Improving resistance to Fusarium root rot [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans.] in Common bean (*Phaseolus vulgaris* L.)

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General abstract

Fusarium root rot (FRR) disease, caused by the fungus *Fusarium solani* f. sp. *phaseoli* (FSP), is an important soil-borne disease reducing common bean (*Phaseolus vulgaris* L.) yields, and hence food security, in Uganda and elsewhere in developing countries where the crop is grown without fungicides. The key aim of this study was to elucidate the significance of bean root rot (BRR), appraise methods for screening germplasm for resistance to FRR, determine the genotypic variability of resistance, and the inheritance of resistance to FRR in common bean. This information was deemed useful in devising an appropriate strategy for breeding FRR resistance in beans.

A participatory rural appraisal (PRA) was conducted in south-western and eastern Uganda to ascertain farmers' awareness of BRR and their influence on preferred bean varieties. Bean root rot is considered to be the most devastating and most recognised disease, especially in south-western Uganda. Control measures for BRR were very minimal, and in some cases, non-existent. Use of resistant varieties to control the disease was not evident, because the most popular varieties were susceptible to the disease. The resistant bean varieties currently available have undesirable characteristics such as small seed size, black seed and late maturity. Large-seeded bean varieties, even though cited as being more susceptible to BRR than the small-seeded varieties, are still very popular. The study highlighted the need for breeding FRR resistance in the large-seeded bean varieties that are highly preferred by farmers.

Four isolates of FSP (FSP-1, FSP-2, FSP-3 and FSP-4) were tested for pathogenicity under screenhouse and laboratory conditions. In addition, three methods of storing and maintaining the viability of FSP isolates were appraised. The isolate FSP-3, was found to be the most pathogenic, resulting in 100% disease incidence on all bean varieties tested, with high severity scores. The potato dextrose agar (PDA) slants stored at 5°C were found to be the best method of storage for pathogenic isolates. The FSP-3 isolate was subsequently utilised for screening bean lines for resistance to FRR.

The influence of soil composition, irrigation frequency, and inoculation technique on the severity of FRR was studied on six bean lines. Interactions of irrigation frequency, soil composition, and bean lines were not significant. The 50% swamp soil:50% forest soil composition and forest soil alone categorized the varieties most distinctly according to their reaction to FRR. Also, the best distinct classification for the varieties was obtained under

treatments that were watered daily and once in a week. Based on economic considerations, the standard forest soil and daily irrigation were subsequently adopted for screening bean germplasm for resistance to FRR. It was also found that sorghum seed as a medium for pathogen inoculation was better than the agar slurry medium.

One hundred and forty seven common bean varieties were evaluated for resistance to FRR (isolate FSP-3) under screenhouse conditions. In order to confirm this resistance, 46 common bean lines selected from the screenhouse trial were further evaluated using natural inoculum in a BRR-infested field. Forty-four varieties comprising ten large-seeded, four medium-seeded and 30 small-seeded varieties showed moderate resistance to FRR; but none were resistant or immune to the disease. Based on adaptability, eight moderately resistant varieties were selected for use as parents in the study of inheritance of resistance to FRR.

A 12 x 12 diallel mating design was utilised to develop 66 F_1 and F_2 populations, plus their reciprocal crosses, with the aim of studying the mode of inheritance of resistance to FRR. The F_1 and F_2 progeny evaluations showed that FRR resistance was mainly governed by additive genes in most populations. However, there were a few crosses which displayed highly significant specific combining ability (SCA) effects, implying that dominant effects were important in some populations. Maternal effects were also highly significant at both the F_1 and F_2 generations, suggesting that resistance was modified by cytoplasmic genes. The non-maternal effects were also significant in some populations, suggesting that the cytoplasmic genes were interacting with nuclear genes. The number of genes governing resistance to FRR varied from two to nine among the eight sources of resistance. The allelism test of resistant x resistant populations, and the observation of continuous distributions of severity scores, suggested the presence of many loci governing FRR resistance in beans. Broad sense heritability of disease resistance varied from 0.22-0.69, while heritability in the narrow sense was estimated as 0.35-0.49 in the populations. These results suggested that selection and backcrossing to both parents would be the best breeding procedures for improving resistance in the popular large-seeded bean varieties in Uganda. However, there could be complications in breeding for resistance to FRR in beans, because resistance was modified by cytoplasmic gene effects and their interaction with nuclear genes in some of the populations.

Declaration

I hereby declare that this thesis, prepared for the Doctor of Philosophy degree, submitted by me to the University of KwaZulu-Natal, is my own original work and has not previously, in its entirety or in part, been submitted to any other university. All sources of materials and financial assistance used for the dissertation have been duly acknowledged.

Signed on …………………. at the University of KwaZulu-Natal, Pietermaritzburg, Republic of South Africa.

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Supervisors Approval

The University of KwaZulu-Natal approves this thesis of Clare Mukankusi Mugisha in fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Breeding

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Dedication

In memory of my late parents, Amos and Margaret Senyakazana

To my husband, Allan Mugisha and our two lovely children, Ian and Tiffany

Acknowledgements

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Introduction[1]

The common bean (*Phaseolus vulgaris* L.) is the most important food legume crop grown worldwide (Wortmann and Allen, 1994; Wortmann et al., 1998; Buruchara, 2006). Beans are considered by many to be the perfect food as they are nutrient dense with high contents of protein, micronutrients, vitamins, dietary fibre, and also have a low glycemic index (Wortmann and Allen, 1994; Bennink, 2005; Widers, 2006). The crop is currently the second most important source of human dietary protein, and the third most important source of calories for over 100 million people in rural and poor urban communities in Africa (Buruchara, 2006). In Uganda, beans provide up to 25% of the total calories and 45% of the total dietary protein, the highest in the world, a figure shared with neighbouring countries Rwanda, Burundi and the Kivu province in the Democratic Republic of Congo (Kirkby, 1987; Pachico, 1993). Green leaves, green pods, and immature and/or dry seeds may all be eaten, because they are very rich in iron and zinc (Kimani et al., 2006). Dry leaves, threshed pods, stalks and bean seeds that do not meet human food quality standards are fed to animals, or used as fuel for cooking, especially in Africa and Asia (Sperling et al., 1996; Buruchara, 2006).

Beans contribute a great deal to improving and sustaining soil fertility due to their ability as legumes to fix nitrogen in the soil. They are hence used in crop rotations, and mixtures with grass in leys and pastures, and as cover crops and green manures (Purseglove, 1968). Thus beans fit well in the farming systems in Uganda and sub-Saharan Africa.

The crop is also an important source of income throughout sub-Saharan Africa, especially for women who grow it both for subsistence and for sale to urban populations (CIAT, 1995). In Uganda, beans are not considered a traditional export crop, with the traditional export crops being coffee, cotton, tea, and tobacco (UEPB, 2006). However, beans are ranked fourth after coffee, maize, and tea in terms of export volume and eighth in terms of export value after coffee, tea, tobacco, maize, cut flowers, cotton, and cocoa beans (UEPB, 2006). Approximately 80% of the Ugandan bean production is consumed domestically while the exported volume is mainly to Kenya (Mauyo et al., 2007) and Rwanda, through informal border trade or relief supply to the World Food Programme (David et al., 1999; UEPB, 2005).

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[1] The style format used in this thesis is that of the Crop Science Journal.

Annual global production of dry beans is estimated at 19.5 million t; Brazil is the highest producer, with an estimated annual production of 4 million t (FAOSTAT, 2007). Production in Africa is estimated at 2.8 million t on 4.8 million ha (FAOSTAT, 2007). East Africa accounts for over 75% of the total production in Africa, and Uganda is second after Kenya, with current production of 424 000 t (FAOSTAT, 2007).

However, even though Uganda is ranked high in bean production, it is ranked among the last five countries in Africa in production per unit area (FAOSTAT, 2007). Over the past 10 years, there has been a steady increase in the area planted to beans in Uganda, from 615 000ha in 1996 to 849 000ha in 2006 (FAOSTAT, 2007). However, production per unit area has been continuously declining. Bean production in the country was estimated at 599kg ha^{-1} in 1999 and 499kg ha^{-1} in 2006 (FAOSTAT, 2007). Decline in production has been attributed to several biotic and abiotic factors of which BRR is one of the major biotic constraints to bean production in Uganda.

Bean root rot (BRR) has been reported to occur in most bean fields throughout the world (Beebe et al., 1981; Burke and Miller, 1983; Abawi and Pastor-Corrales, 1990; Tu and Park, 1993; Park and Tu, 1994). In eastern Africa and many other parts of Africa they are responsible for most bean yield losses (Otsyula and Ajanga, 1994; Pyndji, 1996; Otsyula et al., 1998; Tusiime et al., 2000; Spence, 2003). In Uganda, especially in the south-western highland regions, BRR is one of the most serious constraints to bean production (David et al., 1999; Mukalazi et al., 2001; Spence, 2003; Tusiime, 2003; CIAT, 2005; Opio et al., 2007), with significant losses occurring to susceptible varieties. It 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 neighbour 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.) Snyd. & Hans (FSP) is one of a complex of soil-borne pathogens causing root rots on beans, others being *Pythium* sp, *Rhizoctonia solani* and *Macrophomina phaseoli* (Abawi and Pastor-Corrales, 1990; Rusuku et al., 1997). The pathogen is particularly severe on large-seeded bean genotypes due to a lack of genetic resistance in these seed types (Beebe et al., 1981; Burke and Miller, 1983; Schneider et al., 2001; Román-Avilès and Kelly, 2005). An overemphasis on quality traits in previous breeding programmes, and the consequent reduction in genetic variability

is likely to have contributed to the lack of resistance in the large-seeded bean varieties (Schneider et al., 2001). The intensification of agriculture resulting from the increasing human population that is especially characteristic of the highland regions, could also have led to higher BRR epidemics. It is probable that land fragmentation, due to the high human population density may have resulted in declining soil fertility levels. This could then have led to an imbalance between the beneficial and disease-causing organisms in the soil, hence an increase in root rot pathogen inoculum levels in the soils. Varieties that could previously tolerate the low levels of inoculum have since succumbed to the disease.

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 also predominant, often occurring concurrently with *Pythium* spp. and was alsofound to even be more destructive in screen house tests (Tusiime, 2003). This indicates the need to address Fusarium root rot (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, is the use of bean varieties that are resistant to the most common soil-borne pathogen(s) occurring in the production region (Hall and Nasser, 1996; Otsyula et al., 1998; Abawi et al., 2006). Unfortunately, popular commercial bean varieties currently grown in Uganda are susceptible to the prevailing root pathogens, while known resistant varieties are associated with undesirable characteristics such as late maturity, black seed colour, and small seed size (Rusuku et al., 1997; Otsyula et al., 1998). Large-seeded varieties are the major market class or preferred bean seed types in most parts of Uganda. There is hence a need to improve the resistance of these seed types to FRR, with the involvement of the farmers for whom the varieties are meant. Participatory plant breeding (PPB) has been shown to result in better adoption of new varieties (Weltzien et al., 2003). Previous studies on resistance to *FSP* (Smith and Houston, 1960; Wallace and Wilkinson, 1965; Hassan et al., 1971) were not conclusive as to the mode of inheritance of this character. Knowledge of the inheritance of a trait is critical in designing appropriate breeding

strategies for incorporating such a trait into economically useful populations. This study will hence help in shedding more light on the genetic basis of resistance to FRR.

Objectives of the study

The study aimed at contributing to improved food security by improving resistance to FRR in preferred major market class bean varieties. Specifically the study aimed at
1. Studying farmers awareness and perceptions of BRR and their influence on varietal preferences;
2. Isolating and maintaining a virulent pathogenic isolate of FSP for use in screening common bean (*P. vulgaris* L.) germplasm for resistance to FRR;
3. Developing an effective technique for screening common bean germplasm for resistance to FRR*;*
4. Studying the genotypic variability of common bean (*P. vulgaris* L.) resistance to FRR and identification of sources of resistance;
5. Studying the inheritance of resistance to FSP in common bean.

Organisation of thesis

This thesis is made up of eight sections that include six chapters as shown below:
1. Introduction;
2. Chapter One: Literature review
3. Chapter Two: Farmers' awareness and perceptions of BRR and their influence on bean varietal preferences
4. Chapter Three: Isolation and maintenance of a pathogenic *F. solani* f. sp. *phaseoli* isolate for use in screening common bean (*P. vulgaris* L.) germplasm for resistance to FRR
5. Chapter Four: Developing an effective technique for screening common bean germplasm for resistance to FRR
6. Chapter Five: Studying the genotypic variability of resistance to FRR and identification of sources of resistance
7. Chapter Six: Genetic analysis of resistance to FRR in common bean (*P. vulgaris* L.)
8. An overview of the study

All chapters, except Chapter one (literature review), are written in the IMRAD format, that is, Introduction, Materials and Methods, Results and Discussion. All chapters have a reference list. Hence there may be some limited repetition as well as overlap of content, especially between the references and the Introduction sections of these chapters.

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Chapter One: Literature review

This review of literature provides an overview of the taxonomy, origin and diversity of the common bean. It also gives information on the production constraints facing common bean. An in-depth analysis of Fusarium root rot (FRR) and breeding for resistance to FRR in common bean is also presented.

1.1 Taxonomy of the common bean

The common bean (*Phaseolus vulgaris* L.) belongs to the Angiosperms phylum (flowering plants with the orubs enclosed in a carpel or in several carpels united into an ovary). Over 30 species of *Phaseolus* have been reported from the Americas (Debouck, 1991; 1999). Of these, only five, namely, common bean (*Phaseolus vulgaris* L.), year bean (*Phaseolus polyanthus* Greenman), scarlet runner bean (*Phaseolus coccineus* L.), tepary bean (*Phaseolus acutifolius* A, Gray) and lima bean (*P. lunatus* L.) are known to be domesticated (Gepts and Debouck, 1991; Debouck, 1999; 2000). The common bean (*P. vulgarius*) possesses by far the widest adaptation of all *Phaseolus* spp. with over 85% of the cultivated species falling under this species worldwide (Singh, 2001).

Common beans are classified in the sub-phylum Dicotyledons (embryo with two cotyledons, parallel veined leaves and the stem with the vascular bundles arranged irregularly and cambium usually present), division Magnoliophyta, class Magnoliopsida, family Leguminosae, sub-family Papilionoideae/Fabaceae/Lotoideae (pulse family characterized by edible seeds and pods) and order Leguminales. Common beans are a diploid (2n = 2x = 22) and self-pollinated crop (Rutger and Beckham, 1970; Stoetzer, 1984) possessing complete, papilionaceous flowers with 10 stamens, and an ovary with a long, coiled style and a hairy introrse stigma; the stigma is situated laterally along the inner arc of the curved style, where it intercepts pollen dehiscing from its own anthers. The crop is highly polymorphic, showing considerable variation in growth habit, vegetative characters, flower colour and size, shape and colour of pods and seeds (Purseglove, 1968). There are two major commercial classes of common bean, snap and dry beans (Singh, 2001). Snap beans are also known as string or green beans and are mainly grown for their pods, while dry beans are mainly grown for their seed.

1.2 Origin and genetic diversity of the common bean

It is believed that dry beans, along with maize, squash, and amaranth, probably began as weeds in fields planted to cassava and sweet potatoes in Latin America (Purseglove, 1968). Over the millennia, farmers grew complex mixtures of bean types as a hedge against drought, disease, and pest attacks, a process which has produced an almost limitless genetic array of beans with a wide bean variety of colours, textures, and sizes to meet the growing conditions and taste preferences of many different regions (Purseglove, 1968). The crop was introduced to Africa by Portuguese traders in the 16th century where it was met with great success in the Great Lakes region. Africa is now regarded as a secondary centre of diversity for the crop (Trutmann, 1996). The common bean was domesticated more than 7 000 years ago in two centres of origin, Mesoamerica (Mexico and Central America) and the Andean region (Purseglove, 1968; Harlan, 1975; Evans, 1980; Vargas et al., 1990; Gepts and Debouck, 1991; CIAT, 1995). Hence it is divided into two major genepools, the Middle American and Andean genepools.

According to Evans (1973; 1980), genetic diversity in common bean may be organised into three general classes according to seed size namely, the large-seeded (>40g 100 seed weight^{-1}) Andean genepool and the medium (25-40g 100 seed weight^{-1}) and small (<25g 100 seed weight^{-1}) seeded Middle American genepool. The presence of two genepools is evidenced by differences in seed size (small versus large), "Dl" genes and F_1 incompatibility (Gepts and Bliss, 1985; Vieira et al., 1989), phaseolin seed proteins (Gepts et al., 1986), allozymes (Koenig and Gepts, 1989; Singh et al., 1991c) and DNA markers (Becerra Velasquez and Gepts, 1994; Haley et al., 1994). Within these genepools, landraces sharing certain distinctive morphological, agronomic and adaptive traits, and differing from other groups in allelic frequencies of the genes controlling differences in those traits were defined as races by Singh et al. (1991a). Singh et al. (1991a; 1991b) further divided the Andean and Middle American cultivated genepools into six races: Andean (Chile, Nueva Granada and Peru; large-seeded) and Middle American (Durango and Jalisco; all medium-seeded and Mesoamerican; all small-seeded), based on ecological adaptation and agronomic traits. Beebe et al. (2000) further reported the existence of additional diversity within Middle American races, especially a group of Guatemalan climbing bean accessions that did not group with any of the previously defined races.

Nine major commercial seed types/market classes are grown in Africa. These include the Calima (Rosecoco or red mottled) and the reds (large and small), which together account for about 50% of the production, primarily because of their high market demand. Others are the navy beans, cream-coloured, brown tan, yellow types, purples, white and black beans (Buruchara, 2006).

1.3 Bean production constraints

Even though common bean is adaptable to different cropping systems and has a short growing cycle, it is susceptible to many biotic and abiotic constraints (Schwartz and Pastor-Corrales, 1989; CIAT, 1990; Singh, 1992; Wortmann et al., 1998). Low soil fertility and drought are among the abiotic stresses that are most widely distributed. Deficiencies in soil nitrogen, phosphorous (P) and zinc (Thung, 1990; Karen et al., 2006), and toxicities of aluminium and manganese are particularly disastrous. Low P soils are a major constraint to bean production in regions of Africa and Latin America where farmers lack access to sufficient P fertilizer (Wortmann et al., 1998). Complete crop failures due to drought are very common in dryland conditions (Carlos et al., 2006). Low temperatures below 15°C, as well as frost at the beginning and the end of the growing season in the highlands (above 2 000m elevation) can also reduce yield (Singh, 2001).

Among the biotic stresses, many species of insect pests attack beans both before and after harvest. In Uganda, the major pests include the bean fly (*Ophiomyia phaseoli*, *O. spencerella*, *O. centrosematis;* Diptera: Agromyzidae), foliage beetles (*Ootheca* sp; Coleoptera: Chrysyomelidae), black aphid (*Aphis fabae*; Homoptera: Aphididae), striped beetle (*Alcidodes leucogrammus*; Coleoptera: Curculionidae) and flower thrips (*Megalurothrips sjostedti*; Thysanoptera: Thripidae). Other insect pests attacking beans in Uganda include common whitefly (*Bemisa tabaci*; Homoptera: Aleyrodidae), leaf hoppers (*Empoasca* sp.; Homoptera: Cicadelidae), cutworms (*Agrotis* sp and *Spodoptera* sp; Lepidoptera: Noctuidae), blister beetles (*Mylabris* spp. and *Coryna* spp.; Coleoptera: meloidae), pod borer (*Maruca testularis*; lepidoptera: Pyralidae), American bollworm (*Helicoverpa armigera*; Lepidoptera: Noctuidae) and pod-sucking bugs (*Clavigralla* sp., *Anoplocenemis curvipei*, *Nezara viridula, Piptortus dentipes*) (Abate and Ampofo, 1996; Byabagambi et al., 1999).

Weeds are also an important constraint to bean production due to competition for light, water, space and nutrients (Alteiri and Liebman, 1986; Alemán, 2001). Good weed control may be achieved by a single weeding three weeks after planting. However, major losses in the tropics result when farmers lack sufficient labour for timely hand weeding (Wortmann, 1993). Alemán (2001) reported increased yield of common bean using mechanical and chemical weed control with no or minimum tillage.

Diseases are major constraints to bean production and may be fungal, bacterial or viral in nature. In Uganda, 20 diseases on beans were listed by Hansford (1938), but only 10 of these are considered important. They include common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* Smith), angular leaf spot [*Phaseoriopsis griseolsa* (Sacc) Ferr.], rust (*Uromyces appendiculatus* Pers), bean common mosaic virus (BCMV), and floury leaf spot [*Mycovellosiella phaseoli* (Drummond) Deighton], which are more important in the low altitude high temperature areas. Halo blight (*Pseudomonas syringae* pv. *phaseolica* Burkholder), anthracnose (*Colletotrichum lindemuthianum* Sacc & Magn), aschochyta blight [*Phoma exigua* var. *diversipora* (Bub.) Boerma], and root rots *(Rhizoctonia solani, Pythium* sp. *Fusarium* spp.) are considered more important in the high altitude and low temperature areas of Uganda (Opio et al., 2001).

In Uganda, especially in the south-western highland region, BRR is one of the most serious constraints to bean production (Pyndji, 1996; David et al., 1999; Spence, 2002), with significant losses occurring to susceptible varieties.

1.4 Bean root rots

Bean root rots are widely distributed and economically important on common bean in central and South America, Africa and other areas (Abawi and Pastor-Corrales, 1990; Singh, 2001; Kelly et al., 2002). The disease is caused by soil-inhabiting fungi that cause root rots, and some of which are capable of inciting seed rot and seedling damping-off. These soil-borne fungi include *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans (FSP) that causes Fusarium root rot (FRR); *Rhizoctonia solani* Kuhn that cause Rhizoctonia root rot; *Sclerotium rolfsii* Sacc that causes Sclerotium root rot; *Macrophomina phaseolina* (Tass) Goid that causes Charcol rot; and *Pythium* spp. that causes Pythium root rot. *Fusarium oxysporum* (Schlecht.) f. sp. *phaseoli* Kendrick and Snyder is another important pathogen that takes advantage of damage caused by other root rot pathogens to

enter the vascular system of the plant, causing Fusarium wilt (Kraft et al., 1981; Abawi and Pastor Corrales, 1990; Rusuku et al., 1997; Buruchara et al., 1999;).

Bean root rots have a strong negative impact on bean yield in tropical agro-ecosystems, resulting in significant losses to susceptible varieties, especially if cool and wet weather conditions prevail for the first few weeks after seeding, followed by hot and dry weather (Burke and Miller, 1983; Abawi and Pastor-Corrales, 1990).

1.5 Fusarium root rot (*Fusarium solani* f. sp. *phaseoli)*

Fusarium root rot may cause yield losses of up to 84% (Beebe et al., 1981; Abawi and Pastor-Corrales, 1990; Park and Tu, 1994). Unlike other root-rotting diseases, this pathogen attacks older seedlings and does not cause seed rots or damping-off of seedlings. Symptoms do not appear until a week or more after the seedling emerges. The first symptoms are narrow, long, red to brown streaks on the hypocotyls and taproot (Figure 1.1). The taproot later turns dark brown and cracks often develop lengthwise. It may then shrivel and die, with clusters of fibrous roots developing above the shrivelled taproot (Figure 1.2).

Fig. 1.1 Symptoms of *Fusarium solani* f. sp*. phaseoli* on a bean seedling

Fig. 1.2 Formation of adventitious roots in response to *Fusarium solani* f. sp*. phaseoli* infection

These fibrous roots may keep the plant alive and, under ideal growing conditions, a few above-ground symptoms will be noted. Plants may be stunted, have a pale colour, and grow more slowly than healthy plants, resulting in uneven plant stands (Abawi, 1980; Abawi and Pastor-Corrales, 1990; Hall, 1991; Abawi et al., 2006). Fusarium root rot seems to be favoured by temperatures of 14-24°C, although the optimum is said to be around 21°C (Sippel and Hall, 1982).

Plant damage from FSP is usually increased under environmental conditions that stress plants. These conditions include deep planting, soil compaction (Burke, 1965; Miller and Burke, 1985), cool temperatures, high or low pH, low fertility, pesticide or fertilizer injury, and flooding or extended drought (Burke et al., 1969; 1972; Miller and Burke, 1975; 1977). It has also been noted that there is more damage when *Pythium* spp. occur concurrently with FSP (Pieczarka and Abawi, 1978; Sippel and Hall, 1982; Abawi et al., 2006). A synergistic interaction has been reported to exist between FSP and *Pythium* spp. (Sippel and Hall, 1982), and FSP and root-knot nematodes (Pieczarka and Abawi, 1978), resulting in even higher disease infection levels.

1.5.1 Taxonomy and epidemiology of *Fusarium solani* f. sp. *phaseoli*

The fungus FSP belongs to the *Nectria haematococca – Fusarium solani* species complex section *Martiella* of Fusarium (Booth, 1971; O'Donnell, 2000). It is homothallic, although some strains from *F. solani* are heterothallic (hence the perithecial name *Nectria haematococca*) (Booth, 1971). It is one of the ten (Crowhurst et al., 1991; Suga et al., 2000) or eleven (Shuxian et al., 2000; Cho and Rupe, 2000) formae speciales of *Fusarium solani* [(Teleomorph *Haematonectria haematococca* Syn. *N. haematococca*] (Rossman et al., 1999).

F. solani f. sp. *phaseoli* generally produces only asexual spores (microconidia, macroconidia and thick-walled chlamydospores), although under certain conditions it produces its perithecial stage, *N. heamatococca* (Agrios, 1997). The fungus can overwinter as mycelium or spores in infected or dead plant tissue, and in soil or seed as thick-walled chlamydospores. The spores are easily dispersed by air, equipment, water, and by contact (Nash and Snyder, 1962; Abawi, 1980; Kraft et al., 1981). In soil the pathogen spores are often under the influence of soil fungistasis (Hall, 1991). However, when fungistasis is

reversed, they germinate and penetrate bean tissue directly or through wounds and natural openings (Abawi, 1980; Hall, 1991). Soil fungistasis is reversed when spores are stimulated by nutrients exuded by germinating bean seeds and root tips. The fungus then grows intercellularly throughout the cortical tissues (Kraft et al., 1981). With each successive crop of beans, pathogen population increases and the disease becomes more severe. The pathogen is also capable of colonising roots of non-host crops without causing disease symptoms and colonising organic matter under certain environmental conditions, thereby maintaining or increasing its population in the absence of beans (Abawi, 1980; Hall, 1991).

1.5.2 Management of Fusarium root rot

There are several cultural practices that may help to reduce losses due to bean root rot (BRR) disease, but none has proved completely adequate (Nderitu and Buruchara, 1997; Opio et al., 2001; Abawi et al., 2006). The occurrence of multiple soil-borne pathogens with different mechanisms of pathogenicity has made it difficult to develop a simple and effective disease management programme for FRR (Sippel and Hall, 1982; Abawi et al., 2006). Currently, the management of root rot diseases is possible 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; Abawi, et al., 2006; Opio et al., 2007).

Control of FRR in the greenhouse is often achieved through soil sterilization, use of healthy seed and seed dressing. In the field, loosening compacted soil with sub-soiler chisels before planting has, to date, been the most dependable method of reducing FRR of bean (Burke and Miller, 1983). Others include rotation with non-susceptible crops, ensuring good soil drainage, and soil fertilization, especially with the nitrate form of nitrogen (Kraft et al., 1981; Burke and Miller, 1983; Miller and Burke, 1985; Hall and Nasser, 1996). Most of these methods aim at restricting the activity of the pathogen in the soil and reducing plant stress (Hall, 1996).

Using disease-free or fungicide-treated seed may help reduce losses. Fungicides have been reported to control or reduce bean FRR in glasshouse trials (Mussa, 1986). In the field, however, Abawi and Pastor-Corrales (1990) reported that seed treatment with fungicides such as thiram (Thiram 70 S), benomyl (Benlate), and captafol (Difolatan) was only partially effective, because damage occurred on fibrous roots at some distance from seed

placement. Localized treatments that control seed rot and seedling damping-off help ensure optimal plant populations, which in turn may help counteract yield depressions by root rot (Burke and Miller, 1983).

Biological control of FRR and stem rot has been attempted with some success by incorporating organic materials such as barley straw and chitin into the soil, thus favouring the increase of several fungi and bacteria antagonistic to Fusarium, or by treating seeds or transplants with spores of fungal antagonists, mycorrhizal fungi or antagonistic bacteria. Plants inoculated with the mycorrhiza *Glomus mosseae* in the presence of a root nodulating symbiont *Rhizobium leguminosarum* were found to be more tolerant to FRR (Dar et al., 1997). Similarly, Biegh et al. (1998) found a 34% reduction in pathogenic root rot when soil was inoculated with *R. leguminosarum.* Filion et al. (2002) found that the vesicular arbuscular mycorrhizal fungus *G. intraradices* significantly reduced FRR symptoms. However, none of these biological control methods is currently being used in Uganda as the technology is not sufficiently developed for dissemination to bean growers.

The use of resistant varieties in FRR management offers the best control measure for the disease, and the most suitable option for small-scale farmers. However, it must be supplemented with conditions that do not favour disease development. Miller and Burke (1986) reported an 84% -160% bean yield increase of a resistant dry bean bean line over a susceptible one. In addition, several *P. vulgaris* and *P. coccineus* lines have been reported to be resistant to FRR (Burkholder, 1919; Azzam, 1958; Baggett and Frazier, 1959; Baggett et al., 1965; Burke and Silbernagel, 1965; Wallace and Wilkinson, 1965; Wallace and Wilkinson, 1966; Boomstra et al., 1977; Beebe et al., 1981; Silbernagel, 1987). In combination with other cultural practices, Silbernagel and Mills (1990) reported a lower root rot severity and higher yield of a resistant bean line compared with a susceptible one. Abawi and Pastor-Corrales (1990) and Otsyula and Ajanga (1994) have also reported the importance of resistance in controlling BRR. However, the common and most popular bean varieties currently being grown in Uganda are susceptible to BRR (Pyndji, 1996; Tusiime, 2003; Kalyebara and Kassozi, 2005).

1.6 Genetic improvement of common bean in Uganda

Two main cultivated species of *Phaseolus* are grown in Uganda, namely, *P. vulgaris* and *P. lunatus* L. Others include *P. coccineus* L. (scarlet runner bean) and *P. acutifolius* A. Gray (tepary bean) which are not common in Uganda for large scale production, but are mostly used at research institutes for experimental purposes. The Bean Research Programme at Namulonge Agricultural and Animal Production Research Institute (NAARI) has over 400 accessions of *Phaseolus* species collected from different parts of Uganda.

Bean research in Uganda was started in the 1960s to address protein deficiency problems and especially to combat Kwashiorkor that was prevalent in the banana-based region (Leakey, 1970). By 1968 several bean varieties had been released, reaching a climax with the release of bean line K20 in 1968. To date, K20 is the most widely grown bean line in Uganda (Kalyebara and Kassozi, 2005). The bean line K20 has been given different names depending on the location, viz. "*Nambale"* in Mukono, Mbale, and Sironko, "*Kamenyamigo"* in Masaka and Rakai, "*Kachwekano"* in Kabale, and "*Tanzania"* in Iganga, Mbale, Sironko and Kapchowra. Other released varieties include K131, K132, OBA1, MCM2001 and MCM1015. Between 1970 and 1980 there was little bean research and no bean bean line was released during this period. However, in 1985/86, bean research restarted with the aim of increasing the productivity of beans by developing high-yielding and acceptable bean varieties with resistance to the major pests and diseases, both for the domestic market and for export. Selections from introductions from Burundi, Democratic Republic of Congo, Ethiopia, Kenya, International Center for Tropical Agriculture (CIAT), Malawi, Tanzania, and Rwanda, and locally collected varieties, and hybridization were the main breeding techniques used in the breeding programme in Uganda (Opio et al., 2001). Currently the bean-breeding programme focuses on both yield and the most serious pests and disease, which include bean common mosaic virus (BCMV), common bacterial blight (CBB), angular leaf spot (ALS), anthracnose, and BRR (Opio et al., 2001).

Seed size, seed colour, yield, taste and cooking time are the major characteristics farmers consider before adopting a new bean bean line (David et al., 1997; Mugisa-Mutetika, 1997).

1.7 Breeding for resistance to *Fusarium solani* f. sp. *phaseoli* and large seed size

Improvement of resistance to FSP, especially in large-seeded dry and snap bean types, has been limited, in spite of considerable research efforts to elucidate its genetic control. FRR is a particularly severe disease on large-seeded Andean bean genotypes due to a lack of genetic resistance in these seed types (Dickson, 1973; Wallace and Wilkinson, 1973; Abawi and Pastor-Corrales, 1990; Schneider et al., 2001). In addition, genetic diversity in the cultivated Andean genepools is generally very limited, confounding this problem (Becerra Velasquez and Gepts, 1994; Sonnate et al., 1994; Beebe et al., 2000; Islam et al., 2004). Farmers in Uganda have been forced to abandon growing the popular large-seeded varieties in preference to the small-seeded types due to the root rot epidemic.

It has also been suggested that the components of varietal mixtures (a common practice in Uganda) have been changing over time, with a decrease in diversity due to the root rot problem (Ampaire, 2003). Beebe et al. (1981) found that small and black seeded Middle American varieties were, in general more resistant to FSP than the large and red seeded varieties. It is believed that an overemphasis on the improvement of the quality traits, allied to neglect in the improvement of disease resistance in kidney and snap beans, may be responsible for the intense susceptibility to FSP in these seed types as compared to the small-seeded beans (Gepts, 1998; Schneider et al., 2001; Román-Avilès and Kelly, 2005). Thus, small-seeded genotypes of Middle American origin, although not completely resistant to root rot, are valuable sources of resistance (Beebe et al., 1981; Abawi and Pastor-Corrales, 1990).

While recombination between Andean and Middle American genepools occurs readily, hybrid lethality can result (Koinange and Gepts, 1992). Skewed segregation as a result of this phenomenon is also common and may lead to misinterpretation in inheritance studies. In addition, recovery of essential agronomic characteristics such as adaptation, yield and seed, and pod quality characteristics of cultivars is challenging while introgressing desirable alleles by means of bi-parental crosses between Andean and Middle American genepools of common bean (Singh, 2001). This is probably because genotypes from the large-seeded Andean genepool are distinguished from the small-seeded Middle American genotypes in morphological, biochemical, and molecular characteristics (Gepts, 1988; Haley et al., 1994). Some scientists have reported success in the introgression of FRR resistance into large-

seeded Andean beans from the small-seeded beans of the Middle American genepool (Schneider et al., 2001; Román-Avilès and Kelly, 2005). However, the recovery of essential agronomic characteristics in these populations was not reported.

1.8 Mechanisms of resistance to Fusarium root rot

Despite the differences between the resistance levels in the two genepools, mechanisms associated with host defence responses appear to be involved in resistance to FSP (Schneider et al., 2001; Román-Avilès and Kelly, 2005). Although several mechanisms have been suggested as the physiological basis of resistance to FSP by the common bean, most have not been ascertained.

A hypersensitive reaction to invasion by FSP has been reported by Pierre and Wilkinson (1970). They observed browning of cortical cells in the advent of invasion by the hyphae of FSP, which limited the growth of hyphae in resistant varieties. Browning of the peridium of the roots was also observed, but this was reported not to limit hyphal growth.

A vigorous root system has often been suggested to increase tolerance to root rot (Snapp et al., 2003; Román-Avilès et al., 2004.). The division of carbohydrates between shoots and roots is influenced by both genetic and environmental factors. This may imply that the genes governing root system vigour also influence resistance to root rot such that varieties with genetically vigorous root systems are more resistant to BRR's compared to those with weak root systems.

The colour of seed and hypocotyls has also been related to the level of resistance to FSP. Statler (1970) observed higher resistance to FSP in black seeded varieties and varieties with purple-coloured hypocotyls, and related it to the greater production of phenolic compounds inhibitory to fungal growth in the early stages of seedling growth. Phytoalexins such as phaseolin have been identified and reported to be produced in response to infection by R. solani (Pierre and Bateman, 1967) and FSP (Kendra and Hadwiger, 1989), with production of these phytoallexins being shown to be greater and more rapid in resistant varieties. Similarly Beebe et al. (1981) recorded more resistance to FSP in the small and black seeded varieties compared to large red mottled ones. Selection, either direct or indirect,

aimed at enhancing these traits should allow for rapid improvement of resistance to FRR in Andean bean genotypes.

1.9 Breeding methods for beans

Most, if not all, crop breeding methods, have been employed in common bean. The mass pedigree (Singh et al., 1989, Beebe et al., 1993; Grafton et al., 1993; Singh et al., 1993), pedigree (Kelly et al., 1994a; 1994b), and recurrent backcross methods and their modifications (Miranda et al., 1979; Pompeu, 1980; Pompeu, 1982; Alberini et al., 1983; Bliss, 1993) have been used for common bean improvement. Congruity backcrossing (Haghighi and Ascher, 1988; Urrea and Singh, 1995), single seed descent (SSD) (Urrea and Singh, 1994), recurrent (Duarte, 1966; Sullivan and Bliss, 1983; Kelly and Adams, 1987; Beaver and Kelly, 1994; Singh et al., 1999) and gamete selection (Singh and Teran, 1998) methods have also been used. Urrea and Singh (1994) found that the F_2-derived family method of selection was superior to the SSD and bulk methods commonly used for advancing early generation of hybrid seed yield in the early generation of hybrid populations. Singh and Urrea (1995) and Singh et al. (1990) suggested selection for seed yield in early generations of interracial and intergenepool populations with desirable recombinants. From early generation yield tests (F_2-F_4), Singh and Teran (1998) identified high-yielding and low-yielding populations that eventually produced high-yielding and low-yielding advanced generation (F_7) varieties. In this study, the backcross breeding method was employed for improving resistance to FRR, using the diallel mating design for population development.

1.10 Diallel mating design

The diallel cross refers to a set of all possible matings between several genotypes (Hayman, 1954a; 1954b). The genotypes may be individuals, clones, homozygous varieties, etc. The diallel analysis helps to obtain information on the genetic systems governing the inheritance of attributes to be improved, and hence may help in predicting the performance in subsequent generations by assessing the potential of different crosses in F_1 and F_2 (Dickson, 1967; Dabholkar, 1992). Like other mating designs, diallel mating is a frequently used design for estimating the additive and dominance genetic (polygenic) effects involved in quantitative traits observed in the half- and full-sib progenies generated in plant breeding programmes. The diallel design has additional benefits in that the analysis applies to all the

crosses involved and permits the estimation of parameters for additive, dominance and environmental effects, and allows recognition of non-allelic interactions (Hayman, 1954a; 1954b; Griffing, 1956; Jinks, 1956; Matther and Jinks, 1982; Christie and Shattuck, 1992). In addition, this technique enables the breeder to combine desirable genes that are found in two or more genotypes (Dabholkar, 1992).

There are four basic designs and analysis for the diallel mating design (Christie and Shattuck, 1992), and they include

1. Analysis of general and specific combining ability or Griffing's analysis (Griffing, 1956);
2. Analysis of array variances and covariance's or Hayman and Jinks analysis (Jinks and Hayman, 1953; Hayman, 1954b, Jinks, 1954; 1956);
3. Analysis of additive and dominance effects, also referred to as Gardner and Eberhart's analysis (Gardner and Eberhart, 1966; Eberhart and Gardner, 1966) and;
4. Partial diallel analysis (Gilbert, 1958; Kempthorne and Curnow, 1961).

The present study used Griffing's analysis to determine the combining ability of varieties and characterise the nature and extent of gene action (Christie and Shattuck, 1992). This analysis requires no genetic assumptions (Wright, 1985), and has been shown to convey reliable information on the combining potential of parents (Nienhuis and Singh, 1986).

This design provides breeders with useful genetic information, such as general combining ability (GCA) and specific combining ability (SCA), to help them devise appropriate breeding and selection strategies (Zhang et al., 2001). The GCA and SCA effects help to locate the parents and crosses that will be responsible in bringing about a particular type of gene action (Dabholkar, 1992). General combining ability refers to the mean performance of a line in all its crosses, and is expressed as a deviation from the mean of all crosses (Falconer and Mackay, 1996). It is the average value of all F_1s having this line as one parent, the value being expressed as a deviation from the overall mean of crosses. Any particular cross has an expected value which is the sum of the general combining abilities of its two parental varieties. However, the cross may deviate from this value to a greater or lesser extent. This deviation is called the SCA of the two varieties in combination (Falconer and Mackay, 1996). Differences in GCA have been attributed to additive, additive x additive and higher order interactions of additive genetic effects in the base population, while differences in SCA have been attributed to non-additive genetic variance (Baker, 1978).

Resistance to FRR has been observed to be additive in nature being governed by 3-7 largely dominant genes with major additive effects (Bravo et al., 1969), two to three recessive genes (Azzam, 1958), two genes with recessive duplicate action (McRostie, 1921) or with dominant and recessive epistasis (Smith and Houston, 1960). However, Hassan et al. (1971) reported a shift from additive gene action to partial dominance with length of exposure to the pathogen. Similarly, Wallace and Wilkinson (1966) reported that resistance was dominant, while others simply reported that resistance to FRR was complex (Wallace and Wilkinson, 1965). These findings show a lot of inconsistency, which is probably due to the different sources of resistance that were used as well as the fungal isolates, environmental conditions, and the methods of testing and evaluation in these studies. This study reports further on the inheritance of resistance to FRR in improved populations being developed for Africa.

Heritability (h^2) is a statistical tool used to evaluate the genetic control of traits determined by many loci and can be used to effectively plan strategies for incorporating characters into new cultivars (Falconer and Mackay, 1996). Breeders are interested in heritability for the simple reason that characters with higher values can be improved more rapidly with less intensive evaluation than those with lower heritability. However, heritability estimated is unique to the population being studied and the environmental conditions to which individuals have been subjected (Falconer, 1989; Dabholkar, 1992). Populations which are genetically uniform, such as inbred varieties, are expected to show lower heritability than genetically diverse populations. When heritability is high, more reliance can be placed on mass selection, and when it is low, more emphasis is placed on progeny, sib, or family selection. The heritability is used to estimate the improvement due to selection. The ratio of the genotypic variance (VG) to phenotypic variance (VP) expresses the extent to which individual phenotypes are determined by the genotypes, and is referred to as heritability in the broad sense (H^2), or the degree of determination. Broad sense heritability estimates include additive (VA), dominance (VD) and epistatic (VI) sources of genetic variation. The ratio VA/VP expresses the extent to which the phenotypes are determined by the genes transmitted from the parents, and is termed as heritability in the narrow sense (h^2). It determines the degree of resemblance between relatives and is therefore of greatest importance in breeding programmes (Falconer and Mackay, 1996). Heritability is a reflection of only the additive sources of variation. Environmental variance (VE) forms part of

phenotypic variance and affects the magnitude of heritability; when it is high heritability is low and when it is low heritability is high.

Hassan et al. (1971) reported broad sense heritability (H^2) of resistance to *FSP* of up to 64.3% under greenhouse conditions, and up to 79.7% under field conditions, and narrow sense heritability (h^2) of up to 44.3% in inter-genepool crosses. Schneider et al. (2001) reported an even higher h^2 of resistance to *FSP* of up to 71% in F_4-derived families developed within the same genepool, while Román-Avilès and Kelly (2005) reported h^2 up to 51% in inbred backcross line populations (IBL). The moderate to high heritability estimates suggest that resistance to FRR could be improved by selection.

1.11   Overview of literature review

Most scientists have suggested that resistance to FRR is a quantitative trait that is greatly affected by the environment, and should be analysed as such, with care being taken to control environmental variation as much as possible. The diallel method was hence suggested as a mating design for improving resistance to FRR for this study. The diallel analysis would be able to estimate several genetic parameters such as additive, dominance and environmental effects, and allow recognition of non-allelic interactions. The GCA and SCA effects obtained would help in identifying the parents and crosses that are responsible in bringing about a particular type of gene action and it is these crosses that would be advanced in the next generations.

