

Resistance Levels to Root Rot and Angular Leaf Spot Diseases in Selected High Iron Bean Genotypes

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Abstract

Common bean production is constrained by different diseases the major ones being, Angular Leaf Spot (ALS), bean root rot, anthracnose, Common Bright Bacteria (CBB), Bean Common Cosaic Virus (BCMV) and Bean Common Mosaic Necrotic Virus, (BCMNV). The aim of this study was to identify new and better sources of broad resistance to both bean ALS and Root Rot diseases among nutritional bean varieties. Fifty seven varieties were planted in the screen house of CIAT Africa based at Kawanda Agricultural Research Laboratories Institute (KARL). Virulent inocula actually used at CIAT were used to test these genotypes. Different varieties were resistant to specific isolates but interestingly, only ACC 714 contained broad resistance to both Andean and MesoAmerican isolates of bean Angular Leaf Spot as well as *Fusarium* root rot and *Pythium* root rot at mean, median and mode basis. Since different nutritional bean varieties have varying levels of resistance to different pathogens, it may be possible to pyramid these resistance genes into appropriate background so as to provide durable resistance in biofortified bean genotypes higher in iron and zinc content.

Keywords: Nutritional bean varieties; Pathogens; Resistance; Iron content

Introduction

Biofortification, a practice of enhancing contents of minerals with nutritional significance in food products is regarded as one of the cheap approaches to improve human nutrition [1,2]. Traditionally, crops have been mostly improved for agronomic traits and to a lesser extent for pest and disease resistance. Biofortification is only a recent practice. The most limiting micronutrients in the diets of the rural and urban poor in Rwanda and Uganda are Fe and Zn, resulting into anemia and depressed immunity, respectively [3]. Efforts have been made in this study to breed for increased Fe and Zn in common beans in Rwanda and Uganda. However, successful deployment of high Fe and Zn common beans in both countries will require that such varieties are high yielding but also resistant to some of the most important diseases. The interaction of disease borne pathogens with the crop-bio system complicates the demonstration of superior genotypes across environments, and thus it results into scale or rank shift of trait performance. The bean root rot (*Pythium* sp, *Fusarium solani* fsp. *phaseoli*, *Rhizoctonia solani*, *Macrophomina phaseoli* and *Sclerotium rolfsii*) and angular leaf spot (*Phaeoisariopsis griseola*) are currently regarded the most important bean diseases in Uganda and Rwanda [4]. The plant diseases lead to food deficit and food insecurity. In order to reduce the yield losses due to disease, it is important to define the diseases contributing to reduced yield, accurately estimate the severity of disease and propose the possible solutions. Therefore, this study was carried out to determine the levels of resistance to these two diseases in common bean genotypes bred for high Fe and Zn contents.

Materials and Methods

Research sites and plant materials

This study was carried out in the CIAT Africa screen house based at Kawanda Agricultural Research Laboratories Institute (KARL), Uganda. It was carried out from June to October 2012. 57 bean genotypes were used in the study. These genotypes were regional nutrition nursery breeding lines; advanced G × E stable lines; susceptible checks for root

rot and ALS; low Fe check (CAL 96); and resistance checks for root rot (MLB49-89A/ RWR719) and ALS (MEX54/ BAT332) (Table 1).

Preparation of pathogen inocula, application and disease evaluation

***Fusarium solani* fsp. *phaseoli*:** Inoculum for *Fusarium solani* fsp. *phaseoli* was prepared from isolate FSP-3, the most virulent isolate for *Fusarium* root rot obtained from infected bean fields in the *Fusarium* root rot hot spot in south-western Uganda [5]. *Fusarium* inoculum was isolated and prepared following CIAT's laboratory training manual. The inoculum was reactivated by sub culturing it on a fresh PDA culture media. Sorghum grains were used as a medium for fungal inoculum multiplication. Approximately 400 ml of water to every 300 g of sorghum grains were placed in polyethylene bags, sterilized and allowed to cool for 12 h. A disc of agar bearing *Fusarium* spp. culture was incubated in the polyethylene bags over the sterilized sorghum grains in a sterile environment at a room temperature for 14 days to allow uniform growth. After incubation, *Fusarium* inoculum was mixed with the loam sandy soil previously sterilized by steaming on firewood for four hours and left overnight to cool. Sterile soil and inoculum were mixed in a ratio of 1:8 and put in a wooden flat tray and left to stabilize for seven days. In each tray, was planted 5 test varieties, each in 2 rows. A susceptible check (Cal 96) and a resistant check (MLB49-89A) were included in each tray. Disease assessment was done twenty one days after planting by carefully uprooting all the seedlings planted per variety taking care not to damage roots and hypocotyls, and washing with clean

