

# Identification of sources of resistance to Fusarium root rot among selected common bean lines in Uganda

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## 1 SUMMARY

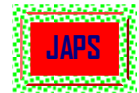
Fusarium root rot (*Fusarium solani* f.sp. *phaseoli*) is one of the most important diseases affecting common beans (*Phaseolus vulgaris* L.). Trials to identify sources of resistance to the pathogen were conducted on 147 bean lines at the National Agricultural Research Laboratories (NARL) at Kawanda in Uganda. The bean lines were from various sources including Uganda, South Africa and the International Centre for Tropical Agriculture in Colombia. A pathogen isolate, FSP-3, was used to produce inoculum for screen house trials through which 46 moderately resistant cultivars were identified. The 46 cultivars were subsequently evaluated in a bean root rot infested field. Data collected included disease severity, colour of hypocotyls, seed size and grain yield, root and shoot weight. Regression analysis showed that field and screen house FRR disease severity data were highly correlated ( $r = 97\%$ ;  $P \leq 0.01$  at 28 DAP data and  $r = 98\%$ ;  $P \leq 0.01$  at 56 DAP). Genotypes differed significantly ( $P = 0.05$ ) between 3.2 (MLB-49-89A) and 8.9 (Apac Ongori) in screen house trials and 4.3 (MLB-49-89A) and 8.8 (RWR2075) under field conditions. Fifteen cultivars were moderately resistant at 28 days after planting with four maintaining this reaction at 56 days after planting under field conditions. Disease resistance was highest among cultivars previously selected for resistance to Fusarium wilt and Pythium root rot indicating presence of quantitative trait loci (QTLs) modifying resistance to more than one root pathogen in some lines. Large-seeded varieties and varieties with green hypocotyls tended to be more susceptible indicating genetic correlation between these traits and resistance to Fusarium root rot. Results confirm the presence of sources of resistance which could be utilized in improving resistance in commercial varieties.

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## 2 INTRODUCTION

Bean root rots have been reported in bean (*Phaseolus vulgaris* L.) production areas throughout the world (Beebe *et al.*, 1981; Abawi

& Pastor-Corrales, 1990; Park & Tu, 1994). In Uganda, especially in the south-western highland regions, bean root rot is one of the



most serious constraints to bean production (CIAT, 2005), with losses of up to 100% (F. Opio Unpublished, Tusiime, 2003) in Uganda and 70% in Rwanda (Buruchara et al., ) occurring on susceptible varieties. The disease has also emerged as the most important constraint to bean production in western Kenya (Otsyula et al., 1998), some regions of the Republic of Rwanda and the Democratic Republic of Congo, that neighbor south-western Uganda (Buruchara et al., 2001), and even in Malawi (Snapp et al., 2006).

*Fusarium solani* (Mart.) Appel and Wollenv. f. sp. *phaseoli* (Burk.) Snyder & Hans (FSP) that causes Fusarium root rot (FRR) is one of a complex of soil-borne pathogens causing root rots on beans, others being *Pythium* sp, *Rhizoctonia solani* and *Macrophomina phaseoli* (Abawi & Pastor-Corrales, 1990; Rusuku et al., 1997). The pathogen has been reported to be particularly severe on large-seeded bean genotypes due to lack of genetic resistance in these seed types (Beebe et al., 1981; Burke & Miller, 1983; Schneider et al., 2001; Román-Avilés & Kelly, 2005). In addition, resistance to FRR has been associated with small seed size, black seed colour, and purple hypocotyls (Statler, 1970; Beebe, 1981), although these correlations have not been conclusive. The bean improvement programme on BRR in Uganda has been targeting *Pythium* root rot (*Pythium* spp.), because it was found to be the most predominant pathogen in the root rot complex in south-western Uganda (Pyndji, 1996; Mukalazi et al., 2001). However, FSP was found to be predominant, often occurring concurrently with *Pythium* spp. and was also found to even be more destructive in screen house tests (Tusiime, 2003). This indicates the need to address FRR if the BRR problem is to be controlled.

Although several measures have been used to control FRR, none has been effective. BRR management has been possible to some extent only through the use of a combination of

control options (cultural, chemical, and biological) which utilize the concept of Integrated Pest Management (Buruchara et al., 2001; Otsyula et al., 2005; Abawi et al., 2006). However, the single most effective and practical management strategy, especially for the resource poor farmers who make up the greatest proportion of Uganda's population, is the use of bean varieties that are resistant to the most common soil-borne pathogen(s) occurring in the production region (Abawi et al., 2006; Opio et al., 2007). Declining soil fertility levels due to over cultivation as a result of increased human population has led to an imbalance between the beneficial and disease-causing organisms in the soil, hence an increase in root rot pathogen inoculum levels. This is especially characteristic of the highland regions. Bean cultivars that previously tolerated the lower levels of inoculum have since succumbed and none of the commercial bean cultivars currently grown are susceptible to bean root rot (Tusiime, 2003; Kalyebara and Kassozi, 2005; Otsyula, 2005). In 2006, two large seeded red common bean genotypes namely NABE 13 (RWR 1946) and NABE 14 (RWR 2075) tolerant to the *Pythium* root rot and low soil fertility condition were released targeting the mid to high altitude areas of Uganda (PABRA, 2007; CIAT 2008). However, the level of resistance to FRR in these genotypes has not been ascertained. Most of the bean genotypes that have been reported to be resistant to FRR are both late maturing, small-seeded, black in color and originating from the Middle American gene pool (Beebe et al., 1981; Abawi & Pastor-Corrales 1990). These characteristics hinder acceptability and adoption in Africa and Uganda in particular, where most bean farmers prefer large-seeded, red mottled or red colored cultivars with bush growth habit (Mukankusi, 2008). Due to a possibility of gene lethality reported to occur when inter-gene pool crosses are made, cultivars from the Middle American gene pool may not be satisfactory parents in the

improvement of resistance to FRR in large-seeded Andean bean cultivars popular in Uganda (Singh, 2001). A further consideration is that the documented sources of resistance to FRR (Abawi and Pastor Corrales 1990) have not specifically been tested for adaptability and resistance to the prevalent FSP isolates in Uganda. In studying the variability of FSP in Uganda, Tusiime (2003) conducted simple pathogenicity tests on one popular commercial variety, K20 (Rosecocco), in Uganda using 57 FSP isolates collected from Uganda, South Africa, Rwanda, Kenya and the international culture collection centre – Centraalbureau voor Schimmecultures (CBS) in the Netherlands. In addition he evaluated 14 popular bean

