IMPROVEMENT OF RESISTANCE TO *FUSARIUM* ROOT ROT THROUGH GENE PYRAMIDING AND VALIDATION OF SSR PVBR87 MARKER IN COMMON BEAN

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DECLARATION

I declare that this is my original work and has never been presented for a degree in this or any other university or institution of higher learning.

Signed..... Date 5th - May - 2011

Obala Jimmy

This thesis has been submitted on our approval as supervisors

Signed..... Date 5-5-2011

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DEDICATION

To Segawa David

And to Ann, Elizabeth and Arthur

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ABSTRACT

Fusarium root rot caused by *Fusarium solani* f. sp. *phaseoli* is among the most serious diseases of the common bean in Uganda causing total crop loss in susceptible cultivars. Studies have indicated that 2-9 genes located at different loci govern resistance to *Fusarium* root rot among different resistance sources. Accumulation of several of these genes from the different sources into a single genetic background has been proposed to result in an increased level of resistance to *Fusarium* root rot, and more effective transfer of this resistance into consumer-preferred cultivars. Use of molecular markers together with phenotypic selection could speed up breeding progress for *Fusarium* root rot resistance. A simple sequence repeat (SSR) PVBR87 marker linked to *Fusarium* root rot resistance quantitative trait loci (QTL) was identified in a previous study but its use in identifying resistant genotypes outside the original two mapping populations has not been determined. This study estimated the number of pyramided *Fusarium* root rot resistance genes from four sources of resistance, their interaction and effectiveness in improving *Fusarium* root rot resistance levels. The study also validated the association of the SSR PVBR87 marker with resistance to *Fusarium* root rot in an additional population.

The study was conducted at the International Centre for Tropical Agriculture (CIAT) based at the National Agricultural Research Laboratories – Kawanda, Uganda. Four *Fusarium* root rot resistant (R) inbred lines: MLB-48-89A (48), MLB-49-89A (49), G2333 (G2) and G685 (G6), and two susceptible (S) lines: K20 and Kanyebwa (Kan) were used in the study. A double cross (DC) was developed from the four resistant parents. The DC F_1 and each resistant parent were crossed to the two susceptible cultivars to form five-parent crosses and single crosses, respectively. Parental, F_1 and F_2 populations were subjected to *Fusarium solani* f. sp. *phasoeli* isolate-3. Twenty one days

after planting, symptom severity was assessed on a scale of 1-9 (varied at 1,2,3,4,5,6,7,8,9). Plants of each cross were grouped into resistant (score 1-4) and susceptible (score 5-9). F_2 plants of K20 x G2 and 49 x Kan were screened with SSR PVBR87 marker. The estimated number of genes and gene interactions were determined using X^2 goodness-of-fit test (P = 0.05) and means were compared by "Students t-test" (P = 0.05). The association of SSR PVBR87 marker to *Fusarium* root rot resistance was determined using X^2 test of independence and single marker regression analysis (P = 0.05).

Two to three genes segregated in the R x R single crosses and at least four genes segregated in the double cross population indicating three of the four resistant parents differed from each other by at least one gene and two of the four parents have at least one gene in common. Genetic effects among the crosses included additive and dominance effects and epistatic interactions. Five-parent crosses performed better than the single crosses, demonstrating the potential of using combined resistance in improving resistance to *Fusarium* root rot in susceptible bean cultivars. The SSR PVBR87 marker showed significant association to *Fusarium* root rot resistance in both K20 x G2 and Kan x 49 indicating its stability in different genetic background but still requires further validation in different environments and in additional genetic backgrounds to determine its use for marker-assisted breeding for improving resistance to *Fusarium* root rot.

The genes responsible for the higher levels of *Fusarium* root rot resistance in the pyramids are not specifically known. It is necessary that these resistance genes be tagged with molecular markers. Tagging of the genes with molecular markers would provide knowledge of their genomic locations, the nature of their interactions and also facilitate the transfer of these genes or alleles, through molecular marker-assisted gene introgression, into other agronomically superior, but *Fusarium* root

rot susceptible cultivars. Since no selection for *Fusarium* root rot resistance or any other desirable agronomic traits was practiced in this study, there is need to select between and within families from among the five-parent cross populations and the single crosses for resistance to *Fusarium* root rot. However, the predominance of non-additive gene effects for *Fusarium* root rot resistance, especially in the five-parent crosses suggests that selection for resistance would be more effective at advanced generations of selfing. The bean parents used in constructing the five-parent cross populations are of diverse seed character, growth habit, maturity period, and have varied response to several abiotic and biotic constraints. There is also need to select for these traits in the populations developed in this study as these traits eventually affect acceptability of any potential new variety. The amounts of phenotypic variation explained by the SSR PVBR87 in two populations were low; hence, there is still need to further validate the marker in additional populations and in several environments to determine its efficacy for marker-assisted breeding for *Fusarium* root rot resistance.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 The common bean

The common bean (*Phaseolus vulgaris* L.) belongs to the family Leguminosae, subfamily Papilionoideae, tribe Phaseoleae, subtribe Phaseolinae (Gepts, 2001). All species of the genus are diploid and most have 22 chromosomes (2n = 2x = 22) with a few species showing an aneuploid reduction to 20 chromosomes (Gepts, 2001). The genetic makeup of the common bean is described by Gept (2001). It is one of the smallest in the legume family at 625 Mbp per haploid genome. Highly repeated sequences comprise some 20% of the genome. A consensus molecular linkage map, correlating some 12 maps, has been established based on molecular and phenotypic markers. Major genes or quantitative trait loci for the domestication syndrome have been located on the linkage map, as have clusters of resistance genes and resistance gene analogs to viral, fungal, and bacterial diseases, and genes for *Rhizobium* nodulation, canning quality, and drought tolerance. In addition, several unmapped genes, especially for disease resistance and seed color and color pattern, have been tagged with molecular markers.

Over 30 species of the genus *Phaseolus* have been reported (Debouck, 1999). Of these, only five, namely; *Phaseolus vulgaris* L. (common bean), *Phaseolus polyanthus* Greenman (year bean), *Phaseolus coccineus* L. (scarlet runner bean), *Phaseolus acutifolius* A. Gray (tepary bean) and *Phaseolus lunatus* L. (lima bean) are known to be domesticated (Debouck, 2000). Among these

species, common bean is by far the widest grown in the world, occupying over 85% of the production area (Singh, 2001). Common bean originated in Latin America and two commercial classes exist; snap and dry beans (Singh, 2001).

Two distinct gene pools, namely Andean and Middle-American, are recognized in the wild (Koinange and Gepts, 1992) and cultivated bean (Evans, 1973). In addition to phaseolin seed protein, an evolutionary marker (Gepts, 1993), morphological and agronomic traits (Singh *et al*., 1991b), allozymes (Singh *et al*., 1991a), molecular markers (Haley *et al*., 1994), and partial reproductive barriers (Gepts and Bliss, 1985) have been used to describe the two gene pools. Genotypes of the Andean gene pool are large-seeded (>40 g per 100 seed weight) while those of the Middle American gene pool are small- (<25 g per 100 seed weight) to medium- (25-40 g per 100 seed weight) seeded (Evans, 1980). Singh *et al*. (1991a) 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.

1.1.2 Importance of common bean

Beans are grown for subsistence agriculture, local, regional and international markets and play an important role in food security and income generation (Buruchara, 2006). Beans account for 75% of the food legumes traded in the world (Broughton *et al*., 2003). Beans constitute an essential part of the diet for over 500 million people in Africa and Latin America and are an important source of protein, vitamins and minerals in the human diet, especially in developing countries (Broughton *et al*., 2003). In Africa, a continent where over 30% of households live below the poverty line (World Bank, 2006), beans are currently valued as 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 (CIAT, 2008). In sub-Saharan Africa Burundi, Ethiopia, Kenya, Rwanda, Tanzania and Uganda are the major producers (FAO, 2005). In Uganda, beans provide up to 25% of the total calories and 45% of the total dietary protein. The crop is also an important source of income in Uganda due to the increasing demands both in the domestic and export markets (NARO, 2000). In addition to its nutritional value, beans also contribute greatly to soil fertility through symbiotic nitrogen fixation (Serraj and Sinclair, 1998)

1.1.3 Common bean production constraints

The common bean suffers from several abiotic and biotic production constraints (Buruchara, 2006). Important abiotic constraints include low soil fertility, mainly deficiency of nitrogen, phosphorus, and zinc, and toxicities from aluminium and manganese (Singh, 2001). Drought affects bean production most strongly in regions with high temperatures (>30°C during daytime and/or >20°C at night) and at low elevations (below 650 m) in the tropical low lands (Singh, 2001).Of the biotic stresses, numerous insect pests attack all parts of common bean during all stages of growth, from seedling to stored product with the major ones being the beanfly (*Ophiomyia* spp), foliage beetles (*Ootheca* spp and *Medythia quaterna*), black bean aphid (*Aphis fabae*), striped beetle (*Alcidodes leucogrammus*), flower thrips (*Megalurothrips sjostedti*), and common white fly (*Bemisia tabaci*) (Abate and Ampofo, 1996). Others include leafhoppers (*Empoasca spp.*), cutworms (*Agrotis* spp. and *Spodoptera* spp.), blister beetles (*Mylabris* spp. and *Coryna* spp.), pod borer (*Maruca testularis*), African bollworm (*Helicoverpa armigera*) and pod sucking bugs (Abate and Ampofo, 1996). The bean weevil *Zabrotes subfasciatus* Boheman, in warm tropical and subtropical environments and *Acanthoscelides obtectus* (Say), in cool and temperate environments, cause severe

losses when dry beans are not properly stored (Wortmann *et al*., 1998). The red spider mite (*Tetrunychus cinnabarinus*) is another serious pest of common bean (Abate and Ampofo, 1996).

Diseases are also a major constraint to bean production and may be bacterial, fungal and viral in nature (Wortmann *et al*., 1998). The diseases include angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferr], rust (*Uromyces appendiculatus* Pers.), bean common mosaic virus (BCMV), common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* Smith) and floury leaf spot [*Mycovellosiella phaseoli* (Drummond) Deighton], which are more important in the low altitude high temperature areas. Anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.], halo blight [*Pseudomonas syringae* pv. *phaseolicola* (Burkh.)], bacterial brown spot (caused by *Pseudomonas syringae* pv. *syringae* van Hall), ascochyta blight [*Phoma exigua* var. *diversipora* (Bub.) Boerma] and the root rots being more important in high altitude and low temperature areas of Uganda (Opio *et al*., 2001).

1.1.4 Bean root rot

Bean root rot (BRR) is a complex of soil-borne fungal pathogens that include *Fusarium solani* f. sp. *phaseoli* (*Fusarium* root rot), *Rhizoctonia solani* (*Rhizoctonia* root rot), *Macrophomina phaseolina* (Charcoal root rot) and *Pythium* spp. (*Pythium* root rot) (Abawi and Pastor-Corrales, 1990). In Uganda, especially in south-western highland regions, root rot is one of the most serious constraints to bean production (Spence, 2002; Mikankusi, 2008), and can cause 100% crop loss in susceptible cultivars (Tusiime, 2003). Root rot caused by *F. solani* f. sp. *phaseoli* (FSP) is considered the most serious and widespread soil-borne pathogen of beans causing yield losses of up to 84% (Park and Tu, 1994). In Uganda, FSP has been found to be predominant often occurring together with *Pythium* spp. and was also found to be even more destructive in screenhouse test (Tusiime, 2003). This highlights the need to address *Fusarium* root rot if BRR problem is to be controlled.

Use of resistant bean cultivars is the most effective and environmentally-friendly control measure for *Fusarium* root rot (CIAT, 2003). Pyramiding of resistance genes from multiple sources could be a way of increasing both the level and duration of resistance to *Fusarium* root rot (Mukankusi 2008), in addition to broadening the genetic base of cultivated common bean (Singh, 2001). Bean breeders are increasingly using molecular approaches, such as marker-assisted breeding, to improve resistance to diseases and tolerance to abiotic stresses (Beaver and Osorno, 2009). Use of such approaches may overcome some of the common limitations encountered by conventional selection for quantitative traits (Asins, 2002; Kelly and Vallejo, 2005) such as *Fusarium* root rot resistance.

1.2 Problem statement

There has been a steady increase in the area planted to beans in Uganda from 615,000ha in 1996 to 849,000ha in 2006 but production per unit area declined from 599kg/ha in 1999 to 499kg/ha in 2006 (FAOSTAT, 2007). The decline in production has been attributed to a number of factors among them are diseases with BRR being identified as one of the major constraints (Wortmann *et al*., 1998; Mukankusi, 2008). Of the BRR diseases, *Fusarium* root rot caused by the fungus *Fusarium solani* f. sp. *phaseoli* (FSP) is considered among the most serious and widespread soil-borne diseases occurring in most bean fields throughout the world (Abawi and Pastor-Corrales, 1990; Harveson *et al*., 2005). It is currently one of the major diseases affecting common bean production in Uganda (Tusiime, 2003) and hence reducing food security. Most of the currently identified sources of resistance to *Fusarium* root rot are of Middle-American origin with only moderate levels of resistance and they have undesirable characteristics such as late maturity, small or black seeded or climbing growth habit (Beebe *et al*., 1981; Burke and Miller, 1983; Mukankusi, 2008). These characteristics limit the acceptability of these bean types by a large percentage of bean farmers in

Africa (Mukankusi, 2008). The large-seeded market class bean genotypes in Uganda such as K20 (Nambale), Kanyebwa and K132 (Kawomera) are highly susceptible to *Fusarium* root rot (Mukankusi, 2008). A previous study in Uganda identified five bean genotypes; MLB-49-89A, RWR719, G2333, G685 and MLB-48-89A as good sources of resistance to *Fusarium* root rot (Mukankusi, 2008). The study showed that variability existed in the number of genes and gene actions governing *Fusarium* root rot resistance in these genotypes, and that the genes were located at different loci. The location of the genes at different loci suggests that accumulation of the different *Fusarium* root rot resistance genes from the different sources into a single line or cultivar can be a way to increase levels of resistance to *Fusarium* root rot (Mukankusi, 2008). It is, however, not known whether combining the different sources of *Fusarium* root rot resistance into a single cultivar would increase the level of resistance to the disease in susceptible market class cultivars. In addition, resistance to *Fusarium* root rot is genetically complex and difficult to evaluate phenotypically, hence, the efficiency of phenotypic selection is low resulting into slow breeding progress (Román-Avilés and Kelly, 2005). Marker-assisted selection (MAS), used to select indirectly for resistant genotypes, may facilitate improvement of disease resistance for a trait like *Fusarium* root rot resistance, where field selection is laborious and destructive sampling is required to identify resistance (Schneider *et al*., 2001; Román-Avilés and Kelly, 2005). Identification of suitable molecular markers linked to *Fusarium* root rot resistance could speed up the breeding progress for the trait. One such marker is the simple sequence repeat (SSR) PVBR87 that has been shown to be linked to resistance to *Fusarium* root rot in two recombinant inbred line (RIL) populations: K132 x MLB-49-89A and K20 x MLB-49-89A, in which MLB-49-89A was the resistance source (Kamfwa, 2010). However, the use of this marker in identifying resistant genotypes outside the two mapping populations has not been determined.

