INHERITANCE OF RESISTANCE TO FUSARIUM WILT (*F. oxysporum f.sp. phaseoli*) AND BREEDING MULTIPLE DISEASE RESISTANT AND MARKETABLE CLIMBING BEANS

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN GENETICS AND PLANT BREEDING

UNIVERSITY OF NAIROBI

FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

JUNE 2008

DECLARATION

This thesis is my original work and has not been submitted to any other university

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DEDICATION

In memory of our beloved son, the late Nelson Manzi-Mazimpaka, fondly called Bob
May your innocent soul continue to rest in Eternal Peace
To your brother and sisters: Dan Ntwali, Diana Munyana and Patience Mutesi
Be inspired to achieve a lot more in education and in life as a whole
You have all it takes to do so:
You have the potential,
You have the opportunity
God bless you.

ACKNOWLEDGEMENTS

This study was sponsored by The Rockefeller Foundation, through the International Centre for Tropical Agriculture (CIAT). Their generous financial and material contributions are greatly acknowledged.

I appreciate the different roles played by the University of Nairobi and CIAT supervisors: Prof. Paul Kimani, Dr. R.D.Narla and Dr. Robin Buruchara in guiding this study to its successful completion.

I deeply recognize the facilitation, advice and encouragement I received from the Director General of the Institute of Science and Agricultural Research of Rwanda (ISAR), Dr. Mark C. Bagabe, the Deputy Director General for Research, Dr Jonas M. Mugabe, the Dean of Faculty of Agriculture, University of Nairobi, Prof. John H. Nderitu, the Chairperson of the Department of Plant Science and Crop Protection, Dr Florence Olubayo and the Coordinator of CIAT, Pan-Africa Bean Research Alliance (PABRA), Dr. Robin A. Buruchara, during the compilation and submission of the thesis.

I am thankful to all the secretarial, pathology and computer laboratories, field or library staff of the College of Agriculture and Veterinary Science, the Board of Postgraduate Studies, University of Nairobi; and of the ISAR-Rubona and Rwerere research stations in Rwanda that assisted me in one way or another during data collection, compilation or during the submission of the thesis. Particular mention goes to Esther, Purity, Gathuma, Njeru, Muthoni and Chege of the University of Nairobi; and Raphael, Eduard and Theoneste of Rubona and Rwerere ISAR research stations in Rwanda.

Last but not least, my special appreciation goes to my dear wife, Aully Tushabe, to our wonderful children: Dan, Diana, and Patience; my brothers: Justine Gatsinzi and Joseph Musonera; sister, Farasia and nieces: Joy and Jane Junior. They were patient understanding, inspiring, and/or backstopped me throughout the long but interesting study.

Best wishes to you all.

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LIST OF ACRONYMS

AEZ: agro-ecological zones

ALS: angular leaf spot

ANOVA: Analysis of variance

ASARECA: Association for Strengthening Agricultural Research in East and Central Africa

ATDT: Agriculture Technology Development and Transfer Project

AYT: Advanced yield trial

BC: Backcross

BCMV: Bean common mosaic virus

BLDA: Bean leaf dextrose agar

ISAR: Institut des Sciences Agronomoques du Rwanda (Institute of Science and Agricultural Research of Rwanda)

C.F: Correction Factor.

CIAT: International Center for Tropical Agriculture

DAI: Days after Inoculation

DF: Degree of freedom

DNA: Deoxyribonucleic acid

FOP*: Fusarium oxysporum f. sp phaseoli*

ECABREN: East and Central Africa Bean Research Network

FW: *Fusarium* wilt

G x E: Genotype by environment interaction

H^2: Heritability

IYT: Intermediate yield trial

ISAR: Institut des Sciences Agronomiques du Rwanda (Rwanda Agricultural Research Institute)

masl: Metres above sea level

MAS: Marker assisted selection

MEX 54: Mexico 54, a bean variety introduced from Mexico resistanct to angular leaf spot

MINAGRI: Ministry of Agriculture and Animal Resources

MLT: Multilocation trial

MMCRW: Code of marketable and multiple constraints resistant Rwanda population

PABRA: Pan Africa Bean Research Alliance

PDA: Potato dextrose agar.

pH: Hydrogen ion concentration

PYT: Preliminary yield trial

R: Resistant disease reaction

R6: Flowering growth stage of beans

R 8: Physiological maturity growth stage of beans

RAPD: Random amplified polymorphic DNA

RF: The Rockefeller Foundation

RR: Root rots

RW: Rwanda RWR: Code for bush bean varieties or lines that are selected at Rubona or Rwerere stations

RWV: General code for climbing (volubile in French) bean varieties or lines developed in Rwanda

S: Susceptible disease reaction

SS: Sum of the squares

SWT: Seed weight

TWA: Tap water agar

USD: United States of America dollar

V_E: Environmental variance

V_G: Genetic variance

V0: Germination vegetative stage of beans

V_P: Phenotypic variance

X^2: Chi-squared analysis

$\sum$: Summation sign

ABSTRACT

Common bean (*Phaseolus vulgaris L.*) is the primary source of dietary protein in Rwanda. Climbing beans are important for intensified production due to their high productivity (3-fold of the bush type) and adaptation under the acute scarcity of cultivable land conditions in Rwanda. However, the commercial climbing bean cultivars: Umubano (G2333), Vuninkingi (G685) and Ngwinurare (59/1-2) lacked multiple resistance to major soil-borne and foliar fungal diseases like, *Pythium* root rot, *Fusarium* wilt, angular leaf spot and anthracnose; and/or market-preferred red-mottled or red seed-types. *Fusarium* wilt *(F. oxysporum f. sp. phaseoli)* in particular caused the abandonment of Umubano cultivar that was the most popularly adopted (over 80%) among the farmers in Rwanda.

The objectives of this study were therefore to:

i) Determine the nature of inheritance of resistance against *Fusarium* wilt disease in climbing beans, and

ii) Select high yielding climbing bean lines that combined multiple resistance against angular leaf spot, anthracnose and root rots with the marketable red or red-mottled seed-types.

To achieve the first objective, two sources of resistance to *Fusarium* wilt: G685 and Flora were crossed to the susceptible cultivar, G2333, in single and backcross arrangement. The parents, progenies of F_1, F_2 and the backcrosses were evaluated for *Fusarium* wilt reaction at a hot-spot screening site in Runyinya location in Rwanda. The trial was replicated in a glasshouse at Kabete field station using potted sterile soil. Injured root tips of 10 day old seedlings were inoculated *Fusarium* isolate (FOP-RW2) at a constration of 10^6 conidia ml^{-1}. The severity of *Fusarium* wilt was rated at 28 days after inoculation using the CIAT scale of 1 - 9: where 1 – 3 represent resistant, 4 - 6 intermediate and 7 – 9 is susceptible reactions.

The Chi-square analysis ($p<5\%$) proved goodness of fit into the Mendelian segregations: the F_1, backcross to the resistant parents and the F_2 progenies from the donor parents were all resistant. All the F_2 of the susceptible and resistant parents segregated in the ratio of 3 resistant: 1 susceptible, while the backcross progenies to the susceptible parent segregated in the ratio of 1 resistant: 1 susceptible. By applying the

ANOVA proposed by Kearsey et al (1996) heritability of resistance against *Fusarium* wilt was 99.7%. Therefore, the inheritance of the resistance / susceptibility to *Fusarium* wilt is conditioned by a major dominant gene that is highly heritable. It is thus achievable by backcross selection.

By observing and re-isolation of the pathogen from infected vascular portions of the lower stems at 28 and 55 days after inoculation, the rate of vertical spread of the pathogen, was found to be 4 times more in the susceptible than in the resistant plants. Damaged tap roots of some susceptible plants developed new secondary adventitious roots on the hypocotyls. This implies that the resistance mechanism to *Fusarium* wilt is related with presence of certain natural or induced physical or chemical barriers in the resistant plants. Tolerance is achieved through formation of compensatory roots in some susceptible plants.

To attain the second objective, multiple-parent crosses were made to create eleven heterogametic (for resistance and seed types) populations. The $F_{1:2}$ plant families and those of subsequent generations were screened for disease resistance under four natural epiphytotic conditions at Gikongoro (root rots), Ntendezi (root rots/anthracnose), Rwerere (anthracnose) and Rubona (angular leaf spot) sites in rotation. Pedigree was used to select resistant lines at each site, using the CIAT rating scale of 1 – 9.

At F_7 the 66 lines that had acceptable yields, diseases resistance and seed type attributes with 8 parental, local and improved checks were planted for advanced replicated yield trial at Rwerere (2300 masl) and Rubona (1700 masl) ISAR research stations.

ANOVA revealed significant G x E interaction and differences in yield and maturity, pods per plant, pod load; and seed mass and size within and across the sites ($p < 5\%$) with higher means observed at Rwerere. Thirty-three elite lines (F_8) that had the most marketable red or red-mottled seed types besides high yield range of 2.5 - 4.5 ton ha^{-1} or 101% to 141% of the improved checks and multiple resistance to at least 2 of the 3 diseases were selected. They offer the opportunity for release to farmers and to replace old climbing bean cultivars in Rwanda in near-future.

CHAPTER ONE

1.0 INTRODUCTION AND JUSTIFICATION

1.1 Dietary importance of beans

The common bean *(Phaseolus vulgaris L.;* 2n = 2x = 22*)*, also variously called food, field, haricot, dry, kidney, French, snap or *Phaseolus* beans, is the most important edible pulse in the world (Debouck, 1999). It is the leading staple after maize in East and Central Africa (ASARECA, 1995). In Africa, about 4 million hectares of beans are cultivated annually with production of 2 million tons (Chrispeels *et al.,* 2003) for more than 100 million consumers; mostly the resource-poor rural and low to medium income earners in urban areas.

In Rwanda, beans are eaten as cooked dry or fresh grain; green leaves or pods by nearly all in Rwanda. They constitute 84% of the pulses and 65% of total plant and (of the scarce and expensive) animal sources of protein diets (Londhono, 1980, Sperling *et al*, 1992; FAOSTAT, 2007). Beans also contribute generous amount of energy (32%); and of micronutrients: iron, zinc and vitamins A and B that promote normal human body and cognitive growth and development. Beans are particularly crucial complements of starchy tuber and cereal dishes in daily diets of the rural resource-poor that make about 91% of the country's population. Due to their diversified nutritional content and predominant protein supply in Rwandan diets, beans are regarded as a near-perfect food (CIAT, 1995), and as the meat for the poor (Sperling *et al*, 1992).

1.2 Production and demand for beans in Rwanda

Currently, beans are intensively cultivated on 320 000 ha, or about 30% of the arable land. Annual production averaged about 232,000 tons between 1980 and 2006 (Fig.1). On-farm productivity (800 kg ha^{-1}) is low and only five times less than the yield potential of climbing beans realised on research stations. Though per capita productivity of beans is one of the highest in eastern and central Africa, Rwanda imports about 60,000 tonnes of additional grain annually from the neighboring countries in order to meet the additional internal consumer demand of 150,000 tonnes (Ferris *et al*, 2002). This is partly caused by elevated per capita consumption of 50 - 60 kg, one of the highest in the world (about 3 times higher than Africa's average).

Ferris *et al* (2002) and the MINAGRI (2004) have projected the demand for beans to double to about 306,850 tons by the year 2020, if the current low trends of production don't improve.

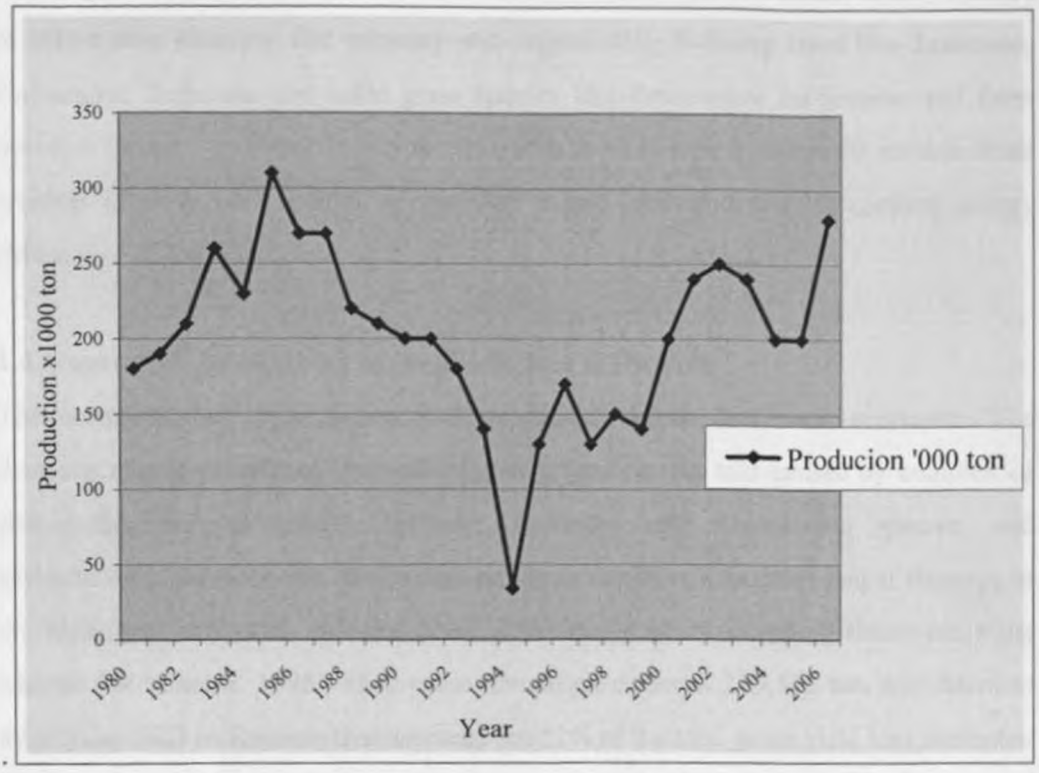


Fig 1. Quantity of bean produced in Rwanda annually between 1980 and 2006 (Source: FAOSTAT, 2007)

1.3 Climbing beans and their importance in Rwanda

Morphologically, the common bean is classified by growth habit into the determinate bush and the indeterminate climbing types. Until recently, improved climbing beans were confined to the higher altitude zones, beyond 1700 masl, but they are fast extending to the lower altitude zones. Their rate of adoption by the farmers has steadily increased from under 5% in 1985 to 65% (Mugabo *et al*, 2005; Musoni *et al*, 2001). The indeterminate morphology and architecture of the climbing beans are positively associated with the high yield potential of 3 to 5 t ha^{-1} (three-fold the yield of the bush beans) and to better tolerance to biotic stresses than the bush beans (Sperling *et al*., 1992).

The higher productivity allows intensified production of beans that in turn saves land on which complementary starchy crops and vegetables are grown (Voss *et al*,

1991) while their staggered maturity growth leads to sequential harvesting and consumption of leaves, pods and grain and ensures diversified nutrition and improved household food supply throughout a growing season. The higher productivity of climbing beans has also induced Rwanda farmers (usually have excess family labour) to invest into planting fast growing and regenerating N-fixing trees like *Leucaena, Calliandra, Sesbania* and some grass species like *Pennisetum* on terraces and farm boarders in order to obtain stakes that in addition protect the soil against erosion, their residues improve soil fertility, supplement animal feeds and provide cooking energy (Musoni *et al*, 2001).

1.4. Constraints of climbing beans production in Rwanda

The constraints of bean production are biotic, abiotic and socio-economic. The diseases: angular leaf spot (*Phaeoisariopsis griseola*), root rots caused by complex of soil pathogens, particularly *Pythium, Fusarium and Rhizoctonia species,* and anthracnose (*Colletotricum lindemuthiunum)* are the most important fungal diseases in the high, wet and cool altitudes (over 1700 masl) where climbing beans are most adapted (Wortmann, 1998). They cause annual yield loss of 219,575 ton, equivalent to 89 million USD in Rwanda that accounts for 52% of the total grain yield loss attributed to all the biotic and abiotic stresses (Trutmann *et al.,* 1993; Wortmann *et al*, 1998).

The lack of varieties that have the appropriate market seed-types leads to slow or poor adoption of the varieties among the farmers, irrespective of having high yields. This in turn contributes to the low productivity. Of the 9 major market classes, nearly all the commercial bean cultivars in Eastern Africa are most popular red-mottled or red-seeded (e.g. Urugezi, RWR 1312 I, GLP 2, CAL 96, Lyamungu 90, K20, Ngwinurare and Lyamungu 85). Besides their culinary attributes, they fetch premiums on urban markets. However, these are largely of the bush type and the proportion of the high yielding climbing types is very insignificant (Kimani *et al*, 2000).

Angular leaf spot, anthracnose, *Pythium* root rot and *Fusarium* wilt diseases may be controlled by seed treatment, good cultural practices, use of resistant varieties and soil fertility improvement (Allen *et al.,* 1996; 1990; Buruchara *et al.,* 1992, Trutmann *et al.,* 1993). While chemical control is the most effective, the fungicides are expensive,

and often not readily available to the resource poor farmers. Besides this, the chemicals are detrimental to the long term stability and sustainability of production, production systems and of the environment (DeVries *et al.*, 2001).

Fallow and crop rotation are more feasible, but are least practised as arable land is diminished in Rwanda (0.7 ha per household) (Chrispeels *et al.* 2003). The use of disease resistant cultivars is therefore more cost-effective and feasible to the small-holder farmers. Due to the holistic and synergic damaging losses on same plant host in the same environments (Kirkby *et al* , 1980), multiple disease resistant cultivars are even more desirable than single resistant ones (Kimani *et al.*, 2000).

1.5 Statement of the problem

The commercial climbing bean cultivars: Umubano (G2333), Vuninkingi (G685) and Ngwinurare (59/1-2) that were released in Rwanda became susceptible to rampant and devastating soil-borne and foliar fungal diseases: angular leaf spot, *Pythium* root rot, *Fusarium* wilt and/or anthracnose, which reduced their yields in farmers' fields. They lacked the most market-preferred red-mottled or red seed-types, thus contributing little to the farmers' income. The chances of their wider adaptation and adoption and commercialization were greatly reduced.

Fusarium wilt *(F. oxysporum f. sp. phaseoli)* in particular caused the abandonment of Umubano, which was the most popularly adopted (over 80%) by the farmers in Rwanda. There is a knowledge gap about the genetics and the inheritance of the resistance against the local strains of the disease in Rwanda and eastern Africa. This complicates the strategy of improvement or breeding and deployment materials that are resistant against the disease in Rwanda and eastern Africa, where the disease is prevalent. The use of multiple disease resistant cultivars with marketable seed traits is more likely to benefit farmers as it lowers production costs, maximizes yields and income earning opportunities.

1.6 Objectives and hypotheses of the study

The general objective of the study was to identify superior climbing bean lines that will subsequently assist farmers to increase bean productivity and incomes.

The specific objectives of the study were:

1. To determine nature of inheritance of *Fusarium* wilt resistance in climbing beans.

2. To select high yielding climbing bean lines that combine multiple resistances against angular leaf spot, anthracnose, *Pythium* root rots and *Fusarium* wilt with red or red-mottled market grain types from segregating populations.

The following hypotheses were made:

1. Resistance against *Fusarium* wilt in climbing beans is simply inherited.

2. Genetic variability and selectable recombinants for resistance against angular leaf spot, anthracnose, *Pythium* root rots, *Fusarium* wilt and, or marketable seed types exist among the developed eleven complex or simple climbing bean breeding populations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of common bean

Phaseolus beans (2n=2x=22) were domesticated in Latin America, about 7,000 to 10,000 years ago. The progenitor was a wild twining vine, *Phaseolus vulgalis var. aborigineus* [Burk] Baudet (Chrispeels *et al.*, 2003; Gepts *et al.*, 1991). They were introduced into Africa by the European and Arab explorers and traders from Brazil and Southern Andes in the 16^{th} to 17^{th} centuries (Debouck *et al.*, 1988). The genus has two major gene pools, Mesoamerican and Andean. Domesticated in the middle Americas (Mexico, El Salvado, Costa Rica), the Mesoamerican gene pool is small seeded (< 25g/100 seeds) while the medium and large seeded Andean gene pool (>25g/100 seeds) were domesticated in the Andean region of Peru, Chile and Argentina (Debouck *et al.*, 1991). There is great diversity in both the Mesoamerican and Andean genotypes in Rwanda. Climbing and bush bean mixtures with multiple genotypes (3 to 27), normally grown by the farmers in Rwanda attest to this. Some landraces, considered as extinct in the Latin American centers of origin exist in the country. Rwanda is considered an important secondary center of bean diversity (Allen *et al.*, 1990; Sperling, 2000).

2.2 Botany and morphology of common bean

The common bean plant has a tap root system that develops secondary and tertiary roots that are often nodulated. The aerial, cylindrical, sub-glabrous or pubescent stem is green, purple or pink in color. It has a raceme inflorescence, which is terminal and auxillary in the determinate and indeterminate stems, respectively. The flower is bilaterally symmetrical, enclosed by green bracteoles while immature. The corolla consists of a standard, two wings and an asymmetrical keel, which sheathes a superior gynoecium with hairy style and androecium of five partially fused and one free stamen. The colors of corolla parts, usually green, white, pink or purple, are characteristic of the bean varieties (Van Schoonohoven *et al*, 1991). The fruit is a pod and dehisces when dry. Placentation is marginal. The color and brilliance; and the round, oval, spherical, or kidney shapes of the seed as well as the structure of the hilum and micropyle differ among varieties. The seed is non-endospermic. Germination is epigeal (Debouck *et al.*,

1988). Primary leaves are simple entire margins, while the secondary leaves are compound trifoliate with reticulate venation. Leaflets have entire margins. They are borne on grooved petioles and rachis, which are pulvinated, stipulated and glabrous (Debouck *et al.*, 1988).

2.3 Biochemical composition and classification of common bean

At 11% moisture content, the dry pulse has 17-30% protein, often with some essential sulphur-amino acids (methionine, cysteine and lysine). The dry seed also contains 57.8% complex sugars, 1.6% fat, iron (about 34-89 ppm), zinc (about 21-54 ppm), fibre (4%) and generous amount of folic acid (Purseglove, 1966; Graham, 1996). Fresh leaves are rich in iron and vitamin A. Higher content of micronutrients in seeds and leaves in certain bean varieties such as Ngwinurare, Gofta, Maharagi Soya has been reported (Kimani *et.al,* 2006; Mamiro *et al*, 2006).

Phaseolus vulgaris L. is a dicotyledonous leguminous plant. The leguminous family (Leguminosae) includes 15 edible pulses such as soybean (*Glycine max L.)* Merr, pigeon pea (*Cajanus cajan L*), cowpea (*Vigna anguiculata L.*) Walp, of which *Phaseolus vulgaris* is the most important worldwide. The family also includes the N-fixing species such as *Sesbania, Calliadra* and *Leucaena species* that are economically important in agroforestry, and provision of the staking wood of climbing beans. *Phaseolus* genus has several species. They include four cultivated relatives: *P. coccineus, P. polyanthus, P. lunatus* and *P. acutifolius* (Hidalgo *et al.*, 1986).

The presence of the quality proteins, phaseolins (globulin I), in their seed coat is characteristic of this genus. Phaseolin 'S'protein is associated with the small seeded, Mesomerican gene pool, while types 'T' and 'C' are commonest among the medium and large seeded Andean gene pools (Singh *et al.*, 1991; 1988). The Andean gene pool also tends to have larger leaves than the Mesoamerican gene pools.

2.3.1 Determinate bush and indeterminate climbing growth habits

In broad terms, beans are classified by the determinate bush and the indeterminate climbing types. The bush beans (Types I, IIa, IIb and IIIa) have shorter erect stems of 30 to 50 internodes and branches that produce terminal reproductive buds. They lack

the ability to climb. Types IIb and IIIa plants terminate into short guides and are semi-determinate and are referred to as semi-climbing. Type I are fully determinate. The bush beans tend to have synchronized early maturity of 70 to 86 days in Rwanda, but this can increase to 120 to 190 days in cooler climates of 13^0 to 15^0 C (Voyster and Desert, 1991). The dwarf growth and bushy growth habit is linked with low potential yields (1.5 – 2.5 ton ha^{-1}) and general poor tolerance to diseases.

On the hand, true climbing beans (Types IIIb, IVa and IVb) develop clear terminal vegetative meristems at flowering, while inflorescences are borne on lateral buds. They have longer, slender and weaker stems with fewer internodes (20 to 30) and branches that terminate into guides, which confer the ability to twine along supports or stakes. Thus, they are also referred to as pole beans. The Type IVb plants tend to climb at earlier (first trifoliate leaf) stages and more vigorously. Type IIIb tend to flower closer to the ground. The climbing beans grow over 2 m tall when staked; otherwise their growth is prostrate. Their growth and development is usually staggered; and at some stages, flowering, pod formation and filling as well as maturation stages occur simultaneously.

2.4 Advantages of climbing beans over bush types

The twining, heavy branching, multiple lateral inflorescence system and enhanced pod-load (up to 10 seeds per pod) of climbing beans are some of the survival relic characteristics of the progenitor of the common bean, a wild twining vine, *Phaseolus vulgaris var. aborigineus* [Burk] Baudet (Chrispeels *et al*., 2003; Gepts *et al.,* 1990). These relics relate to the higher yield potential of climbing beans over the bush type. The clear growth and canopy above ground creates unfavorable microclimates for disease and weed development that make climbing beans more tolerant to biotic stresses than the bush beans. Besides the more rational use of land, the intensification of climbing bean production reduces costs of labor and fertilizer per unit land since the availability and application of fertilizer is low (1.3% of the optimum rate) (Kelly *et al*, 2002, Gahakwa, 2006).