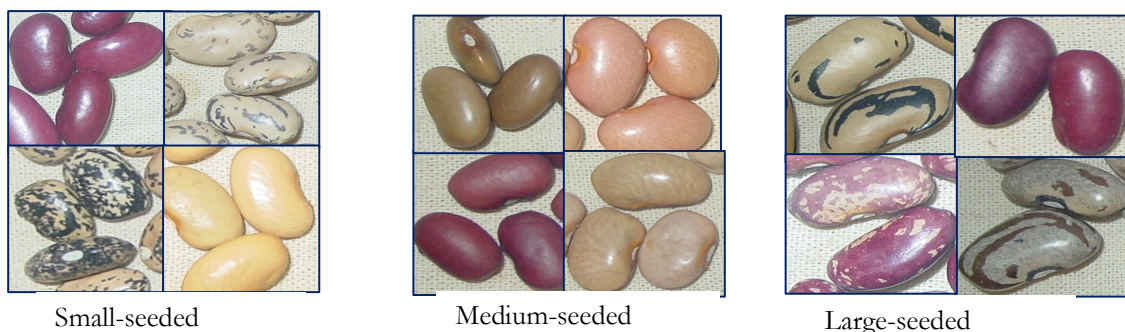
genotypes representing the Andean and Meso-American gene pools, of which none was one of the 41 documented sources of FRR resistance using four selected FSP isolates and found none to be resistant. There is need, therefore, to identify potential sources of resistance adapted to the tropical African environmental conditions.

The present study aimed at identifying sources of resistance to *Fusarium solani* f.sp *phaseoli* for use in improving commercial but susceptible bean cultivars in Uganda. In addition, correlations between FRR resistance and a range of morphological traits were investigated in the materials screened for resistance to FRR.

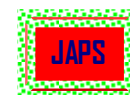
### 3 MATERIALS AND METHODS

**3.1 Genetic materials:** One hundred and forty-four common bean cultivars were assembled and divided into five groups according to their sources. Group 1 (“Pythium Root rot group; PRR”): Included 46 cultivars obtained from CIAT-Africa at NARL in Kawanda. These cultivars had previously been selected for resistance to Pythium root rot (*Pythium ultimum*) and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *phaseoli* (Buruchara & Kimani, 1999; Buruchara & Camacho, 2000; Otsyula et al., 2005). Group 2 included six varieties that are routinely used as are sources of resistance to *F. oxysporum* f. sp. *phaseoli* (FOP) and FOP differential varieties in the Africa region (Abawi and Pastor-Corrales, 1990). Group 3 (“South African group”): Included 31 cultivars were from ARC-Potchefstroom and University of KwaZulu-Natal in

South Africa, Group 4 (“Land race group”): Included 27 Ugandan landraces acquired from the National Crop Resources Research Institute (NaCRRI) in Namulonge, Uganda, and Group 5 (“CIAT group”): Included 43 documented sources of resistance to Fusarium root rot in Colombia (Abawi and Pastor-Corrales, 1990) acquired from CIAT-Colombia. Three commercial but susceptible bean cultivars K20 (Rosecocco), Kanyebeba (landrace), and K132 (CAL96) were used as checks. One cycle of mass selection was conducted in all these materials to remove any off-types and to multiply the seed. The materials consisted of 84 small-seeded cultivars, 24 medium seeded and 39 large-seeded cultivars (Fig 1). Of these, 103 lines had purple hypocotyls and 44 had green hypocotyls.



**Figure 1:** Varying seed sizes and colours of materials evaluated



**3.2 Screen house evaluation:** A *Fusarium solani* f.sp *phaseoli* isolate (FSP-3) obtained from south-western Uganda and isolated at NARL in April, 2005 (Mukankusi, 2008) was used for screening the 147 bean cultivars for resistance to FRR.

**3.2.1 Inoculum preparation:** Pure colonies of the isolate stored on Potato Dextrose Agar (PDA) slants at 5°C were grown on PDA in Petri plates for up to 21 days and used to prepare the disease inoculum. Duran glass bottles (Aldrich, Z305197-10) of 500 ml capacity were partially filled with sorghum seed (2/3 capacity) and 150 ml water added. The bottles were sealed and the contents autoclaved for 1 hour at 120 °C. One plate of the FSP-3 isolate culture was suspended in 4 - 10 ml of sterile and deionised water to make a slurry which was spread evenly onto the surface of the already prepared sorghum medium within the bottles. The bottles were resealed and agitated to mix the slurry with the sterilized sorghum. The mixture was then incubated in the laboratory at 20 – 28 °C for 5d to allow FSP-3 inoculum to grow. The bottles were later opened, but the opening was protected using foil paper to prevent contamination and allow for evaporation of the excess moisture and left to incubate for 21d. After incubation, the bottles were emptied, and the medium slowly dried under room temperature to allow for maturation of the fungal resting spores.

**3.2.2 Soil inoculation:** Wooden trays measuring 0.74m x 0.42m x 0.115m were partially filled (2/3 capacity) with previously sterilized sandy- clay- loam soil collected from a nearby forest. The prepared inoculum was added to the soil at a rate of 500ml of inoculum per tray. A susceptible bean cultivar, CAL96, was planted in the trays and grown for a period of 24-28 days and then uprooted. This served to increase the disease inoculum to 3000-4000 spores/ gm of soil as well as serve as an indicator (Tusiime, 2003) of the inoculum levels in the soil. The infested soil was mixed with fresh soil (2: 1) after every evaluation as a way of diluting the inoculum before the subsequent planting. Each of the five groups was screened separately at different times.

**3.2.3 Trial layout:** The trials were laid out as randomized complete block design (RCBD) with

three replicates (three trays) of 20 plants per replicate (tray) per bean cultivar (total of 60 plants). A replicate was a wooden tray that was planted with five bean cultivars at a time, with each bean cultivar planted in two rows of ten plants each. A susceptible (CAL96) and resistant check (MLB-49-89A) resistant to *Pythium ultimum* was planted in each tray and evaluated at 28 days after planting (DAP). The trials were watered 0-2 times daily depending on the intensity of the sunshine and amount of rainfall. Each trial was repeated once.

**3.2.4 Data recording:** Plant stand per bean cultivar was recorded as the number of standing plants at the time of evaluation. Disease incidence was obtained by uprooting all the standing plants per bean cultivar per replicate and counting the number of plants that exhibited FRR symptoms. This number was expressed as a percentage of the number of plants assessed. FRR severity was assessed by washing the below ground parts of the plant (hypocotyl and roots) under running tap water.

The levels of infection on the roots and hypocotyls were observed, and disease severity assessed based on the 1-9 scale developed at CIAT (Abawi & Pastor-Corrales, 1990) as:

1 = no visible symptoms

3 = light discoloration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions

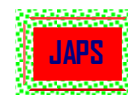
5 = approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm, with some deterioration of the root system

7 = approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting, and reduction of root system

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.

