

**MECHANISM OF INHERITANCE OF RESISTANCE TO *PYTHIUM* ROOT
ROT DISEASE AND TRAITS FOR TOLERANCE TO LOW SOIL
FERTILITY IN COMMON BEAN**

BY

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**A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS
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EXTENDED ABSTRACT

The bean root rot disease mainly *Pythium* spp. is a major problem affecting bean production especially in the major bean producing areas of the Great lakes region including southwestern Uganda, Rwanda, western Kenya, northern and the southern highlands of Tanzania. The problem of *Pythium* bean root rot appears to be made worse by declining soil fertility, resulting from intensive land cultivation by the small holder farmers. Probably for a more sustainable farming system, breeding of common bean genotypes tolerant to both the bean root rot disease and low soil fertility problem would be a welcome improvement. This requires information on the suitable resistant or tolerant germplasm and their nature of inheritance of the resistance genes, which is currently not understood. This research was therefore undertaken in order to: (a) investigate the inheritance of resistance to bean root rot disease caused by *Pythium* spp. in two common bean genotypes RWR 1946 and RWR 2075, and (b) identify the allelic relationship of the resistance genes in these genotypes and RWR 719 (a previously characterized *Pythium* root rot resistance source). In addition, these studies also aimed at: (c) identifying genotypes tolerant to both *Pythium* root rot disease and low soil fertility (low P and Al toxicity), and (d) determining early generation inheritance of selected low phosphorus tolerance-related traits in common bean genotypes RWR 1946 and RWR 2075. Inheritance of resistance to *Pythium* root rot investigated in the F₁, F₂ and backcross populations revealed a single dominant gene that could fully express in several backgrounds and was present at the same locus in the genotypes RWR 1946, RWR 2075 and RWR 719. On the other hand, phenotypic evaluation of the selected known low soil fertility tolerant or susceptible genotypes to identify new sources of *Pythium* root rot

resistance revealed that the BILFA nursery is a potential source of *Pythium* root rot resistance. Assessment of the leaf area, shoot and root dry weights, total root length, lateral and basal roots production, shoot P concentration and P uptake under varying phosphorus availability was performed on 13 common bean genotypes. Results confirmed that genotypes RWR 1946 and RWR 2075 were tolerant to low soil phosphorus availability and responsive to added phosphorus. Unfortunately, when the same genotypes were evaluated under high aluminum saturation of up to 55.2%, they were sensitive to aluminium toxicity. Parental genotypes RWR 1946, RWR 2075, K 132 and their F₁s crosses were evaluated under low and high phosphorus availability to determine early generation inheritance of low phosphorus tolerance related traits. Results revealed that increased lateral and basal root production, total root length and higher shoot growth as traits for low phosphorus tolerance were heritable and were to a great extent likely be due to additive genes. The findings of this study are important because genotypes with tolerance to both *Pythium* root rot disease and low soil phosphorus constraints have been verified. Tolerance or resistance to such two important stresses makes them very good breeding materials since the problem of declining soil fertility is on the increase in the Great lakes region and consequently with likely outbreaks of the bean root rot disease.

DECLARATION

I, Annet Namayanja, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF ABBREVIATIONS AND SYMBOLS

Al :	Aluminium
Al ³⁺ :	Aluminium ion
B :	Boron
bp :	Base pairs
Ca ²⁺ :	Calcium ion
CaCO ₃ :	Calcium carbonate
cm :	Centimeter
cmol/kg:	Centi-mol per kg
Cu:	Copper
CV:	Coefficeint of Variation
DNA:	Deoxyribonucleic acid
F ₁ :	First filial generation
F ₂ :	Second filial generation
Fe:	Iron
g :	Gram
H ⁺ :	Hydrogen ion
ha:	Hectare
H ₂ O:	Water
i.e	that is
K :	Potassium
K ⁺ :	Potassium ion
kg:	kilogram
LSD:	least significance difference

min:	minutes
mg:	milligram
Mg ²⁺ :	Magnesium ion
ml :	milliliter
mm:	millimeter
Mn:	Manganese
N:	Nitrogen
Na ⁺ :	Sodium ion
ng:	nanogram
P:	Phosphorus
pH:	Hydrogen ion concentration
ppm:	parts per million
spp:	species
Sec:	Second
µg:	microgram
µl:	microliter
χ ² :	Chi-square
Zn:	Zinc

CHAPTER ONE

1.0 INTRODUCTION

1.1 Origin and Dispersion of Common Bean

The common bean (*Phaseolus vulgaris* L) is a native of Latin America, about 5,500 to 7,000 years ago with wild populations (Allen *et al.*, 1996; <http://www.cgiar.org/our-research/crop-factsheets/beans/>). According to carbon¹⁴ dating, beans had already been domesticated by BC 2000 (Purseglove, 1968). The crop is now widely cultivated in many parts of the tropics and sub tropics and throughout the temperate region. Beans were taken to Europe in the sixteenth century by the Spaniards and Portuguese and had reached England by 1594. Today, the largest production of the crop is in Latin America especially in Brazil, Mexico, the Andean Zone, Central America, and the Caribbean (<http://www.cgiar.org/our-research/crop-factsheets/beans/>).

The crop became so successful in Africa that the continent is considered to be a secondary center for bean genetic diversity. Africa is the second most important region, producing about 2.5 million metric tons, with most production in Uganda, Kenya, Rwanda, Burundi, Tanzania, and Congo (<http://www.cgiar.org/our-research/crop-factsheets/beans/>). In Africa including East Africa, beans were introduced by the Spanish and Portuguese traders in the sixteenth century (Greenway, 1945). Subsequently the Arab slave traders introduced the crop to the interior. In Uganda beans were introduced in the eighteenth century (Greenway, 1945) and it is reported that various edible beans were grown as green manure in a Kampala plantation. However, serious cultivation of the crop began in the 1920's

when most introductions were made. Today, the crop has the highest annual acreage and production among the grain legumes grown in Uganda (Opio *et al.*, 2001).

1.2 Importance of Common Bean

Common bean is the world's most important food legume, accounting for about 57% of the world's food legume production (CGIAR, 2001). It is the second most important source of calories after maize providing protein, complex carbohydrates, and valuable micronutrients including iron, zinc and folic acid for more than 300 million people in the tropics (<http://www.cgiar.org/our-research/crop-factsheets/beans/>; Kornegay *et al.*, 1996). The crop is grown for its immature edible pods and for the ripe and dry seed (Purseglove, 1968). The leaves are edible and are also used as a pot herb in some parts of the tropics (CGIAR, 2001). In Europe, the United States and other temperate countries, beans are grown mainly for the green immature pods, eaten as a vegetable and are also canned and frozen. Whole dried beans are also cooked with tomato sauce and canned and are usually referred to as baked beans. In eastern and southern Africa, beans rank as the second most important source of human dietary protein and the third most important source of calories after maize and cassava (Pachico, 1993). In these areas, consumption of the crop exceeds 50 kg/per person per year. In Rwanda, beans provide 65% of dietary protein (Kornegay *et al.*, 1996). In Uganda, beans provide about 25% of the total calories and 40% of the protein intake. It plays an important role in fighting against the protein and calorie malnutrition, a problem that would be highly prevalent in the country since the basic food is starchy consisting of sweet potatoes, cassava, millet and maize meal. The 22.1% average protein content of beans is far more than the amount that is found in any of the staple foods (CIAT, 1981).

Beyond their contribution to human nutrition, beans have a considerable economic importance providing income for the smallholder farmer. They are also an attractive crop for farmers because of their adaptability to different cropping systems and short growing cycle (CGIAR, 2001).

1.3 Constraints to Common Bean Production in Uganda

Yields of beans in Uganda average 800 kg/ha, a level significantly lower compared to yields of 1500 - 2500 kg/ha for bush bean varieties often reported from research stations (Ugen and Tukamuhabwa, 2000). The low yield is attributed to a number of factors that include low soil fertility, periodic water stress (drought), insect pests and diseases (Ugen and Tukamuhabwa, 2000). The major field insect pests include the bean stem maggot (*Ophiomyia* spp.), bean aphids (*Aphis fabae*), flower thrips (*Megalurothrips sjostedti*) and foliage beetles (*Oothecca* spp) that occur mostly in Northern Uganda (Opio *et al.*, 2001). In storage, the bean bruchids, *Acanthoscelides obtectus* and *Zabrotes subfasciatus* are very important (Agona, 2000).

Bean diseases include those caused by fungi, bacteria and viruses. Among the most important bean diseases are angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Collectotrichum lindemuthianum*), common bacterial blight (*Xanthomonas campestris pv. phaseoli*), bean common mosaic virus and bean root rot.

The bean root rot disease is one of the major problems threatening the production of common bean in the great lakes region including southwestern Uganda, Rwanda, western Kenya, northern and the southern highlands of Tanzania (Buruchara and Rusuku, 1992; Wortmann *et al.*, 1998). The disease is caused by a complex of

species, which include *Pythium* spp, *Fusarium solani* f.sp. *phaseoli*, *Rhizoctonia solani*, *Sclerotium roflsii* and *Macrophomina phaseolina* (Wortmann *et al.*, 1998; Opio *et al.*, 2001). However, out of these, *Pythium* causes the most severe losses. It is reported that under favorable environmental conditions for pathogen development, a total yield loss can occur due to the disease when susceptible varieties are grown (Buruchara and Rusuku, 1992; Otsyula *et al.*, 2003).

The root rot problem is even made worse by the fact that most of the soils that are prone to the disease are also characterized by declining soil fertility (Abawi *et al.*, 2006). Such soils are very low in soil nutrients due to intensive continuous cultivation that does not allow enough periodic rests (Wortmann *et al.*, 1998; Abawi *et al.*, 2006). The major soil fertility related problems include low available N and P, low availability of exchangeable bases and soil acidity and associated aluminum and manganese toxicities. In Uganda, these problems are mainly characteristic of Kabale and Kisoro in southwestern highlands, where low N and P are ranked among the lowest available nutrients in the soils (Wortmann *et al.*, 1998).

Other important factors include poor agronomic practices, use of low yielding varieties, weeds and socioeconomic and institutional factors which hinder transfer and adoption of improved bean technologies by farmers (Ugen and Tukamuhabwa, 2000).

1.4 Symptoms of *Pythium* Root rot Disease and Low Soil Fertility

In the field, symptoms of *Pythium* root rot disease may often be confused with those of low soil fertility. However on uprooting a plant, one can confirm if it is the

Pythium root rot disease or not, because if the root and lower stem of the bean crop is infected then that is *Pythium* root rot. *Pythium* root rot symptoms normally appear in various forms depending on the time of infection, prevailing environmental conditions and inoculum availability and quantity (Abawi and Pastor-Corrales, 1990). Symptoms may appear as seed rot (before germination), damping off, root rot or leaf yellowing (Abawi and Pastor-Corrales, 1990). Initially symptoms appear as elongated, water-soaked areas on the root and the lower stem tissues, which progress into soft brownish lesions which eventually collapse thereby resulting into plant wilting and death.

On the other hand, low soil fertility symptoms also take various forms, depending on the limiting nutrient. In case of low soil nitrogen, the lower leaves become yellow with chlorosis; for low phosphorus the leaves are small with a dark green colour; while aluminium toxicity is also seen as deformed leaves turning yellow and becoming necrotic.

1.5 Management of *Pythium* Root rot Disease and Low Soil Fertility in Beans

Many control strategies for *Pythium* root rot disease are available including cultural practices, fertilizer applications and genetic resistance (Abawi and Pastor Corrales, 1990; Buruchara, 1991; Otsyula *et al.*, 1998). The cultural control options include crop rotation which keeps the soil inoculum levels of *Pythium* oospores low, planting on ridges which increases aeration and reduces soil moisture, hence reducing *Pythium* oospores (Buruchara, 1991; Buruchara and Rusuku, 1992) and hilling up soil around the stem of bean seedlings. Hilling up of soil around the stem

of the bean seedlings encourages the growth of adventitious roots, allowing the plant to recover from *Pythium* root rot attack.

According to CIAT (1992), application of fertilizers or readily decomposed organic manures has also shown to improve crop yields and tolerance to root rots. The ability of a bean crop to tolerate root rots is related to soil nutrient supply (Otsyula *et al.*, 1998). With high soil fertility, bean grows vigorously and tolerates root rot infections (Otsyula and Ajanga, 1994). This effect appears to be primarily due to the plant's improved ability to obtain adequate nutrients. Beebe *et al.* (2013) also reported that reduced soil quality inhibits root growth and the potential for plant recovery after infection. In addition, genetic resistance is reported as another control strategy (Otsyula and Ajanga, 1994).

On the other hand, for low soil fertility in common bean, currently recommended integrated soil fertility management (ISFM) options include farmyard manure, compost, biomass transfer, green manure and cover crops, liming, phosphate rock and mineral fertilizers in different combinations with organic resources (Lunze *et al.*, 2012). The above mentioned soil management options complemented by utilization of resilient bean germplasm that perform well under low soil fertility conditions is also recommended (Lunze *et al.*, 2012).

1.6 Justification of the Study

Yield losses due to *Pythium* bean root rot disease and low soil fertility can be severe in areas where they occur (Wortmann *et al.*, 1998). Therefore, such losses make it essential that effective management strategies be developed. However, crop rotation

is not practical because of the pressure exerted on land by the rapidly increasing human population. Planting on ridges is labour intensive in systems where beans are traditionally not planted on ridges and intercropped (Otsyula and Buruchara, 2001).

The use of fertilizers is also not practical for both *Pythium* root rot disease and low soil fertility problems, because small holder farmers cannot afford to apply adequate levels of fertilizers since inorganic fertilizers are expensive and organic fertilizers are not readily available in sufficient quantities (Otsyula and Buruchara, 2001; World Bank, 2004; Borlaug, 2006). It is reported that fertilizer use in sub-Saharan Africa is the world's lowest, averaging only 8 kg/ha yearly (<http://www.ifdc.org/...Fertilizer-Summit>).

Genetic resistance appears to be the most appropriate, safe and cost effective control strategy for both problems. This would entail breeding beans that are resistant to the bean root rot disease and also with root characteristics which allow them to use efficiently the scarce nutrients in the prevailing infertile soils; and yet most of the previous breeding and screening efforts have dealt with the root rot and low soil fertility problems independently.

Until recently two genotypes namely RWR 1946 and RWR 2075 resistant to the bean root rot disease and also with tolerance to low soil fertility were identified in the region. Even when grown in soils infested with *Pythium* root rot pathogen, the root system of genotypes RWR 1946 and RWR 2075 are found still growing very vigorously and extensively and are comparable to RWR 719, a popular root rot resistant genotype. It is against this background, that the mechanism of the observed

resistance was further investigated. Their efficient use in any breeding program requires information on their nature and inheritance of resistance genes, which is currently not well known.

Furthermore, the earlier results on low soil fertility tolerances in genotypes RWR 1946 and RWR 2075 have been mainly based on field observations and screening, with possibility of many other confounding factors. Therefore, there was need to reconfirm the observed field reactions using controlled greenhouse techniques. There is also serious need to identify more tolerant genotypes, since the problem of declining soil fertility is on the increase in the region, consequently with likely outbreaks of the bean root rot disease.

1.7 Overall Objective

The overall objective of study was to improve yield of common bean by developing or identifying common bean genotypes resistant to *Pythium* bean root rot disease and also tolerant to low soil fertility conditions.

1.8 Specific Objectives

The specific objectives included the following:

- i. To determine the mechanism of inheritance of resistance to *Pythium* root rot in genotypes RWR 1946 and RWR 2075

- ii. To identify the allelic relationship of the resistance genes in genotypes RWR 1946, RWR 2075 and RWR 719 (known root rot resistance source) using phenotypic and molecular marker techniques
- iii. To evaluate the available bean genotypes tolerant to low soil fertility (low phosphorus and aluminium toxicity) for reaction to *Pythium* root rot disease
- iv. To evaluate the available bean genotypes resistant to *Pythium* root rot disease for reaction to low soil fertility (low phosphorus and aluminium toxicity)
- v. To determine inheritance of low phosphorus tolerance-related traits in common bean genotypes RWR 1946 and RWR 2075

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CHAPTER TWO

GENETIC ANALYSIS OF RESISTANCE TO *PYTHIUM* ROOT ROT DISEASE IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPES

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Abstract

Bean root rot caused by *Pythium* spp. is one of the most important diseases affecting common bean (*Phaseolus vulgaris* L.). A study was carried out to determine the genetics of resistance to *Pythium ultimum* in two common bean genotypes RWR 1946 and RWR 2075, identify the allelic relationship of the resistance genes in these genotypes and evaluate selected genotypes with known tolerance or susceptibility to low soil fertility for reaction to the disease. The resistant genotypes RWR 1946 and RWR 2075 were each crossed to three susceptible genotypes, K 132, Lyamungu and Rushare, to produce F₁ seeds. Part of the F₁ seeds from each cross was used to produce F₂ and backcross seeds. F₁ and F₂ seeds of the crosses RWR 1946 x RWR 2075, RWR 1946 x RWR 719 and RWR 2075 x RWR 719 were also generated. Genotype RWR 719, a known and previously studied *Pythium* root rot resistance source was included mainly for the allelism studies. Some of the low soil fertility-

tolerant genotypes included those from the ‘Bean Improvement for Low Soil Fertility in Africa (BILFA)’ nursery and genotypes from CIAT, Colombia with known tolerance to aluminium toxicity, low soil nitrogen and phosphorus. All parental genotypes, F₁S, F₂S, backcrosses and the low soil fertility-tolerant genotypes were evaluated for *Pythium* root rot severity. The study revealed that resistance to *Pythium ultimum* was controlled by a single dominant gene present at the same locus in all the genotypes. It is concluded that the BILFA nursery is a potential source of *Pythium* root rot resistance.

Key words: allelism – BILFA – dominant gene –PYAA19₈₀₀– qualitative – SCAR marker

Running Head: Resistance to *Pythium* root rot in common bean

Introduction

Pythium bean root rot disease caused by several species is one of the major problems threatening the production of common bean (*Phaseolus vulgaris* L.) in the Great lakes region, including southwestern Uganda, Rwanda, western Kenya, northern and the southern highlands of Tanzania (Buruchara and Rusuku, 1992; Wortmann *et al.*, 1998; Otsyula *et al.*, 2003). Symptoms of the disease may appear as seed rot (before germination), damping off, root rot or leaf yellowing or plant wilting (Abawi and Pastor-Corrales, 1990). Known control strategies for the disease include cultural practices, fertilizer applications and genetic resistance (Abawi and Pastor Corrales, 1990; Buruchara, 1991; Otsyula *et al.*, 1998). The cultural control options include crop rotation, planting on ridges and hilling up soil around the stem of bean seedlings (Buruchara, 1991; Buruchara and Rusuku, 1992). Crop rotation keeps the

soil inoculum levels of *Pythium* oospores low but is not practical because of the pressure exerted on land by the rapidly increasing human population. Planting on ridges increases aeration and reduces soil moisture but is labor intensive in systems where beans are not traditionally planted on ridges and intercropped (Otsyula and Buruchara, 2001). The use of fertilizers is also not practical because farmers cannot afford to apply intensive fertilizers because inorganic fertilizers are expensive and the organic fertilizers are also not readily available in sufficient quantities (Otsyula and Buruchara, 2001) and are bulky. Therefore, genetic resistance appears to be the most appropriate, safe and cost-effective control option.

Most research efforts on the disease have focused on screening of available germplasm to identify sources of resistance. Consequently, a number of resistant genotypes have been identified. The first sources of resistance reported include MLB 49 - 89A, RWR 719, SCAM - 80 – CM/15, AND 1055 and AND 1062 (Buruchara and Rusuku, 1992 ; Buruchara and Kimani 1999). Other potential sources of resistance include the two large-seeded red genotypes RWR 2075 and RWR 1946 (Namayanja *et al.*, 2003; Buruchara *et al.*, 2004). With more research to identify more resistant germplasm, a root rot nursery is now in place, consisting of about 70 lines selected from >6000 germplasm accessions (Buruchara *et al.*, 2009). Efforts have also been initiated in the region to breed specifically for resistance to the disease. For example, Nzungize *et al.* (2011) reported a breeding scheme that was carried out and successfully introgressed *Pythium* root rot resistance genes into commercial bean varieties grown in Rwanda using genotypes RWR 719 and AND 1062 as donor parents.

Breeding for resistance is facilitated if the genetic mechanisms of resistance are known. Currently, there is limited information available on the mode of genetics of resistance to *Pythium* root rot of common bean. Early workers reported that resistance to *Pythium ultimum* was conditioned by polygenic inheritance (Dickson and Abawi, 1974). York *et al.* (1977) proposed similar polygenic inheritance pattern of resistance to seed decay and pre-emergence damping-off in snap bean caused by *Pythium ultimum*. Tu and Parker (1993) observed that *Pythium ultimum* resistance in common beans was complex and not explained by any genetic ratio. Parker and Tu (1994) however, found that the model that best fitted their data involved quantitative mode of inheritance for resistance.

Other studies have shown that resistance is inherited qualitatively. For example, Otsyula *et al.* (2003) showed that a single dominant gene conditioned the inheritance of resistance to *Pythium ultimum* in resistant genotypes RWR 719, AND1062, MLB-49-89A, AND1055 and SCAM-80-CM/15.

Inheritance of resistance to *Pythium* root rot in other crops has been documented. For example, Kumar *et al.* (1991) reported polygenic inheritance of resistance to *Pythium ultimum* in segregating populations between two local resistant varieties of chick pea (*Cicerarietinum* L.), T3 and P436-2 and susceptible introduction C104. Mozaffar *et al.* (2011) reported that a simple additive dominance model accounted for most of the genetic resistance to *Pythium* damping-off and seed rot in two safflower crosses, Aceteria × 34074 and LRV5151 × Arak 2811; whereas Rosso *et al.* (2008) showed

that a single dominant gene conferred resistance to *Pythium* damping-off and root rot in soybean caused by *P. aphanidermatum*.