The higher yield potential and staggered harvest compensate for the relatively late maturity, compared to the bush types, about 75 to 130 days in Rwanda. Consequently,

climbing beans are fast replacing the bush type, not only in Rwanda but also fast spreading from Rwanda to other high potential areas in sub-Saharan Africa (with an acreage of more than 100,000 hectares). Climbing cultivars such as *Umubano,* (G2333*)*, that have a rare combination of high yield and early maturity become even more popular among farmers (Graf *et al.*, 1991).

2.5 Growing environments and practices of climbing beans

In Rwanda, climbing beans have been grown in pockets of northern highlands of Ruhengeri and Gisenyi since they were domesticated 300 to 400 years ago (Allen *et al.*, 1990; 1989). They represent one of the oldest beans based cropping system in Africa. They do better under wet, cool and fertile conditions of high altitude areas between 1400 m and 2300 m above sea level, with mean annual rainfall of 1000 mm to 2000 mm (Table 1). In Rwanda these conditions are fulfilled in about 70% of the land area, comprising of northern, western, southern and parts of Eastern Province. Adequate rainfall supply is the most critical limiting ecological factor for growth of climbing beans (Table 1).

Table 1. Characteristics of climbing bean growing environments in Rwanda

Zone	Altitude (masl)	Rainfall (mm)	Soil type
Impala	1400 – 1900	1300 – 2000	Heavy red
Lake Kivu shores	1460 – 1900	1150 – 1300	Clay-loam surface
Lave Lands	1600 – 2500	1300 – 1600	Volcanic
Summit	1900 – 2500	1300 – 2000	Humic acid
Buberuka	1900 – 2300	1100 – 1300	Lateritic
Central Plateau	1500 – 1900	1100 – 1300	Different humic

Source: Nyabyenda, 1980.

Climbing beans are mostly grown in monoculture, especially at higher altitudes (2000-2300 masl). They are also intercropped with banana, maize, and sorghum. Relay cropping after maize to provide stakes, is also common. They are grown in two major seasons: A (September – January) and B (February – July). An off-season C (May-August) is usually planted in marshy land. Seed broadcasting is more common than row planting. As a compensatory mechanism for plant loss during and after germination,

seed rate is high and variable, ranging between 250,000 and 300,000 seeds per hectare in pure stands on rich soils. This is usually higher than the recommended rate of 200,000 seeds, equivalent to about 40 kg to 70 kg / ha for small and large seeded of improved varieties (Musoni *et al,* 2001). Weeding is done once, rarely twice, in a season as maturing plants choke weeds.

Chemical fertilizers and sprays are rarely, if at all, applied. Animal and farmyard manure is the main sources of soil fertilization, but is also limited by availability due to small animal population in their bean growing zones Rwanda. Fallow and crop rotation are limited by land shortage as about 87.3% farmers own less than 1.0 hectare per household (Mugabo *et al,* 2005). Many farmers also plant local varieties as they lack access to improved varieties. These conservative practices favor occurrence of bean disease and insect pests and loss of soil fertility that result in unstable bean yields.

2.6 Genetics of climbing growth habit

The common bean is highly cleistogamous (over 90%). Artificial out crossing is feasible within races of a cultigen. However, chances for incompatibility increase to a peak with crosses between the Mesoamerican and Andean cultigens; the cultigens and their secondary and tertiary gene pool wild relatives (Debouk *et al.,* 1991, Singh *et al.,* 1991), and other workers observed that the indeterminate climbing ability is dominant to determinate, bush, growth habit. A single completely dominant gene controlled growth habit. There was coupling-phase linkage between determinate and early flowering, and a more distant coupling-phase linking indeterminacy, late flowering and heavy pod-load (and high yield).

2.7 Climbing bean improvement in Rwanda

Traditionally, climbing beans have been grown in Rwanda since their domestication three to four centuries ago (Debouck *et a*l. 1988). However, decades of research on climbing beans in Rwanda since the 1970s attained important results in variety improvement and agronomic management of climbing beans.

2.7.1 Development of the first generation of climbing bean varieties

As the Instutit des Sciences Agronomiques du Rwanda (ISAR) fledged in the 1960s and 1970s, and the importance and relevance of climbing beans in Rwanda became apparent, research was initiated to screen and improve local landraces. Bean varieties from neighboring Congo and Uganda, as well as South America and Europe were also introduced. The local collections and the introduced lines were evaluated for yield, diseases tolerance and for general adaptation in on-station and in on-farm multi-location trials for several years in accordance to the generalized selection scheme shown in Fig.2. The best overall performers in this respect were among the earliest climbing bean varieties that were released to the farmers in Rwanda (Table 2). The table also indicates the origin of the same varieties. Their yield potential ranged from 3 to 3.5 ton per hectare, except Cajamarica that produced up to 5 tons per hectare (Nyabyenda, 1980). As more superior lines were bred in the 1980s, the cultivars were replaced or simply disappeared into farmers' mixtures, except Cajamarica that is still prominently grown to date. None of the varieties released at this time were from a crossing program.

Table 2. Name and origin of the first generation climbing bean varieties in Rwanda

Variety	Origin
Gisenyi 1, Gisenyi 6, Rwerere 14, Var 18, Urunyumba 3, Urunyumba 12, Urunyumba 1	Rwanda
Wulma, C8, C10	DR Congo
Bayo	Uganda
Amarilo-ouro	Angola
Sabre a rames	Belgium
Cajamarica	Peru

Source: Nyabyenda, 1980

2.7.2 Release of second generation of improved climbers

From early in the 1980s to the current period, ISAR and CIAT (International Center for Tropical Agriculture) intensified collaborative breeding and dissemination activities of

climbing beans in Rwanda. Many superior varieties that replaced nearly all the earlier ones were released to farmers. Among the first to be released and disseminated between 1985 and 1990 were the varieties: Umubano (G2333), Vuninkingi (G685), Ngwinurare (59/1-2), Flora de Mayo and Puebla, and Gisenyi 2 bis. They were introductions from CIAT, except Ngwinurare and Gisenyi 2 bis, which were local collections.

They had a high yield potential of 3.0 to 4.5 t/ha (Table 3), or 200 to 300% higher and better tolerance to diseases, than the improved bush types and the local checks (Nyabyenda, 1991). They therefore became more popular among farmers than the bush types and the local climbers (Sperling,, 1993; Woolley *et al., 1*991). These varieties were aggressively disseminated to the farmers, such that by 1990, the adoption of the climbing beans in Rwanda had reached 42% (from below 5% in 1980). The land area under the improved climbing beans was 33,000 hectares or about 17% of the total bean area. The economic benefits from extra harvests translated into USD 12.5 million of additional national income, annually (Sperling *et al*, 1992).

2.7.3 Recently released climbing bean varieties

Breeding and selection continued after the brief interruption by the war and genocide of 1994, resulting in the third generation of improved climbing cultivars. They included: Akezakarigura (CAB19), Mamesa (G2331), Ayinyana (RWV524), Decelaya, Nyiramata (CAB2), and Karera (CAB 28), among others (Musoni, various years). Compared to the previous varieties, CAB19 (navy white), G2331 (yellow seeded) sold 2 to 3 times higher on the urban markets (Ferris et. al, 2002, Rubyogo, 2003). RWV524, the first 'calima' climber (not a true red-mottled), fetched similar premium price (Rubyogo, 2003). The important old and current improved climbing bean varieties used by the farmers in Rwanda are shown in Table 3 (Musoni *et al,* 2001; 2006).

2.8 ISAR bean breeding scheme

Farmer participatory approaches are largely applied in breeding and selection of the released climbing bean varieties. This was reflected in the adaptation and quick

adoption of many of these varieties in Rwanda and in the parts of Central and East Africa where they were introduced from Rwanda.

The source materials are local landraces, introduced nurseries from the PABRA region (Pan Africa Bean Research Alliance) or CIAT. Breeding materials are also generated from local crosses. They are evaluated for tolerance to priority pests and diseases, abiotic stresses, adaptability in different agro-ecological zones (AEZ) and end-users' preferences. Preliminary (PYT), intermediate (IYT) and advanced yield trials (AYT) were conducted on station. Both the on-station and the on- farm multilocation trials (MLT) are replicated and the test entries included a local farmers' mixture and an improved variety as checks. They are conducted during the two main rainy seasons each year.

The PYT is an observation nursery for evaluation of a defined constraint or sets of constraints such as resistance to diseases. The entries usually range between 500 and 1000 lines. These are planted for a single season in unreplicated trial of single rows. They are reduced to about 50 entries that are fed into the IYT, which, after further selection, generates about 23 lines that went into the AYT.

The IYT and AYT are replicated trials laid in randomized complete block design and balanced lattice, respectively. The plot sizes are four 4-m rows with 0.5 m and 0.1 m between rows and hills respectively, and 2 seeds planted in each hill. The AYT selections produced fewer elite lines that are evaluated in 2 phases of multilocation trials on-farm. The experimental plots are fewer but larger (5 rows of 5 m-length) in this case. The most promising lines under the multilocation evaluation are recommended as pre-releases. The IYT, AYT and MLT trials are done over two seasons each year.

Table 3. Climbing bean varieties released by ISAR (1985 – 2001) and their main agronomic and economic characteristics

Variety	Farmers' synonym	Origin	Seed type		Maturity (days)*		Yield (t/ha)	Major use
			Size	Color	M.A.	H.A.		
G2333	Umubano	CIAT	Small	red	90	112	4.5	Food
G685	Vuninkingi	CIAT	Small	red	99	118	4.5	Food
Flor de Mayo	Flora	CIAT	Medium	pink	94	118	4.0	Food
G858	Muhondo 6	CIAT	Medium	yellow	94	118	5.0	food ,cash
Gisenyi 2 bis	Gisenyi	ISAR	Large	zebra	88	111	3.0	food ,cash
Urunyumba 3	Urunyumba	ISAR	Large	red	85	112	3.0	Food
59/1-2	Ngwinurare	ISAR	Large	red	89	-	3.5	food, cash
*NG 224-4	Ikinyamanza	ISAR	Large	zebra	91	-	4.5	food, cash
*RWV 296	Amakwamire	ISAR	Large	zebra	91	-	4.0	food, cash
*RWV 167	Ndamirabashonji	ISAR	Small	red	90	--	4.5	food, cash
*CAB 19	Akezakarigura	CIAT	Small	white	91	-	4.5	Cash
*CAB 2	Nyiramata	CIAT	Medium	white	-	130	4.5	Cash
Decelaya	Decelaya	CIAT	Large	red	87	-	4.0	food ,cash
*CAB 28	Karera	CIAT	Small	white	92	-	4.0	Cash
*LAS 405	Binezeza	CIAT	Large	red	-	120	4.5	food, cash
*RWV 377	Munezero	ISAR	Large	red	-	118	4.0	food, cash
*G2331	Mamesa	CIAT	Medium	yellow	-	120	4.5	food, cash
*RWV 524	Masoyinyana	ISAR	Medium	calima	-	120	4.0	food, cash

Legend: * = third generation released after 1995; - = Data not available or variety not adapted to the mid or high altitude zone
(Source: Nyabyenda, 1991 and Musoni, 2001)

The pre-released lines are then tested in many more locations and the best performers are recommended for release. Breeder and basic seed are produced through an arrangement between ISAR and the National Seed Services, to facilitate diffusion of the new varieties to farmers.

The selection criteria at the various stages include resistance to major biotic and abiotic stresses, plant vigour, maturity duration, yield potential, seed-types, culinary qualities and general adaptability and acceptability under farmers' circumstances (Fig.2). The cycle takes between 3 and 7 years (Fig. 2). The varieties that come from ISAR on station crosses (such as RWV 269, RWV 167, RWV 377 and RWV 524) take longest while selection from local landraces and introductions takes the shortest time into the pipeline. This is because the ISAR bred lines took at least 3 years of selection before they become distinct, stable and uniform, which is a pre-condition for the entry into the general breeding scheme (Fig. 2). Table 4 shows characteristics of the two ISAR stations of Rubona and Rwerere used for primary evaluation of climbing beans.

Table 4. Main on-station screening sites for climbing bean varieties in Rwanda

Station	Location	Altitude (masl)	Rainfall (mm)	Temp (°C)	Soil type	Soil pH	Major diseases
Rubona	South	1650	1171	18.9	Oxisol	5.0	Angular leaf spot
Rwerere	North	2300	1166	15.6	Alfisol-ultisol	5.2	Anthracnose

Source: Ruterana Kamenyero (2000)

2.9 Lack of multiple constraint resistance among climbing beans

All the new and the old varieties excelled principally in high yield advantage, irrespective of market grain attributes. There was, as a result, mixed success of adaptation and adoption at individual variety level because of lack of combined multiple resistance and preferred seed-types. Vuninkingi (G685), a small light red, preferred by farmers for its good yield (for food security) was undesirable in the market (for cash). The reverse was true for the white seeded varieties, CAB 19, CAB 2 and CAB 28. The red-mottled climbing genotypes (ANNEX 5), most preferred for both domestic consumption and in markets were completely lacking among the released varieties.

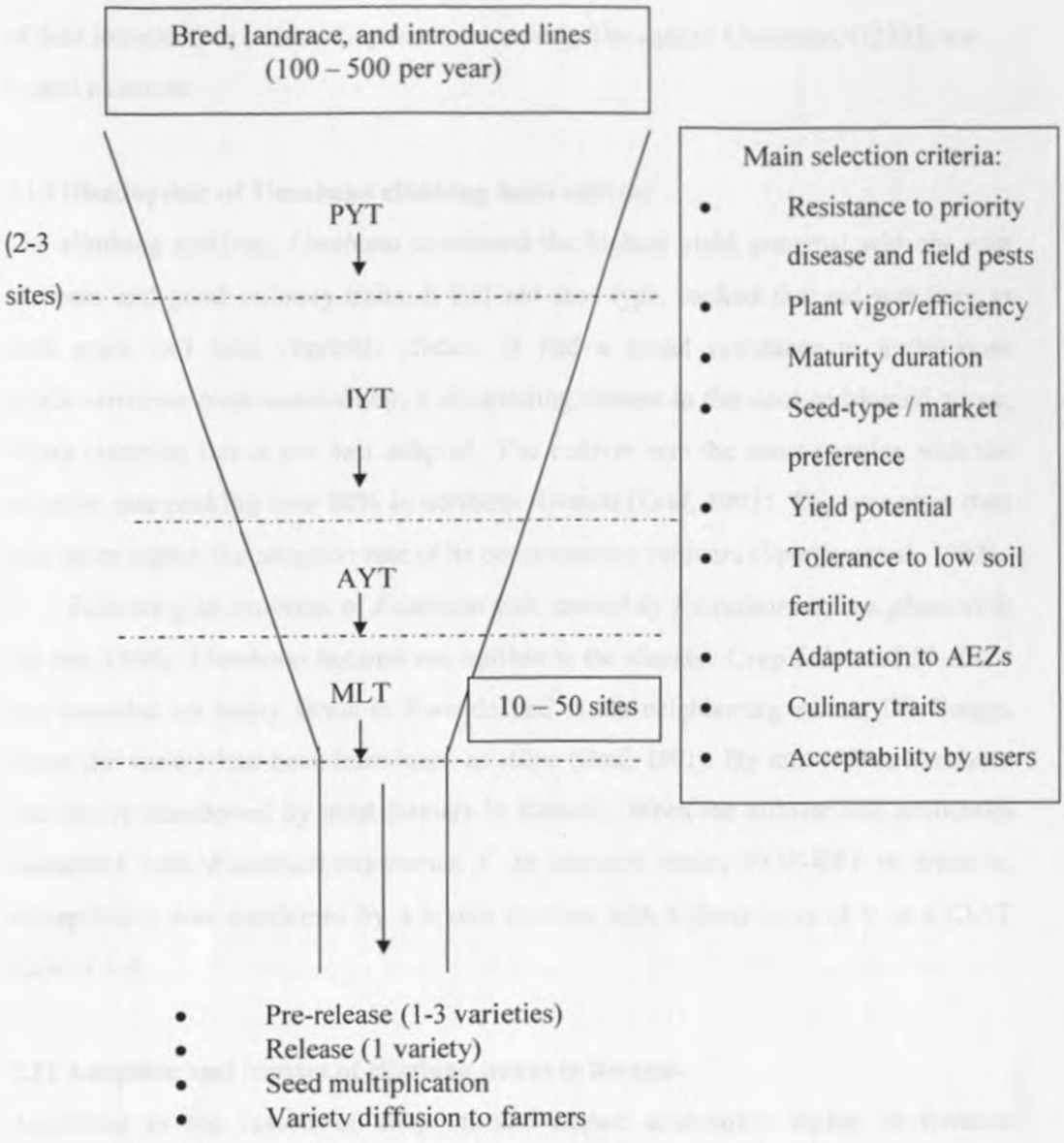


Fig. 2. Generalized participatory breeding scheme followed by ISAR Bean Program

Legend: PYT – Preliminary yield trial; IYT – Intermediate yield trial; AYT – Advanced yield trial; MLT – Multilocation trial

Less attention, too, was given to breeding and selection of varieties that were tolerant to a broader range of biotic and/or abiotic stresses *per se*. Some of the popular varieties (mostly with red seed-types) were susceptible to major diseases in new zones

of their introduction (where they were not tested). The case of Umubano, G2333, is a typical example.

2.10 Disadoption of Umubano climbing bean cultivar

The climbing cultivar, *Umubano* combined the highest yield potential with the rare earliness and good culinary traits. It had red seed type, cooked fast and was tasty in both grain and leaf vegetable dishes. It had a broad resistance to anthracnose (*Colletotrichum lindemuthianum)*, a devastating disease in the cool and humid zones, where climbing beans are best adapted. The cultivar was the most popular, with the adoption rate peaking over 80% in northern Rwanda (Graf, 1991). This was more than four times higher the adoption rate of its contemporary cultivars (Sperling *et al.,* 1992).

Following an outbreak of *Fusarium* wilt, caused by *F.oxysporum f. sp. phaseoli* in the late 1980s, *Umubano* became susceptible to the disease. Crop failure of 50-100% was recorded on many farms in Rwanda and in the neighboring eastern DR Congo, where the variety had been introduced as *Aliya* (Graf, 1991). By mid-1990s, *Umubano* was totally abandoned by most farmers in Rwanda. When the cultivar was artificially inoculated with *Fusarium oxysporum f. sp phaseoli* strain, *FOP-RW1* in Rwanda, susceptibility was confirmed by a severe reaction with highest score of 9 on a CIAT scale of 1-9.

2.11 Adoption and impact of climbing beans in Rwanda

According to the results of adoption and impact assessment studies in Rwanda (Sperling *et al*, 1992, Sperling, 1995), the overall rate of adoption of climbing beans increased from under 5% in early 1980s to 42% and 47% in 1992 and 1995, respectively. It is currently estimated at 65% (Mugabo *et.al* 2005). The adoption ranged between 47% and 90% within two of the four provinces with favourable growing environments, or where research and development projects were most active (CIAT, 1992; Mugabo *et.al,* 2005). The adoption rate of improved climbing bean technologies was independent of wealth and gender status of the farmers. As a result of increased bean productivity that were realized in Rwanda, the farmers were earning extra income

equivalent to US dollars 12.5 million annually after 5 years of the introduction of the improved climbing beans (Sperling *et al*., 1992).

The sharp rise in the adoption of climbing beans in Rwanda and their current expansion in western Kenya and southern-western Uganda since the 1990s was a direct consequence of the root rot complex epidemics that severely attacked and damaged local and improved bush germplasm (CIAT, 1995). The small holder farmers depend more on host resistance as they rarely use chemical sprays and cultural options to combat the fungal diseases, to which the climbing beans are more tolerant.

2.12 Diseases of climbing beans

The most notable diseases of climbing beans are angular leaf spot, and root rot caused by complex of soil pathogens and bean common mosaic virus (BCMV). Anthracnose, ascochyta blight (*Ascochyta phaseolorum*) and halo blight (*Pseudomonas phaseolicola*) are important in higher altitudes (over 1800 m above sea level), while common bacterial blight (*Xanthomonas phaseoli*) is important at lower altitudes. In Rwanda, root rots, anthracnose, and angular leaf spot, come top in terms of damage to beans in general, including the climbing type.

2.12.1 Etiology, epidemiology and symptomatology of root rots, angular leaf spot and anthracnose

2.12.1.1 *Fusarium* wilt (yellows) and *Pythium* root rots

The bean root-rot diseases are ecologically important in Africa and Latin America on small infertile farms, which are intensively cropped without fallow, rotation, and with little use of inputs (Nderitu *et al.,* 1997).

Fusarium yellows are caused by *Fusarium oxysporum* Schiecht *f. sp. phaseoli* Kendrick and Snynder. It produces hyaline, non-septate chlymadospores measuring 6 to15 x 2 to 4µ and macroconidia, which are elongate and curved with three septates. The thick-walled chlymadospores survive for long in the soil and spread the disease via soiled seed or crop residues (Mutitu, 1989). The fungus inflicts necrotic browning of the xylem, early upward chlorosis, epinasty and defoliation, leading to stunting, wilting and death of plant if infection is severe (Allen *et al.,* 1996).

The pathogen is prevalent in South America, United States of America, where 25% to 50% crop damage has been reported (Ogg *et al.,* 2000), and in Africa (Abawi *et al,*1990; Rusuku *et al,*1997), where the 55,200 t annual loss (Wortmann et al,1990) is significant. Fusarium wilt is wide-spread in Rwanda and is most rampant in the southern provinces of Butare, Gikongoro and Cyangugu (Rusuku *et al,* 1997). Its incidence and/or severity is accentuated by dry soils, soil compaction, infertile and poorly drained soils; poor cultural practices and high soil pH of 7-8 (Wickliffe *et al,* 2000). It can also be spread through infected seed.

Pythium infection causes pre-emergence rotting of seeds. The cortex and epidermal tissues of young roots and the hypocotyl region become water soaked and rot resulting in damping off. Bean plants that survive are stunted, chlorotic and/or wilted. The roots of diseased mature plants are discolored, diminished and their less lignified fibrous rootlets are decayed (Abawi *et al,* 1990). The disease is caused by *Pythium* species. *P.ultimum, P. salphingophorum* and *P.torulosum* are the commonest, while *P.ultimum var. ultimum* is the most virulent in Eastern Africa (Buruchara *et al.,* 2003). Its sexual stage is the sporangium, which produces flagellated motile zoospores, by which the pathogen is spread.

Depending on the species, high soil moisture and soil pH (around 7.5); and cool to warm temperatures of 15°C to 30° C, favor the distribution of *Pythium* pathogens. The infective zoospores may survive for 7 days in field soil, while *P. ultimum* survived for 2 years in air dry soil at -18°C (Schwartz *et al.*, 1980). It is contagious through infested debris, soils, and rain run-offs or splashes, or contaminated farm tools and animals (Abawi *et al*, 1990). *Pythium* root rot is the most important in the densely populated and wet regions of Africa. In Rwanda, it accounts for 42.6% of all the root-rot pathogens. It causes 40-60% crop loss (Buruchara *et al* 1992), but 100% crop failure was reported under cool soil temperatures of 16°C - 20°C and excess soil moisture following heavy rains at planting and seedling stages (Pastor-Corrales, 1990; Rusuku *et al* 1997).

2.12.1.2 Angular leaf spot

The causal agent of angular leaf spot (*Phaeoisariposis griseola* (Sacc) Ferr.) is distributed widely in North and South America and in Africa. It is prevalent in bean growing environments which are cool to warm at 16-28°C (optimal 24 ± 2^0C). Sporulation and dissemination are favored by alternating rain/humid, dry and windy weather (Wagara, 1996; Buruchara, 1983). Fungal spores are seed transmitted. They are also disseminated via harvest residual crop, rain splashes and air currents. The spores may survive as long as 140 to 500 days in the soil (Schwartz, 1981).

P.griseola attacks lower surface of plant leaves producing large usually concentric dark brown lesions on primary leaves and smaller angular ones (spots), defined by veins or venules on the trifoliate leaves which are surrounded by chlorotic halos. This usually leads to premature defoliation. The necrotic brown lesions are observed on other aerial plant parts particularly on pods. The seeds inside the pods are infected and become blemished at maturity (Allen, 1987; Allen *et al*, 1996). The whole plant or complete crop may perish when field infestation is severe.

Reported losses attributed to angular leaf spot range from 40% in Argentina. 50% in Wisconsin and Mexico (Sartorato, 2001), 80% in Colombia (Schwartz *et al,* 1979) ; 60% in Ethiopia and 70% in Brazil. It is the most damaging biotic constraint in Rwanda and in Africa, where it causes an estimated annual loss of 384,000 t per year, and 281,000 t in eastern Africa (Wortmann *et al.* 1998).

P.griseola exhibits broad pathogenicity due to somatic mutations and sexual recombination (heterokariosis and parasexualism), which complicates resistance breeding due to gene-for-gene phenomenon. Considerable progress in characterization and virulence typing of the American and African isolates has been made. Different races were associated within bean gene pools and geographical regions (Andean-Latin American, Andean-America, Afro-Andean, Mesoamerican or Mesoamerican–Latin America) (Buruchara *et al.* 2001; Sartrato, 2001). However, some Molecular markers for the resistance genes, identified in cultvars Mex 54 and G10474 have been applied and exploited in marker assisted selection breeding (CIAT, 2003). The inheritance of resistance against angular leaf spot is monogenic and dominant (Mulindwa, 1980;

Namayanja *et al* 2002). However, Singh, (1991) reported a recessive major gene as responsible for the control of its resistance.

2.12.1.3 Anthracnose

Caused by the fungus *Colletotrichum lindemuthianum* (Sacc & Magn) Schrib, anthracnose is ubiquitous in all bean-growing areas in the Tropics and sub-Tropics. High humidity (92%) and cool temperatures of 14-26 °C (optimal 17-20°C) favor its infection and development but is limited by temperatures above 30° C (Thomazella *et al.* 2001; Schwartz *et al.*, 1986). Anthracnose is the most important bean pathogen in the wet and cool to warm central African highlands (Nyabyenda *et al.* 1980). Planting infected seeds on nutrient deficient soils and poor cultural practices common among subsistence poor farmers exacerbates the disease (Mutitu *et al,* 1980). When epiphytotic conditions prevail, economic field loss of 100% is common. The disease reduces production by 247,000 t in eastern Africa annually (Wortmann *et al*, 1998).

