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*Full Length Research Paper*

# A screening technique for resistance to *Fusarium* root rot of common bean

**Clare M. Mukankusi[1,2]*, Rob J. Melis[1], John Derera[1], Robin A. Buruchara[2] and D. Mark[1]**

[1]African Centre for Crop Improvement, School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, P. Bag X01, Scottsville 3209, Pietermaritzburg, South Africa.
[2]International Centre for Tropical Agriculture (CIAT), P. O. Box 6247, Kampala, Uganda.

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**Resistance to *Fusarium* root rot (FRR) in common bean is documented as a quantitative trait and as such is greatly influenced by several environmental factors. A reproducible disease screening technique that considers the selection environment is therefore important in selecting resistant lines. A study was conducted to evaluate soil composition and irrigation frequency on the severity of FRR, using a predominant pathogenic isolate from SW Uganda at the International Centre for Tropical Agriculture (CIAT) in Uganda. Five soil compositions (i) 80% lake sand:20% forest soil, ii) 50% lake sand:50% forest soil, iii) 80% swamp soil:20% forest soil, iv) 50% swamp soil:50% forest soil and v) forest soil alone), and five irrigation frequency levels (once a week, twice a week, three times a week, four times a week, and daily) were evaluated on six common bean varieties with varying levels of resistance to FRR. Forest soil and 50% swamp soil: 50% forest soil (soil composition); daily irrigation and irrigation once a week (irrigation frequency) differentiated test varieties most distinctly, according to their reaction to FRR. In conclusion, a combination of forest soil and daily watering using a pathogenic isolate FSP-3 provided adequate FRR disease levels for disease evaluation and differentiation of bean varieties and was adopted for genetic studies on FRR resistance in beans.**

**Key words:** Common bean, *Fusarium* root rot, resistance, irrigation frequency, screening technique, soil composition.

## INTRODUCTION

*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder and H.N. Hans (FSP) belongs to the *Nectria haematococca- F. solani* species complex section *Martiella* of Fusarium (O'Donnell, 2000). Its main host is the common bean (*Phaseolus vulgaris* L.), on which it causes Fusarium root rot (FRR) disease. The disease causes up to 100% yield loss in susceptible cultivars. The use of resistance is probably the cheapest and most cost-effective control measure against FRR in developing countries.

In aiming to improve resistance to FRR, the choice of an optimum selection environment (one that maximizes the response for the target environment) is critical. Resistance to FRR and other polygenic traits is greatly compounded by environmental factors, hence a limited improvement in disease resistance (Beebe et al., 1981; Sippel and Hall, 1982; Chaudhary et al., 2006). Environmental factors, especially those that stress plants, have been shown to influence resistance to FRR in common bean, making it difficult to identify resistant varieties. These factors include soil compaction (Miller and Burke, 1975, 1977), cool temperatures, soil pH, soil fertility, pesticide or fertilizer injury, soil moisture (Burke, 1965; Miller and Burke, 1985), plant densities (Burke and Barker, 1966), inoculum levels, and the presence of a complex of root rot pathogens (Pieczarka and Abawi, 1978; Sippel and Hall, 1982; Chaudhary et al., 2006). This study aimed at developing an effective and reproducible screening technique for resistance to FRR in common beans. Specific objectives were to; determine an

*Corresponding author. E-mail: c.mukankusi@cgiar.org.

optimum screenhouse irrigation frequency regime to induce adequate disease infection levels for selection of beans for resistance to FRR; determine an optimum soil composition to induce optimum disease infection levels for selection of beans for resistance to FRR in the screenhouse; and to investigate the host-parasite-environment interaction of FSP and common beans.

## MATERIALS AND METHODS

The trial evaluated the effect of the irrigation frequency and soil composition on six bean varieties, MLB-49-89A, Umubano, MLB-17-89A, CIM 9313-1, G 3717, and K20 all having varying levels of resistance to FRR (Mukankusi, 2008). The effect of frequency of irrigation was assessed by varying the number of times the beans were irrigated per week: once a week, twice a week, three times a week, four times a week, and daily. On the day of irrigation, water was applied (till soil was saturated) three times, that is, at 06, 11 and 18h00 and the trail was shed from rain water. Soil composition levels were manipulated by varying the levels of lake sand, forest soil, and swamp soil: i) 80% lake sand: 20% forest soil, ii) 50% lake sand: 50% forest soil, iii) 80% swamp soil: 20% forest soil, iv) 50% swamp soil: 50% forest soil and v) forest soil alone. The study was conducted in the CIAT-Africa screen houses based at Kawanda Agricultural Research Institute (KARL) in Uganda.

A soil analysis test for pH, soil composition, organic matter content and textural classes for the different soil mixtures was done at KARL before the trial was laid out (Table 1). The trial was conducted in the screenhouse as a 6 x 5 x 5 split-split plot experiment with three replications. The varieties were the main factor, soil composition the sub-factor, and the frequency of irrigation, the sub-sub-factor. All the soil types were first dried, crushed, sieved and sterilized by steaming on firewood overnight before being mixed. Infected sorghum seed was used as the medium of pathogen inoculums, as it is the standard method of root rot soil inoculation currently used by CIAT. Inoculum of an FSP isolate obtained from infected bean fields in south-western Uganda (FSP-3) was used (Mukankusi, 2008). Each tray was planted with all the test varieties but with different combinations of soil composition and irrigation frequency. The trial was repeated to confirm the results.

### Disease evaluation and analysis

Disease assessment was done by carefully uprooting all the seedlings planted per variety, taking care not to damage roots and hypocotyls, and washing with clean tap water. The number of plants showing disease symptoms was counted and disease incidence was calculated as the percentage number of plants that exhibited symptoms per line. FRR severity was assessed by observing the roots and hypocotyls and scores given, based on a 1 to 9 disease scale developed at CIAT, (Abawi and 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 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. The data were analysed using a GenStat computer programme to obtain differences in the mean disease severity (Payne et al., 2007).