In addition to using phenotypic screening, molecular markers are used today to detect and track presence of tightly linked genes relating directly to the plant's genotype rather than the phenotype (Guimaraes *et al.*, 2007). These include restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) and sequence characterized amplified region (SCAR) markers (Guimaraes *et al.*, 2007). For *Pythium* root rot, the SCAR markers PYAA19₈₀₀ and PYBA08₃₅₀ were first used and reported to detect the presence of the *Pythium* root rot resistance gene identified in all the resistant genotypes RWR 719, AND 1062, MLB 49-89A, AND 1055, SCAM 80-CM/15 and AND 1062 by Mahuku *et al.* (2007). Nzungize *et al.* (2011) also successfully used the PYAA 19₈₀₀ SCAR marker to select for resistance genes to improve Rwandan susceptible common bean cultivars for *Pythium* root rot resistance, using resistant varieties RWR 719 and AND1062. The objectives of this study were therefore to: i) determine the mechanism of inheritance of resistance to *Pythium ultimum* in common bean genotypes RWR 1946 and RWR 2075, ii) identify the allelic relationship of the resistance genes in genotypes RWR 1946, RWR 2075 and RWR 719 (known root rot resistance source previously studied by Otsyula *et al.* (2003) and iii) evaluate other selected genotypes with known tolerance or susceptibility to low soil fertility (low P, low N, low pH and Al toxicity) for reaction to *Pythium* root rot disease.

Materials and Methods

Genetic materials

Germplasm consisting of three root rot resistant bean genotypes, namely RWR 1946, RWR 2075 and RWR 719 and three susceptible genotypes, K132 (CAL 96), Rushare and Lyamungu 90 was used for the inheritance and allelism studies (Table 1).

Table 1: Characteristics of the bean genotypes used in the study

Genotype	Origin	Seed colour/shape	Growth Habit	Seed Size	Gene pool	Reaction to <i>Pythium</i> root rot
RWR 1946 ¹	Rwanda	Dark red kidney	Determinate	Large	Andean	Resistant
RWR 2075 ¹	Rwanda	Light red kidney	Determinate	Large	Andean	Resistant
RWR 719 ²	Rwanda	Red	Determinate	Small	Meso American	Resistant
K 132 (CAL 96) ¹	CIAT	Red mottled kidney	Determinate	Large	Andean	Susceptible
Rushare ³	Uganda	Red kidney	Determinate	Medium	Andean	Susceptible
Lyamungu 90	Tanzania	Red mottled	Determinate	Large	Andean	Susceptible

4

¹Released varieties in Uganda; ²Released variety in Kenya; ³ Land race in Uganda; ⁴Released variety in Tanzania; Large = >40 g/100 seed weight; medium=(25-40 g/100 seed weight ; small =<25 g per 100 seed weight; RWR = Rwanda Rubona.

In addition, 26 genotypes known to be tolerant to either low soil phosphorus or low soil nitrogen or low pH or to aluminum toxicity from CIAT–Colombia and the

BILFA nursery were evaluated for their reaction to *Pythium* root rot disease. They included the following genotypes: RWR 221, LSA 144, PAN 150, ECAPAN 021, AFR 619, AFR 593-1, ARA 8-5-1, LSA 32, FEB 192, FEB 196, CNF 5520, HM 21-7, M'MFUTALA, ECAPAN 014, M'80LL, RAO 55, AFR 708, CM 9314-36, G 19839, BRB 191, DOR 714, G 19833, MAR 1, G 2333, G 14016 and G 5273. AND 696, a CIAT improved genotype susceptible to low phosphorus but with a large red and cream mottled seed type was also included. Genotypes RWR 719, RWR 1946 and RWR 2075 were included as resistant checks, whereas K132 was the susceptible check. Rojo, a variety from Tanzania was only included in the allelism study using molecular markers.

Population development for inheritance and allelism studies

The resistant genotypes RWR 1946 and RWR 2075 were crossed to each of three susceptible genotypes (as female parents). The following crosses were made: K 132 x RWR 1946, Rushare x RWR 1946, Lyamungu x RWR 1946, K 132 x RWR 2075, Rushare x RWR 2075 and Lyamungu x RWR 2075. Part of the F₁ seeds from each cross was sown in the screen house to produce F₂ seeds and also backcrossed to each of the respective susceptible and resistant parents. Reciprocal crosses were also generated to determine any maternal effects for the trait. For the allelism studies, the following crosses were made: RWR 1946 x RWR 2075, RWR 1946 x RWR 719 and RWR 2075 x RWR 719 to generate F₁ seeds. Part of the F₁ seeds of each cross was also selfed to produce F₂ seeds.

Inoculum production and soil inoculation

One isolate of *Pythium ultimum* (Ms 61) previously characterized and preserved at CIAT-Kawanda was selected for this study. Note that species of *Pythium* previously reported to cause bean root rots in Africa and Latin America are *Pythium ultimum*, *P. irregulare*, *P. aphanidermatum*, and *P. myriotylum*. In East and Central Africa, *P. ultimum* is the most widespread and pathogenic species (Mukalazi *et al.*, 2001; Mukalazi, 2004). Pure colonies of the isolate preserved on water agar were grown on corn meal agar in petri dishes to get active cultures for disease inoculum. Polyethylene bags of 500 ml capacity were partially filled with 160 g of finger millet (*Eleusine spp*) and 160ml of water, which were sealed and autoclaved. After three days, the inoculum was multiplied as follows: 5 mm discs of actively growing hyphae regions of the culture were cut and transferred to the already prepared finger millet medium within the autoclaving bags. The mixture was then incubated in the laboratory at room temperature for three weeks, which allowed high mycelia growth. The infested millet was then mixed with pre-sterilized soil in a ratio of 1:8 v/v (Pyndji *et al.*, 1996) in wooden flat trays of 42cm x 72 cm. Susceptible check variety K132 was planted in the trays and was grown for a period of 21 days to increase the inoculum concentration and then evaluated. A severity score of 9, where approximately 75% or more of the hypocotyl and root tissues were having lesions combined with advanced stages of softening, rotting and severe reduction in the root system indicated the pathogenicity of the inoculum.

Genetic evaluation of resistance to *Pythium ultimum* and Data analysis

Seeds of the different crosses and parents were planted in inoculated soil in wooden trays. For each of the parents and the F₁s, 30 seeds were sown; whereas for the F₂s and the backcrosses the number of seeds depended on availability. For each of the

low soil fertility-tolerant and susceptible bean genotypes, only 30 seeds were planted. On germination, the seedlings were watered twice daily to provide a favorable environment for the pathogen establishment and development (Abawi and Pastor-Corrales, 1990). Three weeks later, seedlings were uprooted and washed with water to remove soil. Severity of root rot symptoms on the tap root was scored visually using the 1-9 CIAT scale (Abawi and Pastor-Corrales, 1990); where **1**= no visible symptoms; **3** = slight discoloration; **5** = moderate, with some deterioration of the root system; **7** = severe combined with considerable softening, rotting and reduction of root system; **9** = more severe with advanced stages of rotting, combined with severe reduction in the root system. Plants with scores of 1 to 3 were considered resistant, whereas those with scores of 4 to 9 were rated as susceptible.

For the inheritance and allelism studies, disease severity data were subjected to qualitative genetic analysis using a Chi-square test (χ^2) for goodness of fit using SAS package (SAS Institute, 1989). Observed segregation was compared to the expected Mendelian ratios. Probability values were calculated using CoStat statistical software (CoStat, 1998-2005). Disease severity data for the low soil fertility tolerant and susceptible genotypes were subjected to analyses using Genstat v.14. software.

Testing allelism using the PYAA19₈₀₀ SCAR marker

DNA was extracted from the youngest bean leaves of two week-old plants of the susceptible genotypes (K 132, Rushare, Rojo and Lyamungu), resistant genotypes (RWR 719, RWR 2075 and RWR 1946), and the F₁S of the crosses RWR 2075 x RWR 719 and RWR 1946 x RWR 719, using procedures adopted by Klimyuk *et al.*

(1993). Polymerase chain reaction (PCR) was performed using a reaction volume of 20µl consisting of a lyophilized premix of 0.2µl/ml, 1µl of 10µM of both the forward and reverse PYAA19 primers, 2µl of 200ng/ul DNA solution and 18µl of sterile double distilled water. The amplification profile was as follows: DNA initial denaturation was at 95°C for 5 min, second denaturation was at 94°C for 20 sec, primer annealing was at 63 °C for 40 sec, extension was at 72° C for 2 min, the total number of cycles was 34 and the final extension step was 1 cycle at 72°C for 10 min and the final hold was at 4°C. After the designated number of cycles and the hold, amplified products were separated on 1.2% agarose containing 10µg/ml ethidium bromide and visualized under the ultra violet light. The gel was photographed (Mahuku *et al.*, 2007).

Testing low soil fertility-tolerant and susceptible genotypes via PYAA19₈₀₀ SCAR marker

In addition to phenotypic screening, DNA of the 27 selected genotypes with known tolerance or susceptibility to low soil fertility was extracted (Klimyuk *et al.*, 1993) and analyzed for presence or absence of a positive band associated with the *Pythium* root rot resistance gene in RWR 719 using the PYAA19₈₀₀ SCAR marker. Genotypes RWR 719, RWR 1946 and RWR 2075 were included as positive controls, whereas K132 was the negative control.

Results

Inheritance of resistance to *Pythium ultimum*

All plants of the genotypes RWR 1946 and RWR 2075 were resistant following artificial inoculation with *Pythium ultimum* strain (MS 61). All plants for genotypes

K 132, Rushare and Lyamungu were susceptible under the same conditions (Tables 2 and 3).

Table 2: Analysis of segregation ratios for resistant (R) to susceptible (S) in parental genotypes Rushare, K 132, Lyamungu, RWR 1946 and their crosses to artificial inoculation with *Pythium ultimum*, strain (MS 61)

Parent/Cross	Generation	Number of plants		Expected ratio	χ^2	Probability
		†R	‡S			
Rushare	PS	0	29			
RWR 1946	PR	30	0			
Rushare x RWR 1946	F ₁	30	0			
Rushare x RWR 1946	F ₂	162	55	3:1	0.0138	0.9065
RWR 1946 x Rushare	F ₁ R	27	0			
RWR 1946 x Rushare	F ₂ R	128	45	3:1	0.0944	0.7587
Rushare x F ₁	BC _S F ₁	37	35	1:1	0.0556	0.8136
RWR 1946 x F ₁	BC _R F ₁	74	0	1:0	0.0000	1
K 132	PS	0	30			
RWR 1946	PR	30	0			
K 132 x RWR 1946	F ₁	23	0			
K 132 x RWR 1946	F ₂	193	64	3:1	0.0013	0.9712
RWR 1946 x K 132	F ₁ R	30	0			
RWR 1946 x K 132	F ₂ R	162	66		1.8947	0.1687
K 132 x F ₁	BC _S F ₁	48	46	1:1	0.0426	0.8365
RWR 1946 x F ₁	BC _R F ₁	81	0	1:0	0.0000	1
Lyamungu	PS	0	29			
RWR 1946	PR	30	0			
Lyamungu x RWR 1946	F ₁	18	0			
Lyamungu x RWR 1946	F ₂	159	52	3:1	0.0142	0.9052
RWR 1946 x Lyamungu	F ₁ R	29	0			
RWR 1946 x Lyamungu	F ₂ R	118	41		0.0524	0.8189
Lyamungu x F ₁	BC _S F ₁	28	26	1:1	0.0741	0.7855
RWR 1946 x F ₁	BC _R F ₁	63	0	1:0	0.0000	1

†R= resistant (scores of 1-3); ‡S= susceptible (scores 4-9); PS = susceptible parent; PR = resistant parent; BC_SF₁= backcross to susceptible parent; BC_R F₁ = backcross to resistant parent; F₁ R = reciprocal cross of the F₁; F₂ R = reciprocal cross of the F₂

Table 3: Analysis of segregation ratios for resistant (R) to susceptible (S) in parental genotypes Rushare, K 132, Lyamungu, RWR 2075 and their crosses to artificial inoculation with *Pythium ultimum*, strain MS 61

Parent/Cross	Generation	Number of plants		Expected ratio	χ^2	Probability
		†R	‡S			
Rushare	PS	0	27			
RWR 2075	PR	30	0			
Rushare x RWR 2075	F ₁	30	0			
Rushare x RWR 2075	F ₂	169	49	3:1	0.7401	0.3896
RWR 2075 x Rushare	F ₁ R	18	0			
RWR 2075 x Rushare	F ₂ R	93	32	3:1	0.0240	0.8769
Rushare x F ₁	BC _S F ₁	24	27	1:1	0.1765	0.6744
RWR 2075 x F ₁	BC _R F ₁	60	0	1:0	0.0000	1
K 132	PS	0	30			
RWR 2075	PR	30	0			
K 132 x RWR 2075	F ₁	23	0			
K 132 x RWR 2075	F ₂	179	58	3:1	0.382	0.5365
RWR 2075 x K 132	F ₁ R	27	0			
RWR 2075 x K 132	F ₂ R	129	42	3:1	0.0175	0.8948
K 132 x F ₁	BC _S F ₁	31	28	1:1	0.0763	0.7824
RWR 2075 x F ₁	BC _R F ₁	58	0	1:0	0.0000	1
Lyamungu	PS	0	29			
RWR 2075	PR	28	0			
Lyamungu x RWR 2075	F ₁	18	0			
Lyamungu x RWR 2075	F ₂	141	48	3:1	0.0159	0.8997
RWR 2075 x Lyamungu	F ₁ R	30	0			
RWR 2075 x Lyamungu	F ₂ R	115	39	3:1	0.0087	0.9257
Lyamungu x F ₁	BC _S F ₁	29	28	1:1	0.0175	0.8948
RWR 2075 x F ₁	BC _R F ₁	56	0	1:0	0.0000	1

†R = resistant (scores of 1-3); ‡S = susceptible (scores of 4-9); PS = susceptible parent; PR = resistant parent; BC_SF₁ = backcross to susceptible; BC_RF₁ = backcross to resistant parent; F₁ R = reciprocal cross of the F₁; F₂ R = reciprocal cross of the F₂

All the F₁ plants of the crosses Rushare x RWR 1946, K 132 x RWR 1946, Lyamungu x RWR 1946, Rushare x RWR 2075, K 132 x RWR 2075 and Lyamungu x RWR 2075 were resistant. All plants were resistant for the backcrosses to the resistant parents. F₁ plants of all the reciprocal crosses were also resistant. Chi-square values revealed good fit to 3:1 ratio; segregation ratios of 3 resistant to 1 susceptible were observed in all the F₂ populations. A ratio of 1 resistant to 1 susceptible was observed for the backcrosses to the susceptible parents. Examples of

the observed resistant and susceptible reactions following inoculation with *Pythium ultimum* are as shown in Figure 1.

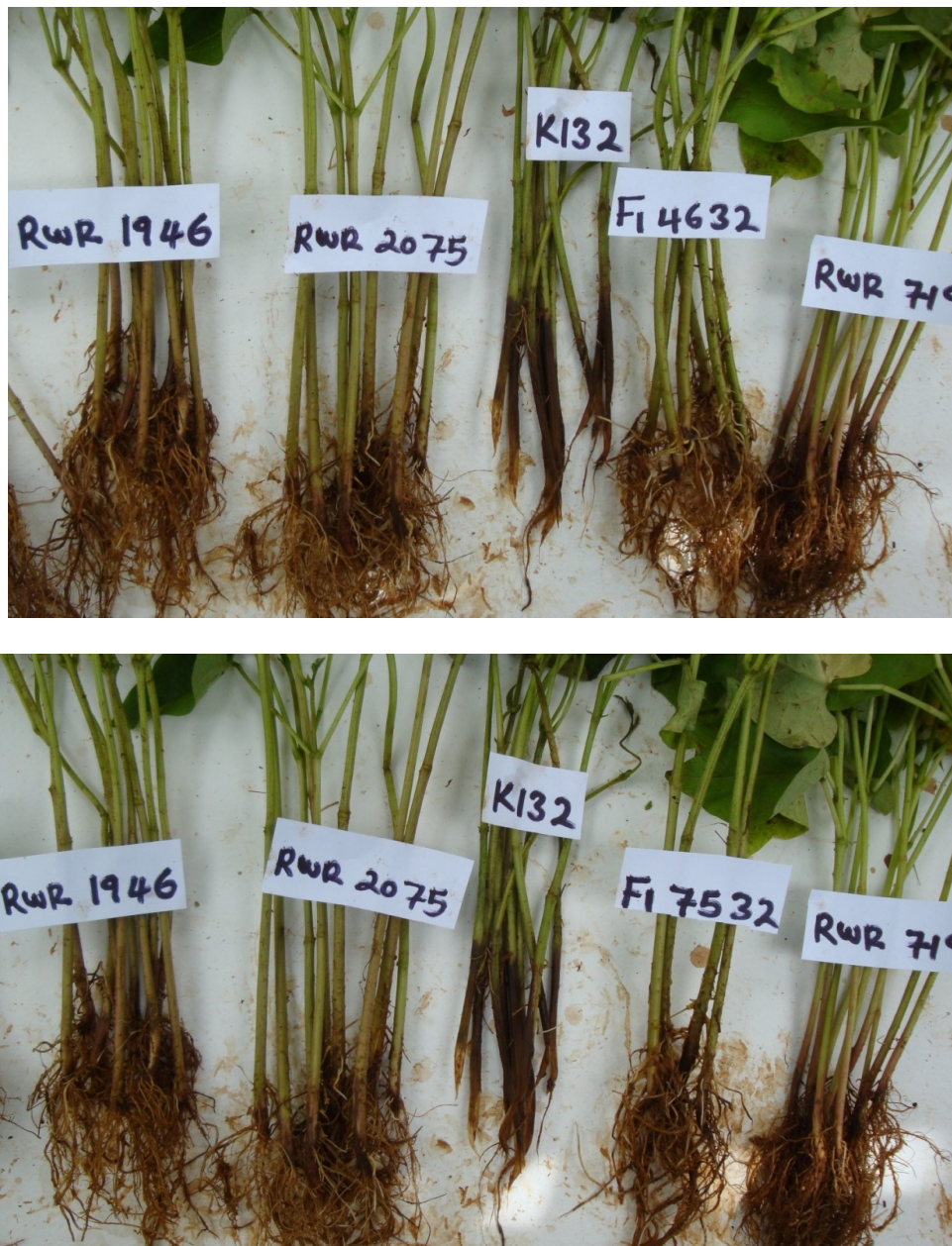


Figure 1: Illustration of a typical resistant reaction to *Pythium* root rot disease on the roots of parental genotypes RWR 1946, RWR 2075 and RWR 719 and the crosses F₁ RWR 1946 x K 132, F₁ RWR 2075 x K 132; while the roots of genotype K 132 show a typical susceptible reaction to the disease on uprooting 3 weeks after inoculation with *Pythium ultimum*, MS 61 in the screen house; (F₁ 4632 = F₁ RWR 1946 x K 132; F₁ 75 32 = F₁ RWR 2075 x K 132)

Allelic relationship of the genes for resistance to *Pythium ultimum*

The F₁ and F₂ plants of the following crosses: RWR 1946 x RWR 719, RWR 2075 x RWR 719 and RWR1946 x RWR 2075 were all resistant (Table 4).

Table 4: Analysis of segregation ratios for resistant (R) to susceptible (S) in the F₁ and F₂ progenies of the crosses RWR 1946 x RWR 2075, RWR 1946 x RWR 719 and RWR 2075 x RWR 719 to artificial inoculation with *Pythium ultimum* (MS 61)

Parent/Cross	Generation	Number of plants	
		†R	‡S
RWR 1946 x RWR 2075	F ₁	39	0
RWR 1946 x RWR 719	F ₁	38	0
RWR 2075 x RWR 719	F ₁	55	0
RWR 1946 x RWR 2075	F ₂	213	0
RWR 1946 x RWR 2075	F ₂	304	0
RWR 2075 x RWR 719	F ₂	276	0

†R = resistant (scores of 1-3); ‡S = susceptible (scores of 4-9)

On using the PYAA19₈₀₀ SCAR marker, the primer amplified a fragment of about 800 bp in all the resistant genotypes, namely RWR 719, RWR 2075 and RWR 1946 and in the F₁s of the crosses RWR 1946 x RWR 2075, K 132 x RWR 1946 and K 132 x RWR 2075 at the same level (Figure 2). This band was absent in all the susceptible genotypes as expected.

