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**Charles RUHIMBANA**

**DEPARTMENT OF BIOTECHNOLOGY**

This thesis was accepted by all jury members or with majority vote on 02/12 /2019.

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Assoc.Prof.Dr.Hasan PINAR signature.........................

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December 2019

ANTALYA

# ÖZET

**RUANDA YAYGIN FASULYELERINDE (*PHASEOLUS VULGARIS* L.) ANTRAKNOZ VE YAYGIN FASÜLYE MOZAIK VIRÜSÜNE KARŞI DIRENÇLILIK IÇIN MOLEKÜLER ISLAH**

**Charles RUHIMBANA**

**Doktora Tezi, Biyoteknoloji Anabilim Dalı**

**Danışman: Prof. Dr. Nedim MUTLU**

**Aralık 2019; 98 sayfa**

Özellikle Doğu ve Orta Afrika'da yaygın fasulye verimini tahrip eden ve genel fasulye üretimini kısıtlayan hastalıklar Fasulye antraknozu ve Fasulye ortak mozaik virüsü (BCMV)'dir. Rwandan gibi yaygın fasülye çeşitlerinde piramit direnci genleri Fasulye antraknozu ve BCMV hastalıkları için mahsul veriminin stabilitesini sağlayacaktır. Bununla birlikte, piramidik genlerin bitki agronomik özellikleri üzerinde yan etkileri olabilir. Bölünmüş Çoğaltılmış Polimorfik Diziler (CAPS) ve Dizilim ile karakterize edilen yükseltilmiş bölgeler (SCAR) belirteçleri, iki antraknoz ve iki BCMV direnç geninin, dört popüler hassas ticari fasulye çeşidinin G54, RWR 1668, RWR 2355 ve RWV 2361 çeşitlerine piramitlenmesi işlemini kolaylaştırmak için kullanılmıştır. Gen piramidi, eşzamanlı geri çaprazlama ve daha sonra hedef genleri sabitlemek için özdeşleştirme yoluyla gerçekleştirilmiştir. Geri melezleme popülasyonlarındaki dirençli genler ve gelişen hatlar, markerler yardımıyla değerlendirilmiştir. Farklı kombinasyonlarda direnç genlerine sahip 92 F2, 52 BC1, 51 BC2, 44 BC3 bitki ve 76 BC3F2 familyası elde edilmiştir. *bc-3* + *Co-1* + *I* ve *bc-3* + *Co-2* + *I* direnç genleri kombinasyonuna sahip Rwandan kuru fasulyeleri için antraknoz ve BCMV direnç genlerinin marker destekli gen piramidi başarıyla gerçekleştirilmiştir ve geliştirilen hatların tüm fasulye antraknoz ırklarına ve BCMV suşlarına baklagiller fasulye antraknozuna ve BCMV hastalıklarına karşı koruyup direnç kazanması beklenmektedir.

Tekrarlı ebeveynler ile ilgili ileri hatlar arasındaki ortalamaların karşılaştırılması; çıkma günleri, çiçeklenme günleri, bitki boyu, bitki başına bakla sayısı, bakla uzunluğu, bakla başına tohum sayısı, fizyolojik olgunluğa kadar gün, bitki başına tohum ağırlığı ve 100 tohum ağırlığı eşleştirilmiş spss t-testi ile yapılmıştır. İki kuyruklu seviyede anlamlı olan en yüksek öğrencinin t-testi değerleri, iki mevsimdeki piramitli genlerin sayısına göre elde edilen 10.45 ve 9.47, ardından bitki başına tohum ağırlığı bakımından elde edilen 5.04 ve 4.89'dur. Öğrencilerin, iki grup arasında çiçeklenme günleri ile ilgili olarak kaydedilen 3.14 ve 4.64 değerleri, birinci ve ikinci mevsimlerde sırasıyla anlamlı olmuştur. İki grup arasındaki bitki başına bakla sayısındaki değişim, birinci ve ikinci sezonda sırasıyla 3.91 ve 3.02 t değerleri ile anlamlı sonuç vermiştir.

Bu çalışmanın diğer bir amacı da, piramit genlerin bitki agronomik özellikleri üzerindeki etkisini korelasyon ve yol katsayısı analizleri ile belirlemektir. Piramit genlerin sayısı ile farklı bitki agronomik karakterleri arasındaki ilişkiyi değerlendirmek için korelasyon ve yol analizleri kullanılmıştır. Piramitlenmiş genlerin sayısı, bakla uzunluğu (-0.33), bakla başına tohum sayısı (-0.342) ve bitki boyu (-0.243) ile önemli derecede negatif olarak ilişkiliydi. Piramit genlerin sayısı ile ortaya çıkma günleri (-0.059), bitki başına bakla sayısı (-0.125), fizyolojik olgunluğa kadar gün sayısı (-0.057) ve 100 tohum ağırlığı arasında anlamlı olmayan negatif korelasyon bulunmuştur (-0.042). Piramitli gen sayısı ile bitki başına tohum ağırlığı (0.128) ve çiçeklenme günleri (0.011) arasında anlamlı olmayan pozitif korelasyonlar göstermiştir. 100 tohumluk ağırlık ve bitki boyu, sırasıyla doğrudan yol katsayılarına (0.957 ve 0.072) eşit olan korelasyon katsayılarına (0.950 ve 0.051) sahipti. Piramitlenmiş genlerin sayısı, bitkilerin tohum ağırlığı üzerinde ortaya çıkma günlerine (-0.017), çiçeklenme günleri (-0.007), bitki boyuna (-0.056), bakla uzunluğuna dolaylı olumsuz etkisi olmuştur. (-0.008), bitki başına bakla sayısı (-0.002), fizyolojik olgunluğa ulaşım günü (-0.013) ve 100 tohum ağırlığı (-0.057) üzerine dolaylı negatif etkileri olmuştur. Piramit genlerin sayısı ve doğrudan etki arasındaki korelasyon katsayısı düşük ve anlamlı bulunmamıştır. Dirençli genlerin piramitleştirilmesi, bağımsız değişkenler vasıtasıyla verimi bağımsız olarak etkileyebilir. Bu nedenle, yetiştiricilerin verim ile ilgili özelliklere sahip olan piramit gen sayısını dikkate almaları önem teşkil etmektedir.

**ANAHTAR KELIMELER:** Bağımlı değişkenler, Bağımsız değişkenler, BCMV, CAPS belirteçleri, *Colletotrichum lindemuthianum*, Gen piramitleşmesi, Korelasyon analizi, MAS, *Phaseolus vulgaris*, SCAR belirteçleri, Yol analizi.

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**ABSTRACT**

**MOLECULAR BREEDING OF RWANDAN COMMON BEANS (*Phaseolus vulgaris L.*) FOR RESISTANCE AGAINST ANTHRACNOSE AND BEAN COMMON MOSAIC VIRUS**

**Charles RUHIMBANA**

**PhD Thesis, Department of Biotechnology**

**Advisor: Prof.Dr.Nedim MUTLU**

**December 2019; 98 pages.**

Bean anthracnose and Bean common mosaic virus (*BCMV*) are the diseases devastating the yield of common beans and constrain common bean production especially in East and Central Africa. Pyramiding resistance genes for bean anthracnose and *BCMV* diseases in Rwandan common bean varieties would ensure stability of the crop yield. However, there might be side effects of pyramided genes on plant agronomic traits. Cleaved Amplified Polymorphic Sequences (CAPS) and Sequence characterized amplified regions (SCAR) markers were used to facilitate the process of pyramiding two anthracnose and two *BCMV* resistance genes into four popular susceptible market-class bean varieties G54, RWR 1668, RWR 2355 and RWV 2361. Gene pyramiding was fulfilled through concurrent backcrossing and then selfing to fix the target genes. Resistance genes in backcross populations and advanced lines were evaluated with the aid of markers. The 92 F2, 52 BC1, 51 BC2, 44 BC3 and 76 BC3F2 families with resistance genes in different combinations were obtained. Marker-assisted gene pyramiding of anthracnose and *BCMV* resistance genes was successfully achieved for Rwandan common beans with *bc-3+Co-1+I* and *bc-3+Co-2+I* resistance genes combination and the lines developed are expected to confer resistance to all bean anthracnose races and *BCMV* strains and protect bean crop against bean anthracnose and *BCMV* diseases.

Analysis of comparisons of means between recurrent parents and their respective advanced lines in regards to; days to emergence, days to flowering , plant height, number of pods per plant, pod length, number of seeds per pod, days to physiological maturity, seed weight per plant and 100-seed weight was done with paired students t-test. The highest student's t-test values significant at the two-tailed level were 10.45 and 9.47 obtained in regard to number of pyramided genes in two seasons, followed by 5.04 and 4.89 obtained in regard to seed weight per plant. The student's t-test values 3.14 and 4.64 recorded in regard to days to flowering between two groups were significant for first and second seasons respectively. The change in number of pods per plant between two groups was significant with t-values 3.91 and 3.02 in first and second season respectively.

Another objective of this study was to determine the effect of pyramided genes on other plant agronomic traits with the help of correlation and path coefficient analyses. Correlation and path analyses were used to assess the relationship between number of pyramided genes and different plant agronomic characters. The number of pyramided genes was significantly negatively correlated with pod length (-0.33), number of seeds per pod (-0.342) and plant height (-0.243). The non-significant negative correlation was found between number of pyramided genes and days to emergence (-0.059), number of pods per plant (-0.125), number of days to physiological maturity (-0.057) and 100-seed weight (-0.042). Non-significant positive correlations were exhibited between number of pyramided genes and seed weight per plant (0.128) and days to flowering (0.011). The 100-seed weight and plant height had correlation coefficients (0.950 and 0.051) almost equal to the direct path coefficients (0.957 and 0.072), respectively. Number of pyramided genes had negative indirect effect on seed weight per plant through days to emergence (-0.017), days to flowering (-0.007), plant height (-0.056), pod length (-0.008), number of pods per plant (-0.002), days to physiological maturity (-0.013) and 100-seed weight (-0.057). The correlation coefficient between number of pyramided genes and direct effect was low and non-significant. Pyramiding number of resistance genes would affect yield through independent variables. Therefore, it is important for breeders to take into consideration the number of pyramided genes with yield related traits.

**KEY WORDS:** Dependent variables, Independent variables, *BCMV,* CAPS markers, *Colletotrichum lindemuthianum*, Gene pyramiding, Correlation analysis, MAS, *Phaseolus vulgaris*, SCAR markers, Path analysis.

**COMMITTEE:** Prof. Dr. Nedim MUTLU

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## PREFACE

The Central and Eastern Africa region is a secondary center of diversity for common bean. The common bean is the most important legume crop in Rwanda occupying an important niche in the Rwanda agricultural sector and farm household economy. Bean per capita consumption is reported to be highest in Rwanda. Pyramiding resistance genes for bean anthracnose and *BCMV* diseases in Rwandan common bean varieties would ensure stability of the crop yield.

I would like to express my sincere gratitude to the following people and organizations that have been the pillars in various ways to make this work a success.

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CURRICURUM VITAE

## DECLARATION

This thesis was written in accordance with Akdeniz University rules and is entirely my own original work and has never been submitted for a degree award in any institution.

02/12/2019

**Charles RUHIMBANA**

# SYMBOLS AND ABBREVIATIONS

**Symbols**

| | |
|---|---|
| × | : Crossing |
| % | : Percentage |
| °C | : Degree Celsius |
| α | : Alpha |
| χ2 | : Chi-Square |

**Abbreviations**

| | |
|---|---|
| A | : Andean |
| BC | : Backcross |
| BC1F1 | : Backcross one filial one generation |
| BC2F1 | : Backcross two filial one generation |
| BC3F1 | : Backcross three filial one generation |
| BC3F2 | : Backcross three filial two generation |
| *BCMNV* | : Bean Common Mosaic Necrosis Virus |
| *BCMV* | ; Bean Common Mosaic Virus |
| bp | : Base pair |
| CAPS | : Cleaved Amplified Polymorphic Sequence |
| CIAT | : International Centre for Tropical Agriculture |
| *Co* | *: Colletotrichium* |
| CTAB | : Cetyl trimethylammonium bromide |
| EDTA | : Ethylenediaminetetraacetic acid |
| eIF | : Eucaryotic Initiation Factor |
| F1 | : Filial one generation |
| F2 | : Filial two generation |
| gDNA | : Genomic Deoxyribose nucleic acid |
| *I* | : Inhibitor gene |
| MA | : Mesoamerican |
| MAS | : Markers-Assisted Selection |
| MDRK | : Michigan Dark Red Kidney |
| MT | : Metric tones |
| NA | : Not available |
| NL | : Netherlands |
| R | : Resistant |
| RAB | : Rwanda Agriculture Board |
| RAPD | : Randomly Amplified Polymorphic DNA |
| S | : Susceptible |
| SCAR | : Sequence Characterized Amplified Region |
| SPSS | : Statistical Package for the Social Sciences |
| TN | : Tanzania |
| VPg | : Virus-protein genome |

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# 1. INTRODUCTION

## 1.1. Background of the Study.

Common bean (*Phaseolus vulgaris* L.) is a true autogamous diploid species, possessing 22 chromosomes with a genome size ranging between 587 Mbp and 637 Mbp (Bernnett and Leitch 1995). The common bean is a nutritionally and economically important food crop grown worldwide. Common beans serve as the main source of human dietary proteins (Mauyo et al. 2007) appreciable levels of B vitamins, iron and Magnesium among other micro-nutrients. Most varieties of dry beans have maturing cycle ranging from 65 to 110 days. The crop grows in many environments ranging from tropical, subtropical to the temperate regions of the world. It was domesticated more than 7,000 years ago from two centers of origin; namely Mesoamerica and the Andean regions (Mamidi et al. 2013). Common beans were introduced in highlands of East Africa by the Portuguese traders about 400 years ago and the highlands are now a secondary center of genetic diversity and since then, it spread into many parts of Africa (CIAT 2001). It is also an important crop grown in East and Central Africa mostly by household farmers (Opio et al. 2001). In Rwanda, common bean is grown nearly in all districts twice a year in diverse cropping systems with limited technology. Rwanda was ranked highest in bean consumption per capita (Blair et al. 2010). Common bean serves as the main source of dietary protein (60%), iron and other micronutrients. The total dry bean production was 455,822 tones, on harvested area of 549,441 ha with yield of 8,296 hg/ha (FAOSTAT 2017) and the government of Rwanda is devoted to improve bean varieties for better yield. Therefore, common beans play an essential role for income generation and food security (Larochelle and Alwang 2014).

Bean common mosaic virus (*BCMV*) belongs to the family of *potyviridae*, the most destructive viruses infecting common bean in the world (Florez –Estevez et al. 2003). Until 1992, based on coat protein, *BCMNV* and *BCMV* were called serotype A and serotype B, respectively (Drijfhout et al. 1978; Morales and Bos 1988; Vetten et al. 1992). Later, TN-1,NL-3, NL-3K, NL-5 and NL-8 strains were identified to belong to *BCMNV* and NL-1 (US-1), NL-2, NL-4 (US-6), NL-6 (US-4), NL-7, US-2 and US-5 strains belong to *BCMV*.

The yield of common bean remains poor in areas growing susceptible cultivars due to *BCMV* disease that later results in an economical problem (Miklas et al. 2006). The *BCMV* is a seed-borne, sap, pollen and transmitted by several aphid species (Kelly et al. 2003). Plant breeding has been promising for developing cultivars with broader host plant resistance to bacteria, fungi and viruses (Coyne and Schuster 1974).

Use of potyvirus-free seeds and good cultural practices programs lessen the dissemination of disease. However, use of resistant cultivars is only best durable and economic effective way to control the pathogen from infecting the crops (Drijfhout 1978; Kelly et al. 1995; Miklas et al. 2000). Resistance to *BCMV* is governed by monogenic dominant inhibitor (*I*) gene and a number of recessive genes namely; *bc-u, bc-1,* $bc\text{-}1^2$, *bc-2,* $bc\text{-}2^2$ and *bc-3* (Strausbaugh et al. 1999). The dominant *I* gene in *P. vulgaris* confers temperature-dependent resistance to a group of *BCMV* races and other potyviruses through

a hypersensitive response and is mapped to linkage group B2 (Freyre et al. 1998). Recessive strain specific resistance genes *(bc-1, bc-1$^2$, bc-2, bc-2$^2$* and *bc-3)* require an independent nonspecific *bc-u* gene to be functional and have been demonstrated to be effective and long lasting in controlling diseases caused by viruses (Drijfhout 1978; Johansen et al. 2001). Common bean genotypes carrying the recessive strain specific resistance *bc-3* gene found to carry homozygous mutations in a PveIF4E coding sequence. The mutated forms of translation initiation factor 4E (eIF4E) and/or its isoform eIF(iso)4E disrupt the interaction between cap-binding proteins and virus-protein-genome linked (VPg) and plants lose susceptibility (Kang et al. 2005; Beauchemin et al. 2007). The *bc-3* gene resides on chromosome B6 and confers resistance to all known strains of *BCMNV* and *BCMV* in the presence of the dominant *I* gene (Kelly et al. 2003). In the *Arabidopsis thaliana*, two proteins namely; AteIF4E-1 and AteIF (iso)4E are associated with potyviral infection and their homologues are linked to potyviral resistance in other plant species (Robaglia and Caranta 2006; Hwang et al. 2009).

The fungus, *Colletotricum lindemuthianum* attacks *Phaseolus* species and causes bean anthracnose disease and the pathogen can stay for up to 5 years in infected seeds (Pastor-Corrales and Tu 1989). Bean anthracnose is a destructive seed-borne disease infecting susceptible bean cultivars particularly in Central and East Africa regions causing severe damage to the stem, leaves and pods which may result into 100% yield loss especially for smallholder farmers in environments favoring this pathogen (Tu 1983).

Environmental factors and infected seeds play important role for the pathogen's spore development and spreading in farms (Tu 1983). In addition to the genetic and environmental factors which challenge the production system of common beans, seed-borne diseases result from fungal and viral diseases of common beans are becoming more problematic in East Africa and are the main cause for yield reductions. *Colletotricum lindemuthianum* has been observed to possess highest pathogenic variation in the centers of origin of its host, the Mesoamerican and Andean regions (Pastor-Corrales et al. 1993).

Thus, there is a need to develop varieties which are resistant to both pathogens. The existence of both bean anthracnose and potyvirus diseases on the bean crop in farmers' fields and the ability of these pathogens to disseminate have greatly undermined conventional breeding leading to severe yield losses in bean varieties that had been previously released with a single pathogen resistance. This has created the need for pyramiding of both bean anthracnose and potyvirus resistance genes which have complementary spectra as a strategy to circumvent the problem of pathogen variability and simultaneously introgress multiple disease resistance genes within the same variety. The advancement of modern molecular marker technology has revolutionized the area of molecular plant breeding and has also widened several aspects of the practical application of marker-assisted gene pyramiding. Use of molecular markers to track pyramided genes also considerably reduces the breeding period involved in the pyramiding program. Both conventional and marker-assisted selection (MAS) were used in this study to effectively screen all populations and also increase precision of selecting for materials with multiple resistance genes against bean anthracnose and *BCMV* diseases. Marker-assisted gene

pyramiding made it possible to breed cultivars with broad resistance to different strains of anthracnose and *BCMV* /*BCMNV*. Use of MAS for indirect selection of specific resistance genes in the absence of the pathogen has been used successfully to breed common bean (Miklas et al. 2006).

The process of pyramiding genes was made more efficient by using both Cleaved Amplified Polymorphic Sequence (CAPS) and Sequence Characterized Amplified Region (SCAR) markers. Simultaneous introgression of bean anthracnose and potyvirus resistance genes is important in generating genotypes that possess multiple disease resistance to the two diseases. The new genotypes developed are resistant to both bean anthracnose and potyvirus diseases and are available as germplasm for national and regional release or for incorporation into breeding programs of other countries.

Yield is a complex character, influenced by several yield related components and environment (Ejara et al. 2017). Selection of plant materials with high yield requires information about interaction between seed yield and other characters contributing to yield for an efficient selection strategy.

Correlation and path-coefficient analyses have been conducted on several crops including common beans (Karasu and Oz 2010; Singh and Singh 2013; Akhshi et al. 2015, AlBallat et al. 2019), maize (Adesoji et al. 2015), rice (Ansari et al. 2010) and tomato (Islam et al. 2010). However, correlation and path analyses for the improvement of common bean in Rwanda where the bean materials obtained from and consumption per capita is high had not been done so far.

### 1.2. Statement of the Problem

The yield of common beans is extremely affected by several pathogens of economic importance, including *C. lindemunthianum* and *BCMV*. Both pathogens cause yield losses in common beans ranging between 5 and 100% depending on races of the respective pathogen, bean cultivars, stage of plantgrowth and environmental factors (Wortmann et al. 1998).

In East African countries especially Tanzania, Sudan and Uganda, yield losses attributed by bean anthracnose disease remain very high (40- 90%) and it attacks seedling, leaf, stem and pods of the plant under climatic condition favorable to the disease (Mudawi et al. 2009). However, in Rwanda there is not any report on gene pyramiding against bean anthracnose and *BCMV* diseases has been published yet.

### 1.3. Justification of the Study

Rwanda has the highest bean consumption per-capita in the world, (40 kg per year). Common beans are a major cash and food crop for the majority of household farmers and consumers (CIAT 2008). Common beans are believed to provide up to 65 percent of the country's national dietary protein intake and 32 percent of caloric intake (Chirwa 2004).

It is grown by about 86 percent of farmers and occupies about 40 percent of arable land in a diverse monoculture or intercropping system, this shows the role it plays in Rwanda Agricultural sector and farm household economy (CIAT 2008). However, the crop shows significantly low grain yield when attacked by pathogens (Wortmann et al. 1998).

The occurrence of pathogens encourages the need to broaden the genetic base of common beans as a crop, to expand the scope of parental bean cultivars with desirable traits as potential sources of resistance genes in breeding programs that fits to the need to overcome the frequent evolution of new races of the pathogens.

Development of well adapted resistant bean cultivars is considered as an effective alternative management option for control of anthracnose and *BCMV* diseases. This study aimed at introgressing genes conferring resistance against anthracnose and *BCMV* diseases into Rwandan popular common bean cultivars to stabilize bean production.

## 1.4. Broad Objective

Reduction of common bean yield losses caused by anthracnose and *BCMV/BCMNV* diseases through incorporation of disease resistance genes into Rwandan common bean cultivars.

## 1.5. Specific Objectives

1. To incorporate genes conferring resistance to bean anthracnose and *BCMV* diseases into common bean (*Phaseolus vulgaris, L.*) genotypes of Rwanda.

2. Use of Markers for selection of beans having resistance genes against bean anthracnose and *BCMV* diseases.

3. Recovery of Rwandan common bean’s genetic background through backcrossing and phenotypic characterization.

4. To develop advanced common bean lines with multiple resistance genes to anthracnose and *BCMV/BCMNV* and fix the multiple resistance genes.

5. Evaluate the effect of multiple resistance genes of bean anthracnose and *BCMV/BCMNV* on plant agronomic characters.

# 2. LITERATURE REVIEW

## 2.1. Origin of Common Beans and Their Races

Common bean belongs to the family Fabaceae, genus *Phaseoulus*, it includes; popping beans, dry beans, green shelled beans and snap beans. The genus has only 50 species with only five domesticated cultigens (McClean et al. 2004; Aragao et al. 2011). Common beans exhibit variations in growth habits; determinate and indeterminate bush types, indeterminate prostrate and extreme climbing types. Seed characteristics such as size, color, shape and brightness of dry bean are important morphological characters that matter for consumer preferences. Linnaeus proposed India as the origin of common beans (Brücher 1988). Mesoamerica was noted as the center of origin for common bean based on the crop diversity and the presence of wild types (Vavilov 1931). Currently, two geographically isolated and genetically (F1 hybrid weaknesses, variation in morphology, phaseolin seed proteins, allozymes and molecular markers) differentiated gene pools are recognized; Mesoamerica and the Andes are recognized in several studies (Singh 1989; Beebe et al. 2001; Mamidi et al. 2013). Singh et al (1991b) further divided the two pools into six races: Andean gene pool, (all large-seeded), Chile, Nueva Granada and Peru; Middle American (MA) gene pool three evolutionary races, Durango (medium-seeded semi-climber), Jalisco (medium-seeded climber) and Mesoamerica (all small-seeded).

## 2.2. Ecology, Distribution and Production of Common Beans

Common bean is a predominantly self-pollinated crop, mostly grown in tropical and subtropical areas of the world. The growing temperatures of the common beans range from 14 to 26°C with about 12 h day-length but some cultivars either through evolution or developed by breeders are adapted to different photoperiod length grown (White and Laing 1989) and the annual precipitation range from 400 to 1600 mm. Most cultivars' life cycles have a range between 100-130 days from germination to seed maturity and grain yield potential (400–5000 kg $ha^{-1}$) (Wortmann et al. 1998). Common beans grow best in loose, well drained, loam soils, rich in organic matter with an average 5-6 acid soil pH (Duke 1983). Common beans do poorly in wet tropics where rain favors growth of pathogens and spread of diseases. Excessive water and frost conditions injure the crop in a few hours and kill them.

Both dry and green beans were grown worldwide with the yield of around 22.8 million MT (metric tons) and 21.37 million MT, and with the average yield of 0.8 and 13.8 Mg t $ha^{-1}$ respectively (FAOSTAT 2014), this proves that common bean is an important food staple to millions of people worldwide (Aragao et al. 2011). Humans eat edible green pods and dry grains of common beans. The dry grains are rich in dietary protein, calories, fibers, vitamin B and micronutrients whch we need for daily life (Bliss 1990). In 2017, dry beans were grown in the USA on harvested area of 814520 ha with an estimated production of 1625900 tons and the average yield was 19961 hg/ha (FAOSTAT 2017). According to the 2007 Census of Agriculture, nearly 14% of the US population consumes dry beans

every day, which implies that the annual average per capita bean consumption was 3 kg year$^{-1}$ (USDA 2007).

