, RWV 2887, and RWV 3006. The resistant and susceptible checks were identified in a study conducted using Rwandan bean varieties selected on isolates trains of Uganda provenance (Mukamuhirwa, et al, 2017). The present study revealed that isolates collected from Rwanda were completely different from isolates collected from Uganda. The current study has permitted to select also resistant and susceptible lines that can be used as checks on Rwandan isolate for pathogenicity test. All lines under this evaluation are newly developed lines or new

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introduced lines that are still under evaluation, and were never been evaluated for root rot disease resistance in Rwanda.

Table3: Selected lines based on low score of three diseases

No	Line	Score (0-1)
55	NUA 566	1.0
5	BOA5-1/8	0.7
7	CAL 96	0.7
15	EQUADOR 299 X G 122164 TU	0.7
16	EQUADOR X ACC 714	0.7
18	G 122164 TU X EQUADOR 299	0.7
24	G 12727 AB 136 X G 122164 TU	0.7
32	IGISUBIZO	0.7
54	NUA 377	0.7
66	RWIBARURA	0.7
69	RWR 1668	0.7
71	RWR 2154	0.7
96	RWV 3006 X G 122164 TU	0.7
98	RWV 3317	0.7
103	SCR 16	0.7
112	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-6	0.7

Table 4: Severity mean of root rot diseases (Sclerotium, Rhizotonia and Fusarium) in common bean lines tested under greenhouse conditions

No	Lines	Sclerotium			Rhizotonia			Fusarium		
		Germinati	Diseas e	Dry weigh	Germinati	Diseas e	Dry weigh	Germinati	Diseas e	Dry weigh

		on mean	score	t	on mean	score	t	on d mean	score	t
1	665SI-4/1	2.0	4.8	0.2	2.0	8.5	0.5	8.0	**4.1**	9.3
2	ACC 714	0.0	5.0	0.0	0.0	9.0	0.0	8.5	**3.8**	8.8
3	ACC 714 T	0.4	4.8	0.9	0.6	8.8	0.0	7.6	**4.3**	7.2
4	BOA5-1/16	0.0	4.9	0.0	5.0	7.5	3.4	6.8	**4.3**	6.0
5	BOA5-1/8	4.0	**4.3**	5.2	7.0	7.1	4.5	8.0	**4.1**	8.5
6	CAB 2	1.0	4.8	0.7	2.2	8.7	0.2	10.4	4.9	13.1
7	CAL 96	0.0	5.0	0.0	9.8	**6.0**	10.3	9.7	**3.8**	14.4
8	CAL 96 T	1.3	4.8	2.9	7.3	**6.3**	8.6	7.0	**3.8**	7.6
9	CIM RM00321 L	3.0	**4.2**	9.1	4.4	8.0	2.0	4.0	5.8	6.1
10	COLTA	0.0	5.0	0.0	2.2	8.6	0.4	7.2	6.7	10.8
11	COLTA X EQUADOR 299	0.0	5.0	0.0	2.0	8.4	1.5	6.0	4.8	12.6
12	COLTA X RWR 2245	0.0	5.0	0.0	3.6	7.9	3.0	4.0	5.0	8.2
13	COLTA XMAC 42	0.0	5.0	0.0	3.4	8.2	0.5	7.0	6.4	5.7
14	EQUADOR 299	0.0	5.0	0.0	1.8	8.5	0.5	8.0	**4.1**	9.4
15	EQUADOR 299 X G 122164 TU	0.0	5.0	0.0	5.0	7.0	4.5	8.0	**3.4**	10.4
16	EQUADOR X ACC 714	0.0	5.0	0.0	7.0	**5.2**	3.9	7.0	**3.6**	7.9
17	EQUADOR X RWR 2245	0.0	5.0	0.0	2.4	8.2	1.7	5.2	4.4	6.8
18	G 122164 TU X EQUADOR 299	2.0	**4.7**	1.8	5.0	**6.4**	4.4	3.8	5.8	3.3
19	G 122164 TU X G 121 727 A B 136	0.0	5.0	0.0	3.4	8.4	-0.2	3.0	6.7	3.5
20	G 122164 TU X MAC 42	0.8	4.9	2.3	5.0	**6.8**	3.9	7.0	4.9	7.9