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No.	Genotypes	Origin	No.	Genotypes	Origin
1	NGWIN × CAB2/2/3/1/1	Rwanda	30	NUA 69	CIAT
2	NGWIN × CAB2 × (RWV3317)	Rwanda	31	KAB06F2.8-12	CIAT
3	MAC 42	Rwanda	32	RWR 2154	Rwanda
4	RWV 3316	Rwanda	33	RWR 2245	Rwanda
5	NUV 219-1	CIAT	34	KAB06F2.8-36	CIAT
6	CAB 2	Rwanda	35	KAB06F8.8-35	CIAT
7	RWV 2359	Rwanda	36	CODMLB 001	CIAT
8	Garukurare	Rwanda	37	HM 21-7	CIAT
9	Kivuzo	Rwanda	38	Ngwaku-Ngwaku.	RDC
10	RWV1129	Rwanda	39	NUA 45	CIAT
11	Ndimirakaguja volubile	Rwanda	40	NUA 59	CIAT
12	Icyana 2	Rwanda	41	NUA 56	CIAT
13	RWV 2361	Rwanda	42	NUA 35	CIAT
14	MAC 44	Rwanda	43	Gitanga	Rwanda
15	RWV 3006	Rwanda	44	Zebra	CIAT
16	RWV 2887	Rwanda	45	ACC 714	CIAT
17	MAC 74	Rwanda	46	CODMLB 033	CIAT
18	Agronome 2	Rwanda	47	ROBA 1	CIAT
19	VRA 4	CIAT	48	SMC 21	CIAT
20	Rugandura	Rwanda	49	SEMC 16	CIAT
21	RWV 2070	Rwanda	50	SMC 18	CIAT
22	Kiangara	DRC	51	SEMC 17	CIAT
23	VCB 81013	CIAT	52	GLP 2	CIAT
24	Gasirida	Rwanda	53	CAL 96 (check: low Fe, root rot susceptible)	Uganda
25	MEX54/ BAT332 resistant check	CIAT	54	DOR 500 (low Fe check)	Uganda
26	MIB 456-High Fe Universal Check	CIAT	55	Maharagi Soya	Rwanda
27	Decelaya 1-Low Fe Check	Rwanda	56	MBC32	CIAT
28	KAB06F2.8-27	CIAT	57	Nyiramogorori2	Rwanda
29	NUA 99	CIAT			

Table 1: Plant materials used in the study of characterization of resistance of selected high iron beans to root rot and Angular Leaf Spot diseases.

Source	Andean Isolate		MesoAmerican Isolate	
	DF	MS	DF	MS
Rep	2	707.77	2	701.30
Genotype	51	784.48***	55	1227.20 ***
Residual	95	70.05	101	364.20
Total	148	324.86	158	668.80
CV		13.49		29.07
Mean		62.02		65.64
LSD		13.57		30.91

***Significant at $p \leq 0.001$.

Table 2: ALS Andean and MesoAmerican isolates mean squares.

tap water. For each variety, twenty plants were evaluated per replicate. FRR severity was assessed by scoring disease on roots and hypocotyls and scoring based on a 1 to 9 disease score [6]. According to this scale, 1=no visible symptoms, 3=light discoloration either without necrotic lesions or with approximately 10% of the hypocotyls and root tissues covered with lesions; 5=approximately 25% of hypocotyls and root tissues covered with lesions but tissues remain firm with deterioration of the root system; 7=approximately 50% of hypocotyls and root tissues covered with lesions combined with considerable softening, rotting and reduction of root system, 9=approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting combined with severe reduction in the root system. The experiment was

conducted in RCBD design with 3 replications. Data were subjected to ANOVA using Genstat [7].