## RESULTS AND DISCUSSION

The repeats of the trial were not significantly different therefore, results were presented for the means of the two trials. The 3-way interaction of bean line x irrigation frequency x soil composition was not significant at P= 0.05 for plant stand and FRR severity, indicating that the lines behaved similarly under different soil composition and moisture level combinations (Table 2) and were not influenced by the different combinations. The irrigation frequency x soil composition interaction was highly significant at P≤0.05 for Fusarium severity and plant stand, indicating that these two factors in combination are important in their effect on FRR severity and plant stand (Table 2).

Generally, 50% swamp soil: 50% forest soil and 80% lake sand: 20% forest soil resulted in the highest FRR severities, while forest soil resulted in the lowest severity levels. The 80% lake sand: 20% forest soil was classified as loamy sand soil and contained the highest proportion of sand and lowest proportion of clay and silt compared to the other soil compositions, while, the 50% swamp soil: 50% forest soil was classified as sandy loam soil with generally a high proportion of clay and silt but lower sand compared to the other three soil compositions (Table 1). The lowest disease severity was obtained on forest soil which was very different from the other soil compositions and was classified as sandy clay loam soil with the lowest levels of sand but the highest levels of clay and silt compared to the other soil compositions. It also had the highest organic matter content, nitrogen (N), phosphorus (P) and potassium (K) levels (Table 1). This could have resulted in the plants thriving and being able to resist the pathogen more than in the other soils that were probably stressful to the young bean seedlings. It is probable that, the higher levels of sand in the 80% lake sand: 20% forest soil, allowed the pathogen to move easily within the soil capillaries and hence reach the bean roots more easily than in forest soil which was relatively more compact due to the higher amounts of clay it contained. Several studies on root rot pathogens have utilised sandy soil because it allowed early development of disease symptoms (Mathre et al., 2003), while other studies have utilized vermiculite (Chaudhary et al., 2006), mixtures of coconut coir and perlite (Snapp et al., 2003; Román Avilès et al., 2004) as these methods also provided representative root rot symptoms, and simplified root extraction. Others still have used the root dipping method, where roots are dipped in a known concentration of spore solution of the pathogen (Perssoni et al., 1997).

In this study, the relative compaction of the soil, levels of soil moisture as well as the availability of nutrients in each soil composition were the major factors that influenced reaction of the different lines to FRR. These findings confirm those of Miller and Burke (1975, 1977 and 1985), Burke and Hall (1991) and Thung and Rao (1999) that root rots are particularly severe under water stressed and compacted soil conditions. Although loose

**Table 1.** Soil compositions evaluated for their effect on *Fusarium* root rot severity on beans.

| Sample | pH | OM | N | P | K | Ca | Mg | Sand | Clay | Silt | Textural class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | …….%……. | | ……………..mgL$^{-1}$……………….. | | | | …………..%……….. | | | |
| Forest soil | 6.3 | 3.07 | 0.18 | 13.6 | 131.3 | 1990.02 | 408.25 | 67.8 | 23.6 | 8.6 | Sandy clay loam |
| 80% lake sand: 20% forest soil | 6.5 | 1.03 | 0.10 | 11.4 | 36.0 | 2208.30 | 451.47 | 89.8 | 7.6 | 2.6 | Loamy sand |
| 50% lake sand: 50% forest soil | 6.4 | 1.18 | 0.11 | 12.6 | 60.3 | 2099.16 | 429.86 | 83.8 | 9.6 | 6.6 | Loamy sand |
| 80% swamp soil: 20% forest soil | 5.1 | 1.40 | 0.12 | 7.5 | 72.7 | 680.34 | 148.94 | 77.8 | 17.6 | 4.6 | Sandy loam |
| 50% swamp soil: 50% forest soil | 5.5 | 1.86 | 0.14 | 10.0 | 96.0 | 1116.90 | 235.38 | 75.8 | 17.6 | 6.6 | Sandy loam |
| Critical value | 5.2 | 3.0 | 0.2 | 5.0 | 150.0 | 350.0 | | | | | |

Om= Organic matter, N = Nitrogen, P= Phosphorus, K= Calcium, Ca= Calcium, M= Magnesium.

**Table 2.** Mean squares for the effect of irrigation frequency and soil composition on severity of *Fusarium* root rot and plant stand of six bean lines.

| Source of variation | DF | *Fusarium* severity(1-9 scale) | Plant stand (%; 28 dap) |
|---|---|---|---|
| Line | 5 | 34.3** | 57408.2** |
| Irrigation frequency | 4 | 157.69** | 13621.1** |
| Soil composition | 4 | 73.82** | 8702.6** |
| Line x irrigation frequency | 20 | ns | 1339.6** |
| Line x soil composition | 20 | ns | 1339.4* |
| Irrigation frequency x soil composition | 16 | 13.44** | 2363.1** |
| Line x irrigation frequency x soil composition | 80 | ns | Ns |
| Total | 899 | | |

* and ** = significant at P= 0.01, and P= 0.001, respectively , ns = not significant at P= 0.05.

soil allow for early development of symptoms due to faster movement for the pathogen (which is preferred for screening trials), compact soils in the long run interferes with the ability of the plant roots to penetrate the soil; hence affecting seedling growth and promoting vulnerability to FRR infection. Optimum fertilisation is necessary if the bean plants are to resist infection from FRR (Román-Avilès et al., 2003). Soil compaction should be minimised and hard pans should be prevented, but if they occur, then they should be broken.

Daily watering and watering once in a week also resulted in the highest *Fusarium* severities, while watering three times in a week resulted in the lowest disease severity (Table 3). Either too little or too much water has been reported to escalate FRR symptoms, as both drought and flooding stress predisposes plants to infection (Miller and Burke, 1975). Too much water results in low aeration, which is stressful to plant roots.