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21	G 12727 AB 136 X ACC714	0.0	5.0	0.0	4.0	7.2	3.7	3.0	5.0	3.8
22	G 12727 AB 136 X COLTA	1.0	4.9	0.5	0.0	9.0	0.0	6.0	7.0	7.3
23	G 12727 AB 136 X EQUADOR 299	2.4	**4.7**	2.5	6.0	7.2	4.7	10.0	5.2	15.0
24	G 12727 AB 136 X G 122164 TU	0.0	5.0	0.0	8.8	**6.2**	7.7	6.8	**2.5**	10.9
25	G 12727 AB 136 X MAC 42	3.2	4.8	0.8	4.6	7.8	1.7	7.6	7.4	11.0
26	G 12727 AB 136 X RWR 2245	0.0	5.0	0.0	3.2	7.9	3.1	6.0	4.6	4.9
27	G 14619 X SEL 1461 F2-2-2-4-4-4/3	0.0	4.9	0.0	5.4	7.3	7.2	7.0	5.2	10.0
28	G122164 TU	0.0	5.0	0.0	6.5	**6.3**	4.0	7.0	4.9	11.5
29	G2331	0.0	5.0	0.0	1.6	8.8	0.2	7.0	5.3	5.4
30	GASIRIDA	2.0	**4.5**	3.9	2.0	8.6	0.7	6.6	5.1	6.8
31	IB ANGA 2	1.0	4.8	3.0	1.8	8.4	0.6	8.4	4.9	9.0
32	IGISUBIZO	2.8	**4.6**	3.8	2.0	8.3	1.1	8.0	**4.0**	10.5
33	KAB06F2-8-27	1.0	4.9	1.0	3.2	8.1	2.4	6.0	**3.9**	8.6
34	MAC 28	0.0	5.0	0.0	3.0	8.1	2.5	4.0	5.5	7.6
35	MAC 42	0.4	5.0	0.0	5.0	7.5	5.0	7.0	6.5	8.7
36	MAC 42 X COLTA	0.0	5.0	0.0	7.0	**5.8**	5.7	8.0	4.6	4.1
37	MAC 44	3.0	**4.3**	3.9	4.0	8.0	3.2	7.0	4.6	7.3
38	MAC 49	3.0	**4.1**	5.3	6.4	7.8	3.7	7.0	4.6	8.7

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39	MAC 9	1.0	4.8	2.5	3.8	7.9	5.6	8.0	4.8	12.8
40	MBC 23	0.0	4.9	0.0	5.0	7.2	4.2	6.6	5.2	5.6
41	MBC 25	2.0	**4.5**	4.1	2.0	8.6	1.2	8.0	5.0	12.9
42	MBC 64	0.0	4.9	0.0	2.0	8.6	0.5	6.9	**4.3**	9.5
43	MBC 71	0.0	5.0	0.0	6.2	**6.8**	4.4	4.4	4.8	4.2
44	MBC 71 X RWR 2245	2.8	4.7	1.1	5.0	7.2	4.9	0.0	6.4	0.0
45	MEXICO 235	0.0	5.0	0.0	0.0	9.0	0.0	4.0	8.9	10.0
46	MEXICO 235 X MBC 71	0.0	5.0	0.0	4.8	7.4	4.7	6.0	6.4	4.9
47	MEXICO 235 X REDRANDSPIONNEER	0.0	5.0	0.0	6.0	7.4	4.2	8.0	5.0	12.7
48	MEXICO 235 X RWV 3006	0.0	5.0	0.0	2.4	8.6	0.5	6.0	4.6	12.0
49	MEXICO 54	0.0	5.0	0.0	1.0	8.8	0.3	8.0	5.0	11.5
50	MEXICO 54 X MEXICO 235	0.0	5.0	0.0	8.0	**5.5**	8.2	5.0	4.8	5.3
51	MWIRASI	2.0	**4.5**	2.2	3.8	8.0	3.3	7.6	5.6	7.1
52	NHYIRAGASAZI	3.4	4.8	0.3	3.0	8.5	1.1	6.6	4.5	9.6
53	NR 1263-1/A	0.0	4.9	0.0	2.0	8.4	1.0	8.0	5.4	6.2
54	NUA 377	3.2	**4.7**	1.7	5.4	7.2	5.0	9.0	**4.2**	10.5
55	NUA 566	1.0	**4.7**	1.5	6.4	**6.9**	4.0	7.0	**4.2**	3.9
56	NYIRAMAGOROLI	5.0	4.8	0.9	0.0	9.0	0.0	7.0	5.6	8.0
57	NYIRAMAGOROLI D	4.0	**4.3**	5.5	2.0	8.1	0.9	6.0	5.1	6.3
58	RED RANDISPIONNEER X G 1287227 AB 136	1.0	5.0	3.9	3.2	8.3	1.4	3.0	5.2	6.1