Pythium sp: Inoculum for *Pythium* was prepared from *P. ultimum* isolate MS 61 from long term storage at CIAT, Kawanda. The inoculum was reactivated by sub-culturing it on a fresh PDA culture media. Finger millet grains were used as a medium for fungal growth. Approximately 200 ml of water to every 300 g of millet grains were placed in polyethylene bags, double sterilized and allowed to cool for 12 h. A disc of agar bearing *Pythium spp* culture was incubated in the polyethylene bags over the sterilized finger millet grains in a sterile environment at a room temperature for 14 days to allow uniform growth.

After incubation, *Pythium* inoculum was mixed with the loam sandy soil previously sterilized earlier as for *Fusarium*. Inoculum and soil were mixed in a 1:8 ratio, put in wooden flat trays and left to stabilize for seven days. Test varieties were planted in these trays as done for *Fusarium solani* above. Again, CAL 96 and RWR719 were included as susceptible and resistant checks respectively. Three weeks after planting, plants were uprooted, washed carefully and immediately evaluated for damage. Disease severity was scored using the 1 to 9 CIAT scale. Twenty plants per replication were evaluated. The experiment was laid out as a RCBD with 3 replications. Data were subjected to ANOVA using Genstat 14th edition.

Phaeoisariopsis griseola: For this pathogen, isolates KAK3, a virulent Andean isolate and isolate 2A a virulent Meso-American isolate were used for testing the 57 genotypes. Isolates were prepared for inoculation following the CIAT laboratory training manual procedures.

Genotypes	AUDPC for Andean isolate	Group	AUDPC for Meso-American isolate	Group
Ngwin × CAB2/2/3/1/1	75.0	mno	63.2	Bcdefghijklmno
Ngwin × CAB2(RWV3317)	75.2	mno	81.6	Jklmnopqrst
MAC 42	29.0	ab	40.4	Abcd
RWV 3316	75.6	no	93.2	Opqrst
NUV 219-1	51.7	efgh	51.3	Abcdefghij
CAB 2	-	-	39.5	Abc
RWV 2359	76.6	o	51.4	Abcdefghij
Garukurare	75.4	mno	64.6	Bcdefghijklmno
Kivuzo	73.7	lmno	103.5	Rst
RWV1129	75.4	mno	53.5	Abcdefghij
Ndimirakaguja vol	75.6	no	78.4	Hijklmnopqrst
Icyana 2	73.0	klmno	87.8	Mnopqrst
RWV 2361	73.0	klmno	74.4	Fghijklmnopqr
MAC 44	60.5	fghijkl	54.0	Abcdefghijk
RWV 3006	64.6	hijklmno	44.8	Abcdefg
RWV 2887	76.0	o	91.6	Nopqrst
MAC 74	65.5	ijklmno	50.6	Abcdefghi
Agronome 2	72.7	klmno	87.0	Mnopqrst
VRA 4	35.2	abcd	42.9	Abcde
Rugandura	60.5	fghijkl	85.5	Lmnopqrst
RWV 2070	23.5	a	35.5	Ab
Kiangara	72.1	klmno	55.5	Abcdefghijkl
VCB 81013	75.0	mno	62.2	Bcdefghijklmn
Gasirida	76.2	o	95.6	Pqrst
MEX54/BAT332 resistant checks	31.3	ab	26.8	A
MIB 456-High Fe Universal Check	76.8	o	98.6	Qrst
Decelaya 1-Low Fe Check	-	-	50.0	Abcdefghi
KAB06F2.8-27	30.5	ab	59.2	Bcdefghijklm
NUA 99	48.4	def	44.0	Abcdef
NUA 69	34.5	abc	45.3	Abcdefg
KAB06F2.8-12	32.5	ab	49.0	Abcdefgh
RWR 2154	73.0	klmno	106.7	T
RWR 2245	56.1	fghij	53.7	Abcdefghij
KAB06F2.8-36	55.4	fghi	105.7	St
KAB06F8.8-35	51.0	efg	78.4	Hijklmnopqrst
CODMLB 001	74.2	mno	72.3	Efghijklmnopq
HM 21-7	71.0	klmno	52.5	Abcdefghij
Ngwaku-Ngwaku	73.6	lmno	54.2	Abcdefghijk
NUA 45	-	-	-	-
NUA 59	41.0	bcde	52.0	Abcdefghij
NUA 56	59.6	fghijk	70.9	Defghijklmnopq
NUA 35	67.5	ijklmno	62.8	Bcdefghijklmno
Gitanga	69.4	jklmno	67.8	Cdefghijklmnopq
Zebra	74.2	mno	106.9	T
ACC 714	31.5	ab	49.4	Abcdefgh
CODMLB 033	35.1	abcd	43.7	Abcdef
Roba 1	75.2	mno	41.0	Abcd
SMC 21	76.2	o	65.9	Bcdefghijklmnop
SEMC 16	-	-	55.7	Abcdefghijkl
SEMC 18	75.5	mno	73.2	Efghijklmnopqr
SEMC 17	62.2	ghijklmn	75.0	Ghijklmnopqrs
GLP 2	-	-	85.0	Klmnopqrst
CAL 96-Low Fe check susceptible check	75.1	mno	90.7	Nopqrst
DOR 500-Low Universal check	74.2	mno	70.7	Defghijklmnopq
Maharagi soya	66.9	ijklmno	50.2	Abcdefghi
MBC32	47.2	cdef	48.9	Abcdefgh
Nyiramogorori2	75.0	mno	80.9	lklmnopqrst
LSD	13.6		30.9	