1.3 Justification of the study

Market class bean cultivars that are resistant to *Fusarium* root rot are needed in Uganda. Accumulation of resistance genes to *Fusarium* root rot into a single genetic background has been suggested as a way to increase levels of resistance in agronomically superior large-seeded bean cultivars such as K20 and Kanyebwa (Mukankusi, 2008). However, there is need to determine whether levels of resistance reached due to pyramided resistance genes are better than that exhibited by single resistance sources. This information would help guide bean breeders in Uganda and elsewhere on whether to incur costs in developing and utilizing a cultivar with such a combination of resistance genes.

Although, it is highly desirable to combine as many resistance genes as possible in a new cultivar in order to make resistance as sustainable as possible, it is hardly feasible to track the accumulated genes in the new cultivar based on phenotypic evaluation data alone (Eibach *et al*., 2007). Suitable molecular markers tagged to the *Fusarium* root rot resistance genes from the different resistance sources may help to overcome this problem. However, markers identified as linked to a trait in a given mapping population needs to be validated before deployment for MAS (Langridge *et al*., 2001; Collard *et al*., 2005; Collard *et al*., 2008). Hence, validation of SSR PVBR87 marker associated with *Fusarium* root rot resistance is a critical step towards the development of marker-assisted breeding for *Fusarium* root rot resistance in common bean in Uganda.

This thesis therefore focuses on; firstly, determining whether resistance genes combined from multiple sources of *Fusarium* root rot resistance would give a higher level resistance than resistance

from single sources of resistance, and secondly, validating SSR PVBR87 marker association with *Fusarium* root rot resistance in independent populations.

1.4 Objectives of the study

The goal of this study was to contribute to the development of bean cultivars with increased and durable resistance to *Fusarium* root rot. The specific objectives were to;

1. Estimate the number of pyramided *Fusarium* root rot resistance genes among four sources of resistance.
2. Determine effectiveness of pyramided resistance genes from different sources in improving levels of *Fusarium* root rot resistance in common bean.
3. Validate the utility of SSR PVBR87 marker in identifying *Fusarium* root rot resistant common bean genotypes outside the mapping population.

1.5 Hypotheses

1. Different number of *Fusarium* root rot resistance genes from different sources can be pyramided in common bean.
2. Pyramided genes for *Fusarium* root rot resistance from different sources are more effective in improving levels of *Fusarium* root rot resistance in susceptible bean cultivars than resistance from single sources.
3. SSR PVBR87 marker associated with *Fusarium* root rot resistance in RIL mapping populations of K132 x MLB-49-89A and K20 x MLB-49-89A can identify *Fusarium* root rot resistant genotypes outside the mapping population.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Fusarium* root rot

The common bean is recognized as the main host of *Fusarium solani* f. sp. *phaseoli* (FSP), which causes *Fusarium* root rot. FSP has also been found to infect other plants, mainly legumes (Abawi, 1980; Gray *et al*., 1999). It has been reported on mung bean (*Vigna radiate* 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 max* L.) on which it causes sudden death syndrome (Abawi, 1980). Although FSP is commonly isolated from bean plant tissue, some isolates may not be pathogenic to beans. Saprophytic forms of FSP species are very common and often occur together with pathogenic species (Hall, 1996; Tusiime, 2003).

Stable resistance depends on the capacity of a line to resist infection from a whole range of pathogen strains in a population (Agrios, 2005). In a study in south-western Uganda, the effectiveness of management of *Fusarium* root rot using resistant cultivars was reported to vary from location to location and sometimes from season to season (Tusiime, 2003). This was thought to be due to strain differences within FSP population. However, when FSP isolates were collected from south-western Uganda and other parts of Africa, and divided into two groups, namely, the “fast-growing” and the “slow-growing”, the fast-growing isolates were non-pathogenic while the slow-growing were very

pathogenic on common bean (Tusiime, 2003). The pathogenic FSP isolate were also found to be highly uniform after molecular analysis, implying that considerable improvements to the disease could be achieved by using only one pathogenic isolate. Mukankusi (2008) tested four pathogenic isolates for pathogenicity, namely, FSP-1, FSP-2, FSP-3 and FSP-4 and found that FSP-3 was the most pathogenic of the four isolates, as it caused 100% disease incidence and severities ranging from 3.6 to 8.6, on a rating scale of 1-9. The current study used FSP-3 as it has been shown to result in good infection levels necessary to differentiate between resistance levels of different cultivars.

Fusarium root rot in common bean is characterized by reddish-brown lesions along the tap roots and lower hypocotyls. Diseased areas of the plant enlarge with age and gradually turn brown. Symptoms may extend up the main root and into the stems of older plants. Longitudinal cracks may develop in older lesions and the cortical tissues may be discoloured and decayed (Figures 1a and 1b). Clusters of fibrous roots may develop above the shrivelled tap root (Figure 1c).

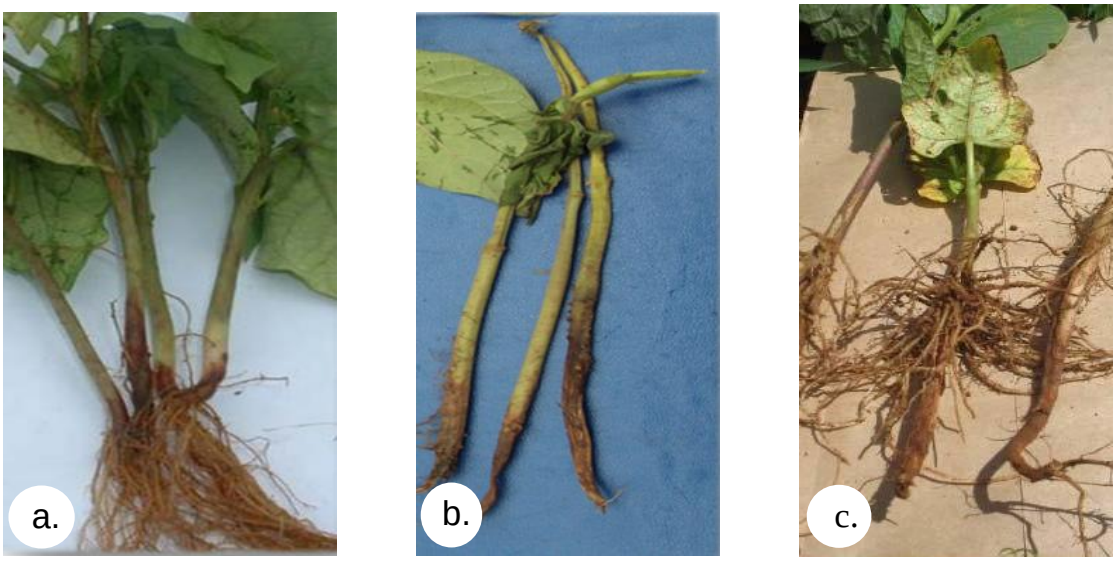

Figure 1: Symptoms of *Fusarium* root rot in common bean

a. reddish brown lesions on taproot and hypocotyls; b. decayed root system; c. clusters of fibrous roots above shriveled tap root.

These fibrous roots may keep the plant alive and, under ideal conditions, a few above-ground symptoms will be noted. Plants may be stunted, have abnormal colour and grow more slowly than healthy plants, resulting in an uneven plant stand (Abawi *et al*., 2006). Plants infected with *Fusarium* root rot are seldom killed but remain stunted and chlorotic, exhibit premature leaf drop and have poor pod fill (Abawi and Pastor-Corrales, 1990; Burke and Hall, 1991; Schneider and Kelly, 2000). The disease usually causes little damage in unstressed plants. However, under conditions of reduced root growth, *Fusarium* root rot can destroy a bean crop and even the highest levels of resistance to the disease will be overcome by the pathogen when roots are flooded or deprived of oxygen for short periods (Burke and Hall, 1991). The disease is particularly severe on large-seeded Andean bean genotypes due to a lack of genetic resistance (Abawi and Pastor-Corrales, 1990; Schneider *et al*., 2001).

Fusarium root rot management options include crop rotation, correcting soil fertility levels and reducing soil compaction and use of resistant cultivars. The disease, however, cannot be completely eliminated from fields because the pathogen survives in soil as chlamydospores for long periods of time (Burke and Hall, 1991) making use of resistant cultivars a more viable alternative method of controlling the disease. Castro *et al*. (2003) and CIAT (2003) have illustrated that genetic resistance is the most economical and environmentally appropriate strategy for disease control in plants.

2.2 Resistance to *Fusarium* root rot

Resistance to *Fusarium* root rot has been found to be quantitative in nature (Miller and Burke, 1985; Schneider and Kelly, 2000). Lack of complete resistance to *Fusarium* root rot and observable differences in levels of susceptibility, support the proposed quantitative inheritance of this trait (Bagget *et al*., 1965). The strong environmental influence on disease incidence and severity ratings

for *Fusarium* root rot provides additional evidence for the complex inheritance of inheritance (Miller and Burke, 1985). Reports from past studies suggest that two to nine genes govern *Fusarium* root rot resistance among different resistance sources (Azzam, 1958; Smith and Houston, 1960; Bravo *et al*., 1969; Hassan *et al*., 1971; Mukankusi, 2008). Similarly, over thirty quantitative trait loci (QTL), with many minor in effect, associated with resistance to *Fusarium* root rot have been reported (Schneider *et al*., 2001; Chowdbury *et al*., 2002; Navarro *et al*., 2003; Román-Avilés and Kelly, 2005; Kamfwa, 2010). These observations emphasize the need to consider *Fusarium* root rot resistance as a quantitative trait (Schneider *et al*., 2001).

Small- and black-seeded Middle-American bean cultivars are generally more resistant to *Fusarium solani* f.sp. *phaseoli* than are the large- and red-seeded cultivars (Beebe *et al.,* 1981). The most preferred market class large-seeded bush bean cultivars in Uganda are highly susceptible to root rot and are continuously becoming difficult to produce (Mukankusi, 2008). The small-seeded genotypes of Middle-American origin although not completely resistant to *Fusarium* root rot have been used to improve resistance in the preferred large-seeded bean genotypes (Beebe *et al*., 1981), however, improvement of resistance to *Fusarium solani* f.sp. *phaseoli*, especially in large-seeded dry and snap bean types, has been limited in spite of considerable research efforts to elucidate its genetic control. *Fusarium* root rot is particularly severe on large-seeded Andean bean genotypes due to lack of genetic resistance in them (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 (Beebe *et al*., 2000; Islam *et al*., 2004). In spite of these limitations, Silbernagel (1987) developed a resistant large-seeded bean cultivar, FR266 that belongs to the Andean gene pool through genetic introgression using a small- and black-seeded variety, N203 of Middle-American origin, as source of resistance. Similarly, Schneider *et al.*, (2001) successfully used FR266 as a

source of *Fusarium* root rot resistance in crosses with beans from the Andean gene pool. The location of several resistance genes at different loci suggests that pyramiding of genes from multiple sources of resistance into a single genetic background could increase the levels of resistance to *Fusarium* root rot among the susceptible market class bean cultivars in Uganda (Mukankusi, 2008).

2.4 Pyramided resistance genes and their interactions

Gene pyramiding is the accumulation of multiple desirable genes from multiple parents into a single genotype (Pedersen and Leath, 1988; Kloppers and Pretorius, 1997; Allard, 1999; Ye and Smith, 2008) A pyramid could be constructed with major genes, minor genes, defeated genes, effective genes, ineffective genes, race-specific genes, nonrace-specific genes, or any other type of host gene that confers resistance (Pedersen and Leath, 1988). Gene pyramiding has been described as an important breeding strategy for disease resistance in crops (Knott, 1989) in which interaction between genes may occur such that resistance of a combination of genes is better than that conditioned by any of the genes individually (Dyck and Sambroski, 1982).

Pyramiding resistance genes can be a solution to improve on both the level of resistance and on durability (Nelson, 1972). Knowledge of the number of genes to assemble is of great importance for gene pyramiding (Ye and Smith, 2008). Gene pyramiding has been successfully applied in combining multiple genes not only for qualitative disease resistance such as bacterial blight resistance (Huang *et al*., 1997) and blast resistance (Hittalmani *et al*., 2000) in rice, but also for quantitative resistance such as stripe rust resistance in barley (Castro *et al*., 2003). Mukankusi (2008) reported that *Fusarium* root rot resistance is governed by two to nine genes located at different loci with the genes having additive, dominance, recessive and epistatic interactions among eight sources of resistance. The presence of several loci suggests that pyramiding of resistance genes

from the different parents could increase levels of resistance to *Fusarium* root rot (Mukankusi, 2008). However, in self-pollinated crops such as the common bean, allelism limits the number of resistance genes that can be combined in a single homozygous plant (Hooker, 1967). Because allelic genes cannot be combined in the same genotype (Ye and Smith, 2008), the present study tested allelic differences among resistance genes to *Fusarium* root rot among four different sources of resistance using the X^2 goodness-of-fit test. Understanding the type of gene action controlling a trait is important in determining when selection is most effective (Jagtap, 1986). For example, when additive effects are larger than the non-additive, selection in the early segregating generations would be effective, while, if the non-additive portion is larger than the additive, the improvement of the trait need intensive selection in the later generations (Ojo *et al*., 2006). Linear relationships between the performance of parents and their progenies (Lynch, 1991; Fenster and Galloway, 2000) were used to determine type of gene interactions conditioning resistance to *Fusarium* root rot in this study.