C. lindemuthianum conidia are spread through infected seed, rains, wind, contact with infected plants and contaminated human and animal bodies (Pastor-Corralles *et al*, 1996). The symptoms of the disease include dark-red to black necrotic lesions along veins, cotyledons, petioles, branches, stems and pods. The lesions on bigger veins and pods develop into sunken cankers and the developing seeds inside pods are also infected and blemished upon maturity (Pastor-Corrales *et al.* 1990).

The anthracnose pathogen is highly variable. Virulence tests using Mesoamerican and Andean isolates by Mwangi (1986) in Kenya, Bashir (2000) in Ethiopia, Kelly (2001) in Mexico, Thomazella *et al* (2001) in Panama, Brazil and others, confirmed the variability of *C. lindemuthianum,* as well as some sources of its resistance. The protection against the *C.lindemuthianum* is conferred by single (Goncalves et al,2001) to several dominant major genes coded as *Co-1* to *Co-7.* The genes are present either in Mesoamerican or Andean genomes (Thomazella, 2001). However, random amplified polymorphic DNA (RAPD) molecular markers for the *C. lindemuthianum* resistance genes in Umubano Co-4 (OAL9 /SAS13) and Co-5 (OAB3) have been characterized. Markers for the genes Co-1, Co-2 and Co-6 present in other cultivars have also been found. Potential exists for the application of marker-assisted selection in improving

breeding efficiency for the elusive anthracnose resistance (Young *et al.,* 1997; Kelly *et al.*, 2001).

2.13 Control strategies of root rot, angular leaf spot and anthracnose diseases

Integrated cultural practices that improved soil drainage, aeration and fertility, planting clean seed, fallow, rotation, rouging and disposal of crop residues, adjusting of sowing dates and proper handling of farm implements are effective in controlling the root and foliar diseases. The application of green manure such as *Calliandra, Sesbania,* or *Tithonia spp,* withered compost or farm yard manure (FYM); and the use of inorganic fertilizers like urea or diammonium phosphate were successfully employed in farmers' fields in Rwanda and western Kenya were effective in the control of root rots (Buruchara *et al,* 1992; Otsyula *et al.* 1998, Musoni, 1999). Planting of a mixture of cultivars and supplementing landrace mixuters with resistant varieties stabilized and improved yield (Pyndji *et al,* 1992; Trutmann *et al,* 1993, 1994).

Chemical seed dressing with benomyl, endosulphan or thiram provides temporary protection to root diseases during early growth stages but the damage is realized later as the root system is established. Chemical foliar sprays with benomyl (1.0 g/liter), thiophanate (2.0 g/liter) or zineb 2.4 g/liter) were the most effective options of managing angular leaf spot (Schwartz *et al.* 1978). Biological control through use of saprophytic fungi has also been reported for *Pythium* species (Allen *et al.,* 1996; Abawi *et al.,* 1990). Antagonistic saprophytes (*Bacillus spp* and *Aspergillus niger)* offer potential to controlling *Fusarium* wilt. However the strategy has not been widely adopted (Mutitu *et al.,* 1989).

However, the chemicals are expensive and hardly available, making this option the least (if at all) applied by the resource-poor bean farmers. The chemicals have negative impacts on the environment and long term sustainability of the cropping systems. The cultural practices are not applied due to land limitations or extra labour and costs.

The use of resistant cultivars is the most important and practical component of the integrated management approach. It is the least demanding and most feasible to the small-holder farmers (Allen *et al.,* 1996). The use of plant hosts that possess multiple

resistance to diseases is even more desirable as many diseases attack and damage the same host together (Kimani *et al.*, 2000).

2.14 Plant host and sources of resistance for root rot, angular leaf spot and anthracnose

Buruchara et al (1992) and Buruchara and Camacho (1999) successfully screened and identified lines with high levels of resistance against root rots in Rwanda. They included Urugezi, RWR 719, SCAM 80 CM/15, MLB-49-89A, MLB-40-89A, RWR 1092 (bush), and the climbers: Vuninkingi (G685), Umubano (G2333), Flora de Mayo, Ngwinurare (59/1-2) and Puebla.

Among the most useful sources of resistance against angular leaf spot that are used in breeding programmes include: Mex 54, MAR I, MAR 2 and G10474. The cultivar G10474 was incompatible with all 13 virulent isolates from Africa while Mex 54 offered resistance to all, but 2, an Andean and a Mesoamerica races in Africa (Buruchara et al. 2000; Mahuku *et al*, 2002).

More durable resistance against anthracnose was achieved through the combination of the Andean Co-1 and the Mesoamerican, Co-4, Co-5 genes (Kelly, 2001). Perry Marrow, Kaboon (Andean), G2333 (Umubano), G2338, SEL1308, AB136 and TU (Mesoamerican), are among the best potential donors for anthracnose resistance (Kelly, 2001). The genes, Co-4, Co-5, and Co-7 present in Umubano (G2333) and Co-6 in AB 136 offer broad-spectrum resistance to known races of *C. lindemuthianum* worldwide. Umubano (G2333) is the best donor of resistance to both Mesoamerican and Andean commercial cultivars (Thomazella *et al*, 2001; Kelly, 2001; Gonclaves-Vidigal *et al*, 2001).

Most of the identified sources are widely used by the farmers and have restored bean production in previously root-rot devastated parts of Rwanda and western Kenya. Others are also used in regional breeding programs, especially those whose markers genes have been mapped) for the improvement of susceptible commercial bean cultivars (Buruchara *et al* 1999; Musoni *et al,* 1999; Sonia *et al*, 2001)

2.15 Inheritance of *Fusarium* wilt resistance

Inheritance of resistance to local isolates of *Fusarium* wilt is not understood. Using a Brazilian and North American isolates of *Fusarium* wilt, Ribeiro *et al.,* (1977 and 1979) identified two genes *Fop 1* (dominant) and *Fop 2* (incompletely dominant) that confer resistance against the isolates, respectively. The lack of conclusive knowledge about the mode of inheritance of *Fusarium* wilt resistance using African and particularly Rwandan variants of the disease make uncertain the breeding and deployment strategy for resistance against the disease in the region. While the continued use of Umubano as a source of anthracnose is desirable, its susceptibility to *Fusarium* wilt limits its usefulness as a source parent and in commercial production until the genetics of resistance against *Fusarium* wilt are fully established.

2.16 Seed market classes and end users' market preferences

Grains of the common bean have different colors, shapes and sizes (market classes or seed-types) that have different commercial, agronomical and ethno-cultural values among the different world communities (Voysest *et al.,* 1991). Farmers, traders, processors and consumers have special preferences of the different seed-types. There are 9 major market classes in Africa. These include small white or navy bean, large whites, small reds, red kidneys, red mottled, sugars, yellows, tans and carioca (Kimani et *al.*, 2000).While the small seeded (<25g/100 seeds) beans are often preferred for better yield within the same bush or climbing plant type (Debouck, 1986), the large seeded (> 40g/100 seeds) are more marketed in Africa (Wortmann *et al.,* 1998). The red-mottled and the red market classes are the most economically important in Africa. They account for 42% of the total dry grain production (Wortmann *et al.* 1998; Kimani *et al.*, 2000). But all the red-mottled and nearly all the red-seeded commercial bean cultivars in Eastern Africa are of the bush type with lower potential yields. Hence, the need to incorporate the large red and red mottled seed marketable traits into the high yielding climbing beans.

2.17 Multiple constraint and market led breeding strategy

All the new and the old varieties excelled principally in high yield advantage, but were deficient in terms of market qualities. They were less plastic in adaptability due to poor

disease (multiple) resistance among other adaptive deficiencies. The success in adoption of the varieties at farm level was thus mixed.

Under the market-led breeding strategy initiated by the East and Central Africa Bean Research Network (ECABREN)) in 2000, the development of improved high yielding climbing bean varieties that combined multiple resistances against major diseases with user-preferred red-mottled or red seeded grain types was prioritized. It was meant to consolidate the food security from higher on-farm productivity (through diseases control) and the generations of income from increased commercialization of the surplus bean produce (Singh et al, 1998). The strategy was the overriding basis for conducting the current study in Rwanda.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Determination of inheritance of resistance against *Fusarium* wilt

3.1.1 Crosses made for the inheritance study

Vuninkingi (G685) and Flora were used as donors of resistance to *F.oxysporum f.sp.phaseoli*. Umubano (G2333) was the susceptible parent. Table 5 gives the characteristics of the parents that were used in the crosses. Pair-wise crosses of (Umubano x Vuninkingi), (Umubano x Flora) and (Vuninkingi x Flora) were made in the green house at Kabete Field Station, University of Nairobi. The F_1 seed of (Umubano x Vuninkingi) and (Umubano x Flora) was harvested separately. It was divided into three sets. One set was kept aside, while the second seed lot was planted in the screen house and the plants were selfed to produce F_2 population. The third lot was planted and was used as female parents in backcrosses (BC) to the susceptible (Umubano), and to the two resistant parents, Vuninkingi and Flora. The F_1 seed of (Vuninkingi x Flora) was planted to raise F_2 progenies. Table 6 shows a summary of the crosses made for this study.

Table 5. Characteristics of the susceptible (G2333) and donor parents used in the crosses for the study of inheritance of Fusarium wilt in climbing beans (Type IVA).

Parent	Code	Seed type	*Diseases reaction				*Maturity (days)		Yield (kg/ha)
			Fus	Pyth	ALS	Anth	M.A	H.A	
Umubano	G2333	small red	S	R	I	R	90	112	4,500
Vuninkingi	G685	small red	R	R	I	R	99	118	4,500
Flora de Mayo	Flora	medium pink	R	R	I	R	94	118	4,000

Legend: Fus = Fusarium wilt, Pyth = Pythium root rot, ALS = Angular leaf spot, Anth = Anthracnose, MA = Mid altitude. HA = High altitude

Table 6. Crosses made to study inheritance of resistance to fusarium wilt

Cross	Generation code
Umubano (S) x Vuninkingi (R)	$F_1$and F_2
(Umubano x Vuninkingi) F_1 x Umubano	BC_1S
(Umubano x Vuninkingi) F_1 x Vuninkingi	BC_1R
Umubano (S) x Flora (R)	F_1 and F_2
(Umubano x Flora) F_1 x Umubano	BC_1S
(Umubano x Flora) F_1 x Flora	BC_1R
Flora (R) x Vuninkingi (R)	F_1 , F_2

R = resistant parents; S = susceptible parent; BC_1 = $Backcross_1$

3.1.2. Crossing techniques and seed handling

The plants were grown in 30 cm diameter clay pots, filled with a mixture of sterilized loam soil and manure at a ratio of 5: 1. Three plants were grown in each pot, with the female plants placed adjacent to the male parents. Crosses were made on vigorous and disease-free plants that had turgid and plump floral parts, in the morning or evening when the weather condition in the greenhouse were cool (Buishand, 1956; Temple *et al.*, 1998). Crosses were made after opening and emasculating young flower buds of the female plants. Using ethanol-sterilized forceps, the keel and petals of the young female bud were slit open by using a pair of forceps and the immature anthers were removed before the internal self pollination had occurred.

After fresh sterilization of the forceps, the mature anther heads from the flowers of male parents were ripped, transferred and brushed to the exposed stigma of the freshly opened young flower bud of the female plants. Pollinated flowers were tagged and labelled to indicate the pedigree and date of pollination. Ten to fifteen flowers of the female plant were pollinated and the rest were pruned. Di-ammonium phosphate fertiliser (0.314 grams per pot) was applied at planting and the plants were well watered each morning and evening until the plants were mature. Dry pods from the respective crosses were harvested separately and hand-threshed. Their seed was bulked, sun-dried, sorted, packaged and kept under cool and air-dry conditions.

3.1.3 Evaluation of *Fusarium* wilt reaction among progenies

The parents, F_1 and F_2 and their backcross progenies were evaluated for resistance against *Fusarium* wilt in an epiphytotic field and with artificial inoculation in a

screenhouse. The objective was to corroborate the disease reaction and the segregation pattern under natural and controlled environments. The field trial was meant to generate recombinants for agronomic characteristics in the segregating populations.

3.1.3.1 Evaluation under field conditions

The site that was selected for the field evaluation of *Fusarium* wilt resistance was in Runyinya location of Butare province in Rwanda. Runyinya lies between latitudes 28° 30′ and 30° 00′ and longitudes 2° 00′ and 2° 30′ within the mid-altitude belt, at 1700 masl. It has a bimodal rainfall of 1100 mm to 1200 mm annually; and a mean temperature range of 18°C to 20° C. The soils are chiefly oxisols with a pH of 5 to 6. Runyinya is a historical and perennial sick-spot, which has been used to successfully screen bean lines for resistance against *Fusarium* wilt. One the earliest attacks of Umubano by the disease occurred here.

Fifty healthy seeds of each of the parents, Umubano and Vuninkingi; and of the F_1, 100 seeds of each of the backcrosses and 250 seeds of the F_2 were sampled from the original seed bulk. They were divided equally and planted in two replications of two rows per replicate. Spacing was 10 cm within rows and 50 cm between rows. One seed was placed in each hill. Planting was done two weeks after the on set of rains so as to suppress the development of other soil borne fungal pathogens, particularly *Pythium* species. This also allowed drier spells that favors the development *Fusarium* wilt pathogens (Mutitu, 1989).

Data was recorded on percentage wilted plants, chlorosis and stunting of foliage and branches, and internal browning of stem and root systems of individual plants at flowering (R 6) and maturity stages (R 8) (CIAT, 1987). The disease severity was rated using the CIAT score scale of 1 - 9: where 1 – 3 is resistant, 4 - 6 moderately resistant and 7 – 9 is susceptible (CIAT, 1987). The mean and variance of disease severity score of each population was determined using the *Function* program of *Excel* computer package in Microsoft office Excel 2003.

The maturity duration was obtained by recording the time between germination (V 0) and maturity (R 8) of 50% of the plants in each entry. One-fifth of the plants were sampled in each row and their total number of pods was averaged and was recorded as

the mean number of pods per plant for each of the entries. The mean number of seeds per pod in each entry was calculated from a sample of 100 pods obtained from a sample of 20 plants of each parent or progeny. In order to estimate and compare the seed size of the parental and the progeny lines, a sample of 100 seeds from each population were weighed and recorded to the nearest one-tenth of a gram using *Sartorius* electronic balance.

3.1.3.2 Evaluation of *Fusarium* wilt in greenhouse

The parents and all their progenies were planted in a completely randomized block design with two replications. Seeds were placed in plastic pots of 25 centimeter diameter in previously autoclaved sand (80%) and soil (20%) mixture (Plate 1a), (Buruchara and Camacho, 1999). Three grams of di-amonium phosphate fertilizer per pot were applied. Plants were watered. The young seedlings were thinned to 5 vigorous plants per pot to achieve the final plant population of 25 for parents and F_1, 125 for F_2 and 100 plants for the backcrosses. Watering continued on daily basis throughout the duration of the experiment.

3.1.3.3 Isolation and culturing *of F.oxysporum f. sp. phaseoli* spores

To isolate the *Fusarium* wilt pathogen, about 1 cm long stem segments of infected Umubano plants in Rwanda were cut close to the hypocotyl. They were washed under tap water, chopped, peeled and split open using a flamed and ethanol-sterilized scapel blade and forceps under a lamina flow hood arrangement. The infected brown vascular tissue was teased into smaller portions. They were sterilized in 2% sodium hypochlolite ($NaHClO_2$) solution for 2 minutes, rinsed twice in distilled water, and dried using sterile blotting paper.

Five portions of infected tissue were then transferred onto sterile potato dextrose agar (PDA) that was inoculated with streptomycin sulphate at 0.4 grams per litre in petri dishe. The plates were incubated at 24 ^{0}C for four days. Portions of the spreading colonies from the plant portions were then transferred onto tap water agar (TWA) and wer furher incubated.

In order to obtain pure culture, single spore micro and megaconidia were identified from the spreading colony on a petri dish using a light microscope at x40 magnification by help of *F. oxysporum f. sp. phaseoli* identification keys and maps. Using a stereomicroscope, single, elongate, curved and septate germinating macroconidia were identified (Plate 1) in the growing colony on tap water agar (TWA). They were isolated and transferred onto fresh potato dextrose agar (PDA) /streptomycin plated petri dishes by help of sterilized wire loop containing a sterile water film. They were incubated for 14 days at 24 ^{0}C for inoculum preparation (Plate 2).

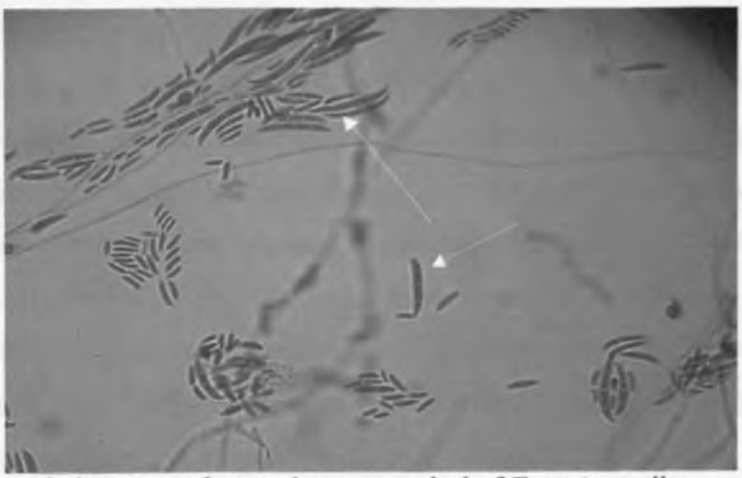


Plate 1. Mass of elongate and curved spores typical of *Fusarium* wilt

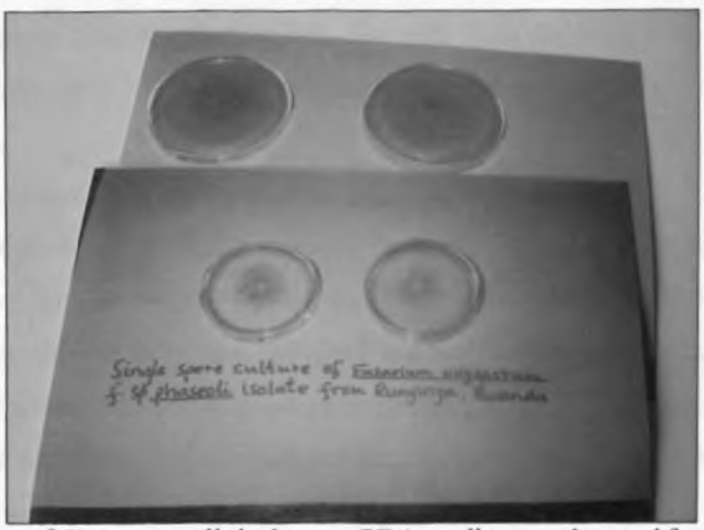


Plate 2. Colony of *Fusarium* wilt isolate on PDA medium as observed from bottom (red) and top (pink) views on the medium

3.1.3.4 Preparation of inoculum of Fusarium wilt isolate

Twenty milliliters of distilled water was added to each plate and its surface scrapped gently using curved ends of a sterile glass rod. The contents of all the petri dishes were aseptically strained through a double layer of cheesecloth into a beaker. The suspension was centrifuged at 5000 revolutions per minute for 10 minutes and the spore pellet rinsed twice in distilled water. The pellet was then uniformly mixed with 50 milliliters of distilled water. The initial spore suspension was diluted further to a final concentration containing 10^6 conidia per milliliter was determined using a haemocytometer.

The method of inoculating roots was a slight modification of the root-dip and transplanting technique model practiced by Ribeiro *et.al* (1979) and Buruchara *et.al* (1999). The young roots and root hairs of intact well-watered ten-day old seedlings were crack-injured by incomplete uprooting of the seedling. Tips of lateral root hairs around the loose hypocotyl were exposed and injured using new scapel blades. Twenty milliliters of the uniform 10^6 conidia per milliliter suspension was poured around the plant so as to flood the root region within two centimeter radius of the hypocotyls. The inoculated plants were firmly secured back into the soil. This minimized plant shock associated with transplanting large numbers of seedlings. Watering of the plants was resumed 24 hours after inoculation. The soil moisture regime was maintained at field capacity throughout (Mutitu *et al*, 1989). The pots were supplied with 3 grams per millimeter of fertilizer (NPK 17:17:17) at an interval of 7 days.

3.1.3.5 Assessment of *Fusarium* wilt damage

In assessing *Fusarium* wilt reaction among inoculated progenies, the appearance of chlorosis, wilting, and necrosis of leaves, branches or stems of all the plants (Plates 3, 4 and 5) were monitored daily and the final disease rating was done after 28 days (Buruchara and Camacho, 1999). Random samples of 20 plants from each segregating population were uprooted, washed observed for above ground symptoms, and then split open with a scapel blade. The external and internal intensity of the brown discolouration was observed and its maximum upward spread along the xylem vessels

from the hypocotyl was measured (Plates 6 and 7). The disease assessment was done using a CIAT severity score scale of 1 – 9, where:

i) 1 - 3 represents resistant reaction with no more than 10% of total foliage being chloritic or wilted and no or light vascular browning;

ii) 4 – 6 stands for intermediate reaction (11% - 50%) foliar chlorosis and wilting with light to slightly severe vascular discolouration); and the scores of

iii) 7 – 9 representing susceptible reaction manifested by more than 50% to 100% foliar yellowing, wilting, defoliation, severe to very severe discolouration or complete death of the plant (van Schoonhoven and Pastor-Corrales, 1987).

The measurement of the vertical spread of the pathogen along with observation of external symptoms was repeated after 55 days using the same procedure above with the remaining plant population. The relative movement (or rate of spread) of the pathogen among the respective resistant and susceptible parents, as well as their progenies was obtained by dividing the highest observed mean disease movement in each generation by the least among them.

Re-isolation of pathogen from damaged vascular tissues was done by taking thirty samples of the longitudinal sections of the xylem from each population (Plates 17 and 18). They were sterilized in sodium hypochlorite ($NaHClO_2$) solution and were aseptically fixed on freshly prepared PDA plated petri dishes and left to incubate at room temperature (22 ^{0}C to 24 ^{0}C) for 4 days. The vertical progression of *Fusarium* wilt inside the xylem was estimated by measuring intervals of the pinkish growth of the re-isolated pathogen on the media/xylem interface (Ribeiro *et al,* 1979; Buruchara *et.al.* 1999) (Plate 9).

The means and variance of severity of each population was determined by Microsoft *Excel* (2003) computer package. The segregation ratio of resistant to susceptible plants in each population was calculated.

The Chi-square analysis was performed using the parental lines, the F_1, F_2 and the two sets of the backcross progenies to determine the goodness-of-fit to the hypothesized Mendelian segregation ratios, by using the formula:

$$X^2 = \frac{\sum [(O - E) - 0.5]^2}{E}$$

where X^2 is the frequency distribution of segregation among the respective populations, O and E are observed and expected frequencies of individual plants of different progenies that showed resistant or susceptible *Fusarium* wilt reaction, and 0.5 is the correction factor for degrees of freedom of less than 2. The computed total X^2 for each population was compared to the tabulated value at 1 degree of freedom (Df) and probability level ($P_{0.01 \geq 0.050}$), so as to determine the level of significance .

The environmental variance (V_E) was computed by using the method recommended by Kearsey *et al* (1996), by obtaining the ratio of the total sum of the squares (SS) to combined degrees of freedom (Df) of the parental homozygous lines, using the formulae:

$$SS = \sum X^2 - C.F. = \sum X2 - \frac{(\sum X)^2}{n}; \quad \text{and} \quad V_E = \frac{SS\ G2333 + SS\ G685}{Df\ G2333 * Df\ G685}$$

where X = individual disease score; C.F.= Correction factor; n = total number of plants evaluated in each parent. Broad sense heritability (H^2bs) was estimated from the values of computed V_E and variance of the F_2 populations as follows:

$$V_P = V_G + V_E; \quad \text{or,} \quad V_G = V_P - V_E, \text{ and,} \quad H^2bs = V_G / (V_G + V_E),$$

where V_P is phenotypic variance, given by the respective variance of the F_2 progenies; V_G is the genetic component of the variance; V_E is the variance due to environmental factors; and H^2_{bs} is the broad sense heritability of the resistant trait.

Plates 3. Observed yellowing leaf symptoms (S) on susceptible parent, Umubano, and normal resistant (R) reaction on Vuninkingi donor parent after artificial inoculation with F. oxysporum f. sp. phaseoli isolate at Kabete, 28 days after inoculation (DAI)

Plates 4. General view of a section of the screenhouse showing vigorous resistant plants (R) and foliar yellowing symptoms susceptible (S) among segregating populations after artificial inoculation with *F. oxysporum f. sp. phaseoli* isolate at Kabete 55 DAI

3.2 Selection of multiple disease resistance and marketable grain types in climbing beans.

3.2.1 Choice of parents, crosses made and selection

Commercial climbing bean cultivars Umubano, Vuninkingi, and Ngwinurare were used as maternal parents. Their important characteristics and those of the donor parents are shown in Table 8.

The bean cultivars SCAM 80 CM/15, Urugezi, RWR 1312 I, Ngwinurare, and Umubano provided the desirable red-mottled or red seed coat colours.

Single, three-way and double crosses that were made to constitute the eleven populations and their codes are shown in Table 9 below. Table 10 on the other hand shows the desired traits incorporated into the respective populations.

Pair-wise crosses were made under greenhouse conditions at ISAR-Rubona during 2000A and 2000B. The same crossing, plant and seed management techniques as described in section 3.1.2 were applied.