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59	RED RANDISPIONNER	0.0	5.0	0.0	3.6	7.8	6.1	7.0	7.2	11.7
60	RED RANDISPIONNER X EQUADOR 299	0.0	5.0	0.0	4.8	**6.9**	4.5	0.0	5.9	0.0
61	RED RANDISPIONNER X MAC 42	0.0	5.0	0.0	4.4	7.6	0.0	4.6	8.3	3.3
62	RED RANDISPIONNER X RWV 3006	0.0	5.0	0.0	8.0	**6.3**	5.5	7.8	5.7	6.5
63	RED RANDSPIONNEER X TU	0.0	5.0	0.0	7.0	**6.4**	7.1	7.0	4.4	10.9
64	REDRANDISPIONEER X MEXICO 235	2.0	**4.6**	5.1	1.4	8.7	0.2	6.0	4.9	4.9
65	RW R 1180	1.0	4.8	1.4	4.0	7.4	3.7	8.0	5.0	7.7
66	RWIBARURA 2	0.0	5.0	2.0	5.0	**6.7**	4.5	7.0	**4.2**	7.9
67	RWK 10	1.0	4.9	0.4	5.2	7.5	2.2	4.4	4.9	6.6
68	RWK 10 X ACC 714	1.0	5.0	0.6	3.0	7.9	3.3	8.0	6.2	8.9
69	RWR 1668	2.0	**4.5**	2.5	7.2	**6.8**	6.0	0.0	5.0	0.0
70	RWR 2154 X SCAM3 F2-7-2	0.0	4.7	0.0	5.2	7.2	3.3	7.8	7.9	7.8
71	RWR 2154	0.0	5.0	0.0	8.6	**6.2**	7.7	9.0	**4.0**	10.4
72	RWR 2245	0.0	5.0	0.0	6.0	7.3	4.4	4.6	**3.7**	7.0
73	RWR 2245 X G12727 AB136	1.0	4.8	1.6	1.0	8.7	1.4	7.0	6.2	7.2
74	RWR 2245 X RED RANDSPIONER	0.0	5.0	0.0	6.0	**6.8**	4.5	6.0	5.0	9.1
75	RWR 2245 X RWR 2245	0.0	5.0	0.0	2.2	8.2	2.2	3.0	6.0	4.2
76	RWR 2245 X RWV 3006	0.0	5.0	0.0	0.4	8.9	0.0	8.0	6.8	10.1