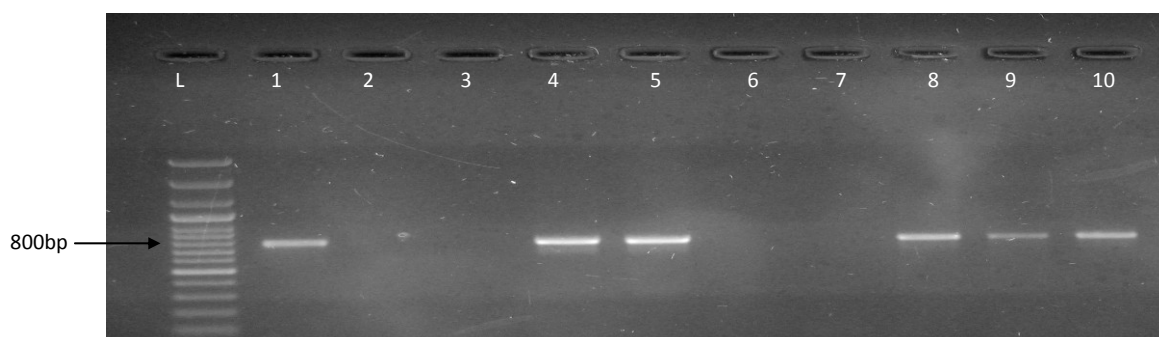


Figure 2: DNA amplification products obtained with PYAA 19 SCAR primer: L- 100bp Ladder, 1- RWR719, 2 - K132, 3 - Rushare, 4 - RWR 2075 , 5 – RWR 1946, 6 - Lyamungu, 7 - Rojo, 8 - F₁ RWR 1946 x RWR 2075, 9 - F₁ K 132 X RWR 1946, 10 - F₁ K 132 x RWR 2075

Reaction of the low soil fertility tolerant and susceptible genotypes following inoculation with *Pythium ultimum* (Ms 61) and DNA amplification using the PYAA19₈₀₀ SCAR marker

Genotypes from the BILFA nursery, such as LSA 144, RWR 221, PAN 150, FEB 192, FEB 196 and the resistant checks, RWR 719, RWR 1946 and RWR 2075 were observed with scores of 1.0 to 3.0 on the CIAT scale of 1 – 9 (Table 5). Genotypes G 19839, AND 696, BRB 191, DOR 714, G 19833, MAR 1, G 2333, G 14016, G5273 and the susceptible check K 132 had scores of 5.0 to 9.0. DNA amplification using the PYAA19₈₀₀ SCAR marker, showed a band of about 800 bp in only genotypes RWR 719, RWR 1946, RWR 2075, ARA-8-5-1, FEB 192, ECAPAN 014, PAN 150, AFR 593-1 and FEB 196 (Figures 3 and 4). The band was absent in the other genotypes.

Table 5: Description for the low soil fertility tolerant genotypes and their observed phenotypic disease scores on the CIAT scale of 1-9 after inoculation with *Pythium ultimum* (Ms 61)

Genotype	Source of seed	Market Class	Gene pool	Known reaction to low soil fertility stress (es)	<i>Pythium</i> root rot disease score on a CIAT scale of 1-9
RWR 719 (R)	BILFA	Red	M	Tolerant to low P (Kimani <i>et al.</i> , 2006)	2.0
RWR 221	BILFA	Pink	M	Tolerant to low P, low N (Wortmann <i>et al.</i> , 1999)	2.0
LSA 144	BILFA	Red	M	Tolerant to low pH (Lunze <i>et al.</i> , 2012)	2.0
RWR 1946 (R)	BILFA	Red	A	Tolerant to low P (Kimani <i>et al.</i> , 2006)	2.0
RWR 2075 (R)	BILFA	Red	A	Tolerant to low P (Lunze <i>et al.</i> , 2012)	2.0
PAN 150	BILFA	Carioca	M	Tolerant to low N, low P (Lunze <i>et al.</i> , 2012)	2.0
ECAPAN 021	BILFA	Red	M	Tolerant to low P (Kimani <i>et al.</i> , 2006)	2.0
AFR 619	BILFA	Red	A	Tolerant to low P (Lunze <i>et al.</i> , 2012)	2.0
AFR 593-1	BILFA	Carioca	M	Tolerant to Al toxicity (Kimani <i>et al.</i> , 2006)	2.0
ARA 8-5-1	BILFA	Carioca	M	Tolerant to Al toxicity (Kimani <i>et al.</i> , 2006)	1.0
LSA 32	BILFA	Carioca	M	Tolerant to low N, low P (Lunze <i>et al.</i> , 2012)	3.0
FEB 192	BILFA	Cream	M	Tolerant to low N, low P (Lunze <i>et al.</i> , 2012)	1.0
FEB 196/008	BILFA	Carioca	M	Tolerant to low N, low P (Lunze <i>et al.</i> , 2012)	1.0
CNF 5520	BILFA	White	M	Low pH (Lunze <i>et al.</i> , 2012)	2.0
HM 21-7	BILFA	Red	M	Tolerant to Al toxicity (Kimani <i>et al.</i> , 2006)	2.0
M'MFUTALA	BILFA	Brown	M	Tolerant to Al toxicity (Kimani <i>et al.</i> , 2006)	2.0
ECAPAN 014	BILFA	Carioca	M	Low P (Kimani <i>et al.</i> , 2006)	2.0
M'80LL	BILFA	Brown	M	Low P (Kimani <i>et al.</i> , 2006)	2.0
RAO 55	BILFA	Red	M	Tolerant to low N, low P (Kimani <i>et al.</i> , 2006; Wortman <i>et al.</i> , 1999)	2.0
AFR 708	BILFA	Red mottled	A	Tolerant to low P, low N (Kimani and Kimani, 2001)	3.0
CM 9314-36	BILFA	Red mottled	M	Tolerant to low N, low P (Kimani and Kimani, 2001; Lunze <i>et al.</i> , 2012)	4.0
G19839	CIAT	Large yellow & red mottled	A	Tolerant to low P (Miller <i>et al.</i> , 2003)	5.0
AND 696	CIAT	Red mottled	A	Susceptible to low P (CIAT, 2000; Cichy <i>et al.</i> , 2009)	6.0
BRB 191	CIAT	Red mottled	A	Tolerant to Al toxicity (Manrique <i>et al.</i> , 2006)	6.0
DOR 714	CIAT	dark red	M	Tolerant to Al toxicity (Manrique <i>et al.</i> , 2006)	7.0
G 19833	CIAT	Large yellow & red mottled	A	Tolerant to low P, Al toxicity (CIAT, 2000; Cichy <i>et al.</i> , 2009; Yan <i>et al.</i> , 1995a; Manrique <i>et al.</i> , 2006)	7.0
MAR 1	CIAT	Cream	M	Tolerant to Al toxicity (Manrique <i>et al.</i> , 2006)	7.0
G 2333	CIAT	Red	M	Tolerant to low P (Miller <i>et al.</i> , 2003)	7.5
G 14016	CIAT	Red mottled	A	Tolerant to Al toxicity (Blair <i>et al.</i> , 2009)	8.0
G 5273	CIAT	Yellow	A	Tolerant to Al toxicity (Manrique <i>et al.</i> , 2006)	9.0
K 132(S)	CIAT	Red mottled	A	Susceptible to low P, low N (Kimani and Kimani, 2001)	9.0
Mean					3.616
CV (%)					7.92
LSD (5%)					0.146

(R) = resistant check for *Pythium ultimum*; (S) = susceptible check for *Pythium ultimum*; CIAT= CIAT-Colombia; BILFA= Bean Improvement for Low Fertility soils in Africa; M = Mesoamerican; A = Andean; low P= low phosphorus; low N= low nitrogen; Al toxicity = aluminium toxicity

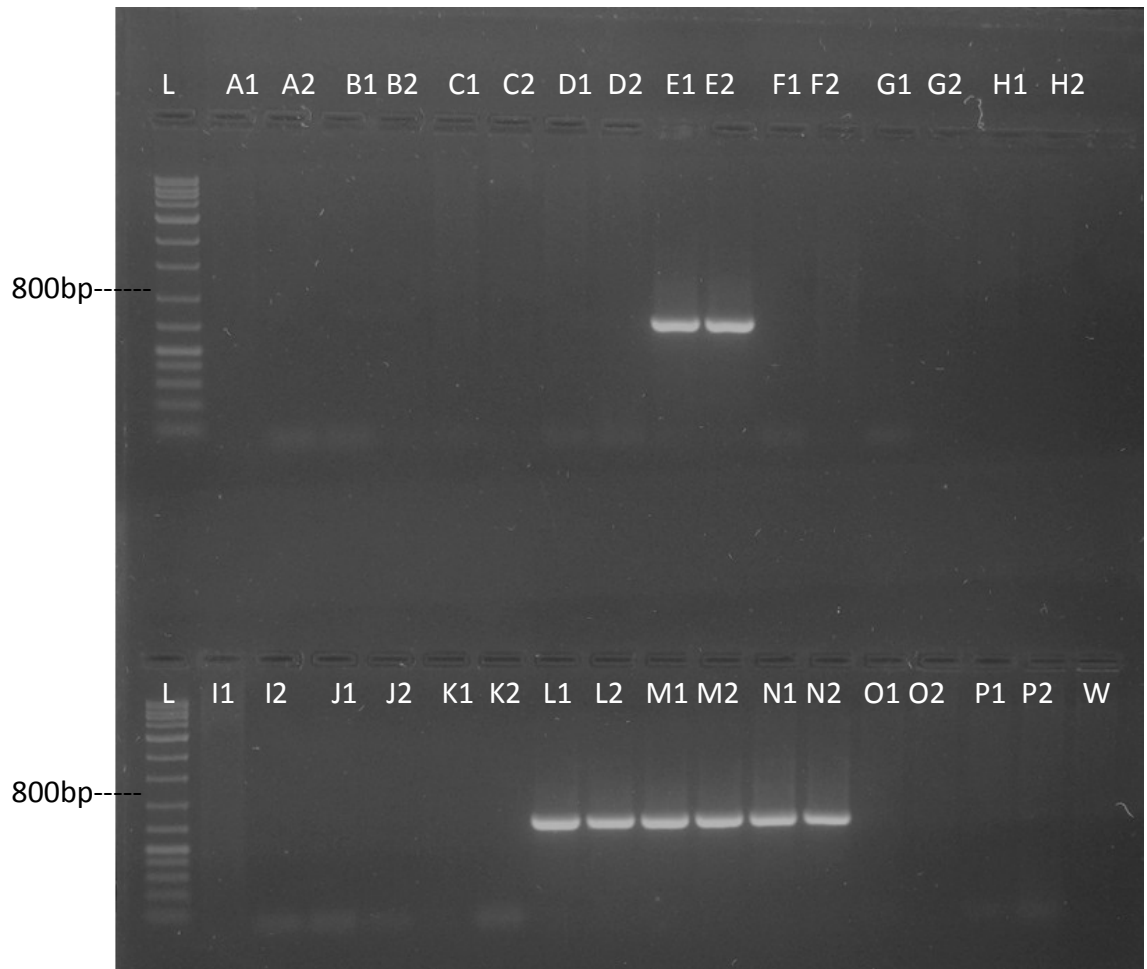


Figure 3: DNA amplification products obtained with PYAA 19₈₀₀ SCAR primer: A1-A2- DOR 714 ; B1-B2- G2333 ; C1-C2- G 19839 ; D1-D2- G5273; E1-E2- RWR 719 ; F1-F2- RWR 221; G1-G2- G19833; H1-H2- AFR 708; I1-I2- MAR 1; J1-J2 – LSA 144 ; K1-K2- G14016; L1-L2-RWR1946; M1-M2- RWR 2075; N1-N2- RWR 10; O1-O2- K132; P1-P2- AND 696; W-water control

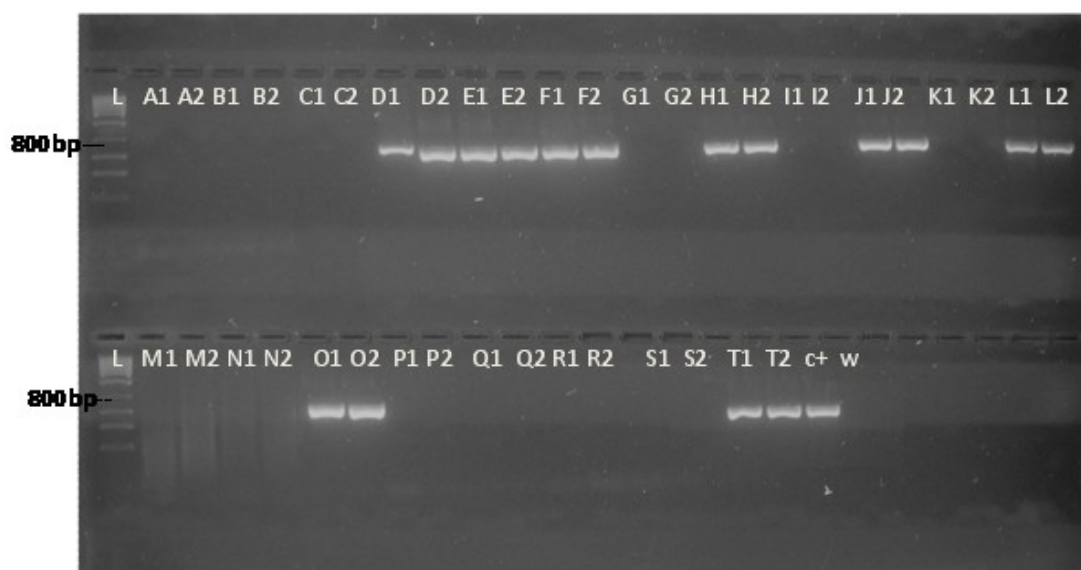


Figure 4: DNA amplification products obtained with PYAA 19₈₀₀ SCAR primer: A1-A2-CIM 9314-36; B1-B2- AFR 708; C1-C2- AFR 619; D1-D2- ARA 8-5-1; E1-E2- FEB 192; F1-F2-CIM 9315-37; G1-G2-T8426F-11-6 ; H1-H2-ECA PAN 014; I1-I2-M' 80LL; J1-J2-PAN 150; K1-K2- ECA PAN 021; L1-L2- AFR 593-1 ; M1-M2- LSA 144 ; N1-N2-HM 21-7; O1-O2- FEB 196/008; P1-P2- CNF 5520; Q1-Q2- M' MFUTALA; R1-R2- LSA 144; S1-S2-RAO 55; T1-T2- PAN 150; c+- Positive control (RWR 719) ; W- water control

Discussion

The observed resistant reaction of all plants of genotypes RWR 1946 and RWR 2075 and the F₁s resulting from their crosses with the susceptible genotypes K 132, Rushare and Lyamungu, suggested that resistance to *Pythium ultimum* was inherited as a dominant trait in the genotypes. Segregation ratios of 3 resistant to 1 susceptible observed in all the F₂ populations also strongly suggested that the resistance in RWR 1946 and RWR 2075 to strain Ms 61 of *P.ultimum* was controlled by a single dominant gene. This was further confirmed by the 1:1 (resistant to susceptible) ratios for the backcrosses to susceptible parents and 1:0 (resistant to susceptible) ratios for the backcrosses to resistant parents. The results of studies on the nature of inheritance greatly depend on the tester genotype used as the susceptible parent among other factors (Pastor-Corrales *et al.*, 1994). However, in the present study,

resistance to *P.ultimum* in RWR 1946 and RWR 2075 was conferred by a single dominant gene in the three different susceptible backgrounds. The stability of this resistance provides a strong basis for their use in breeding programs.

A resistant reaction of all the F₁ plants of all the reciprocal crosses revealed no maternal effect for the trait. A resistant reaction of the F₁ plants of the crosses RWR 1946 x RWR 719, RWR 2075 X RWR 719 and RWR 1946 X RWR 2075 and the lack of segregation in their F₂s indicated that the same locus was responsible for resistance to *Pythium ultimum* in genotypes RWR 1946, RWR 2075 and RWR 719. These results further suggest that the genetic basis for resistance to *Pythium* root rot in common beans may be the same, regardless of the source and may not be confined to gene pools (Buruchara and Mayanja, 2001; Otsyula *et al.*, 2002; Otsyula *et al.*, 2003). Presence of the same locus was further confirmed using the PYAA19₈₀₀ SCAR marker where it amplified a fragment of about 800 bp, in all the three resistant genotypes RWR 719, RWR 2075 and RWR 1946 and in the F₁s of the crosses RWR 2075 x RWR 719 and RWR 1946 x RWR 719. Another possible explanation is that the resistance genes are closely linked. According to Mahuku *et al.* (2007), the SCAR markers PYAA19₈₀₀ and PYBA08₃₅₀ also amplify DNA fragments at the same level in the resistant genotypes RWR 719, AND 1062, MLB 49-89A, AND 1055, SCAM 80-CM/15 and AND 1062.

Phenotypic scores of 1.0 to 3.0 observed among the low soil fertility-tolerant genotypes from the BILFA nursery and the checks RWR 719, RWR 1946 and RWR 2075 implied a resistant reaction to *Pythium ultimum*, Ms 61. Genotypes with scores

of 4.0 to 6.0, such as CM 9314-36, G 19839, AND 696 and BRB 191 were intermediate in reaction. Genotypes DOR 714, G 19833, MAR 1, G5273, G 2333, G 14016 and K 132 with scores of 7.0 to 9.0 are considered susceptible. The results suggested that the BILFA nursery contained sources of *Pythium* root rot resistance. This nursery was a result of screening and selection of common bean germplasm from 15 national and regional bean programs for tolerance to low soil fertility under field conditions following a standardized protocol (Lunze *et al.*, 2012). The resistant checks, RWR 1946 and RWR 2075, are also selections from the BILFA nursery (Namayanja *et al.*, 2003). In addition to low soil fertility tolerance, they were selected and released because of their resistance to *Pythium* root rot disease in south western Uganda. Selection of these two genotypes for resistance to the root rot disease was the basis of evaluating other known low soil fertility-tolerant genotypes, with the hope that new genotypes combining tolerance to both constraints would be identified in this study. The ability of a bean crop to tolerate root rots is related to soil nutrient supply (Otsyula *et al.*, 1998). With high soil fertility, bean grows vigorously and tolerates root rot infections (Otsyula and Ajanga, 1994). This effect appears to be primarily attributable to the plant's improved ability to obtain adequate nutrients. However, most farmers in developing countries cannot afford to apply intensive fertilizers. Therefore, the most sustainable approach would be breeding beans that are resistant to the bean root rot disease and also those that have morphological characteristics that allow them to use efficiently the scarce nutrients in the prevailing infertile soils.

DNA amplification using the PYAA19 SCAR marker of a fragment of 800bp at the same level in some of the genotypes observed with phenotypic scores of 1.0 to 3.0,

namely RWR 1946, RWR 2075, ARA-8-5-1, FEB 192, ECAPAN 014, PAN 150, AFR 593-1 and FEB 196 strongly suggested that the marker could detect the presence of the *Pythium* root rot resistance gene linked to RWR 719 in all these genotypes. Probably all these genotypes possess the same locus conditioning resistance to *Pythium ultimum*, Ms 61 or the resistance genes could be closely linked. The other genotypes rated as resistant to *Pythium ultimum*, Ms 61, such as AFR 708 and RAO 55, which showed no DNA amplification using the PYAA19 SCAR marker might have a different locus for resistance to *Pythium ultimum*, Ms 61.

Conclusion

Inheritance of resistance to *Pythium ultimum* in genotypes RWR 1946 and RWR 2075 was controlled by a single dominant gene that could fully express in several backgrounds. There was no segregation for resistance to *Pythium ultimum* in F₂ populations developed from resistant genotypes (RWR 719, RWR 1946 and RWR 2075), implying that the same resistance gene was present in the genotypes. Given the dominant nature of *Pythium* root rot resistance, the two genotypes should be useful in future bean root rot-resistance-improvement programs.

The findings of this study are important because genotypes RWR 1946, RWR 2075 and RWR 719, which were previously selected for tolerance to low soil fertility, are confirmed to be resistant to *Pythium* root rot. Other genotypes with possible tolerance to both constraints have been identified. Tolerance or resistance to two such important stresses makes them very good donor parents for breeding programs

aimed at developing bean varieties adapted to *Pythium* root rot-prone areas, a problem which is even made worse by the declining low soil fertility conditions common on farmers' fields.

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CHAPTER THREE

GENOTYPIC VARIATION FOR TOLERANCE TO LOW SOIL PHOSPHORUS IN COMMON BEAN UNDER CONTROLLED SCREEN HOUSE CONDITIONS

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ABSTRACT

Production of common bean (*Phaseolus vulgaris*) is often limited by the low availability of soil phosphorus (P). Identification of common bean genotypes adapted to low phosphorus (P) availability is one of the proposed feasible strategies to overcome poor plant growth and production in P-deficient soils. Genetic variation for P response of thirteen common bean genotypes was studied under screen house controlled conditions using triple super phosphate as P source. The common bean genotypes varied in leaf area, shoot and root dry weights, total root length, basal and lateral roots production, shoot P concentration and P uptake under phosphorus deficiency and high phosphorus. All the measured variables were significantly correlated with each other, which in turn correlated to P uptake. Generally the large-seeded genotypes RWR 1946 and RWR 2075 appeared to have

the best growth, hence superior P efficiency under low P availability while at the same time they were more responsive to added P. These results complement the earlier field based observed tolerance to low soil phosphorus of the selected genotypes under the BILFA strategy.

Keywords: BILFA, *Phaseolus vulgaris*, phosphorus, P uptake, shoot dry weight

1.0 INTRODUCTION

Low soil phosphorus is a widespread constraint to common bean production in tropical and sub-tropical soils in Latin America and Africa (Beebe *et al.*, 2006; Beebe *et al.*, 2013; <http://www.ifdc.org/...Fertilizer-Summit>), mostly in soils that have been over cultivated with pH below 5.5 or above 8.0 (Allen *et al.*, 1996). In eastern and southern Africa, it is the most frequently deficient soil nutrient with the supply low in 65% and 80% of the bean production areas according to the Atlas of common bean production in Africa (Wortmann *et al.*, 1998). For example, in Uganda this problem is mainly characteristic of the southwestern highlands, central and eastern tall grass zones, and North short grass zones where associated annual mean losses of 200 kg/ha have been reported (Wortmann *et al.*, 1998). Moderate levels resulting into annual mean losses of 100 kg/ha have also been reported in the western highlands, Mt Elgon, North central, and North West parts of the country (Wortmann *et al.*, 1998). And yet all the above mentioned areas form part of the major bean growing and consumption areas of the country. In Tanzania, the problem is reported in the Northern highlands with very high levels resulting into annual mean losses of 200kg/ha; in the Usambara, Uluguru, West Kigoma, South Lake, the southern highlands and Morogoro moderate levels of mean annual losses of 100kg/ha have also been reported (Wortmann *et al.*, 1998).