**3.2.5 Data analysis:** Averages were computed per bean cultivar, and the data were analysed using the Genstat computer programme (Payne *et al.*, 2007). Bean cultivars were grouped into five classes





based on the severity scores of the disease as: tolerant/resistant reaction = 1-3; moderately resistant = 3.1-5; moderately susceptible = 5.1-6; susceptible = 6.1-7.9; and very susceptible = 8-9. Simple frequency distributions to assess correlations between FRR severity and seed size and FRR severity and hypocotyl color, and FRR and growth habit were done.

**3.3 Field evaluation:** To confirm resistance, 30 cultivars classified as moderately resistant under screen house conditions, and 16 cultivars that were classified as moderately susceptible and susceptible, including six landraces were screened under field conditions at NARL. Three susceptible cultivars (K20, CAL96 and Kanyeowa) were also included. The field used had a high occurrence of root rot pathogens and is continuously used by the CIAT-Africa breeding programme as a bean root rot hot-spot.

**3.3.1 Trial layout:** The trial was laid out as a 7 x 7 lattice square design with three replicates during the short rainy season of 2005 (August-October) and long rain season of 2006 (March-June). The plot was fertilized one week before planting by applying 55 kg N ha<sup>-1</sup>, 66 kg P ha<sup>-1</sup> and 55 kg K ha<sup>-1</sup>. The beans were grown under natural inoculum, but 500gms of the FSP infected soil (3000-4000 spores/gm of soil) that was being used in the screen house was placed in each planting hole at planting time, as a means of increasing the levels of FSP-3 in the soil relative to other soil-borne pathogens if they were present. Each bean cultivar was planted

in 5 rows with each row having 10 plants with 10cm between plants. Rows were spaced at 0.5m from each other and a 1m space was left between replicates. Hand weeding was done twice at 14d after seedling emergence and just before flowering. No irrigation was needed as the rainfall was adequate.

**3.3.2 Field data recording:** Disease was evaluated at 28 and at 56 DAP. At each evaluation, 10 plants per bean cultivar were randomly uprooted from the three central rows of each plot and disease severity rated as described above. Plant stand at 28 and 56 DAP was calculated as the percentage number of plants standing at 28 DAP and 56 DAP, respectively, divided by the number of plants that emerged.

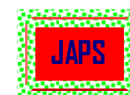
Shoot and root masses were obtained at 28 DAP by separating the uprooted plants into root and shoot portions and drying to constant weight in an oven at 60°C for 24h to obtain shoot and root dry weights. The data were used to compute the shoot: root ratio of the selected cultivars and correlated with FRR severity scores. At maturity, the cultivars were hand harvested, the pods weighed, threshed and seeds weighed to obtain yield data. Yield was calculated as yield per plant, then converted to yield per plot (50 plants), and then to yield per hectare. Disease severity data were transformed using log transformation to obtain least square means and analysed using the general linear model procedure using SAS.

## 4 RESULTS AND DISCUSSION

**4.1 General observations:** Disease evaluations conducted in the screen house were characterised by relatively uniform disease inoculum levels, as one specific isolate of *Fusarium solani* f.sp. *phaseoli* was used, and the technique was simple hence evaluation was rapid. Under field conditions, pests such as bean stem maggot (*Ophiomyia* spp) and probably other soil-borne root rot pathogens (*Pythium* spp. and *Rhizoctonia solani*), and other strains of FSP could have influenced the performance of the cultivars. However, selection under field conditions remains important as it tested the adaptability of the different cultivars to prevailing environmental factors since pathogens do not affect the crop in isolation under field

conditions. In general, the field and screen house disease severity data were highly correlated ( $r=97\%$ ;  $P\leq 0.01$  for the 28 DAP data and  $r=98\%$ ;  $P\leq 0.01$  for the 56 DAP), implying that selection of resistant cultivars may be based on either trial or on both.

**4.2 Disease incidence and severity under screen house conditions:** For all groups, plant stand at 28 DAP was not significantly affected at  $P\leq 0.05$  by FRR at the time of disease evaluation with entries behaving similarly. After 28 DAP, all cultivars, including the resistant check (MLB-49-89A), showed symptoms of FRR but at varying disease severity levels (3.1 - 9 on a 1-9 scale) indicating a lack of immunity to the disease for all



the bean cultivars. Disease severity varied significantly ( $P \leq 0.05$ ) among the 147 bean entries (Table 1). Since there were no significant differences at  $P \leq 0.05$  between disease severity levels on the cultivars between the repetitions of the trials for all the groups, the means of the trials are presented. None of the entries had a mean score  $\leq$

3 on the 1-9 scale; 9.5% had disease severity between 3.1 and 4, while approximately 70% of the entries had disease severities scores of 4.1-7, and 21% had disease severity  $>7$ . The distribution was continuous in nature, which is typical of a quantitative trait (Falconer and Mackay, 1996).

**Table 1:** Analysis of Fusarium root rot severity on 147 common bean (*Phaseolus vulgaris* L.) lines under screen house conditions at Kawanda, Uganda.

| Group             | DF | Plant stand (%) | Mean Squares (FRR severity) |           | Mean FRR Severity |           | SED ( $P=0.05$ ) |           | CV (%)  |           |
|-------------------|----|-----------------|-----------------------------|-----------|-------------------|-----------|------------------|-----------|---------|-----------|
|                   |    |                 | % Scale                     | 1-9 Scale | % Scale           | 1-9 Scale | % Scale          | 1-9 Scale | % Scale | 1-9 Scale |
| CIAT varieties    | 35 | 936.7**         | 326.4*                      | 1.7214**  | 38.5              | 5.5       | 7.0              | 0.7       | 31.4    | 14.8      |
| Landraces         | 28 | NS              | 633.3**                     | 3.5419**  | 42.0              | 7.0       | 5.2              | 0.6       | 36.6    | 19.8      |
| PRR group         | 47 | 1031.2**        | 744.4**                     | 8.263**   | 42.9              | 5.7       | 8.9              | 0.8       | 46.9    | 26.9      |
| South African     | 31 | 2963.5**        | 2301.5**                    | 5.63**    | 44.6              | 5.7       | 6.8              | 0.5       | 31.3    | 18.2      |
| FOP differentials | 6  | 2115.9**        | 676.7**                     | 4.923**   | 38.8              | 5.9       | 4.2              | 0.3       | 25.6    | 12.1      |

DF=Degrees of Freedom; FRR = Fusarium root rot; SED=Standard Error of difference between means; CV= Coefficient of Variation for the means

In the Pythium root rot group, FRR severity scores ranged from 3.2 for MLB-49-89A to 8.4 for DOR 622. Seventeen cultivars in this group were classified as moderately resistant (MR) to FRR, 14 as moderately susceptible (MS), 13 as susceptible (S), and four cultivars as very susceptible (VS). In the South African group, the varieties ranged from moderately resistant to susceptible, with disease severity scores ranging from 3.8 for PAN185 to 7.4 for Mkomazi. Fourteen cultivars were classified as moderately resistant, 10 as moderately susceptible, and 7 as susceptible.