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Chapter Two: Farmers' awareness and perceptions of bean root rots and their influence on bean varietal preferences

Abstract

The awareness and perceptions of farmers on bean root rot (BRR) is likely to affect the type of bean varieties adopted. Farmers in most parts of Uganda prefer the large-seeded bean varieties both for consumption and for market, but these varieties are very susceptible to BRR. Over the years, reports have indicated that farmers were abandoning large-seeded bean varieties in preference for the smaller seeded varieties that seem to be more resistant. The objective of this study was to assess the awareness and perceptions of bean growers on the influence of BRR on the type of bean varieties being grown. A Participatory Rural Appraisal (PRA) was conducted in the districts of Kisoro and Kabale in south-western Uganda, and Mbale and Sironko in eastern Uganda, during April-August, 2005. The study showed that diseases were the most important bean production constraints, others being climate- and soil-related. Of the common diseases, BRR is the most devastating and most widely recognized, especially in south-western Uganda. Bean growers were able to identify BRR, but control measures taken were minimal, probably due to the lack of knowledge and resources. Bean root rots were associated with poor soils, high/excessive rainfall, drought and many other environmental factors, as well as poor crop management practices. Although, the farmers associated BRR mainly with the large-seeded bean varieties, they are still the most popular among the bean growers. Varietal preferences were based on high yielding ability, early maturity, marketability, and disease and drought resistance. Other factors considered important included, taste, bush growth habit, cooking duration, large seed size and seed colour. Generally, large-seeded bean varieties are the most preferred in both regions; however, the percentage of farmers preferring large-seeded varieties was greater in eastern Uganda, while the percentage of farmers preferring small-seeded varieties compared to the large-seeded varieties was greater in south-western Uganda. Farmers that preferred the small-seeded bean varieties based their preferences on the ability to resist pests and diseases and ability to thrive under harsh environments such as excessive rainfall, drought and mist. However, the varieties K20 and K132, both of which are large-seeded and red mottled kidney beans though susceptible to BRR, were the most popular bean varieties grown both for consumption

and sale in the south-western and eastern regions, respectively. This therefore indicated the need to develop bean varieties that have the qualities of the large-seeded varieties but are resistant or tolerant to BRR.

2.1 Introduction

Bean root rots (BRR) have been cited as one of the major causes of low bean yields in the south-western and eastern highland regions of Uganda, with some farmers losing entire crops to the disease (CIAT, 1995; Opio et al., 2001; Ampaire, 2003). A study conducted by UNBP (Uganda National Bean Programme) in Kigezi County in Kisoro district to determine the organisms responsible for root rot, revealed that 80% of bean fields were affected by BRR (Ampaire et al., 2003; Spence, 2003). Several control measures directed at controlling BRR have been developed and applied, but currently none has been found to be adequate. The use of resistant varieties is probably the single most effective control measure that would be a more viable option for the poor rural farmers in Uganda. However, the most popular and preferred bean varieties (red, and red mottled large-seeded varieties) for both consumers and traders are susceptible to BRR (Tusiime et al., 2000; Schneider et al., 2001; Otsyula et al., 2005); hence the acreage grown to these varieties is declining fast due to their susceptibility to BRR (Opio et al., 2001; Kalyebara and Kassozi, 2005). Indeed, an impact study by Kalyebara and Kassozi (2005) showed that in the year 2004, 23% of farmers had abandoned growing K20, 14% abandoned Kanyebwa, while 9% had abandoned K132. Climbing beans have been shown to be more tolerant to BRR compared to the bush type beans and several have been introduced, that is, NABE 7C, NABE 8C, NABE 9C, and NABE 10C in 1999 and NABE 12C in 2003 (Opio et al., 2001; Kalyebara and Kassozi, 2005). However, to date their adoption is very low (Kalyebara and Kassozi, 2005), probably because these varieties were developed without the participation of the farmers, for whom they were meant, thus perhaps lacking some of the qualities required.

The above observations therefore indicate the need to involve farmers in the breeding process as this will help to fit the crop to specific needs and uses within farmers' communities (Ceccarelli et al., 2000), and hence improve cultivar adoption (Horne and Stur, 1997). Farmer participation is a powerful tool to achieve a meaningful orientation of a breeding programme (Weltzien et al., 2003).

Participatory plant breeding involves scientists, farmers, and others, such as consumers, extensionists, vendors, industry representatives, and rural cooperatives in plant breeding research, and it is termed participatory because many actors, and especially the users, can have a research role in all major stages of the breeding and selection process (Sperling et al., 2001). A Participatory Rural Appraisal (PRA) was employed in this study to gather information on the status of BRR under farmers' conditions, and to highlight the need for new improved bean varieties that combine root rot resistance and market class qualities. The PRA enables rural communities to do their own investigations through modelling, diagramming, ranking, and quantification. It allows for learning, from and with, the rural people, eliciting and using their criteria and categories and finding, understanding and appreciating indigenous technical knowledge (Chambers, 1993; Sperling et al., 2001). In PRA, farmers/respondents are able to do the analysis and presentations and to plan and own their outcomes (Chambers, 1993; Scoones and Thompson, 1994a; 1994b). The PRA also allows for direct contact between the investigator and local people in the field.

The objectives of this study were as follows:

1. Assess farmers' awareness of BRR as a constraint to bean production in south-western and eastern Uganda;
2. Assess farmers' preferences of bean varieties and the influence of BRR on types being grown;
3. Assess farmers' perceptions on factors affecting bean yield that may or may not be related to BRR;
4. Assess the incidence and severity of BRR in farmers' fields and,
6. Assess farmers' practices in combating BRR.

2.2 Materials and methods

2.2.1 Study area

The PRA was carried out in two regions of Uganda, namely the south-western and eastern highland regions. Agricultural productivity in the highlands is the highest in the country due to an endowment of fertile volcanic soil, and a cool moist temperate climate (Wortmann and Eledu, 1999; Opio et al., 2001). Four major bean-producing districts of Uganda, namely, Kabale and Kisoro districts from the south-western highlands, and Sironko and Mbale from the eastern highlands, were selected. Two villages from one sub-county were selected per

district, that is, from Kabale district, Ryakarimira and Katabura villages were selected from Rubaya sub-county. In Sironko district, Bunywaka and Bwikhonge villages were selected from Muyembe sub-county. In Mbale district, Makhai and Namwaro villages were selected from Busoba sub-county. However, in Kisoro district the two villages were selected from two sub-counties, namely, Rutare village from Chahi sub-county and Nyarusiza village from Nyarusiza sub-county.

The south-western region accounts for 30% of the total bean production in Uganda (Opio et al., 2001). The region produces high-altitude crops, including Irish potatoes, highland bananas, beans, cowpeas, maize, fruits, sorghum, sweet potatoes, rice, vegetables, and wheat, (Raussen et al., 2002). Climbing beans are mainly produced in the high-altitude areas and bush beans in the lower-altitude areas. Kabale district borders on the districts of Kisoro in the west, Rukungiri to the north, Ntungamo to the east and the Republic of Rwanda to the south (see Appendix 2.1: Map of Kabale district). The district is made up of four counties and 17 sub-counties. The area is predominantly occupied by the Bakiga tribe, although there are a few other ethnic groups found in the area, mainly the Banyarwanda and Bafumbira. Kisoro district is located in the south-western corner of Uganda and borders on Rukungiri district to the north, Kabale district to the east, Rwanda to the south and the Democratic Republic of Congo to the west (see Appendix 2.2: Map of Kisoro district). The district is made up of one county, Bujumbura and 14 sub-counties. There are three main ethnic groups, namely, Bafumbira, Bakiga, and the minority Batwa. Rufumbira and Rukiga are the main languages spoken.

The eastern highland region is very similar in agro-ecology to the south-western highlands, but is made up of a maize-bean system characterised by commercial production of bush beans at low altitude and climbing beans at high altitude, and a banana-coffee system characterized by intercropping beans with bananas and coffee (Wortmann and Eledu, 1999). This region is known for its relatively high level of commercial bean production due to the proximity to bean markets in neighbouring Kenya. It is a major producer of highland bananas, Arabica coffee, maize, wheat, rice, sweet potatoes, fruits, and vegetables. Mbale district borders the Republic of Kenya in the east, Sironko district in the north, Kumi district in the west and Tororo in the south (see Appendix 2.3: Map of Mbale and Sironko districts). The district's indigenous population comprises mainly of the Bamasaba people. Other ethnic groups found in the district include Adholas, Etesots, Banyoli, and Sabiny. The district

comprises of four counties and 28 sub-counties. Sironko district is bordered by Kumi district on the south-west, Nakapiripirit district in the north-east, Mbale district in the south, with the republic of Kenya in the east. About 93% of the district's indigenous population is composed of the Bagisu (Lumasaba tribe) while the other ethnic groups include Sabiny, Iteso, Banyole and Karamajong, among others. The district is made up of two counties and 19 sub-counties.

The study was conducted during the months of April and July, 2005, using both formal surveys and semi-structured interviews (focus group discussions) with the objective of gathering descriptive and numerical data. Semi-structured interviewing refers to a guided conversation in which only the topics are predetermined, and new questions and insights arise as a result of the discussion and visualised analyses.

2.2.2 Surveys

A questionnaire was designed, pre-tested, and executed. The questionnaire involved questions on the background of respondents, bean variety preferences, farmers' perceptions of BRR and management. Fifteen questionnaires per sub-county were pre-tested in Rubaya and Buhara in Kabale district (Figure 2.1). Changes were then made to the questionnaire and the formal survey conducted in all four districts. Visits were organized with the help of CIAT's (International Centre for Tropical Agriculture) staff based in Kabale and Tororo districts, and government extension workers based at the different sub-counties visited. Secondary data on bean production and district data (climate, administration, etc.) was obtained from the district sub-county offices, the Ministry of Agriculture, the National Agricultural Research Organisation (NARO), non-governmental organisations (NGOs) such as AHI (African Highlands Initiative), Africa 2000 Network and Afri-Care, and from literature.

Four enumerators were selected from each district to help in gathering information using the questionnaires. Some of the enumerators were service providers of the National Agricultural Advisory Services (NAADS), teachers, government agricultural extension workers and social workers from NGOs such as Africa 2000. Before conducting the survey, all enumerators underwent training on the objective of the survey and on how to carry out effective interviews.

Thirty bean farmers per district, and hence 120 respondents for the whole survey were interviewed. The respondents were selected in a random and non-random manner (systematic technique and accidental sampling), that is, the fourth household on a particular selected footpath or the owner of a bean field with symptoms of root rot were selected. Interviews were carried out if the respondent was a regular bean grower and had a bean field at the time. The questionnaire involved open-ended questions that allowed the farmers to express themselves in order to gain as much information as possible. Data from the survey was analysed using the statistical programme for social scientists (SPSS Inc., 2002) and Genstat computer programme (Lawes Agric. Trust, 2007).

Fig. 2.1. Pre-testing the questionnaire on perceptions of bean root rot in Kabale district, Rubaya sub-county.

2.2.3 Semi-structured interviews: Focus group discussions (FGDs)

Focus group discussions were carried out in two villages per sub-county per district, with a group comprising at least 15 people. A checklist with predetermined questions was used as an aid to guide the discussions. Discussions were conducted with the help of a facilitator (in most cases a school teacher or a NAADS service provider). The facilitators were able to

speak both English and the local language fluently. Open-ended questions were asked to trigger discussions and questions from farmers were entertained to get everyone involved. Women, particularly, were encouraged to give their views and constructive arguments were allowed. Brainstorming amongst the farmers was allowed to create an atmosphere in which more aspects pertaining to the topic at hand were discussed (see Figures 2.2 and 2.3).

Fig. 2.2. Focus group discussions in Ryakarimira village, Rubaya sub-county, Kabale district.

Fig. 2.3. Focus group discussions in Kisoro, Nyarusiza sub-county.

Farmers were asked to rank their preferences of bean varieties, bean production constraints, bean diseases and causes of these diseases. This was done using the pair-wise ranking (matrix) method, which refers to making comparisons between factors mentioned in pairs and then counting the totals of each. The factor with the highest number of points is

ranked highest and the one with the least is ranked lowest. Figure 2.4 shows an example of a pair-wise ranking sheet used to capture data.

Fig. 2.4 Example of pair-wise ranking sheet (bean diseases).

Samples of diseased plants were shown to the farmers who were not familiar with BRR. Also bean seed varieties differing in size, shape and colour were shown to farmers to allow for visual and verbal assessment of qualities farmers use in selecting a bean bean variety. Transect walks were carried out together with the farmers in nearby bean fields to become familiar with the general state of a selected farmer's field in terms of root rot occurrence in a field situation. Follow-up notes were compiled and personal impressions written down. Photographs of the process were taken.

2.2.4 Observations of incidence and severity of BRR in farmers' fields

Observations were made on ten bean fields per village visited in the four districts. Ten plants were randomly picked in a zigzag pattern from the gardens and observations made on the roots and hypocotyls. Also the general appearance of the bean field was noted. Incidence of BRR was scored as the percentage of the bean fields visited that had plants infected with root rots. Severity of BRR was scored as the average percentage of the root and hypocotyl tissue of ten plants covered by lesions per field visited. Data were analysed using Genstat computer package (Lawes Agric. Trust, 2006).

2.3 Results

2.3.1 Farmers' perceptions of major constraints to bean production

The farmers considered several factors as major constraints to bean production. These included diseases, pests, excessive rainfall, poor soil, soil erosion, lack of stakes and drought (Table 2.1). Other factors included wind, rats, moles, cutworms, and mist.

In general, farmers had similar (P≤ 0.05) perceptions about the importance of diseases, drought, and poor soil to bean production. However, they had different (P≤0.01) perceptions about the importance of other factors on bean production (Table 2.1) across the four districts. Diseases were the most-mentioned constraint to bean production in Kabale, Kisoro, and Mbale, while in Sironko, pests such as beanfly, cutworms, bruchids/bean weevils, and aphids were said to be most prevalent (Table 2.1). Excessive rainfall was considered a major constraint to bean production in Kabale, Kisoro and Sironko, while drought was a major constraint in Mbale compared to other districts (Table 2.1). This could well have been due to Mbale having received less rainfall compared to the other districts in the previous seasons. Soil erosion was considered a problem in Kabale and Kisoro, due to the heavy rains on steep mountain slopes leading to shallow soils, which were said to escalate the BRR problem. Infertile soil was most mentioned in Kisoro and Kabale, compared to Sironko and Mbale (Table 2.1). Lack of stakes was a problem in Kabale and Kisoro only, where climbing beans are more popular than in the eastern region (Table 2.1).

Table 2.1. Percentage (%) of farmers/respondents mentioning different constraints to bean production in four districts of Uganda (2005).

Constraint	South-western Uganda		Eastern Uganda		Overall mean	P value
	Kabale	Kisoro	Mbale	Sironko		
Diseases	93.3	95.0	80.0	73.3	85.5	0.122
Pests	30.0	24.6	77.1	93.3	56.3	0.000
Excessive rainfall	66.7	62.5	22.9	63.3	53.9	0.000
Drought/a lot of sunshine	30.0	25.5	74.0	8.3	34.5	0.093
Soil erosion	36.7	26.7	0.0	0.0	15.9	0.000
Lack of staking material	26.7	30.5	0.0	0.0	14.3	0.000
Poor/infertile soil	16.7	22.1	2.9	6.7	12.1	0.127

2.3.2 Farmers' awareness and perceptions of bean diseases and their causes

In south-western Uganda, most of the farmers found it difficult to differentiate between diseases and pests. At times they mentioned rats, aphids, moles and beanfly as diseases of beans. Farmers described diseases based on the effects on the plant (symptoms), and associated them with environmental factors. Disease symptoms mentioned in Kabale and Kisoro included *Kiniga/ Kirusuka* (root rot), *Okwoma* (wilting or drying up), *Okusaana* (powdery substance on leaves), *Okuhoha* (probably halo blight), *Okusya/okuhisa amababi* (yellowing of leaves), and *Okukokoota amababi* (probably Ascochyta blight) (Table 2.2). They associated BRR with poor soil, overuse of land, over cultivation, too much rainfall, and severe drought (Table 2.2). Similarly, all other diseases were mainly associated with heavy rainfall, drought, and poor soil (Table 2.2).

Table 2.2. Farmers' perceptions of bean diseases and their predisposing factors in south-western Uganda (2005).

Bean diseases[2]	Predisposing factors
1. Root rot (*Kiniga/Kirusuka*)	Poor soil, over-cultivation, severe drought, and excessive rainfall
2. Wilting or drying up (*Okwoma*)	Poor soil and drought
3. Burnt appearance (*Okubabuka/Okusya*)	Excessive rainfall, mist, poor soil, and weeds
4. Powdery substance on leaves (*Okusaana)*	Weeds, lack of field monitoring, and excessive rainfall
5. Halo blight (*Okuhoha*)	Excessive rainfall and mist
6. Yellowing of leaves *(Okuhisa amababi)*	Poor soil, over-cultivation, severe drought, excessive rainfall, and late planting
7. Ascochyta blight (*Okukokoota amababi*)	Poor soil, over cultivation, severe drought, excessive rainfall, and late planting

In eastern Uganda, farmers were better at differentiating between diseases and pests of beans than in south-western Uganda. They described disease symptoms such as curling (*Kakata*) typical of Bean Common Mosaic Virus (BCMV), yellowing, burnt appearance (*Tsumbu)*, leaf spots and blights (*Washa)* root rots and rotting (*Kyenju/Okwishukula),* wilting, stunting typical of BCMV, swollen hypocotyls (probably due to the bean fly), white powder on leaves, flower abortions and white powder on stem and roots (probably

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[2] Words in italics are local names (Rukiga) given to bean diseases

root rot) as major diseases of beans (Table 2.3). Most of the diseases were associated with excessive rainfall, drought, poor soil, insect pests, late planting, etc. (Table 2.3).

Table 2.3. Farmers' perceptions of bean diseases and their predisposing factors in eastern Uganda (2005).

	Disease symptom[3]	Predisposing factors
1.	Rotting (*Okwishukula*)	Excessive rainfall, and insects in the soil
2.	Yellowing (*Yello*)	Excessive rainfall, drought, late planting, pests, infertile soil, and weeds
3.	Drying (*Okukala*)	Pests and drought
4.	Curling/mottling (*Kakata*)	Aphids, late planting, excessive rainfall, drought, and insects in the soil
5.	Burnt appearance (*Tsumbu)*	Excessive rainfall, insects in the soil, and poor soils
6.	Wilting	Pests, drought, and insects in the soil
7.	Leaf blights and spots (*Washa*)	Pests and excessive rainfall
8.	Stunting	Drought, pests, infertile soil, and bad seed
9.	Swollen roots	Bean fly
10.	White powder on leaves	Pests
11.	Flower abortions	Pests and excessive rainfall
12.	White powder on stem and roots when uprooted	Pests and excessive rainfall

2.3.3 Ranking of farmers' perceptions of biotic constraints to bean production

In south-western Uganda, eight biotic constraints that farmers perceive as important for bean production were ranked using the pair-wise rank matrix. They included root rot, burnt appearance, aphids, bean fly, rats and moles, birds, and cutworms. From the ranking, root rot was considered most important in all the villages, followed by burnt appearance then aphids. Rats were not considered very important to succeful bean cultivation (Table 2.4).

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[3] Words in italics are local names (Lumasaba) given to bean diseases

Table 2.4. Pair-wise ranking of farmers' perceptions of biotic constraints to bean production in south-western Uganda (2005).

Biotic constraint	Kabale		Kisoro	
	Ryakarimira	Ntarangama	Nyarusiza	Rutare
Root rot	1	1	1	1
Burnt appearance	2	2	3	3
Aphids	3	3	2	2
Bean fly	4	2	5	6
Rats	5	4	6	5
Cutworm	-	-	4	-
Birds	5	4	5	5

A different type of ranking was done for eastern Uganda, using information from the questionnaires. This was done based on the number of farmers who mentioned the particular disease, and the number was expressed as a percentage of the total number of farmers interviewed. Leaf and pod curling (*Kakata*/BCMV) was ranked highest, followed by yellowing, rotting, burnt appearance, blights, flower abortions, wilting, white powder on roots and hypocotyls (*Kyengu*), swollen roots, and lastly, drying (Table 2.5).

Table 2.5. Ranking of farmers' perceptions of biotic constraints to bean production in eastern Uganda (2005).

Biotic constraint[4]	Sironko		Mbale	
	% Respondents	Rank	% Respondents	Rank
Curling/mottling (*Kakata*)	73.3	1	93.3	1
Yellowing (*Yello*)	53.3	2	50.0	2
Rotting (whole plant) (*Ukwishikula*)	40.0	3	23.3	4
Burnt appearance (*Tsumbu*)	40.0	3	30.0	3
Leaf and pod spots/blights (*Washa*)	33.3	4	16.7	5
Flower abortion	20.0	5	0.0	-
Wilting of plant	13.3	6	10.0	6
White powder on stem and roots (*Kengu*)	13.3	6	0.0	-
Swollen roots (Bean fly)	0.0	-	6.7	7
Drying (*Okukala*)	0.0	-	6.7	7
Flower abortion	0.0	-	3.3	8

Most of the above mentioned factors are symptoms of BRR, although they were made on the above-ground parts of the bean plant and not on the roots. A few farmers (13.3 % in Sironko) observed white mycelia on roots and stem bases of bean plants (white powder on roots), which is a typical symptom of either FRR or Rhizoctonia root rot (Table 2.5).

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[4] Words in italics are local names (Lumasaba) given to bean diseases

2.3.4 Farmers' perceptions of the factors causing bean diseases

Several factors were said to either cause diseases or increase their occurrence and severity. The factors mentioned included poor soil, excessive rainfall, drought, over-cultivation, late planting, lack of fertilizer, lack of pesticide, lack of field monitoring, mist settling on plants, lack of improved varieties, intercropping, soil erosion, and weeds. A pair-wise ranking of these factors showed that poor soil was the most important, followed by excessive rainfall, over-cultivation, late planting, soil erosion, weeds and unkempt bushes, lack of fertilizer, lack of pesticide, lack of field monitoring, and finally mist settling on plants in south-western Uganda (Table 2.6). Ranking in eastern Uganda was similar to that obtained in south-western Uganda, with poor soil being ranked highest, followed by drought, excessive rainfall, lack of improved varieties, lack of pesticides, late planting, over-cultivation, intercropping, and lastly weeds (Table 2.6).

Soil erosion and over-cultivation were considered as major problems in south-western Uganda, but not in eastern Uganda, probably because most bean fields in south-western Uganda are on the mountain slopes, unlike eastern Uganda where production is mostly done in the lowlands. Lack of improved varieties was mentioned only in eastern Uganda, probably because production is more on a commercial basis in this region, making quality and yield capability very important, unlike south-western Uganda, where growing mixtures is very popular.

Table 2.6. Pair-wise ranking of farmers' perceptions of the causes of bean diseases in two bean growing regions in Uganda (2005).

Factor	South-western Uganda	Eastern Uganda
Poor soil	1	1
Drought	2	2
Excessive rainfall	2	3
Over-cultivation	3	7
Late planting	4	6
Soil erosion	4	-
Weeds	5	9
Lack of fertilizer	6	-
Lack of pesticides	7	5
Lack of field monitoring	8	3
Mist	9	-
Bushes	9	-
Lack of improved varieties	-	4
Intercropping	-	8

2.3.5 Farmers' awareness of bean root rots

Bean root rots were recognized by all farmers interviewed in Kabale and Kisoro, while 85.7%, and 86.7% respectively, recognized the disease in Mbale and Sironko (Figure 2.5).
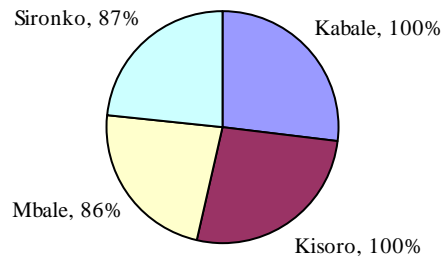
Fig. 2.5. Percentage of bean farmers who could recognize bean root rot in Kisoro, Kabale, Mbale and Sironko (2005).

Bean root rots were not considered as important in eastern Uganda as in south-western Uganda, that is, 37% of the respondents in Mbale and 70% in Sironko considered root rot important, compared to 93% in Kabale and 88% in Kisoro (Figure 2.6). In Kabale and Kisoro, it was ranked as the highest cause of bean yield losses. In Kabale, BRR was referred to as "*Kiniga*" (*Rukiga:* committing suicide by strangulation) and in Kisoro as "*Kirisuka*" (*Rufumbira:* meaning coming home with only a hoe and no harvest). In eastern Uganda, it is called "*Ukwishikwikula*" (yellowing and general sickly appearance), "*Washa*" (burning) or "*Kyengu*"(rotting), depending on what symptoms are seen.
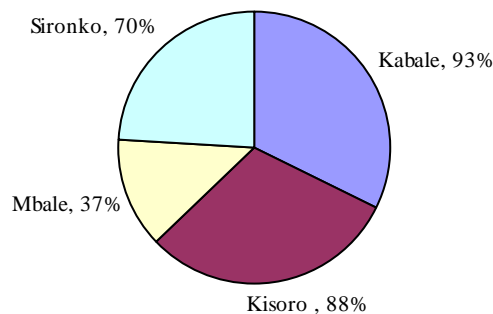
Fig. 2.6. Percentage of farmers who considered bean root rot to be important to bean production in Kabale, Kisoro, Mbale and Sironko Districts (2005).

2.3.6 Farmers' perceptions of symptoms of bean root rot

Bean root rots were mainly observed before flowering, that is, at the 3-4 leaf stage. Farmers recognized BRR based on several symptoms, which included yellowing, drying of the whole plant or roots, wilting, water-soaked roots, stunted growth, brittle roots, small leaves, poor root development, flower drop, weak and reduced root mass, roots, and poor pod set (Table 2.7). Of the symptoms mentioned, plant yellowing was the main symptom farmers associated with BRR, followed by drying-up of the whole plant (Table 2.7). Root rot symptoms were said to be most severe where the soil was considered infertile. In several cases, this occurred in patches in bean fields, with some plants having a yellow colour and others having a healthy green colour. Symptoms would gradually spread to cover the whole field, or in other cases, infected plants would die while others would survive to give some yield. In south-western Uganda, farmers recognized most BRR symptoms on fields located on hillsides, where the soils were shallow, and not in the valleys, where the soil was deep.

Table 2.7. Pooled percentage of respondents over four districts (Kabale, Kisoro, Mbale, and Sironko) in Uganda mentioning different symptoms of baen root rot (2005).

Symptom	% Respondents
Yellowing	76.8
Drying-up of plant	63.2
Drying of roots	27.4
Wilting	26.3
Water-soaked stem and rots/rotten roots and stem	14.7
Stunted growth	13.7
Drop of root hairs	11.6
Leaves shrinking	7.4
Poor root development	5.3
Flower drop and poor flowering	3.2
Few and weak roots	4.2
Lack of pods	2.1

A large percentage of farmers in south-western Uganda associated BRR occurrence with excessive rainfall, and could not conclusively tell the number of times the epidemic occurred in a year. However, a few farmers observed the disease symptoms once a year, usually in the season that received high rainfall, while others, especially in eastern Uganda, observed root rot epidemics every season. A few claimed that root rot

epidemics were unpredictable and occurred unexpectedly, while others associated them with seasons when it was dry (Table 2.8).

Table 2.8. Percentage of farmers mentioning the frequency of occurrence of bean root rot epidemics in four didtricts of Uganda (2005).

Frequency	% Respondents			
	Kabale	Kisoro	Mbale	Sironko
Once a year	23.3	30.0	40.0	43.3
Every season	3.3	2.0	28.6	26.7
Rare	3.3	0.0	0.0	0.0
Unpredictable	13.3	10.0	13.3	20.0
Whenever it is wet (too much rain)	56.8	45.5	0.0	0.0
Whenever it is dry	0.0	0.0	2.9	0.0

2.3.7 Farmers' perceptions of the factors causing bean root rot

The factors farmers associated with the cause of BRR were similar to the ones mentioned for bean diseases as a whole (Section 2.3.3). However, in the case of BRR, excessive rainfall was considered the major predisposing factor, while poor soils were considered most important for all diseases (Section 2.3.3). In addition, drought was considered a major factor in predisposing beans to root rot, especially in eastern Uganda (Table 2.9), while poor soil was ranked as the second and third most important factor that predisposes beans to root rot in south-western and eastern Uganda, respectively. A few farmers said they did not know what caused root rots in beans.

Other factors mentioned included poor soil drainage, shallow soils caused by soil erosion, because most bean fields are on hill slopes in south-western Uganda and over-cultivation of soil caused by land fragmentation, especially in south-western Uganda. A few farmers, especially in Kabale, associated BRR with witchcraft and cultural rituals were said to be performed to control it in case of an epidemic. For example, one respondent claimed that when the disease occurred, a few of the dead and sick plants are uprooted, placed on a boat with all family members and rowed over Lake Bunyonyi, while chanting "Kuka Runiga", meaning "root rot disappear", to their gods.

Table 2.9. Percentage of farmers in four districts ogf Uganda (Kabale, Kisoro, Mbale and Sironko) mentioning different factors that influence the occurrence and severity of bean root rot (2005).

Cause	% Respondents			
	Kabale	Kisoro	Mbale	Sironko
Excessive rain	93.3	89.5	40.0	50.0
Drought	10.0	15.0	45.7	46.7
Poor soil	20.0	26.5	20.0	6.7
Lack of crop rotation	0.0	0.0	8.6	6.7
Water stagnation	20.0	5.0	2.9	10.0
Planting under trees	0.0	0.0	2.9	3.3
Weeds	0.0	5.0	0.0	6.7
Intercropping	0.0	0.0	2.9	0.0
Lack of resistant varieties	0.0	3.5	5.7	0.0
Insects in soil	0.0	0.0	11.4	0.0
Pests	0.0	0.0	20.0	0.0
Witchcraft	3.3	0.0	0.0	0.0
Don't know	3.3	0.0	5.7	10.0

2.3.8 Farmers' practices in combating bean root rots

Most farmers, especially in Kabale and Mbale, did nothing once the disease occurred. However, roguing was the main control practice used, especially in eastern Uganda, while adding farm yard manure was the major control practice for BRR in south-western Uganda. Other control measures included constructing water channels, hand irrigation during drought periods, planting bean variety mixtures, applying ash around infected plants, and terracing. Hilling up and planting mature seed were mainly mentioned in south-western Uganda, while weeding was mentioned in Mbale and Kabale only (Table 2.10). Other control measures mentioned during the FGDs included, addition of inorganic fertilizers (very few are able to afford this), spraying with chemicals (very few farmers spray against root rots but spray mainly against insect pests), timely planting, good quality seed, soil conservation using drainage trenches, fallowing, crop rotation, intercropping, planting improved varieties (resistant varieties, although in most cases these have succumbed to the disease), spreading ash on infected plants, weeding, ridging, roguing, and burying infected plants.

Table 2.10. Percentage of farmers mentioning different control measures for bean root rots in four districts of Uganda (2005).

Control measure	% Respondents			
	Kabale	**Kisoro**	**Mbale**	**Sironko**
Nothing	43.3	45.0	31.4	13.3
Farmyard manure	26.7	32.4	0.0	3.3
Roguing	10.0	12.5	28.6	53.3
Crop rotation	16.7	9.7	8.6	8.6
Intercropping	0.0	0.0	0.0.	3.3
Addition of fertilizer	3.3	5.6	0.0	0.0
Improved varieties	3.3	0.0	2.9	0.0
Fallowing	0.0	0.0	2.9	0.0
Ash	0.0	0.0	2.9	0.0
Weeding	3.3	0.0	11.4	0.0
Traditional methods	10.0	0.0	0.0	0.0
Hilling up	3.3	4.8	0.0	0.0
Planting mature seed	6.6	6.7	0.0	0.0

2.3.9 Farmers' perceptions of the characteristics of a desirable bean variety

Farmers consider several factors in choosing bean varieties to grow, with yield being the most important factor, followed by early maturity, marketability, disease resistance, taste and drought tolerance (Figure 2.7). Other factors considered include bush growth habit (mentioned only in eastern Uganda, as both bush type and climbing types are popular in south-western Uganda), short cooking duration, seed size, especially large seed-sized varieties, light-coloured beans, that is, brown, red or white, storability and resistance to excessive rainfall.
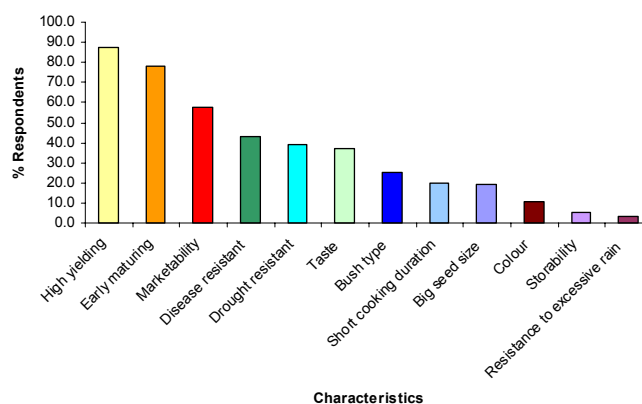
Fig. 2.7. Pooled percentage over four districts (Kabale, Kisoro, Mbale and Sironko) of what bean farmers perceive to be the characteristics of a good bean variety.

2.3.10 Farmers' bean seed size preferences and reasons for preferences

Generally, large-seeded bean varieties were the most preferred in both eastern and south-western Uganda (Figure 2.8), although the percentage was greater in eastern Uganda. The percentage of farmers preferring small-seed varieties was greater in south-western Uganda. This could probably be related to the reports of farmers slowly shifting to preferring the small-seeded varieties because of their resistance to BRR; as BRRs were the major disease in the south-western region, this seems the probable reason.
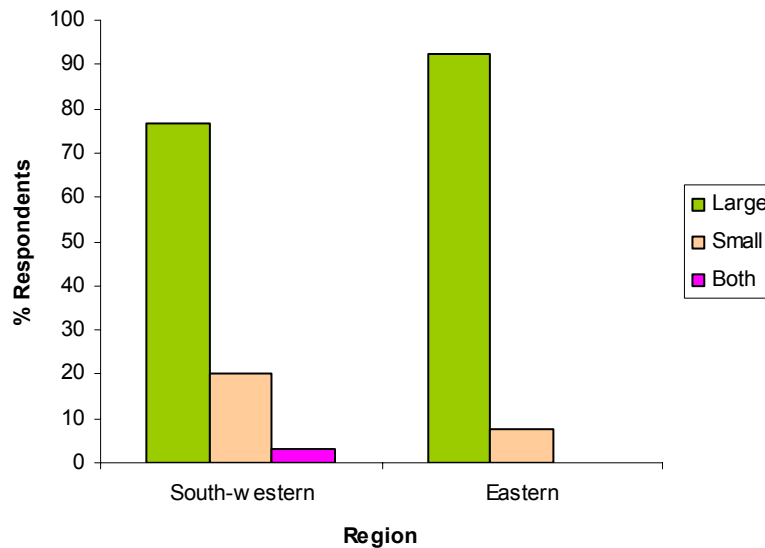
Fig. 2.8. Bean seed size preferred in two regions in Uganda (2005).

Farmers mentioned various reasons as to why they preferred the large-seeded varieties (Figure 2.9). Reasons ranged from the ability of large-seeded beans to give higher yields compared to the smaller seeded varieties, and their preference on the market, to the fact they swell when cooked, meaning that only small amounts are necessary for a meal. Other reasons included a better taste/texture when eaten and a good appearance, especially for farmers who market the beans (Figure 2.9). Some farmers mentioned that large-seeded varieties were less vulnerable to destruction by bruchids in storage. It was also mentioned during the FGDs that the leaves and stems of large-seeded varieties could be eaten, unlike the small-seeded varieties. Also, it was mentioned that the crop of the large-seeded varieties was uniform in its growth compared to small-seeded varieties, which usually mature at different times.
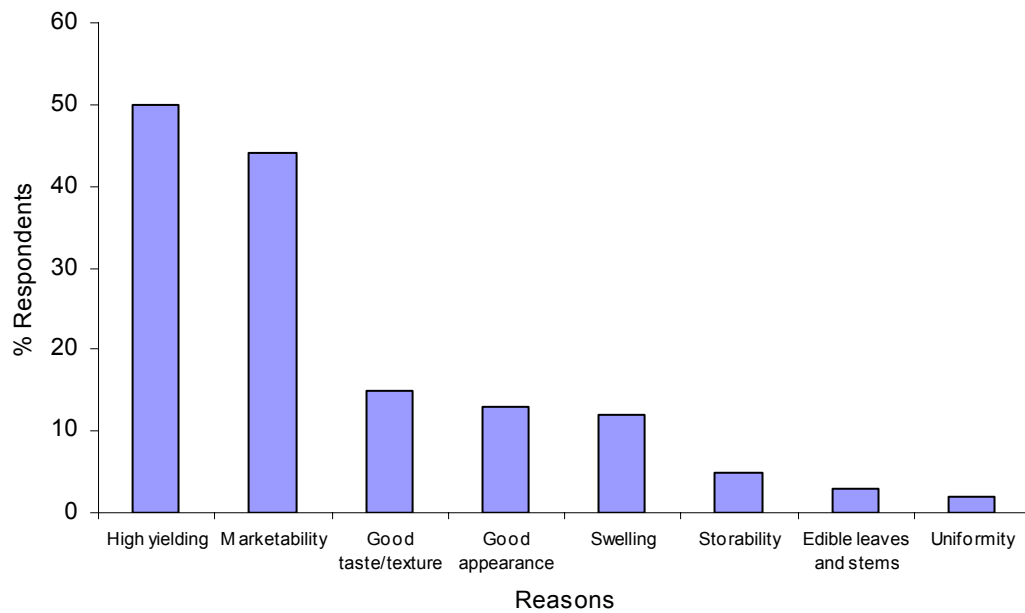
Fig. 2.9. Pooled percentages over four districts (Kabale, Kisoro, Mbale, and Sironko) of respondents giving various reasons for preferring large-seeded bean varieties.

The farmers who preferred the small-seeded bean varieties based their preferences on the ability to resist pests and diseases and to thrive under harsh environments such as excessive rainfall, drought, and mist, when compared to the large-seeded varieties (Figure 2.10).
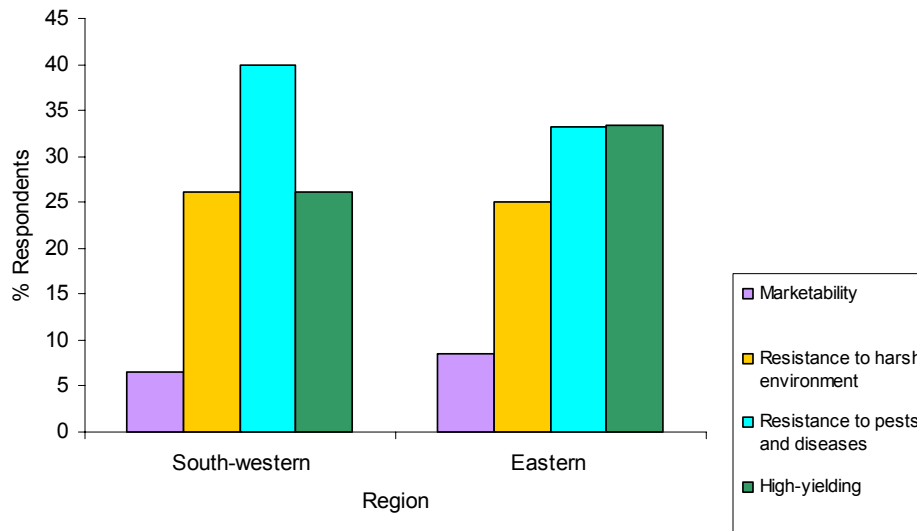
Fig. 2.10. Reasons for preferring small-seeded bean varieties in two bean growing regions in Uganda (2005).

Yield was also mentioned because most of the small-seeded varieties are very high-yielding and hence ensured food security. Marketability was the least mentioned factor for small-seeded bean preferences, probably because these varieties are not as marketable as the large seed types. However, most farmers grew small-seeded varieties for consumption and rarely, if at all, marketed them (Figure 2.10).

2.3.11 Farmers' bean seed colour preferences

Generally, farmers based their preferences of bean seed colour on the colour of soup produced after cooking, marketability, taste, storability, yield, and appearance (Figure 2.11). Light-coloured varieties such as red, red mottled, brown, yellow, and white in comparison to darker-coloured varieties such as dark brown, black, and purple, were the most preferred bean seed types due to the colour of the soup produced after cooking. The red mottled varieties were most preferred, especially in eastern Uganda, followed by brown coloured ones, especially in Kabale. Other types grown included mixtures, mainly in Kabale, black in Mbale, black and white mottled in Kabale and Sironko, white in Mbale and pink in Sironko. A few farmers, mainly those that grew for home consumption, had no colour preference, and grew whatever was available.
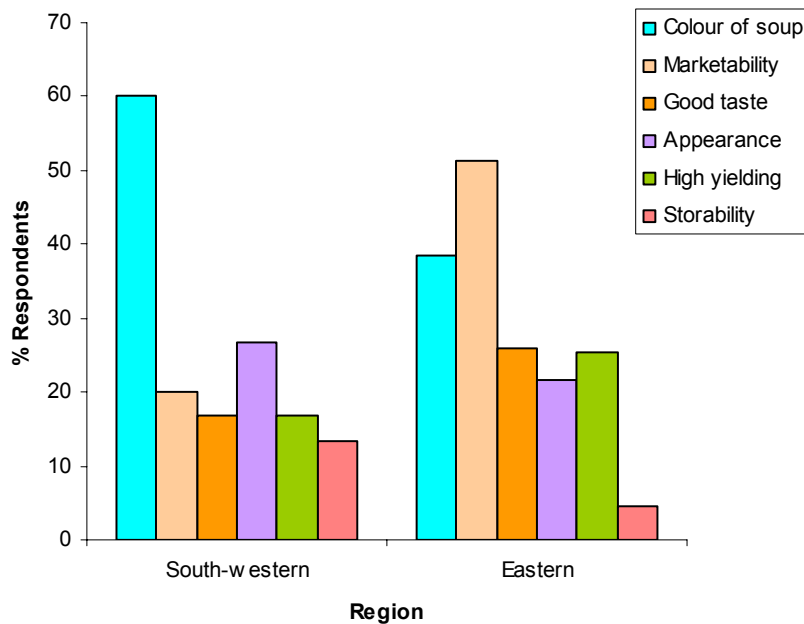
Fig. 2.11. Percentage of farmers giving specific reasons for preferring particular bean seed colour in two bean growing regions in Uganda (2005).

2.3.12 Farmers' perceptions of the relationship between resistance to bean root rot, seed size, and growth habit of bean varieties being grown

As regards resistance to BRR, a large percentage of farmers made some observations on which varieties seemed to resist root rot. In south-western Uganda, 50% of the respondents had observed resistant varieties, while 40-49% had done so in eastern Uganda. In south-western Uganda, resistance to root rot was mainly associated with the small-seeded varieties, that is, 58% in Kabale and 65% in Kisoro while a few related resistance to both the small-seeded and larg-seeded varieties, that is, 29% in Kabale and 14% in Kisoro (Figure 2.12a and b). However, in eastern Uganda, root rot resistance was more associated with large seed size, that is, 33% in Sironko and 45% in Mbale (Figure 2.8 c and d). Some farmers (17% in Sironko and 25% in Mbale) said small seed sized varieties were resistant, while others (17% in Mbale and 25% in Sioronko) mentioned that both small- and large-seeded varieties were resistant. In all the districts, 11% to 25% of the farmers did not associate root rot resistance with seed size (Figure 2.12).

a.
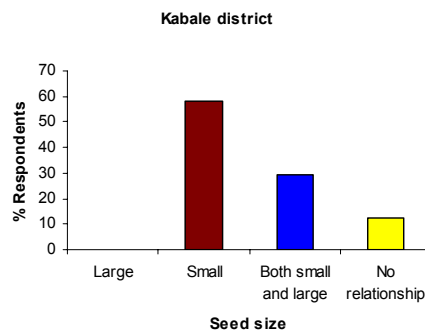
b.
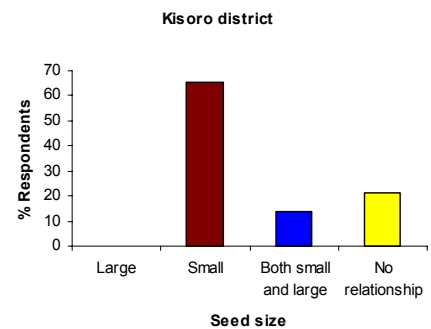
c.
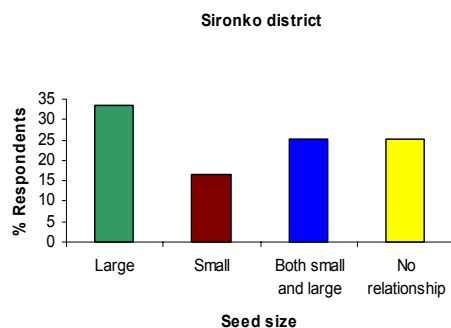
d.
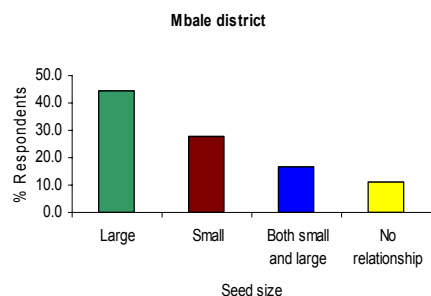
Fig. 2.12. Percentage of farmers in a) Kabale, b) Kisoro, c) Sironko, and d) Mbale districts in Ugand relating different seed sizes to resistance to root rot (2005).