Mature F_1 hybrid seed was harvested from healthy pods. The seeds from each cross were bulked. Thirty seeds were selected randomly from each population bulk. They were planted at Rubona field station in single rows with 20 cm x 50-cm intra and inter-row spacing at the rate of one seed per hill. Parental materials were included between adjacent rows of the F_1 plants. Individual plants within each population that were vigorous and had a disease severity rating of less than 3 on the CIAT score scale of 1 – 9 for angular leaf spot, and were at the same time resistant or tolerant to bean rust and common bacterial blight (two prevalent diseases at Rubona) were selected. The $F_{1.2}$ derived seed of the selected plants in each population were bulked and used as source of progeny selection in the subsequent generations.

In subsequent selections, four sets of thirty seeds were sampled from the bulked $F_{1.2}$ derived seed of the selected plants in each population. They were planted and screened at previously characterized diseases epiphytotic sites at Rubona (angular leaf spot), Rwerere (anthracnose), Gikongoro (root rots) and Ntendezi (root rots and anthracnose) (Table 7). They were planted in two rows using the same spacing plant spacing arrangement as for F_1 plants.

Individual plants that were resistant, with a severity rating of 1 – 3 on a CIAT scale of 1 - 9, against the respective targeted diseases at each site were selected within and

between rows. The method of assessing root rot damage is described in section 3.1.1.5. The assessment of anthracnose and angular leaf spot was based on the percent area of the total surface that was necrotic or wilted as follows:

i) 1 - 3 represents resistant reaction with no more than 10% of total foliage necrotic

ii) 4 – 6 stands for intermediate reaction with11% - 50% foliar necrotic

iii) 7 – 9 representing susceptible reaction manifested by more than 50% to 100% necrotic (van Schoonhoven and Pastor-Corrales, 1987).

Susceptible plants were discarded. The seed of individual plant selections was bulked and advanced as families within populations in the subsequent $F_{2.3\ to}$ $F_{2.6}$ derived generations.

At the $F_{2.3}$ and $F_{2.4}$ derived generations, when segregation into seed colours started, all the plants that had red-mottled, red seeds or various shades of these colours were selected, if they were rated as resistant or tolerant to the angular leaf spot, anthracnose, or *Pythium* and *Fusarium* root rots. Individual plants with other seed types such as black or cream were also selected if they showed high levels of diseases resistance (1 – 3) and high yield potential.

Table 7. Characteristics of four hot spot sites used to evaluate the eleven populations in rotation in Rwanda and the main diseases evaluated at the sites

Site	Altitude (masl)	Rainfall (mm)	Temp °C	Disease Constraint	Disease Pressure	Region
Rubona	1650	1171	18.9	angular leaf spot	High	Southern
Rwerere	2300	1166	15.6	Anthracnose	Moderate	Northern
Ntendezi	1600	1170	19	Root rots and anthracnose	very high	Western
Gikongoro	2300	1100	17	Root rots	very high	Southern

Table 8. Characteristics of the eight parental lines used to generate breeding populations

Genotype	Growth habit	Seed color	Seed size	Anth	ALS	Pythium	Fusarium	Yield (t/ha)
Ngwinurare (59-1/2)	IVA	red	large	I	I	R	R	3.5
Umubamo (G2333)	IVA	red	small	R	I	R	S	4.5
Vunikingi (G685)	IVA	red	small	R	I	R	R	4.5
SCAM 80 CM/15	II	red mottled	medium	R	R	R	R	2.5
Urugezi	II	red mottled	medium	R	R	R	R	2.5
RWR 11312 I	I	red mottled	large	R	I	R	-	1.5
Mex 54	III	pink	large	-	R	-	-	2.5
Puebla	IVA	cream	large	R	R	R	R	4.0

Legend: R= resistant, I= intermediate or moderately resistant, S=susceptible, - means the resistance/susceptibility status is not confirmed or known; the variety chosen for the resistance or seed colour indicated

Table 9. Breeding populations with desired multiple traits recombinations

Population	Code
(Ngwinurare x SCAM 80 CM/15) F_1 x (Ngwinurare x Puebla) F_1	MMCR-RW-1
(Ngwinurare x Puebla) F_1 x Mex 54	MMCR-RW-2
(Umubano x SCAM 80 CM/15) F_1 x (Umubano x Ngwinurare) F_1	MMCR-RW-3
(Umubano x SCAM 80 CM/15) F_1 x RWR13121	MMCR-RW-4
Umubano x Mex 54	MMCR-RW-5
Vuninkingi x Mex 54	MMCR-RW-6
Urugezi x Puebla	MMCR-RW-7
(Umubano x Urugezi) F_1 x Mex 54	MMCR-RW-8
(Vuninkingi x Urugezi) F_1 x Umubano	MMCR-RW-9
Umubano x Vuninkingi	MMCR-RW-10
(Umubano x Vuninkingi) F_1 x Umubano	MMCR-RW-11

Table 10. Seed colour and disese trait combinations in 11 populations constituted from simple and multiple parent crosses at ISAR Rubona station.

Population	Seed colour		Disease resistance			
	Red motlled	Red	Anthracnose	Angular leafspot	Pythium root rot	Fusarium wilt
MMCRW-1	+	+	-	+	+	+
MMCRW -2	-	+	+	+	+	+
MMCRW -3	+	+	-	-	+	+
MMCRW -4	+	+	+	+	+	-
MMCRW -5	-	+	+	+	-	-
MMCRW -6	-	-	+	+	+	+
MMCRW -7	+	-	-	-	+	+
MMCRW -8	+	+	+	+	+	+
MMCRW -9	+	+	+	-	+	+
MMCRW -10	-	+	+	-	+	+
MMCRW -11	-	+	+	-	+	+

+ = presence or - = absence of trait among the progenies.

After each selection cycle at each site, thirty seed samples from each of the individual plant bulks were evaluated in rotation for other target diseases in complementary disease 'hot spots until all the $F_{2.4}$ progenies were evaluated for the four diseases (Singh *et. al*, 1998) (Fig 2). The same pedigree selection procedure was used in all these subsequent selections. Susceptible local variety (*Local mixture*) and a local improved variety were used as checks at each hot-spot site.

At the $F_{2.5}$ generation, the selected individual plant families from the four sites in each of the populations were grown at Rubona and Rwerere in an observatory unreplicated trial with single rows of 3 m each. Further selection for red and red-mottled seed types, plant

vigour and yield was done. The yield potential of the eleven populations was calculated in kg/ha.

3.2.2 Naming of replicated yield trials of selected lines

Sixty-six most promising advanced lines that had red-mottled, red or other seed types that combined disease resistance or tolerance to 2 or all of the diseases (angular leaf spot, anthracnose and/or root rots) were selected from the $F_{2.5}$ and $F_{2.6}$ progenies of the 11 populations. They were code named using the ISAR Bean Program nomenclature of climbing bean varieties of three letter-code of 'RWV' followed by a four-digit number, starting with 2572 and ending with '2864' (Table 25). $F_{2.7}$ lines were evaluated in a preliminary yield trial at Rwerere and Rubona Research Stations.

The experiment was laid out in a randomized complete block design (RCBD) with two replications. Each entry was sown in four 4-meter row plots with a spacing 20 cm within and 50 cm between rows. Two seeds were placed in each hill. All the nine parental materials were included among the selected lines as checks. This gave a total of 75 entries in each replicate. Data was collected on the rate of germination, flowering and maturity duration (50% of plants). The diseases severity of anthracnose and angular leaf spot was rated as described above. Root rot severity was estimated by observing root samples and from the percentage of the plant loss at maturity (Buruchara, 1992).

The two inner rows were sampled and used to estimate the mean number of pods per plant and seeds per pod at maturity stage. They were harvested, weighed and used to estimate the plot yield in grams, which was converted to yield in kilograms per hectare (kg/ha). The seed color and size of the different entries were observed. Seed size was estimated by weighing samples of sun-dried 100 seeds on *Sartorius* electronic balance.

Analysis of variance was performed using Microsoft GENSTAT statistical package (2003) to determine means, variance, least significant differences and coefficient of variation both within and across the two sites. The results of the test lines were compared with the check parents. The most promising high yielding, diseases resistant and red or red mottled traits were identified.

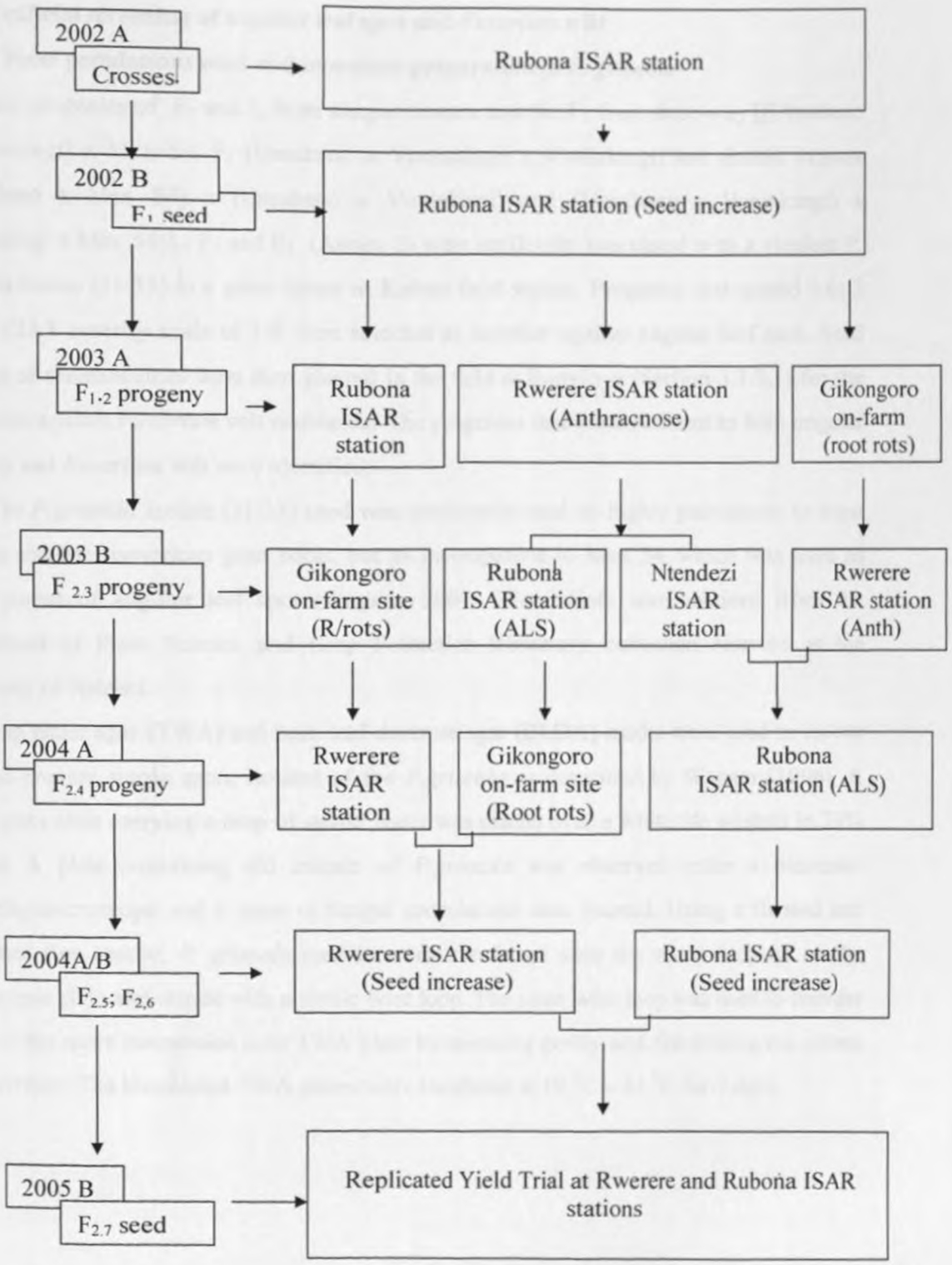


Fig 3. Rotational selection scheme for multiple resistances to angular leaf spot, anthracnose and root rots at different epiphytotic sites.

3.2.3 Artificial screening of angular leaf spot and *Fusarium* wilt

3.2.3.1 Plant populations used and inoculum preparation of *P. griseola*

The plant progenies of F_1 and F_2 from simple crosses, and the F_1 from three-way [(Umubano x Vuninkingi) x Mex 54, F_1 (Umubano x Vuninkingi) x Vuninkingi] and double crosses [(Umubano x Mex 54) x (Umubano x Vuninkingi) and (Umubano x Vuninkingi) x (Vuninkingi x Mex 54)] , F_1 and F_1 (Annex 2) were artificially inoculated with a virulent *P. griseola* isolate (31:33) in a green house at Kabete field station. Progenies that scored 1 to 3 on the CIAT severity scale of 1-9 were selected as resistant against angular leaf spot. Seed samples of resistant lines were then planted in the field at Runyinya (Section 3.1.3.1) for the evaluation against *Fusarium* wilt resistance. The progenies that were resistant to both angular leaf spot and *Fusarium* wilt were identified.

The *P.griseola* isolate (31:33) used was previously rated as highly pathogenic to most Andean and Mesoamerican gene pools, but as incompatible to Mex 54, which was used as donor parent of angular leaf spot (Wagara, 2004). The isolate was obtained from the Department of Plant Science and Crop Protection laboratory collection reserves at the University of Nairobi.

Tap water agar (TWA) and bean leaf dextrose agar (BLDA) media were used to isolate and and prepare single spore isolates of the *P.griseola* as described by Wagara (1996). A sterile glass slide carrying a drop of sterile water was placed over a white tile washed in 70% alcohol. A plate containing old culture of *P.griseola* was observed under a binocular dissecting microscope and a mass of fungal sporulations was located. Using a flamed and moistened fine needle, *P. griseola* conidia were transferred onto the water droplets on the microscopic slide and stirred with a sterile wire loop. The same wire loop was used to transfer a film of the spore suspension onto TWA plate by streaking gently and distributing the spores on its surface. The inoculated TWA plates were incubated at 19 ^{0}C – 21 ^{0}C for 2 days.

Germinating spores on the TWA plates were identified by observing under a compound microscope. Small slabs of the media carrying single germinating conidia were sliced with help of sterile scapel blade. They were transferred onto BLDA in sterile petri dishes and incubated at 21 ^{0}C – 24 ^{0}C in darkness for 14 days (Wagara, 1996). The spores from the mother single spore cultures were multiplied by culturing onto BLDA afresh and were re-incubated for 14 days.

About 20 milliliters of distilled water was added to the BLDA plates containing the 14-day old cultures. The surface was scrabbled gently using the edge of a sterile microscopic slide until a dark suspension of the conidia was formed. The suspension was aseptically strained through a double layer of cheesecloth into a beaker of water. It was stirred until a uniform mixture was obtained. The concentration of the suspension was then adjusted to 2 x 10^6 spores per milliliter using a haemocytometer after dilution with distilled awter.

3.2.3.2 Screening against *Fusarium* wilt and angular leaf spot

The screening and assessment of reaction against *Fusarium* wilt was done at Runyinya site (section 3.1), while it was done in the screenhouse in case of angular leaf spot. In the latter case, fifty seeds of the F_1 (Umubano x Mex 54) plant progenies, 250 seeds of the F_2 and F_1 (Umubano x MEX 54) and 100 seeds of each of F_1 (Umubano x Vuninkingi) x Mex 54, F_1 (Umubano x Vuninkingi) x Vuninkingi, F_1 (Umubano x Vuninkingi) x (Vuninkingi x MEX 54) and F_1 (Umubano x MEX 54) x (Umubano x Vuninkingi) crosses (Annex 2) were sampled. Fifty seeds of each of the parental materials (Umubano, Mex 54 and Vuninkingi) were included as checks. They were planted in 20 cm-diameter plastic pots containing previously autoclaved clay-loam soil after it was left out for 30 days so as to allow the cooling and restoration of microbial activity (re-naturing). The trial was laid out in randomized complete design with two replicates. The pots were watered to field capacity daily.

After 21 days, the primary simple and the first trifoliate leaves of the seedlings were sprayed with the suspension of the inoculum containing 2 x 10^6 of the virulent isolate (31:33) of *P. griseola,* until the whole leaves were wet-dripping by using a half litre Baygon atomizer. The inoculation was done early in the morning when the stomata were open. The lower surfaces of the leaves were mostly targeted. The floor of the screen house was flooded with water every morning and evening to maintain high humidity. The warm (about 24 ^{0}C) and

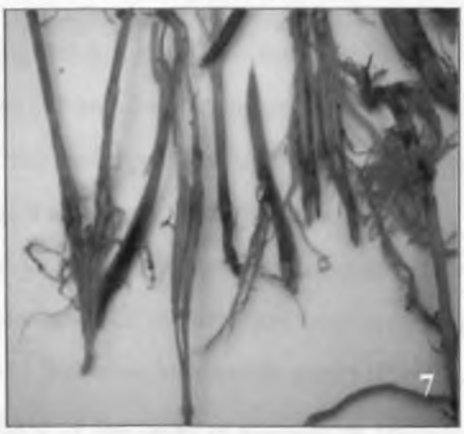
high humid environment was maintained by reinforcing the glass walls with transparent plastic sheeting lining inside the glasshouse.

The development of angular leaf spot symptoms on leaves was monitored daily. The assessment of its severity was made during the third week (14^{th} to 21st day) after the inoculation (Wagara, 1996). The percentage of the total leaf area of the individual plants that was covered by the typical inter-vein spots and lesions was estimated visually. The values obtained were used assess disease severity using the standard CIAT scale of 1 – 9 (van Schoohoven and Pastor-Corrales, 1987). .In this scale, a score of 1= no observed symptoms; less than 10% of leaf area affected by lesions = 3; 25% of the leaf area affected = 5; 50% of leaf area affected = 7; and more than 75% of the leaf area covered by lesions = 9. Scores of 1 to 3 represented a resistant reaction; 4 to 6 intermediate reaction, while 7 to 9 was for susceptible disease reaction (Plate 15).

All the plants that were resistant against angular leaf spot from the different populations were selected. Their seed was bulked. Samples of the seed were obtained and they were used in the field experiment for the selection of those among them that were resistant against *Fusarium* wilt at the same time. The assessment for *Fusarium* wilt resistance in the field was carried out as described under section 3.1.3.1.

Seed samples of lines that were resistant against angular leaf spot were then planted in the field at the Runyinya hot-spot site (Section 3.1.1) for the evaluation against the *Fusarium* wilt resistance. Two hundred and fifty seeds of the F_2 (Umubano x Mex 54) plant progenies and of the F_3 (Umubano x Mex 54) and 100 seeds of each of F_3 (Umubano x Vuninkingi) x (Vuninkingi x Mex 54) and F_3 (Umubano x Mex 54) x (Umubano x Vuninkingi) crosses were planted in 2 replicates. One hundred seeds of Umubano, Vuninkingi and Mex 54 were included in the planting arrangements as checks. The assessment of Fusarium wilt reaction was done using the CIAT scale as described under section 3.1.1.

Plate 5. Stunting, yellowing and wilting symptoms of Fusarium wilt observed on susceptible plants in screenhouse at Kabete after artificial inoculation with F. oxysporum f. sp phaseoli isolate



Plates 6 and 7. External hypocotyl damage (left) and internal vascular penetration and discolouration (right) on susceptible plants due to *F.oxysporum f. sp. phaseoli* inside screenhouse at Kabete field station.

CHAPTER FOUR

4.0 RESULTS

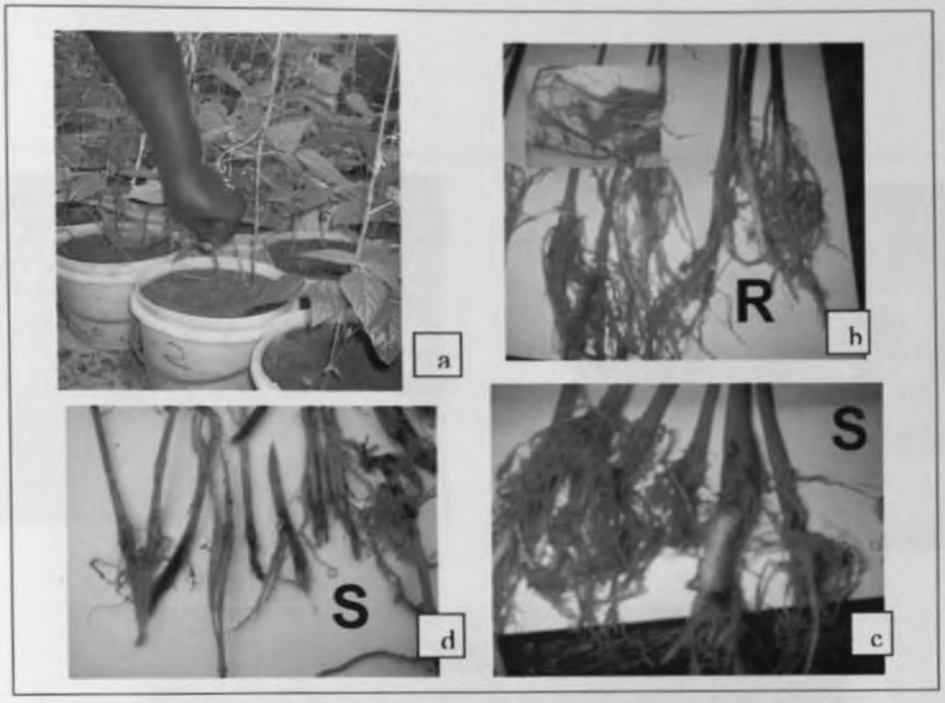
4.1 Inheritance of resistance to *Fusarium* wilt in climbing beans

Yellowing of leaves, stunting and wilting symptoms were observed on susceptible plants, while others remained vigorous with light green or green foliage (Plates 3, 4 and 5). The yellow, wilted or stunted plants had brown discolouration of the hypocotyl region with light to deep brown lesions and cankers that exposed the cortex of the hypocotyls and on tap roots (Plates 6, 7 and 8). The vigorous green plants on the other hand had very light superficial discolouration of the hypocotyls and healthy adventitious roots (Plate 8). In all the cases, the brown discoloration or lesions was very rarely noticed beyond the soil/sand level throughout the eight weeks of the disease evaluation.

However, when the lower stems of sampled plants were dissected after 4 weeks of inoculation, the brown discoloration of different intensity was observed inside the plants. The mean spread of the brown discolouration along the xylem ranged between 2 and 4 cm in Vuninkingi and Flora parents, the F_1 and backcross progenies to resistant parents, as well as among the G685 x Flora F_2 progenies. The movement of the pathogen was greatest in G2333, the backcross (G2333 x G685) x G2333 and (G2333 x Flora) x G2333, and ranged between 4 cm to 8 cm. Similar trend in the movement of the pathogen was observed 8 weeks after inoculation, but the range had increased from 2 – 4 cm to 6.2 - 12 cm and from 4 – 8 cm to 12 - 24 cm among the 2 categories of plants respectively.

The susceptible cultivar, G2333 recorded the highest infection into the vascular tissue by the pathogen into the xylem after the 4 and 8 weeks (10 and 24 cm respectively). This was about 4 times more (or faster) than the spread of the pathogen inside the tissues of the resistant G685 and Flora parents (Table 11 and Figures 4 and 5).

When the cut stem sections were placed on the yeast extract mediam, pathogens grew along the discoloured portions of the sections. The re-isolation of the pathogen occurred along the entire length of the sections in the samples of G2333 and other susceptible plants (Plate 9). The pathogen was recovered at 2 – 3 cm from the base of the stem in sections of resistant parents, G685 and Flora. No growth on the media plate was noticed along the stem sections of the non-inoculated control plants, meaning the browning were caused by infestation of *Fusarium* wilt pathogen in the inoculated plants. Some of the severely damaged hypocotyls developed some secondary adventitious roots (Plates 9)



Plates 8. Root sampling (8a); 8b. roots from resistant plants; 8c and d. roots from susceptible plants, showing external and internal discolouration of the root system due to *F. oxysporum sp. phaseoli* attack in a glasshouse at Kabete.