In the CIAT group, disease severity ranged from 4.3 (G1459) to 6.4 for G4789 and G5533. Nine cultivars were classified as moderately resistant, 21 as moderately susceptible and 11 as susceptible. This group, which consisted of documented sources of resistance to FRR, did not exhibit as much tolerance to the FSP-3 isolate as expected, with most of the cultivars exhibiting moderately susceptible to susceptible reactions. This shows that there is variation in the FSP isolates across the world probably indicating that the FSP-3 isolate used in this study does not exist in

Colombia. This finding also highlights the need to select for resistance to Fusarium root rot in the targeted environment possessing specific FSP isolates. In the landrace group, severity ranged from 5.1 on Hoima-Kaki to 8.9 on Apac Ongori. Four cultivars were classified as moderately susceptible, 18 were classified as susceptible and four as very susceptible. None of the landraces was classified as moderately resistant, indicating the low levels of resistance to FRR in bean cultivars commonly grown by farmers in Uganda. This further highlights the need to improve resistance in the most popular varieties. Among the *F. oxysporum* differentials, severity scores ranged between 3.6 and 6.3 on the cultivars HF-465-63-1 and IPA-1, respectively, and four of these cultivars were classified as moderately resistant.

In all the groups, the susceptible checks had the highest FRR severity, apart from the landrace group where more than 50% of the landraces had a severity rating higher than that of K20 the local susceptible check. Most of the best performing cultivars, i.e., resistant and moderately resistant, were from PRR group which included

lines that had been previously selected for resistance to Fusarium wilt and Pythium root rot. The higher levels of resistance in this group suggest that these cultivars could also have been indirectly selected for FRR resistance because soil-borne pathogens are known to occur concurrently (Sippel & Hall, 1982). This finding also suggests the presence of quantitative trait loci (QTLs) conditioning resistance to more than one root pathogen in specific bean cultivars.

However, several cultivars from the PRR group also exhibited susceptible reactions to FRR indicating that they could be resistant to Pythium root rot but not to FRR, suggesting that the

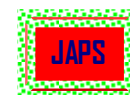
mechanisms of resistance to these two root pathogens are different. From all the groups, 44 cultivars were classified as moderately resistant to FRR, and of these, MLB-49-89A was the most resistant. Ten of the moderately resistant cultivars were large-seeded, of which six were red kidney/Calima types, that is, MLB-17-89A, MLB 22-88B, RWR 1058, GLP 24, RWR 1092, and RWR 2075 (NABE 14) the recently released variety, while four were red speckled sugar beans and included RS5, PAN 148, PAN 128 and PAN 159. Three moderately resistant cultivars were medium-seeded and included MLB-49-89A (black seeded), Umubano (red) and PAN 146 (red speckled).



**Figure 2:** Variation in levels of infection on different bean lines.

**4.3 Disease severity and incidence under field conditions:** In this study, FRR severity ratings, plant stand at 28 and 56 DAP, root weight and root: shoot ratio were significantly different ( $P=0.01$ ) among the 49 cultivars (Table 2) under field conditions. Plant stand at 28 DAP ranged from 6.8% on Kiboga-Yellow to 63.8% on Hoima-Kaki.

At 56 DAP, plant stand ranged from 0.0% for Kiboga-Yellow, Timbavati and Elangeni to 42.7% on PAN 150. The varieties G3717, CIM 9313-1 and PAN 150 maintained their plant stands after 28DAP compared to other cultivars. FRR severity scores ranged from 4.3 on Kabale-White and MLB-48-89A to 8.8 on RWR2075.

**Table 2:** Analysis of 49 bean cultivars' resistance to isolate FSP-3 of *Fusarium solani* f.sp. *phaseoli* under field conditions at Kawanda Agricultural Research Institute, Uganda.

| Conditions at Kawanda Agricultural Research Institute, Uganda. |     |                |               |        |             |         |                |                         |                              |
|--|-----|----------------|---------------|--------|-------------|---------|----------------|-------------------------|------------------------------|
| Source   | df  | Mean squares   |               |        |             |         |                |                         |                              |
|  |     | %<br>emergence | FRR severity* |        | Plant stand |         | Root<br>weight | Root:<br>Shoot<br>ratio | Yield kg<br>ha <sup>-1</sup> |
|  |     |                | 28DAP         | 56DAP  | 28DAP       | 56DAP   |                |                         |                              |
| Reps   | 2   |                |               |        |             |         |                |                         |                              |
| Entries  | 48  | 619.60**       | 2.59**        | 2.11** | 687.6**     | 127.06* | 0.085**        | 11.09**                 | 437028.0**                   |
| Error  | 96  | 362.8          | 1.46          | 2.01   | 132.9       | 72.59   | 0.04           | 4.074                   | 81720                        |
|  | 146 |                |               |        |             |         |                |                         |                              |

\* 1-9 scale data

Five cultivars had disease severity scores ranging between 4 and 5, while three had severity scores of 5-6 (Table 3). All these cultivars were considered resistant to the root rot pathogens that occurred, as well as being adaptable, especially the CIAT cultivars G3717, G1459 and G4795.

Disease severity scores ranged between 3.8 for Hoima-Kaki to 8.2 for RWR868, at 28 DAP. Fifteen bean cultivars had low disease severity under field conditions at 28 DAP with scores of  $\leq 5$  (Table 3). Twenty three cultivars had disease severity greater than the local susceptible checks. At 56 DAP, most of the varieties succumbed to FRR infection, however, the ranking was not affected and was highly correlated to the ranking done at 28 DAP. The cultivars MLB-48-89A, Hoima-Kaki, G3717 and MLB-49-89A maintained their low FRR severity both at 28 and at 56 DAP, while MLB-48-89A had an even lower severity score at 56 than at 28 DAP. Similarly, cultivars G1459, G4795, RIZ 30, PAN128, Mbarara Kanye bwa and Kabale-White had lower *Fusarium* root rot severity at 56 DAP compared to their disease scores at 28 DAP (Table 3). These cultivars showed good adaptability as well as tolerance to the constraints that occurred, including FRR.