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77	RWR 3194	1.2	4.8	1.5	5.0	7.1	4.0	8.0	4.5	10.5
78	RWR 3228	3.8	**4.2**	2.7	3.0	8.1	2.6	7.0	4.6	7.1
79	RWR 390	1.0	4.7	1.9	3.6	8.2	1.8	8.0	4.9	11.4
80	RWV 1129	1.0	4.9	0.4	6.2	**7.0**	3.0	7.0	4.4	9.6
81	RWV 1348	1.0	5.0	0.0	4.0	8.0	2.0	7.0	4.9	5.3
82	RWV 2070	0.0	5.0	0.0	2.0	8.6	1.3	7.6	4.6	9.1
83	RWV 2269	0.0	4.9	0.0	1.6	8.8	0.0	5.4	**4.2**	8.5
84	RWV 2350 A-2B	0.0	4.9	0.0	4.8	7.4	4.7	7.0	5.4	7.3
85	RWV 2352- 1A	0.0	**4.6**	0.0	1.8	8.7	0.8	7.0	5.2	1.8
86	RWV 2357-B-3	0.0	**4.5**	3.6	3.0	8.2	1.8	9.0	5.1	6.7
87	RWV 2361	1.0	5.0	0.0	5.0	7.6	5.5	6.2	**4.3**	8.5
88	RWV 2365-2	0.0	4.9	0.0	4.4	7.5	4.4	7.4	5.2	3.4
89	RWV 23794	0.0	4.7	0.0	1.8	8.7	0.2	8.0	4.5	9.9
90	RWV 2411A- 2	0.0	5.0	0.0	0.0	9.0	0.0	7.8	4.3	2.3
91	RWV 2699-1	0.0	**4.6**	0.0	0.0	9.0	0.0	6.8	4.8	2.4
92	RWV 2828-1	0.0	4.7	0.0	1.2	8.8	0.0	8.0	5.1	8.3
93	RWV 2872	0.0	5.0	0.0	6.0	7.1	4.8	7.0	5.2	8.4
94	RWV 2887	0.0	5.0	0.0	5.8	7.4	1.5	7.8	5.3	7.8
95	RWV 3006	0.0	5.0	0.0	6.8	**6.6**	5.6	10.0	4.6	10.2
96	RWV 3006 X G 122164 TU	0.0	5.0	0.0	5.2	**6.8**	5.8	8.0	**3.2**	12.3
97	RWV 3316	0.0	4.8	1.3	3.0	8.4	1.2	8.0	4.7	9.9
98	RWV 3317	1.0	**4.7**	1.8	6.0	**7.0**	4.1	6.8	4.6	3.0

99	RWV 3346	1.0	4.7	0.0	3.0	8.2	2.6	7.8	5.0	3.1
100	RWV 3347	0.0	**4.3**	5.7	3.0	8.0	1.2	8.0	4.4	8.5
101	SAB 16	0.0	4.8	0.0	2.0	8.3	3.1	7.4	4.4	10.4
102	SC B790	1.6	**4.5**	3.7	4.4	8.2	0.8	9.0	4.6	8.5
103	SCR 16	0.0	4.7	1.3	3.0	8.3	0.6	8.0	**3.5**	8.6
104	SER 13	2.2	4.9	1.0	5.4	7.3	1.8	9.2	4.4	8.4
105	SER 16	1.0	5.0	0.0	5.0	7.5	2.4	9.4	**3.8**	7.9
106	SER 30	1.0	5.0	0.0	6.6	7.2	2.9	4.4	**4.0**	8.1
107	TU	0.0	5.0	0.0	5.0	7.4	3.3	6.0	5.9	6.6
108	TU X G 12727 AB 136	0.0	5.0	0.0	5.0	**6.9**	2.8	6.0	5.4	9.6
109	TU X MBC 71	0.2	5.0	2.2	2.0	8.2	2.4	8.0	5.3	8.4
110	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-2	0.0	4.7	0.0	4.8	7.6	2.6	4.8	4.4	4.1
111	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-4	1.0	**4.6**	0.2	4.6	8.0	1.1	5.0	6.5	3.1
112	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-	0.0	**4.7**	0.0	6.0	**6.8**	4.7	8.0	5.7	8.9