A few farmers, associated resistance to BRR with the type of growth habit of the bean varieties. In south-western Uganda, 45-60% of the respondents who observed resistance to root rot associated the resistance with climbing growth habit, while the rest did not perceive any relationship. In eastern Uganda, less than 5% of the respondents who observed resistance to root rot associated the resistance with climbing growth habit, while the rest said there was no relationship between growth habit and resistance to root rot.

2.3.13 Incidence and severity of bean root rot in farmers' fields

Based on the visual symptoms in the bean fields visited as a whole, and on the hypocotyl and roots of ten plants sampled per field, there were no significant differences ($P \leq 0.05$) between the districts and villages regarding incidence and severity of bean root roots. Generally the incidence of root rots was highest in Kabale and Kisoro (see Figure 2.13) where, in some villages, such as Ryakarimira and Rutare, all the bean fields visited had root rot symptoms. This was followed by Sironko and lastly Mbale which did not have such high BRR incidences in the bean fields visited (Table 2.11).

Fig. 2.13. A farmers' bean field in Kisoro showing yellowing due to bean root rot.

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Bean root rot severities ranged between 10% and 34% based on the observations of root rot symptoms on the plant hypocotyl and root tissue (Table 2.11).

Table 2.11. Incidence and severity of root rots in bean fields in Kabale, Kisoro, Mbale and Sironko districts of Uganda (2005).

District	Village	Incidence (%)	Severity (%)
Kabale	Ryakarimira	100	33.8
	Ntarangama	60	19.5
Kisoro	Nyarusiza	80	27.9
	Rutare	100	34.1
Mbale	Makhai	40	10.0
	Namwaro	50	19.8
Sironko	Bunywaka	50	20.2
	Bwikhonge	80	22.2
Mean		70.0	23.4
S.e.d. ($P \leq 0.05$)			6.7
CV%			42.1

2.3.14 Marketing of beans in south-western and eastern Uganda

Very few farmers, that is, 16% in south-western and 20% in eastern Uganda, produce beans for consumption only while the majority produce for both consumption and sale (Table 2.12).

Table 2.12. Percentage of farmers who sell beans in four districts of Uganda (2005).

Districts	% Respondents		
	Sell to neighbours and traders from home	Sell in markets	Do not sell
Kabale	53.3	73.3	16.7
Kisoro	34.2	76.8	15.0
Mbale	68.6	42.9	20.0
Sironko	90.0	60.0	0.0
Mean	61.5	63.3	12.9

In eastern Uganda, a large percentage of farmers sell their bean produce from their homesteads as traders can easily access the homes, while in south-western Uganda farmers have to carry their produce to the markets as very few traders are able to get to the homesteads due to the hilly terrain. The bean farmers in eastern Uganda also enjoy a ready

market with the bordering country of Kenya. Bean production is thus more of a business for them, unlike their counterparts in south-western Uganda, whose market in Rwanda and the DRC is not so lucrative. Generally bean farmers, especially in south-western Uganda, complained of having poor returns for their produce because of the low prices of beans caused by the lack of a formal marketing system. In most cases, the farmers have no say in price determination and accept whatever the traders have to offer. K132 was reported to earn the highest prices of 150-700Ushs kg^{-1} in Sironko and Mbale, 250-600Ushs kg^{-1} in Kabale and Kisoro; followed by Kanyebwa at 150-600Ushs kg^{-1} in Sironko, 150-400Ushs kg^{-1} in Mbale, 500Ushskg^{-1}in Kabale; and finally, K20 at a price of 100-370Ushs kg^{-1} in Sironko, 150-400Ushs kg^{-1}in Mbale, and 150-450Ushs kg^{-1}in Kabale.

2.4 Discussion

The PRA helped in elucidating farmers' perceptions of various issues related to BRR that will guide future breeding programmes by solving "real problems" rather than solving "perceived problems" that may not be the actual problems. This study was carried out mainly to determine the need for new varieties with improved resistance to BRR, which is one of a complex of pathogens causing BRR. The study assessed farmers' perceptions of BRR, management of bean diseases and their perceptions on the causes of bean root and how they relate BRR to the types of bean varieties being grown. It also assessed the level of BRR infection on farmers' fields. The major characteristics of beans that farmers consider when adopting a new bean variety were also identified. The data was obtained from a formal survey of 120 households/respondents and focus group discussions, with over 240 respondents from four districts in the highland regions of Uganda. Additional data were obtained from secondary sources such as the Ministry of Agriculture, NARO and NGOs involved in bean production.

From the PRA, bean root rots were recognised by farmers as the major constraint to bean production, especially in south-western Uganda. Resistance to BRR, as well as seed quality traits, especially large seed size and light seed colour, were the major traits that needed intervention by breeders. Similarly in Malawi, root rot tolerance and seed quality were considered top priority for genetic improvement (Snapp et al., 2006). However, BCMV was considered the major disease affecting bean production in eastern Uganda.

The study showed that, 40%-100% of the bean fields visited were infected with root rot. The disease was easily recognized by the farmers in Kabale and Kisoro districts in the south-western highlands, where it was associated with low bean production. In Kabale it was referred to as *"Kiniga"* and in Kisoro as "*Kirusuka*". The factors which farmers associated with the cause of BRR were similar to the ones mentioned for all other bean diseases, implying that farmers often recognize diseases as a whole, that is, they tend to consider the general appearance of the whole bean plant and not specific diseases attacking a particular plant part. This is important for researchers to note, as they usually target specific diseases and may be misled by the farmers' responses. Hence, there is a need to probe at some depth to get specific details of the pathogen one is investigating.

Excessive rain, drought and poor soil fertility were the major factors predisposing beans to root rot. Many other factors mentioned as causes of BRR, such as low soil depth/shallow soils due to soil erosion, insects/organisms in the soil, lack of crop rotation, planting under trees, lack of intercropping, lack of fertilizer and farmyard manure, and over-cultivation were all soil-related. This implies that poor soil fertility and soil sanitation were the major causes of BRR. However, even though farmers were able to observe the causes of root rot they were not able to explain the reasons for it. For instance, farmers who associated BRR with excessive rain could not explain why root rot was also observed in drought periods.

Bean root rots are associated with the intensification of agriculture, which has been a result of the increasing human population. The high population characteristic of the highland regions has led to land fragmentation and hence a decline in soil fertility. This has created a scenario where there is an imbalance between the beneficial and disease causing organisms in the soil, and hence an increase in root rot pathogen inoculum levels (Buruchara and Rusuku, 1992; Pyndji, 1996).

It was evident that farmers did not have a clear understanding of the causal organism of BRR. Even though some mentioned insects in the soil, it was probable that they were referring to bean fly or an actual insect, and not a pathogen. The idea of a soil-borne pathogen was poorly understood.

Most of the control measures farmers used to manage BRR were directed to soil management, which further indicates that farmers associate BRR with poor soil. Very few, if any of the farmers, could afford to use inorganic fertilizer on their bean fields, hence adding farm yard manure to soil, especially in south-western Uganda, was a major control measure for the disease. However, difficulties in ferrying manure to far-off fields in the mountains due to the hilly terrain were encountered and hence in most cases manure was never applied at all, or minimal amounts were added. In addition, most farmers lacked domestic animals such as cows and goats from which they could get the farm yard manure. Roguing was a routine measure for any damaged plants and not specifically for BRR, and was the main disease control measure, especially in eastern Uganda. However, very few farmers monitored their bean fields for BRR as land fragmentation was cited as having created excessive distances between homes and gardens, thereby making soil and disease monitoring and management very difficult.

Although a few farmers mentioned the use of improved varieties as a control of BRR, this use was not very evident as most farmers still grew the old bean varieties which were susceptible to bean rot. This could well be because they had not received any good varieties to replace the old varieties. Most of the new varieties currently available to the farmers have not been widely adopted because they were either long-maturing, small-seeded, or climbing in growth habit, hence, requiring staking and easily attacked by birds.

Bean variety preference was generally based on high yield, early maturity period, resistance to pests and diseases, drought tolerance, seed size, taste, cooking time, and seed colour. Farmers associated susceptibility to BRR with large seed size and bush growth habit. Even though the large-seeded bean varieties were the most preferred bean seed types, farmers were slowly abandoning them in preference of the small-seeded ones due to their susceptibility to many diseases, with BRR being the major problem. This was most observed in south-western Uganda, where a good percentage of farmers said they preferred growing small-seeded varieties rather than the large-seeded varieties. Small-seeded varieties were said to be resistant to excessive rainfall, drought, and diseases. The lack of resistance to BRR over the years may well be due to a concentration of both breeding efforts and management practices on other factors, such as seed size and growth habit, rather than pest and disease resistance (Schneider et al., 2001).

As regards the growth habit, climbing beans are said to be more resistant to BRR compared to the bush type beans. In addition, they are generally higher-yielding (2500-4000kg ha^{-1}) than bush beans (1500-2500kg ha^{-1}) (Opio et al., 2001). However, production of climbing beans is hindered by their need for stakes, which are difficult to obtain. Wooden stakes are the common types of stakes used by all farmers but these have a disadvantage in that they are damaged by termites over time, hence complicating the situation and increasing the expenses of growing climbing beans. This therefore indicates a need and an opportunity for the production of non-wooden stakes for beans.

Using the PRA approach, the study was able to obtain important information to help guide interventions aimed at controlling BRR or other bean diseases on farmers' fields. The need to involve farmers in all steps of developing new technologies, that is, new varieties, was highlighted. Such varieties would be met with less rejection than unfamiliar varieties bred elsewhere and introduced without any consideration of the farming community's needs and preferences. Similarly, in Ethiopia the involvement of farmers in bean breeding was shown to improve bean variety development as farmers were capable of identifying superior varieties that met specific requirements within relatively short periods, hence increasing the chances of adoption of the new varieties by other farmers in the community (Asrat et al., 2006). The importance of BRR as a major constraint to bean production was highlighted, hence there is an urgency to provide these farmers with a bean variety with resistance to BRR, as well as one that will be easily adopted to control this disease. Integrated Pest Management (IPM), especially soil IPM, still remains a very important component of controlling bean root rot (CIAT, 2003; Abawi et al., 2006), because soil fertility is a major problem in these regions and also given that disease resistance often reduces over time due to inoculum build-up and poor crop rotation practices.

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Chapter Three: Isolation and maintenance of a pathogenic *Fusarium solani* f. sp. *phaseoli* isolate for use in screening common bean (*Phaseolus vulgaris* L.) germplasm for resistance to Fusarium root rot

Abstract

Several strains of *Fusarium solani* f. sp*. phaseoli* (FSP) that causes Fusarium root rot (FRR) occur in nature with some strains being more pathogenic than others. The objective of this study was to identify a predominant and pathogenic isolate from south-western Uganda for use in a genetics study on resistance to *FSP*. Infected bean plants and soil samples were collected from farmers' fields in Kabale and Kisoro district in south-western Uganda, a region highly affected by bean roo rot (BRR) epidemics. Isolations of the pathogen were done using both selective and Potato Dextrose Agar (PDA) medium. Four *F. solani* f. sp. *phaseoli* isolates (FSP-1, FSP-2, FSP-3 and FSP-4) were tested for pathogenicity under screenhouse and laboratory conditions on one susceptible line, K132, two varieties resistant to Pythium root rot, MLB-49-89A and RWR719, and one line resistant to FRR, G1459.

Three methods of storing and maintaining the viability of *Fusarium* spp. isolates were tested. They included, storing 5mm^2 PDA discs with pure colonies of *FSP* in double-distilled water at 5°C, keeping PDA plates with pure colonies of the pathogen at room temperature and storing PDA slants with pure colonies of the pathogen at 5°C. The viability of the isolate from the three storage methods was tested by sub-culturing from each of these cultures on to fresh PDA plates, and observing the growth of the fungus after six months, one year and two years. Pathogencity testing was also done at each of these times on a susceptible cultivar, K132. The isolate FSP-3 was found to be the most pathogenic resulting in 100% disease incidence on all bean varieties and disease severity in the range of 5.1-8.6 on a 1-9 scale. More isolate samples remained viable from the PDA slants compared to the other two methods. However, pathogenicity was maintained for all these methods. FSP-3 was selected for use in further studies of FRR.

3.1 Introduction

Fusarium solani (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans (FSP) belongs to the *Nectria haematococca-Fusarium solani* species complex section *Martiella* of Fusarium (Booth, 1971; O'Donnell, 2000). The main host of FSP is recognized as common

bean (*Phaseolus vulgaris* L.)*,* on which it causes Fusarium rot rot (FRR) disease. *FSP* attacks older seedlings and does not cause seed rots or damping-off of seedlings. Infection by the pathogen is characterized by narrow, long, red to brown streaks on the hypocotyls and taproot, which later turn dark brown, and cracks often develop lengthwise. The roots may then shrivel and die, with clusters of fibrous roots developing above the shrivelled taproot (see Chapter one, Figures 1.1 and 1.2) and the plant may also eventually die.

Although FSP is commonly isolated from bean plant tissue, some isolates may not be pathogenic to beans. Saprophytic forms of FSP are very common and often occur together with pathogenic FSP species (Hall, 1996; Roy, 1997; Tusiime, 2003). In addition, the pathogen has also been reported to infect other plants, mainly legume crops (Abawi, 1980; Gray, 1991; O'Donnell and Gray, 1995; Gray et al., 1999). It has been reported on mung bean (*Vigna radiata* L.) and green bean (*P. vulgaris L.*) (Gray, 1991; Gray et al., 1999), on lima bean (*P. lunatus* L.), scarlet runner bean (*P. coccineus* L.), adzuki bean (*Vigna angularis* Willd.) and moth bean (*V. aconitifolia* Jacq.). It has also been reported to be pathogenic on garden peas (*Pisum sativum* L.), cowpea (*Vigna unguiculata* L.), and on soybean (*Glycine L. max*), on which it causes sudden death syndrome (Abawi, 1980; O'Donnell and Gray, 1995).

The use of resistance is probably the cheapest and most cost-effective control measure against FRR; however, stable resistance depends on the capacity of the line to resist infection from the whole range of pathogen strains in a population. Previous research on the management of FRR in south-western Uganda using resistant varieties has shown that effectiveness varied from location to location and sometimes season to season (Tusiime, 2003). This was thought to be due to strain differences within the FSP population. However, Tusiime (2003) divided FSP isolates collected from south-western Uganda and other parts of Africa into two major groups, namely, the "slow-growing" and "fast-growing". The grouping was based on morphological and molecular characteristics as well as on pathogenicity to beans. The fast-growing isolates were always light yellowish and non-pathogenic, while the slow-growing were initially buff but developed various shades of blue as potato dextrose agar (PDA) cultures grew old and were very pathogenic to beans. The pathogenic FSP isolates were also found to be highly uniform after molecular analysis, implying that considerable improvement to the disease could be achieved by utilizing only one pathogenic isolate. It is important to be aware of the coexistence of both pathogenic and non-pathogenic

forms of *F. solani* in symptomatic plants, and their differences in order to avoid wasting time and resources on research based on the wrong identity of the pathogen or on a non-pathogenic isolate.

For research purposes, it is necessary to prevent loss of genetic variability and to be able to maintain isolates for a long period of time in their original condition. Fungal isolates are usually preserved in water at room temperature (McGinnis et al., 1974), an easy and economical procedure introduced for fungi by Castellani (1939). However, stability of fungal cells is not ensured by this simple procedure and hence other methods have been developed, such as preservation in soil (Chaudary et al., 2006) or on oil- or water-covered slants; cryopreservation either in liquid nitrogen or at low temperature (-20 and -70°C) (Hwang et al., 1976; Butler 1980; Stalpers et al., 1987; Pasarell and McGinnis, 1992) and lyophilization (American Type Culture Collection, 1991). Cryopreservation in liquid nitrogen and lyophilization are the methods recommended and used by the American Type Culture Collection for long-term storage (American Type Culture Collection, 1991). Studies with plant pathogenic fungi have demonstrated survival of fungi for several years after storage in liquid nitrogen at -196°C (Diaz de Ackermann et al., 1988; Kaise et al., 1989). However, there have been reports of decline in virulence of the pathogenic fungi, as well as in reduced production of spores, after long periods of storage (Hajeck et al., 1995). Repeated *in-vitro* sub-culturing has been shown to decrease the virulence of *Entomophaga maimaiga*, a fungal pathogen of gypsy moth, after long periods of storage. However, virulence was restored when the pathogen was introduced to the hosts again (Hajeck et al., 1995).

In this study, simple and cheap methods were sought for their suitability to store the selected FSP isolate throughout the life of the project. The objectives of the study were
1. To isolate a pathogenic FSP isolate that would be used in the genetic study of resistance to FRR in beans;
2. To identify a simple and cheap storage method that could be used to maintain FSP isolates in a pathogenic state for long periods of time.

3.2 Materials and methods

3.2.1 Sample collection

Bean plants showing symptoms of FSP were collected from Kabale and Kisoro districts in the south-western region of Uganda. Plant samples were collected from over 20 bean fields per district by randomly picking up to 15 infected bean plants per bean field showing disease symptoms on the roots. In each field, samples were collected from predetermined positions using a grid distance of 5-10km in a "W" pattern. Soil samples were also collected from the same spots where the infected plants had been picked, and bulk samples were made up by mixing soil from the same farmer's field. Plants and soil samples were put in different paper bags, labelled and brought back to Kawanda Agricultural Research Institute (KARI), where isolation was conducted in the laboratory.

3.2.2 Isolation of *Fusarium solani* f. sp. *phaseoli* from soil and plant tissue

A protocol adopted from Burgess et al. (1994), with several modifications, was followed in the isolation of the pathogen from plant tissue. The leaf and stem tissue of the sampled bean plants were cut off and discarded, leaving only the hypocotyls and roots. The hypocotyl and root tissues were then washed in running water and blotted dry. Tissues showing typical FRR symptoms were cut into 20-30mm pieces and surface sterilized with 20% NaOCl solution for approximately 1min and then rinsed twice in sterile water. Small pieces of tissue, 2-3mm, were aseptically cut from the edges of the lesions and plated on Nash and Synder selective medium (Appendix 3.1), amended with 2mg l^{-1} benomyl (Hall, 1981) and incubated at 23 ± 2°C for 14d. Isolations from the soil were done by first dissolving 2g of infected soil in 100ml of water and later spreading 0.5ml of suspension onto Nash and Synder selective medium. Cultures were incubated at $20-25^{o}$C for up to 6d.

The growing colonies from plant and soil isolations were later sub-cultured onto PDA medium amended with streptomycin sulphate and aureomycin, and allowed to grow for up to 14d. A further subculture was made from clean colonies onto PDA without antibiotics and incubated at room temperature for 14d. Microscopic examination was used for preliminary confirmation that cultures were true *F. solani* species. After 14d, mono-conidial isolates were prepared for all isolates by dipping a sterile loop in sterile water and using it to slightly touch

the surface of the culture, and then streaked on PDA medium. Mycelial plugs measuring 20mm were aseptically cut from 7d old mono-conidial cultures of each isolate and incubated at 25°C for up to 21d. During this period the cultures were observed for colour changes and production of conidia.

3.2.3 Testing for pathogenicity of *Fusarium solani* f. sp. *phaseoli* isolates

Four FSP isolates were tested for pathogenicity on four bean varieties, namely, a local susceptible check, K132, and three possible resistant varieties, that is, MLB-49-89A and RWR719, resistant to Pythium root rot, and G1459, resistant to FRR (Abawi and Pastor Corrales, 1990). Trials were done in the screenhouse and in the laboratory at KARI.

In the screenhouse trial, inoculum of the different FSP isolates was prepared using sorghum seed as a medium for pathogen growth as follows: Duran graduated laboratory bottles (Aldrich, Z305197-10), 1 000ml or 500ml in capacity were washed and partially filled (1/2-2/3 capacity) with sorghum seed and water. The bottles were sealed and the contents autoclaved for 1hr at 120°C. Using previously purified isolates, two agar plates per 1 000ml bottle were suspended in 60-70ml of sterile, deionised water. The slurry formed was then 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 and water. Bottles containing the inoculated medium were incubated in the laboratory at 20-28°C for 5d to allow FSP to grow. Later, the bottles were opened, but the opening was protected using foil paper to prevent contamination, to allow for evaporation of the excess moisture and nutrient solution, and allowed to incubate for 21d (Figure 3.1). The contents were then emptied and the medium slowly dried to allow maturation of the fungal resting spores. The inoculum was added to pre-sterilized sandy clay loam soil at a rate of 500ml of inoculum per 0.74 x 0.42 x 0.115m^3 trays of sterilized soil (Figure 3.2). NPK fertiliser was applied at a rate of 3x10^{-3}kg ml^{-1} before planting and therafter it was applied every after 7d. A susceptible line, K132 was then planted in the trays for up to 28d, when it was uprooted. This acted as a means of increasing disease inoculum levels in the soil before planting the test materials. Thereafter, the test materials were planted to test the levels of pathogenicity of the different isolates. In all cases, each isolate was prepared separately and care taken to avoid mixing. Symptoms were observed after 28d.

Fig. 3.1. *Fusarium solani* f. sp. *phaseoli* growing on sorghum seed.

Fig. 3.2. Wooden tray planted with different varieties to test pathogenicity to one *Fusarium solani* f. sp. *phaseoli* isolate.

The laboratory trial was done as a quick assessment of the pathogenicity of the different isolates. In this case, the inoculum was prepared by flooding the culture colonies of the pathogen in the Petri dishes/plates with sterile water and scraping the mycelia into the water using a sterile cover slip. The resulting slurry was then filtered through a double layer of muslin cloth. Using a haemocytometer, the concentration in the inoculum was adjusted to between 3 000-4 000 conidial spores per ml of water in 500ml flasks. Seedlings of the different varieties were then placed in the flasks containing different FSP isolates (Figure 3.3). Often symptoms developed after four to five days and evaluation was done after 14d.

Fig. 3.3 Plants growing in water containing different *Fusarium solani* f. sp. *phaseoli* isolates in the laboratory.

3.2.4 Trial layout and disease evaluation

Both trials were laid out in a randomized complete block design (RCBD) and replicated three times. Each of the replicates comprised 20 plants per line per isolate. The trial was repeated twice to reconfirm the pathogenicity of the isolates. For the screenhouse trial, a basal application of 3×10^{-3} kg ml^{-1} of 1:1:1 NPK fertilizer was applied before planting and the trial was watered daily to ensure adequate water at all times.

Fusarium root rot symptoms were assessed at 28d after planting (dap) in the screenhouse trial and 4d in the laboratory. All 20 seedlings planted per line were carefully uprooted, taking care not to damage roots and hypocotyls, and washed with clean tap water. The number of plants showing disease symptoms were 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 the extent of the disease infection:

- 0% = no visible symptoms;
- 25% = approximately a quarter of the hypocotyls and root tissue have lesions but tissue is still firm;
- 50% = approximately half of the hypocotyl and root tissues have lesions with considerable softening/rotting;
- 75%-100%= whole of the hypocotyl and root tissues havie lesions of FRR and root system is in advanced degree of rotting to complete destruction.

In addition, damage was also assessed based on the 1-9 scale developed at the International Centre for Tropical Agriculture (CIAT), (Abawi and Pastor-Corrales, 1990), where:

- 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 (Lawes Agric. Trust, 2007).

3.2.5 Storage and maintenance of pathogenic *Fusarium solani* f. sp. *phaseoli* isolates

The four isolates were stored in three ways (Figure 3.4):

1. One set was stored in double-distilled water, i.e., PDA agar bearing pure colonies of *Fusarium solani* f. sp. *phaseoli* was cut up in 5mm^2 square discs and these were transferred to micropyle bottles containing double-distilled water and kept at 5°C.

2. Another set was grown on PDA agar plates and plates stored on benches in the laboratory at room temperature.

3. Another set was grown on PDA slants and after 7d stored at 5°C.

Fig. 3.4. Methods tested for storage of *Fusarium solani* f. sp. *phaseoli* isolates.

The viability of the FSP-3 was tested after six months, one year and two years by sub-culturing 30 samples per method onto fresh PDA plates and observing the growth. Pathogencity was tested by preparing inoculum for regenerated isolate samples onto sorghum seed, as described in section 3.2.3, and then tested on a local susceptible line, K132, in the screenhouse.

3.3 Results

3.3.1 Isolation of *Fusarium solani* f. sp. *phaseoli*

Several types of cultures were obtained on the PDA medium. The majority of the culture colonies were whitish-creamish or buff, producing a line of colours ranging from blue to various shades of blue through purple to violet as they grew older, while others were mostly cream to light yellow in colour and remaining yellowish with age (Figure 3.5). The buff-coloured isolates grew much slower than the other isolates.

The slow-growing isolates (Figures 3.5 a, d, e and f) produced conidia which were mainly macroconidia in slimy masses, radiating from the centre of colonies. Macroconidia were generally 3 or 4 septate, while a few produced 5 septate macroconidia (Figure 3.6). A few isolates within this group remained predominantly buff, sporulated profusely, but never developed blue-purplish colours, and their spore masses were cream. On successive sub-culturing, some of the slow-growing isolates tended to produce more aerial mycelium and others developed sectors.

a.   b.   c.

d.   e.   f.
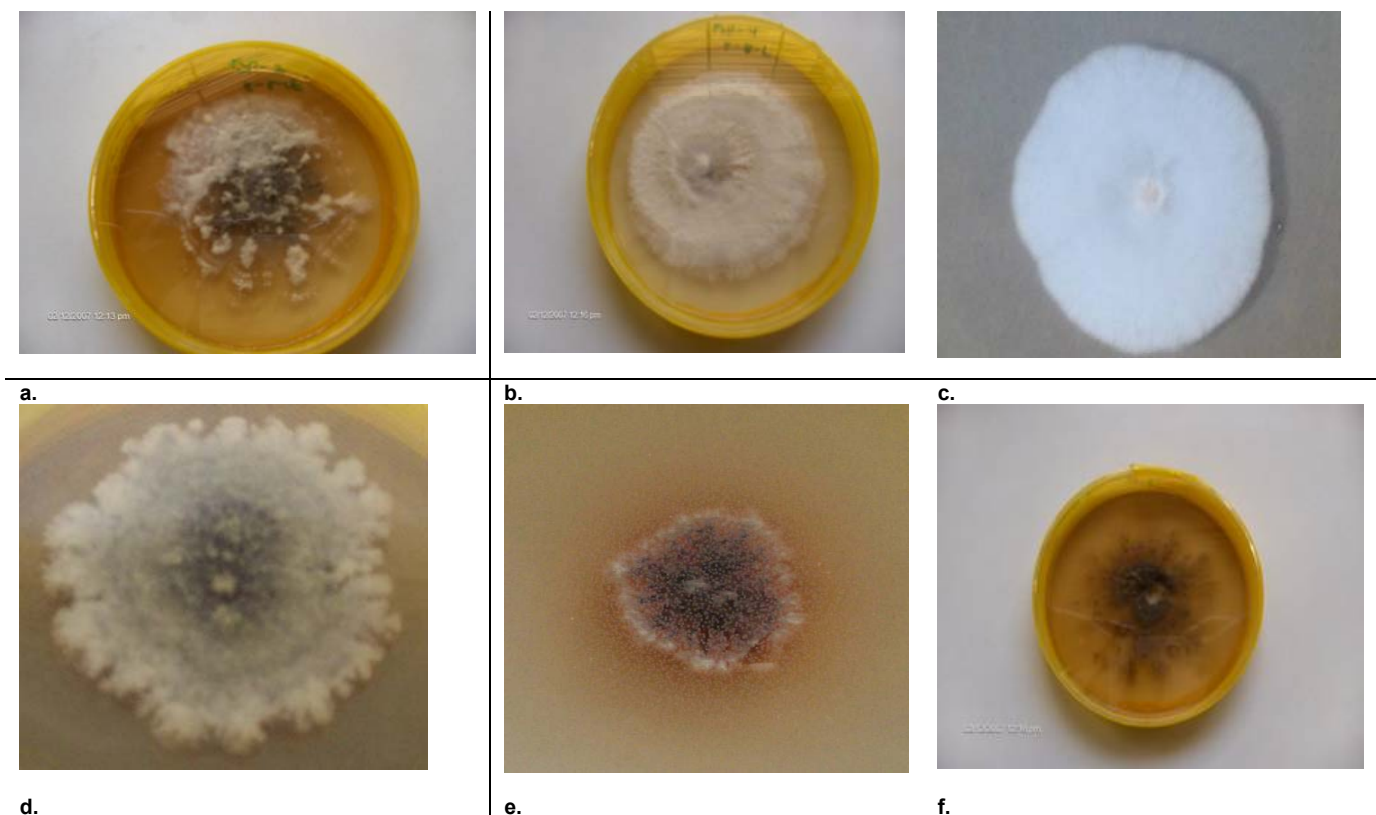
Fig. 3.5. Different types of *Fusarium solani* f. sp. *phaseoli* cultures obtained on PDA medium.

Fast-growing cultures produced sparse mycelia, which mostly remained on the surface of the medium. Old cultures produced concentric rings of yellow slime containing masses of both micro- and macroconidia. Macroconidia were also 3-4 septate (Figure 3.6).

Fig. 3.6. Macroconidia of *Fusarium solani* f. sp. *Phaseoli* with 3-4 septa.

3.3.2 Pathogenicity of *Fusarium solani* f. sp. *phaseoli* isolates

In order to select for pathogenicity and virulence, four isolates (Figures 3.7-3.10) showing the characteristics described for FSP on PDA medium and having the macroconidia shape described above, were tested.

Observations of the cultural characteristics showed that FSP-1 and FSP-3 were purplish-blue in colour, with both the distinctive blue centre and white margin characteristic of pathogenic FSP described by Tusiime (2003) (Figures 3.7 and 3.9). Conidia were produced in slimy masses radiating from the centre of colonies. The other two isolates, FSP-4 and FSP-2, which remained pinkish-white in colour, sporulated profusely, but never developed blue-purplish colours and their spore masses were cream, characteristic of some slow growing FSP species (Figures 3.8 and 3.10).

Fig. 3.7. FSP-1 isolate on PDA.

Fig. 3.8. FSP-2 isolate on PDA.

Fig. 3.9. FSP-3 isolate on PDA.

Fig. 3.10. FSP-4 isolate on PDA.

There were no significant differences (P= 0.05) in FRR incidence and severity between the two sets of trials, and hence means over the trials are presented (Table 3.1). Generally, FRR incidence varied significantly among the four varieties and four isolates, however, the interaction line x isolate did not influence the disease incidence significantly at P= 0.05 (Table 3.1). This implies that the four bean varieties had different resistance levels to FRR and the FSP isolates had different pathogenicity levels but behaved similarly across the the four varieties. In the case of FRR severity, the four bean varieties were not significantly different from each other at P≤0.05 under both laboratory and screenhouse conditions probably due to low inoculum levels of the isolates used in this trail. Similarly, the interaction of the varieties x isolate did not differ significantly (P= 0.05) for Fusarium root severity. However, disease severity due to the four isolates varied significantly (P= 0.001) in all the trials implying that the isolates had different pathogenicity levels.

Table 3.1. Mean squares of the severity of Fusarium root rot on four bean varieties caused by four *Fusarium solani* f. sp. *solani* isolates.

Source	Df	Mean squares					
		Disease incidence		Disease severity			
		Laboratory	Screenhouse	Laboratory		Screenhouse	
				%	1-9 Scale	%	1-9 Scale
Line	3	532.8**	655.4*	450.6	2.15	660.6	2.46
Isolate	3	1342.9*	1468.0**	931.3***	7.66***	1208.7**	10.34**
Line x isolate	9	211.3	244.3	163.5	1.63	255.4	2.14
Error	31	419.7	625.4	320.4	2.09	512.5	3.98
Total	46						

*, **, ***= data significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

The bean line MLB-49-89A exhibited the lowest disease incidence among the four varieties tested, while the local susceptible line, K132 had the highest incidence (Table 3.2). The FSP-3 isolate had the highest disease incidence of 100% on all the bean varieties, both in the screenhouse and in the laboratory. The isolate FSP-1 caused disease incidence of 56.9-67% in the screenhouse trial and 100% incidence on all the varieties in the laboratory. The isolate FSP-4 caused the lowest incidence of FRR in both trials, ranging from 18 to 40% in the screenhouse and 54.8 to 78.5% in the laboratory, while FSP-2 caused disease incidence ranging from 34.9% to 57% in the screenhouse and 62.5 to 77.4% in the laboratory (Table 3.2).

Table 3.2. Fusarium root rot incidence caused by FSP isolates on four bean varieties.

Varieties	Screenhouse					Laboratory				
	FSP-1	FSP-2	FSP-3	FSP-4	Mean	FSP-1	FSP-2	FSP-3	FSP-4	Mean
MLB-49-89A	56.9	53.4	100	18.0	57.1	100	72.0	100	78.5	87.6
G1459	65.0	50.0	100	22.5	59.4	100	77.4	100	65.7	85.8
RWR719	56.9	34.9	100	32.0	56.0	100	62.5	100	54.8	79.3
K132	67.0	57.0	100	40.0	66.0	100	75.0	100	64.5	84.9
Mean	61.5	48.8	100	28.1		100	71.7	100	65.9	
SED$_{Line}$ (P= 0.05)			3.21					4.22		
SED$_{isolates}$ (P= 0.05)			6.13					7.06		
SED$_{isolatexline}$(P= 0.05)			12.5					11.2*		
CV%			35.3					37.4		

* s.e.d (P= 0.05) for mean of varieties.

Disease severity varied from 2.9 on MLB-49-89A to 5.7 on K132 in the screenhouse using the 1-9 scale developed at CIAT, while it varied from 4.1 to 5.6 on the same varieties in the laboratory (Table 3.3). The isolate FSP-3 caused the highest disease severity on all the varieties (Table 3.3), while the isolate FSP-4 caused the lowest severity. The trend of the

pathogenicity of the four isolates obtained from the laboratory was similar to that of the screenhouse trial, even though incidence levels were higher in the former.

Table 3.3. Fusarium root rot severity rating caused by four FSP isolates on four bean varieties.

Varieties	Screenhouse (1-9 scale)					Laboratory (1-9 scale)				
	FSP-1	FSP-2	FSP-3	FSP-4	Mean	FSP-1	FSP-2	FSP-3	FSP-4	Mean
MLB-49-89A	3.5	2.0	3.8	2.1	2.9	4.5	3.1	6.5	2.1	4.1
G1459	6.7	4.5	7.6	3.0	5.6	5.7	4.0	6.9	3.0	4.9
RWR719	3.5	4.5	4.5	2.3	3.7	4.8	3.2	5.1	2.2	4.7
K132	6.8	5.0	7.7	3.2	5.7	6.9	4.3	7.8	3.5	5.6
Mean	5.1	4.0	5.9	2.7		5.5	3.7	6.6	2.7	
SED$_{Line}$ (P= 0.05)			0.49					0.52		
SED$_{isolate}$(P= 0.05)			0.49					0.52		
SED$_{isolatexline}$(P= 0.05)			1.161					1.064		
CV%			30.2					21.8		

* s.e.d (P= 0.05) for mean of varieties; 1 = resistant and 9 = susceptible.

The trend of severity using the percentage scale was similar to that of the trend observed with the 1-9 disease scale, with the isolate FSP-3 still causing the highest severity in both the screenhouse and the laboratory trials. The isolate FSP-4 caused very low severity levels using the percentage scale on all the varieties. High disease severity was observed on the line K132 and the lowest on MLB-49-89A, in both the screenhouse and laboratory (Table 3.4).

Table 3.4. Fusarium root rot severity rating caused by four FSP isolates on four bean varieties.

Varieties	Screenhouse (%)					Laboratory (%)				
	FSP-1	FSP-2	FSP-3	FSP-4	Mean	FSP-1	FSP-2	FSP-3	FSP-4	Mean
MLB-49-89A	24.6	15.2	24.6	9.0	18.4	16.0	20.6	49.0	2.0	21.9
G1459	34.8	25.8	60.7	15.3	37.7	40.0	18.0	34.0	8.5	28.5
RWR719	25.0	29.0	33.0	11.9	24.7	20.0	15.5	44.5	11.3	22.8
K132	49.0	44.2	66.9	12.9	39.7	53.5	32.5	64.5	16.0	38.3
Mean	33.4	28.5	46.3	12.3		32.4	21.7	48.0	9.4	
SED$_{Line}$ (P= 0.05)			5.02					5.49		
SED$_{isolate}$(P= 0.05)			5.02					5.49		
SED$_{isolatexline}$(P= 0.05)			11.91					10.97		
CV%			37.6					32.4		

* s.e.d (P= 0.05) for mean of varieties.

For both experiments (screenhouse and laboratory) there was a tendency for the varieties with purple-coloured hypocotyls, that is, RWR719, MLB-49-89A and G1459, to have an intensified purple colour expressed on to their hypocotyls under FSP-3 when compared to infection by the other isolates (Figures 3.11, 3.12, and 3.13). The purple colour intensity decreased in the order FSP-3>FSP-2>FSP-4 (Figures 3.12, 3.13, 3.14). The disease scores also decreased in the same trend.

Fig. 3.11. Symptoms caused by Isolate FSP-2 at 2w after planting on varieties G1459, MLB-49-89A, K132, and RWR719.

Fig. 3.12. Symptoms caused by Isolate FSP-3 at 2w after planting on varieties G1459, MLB-49-89A, K132, and RWR719.

Fig. 3.13. Symptoms caused by Isolate FSP-4 at 2w after planting on varieties G1459, MLB-49-89A, K132, and RWR719.

3.3.3 Maintenance of FSP-3 isolate

There were no significant differences at P≤0.05 significance level between the pathogenicity of the isolates stored differently for 6mo, 1yr and 2yr, with disease severities ranging from 7.2 to 7.5 on the 1-9 scale. However, there were significant differences in the number of samples that remained viable at the different times of testing among the three methods. More viable samples were recovered from the PDA slants than from the other methods. Samples stored in distilled water at $5^{o}C$ had the least number of viable samples recovered as most had been contaminated with bacteria (Table 3.5). Even though more samples stored on PDA plates were recovered compared to the distilled water, there was a tendency for the PDA to dry out over time and hence a lower number of viable samples were recovered compared to the number recovered from PDA slants.

Table 3.5. Effect of three storage methods on the viability and pathogenicty of FSP-3 isolate after 6mo, 1yr and 2yr.

Time tested after storage	No. of samples tested	No. of viable samples			Average severity on K132
		PDA slants, $5^{o}C$	Double distilled water, $5^{o}C$	PDA plates under room temperature	
6mo	30	26	15	22	7.5
1yr	30	25	10	19	7.3
2yr	30	23	5	10	7.2
S.e.d (P= 0.05)					0.321

3.4 Discussion

Observations of the cultural characteristics of the different isolates showed that FSP-1 and FSP-3 were purplish-blue in colour, with the distinctive blue centre and white margin characteristic of pathogenic FSP isolates as described by Tusiime (2003). The other two isolates, FSP-4 and FSP-2, were white/creamish to pinkish in colour, which is characteristic of some pathogenic FSP isolates. All the isolates had 3-5 septate macro-conidia, having a uniform diameter along their length, and curved or rounded at the apex. The macro-conidia occurred either singly, paired or clamped together, which is characteristic of FSP (Abawi, 1980; Kraft *et al.*, 1981).

The four isolates FSP-1, FSP-2, FSP-3 and FSP-4 differed significantly (P= 0.05) from each other on the level of infection caused on the four varieties. Isolate FSP-3 was the most

pathogenic isolate among the four isolates tested, as it caused 100% disease incidence and severities ranging from 3.6 on the line MLB-49-89A to 8.6 on the local susceptible check K132. The variation in the pathogenicity and morphology of the isolates confirmed the variability among the strains of FSP occurring in Uganda (Tusiime, 2003). Pathogenicity differed amongst these four isolates and resistance levels of the four varieties, with MLB-49-89A being the most tolerant, followed by RWR719, G1459, and finally K132 as the most susceptible. The non significant interaction of the line x isolate on disease incidence and severity implies that any of these isolates or mixture of all these isolates could be utilized in screening for resistance to FRR. However, it is probable that in a mixture form, these isolates may interact with each other to give different results. Hence, for purposes of simplicity one isolate FSP-3, which was the most pathogenic, was chosen for the breeding programme as it would result in good infection levels necessary to differentiate between resistance levels of different varieties.

Higher disease incidence was observed in the laboratory compared to the screenhouse trial. This was probably because, in the laboratory, the pathogen was free floating in the water and could easily infect the plant, while in the screenhouse, the pathogen occurred as mycelia on sorghum seed and hence took longer to infect the plants, resulting in lower incidence at the final evaluation.

There was a tendency for the varieties with purple-coloured hypocotyls, that is, RWR719, MLB-49-89A and G1459, to have an intensified purple colour of hypocotyl tissue under FSP-3 infection. The intensity of the purple colour decreased in the order FSP-3> FSP-1> FSP-2> FSP-4 as did the disease scores.

Storing the isolate on PDA slants at 5°C was the most viable method of storage for FSP amongst the methods tested. Seventy seven percent of the samples stored on PDA slants at 5°C remained viable after two years of storage compared to 33% for the PDA plates stored under room temperature, and 17% of the samples stored in double-distilled water at 5°C. Storing in double-distilled water resulted in contamination with bacteria probably, because our facilities were not sterile enough. There was a tendency for agar to mix with the water making pathogen culture recovery difficult, while PDA plates under room temperature often dried out with time. Keijer et al. (1996) also recommended the use of PDA slants at low temperatures for storage of *Rhizoctonia solani*.

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Chapter Four: Developing an effective technique for screening common bean germplasm for resistance to Fusarium root rot (*Fusarium solani* f. sp. *phaseoli*)

Abstract

Common bean (*Phaseolus vulgaris* L.) resistance to Fusarium root rot (FRR) caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans (FSP) has been documented as a quantitative trait and as such is greatly influenced by environmental factors. This, therefore, suggests the need for a reproducible screening method for selecting resistant germplasm that would be important in the improvement of resistance of the common bean to FRR. The present study was conducted to determine the effect on the severity of FRR of soil composition, irrigation frequency, and inoculation technique for screenhouse evaluation trials. The effects of five soil compositions, and five irrigation frequency levels in one trial, and the effect of two inoculation techniques in another trial, on the severity of FRR on six common bean varieties were investigated at Kawanda Agricultural Research Institute (KARI), Uganda. The first trial was a 6 x 5 x 5 split-split plot with three replications and the second was a 6 x 3 factorial with three replications. The severity of FRR and plant stand were significantly (P≤0.05) affected by different irrigation frequencies, soil compositions and their combinations. However, there were no significant (P≤0.05) differences between the varieties under the different combinations. Planting on 80% lake sand:20% forest soil, or 50% lake sand:50% forest soil, gave the highest disease infection levels in all the varieties, while the lowest disease severity was obtained on forest soil, the commonly used soil composition for screening for root rot pathogens at KARI. The 50% swamp soil:50% forest soil composition and forest soil differentiated the varieties most distinctly into categories according to their reaction to FRR. Furthermore, the best distinct classification for the varieties was obtained under treatments that were watered daily and once week. A combination of forest soil and daily watering provided adequate FRR disease levels necessary for disease evaluation and differentiation between bean varieties of varying resistance levels. From the second trial, using sorghum seed as a medium for pathogen inoculation resulted in adequate FRR infection levels at 4 weeks after planting (wap) for differentiating between bean varieties of varying resistance levels, while using agar slurry on the other hand, resulted in very high infection levels, making differentiation between varieties difficult. A further consideration was that plant stands were higher at 4 wap when sorghum

seed was used as a media for FSP inoculum than when agar slurry was used. This ensured that several plants remainied available for disease evaluation at 4 wap with sorghum seed inoculum than with agar slurry medium. In conclusion, the study showed that interactions of irrigation frequency, soil composition and varieties were not significant. Therefore, taking into consideration the extra costs of labour and time in preparing different soil composition mixtures, the standard method of using forest soil and watering daily with sorghum seed as mode of pathogen inoculation was adopted as a screening technique for further studies on FRR resistance in beans

4.1 Introduction

Several environmental factors, especially those that stress plants, have been shown to affect resistance to Fusarium root rot or FRR [*Fusarium solani* (Mart.) Appel and Wollenv. f. sp. *phaseoli* (Burk.) Snyd. & Hans] (FSP) in common bean (*Phaseolus vulgaris* L.). For example, factors that limit root development, such as soil compaction (Burke et al., 1969, 1972; Miller and Burke, 1975; Miller and Burke, 1977), predispose beans to root rot. Cool temperatures, high or low pH, low soil fertility, pesticide or fertilizer injury, excessive soil moisture or low soil moisture (Burke, 1965; Miller and Burke, 1985), high plant densities or high competition between plants (Burke and Barker, 1966), high inoculum levels, and the presence of a complex of root rot pathogens (Baker, 1970; Pieczarka and Abawi, 1978; Sippel and Hall, 1982; Chaudhary et al., 2006) also affect resistance to FRR in beans.