Table 3: AUDPC values for Andean and Meso American isolates on tested genotypes.

Source of variation	DF	Fusarium root rot isolate			Pythium root rot isolate			
		Mean	Median	Mode	Mean	Mode	Median	
		MS	MS	MS	MS	MS	MS	
Reps	2	10.247 **	25.158 **	50.518 **	2	28.08 ***	36.21 ***	38.838 ***
Genotype	54	8.485***	15.768 ***	17.717 ***	54	13.48***	24.01***	22.62 ***
Residual	106	2.043	5.626	7.533	101	1.731	5.015	4.49
Total	162	4.291	9.248	11.458	157	6.107	11.946	11.163
CV		31.458	55.560	57.988		24.607	41.28	40.53
Mean		4.544	4.269	4.733		5.347	5.42	5.23
LSD		2.314	3.840	4.443		2.131	3.63	3.43

** , ***significant at $P \leq 0.01$ and $P \leq 0.001$ respectively.

Table 4: Means squares for both beans Fusarium and Pythium root rot.

Genotype	Fusarium root rot				Pythium root rot			
	Mean	Median	Mode	DI	Mean	Median	Mode	DI
Ngwin x CAB2/2/3/1/1	5.54	6.66	6.66	65	6.72	8.99	8.99	76
Ngwin x CAB2 x (RWV3317)	6.73	6.99	6.66	73	8.44	8.99	8.99	94
MAC 42	3.79	2.16	1.99	42	6.02	6.66	6.66	66
RWV 3316	4.22	2.49	4.33	47	2.83	1.99	1.99	30
NUV 219-1	4.91	4.33	4.33	54	5.06	2.32	4.32	57
CAB 2	6.05	4.83	6.66	59	4.99	5.92	5.91	69
RWV 2359	7.91	9.19	8.98	88	8.07	8.99	8.99	89
Garukurare	3.93	3.66	4.66	43	2.64	1.99	1.99	29
Kivuzo	5.71	6.66	6.66	62	3.74	2.16	1.99	42
RWV1129	8.51	8.99	8.99	96	8.99	8.99	8.99	100
Ndimirakaguja vol	3.66	2.33	1.99	40	3.06	2.32	1.99	34
Icyana 2	3.48	2.33	4.33	39	2.46	1.99	1.99	28
RWV 2361	4.72	4.33	4.33	51	3.25	2.16	2.32	36
MAC 44	5.47	6.99	6.66	62	6.56	6.99	8.99	75
RWV 3006	3.87	2.66	4.66	40	4.69	4.66	4.32	44
RWV 2887	4.99	4.66	6.66	56	7.14	8.99	8.99	79
MAC 74	4.12	2.33	4.33	47	7.14	8.99	8.99	81
Agronome 2	5.66	5.71	5.99	48	9.14	9.07	9.09	100
VRA 4	4.80	4.49	4.33	55	6.13	5.16	4.99	71
Rugandura	2.00	1.99	1.99	22	2.02	1.99	1.99	22
RWV 2070	7.61	8.99	8.99	84	5.92	5.66	6.66	64
Kiangara	2.03	1.99	1.99	23	2.83	1.99	1.99	31
VCB 81013	3.63	4.33	4.33	41	2.36	1.99	1.99	27
Gasirida	2.99	1.99	1.99	33	5.85	4.99	4.66	63
MLB49-89A/ RWR719 Resistant check	2.13	1.99	1.99	24	2.01	1.99	1.99	22
MIB 456-High Fe Universal Check	2.39	1.99	1.99	27	3.48	2.32	2.32	38
Decelaya 1-Low Fe Check	3.03	1.99	1.99	37	2.58	2.77	2.73	22
KAB06F2.8-27	5.58	4.83	6.66	64	5.19	6.66	6.66	56
NUA 99	6.05	6.99	8.99	67	7.06	6.99	6.66	79
NUA 69	5.56	4.66	6.66	62	7.10	8.99	8.99	78