	6										
113	USCR9 X RWR 2074 X G2333 XRWR 719 F2-1-9	1.0	**4.4**	0.9	3.4	7.9	2.9	6.8	5.3		9.6
114	VRA 17	0.0	4.9	0.0	5.4	**6.9**	6.3		5.5		5.0

Cluster analysis

The distribution of inoculated bean lines based on their mean root rot disease (Sclerotium sp, Rhizotonia sp and Fusarium sp) score is detailed in figures 1, 2 and 3. The high number of bean lines was clustered closer to mean score bar for Fusarium sp. (Figure 1). The bean lines 24, 15 and 96 representing G 12727 AB 136 x G 122164 TU, EQUADOR 299 X G 122164 TU and, RWV 3006 X G 122164 TU respectively revealed the lowest disease score while the bean lines 45, 61, 70, 25, and 59 representing MEXICO 235, RED RANDISPIONNER X MAC 42, RWR 2145 x SCAM3 F2-7-2, G 12727 AB 136 x MAC 42 and RED RANDISPIONNER respectively had the highest disease score (Figure 1). MEXICO 235, RED RANDISPIONNER X MAC 42, RWR 2145 x SCAM3 F2-7-2 showed highly susceptible to Fusarium, while G 12727 AB 136 x G 122164 TU showed strong resistance. The scatter plots were used to show how much bean lines were affected by the pathogen through their distribution based on the disease scores. It showed also the relationship between the disease severity and the bean lines. The genotypes closer to the low score line are considered as resistant, while genotypes which are closer to a high score line, are considered as susceptible. The results of scatter plots revealed that bean varieties performed differently against Fusarium pathogen. In season A 1990, severe symptoms of Fusarium were observed for the first time on the popular climbing bean cultivar G2333. In their study, Buruchara and Camacho (2000) have identified as resistant beans, RWR 950 and G685; and susceptible varieties, G2333 and MLB 48-89A. The resistant varieties are no longer into use in Rwanda, and have disappeared from the production system due to multiple causes. A strong selection strategy is needed for Rwanda Bean Breeding to screen for improved multiple resistance to Fusarium sp., Sclerotium sp., and Rhizotonia sp. Fusarium root rot has caused bean growers in Rwanda to abandon the popular variety Umubano (G2333) (Musoni et al., 2010). Despite its susceptibility, G2333 is still visible along with MLB 48-89A in local mixture commonly used in bean cropping system. G2333 needs a continuous improvement by backcrossing since Fusarium wilt is conditioned by a single heritable major dominant gene and has been used by many Breeders in the region. Susceptible varieties may have genes of interest which confer to other desired phenotype. Therefore, it is important to keep them from disapearing (Musoni et al., 2010).