2.5 Effectiveness of pyramided resistance genes and their action in improving levels of disease resistance

A limited number of scientific reports have been published quantifying the effects of pyramided resistance genes in different plant-pathosystems (Tan *et al*., 2010). In common bean, Terán and Singh, (2009) used gamete selection to pyramid resistance to white mold. Despite the low to intermediate levels of resistance to white mold in eight parents in that study, they were able to obtain an average of 20.5% gain in white mold resistance in two populations. Similarly, Asensio-S.-Manzanerra *et al*. (2005), using gamete selection pyramided resistance to common bacterial blight and halo blight in two dry bean populations and were able to obtain 3 to 25% increase in resistance. Barloy *et al*. (2007) using marker-assisted selection showed a higher level of resistance against

cereal cyst nematodes in wheat when pyramiding resistance genes *CreX* and *CreY*. Several groups have reported on the pyramiding of bacterial blight resistance genes in rice and observed higher resistance levels and obtained additionally, a broader spectrum of resistance (Yoshimura *et al*., 1995; Huang *et al*., 1997; Singh *et al*., 2001; Zhang *et al*., 2006). When pyramiding multiple QTL involved in resistance against barley stripe rust, Richardson *et al*. (2006) reported higher levels of resistance than achieved with individual QTL. However, the study of Sharma *et al*. (2004) did not show improved resistance when pyramiding the brown planthopper resistance genes *Bph1* and *Bph2* in rice, which resulted in a resistance level of the pyramided line equivalent to that of the *Bph1*-single introgression line.

Although it is highly desirable to combine as many resistance genes as possible in a new cultivar, it is hardly feasible to track the accumulation of resistance genes in a new breeding line based only on phenotypic evaluation data (Eibach *et al*., 2007). The use of molecular markers can help to overcome this problem (Luo *et al.,* 2001; Fischer *et al.,* 2004). Use of molecular markers is becoming increasingly important for breeding purposes in a lot of agricultural crops like wheat (Gupta *et al.,* 2005; Yang *et al.,* 2005; Sardesai *et al.* 2005), rice (Sharma *et al.,* 2004, Ashikari and Matsuoka, 2006), maize (Widstrom *et al.,* 2003), but also common bean (Kelly *et al*., 1995; Ragagnin *et al*., 2003; Miklas *et al*., 2003; Miklas *et al.,* 2006). The use of molecular markers is particularly relevant for polygenic traits (Eibach *et al*., 2007) such as resistance against *Fusarium* root rot in common bean. The procedure of developing molecular markers for marker-assisted breeding of crop plants has been discussed with one of the most critical steps involved being marker validation (Langridge *et al*., 2001; Collard *et al*., 2005; Collard *et al*., 2008).

2.6 Validation of molecular markers

Marker validation refers to the process of confirming the effectiveness of markers associated with putative QTL in one population for indicating the target phenotype in independent populations and other germplasm, which incorporate those QTL (Sharp *et al*., 2001; Collins *et al*., 2003; Collard *et al*., 2005; Collard *et al*., 2006). Markers are also validated to test for the presence of the marker on a range of cultivars and other important genotypes (Sharp *et al*., 2001). Studies have warned that there is danger in assuming that marker-QTL linkages will remain in different genetic backgrounds or in different testing environments, especially for complex traits (Reyna and Sneller, 2001) such as *Fusarium* root resistance. This is because detection of QTL-marker linkages can be influenced by several factors such as QTL magnitude, the existence of other linked QTL, the mapping population size, phenotypic assessment accuracy, genotyping errors, lost data and environmental effects (Collard *et al*., 2005; Francia *et al*., 2005). An SSR PVBR87 marker was identified to be linked to a QTL associated with *Fusarium* root rot resistance in two recombinant inbred line populations of K132 x MLB-49-89A and K20 x MLB-49-89A accounting for 34% and 14% of the phenotypic variations in *Fusarium* root rot score, respectively (Kamfwa, 2010). A molecular marker such as SSR PVBR87 linked to a QTL associated with resistance can be utilized to enhance conventional breeding approaches by providing information that breeders can use to make choices of which resistance loci to combine in future bean cultivars (Román-Avilés and Kelly, 2005). The marker can also be used for marker-assisted selection for *Fusarium* root rot resistance as phenotypic selection for the trait is laborious, destructive and strongly influenced by the environment (Schneider *et al*., 2001; Román-Avilés and Kelly, 2005). Thus, for a marker to be most useful in breeding programs, it should reveal polymorphism in different populations derived from a wide range of different parental genotypes (Langridge *et al*., 2001; Fasoula *et al*., 2004). The efficacy of SSR PVBR87 marker

should therefore be tested to determine whether the marker can identify *Fusarium* root rot resistant plants in independent populations.

Two to nine genes located on different loci have been reported to condition *Fusarium* root rot resistance among different resistance sources. Pyramiding of these resistance genes into a single genetic background has been suggested as a way of increasing the levels of resistance to disease, however, allelism limits the number of genes that can be pyramided. It is therefore necessary to test for allelic differences among *Fusarium* root rot resistance genes combined from different sources of resistance. It is also necessary to determine whether levels of resistance attained due to resistance gene pyramiding is better than resistance from the single sources of resistance. Indirect selection based on molecular markers linked to QTL associated with resistance to *Fusarium* root rot could overcome the limitation of phenotypic selection. However, markers identified to be linked to putative QTL for resistance need to be validated before they are deployed in marker-assisted breeding programs. This is because a marker is only useful in breeding programs if it can reveal polymorphism in different populations derived from a wide range of different parental genotypes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at the International Centre for Tropical Agriculture (CIAT) based at the National Agricultural Research Laboratories (NARL) – Kawanda, Uganda.

3.2 Parents and hybridization

Six common bean genotypes that had been fully characterized for resistance to *Fusarium* root rot were used (Table 1). Two single cross F_1 populations derived from the four resistant parents: MLB-48-89A (48), MLB-49-89A (49), G2333 (G2) and G685 (G6), provided by CIAT, were intercrossed to form two related double cross (DC) populations: (G2 x G6) x (49 x 48) and (49 x 48) x (G2 x G6). The F_1 seed of the DC populations was divided into two parts. The first part was crossed to each of the two susceptible cultivars, K20 and Kanyebwa, to form two five-parent cross populations: K20 x [(49 x 48) x (G2 x G6)] and Kan x [(49 x 48) x (G2 x G6)]. Single crosses between each of the four resistant parents and the two susceptible cultivars were also developed namely; K20 x 49, K20 x 48, K20 x G2, K20 x G6, Kan x 49, Kan x 48, Kan x G2 and Kan x G6. In all crosses, as advised by Singh (1994), the susceptible but most popular cultivars were used as seed parents to ensure that they made a 50% genetic contribution to retain as many as possible of their already desirable attributes. The second part of the DC F_1 seed was used for population advancement. All crosses were advanced to F_2 generation by selfing and seed from each cross was harvested in bulk.

Table 1: Parents, their pedigree, estimated number of Fusarium root rot (FRR) resistance genes, origin and agronomic characteristics

Genotype	Pedigree	No. of FRR resistance genes	Origin	Agronomic characteristics
G685 (G6)	Moncure no.12 (PI182007)	3-5	Mexico	Moderately resistant to FRR. Small and red to maroon seeded with climbing growth habit. Drought tolerant. Yield potential: 2,500-4,000kg ha^{-1}. Low marketability.
G2333 (G2)	Gentry 21835 Colorado Teopisca/PI311998	3-5	Mexico	Moderately resistant to FRR. Small and red-seeded with climbing growth habit Yield potential: 2,500-4,000kg ha^{-1}. Low marketability.
MLB-49-89A (49)	A 240 X Inyumba	2-6	DRC	Moderately resistant to FRR. Black and medium seeded with semi-climbing growth habit. Very low marketability.
MLB-48-89A (48)	A 240 X Inyumba	2-3	DRC	Moderately resistant to FRR. Grey and small seeded with semi-climbing growth habit. Low marketability.
K20 (GLP2)	Roseccoco	-	CIAT	Susceptible to FRR. Large and red-mottled seed with bush growth habit. Yield potential: 1500-2500kg ha^{-1}. Marketable and tolerant to most diseases.
Kanyebwa (Kan)	Landrace	-	Uganda	Susceptible to FRR. Large and red-speckled sugar bean with bush growth habit. Tasty and marketable.

Mukankusi (2008)

Each parent per cross combination was planted in 10 buckets. NPK (1:1:1) fertilizer in liquid form was added to the soil at a rate of $3x10^{-3}$kg ml^{-1} a few days before planting and thereafter every 7 days. The plants were watered once a day in the morning. Due to differences in flowering dates of the parents, planting was staggered so as to synchronise flowering. To ensure adequate seed

production four crossing blocks were planted. Crossing was done by hand pollination using the emasculation and hooking method (Buishand, 1956), using all the available flowers. To avoid contamination of new crosses from previous parental lines, forceps used to tease open the flowers were sterilized in 70% ethanol between genotypes. The crossing exercise was carried out between 07:00hrs and 10:00hrs, and after 17:00hrs to target the cooler temperatures at those times. F_1 plants were advanced to F_2 by selfing.

3.3 *Fusarium solani* f. sp. *phaseoli* inoculum, inoculum preparation and management of evaluation trial

An isolate of *F. solani* f. sp. *phaseoli*, FSP-3, obtained from south-western Uganda (Mukankusi, 2008) was used. The isolate had been maintained at the CIAT-laboratory at the National Agricultural Research Laboratories (NARL) – Kawanda on Potato Dextrose Agar (PDA) slants at 5^{o}C. FSP inoculum was produced by sub-culturing the fungus from the slants onto PDA plates by streaking and allowed to grow for a period of up to 21 days. Infested sorghum seed were used as a medium for FSP inoculation in the soil as routinely done at CIAT. Duran glass bottles (Aldrich, Z305197-10) of 500ml capacity were partially filled with sorghum seed (2/3 capacity) and 150ml of water was added. The bottles were then sealed and contents autoclaved for 1 hour at 120 ^{o}C. One PDA plate of the FSP-3 isolate was dissolved in 10ml of sterile deionised water to make slurry. The slurry 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. The mixture was incubated in the laboratory at 20-28^{o}C for 5 days to allow FSP-3 to grow, after which the bottles were opened to allow for evaporation of excess moisture and nutrient solution. After 21 days of incubation, the bottles were emptied, and the medium slowly dried to allow for maturation

of the fungal resting spores. Wooden trays in the screen house measuring (0.74 x 0.42 x 0.115) m^2 were partially filled (2/3 capacity) with steam sterilised loamy sand soil. Prior to planting, prepared inoculum was added to the soil at a rate of one 500ml bottle of inoculum per tray. Liquid NPK fertilizer (1:1:1) was used to fertilize the soil 3-4 days before planting. Assessment for *Fusarium* root rot severity was done 21 days after planting (Hassan *et al*., 1971).

3.4 Scoring for *Fusarium* root rot symptom severity

The severity of *Fusarium* root rot symptoms was assessed by carefully uprooting and washing the below ground parts of the plant (hypocotyls and roots) under running tap water. The severity of *Fusarium* root rot symptom was visually scored by assessing the lower hypocotyl discoloration using a rating scale of 1 - 9 (Chaudhary *et al*., 2006) (Figure 2; Table 2)

Figure 2: Effect of *Fusarium* root rot on bean roots

a. = score 1 and b. = score 9

Table 2: Disease rating scales used for *Fusarium* root rot screening

Disease score	Phenotypic description
1	No apparent infection
2	0.1 – 0.5 cm reddish brown lesion
3	0.5 – 1.0 cm reddish brown lesion covering half of the stem
4	1.0 – 1.5 cm reddish brown to brown lesion
5	1.5 – 2.0 cm brown to dark brown lesion, lesion girdling the stem
6	2.0 – 2.5 cm brown to dark brown lesion, lesion often associated with increasing intensity
7	2.5 – 3.0 cm brown to dark brown lesion, lesion often associated with increasing intensity
8	3.0 – 3.5 cm brown to dark brown lesion, lesion often associated with increasing intensity
9	Dead plant

3.5 Experiment 1: Estimation of number of pyramided *Fusarium* root rot resistance genes and their interaction

3.5.1 Evaluation of parents, F_2 of single crosses, and F_1 and F_2 of double crosses involving Fusarium root rot resistant genotypes

Parental genotypes: 48, 49, G2 and G6; F_2 populations of the crosses: 49 x 48 and G2 x G6; F_1 and F_2 populations of the crosses (G2 x G6) x (49 x 48) and 49 x 48) x (G2 x G6) were evaluated for their reaction to an isolate *F. solani* f. sp *phaseoli*. F_1 of the single-crosses (SC) were not evaluated. Population sizes were 240 seeds per parent, 260 seeds per F_2 SC, 200 seeds per F_1 of the double-crosses (DC) and 560 seeds per F_2 of the DC. In all, there were 3300 seeds in the experiment. Ten seeds were planted per row and there were 11 rows per tray with 15 wooden trays in each of the two replications. In each replication there were 12 rows for each of the four parents, 13 rows each of the two F_2 single cross progenies, 10 each of the two double cross F_1 progenies, 24 each of the two

double cross F_2 progenies and 15 of the susceptible check, K132. The planting arrangement in each replication was as indicated in Appendix 1 with individual trays being used as incomplete blocks. To assess uniformity between trays there was overlap of entries across trays. *Fusarium* root rot symptom severity was assessed 21 days after planting as described in section 3.4 above.