Time-course changes in plant performance have been shown to affect the level of resistance to FRR. Cultivars that appeared to have similar levels of resistance at a young stage differed dramatically at an older stage indicating that resistance of seedlings may not reflect resistance in older plants (Hall & Phillips, 2004). This could probably be due to the difficulty in classifying plants late in the season because the hypocotyls are completely covered with lesions making it necessary to score

on the basis of the depth rather than percentage of infection (Hassan et al., 1971). However, it should be noted that the ratings in the field were in some cases overestimated because they were confounded with the occurrence of other root rot pathogens, especially *Pythium* spp. as well as the effect of bean stem maggot. This made field screening difficult, especially since the target pathogen in the screen house was a single isolate of FSP. It was interesting to note that even if the local landrace Hoima kaki was classified as moderately susceptible under screen house conditions, it was one of the best performers under field conditions highlighting its high level of adaptability and tolerance to the prevailing environmental and biotic constraints in Uganda.

**4.4 Relationship between resistance and agronomic traits:** Generally grain yield was low, ranging from 2-1151 kg ha<sup>-1</sup> (Table 3). Twenty-two cultivars had yields lower than 500 kg ha<sup>-1</sup> while 23 cultivars had yields between 500 to 1,000 kg ha<sup>-1</sup> and two varieties had yields above 1000 kg ha<sup>-1</sup>. The cultivar Elangeni, had the highest yield, followed by 1/MS/11-1, IPA 1, Kabale-White, G3717, and CIM 9313-1. Even though the local susceptible checks and the landraces were adapted to the environment in Uganda, they produced low yields with K20 having the lowest yield of 2 kg ha<sup>-1</sup>. The exception was Kabale-White (955 kg ha<sup>-1</sup>) and Hoima-kaki (733 kg ha<sup>-1</sup>) which had relatively good yields compared to the other cultivars, showing their adaptability as well as tolerance to root rot under field conditions (Table 3). Generally, FRR severity at 56 DAP affected yield ( $r = -0.21$ ;  $P \leq 0.05$ ) more than severity at 28 DAP ( $r = -0.06$ ;  $P \leq 0.05$ ). However, it should be noted that ratings done later in the crop cycle were

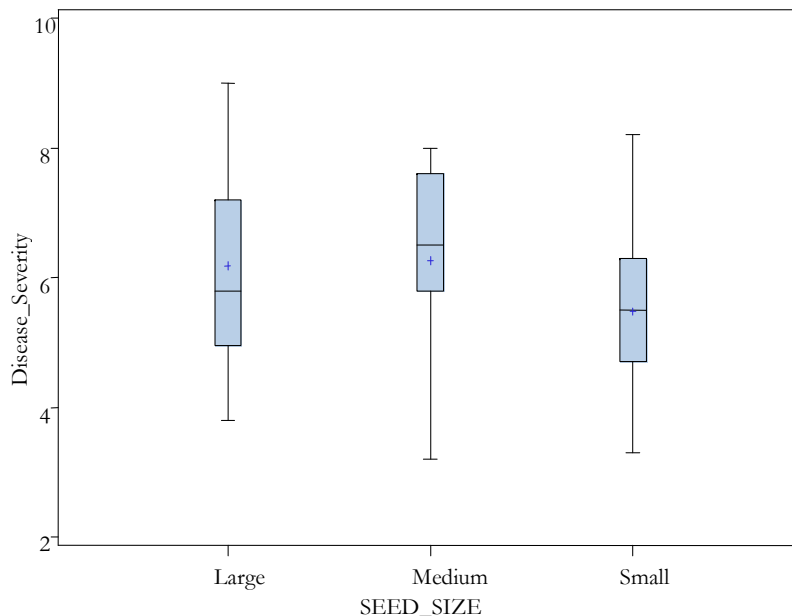


highly confounded by many other soil inhabiting pathogens, as well as bean stem maggot. Due to these factors the correlation between Fusarium root rot and yield could not be ascertained; however, several well adapted cultivars were identified. In addition, the yield data were difficult to interpret because many genotypes were probably not adapted to the tropical climate, and hence were lower yielding.

Root weight and root: shoot ratio was not significantly correlated at  $P \leq 0.05$  to FRR severity for these cultivars as many cultivars which were relatively resistant had small root masses as well as low root to shoot ratios as exemplified by MLB-49-89A, Umubano, and Vuninkingi (Table 1). It has often been suggested that a vigorous root system increases tolerance to root rot (Snapp et al., 2003; Román-Avilès et al., 2004a and b), however, root: shoot weight ratio was not statistically correlated with FRR severity in this study.

Fusarium root rot resistance has been associated with small seed size, as large-seeded bean genotypes are more susceptible (Schneider et al., 2001; Román-Avilès & Kelly, 2005). In this study, the relationship between seed size and FRR was

not statistically tested, however, there was a trend indicating that more of the small-seeded varieties tended to be more resistant to the root rot pathogen than their larger seeded counterparts (Figure 3). The proportion of cultivars with disease severity scores of 3-3.9 was greatest for the small-seeded bean varieties that is, 66.7% small-seeded, 16.7% medium-seeded and 16.7% large-seeded (Figure 2). Similarly, most of the cultivars with disease severity scores of 4-6.9 were small-seeded, that is, 54%, while 30% were large-seeded and 16% were medium-seeded. However, in the classification 7.0-9 and 8.0-9.0 disease scores, 50 and 56% of the varieties were large-seeded, respectively, while the small-seeded made up 27 and 22%, respectively, in these disease classifications (Figure 4). These results are not conclusive on the relationship between seed size and resistance to FRR, as the sample size of large-seeded and medium-seeded varieties was much lower than that of the small-seeded varieties. However, the trend showed skewedness to the susceptible side for the large-seeded varieties, and skewedness to the resistant side for the small-seeded varieties.