Symptoms of phosphorus deficiency in common bean plants can be seen on the leaves where by the young leaves become very small with a dark green colour, while the older leaves senesce prematurely (Allen *et al.*, 1996). In most soil conditions, phosphorus is the least mobile and least available nutrient to plants (Schachtman *et al.*, 1998; Hinsinger, 2001). Some soils such as sandy soils possess low total phosphorus content and are also not able to retain the phosphorus added by fertilizer application according to the WRB soil classification system (Fairhurst *et al.*, 1999; Driessen *et al.*, 2001). However, most soils that have little phosphorus available for the plant may contain considerable amounts of phosphorus but a large proportion is bound to different soil constituents, forming complexes of limited availability (Driessen *et al.*, 2001). On the other hand, some soils with high total phosphorus content fix most of it and will also equally fix a large proportion of the added phosphorus. In both cases, the concentration of phosphate in soil solution is suboptimal for crop production.

Furthermore, the use of fertilizers to correct soil phosphorus deficiency may not be a practical option for the small scale farmers in developing countries because inorganic fertilizers are expensive (World Bank, 2004; Borlaug, 2006). In addition to this, recovery is low because most of the nutrient becomes unavailable due to adsorption, precipitation or conversion to organic forms (Araujo *et al.*, 2005). Worse still, part of the applied P in intensive cropping systems can enter the waterways through runoff and erosion, contributing to pollution of surrounding lakes and marine environments (Tesfaye *et al.*, 2007). Probably an alternative approach to all the above problems is to enhance the plant's efficiency to acquire soil

phosphorus (Shenoy and Kalagudi, 2005; Lynch, 2007; Fageria *et al.*, 2008). Hence the need to identify and use genotypes tolerant to phosphorus deficiency which would also reduce production costs and dependence of farmers on soil amendments.

Tolerance to low phosphorus requires maintenance of plant growth and yields in soils with limited available phosphorus and is reported to occur by two distinct routes namely acquisition efficiency and utilization efficiency (Lynch and Beebe, 1995). Acquisition efficiency is the plant ability to extract phosphorus from the soil and is related to root system traits that increase root surface area or facilitate phosphorus acquisition (Gahoonia and Nielsen, 2003). Utilization efficiency is a function of plant growth, remobilization and physiological traits that translocate phosphorus acquired by the roots into yield. Therefore phosphorus efficiency is defined as the ability of plants to produce higher biomass or yield, and/or take up more phosphorus under inadequate phosphorus conditions (Yan *et al.*, 2006).

Breeding of improved common bean lines with greater phosphorus acquisition and better tolerance to low phosphorus soils is a feasible strategy as shown by a range of inheritance studies (Fawole *et al.*, 1982; Araújo *et al.*, 2005; Beebe *et al.*, 2006; Kimani *et al.*, 2007). In addition, there is adequate genetic variability for tolerance to low phosphorus soils (Beebe *et al.*, 1997; Singh *et al.*, 2003). Several research efforts have focused on screening of available germplasm such as landraces and several improved genotypes (Singh *et al.*, 2003; Cichy *et al.*, 2009; Beebe *et al.*, 2009). Outside Africa, some of the identified sources include the following: i) G19833, a Peruvian landrace with large yellow and red mottled seed and an indeterminate (Type III) growth habit (Yan *et al.*, 1995a; Cichy *et al.*, 2009), ii) G19839, another

Peruvian landrace (Andean gene pool) of indeterminate prostrate growth habit (type III) with a high phosphorus acquisition efficiency (Miller *et al.*, 2003) and iii) G4017, a Brazilian cultivar 'Carioca' (Mesoamerican gene pool) with intermediate prostrate habit (type III), responsive to phosphorus fertilization, and characterized as having intermediate phosphorus efficiency (Miller *et al.*, 2003). While some of the known phosphorus-inefficient breeding lines include i) DOR 364, of Mesoamerican origin and has an indeterminate bush habit (type II), erect stems and small seeds, ii) AND 696 a CIAT improved line from the race Nueva Granada, with a determinate growth habit (Type I) and large red and cream mottled seed (CIAT, 2000).

In Africa, genotypes tolerant to low soil phosphorus have also been selected (Wortmann *et al.*, 1995; Lunze *et al.*, 2002; Lunze *et al.*, 2012). Genotypes tolerant to low soil phosphorus and other soil fertility stresses such as low nitrogen and aluminum toxicity have been put together in nurseries. Nurseries for these genotypes are referred to as BILFA (Bean Improvement for Low soil Fertility soils in Africa). Currently there are seven BILFA nurseries (Kimani *et al.*, 2006). For Example, in BILFA 1 and 11, the low phosphorus lines reported include Carioca, BAT 25, RAO 55, XAN 76 and MMS 224, ACC 433 and Ikinimba (Kimani *et al.*, 2006). Tolerance was reported among mostly the small seeded types. In BILFA 111 the genotypes ARA 4, A 286, AFR 675, AFR 708, AFR 714, AND 871, CIM 9314-3, CIM 9314-36, CIM 9331-1, CIM 9331-3, FEB 192, FEB 196, G 5889, LSA 32, PAN 150, RAB 482, RWR 1873, RWR 1946, RWR 2075, VEF 88(40) L1PYT6 were also reported with tolerance to low P (Kimani *et al.*, 2006). Some of these low soil phosphorus tolerant genotypes have also been reported to be resistant to *Pythium* root rot disease under both field and controlled screen house conditions and

consequently they have been released as commercial varieties in some African countries such as Uganda and Kenya (Namayanja *et al.*, 2003; PABRA, 2007).

Unfortunately, the earlier results on low soil fertility tolerances (such as low phosphorus, low nitrogen, and aluminium toxicity) in most of the BILFA genotypes such as RWR 1946 and RWR 2075 have been mainly based on field screening, with possibility of many other confounding factors. Therefore the objective of this research was to reconfirm the earlier observed field tolerant reaction to low soil phosphorus for selected genotypes from the BILFA nursery, particularly those which are also resistant to *Pythium* root rot disease under controlled screen house conditions.

2.0 MATERIALS AND METHODS

2.1 Genetic Materials and Soil Preparation

The experiment was carried out in the screen house at Sokoine University of Agriculture (SUA), Morogoro, Tanzania following a completely randomized block design, arranged as split plot with the phosphorus levels as the main plots and the genotypes as sub plots. Three replications were used. Thirteen common bean genotypes as described in Table 1 were evaluated under four phosphorus levels /treatments namely: 1) absolute control, 2) control for phosphorus, 3) low phosphorus and 4) high phosphorus.

The substrate was a 6-mm sieved soil collected from the top 10 - 15 cm layer at the botanic gardens of SUA and had originally the following physical and chemical properties: pH of 4.94 (in H₂O), 50.33 % clay, 11 .00 % silt, 38.67 % sand, clay

textural class, 3.5 mg of extractable P /kg of soil (Bray-1- P), 0.21% N, 1.37 mg Cu /kg of soil, 0.86 mg Zn/kg of soil, 48.14 mg Mn/kg of soil, 22.19 mg Fe/kg of soil, 1.073% of organic carbon, cation exchange capacity of 10 cmol/kg of soil, 1.167 cmol Ca²⁺/kg of soil, 1.107 cmol Mg²⁺ /kg of soil, 0.513 cmol K⁺ /kg of soil, 0.25 cmol Na⁺/kg of soil, 0.33 cmol H⁺ /kg of soil and 0.583 cmol Al³⁺ /kg of soil.

Before potting, soil for each treatment was handled separately and mixed thoroughly well. For the absolute control, no fertilizer was added; for the control for phosphorus, only base fertilizers i.e 100 mg K/kg of soil (as potassium chloride), 10 mg Zn /kg of soil (as zinc sulphate) and 1 mg B/kg of soil (as boric acid) were added with no phosphorus; the low phosphorus treatment consisted of 10 mg P/kg of soil as triple super phosphate and the same rate of the above base fertilizers; and the high phosphorus treatment consisted of 160 mg P/kg of soil as tripple super phosphate also with the same rate of the mentioned base fertilisers. For each phosphorus treatment, 4 kg of soil was potted into 4 litre plastic buckets which were later placed in the open air above on a metallic screen house bench. The field water content for each 4 kg plastic bucket was determined by the Savage method (1979) and watering was done to field capacity. Sowing was done when the soil had reached field capacity. One bucket for each phosphorus treatment was kept unplanted to measure soil evaporation losses from the buckets. Five seeds of each genotype were sown and seven days after emergence, thinning was done leaving only three plants per bucket. Watering was done as adequately as possible. For each of the buckets for the control for phosphorus, low phosphorus and high phosphorus treatments, nitrogen fertilisers i.e 200 mg N /kg of soil (as ammonium sulphate) and

200 mg N/kg of soil (as urea) were applied via dilute solution 12 and 25 days after emergence respectively.

Table 1: Characteristics of the common bean genotypes used in the experiment

Genotype	Other name	Market Class	Origin	Nursery/ Source of seed	where commercially grown	Gene pool	Growth Habit
1.MLB 49-89A	KK 15	Black	DRC	Root rot	Kenya, DRC	M	ID
2.RWR 221	NA	Pink	Rwanda	BILFA	NA	M	D
3.LSA 144	NA	Red kidney		BILFA	NA	M	D
4.RWR 719	KK 22	Small red	Rwanda	Root rot	Kenya, Rwanda, Ethiopia	M	D
5.RWR 2075	NABE 14	Large red	Rwanda	BILFA	Uganda	A	D
6. AFR 708	NA	Red mottled	CIAT	BILFA	NA	A	D
7. AND 1055	NA	Red	CIAT/IBN	Root rot	NA	A	D
8. AB 136	NA	Small red		Anthracnose	NA	M	ID
9. RWR 1946	NABE 13	Large red	Rwanda	BILFA	Uganda	A	D
10. AND 1062	NA	Kidney red	CIAT/IBN	Root rot	NA	A	D
11. RWR 10	NA	Red kidney	Rwanda	BILFA	NA	A	D
12. RWR 1059	NA	Red mottled	Rwanda	Root rot	NA	M	D
13. G 19839	NA	Large yellow & Red mottled	Peru	CIAT-Colombia	Peru	A	ID

DRC = Democratic Republic of Congo, M= Mesoamerican; A= Andean; D = Determinate; ID =indeterminate; IBN= International Breeding Nursery at CIAT-Colombia; NA= No other known name currently

2.2 Plant Measurements

At 45 days after planting, the most fully expanded top trifoliolate leaf (including petioles) from one plant per bucket was detached, and leaf length and leaf width measured. For each leaf among the trifoliolate, leaf length was measured from lamina tip to the point of intersection of the lamina and the petiole, along the mid rib of the lamina. Leaf width was measured from end to end between the widest lobes of the lamina perpendicular to the mid rib. These linear measurements were then used to estimate the average leaf area per genotype following the model below as described by Bhatt and Chanda (2003).

$LA \text{ (cm}^2\text{)} = 0.11 + 0.88 (L + W)$, where LA = Leaf area; L= length of the leaf midrib; W= Maximum leaf width.

At mid pod filling stage, which ranged from 48 to 54 days after planting, all shoots (including petioles, leaves, stems) were harvested per bucket and oven dried for 4 days at 70°C. Shoot dry weights were recorded. Roots including nodules were separated from soil by washing with water, left to dry in the open air and then put in the oven at 70°C for 4 days. Root dry weights were recorded. Note that after pod setting, root growth is less intense. Using one representative root sample per bucket, total root length was determined from the base of the hypocotyl along the length of the longest basal with the lateral roots that emerge from them. It is important to note that basal roots arise from the base of the hypocotyl and in conjunction with the lateral roots that emerge from them basal roots usually comprise the majority of the total root length (Vieira *et al.*, 2008). Lateral and basal roots production in response to phosphorus availability was visually determined according to the common bean shovelomics (Jochua, 2013), using a score of 1-9 (1 was excellent and 9 was very poor). Shoot P concentration was determined following the calorimetric determination method as described by Murphy and Riley (1962). P uptake was also calculated as the product of the shoot dry weight and shoot P concentration.

2.3 Data Analysis

Analysis of variance and correlation among variables were performed using GENSTAT v.14. software (VNS International Hempstead, UK). Where means were different significantly, Fisher's protected least significant difference was used. Data for leaf area, shoot and root dry weights for two of the genotypes namely G 19839 and AND 1055 was excluded from the analysis and is therefore not presented.

3.0 RESULTS

3.1 Phosphorus Response

Phosphorus effects were highly significant ($P= 0.05$) on all the variables measured (Table 2) and so was the effect of genotype on all the other variables, except root dry weight, shoot P concentration and P uptake. Only leaf area, total root length, and lateral and basal roots production were influenced by both genotype and P level. In addition, the F values for all the measured variables at either the genotype or P level were higher than the F values for genotype x phosphorus interaction level.

Table 2: Analysis of variance for the different variables evaluated for the common bean genotypes

Variables	Source	Df	SS	MS	F value	P value
1. Leaf area (cm ² /leaf)	P levels	3	1699.589	566.530	231.77	<0.001
	Genotype	10	233.461	23.346	9.55	<0.001
	Gen. x P.	30	219.941	7.331	3.00	<0.001
2. Shoot dry weight (g/plant)	P levels	3	1636.342	545.447	385.62	<0.001
	Genotype	10	71.947	7.195	5.09	<0.001
	Gen. x P.	30	63.903	2.130	1.51	0.073
3. Root dry weight (g/plant)	P levels	3	25.07399	8.35800	100.73	<0.001
	Genotype	10	1.87899	0.18790	2.26	0.021
	Gen. x P.	30	4.22899	0.14097	1.70	0.030
4. Total root length (cm)	P levels	3	597.8134	199.2711	246.37	<0.001
	Genotype	12	65.2484	5.4374	6.72	< 0.001
	Gen. x P.	36	111.6746	3.1021	3.84	<0.001
5. Lateral and basal roots production score	P levels	3	520.949	173.650	97.80	<.001
	Genotype	12	72.103	6.009	3.38	<.001
	Gen. x P.	36	143.718	3.992	2.25	<0.001
6. Shoot P concentration (%)	P levels	3	0.279815	0.093272	33.19	<.001
	Genotype	11	0.063452	0.005768	2.05	0.031
	Gen. x P.	33	0.099623	0.003019	1.07	0.383
7. P uptake (mg P/plant)	P levels	3	10616.69	3538.90	231.77	<.001
	Genotype	11	386.02	35.09	2.30	0.015
	Gen. x P.	33	534.57	16.20	1.06	0.400

Gen. x P. = Genotype x phosphorus interaction; Levels of $P < 0.001$ were highly significant

The mean values for the leaf size, shoot and root dry weights, total root length, lateral and basal roots production scores, shoot P concentration and P uptake for the

13 common bean genotypes evaluated under four different phosphorus levels were highly significant (Table 3). Leaf area increased with increase in phosphorus levels, ranging from 7.63 cm²/leaf to 17.08 cm²/leaf. Shoot dry weight also increased with increase in phosphorus level, being very significantly different with the high phosphorus treatment at 10.175 g/plant. However there was significant difference between the control for P and the absolute control treatments. The root dry weight also increased significantly with increase in phosphorus levels, with mean values ranging from 0.3258 g/plant to 1.4545 g/plant. However there was no significant difference between the low phosphorus and absolute control treatments for the root dry weight. On the other hand, for the total root length highly significant mean values of 22.29 cm and 21.59 cm were observed for the absolute control and high phosphorus treatments respectively. Lateral and basal roots production also varied significantly with excellent average scores of 1.378 recorded at the high phosphorus treatment and poor average score of 6.974 recorded at the control for phosphorus treatment. Shoot P concentration ranged from 0.1157% (control for P) to 0.2323 % (high P). P uptake was also highly variable ranging from 1.647 mg P/plant (control for P) to 22.638 mg P/plant (high P), but was not significantly different between the absolute control and control for P treatments. In general, high phosphorus level resulted into better performance of all the variables that were measured. While for all the variables, the absolute control was always higher than the control for P treatment.

Table 3: Mean values for the leaf area, shoot and root dry weights, total root length, lateral and basal roots production scores, shoot P concentration and P uptake for the 13 common bean genotypes evaluated under four different phosphorus levels

Phosphorus level (P)	Leaf area (cm ² /leaf)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total root length (cm)	Basal and lateral roots production score on a scale 1-9	Shoot P Concentration (%)	P uptake (mg P/plant)
1. Absolute control	9.35 ^c	1.863 ^c	0.5269 ^b	22.29 ^a	4.154 ^b	0.1440 ^b	2.648 ^c
2. Control for P	7.63 ^d	1.408 ^c	0.3258 ^c	15.54 ^b	6.974 ^c	0.1157 ^c	1.647 ^c
3. Low P (10 mg P/kg of soil)	12.37 ^b	3.409 ^b	0.5269 ^b	18.21 ^b	4.538 ^b	0.1444 ^{bc}	4.579 ^b
4. High P (160 mg P/kg of soil)	17.08 ^a	10.175 ^a	1.4545 ^a	21.59 ^a	1.378 ^a	0.2323 ^a	22.6389 ^a
Mean	11.61	4.21	0.715	19.41	4.299	0.1583	7.88
CV%	13.5	28.2	40.3	26.6	30.6	35.1	33.5
LSD (5%)	0.765	0.582	0.1409	2.930	1.470	0.02480	1.828

Means followed by the same letter within the same column are not significantly different (P = 0.05) by Fisher's protected least significant difference test

High P level markedly increased the leaf size (leaf area per leaf), shoot dry weight, total root length, lateral and basal roots production, shoot P concentration and P uptake (Table 4). Extreme phosphorus deficiency strongly reduced the leaf area and shoot dry weight more than the root dry weight. The control for phosphorus treatment had the strongest reduction effect on all the measured plant traits more than the absolute control and the low phosphorus treatments.

Table 4: Magnitude of the effect of phosphorus levels on leaf area, shoot and root dry weights, total root length, lateral and basal roots production, shoot P concentration and P uptake

Magnitude of the effect of phosphorus levels on the measured variables							
Phosphorous level (P)	Leaf area	Shoot dry weight	Root dry weight	Total root length	Lateral and basal roots production	Shoot P concentration	P uptake
1. Absolute control	- 2.26	- 2.35	- 0.188	2.88	0.22	- 0.0144	-5.23
2. Control for P	- 3.98	- 2.81	- 0.389	- 3.87	- 2.60	- 0.0426	-6.23
3. Low P	0.77	- 0.80	- 0.162	-1.20	- 0.17	- 0.0169	-3.30
4. High P	5.57	5.96	0.739	2.18	2.55	0.0739	14.76

3.2 Genotypic Variability

Genotypic variation for each variable under each of the four phosphorus levels is presentable in Figures 1, 2, 3, 4 and Table 5 separately. **Leaf area:** For this variable, both the genotype and P levels as sources of variation were highly significant ($P < 0.001$). Under extreme phosphorus deficiency the leaf area was reduced ranging from 8.60 to 11.43 cm²/leaf (absolute control) and from 5.9 to 9.1 cm²/leaf (control for P). When phosphorus was applied at a rate of 10 mg P/kg of soil (low phosphorus), genotype RWR 1946 and RWR 2075 had the highest leaf area of 16.33 and 15.58 cm²/leaf respectively (Figure 1). At high phosphorus level of 160 mg P/kg of soil, genotypes RWR 1946 had the highest response to phosphorus, followed by genotype RWR 2075 and AFR 708.

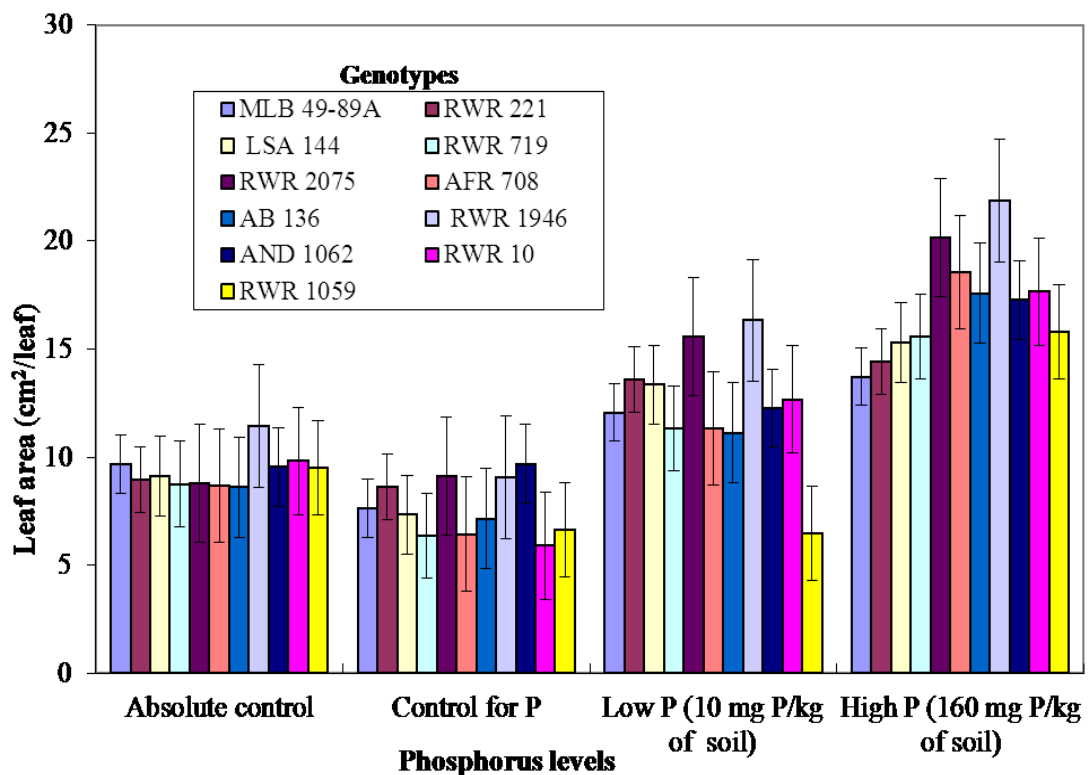


Figure 1: Phosphorus level and genotype interaction effects on leaf area

Shoot dry weight: The genotypes did not have significantly different shoot dry weight under extreme phosphorus deficiency. Highest shoot dry weight of 2.54 g/plant and 2.26 g/plant was observed among genotypes RWR 221 and MLB 49-89A respectively under the absolute control treatment (Figure 2). Under the control for phosphorus treatment, genotypes RWR 1946 and RWR 2075 had the highest weight of 2.49 g/plant and 2.27 g/plant respectively. At a rate of 10 mg P/kg of soil, genotype RWR 1946, had the highest shoot dry weight of 6.34 g/plant. At high phosphorus level of 160 mg P/kg of soil, all the genotypes significantly responded with the highest shoot dry weight of 13.58, 11.33 and 11.21 g/plant being recorded with genotypes RWR 1946, RWR 2075 and RWR 10 respectively.