3.5.2 Data analysis

Frequency distribution curves aided the classification of plants into two categories of resistant and susceptible classes and provided insight into the nature of gene action in the evaluated populations. For Mendelian analysis of segregating populations, plants were categorized into resistant (score of 1-4) and susceptible (score of 5-9). Gene models were developed by taking into consideration the segregation patterns of the SC F_2, DC F_1 and F_2. For example whereas a SC F_2 plant is produced by the selfing of a heterozygous (Rr) F_1 plant, a DC F_1 plant can be produced from a cross between two heterozygous (Rr) SC F_1 plants, or between an heterozygous (Rr) SC F_1 and an homozygous (RR or rr) F_1 plant or between two homozygous RR and rr plants as illustrated for the segregation of a single locus in Table 3.

Table 3: Single locus segregation in the double cross F_1 and F_2 populations resulting from possible crosses between any two types of single cross F_1 plants

Single cross F_1[t]			Single locus segregation in a double-cross population					
			F_1			F_2		
P1 x P2	x	P3 x P4	R_1R_1	R_1r_1	r_1r_1	R_1R_1	R_1r_1	r_1r_1
R_1 r_1	x	R_1 r_1	¼	2/4	¼	3/8	2/8	3/8
R_1r_1	x	r_1 r_1	0	1/2	½	1/8	2/8	5/8
R_1R_1	x	R_1r_1	½	1/2	0	5/8	2/8	1/8
R_1R_1	x	r_1r_1	0	1/1	0	2/8	4/8	2/8

[t]Reciprocal crosses follow the same pattern of segregation therefore are not explicitly shown

There are several possible gene models that can be generated from the segregation in the crosses with two or more loci. For example, a cross between a homozygous (RR) and a heterozygous (Rr) SC F_1 plants for any number of loci (n) would result in an equal proportion of homozygous (RR) and heterozygous (Rr) individuals at each locus but zero homozygous recessive (rr) individuals in the F_1, while in the F_2, a very high number of homozygotes (RR) or heterozygotes (Rr) are produced with only $(1/8)^n$ individuals being homozygous recessive (rr) at all loci. Equally a cross between two homozygous SC F_1 plants (RR x rr) would only produce individuals heterozygous at all loci in the DC F_1 while segregation in the DC F_2 would result in a high frequency of individuals with the R_ genotypes and only $(1/4)^n$ individuals with homozygous (rr) genotype at all loci (Table 3). From these examples, the cross between two heterozygous SC F_1 plants heterozygous at each locus represents the most simple type of cross that could produce adequate number of resistant and susceptible plants that corresponds to the observed segregation in the double-cross populations in this study. This assumption is supported by the illustration of Fehr (1987) that a double cross F_1 segregates the same way as the F_2 of a single cross while a double cross F_2 segregates similarly to a single cross F_3. Therefore two, three and four gene segregation patterns were developed based on the above assumption (Table 4) and these segregation ratios were used for the χ^2 goodness-of-fit test.

Table 4: Expected ratios[y] for the segregation of double cross F_1 and F_2 from a cross of two single cross F_1 plants heterozygous at each segregating loci

No. of loci	Genotype	Phenotype	DC F_1	DC F_2
2 genes with duplicate	R_1_ _ _; _ _ R_2_	R	15/16	55/64
dominant epistasis for	$r_1r_1r_2r_2$	S	1/16	9/64
Resistance	**Expected ratio**	**R:S**	**15:1**	**55:9**
2 dominant genes and	R_1_ _ _ _ _; _ _R_2_ _ _ _ _	R	60/64	440/512
1 recessive genes	$r_1r_1r_2r_2r_3r_3$	R	1/64	27/512
	$r_1r_1r_2r_2R_3R_3$	S	1/64	27/512
	$r_1r_1r_2r_2R_3r_3$	S	2/64	18/512
	Expected ratio	**R:S**	**61:3**	**467:45**
2 dominant genes and	R_1_R_2_ _ _ _ _; _ _ _ _ R_3_R_4_	R	249/256	3745/4096
2 complementary genes	$r_1r_1r_2r_2r_3r_3r_4r_4$	S	1/256	81/4096
	$r_1r_1r_2r_2R_3r_3r_4r_4$	S	2/256	54/4096
	$r_1r_1r_2r_2r_3r_3R_4r_4$	S	2/256	54/4096
	$r_1r_1r_2r_2R_3R_3r_4r_4$	S	1/256	81/4096
	$r_1r_1r_2r_2r_3r_3R_4R_4$	S	1/256	81/4096
	Expected ratio	**R:S**	**249:7**	**3745:351**
2 dominant and 2	R_1_ _ _ _ _ _ _; _ _R_2_ _ _ _ _	R	246/256	3871/4096
recessive genes	$r_1r_1r_2r_2r_3r_3r_4r_4$	R	1/256	81/4096
	_ _ _ _ R_3_R_4_	S	5/256	117/4096
	_ _ _ _$R_3r_3r_4r_4$	S	2/256	54/4096
	_ _ _ _$r_3r_3R_4r_4$	S	2/256	54/4096
	Expected ratio	**R:S**	**247:9**	**3871:225**

DC = double cross; [Y]DC F_1 expected ratio same for single cross F_2 (Fehr, 1987)

Prior to conducting the χ^2 goodness-of-fit test, homogeneity of ratio test was performed to;

i) Assess the difference in segregation between the two replications.

ii) Test for cytoplasmic inheritance in the reciprocal DC F_1 and F_2 populations (Pozniak and Hucl, 2004).

The X^2 test of homogeneity was based on the Mather (1957) model:

$$\chi^2_{Heterogeneity} = \chi^2_{Total} - \chi^2_{Pool}$$

Where;

$$\chi^2_{Total} = \sum_{i=1}^{p}\sum_{i=1}^{p}((o_{ij}-e_{ij})^2/e_{ij})$$

$$\chi^2_{Pool} = \sum_{j=1}^{n}((\sum_{i=1}^{p}o_{ij}-\sum_{i=1}^{p}e_{ij})^2/\sum_{i=1}^{p}e_{ij})$$

p = Number of populations

n = Number of genotypic classes

o_{ij} = Observed frequency of the cell in the i^{th} row and j^{th} column

e_{ij} = Expected frequency of the cell in the i^{th} row and the j^{th} column

Σ = Summation

Where the homogeneity of ratio test indicated no difference in the segregation pattern of a cross between the two replications, data from the replications were added prior to χ^2 goodness-of-fit test. Similarly, where there was no significant deviation in the segregation ratios between the reciprocal DC F_1 and F_2 populations, data from reciprocal crosses were added prior to χ^2 analysis.

The Chi-square value for goodness-of-fit test was calculated using the model of Mather (1957) as follows:

$$\chi^2 = \sum_{j=1}((o_j-e_j)^2/e_j)$$

Where;

χ^2 = Chi-square

n = Number of genotypic classes

o_j = Observed score for the j^{th} plant

e_j = Expected score for the j^{th} plant

Σ = Summation

Additional information on the types of gene action conditioning resistance to *Fusarium* root rot in the resistant x resistant (R x R) crosses were obtained by comparing disease severity mean scores of parents with that of F_1 and F_2 generations. This was based on the following relationships:

(i) If gene action is purely additive F_1 mean should be positioned mid-way between the mean scores of parents of the respective progenies (Fehr, 1987);

(ii) Presence of non-additive gene action, that is, dominance and epistasis, would result in deviations of the progeny means from the mid-parent (MP) values (Fehr, 1987; Hallauer and Miranda, 1988);

(iii) In the absence of epistasis, the F_2 mean score would be intermediate to the MP value and the F_1 mean because the segregating F_2 has a half the heterozygosity and therefore half the mean relative to the F_1 mean (Lynch, 1991);

(iv) In the presence of epistasis the segregating F_2 generation would have a mean score greater or less than the average of the MP and F_1. The mean score of F_2 greater or less than the average of MP and F_1would indicate that genes combined for resistance have either a positive or a negative effect on level of resistance (Lynch, 1991; Fenster and Galloway, 2000).

Means of parents and their progenies were computed using the restricted (residual) maximum likelihood (ReML) analysis in GenStat (Release 12.2 PC/Windows; LAWES Agric Trust, 2010). The outline of the ReML analysis is presented in Table 5.

Table 5: The outline of the ReML analysis of the response of bean genotypes to *Fusarium* root rot

Source of variation	D.f.	Mean square(MS)	F-value	F-prob.
Replication (R)	r-1	RepMS	RepMS/ErMS	
Tray (Ty)	r(ty-1)	TrMS	TrMS/ErMS	
Genotype (G)	(g-1)	GMS	GMS/ErMS	
Error (Er)	Tot.df - Ty.df -G.df	ErMS		
Total (Tot)	tot-1			

Where the mean squares from ReML analysis indicated significant genotype effects, means were compared using a "Student's t-test" for each pair wise comparison of interest, based on the standard error of the difference for that specific pair of entries. The "Student's t-test" was used due to unequal number of individuals among genotypes tested and the use of individual trays as incomplete blocks (Gomez and Gomez, 1984). The value of 't' ($t\text{-}_{calc}$) was calculated as the difference between two means divided by the associated standard error of the difference between pairs (SED) provided directly by the ReML analysis, that is, $t_{calc}= (M_1\text{-}M_2)/SED_{1,2}$, where M_1 and M_2 are the two means being compared, and $SED_{1,2}$ is the SED associated with the two means. Where one of the means of interest was derived from more than one population type, such as a mid-parent (MP), the mean was calculated as: $M_3 = k_1M_1+k_2M_2$. For example, MP = $(½*M_{P1}+½*M_{P2})$, where k_1 and k_2 are the coefficients of mean 1 and mean 2, respectively. The associated SED's were calculated as: SED $(M_3, M_4) = \sqrt{(f^2*SED^2_{1,4} + f^2*SED^2_{2,4})}$. For example, SED for the comparison of F_1 with the MP would be calculated as: SED $(MP, F_1) = \sqrt{\{(0.5^2*SED^2_{P1,F1}) + (0.5^2*SED^2_{P2,F1})\}}$.The above formulae were applied repetitively, as needed, to obtain the variances of means that involved a derived mean as part of the calculation such as $F_2 - (MP+F_1)/2$ involves $(MP+F_1)/2$, which itself involves $MP = ½*M_{P1}+½*M_{P2}$).

3.6 Experiment 2: Effectiveness of pyramided resistance genes and their interactions in improving levels of *Fusarium* root rot resistance

3.6.1 Evaluation of parents, F_1 and F_2 single and five-parent crosses involving Fusarium root rot susceptible parents K20 and Kanyebwa

Parental, F_1 and F_2 of the single crosses and the five-parent cross were planted in screenhouse in wooden trays prepared as described in section 3.3 above. Crosses involving K20 and Kanyebwa are hereafter referred to as K20 population while crosses involving Kanyebwa are referred to as Kanyebwa populations. Population size evaluated per genotype depended on the available number of seed as indicated in Table 6.

Table 6: Number of seeds per genotype planted for evaluation in the K20 and Kanyebwa crosses

	Seed number per genotype	
Generation	**Kanyebwa population**	**K20 population**
Resistant parents	52 – 65	52
Susceptible parent	156	169
DC F_1	65	130
SC F_1	52	39 – 78
FPC F_1	130	208
SC F_2	156	156 – 169
FPC F_2	420	429
Total	1864	2184

DC = double-cross, SC = single-cross, FPC = five-parent cross

The planting arrangement in trays was similar to that described in section 3.5.2 and the test rows contained in each tray are shown in Appendix 2 and Appendix 3 for Kanyebwa and K20

populations, respectively. Scoring for symptom severity was done 21 days after planting using a scale of 1-9 as described in section 3.4.

3.6.2 Data analysis

Deviations of F_1 from the MP and deviations of F_2 mean from the average of MP and F_1 were used to assess the type of gene action conditioning resistance to *Fusarium* root rot in S x R crosses as indicated in the R x R crosses in section 3.5.2. Single-crosses (SC) involving a single resistance source were contrasted with the five-parent crosses (FPC) involving four resistance sources to determine if combined resistance from the four sources gave a better level of resistance than the single sources. The analysis for the comparison of means was similar to that described in section 3.5.2. The outline of the ReML analysis for the response of bean genotypes to FSP is presented in Table 7.

Table 7: Outline of the ReML analysis for the response of bean genotypes to *Fusarium solani* f.sp. *phaseoli*

Source of variation	D.f.	Mean square (MS)	F-value	F-prob.
Tray (T)	t-1	TMS	TMS/ErMS	
Genotype (G)	g-1	GMS	GMS/ErMS	
Error	Tot.df - T.df -G.df	ErMS		
Total (Tot)	tot-1			

Where the mean squares from ReML analysis indicated significant genotype effects, a "Student's t-test" was used for any pair wise comparison as already described in section 3.5.2.

3.7 Experiment 3: Validation of SSR PVBR87 marker for association with *Fusarium* root resistance in independent populations

3.7.1 Plant materials and screenhouse evaluation

Plant materials used in this study included two *Fusarium* root rot susceptible bean genotypes: K20 and Kanyebwa, and two *Fusarium* root rot resistant bean genotypes; G2333 and MLB-49-89A. The polymorphism of the SSR PVBR87 marker between resistant and susceptible parents was first determined in the above genotypes. Crosses were made between cultivars K20 and G2333, and between Kanyebwa and MLB-49-89A. Parents and F_2 progenies of the two crosses were planted in a non-replicated trial in a screenhouse in wooden trays treated as described in section 3.3. Population size was 40 seeds per parent and 217 to 225 seeds for the F_2 depending on the availability of seed.

3.7.2 DNA extraction and molecular marker analysis

Total genomic DNA from young trifoliate leaves of 2-week-old seedlings was isolated according to Mahuku (2004). Polymerase chain reaction (PCR) amplifications for the SSR marker was done in a GeneAmp® PCR system 9700 thermocycler (Applied Bio system) in a 20 µL final volume containing 2 µL of genomic DNA, 1 µL of 10mM of forward primer (CTC ATT GCG TCT ACC AGT GC) and 1 µL of 10mM of reverse primer (CCT AGG TTC CGC AGC ATG T), 0.4 µL of 10mM total dNTPs, 0.2 µL of *Taq* polymerase (5U/µL) and 4.0 µL of 5x Go *Taq* buffer, 1.92 µL of 25mM $MgCl_2$ and 9.48 µL of PCR water. The cycling conditions were an initial denaturation at 95^0C for 5 min followed by 30 cycles of PCR amplification under the following parameters: 1 min at 94^0C, 1 min at 60^0C, and 1 min at 72^0C, followed by a final extension at 72^0C for 5 min. Amplified products, along with 100 bp DNA markers, were separated on 4% high resolution MetaPhor agarose gel in a 1 x TAE buffer and electrophoresed at 120 V for 1 hour using a BIO-

RAD electrophoresis system (BIO-RAD Laboratories, California, USA). Gels were stained with ethidium bromide (Promega, Madison USA) and visualized using Gel Doc 1000 documentation system (BIO-RAD Laboratories, California, USA). Banding patterns were scored with reference to those of parental lines.