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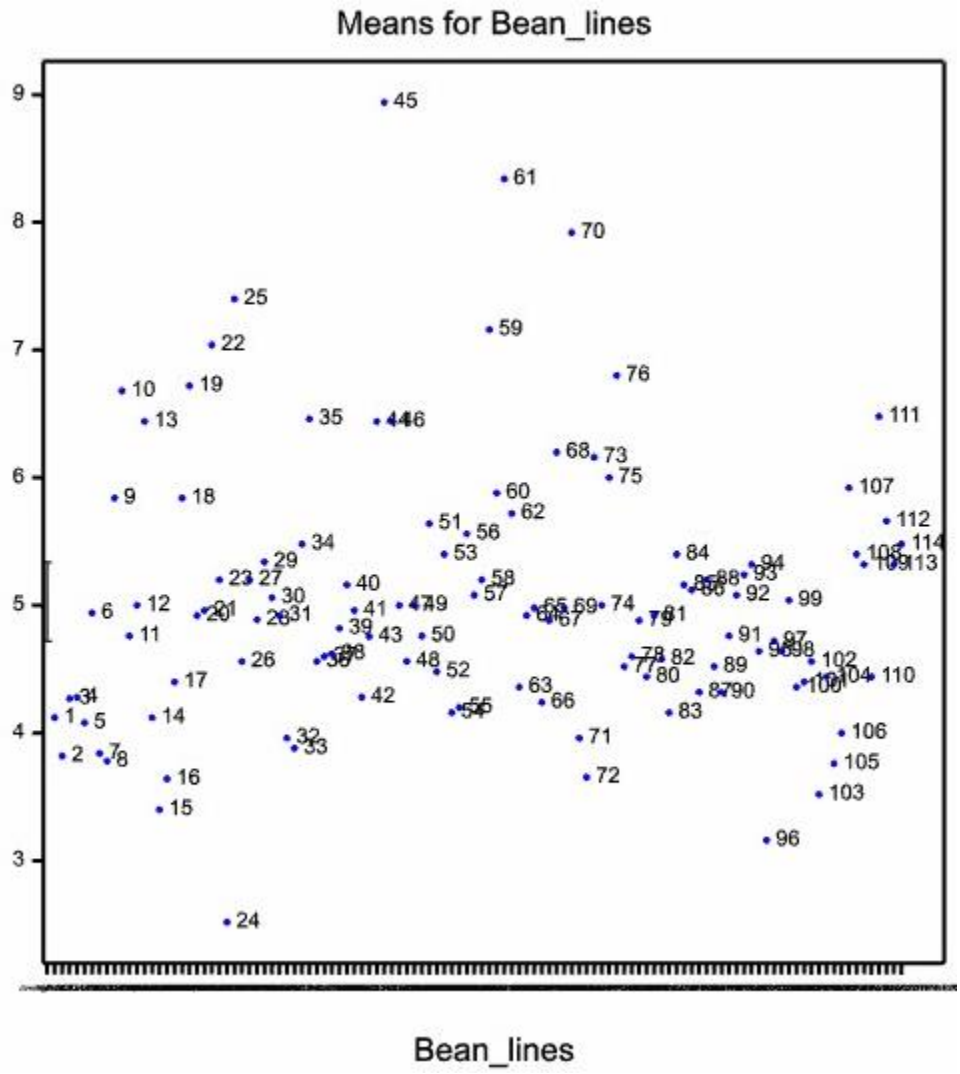

Figure 1: Distribution of inoculated bean lines based on their mean score of Fusarium

The bean lines revealed also a scattered distribution based on disease mean score of Rhizoctonia (Figure 2). The bean lines 16, 50, 36 and 7 standing for EQUADOR x ACC 714, MEXICO 54 x MEXICO 235, MAC 42 x COLTA and CAL 96 showed the lowest mean score, while the bean lines 90, 2, 22, 45, 56, 76 and 91 standing for RWV 2411A-2, ACC 714, G 12727 AB 136 X COLTA, MEXICO 235, NYIRAMAGOROLI, RWR 2245 x RWV 3006 and RWV 2699-1 respectively, revealed the highest mean score (Figure 2). Buruchara et al., (2015) reported that *Rhizoctonia* spp and *Fusarium* spp were the most prevalent in Latin America but also *Sclerotium*

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sp was observed. Their report states that *Rhizoctonia sp* and *Sclerotium sp* was prevalent in the northern production region where *Fusarium sp* was more prevalent in southern region of Latin America. In Africa, *Pythium sp* was more predominant than *Rizoctonia sp* but *Sclerotium sp* was not reported. Incidence and severity of root pathogens varied among fields in the same region (Buruchara et al., 2015). To our knowledge no other study was conducted on *Rhizoctonia* sp. in Rwanda. Our findings constitute the basis of research on *Rhizoctonia sp* in Rwanda. In a survey conducted in seven of ten ex-prefectures of Rwanda during four seasons from 1989 to 1990, it was identified 3 soil born pathogens including *Rhizoctonia solani* in all regions where isolates were collected during the four seasons. The pathogen was isolated with relatively low frequency but regular accross sites and seasons (Rusuku et al., 1997).