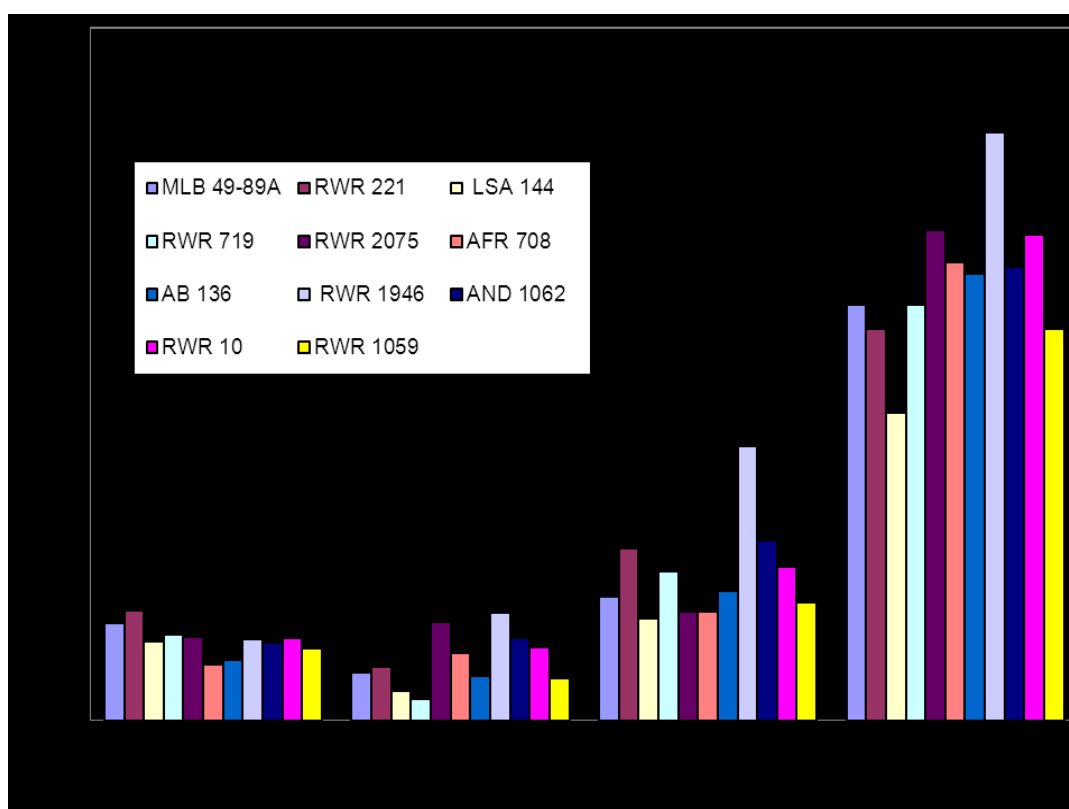


Figure 2: Phosphorus level and genotype interaction effects on shoot dry weight

Root dry weight: Mean genotypic variation for root dry weight was lowest in the control for phosphorus treatment (Figure 3). Under the absolute control and low phosphorus treatments, the genotypes had moderately high root dry weights. A high response in the root dry weight was observed with an increase in phosphorus application (i.e 160 mg P/kg of soil) for all the genotypes. Where the highest root dry weights of 2.093 and 2.075 g/plant were recorded on genotypes RWR 10 and RWR 2075 respectively.

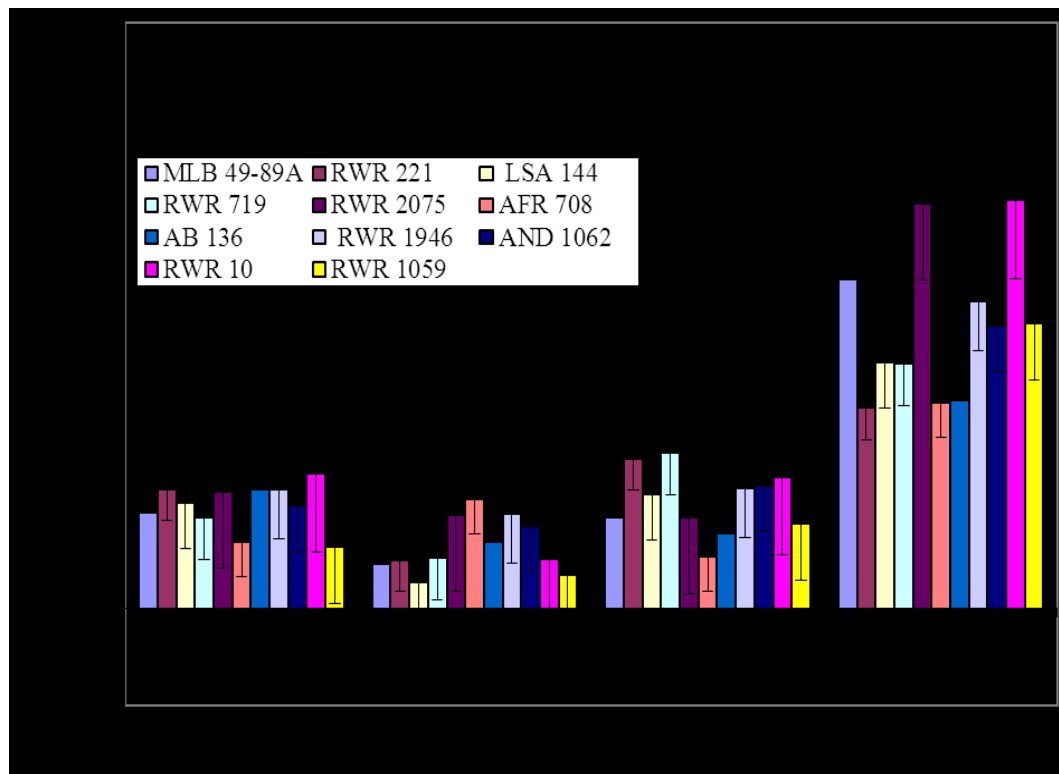


Figure 3: Phosphorus level and genotype interaction effects on root dry weight

Total root length: Highest total root lengths among genotypes were observed in the absolute control (Figure 4), where genotypes RWR 2075, RWR 221, and RWR 1946 had the highest values of 34.50, 28.00 and 25.00 cm respectively. When phosphorus was applied at a rate of 10 mg P/kg of soil, genotypes RWR 221, RWR 1946, AB

136 and G 19839 had the highest total lengths. In genotypes RWR 1946, RWR 2075, MLB 49-89A and AND 1062, an increase in phosphorus level to 160 mg P/kg of soil resulted in increased total root length. In genotypes RWR 719 and AB 136, an increase in phosphorus did not lead to an increase in root length. Genotype RWR 1946 produced nearly the same total root length of 25.00, 22.33 and 22.00 cm under phosphorus deficiency and 28.33 cm under high phosphorus. Phosphorus deficiency reduced the root length in some genotypes, while in other genotypes such as G 19839, RWR 1946, AB 136, root length was increased.

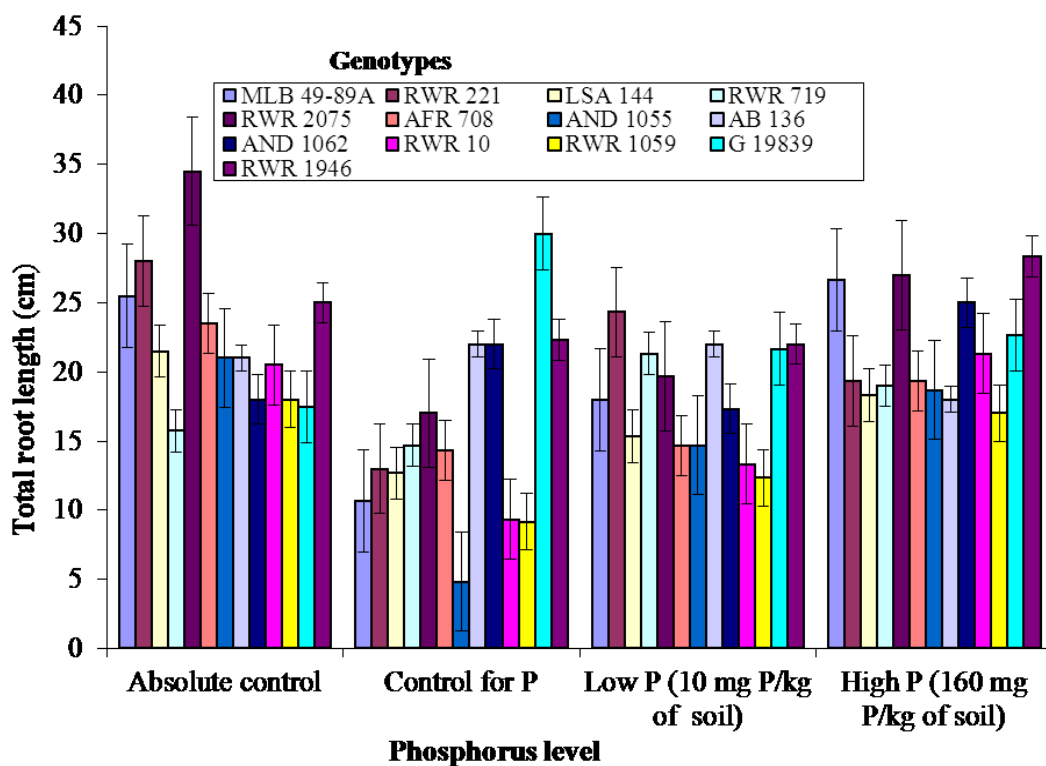


Figure 4: Phosphorus level and genotype interaction effects on total root length

Lateral and basal roots production in response to phosphorus availability:

Generally genotypic variation for production of lateral and basal roots was more evident under phosphorus deficiency than high phosphorus. It was poorest under the control for phosphorus treatment (Table 5). Under the absolute control, genotypes

produced slightly more and longer lateral and basal roots where genotypes such as RWR 1946 and RWR 2075 had vigorous and good root production scores. Under the low phosphorus treatment, some genotypes such as RWR 2075 and AB 136 had more vigorous and increased production of roots than the others. At high phosphorus level of 160 mg P/kg of soil, all genotypes extremely responded in a similar way, with very good root production scores of 1 and 2. The highest mean values for lateral and basal root production scores of 3.208, 3.50 and 3.50 were observed in genotypes RWR 1946, RWR 2075 and G 19839 respectively.

Table 5: Genetic variability for lateral and basal roots production scores in response to phosphorus availability using the common bean shovelomics on a scale of 1-9

Genotype	Phosphorus level				Mean
	Absolute control	Control for P	Low p	High P	
1. MLB 49-89A	6	9	5	1	5.1667 ^{de}
2. RWR 221	4	7	5	1	4.500 ^{cde}
3. LSA 144	5	9	4	1	4.708 ^{cde}
4. RWR 719	4	9	4	1	4.292 ^{bcde}
5. RWR 2075	3	7	3	1	3.500 ^{ab}
6. AFR 708	4	6	5	1	4.042 ^{abc}
7. AND 1055	4	8	7	2	5.167 ^{de}
8. AB136	4	8	3	1	4.208 ^{abcd}
9. AND 1062	5	6	4	2	3.958 ^{abc}
10. RWR 10	6	5	5	1	4.318 ^{cde}
11. RWR 1059	6	7	6	2	5.333 ^e
12. G 19839	4	5	4	1	3.500 ^{ab}
13. RWR 1946	3	6	4	1	3.208 ^a
Mean	4	7	5	1	4.299

CV % = 23.7; LSD (5%) = 1.926; 1 – 9 scale, where 1 was excellent and 9 was very poor; Means bearing the same letters in column 6 are not significantly different

Shoot P concentration: There was significant difference in shoot P concentration for the phosphorus levels. Under the absolute control, genotypes AFR 708, AB 136 and RWR 1946 had the highest shoot P concentration of 0.1976, 0.1999 and 0.1855 % respectively (Figure 5) while LSAA 144 and RWR 221 had the lowest. For the

control for phosphorus treatment, genotypes RWR 2075, RWR 719, AFR 708 and RWR 1946 had the highest values, the lowest being observed with genotype AND 1055. Under low phosphorus treatment, genotypes RWR 2075, LSA 144 and RWR 1946 had the highest shoot P concentration. When phosphorus level was increased to 160 mg P/kg of soil, genotypes AFR 708, LSA 144, RWR 221 and AND 1055 had the highest values.

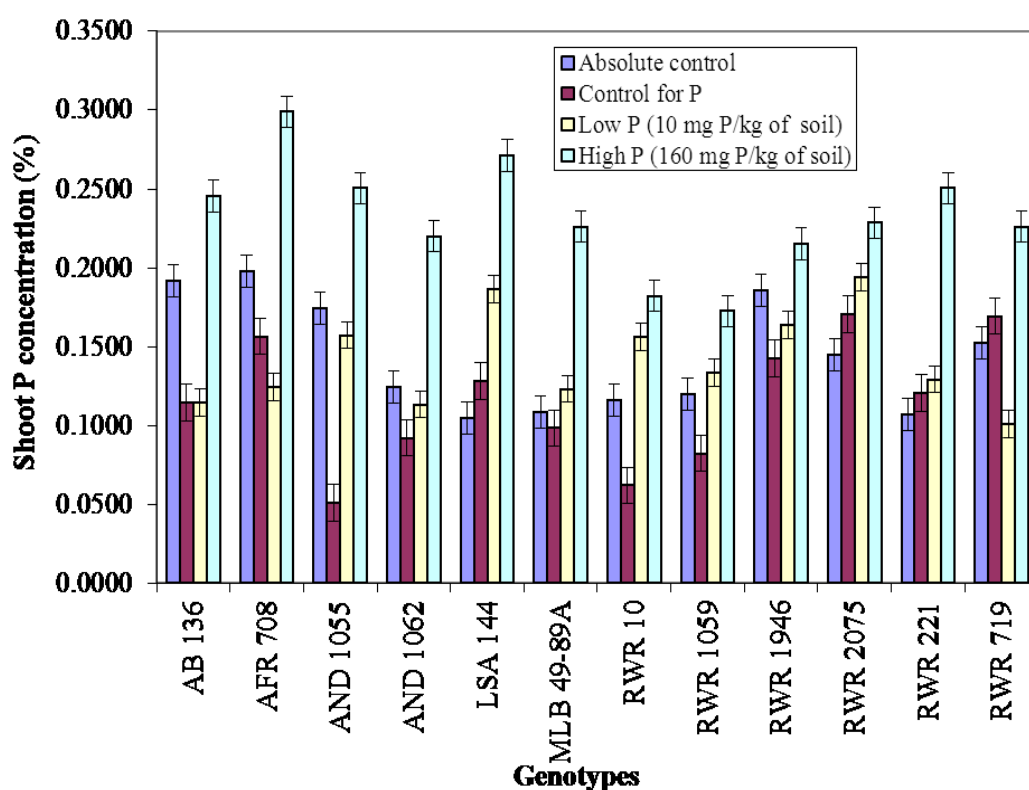


Figure 5: Phosphorus level and genotype interaction effects on shoot P concentration

P uptake: Generally the studied genotypes had the poorest P uptake under the control for phosphorus treatment followed by the absolute control (Figure 6). AND 1055 and RWR 1946 had the highest P uptake under the absolute control. Under the control for phosphorus treatment, genotypes RWR 2075 and RWR 1946 had the highest, with the lowest P uptake being observed in genotype AND 1055. Under the

low phosphorus treatment, genotypes RWR 1946, RWR 221, RWR 10, AND 1062 and RWR 2075 had the highest P uptake. At the high phosphorus treatment, P uptake was highest in genotypes AFR 708, RWR 1946, RWR 2075 and AB 136.

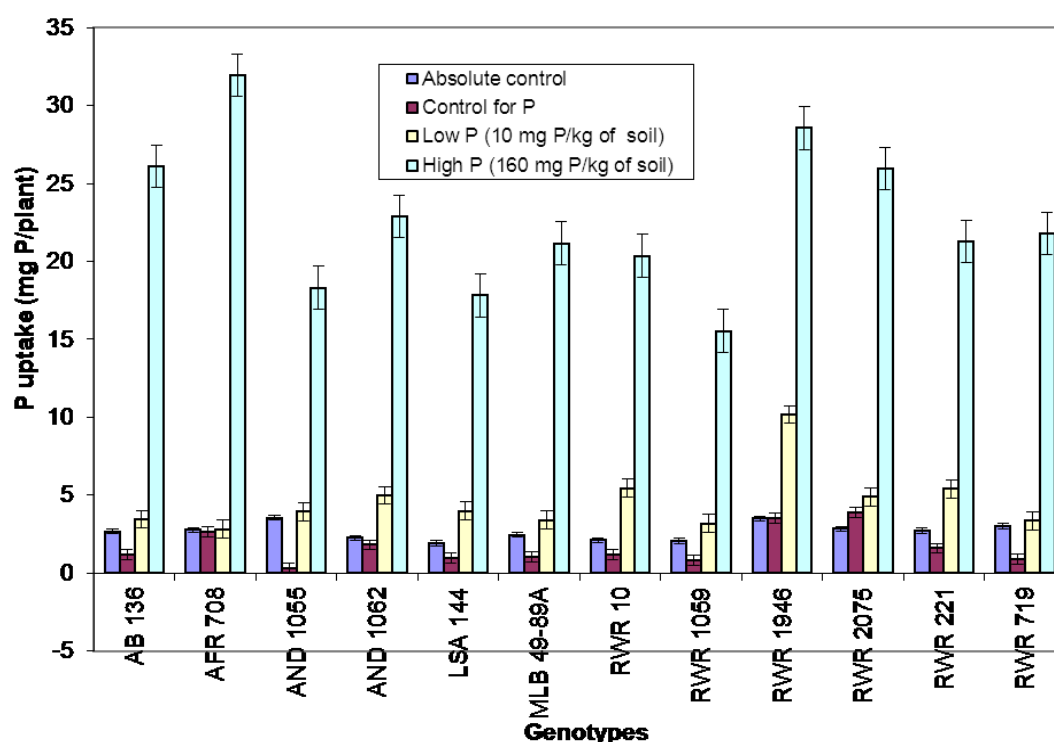


Figure 6: Phosphorus level and genotype interaction effects on P uptake

Simple correlations for measured variables: Correlations between the measured variables were highly significant (Table 6). Genotypes had very weak correlation coefficients with all of the variables under study. Unlike genotypes, a strong positive correlation existed between phosphorus levels and all the variables, except total root length. Leaf area was positively correlated with all variables, and had the highest correlation coefficient with shoot dry weight and the lowest was with total root length. Shoot dry weight was significantly and positively correlated with all

variables, with the highest correlation coefficient being with P uptake and the lowest was with total root length. Root dry weight was significantly and positively correlated with all variables. Total root length had weak correlations with shoot P concentration, genotypes and phosphorus levels. Lateral and basal roots production had a strong positive correlation with the root dry weight. Shoot P concentration was highly and positively correlated with P uptake and shoot dry weight. On the other hand, P uptake was positively and strongly correlated with phosphorus levels, leaf area, shoot and root dry weights, shoot P concentration and lateral roots production. However, it was weakly correlated with genotype.

Table 6: Pearson simple correlations (r-values) between variables measured on 13 common bean genotypes under phosphorus deficiency and high phosphorus

	Genotype	Treatment	Leaf area	Shoot dry weight	Root dry weight	Total root length	Lateral and basal roots scores	Shoot P concentration	P uptake
Genotype	1								
Treatment	0	1							
Leaf area	0.088	0.737**	1						
Shoot dry weight	0.100	0.795**	0.886**	1					
Root dry weight	0.097	0.623**	0.728**	0.824**	1				
Total root length	-0.125	-0.004	0.288**	0.279**	0.342**	1			
Lateral and basal roots scores	0.044	0.518**	0.659**	0.655**	0.719**	0.420**	1		
Shoot P concentration	0.079	0.623**	0.506**	0.728**	0.385**	0.138	0.646**	1	
P uptake	0.04	0.749**	0.836**	0.932**	0.735**	0.250**	0.688**	0.735**	1

** . Correlation is significant at the 0.01 level (2-tailed).

An illustration of the observed tolerance to extreme phosphorus deficiency and low phosphorus (10 mg P/kg of soil) in genotype RWR 1946 is in Figures 7 and 8 respectively.