3.7.3 Phenotypic assessment

Parents and F_2 plants were phenotypically assessed for *Fusarium* root rot symptom severity 21 days after planting as described in section 3.4.

3.7.4 Data analysis

The segregation of SSR PVBR87 marker in the F_2 population was studied by χ^2 goodness-of-fit test to the Mendelian segregation ratio of 1:2:1 as outlined by Gomez and Gomez (1984). The association between the molecular marker and the *Fusarium* root rot resistance category (resistant and susceptible) was determined using a χ^2 test of independence as outlined in Gomez and Gomez (1984). The strength of association between the marker and the phenotypic score was evaluated by single-marker regression analysis, with the regression coefficient ("b") indicating the fraction of the total *Fusarium* root rot score variation accounted for by the marker.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Number of pyramided *Fusarium* root rot resistance genes and their interaction

4.1.1 Segregation in crosses of Fusarium root rot resistant bean genotypes

Frequency distributions of segregating F_1 and F_2 families are presented in Figure 3.

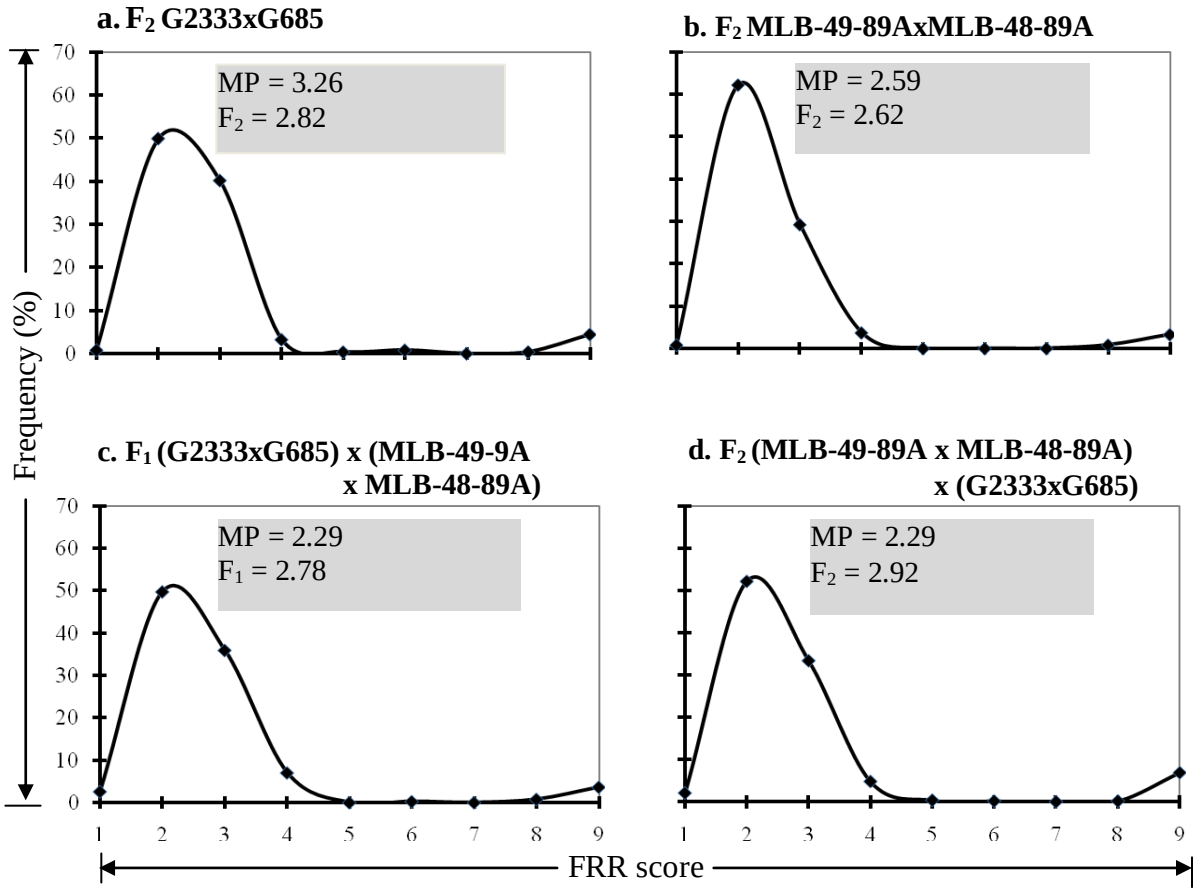

Figure 3: Frequency distribution of *Fusarium* root rot scores in populations of single and double cross mating of common bean genotypes resistant to *Fusarium* root rot

MLB-48-89A (2.64), MLB- 49-89A (2.53), G2333 (3.35) and G685 (3.16); (means in parentheses). Mid-parent, F_1 or F_2 means are indicated in the shaded insert box.

The distributions were discontinuous and skewed towards resistance suggesting that resistance is strongly influenced by non-additive gene interactions. The observed distributions are within the theoretical two to six gene models of Allard (1999). The models were developed under the assumptions of 100% heritability, all gene pairs conditioning a trait have equal effects, no linkage, and that dominance is isodirectional. However, where all gene pairs do not have equal effects, the distribution tends to resemble those produced by fewer genes (Allard, 1999).

Because the frequency distributions grouped the plants into two distinct phenotypic classes in all the crosses (Figure 3), the plants were put into two classes of resistant (R) and susceptible (S) prior to the χ^2 goodness-of-fit analysis. The observed and hypothesized phenotypic class frequencies for resistant and susceptible reactions to *F. solani* f. sp *phaseoli* (FSP) in R x R single-cross F_2 and R x R double-cross F_1 and F_2 are shown in Table 8.

Table 8: Observed and hypothesized phenotypic class frequencies for resistant and susceptible reaction to *Fusarium solani* f. sp. *phaseoli* in F_1 and F_2 single and double cross populations

	Number of plants			Observed ratio	Expected ratio	Number of resistance genes/gene interactions	Goodness-of-fit		HRC [§]	
Generation/cross	Total	R	S	R:S	R:S		χ^2	P	χ^2	P
F_1 DC [a]	384	366	18	20.3:1	15:1	2 genes with duplicate dominant epistasis	1.60	0.21	0.76	0.38
					61:3	2 dominant and 1 recessive genes	0.00	1.00		
					247:9	2 dominant and 2 recessive genes	1.55	0.21		
					249:7	2 dominant and 2 complementary genes	5.51	0.02		
F_2 DC [a]	1061	980	81	12.1:1	55:9	2 genes with duplicate dominant epistasis	36.28	0.00	0.62	0.48
					467:45	2 dominant and 1 recessive genes	1.76	0.18		
					3871:225	2 dominant and 2 recessive genes	9.37	0.02		
					3745:351	2 dominant and 2 complementary genes	1.18	0.27		
F_2 G2 x G6	247	232	15	15.5:1	15:1	2 genes with duplicate dominant epistasis	0.01	0.91	-	-
					61:3	2 dominant and 1 recessive genes	1.06	0.30		
					249:7	2 dominant and 2 complementary genes	10.35	0.00		
F_2 49 x 48	243	233	10	23.3:1	15:1	2 genes with duplicate dominant epistasis	1.89	0.17	-	-
					61:3	2 dominant and 1 recessive genes	0.18	0.67		
					249:7	2 doiminant and 2 complementary genes	1.74	0.19		

DC (double-cross) = (49 x 48) x (G2 x G6); G2 = G2333, G6 = G685, 48 = MLB-48-89A, 49 = MLB-49-89; R = resistant, S = susceptible plants; Chi-square P values greater than 0.05 indicate that observed values were not significantly different from expected values; HRC = Homogeneity of reciprocal crosses. [§]Chi-square P-values greater than 0.05 indicate that reciprocal F_2 populations were homogeneous and data from the two reciprocal populations were pooled; [a] The F_1 and F_2 data of the cross (49 x 48) x (G2 x G6) represents pooled data from reciprocal crosses (49 x 48) x (G2 x G6) and (G2 x G6) x (49 x 48).

The Chi-square (χ^2) test of homogeneity of ratio between the two replications revealed no significant differences in the segregation ratios of the crosses in replication 1 and replication 2 ($P > 0.05$) (Appendix 4) so data was pooled over replications prior to the χ^2 goodness-of-fit analysis. Each of the four populations showed non-significant χ^2 for more than one gene model (Table 8). However, an observed segregation can fit gene models for differing numbers of loci if the population sizes are not adequately large for distinguishing between the fitted ratios (Mather, 1957) as illustrated in (Appendix 5). In addition, an observed segregation may not fit the true genetic model because of the effect of minor genes, modifier genes, epistasis or environmental factors (Estakhr and Assad, 2002). Because of the several possible ratios, the explanation of the χ^2 goodness-of-fit results was based on the gene models which gave the best fit (low χ^2 value with a high P value) to the observed ratio for a specific cross even though a ratio having a slightly higher χ^2 value and a lower but non-significant χ^2 probability does not imply an inadequate fit. Similarly methods have been used by other authors to explain gene models that best fit the observed ratios (Chen and Line, 1992; Estakhr and Assad, 2002).

Segregation in the F_2 population of the cross G2 x G6 fits two of the tested ratios: 15:1 ($\chi^2 = 0.00$, $P = 0.91$) and 61:3 ($\chi^2 = 1.06$, $P = 0.30$) with the best fit to the 15:1 as indicated by the low χ^2 value and high P value (Table 8). The best fit to a 15:1 ratio in this cross suggests that G2 x G3 segregated for at least two genes with duplicate dominant epistasis with one dominant gene present in each parent (Estakhr and Assad, 2002). Segregation in the F_2 population of 49 x 48 fit a 15:1 ($\chi^2 = 1.89$, $P = 0.17$), 61:3 ($\chi^2 = 0.18$, $P = 0.67$) and 249:7 ($\chi^2 = 1.74$, $P = 0.19$). The best fit to 61:3 ratio suggests that the F_2 population of 49 x 48 more likely segregated for at least two dominant genes and one recessive gene for resistance (Chen and Line, 1992).

The χ^2 test of homogeneity of reciprocal crosses in double cross populations revealed no significant deviations ($P > 0.05$) from the segregation ratios in the F_1 and F_2 indicating the absence of detectable cytoplasmic inheritance (Pozniak and Hucl, 2004). Therefore, data from the two reciprocal populations were combined prior to the Chi-square goodness-of-fit analysis of the double cross F_1 and double cross F_2 data (Table 8). The F_1 of the double cross: (49 x 48) x (G2 x G6) fitted a 15:1 ($\chi^2 = 1.60$, $P = 0.21$) and 61:3 ($\chi^2 = 0.00$, $P = 1.00$) suggesting that this population likely segregated for at least one with the best fit to 61:3 ratio dominant gene and two recessive genes for resistance (Chen and Line, 1992) than for two genes as suggested by 15:1 ratio. Segregation in the F_2 of the double cross (49 x 48) x (G2 x G6) fit a 467:45 ($\chi^2 = 1.76$, $P = 0.18$) and 3745:351 ($\chi^2 = 1.18$, $P = 0.27$) ratios indicating segregation for three and four resistance genes, respectively with the four gene ratio providing a slightly better fit.

The adequate fit of only four genes in the double cross compared to the segregation of two genes in the G2 x G6 cross plus three genes in the 49 x 48 cross probably suggests that at least one parent in the G2 x G6 cross have the same or closely linked genes with one parent in the 49 x 48 cross (Chen and Line, 1992). Had the genes that segregated in the individual single crosses been different or distantly linked, a total of five genes should have segregated in the double-cross. Nonetheless, the segregation of more genes in the double cross than in the single crosses in this study provided further evidence that resistance genes to *Fusarium* root rot are located on multiple loci among the different sources of resistance as previously observed by Mukankusi (2008). The results also further indicate that the effects of dominance, recessiveness and epistatic gene interactions condition resistance to *Fusarium* root rot as earlier reported by McRostie (1921), Smith and Houston (1960), Bravo *et al*. (1969), Hassan *et al*. (1971) and Mukankusi (2008).

Although the segregation ratios suggested that two, three and four genes independently segregated in G2 x G6, 49 x 48 and (49 x 48) x (G2 x G6), respectively, based on the gene models which best fit the observed segregation, the actual number of genes involved in each of the crosses cannot be detected by the χ^2 goodness-of-fit test, as already illustrated by observed segregation ratios showing a suitable fit to more than one gene model (Table 8). The difficulty in determining the actual number of genes controlling resistance was expected due to the level of resolution of the method used (Lewers *et al*., 2003). Use of molecular markers tagged to the resistance genes would improve the understanding of the number and effects of genes controlling *Fusarium* root rot resistance in common bean (Schneider *et al*., 2001; Román-Avilés and Kelly, 2005).

4.1.2 Midparent analysis in crosses of Fusarium root rot resistant bean genotypes

Mean squares for symptom scores of bean genotypes challenged with *Fusarium solani* f. sp. *phaseoli* (FSP) are presented in Table 9.

Table 9: Mean squares from ReML analysis for reaction of bean genotypes to *Fusarium solani* f.sp. *phaseoli* in resistant x resistant crosses

Source of variation	D.f.	Mean square
Replication	1	56.45***
Tray	28	1.39***
Genotype	9	2.19***
Error	261	0.57
Total	299	
Grand mean	2.85	
Average SED	0.19	
CV (%)	26.47	

*** = significant at $P \leq 0.001$

The ReML analysis showed significant differences ($P \leq 0.001$) among the evaluated genotypes (Table 9) and therefore means were compared to assess the types of gene interaction controlling *Fusarium* root rot resistance in the evaluated genotypes (Table 10).

