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 Cosatic 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 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 includes 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 with 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 Kiruzo Rwanda 38 Ngwaku-Ngwaku. RDC
10 RWV1129 Rwanda 39 NUA 45 CIAT
11 Ndimirakagju vulgaris Rwanda 40 NUA 59 CIAT
12 Icyna 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 ACC 714 CIAT
16 RWV 2887 Rwanda 45 CODMLB 033 CIAT
17 MAC 74 Rwanda 46 ROBA 1 CIAT
18 Agronome 2 Rwanda 47 Zebra CIAT
19 VRA 4 CIAT 48 SEMC 16 CIAT
20 Rugandura Rwanda 49 SEMC 16 CIAT
21 RWV 2070 Rwanda 50 SEMC 17 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 Nyiramogori2 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 DF | MS | MesoAmerican Isolate DF | MS |
|--------|-------------------|----|-------------------------|----|
| Rep    | 2                 | 707.77 | 2                     | 701.30 |
| Genotype | 51       | 764.48*** | 55                   | 1227.20 *** |
| Residual | 95       | 70.05   | 101                   | 364.20  |
| Total  | 148               | 324.86  | 158                   | 668.80  |
| CV     | 13.49             | 29.07   | 65.64                  |        |
| Mean   | 62.02             | 30.91   |                        |        |

***Significant at p ≤ 0.001.

Table 2: ALS Anden 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]. Acccording 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 × CAB2/3/3/11       | 75.0                     | mno   | 63.2                            | Bcdefghijklmno |
| Ngwin × CAB2(RWV3317)     | 75.2                     | mno   | 81.6                            | Jklnmnopqrst  |
| MAC 42                    | 29.0                     | ab    | 40.4                            | Abcd          |
| RWV 3316                  | 75.6                     | no    | 93.2                            | Opqrst        |
| NUV 219 1                 | 51.7                     | efg   | 51.3                            | Abcdefghijkl  |
| CAB 2                     | -                        | o     | 39.5                            | Abcd          |
| RWV 2359                  | 76.6                     | o     | 51.4                            | Abcdefghijklmno |
| Garukurare                | 75.4                     | mno   | 64.6                            | Bcdefghijklmno |
| Kivu zo                   | 73.7                     | lmn o | 103.5                           | Rpqrst        |
| RWV/1129                  | 75.4                     | mno   | 53.5                            | Abcdefghijklmno |
| Ndimirakaguya vol          | 75.6                     | no    | 78.4                            | Hjiklmnopqrst |
| Icyana 2                  | 73.0                     | kim   | 87.8                            | Mnopqrst      |
| RWV 2361                  | 73.0                     | kim   | 74.4                            | Fghijklmnopqrst |
| MAC 44                    | 60.5                     | fgh   | 54.0                            | Abcdefghijklmno |
| RWV 3006                  | 64.6                     | hijk   | 44.8                            | Abcdefghijklmno |
| RWV 2887                  | 76.0                     | o     | 91.6                            | Nopqrst       |
| MAC 74                    | 65.5                     | ijk   | 50.6                            | Abcdefghijklmno |
| Agronome 2                | 72.7                     | knm   | 87.0                            | Mnopqrst      |
| VRA 4                     | 35.2                     | abcd  | 42.9                            | Abcd          |
| Rugandura                 | 60.5                     | fgh   | 85.5                            | Lmnopqrst     |
| RWV 2070                  | 23.5                     | a     | 35.5                            | Ab             |
| Kiangara                  | 72.1                     | knm   | 55.5                            | Abcdefghijklmno |
| VCB 81013                 | 75.0                     | mno   | 62.2                            | Bcdefghijklmno |
| 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                            | Qqrst         |
| Decelaya 1-Low Fe Check   | -                        | -     | 50.0                            | Abcdefghijklmno |
| KAB06F2.8-27              | 30.5                     | ab    | 59.2                            | Bcdefghijklmno |
| NUA 99                    | 48.4                     | def   | 44.0                            | Abcdefghijklmno |
| NUA 69                    | 34.5                     | abc   | 45.3                            | Abcdefghijklmno |
| KAB06F2.8-12              | 32.5                     | ab    | 49.0                            | Abcdefghijklmno |
| RWR 2154                  | 73.0                     | knm   | 106.7                           | T             |
| RWR 2245                  | 56.1                     | fgh   | 53.7                            | Abcdefghijklmno |
| KAB06F2.8-36              | 55.4                     | fghi  | 105.7                           | St            |
| KAB06F8.8-35              | 51.0                     | efg   | 78.4                            | Hjiklmnopqrst |
| CODMLB 001                | 74.2                     | mno   | 72.3                            | Efgijklmnopqrst |
| HM 21-7                   | 71.0                     | knm   | 52.5                            | Abcdefghijklmno |
| Ngwaku-Ngwaku             | 73.6                     | lmn   | 54.2                            | Abcdefghijklmno |
| NUA 45                    | -                        | -     | -                               | -             |
| NUA 59                    | 41.0                     | bcode | 52.0                            | Abcdefghijklmno |
| NUA 56                    | 59.6                     | fgh   | 70.9                            | Dfghijklmnopqrst |
| NUA 35                    | 67.5                     | iklmno | 62.8                          | Bcdefghijklmno |
| Gitanga                   | 69.4                     | knm   | 67.8                            | Cdefghijklmnopqrst |
| Zebra                     | 74.2                     | mno   | 106.9                           | T             |
| ACC 714                   | 31.5                     | ab    | 49.4                            | Abcdefghijklmno |
| CODMLB 033                | 35.1                     | abcd  | 43.7                            | Abcdefghijklmno |
| Roba 1                    | 75.2                     | mno   | 41.0                            | Abcd          |
| SMC 21                    | 76.2                     | o     | 65.9                            | Bcdefghijklmno |
| SEMC 16                   | -                        | -     | 55.7                            | Abcdefghijklmno |
| SMC 18                    | 75.5                     | mno   | 73.2                            | Efgijklmnopqrst |
| SEMC 17                   | 62.2                     | ghlk   | 75.0                            | Ghihijklmnopqrst |
| GLP 2                     | -                        | -     | 85.0                            | Klnmnopqrst   |
| CAL 96-Low Fe check susceptible check | 75.1                     | mno   | 90.7                            | Nopqrst       |
| DOR 500-Low Universal check | 74.2                     | mno   | 70.7                            | Defghijklmnopqrst |
| Maharagi soya             | 66.9                     | ijk   | 50.2                            | Abcdefghijklmno |
| MBC32                     | 47.2                     | cdef  | 48.9                            | Abcdefghijklmno |
| Nyiramugorizi             | 75.0                     | mno   | 80.9                            | Ijklnmnopqrst |
| LSD                       | 13.6                     | -     | 30.9                            |               |