Figure 7: Tolerance to extreme phosphorus deficiency (no external added P) in genotype RWR 1946 compared with a more sensitive genotype MLB 22-88A (genotype MLB 22-88A was excluded from data analysis, due to missing data, it is therefore only mentioned here in this study)



Figure 8: Tolerance to low soil phosphorus (10 mg P/kg of soil) availability in genotype RWR 1946 compared with G 2333 (genotype G 2333 was excluded from data analysis due to missing data, it is therefore only mentioned here in this study)

4.0 DISCUSSION AND CONCLUSION

The genotypes used in this study varied in leaf area, shoot and root dry weights, total root length, lateral and basal roots production, shoot P concentration and uptake under varying phosphorus availability. These observations confirm that there is genetic variability for response to soil phosphorus availability in common bean (Lynch and Beebe, 1995; Beebe *et al.*, 1997; Broughton *et al.*, 2003; Ochoa *et al.*, 2006). Phosphorus deficiency strongly reduced the leaf area of bean plants implying that genotypes such as RWR 1946 and RWR 2075 observed with the highest leaf area were able to maintain their leaf growth under low phosphorus availability. Leaf area production is important for energy transference and processes for dry matter

accumulation in the crop canopy. According to Trindade *et al.* (2010), low phosphorus supply markedly limits leaf growth in common bean and genotypes able to maintain adequate leaf area at low P could adapt better to limited-P conditions. The observation that low P reduced leaf area is consistent with several earlier findings; for example Lynch *et al.* (1991) and Oliveira (1995) who reported that leaf area development was reduced by phosphorus deficiency in common bean. Lynch and brown (2008) reported that low P availability reduces leaf expansion and branching. Phosphorus deficiency also reduced leaf expansion in cotton (Radian and Eidenbock, 1984). Decreases in leaf area were also observed in soybean plants deprived of P (Fredeen *et al.*, 1989). It is hypothesized that the restricted rate of expansion of individual leaves could result from reduced leaf epidermal cell area (Fredeen *et al.*, 1989), fewer cells per leaf primordia or limited cell elongation (Rodríguez *et al.*, 1998). On the other hand, the significant increase in leaf area observed among all genotypes at 160 mg P/kg of soil, most especially for genotypes RWR 1946 and RWR 2075 confirms that leaf area of bean plants responds to increased P supply mainly by improving the leaf appearance and by enhancing leaf expansion (Lynch *et al.*, 1991).

The decrease in leaf area due to phosphorus deficiency was also accompanied by decrease in shoot dry weight. This is because when leaf expansion is reduced, there is less carbon assimilation which results into low shoot dry weight. Therefore the genotypes observed with higher mean values for the shoot dry weight at low phosphorus availability were more efficient. P efficiency is defined as the ability of plants to produce relatively more yield (either biomass or grain) with suboptimal P

availability or take up more P under inadequate P conditions (Yan *et al.*, 1995a; Yan *et al.*, 2006). The root dry weight also increased with increase in phosphorus level. In the present study, phosphorus deficiency strongly reduced shoot dry weight more than the root dry weight. This is in agreement with other studies that showed that root growth is less affected by phosphorus stress than shoot growth (Fist and Edwards, 1987; Lynch *et al.*, 1991; Oliveira, 1995). This is also in accordance with Brouwer (1962b) who proposed a mechanism by which plants regulate allocation, simply assuming that the organ involved in the acquisition of a resource has priority over that resource. At a sub optimal nutrient supply, shoots (leaves) retain more of the limiting amount of photosynthates, leaving less carbon for root growth. At low nutrient and water availability, roots use relatively more of these resources, leaving less for the shoots (leaves) (Brouwer, 1962b). Consequently, leaf growth is limited by the supply of nutrients and water and less photosynthates are incorporated above-ground. The excess photosynthates are then transported to the root, enhancing root growth relative to that of shoots (Brouwer, 1962b). Also Silva *et al.* (2014) observed an increase in root production in relation to the shoot, in proportion to reductions in the dose of P applied in the plots, with the mean ratios of plots 0.05, 2.00, 4.00 and 8.00 mg L⁻¹ of P being 3.00, 2.73, 2.19 and 0.097, respectively. They further stated that a common response of plants in relation to P deficiency is an increase in the size of the root system in relation to the shoot. Part of this change from dry matter of the shoot to root formation is allometric, Shoot formation ratios normally decrease with root growth since plants with low P supply grow more slowly so that the root may achieve greater indices of development. Some genotypes present a greater coefficient of allometry at low P concentrations (Silva *et al.*, 2014).

Increased production (length and number) of lateral and basal roots and total root length in some genotypes, confirmed that low phosphorus availability modifies root architecture traits such as number and length of lateral roots, primary root length, root branching, enhancement of root hair and cluster root formation, adventitious rooting and top soil foraging in common bean and in other cultivated crops (Borch *et al.*, 1999; Lynch and Brown, 2001; Miller *et al.*, 2003; Kim *et al.*, 2008; Lambers *et al.*, 2011; Jin *et al.*, 2012; Niu *et al.*, 2012). From the present study, lateral and basal roots production was also positively correlated to P-uptake confirming the fact that, lateral roots play an important role in plant adaptation to low phosphorus availability by increasing soil exploration (Zhu *et al.*, 2004; Zhu *et al.*, 2005a), the absorptive surface of the root system (Pérez-Torres *et al.*, 2008) and P solubilization (Lynch, 2007), which results into increased P uptake. Several other studies have also reported that plant roots typically respond to P deficiency through allocation of more carbon to roots resulting in increased root growth, enhanced lateral root formation, greater exploration of the surface soil, increased length and number of root hairs (Liao *et al.*, 2001; Lynch and Brown, 2001; Williamson *et al.*, 2001) that increase P availability. Some genotypes showed increased and vigorous production of lateral and basal roots under low phosphorus availability, others showed the opposite. This is because the response of lateral roots to P deficiency shows genotypic and species variations (Niu *et al.*, 2012). For example in maize, some genotypes show an increase in the number and length of lateral roots while others show the opposite effect (Niu *et al.*, 2012). In arabidopsis low P availability also promotes the development of a highly branched root system characterized by the stimulated formation and emergence of lateral roots and root hairs (Bates and Lynch, 1996; Pérez-Torres *et al.*, 2008; Péret *et al.*, 2011). This is because under low P

concentrations, the mitotic activity is relocated to the sites of lateral root formation, which leads to increased lateral root density (Tyburski *et al.*, 2012) and that each lateral root can produce more secondary, tertiary lateral roots and a complex root system is constructed by the reiteration of a single developmental process.

Consequently because of increased lateral elongation, the total root length increased such that in this study it was also observed that phosphorus deficiency increased the total root length in some genotypes such as G 19839, RWR 1946, AB 136 and RWR 221 and reduced it in others. Genotype RWR 1946 produced nearly the same total root length under phosphorus deficiency and high phosphorus, implying it was phosphorus efficient. Phosphorus-efficient genotypes G2333 and G19839 were also reported to have greater and statistically significant root length under both medium and low phosphorus than the phosphorus-inefficient genotypes (Miller *et al.*, 2003). According to Lynch and Van Beem (1993), a P efficient genotype had a vigorous, highly branched root system with numerous basal roots while a P inefficient genotype had a smaller, less branched root system. Yan *et al.* (1995a) reported that P efficient genotypes had more root length than inefficient genotypes. According to studies by Liao and Yan (2001) also P efficient genotypes had greater root biomass, total root length and smaller average diameter than the P inefficient genotypes under low P conditions.

High and significant correlations between leaf area and shoot dry weight; leaf area and root dry weight; leaf area and P uptake; shoot and root dry weights; shoot dry weight and P-uptake; and root dry weight and P uptake indicate that direction selection for either improved leaf area or shoot dry weight under limited phosphorus

supply would result into increased root dry weight and P uptake, also suggesting that leaf and shoot growth was determined by the amount of phosphorus absorbed (Araujo *et al.*, 2005). Several morphological characters including root and shoot dry weights have been identified as important to low P tolerance in common bean (Wortmann *et al.*, 1995). The present findings on the negative effect of phosphorus deficiency on P uptake suggest that uptake of P also depends on its availability in the soil. The observed variation in P uptake among the genotypes shows the diversity in efficiency to absorb phosphorus from soils of varying availability. Therefore, P uptake would be a good indicator of P acquisition efficiency which is the plant's ability to extract P from the soil.

A positive and high response of all bean genotypes to increased phosphorus levels confirmed that phosphorus is an important macronutrient for common bean growth and production. On the other hand, the absolute control treatment where no fertilizer was added at all resulted into better performance of the measured variables than the control for phosphorus treatment where at least base and nitrogen fertilizers were added but without phosphorus. This is explained by the fact that adding of nitrogen and the base fertilizers alone without phosphorus in the control treatment created a further nutrient imbalance which greatly affected the plant. There by confirming that phosphorus was already the limiting nutrient.

It can be concluded that out of the studied common bean genotypes, some genotypes were more tolerant to low phosphorus availability and greatly responded to added P than others. Generally the large-seeded genotypes RWR 1946 and RWR 2075 appeared to have superior P efficiency under low P availability, while at the same

time they were more responsive to added P. Yan *et al.* (1995) observed that Andean common bean genotypes that have large seeds tend to be more efficient when phosphorus deficiency is present. The root rot resistant genotypes namely RWR 719, MLB 49-89A, AND 1062, AND 1055 and genotypes AFR 708 and RWR 221 were of moderate tolerance while genotypes LSA 144 and RWR 1059 were the least efficient. The controlled screen house study therefore complements the earlier observed field findings for tolerance to low soil phosphorus under the BILFA strategy that had highlighted genotypes such as RWR 2075 (red), AFR 708 (Calima) and many others as tolerant to low soil phosphorus (Lunze *et al.*, 2002; Lunze *et al.*, 2012). In addition, the higher F values observed for all the measured variables at either the genotype or phosphorus level than the F values for genotype x phosphorus interaction level suggest that phosphorus tolerance in the studied genotypes is likely to be stable. The result of this study provides useful information for a breeding program towards obtaining more phosphorus efficient lines. The efficient genotypes can also be recommended for growing in environments of low soil phosphorus.

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CHAPTER FOUR

EARLY GENERATION INHERITANCE OF LOW PHOSPHORUS TOLERANCE-RELATED TRAITS IN COMMON BEAN

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ABSTRACT

Aims: To investigate if morphological traits, in particular increased lateral and basal root production, total root length and higher shoot growth are heritable in the common bean genotypes RWR 1946 and RWR 2075 in order to provide a basis for a genetic improvement program for tolerance to low soil phosphorus availability.

Study design: A completely randomized block design, arranged as a split plot with the low and high phosphorus levels as the main plots and the genetic materials as sub plots was used.

Place and Duration of Study: In the screen house at the National Crops Resources Research Institute (NaCRRI) – Namulonge, between July 2013 and December 2013.

Methodology: Low phosphorus tolerant bean genotypes RWR 1946 and RWR 2075 and the susceptible genotype K132 were crossed to generate F₁ crosses: K132 x RWR1946, K132 x RWR2075 and RWR1946 x RWR2075. The three parental

genotypes and the F₁ crosses were evaluated for shoot growth, lateral and basal roots production and total root length under low and high phosphorus availability.

Results: All the plants for parental genotypes RWR1946 and RWR2075 showed greater lateral and basal roots production, shoot growth and total root length than the plants of genotype K132. Under low phosphorus availability, the response of lateral and basal roots production and shoot growth of the F₁ progenies was similar to parental genotypes RWR1946 and RWR2075 indicating that these traits are heritable in the early generation. Estimated narrow sense heritabilities were 0.60 and 0.75, and 0.45 and 0.51 for lateral and basal root production, and 0.47 and 0.57, and 0.63 and 0.67 for shoot growth under low and high phosphorus respectively. The broad sense heritabilities were 0.38 and 0.43, and 0.30 and 0.54 for lateral and basal root production, and 0.57, and 0.54 for shoot growth under low and high phosphorus respectively.

Conclusion: Narrow sense heritabilities generally higher than broad sense heritabilities strongly suggested that early generation inheritance of increased lateral root production and higher shoot growth in genotypes RWR1946 and RWR2075 was largely due to additive genetic effects. Genetic improvement for low phosphorus availability is likely to be possible using genotypes RWR1946 and RWR2075 as donor parents, while using higher shoot growth and increased lateral and basal root production as selection criteria for desirable genotypes. However, further studies are recommended to better understand the traits in advanced generations and the number of genes involved.

Keywords: [Andean, BILFA, Heritability, Lateral rooting, Shoot growth]

1.0 INTRODUCTION

Common bean (*Phaseolus vulgaris*) is the most important food legume for human consumption worldwide (Broughton *et al.*, 2003), providing high protein content and generous amounts of micronutrients including iron, zinc, folic acid, complex carbohydrates and other essentials (Kornegay *et al.*, 1996). The crop is mostly grown in soils that have been over cultivated and hence characterized with soil mineral deficiency (Wortmann *et al.*, 1998). According to the Atlas of common bean production in Africa, phosphorus (P) is the most frequent deficient soil nutrient with the supply low in 65% and 80% of the bean production areas in eastern and southern Africa (Wortmann *et al.*, 1998). Use of organic and inorganic soil amendments offers the possibility to correct soil phosphorus deficiency, however it has major limitations (Driessen *et al.*, 2001; World Bank, 2004; Araujo *et al.*, 2005; Borlaug, 2006; Tesfaye *et al.*, 2007). Breeding of improved common bean lines with greater phosphorus acquisition and better tolerance to low soil phosphorus is reported as one of the ways to reduce on small scale farmers' dependence on the use of soil amendments (Lynch and Brown, 2012). This is a feasible strategy because genetic variability for tolerance to low phosphorus soils has been identified in common bean (Beebe *et al.*, 1997; Singh *et al.*, 2003). Outside Africa, some of the reported tolerant genotypes include the following: G19833, G19839 and G14017 (Yan *et al.*, 1995a; Miller *et al.*, 2003, Cichy *et al.*, 2009). Whereas in Africa, genotypes tolerant to low soil phosphorus and other fertility stresses such as low nitrogen and aluminium toxicity have been put together in the BILFA (Bean Improvement for Low soil Fertility soils in Africa) nursery (Wortmann *et al.*, 1995; Lunze *et al.*, 2002; Lunze *et al.*, 2012). Examples of the low phosphorus tolerant

lines in the BILFA nursery include: BAT25, RAO55, XAN 76, MMS 224, ACC 433, Ikinimba, ARA 4, AFR 675, AFR 708, AFR 714, AND 871, CIM9314-3, CIM9314-36, CIM9331-1, CIM9331-3, FEB192, FEB196, G5889, LSA32, PAN150, RAB482, RWR1873, RWR1946 and RWR2075 (Kimani *et al.*, 2006; Lunze *et al.*, 2012).

Breeding for resistance or tolerance is facilitated if the mechanisms of resistance or tolerance are known. Consequently, a number of controlled environmental studies have identified several morphological and physiological characters that are important for tolerance to low soil phosphorus (Wortmann *et al.*, 1995). Fageria *et al.* (2008) reported that root traits can be used to improve tolerance to nutrient deficiency and other edaphic stresses such as drought and salinity. Some of the reported root traits include: number and length of lateral roots, primary root length, root branching, enhancement of root hair and cluster root formation, adventitious rooting and top soil foraging (Borch *et al.*, 1999; Lynch and Brown, 2001; Miller *et al.*, 2003; Kim *et al.*, 2008; Lambers *et al.*, 2011, Niu *et al.*, 2012). Liao and Yan (2001) also reported that in terms of root traits, morphological characteristics of the basal and lateral roots contribute more to phosphorus efficiency than those of the tap roots. Lateral roots in particular have been reported to play an important role in plant adaptation to low phosphorus availability by increasing soil exploration (Zhu and Lynch, 2004; Zhu *et al.*, 2005a), the absorptive surface of the root system (Pérez-Torres *et al.*, 2008) and phosphorus solubilization (Lynch, 2007), which results into increased phosphorus uptake. The ability of plants to produce higher biomass (either shoot or root) under inadequate phosphorus conditions is another

measure of phosphorus efficiency in common bean (Yan *et al.*, 2006). According to Araujo *et al.* (2005), selection of bean cultivars with enhanced root growth would be a strategy for increasing phosphorus absorption.

Previous studies on genetic variation for phosphorus response by Namayanja *et al.* (2014) indicated that out of the thirteen common bean genotypes, the large seeded genotypes RWR 1946 and RWR 2075 had the best growth, hence superior phosphorus efficiency under low phosphorus availability based on the measured traits namely leaf area, shoot and root dry weights, total root length, basal and lateral roots production, shoot phosphorus concentration, and phosphorus uptake. These genotypes are probably useful donor parents. However lack of information about genetic control of the observed traits limits their exploitation by a plant breeding program for tolerance to low soil phosphorus availability. Therefore the present study investigated if increased lateral and basal root production, total root length and higher shoot growth under low phosphorus availability were heritable traits in common bean. This would be useful in providing a basis for a genetic improvement program for tolerance to low soil phosphorus availability.

2.0 MATERIAL AND METHODS

2.1 Genetic Materials

The study was conducted in the screen house at the National Crops Resources Research Institute- Namulonge, Uganda. Three Andean parental common bean genotypes, namely RWR1946, RWR2075 and K132 released and commercially grown in Uganda and also contrasting in their phosphorus efficiency were used

(Table 1). The two low phosphorus tolerant genotypes (RWR1946 and RWR2075) and the susceptible genotype (K132) were crossed to generate the F₁ crosses: K132 x RWR1946, K132 x RWR2075 and RWR1946 x RWR2075.

Table 1: Description of the parental bean genotypes used in the study

Genotype	Other name	Market Class	Origin	Reaction to low P	Other desirable attributes
1. RWR 1946*	NABE 13	Red	Rwanda	Tolerant (Kimani <i>et al.</i> , 2006; Namayanja <i>et al.</i> , 2014)	Tolerant to <i>Pythium</i> root rot, marketable seed
2. RWR 2075*	NABE 14	Red	Rwanda	Tolerant (Lunze <i>et al.</i> , 2012; Namayanja <i>et al.</i> , 2014)	Tolerant to <i>Pythium</i> root rot, marketable seed
3. K 132	CAL 96	Red mottled	CIAT	Susceptible (Kimani and Kimani, 2001)	Marketable seed

*First selected from the BILFA (Bean Improvement for Low soil Fertility in Africa) nursery

2.2 Soil Preparation and Planting

The soil substrate used was collected from the National Crops Resources Research Institute, Namulonge and had originally the following physical and chemical properties: pH of 5.7, 4.28 ppm of extractable P (Mehlich- 3), 0.32% N, 7.08 ppm Cu, 1.57 ppm Zn, 262.90 ppm Mn, 253.90 ppm Fe, 6.64% organic carbon, cation exchange capacity of 20.41 cmol/kg of soil, 1795.13 ppm Ca²⁺, 1184.44 ppm Mg²⁺, 82.82 ppm K⁺, and 0.16 cmol Al³⁺/kg of soil (Soil and Plant Analytical Laboratories at NARL,NARO, Uganda).

The experimental design used was a completely randomized block design, arranged as split plot with two phosphorus levels as the main plots and the genetic materials

as subplots. The phosphorus levels included low phosphorus consisting of 10 mg P/kg of soil and high phosphorus consisting of 160 mg P/kg of soil. The source of phosphorus was triple super phosphate fertilizer. Soil for each treatment was handled separately and mixed thoroughly well with the appropriate phosphorus level and basal fertilizers. Basal fertilizers were applied at the following rates, i.e 100 mg K/kg of soil (as potassium chloride), 10 mg Zn /kg of soil (as zinc sulphate) and 1 mg B/kg of soil (as boric acid). The soil for each treatment was then placed in 16 kg plastic containers which were later placed in the open air on a raised metallic screen house bench. The field water content for each 16 kg plastic container was determined by the Savage method (1979) and watering was done to field capacity. Sowing was done when the soil had reached field capacity.

For each of the parents and the F₁s, two 16 kg containers each sown with 15 seeds were used under each of the low and high phosphorus treatments. Six days after emergence, thinning was done leaving only 12 plants in each 16 kg container. This gave a total of 24 plants for each genotype (i.e the 3 parents and the F₁s) under each treatment. Nitrogen fertilisers i.e 200 mg N /kg of soil (as ammonium sulphate) and 200 mg N/kg of soil (as urea) were also applied via dilute solution 12 and 25 days after emergence respectively. Watering was done as adequately as possible.

2.3 Traits Measurements and Analyses

At 30 days after planting, shoot heights were measured for all plants in each 16 kg container. Fifteen days later the shoots (including petioles, leaves, stems) of all plants in each container were harvested and oven dried for 4 days at 70°C. Shoot dry weights were recorded. Roots including nodules were separated from soil by

washing with water, left to dry in the open air and then put in the oven at 70°C for 4 days. Total root length was determined from the base of the hypocotyl along the length of the longest basal with the lateral roots that emerge from them. It is important to note that basal roots arise from the base of the hypocotyl and in conjunction with the lateral roots that emerge from them basal roots usually comprise the majority of total root length (Vieira *et al.*, 2008). Lateral and basal root production in response to phosphorus availability was visually determined according to the common bean shovelomics (Jochua, 2013), using a score of 1-9 (1 was excellent and 9 was very poor).