In crop breeding programmes, the choice of the optimum selection environment (one that maximizes the response for the target environment) is critical, particularly when the trait being targeted is affected by the environment and may be polygenic in nature (Sippel and Hall, 1982; Chaudry et al., 2006). There is often limited improvement of disease resistance for polygenic traits such as resistance to FRR, because inheritance of the trait is compounded by strong environmental effects, the sources of resistance used and the evaluation procedures (Hassan et al., 1971; Boomstra and Bliss, 1977; Beebe et al., 1981; Hall and Philips, 2004).

Different greenhouse screening methods that include variation in the inoculation technique, type of planting medium, moisture content, soil temperature, and fertility of the planting medium have been used to examin FRR resistance in common bean. Bilgi et al. (2006)

compared three methods of inoculating FSP in soil, and found that they all achieved good infection levels and had good correlations to field data. This suggested that they could all be used in an efficient manner to screen germplasm or cultivars for resistance to FRR without necessarily having a field trial. Similarly, Schneider and Kelly (2000) developed a protocol, the accuracy of which was confirmed by the significant correlations between greenhouse and field ratings. However, although good inoculation techniques may be effective in inducing good infection levels and may be correlated to field conditions, other factors such as soil moisture level, low soil fertility and soil composition may still influence disease severity ratings, which may influence the selection of populations for improvement of resistance to FRR. Often breeding programmes have thrown away potential resistant varieties without considering environmental factors (of the selection environment) that may have influenced the reaction of a line to the disease. Currently the breeding programme at KARI uses a uniform screening technique that targets selection for Pythium root rot resistance for all the other root rot pathogens. However, Pythium resistance has been found to be governed by a single dominant gene (Otysula et al., 2005) and may be less affected by the selection environment. The present study therefore aimed at modifying the screening technique currently being used at KARI to suit screening of beans for FRR resistance. The specific objectives were as follows:

1. To determine the optimum irrigation frequency level to induce adequate disease infection levels for selection of beans for resistance to FRR;

2. To determine the optimum soil composition to induce optimum disease infection levels for selection of beans for resistance to FRR;

3. To appraise two commonly used inoculation techniques for screening beans for root rot pathogens on screening for resistance to FRR;

4. To investigate the host-parasite-environment interaction of FSP and common beans.

4.2 Materials and methods

4.2.1 Effect of irrigation frequency and soil composition on expression of resistance to *Fusarium solani* f. sp. *phaseoli* of six bean varieties

The trial evaluated the effect of the irrigation frequency and soil compaction on six bean varieties with varying levels of tolerance to FRR, that is, MLB-49-89A, Umubano, MLB-17-89A, G 3717, CIM 9313-1, and K20 (local susceptible check) (Table 4.1). MLB-49-89A,

Umubano, MLB-17-89A, and CIM 9313-1 were obtained from a nursery that had previously been screened for Fusarium wilt (*Fusarium oxysporum* f. sp. *phaseoli*) and Pythium root rot (*Pythium* sp) (Buruchara and Kimani, 1999; Buruchara and Camacho, 2000; Otsyula et al., 2005), while G3717 is a documented source of resistance to FRR obtained from CIAT (International Centre for Tropical Agriculture)-Colombia (Abawi and Pastor-Corrales, 1990).

Table 4.1. Source and reaction to Fusarium root rot and Pythium root rot of bean lines used in study.

Line	Characteristics	Source/Nursery
1. MLB-49-89A	Resistant to Pythium root rot	CIAT-Africa
2. CIM 9313-1	Resistant to Pythium root rot	CIAT-Africa
3. MLB-17-89A	Resistant to Pythium root rot	CIAT-Africa
4. Umubano	Resistant to Pythium root rot	CIAT-Africa
5. G 3717	Resistant to FRR	CIAT-Colombia
6. K20	Susceptible to FRR	Commercial bean line

Assessing the impact of frequency of irrigation was achieved 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 three times, that is, at 06h00, 11h00 and 18h00. Soil composition levels were manipulated by varying the levels of lake sand referred to simply as sand in the text, forest soil (collected from a nearby forest), and clay collected from a swamp referrred to simply as swamp soil in the text: 80% lake sand:20% forest soil, 50% lake sand:50% forest soil, 80% swamp soil:20% forest soil, 50% swamp soil:50% forest soil and forest soil alone. A soil analysis test for pH, soil composition, and organic matter content was done at KARI soils laboratory before the experiment was laid out (Table 4.2).

Table 4.2. Soil compositions evaluated for their effect on Fusarium root root 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= Phosphprus, K= Pottasium, Ca= Calcium, M= Magnesium.

The trial was conducted in the screenhouse at KARI, and was 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. The swamp soil and forest soil 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 inoculum as it is the standard method of root rot soil inoculation currently used at KARI. Inoculum of the FSP-3 *FSP* isolate that was obtained from infected bean fields in south-western Uganda (see Chapter 3) was used. Pure colonies of the isolate stored on Potato Dextrose Agar (PDA) slants at 5°C were grown on PDA plates for a period of up to 21d and used to prepare the disease inoculum. Duran glass bottles (Aldrich, Z305197-10), of 500ml capacity, were partially filled with sorghum seed (2/3 capacity) and water was added. The bottles were sealed and the contents autoclaved for 1hr at 120°C. One PDA plate of the FSP-3 isolate was suspended in 4-10ml of sterile and deionised water to make a slurry which was spread evenly onto the surface of the already prepared and cooled 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 to grow. After this the bottles were opened but the opening was protected by foil paper to allow for evaporation of the excess moisture. After 21d of incubation, the bottles were emptied and the medium slowly dried to allow maturation of the fungal resting spores.

Wooden trays measuring 0.74 x 0.42 x 0.115m^3 were partially filled (2/3 capacity) with the different soil compositions. The soil was fertilised with NPK (1:1:1) at a rate of 3x10^{-3} kg ml^{-1} every seven days. The prepared inoculum was added to the soil at a rate of 500ml of inoculum per tray. A susceptible line K132 was planted in the trays, grown for a period of up to 28d and was then uprooted. This was a means of increasing disease inoculum as well as a means of testing the inoculum levels in the soil. Each tray was then planted with all the test varieties but with different combinations of soil composition and irrigation frequency. The trial was repeated to confirm the results.

4.2.2 Effect of inoculation technique on expression of Fusarium root rot resistance of six bean lines

This trial investigated the effect of two techniques used for inoculating soil with root rot pathogens currently used at KARI, viz. sorghum seed and agar slurry, on FRR resistance. Five bean lines, MLB-49-89A, MLB-17-89A, G 3717, CIM 9313-1, and K20 were used (Table 4.1). The trial was a 6 x 3 factorial experiment comprising of the six varieties and three inoculation techniques, that is, sorghum seed, agar slurry and no inoculum as a control.

Preparation of the pathogen inoculum using agar slurry was done as follows:- clean colonies of FSP-3 isolate that were on PDA plates were sub-cultured onto new PDA plates by dipping a sterile wire loop in sterile water and slightly touching the surface of the old cultures and then streaking onto the new PDA medium (Figure 4.1). The new cultures were allowed to grow for up to 30d. Thereafter the plates containing the culture colonies were flooded with sterile water and the mycelia was scraped off the PDA media using a sterile cover slip into more steril water. The resulting slurry was then filtered through a double layer of muslin cloth. Using a heamocytometer, the concentration in the inoculum was adjusted to between 3 000 and 4 000 conidial spores per 1 000ml of water which was enough to inoculate one wooden tray.

Fig. 4.1. *Fusarium solani* f. sp. *phaseoli* growing on PDA plates.

The FSP-3 inoculum on the sorghum seed was prepared by partially filling (2/3 capacity) 1 000ml Duran glass bottles (Aldrich, Z305197-10) with sorghum seed and water. The bottles

were sealed and the contents autoclaved for 1h at 120°C. Using previously purified isolates, two agar colonies were suspended in 60-70ml of sterile, deionised water. The slurry formed was then 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. Bottles containing the inoculated medium were incubated in the laboratory at 20-28°C for 5d to allow FSP to grow (Figure 4.2), after which the bottles were opened to allow for evaporation of the excess moisture, and incubated for 21d. Thereafter, the bottles were emptied and the medium slowly dried to allow maturation of the fungal resting spores. The inoculum was added to pre-sterilized soil at a rate of 500ml of inoculum per $0.74 \times 0.42 \times 0.115m^3$ trays of sterilized soil.

Fig. 4.2. *Fusarium solani* f. sp *phaseoli* isolate
FSP-3 growing on sorghum seed.

For both inoculation techniques, a susceptible line, K132, was planted in the trays for a period of up to 28d. It was then uprooted and the intensity of root rot symptoms observed. This acted as a means of increasing disease inoculum as well as a means of testing the inoculum levels in the soil. When uniform infection levels were obtained, the test materials were planted. The trial was replicated three times, with each tray acting as a replication for a treatment combination and repeated to confirm the results.

4.2.3 Data collection and analysis

For each of these trials, data on plant stand and FRR symptom severity were taken at 28d after planting (dap). Plant stand per treatment combination was recorded by counting the

number of plants that were still alive at 28dap. Disease severity from plants in the seedling trays was assessed by carefully uprooting all still-standing plants per treatment combination per replicate. Disease severity was assessed by washing the below ground parts of the plant (hypocotyl and roots) under running tap water. The roots and hypocotyls were split and the levels of infection observed.

Disease severity was assessed based on the percentage of plant tissue infected where:
- 0% = no visible symptoms;
- 25% = approximately a quarter of the hypocotyls and root tissue with lesions but tissue is still firm;
- 50% = approximately half of the hypocotyl and root tissues have lesions with considerable softening/ rotting;
- 75%-100% = whole of the hypocotyl and root tissues having lesions of FRR and root system is in advanced degrees of rotting or completely destroyed.

In addition, the 1-9 CIAT scale (Abawi and Pastor-Corrales, 1990) was used to assess disease severity, where:
- 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 and;
- 9 = approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with severe reduction in the root system.

Means were computed per treatment combination and the data analysed using the Genstat computer programme to obtain differences in the mean disease severity (Lawes Agric. Trust, 2006).

4.3 Results

Trials were not significantly different therefore results are presented for the means of the two trials.

4.3.1 Effect of bean line, irrigation frequency, and soil composition on expression of resistance to Fusarium root rot

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 4.3) 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 4.3).

Table 4.3. 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 (% scale)	Fusarium severity (1-9 scale)	Plant stand (28dap)
Line	5	3716.8**	34.3**	57408.2**
Irrigation frequency	4	18715.4**	157.69**	13621.1**
Soil composition	4	7097.3**	73.82**	8702.6**
Line x irrigation frequency	20	ns	Ns	1339.6**
Line x soil composition	20	ns	Ns	1339.4*
Irrigation frequency x soil composition	16	3276.2**	13.44**	2363.1**
Line x irrigation frequency x soil composition	80	ns	Ns	ns
Total	899			

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

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. 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 (Tables 4.4 and 4.5).

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 (Tables 4.4 and 4.5). A combination of forest soil and applying water application twice a week resulted in the lowest FRR infection (Tables 4.4 and 4.5). Applying water three times a day in 80% swamp soil:20% forest soil also resulted in low disease levels.

Table 4.4. Effect of different soil composition and irrigation frequency combinations on Fusarium root rot severity (1-9 scale).

Soil composition	Irrigation frequency per week					Mean
	Once	Twice	Three times	Four times	Daily	
80% lake sand:20% forest soil	7.6	6.7	4.6	4.5	8.0	6.3
50% lake sand:50% forest soil	6.0	5.4	4.4	5.1	7.0	5.6
Forest soil	4.7	3.6	4.7	5.1	4.8	4.5
80% swamp soil:20% forest soil	5.2	4.3	3.8	6.4	7.4	5.5
50% swamp soil:50% forest soil	6.9	6.1	5.8	6.9	7.1	6.6
Mean	6.1	5.2	4.7	5.6	6.9	
S.e.d $_{soil\ composition}$(P= 0.05)			0.29			
S.e.d $_{irrigation\ frequency}$ (P= 0.05)			0.26			
S.e.d $_{soil\ composition\ x\ irrigation\ frequency}$ (P= 0.05)			0.58			
CV%			30.9			

Table 4.5. Effect of different soil composition and irrigation frequency combinations on the Fusarium root rot severity (percentage of root tissue infected).

Soil type	Irrigation frequency per week					Mean
	Once	Twice a week	Three times	Four times	Daily	
80% lake sand:20% forest soil	65.0	54.5	29.8	31.4	67.5	49.6
50% lake sand:50% forest soil	54.4	43.1	33.5	39.3	63.4	46.7
Forest soil	38.9	25.7	33.9	36.4	38.7	38.3
80% swamp soil:20% forest soil	41.6	28.7	25.6	52.8	59.2	41.6
50%swamp soil:50% forest soil	57.2	45.0	47.8	58.4	60.7	53.8
Mean	51.4	39.4	34.1	43.7	58.0	
S.e.d $_{soil\ composition}$(P= 0.05)			2.64			
S.e.d $_{irrigation\ frequency}$ (P= 0.05)			2.36			
S.e.d$_{soil\ composition\ x\ irrigation\ frequency}$ (P= 0.05)			5.27			
CV%			36.1			

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 4.6).

Table 4.6. Effect of different soil compositions and irrigation frequency combinations on the plant stand (28dap) of bean lines.

Soil type	Irrigation frequency per week					Mean
	Once	Twice a week	Three times	Four times	Daily	
80% lake sand:20% forest soil	44.7	57.6	49.8	70.9	41.9	53.0
50% lake sand:50% forest soil	42.2	52.4	37.8	68.9	68.3	54.0
forest soil	54.8	56.4	58.4	70.9	69.9	62.1
80% swamp soil:20% forest soil	62.4	66.7	68.9	74.4	59.1	66.4
50% swamp soil:50% forest soil	34.3	41.6	55.7	68.1	34.5	46.8
Mean	47.7	54.9	54.1	70.6	54.7	
S.e.d soil composition means			3.08			
S.e.d irrigation frequency (P= 0.05)			2.75			
S.e.d soil composition x irrigation frequency (P= 0.05)			6.16			
CV%			32.7			

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 (Tables 4.7 and 4.8). 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 (Tables 4.7 and 4.8). 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 4.7). For all the lines, the highest plant stands were observed in trays that received water four times in a week (Table 4.7). 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 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 4.7. Effect of different bean lines and irrigation frequency combinations on the plant stand (28dap) of bean lines.

Bean line	Irrigation frequency per week					Mean (Lines)
	Once	Twice a week	Three times	Four times	Daily	
MLB-49-89A	64.8	76.0	85.0	86.2	78.4	78.1
Umubano	73.2	78.6	73.7	92.7	79.4	79.5
K20	54.4	68.0	51.1	90.6	68.0	66.4
MLB-17-89A	20.1	24.6	29.1	33.6	21.3	25.7
G3717	31.3	35.1	43.1	51.2	33.3	38.8
CIM-3133-1	42.3	47.3	44.7	70.4	45.3	50.1
Mean (irrigation frequency)	47.7	54.9	54.5	70.8	54.3	
S.e.d lines			3.37			
S.e.d irrigation frequency (P= 0.05)			2.75			
S.e.d bean line x irrigation frequency (P= 0.05)			6.75			
CV%			32.7			

As shown in Table 4.8 below, 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 the 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 (Table 4.8). 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 4.8. Effect of different bean lines and soil composition combinations on the plant stand (28dap) of bean lines.

Bean line	Soil composition					Mean (bean lines)
	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	77.1	69.6	80.8	89.2	73.6	78.1
Umubano	74.2	78.8	87.1	90.0	67.5	79.5
K20	57.9	56.7	69.2	93.3	55.0	66.4
MLB-17-89A	20.4	27.9	24.6	40.0	15.8	25.7
G3717	35.8	40.4	43.3	39.6	35.0	38.8
CIM-3133-1	52.5	50.4	67.5	46.3	33.8	50.1
Mean (Soil compositions)	53.0	54.0	62.1	66.4	46.8	
S.e.d$_{lines}$ (P= 0.05)			3.37			
S.e.d $_{soil composition}$(P= 0.05)			3.08			
S.e.d $_{bean line x soil composition}$ (P= 0.05)			7.54			
CV%			32.7			

4.3.2 Effect of factor combinations on the ranking of different bean lines for resistance to Fusarium root rot

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 once a week (Figure 4.3). In addition, irrigation once a week and daily resulted in the highest disease scores indicatiing that only a few lines would be able to escape the disease at these irrigation frequencies.
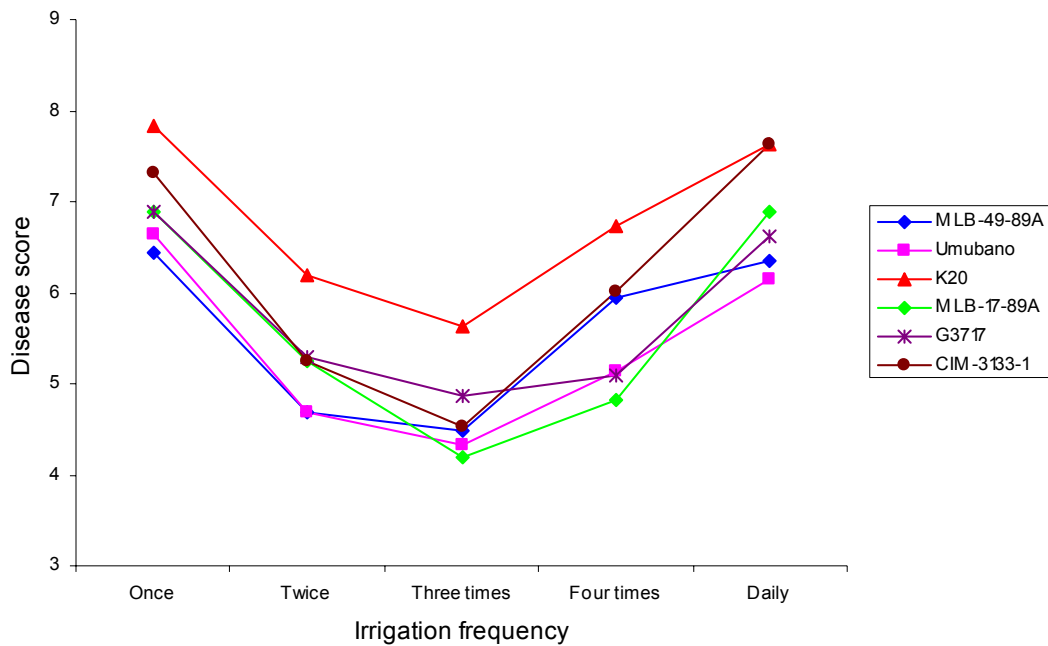
Fig. 4.3. Effect of irrigation frequencies on the reaction of different bean lines to Fusarium root rot.

With regard to soil composition, the 50% swamp soil:50% forest soil differentiated the lines most distinctly according to their reaction to FRR (Figure 4.4). 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 4.4). 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.
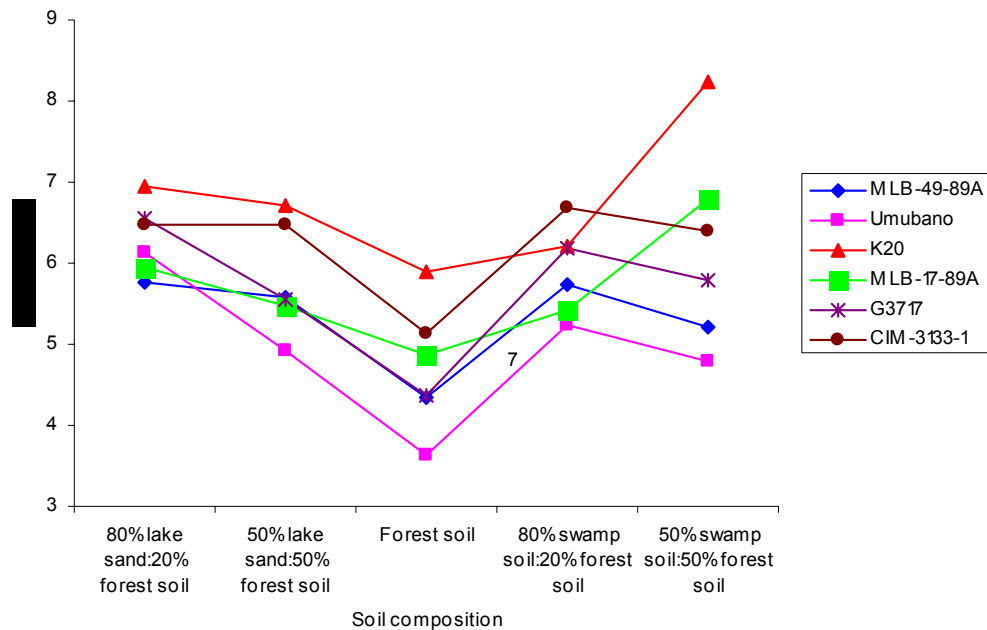
Fig. 4.4. Effect of soil composition on reaction of different bean lines to Fusarium root rot.

As shown in Figure 4.5a-f below, 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 CIM 3133-1 did not vary much, irrespective of the frequency of irrigation (Figure 4.5a-f). Similarly, on 50% lake sand:50% forest soil most of the lines apart from Umubano (Figure 4.5c), MLB-17-89A (Figure 4.5e) and CIM 3133-1 (Figure 4.5f) 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.

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a.

b.

c.

d.

f.

e.
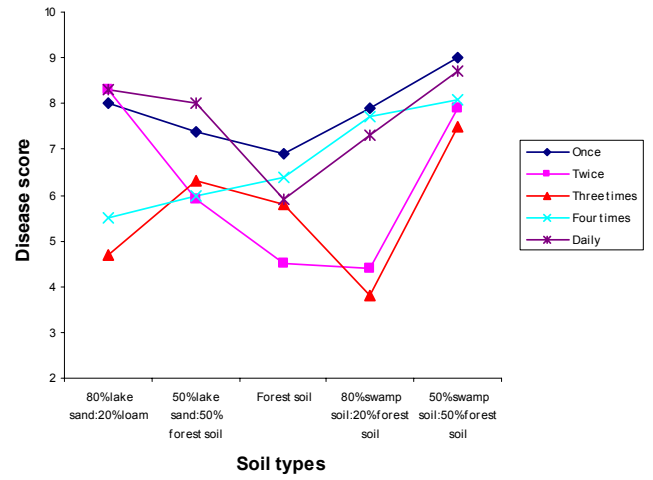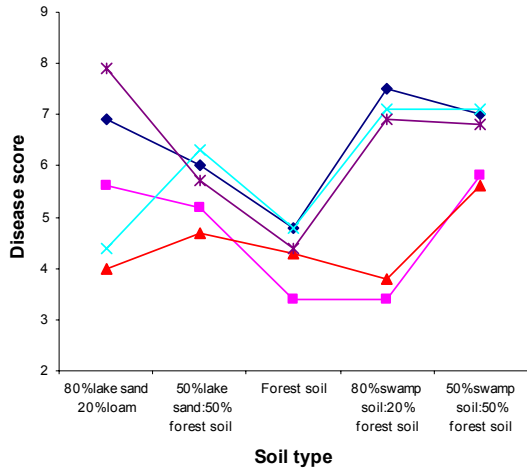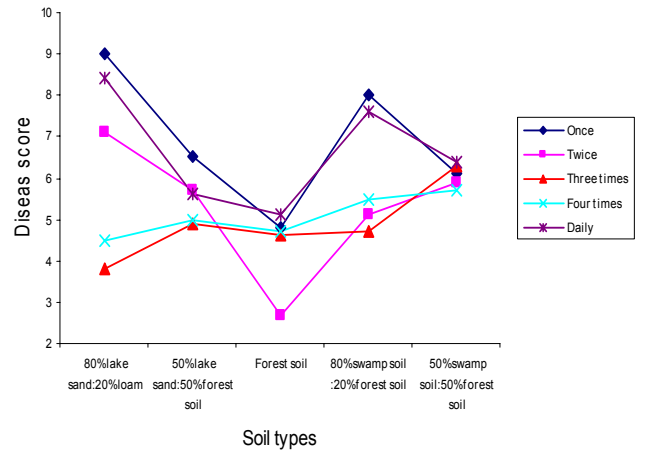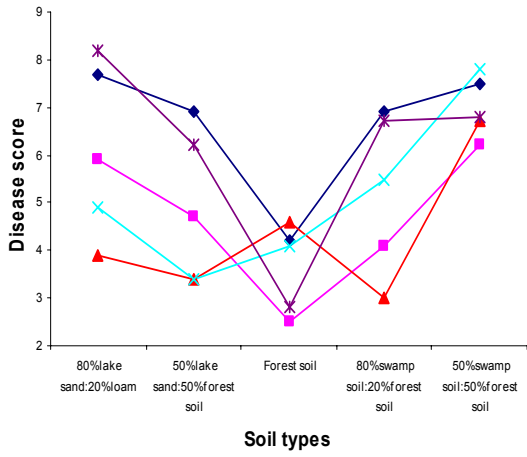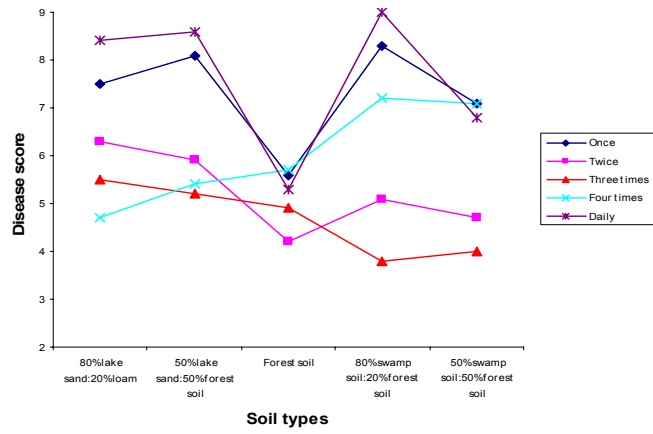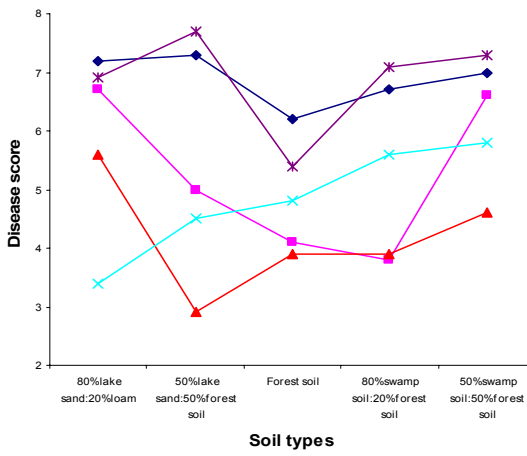
Figure 4.5. Reaction of different bean lines to Fusarium root rot under different soil type and soil moisture level combinations; a. MLB-49-89A, b. K20, c. Umubano, d. G3717, e. MLB-17-89A, f. CIM-3133-1.

4.3.3 Effect of inoculation techniques on expression of Fusarium root rot resistance

The interaction of inoculum technique and bean line on FRR severity was not significant at P=0.05 (Table 4.9), indicating that lines behaved similarly under the two types of inoculation techniques. Similarly, the five bean lines did not dffer significantly (P≤ 0.05) from one another for FRR severity. However, there were significant differences between the plant stands of the different lines at P≤ 0.05. In addition the two inoculation techniques were significantly different at P≤ 0.05 for their the effect on FRR severity levels.

Table 4.9. Mean squares for the effect of inoculation technique on Fusarium root rot severity on six bean lines.

Source of variation	DF	Fusarium severity		Plant stand
		(%)	(1-9 scale)	(28dap)
Bean line x Inoculation technique	4	ns	ns	ns
Bean line	5	ns	ns	1035.4*
Inoculation technique	1	1545.5***	16.26**	4687.5**
Total	29			

*, **, ***= significant at P= 0.05, P= 0.01 and P= 0.001, respectively.

Agar slurry inoculum resulted in more severe FRR symptoms (Table 4.10) than the sorghum seed inoculum. Plant stand was higher under the sorghum inoculum, 88.3%, compared to 63% under Agar slurry inoculum.

Table 4.10. Effect on inoculation techniques on the severity of Fusarium root rot and plant stand at 28dap on six bean lines.

Inoculation technique	Fusarium severity		Plant stand
	%	1-9 Scale	
Sorghum	34.1	4.6	88.3
Agar	48.4	8.1	63.3
S.e.d (P= 0.05)	3.81	0.34	6.07
CV%	33.4	21.1	21.9

4.4 Discussion

This study aimed at developing a screening protocol that would be effective in causing reliable infection levels of FRR and yet at the same time be affordable and easy to apply. The recommendations drawn from this could be utilised in evaluating bean germplasm for resistance to FRR under screenhouse conditions.

The study showed that the highest disease severity was observed on soil that was watered once a week and also that which received water on a daily basis. 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.

As regards soil composition, the highest disease severity and lowest plant stands were recorded on 50% swamp soil:50% forest soil and 80% lake sand:20% forest soil. 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 4.2). 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 4.2). 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.

The relative compaction of the soil, level 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. Soil compaction interferes with root penetration in 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.

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 ustilised 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 concentratioon of spore solution of the pathogen (Perssoni et al., 1997).

In this study, using infested sorghum seed as medium for FSP inoculation in soil was regared a better method for inheritance studies of FRR than agar slurry because it resulted in moderate disease infection levels necceseay for disease eveluation and differentiatingf between bean lines with varying FRR resistance levels. Agar slurry resulted in very high infection levels which made differentiation between lines difficult. Plant stands also remained higher on soil inoculated with sorghum FSP infested seed than in agar FSP slurry, ensuring enough plants for evaluation at the time of disease evaluation at 4 wap. For these reasons, studies reported on in the next chapters relied on sorghum seed as the mode of inoculation of the pathogen into the soil. Several alternative methods for inocula preparation and inoculation techniques for the various root rot pathogens are available (Abawi and Pastor-Corrales, 1990). For example, soil-potato inoculum is effective for screening for resistance to Rhizoctonia root rot (Abawi, 1989; Abawi and Pastor-Corrales, 1990). Seed of grain crops e.g., beet seed colonized by *Rhizoctonia* sp. (Abawi et al., 1985; Abawi et al., 2006) and millet seed for *Pythium* spp. (Pynjdi, 1996; CIAT, 2005) can also be used as an inoculum source and mixed with sterilized soil or placed directly next to the seedling stems near the soil surface.

Irrigation frequency and soil composition affected FRR severity but did not affect the way the different lines reacted to the pathogen. However, the plant stands of the different lines were affected by varying irrigation frequency levels and soil compositions. The commonly used soil composition for screening for root rot pathogens at KARI is forest soil, collected from a nearby forest. Water is applied on a daily basis three times a day. In this study, the lowest disease severity was obtained on forest soil and the highest disease severity on 80% lake sand:20% forest soil, 50% lake sand:50% forest soil and 50% swamp soil: 50% forest soil. This would imply that utilising either of these soil types which resulted in high disease

infection levels would be better options than using the forest soil. However, on 80% lake sand:20% forest soil, FRR severity on the different bean lines was not uniform, being affected by irrigation frequency. In contrast, on 50% swamp soil:50% forest soil and forest soil, the disease severity of FRR on the bean lines was not greatly affected by the irrigation frequency. However, adequate infection levels were obtained when irrigation was done once, four times a week and daily on these soil types. Also lines were well differentiated on 50% swamp soil:50% forest soil and on forest soil compared to the other soil types.

In conclusion, therefore, since the interactions of the factors with the 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, daily irrigation and FSP infested sorghum seed as a medium for inoculum was adopted as the standard screening technique for resistance to FRR and in the inheritance studies of FRR resistance in beans described in the next chapters. This method gave satisfactory disease infection levels and differentiated lines distinctly according to their FRR disease severity level. 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.

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Chapter Five: Genotypic variability of resistance to Fusarium root rot (*Fusarium solani* f. sp. *phaseoli*) and identification of sources of resistance

Abstract

The use of resistant varieties is probably the most effective control measure against for Fusarium root rot (FRR), especially for small-scale farmers who have limited access to fungicides. The objective of this study was to identify sources of resistance to FRR that may be used as parents in improving resistance in three large-seeded and popular bean varieties in Uganda. One hundred and forty seven common bean lines were screened for resistance to the *FSP* FSP-3 isolate under screenhouse conditions at Kawanda Agriculture Research Institute (KARI), during 2005 and 2006. They included 27 Uganda landraces, 31 lines from South Africa, 52 lines from CIAT-Africa, and 34 "resistant" lines from CIAT-Colombia. Three local susceptible lines, K20, K132, and Kanyebwa, were used as checks in the trials. Forty six moderatey resistant lines selected from the screenhouse trial were further evaluated against natural inoculum in a bean root rot (BRR) infested field at KARI, in order to confirm this resistance. Generally, field and screenhouse FRR severity data were highly correlated. Genotypes differed in their degree of sensitivity to FRR under screenhouse conditions, exhibiting a continuous distribution characteristic of quantitative traits. Although none of the lines was immune or highly resistant (severity ≤3 on a 1-9 disease scale) to FRR, some lines showed moderately resistant reactions. MLB-49-89A and HF-465-63-1 were the most resistant with severity scores of 3.2 and 3.6, respectively. Most of the resistant and moderately resistant lines were from the nursery that had undergone previous selection for resistance to Fusarium wilt and Pythium root rot. The resistant lines from CIAT-Colombia did not show high levels of resistance while the landraces were very susceptible to the FSP-3 isolate. The landrace, Hoima-Kaki, however, was only moderately susceptible to FRR, both under screenhouse and field conditions. Fifteen bean lines were moderately resistant and 15 were moderately susceptible to FRR at 28d after planting (dap) under field conditions. Four of these lines, namely, G3717, MLB-49-89A, MLB-48-89A, and Kabale-White, remained moderately resistant at 56dap. Fusarium root rot atings done at 28dap and those done at 56dap were highly correlated. However, most lines succumbed to the disease at 56dap. FRR severity at 56dap was shown to affect yield more than the severity at 28dap. The lines MLB-48-89A, G1459, G4795, RIZ 30, PAN128, Mbarara-Kanyebwa and Kabale-White had lower Fusarium root severity at 56dap compared to the disease scores at 28dap. It was

observed that the small-seeded lines tended to be more resistant to the root rot pathogen compared to the large-seeded lines. In addition, lines with purple hypocotyls tended to be more resistant to FRR than lines with green hypocotyls. However, ratings at 56dap were highly confounded by many other soil inhabiting pathogens as well bean fly. In conclusion, none of the common bean lines screened had high resistant levels but forty four were identified as potential sources of resistance to FRR. Of these ten were large-seeded and four were medium-seeded lines.

5.1 Introduction

Fusarium root rot (FRR) caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans (FSP) probably occurs in most bean fields throughout the world (Beebe et al., 1981; Burke and Miller, 1983; Abawi and Pastor-Corrales, 1990; Tu and Park, 1993; Park and Tu, 1994). It is currently one of the major diseases affecting common bean production in Uganda, causing total crop losses when susceptible lines are grown (Tusiime, 2003). The common bean breeding programme for BRR in Uganda has been mainly targeting Pythium root rot caused by *Pythium* spp., because this pathogen was found to be most predominant in the BRR complex in south-western Uganda (Pyndji, 1996; Mukalazi et al., 2001; Ostyula et al., 2005). However, FSP was subsequently found to be equally predominant and often occurred together with *Pythium* spp. (Tusiime, 2003). It is probable that the slow growing nature of this pathogen (see Chapter 3) could have resulted in its low diagnosis. Bean root rot disease has been shown to be more devastating when the two pathogens occur concurrently (Baker, 1970; Pieczarka and Abawi, 1978; Sippel and Hall, 1982. This may explain the severe epidemics that affect the common bean in south-western Uganda even when Pythium root rot resistant cultivars are planted. This phenomenon thus justifies research addressing resistance to FRR in common bean.

The use of resistant lines is probably the most effective control measure for FRR especially for small-scale farmers with limited access to fungicides (Hassan et al., 1971; Beebe et al., 1981; Hall and Nasser, 1996; Abawi et al., 2006). Several bean lines with resistance to a single or multiple root rot pathogens have been reported in Africa. However, none of the commercial bean lines currently grown exhibit a high level of tolerance to the prevailing root rot pathogens. Complete genetic resistance or immunity to FRR has so far not been

discovered in *P. vulgaris*. However, several *P. vulgaris* and *P. coccineus* lines have been reported to have some resistance to FRR (Wallace and Wilkinson, 1965; 1966; Boomstra and Bliss, 1977; Beebe et al., 1981; Silbernagel, 1987) with N203 (PI 203958) being the first recognised source of resistance (Wallace and Wilkinson, 1965; Wallace and Wilkinson, 1966). Most of these lines are maintained in the gene bank at the International Centre for Tropical Agriculture (CIAT) Cali, Colombia (Abawi and Pastor-Corrales, 1990).

Most of the developed and identified resistant genotypes are either late maturing, small or black seeded with a climbing growth habit (Beebe et al., 1981). Hence they would not easily be accepted by a large percentage of bean farmers in Africa (see Chapter 2). In addition, they may not be satisfactory parents in breeding programmes for improving resistance to FRR in the large-seeded Andean bean lines popular in Uganda. Most of the resistant genotypes originated from the Middle American genepool but inter-genepool crosses can pose a problem when trying to improve resistance in the Andean cultivars (see Chapter 1). However, Silbernagel (1987) developed a resistant large-seeded cultivar, FR266, that belongs to the Andean genepool, using a small and black seeded bean line, N203, as a source of resistance from the Middle American genepool. Schneider et al. (2001) have used this cultivar, FR266, successfully in crosses with beans from the Andean genepool for improvement of resistance to FRR. This suggests that there is the possibility of introgressing resistance genes from the small-seeded, Middle American genepool cultivars into the large-seeded, Andean bean seed types. A further consideration is that the sources of resistance to FRR currently available (Abawi and Pastor Corrales, 1990) are more adapted to the climate in USA and Latin America. They may not be good sources of resistance to FRR in the tropical African environment with probably different pathogens and pathogenic strains. Therefore, this underscores the need to identify potential new sources of resistance that are more adaptable to the tropical African climate and which are resistant to the predominant pathogens and pathogenic strains of this locality.

Screening for resistance to FRR is difficult because the trait is greatly influenced by the environment, and is thought to be genetically complex and difficult to evaluate. The efficiency of phenotypic selection for such a trait is low, resulting in limited progress in breeding for resistance (Román-Avilès and Kelly, 2005). Also for such a trait, field screening is very difficult because of the presence of other root rot pathogens. Greenhouse evaluations are ideal for the characterization of resistance gene(s) to specific pathogens as

they are done under controlled conditions and it is possible to subject test materials exclusively to the pathogen of interest (Schneider and Kelly, 2000; Román-Avilès et al., 2004b). In addition, the greenhouse allows for simultaneous screening of large numbers of plants and lines. However, results from the greenhouse are only dependable in the development of resistant cultivars if they correlate closely to the reaction of bean germplasm under field conditions (Schneider and Kelly, 2000; Hall and Philips, 2004; Bilgii et al., 2006). Field screening under naturally fluctuating conditions may enable the accurate measurement of the reaction of bean germplasm to root rot pathogens and the assessment of the impact of root rots on the quantity and quality of marketable yield (seeds or pods); this would, therefore, allow for selection for local adaptation, reaction to other disease pathogens and pests, and for tolerance to abiotic stresses (Abawi and Widmer, 2000; Abawi et al., 2006).

Genetic correlation refers to the degree of association between traits and how they may enhance selection (Dabholkar, 1992) and is a function of additive gene action (Falconer and Mackay, 1996). Correlation may be due to pleiotropic gene effects or linkage of genes governing inheritance of two or more characteristics (Falconer, 1989). Genetic correlation between traits is an important aspect of plant breeding as it suggests that selection for one trait will simultaneously result in changes of the other trait. It is particularly advantageous if the primary trait is more difficult to evaluate than the secondary trait. Evaluating resistance to FRR requires destructive sampling as well as the use of disease score scales that may vary at different evaluation times and between evaluators. The prospect of a highly correlated trait to resistance to FRR would therefore be a great milestone for breeders interested in this character. Resistance to FRR has been associated with small seed size, black seed color, and purple hypocotyls, although past studies have not been conclusive on these correlations. The present study sought to identify correlations between FRR resistance with a range of morphological traits in the materials screened for resistance to FRR. However, additive genetic correlation is unique to the population under selection and particular environmental conditions and may, as a result, not be extrapolated to other populations (Hallauer and Miranda, 1988). The objectives of this study therefore were as follows:

1. To identify possible sources of resistance to the FSP, isolate FSP-3,
2. To study the correlation between seed size, hypocotyl colour with FRR resistance.

5.2 Materials and methods

5.2.1 Genetic materials

One hundred and forty four common bean lines were assembled from different sources. Fifty six lines were obtained from CIAT-Africa and Kawanda Agricultural Research Institute (KARI). Of these, 46 lines had been previously screened for Pythium root rot and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *phaseoli* (Buruchara and Kimani, 1999; Buruchara and Camacho, 2000; Otsyula et. al., 2005). Six were *F. oxysporum* f. sp. *phaseoli* (FOP) disease differentials. Thirty one lines came from South Africa, 34 from CIAT-Colombia and 27 were local landraces from the Uganda National Bean Programme (UNBP). The lines from CIAT-Colombia are documented sources of resistance to FSP (Abawi and Pastor-Corrales, 1990). Three popular but susceptible bean lines were selected as checks, that is, K20 (Rosecocco), Kanyebwa, and K132 (CAL96) and were supplied by CIAT-Africa (Table 5.1).

One cycle of mass selection was done for all these materials in order to remove any off-types from the seed and to multiply the seed. This was done under controlled conditions in the screenhouse at KARI. Genotypes were of different seed sizes and were divided into nurseries according to their sources and characteristics (Table 5.1).

Table 5.1. Source and seed size categories of bean lines screened against *Fusarium solani* f. sp. *Phaseol.*

Nursery	Source	Seed sizes			Total
		Small	Medium	Large	
1. Pythium root rot	CIAT-Africa	24	12	10	46
2. Sources of resistance	CIAT-Colombia	31	3	0	34
3. Local landraces	UNBP-Uganda	7	8	11	27
4. South African cultivars	ARC Potchestroom and PRO-SEED, RSA	14	1	16	31
5. F.O.P Differentials	CIAT-Africa	6	0	0	6
Controls	CIAT-Africa	0	0	3	3
Total		**84**	**24**	**37**	**147**

5.2.2 Screenhouse evaluation

Isolate FSP-3 of *FSP*, that was obtained from south-western Uganda (see Chapter 3), was used for screening the 147 bean lines for resistance. Pure colonies of the isolate stored on Potato Dextrose Agar (PDA) slants at 5^oC were grown on PDA plates for a period of up to 21d and used to prepare the disease inoculum. Duran glass bottles (Aldrich, Z305197-10) of 500ml capacity were partially filled with sorghum seed (2/3 capacity) and 150ml water was added. The bottles were sealed and the contents autoclaved for 1hr at 120^oC. One PDA plate of the FSP-3 isolate was suspended in 4-10ml 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 $20\text{-}28^oC$ for 5d to allow FSP-3 to grow, after which the bottles were opened (the openings were protected from contamination using foil paper) to allow for evaporation of the excess moisture and nutrient solution. After 21d of incubation, the bottles were emptied, and the medium slowly dried to allow for maturation of the fungal resting spores.

Wooden trays measuring 0.74 x 0.42 x $0.115m^3$ were partially filled (2/3 capacity) with previously sterilized sandy clay loam soil. The soil was fertilised with NPK (1:1:1) at a rate of $3x10^{-3}kg\ ml^{-1}$ before planting and thereafter every after 7d. The prepared inoculum was added to the soil at a rate of 500ml of inoculum per tray. A susceptible bean line, K132, was planted in the trays and grown for a period of up to 28d and then uprooted. This acted as a means of increasing the disease inoculum as well as a means of testing the inoculum levels in the soil. Each of the five nurseries was screened separately at different times using the same soil. However, this resulted in increased soil inoculum levels, such that lines planted after an evaluation were heavily infected due to the very high soil inoculum levels, making further evaluation difficult (Figure 5.1a). Hence, thereafter, the soil was mixed with clean soil after every evaluation as a way of diluting the inoculum before a subsequent planting was done. This was done by mixing one tray of clean soil to two trays of infected soil, resulting in uniform inoculum levels at each evaluation (Figure 5.1b).

Fig. 5.1a. Plants under high inoculum levels of *Fusarium solani* f. sp. *phaseoli* resulting in premature seedling plant death.