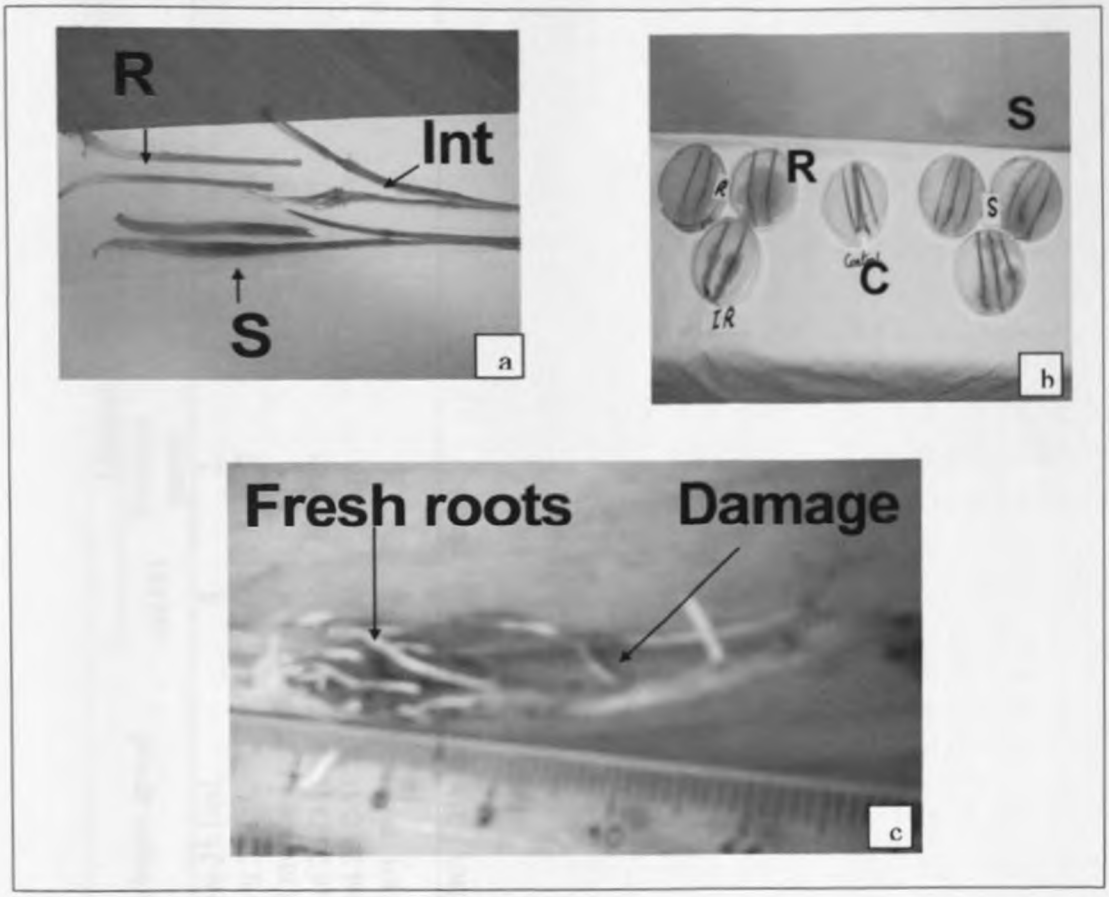


Plate 9. (a) Vertical spread of *Fusarium* wilt in resistant (R) (susceptible (S) and intermediate resistant (Int) ; (b) the re-isolation of *Fusarium* wilt pathogen from infected stem portions in (a), including control (C) on agar plate and (c) showing secondary root development after damage of primary roots by *Fusarium* wilt in a screen house at Kabete

Table 11. Vertical spread of Fusarium wilt into vascular tissue at 28 and 55 days after inoculation (DAI) with *Fusarium oxysporum f.sp. phaseoli* isolate in screenhouse at Kabete

Cross	Description of pathogen spread	Distance moved in cm by Cultivar or progeny						
		G2333	Resistant parent	F_1	F_2	*BC_S	*BC_R	G685 x Flora (F_2)
G2333 (S) x G685 (R_2)	Vertical movement 28 DAI	8	2	3.2	4.5	6.5	4	2
	Vertical movement 55 DAI	24	6.2	7	12	18	11	8
	Longest: Shortest movement 55 DAI	4	1	1	2	3	2	1.3
G2333 (S) x Flora (R_1)	Vertical movement 28 DAI 28	10	3.5	4	5.4	7	4.2	2.2
	Vertical movement 28 DAI 55	23	8	11	20	21	12	9
	Longest : Shortest movement 55 DAI	3	1	1.5	2.5	2.5	1.5	1

BC_S = Backcross to susceptible parent, BC_R = Backcross to resistant parent

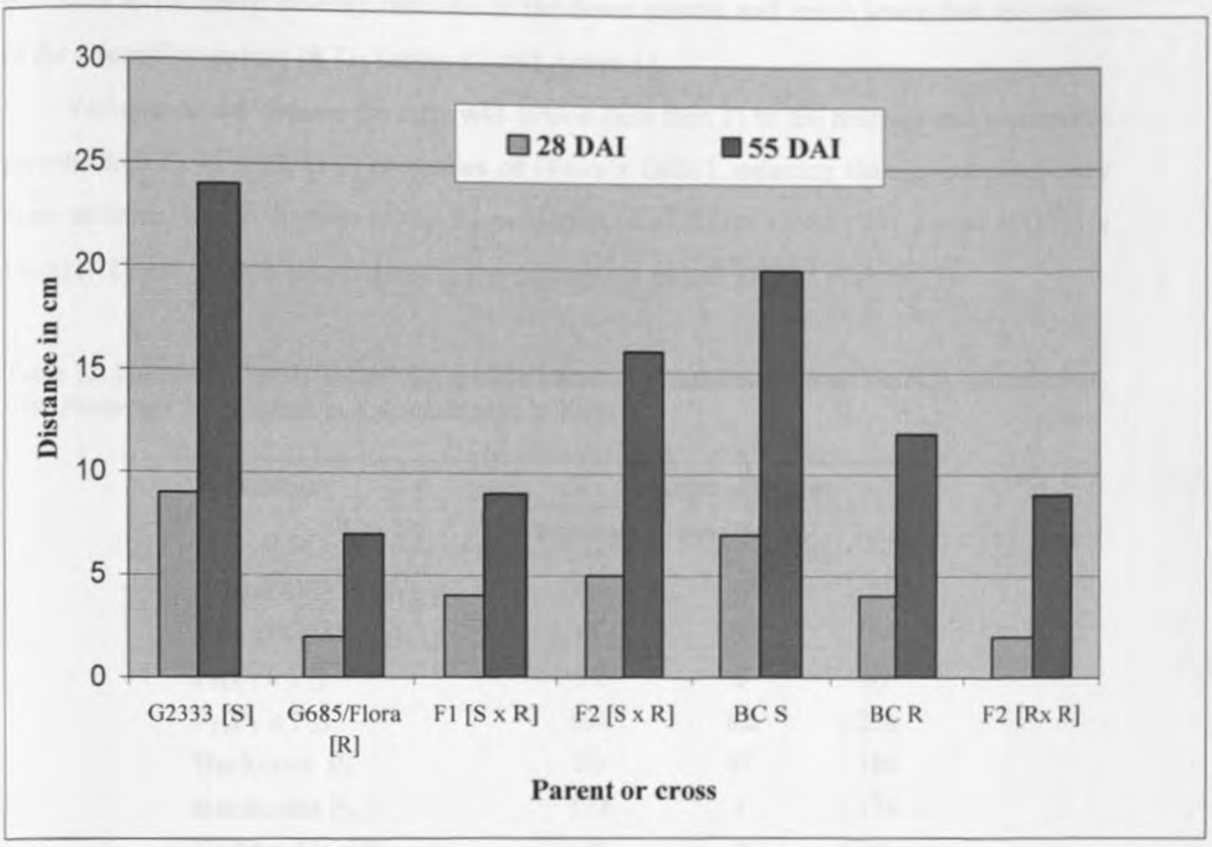


Fig.4. Mean length of vertical penetration in cm of *Fusarium oxysporum f.sp. phaseoli* into xylem of different generations of G2333 x G685 cross at 28 and 56 days after inoculation (DAI) in screenhouse at Kabete. (BCS = Backcross to susceptible parent, BCR = Backcross to resistant parent)

4.1.1 Overall *Fusarium* wilt assessment and Chi-square analysis

All plants of G2333 were susceptible with the severity score ranging between 7 and 9, with a mean of 8.7. The plants of the donor parents, G685 and Flora and all the F_1 progenies showed resistant reaction scores between 1 and 3. About three-quarters of the F_2 progenies of both crosses were resistant and one-quarter was susceptible. The backcross progenies of G2333 segregated in the ratio of 1: 1 for the resistant and susceptible reaction. The backcrosses of both donor parents were significantly resistant to *Fusarium* wilt (Tables 12 and 14; Annex 1).

The mean severity scores of the F_1 of G2333 x G685 and of G2333 x Flora were 2.2 and 2.8 respectively. They were very close to the mean severity score of the donor parents of 1.6 and 2.3 respectively, within the resistant disease severity bracket. The severity score of the F_2 progenies ranged between 1 and 9, with a mean of 3.9 and 4.0 respectively. They were higher

but closer to the mean severity reaction of the donor parents and much lower than the means of the susceptible parent (8.7) (Tables 12 and Annex 1).

Variance of the disease severity was lowest (less than 1) in the resistant and susceptible parents, their F_1 as well as F_2 progenies of (Flora x G685), meaning these populations were more uniform. It was highest in the F_2 progenies of G2333 x G685 (7.8) and of (G2333 x Flora) (7.1) and of both backcrosses to the susceptible parent, G2333 (Tables 13).

Table 12. Number of resistant or susceptible plants or progenies after artificial inoculation with *Fusarium* wilt isolate in a screenhouse at Kabete.

Population	Total no. of plants		
	Resistant	Susceptible	Total
Parent G2333 (P_1)	0	32	32
Parent G685 (P_2)	38	0	38
F_1(P_1 x P_2)	39	0	39
F_2(P_1 x P_2)	194	62	256
Backcoss P_1	89	91	180
Backcross P_2	177	1	178
G685 x Flora F_2	40	0	40
Parent G2333 (P_1)	0	32	32
Parent Flora (P_2)	40	0	40
F1(P_1 x P_2)	40	0	40
F2(P_1 x P_2)	193	67	260
Backcross P_1	86	94	180
Backcross P_2	175	5	180
Flora x G 685 (F_2)	40	0	40
Total	1151	364	1535

The segregation pattern for resistance among the parental lines and the different progenies is depicted in Figure 4 below. When the X^2 analysis was performed, it confirmed segregation of the F_2 into the Mendelian 3 resistant: 1 susceptible. The backcross to the susceptible parent was in agreement with the 1 resistant: 1 susceptible. This was true for both the G2333 x G685 and G2333 x Flora crosses (Tables 14 and 15).

Table 13. Number of plants among parents and their progenies showing the *Fusarium* wilt severity scores 1 to 9 in screenhouse at Kabete.

	Number of plants with disease severity score (1 – 9)										
Generation	1	2	3	4	5	6	7	8	9	Mean	Variance
G2333 (P_1)	0	0	0	0	0	0	3	5	24	8.7	0.426
G685 (P_2)	20	12	6	0	0	0	0	0	0	1.6	0.563
$F_1(P_1 \times P_2)$	10	11	18	0	0	0	0	0	0	2.2	0.730
$F_2(P_1 \times P_2)$	20	75	99	1	0	3	2	3	53	3.9	7.766
Backcross P_1	24	38	27	0	0	3	24	24	40	5.1	9.983
Backcross P_2	32	58	87	0	0	0	1	0	0	2.3	0.758
F_2 (685 x Flora)	5	33	2	0	0	0	0	0	0	1.9	0.174
G2333 (P_1)	0	0	0	0	0	0	1	8	23	8.7	0.286
Flora (P_2)	8	14	18	0	0	0	0	0	0	2.3	0.603
$F_1(P_1 \times P_2)$	2	6	32	0	0	0	0	0	0	2.8	0.295
$F_2(P_1 \times P_2)$	16	67	110	0	1	0	3	30	33	4.0	7.127
Backcross P_1	14	47	25	1	2	2	15	24	50	5.3	10.10
Backcross P_{2b}	21	120	34	0	1	1	1	2	0	2.2	0.946
F_2 (Flora x G685)	2	29	9	0	0	0	0	0	0	2.2	0.251

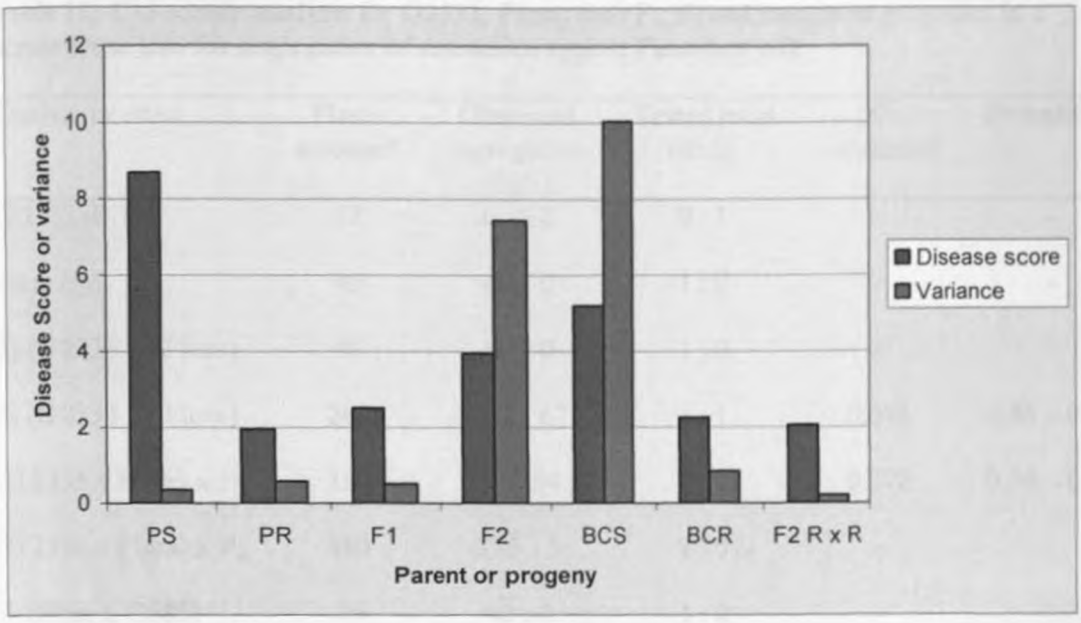


Fig. 5. Mean disease score and variance among susceptible parent (PS), resistant (PR), F1, F2 abd backcross progenies (BCS / BCR and F2 of resistant parents among G2333 x Flora and G2333 x G685 crosses after artificial inoculation with *Fusarium oxsporum f.sp.phaseoli* in screenhouse at Kabete

Table 14. Chi-square analysis for G2333, G685, their F_1, F_2 and backcross progenies from the segregation observed in screenhouse

Cultivar or cross	Plants screened	Observed segregation (R:S)	Tested ratio (R: S)	X^2 calculated	Probability
G 2333 (P_1)	32	0 : 32	0 : 1	-	-
G 685 (P_2)	38	38 : 0	1 : 0	-	-
F_1 (G 2333 x G685)	39	39 : 0	1 : 0	-	-
F_2 (G 2333 x G685)	256	194 : 62	3 : 1	0.0468	0.80 – 0.90
(G 2333 x G 685) x P_1	180	89 : 91	1 : 1	0.0054	0.90 – 0.95
(G 2333 x G 685) x P_2	178	177 : 1	1 : 0	-	-
F_2 (Flora x G685)	40	40 : 0	1 : 0	-	-

Table 15. Chi-square analysis for G2333, Flora, their F_1, F_2 and backcross progenies in a screenhouse trial for segregation of resistance against *Fusarium* wilt

Cultivar or cross	Plants screened	Observed segregation (R:S)	Tested ratio (R: S)	X^2 calculated	Probability
G 2333 (P_1)	32	0 : 32	0 : 1	-	-
Flora (P_2)	40	40 : 0	1 : 0	-	-
F_1 (G 2333 x Flora)	40	40 : 0	1 : 0	-	-
F_2 (G 2333 x Flora)	260	193 : 67	3 : 1	0.046	0.80 – 0.90
(G 2333 x Flora) x P_1	180	86 : 94	1 : 1	0.272	0.50 – 0.70
(G 2333 x Flora) x P_2	180	175 : 5	1 : 0	-	-
F_2 (Flora x G685)	40	40 : 0	1 : 0	-	-

4.1.2 Disease reaction and Chi-square analysis for field trial

The mean severity rating was highest in the susceptible parent, G2333 (score of 8.7) and its backcross progenies (6.4). It was lowest in G685 and among the F_1 progeny (3.7). The variances were least among the non-segregating parents G2333 and G685, and the uniformly non-segregating F_1 progeny (0.782, 1.08 and 1.062 respectively). It was highest in the backcross of G2333 and the F_2 progenies (7.3 and 2.4 respectively) (Fig 5). The mean severity scores of the F_1 progeny were closer to the severity score of the resistant parent (Fig 6).

Chi-square analysis showed no significant differences between the observed and the theoretical segregation ratios of 3 resistant: 1 susceptible among the F_2 progenies. All the F_1 and the backcross progenies to the resistant parent (G685) were resistant. Similarly, there were no significant differences between the observed and the expected segregation ratio of 1 resistant: 1 susceptible among the backcross progenies to the susceptible parent, G2333 (Tables 16).

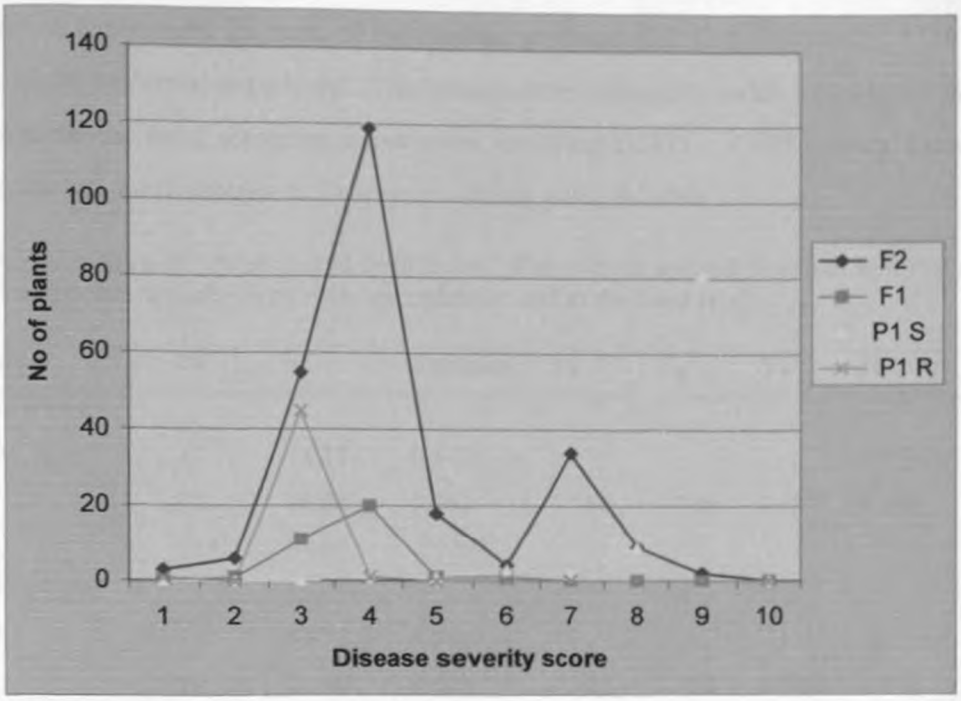


Fig.6. Number of plants showing different *Fusarium* wilt reaction among the parental lines G2333 and G685, the F_1 and F_2 progenies in the field evaluation trial at Runyinya, Rwanda

Table 16. Chi-square analysis for the cross between Umubano and Vunikingi, their F_1, F_2 and backcross progeny following natural infection by *Fusarium oxysporum pv phaseoli* at Runyinya, Rwanda

Cross/Line	Number of plants			Expected	X^2	P-value
	Total	Resistant (R)	Susceptible (S)	(R : S)	(calc)	
Umubano (G 2333) P_1	97	01	96	0 : 1	-	-
Vuninkingi (G 685)P_2	47	47	0	1 : 0	-	-
(G 2333 x G685) F_1	33	31	2	1 : 0	-	-
(G 2333 x G685) F_2	250	183	67	3 : 1	0.341	0.50 – 0.70
(G 2333 x G 685) x P_1	94	43	51	1 : 1	0.522	0.30 – 0.50
(G 2333 x G 685) x P_2	100	89	11	1 : 0	-	-

4.1.3 Heritability of *Fusarium* wilt resistance

The heritability of *Fusarium* wilt resistance was estimated at 99.6% under the artificial inoculation experiment for both of the crosses involving G2333 x G685 and G2333 x Flora under the screenhouse experiment. The broad sense heritability value was slightly higher, at 99.8% under the field screening experiment involving G2333 x G685 crosses. Estimates of the heritability for resistance to *Fusarium* wilt are given in Table 17.

Table 17. Analysis of variance and heritability of resistance against fusarium wilt from observed disease severity scores in screenhouse and in the field trial.

Source	Df	SS	Variance	V_P	V_G	V_E	H_{2bs}	H_{2bs} (%)
*G2333 x G685								
G2333	31	13.22	0.462					
G685	37	20.84	0.563	7.766	7.736	0.0297	0.996	99.6
Total	1147	34.06	0.0297					
*G2333 x Flora								
G2333	31	8.875	0.286					
Flora	39	23.50	0.603	7.127	7.100	0.0268	0.996	99.6
Total	1209	32.375	0..268					
**G2333 x G685								
G2333	96	76	0.792					
G685	46	5	0.108	2.373	2.355	0.0183	0.998	99.8
Total	4416	81	0.0183					

* Under artificial inoculation in screenhouse, and** under natural infestation field conditions

4.1.4 Inheritance of agronomic traits

4.1.4.1 Flower and seed traits

The flowers of both parents, G2333 and G685 were white. All their F_1, F_2 and backcrosses progenies inherited the white flower of the parents as was expected (Table 18). The seeds of the susceptible parent, G2333 were red, while those of the resistant parent, G685 were light red. All the F_1 plants were red seeded, like those of the recipient parent. However, all the F_2 progenies had light red seeds, of the donor parent type. The same pale red colour was predominant among the plants of the backcross progenies. A negligible fraction of their seeds had the red colour of G2333 (less than 0.1%) (Table 18).

Both parents were small seeded, with 100 seed weight of 21.1 g and 21.6 g in case of G2333 and G685 respectively. The seed of the F_1 progenies were small in size (24.4 g per 100

seeds) but bigger than for either parent. However, the mean seed weight of the F_2 plants was 35.3 g in the medium size category, just as it was observed in the backcross progenies of the susceptible parent (32.7 g per 100 seeds) and of the resistant parent (30.6 g per 100 seeds) (Fig. 7).

Table 18. Flower colour and seed colour and seed size of parental lines, F_1, F_2 and backcross progenies grown in the field at Runyinya, Rwanda

Line/Cross	Flower color	Seed color	Seed size
G2333 P_1 S	white	red	small
G685 P_2 R	white	pale red	Small
(P_1 x P_2) F_1	white	red	Small
(P_1 x P_2)F_2	white	pale red	Medium
BCP_1S	white	pale red or red	Medium
BCP_2R	white	pale red or red	Medium

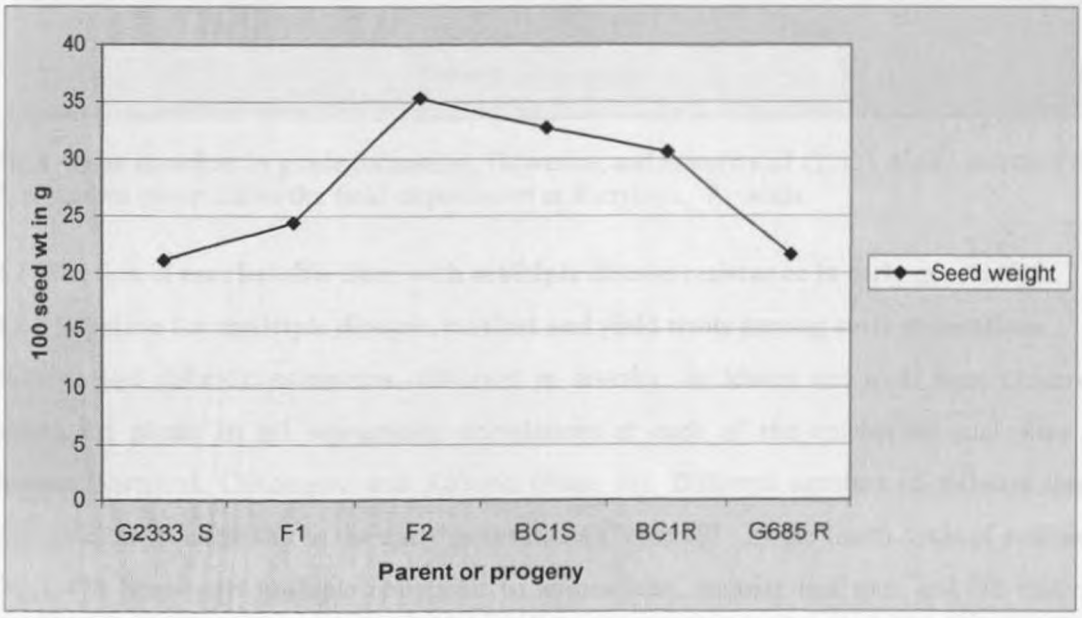


Fig 7. Seed size of parental lines, F_1, F_2 and backcross progenies grown in the field at Runyinya, Rwanda measured as weight of 100 seeds

4.1.4.2 Development of guides and duration to flowering and maturity

The formation of guides or tendrils took 21 and 30 days in G2333 and G685 parents respectively. The mean duration for guide formation was 26 days for the F_1 and 30 days for the F_2 progenies. The duration of flowering was shortest in G2333 and longest in G685 (49 and 53 days respectively). It was intermediate among F_1 (50 days) and F_2 progenies (51 days)

.At 87 days, G2333 was the earliest to mature, while G685 was the latest of the group. The F_1 progeny matured after 88 days while the F_2 progeny attained maturity in 89 days (Fig.8).

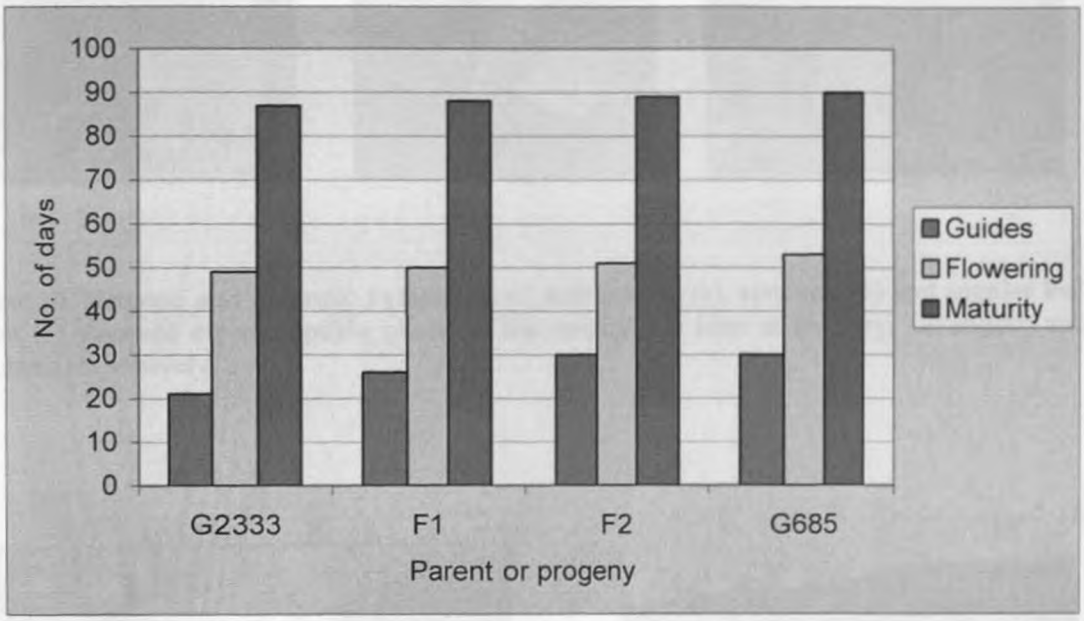


Fig 8. Mean duration in guide formation, flowering and maturity of G2333, G685 and their F_1, F_2 progenies observed in the field experiment at Runyinya, Rwanda.