Canada grew beans on harvested area of 116390 ha with the average yield of 22067 hg/ha and production was 256835 tons in 2017 (FAOSTAT 2017), and was ranked among the top exporters of the common beans in the world. It was not surprising that 30% of bean production is for consumption while 70% is grown for trade markets. Nearly 110000 ha of land area were utilized for common bean production in Alberta, Manitoba and Ontario in Canada (Beebe et al. 2011; FAOSTAT 2011). Latin America countries grew beans on area of 6.8 million ha with a total harvest of 6 million tons in 2010. The main producers of common beans in the region of South America are Brazil and Mexico, and their annual production was 4.1 million tons (Beebe et al. 2011). Common beans are mostly grown in Latin America (except in Argentina) by subsistence farmers whose fields range between 1 and 10 ha. Common bean, maize, potato and rice are grown only for consumption in Latin America. In Latin American countries bean consumption per capita ranges from 6 to 18 kg year-$^{1}$ (Broughton et al. 2003). Brazil was ranked third among top bean producers worldwide (3033017 tons), its consumption is also high but it exports less than 0.1% of its harvest (FAOSTAT 2017). Common beans contribute 9% of dietary protein to the consumers of this crop in Brazil (Gepts et al. 2008). Asia is the largest producer of common beans (46%) and it exports the crop to the world. India in 2017 was on top, with the production of 6390000 tons, followed by Myanmar (5466166 tons) (FAOSTAT 2017). In 2010, production of dry beans was 9.8 million t in China (FAOSTAT 2011). The crop is grown on 1.2 million ha of land owned by families in various parts of the country. China is one of the largest exporters (800000 tons) of common bean in the world (FAOSTAT 2011). Common beans are grown for consumption and trade markets in Myanmar and India. Production and trade markets of dry beans increased and reached the annual exports of around 1.4 MT (Dapice et al. 2011).

The common beans were introduced from Mesoamerica and Andes 8000 years ago and diverged to many parts of the world (Mamidi et al. 2011). They were introduced to Africa 400 years ago and today are grown at 6.4 million ha primarily by small-scale farmers mainly for consumption and revenue, but the crop shows poor yield ranging from 0.35 to 0.75 t ha-$^{1}$ due to low inputs, pests, diseases and environmental constraints (Wortmann et al. 1998; Katungi et al. 2009).

East Africa has the highest bean production in sub-Saharan Africa at 1297000 tons per annum (Wortmann et al. 1999). These include Burundi, D.R. Congo, Ethiopia, Kenya, Rwanda, Tanzania and Uganda.

## 2.3. Nutritional Values

Common beans are important source of calories, high in dietary fibers, protein and vitamin. Common beans contain a large amount of free biotin, an essential cofactor for carboxylases and decarboxylases in metabolic organisms (Knowles 1989). Beans are also essential sources of minerals such as iron, phosphorus, magnesium, manganese, zinc,

copper, calcium and sodium in a lesser degree and are gluten free (Agriculture and (Broughton 2003). Regular consumption of common beans lessens cholesterol and cancer risks.

## 2.4. Breeding of Common Beans

Common beans originated from Andes and Mesoamerica, are now cultivated across the globe in diverse habitats. Breeders develop new cultivars suitable for high yield, harsh–environmental conditions tolerant and disease resistant through crossing parents possessing traits contributing to yield, environmental conditions tolerance and disease resistance respectively.

Breeding programs are established for crop improvements through selecting desirable plant traits and seed types that are economically viable. The process of matching growth habit to changing economy, technology, and environment, is a task for all plant breeders in order to develop improved cultivars that are adapted to specific agronomic production systems and environmental conditions (Acquaah et al. 1991; Brothers and Kelly 1993; Nienhuis and Singh 1985).

Crossing and selection programs to develop improved cultivars are influenced by resources available and training. The germplasms from which cultivars derived are elite x elite crosses, adapted genotypes within gene pools in order to diversity the pools for the improvements in canopy structure for maximizing yield (Zyla et al. 1993).

## 2.5. Pathogens of Common Beans

Both grain yield and quality of common beans are affected by several pathogens of economic importance. Major pathogens include *Bean common mosaic virus*, *Bean common mosaic necrosis virus, Colletotrichum lindemunthianum*, *Phaeoisariopsis griseola*, *Pseudomonas syringae pv. phaseolicola*, *Sclerotinia sclerotiorum, Uromyces appendiculatus* and *Xanthomonas axonopodis pv. phaseoli*. Many of these pathogens cause a yield loss that ranges between 5 and 100% in all parts of the world, depending on races of the respective pathogen, cultivar and its stage of development and environmental factors.

The pathogens caused serious grain yield loss in the past both in Europe and North America (Schwartz et al. 1982; Miklas et al. 2006). The fungal pathogens; *Colletotrichum lindemunthianum, Phaeoisariopsis griseola*, and *Uromyces appendiculatus* caused 1.8 million tons annual yield loss while bacterial pathogens such as; halo blight and common bacterial blight and others caused 0.8 million annual grain yield loss.Both Viruses (*Bean common mosaic virus)* and insect pests (bruchids and aphids) account for 0.4 million and 0.8 million of annual yield losses, respectively (Wortmann et al. 1998).

### 2.5.1. *Colletotrichum lindemuthianum*

#### 2.5.1.1. Introduction

Bean anthracnose is a worldwide destructive seed borne disease infecting common beans at all stages of its growth (Balardin et al. 1997). It can cause serious yield losses by up to 90% in susceptible plants of bean cultivars in tropical or subtropical regions (Tu 1981). Environmental factors favouring its spread are rainfall, temperature < 28°C, and high humidity. Infected seeds play vital role for the development of spores of pathogens and distribution in fields (Tu 1982). Anthracnose symptoms are usually associated with Small reddish-brown, black-sunken spots on the leaves, pods and stems.

#### 2.5.1.2. Taxonomy

The causative agent for bean anthracnose was previously used to have numerous names such as *Gleosporium lindemuthianum*, *Glomerella lindemuthiana, Septoria legunimun*, *Septoria legunimun* var *phaseolorum*, *Colletotrichum lindemuthianum*, *C. lagenarium*. The causative agent of the bean anthracnose disease was first identified in 1875 and today it remains clear that it is a fungus that presents imperfect and perfect forms called *Colletotrichum lindemuthianum* and *Glomerella cyngulata f.sp phaseoli*, respectively (Martínez-Pacheco et al. 2009).

Most authors agreed that *C. lindemuthianum* belongs to the Kingdom Fungi, Phylum Ascomycota, Class Sordariomycetes, Order Phyllachorales, Family, Phyllacholaceae; Genus Colletotrichum, Species Lindemuthiunum. O'Sullivan et al (1998), using Pulse Field Gel Electrophoresis (PFGE), found that total chromosome numbers varied from 9 to 12 and suggested two distinct classes of the chromosomes.

#### 2.5.1.3. Life cycle of *Colletotrichum lindemuthianum*

Bean anthracnose is caused by a hemibiotrophic fungus *Colletotrichum lindemuthianum L* and is the most destructive disease of common bean (Pastor-Corrales and Tu 1989). The seed-borne nature and pathogenic variability of *C.lindemuthianum* allows it to affect plant parts at all stages of plant growth and development, the disease disseminated from contaminated seeds, infected plant debris and/or from weeds (Dillard and Cobb 1993; Tu 1981).

Use of 12 bean differential cultivars (Pastor-Corrales 1991) revealed at least 100 races of *C.lindemuthianum* (Mahuku and Riascos 2004). In Spain, two races 6 and 38 are the most common ones and they undermine the yield of the market class fabada beans (Ferreira et al. 2008). The disease is not only a problem to the subsistence farmers of Latin America and Africa but also for commercial farmers in North America as well.

The fungus is a hemibiotrophic pathogen, meaning that it spends part of its infection cycle as a biotroph, and the other part as a necrotroph in its development phases (Martínez-Pacheco et al. 2009). The spores of *C. lindemuthianum* are dispersed by rain splash, and

later attach to the aerial parts of the bean plant in order to infect the host. the spore germination process begins with the spore adhering to the plant surface under adequate humidity conditions and form a short germ tube which develops an appressorium, as the germ tube grows, it causes an indectation to occur in the cell wall and hyphae elongates to colonize the substrate (Mercure et al. 1994). An infection peg is then able to protrude from the appressorium and penetrate through the cell wall. The aerial mycelia use mechanical force and release proteins (enzymes) for futher colonization; the fungal reproductive structures (spores) are formed during the course of development and stayed dormant until conditions become favourable, disseminated by rain splash to new host (Mercure et al. 1994).

**2.5.1.4. Disease symptomatology**

The initial symptoms appear as a black or dark brown along the veins on the underside of the leaves (Buruchara et al. 2010). The symptoms of bean anthracnose also include black-red sunken cankers, lenticular necrotic lesions containing the acervuli of the pathogen, black brown colored and discolored seeds (Schwartz 2005).

In severe cases, defoliation, fall of the pods and death of the plant can happen. The pathogen infects leaves, stems, and pods of the susceptible bean cultivars plants. Seed borne infection usually induces dark brown to black eye-shaped lesions longitudinally on the hypocotyls and cotyledons. In severe conditions, young pods may wither and dry prematurely. The pathogens may penetrate the seed coat and get established within the seed which, when planted serve as the source of infection in the succeeding crop (Buruchara et al. 2010).

**2.5.1.5. Disease epidemiology**

Early seedling infection often leads to high disease severity on the same plant and greater chances for spread and infection of neighboring plants (Zaumeyer and Thomas 1957). Spread of the disease from a focus to other susceptible plants is influenced by environmental factors responsible for inoculum dispersal, such as rain-splash or wind-driven rain and cultural practices such as intercropping and growing of mixtures (Tu 1983). *C. lindemuthianum* can survive between seasons on seeds and plant debris (CAB International 1998).

Length of survival is influenced by environment, especially moisture and temperature. The fungus remains viable up to five years in air-dried pods or seeds stored at 4°C. High relative humidity or free water is essential for dissemination and germination of conidia, infection, incubation and subsequent sporulation (CAB International 2004).

The disease is most severe in moderate temperatures of between 13-26°C (Zaumeyer and Thomas 1957), 17-22°C (Holliday 1980), 17-24°C (Tu and McNaughton 1980) and under wet conditions. It thrives in relatively cool and wet regions of the tropics

and sub-tropics, and is endemic in southern and central Brazil, Mexico, Central and East Africa (Pastor-Corrales et al. 1995).

### 2.5.1.6. Disease control and disease resistance mechanisms in plant species

Planting of disease free seed produced under strictly controlled conditions such as clean seeds and seed treatment with hot water at 50$^{o}$C for 20 minutes is an effective strategy to control *C. lindemuthianum* without affecting seed viability (Araya et al. 1987). Burial of plant debris, removal of diseased basal leaves at weeding and crop rotation also decrease disease incidence (Truttman and Kayitare 1991). Use of cultivar mixtures plays an important role in buffering against the disease and stabilizing yields (Truttman et al. 1993).

Chemical control of anthracnose also has been reported to be effective with satisfactory results reported after use of foliar application of benomyl, carbendazim, difolatan, zaneb, captafol and maneb. Chemical control is however, limited by possible development of resistant biotypes, limited effectiveness and high costs (Tu and McNaughton 1980; and Pastor-Coralles and Tu 1989).

Ancients believed that plant diseases were caused by supernatural forces and poisonous vapors (Whetzel 1918). Recognition of the role of plant disease causing agents took centuries for scientists to establish the link between pathogens and disease, and it depended largely upon the development of new various techniques for their study. The agents that cause plant diseases are viruses, bacteria, fungi, protozoa and nematodes, or damage results from insects attack. Understanding of plant genetics allowed researchers to identify sources of heritable resistance, resistance genes (R genes) (Rhoades1935, Bushnell 2012).

Numerous classes of R genes encode complex regulatory systems that control plant defense responses against invasion of pathogens (Antolín-Llovera, 2012). Mechanisms for resistance using R genes largely build upon the gene-for-gene model. (Hammond-Kosack, 1996, 1997). The phases of plant immune system response include pathogen detection, signal transduction, or defense response. However, the zig-zag model showed that plant's detection systems and pathogen's evasion techniques keep evolving (Jones and Dangl 2006).

### 2.5.1.7. Bean anthracnose resistance genes

Use of resistant cultivars is environmentally-safe, socially-acceptable, easiest for farmers to adopt and provides a long lasting solution to the economic losses of beans caused by anthracnose (Goncalves-Vidigal et al. 1997; Mahuku et al. 2002)

A number of genes conferring resistance to bean anthracnose with Co-symbols have been reported in common bean, namely; *Co-1, Co-u, Co-3/9, Co-5, Co-4, co-8* and *Co-2* (Kelly and Vallejos 2004; Campa et al. 2005; Geffroy et al. 2008; Campa et al. 2009). Studies reveal that the anthracnose resistance loci are organized as clusters in the genome

(Geffroy et al. 1999; Rodrı´guez-Sua´rez et al. 2007, 2008; David et al. 2009; Campa et al. 2010).

Resistance of common bean to *Colletotrichum lindemuthianum* is controlled by a single or few major race-specific Co-genes, some exist as resistance gene clusters and due to several forms of races of the pathogens durable resistance to anthracnose requires pyramiding of race-specific anthracnose resistance genes into a common bean background (Mahuku et al. 2002; Lacanallo et al. 2010).

All bean anthracnose resistance genes are dominant except recessive co-8 gene and multiple alleles exist at the *Co-1, Co-3* and *Co-4* and *Co-5* loci. The resistance genes *Co-2* to *Co-11* are Meso-American and the genes *Co-1*, *Co-1*$^2$, *Co-1*$^3$, *Co-12, Co-13, Co-14, Co-w,* and *Co-x* are primarily from the Andean gene pool in origin (Geffroy et al. 1998; Ferreira et al. 2012) (Table 2.1).

Resistance loci have been mapped to the eight chromosomes Pv01, Pv02, Pv03, Pv04, Pv07, Pv08, Pv09 and Pv11 in addition to other genes Co-u, Co-w, Co-x, Co-y, Co-z and CoPv02c. G 2333 bean cultivar carrying *Co-4*$^2$, *Co-5* and *Co-7* genes could be used as a source of resistance in the bean breeding program since it is highly resistant to different races of *C. lindemuthianum* in Africa, Brazil, Europe and North America (Pastor-Corrales and Tu 1989; CIAT 1990). PI 207262 cultivar possesses two genes and could be adopted for use in breeding programs aiming at improving resistance to anthracnose (Poletine et al. 2000). Molecular markers linked to major race-specific resistance *Co*-genes localized in different *Co*-gene cluster regions are shown in Table 2.1.

The use of DNA markers linked to race-specific resistance genes for bean anthracnose have been proven most useful to underpin marker-aided selection in different market classes belonging to the Middle-American and Andean gene pools and these provide an opportunity to enhance disease resistance through gene pyramiding. Vallejo and Kelly 2001 developed SCAR marker (SAB 3) linked to Co-5 gene in TU, SEL 1360, G2333 and G 2338 bean cultivars.

**Table 2.1.** Anthracnose resistance genes, their sources and linked markers

| Gene symbols | | Genetic sources | Gene pool | Linked markers | Map location | References |
|---|---|---|---|---|---|---|
| New | Origin | | | | | |
| *Co-1* | A | MDRK | A | $OF10_{530}$ | B1 | Mc Rostie 1919,Vallejo and Kelly 2001 |
| *CO-1²* | | Kaboon | | $SE_{ACT}/M_{CCA}$ | | |
| *Co-1³* | | Perry marrow | | | | |
| *Co-1⁴* | | AND 277 | | | | |
| *Co-1⁵* | | Widusa | | $OAI8_{1500}$ | | |
| *Co-2* | Are | Cornell-49242 | MA | $OQ_{1440}$<br>$OH20_{450}$<br>$B355_{1000}$ | B11 | Mastenbroek 1960<br>Adam-Blondon et al. 1994<br>Young and Kelly 1996b |
| *Co-3* | Mexique 1 | Mexico 222 | MA | NA | B4 | Bannerot 1965 |
| *Co-3²* | | Mexico 227 | MA | | | Fouilloux 1978 |
| *Co-3³* | | Co-9 | | BAT 93 | | |
| *Co-4* | Mexique 2 | TO | MA | SAS13,SH18 | B8 | Young et al. 1998 |
| *Co-4²* | | SEL 1308 | MA | SBB14, OC8 | | |
| *Co-4³* | | PI 207262 | MA | OY20 | | |
| *Co-5* | Mexique 3 | TU | MA | OAB3 450 | B7 | Vallejo and Kelly 2001 ; Young and Kelly 1996a |
| *Co-5²* | | SEL 1360 | MA | SAB3 | | |
| *Co-6* | Q | AB136 | MA | $OAH1_{780}$<br>$OAK20_{890}$ | B7 | Vidigal 1994 |
| *Co-7* | | MSU-7<br>G2333 | MA | NA | | Young et al. 1998 |
| *Co-8* | NA | AB136 | MA | OPAZ20 | NA | Alzate-marin et al. 2001 |
| *Co-9* | NA | BAT 93 | MA | SB12 | B4 | Geffroy et al. 1999 |
| *Co-10* | NA | Eauro-Negro | MA | F10 | B4 | Alzate-marin et al. 2003b |
| *Co-11* | NA | Mitchelite | MA | NA | NA | |
| *Co-13* | NA | Jalo Listras | A | $OPV20_{680}$ | Pv13 | Goncalves-Vidigal et al. 2009 |

NA: Not Available; A: Andean, MA: Meso American; Modified source: Kelly et al (2010)

### 2.5.2. *BCMV* and *BCMNV*

#### 2.5.2.1. Introduction and Taxonomy of *BCMV* and *BCMNV*

*BCMV* and *BCMNV* belong to the genus *potyvirus* and are the plant viruses with positive-sense RNA genomes, and 70% of single-stranded RNA viruses infect and undermine the yield common bean in different part of the world. The genus is largest in family *potyviridae* comprising 146 virus species (ICTV 2013; Ivanov et al. 2014)

The taxonomy of family *potyviridae* has undergone major changes since the invention of agriculture and it is believed that the movement of people into new arable land and the set up of trade routes all over the world contributed to spread and evolution of potyviruses (Gibbs et al. 2008 ab).

Bean common mosaic virus (*BCMV*) is an economically important pathogen of common bean (*Phaseolus vulgaris L.*) genotypes. The Bean common mosaic virus (*BCMV*) and Bean common mosaic necrosis virus (*BCMNV*) are transmitted through pollen, seeds and aphids, have an effect on bean production by significantly lowering the yield (Drijfhout 1978; Wax 1987). BCM and BCMN viruses have a wide distribution in many parts of the

world, Europe (Drijfhout 1978; Schmidt 1987), U.S (Kelly et al. 1983; Provvidenti et al. 1984), and Africa (Silbernagel et al. 1986; Spence and Walkey 1995; NjauandLyimo 2000). *BCMV* and *BCMNV* diseases can cause yield losses of 35-100 %, depending on cultivars, environment, time and source of the virus (Hampton 1975; Wortmann et al. 1998; Worrall et al. 2015). The yield losses of common bean attributed to *BCMV* and *BCMNV* diseases remains an economical problem in areas growing susceptible cultivars (Miklas et al. 2006). The *BCMV* and *BCMNV* are seed-borne, sap, pollen and transmitted by several aphid species (Kelly et al. 2003). They infect common beans and cause similar symptoms such as mosaic, malformation of leaves and pods, dwarfing, leaf curling, chlorosis and black root often causing growth reduction (Morales 2006).

Trueman et al (2008) reported that *BCMV* lineage group originated in South and East Asia. Prior to 1934, all strains of *BCMV* and *BCMNV* were pathogenically considered identical and assigned different names Bean virus 1, Bean mosaic virus, and Phaseolus virus 1 (Morales and Bos 1988).

Coat protein (CP) serology, its proteolytic digest and the differential reactions of many bean cultivars to infection led to the classification of *BCMV* strains into serotypes A and B (Drijfhout et al. 1978; Vetten et al. 1992) which were later named *BCMNV* and *BCMV*, respectively (Berger et al. 1997).

#### 2.5.2.2. Genome organization

*BCMV* and *BCMNV* are monopartite RNA viruses whose genomic RNA molecule is approximately 10 kb long, it is polyadenylated at the 3′ end and covalently linked virus-protein-genome (VPg) at the 5′ end, helically surrounded by CP to form flexuous rod-shaped virions (Ivanov et al. 2014) (Figure 2.1).

**Figure 2.1.** The positive-sense single-stranded RNA genome of *BCMV* and *BCMNV*

#### 2.5.2.3. Origin and geographic distribution

Gibbs et al (2008) reported that *BCMV* originated from South and East Asia, spread worldwide with contaminated seeds and aphids in non-persistent manner wherever legumes are grown. Spence and Walkey (1995) conducted a survey in African countries and found that *BCMNV* was endemic with various strain diversity and concluded that *BCMNV* most likely evolved from Central or Eastern Africa. Seed transmission of *BCMV* and *BCMNV* has spread both viruses to many regions including: Europe (Sa´iz et al. 1995; Pasev et al. 2014), North America (Provvidenti et al. 1984; Tu 1986; Flores-Este´vez et al. 2003) and

South America (Melgarejo et al. 2007). Around half of the potvirus populations found in Australia are indigenous virus species in native and colonized wild plants, which could conceivably pose a threat to cultivated plants in the future (Gibbs, Ohshima, et al. 2008; Coutts et al. 2011; Kehoe et al. 2014).

#### 2.5.2.4. Seed transmission and Aphid-mediated Transmission

The strains of both *BCMV* and *BCMNV* can be transmitted by pollen, contaminated seed and contaminated equipment between fields and by viruliferous aphids; *Macrosiphum solanifolii, M. pisi, M. ambrosiae, Myzus persicae, Aphis rumicis, A. gossypii, A. medicaginis, Hyalopterus atriplicis*, and *Rhopalosiphum pseudobrassicae* (Zaumeyer and Meiners 1975; Zettler and Wilkinson 1966). Seed transmission of both viruses was revealed by electron microscope and it revealed that *BCMV* particles were present in cells and tissues of infected plants (Hoch and Provvidenti 1978). Seeds of common beans can retain infectious pacticles of *BCMV* and *BCMNV* for at least 30 years and this stability is the most important factor in long-distance spread of potyviruses around the world (Pierce and Hungerford 1929). The rate at which *BCMV* and *BCMNV* are transmitted through seed transmission is irregular and depends upon bean cultivar, potyvirus strain, stage of infection and plant age at the time of infection (Sastry 2013).

The rates of peanut seed infection with the *BCMV* strain PStV in Georgia (USA), decreased from 37% in summer to 18% in winter, also it decreased from 19% to 11% in a Spanish cultivar (Demski and Warwick 1986). When viruliferous aphid population is high, it can result in yield losses as high as 100% (Galvez and Morales 1989). Seeds produced by plants infected prior to flowering are always account for the higher transmission rates (Udayashanka et al. 2012). Aphids retain potyviruses for viral acquisition and transmission on their stylets for only a limited time (Westwood and Stevens 2010). They transmit viruses in non-persistent manner and spread on short distances.

#### 2.5.2.5. Resistance genes against *BCMV* /*BCMNV* diseases

A number of methods are recommended in the control of these two bean diseases such as crop rotation, removal of contaminated plant materials, use of fungicides and resistant varieties (Pastor-Corrales et al. 1994). Chemical control elevates production costs and is not eco-friendly. The use of resistant cultivars provides farmers a low-price alternative for managing the diseases and boosting their production. The use of chemicals lessens the spread of aphid vectors, the carriers of both viruses and seed certification programs may reduce the viral inoculum levels, however, a long lasting solution to address the economic losses of beans caused by potyvirus diseases would come from planting resistant cultivars.

Table 2.2 shows DNA markers linked to resistance genes for *BCMV* and *BCMNV* that have been proven most useful to underpin marker-aided selection in different market classes belonging to the Middle American and Andean gene pools and these provide an opportunity to enhance disease resistance through gene pyramiding (Haley et al. 1994;

Melotto et al. 1996; Morales and Kornegay 1996). The best way to prevent the *BCMV* disease is to develop genetically resistant cultivars and the genes confer resistance are single dominant (*I*) and four recessive (*bc-u, bc-1, bc-2, bc-3*) genes (Drijfhout, 1978; Kelly et al. 1995). Pyramiding of specific resistance genes in a single cultivar is strategy to achieve durable resistance in the common beans against *BCMV*.

The hypersensitive resistance response to *BCMNV* and *BCMV* conditioned by the dominant inhibitory (*I*) gene has been noted for the last half century (Kelly 1997; Ali 1950) whereas the recessive strain specific resistance genes *(bc-1, bc-1*$^{2}$, *bc-2, bc-2*$^{2}$ and *bc-3)* that require an independent helper nonspecific *bc-u* gene to be functional have been demonstrated to be effective and long lasting in controlling diseases by restricting virus replication or movement within the plant (Drijfhout 1978; Kelly 1997; Johansen et al. 2001; Kelly et al. 2003).

Common bean genotypes carrying the recessive strain specific resistance *bc-3* gene found to carry homozygous mutations in a PveIF4E coding sequence. The mutated forms of translation initiation factor 4E (eIF4E) and/or its isoform eIF(iso)4E disrupt the interaction between VPg and eIF4E therefore plants loose susceptibility (Kang et al. 2005; Beauchemin et al. 2007). In the *Arabidopsis thaliana*, two proteins namely; AteIF4E-1 and AteIF(iso)4E are associated with potyviral infection and their homologues are linked to potyviral resistance in other plant species (Robaglia and Caranta 2006; Hwang et al. 2009). The *bc-3* gene resides on chromosome B6 and confers resistance to all known strains of *BCMNV* and *BCMV* in the presence of the dominant *I* gene (Kelly et al. 2003). The dominant *I* gene confers extreme resistance against all strains of bean common mosaic virus when temperature stays below 30°C (Ali 1950; Fisher and kyle 1994; Collmer et al. 2000). The *I* gene prevents the spread of pathogens through induced cell death (Collmer et al. 2000; Cadle-davidson and Jahn 2005).