Fig. 5.1b. Plants under sufficient innoculum levels of *Fusarium solani* f. sp. *Phaseoli* that ensures disease infection but maintains plant stand.

i) Experimental design and management

The trials were laid out as randomised complete block design (RCBD) with three replicates (three trays) of 20 plants per replicate (tray) per bean line. A replicate was a wooden tray that was planted with five bean lines at a time, with each bean line planted in two rows of ten plants each. A susceptible check was planted in each tray and evaluation was done at 28d after planting (dap). Watering of the trials was done 1-3 times daily depending on the intensity of the sunshine and amount of rainfall. Each of the trials was repeated.

Plant stand per bean line was recorded as the number of plants at the time of evaluation. Disease incidence was obtained by uprooting all the standing plants per bean line 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.

Disease 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 percentage of plant tissue infected where

- 0% = no visible symptoms;
- 25% = approximately a quarter of the hypocotyls and root tissue have lesions but tissue is still firm;

- 50% = approximately half of the hypocotyl and root tissues have lesions, with considerable softening/rotting;
- 75%-100% = the whole of the hypocotyl and root tissues have FRR lesions and the root system is in an advanced degree of rotting close or completely destroyed.

In addition, the 1-9 scale used at CIAT (Abawi and Pastor-Corrales, 1990) was also used to assess disease severity, where

- 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.

Averages were computed per bean line, and the data were analysed using the Genstat computer programme to obtain differences in the mean disease severity and to rank the lines for resistance to FRR (Lawes Agric. Trust, 2006).

ii) Classification of bean lines for resistance to Fusarium root rot

Classification of bean lines was based on the severity of the disease. Bean lines were grouped into five classes as follows:

1. Tolerant/resistant reaction = 0-15% or CIAT scale of 1-3;
2. Moderately resistant = 15.1-25% or CIAT scale of 3.1-5;
3. Moderately susceptible = 25.1-40% or CIAT scale of 5.1-6;
4. Susceptible = 40.1-50% or CIAT scale of 6.1-7.9;
5. Very susceptible = >50% or CIAT scale of 8-9.

5.2.3 Screening of selected bean lines under field conditions

To confirm resistance, 46 selected bean lines were screened under field conditions at KARI. The field used has a high occurrence of BRR pathogens and is continuously used by the CIAT-Africa breeding programme as a BRR hot-spot. The trial was laid out as an RCBD with three replicates during the short rainy season of 2005 (August-October) and long rain season of 2006 (March-June). A basal fertiliser was added one week before planting at a rate of 55kg N ha^{-1}, 66kg P ha^{-1} and 55kg K ha^{-1}. The trial was grown under natural inoculum, but the FSP infected soil that was being used in the screenhouse was placed below each seed on planting, as a means of increasing the levels of *FSP* in the soil relative to other soil-borne pathogens. Each bean line was planted in 5 rows with each row having 10 plants with 0.1m between plants. Rows were spaced at 0.5m between rows and a 1m space was left between replicates. Hand weeding was done twice at 14d after seedling emergence and just before flowering to control weeds. No irrigation was done as the rainfall was adequate.

Disease evaluation was done at 28dap and at 56dap. At each evaluation, 10 plants per bean line were randomly uprooted from the three central rows of each plot and disease severity rating was assessed, based on the 1-9 scale discussed under Section 5.2.2.1. Plant stand at 28dap was calculated as the percentage number of plants standing at 28dap, divided by the number of plants that emerged. Plant stand at 56dap was calculated as the percentage number of plants standing at 56dap, divided by the number of plants that remained after the first evaluation.

Shoot and root masses were obtained at 28dap 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 lines and correlated with FRR severity scores.

At harvest maturity, the lines 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. Data were analysed using the Genstat computer programme (Lawes Agric. Trust, 2006).

5.3 Results

Analysis of variance (ANOVA; see Appendices 5.1-5.6) obtained for FRR severity using the two rating scales used in this study, that is, the 1-9 scale and the percentage of root and hypocotyls tissue infected, gave similar trends of the mean squares and ranking of lines. This indicated that both rating scales were accurate and either one might be used to differentiate between the bean lines according to resistance to FRR. They were also highly correlated, with a correlation coefficient "r" of 83.7% at P= 0.001 (Spearman's rank correlation). However, discussion is based on the 1-9 scale data, as it had lower CVs; however the percentage data is also presented because it was also used in selecting resistant lines.

5.3.1 Incidence and severity of Fusarium root rot on bean lines under screenhouse conditions

Plant stand at 28dap was not significantly (P≤0.05) different among the entries, indicating that it was not affected by FRR at the time of disease evaluation. There were no significant differences (P≤0.05) between the disease severities of the lines between the two trials for all the nurseries and hence the means of the trials are presented. There was 100% disease incidence for all lines, indicating a lack of immunity to the disease for all the bean lines. However, disease severity varied significantly (P≤0.05) among the 147 bean entries (Appendix 5.1-5.5). Using the 1-9 scale, none of the entries had a mean score ≤ 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 in the range of 4.1-7, and 21% had disease severity >7 (Figure 5.2). This continuous distribution is typical of a quantitative trait and followed an almost normal distribution (R^2 = 0.87).
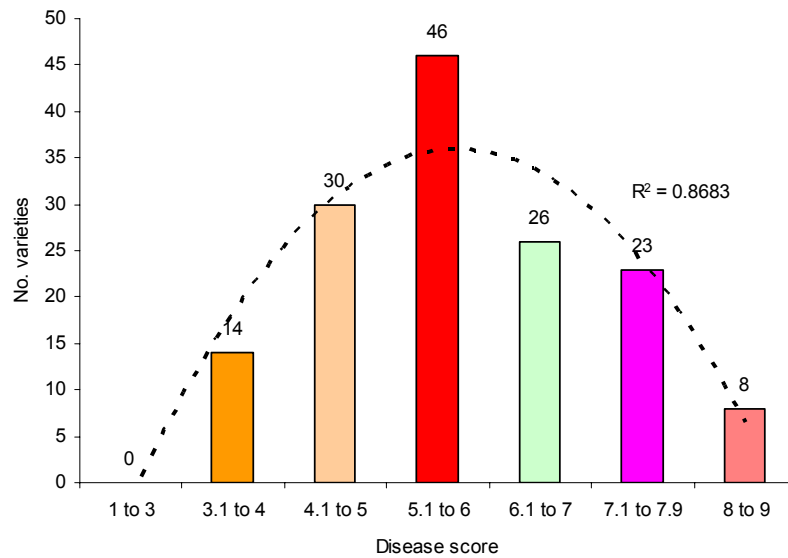
Fig. 5.2. Variation in Fusarium root rot severity on 149 bean lines in screenhouse trial.

In the Pythium root rot nursery, FRR severity ranged between a mean of 15.0% on the bean line MLB-49-89A and 75.6% on DOR 622, using the percentage scale and 3.2 (MLB-49-89A) and 8.4 (DOR 622) using the 1-9 scale (Table 5.2). Seventeen lines in this nursery were classified as moderately resistant (MR). They included MLB-49-89A, MLB-48-89A, MLB-17-89A, MLB-22-89A, Umubano/G2333, RWR719, RWR 1058, Vuninkingi/G685, FEB 181, CIM 9313-1, CIM 9314-1, EC-DE-HAR, 1/MS 11-1, 297/8, GLP 24, RWR 1092, and RWR 2075, with MLB-49-89A being the most resistant of these. Fourteen lines were classified as moderately susceptible (MS), 13 as susceptible (S), and four lines were classified as very susceptible (VS). None were classified as resistant.

The South African nursery (Table 5.3) had a range of moderately resistant to susceptible bean lines, with disease severity scores ranging from 21.2% on PAN185 to 63.3% on Mkomazi; and 3.8 (PAN 185) to 7.4 (Mkomazi) on the 1-9 scale. Fourteen lines were classified as moderately resistant and included Teebus RR1, OPS-KW1, OPS-RS4, RS5, Umtata, PAN185, PAN148, PAN128, PAN159, PAN146, Outeniqua, Timbavati, Imbali and Tongati. None of the lines were classified as resistant.

The CIAT nursery, which consisted of documented sources of resistance to FRR, did not exhibit as much tolerance to the FSP-3 isolate disease as expected, with most of the lines having moderately resistant to susceptible reactions. This indicates the possibility of

environmental differences in the screening environment, possible differences in screening techniques, as well as in the isolate that was used. Severity ranged from 23.4% on G3717 (Ica Tui) to 51.7% on G5334 (Bico de Ouro), and scores of 4.3 (G1459) to 6.4 on G4789 and G5533. Nine lines, G1459 (Jamapa), G3717 (Ica Tui), G4481 (Porillo no1), G4795 (Porrillo Sintetico), G 5149 (Jamapa), G4830 (Rio Tibagi), G5473 (Nep 2), G21950 (Bico de Ouro), and G9384 (Sutter Pink), were classified as moderately resistant.

A large percentage of the local landraces exhibited susceptible to very susceptible reactions to the disease. However, four lines were classified as moderately susceptible and they included Hoima-Kaki, Masaka-Manyigamulimi, Kayunga-Special K132 and Kabale-White. Severity ranged from 22.6% on Hoima-Kaki to 60.4% on Apac Ongori on the percentage scale and scores ranged between 5.1 and 8.9 on the 1-9 scale (Table 5.5). The high severities observed in the landrace nursery is an indication of the lack of resistance to FRR in the commonly grown lines in Uganda.

Table 5.2. Reaction of common bean lines from the Pythium root rot nursery to FSP-3 isolate in screenhouse.

Entry	Fusarium severity		Seed size	Seed colour	Hypocotyl colour	Classification*
	(%)	1-9 scale				
1. MLB-49-89A	15.0	3.2	Medium	Black	Purple	MR
2. MLB-40-89A	64.0	7.5	Small	Chocolate-yellow	Green	VS
3. MLB-48-89A	29.1	4.7	Small	Grey	Purplish	MR
4. MLB-39-89A	61.7	8.0	Small	Chocolate yellow	Green	VS
5. MLB-17-89A	27.7	3.8	Large	Calima		MR
6. MLB-36-89A	63.1	7.5	Small	Cream mottled	Green	VS
7. MLB 22-88B	33.8	4.8	Large	Calima		MR
8. Scam80-cm/15	39.6	5.8	Medium	Calima	Green	MS
9. Scam 80-cm/5	48.4	6.0	Medium	Calima	Green	S
10. K131/ MCM 5001	52.0	6.8	Small	Carioca	Green	S
11. Umubano/ G2333	25.1	3.9	Medium	Red	Purple	MR
12. G685/Vuninkingi	25.8	3.8	Small	Red/Maroon	Purple	MR
13. RWR719	25.5	4.5	Small	Red	Purple	MR
14. RWR 868	34.3	5.6	Large	Calima	Green	MS
15. APN 154	34.3	5.5	Small	Black	Purple	MS
16. Urugezi.	39.9	5.8	Medium	Calima	Green	MS
17. RWR 1059	35.2	5.3	Medium	Calima		MS
18. MCD 221	39.1	5.8	Large	Calima		MS
19. AND 1064	56.2	6.2	Large	Kidney red	Green	S
20. MAM 38	48.4	6.0	Small	Pinkish	Green	S
21. UBR (95) 25	60.3	7.4	Small	Cream	Green	S

Table 5.2. Reaction of common bean lines from the Pythium root rot nursery to FSP-3 isolate in screenhouse.

Entry	Fusarium severity		Seed size	Seed colour	Hypocotyl colour	Classification*
	(%)	1-9 scale				
22. CIM 9314-1	26.3	3.9	Small	Grey	Green	MR
23. CIM 9313-1	22.2	3.3	Small	Pinkish	Green	MR
24. EC-DE-HAR	37.1	4.9	Small	Cream	Green	MR
25. DFA 54	42.2	5.3	Small	White		S
26. FEB 181	22.8	3.5	Small	Red	Purple	MR
27. 311/7	50.1	7.2	Small	Red	Green	S
28. 1/MS 11-1	30.2	4.4	Small	Cream mottled		MR
29. DOR 711	62.7	7.6	Medium	Red	Purple	S
30. 106/1	42.2	5.7	Small	Red	Green	MS
31. GLP X 92	50.7	6.8	Medium	Pinto	Green	S
32. DOR 622	75.6	8.4	Small	Black	Purple	VS
33. Mexico 54	56.4	6.5	Medium	Grey	Purple	S
34. 217/2	41.9	5.7	Small	Black	Purple	MS
35. GLP 585	43.6	5.6	Small	Red	Purple	MS
36. 297/8	39.6	4.2	Medium	Cream		MR
37. CC 547	64.6	7.8	Medium	Cream mottled	Green	S
38. A686	40.6	5.5	Small	Cream mottled	Green	MS
39. A 797	60.1	7.3	Small	Cream		S
40. 302/7	41.6	5.9	Small	Black		MS
41. DOR 708	45.8	5.9	Medium	Red	Purple	MS
42. VAX 2	52.5	6.6	Small	Cream mottled	Green	S
43. RWR 1058	25.8	4.0	Large	Cream mottled	Green	MR
44. GLP 24	26.9	4.2	Large	Kidney red		MR
45. RWR 1092	32.0	4.6	Large	Kidney red	Purple	MR
46. RWR 2075	26.9	4.1	Large	Kidney red	Purple	MR
K20 (susceptible control)	74.2	8.8	Large	Kidney Red	Green	VS
K132/CAL 96 (susceptible control)	65.0	8.2	Large	Calima	Green	VS
Mean	42.9	5.7				
S.E.D (P= 0.05)	8.9	0.8				
CV%	46.9	26.9				

*Where; 1-3 = Resistant reaction, 3.1-5 = Moderately resistant, 5.1-6 = Moderately susceptible; 6.1-7.9 = Susceptible, 8-9 = Very susceptible.

Table 5.3. Reaction of common bean lines from the South African nursery to the FSP-3 isolate in screenhouse (% severity and 1-9 scale).

Entries	Identification (Source or Pedigree)	Fusarium severity (%)	scale	Seed size	Seed colour	Hypocotyl colour	Classification*
1. Teebus	Gallaroy/White Dutch Princess	51.9	6.7	Small	White	Green	S
2. Teebus RR1	Teebus*3/BelDak-RR-2	32.2	4.5	Small	White	Green	MR
3. OPS-KW1	Cambsel 14/C20//TC 1158-3-D4	28.2	4.2	Small	White	Green	MR
4. Kranskop	(Bonus///Redlands Autumn Crop//Bonus*2/UI51)(Bonus///Redlands Greenleaf C//Bonus*2/UI50)	46.3	5.7	Large	Red speckled	Green	MS
5. Jenny	Kranskop////Redlands Autumn Crop///Bonus//UI 51/Bonus	46.1	5.1	Large	Red speckled	Green	MS
6. OPS-RS1	Kranskop////Redlands Autumn Crop///Bonus//UI 51/Bonus	46.4	5.4	Large	Red speckled	Green	MS
7. OPS-RS2	Kranskop/PAD 61	49.7	5.8	Large	Red speckled	Green	MS
8. OPS-RS4	Sug 55//AND 308/Kranskop///SUG 84	35.8	4.8	Large	Red speckled	Green	MR
9. RS5	(Kranksop/Enseleni)*2//SUG 70	27.5	4.6	Large	Red speckled	Green	MR
10. Bonus	Selection from South African landrace "VanZyl Sugar"	46.8	5.4	Large	Red speckled	Green	MS
11. Mkuzi (A 286)	G4017/G4830; G4017="Carioca"; G4830=RioTibagi both from Brazil	58.4	6.9	Small	Carioca	Purple	S
12. Enseleni	PRO-SEED	45.0	5.4	Medium	Red speckled	Green	MS
13. Kranskop-HR 1	Kranskop*5/Edmund	49.8	5.8	Large	Red speckled	Green	MS
14. Umgeni	PRO-SEED	61.3	7.1	Medium	Red speckled	Green	S
15. Mkomazi	PRO-SEED	63.3	7.4	Small	Red speckled	Green	S
16. Gadra	PRO-SEED	51.3	6.9	Large	Red speckled	Green	S
17. Umtata	PRO-SEED	36.6	4.8	Small	Yellow	Green	MR
18. PAN 185	PANNAR seed company cultivar, pedigree unknown, closely related to Teebus	21.2	3.8	Small	White	Green	MR
19. PAN 148	PANNAR seed company cultivar, pedigree unknown, closely related to Kranskop	38.8	4.5	Large	Red speckled	Green	MR
20. PAN 116	PANNAR seed company cultivar	46.5	5.6	Large	Red speckled	Green	MS
21. PAN 128	PANNAR seed company cultivar	26.7	3.9	Large	Red speckled	Green	MR
22. PAN 159	PANNAR seed company cultivar	30.5	4.3	Large	Red speckled	Green	MR
23. PAN 146	PANNAR seed company cultivar	33.7	4.5	Medium	Red speckled	Green	MR
24. PAN 150	PANNAR seed company cultivar	41.0	5.6	Large	Red speckled	Green	MS
25. DBS 310	DBS cultivar, pedigree unknown	43.8	5.8	Large	Red speckled	Green	MS
26. DBS 360	DBS cultivar, pedigree unknown	59.1	7.1	Large	Red speckled	Green	S
27. Outeniqua	PRO-SEED	22.4	3.7	Small	White	Green	MR
28. Timbavati	PRO-SEED	31.2	4.6	Small	White	Green	MR
29. Imbali	PRO-SEED	32.7	4.7	Small	White	Green	MR

Table 5.3. Reaction of common bean lines from the South African nursery to the FSP-3 isolate in screenhouse (% severity and 1-9 scale).

Entries	Identification (Source or Pedigree)	Fusarium severity		Seed size	Seed colour	Hypocotyl colour	Classification*
		(%)	scale				
30. Elangeni	PRO-SEED	56.1	6.7	Small	White	Green	S
31. Tongati	PRO-SEED	28.0	4.0	Small	White	Green	MR
K20	Susceptible checks	69.3	8.4	Large	Red kidney	Green	VS
K132	Susceptible checks	58.9	7.8	Large	Red kidney	Green	S
Kanyebwa	Susceptible checks	55.3	7.5	Large	Red speckled	Green	S
Mean		44.6	5.7				
S.E.D (P= 0.05)		6.76	0.49				
CV (%)		31.3	18.2				

*Where; 1-3 = Resistant reaction, 3.1-5 = Moderately resistant, 5.1- 6 = Moderately susceptible; 6.1-7.9 = Susceptible, 8-9 = Very susceptible.

Table 5.4. Reaction of common bean lines from the CIAT-Cali nursery to FSP-3 isolate in screenhouse (% severity and 1-9 scale).

Entries	Identification	FSP severity (%)	FSP severity 1-9 scale	Seed ize	Seed colour	Hypocotyl colour	Classification
1. G 1459	Jampa	26.1	4.3	Small	Black	Purple	MR
2. G 1741	Porillo no1	45.8	5.4	Small	Black	Purple	MS
3. G 3018	Jamapa	35.1	5.6	Small	White	Green	MS
4. G 3645	Jamapa	42.9	5.9	Small	Purplish black	Purple	MS
5. G 3715	Porillo1	46.0	6.4	Small	Black	Purple	S
6. G 3717	Ica Tui	23.4	4.6	Small	Black	Purple	MR
7. G 4449	Pinto u.i. 114	48.3	6.0	Medium	Black	Green	S
8. G 4454	IcaTui	30.9	5.1	Small	Black	Purple	MS
9. G 4456	Jamapa	41.5	5.9	Small	Black	Purple	MS
10. G1459	Jampa	26.1	4.3	Small	Black	Purple	MR
11. G 1741	Porillo no1	45.8	5.4	Small	Black	Purple	MS
12. G 3018	Jamapa	35.1	5.6	Small	White	Green	MS
13. G 3645	Jamapa	42.9	5.9	Small	Purplish black	Purple	MS
14. G 3715	Porillo1	46.0	6.4	Small	Black	Purple	S
15. G 3717	Ica Tui	23.4	4.6	Small	Black	Purple	MR
16. G 4449	Pinto u.i. 114	48.3	6.0	Medium	Black	Green	S
17. G 4454	IcaTui	30.9	5.1	Small	Black	Purple	MS
18. G 4456	Jamapa	41.5	5.9	Small	Black	Purple	MS
19. G 4461	Porillo no1	45.8	5.8	Small	Black	Green	MS
20. G 4481	Porillo no1	34.5	4.7	Small	Black	Purple	MR
21. G 4495	Porrillo Sintetico	26.5	5.3	Small	Black	Purple	MS
22. G 4497	Cubagua	49.6	6.3	Small	Black	Purple	S
23. G 4789	Honduras 46	44.7	6.4	Small	Black	Green	S
24. G 4791	Porrillo no1	38.8	5.4	Small	Deep maroon	Green	MS
25. G 4795	Bico de Ouro	38.5	4.1	Small	Black	Purple	MR
26. G 4830	Rio Tibagi (lote 10)	46.6	5.0	Small	Black	Purple	MR
27. G 5043	Bico de Ouro	38.9	5.7	Small	Cream	Purple	MS
28. G 5108	Bico de Ouro	35.6	6.3	Small	Brown	Purple	S
29. G 5149	Jamapa	30.7	4.7	Small	Black	Green	MR
30. G 5165	Black Turtle soup	40.3	5.6	Small	Black	Purple	MS
31. G 5196	Criollo Pacuar 2	39.4	5.1	Small	Black	Green	MS
32. G 5256	Venezuela 54	34.6	5.5	Small	Black	Purple	MS
33. G 5334	Bico de Ouro	51.7	6.2	Small	Cream-brown	Green	S
34. G 5448	Honduras 46	38.5	6.1	Small	Deep maroon	Purple	MS
35. G 5473	Nep 2	38.6	5.0	Small	White	Green	MR
36. G 5533	Bico de Ouro	41.0	6.4	Small	Cream-brown	Green	S
37. G 5694	Cornell 49-242	38.3	5.7	Small	Black	Purple	MS
38. G 5749	Venezuela 54	33.7	5.5	Small	Black	Purple	MS
39. G 9384	Sutter Pink	24.5	4.9	Small	Pink	Purple	MR
40. G 9508	Bico de Ouro	34.4	5.3	Small	Brown	Green	MS
41. G 21796	Nw410	49.8	6.2	Medium	Cream-mottled	Green	S
42. G 21950	Bico de Ouro	40.6	5.0	Small	Greyish-brown	Green	MR
43. G 23376	Nw 590	36.1	6.5	medium	Cream-	Green	S
K20 (susceptible check)	Roseccoco	55.1	7.1				
MLB-49-89A (resistant check)	A40 x Inyumba	18.2	3.2				
Mean		38.5	5.5				
S.E.D (P= 0.05)		7.00	0.66				
CV(%)		31.4	14.8				

*Where; 1-3 = Resistant reaction, 3.1-5 = Moderately resistant, 5.1- 6 = Moderately susceptible; 6.1-7.9 = Susceptible, 8-9 = Very susceptible.

Table 5.5 Reaction of common bean lines from the Uganda landrace nursery to the FSP-3 isolate in screenhouse.

Entries	FSP Severity		Seed size	Seed colour	Hypocotyl colour	Classification *
	%	1-9 scale				
1. Kayunga-Kayinja	33.0	6.4	Small	Red	Green	S
2. Mukono-Kayinja owamabala	45.4	8.0	Medium	Red mottled	Green	VS
3. Bushenyi-Purple	52.2	7.8	Large	Purple	Purple	S
4. Apac-Ongori	60.4	8.9	Large	Red mottled	Green	VS
5. Masindi-OBAI	49.9	8.0	Medium	Red mottled	Green	VS
6. Masaka-Manyigamulimi	27.3	5.8	Large	Red mottled	Green	MS
7. Bushenyi-Nambale	47.1	6.9	Large	Red mottled	Green	S
8. Kayunga-Special K132	37.6	6.4	Large	Red mottled	Green	MS
9. Kiboga-OBAI/ Nambale omumpi	50.9	7.6	Medium	Red mottled	Green	S
10. Mbarara-Kanyebwa (Cream)	32.5	6.5	Medium	Sugar bean	Green	S
11. Bushenyi-Kanyebwa omuwanvu	44.6	7.2	Large	Sugar bean	Green	S
12. Masaka-Kanyebwa	52.4	7.9	Medium	Sugar bean	Green	S
13. Masaka-Kyenvu	42.7	6.5	Large	Yellow	Green	S
14. Bushenyi-Coffee small	44.6	7.3	Small	Coffee	Green	S
15. Bushenyi-Large coffee	44.2	7.2	Large	Coffee	Green	S
16. Kiboga-Yellow	46.6	7.5	Small	Yellow	Green	S
17. Hoima-Large yellow	57.4	7.7	Large	Yellow	Green	S
18. Mbarara-Kahura	56.0	8.2	Small	Red	Green	VS
19. Mukono-Red	45.4	7.6	Medium	Red	Green	S
20. Bushenyi-Nakyewegola	45.7	7.5	Large	Red mottled	Green	S
21. Kabale-White	35.5	5.6	Small	White	Green	MS
22. Apac-White	36.3	6.6	Small	White	Green	S
23. Hoima-Kaki	22.6	5.1	Small	Brown	Green	MS
24. Mpigi-Nakawunde	40.1	7.2	Large	Black striped	Green	S
25. Lira-Cream	29.4	6.0	Small	Cream	Purple	S
26. Mbale-Sonia	34.8	6.5	Medium	Pink-purplish	Green	S
27. Mpigi-Carolina	36.2	7.1	Medium	Red mottled	Green	S
K20 (Susceptible check)	48.2	7.0	Large	Red mottled	Green	S
MLB-49-89A (Resistant check)	18.0	3.2	Small	Black	Green	MR
Mean	42.0	7.0				
S.E.D (P= 0.05)	5.19	0.55				
CV (%)	36.6	19.8				

*Where; 1-3 = Resistant reaction, 3.1 - 5= Moderately resistant, 5.1- 6 = Moderately susceptible; 6.1- 7.9 = Susceptible, 8-9 = Very susceptible.

Among the *F. oxysporum* differentials, severity ranged between 19.4 and 46.5% and scores ranged between 3.6 and 6.3 on the lines HF-465-63-1 and IPA-1, respectively (Table 5.6). Four of these lines were classified as moderately resistant.

Table 5.6. Reaction of seven *Fusarium oxysporum* f. sp. *phaseoli* differentials to the FSP-3 isolate in the screenhouse (% severity and 1-9 scale).

Entries	FSP Severity		Seed size	Seed colour	Hypocotyl colour	Reaction*
	%	1-9 scale				
1. Calima	39.3	5.2	Small	Pale-cream to buff	Green	MS
2. Riz 30	26.7	4.3	Small	Pale-cream to buff	Purple	MR
3. A 211	26.3	3.8	Small	Whitish	Purple	MR
4. IPA 1	46.5	6.3	Small	Brown, pale to dark	Purple	S
5. HF-465-63-1	19.4	3.6	Small	Brown, pale to dark	Purple	MR
6. BAT 477	25.4	4.4	Small	Brown, pale to dark	Green	MR
K20 (susceptible check)	44.2	7.2	Large	Red mottled kidney	Green	VS
K132 (susceptible check)	54.3	9.0	Large	Red mottled kidney	Green	VS
Kanyebwa (susceptible check)	67.5	9.0	Large	Red speckled sugar bean	Green	VS
Mean	38.8	5.9				
S.E.D (P= 0.05)	4.2	0.3				
CV (%)	25.6	12.1				

*Where; 1-3 = Resistant reaction, 3.1 - 5= Moderately resistant, 5.1- 6 = Moderately susceptible; 6.1-7.9 = Susceptible, 8-9 = Very susceptible.

In all the nurseries, the susceptible checks had the highest FRR severity, apart from the landrace nursery where more than 50% of the landraces had a severity higher than that of K20, the local susceptible check. A large number of the moderately resistant lines were from the Pythium nursery and Fusarium wilt (*F. oxysporum* f. sp. *phaseoli*) differentials. This is probably because they had been previously selected for Pythium root rot and Fusarium wilt resistance under field conditions and, in so doing, may have been indirectly selected for FRR resistance as these pathogens often occur concurrently. Forty four lines were classified as moderately resistant to FSP, and of these, MLB-49-89A was the most resistant. Ten of the moderately resistant lines were large-seeded lines, 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, while four were red speckled sugar beans and included RS5, PAN 148, PAN 128 and PAN 159. Three moderately resistant lines were medium-seeded and included MLB-49-89A, Umubano and PAN 146. Figure 5.3 shows different bean lines with different bean severity levels, highlighting the resistance to FRR of MLB-49-89A, Umubano, MLB-48-89A, and Umgeni compared to the local checks, K132 and K20.

Fig. 5.3. Variation in levels of infection on different bean lines.

5.3.3 Severity of Fusarium root rot on selected common bean lines under field conditions

Thirty lines classified as moderately resistant under screenhouse conditions, plus sixteen lines that were classified as moderately susceptible or susceptible, including six landraces were screened under field conditions at KARI. The first season of the field trials was greatly affected by bean fly (*Ophiomya* spp.), resulting in very low plant stands at 28dap and hence the following discussion is based mainly on the second season data. Scoring for FRR symptoms proved difficult because it was compounded by other root rot pathogens, especially *Pythium* spp. and *Rhizoctonia solani,* and bean fly damage. However, FRR severity, plant stand at 28dap and 56dap, root weight and root:shoot ratio were significantly different at P= 0.01 among the 49 lines (Appendix 5.6).

Disease severity under field conditions ranged from 3.8 on Hoima-Kaki to 8.2 on RWR868 on the 1-9 scale, at 28dap. Fifteen bean lines had low disease severity under field conditions at 28dap, even with all constraints considered, having scores of ≤ 5 on the 1-9 scale. They included G4795, G3717, G5149, 1/MS/11-1, MLB-49-89A, Vuninkingi, 311/7, MLB-48-89A,

APN 154, 217/2, A211, HF-465-63-1, Hoima-Kaki, TeebusRR1, and Imbali (Table 5.7). However, some lines were very susceptible to FRR even when compared to the susceptible checks with 23 lines having disease severity greater than the local susceptible checks. Plant stand at 28dap ranged between 6.8% on Kiboga-Yellow and 63.8% on Hoima-Kaki.

At 56dap, plant stand ranged between 0.0% for Kiboga-Yellow, Timbavati and Elangeni and 75% on G3717. The lines Kiboga-Yellow, Timbavati and Elangeni had all died by 56dap probably due to the combination of root rot infection and bean fly damage. Fifteen lines recorded higher plant stand at 56dap compared to 28dap indicating that few plants died during the 28d interval; these were thus considered tolerant to the prevailing environmental factors. They included G3717, G5108, 1/MS/11-1, MLB-17-89A, Umubano, Vuninkingi, IPA-1, APN154, 217/2, RIZ-30, MLB-48-89A, PAN150, and K20. FRR severity scores ranged from 4.3 on Kabale-White and MLB-48-89A to 8.8 on RWR2075. Five lines had disease severity ranging between 4 and 5 on the 1-9 scale and included G4795, G3717, MLB-49-89A, MLB-48-89A, and Kabale-White, while three had severity in the range of 5-6, that is, G1459, Hoima-Kaki, and Mbarara Kanyebwa (Table 5.7). All these lines were considered resistant to the root rot pathogens that occurred, as well as being adaptable, especially the CIAT lines, G1459 and G4795 that had been exposed to different climate. Most of the lines that had low severities at 28dap, succumbed to the disease over time. However, the lines MLB-48-89A, Hoima-Kaki, G3717, and MLB-49-89A maintained their good performance (moderately resistant classification) both at 28dap and at 56dap, with MLB-48-89A having an even lower severity score at 56dap (4.3) compared to 28dap (4.9) on the 1-9 scale. Similarly, G1459, G4795, RIZ 30, PAN128, Mbarara kanyebwa, and Kabale-White had lower FRR severity at 56dap than at 28dap (Table 5.7).

As shown in Table 5.7, bean yield was very low, ranging between 168 and 1 312kg ha^{-1}. The bean line 1/MS/11-1 had the highest yield, followed by Kabale-White, GLP585, and then Umubano. All these lines had yields above 1 000kg ha^{-1}. Even though the local susceptible checks and the landraces are adapted to the environment in Uganda, they had very low yields with K20 having the lowest yield of 168kg ha^{-1}. However, the local landraces, Hoima-Kaki and Kabale-White, had relatively good yields compared to the other lines, showing their adaptability as well as tolerance to BRR under field conditions. Twenty one lines had yields lower than 500kg ha^{-1} while twenty lines had yields between 500-1 000kg ha^{-1}. Generally, FRR severity at 56dap affected yield (r=0.34) more than severity at 28dap (r=0.09). FRR

ratings of the field and screenhouse were highly correlated, that is, 97% to the 28dap data and 98% for the 56dap, implying that selection of resistant lines may be based on either trial or on both.

Table 5.7. Plant emergence, plant stand, Fusarium severity and yield of 49 bean entries under field conditions.

Entry	Nursery	(%) emergence	Plant stand (%)		Fusarium severity		Yield (kg ha^{-1})	*classification	
			28dap	56dap	28dap	56dap		28dap	56dap
1. G1459	CIAT	80.0	51.5	31.7	5.8	5.2	189.5	MS	MS
2. G4795	CIAT	94.0	56.0	56.7	4.7	5.0	566.7	MR	MS
3. G9384	CIAT	96.0	34.6	7.5	5.8	7.0	196.3	MS	S
4. G3717	CIAT	68.7	53.5	75.0	3.9	4.9	891.1	MR	MR
5. G5149	CIAT	88.7	48.2	36.7	4.8	5.8	374.1	MR	MS
6. G5108	CIAT	78.7	51.5	55.0	5.3	7.7	448.9	MS	S
7. 1/MS/11-1	Pythium	75.3	35.7	43.3	4.4	7.0	1312.0	MR	S
8. CIM 9313-1	Pythium	48.0	93.2	45.0	6.0	7.4	520.3	S	S
9. MLB-17-89A	Pythium	98.0	14.5	30.0	6.5	7.8	543.2	S	S
10. MLB-49-89A	Pythium	97.3	51.5	30.0	4.4	4.8	899.0	MR	MR
11. UMUBANO	Pythium	78.0	42.4	50.0	6.3	5.7	1074.0	S	MS
12. VUNINKINGI	Pythium	82.7	31.3	42.5	4.3	6.6	770.3	MR	S
13. SCAM 80-CM/15	Pythium	65.3	30.9	21.7	6.1	6.4	418.9	S	S
14. 311/7	Pythium	69.3	30.0	25.0	4.9	6.9	532.4	MR	S
15. MLB-48-89A	Pythium	100.0	46.6	46.7	4.9	4.3	356.2	MR	MR
16. RWR719	Pythium	92.0	45.2	30.0	5.4	6.3	795.1	MS	S
17. CIM 9314-1	Pythium	78.6	56.7	24.5	8.1	7.5	243.5	VS	S
18. GLP 24	Pythium	86.5	37.2	16.5	6.5	7.8	657.3	S	S
19. RWR 2075	Pythium	99.4	34.8	25.2	8.0	8.8	196.7	VS	VS
20. RWR 1058	Pythium	100.0	45.7	19.6	7.1	7.0	345.8	S	S
21. RWR 1059	Pythium	54.7	11.5	20.0	7.3	7.7	188.0	S	S
22. FEB 181	Pythium	93.5	47.7	33.5	6.3	7.3	705.4	S	S
23. RWR 868	Pythium	57.3	22.1	18.3	8.2	8.2	356.9	VS	VS
24. APN 154	Pythium	67.3	54.4	55.0	4.4	6.4	962.6	MR	S
25. GLP 585	Pythium	80.0	51.9	50.0	6.5	6.6	1080.4	S	S
26. 217/2	Pythium	98.7	27.7	35.0	4.1	7.2	732.0	MR	S
27. A211	F.O.P	88.7	55.1	51.7	4.1	8.2	283.7	MR	VS
28. HF-465-63-1	F.O.P	99.3	48.4	43.3	4.5	7.4	399.8	MR	S
29. RIZ 30	F.O.P	99.3	49.4	56.7	5.9	5.2	922.7	MS	MS
30. IPA 1	F.O.P	84.9	32.4	38.4	5.3	6.8	865.1	MS	S
31. Hoima-Kaki	Landrace	87.3	63.8	38.3	3.8	5.3	868.5	MR	MS
32. Lira-Cream	Landrace	80.7	36.8	21.7	7.0	7.6	420.0	S	S
33. Masaka-Manyigamulimi	Landrace	74.7	53.9	51.7	6.0	7.5	353.9	S	S
34. Mbarara-Kanyebwa	Landrace	95.3	41.3	16.7	5.2	5.0	541.3	MS	MS
35. Kabale-White	Landrace	78.7	63.6	36.7	5.8	4.3	1135.9	MS	MR
36. Kiboga-Yellow	Landrace	74.0	8.0	0	6.1	-	-	S	na
37. RS5	SA	82.0	51.8	31.7	5.2	6.6	856.1	MS	S
38. OPS-KW1	SA	87.3	51.8	25.0	6.0	6.5	717.1	MS	S
39. Teebus RR1	SA	81.3	52.6	23.3	3.7	5.3	561.3	MR	MS
40. Timbavati	SA	90.7	6.9	0.0	7.2	na	na	S	na
41. Elangeni	SA	73.3	24.1	0.0	7.1	na	na	S	na
42. Imbali	SA	75.3	24.4	22.5	4.7	6.3	424.9	MR	S
43. PAN 128	SA	67.5	60.7	6.0	6.8	5.6	444.8	S	MS
44. PAN 185	SA	76.4	41.4	22.5	5.8	7.9	197.8	MS	S
45. PAN 150	SA	57.3	64.0	66.7	5.2	6.7	738.4	MS	S

Table 5.7. Plant emergence, plant stand, Fusarium severity and yield of 49 bean entries under field conditions.

Entry	Nursery	(%) emergence	Plant stand (%)		Fusarium severity		Yield (kg ha⁻¹)	*classification	
			28dap	56dap	28dap	56dap		28dap	56dap
46. Quteniqwa	SA	90.6	36.2	16.8	5.2	7.7	346.8	MS	S
Kanyebwa(Susceptible check)	Controls	91.3	54.0	6.3	5.6	7.3	368.9	MS	S
K132 (Susceptible check)	Controls	68.7	6.8	10.0	6.8	7.2	472.0	S	S
K20 (Susceptible check)	Controls	94.0	28.1	50.0	5.7	8.0	168.0	MS	VS
S.E.D (P= 0.05)		15.55	9.412	6.952	0.986	0.887	233.4		
CV%		23.9	33.7	44.3	32.1	35.6	46.4		

*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.

MLB-17-89A, RWR868, and SCAM-80-CM/15 had the highest root masses, while Elangeni, Timbavati and RWR719, had the lowest root masses (Table 5.8). However, even though MLB-17-89A had the highest root mass at 28dap, its root to shoot mass ratio was relatively low compared to RWR719 which had a low root mass but high root:shoot mass ratio (Table 5.8). In this study, root mass, or root mass to root mass ratio, was not correlated to Fusarium severity, as lines which were relatively resistant had small root masses as well as low ratios as exemplified by MLB-49-89A, Umubano, and Vuninkingi. Root weight and root:shoot ratio was not significantly correlated (P= 0.05) to FRR severity for these lines.

Table 5.8. Root weight and root:shoot weight ratio of selected bean lines at 28dap.

Entry	Nursery	Root weight (g/10plants)	Root: Shoot weight ratio (g/10plants)
G1459	CIAT	0.63	0.19
G4795	CIAT	0.63	0.12
G9384	CIAT	0.60	0.15
G3717	CIAT	0.70	0.19
G5149	CIAT	0.47	0.12
G5108	CIAT	0.60	0.15
1/MS/11-1	Pythium	0.60	0.19
CIM 9313-1	Pythium	0.83	0.19
MLB-17-89A	Pythium	1.00	0.14
MLB49-89A	Pythium	0.60	0.13
Umubano	Pythium	0.70	0.15
Vuninkingi	Pythium	0.40	0.09
SCAM 80-CM/15	Pythium	0.97	0.20
311/7	Pythium	0.83	0.17
MLB-48-89A	Pythium	0.77	0.16
RWR719	Pythium	0.40	0.17
CIM 9314-1	Pythium	n/a	n/a
GLP 24	Pythium	n/a	n/a
RWR 2075	Pythium	n/a	n/a
RWR 1058	Pythium	n/a	n/a
RWR 1059	Pythium	0.67	0.13

Table 5.8. Root weight and root:shoot weight ratio of selected bean lines at 28dap.

Entry	Nursery	Root weight (g/10plants)	Root: Shoot weight ratio (g/10plants)
FEB 181	Pythium	n/a	n/a
RWR 868	Pythium	0.97	0.21
APN 154	Pythium	0.50	0.15
GLP 585	Pythium	0.63	0.18
217/2	Pythium	0.87	0.20
A211	F.O.P	n/a	n/a
HF-465-63-1	F.O.P	n/a	n/a
RIZ 30	F.O.P	n/a	n/a
IPA 1	F.O.P	n/a	n/a
Hoima-Kaki	Landrace	0.73	0.18
Lira-Cream	Landrace	0.80	0.19
Masaka-Manyigamulimi	Landrace	0.80	0.16
Mbarara-Kanyebwa	Landrace	0.83	0.20
Kabale-White	Landrace	0.93	0.17
Kiboga-Yellow	Landrace	n/a	0.16
RS5	SA	0.80	
OPS-KW1	SA	0.73	0.14
Teebus RR1	SA	0.50	0.17
Timbavati	SA	0.40	0.16
Elangeni	SA	0.30	0.07
Imbali	SA	0.50	0.18
PAN 128	SA	n/a	n/a
PAN 185	SA	n/a	n/a
PAN 150	SA	0.60	0.20
Outeniqua	SA	n/a	n/a
Kanyebwa	Control	0.73	0.16
K132	Control	0.77	0.14
K20	Control	0.65	0.14
S.E.D (P= 0.05)		0.16	0.03
CV%		29.0	25.2

n/a = data not recorded.

5.2.4 Relationship between seed size and resistance to Fusarium root rot

Correlation between seed size and FRR severity scores could not be calculated because the individual seed weights for the different varieties were not recorded. Since the varieties were already classified in the respective seed size categories (small, medium or large) from their source nurseries only proportions could be estimated. The proportion of varieties with disease severity scores of 3-3.9 was greatest for the small-seeded bean varieties, that is, 66.7% small-seeded, 16.7% were medium-seeded and 16.7% were large-seeded (Figure 5.4). Similarly, most of the varieties 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 5.4). These results were not conclusive on the relationship between seed size and resistance 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 (Figure 5.4).
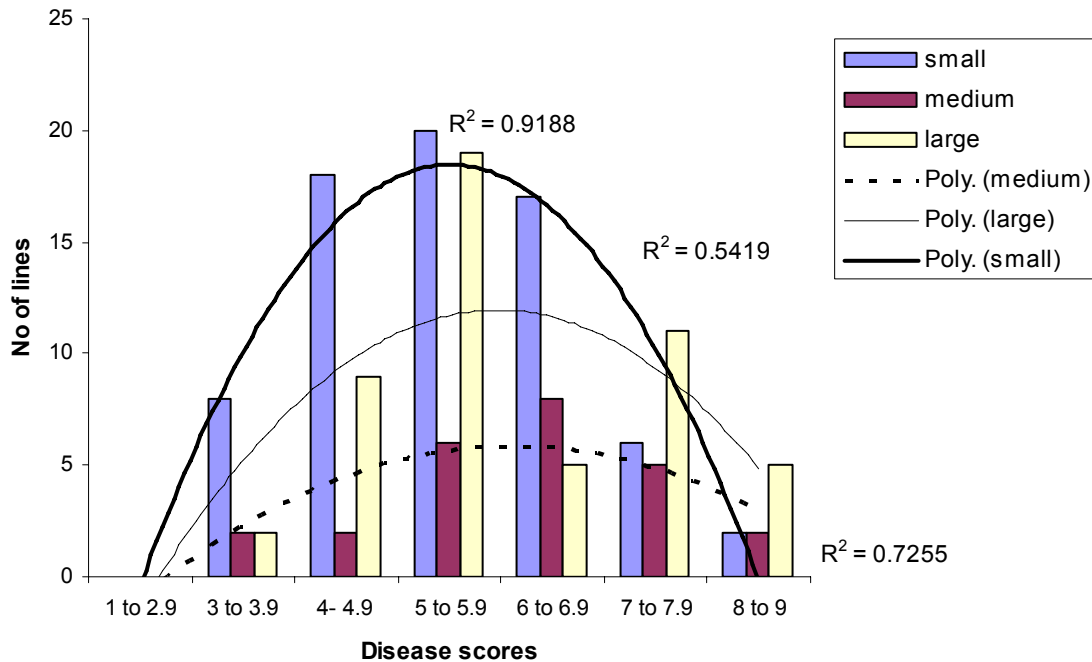
Fig. 5.4. Relationship between seed size and resistance to Fusarium root rot.