4.2 Selection of marketable lines with multiple disease resistance in early generations

4.2.1 Selection for multiple disease, market and yield traits among early generations

Necrotic and chloritic symptoms, different in severity, on leaves and roots were observed among the plants in all segregating populations at each of the epiphytotic trial sites of Rwerere/Ntendezi, Gikongoro and Rubona (Plate 10). Different numbers of resistant lines, were selected at each site in the early generations (Table 19). In the fourth cycle of selection ($F_{2.4}$), 475 lines with multiple resistance to anthracnose, angular leaf spot, and the root rot diseases were selected across the sites (Table 20). The susceptible checks had disease severity rating of 9 at the three sites at every cycle of selection. Only plants with disease severity rating of 1 – 4 on the CIAT scale of 1 – 9 were selected. The disease selection pressure was consistently high at Gikongoro, Cyangugu and Rubona, but was moderate at Rwerere. This was shown by high loss of plant stand at these sites (Plate 11).

Plate 10. Necrotic and chlorotic symptoms of anthracnose (a), root rots (b) and angular leaf spot (c) observed on susceptible plants at the epiphytotic sites at Rwerere, Gikongoro and Rubona respectively.

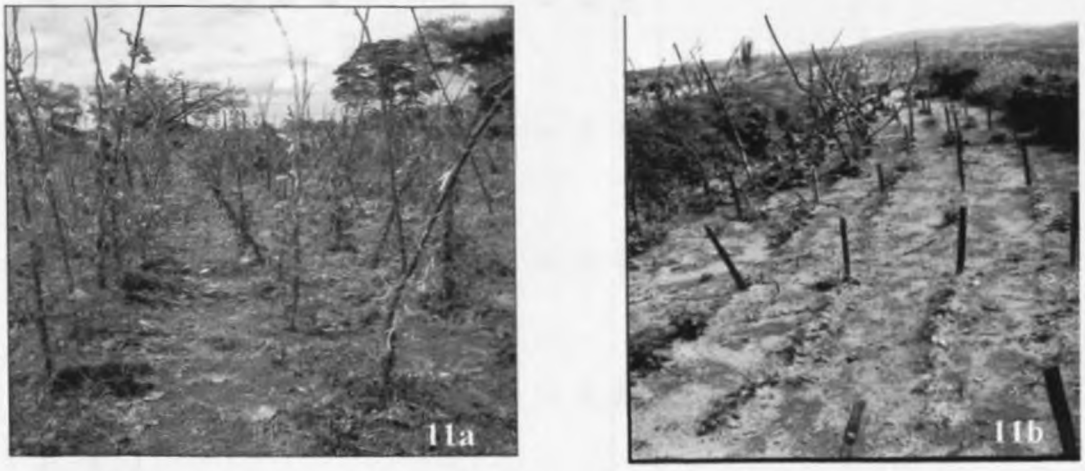


Plate 11. Loss of plant stand due to high disease pressure observed at Ntendezi due to anthracnose (11a) and at Gikongoro due to root rots (11b) during evaluation of segregating populations for resistance against the diseases.

Table 19. Number of F_2 plants, F_3 families and F_4 lines selected for resistance to angular leafspot (Rubona), anthracnose (Rwerere/Cyangugu), root rots (Gikongoro/Cyangugu) and Fusarium wilt (Gikongoro/Cyangugu) from 11 popultions

	F_2				F_3				F_4			
Population	Rubona	Rwerere	Cyangugu	Gikongoro	*Rubona*	*Rwerere*	*Cyangugu*	*Gikongoro*	Rubona	Rwerere	Cyangugu	Gikongoro
MMCRW-1	5	39	18	8	20	16	0	35	10	74	37	0
MMCRW-2	3	42	9	8	19	12	30	23	20	37	5	12
MMCRW-3	3	25	16	3	18	3	23	21	0	10	0	0
MMCRW-4	8	22	11	12	22	21	24	21	22	51	45	7
MMCRW-5	3	17	16	21	26	28	40	58	31	24	2	0
MMCRW-6	2	24	7	6	19	16	37	2	3	0	5	0
MMCRW-7	6	8	3	21	22	32	5	3	0	11	0	0
MMCRW-8	2	10	13	7	21	7	15	2	0	1	0	8
MMCRW-9	2	9	3	*10*	0	12	19	3	4	0	2	0
MMCRW-10	22	13	2	*7*	22	7	0	0	0	36	0	0
MMCRW-11	26	20	4	*6*	17	7	18	0	0	18	0	0
Total	82	229	102	109	206	161	211	168	86	301	96	37

Table 20. Number of F_4 generation lines selected per population that possess multiple disease resistance or tolerance to angular leaf spot, anthracnose and root rots

Population	Number of multiple resistant lines
MMCRW-1	121
MMCRW-2	74
MMCRW-3	10
MMCRW-4	125
MMCRW-5	57
MMCRW-6	8
MMCRW-7	11
MMCRW-8	9
MMCRW-9	6
MMCRW-10	36
MMCRW-11	18
Total	475

F_2 populations segregated for seeds with red, red-mottled, pink, purple, tan, white or black colours of small, medium or large sizes. Among the $F_{2:5}$ plant families that were selected, populations MMCRW-1, MMCRW-3, MMCRW-8 and MMCRW-9 produced the largest numbers of the red and / or red-mottled seed types (Table 21). They generated a total of 37 and 45 red-mottled and red seeded lines respectively, or 36% of all the selections by seed types at this stage. All the red and red-mottled seeds of the selected plants were medium or large in size.

Forty of the 45 (89%) red-mottled or red seeded lines above combined multiple resistances (severity score of 1 – 3) against anthracnose, angular leaf spot and root rots. Of the remaining 5 lines, 2 were resistant to anthracnose and angular leaf spot and tolerant (severity score of 4 – 6) to angular leaf spot.

Table 21. Number of selected lines among 11 populations that were red, red-mottled or of other market seed types at F_5 generation

Population	Market class			
	Red-mottled	Red	Other	Total
MMCRW-1	10	29	10	49
MMCRW-2	0	1	6	7
MMCRW-3	6	4	1	11
MMCRW-4	7	6	0	13
MMCRW-5	0	5	43	48
MMCRW-6	0	2	8	10
MMCRW-7	0	4	33	37
MMCRW-8	10	0	6	16
MMCRW-9	6	0	7	13
MMCRW-10	0	0	18	18
MMCRW-11	0	0	4	4
Total	37	45	146	226

4.2.2 Yield, pods per plant and maturity duration of early generations

Under early yield testing of $F_{2.5}$ populations at Rubona and Rwerere, the highest yielding populations were MMCRW-3and MMCRW-6 at Rwerere; and MMCRW-5 and MMCRW-8 at Rubona. They had mean yields of 3.2 and 2.3 ton ha^{-1}, respectively. .Populations MMCRW-1, MMCRW-3, MMCRW-6, MMCRW-8, and MMCRW-11 were the best yielding at Rwerere. Their average yields ranged from 2.4 to 3.2 ton ha^{-1} .The highest yielding populations at Rubona were MMCRW-1, MMCRW-2, MMCRW-8, MMCRW-10, and MMCRW-11. Their yield ranged between 2.2 to 2.3 ton ha^{-1} .The populations MMCRW-1, MMCRW-11 and MMCRW-8 were high yielding at both Rwerere and Rubona sites. The mean yields were higher at Rwerere than at Rubona (Table 22)

The average number of pods per plant varied from 33 in population MMCRW-3 to 98 in population MMCRW-6 with a mean number of 49 pods. The number of seeds per pod also varied from 6 in population MMCRW-11 to 10 grains among MMCRW-4 and MMCRW-10 populations with a mean of 6 grains. The population that had the high

numbers of pods per plant or seeds per pod were not necessarily the highest best yielding (Table 21).

The mean maturity duration was also longer at Rwerere than at Rubona site. The mean duration ranged between 99 to 107 days at Rubona and 97 and 117 days at Rwerere. The earliest populations were MMCRW-4 and MMCRW-5, while the latest maturing populations were MMCRW-7 and MMCRW-10 at Rwerere and Rubona, respectively (Table 21). All the populations produced plants of climbing bean growth habit, irrespective of having some parents of the bush types.

Table 22. Mean grain yield, duration to maturity, pods plant-^{1} and seeds pod-^{1} of a random sample of 50% of F_5 lines from 11 of populations grown at Rwerere and Rubona ISAR stations

Population	No. of lines	Yield (kg / ha)		Days to maturity		Pods plant-^{1}	Seeds pod-^{1}
		Rwerere	Rubona	Rubona	Rwerere		
MMCRW-1	49	2400	2200	100	104	42	8
MMCRW-2	7	2000	2300	101	118	35	7
MMCRW-3	11	3200	1600	102	100	33	7
MMCRW-4	13	1600	1900	101	97	47	10
MMCRW-5	48	2500	2300	99	117	43	7
MMCRW-6	10	3200	1900	101	97	98	8
MMCRW-7	37	1900	1700	100	123	49	7
MMCRW-8	16	3000	2300	102	104	49	10
MMCRW-9	13	1400	2000	103	105	46	7
MMCRW-10	18	2100	2300	107	114	53	10
MMCRW-11	4	2500	2200	106	116	42	6
Mean	22	2345	1973	102	109	49	8

4.2.3 Selection from advanced lines in replicated yield trials

By the F_6 generation, 66 of the lines selected for multiple resistance were also homogeneous in terms of seed types and agronomic traits. The ANOVA analysis revealed significant differences ($P_{0.01>0.05}$) among results of their yield, flowering and maturity durations, seed size (100 seed weight), seeds per pod and pods per plant.

The following sections describe the results, which are also summarized together in Tables 23 – 31. The results of the analysis of variance of the data collected from the above traits are presented in (Annex 5).

4.2.3.1 Flowering and maturity duration of advanced lines

There were highly significant differences for duration to 50% flowering and maturity (Annex 5.2 and 5.3). In general, lines flowered earlier at Rubona than at Rwerere with means of 45 and 56 days at the respective sites. The flowering duration ranged between 38 to 49 days and 52 - 64 days. Two red seeded lines flowered earliest. They were RWV 2573 (38 days) and RWV 2575 (42 days) at Rubona site. The same lines were among the early flowering lines (within 55 days) at Rwerere site. The red-mottled lines RWV 2699 was the earliest to flower at Rubona site (41 days). The other red-mottled lines: RWV 2698, RWV 2698, RWV 2698, RWV 2679, RWV 2680, RWV 2673, RWV 2686 also flowered relatively early at Rubona, taking 43 days (Tables 25 – 28).

There were significant differences in duration to maturity (Annex 5). Lines tended to mature earlier at Rubona (86 days) than at Rwerere (100 days). The earliest lines matured in 80 days at Rubona and 95 days Rwerere. The latest lines took 95 days at Rubona and 107 days at Rwerere. Early maturing red-seeded lines included RWV 2616 (80 days), RWV 2573, RWV 2606 (82 days), and RWV 2575 and RWV 2580 which matured after 83 days at Rubona. The same lines also matured early at Rwerere (99 days) except RWV 2575 which matured in 103 days. On the other hand, 10 to 14 of the red mottled lines, matured early at Rubona, or Rwerere or both.

They took less than 85 days at Rubona and less than 100 days at Rwerere station. The lines RWV 2678, RWV 2681 and RWV 2701 were matured earliest, within 82 and 97days at Rubona and Rwerere, respectively(Tables 25 – 28).

4.2.3.2 Number of pods per plant and seeds per pod among advanced lines

There were significant differences in the mean number of pods per plant within and across sites (Annex 5.4 and 5.5). However, the mean pod number per plant was slightly higher at Rwerere than at Rubona. The highest numbers of pods per plant recorded were 33, 26 and 20 at Rubona for RWV 2670, RWV 2692 and RWV 2679 respectively. All the lines are red-mottled. The number of seeds per pod ranged between 4 and 10 at both sites. The mean number of seeds per pod was 6 at both sites. Twenty eight lines had more than 6 seeds per pod at Rubona site. The number of the advanced lines that had more than 6 seeds per pod was 35 at Rwerere station (Tables 25 – 28).

4.2.3.3 Selection for seed colour and size

All the 66 new advanced lines were either red-mottled, red, purple and pink, cream or black seeded. There were 30 red-mottled lines and 13 red seeded. Twenty-three lines had purple, cream or black seeds. Population MMCRW-1 generated the highest number of the red and red-mottled market seed types (70%) among the new lines (Table 23; Annex 5).

Of the 43 marketable red seeded and red-mottled lines, 42 were large seeded (over 40 g per 100 seeds) or medium (25 - 40 g per 100 seeds). They represented about 70% of all the new lines that were large or medium seeded (Tables 24). Population MMCRRW-1 contributed the bulk of the 70% of the large seeded red and red-mottled seed types.

Table 23. Number of advanced lines of different market classes among 11 populations

Population	Reds	Red-mottled	Purples	Cream	Black	Total
MMCRW-1	11	18	4	3	2	39
MMCRW-2	0	0	1	1	0	2
MMCRW-3	0	4	0	0	0	4
MMCRW-4	0	1	0	0	0	1
MMCRW-5	1	0	2	0	3	6
MMCRW-6	0	0	4	0	0	4
MMCRW-7	0	3	0	0	1	4
MMCRW-8	0	0	0	0	0	0
MMCRW-9	0	4	0	0	0	4
MMCRW-10	0	0	2	0	1	2
MMCRW-11	0	0	0	0	0	0
Total	13	30	12	4	7	66

Table 24. Distribution of seed size among different grain colors from the eleven populations

100 seed weight	Red	Red-mottled	Purple	Cream	Black	Total
Large (> 40 g)	8	16	1	0	0	25
Medium (25–40 g)	4	14	9	4	6	37
Small (< 25 g)	1	0	2	0	1	4
Total	13	30	12	4	7	66

4.2.3.4 Yield potential of advanced lines of different seed market types

The differences in yield across the two sites were highly significant at P = 1 % level (Annex 5.1). Overall, the mean yield at Rwerere of 2564 kg ha^{-1} was higher than the mean yield at Rubona (2036 kg ha^{-1}). Grain yield varied from 1000 kg ha^{-1} to 2000 kg ha^{-1} at Rubona, and from 2000 kg ha^{-1} to 3000 kg ha^{-1} at Rwerere. Seventeen entries at Rwerere and 9 at Rubona exceeded a yield of 3000 kg ha^{-1}

Thirty-five (about 53 %) of the new lines had a yield range between 2000 – 4000 kg per ha at Rubona. They exceeded the mean yield of their parents, local and improved checks. The number of new lines that surpassed the mean yield of the parents at Rwerere was 60 (about 91 %). However, only 30 of them (abut 46%) produced higher yields than the local and improved checks (2663 kg ha^{-1}) at Rwerere. Their yield ranged between 3000 kg ha^{-1} to more than 4000 kg ha^{-1}).

4.2.3.5 Yield of marketable red and red-mottled seed types

The mean yield of the new red seeded lines was 2232 k g ha-^{1} at Rubona and 2771 kg ha^{-1} Rwerere. The mean yield of the red-mottled seeded lines was 2416 kg ha-^{1} at Rubona and 2521 kg ha^{-1} at Rwerere sites. These were higher than the mean yield values of the parents, of the checks at both sites and for other market classes selected (Table 25, Fig 9).

4.2.3.6 Mean yield of other market seed types

At the Rubona site, the mean of the purples, creams and the black seeded lines were 1421 kg ha-^{1}, 1385 kg ha-^{1}, and 1771 kg ha-^{1}, respectively. They were lower than the mean yield of the parental lines and of check varieties, except for black seeded lines (Fig 9)

At Rwerere, the purple and black seeded lines (mean 2799 kg ha^{-1} and 2736 kg ha^{-1}) surpassed the mean yield of the parents and of the checks. The mean yield of the cream seeded (2306 kg ha^{-1}) was higher than the parental mean but lower than that of the checks at the same site (Fig.9)

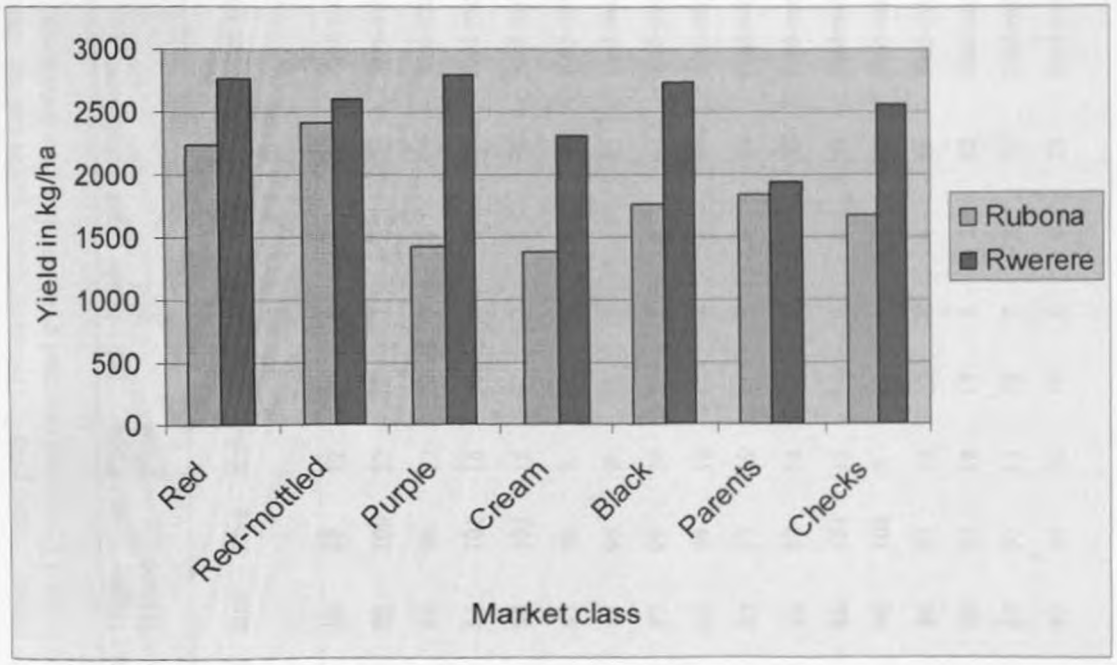


Fig 9. Mean yield of the different market classes among new lines and check varieties n the replicated yield trial at Rubona and Rwerere sites

Table 25. Days to flower, maturity, pods plant-[1], seed pod-[1], 100-seed mass, grain colour, diseases score and grain yield of 30 new red-mottled climbing bean lines selected from 11 populations and evaluated at two locations.

Population	Variety	Days to flower		Days to mature		Pods per plant		Seeds per pod		Seed-type		Disease score			Yield (Kg/HA)		
		Rub*	Rwe	Rub	Rwe	Rub	Rwe	Rub	Rwe	100 seed mass	Seed color	Anth	ALS	Root rot	Rub	Rwe	Mean
MMCRW-9	RWV2639	46	54	89	97	12	10	9	8	36	Red-mottled	1	5	4	2675	1950	2313
MMCRW-4	RWV2670	46	59	90	100	33	19	7	6	33	Red-mottled	1	6	4	3845	2125	2985
MMCRW-1	RWV2673	42	58	85	98	12	22	5	6	49	Red-mottled	1	4	6	1655	2225	1940
MMCRW-9	RWV2675	47	59	84	101	18	10	6	6	34	Red-mottled	1	2	6	1720	1650	1685
MMCRW-9	RWV2676	43	61	89	101	15	9	7	8	36	Red-mottled	1	3	4	3650	2350	3000
MMCRW-9	RWV2677	48	57	95	98	9	14	6	6	44	Red-mottled	1	5	5	1980	2675	2328
MMCRW-1	RWV2678	46	55	82	97	9	15	5	5	45	Red-mottled	1	5	6	2225	2225	2225
MMCRW-1	RWV2679	42	55	87	99	20	12	7	6	43	Red-mottled	1	6	6	1970	2725	2348
MMCRW-1	RWV2680	42	55	83	99	16	14	6	6	44	Red-mottled	1	4	4	2440	3200	2820
MMCRW-1	RWV2681	42	59	83	97	19	11	5	6	44	Red-mottled	1	3	4	3070	2675	2873
MMCRW-1	RWV2682	46	55	84	97	14	13	7	7	46	Red-mottled	1	4	4	3020	1800	2410
MMCRW-1	RWV2683	49	59	86	101	15	14	5	8	34	Red-mottled	1	6	4	2275	2475	2375
MMCRW-1	RWV2685	45	59	90	104	7	13	5	8	26	Red-mottled	1	4	6	1485	2425	1955
MMCRW-1	RWV2686	42	55	86	97	14	13	6	6	45	Red-mottled	1	3	2	3730	2315	3023
MMCRW-1	RWV2687	44	52	80	97	18	17	5	7	42	Red-mottled	1	4	6	1820	2650	2235
MMCRW-5	RWV2691	46	55	87	97	11	10	7	8	35	Red-mottled	1	4	4	2035	2825	2430
MMCRW-1	RWV2692	44	56	92	99	26	16	6	6	28	Red-mottled	1	5	6	3665	2450	3058

MMCRW-1	RWV2693	49	59	82	95	12	14
MMCRW-7	RWV2694	46	55	86	101	17	12
MMCRW-7	RWV2695	46	62	81	102	17	14
MMCRW-1	RWV2697	45	59	92	106	14	10
MMCRW-3	RWV2698	42	54	91	107	11	14
MMCRW-1	RWV2699	41	54	82	101	14	15
MMCRW-1	RWV2701	44	55	83	97	11	11
MMCRW-3	RWV2702	43	53	89	97	14	18
MMCRW-3	RWV2708	46	61	90	107	17	11
MMCRW-1	RWV2763	44	59	89	97	8	12
MMCRW-1	RWV2855	49	55	89	107	13	16
MMCRW-1	RWV2709*	48	59	83	102	12	8
MMCRW-1	RWV2710*	44	57	92	104	13	17
Parent	59/1-2	46	55	86	97	15	8
Parent	G2333	45	55	91	99	8	15
Parent	G685	46	58	91	103	16	10
Checks	Local mixture	46	53	84	99	7	12
Parent	Mex54	46	59	91	99	9	13
Parent	RWR1312	46	56	82	102	12	13
Parent	Scam 80 CM/15	42	55	82	101	12	13
Parent	Urugezi	44	55	85	97	16	15
Check (Rub)	CAB19	46	-	85	-	5	-
Check (Rwe)	G2331	-	61	-	106	-	19

* Rub = Rubona, Rwe = Rwerere, anth = anthracnose and ALS =

5	7	45	Red-mottled	1	4	6	2445	1725	2085
7	7	29	Red-mottled	3	3	2	2220	3100	2660
5	6	29	Red-mottled	2	4	4	3005	3025	3015
6	7	37	Red-mottled	1	4	4	2625	3400	3013
7	6	42	Red-mottled	1	4	4	2565	2275	2420
7	6	46	Red-mottled	1	4	4	2595	2625	2610
5	5	51	Red-mottled	1	4	4	2210	2375	2293
5	5	47	Red-mottled	1	3	4	2030	2150	2090
7	9	29	Red-mottled	1	6	4	2775	3250	3013
5	7	28	Red-mottled	1	5	6	1280	2525	1903
5	7	35	Red-mottled	1	6	4	1410	2875	2143
7	7	35	Red-mottled	1	5	4	1355	2575	1965
8	8	41	Red-mottled	1	6	2	2700	2975	2838
6	7	47	Red	1	5	3	2690	3075	2883
8	9	21	Red	1	6	4	1535	2300	1918
10	9	28	Red	1	6	2	2390	2525	2458
6	5	32	mixture	1	6	6	1005	2550	1778
6	6	36	Pink	1	2	4	925	2325	1625
6	5	46	r/mottle	1	5	4	1655	1050	1353
4	5	37	Red/mottled	1	3	2	1800	1315	1558
5	4	40	Redmottled	1	5	4	1870	950	1410
7	-	27	White	1	8	6	2350	-	-
-	6	-	Yellow	1	4	3	-	2775	-

= angular leafspot

Table 26. Days to flower, maturity, pods plant-[1], seed pod-[1], 100-seed mass, grain colour, diseases score and grain yield of 13 new red climbing bean lines selected from 11 populations and evaluated at two locations.