Recessive gene *bc-u* is only required for the expression of other specific resistance genes, *bc-1*, *bc-1*$^{2}$*, bc-2, bc-2*$^{2}$, and *bc-3* (Drijfhout 1978; Kelly et al. 1995). The mechanism of the resistance conferred by recessive genes to potyviruses is through disruption of the interaction between eIF4E protein and the VPg protein covalently linked to the 5’ terminus of a potyvirus genome (Naderpour et al. 2010; Hart and Griffiths 2013).

**Table 2.2.** Dominant and recessive resistance genes and their linked markers in Common beans

| Gene | Linked markers | Map location | References |
|---|---|---|---|
| *bc-1* | | | Strausbaugh et al. 1999 |
| *bc-1*$^2$ | SBD5 | B3 | Miklas 2000 |
| *bc-2* | | | |
| *bc-2*$^2$ | | | |
| *bc-3* | SCAR $OC11_{350/420}$ | | Johnson et al. 1997 |
| | RAPD $OC20_{460}$ | | |
| | RAPD $OG6_{595}$ | | |
| | $E_{ACA}M_{CGG}$ STS, SG6 | | Mukeshimana et al. 2005 |
| | CAPS-eIF4E$^2$-*Rsal* | B6 | Naderpour et al. 2010 |
| *bc-u* | | B3 | Strausbaugh et al. 1999 |
| *I* | RAPD $OS13_{690}$ | | Haley et al. 1994b |
| | *BCMV* -48289723-CAPS | B2 | Bello et al. 2014 |

The objective of the study was to introgress different genes conferring wider resistance spectra against *C. lindemuthianum* races and *BCMV* into the Rwandan common beans using molecular markers.

# 3. MATERIALS AND METHODS

## 3.1. Plant Materials and Their Growth Habits

Seeds from four recurrent populations of *P. vulgaris*; G54, RWR 1668, RWR 2355, and RWV 2361 were obtained from RAB (Rwanda Agriculture Board, Rwanda) and donor parents; Ac-hensal and USCR-7, carrying a combination of *bc-3,Co-1,Co-2* and *I* resistance genes, were obtained from National Plant Germplasm System (GRIN),USAID,USA.

The donor parents namely Ac-hensal specifically carried *bc-3, Co-1* and *I* resistance genes while USCR-7 carried *bc-3, Co-2* and *I* resistance genes (Figure 3.2). Growth habits of plant materials were taken into consideration (Table 3.1). The Ac-hensal seeds are small, round and white in color. The G54 seeds are small, navy white, and kidney shape. The RWR 1668 seeds are large and dark red. The RWR 2355 seeds are medium and cranberry in color. The RWV 2361 seeds are medium-sized seeds, pinto in color. The USCR-7 seeds are large, pinto color (Figure 3.1).

## 3.2. Experimental Site, Crossing and Management Measures

The entire field experiments were conducted at Akdeniz University green house, Antalya, Turkey. The present study was conducted in six successive seasons (fall and spring, 2016-2019). The planting dates include; September 2016, February 2017, September 2017, February 2018, September 2018, and the last experiment was February, 2019.

The six parents were crossed through emasculation process. Seeds were sown manually in lines for ease of drip irrigation purpose in a prepared soil supplied with fertilizer. Plowing and harrowing exercises were performed before sowing season starts. The frequency of drip irrigation largely depended on the weather and was held when necessary. NPK fertilizer was applied twice in each season.

Hand weeding and other good agronomic practices were performed when necessary. Insecticides (Confidor, Mospilan, Admiral and Agrimec) were bimonthly sprayed on crops. Flower buds that were near to open were opened with forceps, hand pollinated, closed to maintain humidity, and the activity was conducted at either morning or evening hours. Every pollinated flower was labelled and tagged, and nurtured until its pod is harvested. The dried pods from crosses were harvested at the end of each season and their respective seeds were kept in seed bags (Figure 3.2).

**Figure 3.1.** Parental seeds used in the experiment

**Table 3.1.** Characteristics of parental plant materials used in study

| Cultivar | Growth habit | Gene pool | Seed color | Response to Anthracnose and *BCMV* |
|---|---|---|---|---|
| Ac-hensal* | Bush | MA | White | + |
| G 54 | Semi-climber | MA | Navy white | - |
| RWR 1668 | Bush | A | Dark red | - |
| RWR 2355 | Semi-climber | A | Cranberry | - |
| RWV 2361 | Climber | A | Pinto | - |
| USCR-7+ | Bush | A | Pinto | + |

A: Andean, MA; Mesoamerican.

+: Resistant to Anthracnose and *BCMV*, -: Susceptible to Anthracnose and *BCMV*

*= *bc-3, Co-1* and *I genes*, += *bc-3, Co-2* and *I* genes

**Figure 3.2.** Seed bags

### 3.3. Segregating Populations and Advanced Lines Development

Segregating populations derived from crosses between dry beans were developed; namely G54×Ac-hensal, RWR 1668×Ac-hensal, RWR 2355× Ac-hensal and RWV 2361× USCR-7. The F1 generation individuals were both self-pollinated to produce F2 and backcrossed to their respective recurrent parents to generate BC1, BC2, and BC3 populations. Segregating and advanced line development populations developed were; 92 F2, 52 BC1F1, 51 BC2F1, 44 BC3F1 and 76 BC3F2 (Figure 3.3).

Pollination was made manually with emasculation during crossing exercises through out entire experiments. Crosses were made through emasculation of the opened flowers from resistant plants viz; USCR-7 and Ac-hensal cultivars that carry *bc-3, Co-1, Co-2* and *I* genes, followed by transfer of pollens to the stigmas of recipient plants, recurrent plant materials. Eventually buds were closed to minimize unintended crossing and to sustain humidity around the stigmas.

### 3.3. DNA Extraction and Molecular Markers

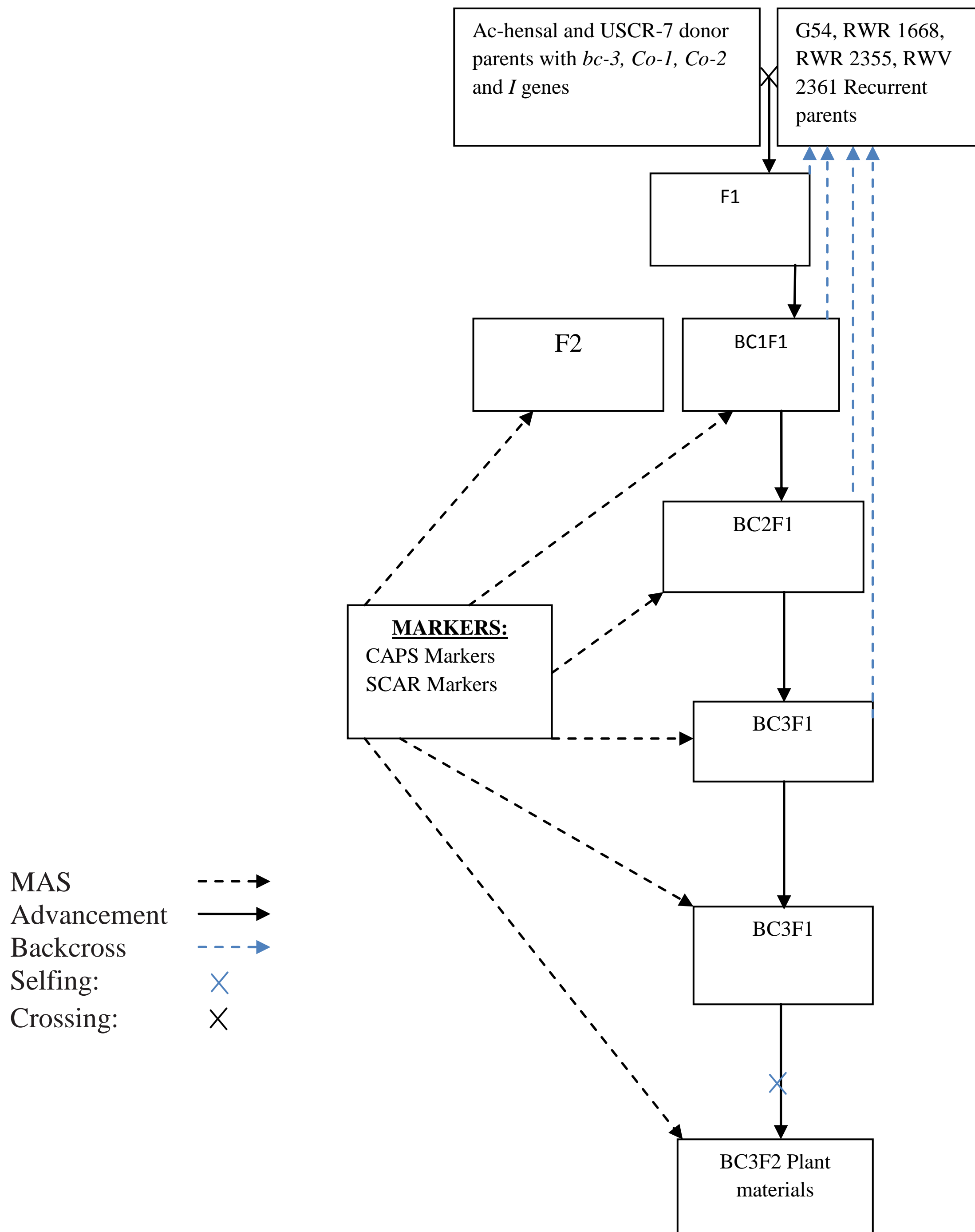
#### 3.3.1. DNA extraction

The gDNA of parents, F2 and BC populations was extracted from their respective young leaves of seedlings collected from greenhouse (Figure 3.4). A few milligrams of fresh tissues were ground and dispersed in 500 µL of extraction buffer [1.4 M of NaCl, 20 mM of EDTA, 100 mM of Tris–HCl (pH 8), 2% CTAB, and plus 0.2 % beta-mercaptoethanol added just before use] (Doyle and Doyle 1990) with minor modifications (Figure 3.5).

The suspension was mixed well, incubated at 65°C for 2 h, the homogenate was extracted with 500 µL chloroform–isoamyl alcohol (24:1), mixed gently for 1 min, centrifuged for 30 min at 13100$g_n$ in a microcentrifuge. After centrifugation, the upper (aqueous) phase was transferred to a new set of 1.5 mL microtubes and precipitated with 350 µL ice-cold isopropanol at −20 °C for overnight. The pellet formed after centrifugation at 13100$g_n$ for 10 min was washed twice with 200 µL of 70 % ethanol. The resulting pellet was set to dry for approximately 30 min with the tubes inverted over a filter paper at room

temperature and then resuspended in 100 µL sterile distilled water; the tubes were incubated at 37ºC for 30 min and the DNA was stored at −20 °C until use.

**Figure 3.3.** Schematic diagram showing a breeding strategy used to develop advanced common bean lines pyramided with *BCMV* /*BCMNV* and anthracnose resistance genes



### 3.3.2. Molecular markers

Cleaved Amplified Polymorphic Sequence (CAPS) and Sequence Characterized Amplified Regions (SCAR) markers were used to indirectly select *BCMV* /*BCMNV* and bean anthracnose resistance genes, respectively (Table 3.2). The primers were ordered from the Thermo Scientific Company. 1 kb DNA ladder designed for determining the size of DNA was used (Table 3.2).

**Figure 3.4.** Collection of young leaves for DNA extraction

**Figure 3.5.** DNA extraction

### 3.3.2.1. CAPS Markers

CAPS technique which works on the principle that a single base changes between individuals can create or abolish restriction endonuclease sites. The CAPS assays utilize DNA fragments amplified by the PCR that are digested with a restriction endonuclease to display a restriction fragment length polymorphisms, the fragments were fractionated by agarose gel after digestion with an appropriate restriction enzyme (Tragoonrung et al. 1992).

CAPS and SCAR markers were utilized in marker-assisted selection; ENM CAPS tagged *bc-3* allele, *BCMV*-48289723-CAPS tagged *I* resistance allele (Table 3.2). Number of genes, 0 to 3 in both segregating populations and advanced lines were selected using markers and fixed through selfing up to BC3F3 generations.

**Table 3.2.** Molecular marker sequences used for selection of *I* , *bc-3, Co-1* and *Co-2* genes

| Gene | Marker type | Enzyme | Primer sequence (5'---3') | LG | Fragment (bp) | Reference |
|---|---|---|---|---|---|---|
| *I* | CAPS | *TaqI* | F: AGGAGGAAGAACGGTGGTC | 2 | 311(201/110) | Bello et al. 2014 |
| | | | R: TTTGGTGGTAATTTGAAAATGG | | | |
| *bc-3* | CAPS | *RsaI* | F: ACCGATGAGCAAAACCCTA | 6 | 541(381/160) | Naderpour et al. 2010 |
| | | | R: CAACCAACTGGTATCGGATT | | | |
| *Co-1* | SCAR (STK-1) | | F: AAAACATTTGGATTCGCTAC | 1 | 110 | Chen et al. 2017 |
| | | | R: AACTATTGGACAAGGGATG | | | |
| *Co-2* | SCAR(SQ4) | | F: CCTTAGGTATGGTGGGAAACGA | 11 | 1440 | Genchev et al. 2014 |
| | | | R: TGAGGGCGAGGATTTCAGCAAGTT | | | |
| *Co-2* | SCAR(SCH20) | | F: GGGAGACATCCATCAGACAACTCC | 11 | 450(260/136) | |
| | | | R: GGGAGACATCTTCATTTGATATGC | | | |

L G: linkage group; F: Forward primer; R: Reverse primer, bp: base pair
CAPS: Cleaved Amplified Polymorphic Sequence.
SCAR: Sequence Characterized Amplified Region
bp: base pair

The primers were synthesized based on the sequence information available in databank. CAPS markers are co-dominant, locus specific and have been used to distinguish between plants that are homozygous or heterozygous for alleles, and the assays are reproducible. The use of restriction enzymes to detect polymorphic nucleotides by the loss or gain of a restriction enzyme recognition site is called CAPS (Konieczny and Ausubel 1993) and used to increase the number of polymorphisms detected by a single marker.

### 3.3.2.2. Materials for markers analysis.

1. 2.5 mM dNTPs; 2.5 mM dATP, 2.5 mM dTTP, 2.5 mM dCTP, 2.5 mM dGTP Nucleotides were diluted from commercial grade 100 mM solutions, and stored in aliquots at -20$^0$ C.
2. Forward and reverse primers (see table 3.2).
3. Taq Polymerase
4. Taq polymerase buffer
5. $MgCl_2$
6. Restriction endonucleases (*RsaI* and *TaqI*)
7. Restriction endonuclease buffer(Tangoo)
8. Water bath
9. PCR tubes
10. Thermal cycler
11. Distilled water
12. DNA template

### 3.3.2.3. CAPS reaction

*BCMV-48289723-CAPS* and ENM CAPS markers were used to identify *I* and *bc-3* genes, respectively (Table 3.2). A total of two CAPS markers were screened against parents and progenies. The PCR products generated were digested with *RsaI* and *TaqI* enzymes in separate sets (Table 3.2).

PCR reactions were performed in a total volume of 12 µL containing 1X *Taq* buffer $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1 mM dNTPs, 5 Units of Taq polymerase, 4 µM of each primer and 2 µL gDNA in a PTC-200 thermocycler (Bio-Rad, Hercules, CA).

PCR Amplifications involved initial denaturation step at 95°C for 5 min, programmed for 35 cycles at 95°C for 1 min; 53°C for 1 min; 72°C for 2 min and a final phase at 72°C for 10 min. Eight µL of PCR amplicon was digested with *RsaI* and *TaqI* enzymes separately in a final volume of 15 µL, incubated for 2 h at 37°C and 65°C, respectively (Table 3.3).

### 3.3.2.4. DNA quantification

Loading dye was mixed with PCR products, the dye serves two main purposes, it is for visual tracking of DNA migration during electrophoresis and rendering the samples denser than the running buffer. The products were separated on a 2% agarose gel with ethidium bromide, run in 1X TBE buffer at 120 V for 1.5 h. The DNA fragments were viewed under ultraviolet light and the image was captured using the *DNR* Bio-imaging systems.

**Table 3.3.** Restriction digestion protocol

| Add: | | Add: | | Add: | |
|---|---|---|---|---|---|
| Nuclease-free water | (6.0 µL) | Nuclease-free water | (6.0 µL) | Nuclease-free water | (6.0 µL) |
| 10X Buffer Tango | (1.0 µL) | 10X Buffer Tango | (1.0 µL) | 10X Buffer Tango | (1.0 µL) |
| TaqI | (0.5 µL) | RsaI | (0.5 µL) | DdeI or HpyF31 | (0.5 µL) |
| PCR amplicons | (8.0 µL) | PCR amplicons | (8.0 µL) | PCR amplicons | (8.0 µL) |
| Mix gently and spin down | | Mix gently and spin down | | Mix gently and spin down | |
| for a few seconds. | | for a few seconds | | for a few seconds | |
| Incubate in a capped | | Incubate in a capped | | Incubate in a capped | |
| vial at 65°C for 2 h | | vial at 65°C for 2 h | | vial at 65°C for 2 h | |

### 3.3.2.5. SCAR reaction

STK-1, SQ4 SCAR markers were used to identify *Co-1* and *Co-2* genes (Table 3.2). A total of two SCAR primers were screened against parents, F2 and BC progenies. PCR reactions were performed in a total volume of 15 µL containing 1X *Taq* buffer $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1 mM dNTPs, 5 Units of Taq polymerase,4 µM of each primer and 2 µL gDNA in research a PTC-200 thermocycler (Bio-Rad, Hercules, CA).

The amplification conditions with the SQ4 marker are as follows: one cycle of 3 min at 94°C, followed by 35 cycles of 10 sec at 94°C, 40 sec at 60°C, 1 min at 72°C, and final elongation for 10 min at 72°C. The amplified products were visualized on 2% agarose gel.

PCR Amplification conditions with the STK-1 marker are as follows: one cycle of 3 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 50°C for 30 sec; 72 °C for 1 min and a final phase at 72°C for 10 min. PCR products were separated on a 2% agarose gel with ethidium bromide, run in 1XTBE buffer at 120 V for 1.5 h. The DNA fragments were viewed under ultraviolet light and the image was captured using the *DNR* Bio-imaging systems.

## 3.4. Phenotypic, Morphological and Agronomic Observations

The seeds were sown in plots consisting of 8 rows of 20 m long, spaced 0.5 m from each other, with an average density of 40 plants per line. Observations were recorded at the appropriate developmental stages of plant growth and development. They were characterized as per the minimal descriptors. Ten plants from the rows of each genotype

were randomly taken and labeled; descriptors of common bean were evaluated according to International Board for Plant Genetic Resources descriptor list (1982).

The collected plants were evaluated for the agronomic traits as follows; observations on number of days to emergence (d), plant height (cm), days to flowering (d), pod length (cm), number of pods per plant, number of seeds per pod, days to physiological maturity, 100-seed weight (g), number of pyramided genes and these were randomly recorded from 10 plants.

### 3.4.1. Number of days to emergence and germination percentage

The number of days to emergence in different cultivars of parents and BC progenies was recorded by counting in the field from date of sowing until the seed germinates.

$$\text{Germination percentage} = \frac{\text{total number of germinated seeds}}{\text{total number of seeds sown}} \times 100\%$$

### 3.4.2. Plant height

The average plant height of the cultivars was measured and expressed in centimeters, which was taken after 45 days of emergence, measured from the cotyledon scar to the plant tip. Ten plants from randomly selected parents and their respective BC progenies were chosen for the evaluation of plant height.

### 3.4.3. Days to flowering and flower characteristics

Average number of days to flowering was estimated by counting number of days from sowing day to the date on which nearly 50% of sampled parent materials and their respective BC progenies in all cultivars had set at least one flower. The colors of freshly opened flowers were also recorded.

### 3.4.4. Pod characteristics

The average Pod length was measured from exterior distance of the pod apex to the peduncle in centimeters of the largest fully expanded mature pods. This was recorded from ten random plant materials of parents and BC progenies. The number of pods per plant material in both parents and their respective BC progenies was recorded.

### 3.4.5. Days to physiological maturity

Average number of days from sowing to physiological maturity was estimated as the number of days from emergence until 90% of pods of the sampled plants have changed the color of their pods.

### 3.4.6. Seed characteristics.

Variation in morphological seed descriptors was evaluated; these include seed color (primary and secondary), number of seed colors (one, two, more than two), distribution of secondary seed color, seed shape and brightness of seed were recorded.

The average number of seeds per pod was counted from 10 randomly selected mature pods, seed weight per plant was recorded from 10 randomly selected plants and 100-seed weight was measured. An average 100-seed weight in grams was recorded on a randomly drawn sample of 100 sun and air dried seeds. Extrapolation was done to the plants that had less than100 seeds.

## 3.5. Statistical Analysis

### 3.5.1. Molecular data

Goodness-of-fit test was performed at the significance level of 0.05 to check for deviation from the expected ratio 3R:1S (R: resistance allele, S: susceptible allele) for, Co-1, Co-2 and *I* genes and 1R:3S for *bc-3* gene in the F2 population and 1R:1S for the four genes in BC populations with both CAPS and SCAR markers. The *p-values* were calculated from *chi*-square scores.

### 3.5.2. Field Data analysis

The Student t-test was computed for its statistical significance at the confidence level of 95 %. The correlation coefficients were calculated for their statistical significance at the confidence levels of 99 % and 95 %. The degree of association (correlation coefficients) of various traits was calculated through correlation analysis. The calculated values of correlation coefficient were compared with tabulated Pearson's-r value at n-2 degree of freedom where "n" is the number of observations, statistical analyses were performed with the help of Excel and SPSS.

Correlation matrix and path data analyses were done on days to emergence, plant height, days to flowering, pod length, number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weigh (g), days to physiological maturity, number of pyramided genes and seed weight per plant.

The raw data were converted into standardized data before path analysis. Unlike regression of raw data that results into concrete regression coefficients, standardized variables give partial regression coefficients or direct path coefficients. This study contains responsive variable, the seed weight per plant and independent variables (the yield related components). The equation $x^* = (x-\ddot{x})/\sigma$ was applied to convert raw data into standardized data (Akintunde, 2012).

Where $x^*$ = Standardized variable,

x = Its respective un-standardized variable,

ẍ = Mean of un-standardized individual variables

σ = Standard deviation of un-standardized individual variables

Correlations examined the associations between individual yield related components and the path analysis examined the direct contributions and indirect effects through other variable to the effect variable.

Path coefficient analysis was done based on the correlation coefficients. Seed weight per plant was the dependent variable and the rest were independent variables. The path analysis was carried out according to Akintunde (2012).

$rij = pij + \Sigma r_{ik} p_{kj}$

where, rij = is the correlation coefficient resulted from mutual association between the independent variables (i) and the dependent variable (j), Pij is the component of direct effects of the independent trait (i) on the dependent variables (j) and $\Sigma\ r_{ik}p_{kj}$ is the sum of yield related components of the indirect effects of an independent variable (i) on the dependent variable (j) through all other independent variables (k).

The residual effect plays an important role in validating the regression model, it refers to the contribution of unknown factors, determines how best the independent variables stand for the variability of the dependent variable. The residual effect was calculated using excel.

# 4. RESULTS AND DISCUSSION

## 4.1. Introgression and Inheritance of Resistance Genes

Developing bean cultivars with a complex resistance to all races of bean anthracnose and various strains of *BCMV* identified in East Africa is one of the most effective ways of controlling these important pathogens and this can be achieved by pyramiding of the resistance genes in a single genetic background.

The crosses were performed between G54 × Ac-hensal, RWR 1668 × Ac-hensal, RWR 2355 × Ac-hensal and RWV2361 × USCR-7 (Figure 3.2). Resistant genes against anthracnose and *BCMV* were introgressed into lines G54, RWR 1668, RWR2355 and RWV 2361 through backcross programs (Figure 3.3), and advanced breeding lines were obtained. Figure 4.2 shows seed phenotypes of the donor parents, recurrent parents and the advanced lines. Parents; Ac-hensal and USCR-7 were the sources of *bc-3*, *Co-1*, *Co-2* and *I* resistances genes conferring resistance to races of *C. lindemuthianum* and various strains of *BCMV*. Both donor and recurrent parents served as controls.

The breeding program was made in various steps (Figure 3.3). The first step was making the cross between donor and recurrent parents. In theory, all F1 plants were expected to be resistant since resistance genes were dominant except plants with *bc-3* gene that is recessive.

The part of second step was selfing to develop F2. The results from the segregating populations carried out with 92 F2 offspring populations from crosses between the G54 × Ac-hensal, RWR 1668 × Ac-hensal, RWV2361 × Ac-hensal and RWR 2355 × USCR-7 cultivars were obtained.

The inheritance study in the F2 offspring developed from selfing of F1 generations showed a good fit ($\chi^2 = 0.93$; $p = 0.33$) to a segregation ratio of 1:3 confirming the presence of single recessive resistance gene, *bc-3*. The SCAR marker analysis of the F2 population showed a good fit of 3R:1S ($\chi^2 = 0.125$; $p = 0.26$) for *Co-1* gene, 3R:1S ($\chi^2 = 0.89$; $p = 0.35$) for *Co-2*. The CAPS analysis segregation ratio supported a good fit of 3R:1S ($\chi^2 = 0.93$; $p = 0.33$) for *I* gene, confirming the independence of single dominant resistance genes (Table 4.2). Another part of second step was backcross to develop BC1F1. The F1 plants were backcrossed to their respective recurrent parents in the greenhouse to develop BC1F1. In backcross generation, resistant plants were identified and selected using molecular markers. At this step of backcross, the BC1F1 plants showed nearly a 1R:1S ratio segregation with the aid of markers (Table 4.3). The inheritance study in the BC1F1 generation showed deviation ($\chi^2 = 4.92$; $p = 0.03$) to a segregation ratio of 1:3 for *bc-3* possibly due to a small size of BC1F1 population used in the study. The SCAR marker analysis of the BC1F1 generation showed a good fit of 1R:1S ($\chi^2 = 1.78$; $p = 0.18$) for *Co-1* gene, and 1R:1S ($\chi^2 = 1.00$; $p = 0.32$) for *Co-2* loci. The CAPS analysis segregation ratio supported a good fit of 1R:1S ($\chi^2 = 0.31$; $p = 0.58$) for *I* gene, confirming the independence

of single dominant resistance genes (Table 4.3). The resistant plants were selected in the breeding program, and the segregating populations showed a 1 resistant: 1 susceptible ratios segregation in BC populations. This indicates the introgression of independent resistance genes.