KAB06F2.8-12	4.52	3.83	2.33	50	6.84	6.99	6.66	73
RWR 2154	4.95	4.66	6.66	58	7.65	8.99	8.99	85
RWR 2245	5.84	4.33	4.33	68	3.57	1.99	1.99	40
KAB06F2.8-36	5.23	4.66	6.66	59	8.91	8.99	8.99	98
KAB06F8.8-35	4.73	3.33	4.33	50	6.17	4.66	4.32	71
CODMLB 001	3.51	2.66	2.33	39	7.42	8.99	8.99	81
HM 21-7	3.49	4.33	4.33	48	4.20	2.32	2.32	47
Ngwaku-Ngwaku	3.11	2.33	2.33	32	4.74	5.92	5.91	58
NUA 45								
NUA 59	6.91	6.99	8.99	70	7.39	6.99	8.99	81
NUA 56	7.96	8.99	8.99	89	8.56	8.99	8.99	95
NUA 35	6.86	8.99	8.99	76	8.48	8.60	8.62	98
Gitanga	2.00	1.99	1.99	22	1.99	1.99	1.99	22
Zebra	2.65	1.99	1.99	28	3.21	1.99	1.99	36
ACC 714	2.00	1.99	1.99	22	2.18	1.99	1.99	24
CODMLB 033	3.89	2.33	4.33	43	5.87	4.32	4.32	62
Roba 1	2.33	2.33	2.33	26	3.26	2.32	4.32	37
SMC 21	3.66	1.99	1.99	41	6.40	6.99	6.99	67
SEMC 16	4.76	4.49	4.33	52	5.24	5.82	6.99	63
SMC 18	3.68	2.33	1.99	41	5.58	4.32	4.32	53
SEMC 17	4.96	4.66	4.33	55	6.81	6.99	6.99	75
GLP 2								
CAL 96-Low Fe check susceptible check	7.56	8.99	8.99	86	8.61	8.99	8.99	96
DOR 500-Low Universal check	3.22	2.33	4.33	36	5.57	4.32	4.32	63
Maharagi soya	3.54	1.99	1.99	40	3.36	2.32	2.32	37
MBC32	5.45	6.66	6.66	61	6.60	6.16	8.99	73
Nyiramogorori2	2.03	1.99	1.99	23	2.01	1.99	1.99	22

DI=Disease Index.

Table 5: Means, modes and medians values for both beans *Fusarium* and *Pythium* root rot.

Forest black soil, lake sandy and decomposed farm yard manure were mixed in a ratio of 3:1:1 (currently used by CIAT) and was used in the screening. Test beans were planted in 5 L buckets and arranged in a RCBD with 3 replicates. Twenty-one (21) days after planting, bean leaves were inoculated using a hand sprayer, by misting inoculum onto lower surfaces of leaves when beans had developed two trifoliolate leaves. Plants were thereafter covered with polyethylene bags maintained that way for three days.

On the appearance of ALS symptoms, the disease severity was assessed every three days for seven times. Four to five plants were evaluated per variety per replication. For the Andean isolate, genotypes CAL96 and MEX54 were included in the trial as susceptible and resistant checks respectively. MCM5001 and BAT332 were used as susceptible and resistant checks respectively for Meso-American isolate. The disease was rated according to the CIAT 1 to 9 scales CIAT [8] and the data were subjected to ANOVA using Genstat 14th edition.