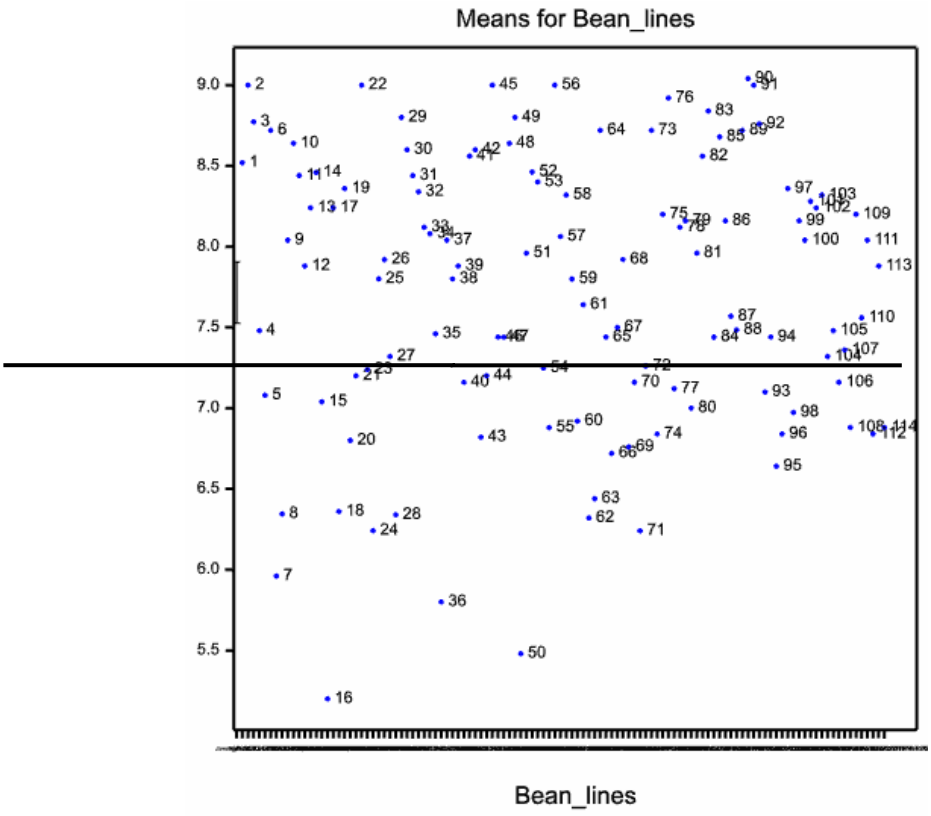

Figure 2: Distribution of inoculated bean lines based on their mean score of *Rhizoctonia* sp.

The majority of inoculated bean lines were clustered near the mean score for *Sclerotium* sp. (Figure 3). The bean lines of 38, 9, 78, 5, 37,57, and 100, representing MAC 49, CIM RM00321 L, RWR 3228, BOA5-1/8, MAC 44, NYIRAMAGOROLI D, and RWV 3347, respectively revealed the lowest disease scores (Figure 3). The bean lines of 68, 58, and 109 representing RWK 10 x ACC 714, RANDISPIONNEER x G 1287227 AB 136, and TU x MBC 71, respectively, showed the highest disease scores (Figure 3). According to the surveys conducted

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in Rwanda during four seasons of 1989-1990, *Sclerotium rolfsii* was dentified only in one season of 1990 (Rusuku et al., 1997). In this study, the majority of bean lines were distributed between Sclerotium severity of 4.4 and 5.0. Given the serious threat of Sclerotium on the bean production, it was suggested further investigation (Michigan State Univesity, 2016)