Miller and Burke (1977) reported a depression in yield due to water logging in a field with a history of FRR and concluded that, the aggravation of root rot was the principal cause of plant stunting under wet conditions.

A combination of 80% lake sand: 20% forest soil, 80% swamp soil: 20% forest soil, 50% swamp soil: 50% forest soil with daily watering, and 80% lake sand: 20% forest soil with water once a week resulted in very high disease levels (Table 3). A combination of forest soil and applying water application twice a week resulted in the lowest FRR infection (Table 3). Applying water three times a day in 80% swamp soil: 20% forest soil also resulted in low disease levels. The highest plant stands were observed in the treatment that received water four times in a week and in 80% swamp soil: 20% forest soil, while a combination of 50% swamp soil: 50% forest soil with watering once a week or daily had the lowest plant stands (Table 3). The interactions, bean line x irrigation frequency and bean line x soil composition, were significant (P=0.05) for their effects on plant stand, indicating that the reaction of the different lines were significantly affected by different soil compositions and irrigation frequencies (Table 2).

The plant stands of the individual lines were also significantly different (P=0.05) from each other, with Umubano having the highest plant stand, followed by MLB-49-89A while MLB-17-89A had the lowest plant stand. There were no significant differences (P=0.05) between applying water twice a week, three times a week or daily on the plant stand of the different lines (Table 2). For all the lines, the highest plant stands were observed in trays that received water four times in a week (Table 4). Daily watering also resulted in high plant stands for the lines MLB-49-89A, Umubano, and K20, while it resulted in low plant stands for the bean line G3717 and

**Table 3**. Effect of different soil composition and irrigation frequency combinations on *Fusarium* root rot severity and plant stand.

| Soil composition | *Fusarium* root rot severity (1-9 scale) | | | | | | Plant stand (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Once | Twice | Three times | Four times | Daily | Mean | Once | Twice | Three times | Four times | Daily | Mean |
| 80% lake sand:20% forest soil | 7.6 | 6.7 | 4.6 | 4.5 | 8.0 | 6.3 | 44.7 | 57.6 | 49.8 | 70.9 | 41.9 | 53.0 |
| 50% lake sand:50% forest soil | 6.0 | 5.4 | 4.4 | 5.1 | 7.0 | 5.6 | 42.2 | 52.4 | 37.8 | 68.9 | 68.3 | 54.0 |
| Forest soil | 4.7 | 3.6 | 4.7 | 5.1 | 4.8 | 4.5 | 54.8 | 56.4 | 58.4 | 70.9 | 69.9 | 62.1 |
| 80% swamp soil:20% forest soil | 5.2 | 4.3 | 3.8 | 6.4 | 7.4 | 5.5 | 62.4 | 66.7 | 68.9 | 74.4 | 59.1 | 66.4 |
| 50% swamp soil:50% forest soil | 6.9 | 6.1 | 5.8 | 6.9 | 7.1 | 6.6 | 34.3 | 41.6 | 55.7 | 68.1 | 34.5 | 46.8 |
| Mean | 6.1 | 5.2 | 4.7 | 5.6 | 6.9 | | 47.7 | 54.9 | 54.1 | 70.6 | 54.7 | |
| S.e.d soil composition | | | 3.08 | | | | | | | 0.29 | | |
| S.e.d irrigation frequency | | | 2.75 | | | | | | | 0.26 | | |
| S.e.d soil composition x irrigation frequency | | | 6.16 | | | | | | | 0.58 | | |
| CV% | | | 32.7 | | | | | | | 30.9 | | |

**Table 4.** Effect of different bean lines and irrigation frequency combinations on the plant stand (28 dap) of bean lines.

| Bean line | Irrigation frequency per week | | | | | Mean | Soil composition | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Once | Twice a week | Three times | Four times | Daily | | 80% lake sand: 20% forest soil | 50% lake sand: 50% forest soil | Forest soil | 80% swamp soil: 20% forest soil | 50%swamp soil: 50% forest soil | |
| MLB-49-89A | 64.8 | 78.1 | 76.0 | 85.0 | 86.2 | 78.4 | 77.1 | 69.6 | 80.8 | 89.2 | 73.6 | 78.1 |
| Umubano | 73.2 | 79.5 | 78.6 | 73.7 | 92.7 | 79.4 | 74.2 | 78.8 | 87.1 | 90.0 | 67.5 | 79.5 |
| K20 | 54.4 | 66.4 | 68.0 | 51.1 | 90.6 | 68.0 | 57.9 | 56.7 | 69.2 | 93.3 | 55.0 | 66.4 |
| MLB-17-89A | 20.1 | 25.7 | 24.6 | 29.1 | 33.6 | 21.3 | 20.4 | 27.9 | 24.6 | 40.0 | 15.8 | 25.7 |
| G3717 | 31.3 | 38.8 | 35.1 | 43.1 | 51.2 | 33.3 | 35.8 | 40.4 | 43.3 | 39.6 | 35.0 | 38.8 |
| CIM-3133-1 | 42.3 | 50.1 | 47.3 | 44.7 | 70.4 | 45.3 | 52.5 | 50.4 | 67.5 | 46.3 | 33.8 | 50.1 |
| Mean | 47.7 | 54.9 | 54.5 | 70.8 | 54.3 | | 53.0 | 54.0 | 62.1 | 66.4 | 46.8 | |
| S.e.d lines | | | 3.37 | | | | | | 3.37 | | | |
| S.e.d irrigation frequency | | | 2.75 | | | | | | 3.08 | | | |
| S.e.d bean line x irrigation frequency | | | 6.75 | | | | | | 7.54 | | | |
| CV% | | | 32.7 | | | | | | 32.7 | | | |