Data analysis

Area under Disease Progress Curve (AUDPC) based on mean, was

calculated for ALS while the mean mode, median and disease index [9] were used for *Pythium* and *Fusarium* root rot.

The AUDPC value for each genotype was calculated by trapezoidal integration [10] and is given by: $AUDPC = \sum ((X_i + X_{i+1}) / 2) (t_{i+1} - t_i)$ in which: X_i and X_{i+1} is the disease severity for two consecutive assessments, and $t_{i+1} - t_i$ the interval between two consecutive assessments [11]. The disease index for each variety was calculated according to Kobriger et al. [9] as: Disease Index = $(\sum(\text{disease class} \times \text{number of plants in class}) / ((\text{total plants}) \times 9) \times 100)$ Statistical Analysis was performed by the ANOVA statistical procedure of Genstat GenStat 14th Edition.

Results

Angular leaf spot (ALS)

Tested varieties had a significant differences on resistance to ALS for both Andean and Mesoamerican isolates ($P < 0.001$) (Table 2).

However, genotypes with the same letter were not significantly different (Table 3). Fourteen genotype including MAC 42, NUV 219-

1, MAC 44, VRA 4, RWV 2070, KAB06F2.8-27, NUA 99, NUA 69, KAB06F2.8-12, RWR 2245, NUA 59, ACC 714, CODMLB 033, MBC32, and resistant checks (Mex54/BAT332) were identified as resistant to both Andean and Meso-American bean angular leaf spot isolates.

Root rot

A high and significant ($P < 0.001$) difference in resistance to *Fusarium* root rot and *Pythium* root rot isolates was observed among the tested genotype (Table 4). However, the means, modes and medians for both *Fusarium* and *Pythium* root rot isolates were not significantly different (Table 5). Seven genotype; Rugandura, Kiangara, Decelaya 1, Gitanga, Zebra, ACC 714, Nyiramogorori2 were identified as resistant varieties to both *Fusarium* root rot and *Pythium* root rot.

Discussion

In this study, genotypes that make up the regional nutritional nursery were screened to identify new and better sources of resistance to both bean angular leaf spot and root rot diseases. Genotypes exhibited different reactions to the different diseases. Only ACC 714 exhibited resistance to both Andean and MesoAmerican isolates of bean angular leaf spot and also to *Fusarium* and *Pythium* root rot. This genotype is highly recommended for both nutritional and multi-resistance breeding program. The results also indicate that resistance to different pathogens can be pyramided into appropriate background to provide broad spectrum resistance in iron and zinc biofortified bean genotypes.

The results of this study showed that some of the nutritional bean genotypes have good levels of resistance to a particular pathogen. In 2007, Wagara and Kimani [12] reported that some of the nutrient rich bean varieties evaluated in Kenya possessed good level of resistance to major diseases occurring in farmer fields. For example, they reported that Kiangara had high to moderate resistance to major biotic constraints in Kenya. In the current study, Kiangara exhibited an intermediate level of resistance to both ALS and root rot. Results suggest that selecting biofortified beans for broad resistance to diseases in plant breeding programs is possible; and that this can result in significant amounts of genetic resources for multiple purposes with minimal resources. Thus, our results support the fact that breeding for higher iron as well as high zinc content and multiple resistance in common beans could contribute significantly to improving the life status of individuals dependent on beans as staple foods [13].

Conclusion and Recommendations

The study revealed variations among the screened nutritional bean varieties according to resistance to different pathogens. This implied the potential for utilization of some of these varieties to pyramid useful disease resistance and high Fe and Zn content quantitative trait loci into appropriate background to provide durable resistance in bean genotypes higher in iron and zinc content. The variety ACC 714 was attributed to a broad resistance among 57 genotypes screened for bean angular leaf spot (*Pseudocercospora griseola*) and bean root rot (*Pythium ultimum* and *Fusarium solani* fsp. *phaseoli*) under inoculation in the screenhouse. Efforts should be made to promote selection of genotypes that combine high capacity to accumulate high iron and zinc content in their seed and high level of resistance to major stresses.

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