As a result of the breeding programs, four cultivars resistant against bean anthracnose and *BCMV* were developed; the advanced lines of G 54 BC developed from the donor Ac-hensal and G54 P, RWR 1668 BC from line Ac-hensal and RWR 1668 P, RWR 2355 BC from the line Ac-hensal and RWR 2355P, RWV2361 BC short and tall both from the line USCR-7 and RWV2361 P. Figure 4.2 shows the two donor genotypes (Ac-hensal and USCR-7), the recurrent parents (G54 P,RWR 1668 P, RWR2355P and RWV 2361 P) and the advanced breeding lines and their seed coats color recovery.

The third step was the backcross of BC1F1 to their respective recurrent parents in the greenhouse to develop BC2F1 populations. Identification of BC1F1 plants with resistance genes was carried out with the aid of molecular markers. At this step selection was done with the aid of markers and crosses were made through emasculation. A total of 51 BC1F1 plants were involved in backcross to their respective recurrent parents in the greenhouse to develop BC2F1 (Figure 3.3).

The forth step was the backcross of BC2F1 to their respective recurrent parents in the greenhouse to develop BC3F1. Identification of BC2F1 plants with resistance genes was carried with the aid of markers. At this step selection was done with the aid of markers and crosses were made through emasculation. All backcross processes were designed to recover the recurrent genome content and seed coat color (Figure 4.2).

The fifth step was the selfing of 44 BC3F1 to develop BC3F2. Identification of BC3F1 plants with resistance genes was carried out with the aid of markers before the plant set the flowers. At this step selection was done with the aid of markers and self-pollination took place. All backcross processes were designed to recovering the recurrent genome content and their respective seed coat color, but selfing was done purposely to achieve homozygosity for the genes of interest (Figure 3.3).

In each backcross generation (BCnF1), selections were carried with the aid of markers; CAPS and SCAR markers. Resistant plants against bean anthracnose were analyzed with the SCAR markers; STK-1 linked to gene *Co-1* and SQ4 linked to gene *Co-2*, and those showing the expected amplification product were selected. Resistant plants against *BCMV* were analyzed with the CAPS markers; ENM-CAPS specific to *bc-3,* BCMV-48289723-CAPS specific to *I* gene and those showing the amplification product after digestion with restriction enzymes were selected.

The last step of breeding program was selfing of 76 BC3F2 to develop BC3F3 generation for fixing all resistance genes acquired. The plants were evaluated with molecular markers, and a segregation ratio corresponding to resistance genes was determined with Chi-square test. At this step of selfing, the BC3F2 plants showed nearly a

3R:1S ratio segregation with the aid of markers (Table 4.4). The inheritance study in the BC3F2 generation revealed deviation ($\chi^2 = 7.02$; $p = 0.01$) to a segregation ratio of 3:1 for *I* possibly due to a small size of one genotype in four genotypes evaluated in this study. The SCAR marker analysis of the BC3F2 generation showed a good fit of 3R:1S ($\chi^2 = 0.67$; $p = 0.41$) for *Co-1* gene, and 3R:1S ($\chi^2 = 1.26$; $p = 0.26$) for *Co-2* loci. The CAPS analysis segregation ratio supported a good fit of 1R:3S ($\chi^2 = 0.47$; $p = 0.43$) for *bc-3* gene, confirming the independent segregation of resistance genes (Table 4.4). By this breeding program we aimed at achieving broad-spectrum resistance in an individual plant with stable traits.

Our findings are in agreement with the segregation ratio observed in the population developed from Andecha x Mexico 222 crosses, inoculated with *C.lindemuthianum* race 38 that produced a F2 segregation ratio of 3R:1S (Méndez-Vigo et al. 2005). In contrary, adjusted resistance segregation ratio of 1R:3S in the F2 offspring resulted from Tuscola (R) x Montcalm (S) cross and Michelite x MDRK cross that were inoculated with *C. lindemuthianum* race beta was reported (Cardenas et al 1964; Muhalet et al 1981). The results implied reverse dominance. Reversal of dominance occurs as a result of a multi-allelic series residing at the same locus (Kelly and Vallejo 2004).

## 4.2. Molecular Marker Analysis

Markers are used in indirect selection of crops with target genes. Markers for disease resistance are efficient, neutral, not affected by incomplete or masked expression or low heritability or environment, provide the advantage of allowing selection for resistance in the absence of the pathogen and scored at any stage of plant growth and development. Breeding for multiple disease resistance is greatly facilitated by markers linked to various resistance genes. Marker-assisted selection dictates indirectly selection for one or more highly desirable disease resistance genes tightly linked to the genes of interest (Melchinger 1990).

Various breeding strategies have been devised for accumulation of multiple disease resistance traits into breeding lines. However, in some cases, resistance genes are lost during segregation in segregating populations and use of markers ensures durable stable resistance through tagging genes in breeding lines.

It is clear that single resistance gene looses resistance easily but gene pyramiding provides resistance against many races of pathogens for many years. Spring wheat cultivars possessing six resistance genes provide durable resistance against stem rust in North America (Schafer and Roelfs 1985).

The SCARs are PCR-based markers that represent gDNA fragments at genetically defined loci and were first developed for downy mildew resistance genes in lettuce. They were identified by PCR reaction using sequence specific oligonucleotide primers (Paran and Michelmore 1993).

Development of SCAR markers involves cloning and sequencing of the amplified specific band products of arbitrary marker techniques with the two ends of the cloned products. The sequence is thereafter utilized to design specific primer pairs which amplify major specific bands of the size similar to that of cloned fragment. SCAR experiments are rapid, easy to perform and reproducible. SCAR markers are both co-dominant and dominant, specific, more informative and may contain high-copy number and spread all over genomic sequences within the amplified region. The SCAR marker gels are cleaner, readable and easier to score.

Markers have been utilized to tag targeted genes in common bean (Nodari et al. 1993; Haley et al. 1993; Degremont and Vallejos, 1994; Johnson and Gepts 1994; Jung et al. 1994), rice (Mohan et al. 1994), and wheat (Schachermayr et al. 1994), lettuce (Paran et al. 1991), pepper (Tanksley et al. 1988), potato (Ritter et al. 1991), and tomato (Nienhuis et al. 1987; Sarfatti et al. 1991; Williamson et al. 1994).

The six bean genotypes were analyzed using a set of four molecular markers linked to their respective resistance alleles; the alleles were introgressed and pyramided into breeding lines. Table 3.2 shows the set of four molecular markers utilized and their gene products. The expression of the bands was consistent in donor parents with amplification products of 381 and 160 bp for markers ENM-CAPS, 201 and 110 for BCMV-48289723, 110 bp for STK-1 and 1440 bp for SQ4 linked to the *bc-3*, *I*, *Co-1* and *Co-2* resistance genes, respectively (Table 3.2).

The PCR amplification products for SCAR markers were absent in the four recurrent parents. The PCR amplification products for CAPS markers were present in all parents, but restriction digestion products were absent in recurrent parents. The expression of molecular markers was consistent with the segregating and backcross populations.

The primer pair BCMV-48289723 F/R and ENM-FWe/RVe were utilized in order to amplify a fragment of genomic DNA (gDNA) containing the polymorphic *TaqI* and *RsaI* sites, and PCR amplification generated 311 and 541 bp, respectively. Digestion of the PCR products from donor parents (Ac-hensal and USCR-7) with restriction endonucleases resulted in cleavage into resistance allele bands of 201 and 110 bp for *TaqI* and 381 bp and 160 bp fragments for *RsaI*.

In contrast, PCR products from susceptible genotypes (G54, RWR 1668, RWR 2355 and RWV 2361) remained uncut by *TaqI* and *RsaI* due to the modification of the restriction sites (Figure 4.1). These allele-specific assays detected *bc-3* (*bc-3bc-3*) and *I* resistance alleles (*II*), *Bc-3Bc-3* and *ii* in all susceptible alleles evaluated with the CAPS markers. The markers were able to distinguish between resistant and susceptible individuals in parents, segregating populations and advanced lines (Figure 4.1). The primer BCMV-48289723-F/R amplified DNA fragment and generated 311 bp, which in turn after digestion resulted in cleavage into 301, 201 bp and 110 bp fragments in heterozygote individuals (Figure 4.1). The observed polymorphism of BCMV-48289723-CAPS marker

in this study resembles what was reported previously associated with resistance to bean common mosaic virus resistance in common bean (Bello et al. 2014).

The primer pair ENM-FWe/RVe was chosen in order to amplify a fragment of gDNA containing the polymorphic *RsaI* site as predicted from *P. vulgaris* and the *A. thaliana* (Lellis et al. 2002; Naderpour et al. 2010). The primer pair ENM-FWe/RVe on the *P. vulgaris* gDNA generated an expected size of 541 bp fragment from both parents and segregating populations evaluated. The co-dominance and fragment length polymorphisms were displayed after restriction digestion of their respective PCR products.

Digestion of PCR fragments with *RsaI* from *PveIF4E*$^{2}$ cultivars, including Ac-hensal and breeding lines resulted in cleavage into 381 bp and 160-bp, the fragments were fractionated by agarose gels (Tragoonrung et al. 1992; Konieczny and Ausubel 1993) and all predicted to carry the *bc-3* gene. Digestion of PCR fragments by *RsaI* enzyme from *PveIF4E*$^{1}$ cultivars, including G54, RWR 1668, RWR 2355 and RWV 2361 parents resulted into uncut fragment band of 540 bp by size. Heterozygote individuals found to have both susceptible (541 bp) and resistance alleles (381 bp and 160 bp) (Figure 4.1).

Our results are in favor of previous results published by Bello et al (2014) on the CAPS markers co-segregated with the *I* gene in the full BeanCAP panel and a RIL population. The observed polymorphism of ENM-CAPS marker specific for *bc-3* resistance allele in this study resembles what was reported previously associated with resistance to potyviruses in *P.vulgaris* and other species: *C. annuum*, *L. sativa* and *P. sativum* (Robaglia and Caranta 2006; Beauchemin et al. 2007; Naderpour et al. 2010).

Segregating populations arising from crosses between G54XAc-hensal, RWR 1668xAc-hensal, RWR 2355xAc-hensal and RWV 2361xUSCR-7 were importantly used to study inheritance patterns of resistance genes in diverse genetic backgrounds. The marker analysis was carried out on 92 F2, 52 BC1F1, 51 BC2F1, 44 BC3F1 and 76 BC3F2 populations. The chi square test was calculated in 92 F2 and 51 BC1F1 and 76 BC3F2 populations.

The 28 F2 population of G54 showed a good fit to the expected 1:3 ratio ($p$ = 0.66) for *bc-3,* a good fit to the expected 3:1 ratio ($p$ = 0.66) for *I* gene and a good fit to the expected 3:1 ($p$ = 0.08) for *Co-1*gene. The 20 F2 population of RWR 1668 showed a good fit to the expected 1:3 ratio ($p$ = 0.61) for *bc-3,* a good fit to the expected 3:1 ratio ($p$ = 0.61) for *I* gene and a good fit to the expected 3:1 ($p$ = 1.0) for *Co-1*gene. The 20 F2 population of RWR 2355 showed a good fit to the expected 1:3 ratio ($p$ = 0.6) for *bc-3,* a good fit to the expected 3:1 ratio ($p$ = 0.6) for *I* gene and a good fit to the expected 3:1 ($p$ = 1.0) for *Co-1*gene. The 24 F2 population of RWR 2361 showed a good fit to the expected 1:3 ratio ($p$ = 0.64) for *bc-3,* a good fit to the expected 3:1 ratio ($p$ = 0.34) for *I* gene and a good fit to the expected 3:1 ($p$ = 0.34) for *Co-2* gene (Appendix table 6.1). The 17 BC1F1 population of G54 showed a good fit to the expected 1:1 ratio ($p$ = 0.09) for *bc-3,* a good fit to the expected 1:1 ratio ($p$ = 0.81) for *I* gene and a good fit to the expected 1:1 ($p$ = 0.23) for *Co-1*gene. The 10 BC1F1 population of RWR 1668 showed a good fit to the expected

1:1 ratio (*p* = 0.53) for *bc-3,* a good fit to the expected 1:1 ratio (*p* = 1.0) for *I* gene and a good fit to the expected 1:1 *(p* = 0.53) for *Co-1*gene. The 9 BC1F1 population of RWR 2355 showed a good fit to the expected 1:1 ratio (*p* = 0.6) for *bc-3,* a good fit to the expected 1:1 ratio (*p* = 0.31) for *I* gene and a good fit to the expected 1:1 *(p* = 0.74) for *Co-1*gene (Appendix table 6.2).The 16 BC1F1 population of RWR 2361 showed a good fit to the expected 1:1 ratio (*p* = 0.64) for *bc-3,* a good fit to the expected 1:1 ratio (*p* = 0.31) for *I* gene and a good fit to the expected 1:1 *(p* = 0.31) for *Co-2* gene (Appendix table 6.2). All the results from breeding lines (segregating populations) confirmed independent inheritance of resistance genes.

The use of molecular markers is of great interest in plant breeding for the identification and selection of plants with target genes. In this study, two characterized amplified regions (SCARs) molecular markers showed polymorphism in the parents, F2 and backcross populations. The SCAR markers evaluated are shown in Table 3.2 and their known associations with resistance genes are included.

The presence of *Co-1* and *Co-2* resistance genes was confirmed using pair of markers STK-1 and SQ4 respectively. Cultivars 'Ac-hensal' and'USCR-7' are sources of *Co-1* and *Co-2,* respectively. The primer pairs STK-1 and SQ4 SCAR were utilized in order to amplify a fragment of genomic DNA (gDNA) containing the *Co-1* and *Co-2* resistance alleles. The PCR Amplification with the primer pairs STK-1 F/R and SQ4 F/R on the *P. vulgaris* gDNA template generated 110 and 1440 bp, respectively. However, DNA fragments from susceptible genotypes (G54, RWR 1668, RWR 23355 and RWV 2361) remained un amplified by PCR due to lack of resistance allele sequence (Figure 4.1).

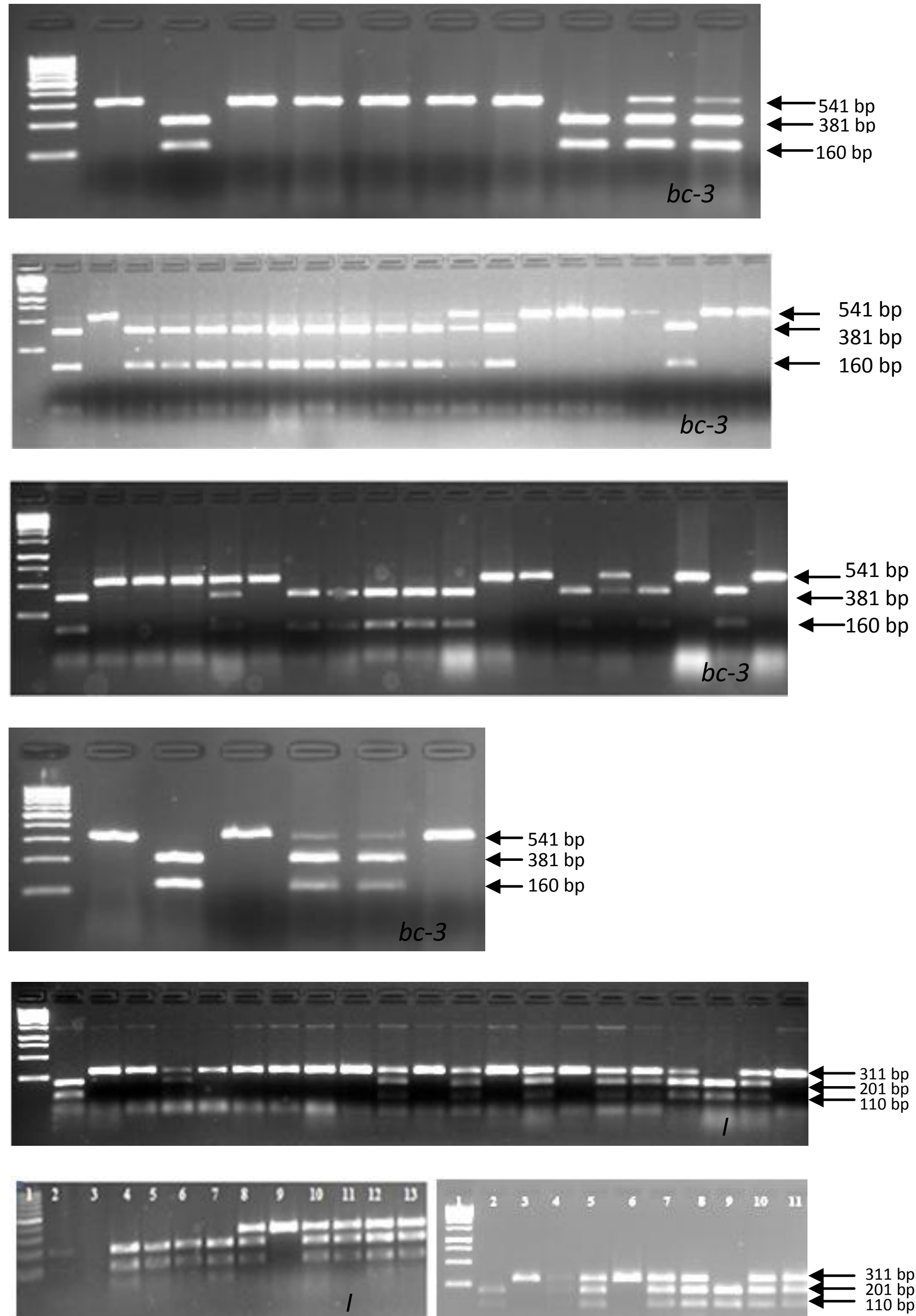
The markers STK-1 linked to *Co-1* and SQ4 linked to *Co*-2 indicated a good fit to the expected 3:1 and 1:1 ratios, in both F2 and BC1F1 populations, respectively (Table 4.2).

The STK-1 is a co-dominant marker, able to distinguish between heterozygote from homozygote genotypes in segregating populations and in advanced progenies (Figure 4.1). The STK-1 assays yielded consistent polymorphic bands between resistant and susceptible, homozygote and heterozygote individual plants.

The SQ4 marker was able to distinguish between resistant versus susceptible genotypes in parents, F2, and backcross populations. It is a dominant marker, yielded consistent polymorphic bands in all materials tested; however it didn't distinguish homozygote from heterozygote resistant individual plants (Figure 4.1).

The gene loci of *Co-1* and *Co-2* are all known to confer resistance to races alpha-17, beta 130, delta 23,gamma 102, 74, 258, 264, 339 and 343 (Melotto and Kelly 2000; Alzate-Marin et al. 2001b, 2003a; Rodrı´guez-Sua´ rez et al. 2008). The observed polymorphism of STK-1 marker in this study resembles what was reported previously associated with resistance to bean anthracnose (Chen et al. 2017). The STK-1 primer was chosen in order to amplify a fragment of gDNA containing the resistance allele as predicted from *P. vulgaris*

(Chen et al. 2017). The findings of SQ4 marker in this study resembles what was reported previously associated with resistance to bean anthracnose in *P.vulgaris* (Genchev et al. 2010).



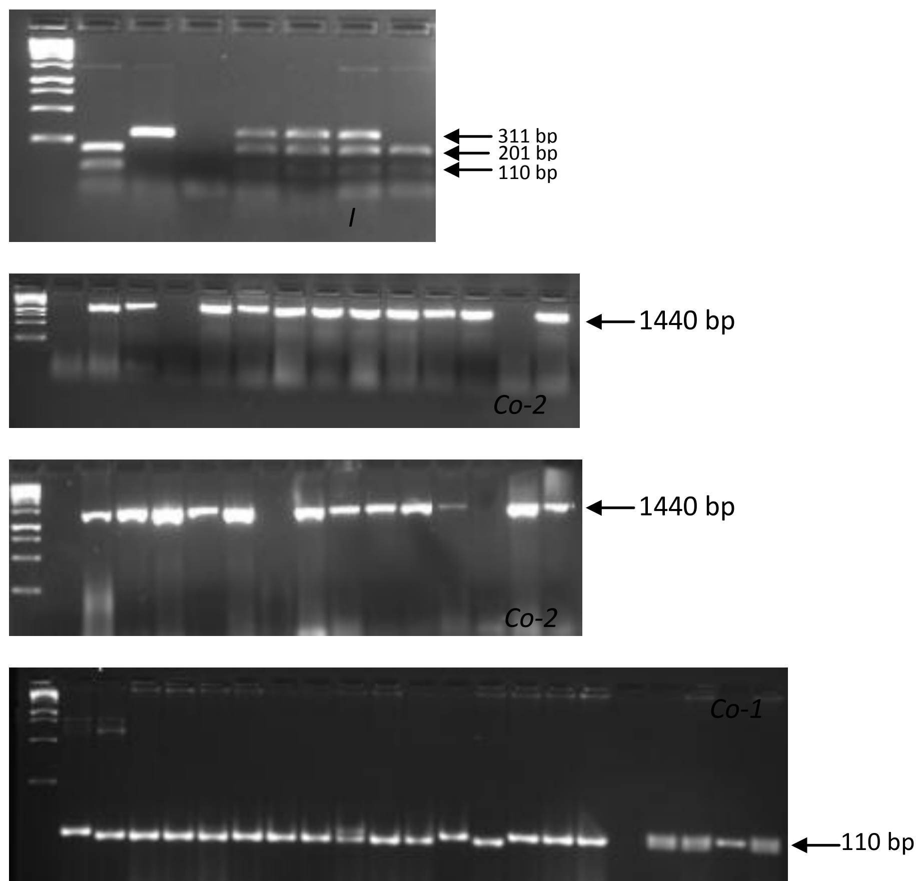



**Figure 4.1.** A representative figure showing segregation of resistance genes by CAPS markers in F2 and backcross populations

Lane 1, DNA ladder; Lanes 2 and 3: donor and recurrent parents in all plates. Lanes from 4 through the right side represent the F2 and BC lines. Both 381 bp and 160 bp: Resistance bands (*bc-3*/*bc-3*); 541 bp: susceptibility band (*Bc-3*/-). Both 201 bp and 110 bp: Resistance bands (*I* gene); 311 bp: susceptibility band (*i/i*).110 bp: Resistance band (*Co-1* gene); 1440 bp: Resistance band (*Co-2* gene).

The challenge encountered in utilizing $SQ4_{1440}$ SCAR marker was provision of limited information at the locus it tags because of its dominance nature. It doesn't distinguish between the heterozygote from the homozygote genotypes in a segregating population. Although, the marker was sufficient to identify resistant individuals among segregating BC populations in which only heterozygote resistant individuals existed, it lacked the power to detect homozygous resistant ones in the selfed generations (F2, BC3F2). The co-dominant markers provided larger information since they distinguish between homozygote and heterozygote genotypes and this eliminates the necessity of further genotyping for the fixed alleles rather dealing with fewer segregating alleles in subsequent generations (Piepho and Koch 2000).

## 4.3. Development of Pyramided Lines

Inheritance pattern of bean anthracnose and *BCMV* resistance genes in populations developed from the crosses between Ac-hensal and USCR-7 as donor parents and G54, RWR 1668, RWR 2355,and RWV2361 as recurrent parents was studied. The parent genotypes, F2 and BC1, BC2 and BC3 and advanced lines were evaluated for bean anthracnose and *BCMV* disease resistance genes Using CAPS and SCAR Markers.

Pyramiding of resistance genes was carried out in four breeding lines and the entire breeding program for introgression, pyramiding and fixing the desired genes lasted for 3 years (Figure 3.3). Lines G54, RWR 1668 and RWR 2355 showed three genes combinations, Viz; one against anthracnose (*Co-1*) and two resistance genes against *BCMV* (*bc-3* and *I*); while line RWV 2361 had a combination one against anthracnose (*Co-2*) and two resistance genes (*bc-3* and *I*) (Figure 4.1).

The presence of resistance genes was expressed with the aid of molecular markers; ENM-CAPS marker linked to *bc-3* resistance allele, SQ4 linked to gene *Co-2*, and the *BCMV-48289723-CAPS* linked to locus *I*, respectively. Segregation to bean anthracnose and *BCMV* observed in an F2, BC populations and advanced lines confirmed independent inheritance of resistance genes.

Breeding lines ; G 54 BC, RWR 1668 BC, RWV 2355 BC, and RWR 2361 BC of BC3F2 generation showed either three genes combinations or two genes combinations against anthracnose and *BCMV* were selected (Figure 4.1).

The gene combinations were expressed with the aid of molecular markers STK-1, ENM-CAPS, BCMV-48289723-CAPS and SQ4. Determination of gene combinations was important in order to develop advanced lines with best gene combinations.

The best combination attained was *bc-3+ Co-1+I* and *bc-3+Co-2+I* and observed in BC advanced lines, however heterozygosity of genes was observed in best combinations.

The BC3F2 progenies selected from the homozygous resistant plants (genotype *Co-1Co-1* or *Co-2Co-2* were evaluated for the expression of the molecular markers ENM-CAPS, linked to *bc-3* gene and marker *BCMV*-48289723-CAPS linked to *I* gene. These were in homozygote or heterozygote forms (*-bc-3/I-*).