MLB-17-89A. Watering once a week resulted in the lowest plant stands for G3717, MLB-17-89A and MLB-49-89A, while Umubano maintained a relatively high plant stand in this treatment (Table 4). As shown in Table 3, 80% lake sand: 20% forest soil, 50% lake sand: 50% forest soil, and 50% swamp soil: 50% forest soils were not significantly different from each other in their effects on the plant stand of the different lines. The highest plant stand was recorded on 80% swamp soil: 20% forest soil, while the lowest plant stands were recorded on 80% lake sand: 20% forest soil

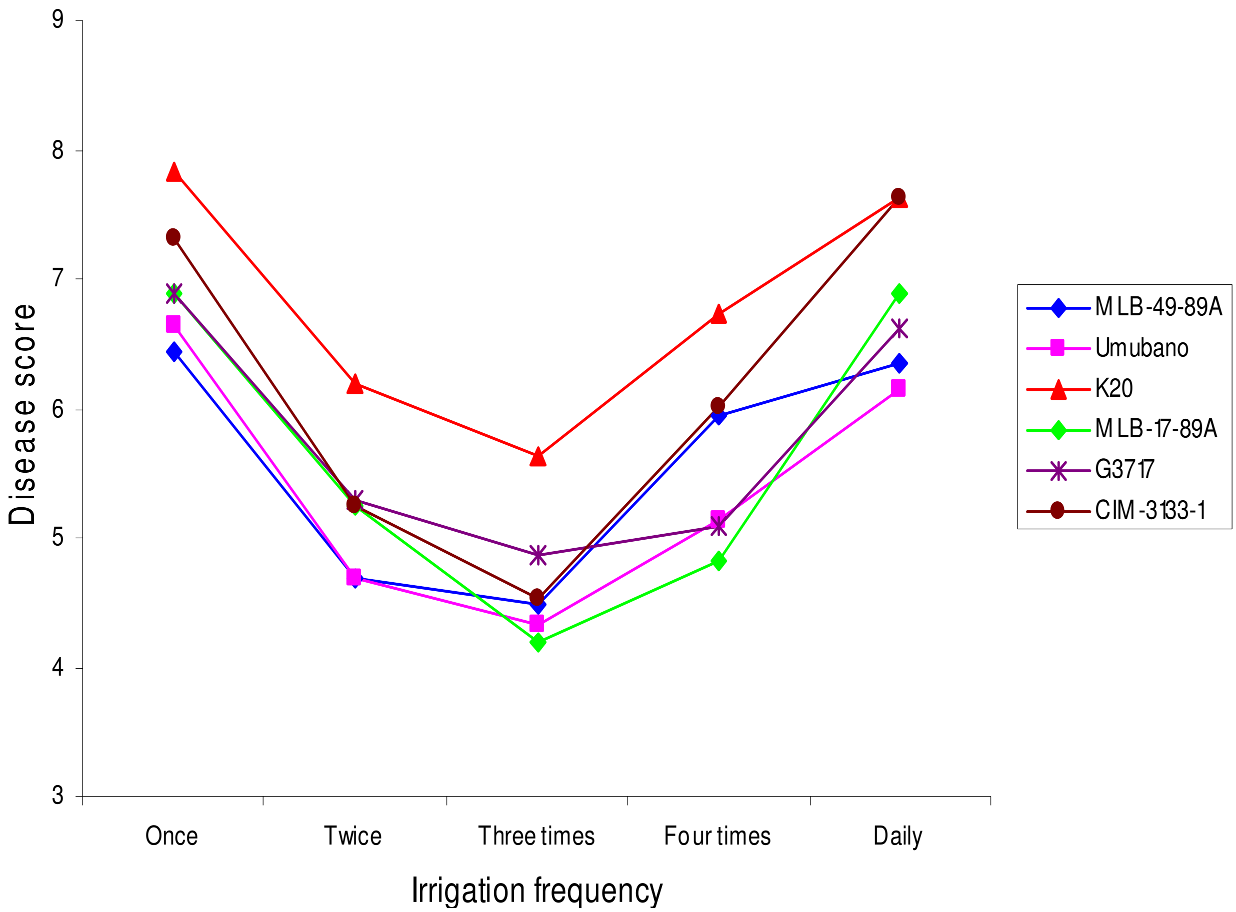



**Figure 1.** Effect of irrigation frequencies on the reaction of different bean lines to Fusarium root rot.

(Table 3). The forest soil also had relatively higher plant stands for all the lines. The local susceptible check, K20, had the highest plant stand (93.3%) on the 80% swamp soil: 20% forest soil (Table 4). The varieties CIM 3133-1, G3717 and MLB-17-89A, and had the lowest plant stands in general (Table 4).

Though the interaction between the three factors, lines, soil composition and irrigation frequency levels, was not significant at P=0.05 for disease severity, the ranking of the different lines according to their reaction to FSP varied with the different factor combinations. Generally, all the five watering regimes were able to differentiate the lines regarding their reaction to FRR, although the most distinct differentiation of the lines was obtained under treatments that were irrigated daily and those irrigated once a week (Figure 1). In addition, irrigation once a week and daily; resulted in the highest disease scores indicating that only a few lines would escape the disease at these irrigation frequencies. With regard to soil composition, the 50% swamp soil: 50% forest soil differentiated the lines most distinctly according to their reaction to FRR (Figure 2).