Population	Variety	Days to flower		Days to mature		Pods per plant		Seeds per pod		Seed-type		Disease score			Yield (Kg/Ha)		
		Rub*	Rwe*	Rub	Rwe	Rub	Rwe	Rub	Rwe	100 s.wt	Seed color	Anth	ALS	Root rot	Rub	Rwe	Mean
MMCRW-1	RWV2572	47	55	87	103	10	11	10	6	46	Red	1	3	4	1735	4175	2955
MMCRW-1	RWV2573	38	55	82	99	18	14	5	6	40	Red	1	4	2	3310	3475	3393
MMCRW-1	RWV2575	42	55	83	103	15	10	5	6	42	Red	1	4	2	2690	2925	2808
MMCRW-1	RWV2576	44	57	82	97	12	16	6	5	50	Red	1	5	4	2190	2775	2483
MMCRW-1	RWV2577	45	55	85	97	13	20	6	7	42	Red	1	6	4	2575	2600	2588
MMCRW-1	RWV2580	46	53	83	97	12	12	5	5	39	Red	1	5	3	3345	1950	2648
MMCRW-1	RWV2581	44	53	85	97	15	15	5	6	52	Red	1	4	3	3095	3000	3048
MMCRW-1	RWV2594	45	52	85	101	13	13	10	6	49	Red	1	4	4	2290	2975	2633
MMCRW-1	RWV2599	45	54	91	99	11	11	6	6	51	Red	1	4	2	2520	2750	2635
MMCRW-1	RWV2600	47	55	87	103	5	14	5	6	29	Red	1	5	7	595	3175	1885
MMCRW-1	RWV2606	47	55	82	99	12	18	6	6	51	Red	1	4	2	2610	2350	2480
MMCRW-1	RWV2613	45	57	89	99	9	12	7	8	22	Red	1	4	6	995	1700	1348
MMCRW-5	RWV2616	47	59	80	99	7	13	5	6	27	Red	1	3	4	1065	2175	1620

Parent	59/1-2	46	55	86	97	15
Parent	G2333	45	55	91	99	8
Parent	G685	46	58	91	103	16
Checks	Local mixture	46	53	84	99	7
Parent	MEX54	46	59	91	99	9
Parent	RWR1312	46	56	82	102	12
Parent	Scam 80 CM/15	42	55	82	101	12
Parent	Urugezi	44	55	85	97	16
Check (Rub)	CAB19	46	-	85	-	5
Check (Rwe)	G2331	-	61	-	106	-

* Rub= Rubona, Rwe= Rwerere, anth=anthracnose and ALS=

8	6	7	47	Red	1	5	3	2690	3075	2883
15	8	9	21	Red	1	6	4	1535	2300	1918
10	10	9	28	Red	1	6	2	2390	2525	2458
12	6	5	32	mixture	1	6	6	1005	2550	1778
13	6	6	36	Pink	1	2	4	925	2325	1625
13	6	5	46	r/mottle	1	5	4	1655	1050	1353
13	4	5	37	Red/mottle	1	3	2	1800	1315	1558
15	5	4	40	r/mottle	1	5	4	1870	950	1410
-	7	-	27	white	1	8	6	2350	-	-
19	-	6	-	yellow	1	4	3	-	2775	-

= angular leafspot

Table 27. Days to flower, maturity, pods plant-^{1}, seed pod-^{1}, 100-seed mass, grain colour, diseases score and grain yield of 12 new pink and purple climbing bean lines selected from 11 populations and evaluated at two locations.

Population	Variety	Days to flower		Days to mature		Pods per plant		Seeds per pod		Seed-type		Disease score			Yield (Kg/Ha)		
		Rub*	Rwe*	Rub	Rwe	Rub	Rwe	Rub	Rwe	100-Seed mass	Seed color	Anth	ALS	Root rot	Rub	Rwe	Mean
MMCRW-1	RWV2713	46	59	90	97	14	11	6	7	43	Pink	1	3	4	1870	2190	2030
MMCRW-6	RWV2715	46	55	80	103	11	15	5	8	29	Pink	1	2	6	910	2550	1730
MMCRW-6	RWV2716	44	52	83	98	7	15	7	9	30	Pink	1	4	6	1200	3475	2338
MMCRW-6	RWV2734	46	57	82	98	11	14	7	7	32	Pink	1	4	5	1560	3125	2343
MMCRW-1	RWV2844	47	57	96	104	9	14	4	8	33	purple	1	4	2	1005	2375	1690
MMCRW-6	RWV2776	42	55	86	103	14	15	7	5	31	Pink	1	5	4	1960	3025	2493
MMCRW-1	RWV2632	49	55	87	99	11	24	7	7	36	Pink	1	4	4	1430	2600	2015
MMCRW-1	RWV2642	47	58	91	101	8	13	5	9	30	purple	1	4	6	1345	2275	1810
MMCRW-10	RWV2654	46	55	85	101	14	13	8	9	30	Pink	1	4	4	1955	3725	2840
MMCRW-10	RWV2655	42	63	85	101	16	14	7	8	28	Pink	1	3	6	1525	3250	2388
MMCRW-5	RWV2664	48	64	81	106	9	16	5	8	29	Pink	1	3	6	1325	2525	1925
MMCRW-5	RWV2788	41	55	81	104	6	12	6	7	24	purple	1	6	4	975	2275	1625
Parent	59/1-2	46	55	86	97	15	8	6	7	47	Red	1	5	3	2690	3075	2883

Parent	G2333	45	55	91	99	8
Parent	G685	46	58	91	103	16
Checks	Local mixture	46	53	84	99	7
Parent	Mex54	46	59	91	99	9
Parent	RWR1312	46	56	82	102	12
Parent	Scam 80CM/15	42	55	82	101	12
Parent	Urugezi	44	55	85	97	16
Check (Rub)	CAB19	46	-	85	-	5
Check (Rwe)	G2331	-	61	-	106	-

* Rub= Rubona, Rwe= Rwerere, anth=anthracnose and ALS=

15	8	9	21	Red	1	6	4	1535	2300	1918
10	10	9	28	Red	1	6	2	2390	2525	2458
12	6	5	32	mixture	1	6	6	1005	2550	1778
13	6	6	36	Pink	1	2	4	925	2325	1625
13	6	5	46	Red/mottle	1	5	4	1655	1050	1353
13	4	5	37	Red/mottle	1	3	2	1800	1315	1558
15	5	4	40	r/mottle	1	5	4	1870	950	1410
-	7	-	27	white	1	8	6	2350	-	-
19	-	6	-	yellow	1	4	3	-	2775	-

= angular leafspot

Table 28 Days to flower, maturity, pods plant-^{1}, seed pod-^{1}, 100-seed mass, grain colour, diseases score and grain yield of 11 new cream or black climbing bean lines selected from 11 populations and evaluated at two locations.

Population	Variety	Days to flower		Days to mature		Pods per plant		Seeds per pod		Seed-type		Disease score			Yield (kg ha-^{1})		
		Rub*	Rwe*	Rub	Rwe	Rub	Rwe	Rub	Rwe	100-Seed mass (g)	Seed color	Anth*	ALS*	Root rot	Rub	Rwe	Mean
MMCRW-1	RWV2813	47	55	87	106	15	12	6	7	39	Cream	1	5	4	1300	2500	1900
MMCRW-1	RWV2814	46	57	87	102	7	10	7	6	36	Cream	1	4	4	1615	2500	2058
MMCRW-1	RWV2828	48	57	83	99	9	13	4	8	26	Cream	1	3	6	1035	2075	1555
MMCRW-2	RWV2837	47	61	89	104	11	17	7	7	26	Cream	1	7	4	1595	2150	1873
MMCRW-5	RWV2840	47	55	82	104	11	14	5	8	26	Black	1	6	6	2375	3750	3063
MMCRW-10	RWV2777	46	55	89	106	16	13	6	6	35	Black	1	4	4	1400	1575	1488
MMCRW-7	RWV2851	46	57	82	100	11	14	7	7	28	Black	1	3	4	1695	2975	2335
MMCRW-5	RWV2852	42	55	90	105	8	14	6	8	34	Black	1	3	4	1490	2950	2220
MMCRW-1	RWV2772	43	55	86	104	10	11	6	6	24	Black	1	6	4	1400	2325	1863
MMCRW-5	RWV2856	46	60	92	103	11	15	6	7	32	Black	1	4	3	2405	3275	2840
MMCRW-1	RWV2864	43	59	84	101	6	17	7	7	34	Black	1	6	6	1635	2300	1968
Parent	59/1-2	46	55	86	97	15	8	6	7	47	Red	1	5	3	2690	3075	2883

Parent	G2333	45	55	91	99	8
Parent	G685	46	58	91	103	16
Checks	Local mixture	46	53	84	99	7
Parent	Mex 54	46	59	91	99	9
Parent	RWR1312	46	56	82	102	12
Parent	Scam 80CM/15	42	55	82	101	12
Parent	Urugezi	44	55	85	97	16
Check (Rub)	CAB19	46	-	85	-	5
Check (Rwe)	G2331	-	61	-	106	-

* Rub= Rubona, Rwe= Rwerere, anth=anthracnose and ALS=

15	8	9	21	Red	1	6	4	1535	2300	1918
10	10	9	28	Red	1	6	2	2390	2525	2458
12	6	5	32	mixture	1	6	6	1005	2550	1778
13	6	6	36	Pink	1	2	4	925	2325	1625
13	6	5	46	r/mottle	1	5	4	1655	1050	1353
13	4	5	37	red/mottle	1	3	2	1800	1315	1558
15	5	4	40	r/mottle	1	5	4	1870	950	1410
-	7	-	27	white	1	8	6	2350	-	-
19	-	6	-	yellow	1	4	3	-	2775	-

= angular leafspot

Plates 12. Replicated yield trials of 66 new lines, 7 parents and 2 checks at ISAR Rwerere research station with (below) a late and early maturity lines among them.

Plate 13. A late maturing line at Rwerere

Plate 14. Early maturing line at Rwerere

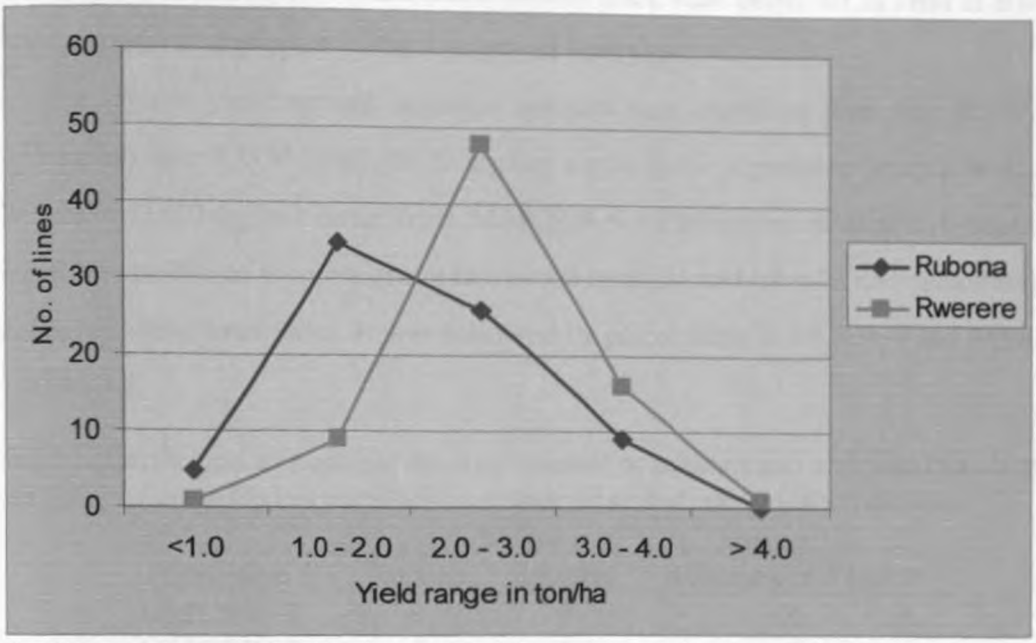


Fig. 10. Frequency distribution of yield among the lines and check varieties grown at Rubona and Rwerere.

4.2.3.7 Marketable seed types, multiple disease resistance and high yielding lines

There were 13 and 11 red or red mottled advanced lines that exceeded the yield of improved checks CAB 19 at Rubona (2500 kg ha $^{-1}$) and G2331 at Rwerere (2700 kg ha $^{-1}$) at Rubona and Rwerere sites. Eight of these exceeded the yield of the checks at both sites (Tables 29). The same marketable and high yielding lines had multiple resistance against the angular leaf spot, root rots and anthracnose with disease severity rating of 1 – 4 on the CIAT scale of 1 – 9.

Table 29. Distribution of high yielding climbing bean lines with multiple disease resistance at Rubona and Rwerere.

Market class	Number of lines		
	Rubona	Rwerere	Rubona and Rwerere
Red	5	6	4
Red-mottled	8	5	4
Purples	0	1	0
Cream	0	0	0
Black	1	3	1
Total	14	15	9

The multiple resistant and tolerant black seeded lines were better off in yield at Rwerere, while the cream and purples yielded poorly at both sites.

The highest yielding and multiple resistant new climbing bean line, RWV 2572 (4175 kg/ha) and RWV 2682 (3730 kg/ha) came from population MMCRW-1, while RWV 2676 (3650 kg/ha) came from MMCRW-9. Population MMCRW-1 contributed the highest number of the new multiple disease resistant and tolerant and marketable high yielding climbing bean lines. It was followed by populations MMCRW-7 and MMCRW-5 (Tables 30).

Table 30 Distribution of multiple disease resistant or tolerant and high yielding climbing bean lines among different populations evaluated at Rubona and Rwerere sites

Population	Rubona	Rwerere	Rubona and Rwerere
MMCRW-1	9	9	6
MMCRW-2	1	0	0
MMCRW -3	0	0	0
MMCRW -4	0	0	0
MMCRW -5	1	2	1
MMCRW -6	0	0	0
MMCRW -7	2	3	2
MMCRW -8	0	0	0
MMCRW -9	1	0	0
MMCRW -10	0	1	0
MMCRW-11	0	0	0
Total	14	15	9

Tables 31, 32 and 33 below give the details of the names, yield (% over checks) and diseases reaction of the obtained red, red mottled, purple, black and cream lines among the eleven populations within and across the two sites of Rwerere and Rubona.

Table 31. Yield advantage of new marketable climbing bean lines with multiple disease resistance over commercial checks at Rubona

Seed colour	Population	Variety	Disease severity			Yield increase over check (%)
			Anth.	ALS	Root rot	
Red	MMCRW-1	RWV 2573	1	4	2	141
		RWV 2575	1	4	2	126
		RWV 2581	1	4	3	131
		RWV 2599	1	4	2	107
		RWV 2606	1	4	4	111
Red-mottles	MMCRW-9	RWV 2676	1	4	3	155
	MMCRW-1	RWV 2680	1	3	4	103
		RWV 2681	1	4	4	130
		RWV 2682	1	3	4	158
		RWV 2697	1	4	4	109
	MMCRW-2	RWV 2698	1	4	4	110
	MMCRW-7	RWV 2694	1	4	2	129
		RWV 2695	1	4	4	112
Black	MMCRW-5	RWV 2856	1	3	4	102

Table 32. Yield advantage of new marketable climbing bean lines with multiple disease resistance over commercial checks at Rwerere.

Seed colour	Population	Variety	Disease severity			Yield advantage (%)
			Anth.	ALS	Root rot	
Red	MMCRW-1	RWV 2572	1	3	4	151
		RWV 2573	1	4	2	125
		RWV 2575	1	4	2	105
		RWV 2581	1	4	3	108
		RWV 2594	1	4	4	107
		RWV 2599	1	4	2	100
Red-mottles	MMCRW-1	RWV 2680	1	3	4	115
		RWV 2691	1	3	4	101
		RWV 2697	1	4	4	122
	MMCRW-7	RWV 2694	1	4	2	111
		RWV 2695	1	4	4	109
Purples	MMCRW-10	RWV 2654	1	4	4	134
Black	MMCRW-7	RWV 2851	1	3	4	107
	MMCRW-5	RWV 2852	1	3	4	106
	MMCRW-5	RWV 2856	1	3	4	118

Table 33. Yield advantage of new marketable climbing bean lines with multiple disease resistance over commercial checks at Rwerere and Rubona.

Seed colour	Population	Variety	Disease severity			Yield advantage over check (%)	
			Anth	ALS	Root rot	Rubona	Rwerere
Red	MMCRW-1	RWV 2573	1	4	2	141	125
		RWV 2575	1	4	2	126	105
		RWV 2581	1	4	3	131	108
		RWV 2599	1	4	2	107	100
Red-mottles	MMCRW-1	RWV 2680	1	3	4	103	115
		RWV 2697	1	4	4	109	122
	MMCRW-7	RWV 2694	1	4	2	129	111
		RWV 2695	1	4	4	112	109
Black	MMCRW-5	RWV 2856	1	3	4	102	118

4.2.4 Selection of resistance against angular leaf spot and *Fusarium* wilt

After 14 days of inoculation, necrotic inter-vein spots of different magnitude were observed on the leaf lamina of the plant progenies. In susceptible plants, defoliation of inoculated leaves occurred. Inoculated leaves of resistant plants had smaller surface area tarnished or of damaged tissue. Plate 11 shows the reaction of different inoculated plant leaves, and how they were used to rate the reaction. Annex 3 shows the number of resistant lines that combined resistance for both angular leaf spot and *Fusarium* wilt. They were 185 altogether. Annex 4 shows the reaction of the susceptible and resistant parents to the diseases at the Kabete and Runyinya trial sites. MEX 54 was resistant to both angular leaf spot and *Fusarium* wilt, while Umubano (G2333) expressed susceptible reaction against *Fusarium* wilt and intermediate reaction to angular leaf spot. Vuninkingi (G685) was resistant to *Fusarium* wilt and intermediate to angular leaf spot. The following plates show the ratings of the angular leaf reaction in the screenhouse evaluation at Kabete.

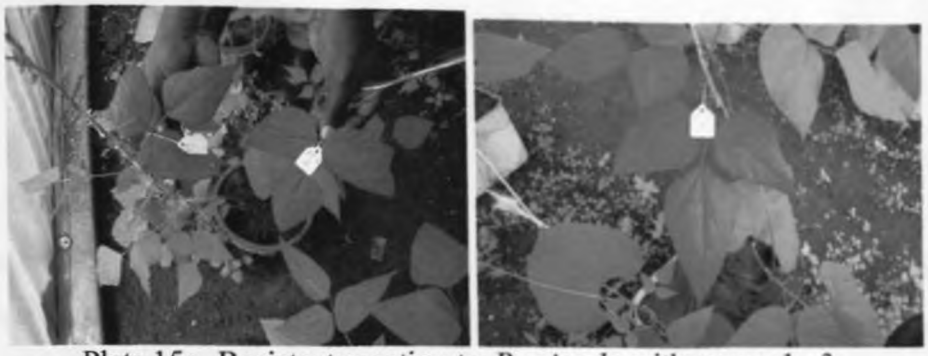


Plate 15a. Resistant reaction to *P.griseola* with scores 1 - 3

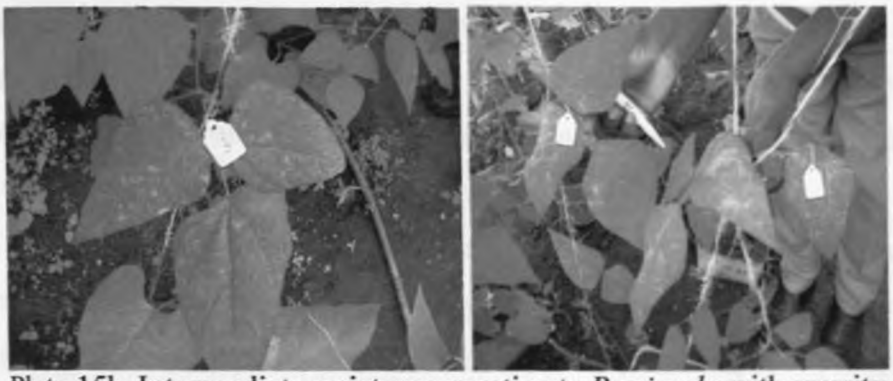


Plate 15b. Intermediate resistance reaction to *P.griseola* with severity scores of 4 - 6

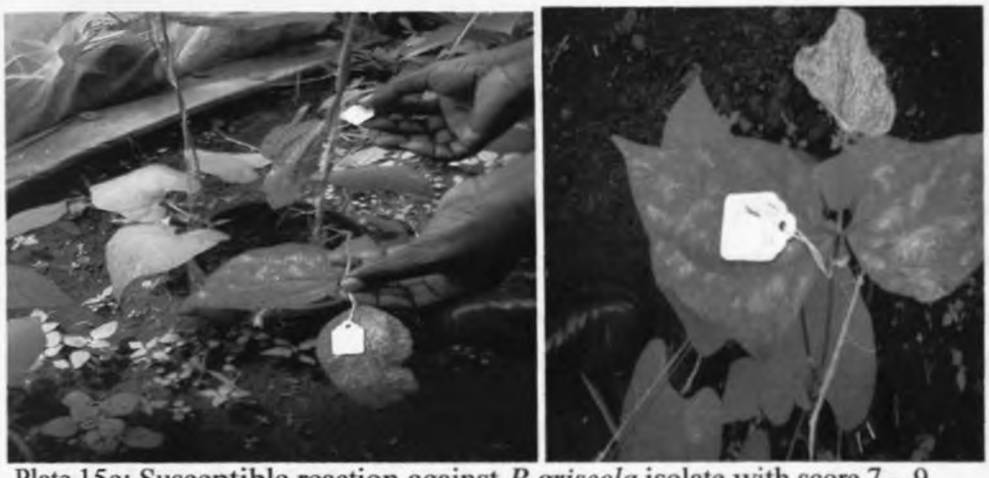


Plate 15c: Susceptible reaction against *P.griseola* isolate with score 7 – 9

Plate 15. Samples of plants showing severity reaction against angular leaf spot (P.griseola) isolate 31:33 and its rating after 14 days of inoculation in the glasshouse at Kabete field station.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Inheritance of resistance against *Fusarium* wilt

The investigation into the brown discoloration of the root system, the hypocotyl and the xylem and the re-isolation corroborated the externally visible yellowing, wilting and/or death symptoms and facilitated better assessment of disease development in test plants. This was important in view of the fact that, the yellowing symptoms were confined to the lower foliage of the plant, except in cases where the plants were wilted or dead completely. It also ruled out the possibility that the yellowing or death symptoms were due to low N or other nutritional disorders.

The vascular and external damage symptoms produced by the pathogen Fusarium *oxsporum f. sp. phaseoli* was more apparent in the susceptible than the resistant plants. There was a close correlation between the external yellowing and wilting symptoms and the internal movement and destruction of the vascular tissues of the sampled plants. The results suggested that external symptoms can be relied upon to assess the severity of *Fusarium oxsporum f. sp. phaseoli* without necessarily resorting to the destructive sampling when the soil nutritional factors are ideal.

The higher rate of penetration of *F. oxsporum f. sp. phaseoli* into the vascular tissue of the susceptible plants (G2333) compared to the samples of resistant ones (such as G685) suggested presence or formation of some obstacles (physical or chemical) on the surface of the plants or inside the vascular tissues that hinder or slow down multiplication of .*F. oxsporum f. sp. phaseoli* spores and their movement inside the resistant plants during and after the infection.

When Ribeiro and Hagedorn (1979) cut stem transverse sections at intervals of 3 cm, 3-6 cm and 6-9 cm after 30 days of inoculation and incubated them on plated media, they were able to re-isolate *F. oxsporum f. sp. phaseoli* from 50%, 30% and 0% infections of the inoculated and resistant plants respectively. The proportion of infected

plants was much higher at 100%, 95% and 85% along the same section intervals in case of the susceptible parents.

Results of this study are consistent with those of Buruchara and Camaco (1999). While the upward colonisation of the vascular tissue by *.F. oxsporum f. sp. phaseoli* was 0% in case of the resistant cultivar, G685, the pathogen was recovered in all (100%) of the sampled susceptible (G2333) plants at a 30 cm interval after 55 days of inoculation.

These results led to the plausible assumption that the resistance against the pathogen was expressed by a restriction of the growth and / or systemic distribution of the pathogen into the vascular tissue of inoculated plants. It was suggested that the formation of mechanical or chemical barriers (oclusions and fungistatic substances) inside the xylem from the initial point of the penetration of the pathogen as the possible reasons for the slow spread of the pathogen in susceptible genotypes.

5.1.2 Generation means and variance of the F_1, F_2, and backcross populations

The observed generation means of disease severity confirmed that G2333 (score 8.7) was very susceptible to infection by *F. oxysporum f.sp. phaseoli.* In contrast, G685 and Flora showed resistant reactions (score less than 3.0) both in the field trial and under artificial inoculation with the *F. oxsporum f. sp. phaseoli* isolate. These results closely agree with the severity rating that was observed by Buruchara and Camacho (1999), with a severity rate of 9 for G2333, 1.3 and 2.6 for G685 and Flora, respectively.

The mean disease severity rate of the F_1 progenies in the screenhouse trials was 2.2 and 2.8 for the G2333 x G685 and G2333 x Flora crosses, showing that the F_1 inherited the *Fusarium* wilt resistance trait of the donor parents. However, in the case of the field trials, the mean disease ratings of the resistant parent and of the segregating progenies were slightly higher than the corresponding values in the screenhouse trial. It is possible that the stunting, yellowing or wilting symptoms were not due to *Fusarium* wilt infestation alone as the other soil-borne root rot pathogens such as *Pythium species* and *F. solani* that produce similar symptoms may have enhanced severity under field conditions.

Therefore, while the severity rating of G685 and the F_1 progeny were 1.6 and 2.2 under artificial inoculation, their disease scores were higher at 3 and 3.7 under field conditions respectively. Similarly, the disease severity rating of the F_2 (G2333 x G685) was higher in the field at 4.4 compared to 3.9 under screenhouse conditions.