Analysis of variance and correlation among measured traits were performed using GENSTAT v.14. software (VNS International Hemstead, UK). When means were different significantly, Fisher's protected least significant difference was used. Broad sense heritability (H^2) and narrow sense heritability (h^2) were determined as below;

$$H^2 = \frac{\text{Genotypic variance (VG)}}{\text{Phenotypic variance (VP)}} ; \quad h^2 = \frac{\text{Additive Variance (VA)}}{\text{Phenotypic Variance (VP)}}$$

Broad sense heritability includes all components of genetic variance. While narrow sense heritability includes only the additive genetic variance and it is the proportion of variability that can be passed on from parent to offspring, and it is the form of heritability that is of interest to plant breeders, since it measures the resemblance of progenies to their parental genotypes (<http://www.lopdf.net/.../Lecture-18-Genetics-of-complex-traits-quantitative-gene>).

3.0 RESULTS AND DISCUSSION

3.1 Phosphorus Response and Genotypic Variability

Analysis of variance for the shoot growth (height and dry weight), increased lateral and basal root production and total root length evaluated for the three parental genotypes and their F₁ progenies revealed that phosphorus effects (P levels) were highly significant ($P < 0.05$) on all the traits measured except on the lateral root production (Table 2). In contrast, effect of genotype was highly significant ($P < 0.05$) on all the traits which suggested the existence of genetic variation for phosphorus efficiency among the studied materials. Only total root length was influenced by both genotype and phosphorus level.

Table 2: Analysis of variance for the different traits evaluated for the three Andean parental genotypes and their F₁ progenies

Traits	Source	Df	MS	F value	P value
1.Shoot dry weight	P levels	1	585.1640	678.49	<.001
	Genotype	5	13.206	15.31	<.001
	Gen. x P.	5	0.4968	0.58	0.718
2.Shoot height	P levels	1	1223.834	174.09	< .001
	Genotype	5	156.116	21.21	< .001
	Gen. x P.	5	7.362	1.05	0.413
3.Total root length	P levels	1	105.062	47.71	<.001
	Genotype	5	29.007	12.62	< .001
	Gen. x P.	5	16.796	7.31	< .001
4. Lateral and basal root production scores	P levels	1	8.028	6.72	0.016
	Genotype	5	17.45	14.61	< .001
	Gen. x P.	5	4.161	3.48	0.017

Gen. x P. = Genotype x phosphorus interaction; Levels of $P < 0.001$ were significant

The higher F values observed for all the measured traits at either the genotype or phosphorus level than the F values for genotype x phosphorus interaction level suggests that the observed tolerance to phosphorus is likely to be stable.

In general, all the measured traits increased with increase in phosphorus level (Table 3). Under low phosphorus, shoot height ranged from 13.25 to 29.00 cm, while under high phosphorus level it ranged from 28.27 to 40.83 cm. El-Gizawy and collaborators (Silva *et al.*, 2014) reported that an increase of 29% in plant height was observed in common bean in plots fertilized with 30 kg of phosphate (P_2O_5) compared to the control without application of the nutrient. Shoot dry weight also increased with increase in phosphorus level, ranging from 3.01 to 6.59 g plant⁻¹ at low phosphorus and from 10.63 to 15.35 g plant⁻¹ under high phosphorus. On the other hand, for the total root length, mean values ranging from 5.33 to 15.83 cm and from 12.33 to 17.33 cm were observed for the low and high phosphorus treatments respectively. These observations confirm that phosphorous is important for plant nutrition in common bean. It is generally a key component for energy generation in plants and the observed growth is the result of the increase in cell division due to application of phosphorus, raising the quantity of ATP in the growth centers as reported by Zafar and collaborators (Silva *et al.*, 2014). Lateral and basal roots production scores ranged from 1.67 to 8.67 and from 4.0 to 7.0 under low and high phosphorus availability respectively on the scale of 1 – 9, where 1 was excellent and 9 was very poor.

Table 3: Shoot growth (height and dry weight), lateral and basal root production and total root length of the parental genotypes and their F₁ progenies evaluated under low and high phosphorus levels

Phosphorus level	Genotype	Shoot height (cm)	Shoot dry weight (g plant ⁻¹)	Total root length (cm)	Lateral and basal root production on a scale of 1-9
1.Low	1.RWR 1946	28.00	6.36	12.67	1.67
	2.RWR 2075	27.67	6.29	12.00	2.33
	3.K 132	13.25	3.01	5.33	8.67
	4. F ₁ 3246	26.83	6.10	11.00	4.00
	5. F ₁ 3275	29.00	6.59	15.83	4.00
	6. F ₁ 4675	23.75	5.40	11.00	4.00
2. High	1.RWR 1946	36.17	13.60	15.33	4.00
	2.RWR 2075	38.50	14.47	16.00	5.33
	3.K 132	28.27	10.63	13.00	7.00
	4. F ₁ 3246	39.03	14.67	12.33	5.00
	5. F ₁ 3275	40.83	15.35	14.33	5.00
	6. F ₁ 4675	35.67	13.41	17.33	4.00
	Mean	30.58	9.66	13.01	4.58
	LSD (5%)	4.576	1.609	2.514	1.84
	CV (%)	8.8	9.8	11.4	23.8

Under low phosphorus of 10 mg P/kg of soil, the efficient genotypes RWR 1946 and RWR 2075 and their F₁ progenies were observed with higher shoot growth, total root length and more lateral and basal roots than the inefficient genotype K132. This is in agreement with earlier researchers who reported that phosphorus efficient genotypes had more root length than inefficient genotypes under low phosphorus conditions (Yan *et al.*, 1995a; Liao and Yan, 2001) According to Lynch and Van Beem (1993), a phosphorus efficient genotype had a vigorous, highly branched root system with numerous basal roots while an inefficient genotype had a smaller, less branched root system. On the other hand, under high phosphorus availability of 160 mg P/kg of soil, the efficient genotypes and the F₁ progenies did not show a very big difference from the inefficient genotype K 132 for all the measured traits. Suggesting that the traits were more important for genetic variation under low

phosphorus conditions than under high phosphorus. Coltman and collaborators (Pessarakli, 2011) also reported that at low levels of available phosphorus, total root length, root weight and root hairs were important traits in genetic variation of tomato and that when plants were grown with adequate levels of available phosphorus, these traits were not important.

3.2 Heritability of Increased Lateral and Basal Roots Production and Shoot Growth in Parental Genotypes RWR1946 and RWR2075 in the Early Generation

The performance of the F₁ progenies namely K132 x RWR1946, K132 x RWR2075 and RWR1946 x RWR2075 was generally similar to parental genotypes RWR1946 and RWR2075 for all the measured traits (Table 4), indicating that these traits are heritable in the early generation. From the present study, this performance was more visible under the low phosphorus conditions than under high phosphorus as already mentioned in section 3.1 above.

Table 4: Shoot growth (height and dry weight), lateral and basal root production and total root length of the parental genotypes and their F₁ progenies evaluated under low and high phosphorus levels

Genotype	Shoot height (cm)	shoot dry weight (g plant ⁻¹)	Lateral and basal roots production scores on a scale 1-9	Total root length (cm)
1. RWR1946	32.08 ^a	9.98 ^{ab}	2.83 ^a	14.00 ^{ab}
2. RWR2075	33.08 ^a	10.31 ^{ab}	3.83 ^b	14.00 ^{ab}
3. K132	20.76 ^b	6.819 ^c	7.83 ^c	9.17 ^c
4. F ₁ K132 x RWR1946	32.93 ^a	10.39 ^{ab}	4.50 ^{bc}	11.67 ^{bc}
5. F ₁ K132 x RWR2075	34.92 ^a	10.97 ^a	4.50 ^{bc}	15.08 ^a
6. F ₁ RWR1946 x RWR2075	29.71 ^a	9.40 ^b	4.00 ^b	14.17 ^{ab}
Mean	30.60	9.66	4.58	13.01
LSD (5%)	4.47	1.57	1.84	2.56
CV%	8.7	9.6	23.8	11.6

Means followed by the same superscript letter within the same column are not significantly different (P= 0.05) by Fisher's protected least significant difference test

Studies by Liao and Yan (2001) using the F₉ recombinant inbred lines derived from parental genotypes DOR364 and G19833, confirmed that basal root morphological characteristics including biomass, length and surface are inheritable. An illustration of increased lateral and basal root production and higher shoot growth under low phosphorus conditions of the F₁ progenies compared with their parental genotypes is in Figures 1, 2a and 2b.



Figure 1: Lateral and basal root production of: (a) parental genotypes RWR1946 and K132 and their F₁ K132 x RWR1946, (b) parental genotypes RWR2075 and K132 and their F₁ K132 x RWR2075 at 45 days after planting (DAP) under the low phosphorus treatment (Low P): F₁ 3246 = F₁ K132 x RWR1946; F₁ 3275 = F₁ K132 x RWR2075

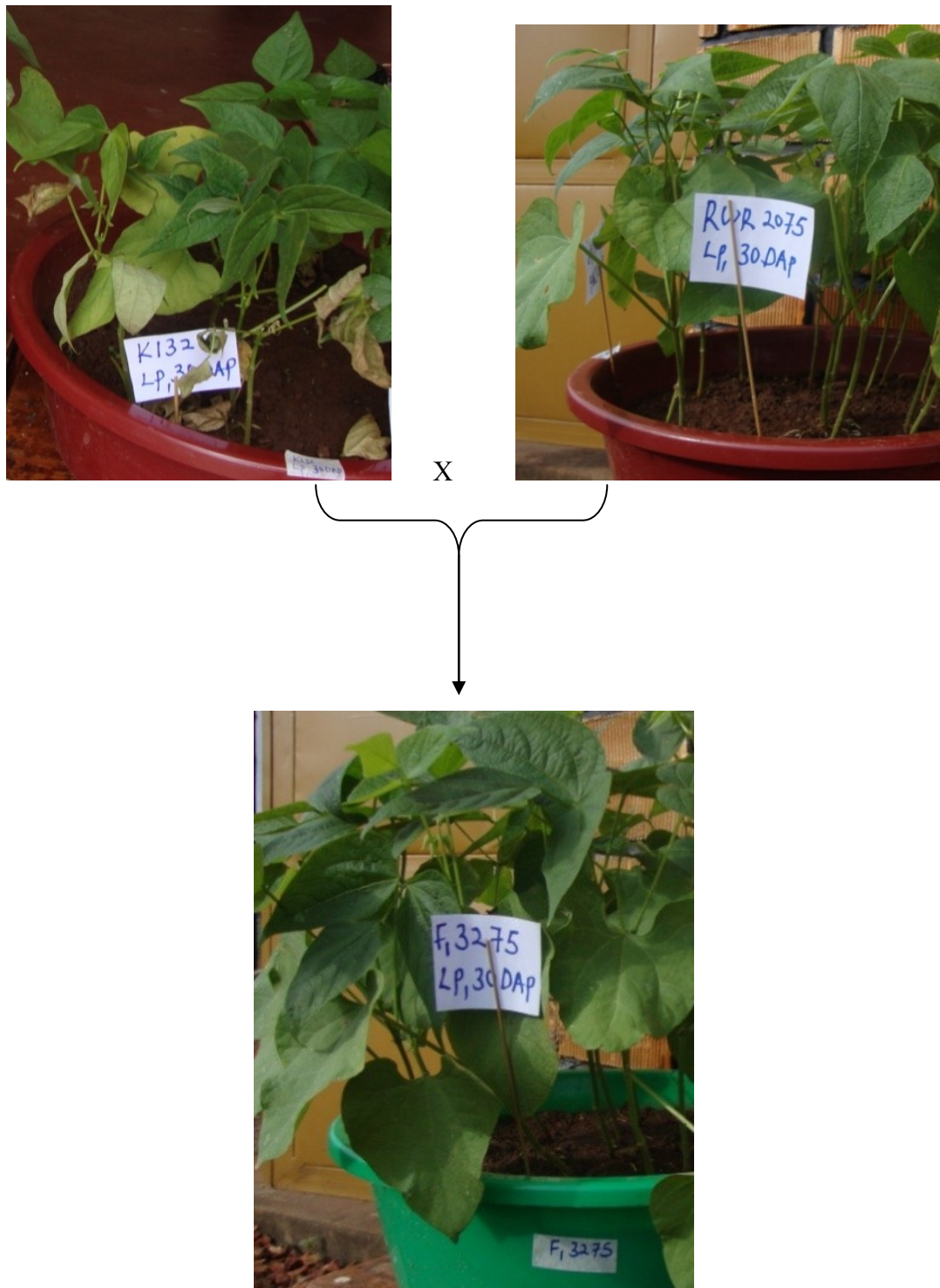


Figure 2a: Shoot appearance of parental genotypes K132, RWR2075, and the crosses F_1 K132 x RWR2075 at 30 days after planting (DAP) under the low phosphorus treatment (LP): F_1 32 75 = F_1 K132 x RWR2075



Figure 2b: Shoot appearance of parental genotypes K132, RWR1946 and the crosses F₁ K132 x RWR1946 at 30 days after planting (DAP) under the low phosphorus treatment (LP): F₁ 3246 = F₁ K132 x RWR1946;

Furthermore, estimated narrow sense heritabilities were 0.60 and 0.75, and 0.45 and 0.51 for lateral and basal root production, and 0.47 and 0.57, and 0.63 and 0.67 for shoot growth under low and high phosphorus respectively (Table 5). The broad sense heritabilities were 0.38 and 0.43, and 0.30 and 0.54 for lateral and basal root production, and 0.57, and 0.54 for shoot growth under low and high phosphorus respectively.

Table 5: Heritability of increased lateral and basal root production and shoot growth in the F₁ progenies

Phosphorus level	F₁ generation	Heritability (%)	Increased lateral and basal root production	Shoot growth
Under low phosphorus	K132 x RWR1946	Broad sense heritability	0.38	0.57
		Narrow sense heritability	0.75	0.57
	K132 x RWR2075	Broad sense heritability	0.43	0.57
		Narrow sense heritability	0.60	0.47
Under high phosphorus	K132 x RWR1946	Broad sense heritability	0.30	0.54
		Narrow sense heritability	0.45	0.67
	K132 x RWR2075	Broad sense heritability	0.54	0.54
		Narrow sense heritability	0.51	0.63

Obtained estimates of narrow sense heritability generally higher than broad sense heritability strongly suggested that early generation inheritance of increased lateral root production and higher shoot growth as traits for tolerance to low phosphorus availability in genotypes RWR1946 and RWR2075 was largely due to additive genetic effects. Heritability of several root traits has been reported in common bean and evidence indicates that root growth in common bean is heritable. For example,

Fawole *et al.* (1982) evaluated six bean crosses in a nutrient solution and obtained estimates of broad sense heritability from 69 to 90 % for root mass. Araújo *et al.* (2005) evaluated two crosses between bean cultivars under limited soil phosphorus supply and they estimated moderate broad sense heritability ranging from 0.55 to 0.51 for root area and 0.51 to 0.61 for root mass, with predominance of additive variance. While Ochoa *et al.* (2006) found narrow sense heritability ranging from low to high for adventitious root traits. Studies by Kimani *et al.* (2007) on inheritance of low soil phosphorus associated traits in common bean genotypes AFR708, CIM9314-36 and CAL143 revealed high general combining ability and specific combining ability with predominance of additive genetic variance. Jochua (2013) reported moderately high narrow sense heritability of root hair length from basal roots in population SEA5 x SXB 418 ($h^2= 0.69$) and population VAX 1 x SXB 418 ($h^2= 0.71$) using parent – offspring regression co-efficient (b) of F₄ progeny family means on F₃ parental values of SEA5 x SXB 418 and VAX 1 x SXB 418 populations. Heritability estimates of root biomass in common bean, with the predominance of additive over dominance effects as reported by earlier researchers (Fawole *et al.*, 1982; Araújo *et al.*, 2005; Kimani *et al.*, 2007), indicates that enhanced root growth is heritable and capable of being fixed through selection into breeding lines. Similarly from their findings, Zhu and Lynch (2004) made a conclusion that enhanced lateral rooting under phosphorus stress is useful trait that may be harnessed for selecting and breeding of more phosphorus efficient maize genotypes.

3.3 Simple Correlations Between Measured Traits for the Three Parental Genotypes and their F₁ Progenies Under Low Phosphorus Availability

Observed correlations between shoot height and all the measured traits were positive and significant (Table 6) under low phosphorus availability. Shoot dry weight also had positive and significant correlations with lateral roots production and total root length. Lateral roots production also had positive and significant correlation with genotype, shoot height and shoot dry weight. Total root length was positively and significantly correlated with all the traits.

Table 6: Pearson simple correlations (r-values) between measured traits on RWR 1946, RWR 2075, K 132 and their F₁ progenies under low phosphorus availability

	Genotype	Shoot height	Shoot dry weight	Lateral roots production	Total root length
Genotype	1				
Shoot height	0.102	1			
Shoot dry weight	0.102	1.000**	1		
Lateral roots production	.589*	.684**	.684**	1	
Total root length	0.025	.890**	.889**	.566*	1

*. Correlation is significant at the 0.05 level (2-tailed).

** . Correlation is significant at the 0.01 level (2-tailed).

This probably offers the possibility of indirect selection for root growth (increased lateral and basal root production and total root length) via higher shoot growth (shoot height and shoot dry weight) under limited phosphorus availability. Indirect selection is important because relying on root traits such as lateral rooting in a breeding program has challenges given the fact that their evaluation involves destructive methods (Trindade and Araujo, 2014). Hence the need for indirect selection of root growth through other plant traits such as higher shoot growth.

Araújo *et al.* (2005) observed high phenotypic and genotypic correlations between shoot mass and root mass in backcross bean families grown at a low soil phosphorus supply. In their studies, Araújo *et al.* (2005) further suggested that selection of bean cultivars with enhanced root growth would be a strategy for increasing phosphorus absorption and that direct selection for higher shoot growth of bean plants under limited soil phosphorus supply would result into increased root mass and phosphorus uptake. Trindade and Araujo (2014) also found significant correlations between shoot mass and root mass and proposed that indirect selection of bean lines with improved root growth via higher shoot growth should be performed at mid-pod filling stages. Previous studies by Namayanja *et al.* (2014) on the genotypic variation for tolerance to low phosphorus availability in 13 common bean genotypes also indicated significant and positive correlation between shoot dry weight and lateral and basal root production.

4.0 CONCLUSION

In conclusion the results of these studies are consistent with earlier findings which reported that under low phosphorus conditions, efficient common bean genotypes tend to have higher shoot growth and increased root traits (Cichy *et al.*, 2009). This implies that these traits are useful selection criteria in a breeding program for tolerance to low soil phosphorus availability. The study also revealed that increased lateral and basal roots production, total root length and higher shoot growth were heritable traits in the studied genotypes under low phosphorus availability. This suggests that there is potential for genetic improvement using these traits to select for desirable genotypes. The study further revealed positive and significant correlations between all the measured traits, hence suggesting the possibility of

indirect selection for the studied root traits via higher shoot growth. The results of the present study are important because as reported by previous researchers, genetic improvement for tolerance to low soil phosphorus is a suitable approach to improve yield since the seeds from improved varieties have larger possibilities of reaching farmers in rural areas in developing countries than fertilisers. However, further studies are recommended to better understand the traits in advanced generations starting with F_2 - F_9 RILS and also to understand the number of genes involved.

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CHAPTER FIVE**RESPONSE OF TWO LOW SOIL PHOPHORUS TOLERANT COMMON
BEAN GENOTYPES TO ALUMINIUM TOXICITY**

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ABSTRACT

Common bean is more sensitive to aluminium toxicity compared to other crops. In the present study, performance of two common bean genotypes, RWR 1946 and RWR 2075 previously characterized as tolerant to low soil phosphorus was evaluated in one soil characterized with relatively high amounts of exchangeable aluminium (Al) ranging up to 55.2 % Al saturation and in another with 14.7%. In the presence of aluminium toxicity (55.2 % Al saturation), very low values of 0.55 and 0.81 g/plant (for shoot dry weight), 0.14 and 0.21 g/plant (for root dry weight), 7.5 and 6.33 cm (for total root length) and 1.38 and 2.89 mg P/plant (for P uptake) were observed for genotypes RWR 1946 and RWR 2075 respectively. Relatively high values of 6.1 and 4.5 g/plant, 0.8 and 0.9 g/plant, 24.4 and 24.5 cm, 11.4 and 9.4 mg P/plant were observed for the same measured traits respectively in the absence of aluminium toxicity (14.7% Al saturation). Out of the measured traits, total root length and P-uptake appeared to be the most affected by presence of high aluminium levels and are therefore useful selection criteria for tolerance to aluminium toxicity.

Key words: aluminium saturation, P-uptake, tolerance, total root length

1.0 INTRODUCTION

Aluminium (Al) toxicity is reported as an important and wide spread constraint of common bean in acid soils with pH 4.5-5.0 resulting into 30 - 60 % reduction in production (Wortmann *et al.*, 1998; Thung and Rao, 1999; Broughton *et al.*, 2003). Common bean is considered to be more sensitive to aluminium toxicity compared to other crops (Thung and Rao, 1999). Other crops such as rice (*Oryza sativa*, L.), peanut (*Arachis hypogea*), sweet potato and cassava are considered to be more tolerant (Fageria *et al.*, 1988; O'Sullivan *et al.*, 1997). For example, the critical toxic level of percent aluminium saturation in the soil for common bean is reported to be 20%, while that of rice is > 45% (Fageria *et al.*, 1988).

In common bean, aluminum toxicity can be observed easily in the root system to which it causes morphological abnormalities (Rangel *et al.*, 2008). Root symptoms include root thickening, inhibition of lateral roots and root hairs, and destruction of root epidermal and cortical cells (Rangel *et al.*, 2008). In case of severe toxicity, the bean plants will not produce the principal roots but rather roots similar to gramineous roots. The leaves dry up as if herbicide had been applied (Schoonhoven and Voysest, 1991). Once the development of the root is greatly affected, a temporary dry spell occurs and it reduces bean growth. Worse still if such a dry spell occurs immediately after germination, the plant may die. The aerial parts of the plant become so affected because the products of metabolism are consumed to produce new roots instead of producing the aerial part (Schoonhoven and Voysest,

1991). It is reported that since the root system is often affected, aluminum toxicity also increases the risk of drought under rain fed conditions in older plants (Rangel *et al.*, 2007; Blair *et al.*, 2009).