K20 ranked highest followed by CIM 3133-1, then by G3717, MLB-49-89A, MLB-17-89A and lastly Umubano with the least FRR severity. Similarly, on forest soil, four lines were distinctly differentiated from each other in comparison to the other soil compositions (Figure 2). This therefore, indicates that 50% swamp soil: 50% forest soil or the forest soil should be the soil compositions of choice in screening for resistance to FRR, as their use resulted in lines showing different resistance levels making selection for FRR disease resistance easier. Very high infection levels obtained under 80% lake sand: 20% forest soil and 50% lake sand: 50% forest soils are not desirable in inheritance studies, as it becomes difficult to differentiate between the resistant and susceptible lines. From Figure 3a to f, the lines behaved differently under different soil composition and irrigation frequency combinations. Generally, on 80% lake sand: 20% forest soil, and 80% swamp soil: 20% forest soil; the different bean line's reaction to FRR varied greatly under the different irrigation frequencies when compared to the other soil compositions. Under forest soil and 50% swamp soil: 50% forest soil, Fusarium severity scores for the lines, MLB-49-89A, K20, Umubano G3717, and CIM3133-1 did not vary much, irrespective of the frequency of irrigation

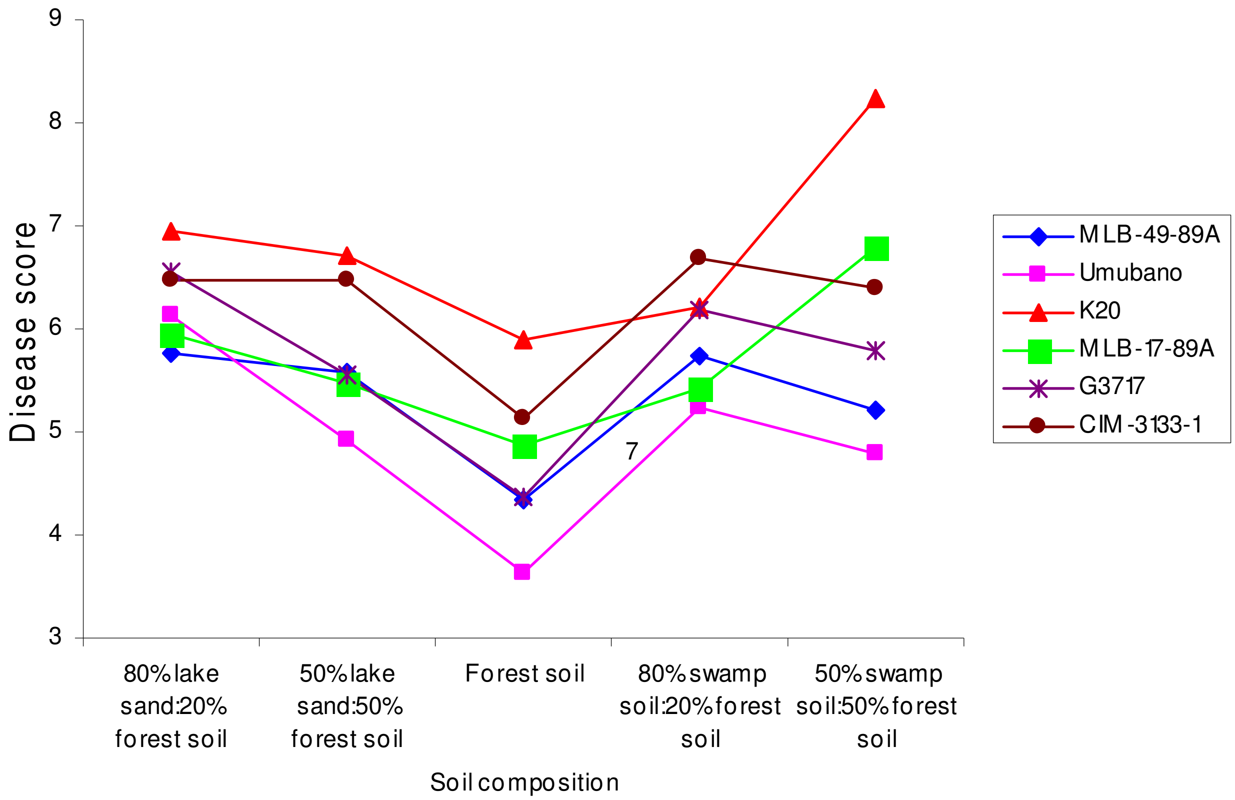



**Figure 2.** Effect of soil composition on reaction of different bean lines to Fusarium root rot.

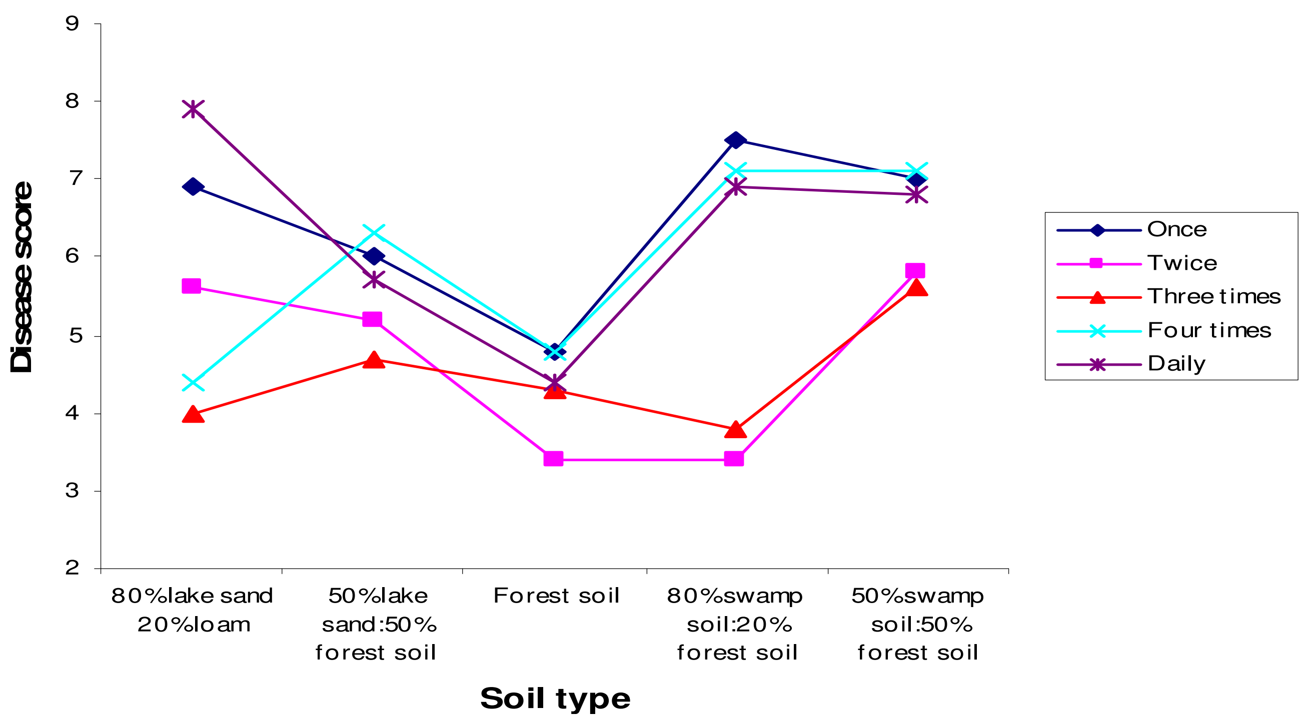
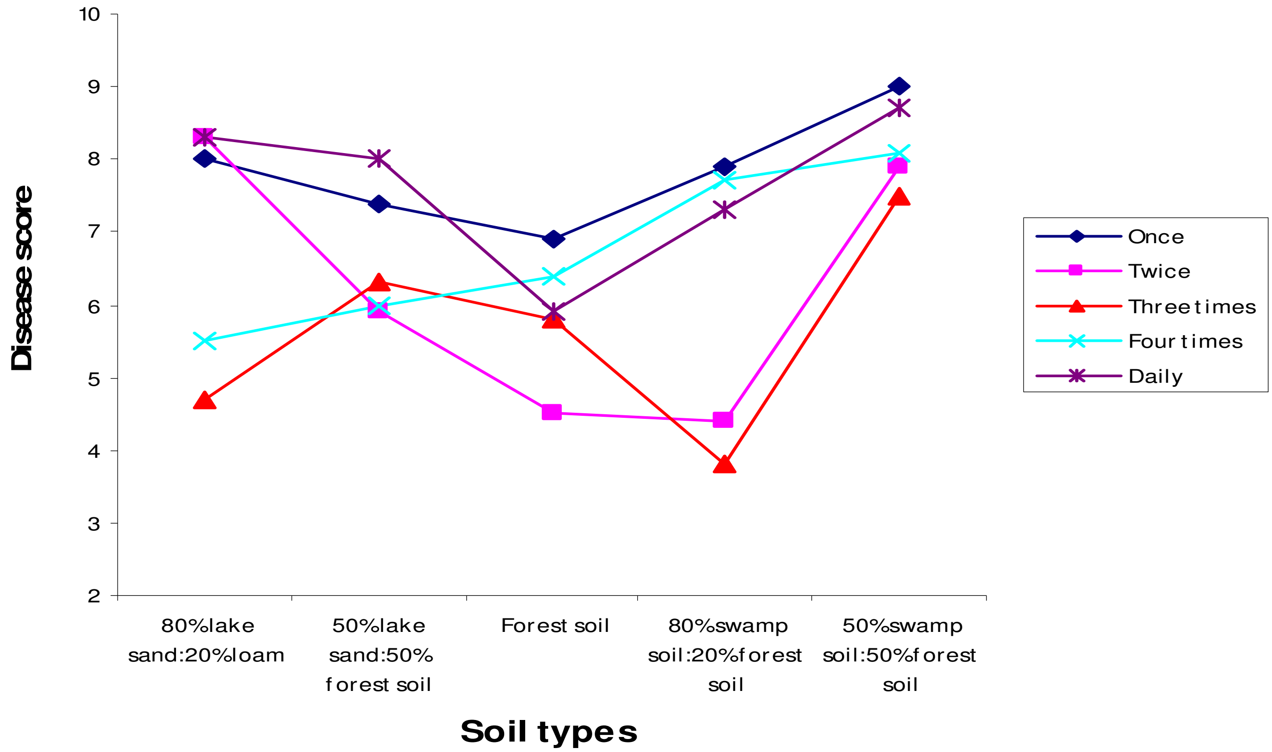
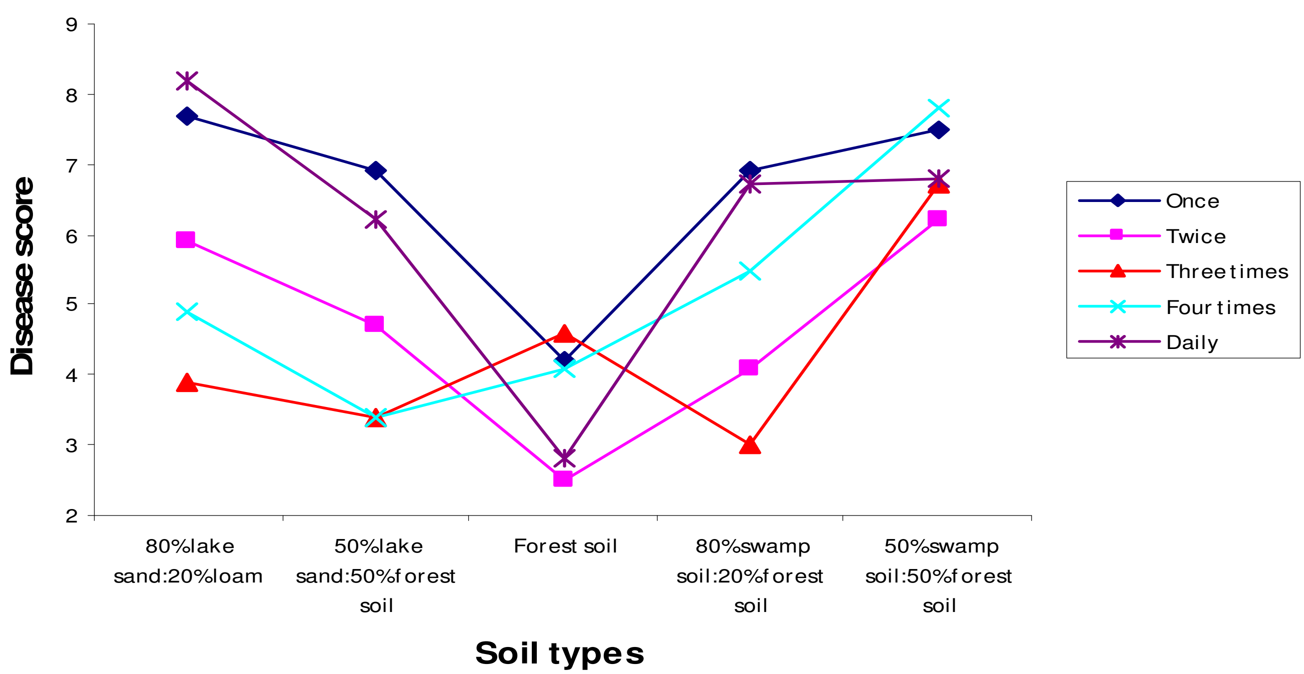