5.1.3 Heritability of resistance to *Fusarium* wilt trait

The observed high heritability value of nearly 100% is a further proof that the resistance against *Fusarium* wilt is inherited qualitatively rather than quantitatively. Its expression is least affected by the surroundings, as shown by very low values of the environmental variance, V_E of nearly 0 and high genetic variances, V_G

5.1.4 Major dominant gene inference

The results of this study suggest that the inheritance of resistance against *Fusarium* wilt disease is determined by a major dominant gene. This is so because, the X^2 probability values indicate that there were no significant differences between the observed and the expected segregation ratios of resistant: susceptible plants. There was agreement of F_2 generation progenies of (G2333 x G685) and of (G2333 x Flora) segregation in the expected ratio of 3 resistant: 1 susceptible. On the other hand, the segregation pattern among the F_1 backcross progenies of the same (G2333 x G685) x G2333 and (G2333 x Flora) x G2333 was 1 resistant: 1 susceptible. Similarly, the segregation of the backcross F_1 (G2333 x G685) x G685 and of F_1 (G2333 x Flora) x Flora progenies were in agreement with the expected Mendelian ratio of 1 resistant: 1 susceptible.

However, the agreement with the expected Mendelian segregation ratio was less evident in the case of the field evaluation trial of the F_2 progenies (3 resistant: 1 susceptible); backcross to susceptible parent progenies (1 resistant: 1susceptible) and to the resistant parent (1resistant: 0 susceptible) of the original G2333 x G685. This could be attributed to the more variable field testing conditions such as inoculum load in the soil. All the F_2 progenies of the Flora x G685 (resistant donor parents) were found to be highly resistant to the *Fusarium* wilt attack with a mean severity score of 2.0. It

compared closely with the resistant reaction that was recorded for either of the parents. The result suggested presence of a single allelic pair of resistance/recessive genes where resistance gene is completely dominant to its recessive allele.

Ribeiro and Hagedorn (1979) observed similar segregation pattern of 3 resistant: 1 susceptible among the F_2 generation progenies and of 1 resistant:1 susceptible among the BC_1 of susceptible and 1 resistant: 0 susceptible in the $BC_1$to the resistant parent. They had inoculated the progenies raised from crosses of the resistant parents Tenderette, Pintado and Early Gallatin with the susceptible bush cultivar, Blue Lake 274 (BBL 274) with a Brazilian pathogenic isolate of *F. oxsporum f. sp. phaseoli.* They designated a single completely dominant gene, *Fop 1* and its recessive allele *Fop*$^+$ *1* as being the alleles that confer the resistance or susceptibility to *F. oxsporum f. sp. phaseoli* attack in *Phaseolus* beans.

They, however, observed a modified monohybrid segregation pattern of 1 resistant:2 intermediate:1 susceptible among the F_2 progenies of the susceptible x resistant parents when the inoculation was repeated by using the North American race of the same pathogen. This suggested the presence of another resistant gene; they coded as *Fop 2,* that was incompletely dominant to its recessive allele, *Fop*$^+$ *2.*

Considering the close similarity of the levels of disease incitement in the same cultivars (G2333, G685 and Flora) caused by the current Rwanda isolate (designated as *FOP-RW2*) and *FOP-RW1* that was used by Buruchara and Camacho (1999), it is possible that the two isolates are physiologically the same. It is also possible the Rwanda isolates (*FOP-RW1* and *FOP-RW2)* also belong to the same physiological race group as the Southern American isolate studied by Ribeiro and Hagedorn (1979). The similarity is supported further by the fact that the 3 cultivars: G2333, G685 and Flora that were used under the current study were introduced from Southern America. This points to the likely possibility of co-evolution between the South American *F. oxsporum f. sp. phaseoli* pathogen and the genotypes.

Thus, subject to allelic tests using both sets of parental materials from the current and Rebeiro et al's trial, the results of this study suggest that the same completely

dominant gene, *Fop 1* and its recessive allele *Fop*$^{+}$ *1* are involved in the determination of the resistance and susceptibility reactions to the Rwandan isolate, *FOP-RW2* (and *FOP-RW1*), of *Fusarium oxsporum f. sp. phaseoli* in the climbing beans

5.1.5 Inheritance of agronomic traits

5.1.5.1 Seed and flower colours

As expected, the G2333 x G685 F_1 plants and those in subsequent generations inherited the white flower colour of both parents. It is difficult to make any plausible inference about the inheritance of the flower colour since both parents had the same white phenotype. On the other hand, the colour was more likely to be qualitative trait, controlled by major genes, as was observed in the pioneer Mendelian work. They were not affected by the environmental changes over two generations, like other qualitative traits.

The intense red colour and small size of G2333 female parent was conspicuously dominant in all the F_1 hybrid generation, regardless of the other parent, Flora or G685. (it was impossible to distinguish between the parent and the F_1 until after planting). This suggested that red seed colour is dominant over pink colour. However, maternal effect cannot be ruled out (xenia effect). However, there was no consistent observable pattern of segregation of seed colour among the F_2 seed and of the backcrosses of G2333 x G685 though there was more tendency to the pale red or pink colours of G685 and Flora parents, with only a negligible proportion of the F_2 seed having the red and shiny colour of G2333. In the absence of a clear Mendelian segregation pattern, and as observed by others, the inheritance of seed colour is of the quantitative nature and may be controlled by allelic and non allelic interactions between oligogenes and polygenes and involving some modifier gene effects.

Although the F_1 seed size, as estimated from the 100 seed mass was intermediate of their parents, it was surprising that the F_2 seeds of the same G2333 x G685 cross were larger than that of their parents. This suggested transgressive gene interactions responsible for the observed segregation for seed size among the progenies.

5.1.5.2 Duration to flowering and maturity duration

The intermediate flowering and maturity durations of the F_1 hybrids between the late and the early maturing parents agree with literature by various authors that most traits of agronomic interests such as maturity and flowering durations polygenically inherited, controlled by additive gene interactions.

The cultivar G685 was the latest, while G2333 was the earliest to mature. The duration to maturity for the two cultivars were less than 90 and 99 days respectively (Nyabyenda 1991), although Runyinya site lies within the same altitude with Rubona. This is most likely attributed to the late planting time that coincided with warmer and drier weather; and suggesting further the maturity trait is polygenic since it is influenced by the environment.

5.2 Selection of high yielding multiple disease resistance and marketable bean lines

5.2.1 Resistance to diseases at early generations

The large number of 475 lines is selected for multiple resistance in early generations is attributable to the fact that variation for the resistance and tolerance to the diseases existed among all the populations. The breeding populations MMCRW-1 and MMCRW-4 contributed 75% of the selected resistant lines, meaning greater variability for the diseases resistance existed in the two populations. It confirms the fact that the parents that were used in the crosses were variable with respect to resistance to the diseases under the study. They were also variable for the main sources of the more desirable red and red-mottled seed types, since the seed colours were obtained among respective progenies.

The high number of selected lines selected at Rwerere (65% of the selected 475) at the F_4 generation is related with the unusually low anthracnose selection pressure that was consistently observed at this site.

On the other hand, the comparatively smaller number of selected plant families, especially at the F_3 and F_4 generations, were due to the high pressure of root rots and angular leaf spot at Gikongoro, Ntendezi (Cyangugu), and Rubona sites.

Although the severity rating at each stage of selection of plant progenies at successive generations was stringent (based on severity rating score was 1-3), cases of lower tolerance and higher susceptibility kept emerging at the next cycle of selection.

As an example, among the advanced materials, the severity rating scores of root rot and angular leaf spot ranged from 3 – 6 with a mode of 4, compared to the severity ratings of 2 or 3 among the resistant check parents. Segregation for the reaction to the different diseases was continued even at the advanced stages suggesting minor genes may have contributed to resistance of the selected lines. This is expected in view of the multiple alleles that were involved, especially among the populations that were derived from the multiple parent crosses.

However, the marked reduction in the numbers of selected advanced lines in many populations was also influenced by difficulties associated with the necessity for combining other agronomic and market attributes such as plant vigor, acceptable maturity, yields, and grain size and color besides high levels of resistance (score of 1 – 3) in a single genotypes. This partly explains why population MMCRW-1, that proved good in many of these attributes, was the most successful progenitor in terms of numbers of selected new climbing bean lines.

5.2.2 Selection for marketable grain types

While the red seeded plants came from nearly all the populations (irrespective of the parental sources), the red-mottled seeded plants originated from populations in which at least one of the parents was red-mottled.

In this respect, the cultivar SCAM 80 CM / 15 was the best donor of red-mottled seed coat color (populations MMCRW-1, MMCRW-3 and MMCRW-4) in comparison with the other adapted and donor varieties, Urugezi and RWR 1312 I which contributed the red mottled grain type in populations MMCRW-4, MMCRW-7, MMCRW-8 and MMCRW-9. In advanced selections, the SCAM 80 CM / 15 crosses maintained larger contributions of the red-mottled seed coat color (77%).

The population MMCRW-4 (Umubano x SCAM 80 CM / 15 x RWR 1312 I) was less effective because their red-mottled seeded progenies tended towards the determinate growth habit of the two donor type II parents, that were consistently selected against. Overall, the population MMCRW-1 in which SCAM 80 CM / 15 the donor was the most successful contributor of the two desirable market seed traits of red and red-mottled.

The other shades of colors, notably pink, purple and cream or tans among the young and advanced progenies were to be expected, considering that all parents were either red or tan or various shades of these colors. However, it what was surprising is the appearance of the black-seeded lines from the populations MMCRW-1, MMCRW-5, MMCRW-7 and MMCRW-10 when none of the parental lines was black-seeded, but was probably due to complementary gene action.

This could be due to hereditary factors from ancestors since the parents are either landraces or introduced lines whose pedigree is not documented. Epistasis and modifier gene effects could be in action as well. Contrary to the objectives of the study, some lines with less preferred grain types, such as black, were retained for their exceptional agronomic attributes, particularly disease tolerance and / or yield potential.

5.2.3 Yield and market potentials and diseases resistance of new lines

The higher yields of early and advanced populations that was observed at Rwerere agrees with earlier observations, that climbing beans are more adapted to the higher and cooler altitudes zones (Nyabyenda, 1991; Musoni et. al. 2000). However, there were new climbing bean lines that performed better at the lower altitude at Rubona others that were more plastic and performing equally well at both sites, because of specific inherent adaptive factors. In the past, ISAR released climbing bean varieties with potential yield ranging between 3.5 and 5.0 ton / ha (Nyabyenda, 1991; Musoni et. al. 2000). Several new climbing bean lines fall within this acceptable range. However, several new lines had higher yields than that of previously released varieties that were used as checks (CAB 19 and G23331) at Rubona and Rwerere. However, few of the new developed climbing bean exceeded the yields of 3.5 ton/ha as expected of climbing beans.

The observed lower yield results were not strange, considering that the evaluations were done without external inputs (Musoni *et al,* 2000) such as fertilizer or sprays. The new climbing bean lines that had higher yields than the improved checks and the commercial lines are likely to replace some old cultivars in future. This is particularly true in case of the new multiple disease resistant and more marketable red-mottled and red seeded climbing bean lines, which surpassed the yield of the parents and the checks by between 114% and 133%.

5.2.5 Maturity duration of new climbing bean lines

As observed by Nyabyenda (1991), and by Musoni *et al* (2000), the mean yields, flowering and maturity durations of climbing bean varieties increased with altitude. The same trends have been observed in the current study, where by flowering, maturity and mean yields were higher at Rwerere (2300 m) than at Rubona (1650 m) sites.

Although there were some exceptions among the new lines, the maturity durations of both the populations and the new advanced climbing bean lines were within the same range of 90 to 120 days, and were consistent with previous maturity data of the climbing bean varieties at Rubona and Rwerere research stations.

However, the study succeeded to identify 14 new marketable red-mottled climbing bean lines that have shorter maturity duration of 85 days at Rubona and less than 100 days at Rwerere or both sites. The new multiple disease resistant red-mottled lines: RWV 2678, RWV 2681 and RWV 2701 that matured earliest, within 82 and 97days at Rubona and Rwerere, respectively were in particular identified by the study.

5.2.6 Multiple resistances against angular leaf spot and *Fusarium* wilt

The study succeeded in obtaining a large number of climbing bean lines that are recombinant for angular leaf spot and *Fusarium* wilt resistance among the 5 study populations. It confirmed the resistance of Mex 54 to angular leaf spot (Namayanja, 2002). It also proved to be resistant against *Fusarium* wilt, like the better known resistant source, Vuninkingi (Buruchara and Camacho, 1999).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

The inheritance study found out that the resistance against *Fusarium oxysporum f.sp. phaseoli*, the causal agent of *Fusarium* wilt (yellows), in climbing beans is a qualitative trait. It is conditioned by a major dominant gene. This was so because of the agreement between the observed and expected Mendelian segregation ratios of 3 resistant: 1 susceptible among all the F_2 progenies; and of 1 resistant: 1 susceptible among the backcross F_1 to the susceptible parent. The improvement of resistance against the disease can therefore be achieved through backcrossing method of selection.

The uniform non-segregation resistant reaction among the F_2 of the resistant source parents confirms the resistance against *Fusarium* wilt is conditioned by one gene. The calculated broad sense heritability of 99.6% infers that the resistance trait is highly heritable and it confirms further the qualitative nature of the resistance trait. The dominance gene action was more prominent than additive as the F_1 progenies reacted closest to the donor parents other than the susceptible parent in terms of disease severity scores.

The inheritance of seed size among progenies was found to be transgressive above the mean size of the parents, suggesting over-dominance due to complementary additive allelic gene interaction. Flowering and maturity durations were intermediate between the parents, implying the traits are controlled by additive polygenic interactions.

Because of the observed slow entry, movement and spread of Fusarium wilt pathogen inside the vascular tissues (phloem and xylem) of resistant plants; the mechanism of resistance against the disease is related with the presence of certain natural or induced restrictive physical or chemical barriers to the pathogen outside or inside the resistant plants. Tolerance is achieved through formation of compensatory adventitious roots in susceptible plants, as they were observed on hypocotyls of damaged tap roots of some susceptible plants, but none on the resistant ones.

In the second study, it was found out that multiple parent crosses were useful in creating climbing bean populations that were highly variable for recombination for multiple disease resistance: angular leaf spot, anthracnose, root rots (*Pythium* and *Fusarium* wilt) seed colour and seed sizes, since a number of lines with various recombinations to the same traits were selectable. Of the selected new advanced climbing bean lines, twenty nine were multiple disease resistant and high yielding, with yield potentials ranging from 101 to 141% of the yields of improved checks. They were also potentially adaptable to high and / or mid altitude zones in Rwanda. Twenty-four of the climbing bean lines combined the multiple resistance and high yield with the desired more marketable large red or red mottled grain types.

The varieties: RWV 2572 , RWV 2682 , and RWV 2676 were selected as the most outstanding for high yield, multiple disease resistance and marketable seed traits, while the new climbing bean lines RWV 6878, RWV 2681 and RWV 2701 were selected for early maturity besides multiple disease resistance, high yield potential and marketable grain types.

One of the bush parents, SCAM 80 CM/15, was the most successful donor of the most preferred red mottled grain type. The cultivar Ngwinurare contributed the greatest number of the large red seed types. This was because the populations that had one or both source parent(s) like MMCRW-1, generated the greatest number of the red or red mottled lines. The cultivars MEX 54, Vuninkingi and Flora were good sources of resistance against angular leaf spot and *Fusarium* wilt as shown by selected lines combining the resistance to the two diseases after the artificial inoculations with isolates of the diseases and in the field screening trials. These cultivars should be routinely used as sources of choice for the marketable red-mottled and large red seed coat colours and of resistance against the diseases in breeding programs.

The indeterminate climbing growth habit was completely dominant to the determinate bush type as no progenies of the bush types were observed in all populations even though bush beans were used as source parents in the nine of the eleven populations that were studied. Therefore, bush beans may be used as donors of desirable traits to

commercial climbing bean types without any fear of losing the yield and other agronomic advantages of the climbers due to these dominancy effects.

From both studies, it is recommended that:

i. Improvement of susceptible commercial cultivars such as Umubano for resistance against *Fusarium* wilt may be initiated and achieved by backcross breeding and selection strategy.

ii. Further work could be done to establish and validate molecular markers for the gene resistance against *Fusarium* wilt in beans.

iii. Further work could be done to establish fully the nature of the mechanism of resistance in climbing beans.

iv. All the 29 new multiple diseases resistant and marketable climbing bean lines are evaluated in many agro-ecological zones under multilocation and farmer participatory evaluation for wider adaptability and acceptability and eventual release to farmers.

v. Marker assisted selection should be used simultaneously to confirm the incorporated resistances against the four diseases.

vi. In particular, the lines should be screened for the presence of resistant markers of the genes Co-*4* and *Co-5* (present in one of the parents, G2333) that confer broad resistance against anthracnose (*C. lindemuthiunum)*; and the markers for the resistant genes against angular leaf spot *(P.griseola)* that exist in another parent, MEX 54 that used in the crosses.

7.0 REFERENCES

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8.0 ANNEXES

ANNEX 1. Number of resistant or susceptible plants or progenies after artificial inoculation with F. oxysporum f.sp.phaseoli isolate in a screenhouse at Kabete.

	REP I		REP II		Total no. of plants		
Population	Resistant	Suscept	Resistant	Suscept	Resistant	Suscept	Total
G2333 (P_1)	0	16	0	16	0	32	32
G685 (P_2)	20	0	18	0	38	0	38
F_1(P_1 x P_2)	19	0	20	0	39	0	39
F_2(P_1 x P_2)	96	30	98	32	194	62	256
BCP_1	47	43	42	48	89	91	180
BCP_2	87	1	90	0	177	1	178
G685xFlora	20	0	20	0	40	0	40
G2333 (P_1)	0	16	0	16	0	32	32
Flora (P_2)	20	0	20	0	40	0	40
F1(P_1 x P_2)	20	0	20	0	40	0	40
F2(P_1 x P_2)	100	30	93	37	193	67	260
BCP_1	52	38	34	56	86	94	180
	86	4	89	1	175	5	180
FloraxG 685	20	0	20	0	40	0	40
	587	178	564	206	1151	384	1535
TOTAL	770		765		1535		

ANNEX 2. Crosses designed for artificial screening of resistance to P. griseola and F. oxysporum f.sp.phaseoli

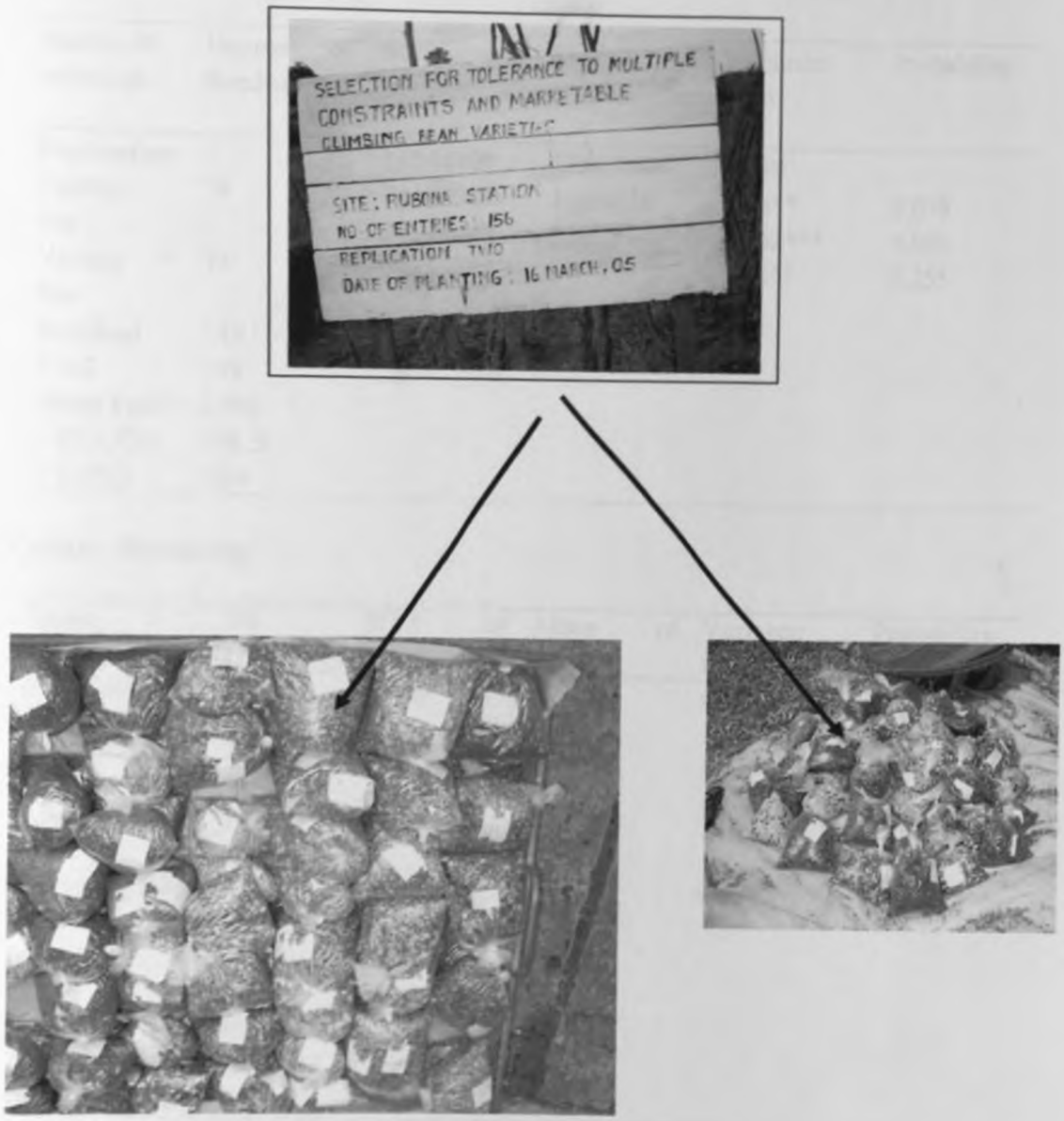
.Cross
(Umubano x MEX 54) F_2
(Umubano x MEX 54) F_3
[(Umubano x MEX 54) F_1 x (Umubano x Vuninkingi) F_1] F_2
[(Umubano x Vununkingi) F_1 x (Umubano x MEX 54) F_1] F_2
Umubano
Vuninkingi
MEX 54

ANNEX 3. Number of new climbing bean lines with combined resistance against angular leaf spot and *Fusarium* wilt resistances after artificial selection at Kabete (angular leaf spot) and field selection at Runyinya (fusarium wilt)

Cross/Line	Number of plants		
	Germinated	Resistant (1-3)	Intermediate / susceptible (4-9)
(Umubano x Mex 54) F_2	220	57	163
(G 2333 x Mex 54) F_3	225	79	146
[(Umubano x MEX 54) F_1 x (Umubano x Vuninkingi) F_1] F_3	100	29	61
[(Umubano x Vununkingi) F_1 x (Umubano x MEX 54) F_1] F_3	95	20	75
Total resistant		185	

ANNEX 4. Number of individual plants among the check parents that show resistant and non-resistant reactions against angular leaf spot after artificial inoculation at Kabete and Fusarium wilt after field evaluation at Runyinya hot spot site.

Parental line	Constraint tested	Number of plants		
		Germinated	Resistant (1-3)	Intermediate / Susceptible (4-9)
MEX 54	Fusarium wilt	50	48	2
	Angular leaf spot	50	50	0
Umubano	Fusarium wilt	97	-	97
	Angular leaf spot	50	11	39
Vuninkingi	Fusarium wilt	47	46	1
	Angular leaf spot	50	22	28



Annex 5. Early generation yield trial of $F_{2:5}$ lines at Rubona station (top) and segregation for the desired marketable red mottled and red (bottom left) and other seed market classes (bottom right).

ANNEX 6: Results of analysis of variance for yield, flowering, maturity durations, number of seeds per pod and pods per plant of advanced bean lines grown at Rubona and Rwerere sites (from Genestat computer package; NS = differences not significant, * = differences are slightly significant; *** = differences are highly significant).

6.1 Variate: Yield (kg ha^{-1})

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	Probability
Replication	1	11544408	11544408	15.59	
Variety	74	74403389	1005451	1.36**	0.059
Site	1	21929440	21929440	29.62***	<.001
Variety * Site	74	62216185.	840759	1.14**	0.255
Residual	149	110314142	740363		
Total	299	280407564			
Mean yield	2300				
LSD (5%)	196.3				
CV (%)	16.4				

6.2 Variate: Flowering

Source	Df	Sum of squares	Mean of squares	Variance ratio	Probability
Replication	1	57.20	57.20	4.94	
Variety	74	1037.09	14.01	1.21*	0.163
Site	1	9987.87	9987.87	3.07***	<.001
Variety * Site	74	636.38	8.60	0.74^{NS}	0.923
Residual	149	1724.30	11.57		
Total	299	13442.84			
Mean	55				
LSD (5%)	4.7				
CV (%)	6.7				

6.3 Variate: Maturity duration

Source	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	Probability
Replication	1	73.01	73.01	1.74	
Variety	74	4945.59	66.83	1.59***	0.009
Site	1	14700.00	14700.00	349.72***	<.001
Variety * Site	74	3979.00	53.77	1.28**	0.104
Residual	149	6262.99	42.03		
Total	299	29960.59			
Mean	93				
LSD (5%)	9.1				
CV (%)	7				

6.4 Variate: Pods plant $^{-1}$

Source	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	Probability
Replication	1	117.81	117.81	4.06	
Variety	74	2198.68	29.71	1.02*	0.442
Site	1	100.92	100.92	3.48**	0.064
Variety * Site	74	2101.08	28.39	0.98*	0.532
Residual	149	4319.19	28.99		
Total	299	8837.68			
Mean	12.5				
LSD (5%)	7.5				
CV (%)	41.8				

6.5 Variate: Grain pod^{-1}

Source	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	Probability
Replication	1	1.920	1.920	0.79	
Variety	74	263.347	3.559	1.47**	0.025
Site	1	21.333	21.333	8.80***	0.004
Variety * Site	74	186.667	2.523	1.04*	0.412
Residual	149	361.080	2.423		
Total	299	834.347			
Mean	6				
LSD (5%)	2.1				
CV (%)	25.1				