Table 10: *Fusarium* root rot symptom severity mean scores of parental, F_1 and F_2 genotypes and their comparisons in resistant x resistant crosses

Cross	P1	P2	P3	P4	MP	F_1	F_2	F_1-MP	F_2-MP	$F_2 - ((MP+F_1)/2)$
SC1[a]	3.35	3.16	-	-	3.26	-	2.82	-	-0.44^{ns}	-
SC2[a]	-	-	2.53	2.64	2.59	-	2.62	-	0.04^{ns}	-
DC1	3.35	3.16	2.53	2.64	2.92	2.92	3.06	0.04^{ns}	0.14^{ns}	0.14^{ns}
DC2	3.35	3.16	2.53	2.64	2.92	2.63	2.75	-0.29^{ns}	-0.17^{ns}	-0.02^{ns}

[a]F_1 progeny were not evaluated;P1 = G2, P2 = G6, P3 = 48, P4 = 49, SC = single cross, SC1 = G2 x G6, SC2 = 49 x 48, DC1 = (G2 x G6) x (49 x 48), DC2 = (49 x 48) x (G2 x G6). ns =deviation not significantly different from zero at P = 0.05

All crosses showed non-significant deviations ($P > 0.05$) of F_1 from MP, F_2 from MP and F_2 from the average of MP and F_1 (Table 10). The result indicate that resistance to *Fusarium* root rot is additive in nature, which is consistent with reports of previous studies (Mukankusi, 2008).

A higher number of resistance genes segregated in the double-cross population than in the individual single-crosses, indicating that the genes were located at different loci among the four parents and that the double-cross pyramided these genes into a single genetic background. This is in acceptance of the study hypothesis that different number of genes for *Fusarium* root rot resistance from different sources can be pyramided in common bean

4.2 Effectiveness of pyramided resistance genes and their interactions in improving levels of *Fusarium* root rot resistance in common bean

Mean squares for the symptom scores of bean genotypes challenged with FSP are presented in Table 11. Significant mean squares for the genotypes showed that the crosses were highly significantly ($P < 0.01$) different from each other.

Table 11: Mean squares for the reaction of bean genotypes to *Fusarium solani* f. sp. *phaseoli* in susceptible x resistant crosses

Source of variation	Kanyebwa population		K20 population	
	D.f.	Mean square	D.f.	Mean square
Tray	12	4.71**	15	3.38***
Genotype	18	19.86***	18	29.94***
Error	104	1.58	141	1.18
Total	134		174	
Grand mean		5.26		4.49
Average SED		0.66		0.51
CV (%)		23.91		24.19

** and *** = significant at $P = 0.01$ and $P \leq 0.001$

The ReML analysis showed significant ($P < 0.001$) genotype effects (Table 11) and therefore means were compared to assess the type of gene interactions conditioning resistance to *Fusarium* root rot in susceptible (S) x resistant (R) crosses (Table 12). *Fusarium* root rot symptom severity mean scores of S x R single and five-parent crosses, involving single and multiple resistance sources, respectively, were also compared (Table 12).

Table 12: *Fusarium* root rot symptom severity mean scores of parental, single cross and five-parent cross F_1 and F_2 and their comparisons

Cross	P_S	P_R	MP	F_1	F_2	F_1-MP	F_2-((MP+F_1)/2)	FPC_{F1}- SC_{F1}	FPC_{F2} - SC_{F2}
Kan x 48	9.00	3.15	6.09	5.71	6.16	-0.38^{ns}	0.30^{ns}	-0.63^{ns}	-0.75^{*}
Kan x 49	9.00	3.38	6.20	4.88	6.26	-1.32^{**}	0.75^{*}	0.20^{ns}	-0.85^{**}
Kan x G2	9.00	4.09	6.56	6.04	6.98	-0.52^{ns}	0.60^{ns}	-0.96^{ns}	-1.58^{***}
Kan x G6	9.00	4.28	6.65	5.22	6.63	-1.43^{**}	0.65^{*}	-0.14^{ns}	-1.22^{**}
FPC_{Kan}	9.00	3.80	6.38	5.08	5.41	-1.30^{***}	-0.35^{ns}		
K20 x 48	9.00	3.88	6.44	4.98	5.10	-1.46^{***}	-0.61^{ns}	-0.61^{ns}	-0.73^{**}
K20 x 49	9.00	2.57	5.78	4.77	5.22	-1.01^{*}	-0.06^{ns}	-0.41^{ns}	-0.85^{***}
K20 x G2	9.00	3.42	6.21	4.40	5.29	-1.81^{***}	-0.01^{ns}	-0.03^{ns}	-0.92^{***}
K20 x G6	9.00	3.28	6.14	4.43	5.40	-1.71^{***}	0.11^{ns}	-0.06^{ns}	-1.03^{***}
FPC_{K20}	9.00	3.08	6.14	4.37	4.38	-1.77^{***}	-0.88^{***}		

FPC = five-parent cross; SC = single-cross; FPC_{Kan} = Kan x [(49 x 48) x (G2 x G6)]; FPC_{K20} = K20 x [(49 x 48) x (G2 x G6)]; Kan =Kanyebwa; 48 = MLB-48-89A; 49 = MLB-49-89A; G2 = G2333; G6 = G685; P_R and P_S = means of resistant and susceptible parents, respectively; P_S for the FPC was the mean for the double-cross F_1; F_1 and F_2 = means of F_1 and F_2 generations, respectively; MP = mid-parent value; F_1-MP = F_1 deviation from MP; F_2-(MP+F_1)/2 = mean deviation of F_2 from the average of MP and F_1, FPCF1-SC_{F1} and FPC_{F2}-SC_{F2} = mean deviations SC_{F1} and SC_{F2} from FPC_{F1} and FPC_{F2}, respectively; ns = not significant at P = 0.05; *, ** and *** = significant at P = 0.05, P = 0.01 and P $\leq$ 0.001, respectively.

4.2.1 Gene interactions in single and five-parent crosses

Two crosses, Kan x 48 and Kan x G2, showed non-significant deviation of the F_1 mean from the MP and non-significant deviation of F_2 mean from the average of MP and F_1 ($P > 0.05$) (Table 12) suggesting that additive gene effects were probably more important than non-additive effects (Salman and Heyne, 1987; Fenster and Galloway, 2000). Three crosses: Kan x 49, Kan x G6 and K20 x [(49 x 48) x (G2 x G6)] showed significant deviations of the F_1 mean from the MP ($P < 0.05$) as well as a significant deviations of the F_2 mean from the average of MP and F_1 ($P < 0.05$) (Table 12) suggesting a major contribution by non-additive gene action particularly epistasis (Marani, 1968; Fenster and Galloway, 2000). Two of the crosses: Kan x 48 and Kan x 49 had non-significant deviations of the F_1 from the MP and of F_2 from the average of the MP and F_1 (Table 12). The remaining five crosses: Kan x [(49 x 48) x (G2 x G6)], K20 x 48, K20 x 49, K20 x G2 and K20 x G6 all had significant deviations of the F_1 mean from the MP ($P < 0.05$), and non-significant deviations of the F_2 mean from the average of the MP and F_1 ($P > 0.05$) (Table 12) suggesting non-additive gene action, particularly dominance for resistance to *Fusarium* root rot (Marani,1968; Hassan *et al*., 1971; Fehr, 1987; Fenster and Galloway, 2000).

The frequency distributions in the F_2 populations of single and five-parent crosses were completely discontinuous with the presence of two distinct phenotypic classes of resistant and susceptible individuals (Figures 4 and 5), which is consistent with the predominance of non-additive gene action, that is, dominance and epistasis for resistance to *Fusarium* root rot in these crosses. The distribution was skewed towards susceptibility in the Kanyebwa populations (Figure 4), while in the K20 populations it was skewed towards resistance in the $FPCK_{20}$ (Figure 5a), in contrast to an almost equal proportion of resistant to susceptible plants occurring in all the SC in the K20 population (Figures 5b-e). The observed distribution patterns possibly illustrate the complexity of the genetic resistance to

Fusarium root rot in the common bean as previously reported by Hassan *et al*. (1971) and Mukankusi (2008).

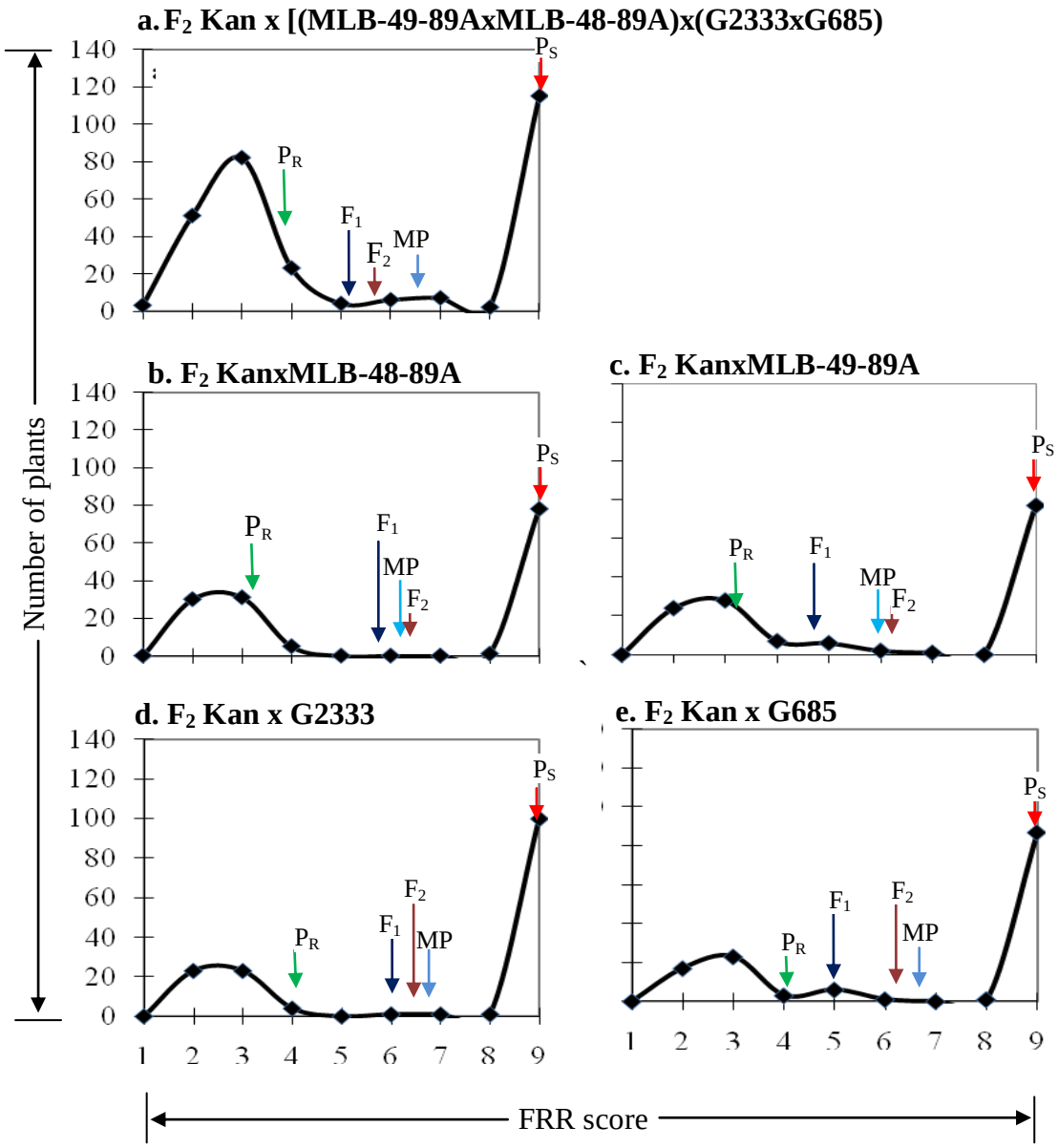

Figure 4: Frequency distribution of F_2 populations in Kanyebwa population

P_S = susceptible parent: Kan = Kanyebwa; P_R = resistant parent

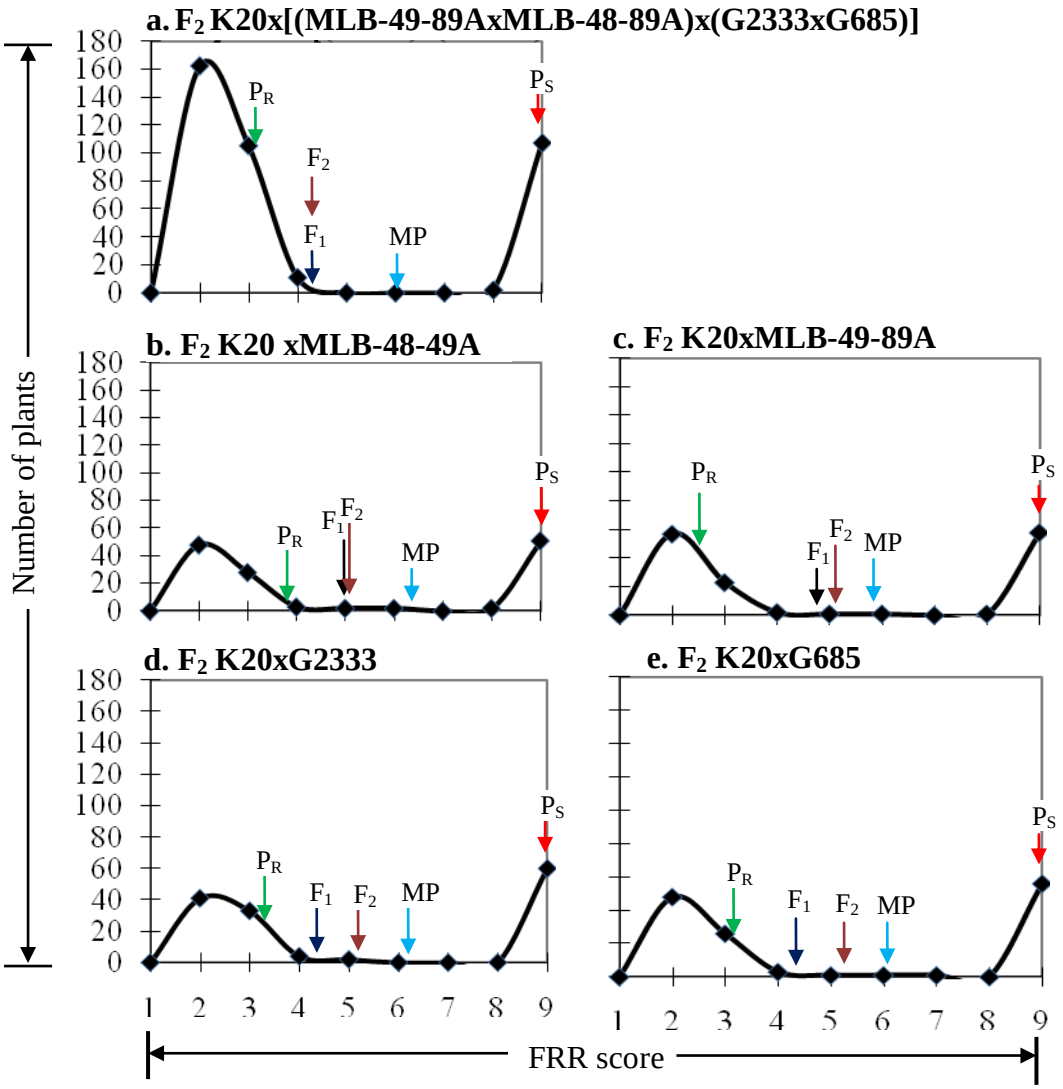

Figure 5: Frequency distribution of F_2 populations in the K20 population

a = Five-parent cross, b – e = Single crosses; P_S = susceptible parent: K20; P_R = resistant parent