**Figure 3a.** Reaction of different bean lines to *Fusarium* root rot under different soil type and soil moisture level combinations. (a) MLB-49-89A

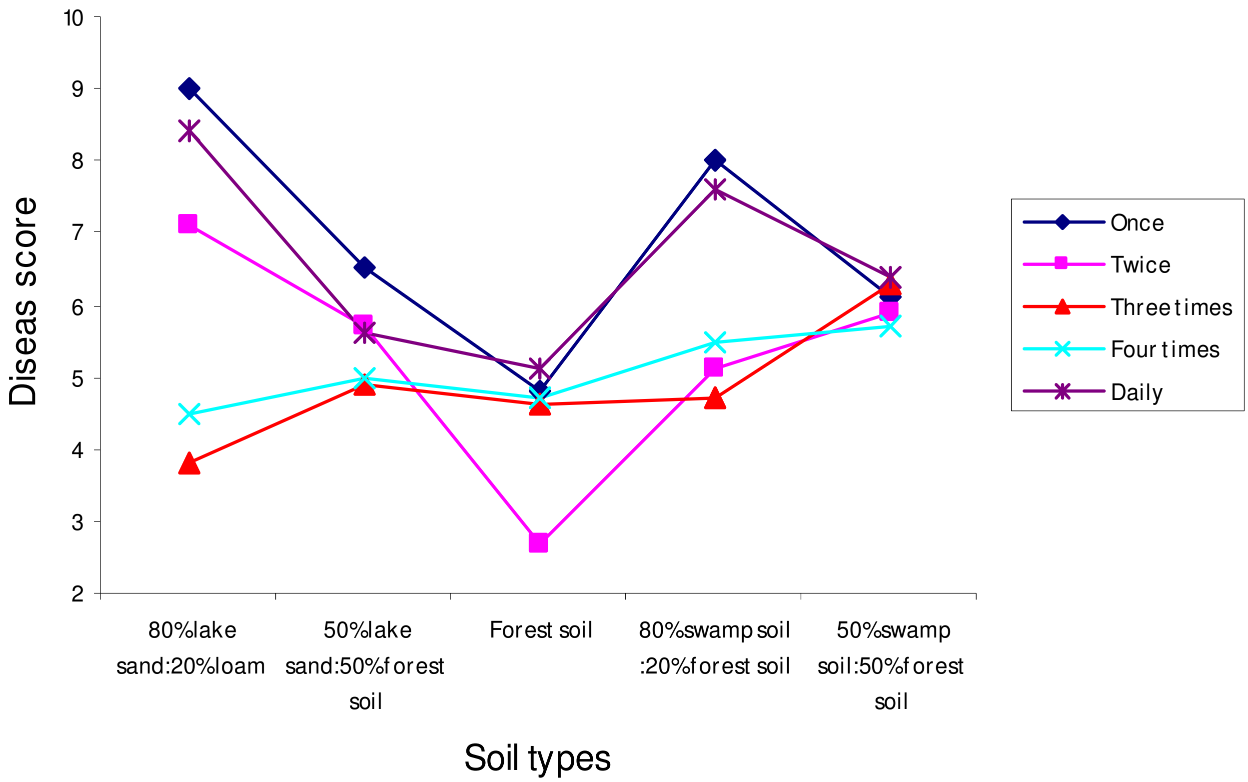



b

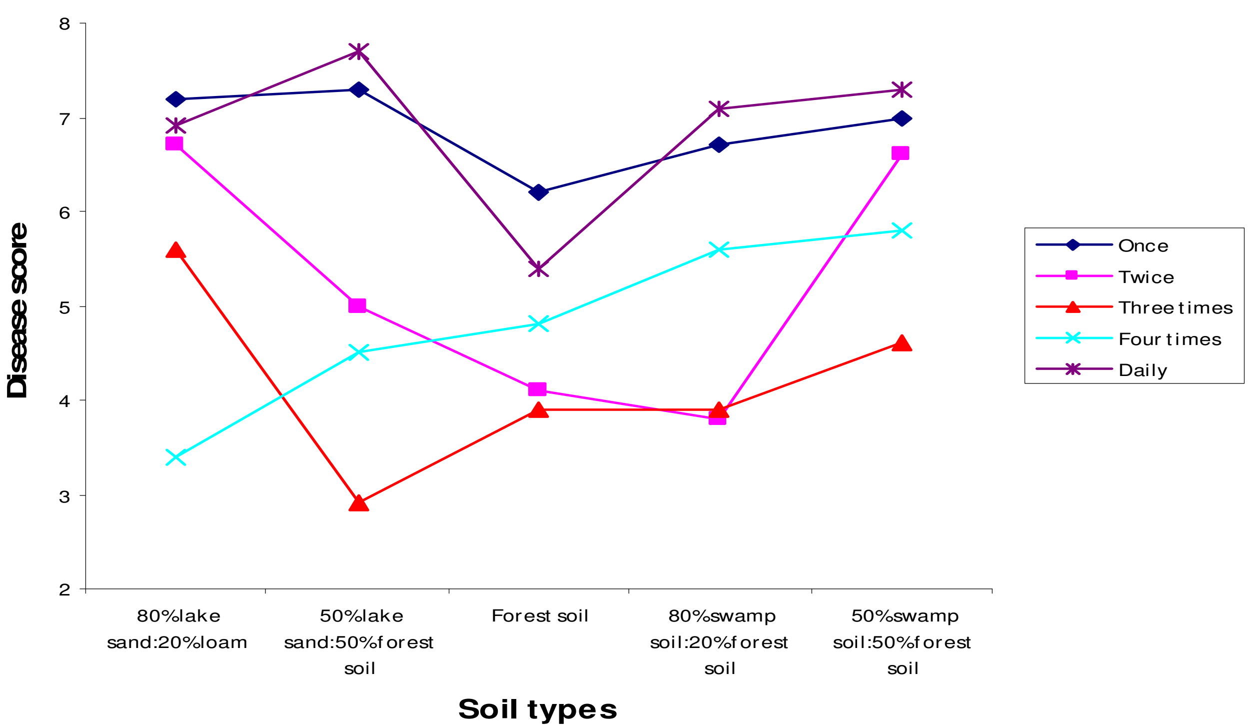



c

**Figures 3b and c.** Reaction of different bean lines to *Fusarium* root rot under different soil type and soil moisture level combinations. (b) K20, (c) Umubano.

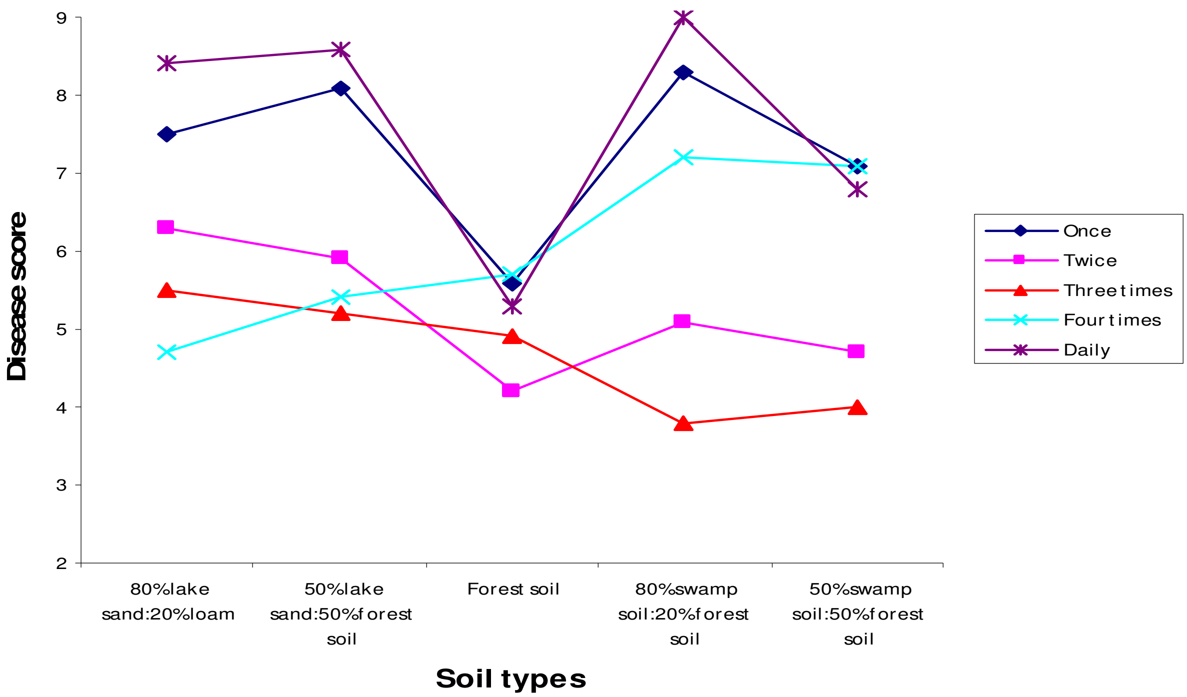



d




e

**Figures 3d and e.** Reaction of different bean lines to *Fusarium* root rot under different soil type and soil moisture level combinations. (d) G3717, (e) MLB-17-89A.




f

**Figure 3f.** Reaction of different bean lines to *Fusarium* root rot under different soil type and soil moisture level combinations. (f) CIM-3133-1.

(Figure 3a to f). Similarly, on 50% lake sand: 50% forest soil most of the lines apart from Umubano (Figure 3c), MLB-17-89A (Figure 3e) and CIM 3133-1 (Figure 3f) had FRR severity scores, that did not differ very much. Therefore, either forest soil or 50% swamp soil: 50% forest soil would be the soil composition of choice for screening for resistance to FRR, as the lines maintained similar resistance levels irrespective of the amount of water they received. Generally, daily irrigation resulted in the highest disease scores in all lines.

## Conclusion

The most distinct differentiation of the bean lines according to the FRR resistance levels was obtained under treatments that were irrigated daily and those irrigated once a week (irrigation frequency) and in 50% swamp soil: 50% forest soil and 100% forest soil (soil composition). However, the interactions of the factors (irrigation frequency and soil composition levels) with the bean lines were not significant and bearing in mind the extra costs of labour and time in preparing different soil composition mixtures, the standard forest soil and daily irrigation was adopted as the standard screening technique for resistance to FRR. This method gave satisfactory disease infection levels and differentiated lines distinctly according to their FRR disease severity levels.

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