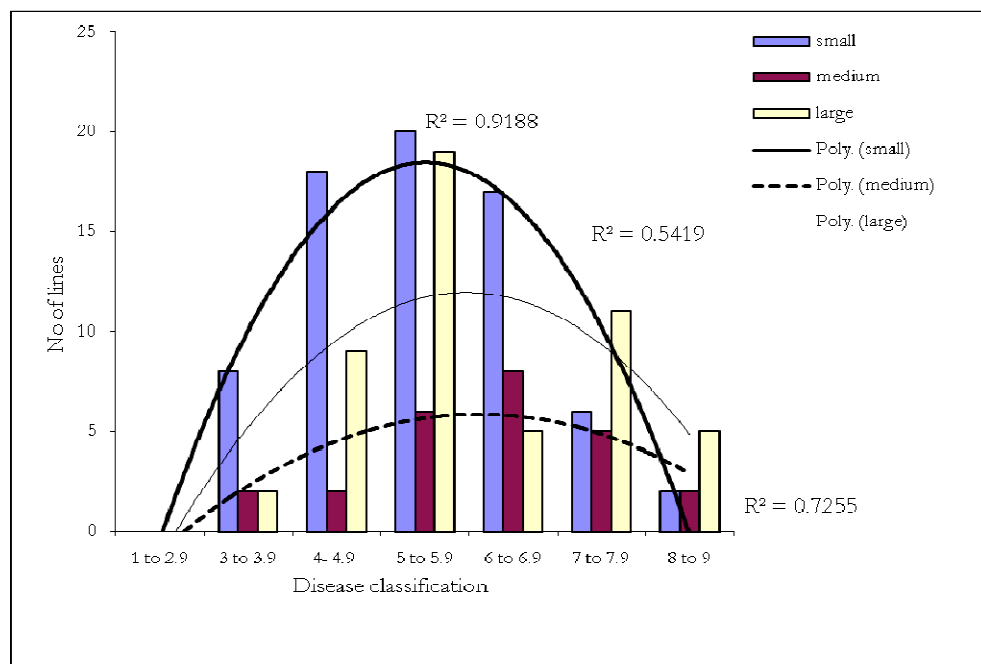


**Figure 3:** Box plot showing skewedness of different seed sizes in relation to FRR severity

Most of the small-seeded varieties are from Middle-American gene pool posing a problem in introgression of resistance into Andean bean types. However, they have been utilised successfully in some breeding programs. For example, Silbernagel (1987) developed a resistant large-seeded cultivar, FR266, belonging to the Andean gene pool, using a small and black seeded bean cultivar, N203, from the Middle American gene pool as the resistance source. Schneider et al. (2001) further used cultivar FR266 successfully in crosses with beans from the Andean gene pool for improvement of resistance to FRR, suggesting a possibility of introgressing resistance genes from the small-seeded Middle American gene pool cultivars into the large-seeded Andean bean seed types.

A large number of varieties with disease severity scores of 3-3.9 had purple hypocotyls (67%). For all the other disease severity categories, the varieties with green hypocotyls had the highest percentages.

None of the purple coloured varieties had severity scores greater than 7.9 (Figure 3). Generally, both groups showed an almost normal distribution of disease scores ( $R^2 = 81$  and  $87\%$  for varieties with green and those with purple hypocotyls, respectively). However, the distribution of FRR severity on the varieties with purple hypocotyls was skewed to the resistant side while that of the varieties with green hypocotyls was skewed to the susceptible side (Figure 4). This observation supports previous studies which showed that the color of seed and hypocotyls was related to the level of resistance to FRR. For example, Statler (1970) observed higher resistance to FRR in black seeded varieties and varieties with purple coloured hypocotyls and related the pigmentation to the greater production of phenolic compounds inhibitory to fungal growth in the early stages of seedling growth.



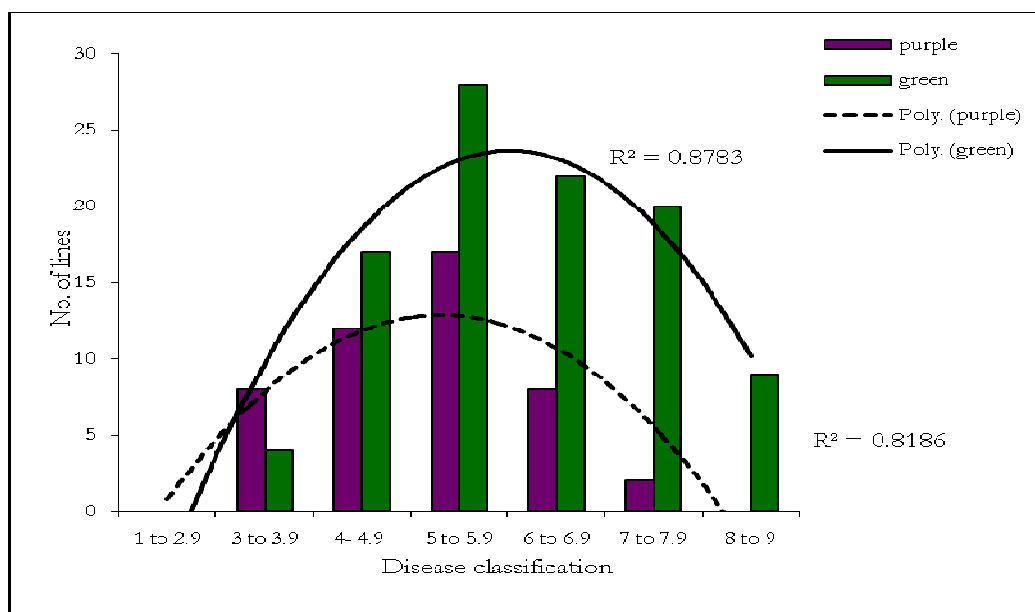
**Figure 4:** Relationship between seed size and resistance to Fusarium root rot in 147 bean lines assessed at Kawanda, Uganda.

Phytoalexins such as phaseolin have been identified and reported as being produced in response to

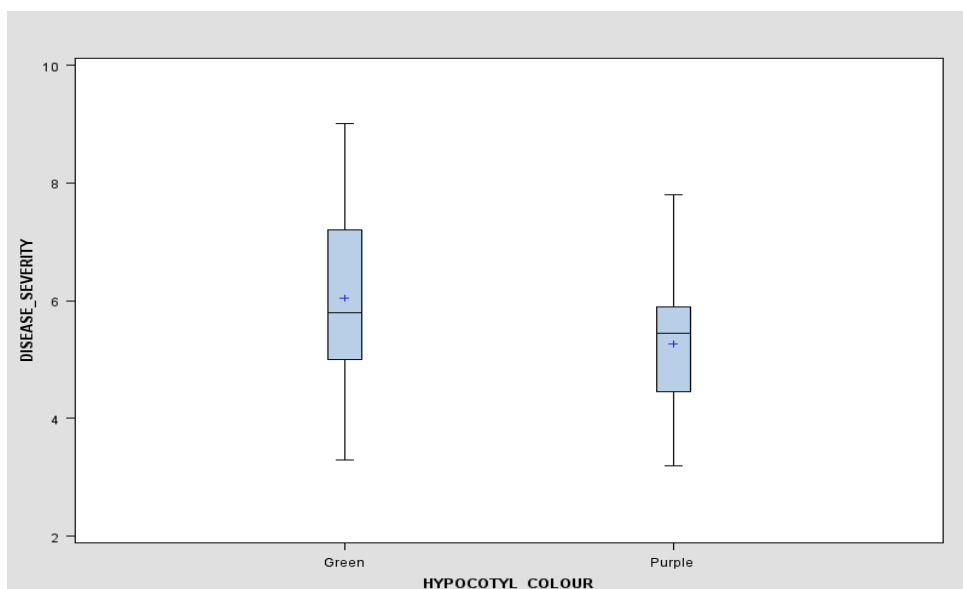
infection by *R. solani* (Pierre & Bateman, 1967) and *Fusarium solani* f.sp. *phaseoli* (Kendra & Hadwiger,

1989). Production of these phytoalexins has been shown to be greater and more rapid in resistant cultivars. Purple-coloured hypocotyls could possibly have higher levels of phytoalexins and hence may

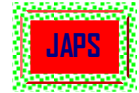
indicate some maternal effects on resistance to FRR. However, these results are not conclusive as the numbers of the green and purple hypocotyl cultivars evaluated in this study was not equal.