The 76 individual plants were analyzed for the three or two molecular markers. A total of 52 BC families were obtained with two or three gene combinations as shown in table 4.1. Ten families possessed three genes for both anthracnose and *BCMV* resistance genes, forty two families inherited two genes conferring resistance to both anthracnose and BCMV

All plants expressed a combination of two genes include *bc-3+ Co-1* , *bc-3+ Co-2* , *bc-3+ I*, *Co-1+ I*, *Co-2+I* and the three genes combinations include *bc-3+Co-1+I, bc-3+Co-2 + I* were identified and taken care of. The individual plants lacking any

combination of genes were identified, uprooted and discarded at early stages of their course of growth.

In each generation, resistance genes were confirmed with molecular markers. Pyramiding of resistance genes was conducted with both SCAR and CAPS markers include STK-1, SQ4, ENM-CAPS and *BCMV* -48289723-CAPS.

Among the 76 BC3F2generation individuals, seven families possessed a combination of *bc-3+Co-1+I* resistance alleles, three families possessed a combination of *bc-3+Co-2+I* resistance, twelve families possessed a combination of *Co-1+I* resistance, six families possessed a combination of *Co-2+I*, ten families possessed a combination of *bc-3+ Co-1*, two families possessed a combination of *bc-3+ Co-2*, conferring resistance to anthracnose and *BCMV*, and a family of twelve individuals with *bc-3+I* conferring resistance to all strains of *BCMV* were selected for future use (BC3F3). The rest of families inherited one or none of the target genes were uprooted and discarded (Table 4.1).

**Table 4.1.** Resistance genes combinations

| Number of families | Gene combinations |
|---|---|
| 7 | *bc-3+Co-1+I* |
| 3 | *bc-3+Co-2+I* |
| 10 | *bc-3+Co-1* |
| 2 | *bc-3+ Co-2* |
| 12 | *bc-3+ I* |
| 12 | *Co-1+I* |
| 6 | *Co-2+I* |

I = Inhibitor, Co = Colletotrichum

In choosing a suitable source of resistance gene, it is necessary to take into account the seed coat color and the presence of useful breeding traits to the maximum possible degree. Besides resistance against bean anthracnose, backcross varieties developed also possess resistance to a number of races of *BCMV* pathogens and valuable agronomic traits (Figure 4.2).

Diversification of resistance genes is essential for durable resistance. It is necessary to diversify the sources of durable multiple resistance against highly variable races *C. lindemuthianum* and *BCMV* (Balardin et al. 1997; Geffroy et al. 2008).

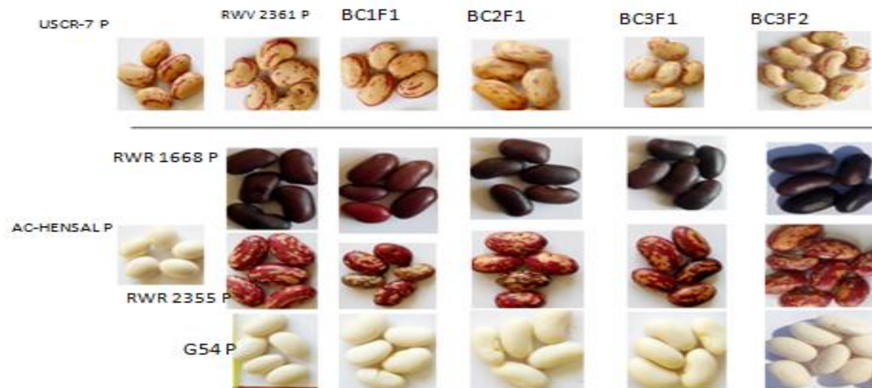



**Figure 4.2**. Seed color recovery.

Previous studies reported by Viteri and Linares (2019) on multiple resistances genes involve one recessive gene and two independent complementary recessive genes controlling ashy stem blight resistance in Andean common beans.

Ragagnin et al (2009) successfully utilized markers to multiple resistance genes for bean rust, anthracnose and angular leaf spot into carioca market-type bean cultivar 'Ruda' and indicated that productive pyramided lines.

Similarly, Suh et al (2013) successfully pyramided three resistance bacterial blight resistance genes *Xa4, Xa5* and *Xa21* into a popular but susceptible elite rice cultivar 'Mangeumbeyo'.

The genetic segregation ratio for *bc-3* in this study was further supported by Kelly et al (1995) and Mukeshimana et al (2005) who reported introgression of a single gene, monohybrid inheritance in a broader array of germplasm. This strongly suggests that the function of *I* gene is independent of *bc-3* in germplasm possessing both and vice-versa. Inheritance of *I* gene as an independent gene was further confirmed in 51 BC1F1 lines with the observed ratios closely fit a 1R:1S ratio (Table 4.3).

The findings of this study are in agreement with findings reported by Drijfhout (1978) who demonstrated that the *bc-3* gene does not require the *bc-u* gene for expression of activity in the presence of the *I* gene.

Resistance genes against anthracnose and *BCMV* were introgressed and pyramided into Rwandan common beans by the use of molecular breeding methods. As a result, backcross lines carrying resistance genes were obtained. The two resistance genes against anthracnose and two against *BCMV* were introgressed and pyramided into Rwandan beans.

**Table 4.2.** Chi square analysis of F2 segregating populations for *bc-3*, *Co-1*, *Co-2* and *I* genes

| Genes (F2) | Expected | Observed | $\chi 2$ | *p*-value |
|---|---|---|---|---|
| *bc-3* | 23:69 | 19:73 | 0.93 | 0.33 |
| *Co-1* | 51:17 | 47:21 | 1.25 | 0.26 |
| *Co-2* | 18:06 | 20:04 | 0.89 | 0.35 |
| *I* | 69:23 | 73:19 | 0.93 | 0.33 |

All *p-values* are greater than 0.05

**Table 4.3.** Chi square analysis of BC1F1 segregating populations for *bc-3*, *Co-1*, *Co-2* and *I* genes

| Genes (BC1F1) | Expected | Observed | $\chi 2$ | *p*-value |
|---|---|---|---|---|
| *bc-3* | 26:26 | 18:34 | 4.92 | 0.03 |
| *Co-1* | 18:18 | 14:22 | 1.78 | 0.18 |
| *Co-2* | 08:08 | 06:10 | 1.0 | 0.32 |
| *I* | 26:26 | 24:28 | 0.31 | 0.58 |

All *p-values* are greater than 0.05 except 0.03 of *bc*-3

**Table 4.4.** Chi square analysis of BC3F2 segregating populations for *bc-3*, *Co-1*, *Co-2* and *I* genes

| Genes (BC3F2) | Expected | Observed | $\chi 2$ | *p*-value |
|---|---|---|---|---|
| *bc-3* | 19:57 | 16:60 | 0.47 | 0.43 |
| *Co-1* | 37.5:12.5 | 35:15 | 0.67 | 0.41 |
| *Co-2* | 19.5:6.5 | 17:9 | 1.26 | 0.26 |
| *I* | 57:19 | 47:29 | 7.02 | 0.01 |

The findings of this study on the inheritance of *Co-2* anthracnose resistance gene is in agreement with the results previously reported by Rodrı´guez-Sua´rez et al (2007) on line A252 carrying one dominant resistant gene against race 38 located in cluster and segregates independently of two independent dominant genes located in clusters *Co-3*/9.

Similarly, Aylesworth et al (1983) reported the *Co-2* gene in A1183 and A1878 lines introgressed from donor line Sanilac BC6-Are, with the help of amplification products of markers SQ41440 and $SCH20_{1000}$, linked to loci *Co-2* present in lines. The results of this study on the inheritance of *bc-3* and *I* resistance genes are in agreement with *I* and *bc-3* findings, used to introgress genetic resistance to BCM in fabada line A25 (Ferreira et al. 2012). Our *bc-3* findings are in favor of the *PveIF4E2* allele found only in the four genotypes reported to carry *bc-3* resistance (Naderpour et al. 2010).

### 4.4. Morphological Studies and Yield Evaluation of Dry Beans

Germplasm characterization, morphology, agronomic traits, cultural preferences, all are useful aspects in breeding industry for development of plant genetic resources in order to satisfy the needs of farmers (Singh 1982; Hammer et al. 1999; Pereira et al. 2005; Stoilova 2007).

Phenotypic markers have been vital for crop genetic evolution analysis, germplasm evaluation and revealing better varieties for farmers (Gepts 1993; Bretting and Widrlechner 1995; Gilliland et al. 2000; Rolda´n-Ruiz et al. 2001).

Both growth habits and seed traits contribute to the genetic variability and are useful for selection of common bean cultivars in breeding programs (Purseglove 1976; Singh et al. 1991a; 1991b). The objective of this study was to evaluate phenotypic, morphological and agronomic characters between parents and their BC progenies at different stages, a strategy to monitor bean’s genetic background recovery, evaluate the effect of pyramided genes on yield related traits.

#### 4.4.1. Growth habit

The growth habit of six cultivars in the study, three were bush type, two semi-climbing and two were climbing type. However, there was a part of RWR 2361 family progenies inherited bush nature from donor parents.

#### 4.4.2 Number of days to emergence and germination percentage

The average number of days to emergence in G54, RWR 1668, USCR-7 and Ac-hensal genotypes, both the parents and advanced BC progenies recorded by counting from date of sowing was 13 days with standard deviation 0.75.

The Ac-hensal, RWR-1668 BC, RWR 2361 P genotypes exhibited 100 % germination, followed by RWR 1668 P, RWR 2355 P, RWR 2361 BC, USCR-7 that exhibited above 90%, and the third category exhibited above 80% included G54 P, G54 BC, RWR 2355 BC. There was no significant variation in germination percentage among the genotypes (Figures 4.3 and 4.4).

### 4.4.3. Plant height

The average plant height after 45 days of sowing between parents and their respective advanced BC lines in all genotypes was 16.6 cm. The lowest plant height was observed in Ac-hensal (6.5 cm). The highest plant height was observed in RWV 2361 P (71.0 cm).The plant height standard deviation was 7.74 cm.

### 4.4.4. Days to flowering and flower characteristics

Average days to flowering recorded by counting number of days from sowing day to the date on which nearly 50% of sampled parent materials and their respective advanced BC progenies was 58 days, and the standard deviation was 0.82. Implying that, there was no variation among genotypes themselves (Figure 4.4). The colors of freshly opened flowers were white in all cultivars except USCR-7 that had pink. Some progenies from crosses also exhibited pink color especially RWR 2361 progenies.

**Figure 4.3.** Plates for germination percentage

### 4.4.5. Pod length and Number of pods per plant

The measurement of pod length in centimeter was recorded, the longest was found in RWV 2361 P (16.1 cm) and the smallest had 6.8 cm from Ac-hensal. The average pod length in all genotypes was 12.5 cm and standard deviation was 2.40 cm.

There was no significant variation in number of pods per plant found between parents and their respective progenies. The average number of mature pods per plant at harvest time recorded was 23 pods and the standard deviation was 9.8.

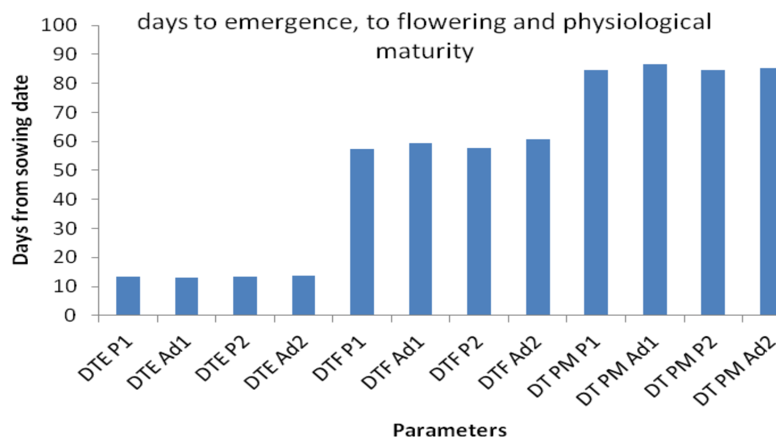



**Figure 4.4.** Comparison of agro-morphological traits between parents and their advanced lines

**Figure 4.5.** Pod characteristics and Physiological maturity

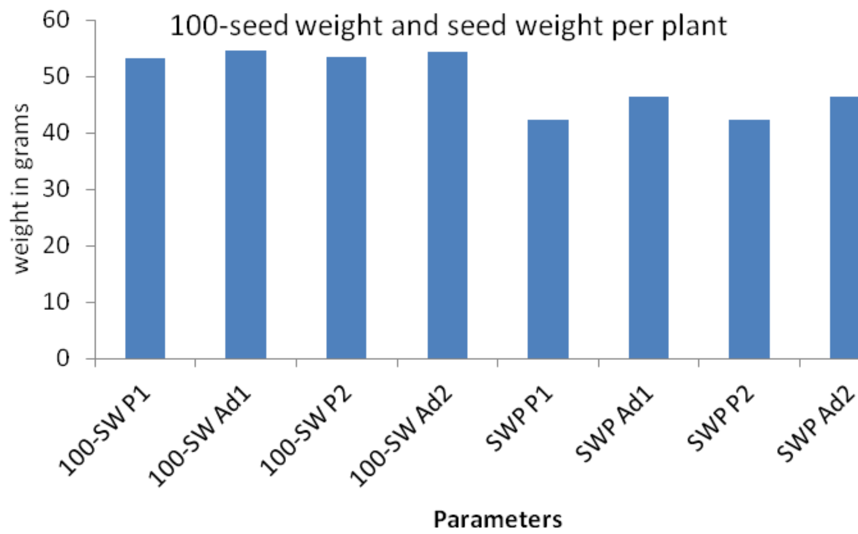



**Figure 4.6.** 100-seed weight and seed weight per plant

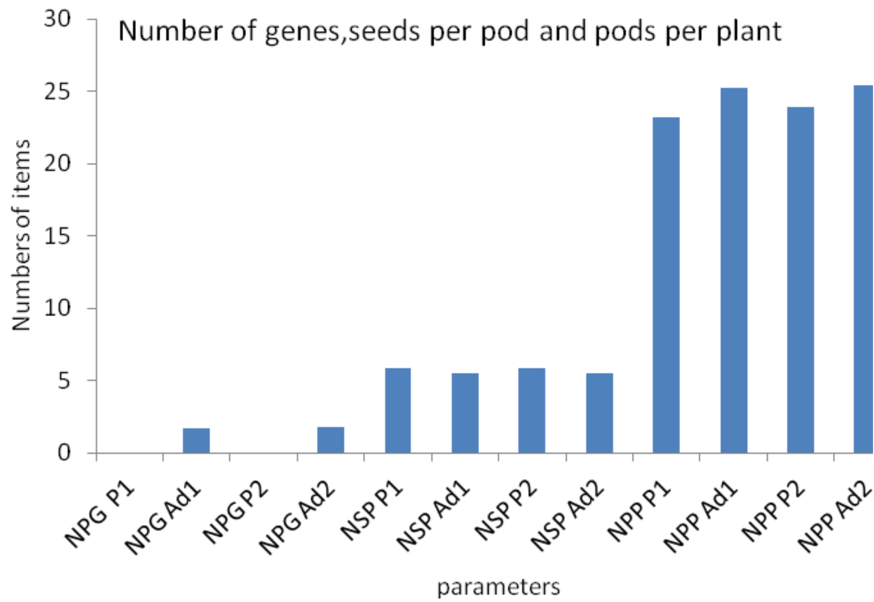



**Figure 4.7.** Number of pyramided genes, seeds per pod and pods per plant

### 4.4.6. Days to physiological maturity

Average number of days from sowing until 50% of the sampled plants have changed the color of their pods was 84 days and the standard deviation was 5.25. The days to physiological maturity were relatively less in Ac-hensal (78 days) and were many in RWR 2361 P (95 days) (Figures 4.4 and 4.5).

### 4.4.7. 100-Seed weight

The records of dry 100-seeds weight (g) data of parents and their advanced BC progenies in the breeding program were taken. The lowest 100-seed weight was recorded in donor parent Ac-hensal (27.2 g) and the highest 100-seed weight was recorded in USCR-7 (73.8 g). The average 100-seed weight from six genotypes was 53 g and the standard deviation was 11.58 (Figure 4.6).

### 4.4.8. Number of seeds per pod and number of seeds per plant

Number of seeds per pod was counted as an average number of seeds from 10 randomly taken mature pods. The highest average pod number was 7 seeds, recorded from RWR 2361 P, the lowest seed number per pod was 3 seeds, recorded in Ac-hensal genotype. The average number of seeds per pod in all genotypes was 5 seeds and the standard deviation was 0.82 (Figure 4.7).

The seed weight per plant from 10 randomly taken plants was recorded. The highest seed weight per plant was 48 g, recorded in RWV 2361 genotype, and the lowest seed weight per plant was recorded in Ac-hensal 14 g. The average seed weight per plant from all genotypes was calculated and found 44 g with standard deviation 9.69 (Figure 4.8).

### 4.4.9. Number of pyramided genes

Number of pyramided genes was counted with the help of markers and evaluation considered 10 randomly plants in every genotype. The donor parents had the highest gene number (3 genes), the susceptible parents had zero genes, and breeding lines expressed varying number of genes from 0 to 3 (Figure 4.1). The average number of genes was calculated and found to be 1.3 with standard deviation 1.26 (Figure 4.7).

**Figure 4.8.** Seed characteristics

## 4.5. Analysis of Comparisons of Means With Student's t-Test

Analysis of comparisons of means between recurrent parents and their respective advanced lines in regards to; days to emergence, days to flowering , plant height, number of pods per plant, pod length, number of seeds per pod, days to physiological maturity, seed weight per plant and 100-seed weight was carried out with paired student's t-test.

The null hypothesis ($H_0$) used in student's t-test was stated that no difference in agro-morphological traits between recurrent parents and their respective advanced lines. The alternative hypothesis ($H_1$) stated that, there was a difference in agro-morphological traits between parents and their respective advanced lines.

The highest student's t-test values significant at the two-tailed level were 10.45 ($p$ = 0.000) and 9.47 ($p$ = 0.000) obtained in regard to genes in two seasons, followed by change in seed weight per plant between two groups with $p$-values 0.000 and 0.000 in first and second season respectively. The $p$-values 0.003 and 0.000, recorded in regard to days to flowering between two groups were significant for first and second seasons respectively. The change in number of pods per plant between two groups was significant with $p$-values

0.000 and 0.004 in first and second seasons respectively. The change in days to emergence between two groups; parents and their advanced lines was significant with *p*-values 0.003 in second season, but non significant in season one with *p*-value -0.752. Also the change in days to physiological maturity between two groups; parents and their advanced lines was significant with *p*-values 0.041 in first season, but non significant in season two with *p*-value 0.356 (Table 5).

In brief, the paired t-test for multiple comparisons between recurrent parents and their respective advanced lines revealed that the corresponding *p*-value was less than the level of significance ($p = 0.05$) for days to flowering, number of pyramided genes, number of pods per plant and seed weight per plant was significant ($P < 0.05$). There are many reasons for these outcomes. Successful introgression of genes into susceptible beans led to significant change between two groups in regard to number of pyramided genes. Secondary, there might be beneficial interactions of genes from two distinct parents and this significantly led change in seed weight per plant. The results that are significant are marked with yellow color while non significant are not marked (Table 4.5).

### 4.6. Correlation Analysis

Seed yield is a quantitative trait, influenced by several genes and other related traits, and environment (Ejara et al. 2017). The knowledge of association among the complex traits for the simultaneous improvement of yield and its related components enables the breeders to understand the changes that occur in a given trait in function of the selection done on another.

Correlation is important for selecting trait of interest that presents low heritability, less genetic, traits that are normally harder to measure or identify. When selecting trait with high heritability, that is easily identified and highly correlated with the desired trait, the breeder can obtain quicker progress than with direct selection.

The magnitude of correlation coefficient and interpretation between two or more traits may be the result of the effect of a group of other traits on them. Therefore, correlation studies among traits do not permit definitive conclusion on the relation of cause and effect (Cruz and Regazzi 1997).

**Table 4.5.** Analysis of comparisons of means with student's t-test

| Variables | Means | Std.Dev | Std.Error .Means | 95% confidence interval of the difference | | t | Df | Sig.(2-tailed) |
|---|---|---|---|---|---|---|---|---|
| | | | | Upper | Lower | | | |
| **DTEAd1 – DTEP1** | -.0750 | 1.49164 | .23585 | .40205 | .552 | **-.318** | 39 | .752 |
| **DTEAd2 - DTEP2** | .3500 | .69982 | .11065 | -.57381 | -.126 | **3.163** | 39 | .003 |
| **DTFAd1 - DTFP1** | 2.0250 | 4.10433 | .64895 | -3.33763 | -.712 | **3.120** | 39 | .003 |
| **DTFAd2 - DTFP2** | 2.9250 | 3.98322 | .62980 | -4.19889 | -1.651 | **4.644** | 39 | .000 |
| **PHAd1 - PHP1** | 1.5000 | 5.41574 | .85630 | -3.23204 | .232 | **1.752** | 39 | .088 |
| **PHAd2 - PHP2** | 1.4975 | 5.89174 | .93157 | -3.38177 | .387 | **1.608** | 39 | .116 |
| **NPGAd1 - NPGP1** | 1.6750 | 1.11832 | .17682 | -2.03266 | -1.317 | **9.473** | 39 | .000 |
| **NPGAd2 - NPGP2** | 1.7750 | 1.07387 | .16979 | -2.11844 | -1.432 | **10.45** | 39 | .000 |
| **NSPAd1 – NSPP1** | -.3000 | 1.04268 | .16486 | -.03346 | .633 | **-1.820** | 39 | .076 |
| **NSPAd2 - NSPP2** | -.3250 | 1.84513 | .29174 | -.26510 | .915 | **-1.114** | 39 | .272 |
| **PLAd1 - PLP1** | -.3575 | 2.28696 | .36160 | -.37391 | 1.089 | **-.989** | 39 | .329 |
| **PLAd2 - PLP2** | -.6825 | 4.42417 | .69952 | -.73242 | 2.097 | **-.976** | 39 | .335 |
| **NPPAd1 - NPPP1** | 2.0000 | 3.23443 | .51141 | -3.03442 | -.966 | **3.911** | 39 | .000 |
| **NPPAd2 - NPPP2** | 1.5750 | 3.29637 | .52120 | -2.62923 | -.521 | **3.022** | 39 | .004 |
| **HSWAd1 - HSWP1** | 1.5150 | 5.48385 | .86707 | -3.26882 | .239 | **1.747** | 39 | .088 |
| **HSWAd2 - HSWP2** | 1.0450 | 6.44308 | 1.01874 | -3.10560 | 1.016 | **1.026** | 39 | .311 |
| **DTPAd1 - DTPP1** | 1.9250 | 5.77078 | .91244 | -3.77059 | -.079 | **2.110** | 39 | .041 |
| **DTPAd2 - DTPP2** | .8000 | 5.41224 | .85575 | -2.53092 | .931 | **.935** | 39 | .356 |
| **SWPAd1 - SWPP1** | 4.2150 | 5.28862 | .83620 | -5.90638 | -2.524 | **5.041** | 39 | .000 |
| **SWPAd2 - SWPP2** | 4.2375 | 5.48526 | .86730 | -5.99177 | -2.483 | **4.886** | 39 | .000 |

P1 = Parents in season one, P2 = Parents in season one, Ad1 = Advanced lines in season one, Ad2 = Advanced lines in season two. DTE= days to emergence, DTF = days to flowering, PH= plant height, NSP = number of seeds per pod, NPG = number of pyramided genes, PL = pod length, NPP = number of pods per plant, DPM = days to physiological maturity, 100-SW = 100-seed weight, SWP = seed weight per plant.

Correlation determines the relationship between traits, but path analysis partitions correlation into direct and indirect effects of different yield related components on seed yield. In addition to the direct selection of complex traits, studying the association between seed yield and its components to perform the indirect selection of traits related to seed yield is of paramount (Ahmed and Kamaluddin 2013).

Correlation and path analyses clarify the relationship between various traits with seed yield, which are important for effective selection procedures designed to improve seed yield. Correlation and path coefficient analyses are used together to understand the cause and effects relationship between seed yield and its components to identify the traits which maybe considered as indirect selection criteria (Khan et al. 2003).

Correlation and path coefficient analyses in the common bean between grain yield and other related components have been previously studied (Mohamed 1997; Gonçalves et al. 2003; Bhushan et al. 2008; Salehi et al. 2010; Ahmed and Kamaluddin 2013; Singh and Singh 2013; Akhshi et al. 2015; Gonçalves et al. 2017; Panchbhaiya et al. 2017).

The relationships among various traits were statistically analyzed through correlation and path coefficients (Weher and Moorthy 1952; Akintunde 2012). The correlation matrix on seed weight and other related components was shown in table 4.6. Significant and strong positive correlations were found between seed weight per plant and 100-seed weight (r= 0.950; $p$ = 0.000), days to emergence (r = 0.591, $p$ = 0.000), pod length (r = 0.513, $p$ = 0.000). Significant and moderate positive correlations were found between seed weight per plant and days to flowering (r = 0.261, $p$ = 0.009), days to physiological maturity (r = 0.229, $p$ = 0.022) and number of seeds per plant (r = 0.217, $p$ = 0.030) (Table 4.6).

Non-significant with lower positive correlation was found between seed weight per plant and number of pyramided genes (r = 0.128, $p$ = 0.205). Non-significant with lower negative correlation was found between seed weight per plant and number of pods per plant (r= -0.111, $p$ = 0.273). Non-significant with no correlation was found between seed weight per plant and plant height (r = 0.071, $p$ = 0.480) (Table 4.6).

100-seed weight, days to emergence, pod length and days to flowering exhibited the highest positive correlation with seed weight per plant, indicating the dependence of seed weight per plant on these characters (Table 4.6).

The heavier 100-seed weight implies higher seed weight per plant, the longer and larger pods accommodate more and larger seeds per pod, thus heavier seed weight per plant as well. The parameter of number of seeds per pod showed a positive correlation, which implies that the more the number of seeds in a pod the higher overall seed weight per plant as well.