5.3.5 Relationship between hypocotyl and resistance to Fusarium root rot

As regards hypocotyl colour, more of the 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 on the 1-9 scale (Figure 5.5). The distribution of FRR severity of 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 5.5). Generally, both groups showed an almost normal distribution of disease scores (R^2= 81-87%).
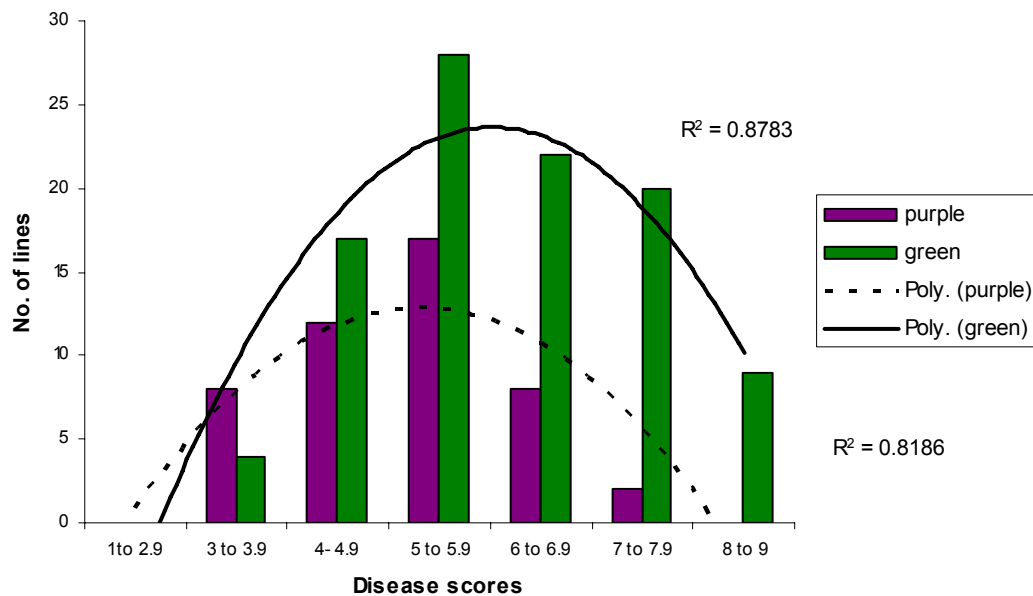
Fig. 5.5. Relationship between bean line hypocotyl colour and Fusarium root rot resistance.

5.4 Discussion

The objective of this study was to identify sources of resistance to FRR that may be used as parents in improving resistance in three large-seeded and popular bean varieties in Uganda. One hundred and forty seven common bean varieties were screened using one *F.* f. sp. *phaseoli* isolate, FSP-3, under controlled conditions in a screenhouse at KARI during 2005 and 2006. In order to confirm this resistance, selected varieties from the screenhouse trial were further evaluated under natural conditions, in a field known to be highly infected with root rot pathogens.

The soil-based screenhouse screening technique required little maintenance and hence was found to be inexpensive. It also permitted the evaluation of large populations at one time. Time and labour constraints were also minimized since the time from planting to evaluation took up to 28d and only daily watering and optional additional fertilization were required. Disease evaluations done in the screenhouse had the advantage over screening done in the field because disease inoculum levels were uniform, one specific isolate was used, the techniques used were simple, and evaluation was rapid. On the other hand, in the field, different root rot pathogens occurred (*Pythium* spp. and *Rhizoctonia solani*), as well as bean fly (*Ophiomya* spp.), other strains of FSP and many other soil inhabiting organisms that may

have influenced the performance of the varieties. Despite this, the field and screenhouse data were highly correlated, that is, 97% to the 28dap data and 98% for the 56dap. Several scientists have also reported high correlations between the field and screenhouse results (Schneider et al., 2001; Román-Avilès and Kelly, 2005). This implies that selection for resistance may be done under any of these conditions. However, replication, statistical procedures and good control of environmental factors are essential in identifying varieties resistant to FRR (Wallace and Wilkinson, 1965; Hassan et al., 1971; Boomstra and Bliss, 1977; Schneider et al., 2001; Román-Avilès and Kelly, 2005). For this reason, screenhouse evaluation would be recommended, because field resistance can be predicted from the screenhouse results. .

The 147 common bean genotypes differed in their degree of sensitivity to FRR under screenhouse conditions. Although none of the varieties was immune, some varieties showed good resistant reactions. Thirty six bean lines were moderately resistant to FSP, with MLB-49-89A being the most tolerant among them. Most of the good performing varieties, that is, resistant and moderately resistant varieties were from the nursery that had been selected for resistance to Fusarium wilt (*F. oxysporum* f. sp. *phaseoli*) and Pythium root rot (*Pythium* spp.). The higher levels of resistance in this nursery suggest that these varieties could also have been selected indirectly for FSP resistance because soil-borne pathogens are known to occur concurrently (Sippel and Hall, 1982). The documented sources of resistance to FRR from CIAT, Colombia did not show high levels of resistance to the FSP-3 isolate, probably due to differences in the screening environment or the pathogenic isolate used. This highlights the need for new sources of resistance adaptable to the region of interest. As expected the majority of the local landraces were very susceptible to the FSP-3 isolate, indicating the low levels of resistance in bean varieties currently being grown by rural farmers in Uganda. It also highlights the need to breed new and resistant varieties. However, the landraces, Hoima-Kaki and Kabale-White were moderately resistant and moderately susceptible to Fusarium resistance, respectively, both in the field and in the screenhouse at 28dap, indicating the availability of some sources of resistance even among the local bean varieties grown by farmers. Unfortunately, Hoima-Kaki is small-seeded and brown in colour, while Kabale-White is small-seeded and white in colour, and are only grown for home consumption because these attributes render them unmarketable (see Chapter 2). However, these varieties could be used as sources of resistance in breeding for resistance to FRR.

In the field, 15 varieties were classified as moderately resistant at 28dap under field conditions and five, that is, G4795, G3717, MLB-49-89A, MLB-48-89A, and Kabale-White at 56dap. These showed good adaptability as well as tolerance to the constraints that occurred, including FRR. However, several varieties showed more susceptible reactions under field conditions than under screenhouse conditions, probably because they were exposed to harsher conditions and because most of them, compared to the local varieties, were not as well adapted to the tropical climate to which they were subjected. In addition, they were also challenged by other pathogens.

Time-course changes in plant performance have been shown to affect the level of resistance to FRR as cultivars that appeared to have similar levels of resistance at 28d differed dramatically at 56dap. Thus, the resistance of seedlings may not reflect the resistance of older plants (Hall and Phillips, 2004). In this study, field rating for FRR was done at 28dap, and at 56dap, while screenhouse rating was done at 28dap only. FRR ratings done at 28dap and those done at 56dap were highly correlated. Even though most of the varieties succumbed to FRR over time, for some varieties the increment was not high. Superior varieties maintained their superiority, thus screenhouse results may still be used for the selection of superior varieties. The varieties MLB-48-89A, Hoima-Kaki, G3717 and MLB-49-89A maintained their low FRR severity at 28dap and at 56dap, with MLB-48-89A having an even a lower severity score at 56dap. Similarly, G1459, G4795, RIZ 30, PAN128, Mbarara Kanyebwa and Kabale-White had lower Fusarium root severity at 56dap compared to the disease scores at 28dap. Hassan et al. (1971) reported a shift from additive gene action for younger plants to partial resistance for older plants, and suggested that, in the absence of any other confounding pathogens, varieties showing resistant reactions at a young age should be left in the field for as long as possible to allow the full expression of resistance. Resistance in MLB-48-89A could probably be due to partial dominance. However, the ratings in the field were in some cases overestimated due to the occurrence of other root rot pathogens, especially *Pythium* spp. as well as the effect of bean fly; these make field screening difficult, especially if the target is a single isolate of a particular pathogen. It is for this reason that the early breeding programme for FRR was carried out exclusively in the screenhouse because the resistance being studied was against a single isolate.

Fusarium root rot resistance has been associated with small seed size, with the large-seeded bean varieties being more susceptible (Schneider et al., 2001; Román-Avilès and Kelly, 2005). In this study, the relationship between seed size and FRR was not statistically tested. However, there was a trend that indicated that more of the small-seeded varieties tended to be more resistant to the root rot pathogen than their larger seeded counterparts. Similarly, Beebe et al. (1981) reported higher resistance to FRR in the small and black seeded varieties compared to large red mottled ones. In previous studies, the colour of seed and hypocotyls was related to the level of resistance to FRR. Statler (1970) observed higher resistance to FRR in black seeded varieties and varieties with purple coloured hypocotyls and related it to the greater production of phenolic compounds inhibitory to fungal growth in the early stages of seedling growth. Phytoallexins such as phaseollin have been identified and reported as being produced in response to infection by *R. solani* (Pierre and Bateman, 1967) and FSP (Kendra and Hadwiger, 1989). Production of these phytoallexins has been shown to be greater and more rapid in resistant varieties. Purple-coloured hypocotyls could possibly have higher levels of pytoallexins and hence may indicate some maternal effects of resistance to FRR.

Root: shoot weight ratio was not statistically correlated with FRR severity in this study. It has often been suggested that a vigorous root system increases tolerance to root rot (Burke and Barker, 1966; Snapp et al., 2003; Román-Avilès et al., 2004 a and b). The division of carbohydrates between shoots and roots is influenced by both genetic and environmental factors and it was thought that the genes governing root system vigour also influence resistance to root rot, with the result that, varieties with genetically vigorous root systems are more resistant to BRR compared to those with weak root systems. However, this was not found to be the case in this study. No conclusions have been drawn from this study, but with a greater number of screened varieties this assumption could be confirmed or disapproved.

Yield varied from 168kg ha^{-1} for the local susceptible bean line K20, to 1 312kg ha^{-1} for 1/MS/11-1, with a mean of 574.8kg ha^{-1}. Disease severity at 56dap was shown to affect yield more than the ratings done at 28dap. However, it should be noted that ratings done later in the life of the crop were highly confounded by many other soil inhabiting pathogens, as well as bean fly. Due to this, the correlation between Fusarum root rot and yield could not be ascertained; however, several well adapted varieties were identified. In addition, the yield

data were difficult to interpret because many genotypes were probably not adapted to the tropical climate, and hence, could not express this trait well.

In conclusion, 44 common varieties were identified as potential sources of resistance to FRR due to their performance under both screenhouse and field conditions. Of these ten were large-seeded and may be recommended for use by the Uganda National Bean Programme (UNBP). However, even though none of the varieties exhibited very high resistance levels, eight varieties were selected as parents in a study of inheritance of resistance to *FSP* described in the next chapter.

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Chapter Six: Genetic analysis of resistance to Fusarium root rot (*Fusarium solani* f. sp. *phaseoli*) in common bean (*Phaseolus vulgaris* L.)

Abstract

The deployment of resistant varieties is probably the best management option for Fusarium root rot (FRR) caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans (FSP)*,* one of the major diseases affecting common bean production in Uganda. The objective of this study was to determine the mode of inheritance of resistance to FRR. A 12 x 12 diallel mating design was used to develop 132 F_1 and F_2 populations, including reciprocal crosses. Resistance to FRR was found to be additive in nature because the GCA[5] effects were highly significant (P≤0.01) in both F_1 and F_2 generations. The lines, RWR719, Vuninkingi, MLB-49-89A, Umubano and MLB-48-89 having negative GCA in all generations, and would be recommended for use as sources of BRR resistance in the bean improvement program in Uganda. In addition, the F_2 populations did not show any distinct segregation patterns, but had continuous distributions, indicating the quantitative nature of resistance to FRR. Even though overall SCA effects were not significant (P≤0.05), two crosses had high, negative, and significant SCA effects (K20 x MLB-49-89A and Umubano x Vuninkingi). In addition, negative heterosis was observed for most of the R x R and R x S crosses in this study. Maternal effects were highly significant in both the F_1 and F_2 generations, suggesting the importance of cytoplasmic genes on resistance to FRR. Non-maternal effects were also significant in some populations, suggesting that the cytoplasmic genes were interacting with nuclear genes. Evaluation of F_1 and F_2 generations showed that FRR resistance was governed by recessive genes for most of the resistant parents. However, there was evidence of more resistance genes in the bean line MLB-49-89A than in the other resistant parents. Broad sense heritability (H) varied from 0.22-0.69 among the crosses, while heritability in the narrow sense (h^2) among the crosses was estimated as was 0.348-0.49. The number of genes governing resistance to FRR varied from two to nine among the eight sources of resistance. The allelism test of resistant x resistant populations and the observation of continuous distributions of severity scores, suggested the presence of many loci governing FRR resistance in beans. Therefore, selection should develop improved population for resistance to FRR. Selection with multiple backcrosses alternating between the recurrent parent and donor parent would be the best breeding procedure for

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[5] Abreviations: GCA = General combining ability, SCA = Specific combining ability

improving resistance to FRR. However, there could be complications because the resistance is modified by cytoplasmic gene effects and their interaction with nuclear genes in some of the populations.

6.1 Introduction

Fusarium root rot (*Fusarium solani* f. sp. *phaseoli)* (FRR) is one of the major diseases affecting common bean production in Uganda, especially in the highland regions in the south-western and eastern parts of the country. These regions are the major bean producing regions and are characterised by high bean cropping intensity leading to poor soils and high pathogen inoculum levels and hence frequent bean root rot (BRR) epidemics.

Planting resistant varieties is probably the best management option for the disease, particularly for small scale farmers who make up the greatest proportion of bean growers in Uganda. Large-seeded bean varieties are the most popular and preferred bean types in the greater part of the country. These are usually red or red-mottled in colour but yellow, white and patterned types are also common. FRR has been found to be very severe in the large-seeded bean varieties probably (Burke and Miller, 1983; Otsyula et al., 1998; Schneider et al., 2001).

Several researchers have suggested that resistance to *F. solani* f. sp. *phaseoli* (FSP) in the common bean is quantitatively inherited and greatly influenced by the environment (Baggett et al., 1965; Dickson and Boettger, 1977; Miller and Burke, 1985; Schneider and Kelly, 2000), hence the slow progress in the improvement of the resistance. Some researchers have suggested that the inheritance of FRR disease resistance is complex, with susceptibility being dominant to resistance (Boomstra, 1975). McRostie (1921) concluded that two duplicate recessive FRR resistance genes were involved in a cross of flat marrow and robust pea bean in field and greenhouse tests. Similarly, a cross between scarlet runner bean (*Phaseolus coccineus)* line no. 2014 and PI 165435 as resistant parents, and a susceptible common bean (*Phaseolus vulgaris*) parent, OSC22, indicated that there were 2-3 major recessive genes governing resistance in these varieties (Azzam, 1958). In 1960, Smith and Houston reported that resistance was governed by one recessive and one dominant gene from crosses involving 10 susceptible and seven resistant common bean varieties, including N203 (PI 203958), the first recognised source of resistance to FRR.

However, FRR resistance from *P. coccineus* was reported to be dominant over susceptibility in a cross of *P. vulgaris* x *P. coccineus* (Yerkes and Freytag, 1965). Bravo et al. (1969) also suggested three or more dominant genes in the sources of resistance, N203 and *P. coccineus*, while Hassan et al. (1971), reported four dominant genes in N203. However, no true breeding line was obtained from these crosses, which would indicate that the inheritance of resistance to FRR was more complex than previously reported. Plant age and testing procedures, including inoculum levels, were shown to influence the results of these inheritance studies. Most of the conventional breeding studies of the inheritance of resistance to FSP involved experimental designs that were more appropriate for the analysis of qualitative rather than for quantitative traits (Smith and Houston, 1960; Wallace and Wilkinson, 1965; Hassan et al., 1971) hence the results may not have been conclusive. However, gene action governing resistance to FRR in common bean was found to be additive in nature, especially in the screenhouse trials. In cases where older plants were scored (field trials), gene action shifted to partial dominance (Hassan et al., 1971). Recent studies that used quantitative trait analysis have indicated nine QTLs to be significantly associated with FRR resistance and explaining 5-53% of the total phenotypic variation (Román-Avilès and Kelly, 2005).

Estimates of additive genetic variation in a population are important for accurate selection and prediction of genetic gain. However, these estimates may be confounded with other sources of environmental or genetic variation. Maternal effects are one of the factors that may lead to over-estimation or under-estimation of the additive genetic variance (Roach and Wulf, 1987; Shaw and Byers, 1998; Gustavo et al., 2003). Past studies on the inheritance of resistance to FRR did not consider maternal effects as a factor that may inflate or reduce the resistance levels in the F_1 generation. Variation in seed, seedling, and adult traits caused by maternal effects can have important consequences for the biological behaviour of an individual (Roach and Wulff, 1987). Maternal effects refer to the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987). Maternal effects are most common in the early stages of the life cycle of a plant and may influence the selection for resistance done at an early stage, such as in this study. It is therefore important to estimate the maternal effects in the parents that were used in this study by estimating reciprocal cross effects of populations developed.

An understanding of allelic relationships between the resistance genes in different sources of resistance may help to refine the selection of resistance genes for use in the breeding programme and avoid the over-deployment of a single locus. Therefore, allelism tests are crucial to the identification of the resistance genes to be used in the improvement of resistance to FRR in common bean. The joint action of favourable combinations of genes at different loci could result in heterosis (Jinks, 1954; 1956). Heterosis is the phenomenon that occurs when the mean performance of the F_1 generation, obtained by crossing two genotypes, is superior to the mean performance of the better or worse parent (heterobeltiosis), or to the mid-parent (relative heterosis) (Dabholkar, 1992). Heterosis may be measured by the amount by which the mean performance of F_1 exceeds the better parent or mid-parent. The amount of heterosis following a cross between two particular varieties or populations depends on the square of the difference of gene frequency between the populations. If the parents crossed do not differ in gene frequency there will be no heterosis (Coors, 1999).

Since knowledge of inheritance is critical in designing appropriate breeding strategies for incorporating a particular trait into economically useful populations, studies of crosses involving twelve parents with varying levels of resistance to FRR were conducted. Populations were developed and their performance was analysed using Griffing's (1956) analysis of diallel mating designs appropriate for quantitative traits to obtain additional information about the inheritance of resistance to FRR. The results were used to select promising crosses that would yield improved populations for resistance to FRR. The major objective of the study was to determine the mode of inheritance of resistance to FRR, while the specific objectives were as follows:

1. To study the gene action governing resistance to FRR in beans;
2. To estimate the number of genes governing resistance to FRR in common bean crosses;
3. To determine the combining ability among 12 common bean varieties for FRR resistance;
4. To estimate the role of maternal effects controlling resistance to FRR in beans;
5. To estimate narrow sense heritability (h^2) for resistance to FRR in common bean populations;
6. To estimate gene dosage and heterosis for resistance to FRR in the F_1 generation of common bean crosses;

7. To determine the allelic relationship between the resistance genes in common bean.

6.2 Materials and methods

6.2.1 Germplasm

Nine inbred varieties were selected as sources of resistance to FRR after having been screened for resistance to the FSP-3 *F. solani* f. sp. *phaseoli* (FSP) isolate at Kawanda Agricultural Research Institute (KARI) in Uganda (see Chapter 5). They included, RWR719, Vuninkingi, Umgeni, MLB-49A-89A, MLB-48A-89A, Umubano, G4795, G1459 and Hoima-Kaki (Table 6.1). The screening trials showed that these varieties had varying levels of resistance to FRR, with MLB-49A-89A being the most resistant, followed by RWR719, Vuninkingi, Umubano, Hoima-Kaki, G4795, G1459 and Umgeni. The number of resistance genes to FRR in these varieties was not known because it has not been studied before. However, Vuninkingi, RWR719 and MLB-49-89A have been documented to have genes for resistance to Pythium root rot (*Pythium* spp.) and Fusarium wilt (*Fusarium oxysporum* (Schlecht.) f. sp. *phaseoli* Kendrick & Snyder) (Otsyula et al., 1998; Buruchara and Camacho, 2000; Otsyula et al., 2005). Umubano (G2333) has been documented to be resistant to FSP and anthracnose (*Collectotrichum lindemuthianum* Sacc & Magn), but is very susceptible to Fusarium wilt (Buruchara and Camacho, 2000). G4795/Porrillo Sintetico and G1459/Jampa are documented sources of resistance obtained from CIAT-Colombia (Abawi and Pastor Corrales, 1990). The varieties MLB-48-89A and Umubano are very susceptible to Fusarium wilt (Buruchara and Camacho, 2000). The bean line Umgeni, was susceptible to FRR in Uganda (see Chapter 5) but had been reported to be tolerant to Fusarium wilt (R. Melis, South Africa, personal communication). Three large-seeded, popular, commercial, but susceptible Ugandan bean varieties, K20, K132 and Kanyebwa (Table 6.1), were also included. These varieties also had varying levels of susceptibility to FRR, with K132 being the most susceptible, followed by Kanyebwa and lastly K20. The detailed descriptions of this germplasm are presented in Table 6.1.

Table 6.1. Characteristics of bean parents used in the inheritance study.

Varieties	Source population	*FSP resistance reaction	Agronomic characteristics	Origin
1. K20/GLP2	Rosecoco	Very susceptible	Large and red-mottled seed with bush growth habit Yield potential: 1 500-2 500kg ha^{-1} Marketable Tolerant to most diseases but susceptible to bean root rot, bean fly and drought	CIAT
2. K132/CAL96	Calima-2 X Argentino	Very susceptible	Large and red-mottled seed with bush growth habit Yield potential: 1 500-2 000kg ha^{-1} Marketable Susceptible to bean root rot, bean fly and drought	CIAT
3. Kanyebwa	Landrace	Very susceptible	Large and red-speckled sugar bean with bush growth habit Susceptible to bean root rot, bean fly and drought Tasty and marketable	Uganda
4. Umubano (G2333)	Gentry 21835 Colorado Teopisca/PI311998	Moderately tolerant	Small and red-seeded with climbing growth habit Yield potential: 2 500-4 000kg ha^{-1} Low marketability	Mexico
5. Vuninkingi/ G685	Moncure no.12 (PI182007)	Moderately tolerant	Small and red to maroon seeded with climbing growth habit Drought tolerant Yield potential: 2 500-4 000kg ha^{-1} Low marketability	Mexico
6. RWR719	Cyunyu x Kermes	Moderately Tolerant	Small and red seed with bush growth habit Resistant to bean root rot Low marketability due to small seed size	Rwanda
7. Umgeni	*n/a	Susceptible	Medium and red-speckled sugar bean with bush growth habit	South Africa
8. G1459	Jamapa (PI268110)	Moderately tolerant	Black and small seed with climbing growth habit	CIAT
9. G4795	Porrillo sintetico No.1	Moderately tolerant	Black and small seed with climbing growth habit	CIAT
10. Hoima-Kaki	Local landrace	Moderately susceptible	Brown and small seed with bush growth habit	Uganda
11. MLB-49-89A	A 240 X Inyumba	Moderately tolerant	Black and medium seed with semi-climbing growth habit Very low marketability	DRC
12. MLB-48-89A	A 240 X Inyumba	Moderately tolerant	Black and small seed with semi-climbing growth habit Low marketability	DRC

*FSP: Fusarium solani f. sp. phaseoli.
*n/a-pedigree not known (proprietary inbred).

6.2.2 Population development

A 12 parent diallel mating design with reciprocals was conducted in a screenhouse at KARI in Uganda. There was no crossing between similar parents, resulting in 66 F_1 and 66 reciprocal progeny families (Table 6.2). These 132 full sib populations were advanced to F_2 population by selfing.

Table 6.2. A 12 x 12 diallel mating scheme of common bean varieties used for an inheritance study of Fusarium root rot resistance at KARI in Uganda.

Parents	K2	K3	KN	UB	M49	RW	M48	G1	G4	VN	UM	HK
K2		X	x	x	X	x	x	x	x	x	x	x
K3	X		x	x	X	x	x	x	x	x	x	x
KN	X	X		x	X	x	x	x	x	x	x	x
UB	X	X	x		X	x	x	x	x	x	x	x
M49	X	X	x	x		x	x	x	x	x	x	x
RW	X	X	x	x	X		x	x	x	x	x	x
M48	X	X	x	x	X	x		x	x	x	x	x
G1	X	X	x	x	X	x	x		x	x	x	x
G4	X	X	x	x	X	x	x	x		x	x	x
VN	X	X	x	x	X	x	x	x	x		x	x
UM	X	X	x	x	X	x	x	x	x	x		x
HK	X	X	x	x	X	x	x	x	x	x	x	

K2 = K20, K3 = K132, KN = Kanyebwa, M49 = MLB-49-89A, M48 = MLB-48-89A, RW = RWR719, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki, UB = Umubano.

Planting of the crossing block was done in previously sterilized sandy loam soil that was collected from a nearby forest in 8L buckets. Each parent per cross combination was planted in 10 buckets. NPK (1:1:1) fertilizer in liquid form was added to the soil at rate of $3x10^{-3}kgml^{-1}$ a few days before planting and thereafter every after 7d. The plants were watered three times a day at 06h00, 11hr00 and 17hr00. Due to the different flowering dates of the parents, planting was staggered so as to synchronise flowering. To ensure adequate seed for advancement and evaluation, seven crossing blocks were planted. Crossing was done by hand pollination uing the emasculation and hooking method (Buishand, 1956), using all the available flowers in order to produce adequate seed for the screening trials. Care was taken to avoid contamination of the new crosses with pollen from the previous parental bean line by sterilising the forceps used to tease open the flowers in 70% alcohol.

The crossing exercise was carried out between 07.00hrs and 10.00hrs, and after 17.00hrs to use the cool weather conditions at those times.

6.2.3 Evaluation of developed populations to FSP-3 isolate

The F_1 and reciprocal diallel populations were planted in a screenhouse in $0.74\times0.42\times0.115m^3$ wooden trays containing pre-sterilized soil that was inoculated with the Fusarium isolate, FSP-3. The soil was fertilised with 1:1:1 NPK fertilizer at rate the of 3×10^{-3} $kgml^{-1}$. Fifty to sixty plants per cross, with reciprocal seed being considered as a separate cross from the respective F_1 seed, were evaluated. Each tray was planted with two rows of five crosses plus a row of a susceptible and resistant checks, K20 and MLB-49-89A, respectively. The trial was laid out as a randomised complete block design (RCBD) with three replications, each having 20 plants per cross. FRR severity was assessed by making observations of the root and hypocotyl tissue using two disease severity rating scales, that is, one based on percentage of hypocotyls and root tissue affected/ extent of infection, where:

- 0% = no visible symptoms;
- 25% = approximately a quarter of the hypocotyls and root tissue have lesions but tissue is still firm;
- 50% = approximately half of the hypocotyl and root tissues have lesions with considerable softening/ rotting;
- 75%-100%= the whole of the hypocotyl and root tissues have lesions of FRR and the root system is in an advanced stages of rotting, to complete root destruction.

The second severity rating scale was based on the 1-9 scale developed at CIAT (Abawi and Pastor Corrales, 1990), where:

- 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.

A total of 200-300 F_2 seed per cross (including reciprocals) was planted in inoculated soil in wooden trays to assess their reaction to FRR, as described for the F_1 populations. F_2 and reciprocal seed were planted in separate trays (100-150 seed per tray) and considered as separate crosses, as for the F_1 trial. This trial was not replicated, with each cross being planted in a tray together with a susceptible (K20) and resistant (MLB-49-89A) check. FRR severity was assessed by carefully uprooting each plant at 28d after planting (dap) and taking disease severity scores as described for the F_1 population above. For ease of interpretation of the segregation of resistant (R) x susceptible (S) populations at F_2, resistance was classified into three main divisions as follows:

1. Resistant = score of 1-4 on the 1-9 scale;
2. Moderately resistant = 5-6 on the 1-9 scale;
3. Susceptible = 7-9 on the 1-9 scale.

6.2.4 Data analysis

Several analyses were done to estimate the combining abilities of the parents, heritability, gene action, number of genes and loci governing resistance to FRR as well as to estimate heterosis in the crosses as discussed below.

6.2.4.1 Diallel analysis (combining ability analysis)

The data were analyzed using the Diallel SAS05 computer programme developed by Zhang et al. (2005) using Model I and Method Three of Griffing (1956) to determine the value of the general combining ability (GCA) and specific combining ability (SCA) effects of the different varieties and crosses. This method is expected to provide unbiased estimates of population parameters (Griffing, 1956; Dabholkar, 1992; Singh and Chaudhary, 2004). A fixed model was used because there were few bean parents (12). The statistical model for this analysis was as follows:

$$Yijk= \mu + g_i + g_j + s_{ij} + r_{ij} + b_k + (bv)_{ijk} + e_{ijkl}; \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(1)$$
$$V= g_i + g_j + s_{ij} + r_{ij}\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(2)$$

where μ is the population mean effect, g_i is the GCA effect of the ith parent, g_j is the GCA effect of the jth parent, s_{ij} is the SCA effect of the ij^{th} genotype, r_{ij} is the reciprocal effect of the

ij^{th} genotype, b_k is the effect of kth block, $(bv)_{ijk}$ is the interaction of ij^{th} genotype with the kth block and e_{ijkl} is the environmental effect of the $ijkl^{th}$ observation. Components of the reciprocal effects were also estimated, that is, maternal and non-maternal effects.

Six populations in the F_1 and F_2 generation were missing and hence the data for these crosses were estimated using Eckhardt's method of prediction of missing values of single crosses (Eckhardt, 1942).

6.2.4.2 Estimation of narrow sense heritability (h^2) for resistance to Fusarium root rot

A parent-offspring regression model (Vogel, et al., 1980) was used to estimate h^2 as follows:

$$Y_i = a + b_*X_i + E_i \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(3)$$

Where:

Y_i = Performance of offsprings of ith parent;

a = Mean performance of all parents evaluated;

b = Linear regression coefficient;

X_i = Performance of the ith parent;

E_i = Experimental error associated with the measurement of X_j.

The regression coefficient as a means of estimating the heritability of a character was based on the following assumptions:

1. The organism is diploid with solely Mendelian inheritance,
2. The genetic population is mating at random. Random mating was ensured by hand pollination between all parents used in the diallel set,
3. There is no linkage among the genes controlling the trait,
4. The offspring are non-inbred and
5. There is no environmental correlation among the offspring

The means for the parents and offspring were plotted against each other and the regression coefficient "*b*" calculated, i.e.,

$$h^2 = 4VA/VP \text{ and } "b" = h^2. \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(4)$$

h^2 = Narrow sense heritability

VA = Variance due to additive gene effects

VP = Total phenotypic variance

"*b*" = Regression coefficient

In addition, heritability was also estimated from the ratio of the variance components of analysis of variance as follows:

$h^2 = \sigma^2A/ \sigma^2A + \sigma^2D + \sigma^2$, which is equivalent to σ^2A/ σ^2P .... (5)

Where:

σ^2A = Variance due to additive gene effects

σ^2D = Variance due to dominance gene effects

σ^2 = Environmental error variance

σ^2P = Total phenotypic variance

Since the bean parents used in this study were fixed varieties, the inbreeding coefficient (F) was equal to one, hence the variance components σ^2g and σ^2s were generated by diallel SAS (Zhang et al., 2005), and used to estimate σ^2A and σ^2D follows:

$\sigma^2A = 2 \times \sigma^2g$ ....(6)

$\sigma^2D = 2 \times \sigma^2s$ ....(7)

6.2.3.3 Estimation of number of loci and genes governing Fusarium root rot resistance

The number of loci and number of genes governing FRR resistance were determined using the original Castle Wright method (K_{cw}); Equation 8, and modifications by Bjarco and Line; Equation 9 (Bjarco and Line, 1987; Zeng et al., 1990; Das and Griffey, 1994).

At F_2 generation:

$n = (GR)^2 [1.5-2 h(1 - h)]/ 8 [VF_2 - (V_{PS} + V_{PR} + 2V_{F1})4]$ ....(8)

$K_{cw} = D^2/8VG = D^2/8[VF_2 - (V_{PS} + V_{PR} + V_{F1})4]$ ....(9)

Where:

n = estimated number of segregating genes estimated by Bjarco and Line Formula;

K_{cw} = Number of loci estimated by the original Castle – Wright formula;

GR = Genotype range;

P_R = Mean of resistant parent;

P_S = Mean of susceptible parent;

F1$_M$ = Mean of F$_1$ progenies;

V$_{PR}$, V$_{PS}$ = Variance of resistant and susceptible parents, respectively;

VF$_1$, VF$_2$ = Variance of F$_1$ and F$_2$ generations, respectively;

h = (F1$_M$-P$_R$)/(P$_s$-P$_R$);

D   = Difference in parental mean (P2 - P1);

VG = Genotypic variance;

The above formulae are based on the assumptions as per Lande (1981) and Zeng et al. (1990):

1. One parent contains all the trait increasing alleles and the other contains all the trait decreasing alleles

2. All crosses are obtained by mating individuals chosen at random in appropriate populations, and

3. The segregating genes are not linked and are in random combinations.

The presence of linkage, dominance, or unequal effects at different loci will result in an underestimation of the actual number of segregating genes present, while the presence of epistasis may cause either an overestimation or an underestimation of the actual number of segregating genes (Lande, 1981; Zeng et al., 1990).

In this study, the genotypic range (GR) was estimated using the phenotypic range of the segregating population which does not assume that segregating genes come from a single parent and can hence be applied to resistant x resistant crosses as well as resistant x susceptible crosses (Zhang et al., 2001); while the D is the difference between the parents. Genotypic variance was estimated by subtracting environmental variance from the phenotypic variance of segregating populations. Standard errors for the estimated number of genes by these methods (genotypic range based on progeny segregation) were not estimated because there is no suitable method available in the literature to do this.

6.2.3.4 Heterosis and heterobeltiosis of resistance in F_1 generation to Fusarium root rot

In this study heterosis was determined for the F_1 populations that involved the three local susceptible varieties namely, K20, K132 and Kanyebwa; and the nine sources of resistance, namely, MLB-49-89, RWR719, Umubano, MLB-48-89A, Vuninkingi, G1459, G4795, Umgeni, and Hoima-Kaki. Mid-parent heterosis was estimated as:

H= [h] - [d] ....(10)

Where:

    h = Departure of the heterozygote from the mid point and reflects the dominance properties of genes;

    D = Departure of homozygote phenotype from the mid point.

Mid-parent heterosis was calculated as: MPH = (F1-MP)/MP x 100; = where F1 is the mean performance of the F1 hybrid and MP is the mean of the two inbred parents.

Similarly, heterobeltiosis was obtained as the differences in the mean performance of the mean of the F_1 to either the resistant or the susceptible parent, that is;

BPH (Better parent heterosis) = (F1-BP)/BP x 100

WPH (Worst perent heterosis) = (F1-WP)/WP x 100

where BP is the mean of the better/resistant parent and WP is mean of worse/susceptible parent

6.2.3.5 Allelism test for Fusarium root rot resistance genes from several potential sources of resistance

Segregation ratios for each of the 16 R x R crosses shown in Table 6.3 were computed. Using the 1-9 scale data, disease score ratings of 1-2.9 were considered resistant, 3.0-4.9 as moderately resistant, 5.0-5.9 as moderately susceptible, 6.0-7.9 as susceptible, and 8.0-9.0 as highly susceptible.

Table 6.3. Sixteen crosses developed for testing the allelic interaction of resistance genes to Fusarium root rot.

Crosses	
1. RWR719 x MLB-49-89A	2. MLB-48-89A x Vuninkingi
3. RWR719 x MLB-48-89A	4. MLB-48-89A x Umubano
5. RWR719 x Vuninkingi	6. MLB-48-89A x G4759
7. RWR719 x Umubano	8. MLB-48-89A x Hoima-Kaki
9. RWR719 x G4759	10. Vuninkingi x Umubano
11. MLB-49-89A x MLB-48-89A	12. Vuninkingi x G4759
13. MLB-49-89A x Vuninkingi	14. Umubano x G4759
15. MLB-49-89A x Umubano	
16. MLB-49-89A x G4759	

Several different genetic hypotheses were tested for significance for each population using the chi-square goodness of fit test in the Genstat computer programme (Genstat 9.1 Release). The chi-square goodness of fit test was used to determine the departure of the observed frequencies from the hypothesized frequencies, based on a critical value of 5.991 for two degrees of freedom at the 0.05 probability level. Eleven phenotypic classes were tested (Strickberger, 1976; Singh and Chaudhary, 2004; Caixeta et al., 2005): 1:0 (alleles on same locus); 15:1 (two independent dominant genes); 9:7 (two complementary dominant genes); 13:3 (two epistatic genes, one dominant and one recessive); 63:1 (three independent dominant genes); 57:7 (one dominant and two complementary genes); 27:37 (three complementary dominant genes); 61:3 (two dominant and one recessive gene), 49:15 (one dominant and two recessive genes); and 249:7 (two dominant and two complementary genes).

6.3 Results

The model (R^2) accounted for 60.9% with the 1-9 scale and 60.6% when the percentage scale was used. This implies that either one of the two scales could be used to rank and differentiate the genotypes. Discussion of results of this study was based more on the 1-9 scale data because it had a smaller coefficient of variation (CV %) than the percentage data, that is, 24% vs 35% in F_1 (Table 6.4).

6.3.1 Gene action determining Fusarium root rot resistance

The analysis of variance for the 132 populations showed that the crosses were highly significantly different from each other at $P \leq 0.01$ at F_1 and F_2 generations (Table 6.4). The

GCA effects were highly significant at P ≤ 0.01 significance level, while the SCA effects were not significant at P ≤ 0.05. GCA effects accounted for 68% of the phenotypic variance observed at F_1 and 76% at F_2 generations, while SCA effects accounted for only 5% of the total variance at both generations. This indicated that additive gene action was far more important in determining resistance to FRR than dominant gene action.

Table 6.4. Mean squares for the ANOVA for Fusarium root rot severity.

Source	DF	Mean square			
		F_1		F_2	
		†scale	percentage	†scale	percentage
GCA	11	35.16****	3772.07****	7.1934****	1090.24*****
SCA	54	ns	ns	ns	ns
Reciprocals	66	2.59*	294.03**	0.861**	100.60
Maternal effects	11	6.37****	661.07***	2.324***	257.14***
Non-maternal effects	55	ns	ns	0.568	69.29
R^2		60.91	60.57		
CV(%)		24.42	35.09		

† 1-9 scale, ns=not significant, *, **, ***, **** = significant at P= 0.05, P= 0.01, P= 0.001, and P=0.00001.

Generally, the GCA effects were very high relative to SCA effects, in both generations; hence, predictability based on GCA was high. That is, 2GCA/(2GCA+SCA) = 0.968, implying that the performance of a single cross progeny could be predicted based on the GCA of its parents (Falconer and Mackay, 1996). The insignificant SCA effects in the analysis of variance also indicated that there were few specific cross combinations which had a resistance higher or lower than expected from the resistance level of the parent and the GCA effect. This implies that the most resistant progeny may be produced by crossing the two parents with the highest GCA effects.

Reciprocal effects were significant at P≤ 0.05, with the maternal effects being more significant at P ≤ 0.05 than the non-maternal effects, indicating that they are important in determining FRR resistance (Table 6.4). They accounted for 6% of the total phenotypic variation. The non-maternal effects were high in the F_2 generation for both the scale and percentage data, indicating the importance of the cytoplasmic x nuclear gene interaction effects in resistance to FRR.

6.3.2 Estimation of combining ability effects for developed crosses

Generally, negative GCA effects were desirable in this study because they indicated the bean line's contribution to resistance to FRR, while positive GCA effects were not desirable

because they indicated the bean line's contribution to susceptibility. In the F_1 generation, K20, K132, Kanyebwa, Umgeni and Hoima-Kaki had significant (P= 0.01) positive GCA effects for both scale and percentage data (Table 6.5). This suggested that these varieties contributed to susceptibility to FRR in the crosses that involved them. Vuninkingi displayed the highest significant negative (P= 0.01) GCA value at the F_1 generation, followed by RWR719 and MLB-49-89A. Crosses involving these varieties also had low FRR severities (Table 6.14). This suggests that they may be the best sources of resistance in that order among the 12 parents (Table 6.5). The varieties Umubano, G4795, and MLB-48-89A had insignificant negative (P= 0.05) GCA effects. This observation suggested that they were effective sources of resistance to FRR. G1459 had a low positive GCA, indicating that it is not an effective source of resistance but may have some resistance genes to FRR (Table 6.5).

Table 6.5. General combining ability effects of 12 bean parents for resistance to Isolote FSP-3 isolate of *Fusarium solani* f. sp. *phaseoli* in F_1 generation.

Parent	1-9 scale	Percentage scale
K20/GLP2	0.80***	6.88***
K132/Cal96	0.98***	10.32***
Kanyebwa	0.89***	10.43***
Umubano/G2333	-0.25	-2.61
MLB-49-89A	-1.01***	-9.29***
RWR719	-1.18***	-11.50***
MLB-48-89A	-0.30	-2.16
G1459	0.10	0.23
G4795	-0.05	-0.37
Vuninkingi/ G685	-1.00**	-12.10***
Umgeni	0.48**	4.35*
Hoima-Kaki	0.53**	5.81***
S.e.d (P= 0.05)	0.20	1.699

*, **, *** = significant at P= 0.05, P= 0.01 and P= 0.001, respectively.

Since the F_2 trial was not replicated, it was not possible to differentiate between the GCA values of the parents based on the significance levels. However, they were plotted against each other to highlight the magnitude of the differences from zero (Figure 6.1). In the F_2 generation, K20, Kanyebwa, K132, Umgeni, and Hoima-Kaki had high positive GCA values similar to the F_1 generation (Figure 6.1). MLB-49-89A had the highest negative GCA value followed by Vuninkingi, RWR719, MLB-48-89A, and Umubano at the F_2 generation (Figure 6.1). In addition, crosses involving these varieties had low FRR severities, suggesting that they could be effective sources of resistance among the 12 parents (Table 6.14). G4795 displayed positive GCA effects at this generation (Figure 6.1), yet it had a negative GCA in

the F_1 generation. This indicates that the bean line may possess genes that contributed to resistance at the F_1 generation, but due to a possible gene interaction in the F_2 it contributed to susceptibility in this generation. The bean line G1459 displayed high positive GCA effects at the F_2, indicating that it may not be an effective source of resistance (Figure 6.1). Umgeni and Hoima-Kaki, even though included in the mating scheme as resistant parents, had positive GCA values at both generations. This suggests that both varieties are not good donors of additive resistance genes for crossing with Ugandan bean varieties, especially for Umgeni which was susceptible as a parent. However, in the case of Hoima-Kaki, it is probable that susceptibility was dominant to resistance in crosses involving this bean line, suggesting that the resistance genes in the bean line are recessive in nature.
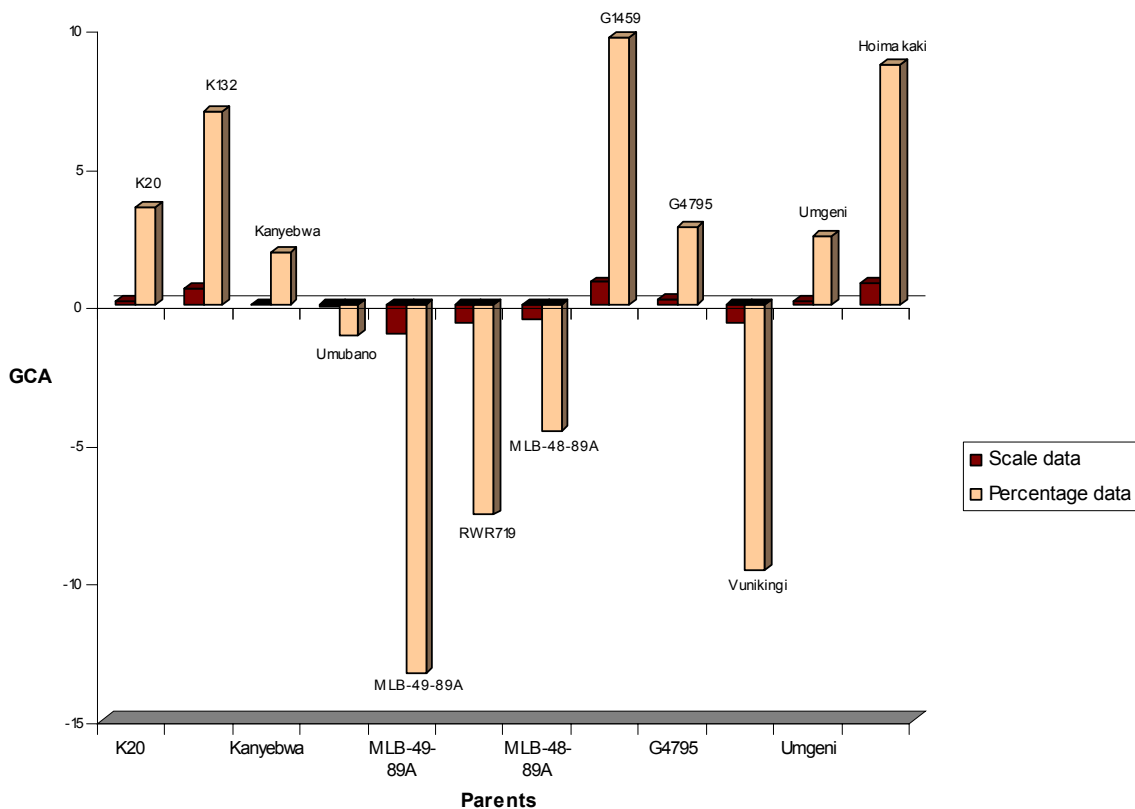
Fig. 6.1. General combining ability effects of 12 parents for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli* in the F_2 generation.