Poor crop growth due to aluminum toxicity can be corrected by several agronomic methods including liming, addition of green manures and animal wastes (Haynes, 1984; Hue, 1992; Bereket *al.*, 1995; Haynes and Mokolobate, 2001). Lime neutralizes the toxicity and provides Ca^{2+} or Ca^{2+} and Mg^{2+} (Schoonhoven and Voysest, 1991). While components in the organic matter from the green manures and animal wastes are capable of binding free Al into non-toxic complexes. The organic matter also helps in slowing the rate of acidification of the soil. Unfortunately, the practice of liming acid soils, i.e., applying CaCO_3 , in order to raise soil pH and precipitate exchangeable Al as insoluble hydroxy – Al often requires large quantities of lime e.g. 2–10 tonnes per hectare to achieve adequate growth of many crops (Haynes and Mokolobate, 2001). On the other hand, organic residues such as animal manures, composted wastes and grass and crop residues are usually readily available to farmers, but sometimes in limited amounts. Probably identification and use of genotypes adapted to aluminum toxicity can reduce production costs and dependence of farmers on soil amendments. Consequently research efforts have focused on screening of available germplasm such as landraces and several improved genotypes using green house, growth chamber, nutrient solutions and or field screening methods. Some of the genotypes identified as tolerant to aluminum toxicity include G19833, BRB 191, G5273, MAM49, Quimbaya (Singh *et al.*, 2003; Rangel *et al.*, 2005; Rangel *et al.*, 2008; Cichy *et al.*, 2009; Blair *et al.*, 2009). Research efforts from the BILFA (Bean Improvement for

Low soil Fertility soils in Africa) strategy have also identified some genotypes tolerant to aluminum toxicity including VTTT 923-6-1, HM 21-7, AFR 593-1, MwaSole, ARA-8-5-1, AND 932-A-1, and BZ 12984-C-1(Kimani *et al.*, 2006). According to Butare *et al.* (2011) some accessions of *Phaseolus coccineus*. L. or runner beans (G35346-2Q and G35464-5Q) have also demonstrated greater level of Al-resistance

Another serious problem of aluminium toxicity is that it decreases nutrient availability (Rao, 2001). As a result, nutrient stresses are often common in plants suffering from Al toxicity. For example phosphorus (P) deficiency symptoms are common in plants suffering from Al toxicity. This is because Al in the hydrous oxide form has the ability to adsorb P onto the surface. Thus, much of the added P is 'fixed' and is not readily available for crop use. Therefore amelioration of Al toxicity normally results in greatly increased P uptake by plants even though the availability of soil P may be unchanged or even decreased (Haynes, 1982). Schoonhoven and Voysest, (1991) reported that the effect of aluminium toxicity is always related to deficiency of phosphorus. Such that normally soils with aluminum toxicity are also low in phosphorus content. Plants that suffer from aluminum toxicity have slight root development and hence less volume to explore for phosphorus. Naidoo (1977) suggested that beans with Al tolerance possess the ability to maintain sufficient phosphorus to the aerial part of the plant. In some regions, such as Latin America, both phosphorus deficiency and aluminum toxicity have been classified as the main nutritional problems of beans which cause low bean productivity (Schoonhoven and Voysest, 1991). Probably selection and breeding of common bean genotypes adapted to both aluminum toxicity and low phosphorus

availability would be a useful strategy. A few genotypes such as G 19833, with a large yellow and red mottled seed type have been reported to be tolerant to both aluminum toxicity and low soil phosphorus (Yan *et al.*, 1995a; Manrique *et al.*, 2006; Cichy *et al.*, 2009). Therefore the objective of this research was to evaluate the two elite large seeded common bean genotypes RWR 1946 and RWR 2075 previously confirmed to be tolerant to both low soil phosphorus and *Pythium* root rot disease for reaction to aluminum toxicity under controlled screen-house conditions.

2.0 MATERIALS AND METHODS

Two separate experiments were conducted. Experiment 1 examined the performance of common bean genotypes in the presence of aluminium toxicity using controlled screen house conditions. In Experiment 2, the performance of common bean genotypes was examined in the absence of aluminium toxicity again using controlled screen-house conditions.

2.1 Experiment 1: Performance of common bean genotypes at high aluminium saturation (55.2 %)

This experiment investigated the performance of five common bean genotypes at high level of aluminium saturation. The genotypes included: i) two previously characterized low phosphorus tolerant genotypes RWR 1946 and RWR 2075 (Kimani *et al.*, 2006; Lunze *et al.*, 2012), ii) K132, a large red mottled variety of CIAT origin also referred to as CAL 96, and iii) two aluminium tolerant genotypes MAR 1 and G 5273 (Manrique *et al.*, 2006; Blair *et al.*, 2009) which were included as checks.

Soil preparation and potting

The soil sample was collected from the 15 - 35 cm subsoil layer at Magadu farm of Sokoine University of Agriculture, Morogoro, Tanzania from a site previously characterized with aluminium toxicity. The soil was left to air dry for four days and it was then sieved using 6 mm sieve. Analysis of the physical and chemical properties was done on a 2mm sieved soil to establish the aluminium level. The soil was characterized by high amounts of exchangeable aluminium ranging up to 55.2 % Al saturation (Table 1). Al saturation was calculated as the ratio of exchangeable Al divided by the sum of basic cations plus Al, and H (Fox, 1979) and expressed as a percentage. i.e

$$\text{Al saturation} = \left[\frac{\text{Al}^{+3}}{\text{Ca}^{+2} + \text{Mg}^{+2} + \text{Na}^{+} + \text{K}^{+} + \text{Al}^{+3} + \text{H}^{+}} \right] \times 100.$$

Aluminum saturation can be used as an indicator for Al toxicity in the soil solution (Evans and Kamprath, 1970; Fox, 1979).

Table 1: Initial soil physical and chemical properties of the soil taken from the 15 - 35 cm sub soil layer at Magadu farm, SUA

Parameter	Magnitude
pH (in H ₂ O)	4.07
Clay (%)	61.67
Silt (%)	1
Sand (%)	37.33
Textural class	Clay
Organic carbon (%)	0.71
Total N (%)	0.18
P (Bray-1 method) mg /kg of soil	2.72
CEC (cmol/kg)	10.73
Exchangeable bases (cmol /kg of soil):	
Ca ²⁺	1.23
Mg ²⁺	0.79
K ⁺	0.2
Na ⁺	0.17
Exchangeable acidity (cmol /kg of soil)	
H ⁺	0.35
Al ³⁺	3.37
Al saturation (%)	55.2
Zn (mg /kg of soil)	1.52
Mn (mg /kg of soil)	31.84
Fe (mg /kg of soil)	17.66

After establishing the Al level of the soil, phosphorus at a rate of 160 mg P/kg of soil as triple super phosphate was added to the soil and mixed thoroughly well. Following a completely randomized block design with three replications, 4 kg of soil was then potted into 4 litre plastic buckets and later placed in the open air above on a metallic screen house bench. The following basal fertilisers were then added via dilute solution to each 4 kg bucket: 100 mg K/kg of soil (as potassium chloride), 10 mg Zn /kg of soil (as zinc sulphate) and 1 mg B/kg of soil (as boric acid). The field water content of the soil was determined by the Savage method (1979) and watering was done to field capacity. Sowing was done when the soil had reached field capacity. One bucket was kept unplanted to measure soil evaporation losses from the buckets. Five seeds of each of the five genotypes were sown and 7 days after

emergence, thinning was done leaving only three plants per bucket. Watering was done as adequately as possible. Nitrogen fertilisers i.e 200 mg N /kg of soil (as ammonium sulphate) and 200 mg N/kg of soil (as urea) were applied via dilute solution 12 and 25 days after emergence respectively.

2.2 Experiment 2: Performance of common bean genotypes at low aluminium saturation (14.7 %)

In the second experiment only two genotypes namely RWR 1946 and RWR 2075 were used to specifically compare their performance at low aluminum saturation.

Soil preparation and potting

The soil sample was taken from the top 10 - 15 cm layer from botanic garden of SUA, where there is no aluminium toxicity and was left for air drying for three days. Analysis of the physical and chemical properties of the soil was done on a 2mm sieved soil to establish the actual aluminum levels and revealed low amounts of exchangeable aluminium ranging up to 14.7 % Al saturation (Table 2).

Table 2: Initial soil physical and chemical properties of the soil taken from the top 10 - 15 cm layer at botanic garden of SUA

Parameter	Magnitude
pH (in H ₂ O)	4.94
Clay (%)	50.33
Silt (%)	11
Sand (%)	38.67
Textural class	Clay
Organic carbon (%)	1.07
Total N (%)	0.21
P (Bray-1 method) mg /kg of soil	3.5
CEC (cmol/kg)	10
Exchangeable bases (cmol /kg of soil):	
Ca ²⁺	1.17
Mg ²⁺	1.11
K ⁺	0.51
Na ⁺	0.25
Exchangeable acidity (cmol /kg of soil)	
H ⁺	0.33
Al ³⁺	0.58
Al saturation (%)	14.7
Cu (mg /kg of soil)	1.37
Zn(mg /kg of soil)	0.86
Mn (mg /kg of soil)	48.14
Fe (mg /kg of soil)	22.19

The soil was arranged into four treatments namely: 1) absolute control, where no fertilizer was added, 2) control treatment where only base fertilizers i.e 100 mg K/kg of soil (as potassium chloride), 10 mg Zn /kg of soil (as zinc sulphate) and 1 mg B/kg of soil (as boric acid) were added, 3) low phosphorus treatment which consisted of 10 mg P/kg of soil as triple super phosphate and the same rate of the above base fertilizers, and 4) high phosphorus treatment which consisted of 160 mg P/kg of soil as tripple super phosphate also with the same rate of the mentioned base fertilisers. A completely randomized block design was used with three replications. For each treatment, 4 kg of soil was potted into 4 litre plastic buckets. Sowing was done as in the first experiment. Nitrogen fertilisers were also added at the same rates and application regimes as in experiment 1.

2.3 Plant measurements

For both experiments, harvesting was done at 45 days after planting. All shoots (including petioles, leaves, stems) were harvested per bucket and oven dried for 4 days at 70°C. Shoot dry weights were recorded. Roots were separated from soil by washing with water, left to dry in the open air and then put in the oven at 70°C for 4 days. Root dry weights were recorded. Using one representative root sample per bucket, total root length was determined from the base of the hypocotyl along the length of the longest basal with the lateral roots that emerge from them. Shoot P concentration was determined following the procedure described by Murphy and Riley (1962). P uptake was also calculated as the product of the shoot dry weight and shoot P concentration.

2.4 Data analysis

Analysis of variance was performed using GENSTAT v.14. software (VNS International Hempstead, UK). Where significant differences between means were found, Fisher's protected LSD was used for comparison of means.

3.0 RESULTS

Experiment 1: Performance of common bean genotypes at high aluminium saturation (55.2 %)

At high aluminium saturation, shoot dry weight ranged from 0.55 to 0.97 g/plant, with genotype G 5273 having the highest value (Table 3). Root dry weight ranged from 0.07 to 0.28 g/plant. Genotype G5273 had the highest root dry weight followed by genotypes K 132 and RWR 2075. Genotypes MAR 1 and RWR 1946 had the most affected root dry weight in general. Total root length ranged from 4.5 and

10.33 cm per plant and was highest in genotype K 132 followed by G 5273. P-uptake was highest for genotype RWR 2075. Generally genotype G 5273, previously characterized to be tolerant to aluminum toxicity had relatively higher shoot and root dry weights, P-uptake and total root length than most genotypes. On the other hand, shoot P concentration did not vary significantly among the studied genotypes.

Table 3: Shoot and root dry weights, total root length, shoot P concentration and P uptake of five common bean genotypes evaluated under high Al saturation (55.2%)

Genotype	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total root length (cm)	Shoot P Concentration (%)	P uptake (mg P/Plant)
1. MAR 1	0.55	0.07 ^c	4.50	0.28	1.51
2. RWR 2075	0.81	0.21 ^{ab}	6.33	0.37	2.89
3. K 132	0.83	0.23 ^{ab}	10.33	0.22	1.68
4. RWR 1946	0.55	0.14 ^{bc}	7.50	0.24	1.38
5. G 5273	0.97	0.28 ^a	9.17	0.23	2.26
F from ANOVA:					
Genotype	0.070^{ns}	0.004	0.20^{ns}	0.27^{ns}	0.12^{ns}

Means followed by the same letter within the same column are not significantly different ($P = 0.05$) by Fisher's protected least significant difference test ; ns = not significantly different at $P = 0.05$

Illustration of sensitivity to aluminum toxicity of common bean genotypes evaluated in experiment 1 expressed as the drying of leaves as if herbicide has been applied is shown in Figure 1 below:



Figure 1: Symptoms of aluminium toxicity expressed as the drying of leaves as if herbicide has been applied on common bean genotypes (RWR 2075, RWR 1946, K 132 and G5273).

Experiment 2: Performance of common bean genotypes at low aluminium saturation (14.7 %)

At low aluminium saturation, shoot dry weight for the two genotypes ranged from 4.5 to 6.1 g/plant (Table 4). Root dry weight ranged from 0.8 to 0.9 g/plant, total root length ranged from 24.4 to 24.5cm, shoot P concentration was the same for the two genotypes and P-uptake ranged from 9.4 to 11.4 mg P/plant.

Table 4: Shoot and root dry weights, total root length, shoot P concentration and P-uptake of genotypes RWR 1946 and RWR 2075 at low aluminium saturation

Genotype	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total root length (cm)	Shoot P Concentration (%)	P uptake (mg P/plant)
1. RWR 2075	4.5	0.9	24.5	0.2	9.4
2. RWR 1946	6.1	0.8	24.4	0.2	11.4
F from ANOVA:					
Phosphorus	125.08*	24.44*	7.25 ^{ns}	3.68 ^{ns}	156.75*
Genotype	12.63 ^{ns}	0.42 ^{ns}	0.00 ^{ns}	0.26 ^{ns}	5.08 ^{ns}
Genotype x P.	4.30 ^{ns}	1.22 ^{ns}	3.06 ^{ns}	1.20 ^{ns}	1.88 ^{ns}

*significant at $P \leq 0.05$ by Fisher's protected least significant difference test ; ns = not significant; Genotype x P. = Genotype x phosphorus

4.0 DISCUSSION AND CONCLUSIONS

Very low values observed for the shoot and root dry weights, total root length and P-uptake in the soil with high Al saturation (55.2%) indicate that the presence of high or toxic levels of aluminium affects the performance of these traits in common bean. Reduction in the growth of roots and shoots is one of the physiological effects of aluminium reported (Foy and Brown, 1964; Edwards *et al.*, 1981; Fageria, 1982). Reduction of root growth is the most widely recognized symptom of Al toxicity resulting from interference of aluminium with cell division in tap root and lateral roots (Naidoo, 1977).

These results also clearly indicated that P-uptake was reduced by presence of aluminum. Genotypes RWR 2075 and RWR 1946 were observed with P-uptake of 2.89 and 1.38 mg P/plant at high Al saturation (55.2%) compared with P-uptake of 9.4 and 11.4 mg P/plant at low Al saturation (14.7 %) respectively. Inhibition of the uptake and utilization of most of the essential nutrients is reported among the biochemical effects of aluminum toxicity (Fageria *et al.*, 1982; Fageria, 1985).

The results of this study further confirm that common bean is sensitive to high aluminium saturation. Out of the measured traits, total root length and P-uptake were the most affected traits. Total root length is one of the five root traits that has been previously recommended as selection criteria to distinguish between Al- resistant and Al-sensitive genotypes (Blair *et al.*, 2009). Based on this study, we also recommend the use of P-uptake in addition to the root traits (elongation rate of primary root, total length, average root diameter, and specific root length) as a selection criterion.

Although there were slight differences among the genotypes RWR 1946 and RWR 2075 in terms of their shoot and root dry weights, total root length and P-uptake at high aluminium saturation, there was a trend suggesting that both genotypes were affected by aluminium toxicity. Further studies are recommended to establish the appropriate integrated management approaches so as to ameliorate the soils at Magadu part of SUA farm from Al toxicity so as to allow the production of common bean.

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CHAPTER SIX

GENERAL CONCLUSION AND RECOMMENDATIONS

Diseases, especially fungal pathogens are major and universal constraints affecting common bean production (Schwartz and Pastor-Corrales, 1989; Wortmann *et al.*, 1998). Among the fungal pathogens, root rots especially those caused by *Pythium* spp. are ranked as a greater problem (Abawi and Pastor-Corrales, 1990; Beebe *et al.*, 2011a). The root rots problem has been worsened by the declining soil fertility resulting from intense cultivation due to increasing population pressure (Wortmann *et al.*, 1998). Breeding genotypes tolerant to both constraints is a strategy that would probably contribute to addressing these problems. However, this is hampered by lack of adequate information on the available germplasm tolerant to both constraints and on the nature of inheritance of resistance genes. Therefore studies reported in this thesis were carried out in order to address those knowledge gaps. The following results were obtained and their implications are discussed.

Inheritance of resistance to *Pythium* root rot in genotypes RWR 1946 and RWR 2075 investigated in the F₁, F₂ and backcross populations revealed a single dominant gene that could fully express in several backgrounds. Allelism test carried out to determine the relationship of the resistance genes in these two genotypes and in RWR 719, a known and previously characterized *Pythium* root rot resistance source using phenotypic and molecular marker techniques showed that genotypes RWR 1946, RWR 2075 and RWR 719 had the same resistance locus. Given the dominant

nature of the resistance, the two genotypes should be useful in bean root rot-resistance-improvement programs.

Identification of new potential sources of germplasm tolerant to both *Pythium* root rot disease and low soil fertility constraints was a major component of this research. To achieve this, selected genotypes with known tolerance to low soil fertility (low P, low N, Al toxicity) were evaluated for their reaction to *Pythium* root rot disease using both phenotypic and molecular marker techniques. In addition, germplasm with known tolerance to *Pythium* root rot disease was evaluated for adaptability to low soil fertility, particularly low soil phosphorus. Genotypes RWR 1946 and RWR 2075 were also evaluated for reaction to aluminium toxicity. Results indicated that the BILFA (Bean Improvement for Low soil Fertility soils in Africa) nursery had some potential sources of resistance to *Pythium* root rot. On the other hand, the root rot resistant genotypes namely RWR 719, MLB 49-89A, AND 1062, AND 1055 were of moderate tolerance to low phosphorus availability. Genotypes RWR 1946 and RWR 2075 were confirmed to possess tolerance to both low phosphorus availability and *Pythium* root rot. Tolerance to both *Pythium* root rot disease and low phosphorus availability in genotypes RWR 1946 and RWR 2075 is particularly of interest because the two genotypes possess Andean seed types, unlike other stress tolerant genotypes such as RWR 719 which are Meso American. In common bean, resistance to major stresses has been found in mostly the Meso American backgrounds, which are not preferred by many consumers in the East Africa region. Consequently this has limited the adoption of some multiple stress tolerant genotypes in some parts of this region. For example, this is the reason why genotype

RWR 719 which is MesoAmerican was never adopted by farmers in the moist highlands of Uganda, where *Pythium* root rot is a major problem. Surprisingly this same genotype, RWR 719 despite being Mesoamerican was adopted in western Kenya. Possession of Andean seed types and tolerance to both *Pythium* root rot disease and low soil phosphorus availability in the genotypes RWR 1946 and RWR 2075, provides a useful base for introgressing into them other specific lacking disease resistance genes such as anthracnose and bean common mosaic necrotic virus that have been reported as serious and emerging constraints to the current use of the genotypes in Uganda. Genotypes RWR 1946 and RWR 2075 were however sensitive to aluminium toxicity, but still they are useful in areas that are not affected by this problem.

Parental genotypes RWR 1946, RWR 2075, K 132 and their F₁s progenies were evaluated under low and high phosphorus to determine early generation inheritance of selected low phosphorus tolerance related traits. Results indicated that increased lateral and basal root production, total root length and shoot growth were heritable traits and were to a great extent likely be due to additive genes. The fact that the traits were heritable means that genetic improvement for low phosphorus availability was possible using genotypes RWR 1946 and RWR 2075 as donor parents. Higher shoot growth and increased lateral and basal root production would then be used as selection criteria for desirable genotypes. Previous studies have reported several important and heritable morphological traits for tolerance to low phosphorus in common bean including root traits and shoot growth (Miller *et al.*, 2003; Araujo *et al.*, 2005; Trindade and Araújo, 2014).

Overall, the information generated through this research will be of direct use to researchers in the Great lakes region. However, further studies are recommended to 1) determine if there is any genetic association of the observed tolerance to low soil phosphorus availability and resistance to *Pythium ultimum* in the genotypes RWR 1946 and RWR 2075 and 2) understand inheritance of the studied low phosphorus tolerance related traits in advanced generations starting with the F₂ up to F₉ recombinant in bred lines and the number of genes involved.

There is also need to introgress *Pythium* root rot resistance into particularly the Andean: a) low phosphorus tolerant genotypes such as G 19839 (large yellow and red mottled) and G 19833 (large yellow and red mottled), and b) and the aluminum tolerant genotypes such as G 14016 (red mottled) and G 5273 (yellow) so as to enhance potential for their use and adoption in the Great lakes region.

Whereas for the genotypes confirmed to be tolerant to both *Pythium* root rot and low phosphorus, such as AFR 708 there is need to evaluate them for their agronomic and farmer acceptability traits and subsequent release to the farming communities, in countries where they have not been released.

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