Positive correlations on days to flowering and physiological mature in this study are in agreements with previous studies done by Malik et al (2006), who reported positive

correlation of bean yield with days to 50% flowering and days to 50% maturity, implying that delay in these yield related components would increase the yield. Therefore, interaction between phenotypic, morphological, agronomic traits and disease resistance genes was evaluated and did not reveal any yield penalty among the pyramided lines.

Significant and positive associations between seed weight per plant and plant height, days to 50% flowering, days to maturity, number of pods per plant and yield/plant reported by Sirohi et al (2007).

Sofi et al (2011) reported that seed yield was significantly associated with number of pods per plant followed by 100-seed weight, seeds per pod and plant height. Results are in conformity with the findings of Bhushan et al (2008), Singh et al (2009) and Sofi et al (2011).

Udensi and Ikpeme (2012) and Islam et al (2015) reported that the pod size contributes to the overall yield in common bean and should be looked at during selection. Mahbub et al (2015) and Aditya et al (2011) reported significant and positive correlations between yield and its related components and suggested that improvement in the value of one trait, contributes the value of yield as well.

Similar findings on positive correlation between plant height (0.071) and yield was reported by Arslan (2007), in that positive correlation was found between seed yield and plant height in Safflower, contrary to our findings, negative significant relationship was obtained between seed yield and 100-seed weight (Arslan 2007).

The correlation of the yield and yield contributing components indicated that the number of pods per plant was significantly positively associated with days taken to set flowers, pod length and seed yield per plant. Berrocal et al (2002), Upadhyay (2001), Vasic et al (1997) and Chaubey et al (2012) reported similar types of findings in French bean. These traits were positively significantly associated with each other.

Therefore, the positively correlated yield attributes, days taken to set flowers, pod length, seed yield/plant should be considered as essential parameters for selection in breeding program targeted for high yield in dry bean. Similar results were observed by Singh (1993) and Vasic et al (1997). This relationship indicated that increase or decrease in number of pods per plant directly reflected in the length of pod.

Similar positive correlations on number of pods per plant, number of seeds per pod, pod height, days to flowering, days to maturity, plant height, 100-seed weight were reported (Ayub and Amjad 2000; Iqbal et al. 2003; Türkeç 2005; Arshad et al. 2006; Malik et al. 2007; Sarutayophat 2012; El-Mohsen et al. 2013; Mili 2013; Arshad et al. 2014; Ghanshyam et al. 2015). In contrast, Rajkumar et al (2010) and Arshad et al (2006) reported a significant negative correlation of yield with days to flowering and maturity, and negative correlations for 100-seed weight with days to flowering, maturity, plant height and number of nodes per plant.

Number of pyramided genes were significantly negatively associated with plant height (-0.243), number of seeds per pod (-0.342), pod length (-0.330). Non-significant negative correlation was exhibited between number of pyramided genes and days to emergence (-0.059), number of pods per plant (-0.125), days to physiological maturity (-0.057) and 100-SW (-0.042).

Similarly, pyramided genes were negatively associated with days to physiological maturity, plant height and number of seeds/pod. This indicates that any delay with respect to these characters could result in decreased yields (Malik et al. 2007).

The findings on correlations between number of pyramided genes and other traits evaluated were in agreement with Kiryowa et al (2015) who reported that pyramided genes significantly negatively associated with number of pods per plant and number of seeds per plant. On contrary, Kiryowa et al (2015) reported that pyramided genes significantly negatively associated with seed weight per plant. This suggests that gene pyramiding may affect number of pods per plant, number of seeds per plant and eventually a slight yield penalty resulted from gene pyramiding.

**Table 4.6.** Estimates of correlation matrix between number of pyramided genes and agronomic traits

| | SWP | DTE | DTF | PH | NPG | NSP | PL | NPP | DPM | 100-SW |
|---|---|---|---|---|---|---|---|---|---|---|
| SWP | 1 | | | | | | | | | |
| DTE | 0.591** | 1 | | | | | | | | |
| DTF | 0.261** | 0.105 | 1 | | | | | | | |
| PH | 0.071 | .070 | 0.450** | 1 | | | | | | |
| NPG | 0.128 | -0.059 | 0.011 | -0.243* | 1 | | | | | |
| NSP | 0.217* | 0.020 | 0.229* | 0.429** | -0.342** | 1 | | | | |
| PL | 0.513** | 0.236* | 0.314** | 0.566** | -0.330** | 0.814** | 1 | | | |
| NPP | -0.111 | -0.157 | 0.341** | 0.814** | -0.125 | 0.509** | 0.545** | 1 | | |
| DPM | 0.229* | 0.073 | 0.829** | 0.414** | -0.057 | 0.360** | 0.414** | 0.409** | 1 | |
| 100-SW | 0.950** | 0.608** | 0.295** | 0.072 | -0.042 | 0.240* | 0.541** | -0.152 | 0.221* | 1 |

** Significant ($P<0.01$), * Significant ($P<0.05$), ns = not significant. DTE= days to emergence, *DTF* = days to flowering, *PH*= plant height, *NSP* = number of seeds per pod, *NPG* = number of pyramided genes, *PL* = pod length, *NPP* = number of pods per plant, DPM = days to physiological maturity, *100-SW* = 100-seed weight, *SWP* = seed weight per plant.

## 4.7. Estimation of Path Coefficients

Partitioning correlation coefficients into direct and indirect cause of association provides information on the actual contribution of independent variables with respect to a dependent variable, this is because the level of degree of correlation between two variables might happen due to the indirect effect of a third variable (Cruz et al. 2012). In the present study, seed weight per plant was considered as responsive variable (dependent) and the rest were independent variables.

The path analysis is performed to determine the direct and indirect contribution of each character to the seed yield (Berhe et al. 1998). Any component of yield may affect yield directly and /or indirectly (Doust et al. 1983). Path analysis is used to determine the amount of direct and indirect effects of independent variables on the dependent variable (Ulukan et al. 2003).

Path analysis permits the splitting of the correlation coefficients into direct and indirect effects of various traits on a basic variable whose estimate is obtained by multiple regression equations where the variables are previously standardized (Akintunde 2012).

Path analysis is necessary to analyze the cause and effect relationship between dependent and independent variables to entangle the nature of the relationship between the variables providing clearer pictures of trait associations (Sidramappa et al. 2008). Path coefficient analysis provides an exact figure of the relative importance of direct and indirect influences of each of the component characters on seed yield and it would help plant breeders to determine efficient selection strategy. Path coefficient analysis partitions direct and indirect factors of correlation coefficients. Thus, it becomes necessary to study path coefficient analysis. Path coefficient analysis is a statistical technique designed to quantify the interrelationship among different components and their direct and indirect effects.

Data in Table 4.7 illustrated maximum direct effect of 100-seed weight (0.957) on seed weight per plant, this suggests the importance of this yield related component as selection criterion for high yield in common bean. Lower direct effects were observed in plant height (0.051), number of pyramided genes (0.200), pod length (0.024), number of pods per plant (0.015), days to physiological maturity (0.131) and days to emergence. However, days to flowering (-0.160) had lower negative direct effect on seed weight per plant.

The 100-seed weight had the largest positive direct effect on seed weight per plant along with the largest correlation coefficient. The yield related component with both high significant positive correlation (0.95) and high direct effect (0.96) indicates its usefulness in selection programs. Plant height showed stable correlation between direct effect (0.051) and correlation coefficient (0.072) (Table 4.7).

These characters have direct positive effects on seed weight per plant, indicating that these are the main contributors to yield for common bean plants. Therefore, during

selection the main emphasis should be given to 100-seed weight for producing high yielding common bean genotypes. On the contrary, days to flowering (-0.160) showed a minor negative direct effect on seed weight per plant (Table 4.7).

All yield related components had positive indirect effects on seed weight per plant through number of days to emergence (0.589), days to flowering (0.264), plant height (0.072), number of seeds per pod (0.217), number of pyramided genes (0.111), pod length (0.512), days to physiological maturity (0.228), 100-seed weight (0.950), except number of pods per plant (-0.107) (Table 4.7).

The significant difference between direct effects and correlation coefficients in the number of days to emergence, number of days to flowering, number of pyramided genes, number of seeds per pod, pod length and days to physiological maturity was noted, indicating that the indirect effects are the cause of the difference.

In the case of days to flowering, number of seeds per pod with negative direct effects along with positive correlation coefficients, it implies that the indirect effects are the cause of the positive correlation. In contrary the direct effects and correlation coefficients in the plant height, 100-seed weight did not show significant difference.

Path analysis results on 100-seed weight and plant height are in agreement with findings reported by Tuncturk and Ciftici et al (2004), Peksen and Gulumser (2005), and Ahmadzadeh et al (2012).

The seed weight per plant was indirectly negatively affected by number of pod per plant (-0.107). This result is in agreement with previous findings reported by (Tofiq 2016). This result demonstrates that for common bean selection on the basis of these traits might lead to a yield compromise (Malik et al. 2007).

The residual effect on seed yield per plant was low (0.061) and this indicates that the traits under study could be used to determine any effects on seed yield (Chandel et al. 2014). Significant indirect effects on yield were 0.264** and 0.228* showed by the yield related components, days to maturity and days to flowering. Previous similar results were obtained by Sharma et al (1983).

Plant height and number of pods per plant had negative direct effect on seed weight per plant, but with positive indirect effect on seed weight per plant. This positive effect could be due to the fact that any positive indirect effects nullified any direct negative effects that plant height and number of pods per plant might have on the seed weight per plant.

The findings suggest that a highly significant positive correlation, with the highest positive direct effect were observed in the 100-seed weight followed by days to physiological maturity, the others like days to emergence, days to flowering, number of seeds per pod and pod length had significant correlation coefficients, too.

Therefore, 100-seed weight followed, days to physiological maturity, days to emergence, days to flowering, number of seeds per pod and pod length, pods per plant and pod length can be considered as critical criteria for improving yield (Table 4.7).

Similar conclusions were found in other studies (Iqbal et al. 2003; Chettri et al. 2003; Malik et al. 2006; Ghanshyam et al. 2015).

Selecting for crop yield improvement, characters which showed negative correlations or non-significant positive correlations, could be problematic with respect to combining these characters to produce a genotype with increased yields. It is recommended to discard them to inhibit their harmful effects to the crop yield (Henry and Krishna 1990; Akinyele and Osekita 2006). Breaking undesirable linkages, bi-parental mating, mutation breeding or diallel selective mating processes are recommended (Ghafoor et al. 1990).

**Table 4.7.** Direct and indirect path coefficients for seed weight per plant

| Variables | DTE | DTF | PH | NPG | NSP | PL | NPP | DPM | 100-SW |
|---|---|---|---|---|---|---|---|---|---|
| DTE | **0.016** | | | | | | | | |
| DTF | -0.018 | **-0.160** | | | | | | | |
| PH | 0.001 | 0.023 | **0.051** | | | | | | |
| NPG | -0.017 | -.007 | -0.056 | **0.200** | | | | | |
| NSP | -0.000 | -0.001 | -0.002 | 0.001 | **-0.004** | | | | |
| PL | 0.0054 | 0.008 | 0.014 | -0.008 | 0.021 | **0.024** | | | |
| NPP | -0.003 | 0.005 | 0.012 | -0.002 | 0.008 | 0.008 | **0.015** | | |
| DPM | 0.011 | 0.109 | 0.056 | -0.013 | 0.047 | 0.055 | 0.055 | **0.131** | |
| 100-SW | 0.593 | 0.285 | 0.069 | -0.057 | 0.227* | 0.516 | -0.142 | 0.211 | **0.957** |
| SWP (r) | 0.589** | 0.264** | 0.072 | 0.111 | 0.217* | 0.512** | -0.107 | 0.228* | 0.950** |

** High significant (P<0.01), * Significant (P<0.05), ns = not significant. DTE= days to emergence, *DTF* = days to flowering, *PH*= plant height, *NSP* = number of seeds per pod, *NPG* = number of pyramided genes, *PL* = pod length, *NPP* = number of pods per plant, DPM = days to physiological maturity, *100-SW* = 100-seed weight, *SWP* = seed weight per plant. Residual = $1 - \sqrt{R^2} = 0.061$. The sum of direct and indirect path coefficients appears at the bottom row of the table. Direct path coefficients appear diagonally in bold. The rest of the coefficients are indirect coefficients.

## 4.8. Summary

Results showed that recurrent plants all had susceptible allele, while all donor parents had resistance alleles as shown in figure 4.1. In theory, all F1 generation plants were resistant to bean anthracnose and *BCMV* diseases, except F1 plants with *bc-3*. The segregation data obtained from F2 and BC populations showed that our breeding lines carried a combination of three or two resistance genes.

The present study showed that both CAPS and SCAR markers can be utilized to pyramid multiple bean anthracnose and *BCMV* resistance genes into susceptible bean cultivars. Breeding programs were designed on the basis of available knowledge on resistance genes and molecular markers linked to their corresponding genes.

Two resistance genes *Co-1* and *Co-2* against anthracnose were introgressed into Rwandan common beans. The source of resistance genes *Co-1* and *Co-2* was from line Ac-hensal and USCR-7, respectively. Also recessive *bc-3* and dominant *I* genes are against *BCMV* and were introgressed into Rwandan common beans. Both Ac-hensal and USCR-7 genotypes possessed *bc-3* and *I* genes.

Seed yield is a quantitative trait, results from multiple interactions between several genes, yield related traits, and environment. The knowledge of association among the complex traits for the simultaneous improvement of yield and its components enables the breeders to understand the changes that occur in a determined trait in function of the selection practiced on another.

Correlation analysis can help when examining selection criteria for improving yield through direct selection of its component traits, which are highly correlated.

Considering the associations of characters studied with seed yield, the 100-seed weight per plant, days to emergence, days to physiological maturity, number of seeds per plant, pod length and days to physiological maturity are important characters to be considered when breeding to improve the yield of common bean.

Plant height and number of pods per plant had negative direct effect on seed weight per plant, but with positive indirect effect on seed weight per plant. Positive indirect effects nullified direct negative effects that plant height and number of pods per plant might have on the seed weight per plant.

The findings suggest that a highly significant positive correlation, with the highest positive direct effect were observed in the 100-seed weight followed by days to physiological maturity, the others like days to emergence, days to flowering, number of seeds per pod.

The results of the present study show that selection of high yielding common bean genotypes would be possible by carefully balancing the pod length, number of pods per plant, number of seeds/plant and 100-seed weight, with plant heights and days to flowering

and maturity. These results should be considered when determining the selection criteria for future varietal improvement of yield of common bean.

The residual effect on seed yield per plant was low and this indicates that the traits under study could be used to determine any effects on seed yield

The correlation coefficients have shown high significant correlation between 100-seed weight and days to physiological maturity with seed weight per plant. The path analysis has reconfirmed that the 100-seed weight and days to physiological maturity as the yield related components that cause a higher favorable direct effect on seed weight per plant and can be used for indirect selection toward increasing common bean productivity.

## 5. CONCLUSION

The Central and Eastern Africa region is a secondary center of diversity for common bean. The common bean is the most important legume crop in Rwanda occupying an important niche in the Rwanda agricultural sector and farm household economy. Bean per capita consumption is reported to be highest in Rwanda.

Among dry bean cultivars evaluated, two were climbing type and the remaining were bush and semi-climbing types. Pod length and seed weight were found to be higher in bush type. Molecular marker data need to be tested for disease reaction under field condition.

Bean production is constrained by diseases that go undiagnosed among them bean anthracnose and bean common mosaic virus diseases are rampant in the region causing severe yield losses.

Bean common mosaic virus is one of the most destructive viral diseases of common bean worldwide. Breeders should deploy a combination of resistance genes; *I* and *bc-3* genes to confer broader spectrum resistance against potyvirus infection.

The resistance genes incorporated in cultivars play a central role in bean protection and will contribute in stabilization of yield against bean common mosaic potyvirus attack in Central and East Africa region. The CAPS and SCAR markers used in this study have proven to be useful for detecting resistance alleles in individual plants from both Andean and Mesoamerican gene pools in early breeding cycles.

Molecular markers increase the importance of indirect selection in plant breeding. The relative efficiency of selection for a marker is due to not affected by incomplete or masked expression or low heritability, selection for resistance in the absence of the pathogen, scored at any stage of plant development.

Markers assist breeders in pyramiding of resistance genes, thus improving durability of multiple resistance genes. Markers tightly linked to disease resistance genes have been reported in various crops and help in the development of more disease-resistant cultivars. SCAR and CAPS markers are simpler, more efficient and designed to overcome reproducibility problems.

The application of molecular markers to breeding represents an excellent example of the successful use of indirect selection for disease resistance. The seed-borne nature of bean anthracnose and *BCMV* diseases, through the unregulated cross boarder seed trade, environmental factors and recombination, through co-evolution with their wide hosts, *Colletotrichum lindemuthianum* and Bean common mosaic virus have become diverse.

All contribute to spread and emergence of new races within the region of East and central Africa. This indicates the need for the bean breeders to be able to breed for broad

and durable resistance to the pathogens. Durable resistance through marker-assisted gene pyramiding is a strategy that would confer a more long-term resistance.

Use of cultivars resistant to them plays an important role in stopping the spread of the disease and stabilizing yields.

This study therefore, purposed to i) To incorporate genes conferring resistance to bean anthracnose and *BCMV* diseases into common bean (*Phaseolus vulgaris, L.*) genotypes of Rwanda. ii) Use of markers for selection of beans having resistance genes to bean anthracnose and *BCMV*. iii) Recovery of Rwandan common bean's genetic background through backcrossing and phenotypic characterization.

iv) To develop advanced common bean lines with multiple resistance genes to anthracnose and Bean common Mosaic Virus and fix the multiple resistance genes. v) Evaluate the effect of multiple resistance genes of bean anthracnose and *BCMV* on plant agronomic characters. The study made the following findings;

The choice of genes to pyramid should base on the mode of action of individual genes in a host background and the interactions with others. It is advisable to deploy many genes at a time to ensure the longevity of resistance against pathogens displayed wide variations. Therefore, with highly variable pathogens like *C. lindemuthianum* and *BCMV*, it is important to diversify the sources of resistance to effectively manage the diseases.

Gene pyramiding for broad resistance against *C. lindemuthianum* two anthracnose resistance genes and two *BCMV* resistance genes was successfully introgressed using CAPS and SCAR markers. In this study, the cultivars Ac-hensal and USCR-7 are the best sources of resistance to bean anthracnose and *BCMV* diseases, they possess resistance genes *bc-3+ Co-1+ I,* and *bc-3+ Co-2+I,* respectively, conferring the broadest resistance against the bean anthracnose and *BCMV.*

It is recommended to combine all four resistance genes into the Mesoamerican and Andean gene pools. The CAPS markers; *BCMV-48289723-CAPS* and ENM-CAPS used are co-dominant in nature tagging *I and bc-3* genes, respectively. SCAR markers; STK-1 and SQ4 used showed co-dominance and dominance nature tagging *Co-1*and *Co-2* genes, respectively.

Markers were efficient, informative, stable and have a high precision. The markers are specific to target genes and there was no possibility to lose genes during recombination. They are suitable markers for a successful gene pyramiding program.

Both single and pyramided genes play a central role in controlling crop diseases; however the choice relies on pathogen diversity and the interaction of different resistance genes. In conditions where pathogen diversity is high, gene pyramiding provides durable resistance in protecting a crop than a single gene resistance that is easily broken.

Gene pyramiding has been successful in the control of many crops against various pathogens, this includes wheat stem rust resisted for over 50 years but later threatened by the new evolved stem rust race Ug99, indicating that gene pyramids confer resistance for a long time, but not for permanent basis (Mundt 2014).

Pyramids; *bc-3+Co-1+I*, *bc-3+Co-2+I, bc-3+Co-1, bc-3+Co-2* and *bc-3+I resistance genes combinations* conferring resistance to all *C. lindemuthianum* races and *BCMV* varieties were established.

Yield is a complex character controlled by various genes and environment, has a low heritability. Selection of plant materials with high yield requires prior information about interaction between seed yield and other characters contributing to yield and is a key to develop high performance varieties, efficient selection strategy in breeding program.

Both correlation and path analyses revealed that number of pyramided genes had non-significant positive correlation with seed weight per plant, but had a significant negative correlation with plant height, number of pods per plant and number of seeds per plant.

Path analysis revealed that number of genes pyramided had negative indirect effects on seed weight per plant via days to emergence, days to physiological maturity, plant height,number of seeds per pod, pod length, number of pods per plant, days to physiological maturity, and 100-seed weight. The positive direct effect nullifies all negative indirect effects and makes positive correlation on the seed weight per plant.

However, correlation and path analyses for the improvement of common bean in Rwanda where the study materials obtained from and per capita consumption is high had not been done before. Correlation coefficient analyses revealed that the most important characters contributing to seed yield per plant that selection should base on are 100-seed weight, pod length, number of pods per plant, number of seeds/plant and plant height.

Correlation and path-coefficient analyses revealed that number of pyramided genes had no effect on seed yield per plant. Breeders should be able to select pyramids with complementary resistance genes to minimize any possibility of yield penalty to which under certain conditions undesirable junk might cause.

Correlation and path-coefficient analyses have been conducted on several crops, these include; common bean (AlBallat et al. 2019), maize (Adesoji et al. 2015), rice (Ansari et al. 2010) and tomato (Islam et al. 2010).

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# 7. APPENDICES

To have best choice of yield related components for selection of genotypes under detailed and planned breeding program for higher yield, the association of yield related component with seed weight per plant and their exact contribution through direct and indirect effects are of great importance.

Yield is a complex character controlled by various genes and environment. It is important to understand the nature of associations among yield related components, cause and effect of the yield related components on seed weight per plant and this is the key to develop high performance varieties, efficient selection strategy in breeding program.