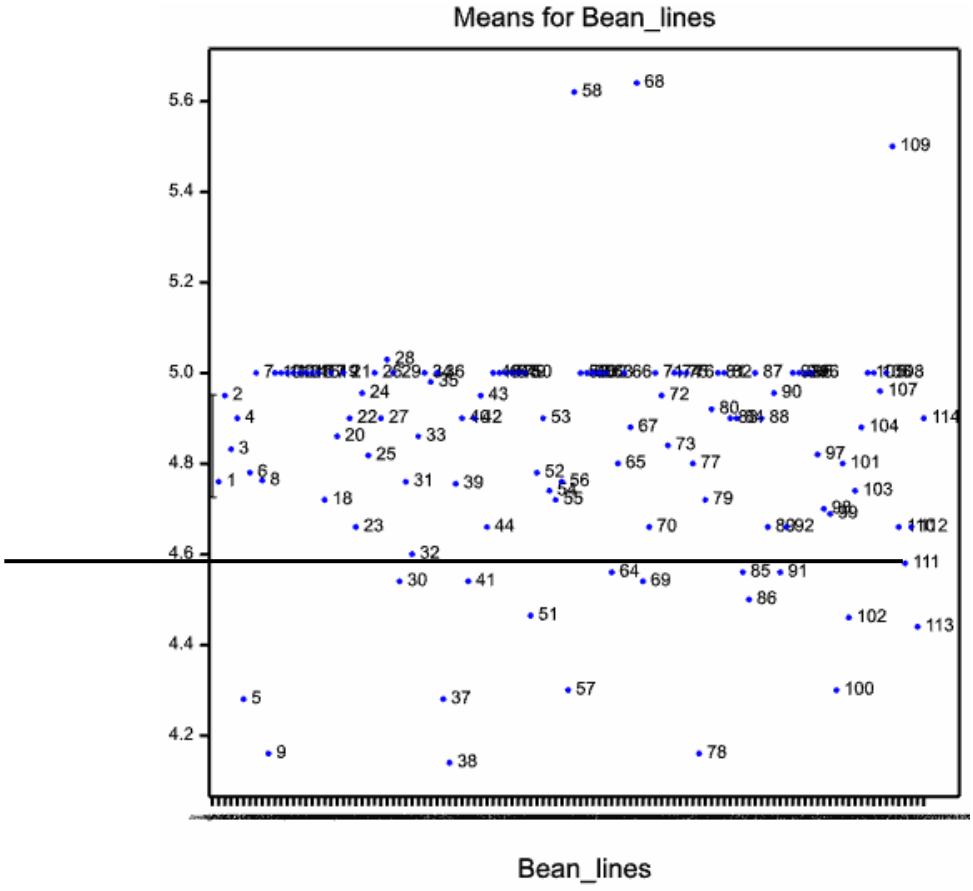

Figure 3: Distribution of inoculated bean lines based on their mean scores of *Sclerotium sp*

4. CONCLUSION

The origin of the isolate may play a major role in inducing stress to bean crop though many other factors may be considered. Difference in quantity of inoculum producing symptoms indicates that different strains may be favorable in different environmental conditions. High inoculum levels in soil are likely to cause severe disease infections and lower crop yields. The genotypes

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evaluated in this study have different genetic makeup. Among isolates collected across Rwanda Agro-ecological zones, *Rhizoctonia sp* and *Sclerotium sp* were more aggressive than *Fusarium sp*. Therefore, understanding root rot pathogen biology and disease risks in an evolutionary context can support breeding for root rot diseases resistance in common beans. Climate changes also affect soil borne pathogen density, diversity, and severity. As a consequence, disease occurrence may shift according to climate changes. The current study showed that *Sclerotium sp* requires more attention than *Rhizoctonia sp* and *Fusarium sp*. Resistance and susceptibility of the bean varieties may change with space, time and age of the genotye but the present study revealed that isolates collected from Rwanda are completely different to isolates collected from elsewhere. The study has contributed in identifying new bean lines that can be used as resistant and susceptible check while studying Rwandan isolates. The present findings constitute the basis of research on Rhizoctonia sp in Rwanda. However, molecular marker for *Fusarium sp, Pythuim sp, Rhizoctonia sp* and *Sclerotium sp* are highly needed to increase the success of transferring resistance from the Mesoamerican genepool to Andean germplasm. Development of breeding program based on marker assisted breeding for *Sclerotium sp* is highly recommended to speed up the selection for *Sclerotium* sp resistance in Rwanda.

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