**Figure 5:** Relationship between hypocotyl color and Fusarium root rot resistance in 147 bean lines assessed at Kawanda, Uganda.



**Figure 6:** Box plot showing skewedness of hypocotyls color in relation to Fusarium root rot severity



## 5 CONCLUSION

This study showed that there is substantial variation in resistance to FRR but that previously documented sources of resistance to FRR succumbed to the pathogenic isolate FSP-3 in Uganda. This finding highlights the effects of differences in the screening environment and techniques, suggesting the need to identify resistance in the predominant environment and using isolates from the targeted areas. The study identified 15 potential sources of resistance to FRR that may be recommended for use by the Uganda

National Bean Breeding Programme. In addition, the presence of QTLs governing resistance to more than one root pathogen in specific cultivars suggests the possibility of improving resistance to multiple root pathogens if such cultivars are utilised as sources of resistance. Correlations between seed size, hypocotyls colour and resistance to FRR were observed but further large-scale screening with emphasis on these traits is needed to clearly elucidate any putative correlations.

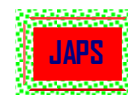
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Agriculture (CIAT), National Agricultural research Laboratories (NARL), Kawanda in Uganda.

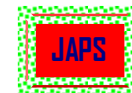
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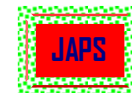


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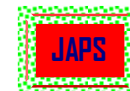


**Table 3:** Plant emergence, plant stand, Fusarium severity and yield of 46 selected bean entries under field conditions

| Entry             | Group   | (%)<br>emergence | Plant stand<br>(%) |       | Fusarium severity<br>(1-9 scale) |       | *classification |       | Yield<br>(kg ha <sup>-1</sup> ) | Root<br>weight<br>(g/10pl) | Root: Shoot<br>weight ratio<br>(g/10pl) |
|-------------------|---------|------------------|--------------------|-------|----------------------------------|-------|-----------------|-------|---------------------------------|----------------------------|---|
|                   |         |                  | 28dap              | 56dap | 28dap                            | 56dap | 28dap           | 56dap |                                 |                            |   |
| 1. G1459          | CIAT    | 80.0             | 51.5               | 16.3  | 5.8                              | 5.2   | MS              | MS    | 365<br>ebdacf                   | 0.63                       | 0.19                                    |
| 2. G4795          | CIAT    | 94.0             | 56.0               | 31.8  | 4.7                              | 5.0   | MR              | MS    | 554<br>ebdac                    | 0.63                       | 0.12                                    |
| 3. G9384          | CIAT    | 96.0             | 34.6               | 2.6   | 5.8                              | 7.0   | MS              | S     | 234<br>ebdcf                    | 0.60                       | 0.15                                    |
| 4. G3717          | CIAT    | 68.7             | 53.5               | 40.1  | 3.9                              | 4.9   | MR              | MR    | 951 bdac                        | 0.70                       | 0.19                                    |
| 5. G5149          | CIAT    | 88.7             | 48.2               | 17.7  | 4.8                              | 5.8   | MR              | MS    | 480<br>ebdac                    | 0.47                       | 0.12                                    |
| 6. G5108          | CIAT    | 78.7             | 51.5               | 28.3  | 5.3                              | 7.7   | MS              | S     | 636<br>ebdac                    | 0.60                       | 0.15                                    |
| 7. 1/MS/11-1      | Pythium | 75.3             | 35.7               | 15.5  | 4.4                              | 7.0   | MR              | S     | 1123b                           | 0.60                       | 0.19                                    |
| 8. CIM 9313-1     | Pythium | 48.0             | 93.2               | 41.9  | 6.0                              | 7.4   | S               | S     | 860bdac                         | 0.83                       | 0.19                                    |
| 9. MLB-17-89A     | Pythium | 98.0             | 14.5               | 4.4   | 6.5                              | 7.8   | S               | S     | 722bdac                         | 1.00                       | 0.14                                    |
| 10. MLB-49-89A    | Pythium | 97.3             | 51.5               | 15.5  | 4.4                              | 4.8   | MR              | MR    | 205 edcf                        | 0.60                       | 0.13                                    |
| 11. UMUBANO       | Pythium | 78.0             | 42.4               | 21.2  | 6.3                              | 5.7   | S               | MS    | 564<br>ebdac                    | 0.70                       | 0.15                                    |
| 12. VUNINKINGI    | Pythium | 82.7             | 31.3               | 13.3  | 4.3                              | 6.6   | MR              | S     | 390<br>ebdac                    | 0.40                       | 0.09                                    |
| 13. SCAM 80-CM/15 | Pythium | 65.3             | 30.9               | 6.7   | 6.1                              | 6.4   | S               | S     | 599<br>ebdac                    | 0.97                       | 0.20                                    |
| 14. 311/7         | Pythium | 69.3             | 30.0               | 7.5   | 4.9                              | 6.9   | MR              | S     | 494<br>ebdac                    | 0.83                       | 0.17                                    |
| 15. MLB-48-89A    | Pythium | 100.0            | 46.6               | 21.8  | 4.9                              | 4.3   | MR              | MR    | 655 bdac                        | 0.77                       | 0.16                                    |
| 16. RWR719        | Pythium | 92.0             | 45.2               | 13.6  | 5.4                              | 6.3   | MS              | S     | 350<br>ebdacf                   | 0.40                       | 0.17                                    |
| 17. CIM 9314-1    | Pythium | 78.6             | 56.7               | 13.9  | 8.1                              | 7.5   | VS              | S     | 989 ba c                        | -                          | -                                       |
| 18. GLP 24        | Pythium | 86.5             | 37.2               | 6.1   | 6.5                              | 7.8   | S               | S     | 471<br>ebdac                    | -                          | -                                       |
| 19. RWR 2075      | Pythium | 99.4             | 34.8               | 8.8   | 8.0                              | 8.8   | VS              | VS    | 464                             | -                          | -                                       |