Even though the SCA effects were not significant in both generations, eight crosses displayed significant SCA effects at P≤ 0.05 (Table 6.6) at F_1. The SCA effects for the crosses K20 x MLB-49-89A and Umubano x Vuninkingi were negative and significant at P≤0.05, indicating the presence of non-additive gene effects impacting on FRR resistance in

these crosses. The SCA effects for MLB-49-89A x G1459 and RWR719 x Vunikigi were positive and significant at P≤0.05, indicating the presence of non-additive gene action governing susceptibility to FRR in these crosses.

Table 6.6. Specific combining ability effects of F_1 bean crosses for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli.*

	K3	Kan	UB	M49	RW	M48	G1	G4	VN	UM	HK
K2	0.69	0.35	0.00	-1.17*	-0.45	0.19	-0.34	-0.57	0.01	0.50	0.79
K3		-0.70	0.44	-0.85	-0.53	0.17	-0.89	-0.65	0.15	0.51	0.26
KN			-0.24	-0.05	-0.87	-0.49	-0.68	0.56	0.45	-0.30	0.57
UB				-0.47	0.15	0.82	-0.50	0.11	-1.20*	0.17	0.71
M49					0.92	-0.08	1.41**	0.19	0.25	0.35	-0.51
R719						0.09	-0.10	0.67	1.08*	-0.47	-0.49
M48							0.12	-0.33	-0.37	-0.43	0.31
G1								0.70	0.08	0.36	-0.18
G4									0.53	-0.36	-0.84
VN										-0.34	-0.64
UM											-0.54
S.e.d (P= 0.05)					0.485						

K2 = K20, K3 = K132, KN = Kanyebwa, UB = Umubano, M49 = MLB-49-89A, RW = RWR719, M48 = MLB-48-89A, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki ; *, **,   *** = significant at P= 0.05, P= 0.01 and P= 0.001, respectively.

In the F_2 generation, the cross K20 x RWR719 had the highest negative SCA effects, suggesting the presence of non-additive gene effects impacting on FRR resistance in this cross, while the cross MLB-49-89A x RWR719 had the highest positive SCA effect indicating non-additive gene action governing susceptibility in this cross (Table 6.7).

Table 6.7. Specific combining ability effects of the F_2 generation for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli.*

	K3	KN	UB	M49	RW	M48	G1	G4	VN	UM	HK
K2	0.43	0.87	-0.27	0.64	-1.09	0.15	0.26	-0.02	-0.38	-0.21	-0.41
K3		-0.52	0.79	-0.20	-0.68	-0.34	0.28	0.39	-0.71	0.45	0.09
KN			-0.49	-0.38	0.23	0.07	-0.86	0.56	0.50	-0.63	0.66
UB				-0.67	0.25	-0.71	-0.09	0.32	0.76	0.13	-0.02
M49					2.25	-0.55	0.01	-0.22	-0.58	0.04	-0.32
RW						-0.04	-0.07	-0.30	0.33	-0.24	-0.65
M48							0.02	-0.11	0.63	0.80	0.10
G1								-0.24	-0.20	0.78	0.11
G4									-0.13	-0.32	0.08
VN										-0.68	0.47
UM											-0.67

K2 = K20, K3 = K132, KN = Kanyebwa, UB = Umubano, M49 = MLB-49-89A, RW = RWR719, M48 = MLB-48-89A, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki.

6.3.3 Reciprocal cross effects on Fusarium root rot resistance

The reciprocal crosses Hoima-Kaki x K20, Vuninkingi x Kanyebwa and G4795 x Kanyebwa had significant positive reciprocal effects in the F_1 generation, as shown in Table 6.8. This implies that Fusarium severity was higher when K20 and Kanyebwa were the maternal parents in these crosses and lower when Hoima-Kaki, Vuninkingi and G4795 were the maternal parents. This suggested that the cytoplasmic genes of K20 and Kanyebwa contributed to susceptibility to FRR in these crosses. The reciprocal effects for the crosses Umgeni x K132, MLB-48-89A x Umubano, G1459 x MLB-49-89A and MLB-49-89A x Umubano, were significant and negative, indicating that FRR severity was lower when K132, Umubano and MLB-49-89A were the maternal parents in these crosses. This implies that the cytoplasmic genes in K132, Umubano, and MLB-49-89A contributed to resistance to FRR in these crosses. The reciprocal effects may further be explained by the maternal and non-maternal effects generated by Diallel-SAS05 (Table 6.9).

Table 6.8. Reciprocal effects of F_1 bean crosses for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli*.

	K2	K3	KN	UB	M49	RW	M48	G1	G4	VN	UM
K3	-0.33										
KN	-0.53	0.23									
UB	0.58	0.13	0.90								
M49	0.05	0.28	0.30	0.16							
RW	-0.12	0.68	-0.03	0.07	-0.10						
M48	0.43	-0.19	-0.83	-1.53**	-0.04	-0.46					
G1	-1.09	-0.29	-0.18	-0.37	-1.61**	-0.01	0.48				
G4	-0.15	-0.58	2.17***	-0.24	-0.93	-0.66	0.29	0.01			
VN	0.72	0.79	1.27*	-0.32	0.39	0.43	0.27	0.38	0.66		
UM	0.13	-1.37*	0.97	-0.60	-0.13	-0.18	-0.51	-0.14	-0.52	-0.31	
HK	1.30*	-0.20	0.51	0.71	-0.11	-0.11	0.28	0.70	0.87	0.44	0.48
S.e.d (P= 0.05)						0.536					

*, **, *** = significant at P= 0.05, P= 0.01, and P= 0.001, K2 = K20, K3 = K132, KN = Kanyebwa, UB = Umubano, M49 = MLB-49-89A, RW = RWR719, M48 = MLB-48-89A, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki.

In the F_1 generation, maternal effects were significant and negative (P= 0.05) for Hoima-Kaki, Umubano and Vuninkingi, with Hoima-Kaki having the highest negative and significant (P= 0.05) maternal effects, followed by Vuninkingi, then Umubano (Table 6.9). This indicated that the cytoplasm of these varieties contributed to their resistance to FRR at F_1. Kanyebwa and G1459 had significant positive maternal effects, indicating that the cytoplasm of these varieties contributed to the susceptibility of these varieties to FRR.

The crosses MLB-49-89A x G1459 and K132 x Umgeni had significant negative non-maternal effects, suggesting that the interaction of cytoplasmic and nuclear genes of these varieties contributed to resistance in these crosses (Table 6.9). In addition, in the case of MLB-49-89A x G1459, the maternal effect for MLB-49-89A, even though non-significant (P= 0.05), was negative while that of G1459 was positive (Table 6.9). This may therefore indicate that even though cytoplasm of the parent G1459 contributed to the susceptibility of resistance in this cross and the cytoplasm of MLB-49-89A contributed to resistance in the same cross, their interaction resulted in a contribution to resistance in the cross. Similarly for the negative non-maternal effects observed in the cross K132 x Umgeni, both parents had negative maternal effects, hence the interaction of their cytoplasmic genes contributed even more to the resistance of the cross.

The cross Kanyebwa x G4795 had significant positive, non-maternal effects at P= 0.05 (Table 6.9) indicating that the interaction of cytoplasmic and nuclear genes of these varieties contributed to susceptibility to FRR in these crosses because both parents had positive maternal effects.

Table 6.9. Maternal and non-maternal effects of 12 bean parents for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli* at F_1 generation.[6]

	K2	K3	KN	UB	M49	RW	M48	G1	G4	VN	UM	HK
K2	**0.06**	-0.40	-0.15	0.21	-0.29	-0.30	0.65	-0.82	-0.12	0.28	0.10	0.83
K3		**-0.01**	0.69	-0.17	0.02	0.57	0.17	0.05	-0.48	0.43	-1.08*	-0.60
KN			**0.44****	0.13	-0.43	-0.61	-0.66	-0.29	1.81***	0.45	0.81	-0.34
UB				**-0.31***	0.19	0.26	-0.94	0.27	0.16	-0.39	-0.01	0.61
M49					**-0.28**	0.05	0.51	-0.99*	-0.56	0.29	0.43	-0.24
RW						**-0.12**	-0.06	0.46	-0.44	0.18	0.22	-0.40
M48							**0.28**	0.53	0.10	-0.38	-0.51	-0.41
G1								**0.33***	-0.23	-0.32	-0.18	-0.03
G4									**0.09**	0.20	-0.33	0.38
VN										**-0.37***	0.34	0.40
UM											**-0.28**	-0.21
HK												**-0.41****
S.e.d$_{ME}$(P= 0.05)						0.148						
S.e.d$_{NM}$ (P= 0.05)						0.489						

*, **, *** = significant at P= 0.05, P= 0.01, and P= 0.001, NM= Non-maternal effects, ME = Maternal effects; K2 = K20, K3 = K132, KN = Kanyebwa, UB = Umubano, M49 = MLB-49-89A, RW = RWR719, M48 = MLB-48-89A, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki.

In the F_2 generation, reciprocal effects persisted, with the cross Hoima-Kaki x Vuninkingi having the highest negative reciprocal effect, followed by G1459 x RWR719, MLB-49-89A x

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[6] Above Diagonal are the non-maternal effects and In Diagonal are the maternal effects.

K20, and G4795 x MLB-49-89A. Similarly the crosses Umgeni x K132 and G4795 x G1495 had the highest positive reciprocal effects (Table 6.10).

Table 6.10. Reciprocal effects based on analysis of the F_2 generation among 12 parents for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli*.

	K2	K3	KN	Umb	M49	RW	M48	G1	G4	VN	UM
K3	0.70										
KN	-0.50	-0.65									
UB	0.60	0.40	-1.00								
M49	-1.25	0.25	-0.55	0.50							
RW	-0.20	0.45	0.35	0.90	0.05						
M48	-0.80	-0.25	-0.15	0.50	-0.40	-0.30					
G1	-1.05	-0.30	-0.25	-0.35	-1.00	-1.50	-0.75				
G4	-0.35	0.20	0.75	0.95	-1.25	-0.95	-0.30	1.20			
VN	-0.35	0.55	-0.55	1.05	0.35	0.15	-0.10	0.90	-0.25		
UM	-0.50	1.20	-0.20	-0.70	-0.65	-0.05	0.05	0.75	-0.15	0.25	
HK	0.05	-0.50	-0.75	0.10	0.15	-0.60	0.10	0.35	0.00	-1.55	0.65

K2 = K20, K3 = K132, KN = Kanyebwa, UB = Umubano, M49 = MLB-49-89A, RW = RWR719, M48 = MLB-48-89A, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki.

The trend of maternal effects in the F_2 generation was different from that observed in the F_1 generation, with RWR719 having the highest negative maternal effects, followed by K20, then Vuninkingi, MLB-49-89A and lastly, Kanyebwa (Table 6.11). This showed that the varieties Vuninkingi, RWR719 and MLB-49-89A maintained their negative maternal effects from the F_1. However, K20 and Kanyebwa had positive maternal effects from the F_1 generation. The bean line G1459 had the highest positive maternal effect, followed by Umubano and Hoima-Kaki. The varieties Umubano and Hoima-Kaki had negative maternal effects in the F_1 generation, while G1459 maintained its high positive maternal effect from F_1. The negative reciprocal effects in the F_2 generation observed in the cross Hoima-Kaki x Vuninkingi and MLB-49-89A x K20 (Table 6.10) may be explained by the high, negative non-maternal effects of these crosses (Table 6.11). The negative reciprocal effects in the F_2 generation of the crosses, G1459 x RWR719 and G4795 x MLB-49-89A, may be explained by high negative maternal effects of RWR719 and MLB-49-89A (Table 6.11), while the high positive maternal effects observed in the cross Umgeni x K132, may be explained by the high non-maternal effect of this cross. The high positive reciprocal effect (Table 6.10) in the cross G4795 x G1495 is explained by the high positive maternal effects of G1459 which contributed to the susceptibility of that cross to FRR (Table 6.11).

Table 6.11. Maternal and non-maternal effects of 12 bean parents for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli* in F_2 generation.[7]

	K2	K3	KN	UB	M49	RW	M48	G1	G4	VN	UM	HK
K2	**-0.40**	1.16	-0.20	0.15	-0.99	-0.20	-0.36	0.05	0.00	-0.20	-0.04	0.62
K3		**0.05**	-0.80	0.69	0.05	-0.00	-0.27	0.34	0.09	0.24	1.20	-0.39
KN			**-0.10**	-0.55	-0.59	0.05	-0.02	0.55	0.80	-0.70	-0.04	-0.48
UB				**0.35**	0.01	0.15	0.19	0.00	0.55	0.45	-0.99	-0.08
M49					**-0.14**	-0.21	0.23	-0.16	-0.16	0.23	-0.45	0.45
RW						**-0.40**	0.13	-0.40	-0.64	0.29	0.40	-0.03
M48							**0.03**	-0.08	-0.39	-0.39	0.07	0.23
G1								**0.70**	0.45	-0.05	0.10	-0.18
G4									**-0.05**	-0.45	-0.04	0.22
VN										**-0.25**	0.56	-1.13
UM											**0.05**	0.76
HK												**0.17**

* significant at P= 0.05, ** significant at P= 0.001, *** = significant at P=0.0001, nm= Non-maternal effects, m = Maternal effects; K2 = K20, K3 = K132, KN = Kanyebwa, UB = Umubano, M49 = MLB-49-89A, RW = RWR719, M48 = MLB-48-89A, VN = Vuninkingi, G1= G1459, G4 = G4795, UM = Umgeni, HK = Hoima-Kaki.

6.3.4 Estimation of narrow sense heritability of resistance to Fusarium rot rot

The mid-parent offspring regression analysis was significant (P= 0.01) with a regression coefficient "*b*" of 0.38±1.04 with the 1-9 scale data and 0.492±0.07 with the percentage data (Table 6.12, Figure 6.2).

Table 6.12. Regression analysis of F_2 crosses on parental F_1 scores.

Source of variance	DF	Mean squares	
		Scale	Percentage
Regression	1	32.843**	5988.5***
Residual	130	1.085	124.0
Total	131	1.328	168.7
"b"		0.38±1.04	0.492±0.07

** Significant at P= 0.001.

The regression coefficient "*b*" is an estimate of the narrow sense heritability according to Vogel et al. (1980) and Falconer and Mackay (1996). F_2 data indicated that 18-26% of the total variation in the mean scores of F_2 population was accounted for by the parental F_1 scores (Figure 6.2). This is very low, suggesting that the environmental effects impacting on resistance to FRR, were very high. Therefore, resistance expression in the F_2 generation could not be reliably predicted based on the F_1 performance. This is shown clearly by the scatter plots in Figure 6.1 a and b.

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[7] Above Diagonal are non-maternal effects and In Diagonal are the maternal effects
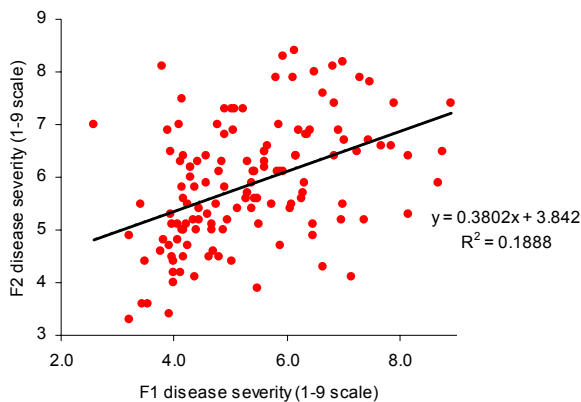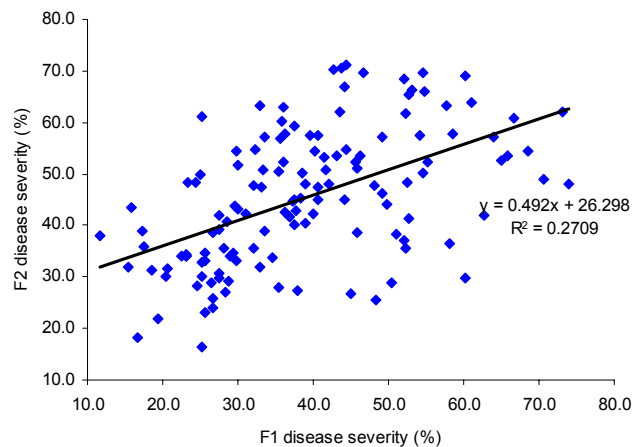
Fig. 6.2. Regression of FRR severity, F2 progeny scores on the F1 scores for 132 populations.

The diallel SAS-05 computer programme estimated $\sigma^2 g$, $\sigma^2 s$, $\sigma^2 R$, $\sigma^2 E$ (Table 6.13) and based on Equations 6 and 7, $\sigma^2 A$ and $\sigma^2 D$ were calculated: $\sigma^2 P$ was calculated as $\sigma^2 A$ + $\sigma^2 D$+ $\sigma^2 R$+ $\sigma^2 E$. Heritability (h^2) was then estimated based on Equation 5 as 0.348387 for scale data and 0.3435973 for the percentage data.

Table 6.13. Estimation of $\sigma^2 g$, $\sigma^2 s$ and $\sigma^2 R$ from DIALLELSAS-05.

Source of variance	df	Variance (V)	
		scale	Percentage
V(g) GCA	54	0.54778****	58.7612***
V(s) SCA	262	0.09992	9.9422
V (R) Reciprocal	262	0.14926**	17.88**
VE		1.70000	186.7439

*significant at P= 0.01, ****Significant at P =0.0001.

6.3.5 Frequency distribution of severity scores in R x S crosses

Segregation ratios of the 27 populations and their reciprocals, involving the eight different sources of resistance and three susceptible varieties (K20, K132 and Kanyebwa) gave a continuous distribution, but could not be fitted into definite genetic ratios, indicating the complexity of inheritance of resistance (Table 6.14). Most of the F_2 populations gave nearly continuous distributions (indicating the presence of additive resistance genes) for the F_2 generation with the exception of K132 x Umubano, G4795 x Kanyebwa, K132 x Umgeni and Kanyebwa x Hoima-Kaki where there were no resistant plants (Table 6.14), indicating skewedness to the susceptible side. This may indicate that the resistant genes to FRR in the parents, Umubano, G4795 and Umgeni, are additive and recessive in nature, or have less effects compared to the resistant genes in the other resistance sources. For most of the crosses there were more susceptible plants than resistant plants with the exception of the reciprocal crosses MLB-49-89A x K20, RWR719 x K20, Vuninkingi x K20, MLB-49-89A x Kanyebwa and Umubano x Kanyebwa, where the number of resistant plants was greater than the number of susceptible plants. It is envident that the maternal effects in MLB-49-89A, RWR719, Vuninkingi and Umubano were responsible for these distributions (Table 6.14).

In addition, crosses that involved MLB-49-89A, RWR719, Vuninkingi, Umubano and MLB-48-89A, in that order, resulted in the lowest Fusarium severity scores both in the F_1 and F_2 generation showing that they were the best sources of resistance to FRR (Table 6.14). Similarly, the lowest disease severity scores were obtained when these varieties were crossed with each other. The varieties G1459 and G4795 also resulted in relatively low severity reactions, while crosses with Umgeni and Hoima-Kaki resulted in relatively high severity scores. The cross K20 x MLB-49-89A resulted in the lowest severity score among the F_1 crosses that involved the three susceptible varieties. This was followed by the crosses Kanyebwa x RWR719, K20 x RWR719, Kanyebwa x MLB-48-89A, K132 x MLB 49-89A. F_1 mean severity was higher than the F_2 mean severity for only seven crosses, indicating that susceptibility was dominant over resistance in these crosses. It could also imply that there were a greater number of additive susceptibility genes than resistance genes in these crosses. The crosses Umubano x K132, MLB-49-89A x K20, MLB-49-89A x K132, all crosses of G4759 and G1459 with the three susceptible varieties had F_2 mean severity greater than the F1. This indicated that in these varieties resistance was dominant over

susceptibility. It could also suggest that, in the absence of dominance, there were more additive resistance genes than susceptibility genes. Crosses with Kanyebwa as a maternal parent (except in crosses with MLB-49-89A, Umubano and Vuninkingi) had F_1 values lower than the F_2 mean, indicating the significant role of maternal effects on resistance to FRR in these varieties (Table 6.14).

Table 6.14. Segregation of resistance to Fusarium root rot resistance in (S x R) F_2 and their reciprocal (R x S) crosses involving K20, K132 and Kanyebwa.

Crosses	Mean Fusarium severity (1-9 scale)**		No. F_2 plants in each segregation class			Total number of F_2 plants assessed
	F1*	F2	R[†]	MR[††]	S[†††]	
1. K20 x MLB-49-89A	4.0	6.9	8	36	77	120
MLB-49-89A x K20	3.9	4.4	21	77	15	113
2. K20 x RWR719	4.5	4.5	62	18	35	114
RWR719 x K20	3.9	4.1	33	75	23	131
3. K20x Umubano	6.5	6.3	20	72	30	122
Umubano x K20	5.3	5.1	12	42	87	141
4. K20 x MLB-48-89A	6.5	6.5	20	60	21	101
MLB-48-89A x K20	5.6	4.9	2	48	57	107
5. K20 x G1459	4.8	8.2	0	12	119	131
G1459 x K20	7.0	6.1	11	33	42	86
6. K20 x G4795	5.4	6.6	11	45	72	128
G4795 x K20	5.7	5.9	3	12	12	27
7. K20 x Vuninkingi	5.9	5.4	6	84	38	128
Vuninkingi x K20	4.4	4.7	21	50	15	86
8. K20 x Umgeni	7.0	6.5	6	48	80	134
Umgeni x K20	7.2	5.5	18	53	41	111
9. K20 x Hoima-Kaki	8.7	6.4	17	5	41	62
Hoima-Kaki x K20	6.0	6.5	2	60	72	134
10. K132 x MLB-49-89A	4.7	5.0	23	29	33	84
MLB-49-89A x K132	4.2	5.5	30	66	29	125
11. K132 x RWR719	5.3	4.7	17	15	39	71
RWR x K132	3.9	5.6	0	0	0	0
12. K132 x Umubano	6.6	6.8	0	17	75	92
Umubano x K132	6.4	7.6	9	38	84	131
13. K132 x MLB-48-89A	6.1	5.9	5	59	42	105
MLB-48-89A x K132	6.3	5.4	29	32	50	110
14. K132 x G1459	5.2	7.9	0	21	110	131
G1459 x K132	5.8	7.3	11	6	68	84
15. K132 x G4795	5.0	6.9	11	9	72	92
G4795 x K132	6.2	7.3	3	39	83	125
16. K132 x Vuninkingi	6.3	4.6	21	95	17	132
Vuninkingi x K132	4.7	5.7	29	17	45	90
17. K132 x Umgeni	5.9	5.9	0	77	57	134

Table 6.14. Segregation of resistance to Fusarium root rot resistance in (S x R) F$_2$ and their reciprocal (R x S) crosses involving K20, K132 and Kanyebwa.

Crosses	Mean Fusarium severity (1-9 scale)**		No. F$_2$ plants in each segregation class			Total number of F$_2$ plants assessed
	F1*	F2	R[+]	MR[++]	S[+++]	
Umgeni x K132	8.7	8.3	0	3	54	57
18. K132 x Hoima-Kaki	6.9	7.9	0	15	87	102
Hoima Kaki x K132	7.3	6.9	14	17	66	96
19. Kanyebwa x MLB-49-89A	5.5	5.0	32	65	29	125
MLB-49-89A x Kanyebwa	4.9	3.9	62	48	17	126
20. Kanyebwa x RWR719	4.1	5.1	20	27	39	86
RWR719 x Kanyebwa	4.2	5.8	9	83	33	125
21. Kanyebwa x Umubano	6.6	6.3	21	33	75	129
Umubano x Kanyebwa	4.8	4.3	51	48	23	122
22. Kanyebwa x MLB-48-89A	4.6	5.6	24	69	45	138
MLB-48- x Kanyebwa	6.3	5.3	9	59	48	116
23. Kanyebwa x G1459	5.5	6.1	5	62	69	135
G1459 x Kanyebwa	5.8	5.6	14	65	38	116
24. Kanyebwa x G4795	8.9	5.9	15	63	59	137
G4795 x Kanyebwa	4.6	7.4	0	24	84	108
25. Kanyebwa x Vuninkingi	7.0	6.3	15	75	38	128
Vunkingi x Kanyebwa	4.4	5.2	0	0	0	0
26. Kanyebwa x Umgeni	7.4	5.6	12	65	42	119
Umgeni x Kanyebwa	5.4	5.2	29	63	14	105
27. Kanyebwa x Hoima-Kaki	7.8	8.1	0	11	107	117
Hoima x Kanyebwa	6.8	6.6	0	50	66	116

* Number of F1 plants varied 60-120 plants, **1-9 scale 1= resistant, 9= susceptible; R[+]= Resistant, MR[++]= Moderately Resistant, S[+++]= Susceptible.

6.3.6 Heterosis and gene dosage effects for resistance to Fusarium root rot observed at the F$_1$ generation

Negative heterosis is desirable as it indicates the superiority of the F$_1$ to either mid-parent, susceptible parent and resistant parent. Even though heterosis may not be considered important in common bean, a self-pollinating crop, it may be used to understand the contribution of different parents to the trait of concern and in so doing, help in the selection of desirable crosses in a breeding scheme, that is, those with high negative heterosis.

Eighteen out of 27 S x R F$_1$ crosses had negative relative/mid-parent heterosis while 24 out of 27 R x S reciprocal crosses had negative heterosis. The negative heterosis varied from -1.4 to -42.2. R x S crosses had higher heterosis levels compared to the S x R crosses (Table 6.15) indicating that higher resistance levels were obtained when the resistant parents were used as mothers than vice versa. This was consistent with observation of

maternal effects. Crosses with negative heterosis show the presence of joint action of a favourable combination of genes at different loci.

The R x S and S x R crosses had negative heterobeltiosis to the susceptible/worse parent, (Table 6.15), while the majority had positive heterobeltiosis to the resistant/better parent, with the exception of crosses involving Umgeni as a resistant parent and crosses with RWR719 as a female parent as they exhibited negative heterobeltiosis to the better parent. The positive heterobeltiosis observed for the R x S and S x R populations indicated that the F_1 generation had higher infection levels than the resistant parent and may suggest the involvement of overdominance effects in favour of susceptibility in these crosses. The negative heterosis observed with crosses with Umgeni indicated that even though this bean line may not have been a good source of resistance (Table 6.15), it still resulted in a better offspring. The negative heterosis observed for reciprocal crosses involving RWR719 indicated that higher resistance levels were observed when RWR719 was a mother than when it was father. Hence, it indicated that this line possesses cytoplasmic genes that confer resistance to FRR. Most F_1 crosses had disease severity levels closer to one parent than to the other, indicating the importance of partial dominance gene effects in these populations. A few crosses exhibited almost complete dominance for resistance to FRR, e.g., K20 x RWR719 and Kanyebwa x RWR719, while others exhibited complete dominance for susceptibility to FRR, e.g., K20 x Hoima-Kaki.

Table 6.15. Mid-parent heterosis and heterobeltiosis observed on the F_1 (R x S) and their reciprocal (S x R) crosses for resistance to Fusarium root rot. [8]

Crosses*		Mean score of parents*		F1 mean score*	Mid parent score*	Mid parent heterosis (%)	Heterobeltiosis (%)	
		R	S				R	S
1.	K20 x MLB-49-89A	3.2	7.5	4	5.35	-25.2	15.0	-46.7
	MLB-49-89A x K20	3.2	7.5	3.9	5.35	-27.1	13.1	-48.0
2.	K20 x RWR719	4.5	7.5	4.5	6.00	-25.0	0.0	-40.0
	RWR719 x K20	4.5	7.5	3.9	6.00	-35.0	-10.0	-48.0
3.	K20x Umubano	3.9	7.5	6.5	5.70	14.0	45.6	-13.3
	Umubano x K20	3.9	7.5	5.3	5.70	-7.0	24.6	-29.3
4.	K20 x MLB-48-89A	4.7	7.5	6.5	6.10	6.6	29.5	-13.3
	MLB-48-89A x K20	4.7	7.5	5.6	6.10	-8.2	14.8	-25.3
5.	K20 x G1459	4.3	7.5	4.8	5.90	-18.6	8.5	-36.0
	G1459 x K20	4.3	7.5	7	5.90	18.6	45.8	-6.7
6.	K20 x G4795	4.1	7.5	5.4	5.80	-6.9	22.4	-28.0

[8]Crosses involving Umgeni behaved like S xS crosses because it was not an effective source of resistance to Fusarium root rot.

Table 6.15. Mid-parent heterosis and heterobeltiosis observed on the F$_1$ (R x S) and their reciprocal (S x R) crosses for resistance to Fusarium root rot. [8]

Crosses*		Mean score of parents*		F1 mean score*	Mid parent score*	Mid parent heterosis (%)	Heterobeltiosis (%)	
		R	S				R	S
	G4795 x K20	4.1	7.5	5.7	5.80	-1.7	27.6	-24.0
7.	K20 x Vuninkingi	3.8	7.5	5.9	5.65	4.4	37.2	-21.3
	Vuninkingi x K20	3.8	7.5	4.4	5.65	-22.1	10.6	-41.3
8.	K20 x Umgeni	7.1	7.5	7	7.30	-4.1	-1.4	-6.7
	Umgeni x K20	7.1	7.5	7.2	7.30	-1.4	1.4	-4.0
9.	K20 x Hoima-Kaki	5.1	7.5	8.7	6.30	38.1	57.1	16.0
	Hoima-Kaki x K20	5.1	7.5	6	6.30	-4.8	14.3	-20.0
10.	K132 x MLB-49-89A	3.2	9	4.7	6.10	-23.0	24.6	-47.8
	MLB-49-89A x K132	3.2	9	4.2	6.10	-31.1	16.4	-53.3
11.	K132 x RWR719	4.5	9	5.3	6.75	-21.5	11.9	-41.1
	RWR x K132	4.5	9	3.9	6.75	-42.2	-8.9	-56.7
12.	K132 x Umubano	3.9	9	6.6	6.45	2.3	41.9	-26.7
	Umubano x K132	3.9	9	6.4	6.45	-0.8	38.8	-28.9
13.	K132 x MLB-48-89A	4.7	9	6.1	6.85	-10.9	20.4	-32.2
	MLB-48-89A x K132	4.7	9	6.3	6.85	-8.0	23.4	-30.0
14.	K132 x G1459	4.3	9	5.2	6.65	-21.8	13.5	-42.2
	G1459 x K132	4.3	9	5.8	6.65	-12.8	22.6	-35.6
15.	K132 x G4795	4.1	9	5	6.55	-23.7	13.7	-44.4
	G4795 x K132	4.1	9	6.2	6.55	-5.3	32.1	-31.1
16.	K132 x Vuninkingi	3.8	9	6.3	6.40	-1.6	39.1	-30.0
	Vuninkingi x K132	3.8	9	4.7	6.40	-26.6	14.1	-47.8
17.	K132 x Umgeni	7.1	9	5.9	8.05	-26.7	-14.9	-34.4
	Umgeni x K132	7.1	9	8.7	8.05	8.1	19.9	-3.3
18.	K132 x Hoima-Kaki	5.1	9	6.9	7.05	-2.1	25.5	-23.3
	Hoima Kaki x K132	5.1	9	7.3	7.05	3.5	31.2	-18.9
19.	Kanyebwa x MLB-49-89A	3.2	9	5.5	6.10	-9.8	37.7	-38.9
	MLB-49-89A x Kanyebwa	3.2	9	4.9	6.10	-19.7	27.9	-45.6
20.	Kanyebwa x RWR719	4.5	9	4.1	6.75	-39.3	-5.9	-54.4
	RWR719 x Kanyebwa	4.5	9	4.2	6.75	-37.8	-4.4	-53.3
21.	Kanyebwa x Umubano	3.9	9	6.6	6.45	2.3	41.9	-26.7
	Umubano x Kanyebwa	3.9	9	4.8	6.45	-25.6	14.0	-46.7
22.	Kanyebwa x MLB-48-89A	4.7	9	4.6	6.85	-32.8	-1.5	-48.9
	MLB-48- x Kanyebwa	4.7	9	6.3	6.85	-8.0	23.4	-30.0
23.	Kanyebwa x G1459	4.3	9	5.5	6.65	-17.3	18.0	-38.9
	G1459 x Kanyebwa	4.3	9	5.8	6.65	-12.8	22.6	-35.6
24.	Kanyebwa x G4795	4.1	9	8.9	6.55	35.9	73.3	-1.1
	G4795 x Kanyebwa	4.1	9	4.6	6.55	-29.8	7.6	-48.9
25.	Kanyebwa x Vuninkingi	3.8	9	7	6.40	9.4	50.0	-22.2
	Vunkingi x Kanyebwa	3.8	9	4.4	6.40	-31.3	9.4	-51.1
26.	Kanyebwa x Umgeni	7.1	9	7.4	8.05	-8.1	3.7	-17.8
	Umgeni x Kanyebwa	7.1	9	5.4	8.05	-32.9	-21.1	-40.0
27.	Kanyebwa x Hoima-Kaki	5.1	9	7.8	7.05	10.6	38.3	-13.3
	Hoima x Kanyebwa	5.1	9	6.8	7.05	-3.5	24.1	-24.4

*1-9 scale; 1= resistant, 9 = susceptible.

Generally heterosis was low amongst the R x R crosses compared to the R x S and S x R crosses, with the majority of the crosses having positive heterosis. This indicates the superiority of these varieties as sources of resistance but also highlights the possibility of the resistance genes being recessive in nature and occurring at different loci. Eight of the R x R crosses had negative heterosis, varying from -1.8% to -17.4% (Table 6.16). All the S x S crosses had high and negative heterosis (Table 6.16). This may imply that the susceptible parents also possessed some minor and recessive genes for resistance to FRR. The data in Table 6.19 also showed that the levels of resistance differed among the resistant parents and that some R x R crosses produced less resistant progeny at the F_1. This therefore, suggests that resistance could be improved by selecting from these crosses.

Table. 6.16. Heterosis observed at F_1 R x R and S x S crosses for resistance to Fusarium root rot. [9]

	Cross		P1 mean score *	P2 mean score*	F1 Mean score*	Mid-parent heterosis(%)
1.	RWR719 x MLB-49-89A	R x R	4.5	3.2	6.4	66.2
	MLB-49-89A x RWR719	R x R	3.2	4.5	6.5	68.8
2.	RWR719 x MLB-48-89A	R x R	4.5	4.7	4.4	-4.3
	MLB-48-89A x RWR719	R x R	4.7	4.5	5.0	8.7
3.	RWR719 x Vuninkingi	R x R	4.5	3.8	5.1	22.9
	Vuninkingi x RWR719	R x R	3.8	4.5	4.8	15.7
4.	RWR719 x Umubano	R x R	4.5	3.9	4.5	7.1
	Umubano x RWR719	R x R	3.9	4.5	6.3	50.0
5.	RWR719 x G1459	R x R	4.5	4.3	4.5	2.3
	G1459 x RWR719	R x R	4.3	4.5	7.5	70.5
6.	RWR719 x G4759	R x R	4.5	4.5	4.2	-6.7
	G4759 x RWR719	R x R	4.5	4.5	6.1	35.6
7.	RWR719 x HoimaKaki	R x R	4.5	5.1	4.8	0.0
	Hoima-Kaki x RWR719	R x R	5.1	4.5	6.0	25.0
8.	RWR719 x Umgeni	R x R	4.5	7.1	5.1	-12.1
	Umgeni x RWR719	R x R	7.1	4.5	5.2	-10.3
9.	MLB-49-89A x MLB-48-89A	R x R	3.2	4.7	3.4	-13.9
	MLB-48-89A x MLB-49-89A	R x R	4.7	3.2	4.2	6.3
10.	MLB-49-89A x Vuninkingi	R x R	3.2	3.8	4.0	14.3
	Vuniking x MLB-49-89A	R x R	3.8	3.2	3.3	-5.7
11.	MLB-49-89A x Umubano	R x R	3.2	3.9	3.6	1.4
	Umubano x MLB-49-89A	R x R	3.9	3.2	4.6	29.6
12.	MLB-49-89A x G4759	R x R	3.2	4.5	3.6	-6.5
	G4795 x MLB-49-89A	R x R	4.5	3.2	6.1	58.4
13.	MLB-49-89A x HoimaKaki	R x R	3.2	5.1	5.5	32.5
	Hoima-Kaki x MLB-49-89A	R x R	5.1	3.2	5.2	25.3
14.	MLB-49-89A x Umgeni	R x R	3.2	7.1	4.4	-14.6

[9]Crosses involving Umgeni behaved as R x S and S x R crosses because it was not an effective source of resistance to Fusarium root rot.

Table. 6.16. Heterosis observed at F_1 R x R and S x S crosses for resistance to Fusarium root rot. [9]

Umgeni x MLB-49-89A	R x R	7.1	3.2	5.7	10.7
15. MLB-48-89A x Vuninkingi	R x R	4.7	3.8	5.3	24.7
Vuniginki x MLB-48-89A	R x R	3.8	4.7	5.5	29.4
16. MLB-48-89A x Umubano	R x R	4.7	3.9	4.1	-4.7
Umubano x MLB-48-89A	R x R	3.9	4.7	5.1	18.6
17. MLB-48-89A x G4759	R x R	4.7	4.5	5.2	13.0
G4795 x MLB-48-89A	R x R	4.5	4.7	5.8	26.1
18. MLB-48-89A x Hoima-Kaki	R x R	4.7	5.1	6.4	30.6
Hoima-Kaki x MLB-48-89A	R x R	5.1	4.7	6.2	26.5
19. MLB-48-89A x Umgeni	R x R	4.7	7.1	6.4	8.5
Umgeni x MLB-48-89A	R x R	7.1	4.7	6.3	6.8
20. Vuninkingi x Umubano	R x R	3.8	3.9	4.9	27.3
Umubano x Vuninkingi	R x R	3.9	3.8	7.0	81.8
21. Vuninkingi x G4759	R x R	3.8	4.5	5.6	34.9
G4795 x Vuninkingi	R x R	4.5	3.8	5.1	22.9
22. Vuninkingi x Hoima-Kaki	R x R	3.8	5.1	5.0	12.4
Hoima-Kaki x Vuninkingi	R x R	5.1	3.8	8.1	82.0
23. Vuninkingi x Umgeni	R x R	3.8	7.1	5.0	-8.3
Umgeni x Vuninkingi	R x R	7.1	3.8	4.5	-17.4
24. Umubano x G4759	R x R	3.9	4.5	7.3	73.8
G4795 x Umubano	R x R	4.5	3.9	5.4	28.6
25. Umubano x Hoima-Kaki	R x R	3.9	5.1	6.7	48.9
Hoima-Kaki x Umubano	R x R	5.1	3.9	6.5	44.4
26. Umubano x Umgeni	R x R	3.9	7.1	5.4	-1.8
Umgeni x Umubano	R x R	7.1	3.9	6.8	23.6
27. G4759 x Hoima-Kaki	R x R	4.5	5.1	7.0	45.8
Hoima-Kaki x G4759	R x R	5.1	4.5	7.0	45.8
28. G4759 x Umgeni	R x R	4.5	7.1	5.8	0.0
Umgeni x G4759	R x R	7.1	4.5	6.1	5.2
29. Hoima-Kaki x Umgeni	R x R	5.1	7.1	6.1	0.0
Umgeni x Hoima-Kaki	R x R	7.1	5.1	7.4	21.3
30. K20 x K132	S x S	7.5	9.0	7.8	-5.5
K132 x K20	S x S	9.0	7.5	6.4	-22.4
31. K20 x Kanyebwa	S x S	9.0	9.0	6.4	-28.9
Kanyebwa x K20	S x S	9.0	9.0	7.4	-17.8
32. K132 x Kanyebwa	S x S	9.0	9.0	5.3	-41.1
Kanyebwa x K132	S x S	9.0	9.0	6.6	-26.7

*1-9 scale; 1= resistant, 9 = susceptible.

6.3.7 Estimation of the number genes governing Fusarium root rot and broad sense heritability (H) in F_2 S X R crosses

Based on the original Castle-Wright analysis (Zeng et al., 1990) and methods used by Bjarko and Line (1988) and Das and Griffey (1994) for estimating the number of genetic

factors governing a trait, different numbers of genes were important for resistance to FRR, depending on the cross. The two methods used in estimating the number of genes did not differ greatly, indicating that either method could be used. The mean of the two formulae was used to explain the results below. The number of genetic factors in MLB-49-89A were estimated to be 2-6 genes; RWR719, 2-3 genes; Vuninkingi, 3-5 genes; Umubano, 3-5 genes; MLB-48-89A, 2-3 genes; G1459, 2 genes; G4795, 2-9 genes; Umgeni, 2-3 genes and Hoima-Kaki, 1-5 genes (Table 6.17).

In addition, estimates of VF_2 and VE (see Equation 8) were used to estimate heritability of the different crosses. Broad sense heritability was low (0.22-0.69), with the highest being recorded for the cross Kanyebwa x G1459 (h^2=0.69). Negative heritability (H) was observed in crosses having significant maternal effects (Table 6.17), for example, Kanyebwa x MLB-49-89A, K132 x Umubano, K20 x Vuninkingi, K132 x Umgeni. Kanyebwa x MLB-49-89A, K132 x Umubano, K20 x Vuninkingi (0.28), K132 x Umgeni.

Table 6.17. Estimation of broad sense heritability (H) and number of genes controlling resistance to Fusarium root rot in 29 F_2 populations.

Susceptible parent	Resistant parent	n	K_{CW}	Mean	Heritability (H)
K20	MLB-49-89A	6.55	6.00	6.28	0.22
K132	MLB-49-89A	2.16	2.15	2.16	0.44
Kanyebwa	MLB-49-89A	-5.39	-5.34	-5.37	-0.86
K20	RWR719	3.18	2.81	3.00	0.25
K132	RWR719	3.47	3.47	3.47	0.35
Kanyebwa	RWR719	1.68	1.64	1.66	0.49
K20	Vuninkingi	-2.78	-2.77	-2.78	-0.52
K132	Vuninkingi	3.03	2.89	2.96	0.36
Kanyebwa	Vuninkingi	5.39	4.75	5.07	0.33
K20	Umubano	3.81	3.69	3.75	0.26
K132	Umubano	-2.49	-2.45	-2.47	-0.92
Kanyebwa	Umubano	5.61	5.38	5.50	0.23
K20	MLB-48-89A	2.87	2.78	2.83	0.38
K132	MLB-48-89A	3.18	3.08	3.13	0.36
Kanyebwa	MLB-48-89A	2.23	2.15	2.19	0.56
K20	G1459	1.84	1.71	1.78	0.52
K132	G1459	2.12	2.11	2.12	0.57
Kanyebwa	G1459	1.76	1.76	1.76	0.69
K20	G4795	1.77	1.61	1.69	0.45
K132	G4795	4.19	4.17	4.18	0.35
Kanyebwa	G4795	10.23	7.15	8.69	0.26
K20	Umgeni	1.90	1.73	1.82	0.47
K132	Umgeni	-1.71	-0.84	-1.28	-1.33
Kanyebwa	Umgeni	3.25	2.68	2.97	0.42

K20	Hoima-Kaki	1.47	1.29	1.38	0.58
K132	Hoima-Kaki	2.34	2.32	2.33	0.54
Kanyebwa	Hoima-Kaki	5.27	4.35	4.81	0.50

n=number of genes according to Bjarco and line formula.

Kcw= number of genes according to the original Castle Wright formula.

$H = (VF_2 - VE)/VF_2$.