Table 3: AUDPC values for Andean and Meso American isolates on tested genotypes.
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    | 2.84   | 1.99   | 1.99 | 29    |
| Kivuzo                                        | 5.71   | 6.66   | 6.66 | 62    | 3.74   | 2.16   | 1.99 | 42    |
| RWV1129                                       | 6.51   | 8.99   | 8.99 | 96    | 8.99   | 8.99   | 8.99 | 100   |
| Ndimirakagujia 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 | 46    | 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    |
Table 5: Means, modes and medians values for both beans Fusarium and Pythium root rot.

| Variety         | Mean Mode | Median | Disease Index |
|-----------------|-----------|--------|---------------|
| CAL96-Low Fe check | 5.87     | 4.32   | 4.32          |
| DOR 500-Low Universal check | 3.68 | 2.33   | 4.32          |
| Maharagi soya    | 4.32     | 2.33   | 4.32          |
| MBC32            | 5.45     | 6.66   | 8.99          |
| Nyiramogorori2   | 6.91     | 6.91   | 8.99          |
| GLP 2            | 6.91     | 6.91   | 8.99          |

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: \( \text{AUDPC} = \frac{1}{2} (X_0 + X_n) \times (T_{n} - T_0) \) in which: \( X_0 \) and \( X_n \) is the disease severity for two consecutive assessments, and \( T_{n} - T_0 \), the interval between two consecutive assessments [11]. The disease index for each variety was calculated according to Kobriger et al. [9] as: Disease Index = \( \frac{1}{9} \sum (disease \ class \times \ number \ of \ plants \ in \ class) \times 100 \) Statistical Analysis was performed by the ANOVA statistical procedure of Genstat GenStat 14th Edition.

Results

Augural 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-
to both Fusarium Zebra, ACC 714, Nyiramogorori2 were identified as resistant varieties (Table 5). Seven genotype; Rugandura, Kiangara, Decelaya 1, Gitanga, Fusarium genotype (Table 4). However, the means, modes and medians for both root rot and Pythium root rot isolates was observed among the tested nursery were screened to identify new and better sources of resistance to bean angular leaf spot and also to 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 Meso-American 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 identified in 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 on 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 (Psuedocercospora 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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