The results of the current study indicate the presence of additive and dominant effects and epistatic interactions of genes for resistance to *Fusarium* root rot and are consistent with results of previous studies as reported by Smith and Houston (1960), Bravo *et al*. (1969), Hassan *et al*. (1971) and Mukankusi (2008).

4.2.2 Effectiveness of pyramided resistance genes in improving levels of resistance to Fusarium root rot in susceptible bean cultivars

In both the Kanyebwa and K20 populations the five-parent cross (FPC) F_1 mean had insignificant negative deviations from the single-cross (SC) F_1 mean ($P > 0.05$) while the F_2 of both FPC had a significant negative deviation from the SC means, indicating lower symptom severity in the FPC than in the SC ($P < 0.05$) (Table 12). The F_2 frequency distributions also showed that the FPC in both Kanyebwa and K20 populations had higher proportions of resistant plants than any of the SC in the respective populations (Figures 4 and 5). Epistasis seemed to have had a major contribution to the lower *Fusarium* root rot symptom severity in the FPC relative to that in the SC. This is because even though the FPC in the Kanyebwa population had a non-significant deviation of the F_2 mean from the average of MP and F_1 ($P > 0.05$), it was the only cross with a negative F_2 deviation in the Kanyebwa population (Table 12). Similarly, even though all crosses in the K20 population had negative F_2 deviations, it was only the FPC that showed a significant negative deviation of the F_2 mean from the average of the MP and F_1 ($P < 0.01$) (Table 12). A positive deviation of the F_2 mean from the average of MP and F_1 would indicate that epistasis had a detrimental effect for resistance to *Fusarium* root rot, that is, it favored susceptibility while a negative deviation would indicate that epitasis had a beneficial effect, which favored resistance (Fenster and Galloway, 2000). Therefore, epistatic effects seemed to have made more contributions than dominance effects to the better performance of the FPC relative to the SC as indicated by the predominance of beneficial epistatic effects in the FPC than in the SC (Table 12). It is therefore likely that the FPC had more beneficially interacting loci than did the SC.

The better performance of the FPC over the SC demonstrates that combining resistance genes from different *Fusarium* root rot resistance sources can provide a better source of resistance than using single sources of resistance. It also supports the observation by Mukankusi (2008) that resistance genes for *Fusarium* root rot are located on different loci among different sources of resistance and that combining these loci would lead to increased levels of resistance beyond what can be achieved by using each of the resistance sources individually. Therefore the study hypothesis that pyramided resistance genes to *Fusarium* root rot from different sources are more effective in improving levels of resistance to *Fusarium* root rot in susceptible bean cultivars than resistance from single sources is accepted.

4.3 Validation of the SSR PVBR87 marker for association with *Fusarium* root rot resistance in common bean

4.3.1 Polymorphism of SSR PVBR87 marker

Polymorphism of the SSR PVBR87 marker in *Fusarium* root rot susceptible and resistant bean lines is presented in Figure 6.

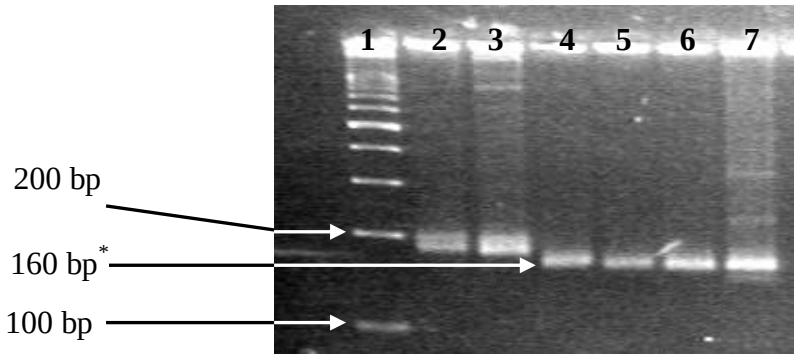

Figure 6: Polymorphism of SSR PVBR87 marker in *Fusarium* root rot susceptible and resistant bean lines

Lane 1: 100 bp DNA ladder, 2: susceptible parent (Kanyebwa), 3: susceptible parent (K20), 4-5: resistant parent (MLB-49-89A), 6-7: resistant parent (G2333), * Approximate fragment size.

The SSR PVBR87 marker was polymorphic between susceptible and resistant parents. The marker produced a 200 bp fragment in the susceptible bean line and a fragment of approximately 160 bp in the resistant line. The fragment size of the marker allele corresponding to the resistant line is reported as approximate because this study used a 100 bp DNA ladder (Figure 6) which could not indicate the exact size of fragments between 100 pb and 200 bp.

4.3.2 Screening F_2 populations with SSR PVBR87 marker

DNA amplification products obtained with the SSR PVBR87 marker in F_2 populations of K20 x G2 and Kan x 49 are presented in Figures 7 and 8, respectively. The marker produced two homozygous marker genotypes, the first with 200 base pairs (bp) corresponding to the susceptible line and the second with approximately 160 bp corresponding to the resistant line.

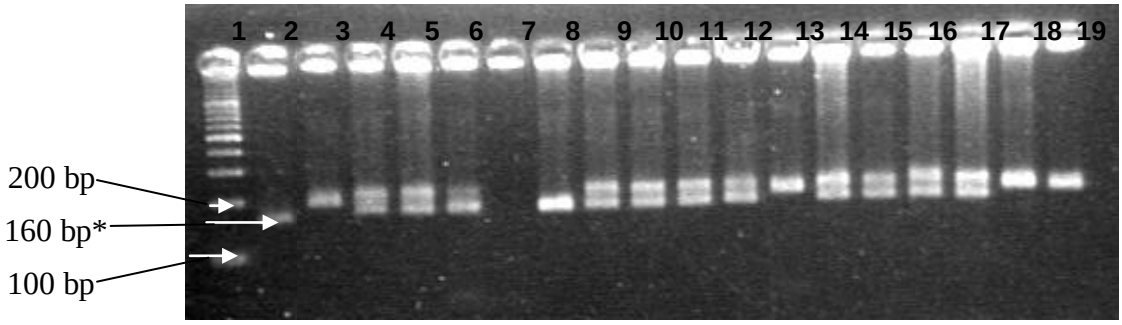

Figure 7: DNA amplification products obtained with SSR PVBR87 marker in F_2 K20 x G2333

Lane 1: 100 bp DNA ladder; 2: resistant parent (G2333); 3: susceptible parent (K20), 4-18: F_2 (K20 x G2333); * Approximate fragment size

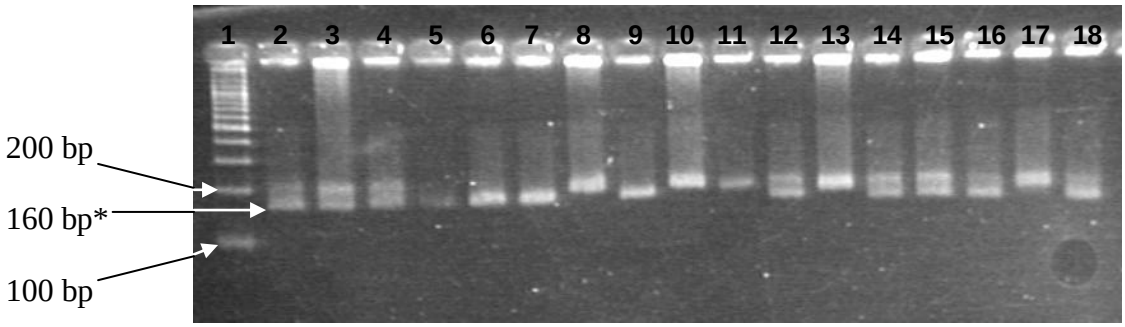

Figure 8: DNA amplification products obtained with SSR PVBR87 marker in F_2 population of Kanyebwa x MLB-49-89A

Lane 1: 100 bp DNA ladder; 2-7 & 10-18: F_2 (Kanyebwa x MLB-49-89A); 8: susceptible parent (Kanyebwa); 9: resistant parent (MLB-49-89A); * approximate fragment size

4.3.3 Segregation of the SSR PVBR87 marker in F2 populations of K20 x G2333 and Kanyebwa x MLB-49-89A

Segregation of SSR PVBR87 marker in F_2 populations of K20 x G2 and Kan x 49 are presented in Table 13.

Table 13: Chi-square (χ^2) test for Mendelian segregation ratio (1:2:1) of SSR PVBR87 marker in F_2 populations of K20 x G2333 and Kan x MLB-49-89A

	Observed number of plants			Expected ratio			
Population	**HR**	**Het**	**Hr**	**HR : Het : Hr**	**χ^2**	**D.f.**	**Prob.**
K20 x G2	37	63	41	1 : 2 : 1	1.82	2	0.40
Kan x 49	46	57	31	1 : 2 : 1	6.34	2	0.04

HR = homozygous for the R allele from resistant parent, Het = segregating and Hr = homozygous for r allele from the susceptible parent; χ^2 = Chi-square; D.f. = degrees of freedom.

Whereas segregation of the marker in K20 x G2 population showed a good fit to the 1:2:1 ratio (χ^2 = 1.84, P = 0.40, D.f. = 2) (Table 13), segregation of the marker in Kan x 49 showed a lack of fit to the expected 1:2:1 segregation ratio in the F_2 generation (χ^2 = 6.34, P= 0.04), indicating distortion of the marker segregation (Table 13). Distortion of marker segregation is a phenomenon in which codominant markers like SSR do not follow a typical Mendelian ratio such as 1:2:1 expected from

an F_2 population (Xu and Hu, 2009) and may be attributed to preferential transmission of either paternal or maternal alleles to the progeny, which can occur when the genetic background of the two parents is very different (Grisi *et al*., 2007). Kan and 49 come from two different gene pools, Andean and Middle-American gene pools, respectively (Mukankusi, 2008).

4.3.4 Test of independence for segregation of the SSR PVBR87 marker with Fusarium root rot resistance in two F_2 populations of common bean

The result of the χ^2 square test of independence of SSR PVBR87 marker segregation from *Fusarium* root rot score in two common bean F_2 populations is presented in Table 14.

Table 14: Test of independence for the segregation of the SSR PVBR87 marker with *Fusarium* root rot resistance in the F_2 populations of common bean

Population	Marker genotype	Observed (o)		Expected (e)		$(o-e)^2/e$				
		R	S	R	S	R	S	$\Sigma\{(o-e)^2/e\}$	D.f.	χ^2
K20 x G2	HR	32	5	25.5	11.5	1.7	3.7	5.4		
	Het	49	14	43.3	19.7	0.7	1.6	2.4		
	Hr	16	25	28.2	12.8	5.3	11.6	16.9	2	24.7***
Kan x 49	HR	41	4	34.8	10.2	1.1	3.7	4.8		
	Het	40	14	44.1	12.9	0.4	1.3	1.7		
	Hr	22	9	24.0	7.0	0.2	0.6	0.7	2	7.3*

R and S = resistant and susceptible classes, where: R = 1- 4 and S = 5-9 on 1-9 scale; R and r = marker alleles from resistant and susceptible parents, respectively; HR = homozygous for the R allele from resistant parent, Het = segregating and Hr = homozygous for r allele from the susceptible parent; χ^2 = Chi-square; * and *** = significant at P = 0.05 and P ≤ 0.001; D.f. = degrees of freedom.

The χ^2-values for independence of the marker and the *Fusarium* root rot score in F_2 populations of K20 x G2 and Kan x 49 were significant (P<0.0001 and P = 0.03, respectively) (Table 14). These results indicate that there is an association between the marker and *Fusarium* root rot score in the two populations.

4.3.5 Relationship between the Fusarium root rot score and the SSR PVBR87 marker

The results of the χ^2 test of independence were confirmed by single-marker regression analysis. The regression of *Fusarium* root rot score on the SSR PVBR87 marker was significant in the two F_2 populations; that is K20 x G2 and Kan x 49 ($P < 0.001$ and $P = 0.03$, respectively), indicating an association between the marker and *Fusarium* root rot score (Figures 9 and 10). In the F_2 populations the R^2-values of 0.13 in K20 x G2 and 0.04 in Kan x 49 indicate that the QTL for *Fusarium* root rot resistance linked to the SSR PVBR87 marker contributed 13% and 4%, respectively, of the total variation in *Fusarium* root rot score among the F_2 plants.