6.3.8 Allelism test for Fusarium root rot resistance genes from several potential sources of resistance

The chi-square test (X^2) results for the goodness of fit of the phenotypic classes of F_2 segregants is presented in Table 6.18. Four out of the 11 ratios were fitted. The test indicated the presence of one dominant and two recessive genes in the cross MLB-49-89A x Vuninkingi, and two complementary dominant genes in the cross MLB-49-89A x G4795. Three complementary dominant genes were suggested by the chi square test in the crosses RWR719 x Vuninkingi, RWR719 x Vuninkingi, MLB-48-89A x Umubano, and MLB-48-89A x G4795. All the other crosses had more than three genes involved and did not fit into any of the ratios tested. The involvement of more than three genes is explained by the continuous distribution exhibited by their progeny, suggesting the importance of polygenic inheritance in these crosses.
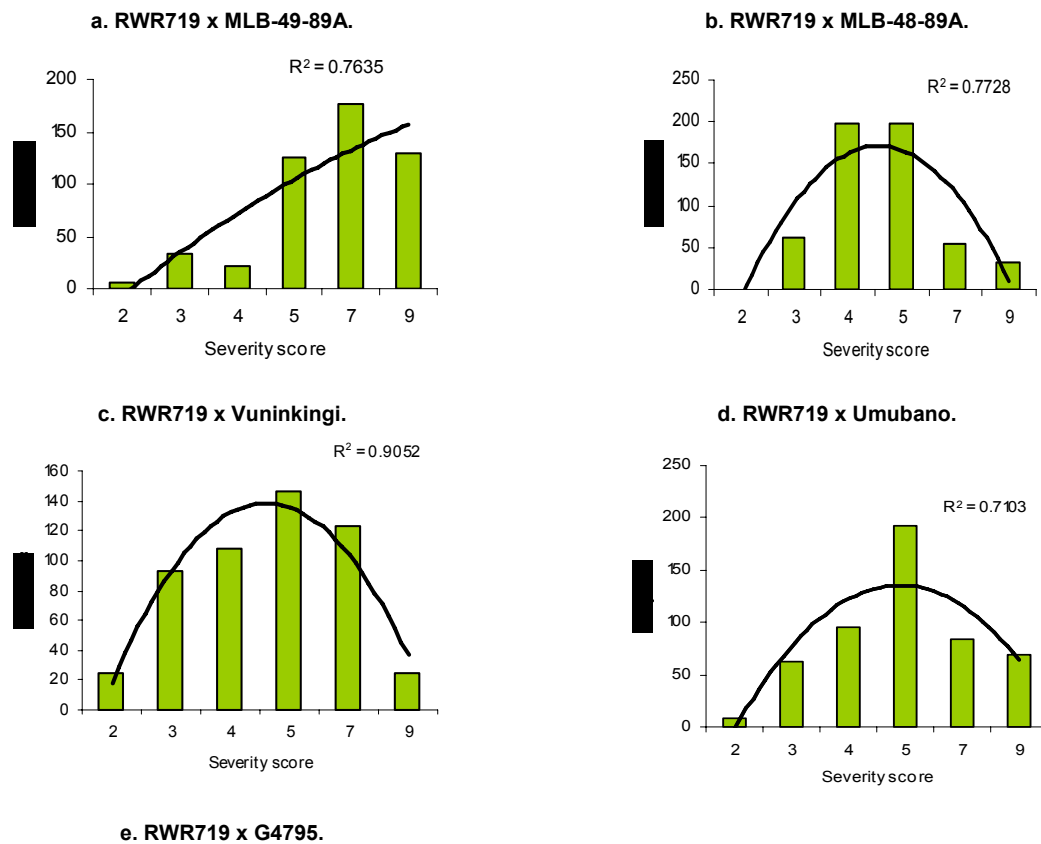
Table 6.18. Chi square testing for goodness of fit of phenotypic classes in F_2.

Cross	Hypothesis	X^2 Value	Df	P value	Implication
MLB-49-89A x Vuninkingi	49:15	0.93	1	0.336	one dominant and two recessive genes
MLB-49-89A x G4759	9:7	0.49	1	0.482	two complementary dominant genes
MLB-49-89A x Umubano	57:7	0.01	1	0.931	one dominant and two complementary genes
MLB-49-89A x MLB-48-89A	57:7	0.60	1	0.438	one dominant and two complementary genes
MLB-48-89A x G4759	27:37	0.16	1	0.693	three complementary dominant genes
RWR719 x Vuninkingi	27:37	0.29	1	0.591	three complementary dominant genes

F_2 populations that involved RWR719 (Figure 6.2a, b, c, d and e) had continuous distribution. This indicated the action of more than one or two additive resistance genes in these crosses. However, the cross RWR719 x MLB-49-89A (Figure 6.2a) was skewed to the susceptible side indicating the presence of major additive recessive susceptibility and resistance genes in both these varieties, probably located within the same quantitative trait loci (QTL) because they only expressed themselves in the F_2. It also implied that the resistance genes were fewer than the susceptibility genes in this cross.

Most of the crosses with MLB-49-89A, that is, MLB-49-89A x MLB-48-89A (Figure 6.3a), MLB-49-89A x Vuninkingi (Figure 6.3b), MLB-49-89A x Umubano (Figure 6.3c), and MLB-49-89A x G4795 (Figure 6.3d) tended towards resistance which indicated the presence of major additive resistance gene effects, probably located at the same locus in these bean varieties. However, the crosses MLB-49-89A x RWR719 (Figure 6.2a) tended towards susceptibility

indicating the possibility of recessive genes for resistance and susceptibility in MLB-49-89A and RWR719, probably located within the same quantitative trait loci (QTL). The distribution of MLB-49-89A x G4795 (Figure 6.3d) was discontinuous (R^2=0.27) because there seemed to be two distinct classes of susceptible and resistant plants in this cross which may indicate the presence of recessive resistance gene(s) in either MLB-49-89A or G4795 or recessive susceptibility gene(s) in both parent and the presence of probably two loci governing resistance to FRR in these varieties.
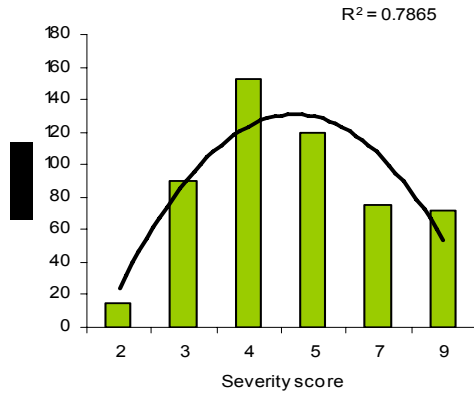

a. RWR719 x MLB-49-89A.

b. RWR719 x MLB-48-89A.

c. RWR719 x Vuninkingi.

d. RWR719 x Umubano.

e. RWR719 x G4795.

Fig. 6.2. F_2 phenotypic segregation for R x R crosses involving RWR719.
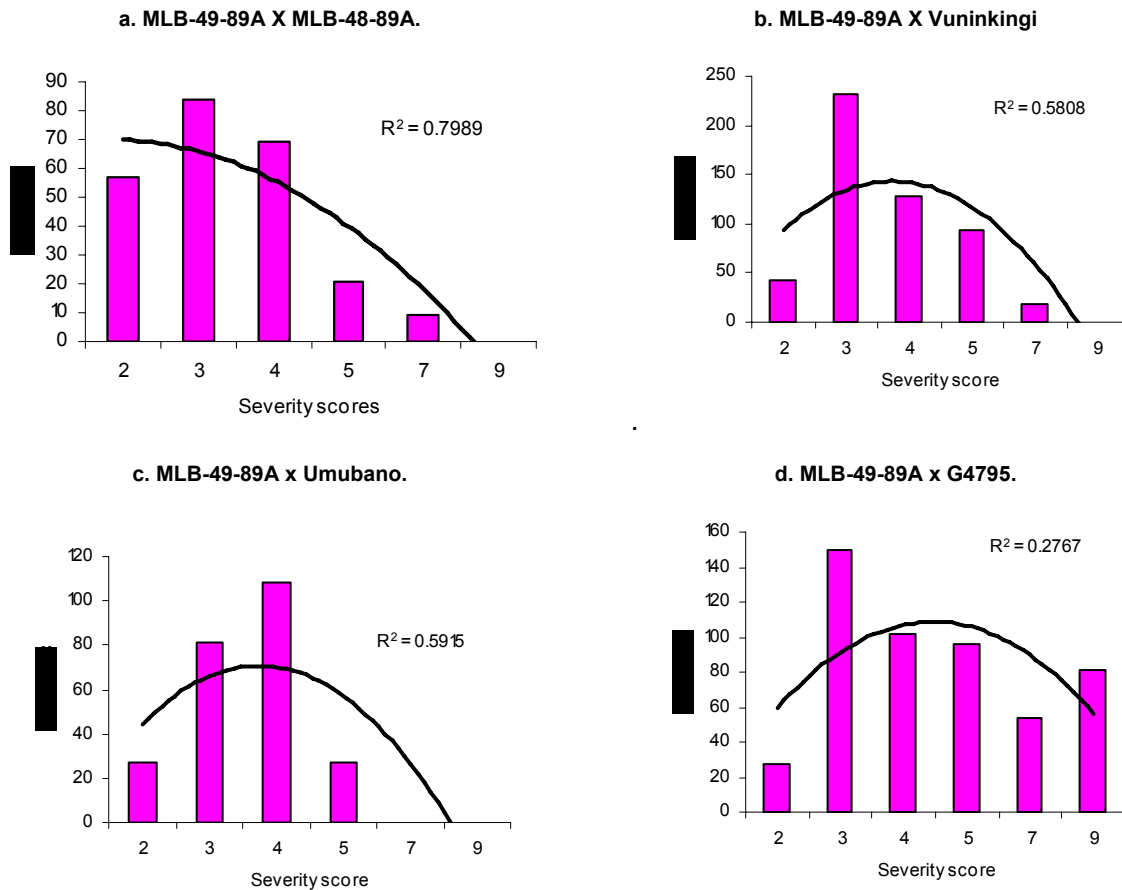

Fig. 6.3. F_2 phenotypic segregation for R x R crosses involving MLB-49-89A.

Crosses involving MLB-48-89A had a continuous distribution of severity scores for some of the crosses, such as MLB-49-89A x MLB-48-89A (Figure 6.3a), tending towards resistance, while the cross MLB-48-89A x Vuninkingi (Figure 6.4a) and MLB-48-89A x G4795 (Figure

6.4c), tended towards susceptibility. The crosses MLB-48-89A x Umubano (Figure 6.4b) had a discontinuous distribution (R^2=0.14), with the appearance of two distinct separate classes of both resistant and susceptible plants at F_2. It is probable that the resistance genes were on different loci in the two bean varieties.
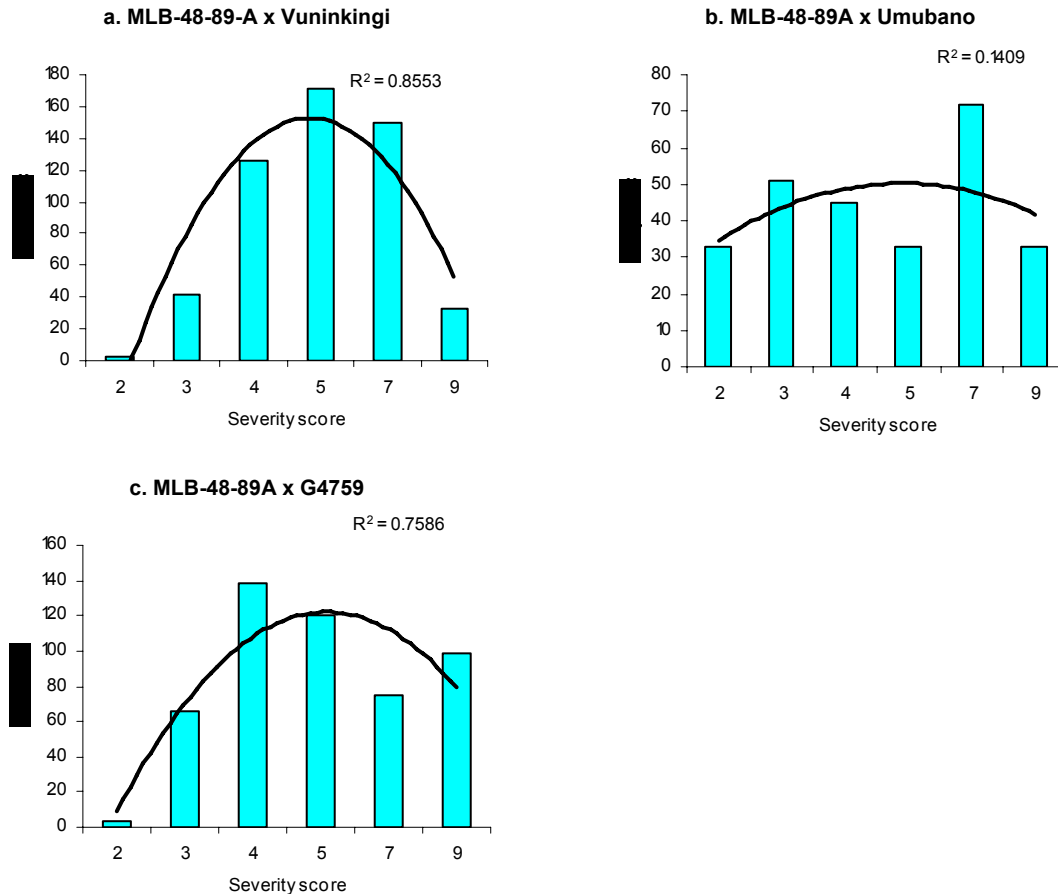
a. MLB-48-89-A x Vuninkingi

$R^2 = 0.8553$

b. MLB-48-89A x Umubano

$R^2 = 0.1409$

c. MLB-48-89A x G4759

$R^2 = 0.7586$

Fig. 6.4. F_2 phenotypic segregation for R x R crosses involving MLB-48-89A.

Crosses involving Vuninkingi (Figures 6.2c, 6.3b, 6.4a, 6.5a and 6.5b) had continuous distributions, indicating the presence of additive resistance genes in these varieties. Most of the populations with Vuninkingi as a parent tended towards susceptibility, with the exception of that with RWR719, which was >90% continuous, and MLB-49-89A, which tended towards resistance. This, therefore, indicated that MLB-49-89A had more additive resistance genes than all the other resistant parents, and that the genes for resistance to FRR are recessive in nature.
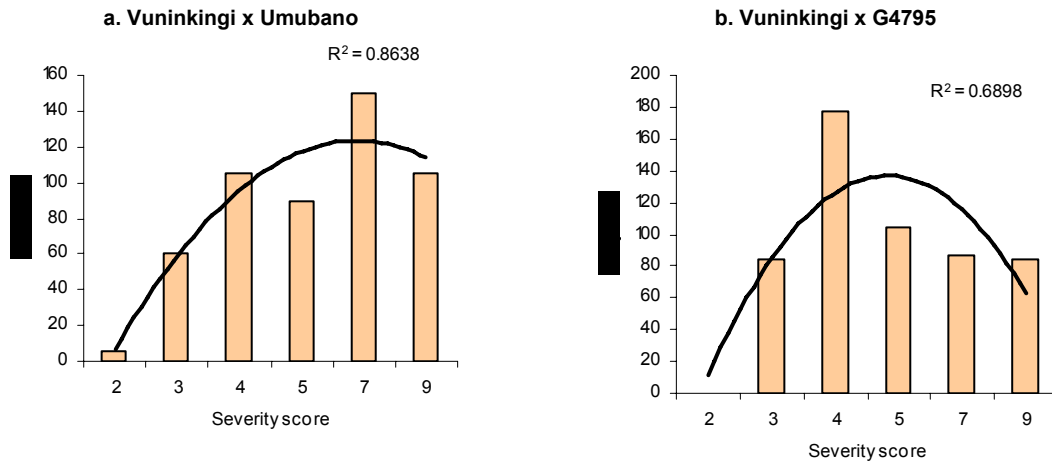
Fig. 6.5. F_2 phenotypic segregation for R x R crosses involving Vuninkingi.

The crosses involving Umubano, that is, RWR719 x Umubano (Figure 6.2d), Vuninkingi x Umubano (Figure 6.5a), and Umubano x G4759 (Figure 6.6), also tended towards susceptibility, while the cross MLB-49-89A x Umubano (Figure 6.3c), tended towards resistance. This again showed that MLB-49-89A had a greater number of resistance genes than any of the other parents. The cross MLB-48-89A x Umubano (Figure 6.4b) had almost equal numbers of both resistant and susceptible plants in the F_2 generation, which indicated the possibility of two loci governing resistance in this cross.

Crosses involving G4795 (Figures, 6.2e, 6.4c, 6.5b, and 6.6) were continuous in nature indicating the involvement of many additive genes, with the exception of MLB-49-89A x G4795, which was discontinuous (Figure 6.3d) as already discussed. Results indicate two distinct classes of susceptible and resistant plants which suggested the presence of a recessive resistance gene or recessive susceptibility gene(s) governing resistance to FRR in both MLB-49-89A and G4795, probably located at two loci in these varieties. The cross Umubano x G4795 (Figure 6.6) was skewed to the susceptible side, indicating that the resistance genes in these varieties were recessive in nature and possibly at the same locus.
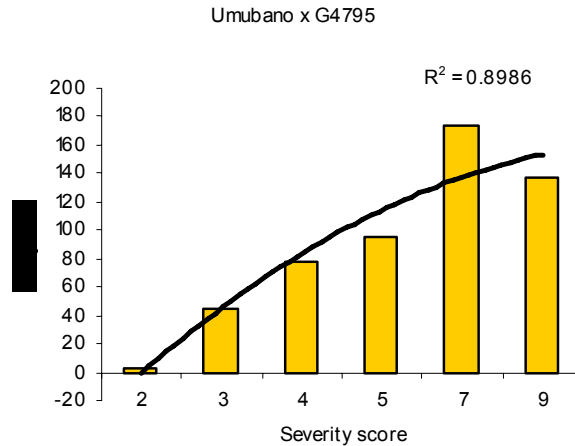
Fig. 6.6. F_2 phenotypic segregation for Umubano x G4795.

In summary, F_2 populations that involved RWR719 had skewed continuous distribution (Table 6.19). This indicated the action of additive recessive resistance genes in these populations, with the possibility of more than one locus. Populations with MLB-49-89A tended towards resistance which indicated the presence of many additive resistance genes in this line. However, the distribution of the RWR719 x MLB-49-89A indicated that the number of susceptibility genes in this combination was greater than the number of resistance genes, hence the susceptible reaction in the F_2. MLB-49-89A x G4795 showed two distinct classes of susceptible and resistant plants, probably indicating the presence of two loci governing resistance to FRR in these varieties. Crosses involving MLB-48-89A had continuous distribution, with some of crosses tending towards resistance while others tended towards susceptibility, thus indicating the presence of more than one locus. The cross MLB-48-89A x Umubano had a discontinuous distribution, with the appearance of two distinct separate classes of both resistant and susceptible plants in the F_2 generation which indicated the presence of two loci (Table 6.19). All crosses involving Vuninkingi had continuous distributions, indicating the presence of several additive genes on different loci. Crosses involving Umubano either tended towards resistance or towards susceptibility, depending on the parents indicating the presence of more than one locus. Crosses involving G4795 were continuous in nature, indicating the involvement of many additive genes; the exception was MLB-49-89A x G4795, the distribution of which discontinuous with two distinct classes of susceptible and resistant plants, indicating the presence of two loci governing resistance to FRR in these varieties (Table 6.19).

Table 6.19. Distribution of F$_2$ populations used in testing allelism of resistance genes to Fusarium root rot.

Line	Populations skewed		Distribution	
	S	R	Continuous	Discontinuous
RWR719	MLB-49-89A		Vuninkingi Umubano G4795	
MLB-49-89A	RWR719	MLB-48-89A Vuninkingi Umubano		G4795
MLB-48-89A		MLB-49-89A	RWR719 Vuninkingi G4795	Umubano
Vuninkingi	Umubano	MLB-49-89A	RWR719 G4795 MLB-48-89A	
Umubano	Vuninkingi G4795	MLB-49-89A	RWR719	MLB-48-89A
G4759	Umubano		Vuninkingi MLB-48-89A RWR719	MLB-49-89A

6.4 Discussion

This study used a 12 x 12 diallel mating design to develop 66 F$_1$ and F$_2$ populations plus their reciprocal crosses, as a means of designing an appropriate breeding strategy for incorporating *Fusarium solani* f. sp. *phaseoli* resistance into three commercial and popular bean varieties in Uganda. In addition the populations developed were used to obtain information on the inheritance of resistance to FRR. F$_1$ and F$_2$ data indicated that resistance to FRR was a recessive trait, with the resistant parents having varying numbers of resistance genes. There was evidence to suggest that the line MLB-49-89A had the greatest number of additive resistance genes compared to all the other parents. The S x R and R x S F$_2$ populations did not show any distinct segregation patterns, even though for most of the populations the distribution was continuous which indicated that inheritance of resistance to FRR was complexity. The results indicate the presence of additive genes, with small effects for most of the crosses, implying that resistance to FRR was additive in nature. Other scientists, using different populations, have also found that resistance to *FSP* was additive in nature (Hassan et al., 1971; Boomstra and Bliss, 1977; Schneider et al., 2001; Román-Avilès and Kelly, 2005). The continuous distribution in the F$_2$ generation is evidence of this, as well as the analysis of variance. The GCA effects were highly significant (P≤0.05) in both

the F_1 and F_2 generations indicating significance of additive gene effects. The lines RWR719, Vuninkingi, MLB-49-89A, Umubano, and MLB-48-89A had negative GCA effects at all generations. This implied that they were effective sources of resistance in these populations and would be recommended as sources of resistance for FRR in the bean improvement program in Uganda. The crosses, MLB-49-89A X K20, RWR719 X K20, Kanyebwa x MLB-49-89A and Umubano X Kanyebwa had the lowest FRR severity scores at both F1 and F2 generations and should be recommended for advancement in the breeding program for FRR. The lines G1459, G4795, Hoima-Kaki and Umgeni were not effective sources of resistance because they had either positive or very low negative GCA effects, but they may still be considered sources of resistance as the GCA values were better than those of the susceptible parents. The susceptible parents, K20, Kanyebwa, and K132, had very high positive GCA effects in the F_1 generation which indicated that that they have susceptibility genes. In the F_2 generation, K20 and K132 still maintained high positive GCA effects but Kanyebwa had a very low negative GCA effect. The negative GCA effect for Kanyebwa was unexpected because this line is very susceptible to FRR. However, it is probable that this line posses some additive recessive resistance genes that were only able to manifest themselves in the F_2 generation. In addition, this line had high maternal effects, which indicated that its cytoplasm could also have contributed to the resistance or susceptibility observed in the crosses in which it was involved.

Two crosses had high negative and significant ($P \leq 0.05$) SCA effects, that is, K20 x MLB-49-89A and Umubano x Vuninkingi, which indicated the presence of non-additive gene effects for FRR resistance in these crosses. It is probable that either one of the parents in these crosses possesses some dominant resistance genes. The SCA effects for MLB-49-89A x G1459 and RWR719 x Vuninkingi were positive and significant at $P \leq 0.05$, indicating non-additive gene action governing susceptibility to FRR in these crosses. This suggested that either one of the parents in these crosses possesses dominant susceptibility genes. These findings tally with past studies that suggested that inheritance of FRR resistance was complex with some studies which indicated that susceptibility was dominant to resistance (Boomstra and Bliss, 1977), while others suggested that resistance to FRR was dominant over susceptibility (Yerkes and Freytag, 1956).

Reciprocal effects were significant in these populations which indicated the role of maternal and non-maternal effects in modifying resistance to FRR. However, they accounted for only

5% of the total phenotypic variation and hence they were not large enough to influence the estimates for GCA and SCA effects in most of the parents and crosses. Reciprocal effects are associated with cytoplasmic inheritance from the female parent. However, accidental self-pollination may be one of the reasons for the significant reciprocal and maternal effects (Dudley, 1963). The maternal effects were highly significant compared to the non-maternal effects, indicating that for some varieties, the cytoplasmic genes contributed to the resistance observed. Negative maternal effects were observed for K132, Umubano, MLB-49-89A, RWR719, Vuninkingi, Umgeni, and Hoima-Kaki in the F_1 generation. This implied that the cytoplasm of these varieties contributed to the resistance that was observed in the crosses that involved these varieties. In the F_2 generation, the maternal effects of K20, Kanyebwa, MLB-49-89A, RWR719, G4795, and Vuninkingi were negative. Kanyebwa and G1459 had significant positive maternal effects in the F_1 generation, which indicated that the cytoplasm of these varieties contributed to the susceptibility of these varieties to FRR. The negative maternal effects of the varieties MLB-49-89A, RWR719, and Vuninkingi persisted into the F_2 generation, while those of K132, Umubano, Umgeni and Hoima-Kaki did not persist. The high positive maternal effects of G1459 were persistent into the F_2 generation. This implies that crosses involving MLB-49-89A, RWR719, Vuninkingi, and G1459 should be monitored further to observe the persistence of the maternal effects in the next generations. It also suggests that populations involving these varieties as maternal parents should be advanced further over their reciprocal crosses to enhance levels of resistance to FRR. However, maternal effects are sources of error because they are non-Mendelian in nature, though they may persist into advanced generations. Environmental maternal effects reduce the precision of genetic studies and slow down the response to selection, while cytoplasmic or nuclear genetic maternal effects will inflate the amount of genetic variance, and slow the response to selection (Roach and Wulff, 1987). Past studies on the inheritance of resistance to FRR did not consider maternal effects as a component of the additive variance (Hassan et al., 1971; Boomstra and Bliss, 1977; Schneider et al., 2006; Román-Avilès and Kelly, 2005) and it may indicate that the heritabilities estimated from those studies were escalated by the maternal effects, and could have been even much lower than those estimated.

Broad sense heritability (H), which indicates the proportion of the F_2 variance attributable to the genetic segregation, was estimated for all the populations that involved the susceptible parents and it varied from 0.22-0.69. Heritability in the narrow sense (h^2) was estimated by the components of analysis of variance as 0.34. The heritability estimated by the regression

coefficient was 0.38±1.04 and 0.49±0.07 based on the 1-9 scale and percentage scale respectively. The heritability estimated by regression in the F1 and F2 generations could be regarded as broad sense because the combining ability values in the F2 may be inflated by the heterosis, and linkage disequilibrium is greatest in these generations. Linkages can be broken by random mating in the later generations (beyond F4). Simmonds (1981), Boss (1993) and Falconer and Mackay (1996) suggested that heritability determined by the regression coefficient, in the case of random mating, offered a more secure approach to h^2 than the partitioning of variance. The F_2 data indicated that 18-26% of the total variation in the mean scores of F_2 crosses was accounted for by the parental F_1 scores, indicating that there was a high environmental variance in the F_1 generation hence the low heritability. However, the estimate of the heritability from the ANOVA could be assumed to be accurate because the error variance due to the environmental and maternal effects that could have led to an overestimation of the additive variance, were estimated and included as components of the phenotypic variance. Generally, the low heritability (h^2) estimates obtained suggest that the heritability pattern of resistance to FRR observed was influenced by the environment, the sources of resistance used, the evaluation procedures and the age of the plants evaluated (Hassan et al., 1971; Boomstra and Bliss, 1977; Hall and Philips, 2004). Also, In addition, the inclusion of reciprocal cross effects in the estimation of heritability of resistance to FRR in this study helped to explain the lower heritability estimates obtained. As mentioned above, maternal effects reduce the precision of genetic studies because they inflate the amount of genetic variance but slow the response to selection (Roach and Wulff, 1987). Hassan et al. (1971) reported broad sense heritability of resistance to FRR varying from 61.5% to 64.3% under greenhouse conditions and 77.9 to 79.7% under field conditions while narrow sense heritability varied from 25.9% to 44.3% for inter-genepool crosses. However, Schneider et al. (2001) reported higher narrow sense heritability for resistance to FRR, varying from 48 to 71% in F_4-derived families developed within the same genepool. Similarly, Román-Avilès and Kelly (2005) reported h^2 values of resistance to FRR ranging from 44% to 51% in red kidney inbred backcross line populations (IBL) and 35 to 51% in cranberry (IBL) populations. The heritability estimates of 34-49% obtained in this study are low but adequate for effective selection but indicate the need for progeny testing and evaluation of root rot as a quantitative character if good breeding progress is to be achieved (Hassan et al., 1971).

The number of genes governing resistance to FRR was estimated using the original Castle-Wright method and a modified version of this method that estimates environmental variance. These formulae have been used by several scientist in estimating the number of genes governing traits (Zhang et al., 2001; Das et al., 2004; Santos and Simon, 2006; Han et al., 2006). The number of genes varied from 2-9 among the resistant parents. Several studies on resistance to FRR have reported 2-4 resistance genes, for example, two duplicate recessive genes were reported in crosses between the bean varieties flat marrow and robust pea bean by McRostie (1921), while three recessive genes were reported in PI 165435 and *P. coccineus* line no. 2014 5 (Azzam, 1958). Smith and Houston (1960) reported one recessive and one dominant gene from crosses involving 10 susceptible and seven resistant varieties, while Bravo et al. (1969) suggested three or more dominant genes in the sources of resistance, N203 and *P. coccineus* Hassan et al. (1971), indicated four dominant genes in N203 (the first recognised source of resistance to FRR).

Allelism tests highlighted the lilkelihood of many loci governing resistance to FRR resistance. Ratios testing the presence of up to three resistance genes were fitted in the chi square test of goodness of fit. Only six out of the 14 populations tested fitted some of these ratios. All the F_2 R x R populations exhibited continuous distributions, indicating the role of many loci governing resistance to FRR. The complexity of the distributions also highlighted the complexity of the nature of resistance to FRR in these populations. These results suggested that gene acumulation of the resistance genes on different loci from the eight parents would result in increased disease resistance levels and produce varieties with durable resistance. The resistance genes in these populations are both Middle-American and Andean in origin, hence, if pooled together in one genoptype, they would result in durable and broad spectrum disease resistance (Young and Kelly, 1996; Pastor-Corrales et al., 1998).

Several R x S crosses exhibited negative heterosis to the mid-parents as well as to the susceptible/worse parent, while the level of negative heterosis varied amongst the R x R crosses, and was relatively lower than that for R x S crosses. One of the two S x S crosses had negative heterosis. All the R x S crosses, with the exception of crosses involving Umgeni as a resistant parent and crosses that had RWR719 as a mother, had positive heterosis to the resistant/better parent indicating that the F_1 had higher infection levels than the resistant parent. This indicates the presence of dominant genes for resistance to FRR

resistance. Flintham et al. (1997) stated that heterozygosity is an important prerequisite for heterosis because heterosis can arise when overdominance at a given locus is a principal cause. Others, however, believe that dominance and epistasis are the underlying genetic basis of heterosis. Loci with no dominance do not cause heterosis. Heterosis in beans may not be important in pure line breeding, because the dominance effects cannot be fixed, but heterosis can be used to estimate the gains from different crosses. However, residual heterosis in the F2 may inflate combining ability values and give a false perception of the gains that could be made in later generations

In conclusion, in screening of the 12 x 12 diallel, resistance to FRR was governed by additive gene action, with a degree of dominance in a few crosses. Resistance was shown to be governed by 2-9 additive genes, with MLB-49-89A, Umubano, and Vuninkingi probably having dominant genes with recessive minor genes, while the other sources of resistance had mainly recessive resistance genes. The heterosis values obtained in these crosses as well as the F_2 progeny distribution also demonstrated this. Resistance genes in these populations were also shown to be located on more than one locus. Heritability estimates obtained for FRR resistance further indicate quantitative nature of this trait. The influence of maternal effects on the trait were also highlighted implying that care must be taken in selecting populations for improving resistance to FRR to avoid delays in achieving progress due to complications posed by maternal and non-maternal effects.

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Chapter Seven: Overview of the study

The common bean (*Phaseolus vulgaris* L.) is the most important food legume crop grown worldwide including Uganda. However, production in Uganda is greatly affected by several constraints. Bean root rot is one of the major biotic constraints to bean production. *Fusarium solani* (Mart.) Appel and Wollenv. f. sp. *phaseoli* (Burk.) Snyd. & Hans, is one of a complex of soil-borne pathogens causing root rots on beans. The study aimed at contributing to food security in Uganda by improving resistance to FRR in major market class bean varieties, which were preferred by local farmers. The study included a participatory rural appraisal (PRA) to identify farmers' perceptions of FRR; pathogen identification; development of a technique for use in screening beans for resistance to FRR; screening germplasm for resistance; and genetic analysis of resistance to FRR in the identified sources. The major findings from the study were as follows:

- The importance of involving rural farmers in devising technologies meant for them was highlighted in the PRA. The PRA also established the need for improved market class bean varieties with resistance to bean root rot (BRR) that meet farmers specifications. Generally, farmers confirmed their preference for large-seeded varieties over small-seeded ones.

- Bean root rot occurrence was wide-spread in the bean fields visited, especially in south-western Uganda. Farmers generally associated the disease with poor soils, excessive rainfall, drought, and poor crop management practices. Most farmers did not attempt to control the diseases; however, a few farmers practiced roguing of infected plants and adding farmyard manure to soil.

- The use of resistant varieties to control the disease was not recognised by over 90% of the farmers. This was probably because the most popular varieties were susceptible to BRR and the newly introduced resistant varieties did not have the seed qualities that the farmers preferred.

- High yield, marketability, resistance to diseases, tolerance to excessive rainfall and drought, and taste were the most important criteria considered before adoption of new bean varieties by farmers. Large-seeded bean varieties, even though recognised as being more susceptible to BRR than small-seeded varieties, were still

popular. Bush beans were the preferred type in both regions because they required less time, labour and materials for their production. Climbing beans were also grown by farmers in south-western Uganda for their high yields and tolerance to major diseases.

- Among four Fusarium isolates tested, FSP-3 was the most pathogenic, resulting in 100% disease incidence on all bean varieties, and severity in the range of 5.1-8.6 on a 1-9 scale. Storing pathogenic isolates on potato dextrose agar (PDA) slants at $5^{o}C$ was found to be the best method for maintaining viable pathogenic isolates.

- Interaction between irrigation frequency, soil composition and line did not affect ranking of varieties for their resistance to FRR. Using soil collected from a nearby forest, with daily irrigation, was adopted as a standard screening technique for FRR.

- Using sterilised sorghum seed as a medium for pathogen inoculation was found to be a suitable method for screening bean germplasm for resistance to FRR.

- Forty four common bean varieties were identified as potential sources of resistance to FRR due to their moderate resistance to the disease, under both screenhouse and field conditions. Ten of these were large-seeded. "Resistant" varieties from CIAT-Colombia were among the susceptible varieties when screened in Uganda, indicating that resistance was dependent on the environment and that local sources would be more stable and effective in breeding for resistance in farmers' preferred varieties. None of the varieties tested were classified as highly resistant or immune, suggesting the need to improve the level of resistance in the best varieties.

- Small-seeded common bean varieties tended to be more resistant to FRR than their larger-seeded counterparts. Also, varieties with purple hypocotyls tended to be more resistant to FRR than varieties with green hypocotyls, indicating a possible correlation between resistance to FRR and seed size, and hypocotyl colour.

- The general combining ability (GCA) effects were highly significant in both the F_1 and F_2 generations, indicating that FRR resistance was governed mainly by additive gene action. The varieties RWR719, Vuninkingi, MLB-49-89A, Umubano and MLB-48-89

had negative GCA effects in all generations; hence they would be the most effective sources of resistance in this population.

- Resistance to FRR was highly affected by maternal effects at both F_1 and F_2 generations, indicating that, for some varieties the cytoplasmic genes contributed to the resistance observed. Non-maternal effects were significant for some crosses, indicating that there was an interaction between cytoplasmic genes and nuclear genes on resistance to FRR. The specific combining ability effects (SCA) effects were not significant, indicating that non-additive gene action played a minor role in controlling the resistance to FRR in most of the bean varieties. A few F_1 crosses displayed significant SCA effects, indicating the importance of dominance gene action in these crosses. The analyses of gene dosage effects showed that most crosses had severity scores intermediate to the two parents supporting the observation of predominance of additive gene action; or closer to one parent supporting the observation of partial dominance for resistance to FRR. It was also observed that only a few F_1 crosses had progeny with resistance levels similar to one parent, indicating the importance of complete dominance gene effects for resistance or susceptibility in those crosses.

- The number of genes governing resistance to FRR was estimated to vary from two to nine among the eight sources of resistanc. The R x R populations exhibited continuous distributions of disease severity scores of the crosses, indicating that many loci govern resistance to FRR. Negative heterosis was observed for most of the R x R, and R x S crosses in this study, implying that some resistance genes exhibited dominance effects. The S x S crosses yielded progeny with lower severity scores than the better parents, suggesting the presence of minor resistance genes in these populations.

- Estimates of heritability of resistance varied from 0.22 to 0.69, depending on the method used. Broad sense heritability varied from 0.22 to 0.69, while heritability in the narrow sense was 0.34 with the partitioning of the variance components, and 0.38±1.04 to 0.49±0.07 with the mid-parent offspring regression analysis. Thus the h^2 was generally low due to high environmental variance which would affect the repeatability of results and reduce the selection progress.

The above findings have several implications for future breeding strategies for resistance to FRR in bean. First and foremost, the involvement of farmers proved a critical aspect for confirming the need for improved bean varieties with root rot resistance; and confirming that farmers preferred large-seeded varieties which should be bred for improved Fusarium resistance. Furthermore, the continued involvement of farmers in the next phases of the breeding programme would ensure that the new varieties produced would have a greater chance of being adopted. Resistant varieties, however, should be components of an Integrated Pest Management (IPM) strategy for BRR, especially soil IPM. This is because soil fertility is a major problem in bean growing areas in Uganda. In addition, there is the reality that the effectiveness of disease resistance often reduces after some time due to lack of crop rotations and increased pathogen inoculum levels in the soil validating the need for IPM.

The isolation of pathogenic *FSP* was a time-consuming process in this study, making the isolation of the FSP-3 isolate a milestone in the breeding process. In breeding beans for resistance to FRR, it is important to be aware of the coexistence of both pathogenic and non-pathogenic forms of *F. solani* in symptomatic plants and to clearly identify the strains, in order to avoid wastage of time and resources on research based on the use of a non-pathogen.

From the screening and inheritance studies, resistance to FRR was found to be greatly influenced by the environment, making it difficult to evaluate phenotypically. The testing environment and the evaluation procedures may affect the selection of resistance plants. Also, the sources of resistance used, the fungal isolates, and the age of plants at evaluation time have all been shown to affect the inheritance of resistance to FRR. This implies that the efficiency of phenotypic selection for such a trait is low, resulting in limited progress in breeding for resistance. In addition, field screening would even be more difficult because of the presence of other root rot pathogens. Nevertheless, resistance expression of bean varieties in the field and screenhouse were highly correlated, indicating that germplasm could be screened under either conditions. The observation that germplasm that had been previously selected for resistance to *Pythium* spp. were among the most resistant to FRR, suggested that field screening might be useful in identifying germplasm with resistant to all

the other important disease caused by the root rot complex. The screenhouse can then be used for confirmation tests of resistance to the individual diseases.

Disease severity was shown to be lower on older plants than younger plants for some varieties, indicating a probable shift in gene action for resistance to FRR. This may also imply that populations showing some levels of resistance at a young age should be evaluated again at an older age to properly classify the gene action observed. Also, due to the problems caused by environmental variation, indirect selection for morphological traits related to FRR resistance would be helpful in reducing the amount of time spent in disease evaluation. More studies should be done on correlations between resistance to FRR and seed size, seed colour, hypocotyl colour, and growth habit. In addition, quantitative trait loci analysis (QTL) using molecular markers would probably be useful in making selection for resistance more effective and less time and labour intensive. QTL analysis would facilitate in solving some of the problems faced in the conventional breeding methods for FRR resistance as it is greatly influenced by the environment and requires destructive sampling for disease evaluation.

Maternal effects were highly significant in determining resistance to FRR in beans, signifying the contribution of cytoplasmic genes to resistance to FRR. Maternal effects are sources of error because they reduce the precision of genetic studies, and slow down the response to selection, because they inflate the amount of genetic variance and may persist into advanced generations. Therefore, in research to improve resistance to Fusarium root rot it is imperative to conduct reciprocal crosses as a means of identifying lines with high negative maternal effects. Populations involving these varieties should be monitored for persistence of the maternal effects in the next generations. This also implies that parents with high maternal effects that contribute to resistance to FRR should be used as females in the crossing program because their offspring are likely to have higher resistance levels.

Heritability estimates obtained for FRR resistance were low in this study, indicating the quantitative nature of this trait and the influence of the environment on this character. Nevertheless, the heritability (h^2) estimate of 34-49% obtained in this study is adequate for effective selection but it indicates the need for progeny testing and evaluation of root rot as a quantitative character, if good breeding progress is to be achieved. Selection with multiple

backcrosses, alternating between the recurrent parent and donor parent is probably the best breeding procedure for improving resistance to FRR.

Lastly, the presence of many loci governing resistance to FRR suggests that accumulation of resistance genes from the eight parents would result in increased disease resistance levels and production of bean lines with durable resistance against FRR. Also, given that the BRR disease is caused by a complex of pathogens (*Pythium* spp., *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *phaseoli*), it would be of interest to identify populations that are resistant to other root rot pathogens in this complex through screening succesive generations for quantitive resistance to them.

In conclusion, the findings clearly show the potential to obtain and improve locally adapted and farmer preferred bean varieties for resistance to FRR through selection from developed populations. Rapid breeding progress could be realised by careful control of the test environment, and taking care of which parent should be used as female or male in designing crosses during the breeding; because the heritability was generally low, and reciprocal effects due to maternal and non-maternal effects were highly significant in modifying resistance and susceptibility in the bean populations under study.

Appendices

Appendix 2.1 Map of Kabale district
Source: ICRAF, 2002

Appendix 2.2 Map of Kisoro district

Appendix 2.3 Map of Mbale and Sironko districts (eastern Uganda)

Appendix 3.1: Nash and Snyder Medium

Colonies begin to develop after about four to seven days.
Basal medium in 1 000ml of water:

Ingredients	Amounts
Agar	20.0g
Peptone	15.0g
KH2PO4	1.0g
MgSO4.7H20	0.5g
PCNB 75% w/w	1.0g (Pentachloro-nitrobenzene)

The basal medium is autoclaved and cooled to about 55°C before adding,

Streptomycin sulfate	1.0g
Neomycin sulfate	0.12g
Benelate/Benomyl	$2mgl^{-1}$

Appendix 5.1 Mean squares for resistance to FSP-3 of CIAT varieties

Source of variation	d.f.	m.s.	
		Percentage scale	1-9 scale[10]
Trial stratum	1	13303.2	
REP stratum	2	149.3	0.1244
Trial .REP stratum	2	344.6	
CIAT varieties	35	326.4**	1.7214**
Residual	175	147.0	0.6538
Total	215		

Appendix 5.2 Mean squares for resistance to FSP-3 of Uganda local landraces

Source of variation	d.f.	m.s.	
		Percentage scale	1-9 scale
Trial stratum	1	13107.3	64.56
REP stratum	2	747.2	0.4151
Trial .REP stratum	2	144.2	1.815
Landraces	28	633.3**	3.5419**
Residual	140	233.7	0.8806
Total	173		

Appendix 5.3 Mean squares for resistance to FSP-3 of Pythium root rot nursery

Source of variation	d.f.	m.s.	
		Percentage scale	1-9 scale
Trial stratum	1	29968.6	232.87
REP stratum	2	762.0	4.374
Trial .REP stratum	2	8233.3	64.661
Entries	47	744.4**	8.263**
Residual	235	238.7	2.109
Total	287		

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[10] Data collected for only one trial

Appendix 5.4 Mean squares for resistance to FSP-3 of the South African nursery[11]

Source of variation	d.f.	m.s. Percentage scale	1-9 scale
REP stratum	2	755.1	12.36
Entries	35	2301.5**	5.63
Residual	70	184.2	3.12
Total	107		

Appendix 5.5 Mean squares for for resistance to FSP-3 of the *F. oxyporum* f. sp. *phaseoli* differentials[12]

Source of variation	d.f.	m.s. Percentage scale	1-9 scale
REP stratum	2	370.78	8.230
Entries	6	676.70**	4.923**
Residual	12	95.10	2.19
Total	20		

Appendix 5.6 Mean squares for resistance to FSP-3 of 49 bean varieties screened under field conditions at Kawanda Agricultural Research Institute

Source	df	Mean squares							
		% emergence	FRR severity* 28dap	56dap	Plant stand 28dap	56dap	Root weight	Root: Shoot ratio	Yield kg ha^{-1}
Reps	2	280.2	8.747	6.553	126.0	182.59	0.02449	4.94	18083
Entries	48	619.6**	2.588**	2.112**	687.6**	127.06*	0.08466**	11.087**	437028.0**
Error	96	362.8	1.46	2.006	132.9	72.59	0.03977	4.074	81720
	146								

* 1-9 scale data

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[11] Data presented for one season
[12] Data for one season