|                              |          |       |      |      |     |     |    |    |                    |      |      |
|------------------------------|----------|-------|------|------|-----|-----|----|----|--------------------|------|------|
| 20. RWR 1058                 | Pythium  | 100.0 | 45.7 | 9.0  | 7.1 | 7.0 | S  | S  | ebdac<br>461       | -    | -    |
| 21. RWR 1059                 | Pythium  | 54.7  | 11.5 | 2.3  | 7.3 | 7.7 | S  | S  | ebdac<br>555       | 0.67 | 0.13 |
| 22. FEB 181                  | Pythium  | 93.5  | 47.7 | 16.0 | 6.3 | 7.3 | S  | S  | ebdac<br>335       | -    | -    |
| 23. RWR 868                  | Pythium  | 57.3  | 22.1 | 4.0  | 8.2 | 8.2 | VS | VS | ebdacf<br>206 edcf | 0.97 | 0.21 |
| 24. APN 154                  | Pythium  | 67.3  | 54.4 | 29.9 | 4.4 | 6.4 | MR | S  | 803bdac            | 0.50 | 0.15 |
| 25. GLP 585                  | Pythium  | 80.0  | 51.9 | 26.0 | 6.5 | 6.6 | S  | S  | 728 bdac           | 0.63 | 0.18 |
| 26. 217/2                    | Pythium  | 98.7  | 27.7 | 9.7  | 4.1 | 7.2 | MR | S  | 741 bdac           | 0.87 | 0.20 |
| 27. A211                     | F.O.P    | 88.7  | 55.1 | 28.5 | 4.1 | 8.2 | MR | VS | 464                | -    | -    |
| 28. HF-465-63-1              | F.O.P    | 99.3  | 48.4 | 21.0 | 4.5 | 7.4 | MR | S  | ebdac<br>398       | -    | -    |
| 29. RIZ 30                   | F.O.P    | 99.3  | 49.4 | 28.0 | 5.9 | 5.2 | MS | MS | ebdac<br>616       | -    | -    |
| 30. IPA 1                    | F.O.P    | 84.9  | 32.4 | 12.4 | 5.3 | 6.8 | MS | S  | ebdac<br>989 bac   | -    | -    |
| 31. Hoima-Kaki               | Landrace | 87.3  | 63.8 | 24.4 | 3.8 | 5.3 | MR | MS | 733 bdac           | 0.73 | 0.18 |
| 32. Lira-Cream               | Landrace | 80.7  | 36.8 | 8.0  | 7.0 | 7.6 | S  | S  | 482                | 0.80 | 0.19 |
| 33. Masaka-<br>Manyigamulimi | Landrace | 74.7  | 53.9 | 27.9 | 6.0 | 7.5 | S  | S  | ebdac<br>416       | 0.80 | 0.16 |
| 34. Mbarara-Kanyebwa         | Landrace | 95.3  | 41.3 | 6.9  | 5.2 | 5.0 | MS | MS | ebdac<br>478       | 0.83 | 0.20 |
| 35. Kabale-White             | Landrace | 78.7  | 63.6 | 23.3 | 5.8 | 4.3 | MS | MR | 955 bdac           | 0.93 | 0.17 |
| 36. Kiboga-Yellow            | Landrace | 74.0  | 8.0  | 0.0  | 6.1 | -   | S  | -  | 161 edf            | -    | -    |
| 37. RS5                      | SA       | 82.0  | 51.8 | 16.4 | 5.2 | 6.6 | MS | S  | 826 bdac           | 0.80 | 0.16 |
| 38. OPS-KW1                  | SA       | 87.3  | 51.8 | 13.0 | 6.0 | 6.5 | MS | S  | 345                | 0.73 | 0.14 |
| 39. Teebus RR1               | SA       | 81.3  | 52.6 | 12.3 | 3.7 | 5.3 | MR | MS | ebdacf<br>598      | 0.50 | 0.17 |
| 40. Timbavati                | SA       | 90.7  | 6.9  | 0.0  | 7.2 | -   | S  | -  | ebdac<br>731 bdac  | 0.40 | 0.16 |
| 41. Elangeni                 | SA       | 73.3  | 24.1 | 0.0  | 7.1 | -   | S  | -  | 1151 a             | 0.30 | 0.07 |



|                       |          |       |      |      |      |      |    |    |               |      |      |
|-----------------------|----------|-------|------|------|------|------|----|----|---------------|------|------|
| 42. Imbali            | SA       | 75.3  | 24.4 | 5.5  | 4.7  | 6.3  | MR | S  | 242<br>ebdacf | 0.50 | 0.18 |
| 43. PAN 128           | SA       | 67.5  | 60.7 | 3.6  | 6.8  | 5.6  | S  | MS | 730 bdac      | -    | -    |
| 44. PAN 185           | SA       | 76.4  | 41.4 | 9.3  | 5.8  | 7.9  | MS | S  | 565<br>ebdac  | -    | -    |
| 45. PAN 150           | SA       | 57.3  | 64.0 | 42.7 | 5.2  | 6.7  | MS | S  | 544<br>ebdac  | 0.60 | 0.20 |
| 46. Quteniqwa         | SA       | 90.6  | 36.2 | 6.1  | 5.2  | 7.7  | MS | S  | 654ebdac      | -    | -    |
| Kanyebwa(Susceptible) | Controls | 91.3  | 54.0 | 3.4  | 5.6  | 7.3  | MS | S  | 68 ef         | 0.73 | 0.16 |
| CAL96 (Susceptible)   | Controls | 68.7  | 6.8  | 0.7  | 6.8  | 7.2  | S  | S  | 63 ef         | 0.77 | 0.14 |
| K20 (Susceptible)     | Controls | 94.0  | 28.1 | 14.1 | 5.7  | 8.0  | MS | VS | 1.649 f       | 0.65 | 0.14 |
| S.E.D (P = 0.05)      |          | 15.55 | 9.41 | 5.36 | 0.99 | 0.89 |    |    | 12.3          | 0.16 | 0.03 |
| CV%                   |          | 23.9  | 33.7 | 30.2 | 22.1 | 35.6 |    |    | 22.8          | 29.0 | 25.2 |

\*Where; 1-2.9 = Tolerant/resistant reaction, 3.0 - 4.9= Moderately resistant 5.0- 5.9 = Moderately susceptible; 6.0-7.9 = Susceptible 8-9 = Very susceptible. na = data not available. Groups; CIAT= Includes documented sources of resistance to FRR from CIAT-Colombia, Pythium involves lines previously selected for resistance to Pythium root rot obtained from CIAT-Africa, F.O.P are Fusarium oxysporum f.sp. phaseoli differential varieties, SA are cultivars obtained from South Africa