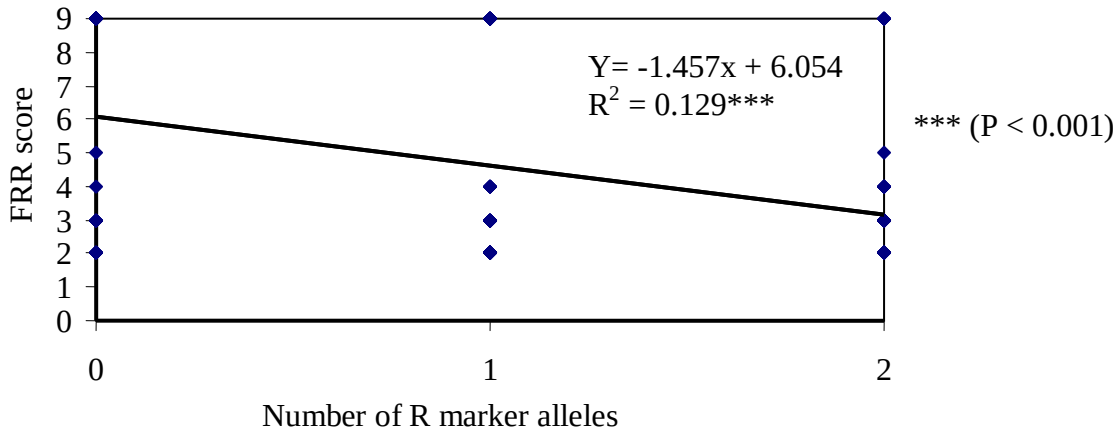

Figure 9: Regression of *Fusarium* root rot score on SSR PVBR87 marker in F_2 population of K20 x G2333

0 = no R allele, 1 = one R allele and 2 = two R alleles

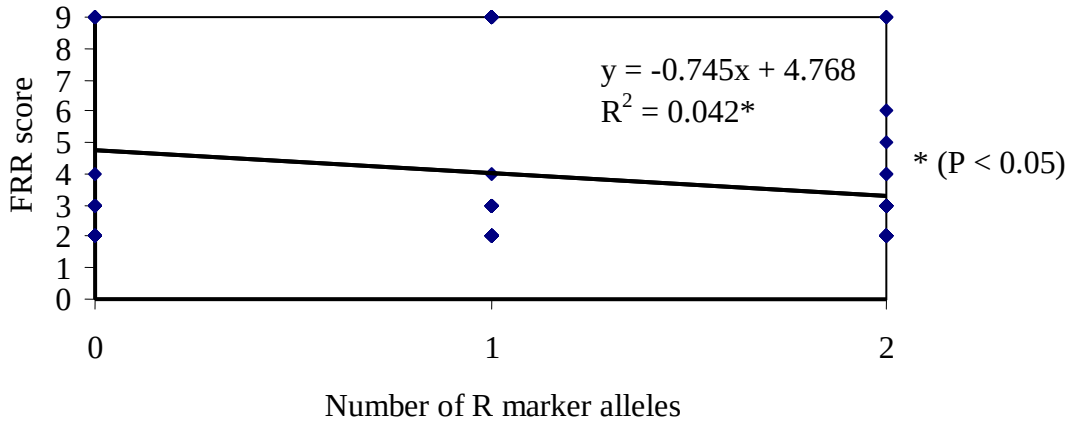

Figure 10: Regression of *Fusarium* root rot score on SSR PVBR87 marker in F_2 population of Kan x MLB-49-89A

0 = no R allele, 1 = one R allele and 2 = two R alleles

This study validated the marker in two independent F_2 populations: Kan x 49 and K20 x G2 and the results indicated the marker was associated with *Fusarium* root rot resistance in both populations, though its contribution to phenotypic variation differed. This difference is probably due to the effect of parental background effect (Doebley *et al*., 1995; Young, 1996). The significant association of this marker to *Fusarium* root rot resistance in two different genetic backgrounds as well as in the two original mapping populations shows that SSR PVBR87 is commonly associated with *Fusarium* root rot (Collard *et al.*, 2005, Sinha *et al*., 2006). Stability of marker-trait association is important in marker-assisted selection because it improves the usability of the marker in different genetic background (Collard *et al*., 2005). The association of the SSR PVBR87 marker with *Fusarium* root rot resistance in G2 is a possible indication that the QTL linked to this marker in 49 as the source of *Fusarium* root rot resistance (Kamfwa, 2010) could be the same in several genetic backgrounds (Khan *et al*., 2007). This result may support the hypothesis that different *Fusarium* root rot resistance sources might possess similar genes or resistance mechanisms associated with known defense response genes in *Phaseolus vulgaris* (Miklas *et al*., 2006).

Validation of the SSR PVBR87 marker showed that the marker can identify resistant genotypes outside the original QTL mapping population from which it was identified providing evidence that the marker is associated with *Fusarium* root rot resistance in at least some crosses of different genetic backgrounds. Thus, the study hypothesis that SSR PVBR87 marker linked to QTL for *Fusarium* root rot in two RIL mapping populations of K132 x MLB-49-89A and K20 x MLB-49-89A can identify *Fusarium* root rot resistant genotypes outside the mapping population is accepted.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

A higher number of *Fusarium* root rot resistance genes segregated in the double-cross population than in the individual single-crosses, indicating that the genes were located at different loci among the four parents and that the double-cross pyramided these genes into a single genetic background.

Fusarium root rot resistance combined from four different sources was more effective in improving levels of resistance in susceptible bean cultivars K20 and Kanyebwa than resistance transmitted from each of the four sources individually. This demonstrates the potential of using gene pyramiding to improve levels of *Fusarium* root rot resistance in susceptible but locally adapted commercial bean cultivars in Uganda.

The results of this study also indicated that *Fusarium* root rot resistance in the bean genotypes tested involved substantial non-additive gene effects: that is, dominance and epistasis. These non-additive effects could lower the expected progress from selection during early segregating generations and thus, lower the gain anticipated from continued inbreeding.

Validation of the SSR PVBR87 marker showed that the marker can identify resistant genotypes outside the original QTL mapping population from which it was identified providing evidence that the marker is associated with *Fusarium* root rot resistance in at least some crosses of different genetic backgrounds.

5.2 Recommendations

The genes responsible for the higher levels of *Fusarium* root rot resistance in the pyramids are not specifically known. It is necessary that these resistance genes be tagged with molecular markers. Tagging of the genes with molecular markers would provide knowledge of their genomic locations, the nature of their interactions and also facilitate the transfer of these genes, through molecular marker-assisted gene introgression, into other agronomically superior, but *Fusarium* root rot susceptible cultivars.

Since no selection for *Fusarium* root rot resistance or any other desirable agronomic traits was practiced in this study, there is need to select between and within families from among the five-parent crosses and the single crosses for resistance to *Fusarium* root rot. However, the predominance of non-additive gene effects for *Fusarium* root rot resistance, especially in the five-parent crosses suggests that selection for resistance would be more effective at advanced generations of selfing.

The bean parents used in constructing the five-parent cross populations are of diverse seed character, growth habit, maturity period, and have varied response to several abiotic and biotic constraints. There is also need to select for these traits in the populations developed in this study as these traits eventually affect acceptability of any potential new variety.

The amounts of phenotypic variation explained by the SSR PVBR87 marker in the two populations were low; hence, there is still need to further validate the marker in additional populations and in

several environments to determine its efficacy for marker-assisted breeding for *Fusarium* root rot resistance.

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APPENDICES

Appendix 1: Layout of experiment I for one replications

			Number of rows per genotype[κ]										
				Parents				Single cross(SC) F_2		Double cross (DC) F_1		Double cross (DC) F_2	
TC	**R/T[¥]**	**T/TC**	**K132[κ]**	**G2**	**G6**	**48**	**49[§]**	**G2xG6**	**49x48**	**D1**	**D2**	**D1**	**D2**
1	11	3	3	3	3	3	3	3	3	3	3	3	3
2	12	3	3	-	-	-	3	3	3	3	3	9	9
3	11	2	2	2	2	2	2	2	2	4	4	-	-
4	11	2	2	2	2	2	2	2	2	-	-	4	4
5	11	1	1	1	1	1	1	3	3	-	-	-	-
6	11	2	2	2	2	2	2	-	-	-	-	12	-
7	11	2	2	2	2	2	2	-	-	-	-	-	12
	Total	15	15	12	12	12	12	13	13	10	10	28	28

TC = tray category; R/T = number of rows per tray; [¥] Each row in a tray was planted with 10 seeds; T/TC = number of trays per tray category; G2 =G2333, G6 = G685, 48 = MLB-48-89A, 49 = MLB-49-89A, DC1 = (G2 x G6) x (49 x 48) F_1, DC2 = (49 x 48) x (G2 x G6) F_1

[κ] Susceptible check [§] Used as parent and resistant check

[¥]Each row was planted with 10 seeds

Appendix 2: Layout of experiment II: Kanyebwa population

			Number of rows per genotype																	
			Parent (P)					D F_1		FP F_1		Single cross (S) F_1				FP F_2		Single cross (S) F_2			
TC	R/T[¥]	T/TC	P1	P2	P3	P4	P5	D1	D2	FP1	FP2	S1	S2	S3	S4	FP1	FP2	S1	S2	S3	S4
1	10	2	2	1	1	-	-	-	-	-	-	2	2	-	-	2	2	2	2	2	2
2	10	2	2	-	-	1	1	-	-	-	-	-	-	2	2	2	2	2	2	2	2
3	10	2	2	1	1		-	2	2	-	-	-	-	-	-	2	2	2	2	2	2
4	11	2	2				1	2	-	2	2	-	-	2	-	2	2	2	1	1	2
5	11	2	2	1	1		1	-	-	2	-	2	2	-	-	2	1	2	1	4	-
6	11	1	1	-	-	1	1	-	1	-	1	-	-	-	1	-	1	2	2	-	-
7	11	1	1	1	1	1	-	1	1	-	1	-	-	-	-	-	2	-	-	-	2
8	11	1	1			1	1		1						1		1		2	1	2
Total		13	13	4	4	4	5	5	5	4	4	4	4	4	4	10	13	12	12	12	12

TC = tray category; R/T = number of rows per tray; [¥] Each row in a tray was planted with 13 seeds; T/TC = number of trays per tray category; P1 = Kanyebwa (Kan); P2 = G2333 (G2); P3 = G685 (G6); P4 = MLB-48-89A (48); P2 = MLB-49-89A (49); D = double cross; FP = five-parent cross; D1 = (G2 x G6) x (49 x 48); D2 = (49 x 48) x (G2 x G6); FP1 = Kan x [(G2 x G6) x (49 x 48)]; FP2 = Kan x [(49 x 48) x G2 x G6); S1, 2, 3 and 4 = Kan x 48, Kan x 49, Kan x G2 and Kan x G6, respectively

[¥] Each row in a tray was planted with 13 seeds

Appendix 3: Layout of experiment II: K20 population

			Number of rows per genotype																		
			Parent (P)					D F_1		FP F_1		Single cross (S) F_1				FP F_2		Single cross (S) F_2			
TC	R/T[¥]	T/TC	P1	P2	P3	P4	P5	D1	D2	FP1	FP2	S1	S2	S3	S4	FP1	FP2	S1	S2	S3	S4
1	12	4	4	1	1	1	1	-	-	-	-	4	4	4	4	4	4	4	4	4	4
2	12	2	2	1	1	-	-	2	2	2	2	-	-	-	-	2	2	2	2	2	2
3	12	2	2	-	-	1	1	2	2	2	2	-	-	-	-	2	2	2	2	-	1
4	12	2	2	1	1	-	-	-	2	2	2	2	1	-	-	-	-	2	2	2	2
5	12	2	2	-	-	1	1	-	-	2	2	-	1	-	-	2	2	2	2	2	2
6	12	3	3	1	1	1	1	2	1	-	-	-	-	-	-	6	4	2	2	2	2
7	12	1	1	-	-	-	-	4	3	-	-	-	-	-	-	2	2	-	-	-	-
	Total	16	16	4	4	4	4	10	10	8	8	6	6	4	4	18	16	14	14	12	13

TC = tray category; R/T = number of rows per tray; [¥] Each row in a tray was planted with 13 seeds; T/TC = number of trays per tray category; P1 = K20; P2 = G2333 (G2); P3 = G685 (G6); P4 = MLB-48-89A (48); P2 = MLB-49-89A (49); D = double cross; FP = five-parent cross; D1 = (G2 x G6) x (49 x 48); D2 = (49 x 48) x (G2 x G6); FP1 = K20 x [(G2 x G6) x (49 x 48)]; FP2 = K20 x [(49 x 48) x G2 x G6); S1, 2, 3 and 4 = K20 x 48, K20 x 49, K20 x G2 and K20 x G6, respectively; [¥] Each row in a tray was planted with 13 seeds.

Appendix 4: X^2 homogeneity of ratio test between replications

Cross	X^2 HR	Probability
F_1(G2 x G6) x (49 x 48)	-1.531	0.216
F_1(49 x 48) x (G2 x G6)	-1.826	0.177
F_2(G2 x G6) x (49 x 48)	-1.827	0.176
F_2(49 x 48) x (G2 x G6)	-1.454	0.228
F_2 G2 x G6	-1.108	0.293
F2 49 x 48	-1.677	0.195

X^2HR = X^2 value for homogeneity of ratio

Appendix 5: Estimated population size required for distinguishing between two possible two-class segregation ratios at 95% confidence level

Ratios to be distinguished		
x:1	y:1	Minimum population size, n*
15:1	61:3	3196
15:1	249:7	510
61:3	249:7	1411

* Calculated using Bailey (1961) model: $n \geq \{[(1 + \sqrt{xy})/(\sqrt{x} - \sqrt{y})]\}*(X^2\alpha,1)$, where: n = number of individuals in an F_2 family; x and y = x:1 and y:1 which are the segregation ratios to be distinguished; $(X^2\alpha,1)$ = one-tailed Chi-square value at a given probability, α; $\alpha = 0.05$.