**Appendix 7.1.** Standardized data for analysis of season one

| *Y* | *x1* | *x2* | *x3* | *x4* | *x5* | *x6* | *x7* | *x8* | *x9* |
|---|---|---|---|---|---|---|---|---|---|
| 1.281 | -2.211 | -0.977 | -1.175 | -1.854 | -2.223 | -0.543 | -0.821 | -2.313 | -2.142 |
| 1.281 | -1.158 | -1.121 | -1.237 | -0.634 | -2.001 | -0.219 | -0.821 | -2.305 | -2.100 |
| 1.281 | -1.158 | -1.121 | -1.237 | -1.854 | -2.311 | -0.651 | -1.392 | -2.448 | -2.390 |
| 1.281 | -0.105 | -0.977 | -1.237 | 0.585 | -1.956 | -0.651 | -1.012 | -2.364 | -2.028 |
| 1.281 | -1.158 | -1.121 | -1.114 | -3.073 | -2.800 | -0.327 | -1.012 | -2.110 | -2.410 |
| 1.281 | -1.158 | -0.833 | -1.114 | -0.634 | -1.823 | -0.651 | -1.012 | -2.279 | -2.007 |
| 1.281 | -1.158 | -1.121 | -1.089 | -1.854 | -2.400 | -0.219 | -0.821 | -2.364 | -1.976 |
| 1.281 | -1.158 | -1.121 | -1.225 | -1.854 | -2.711 | -0.111 | -0.821 | -2.448 | -2.286 |
| 1.281 | -2.211 | -0.833 | -1.262 | -0.634 | -2.311 | -0.327 | -0.821 | -2.279 | -2.286 |
| 1.281 | -0.105 | -0.833 | -1.237 | -1.854 | -2.445 | -0.435 | -0.821 | -2.533 | -1.924 |
| 1.281 | -0.105 | 0.316 | -0.743 | -0.634 | 0.353 | -0.760 | -0.251 | 1.527 | 2.055 |
| 1.281 | 0.947 | 0.603 | -0.619 | 1.805 | 0.885 | -0.651 | 0.129 | 1.696 | 1.900 |
| 1.281 | 0.947 | 0.316 | -0.619 | 0.585 | 0.574 | -0.543 | 0.129 | 1.611 | 1.817 |
| 1.281 | -0.105 | 0.316 | -0.496 | -1.854 | 0.042 | -0.760 | 0.129 | 1.696 | 1.848 |
| 1.281 | 0.947 | 0.029 | -0.434 | -0.634 | 0.219 | -0.760 | 0.129 | 2.017 | 1.951 |
| 1.281 | 0.947 | 0.029 | -0.434 | -0.634 | 0.397 | -0.543 | 0.129 | 1.679 | 2.210 |
| 1.281 | 0.947 | -0.402 | -0.619 | 0.585 | 0.131 | -0.327 | 0.319 | 1.628 | 1.951 |
| 1.281 | 0.947 | 0.029 | -0.372 | 0.585 | 0.131 | -0.435 | 0.319 | 1.636 | 1.869 |
| 1.281 | -0.105 | 0.316 | -0.496 | -0.634 | 0.131 | -1.084 | 0.319 | 1.848 | 1.951 |
| 1.281 | 0.947 | 0.316 | -0.619 | -0.634 | 0.219 | -0.327 | 0.319 | 1.780 | 1.848 |
| -0.941 | -1.158 | 1.897 | 1.853 | -0.634 | 0.086 | 0.753 | 1.841 | -0.080 | 0.091 |
| -0.941 | -0.105 | 1.753 | 1.729 | 0.585 | 0.841 | 1.293 | 2.031 | 0.173 | -0.219 |
| -0.941 | -0.105 | 1.753 | 1.791 | -0.634 | 1.374 | 0.969 | 1.651 | 0.004 | -0.250 |
| -0.941 | -0.105 | 1.753 | 1.432 | 0.585 | 1.018 | 1.617 | 1.841 | -0.080 | -0.188 |
| -0.941 | -0.105 | 1.753 | 0.703 | 1.805 | 1.906 | 1.401 | 1.841 | 0.004 | -0.322 |
| -0.941 | -0.105 | 1.897 | 1.729 | 1.805 | 1.818 | 2.049 | 1.841 | -0.249 | -0.219 |
| -0.941 | 0.947 | 1.753 | 1.803 | 0.585 | 0.930 | 1.833 | 1.651 | 0.089 | -0.219 |
| -0.941 | -3.263 | 1.897 | 0.926 | 1.805 | 0.752 | 1.617 | 2.031 | -0.080 | -0.271 |
| -0.941 | -0.105 | 1.466 | 0.988 | 0.585 | 1.063 | 1.833 | 1.080 | 0.089 | 0.091 |
| -0.941 | -0.105 | 1.753 | 1.358 | 1.805 | 1.285 | 1.833 | 2.031 | 0.343 | -0.343 |
| -0.941 | -1.158 | -0.977 | -0.248 | -0.634 | -0.757 | -1.300 | -1.772 | 0.427 | 0.143 |
| -0.941 | -0.105 | -1.121 | -0.372 | -0.634 | 0.131 | -1.408 | -1.772 | 0.089 | 0.008 |
| -0.941 | -0.105 | -1.121 | -0.681 | -0.634 | -0.313 | -1.192 | -1.772 | -0.114 | 0.091 |
| -0.941 | 0.947 | -0.833 | -0.557 | 0.585 | 0.353 | -0.976 | -1.772 | 0.089 | 0.091 |
| -0.941 | 0.947 | -0.977 | -0.187 | -0.634 | -1.201 | -1.192 | -1.772 | 0.258 | -0.219 |
| -0.941 | 0.947 | -0.977 | -0.681 | 0.585 | 0.574 | -1.408 | -1.772 | 0.427 | -0.219 |
| -0.941 | 0.947 | -1.121 | -0.310 | -0.634 | 0.131 | -1.192 | -1.772 | 0.427 | -0.302 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| -0.941 | 0.947 | -0.402 | -0.434 | -0.634 | -1.201 | -1.192 | -1.582 | 0.427 | -0.147 |
| -0.941 | 0.947 | -0.833 | -0.434 | 0.585 | -0.313 | -1.084 | -1.582 | 0.089 | 0.112 |
| -0.941 | -1.158 | -0.546 | -0.310 | 0.585 | 0.574 | -1.192 | -1.202 | 0.173 | -0.250 |
| -0.941 | 0.947 | -0.402 | -0.434 | -0.634 | -0.491 | -0.327 | -1.012 | 0.427 | -0.250 |
| -0.941 | 0.947 | -0.402 | -0.557 | 0.585 | 0.841 | 0.105 | -0.441 | 0.089 | -0.219 |
| -0.941 | 0.947 | -0.690 | -0.669 | -0.634 | 0.131 | 0.321 | 0.129 | 0.343 | -0.322 |
| -0.941 | 0.947 | -0.690 | 0.209 | 0.585 | 0.308 | 0.105 | 0.129 | 0.427 | -0.219 |
| -0.941 | -0.105 | -0.402 | -0.174 | 1.805 | 1.018 | -0.327 | 0.129 | 0.596 | -0.322 |
| -0.941 | -1.158 | -0.259 | 0.580 | 1.805 | 1.018 | -0.327 | 0.129 | 0.512 | 0.194 |
| -0.941 | -3.263 | -0.259 | 0.332 | 0.585 | 0.619 | -0.003 | 0.129 | 0.258 | -0.219 |
| -0.941 | 0.947 | -0.259 | -0.162 | 0.585 | 0.796 | 0.321 | 0.129 | 0.512 | -0.271 |
| -0.941 | 0.947 | -0.259 | -0.137 | 0.585 | 0.042 | -0.111 | 0.129 | 0.089 | -0.240 |
| -0.941 | 0.947 | -0.259 | -0.100 | 0.585 | -0.003 | 0.105 | 0.129 | 0.258 | -0.333 |
| -0.941 | 0.947 | -0.546 | -0.273 | -0.634 | -0.313 | -0.003 | -0.821 | -0.419 | -0.395 |
| -0.941 | 0.947 | -0.402 | -0.644 | 0.585 | 0.131 | -0.327 | 0.129 | -0.334 | -0.426 |
| -0.941 | -0.105 | -0.833 | -0.360 | 0.585 | 0.574 | -0.327 | -0.441 | -0.672 | -0.374 |
| -0.941 | 0.947 | -0.833 | -0.335 | 0.585 | 0.131 | -0.435 | 0.129 | -0.841 | -0.426 |
| -0.941 | 0.947 | -0.690 | -0.150 | -0.634 | -0.269 | -0.327 | 0.129 | -0.334 | -0.322 |
| -0.941 | 0.947 | -0.690 | -0.582 | 0.585 | 0.796 | -0.327 | 0.129 | -0.867 | -0.033 |
| -0.941 | 0.947 | -0.690 | -0.298 | 0.585 | -0.313 | -0.219 | 0.129 | -0.757 | -0.116 |
| -0.941 | -0.105 | -0.690 | -0.286 | 0.585 | -0.757 | -0.327 | 0.129 | -0.351 | -0.322 |
| -0.941 | -0.105 | -0.690 | -0.582 | -0.634 | -0.757 | -0.003 | 0.510 | -0.419 | -0.260 |
| -0.941 | -0.105 | -0.833 | -0.520 | 0.585 | 0.574 | 0.213 | 0.129 | -0.419 | -0.219 |
| 1.281 | 0.947 | 1.753 | -0.162 | -0.634 | -0.313 | -0.868 | 0.129 | 0.478 | 0.773 |
| 0.541 | 0.947 | 1.753 | -0.409 | 0.585 | -0.091 | -1.084 | 1.460 | 0.478 | 0.618 |
| 0.541 | 0.947 | 1.753 | -0.421 | -1.854 | -0.757 | -1.192 | 1.460 | 0.427 | 0.453 |
| 0.541 | 0.947 | 1.753 | -0.298 | -0.634 | -0.313 | -1.084 | 1.460 | 0.258 | 0.515 |
| 0.541 | -0.105 | 1.753 | -0.372 | 0.585 | 0.131 | -0.976 | 1.460 | 0.393 | 0.008 |
| -0.200 | -0.105 | 1.753 | -0.619 | -0.634 | -0.313 | -0.868 | 1.460 | 0.427 | 0.091 |
| -0.941 | 0.947 | 1.897 | -1.114 | 0.585 | -0.091 | -1.192 | 1.460 | 0.427 | -0.136 |
| 0.541 | 0.947 | 1.897 | -0.248 | -0.634 | -0.047 | -1.192 | 2.031 | 0.258 | -0.012 |
| 0.541 | 0.947 | 1.753 | -0.496 | 0.585 | 0.131 | -1.192 | 1.460 | 0.427 | 0.215 |
| 1.281 | 0.947 | 1.753 | -0.248 | -0.634 | -0.313 | -1.084 | 1.460 | 0.343 | 0.380 |
| 0.541 | -0.105 | 0.316 | -0.125 | 1.805 | 1.285 | 1.833 | 0.129 | -0.249 | -0.219 |
| 0.541 | -0.105 | 0.316 | 3.212 | -0.634 | 0.131 | 2.049 | 0.129 | -0.334 | -0.250 |
| 1.281 | -0.105 | 0.316 | 2.594 | 0.585 | 0.574 | 2.266 | 0.129 | -0.334 | -0.147 |
| 0.541 | 0.947 | 0.316 | 2.471 | -1.854 | -1.201 | 1.833 | 0.129 | -0.249 | -0.116 |
| -0.941 | -0.105 | 0.316 | 1.853 | 0.585 | -0.003 | 1.941 | -0.061 | -0.436 | 0.050 |
| 0.541 | -0.105 | 0.460 | 1.853 | -0.634 | 0.042 | 2.266 | 0.129 | -0.419 | -0.322 |
| -0.200 | -0.105 | 0.316 | 1.828 | 0.585 | 0.486 | 1.509 | 0.129 | -0.419 | 0.060 |
| 0.541 | -0.105 | 0.316 | 1.939 | -0.634 | -0.313 | 2.049 | 0.129 | -0.452 | -0.271 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1.281 | -0.105 | 0.316 | 1.952 | 0.585 | 1.018 | 1.941 | 0.129 | -0.419 | -0.219 |
| -0.941 | -1.158 | 0.603 | 2.940 | -0.634 | 0.885 | 2.049 | 0.129 | -0.249 | -0.167 |
| 0.541 | -1.158 | -0.402 | -0.162 | 1.805 | 1.107 | 0.213 | -0.821 | 0.765 | 1.435 |
| 0.541 | -1.158 | -0.402 | -0.421 | -0.634 | 0.841 | -0.003 | -0.821 | 0.740 | 1.094 |
| -0.941 | -0.105 | -0.402 | -0.286 | 0.585 | 0.574 | -0.003 | -0.821 | 0.630 | 1.021 |
| 1.281 | 0.947 | -0.259 | -0.545 | -0.634 | 0.796 | 0.213 | -0.821 | 0.732 | 0.814 |
| 1.281 | 0.947 | -0.402 | -0.224 | 0.585 | 1.462 | -0.003 | -0.821 | 0.748 | 0.990 |
| -0.200 | 0.947 | -0.402 | -0.656 | -0.634 | 1.018 | 0.213 | -0.251 | 0.715 | 0.825 |
| 0.541 | 0.947 | -0.402 | 0.048 | 0.585 | 0.574 | -0.219 | -0.251 | 0.596 | 0.711 |
| -0.941 | 0.947 | -0.259 | 0.431 | -0.634 | 0.131 | 0.213 | -0.251 | 0.681 | 0.897 |
| 0.541 | 0.947 | -0.402 | -0.286 | 1.805 | 1.462 | -0.003 | -0.251 | 0.774 | 0.732 |
| 1.281 | -0.105 | -0.402 | -0.372 | 0.585 | 0.574 | 0.105 | -0.441 | 0.765 | 0.856 |
| -0.941 | -0.105 | -0.833 | -0.001 | 0.585 | 0.131 | -0.327 | -0.251 | -0.207 | 0.494 |
| 0.541 | -0.105 | -0.833 | -0.001 | -0.634 | -0.313 | -0.327 | -0.251 | -0.165 | 0.194 |
| -0.941 | -2.211 | -0.690 | 0.122 | -0.634 | -0.757 | -0.003 | -0.251 | -0.165 | -0.147 |
| 0.541 | -2.211 | -0.833 | -0.496 | 0.585 | 0.131 | -0.111 | -0.251 | -0.249 | -0.136 |
| -0.941 | -1.158 | -0.833 | -0.125 | -0.634 | -0.313 | -0.003 | -0.251 | -0.334 | -0.322 |
| -0.941 | -1.158 | -0.833 | -0.001 | 0.585 | 0.131 | -0.111 | -0.251 | -0.080 | -0.219 |
| 1.281 | -0.105 | -0.833 | -0.248 | -0.634 | -0.757 | 0.105 | -0.251 | -0.249 | -0.219 |
| -0.941 | 0.947 | -0.833 | -0.273 | 0.585 | -0.313 | 0.213 | -0.251 | 0.004 | -0.012 |
| 1.281 | -0.105 | -0.690 | -0.014 | -0.634 | -0.757 | 0.213 | -0.251 | -0.080 | -0.147 |
| 1.281 | -0.105 | -0.833 | -0.248 | 0.585 | 0.131 | 0.105 | -0.251 | -0.249 | -0.374 |
| **0.000**[a] | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** |
| **1.000**[b] | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |

**a: Mean, b: Standard deviation**

**Appendix 7.2.** Standardized data for analysis of season two

| *Y* | *x1* | *x2* | *x3* | *x4* | *x5* | *x6* | *x7* | *x8* | *x9* |
|---|---|---|---|---|---|---|---|---|---|
| 1.252 | -1.950 | -1.280 | -1.126 | -0.596 | -0.452 | -1.489 | -0.327 | -2.283 | -2.172 |
| 1.252 | -1.950 | -1.280 | -1.168 | -0.310 | -0.452 | -1.349 | -1.788 | -2.370 | -2.070 |
| 1.252 | -1.950 | -1.128 | -1.404 | -0.596 | -1.356 | -1.664 | -1.240 | -2.283 | -2.121 |
| 1.252 | -1.950 | -1.280 | -1.348 | -0.500 | -0.452 | -1.559 | -1.606 | -2.239 | -1.967 |
| 1.252 | -1.950 | -0.824 | -1.265 | -0.214 | -1.356 | -1.804 | -1.423 | -2.300 | -2.377 |
| 1.252 | -1.950 | -1.280 | -1.126 | -1.263 | 0.452 | -1.174 | -1.240 | -2.300 | -1.885 |
| 1.252 | -3.267 | -1.128 | -1.265 | -0.310 | -1.356 | -1.804 | -0.875 | -2.283 | -2.172 |
| 1.252 | -0.632 | -1.128 | -1.237 | -0.500 | -2.260 | -2.223 | -1.240 | -2.283 | -2.326 |
| 1.252 | -1.950 | -0.519 | -1.279 | -0.405 | -0.452 | -1.769 | -1.788 | -2.318 | -2.018 |
| 1.252 | -1.950 | -0.824 | -1.404 | -0.500 | -1.356 | -1.734 | -1.058 | -2.379 | -2.100 |
| 1.252 | 0.685 | 0.242 | -0.848 | -0.786 | 0.452 | 0.573 | -0.875 | 1.828 | 2.134 |
| 1.252 | 0.685 | 0.394 | -0.376 | -0.691 | -0.452 | 0.224 | 0.038 | 1.740 | 1.806 |
| 1.252 | 0.685 | 0.242 | -0.432 | -0.596 | -0.452 | 0.259 | 0.038 | 1.740 | 1.827 |
| 1.252 | 0.685 | -0.215 | -0.709 | -0.786 | -0.452 | 0.398 | -0.144 | 1.696 | 1.929 |
| 1.252 | 0.685 | -0.215 | -0.432 | -0.691 | 0.452 | 0.503 | 0.038 | 1.688 | 1.888 |
| 1.252 | 0.685 | -0.062 | -0.515 | -0.596 | 0.452 | 0.294 | -0.144 | 1.836 | 2.237 |
| 1.252 | 0.685 | -0.671 | -0.668 | -0.500 | -0.452 | 0.259 | 0.038 | 1.723 | 2.063 |
| 1.252 | -0.632 | -0.062 | -0.293 | -0.405 | -1.356 | -0.126 | 0.038 | 1.740 | 1.878 |
| 1.252 | 0.685 | 0.242 | -0.570 | -0.310 | -0.452 | 0.154 | 0.038 | 1.740 | 1.899 |
| 1.252 | 2.003 | 0.394 | -0.473 | -0.214 | -0.452 | 0.154 | 0.038 | 1.784 | 1.991 |
| -0.970 | -0.632 | 1.764 | 1.930 | 1.596 | 0.452 | 0.678 | 1.865 | -0.184 | -0.121 |
| -0.970 | -0.632 | 1.917 | 1.791 | 1.120 | 1.356 | 0.748 | 1.682 | -0.271 | -0.019 |
| -0.970 | -0.632 | 1.308 | 1.166 | 1.596 | 1.356 | 1.272 | 0.952 | -0.184 | -0.142 |
| -0.970 | -0.632 | 1.460 | 0.402 | 1.501 | 1.356 | 1.167 | 1.682 | -0.140 | -0.275 |
| -0.970 | -0.632 | 1.612 | 0.749 | 1.406 | 2.260 | 1.552 | 1.865 | -0.210 | -0.306 |
| -0.970 | -0.632 | 1.308 | 1.652 | 2.073 | 1.356 | 1.377 | 1.682 | -0.289 | -0.378 |
| -0.970 | -0.632 | 1.764 | 1.332 | 1.596 | 0.452 | 0.923 | 1.500 | -0.184 | -0.326 |
| -0.970 | -0.632 | 1.764 | 0.818 | 1.787 | 0.452 | 0.783 | 1.317 | -0.088 | -0.224 |
| -0.970 | -1.950 | 1.917 | 1.374 | 1.787 | 1.356 | 1.202 | 1.134 | -0.201 | -0.224 |
| -0.970 | -1.950 | 1.764 | 1.652 | 1.882 | 1.356 | 1.272 | 1.865 | -0.166 | -0.091 |
| -0.970 | 0.685 | -1.280 | -0.293 | -1.358 | -1.356 | -1.524 | -2.336 | 0.472 | 0.217 |
| -0.970 | 0.685 | -0.824 | -0.432 | -1.263 | -0.452 | -0.126 | -1.971 | 0.341 | -0.039 |
| -0.970 | 0.685 | -0.824 | -0.848 | -1.072 | -0.452 | -0.336 | -1.240 | 0.428 | -0.019 |
| -0.970 | 0.685 | -0.671 | -0.529 | -0.882 | -1.356 | -0.825 | -1.606 | 0.446 | 0.217 |
| -0.970 | 0.685 | -0.824 | -0.140 | -1.263 | -1.356 | -1.174 | -1.788 | 0.341 | -0.285 |
| -0.970 | 0.685 | -0.976 | -0.848 | -1.358 | -2.260 | -1.594 | -1.788 | 0.385 | -0.214 |
| -0.970 | 0.685 | -0.671 | -0.154 | -1.358 | -0.452 | -0.650 | -1.240 | 0.420 | -0.162 |
| -0.970 | 0.685 | -0.367 | -0.182 | -1.263 | -0.452 | -0.475 | -1.788 | 0.446 | -0.039 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| -0.970 | -0.632 | -0.824 | -0.432 | -0.882 | -0.452 | -0.930 | -1.058 | 0.385 | -0.019 |
| -0.970 | -0.632 | -0.824 | -0.154 | -0.882 | 0.452 | 0.923 | -1.240 | 0.420 | -0.121 |
| -0.970 | 0.685 | -0.215 | -0.362 | -0.119 | 0.452 | 0.189 | -1.058 | 0.385 | -0.132 |
| -0.970 | 0.685 | -0.215 | -0.570 | 0.167 | 1.356 | 1.167 | -0.875 | 0.341 | -0.039 |
| -0.970 | 0.685 | -0.062 | -0.598 | 0.262 | 0.452 | 0.398 | 0.221 | 0.253 | -0.378 |
| -0.970 | 0.685 | -0.367 | 0.263 | -0.024 | 1.356 | 1.377 | 0.038 | 0.428 | -0.224 |
| -0.970 | 0.685 | -0.215 | 0.055 | -0.214 | 0.452 | 0.713 | 0.038 | 0.341 | -0.265 |
| -0.970 | -0.632 | -0.367 | 0.541 | -0.310 | 0.452 | 0.853 | 0.221 | 0.253 | -0.224 |
| -0.970 | -0.632 | -0.215 | 0.277 | 0.167 | 1.356 | 1.167 | 0.038 | 0.341 | -0.306 |
| -0.970 | 0.685 | -0.215 | -0.293 | 0.262 | 0.452 | 0.573 | 0.038 | 0.341 | -0.224 |
| -0.970 | -0.632 | -0.215 | -0.015 | 0.262 | -0.452 | 0.014 | -0.875 | 0.428 | -0.173 |
| -0.970 | 0.685 | -0.367 | -0.182 | 0.071 | 0.452 | 1.028 | -0.144 | 0.385 | -0.326 |
| -0.970 | 0.685 | -0.976 | -0.015 | -0.310 | -0.452 | -0.825 | -0.875 | -0.446 | -0.347 |
| -0.970 | 0.685 | -0.824 | -0.459 | -0.214 | 0.452 | 0.364 | 0.038 | -0.709 | -0.491 |
| -0.970 | -1.950 | -0.976 | -0.154 | -0.405 | 0.452 | 0.608 | 0.404 | -0.359 | -0.409 |
| -0.970 | 0.685 | -0.976 | -0.195 | -0.500 | 0.452 | 0.643 | 0.586 | -0.490 | -0.326 |
| -0.970 | 0.685 | -1.128 | 0.263 | -0.214 | -0.452 | -0.405 | 0.769 | -0.438 | -0.214 |
| -0.970 | -0.632 | -0.976 | -0.529 | -0.214 | -0.452 | -1.070 | 0.404 | -0.481 | -0.326 |
| -0.970 | 0.685 | -0.976 | -0.043 | -0.310 | 0.452 | 0.224 | 0.586 | -0.464 | -0.173 |
| -0.970 | 0.685 | -1.128 | -0.154 | -0.214 | -0.452 | -0.895 | 0.038 | -0.455 | -0.214 |
| -0.970 | -0.632 | -0.824 | -0.570 | -0.310 | 0.452 | 0.224 | 0.586 | -0.455 | -0.224 |
| -0.970 | -1.950 | -0.976 | -0.612 | -0.214 | 1.356 | 1.098 | 0.038 | -0.429 | -0.326 |
| 0.511 | 0.685 | 1.764 | -0.432 | -0.977 | -1.356 | -1.105 | 0.038 | 0.428 | 0.596 |
| 1.252 | 0.685 | 1.917 | -0.709 | -1.072 | -0.452 | -1.035 | 1.865 | 0.428 | 0.391 |
| 0.511 | 0.685 | 1.764 | -0.376 | -0.882 | -2.260 | -1.279 | 1.865 | 0.411 | 0.289 |
| 0.511 | 0.685 | 1.764 | -0.570 | -0.977 | -0.452 | -0.825 | 0.952 | 0.245 | 0.391 |
| -0.230 | 0.685 | 1.917 | -0.320 | -1.072 | -1.356 | -1.314 | 0.952 | 0.516 | 0.371 |
| -0.970 | -0.632 | 1.764 | -0.570 | -0.882 | -1.356 | -1.209 | 0.952 | 0.463 | 0.268 |
| 1.252 | 0.685 | 1.764 | -1.265 | -0.882 | -0.452 | -1.000 | 1.500 | 0.437 | 0.217 |
| 0.511 | 0.685 | 1.764 | -0.293 | -1.072 | -0.452 | -1.349 | 1.682 | 0.411 | 0.155 |
| 1.252 | 0.685 | 1.764 | -0.265 | -1.263 | -1.356 | -1.279 | 1.865 | 0.437 | 0.289 |
| -0.970 | -0.632 | 1.917 | -0.154 | -1.072 | -1.356 | -1.209 | 0.952 | 0.446 | 0.330 |
| 1.252 | -0.632 | 0.547 | 0.124 | 2.454 | 1.356 | 1.272 | 0.586 | -0.306 | -0.275 |
| -0.230 | 0.685 | 0.547 | 3.318 | 2.073 | 1.356 | 1.028 | 0.404 | -0.446 | -0.429 |
| -0.970 | 0.685 | 0.394 | 1.791 | 1.978 | 1.356 | 1.237 | 0.769 | -0.403 | -0.326 |
| 1.252 | 0.685 | 0.394 | 3.180 | 2.168 | 0.452 | 1.132 | 0.038 | -0.341 | -0.224 |
| 0.511 | 0.685 | 0.547 | 0.193 | 2.073 | -1.356 | -1.174 | -0.144 | -0.359 | -0.183 |
| 0.511 | -0.632 | 0.547 | 2.791 | 2.168 | 1.356 | 1.342 | -0.144 | -0.481 | -0.224 |
| 0.511 | 0.685 | 0.699 | 2.485 | 1.501 | 1.356 | 1.132 | 0.038 | -0.403 | -0.070 |
| -0.230 | 0.685 | 0.699 | 1.027 | 1.596 | 0.452 | 0.643 | 0.038 | -0.403 | -0.224 |
| 1.252 | 0.685 | 0.547 | 2.902 | 1.787 | 1.356 | 1.307 | 0.586 | -0.665 | -0.326 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| -0.970 | -0.632 | 0.547 | 3.874 | 1.692 | 0.452 | 0.888 | 0.038 | -0.359 | -0.275 |
| 0.511 | 0.685 | -0.367 | -0.293 | 0.071 | 0.452 | 0.398 | -0.327 | 0.778 | 1.212 |
| 1.252 | 0.685 | -0.367 | -0.570 | 0.167 | 1.356 | 1.167 | -0.144 | 0.734 | 1.191 |
| 1.252 | 0.685 | -0.215 | -0.293 | -0.024 | 0.452 | 0.398 | 0.038 | 0.673 | 0.945 |
| 0.511 | 0.685 | -0.367 | -0.598 | -0.214 | 1.356 | 0.958 | 0.038 | 0.761 | 0.924 |
| -0.230 | 0.685 | -0.671 | 0.124 | -0.024 | 1.356 | 1.098 | 0.038 | 0.708 | 1.007 |
| 0.511 | 0.685 | -0.367 | 0.055 | -0.024 | 0.452 | 0.608 | 0.404 | 0.691 | 1.037 |
| 0.511 | 0.685 | -0.519 | 0.263 | -0.024 | 0.452 | 0.259 | 0.769 | 0.647 | 0.801 |
| 1.252 | 0.685 | -0.367 | 0.402 | 0.167 | -0.452 | 0.014 | 0.038 | 0.734 | 0.863 |
| -0.970 | -0.632 | -0.367 | -0.043 | -0.024 | 1.356 | 1.342 | -0.510 | 0.787 | 1.058 |
| -0.230 | -0.632 | -0.671 | -0.154 | -0.119 | 0.452 | 0.608 | -0.510 | 0.647 | 0.863 |
| 1.252 | 0.685 | -0.519 | 0.263 | -0.310 | -0.452 | -0.126 | -0.327 | -0.263 | 0.145 |
| 0.511 | 0.685 | -0.671 | -0.459 | -0.214 | -1.356 | -0.825 | -0.144 | -0.184 | 0.145 |
| -0.230 | -0.632 | -0.367 | -0.154 | 0.071 | -0.452 | -0.091 | -0.144 | -0.140 | -0.173 |
| 0.511 | 0.685 | -0.519 | -0.348 | -0.214 | 0.452 | 0.049 | -0.144 | -0.315 | -0.214 |
| 0.511 | 0.685 | -0.519 | -0.015 | -0.024 | -0.452 | 0.014 | -0.144 | -0.376 | -0.409 |
| 1.252 | 0.685 | -0.367 | -0.154 | -0.119 | -0.452 | -0.196 | -0.327 | -0.289 | -0.388 |
| -0.970 | 0.685 | -0.519 | -0.293 | -0.024 | -1.356 | -1.000 | -0.144 | -0.289 | -0.326 |
| -0.970 | 0.685 | -0.519 | -0.154 | 0.071 | -0.452 | -0.126 | 0.038 | -0.228 | 0.114 |
| 1.252 | -0.632 | -0.519 | -0.195 | -0.024 | -0.452 | -0.091 | 0.586 | -0.166 | 0.145 |
| -0.230 | 0.685 | -0.671 | -0.293 | -0.119 | 0.452 | 0.503 | -0.144 | -0.376 | -0.214 |
| **0.000[a]** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** |
| **1.000[b]** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |

**a: Mean, b: Standard deviation**

**Appendix 7.3.** Cycle of marker assisted selection among parents and their respective F2 progenies

| Sample No: | Genotype | Markers and genes evaluated | | | |
|---|---|---|---|---|---|
| | | STK-1 | SQ4 | *BCMV* -48289723-CAPS | ENM-CAPS |
| | | *Co-1* | *Co-2* | *I* | *bc-3* |
| 1 | AC-HEN | + | | + | + |
| 2 | AC-HEN | + | | + | + |
| 3 | AC-HEN | + | | + | + |
| 4 | AC-HEN | + | | + | + |
| 5 | AC-HEN | + | | + | + |
| 6 | AC-HEN | + | | + | + |
| 7 | AC-HEN | + | | + | + |
| 8 | AC-HEN | + | | + | + |
| 9 | AC-HEN | + | | + | + |
| 10 | AC-HEN | + | | + | + |
| 11 | USCR-7 | | + | + | + |
| 12 | USCR-7 | | + | + | + |
| 13 | USCR-7 | | + | + | + |
| 14 | USCR-7 | | + | + | + |
| 15 | USCR-7 | | + | + | + |
| 16 | USCR-7 | | + | + | + |
| 17 | USCR-7 | | + | + | + |
| 18 | USCR-7 | | + | + | + |
| 19 | USCR-7 | | + | + | + |
| 20 | USCR-7 | | + | + | + |
| 21 | G 54 | - | - | - | - |
| 22 | G 54 | - | - | - | - |
| 23 | G 54 | - | - | - | - |
| 24 | G 54 | - | - | - | - |
| 25 | G 54 | - | - | - | - |
| 26 | G 54 | - | - | - | - |
| 27 | G 54 | - | - | - | - |
| 28 | G 54 | - | - | - | - |
| 29 | G 54 | - | - | - | - |
| 30 | G 54 | - | - | - | - |
| 31 | RWR 1668 | - | - | - | - |
| 32 | RWR 1668 | - | - | - | - |
| 33 | RWR 1668 | - | - | - | - |
| 34 | RWR 1668 | - | - | - | - |
| 35 | RWR 1668 | - | - | - | - |
| 36 | RWR 1668 | - | - | - | - |
| 37 | RWR 1668 | - | - | - | - |
| 38 | RWR 1668 | - | - | - | - |
| 39 | RWR 1668 | - | - | - | - |
| 40 | RWR 1668 | - | - | - | - |
| 41 | RWR 2355 | - | - | - | - |
| 42 | RWR 2355 | - | - | - | - |
| 43 | RWR 2355 | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| 44 | RWR 2355 | - | - | - | - |
| 45 | RWR 2355 | - | - | - | - |
| 46 | RWR 2355 | - | - | - | - |
| 47 | RWR 2355 | - | - | - | - |
| 48 | RWR 2355 | - | - | - | - |
| 49 | RWR 2355 | - | - | - | - |
| 50 | RWR 2355 | - | - | - | - |
| 51 | RWR 2361 | - | - | - | - |
| 52 | RWR 2361 | - | - | - | - |
| 53 | RWR 2361 | - | - | - | - |
| 54 | RWR 2361 | - | - | - | - |
| 55 | RWR 2361 | - | - | - | - |
| 56 | RWR 2361 | - | - | - | - |
| 57 | RWR 2361 | - | - | - | - |
| 58 | RWR 2361 | - | - | - | - |
| 59 | RWR 2361 | - | - | - | - |
| 60 | RWR 2361 | - | - | - | - |
| | **G54 F2** | | | | |
| 61 | F1XF1 | + | | ± | - |
| 62 | F1XF1 | - | | + | - |
| 63 | F1XF1 | ± | | ± | ± |
| 64 | F1XF1 | ± | | ± | ± |
| 65 | F1XF1 | - | | - | - |
| 66 | F1XF1 | ± | | + | - |
| 67 | F1XF1 | ± | | - | ± |
| 68 | F1XF1 | - | | ± | + |
| 69 | F1XF1 | - | | ± | ± |
| 70 | F1XF1 | + | | + | ± |
| 71 | F1XF1 | - | | ± | ± |
| 72 | F1XF1 | - | | ± | + |
| 73 | F1XF1 | ± | | - | + |
| 74 | F1XF1 | ± | | + | ± |
| 75 | F1XF1 | - | | - | - |
| 76 | F1XF1 | ± | | + | + |
| 77 | F1XF1 | + | | ± | ± |
| 78 | F1XF1 | ± | | ± | ± |
| 79 | F1XF1 | - | | ± | - |
| 80 | F1XF1 | - | | - | - |
| 81 | F1XF1 | + | | ± | ± |
| 82 | F1XF1 | ± | | + | ± |
| 83 | F1XF1 | - | | ± | ± |
| 84 | F1XF1 | ± | | ± | ± |
| 85 | F1XF1 | + | | + | - |
| 86 | F1XF1 | ± | | + | + |
| 87 | F1XF1 | ± | | ± | + |
| 88 | F1XF1 | - | | - | - |

| | | | | |
|---|---|---|---|---|
| | **RWR 1668** | | | |
| 89 | F1XF1 | - | ± | - |
| 90 | F1XF1 | - | ± | - |
| 91 | F1XF1 | ± | - | - |
| 92 | F1XF1 | ± | ± | + |
| 93 | F1XF1 | + | ± | - |
| 94 | F1XF1 | ± | - | ± |
| 95 | F1XF1 | ± | - | - |
| 96 | F1XF1 | - | ± | + |
| 97 | F1XF1 | + | ± | ± |
| 98 | F1XF1 | ± | + | ± |
| 99 | F1XF1 | ± | + | + |
| 100 | F1XF1 | ± | - | - |
| 101 | F1XF1 | - | ± | ± |
| 102 | F1XF1 | + | ± | ± |
| 103 | F1XF1 | + | + | + |
| 104 | F1XF1 | ± | - | - |
| 105 | F1XF1 | ± | + | - |
| 106 | F1XF1 | ± | ± | ± |
| 107 | F1XF1 | - | ± | - |
| 108 | F1XF1 | + | ± | ± |
| | **RWR 2355 F2** | | | |
| 109 | F1XF1 | ± | ± | ± |
| 110 | F1XF1 | ± | - | - |
| 111 | F1XF1 | - | ± | - |
| 112 | F1XF1 | + | + | - |
| 113 | F1XF1 | + | ± | - |
| 114 | F1XF1 | + | ± | - |
| 115 | F1XF1 | - | + | ± |
| 116 | F1XF1 | ± | ± | ± |
| 117 | F1XF1 | ± | + | + |
| 118 | F1XF1 | - | + | + |
| 119 | F1XF1 | ± | ± | - |
| 120 | F1XF1 | ± | - | + |
| 121 | F1XF1 | ± | ± | - |
| 122 | F1XF1 | + | ± | ± |
| 123 | F1XF1 | ± | + | - |
| 124 | F1XF1 | ± | - | ± |
| 125 | F1XF1 | + | ± | ± |
| 126 | F1XF1 | + | - | - |
| 127 | F1XF1 | - | ± | + |
| 128 | F1XF1 | - | + | ± |
| | **RWV 2361 F2** | | | |
| 129 | F1XF1 | + | ± | ± |
| 130 | F1XF1 | - | + | + |
| 131 | F1XF1 | + | - | - |
| 132 | F1XF1 | + | - | - |
| 133 | F1XF1 | + | + | - |
| 134 | F1XF1 | - | + | ± |
| 135 | F1XF1 | + | + | - |

| | | | | |
|---|---|---|---|---|
| 136 | F1XF1 | + | ± | ± |
| 137 | F1XF1 | + | + | ± |
| 138 | F1XF1 | + | - | - |
| 139 | F1XF1 | + | + | + |
| 140 | F1XF1 | + | ± | ± |
| 141 | F1XF1 | - | ± | ± |
| 142 | F1XF1 | + | + | - |
| 143 | F1XF1 | + | + | - |
| 144 | F1XF1 | + | ± | + |
| 145 | F1XF1 | + | ± | ± |
| 146 | F1XF1 | + | ± | - |
| 147 | F1XF1 | + | + | - |
| 148 | F1XF1 | + | ± | - |
| 149 | F1XF1 | + | + | + |
| 150 | F1XF1 | - | ± | ± |
| 151 | F1XF1 | + | - | + |
| 152 | F1XF1 | + | ± | - |

*STK-1* = Co dominant marker linked to *Co-1*, *SQ4* = A dominant marker linked to *Co-2*

ENM-CAPS = Co dominant marker linked to *bc-3, BCMV-48289723-CAPS* = Co dominant marker linked to *I*

+ = Presence of marker, - = Absence of marker, ± = Heterozygous for the marker.

**Appendix 7.4.** Cycle of marker assisted selection among BC1F1 progenies

| Sample No:Genotype | | Markers and genes evaluated | | | |
|---|---|---|---|---|---|
| | | STK-1 *Co-1* | SQ4 *Co-2* | *BCMV* -48289723-CAPS *bc-3* | ENM-CAPS *I* |
| 1 | G 54X F1 | ± | | ± | ± |
| 2 | G 54x F1 | - | | - | ± |
| 3 | G 54x F1 | - | | - | - |
| 4 | G 54x F1 | - | | - | - |
| 5 | G 54x F1 | ± | | - | ± |
| 6 | G 54x F1 | - | | ± | ± |
| 7 | G 54x F1 | - | | - | ± |
| 8 | G 54x F1 | ± | | ± | ± |
| 9 | G 54x F1 | - | | - | - |
| 10 | G 54x F1 | - | | - | ± |
| 11 | G 54X F1 | ± | | - | - |
| 12 | G 54x F1 | - | | - | - |
| 13 | G 54x F1 | - | | - | - |
| 14 | G 54x F1 | - | | ± | ± |
| 15 | G 54x F1 | - | | - | - |
| 16 | G 54x F1 | ± | | - | - |
| 17 | G 54x F1 | ± | | ± | ± |
| 1 | RWR 1668X F1 | ± | | - | ± |
| 2 | RWR 1668X F1 | ± | | ± | ± |
| 3 | RWR 1668X F1 | - | | ± | ± |
| 4 | RWR 1668X F1 | - | | - | - |
| 5 | RWR 1668X F1 | ± | | - | - |
| 6 | RWR 1668X F1 | - | | - | - |
| 7 | RWR 1668X F1 | - | | - | - |
| 8 | RWR 1668X F1 | - | | ± | ± |
| 9 | RWR 1668X F1 | ± | | ± | ± |
| 10 | RWR 1668X F1 | - | | - | - |
| 1 | RWR 2355X F1 | - | | - | ± |
| 2 | RWR 2355X F1 | ± | | - | ± |
| 3 | RWR 2355X F1 | - | | - | - |
| 4 | RWR 2355X F1 | - | | - | - |
| 5 | RWR 2355X F1 | ± | | ± | ± |
| 6 | RWR 2355X F1 | - | | - | - |
| 7 | RWR 2355X F1 | ± | | - | - |
| 8 | RWR 2355X F1 | ± | | ± | ± |
| 9 | RWR 2355X F1 | - | | ± | ± |
| 1 | RWR 2361X F1 | | + | ± | ± |
| 2 | RWR 2361X F1 | | + | - | - |
| 3 | RWR 2361X F1 | | - | ± | ± |
| 4 | RWR 2361X F1 | | - | - | - |
| 5 | RWR 2361X F1 | | - | - | - |
| 6 | RWR 2361X F1 | | + | ± | - |

| | | | | |
|---|---|---|---|---|
| 7 | RWR 2361X F1 | + | ± | ± |
| 8 | RWR 2361X F1 | - | - | - |
| 9 | RWR 2361X F1 | - | - | - |
| 10 | RWR 2361X F1 | - | - | - |
| 11 | RWR 2361X F1 | + | - | - |
| 12 | RWR 2361X F1 | + | ± | ± |
| 13 | RWR 2361X F1 | - | - | - |
| 14 | RWR 2361X F1 | - | - | - |
| 15 | RWR 2361X F1 | - | ± | ± |
| 16 | RWR 2361X F1 | - | - | - |

STK-1 = Co dominant marker linked to *Co-1*, SQ4 = A dominant marker linked to *Co-2*

ENM-CAPS = Co dominant marker linked to *bc-3, BCMV-48289723-CAPS* = Co dominant marker linked to *I*

+ = Presence of marker, - = Absence of marker, ± = Heterozygous for the marker.

**Appendix 7.5.** Cycle of marker assisted selection among BC2F1 progenies

| Sample No: | Genotype | Markers and genes evaluated | | | |
|---|---|---|---|---|---|
| | | STK-1 *Co-1* | SQ4 *Co-2* | *BCMV* -48289723-CAPS *bc-3* | ENM-CAPS *I* |
| 1 | G 54X BC1F1 | - | | - | - |
| 2 | G 54x BC1F1 | - | | - | - |
| 3 | G 54x BC1F1 | - | | ± | ± |
| 4 | G 54x BC1F1 | - | | - | - |
| 5 | G 54x BC1F1 | ± | | - | ± |
| 6 | G 54x BC1F1 | ± | | ± | ± |
| 7 | G 54x BC1F1 | - | | - | - |
| 8 | G 54x BC1F1 | - | | ± | ± |
| 9 | G 54x BC1F1 | - | | ± | ± |
| 10 | G 54x BC1F1 | - | | - | - |
| 11 | G 54X BC1F1 | - | | - | - |
| 12 | G 54x BC1F1 | ± | | ± | ± |
| 13 | G 54x BC1F1 | ± | | ± | - |
| 14 | G 54x BC1F1 | - | | - | - |
| 1 | RWR 1668X BC1F1 | - | | ± | - |
| 2 | RWR 1668X BC1F1 | - | | ± | ± |
| 3 | RWR 1668X BC1F1 | - | | - | - |
| 4 | RWR 1668X BC1F1 | ± | | - | - |
| 5 | RWR 1668X BC1F1 | - | | ± | ± |
| 6 | RWR 1668X BC1F1 | - | | - | - |
| 7 | RWR 1668X BC1F1 | ± | | - | ± |
| 8 | RWR 1668X BC1F1 | ± | | ± | ± |
| 1 | RWR 2355x BC1F1 | ± | | ± | ± |
| 2 | RWR 2355x BC1F1 | - | | - | - |
| 3 | RWR 2355x BC1F1 | + | | + | + |
| 4 | RWR 2355x BC1F1 | ± | | ± | ± |
| 5 | RWR 2355x BC1F1 | - | | - | - |
| 6 | RWR 2355x BC1F1 | - | | - | - |
| 7 | RWR 2355x BC1F1 | - | | - | ± |
| 8 | RWR 2355x BC1F1 | - | | - | - |
| 9 | RWR 2355x BC1F1 | - | | - | - |
| 10 | RWR 2355x BC1F1 | - | | ± | ± |
| 11 | RWR 2355x BC1F1 | - | | - | - |
| 12 | RWR 2355x BC1F1 | - | | - | - |
| 13 | RWR 2355x BC1F1 | - | | ± | ± |
| 14 | RWR 2355x BC1F1 | ± | | ± | ± |
| 15 | RWR 2355x BC1F1 | ± | | - | - |
| 16 | RWR 2355x BC1F1 | - | | - | - |
| 17 | RWR 2355x BC1F1 | - | | ± | ± |
| 18 | RWR 2355x BC1F1 | - | | ± | ± |
| 1 | RWR 2361x BC1F1 | | + | - | + |
| 2 | RWR 2361x BC1F1 | | + | - | - |

| | | | | |
|---|---|---|---|---|
| 3 | RWR 2361x BC1F1 | + | ± | ± |
| 4 | RWR 2361x BC1F1 | - | - | - |
| 5 | RWR 2361x BC1F1 | + | ± | - |
| 6 | RWR 2361x BC1F1 | - | - | - |
| 7 | RWR 2361x BC1F1 | + | ± | ± |
| 8 | RWR 2361x BC1F1 | - | ± | ± |
| 9 | RWR 2361x BC1F1 | - | - | ± |
| 10 | RWR 2361x BC1F1 | - | - | - |
| 11 | RWR 2361x BC1F1 | + | ± | ± |

STK-1 = Co dominant marker linked to *Co-1*, SQ4 = A dominant marker linked to *Co-2*

ENM-CAPS = Co dominant marker linked to *bc-3, BCMV-48289723-CAPS* = Co dominant marker linked to *I*

+ = Presence of marker, - = Absence of marker, ± = Heterozygous for the marker.

**Appendix 7.6.** Cycle of marker assisted selection among BC3F1 progenies

| Sample No: | Genotype | Markers and genes evaluated | | | |
|---|---|---|---|---|---|
| | | STK-1 | SQ4 | ENM-CAPS | *BCMV* -48289723-CAPS |
| | | *Co-1* | *Co-2* | *bc-3* | *I* |
| 1 | G 54X BC2F1 | | | ± | ± |
| 2 | G 54x BC2F1 | - | | ± | ± |
| 3 | G 54x BC2F1 | - | | - | - |
| 4 | G 54x BC2F1 | - | | - | - |
| 5 | G 54x BC2F1 | - | | ± | ± |
| 6 | G 54x BC2F1 | - | | ± | ± |
| 7 | G 54x BC2F1 | - | | - | - |
| 8 | G 54x BC2F1 | ± | | ± | ± |
| 9 | G 54x BC2F1 | - | | - | - |
| 10 | G 54x BC2F1 | - | | - | - |
| 11 | G 54X BC2F1 | ± | | - | - |
| 12 | G 54x BC2F1 | ± | | ± | ± |
| 13 | G 54x BC2F1 | - | | ± | - |
| 14 | G 54x BC2F1 | ± | | - | - |
| 1 | RWR 1668X BC2F1 | ± | | ± | ± |
| 2 | RWR 1668X BC2F1 | - | | - | - |
| 3 | RWR 1668X BC2F1 | - | | + | - |
| 4 | RWR 1668X BC2F1 | - | | - | - |
| 5 | RWR 1668X BC2F1 | - | | - | - |
| 6 | RWR 1668X BC2F1 | - | | ± | ± |
| 7 | RWR 1668X BC2F1 | ± | | ± | ± |
| 8 | RWR 1668X BC2F1 | ± | | - | - |
| 1 | RWR 2355x BC2F1 | - | | ± | ± |
| 2 | RWR 2355x BC2F1 | - | | - | - |
| 3 | RWR 2355x BC2F1 | - | | ± | - |
| 4 | RWR 2355x BC2F1 | ± | | ± | ± |
| 5 | RWR 2355x BC2F1 | - | | - | - |
| 6 | RWR 2355x BC2F1 | - | | - | - |
| 7 | RWR 2355x BC2F1 | - | | ± | ± |
| 8 | RWR 2355x BC2F1 | - | | ± | ± |
| 9 | RWR 2355x BC2F1 | - | | - | - |
| 10 | RWR 2355x BC2F1 | ± | | ± | ± |
| 11 | RWR 2355x BC2F1 | ± | | - | - |
| 12 | RWR 2355x BC2F1 | - | | - | - |
| 1 | RWR 2361x BC2F1 | | + | ± | ± |
| 2 | RWR 2361x BC2F1 | | + | - | - |
| 3 | RWR 2361x BC2F1 | | + | ± | ± |
| 4 | RWR 2361x BC2F1 | | - | - | - |
| 5 | RWR 2361x BC2F1 | | + | - | ± |
| 6 | RWR 2361x BC2F1 | | - | - | - |
| 7 | RWR 2361x BC2F1 | | - | - | - |

| | | | | |
|---|---|---|---|---|
| 8 | RWR 2361x BC2F1 | - | ± | ± |
| 9 | RWR 2361x BC2F1 | - | ± | ± |
| 10 | RWR 2361x BC2F1 | + | - | - |

STK-1 = Co dominant marker linked to *Co-1*, SQ4 = A dominant marker linked to *Co-2*

ENM-CAPS = Co dominant marker linked to *bc-3, BCMV-48289723-CAPS* = Co dominant marker linked to *I*

+ = Presence of marker, - = Absence of marker, ± = Heterozygous for the marker.

**Appendix 7.7.** Cycle of marker assisted selection among BC3F2 progenies

| Sample No: | Genotype | Markers and genes evaluated | | | |
|---|---|---|---|---|---|
| | | STK-1 | SQ4 | ENM-CAPS | *BCMV* -48289723-CAPS |
| | | *Co-1* | *Co-2* | *I* | *bc-3* |
| | **G 54** | | | | |
| 1 | BC3F1xBC3F1 | ± | | + | - |
| 2 | BC3F1x BC3F1 | ± | | ± | + |
| 3 | BC3F1x BC3F1 | - | | ± | ± |
| 4 | BC3F1x BC3F1 | ± | | - | ± |
| 5 | BC3F1x BC3F1 | - | | - | + |
| 6 | BC3F1x BC3F1 | - | | - | + |
| 7 | BC3F1x BC3F1 | - | | ± | - |
| 8 | BC3F1x BC3F1 | + | | + | + |
| 9 | BC3F1x BC3F1 | - | | ± | - |
| 10 | BC3F1x BC3F1 | ± | | - | ± |
| 11 | BC3F1x BC3F1 | + | | - | ± |
| 12 | BC3F1x BC3F1 | ± | | ± | - |
| 13 | BC3F1x BC3F1 | + | | - | - |
| 14 | BC3F1x BC3F1 | + | | - | ± |
| 15 | BC3F1x BC3F1 | + | | ± | + |
| 16 | BC3F1x BC3F1 | - | | - | - |
| 17 | BC3F1x BC3F1 | ± | | - | ± |
| 18 | BC3F1x BC3F1 | ± | | - | ± |
| 19 | BC3F1x BC3F1 | + | | + | - |
| 20 | BC3F1x BC3F1 | ± | | + | + |
| | **RWR 1668** | | | | |
| 1 | BC3F1xBC3F1 | ± | | + | + |
| 2 | BC3F1x BC3F1 | - | | + | - |
| 3 | BC3F1x BC3F1 | - | | - | + |
| 4 | BC3F1x BC3F1 | + | | + | - |
| 5 | BC3F1x BC3F1 | ± | | - | ± |
| 6 | BC3F1x BC3F1 | ± | | ± | - |
| 7 | BC3F1x BC3F1 | ± | | + | - |
| 8 | BC3F1x BC3F1 | - | | + | - |
| 9 | BC3F1x BC3F1 | + | | - | ± |
| 10 | BC3F1x BC3F1 | - | | + | + |
| 11 | BC3F1x BC3F1 | + | | + | - |
| 12 | BC3F1x BC3F1 | ± | | + | - |
| | **RWR 2355** | | | | |
| 1 | BC3F1XBC3F1 | + | | + | + |
| 2 | BC3F1x BC3F1 | ± | | - | ± |
| 3 | BC3F1x BC3F1 | ± | | + | ± |
| 4 | BC3F1x BC3F1 | - | | + | ± |
| 5 | BC3F1x BC3F1 | + | | - | - |
| 6 | BC3F1x BC3F1 | + | | + | - |
| 7 | BC3F1x BC3F1 | ± | | - | ± |
| 8 | BC3F1x BC3F1 | - | | + | ± |

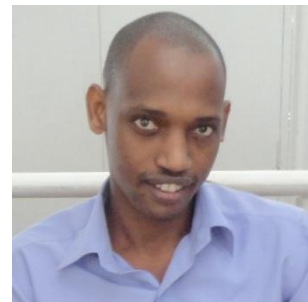
| | | | | |
|---|---|---|---|---|
| 9 | BC3F1x BC3F1 | ± | - | ± |
| 10 | BC3F1x BC3F1 | - | + | - |
| 11 | BC3F1x BC3F1 | + | - | - |
| 12 | BC3F1x BC3F1 | - | - | ± |
| 13 | BC3F1x BC3F1 | ± | + | - |
| 14 | BC3F1x BC3F1 | - | + | + |
| 15 | BC3F1x BC3F1 | ± | - | ± |
| 16 | BC3F1x BC3F1 | ± | ± | - |
| 17 | BC3F1x BC3F1 | ± | ± | ± |
| 18 | BC3F1x BC3F1 | + | + | + |
| | **RWR 2361** | | | |
| 1 | BC3F1 x BC3F1 | - | + | ± |
| 2 | BC3F1x BC3F1 | + | ± | ± |
| 3 | BC3F1x BC3F1 | + | ± | - |
| 4 | BC3F1x BC3F1 | + | - | ± |
| 5 | BC3F1x BC3F1 | + | + | ± |
| 6 | BC3F1x BC3F1 | - | - | + |
| 7 | BC3F1x BC3F1 | + | - | ± |
| 8 | BC3F1x BC3F1 | + | + | - |
| 9 | BC3F1x BC3F1 | + | + | ± |
| 10 | BC3F1x BC3F1 | - | - | ± |
| 11 | BC3F1x BC3F1 | - | ± | - |
| 12 | BC3F1x BC3F1 | - | - | ± |
| 13 | BC3F1x BC3F1 | + | + | - |
| 14 | BC3F1x BC3F1 | + | ± | + |
| 15 | BC3F1x BC3F1 | + | + | ± |
| 16 | BC3F1x BC3F1 | - | - | ± |
| 17 | BC3F1x BC3F1 | - | - | ± |
| 18 | BC3F1x BC3F1 | + | + | - |
| 19 | BC3F1x BC3F1 | + | ± | - |
| 20 | BC3F1x BC3F1 | - | - | ± |
| 21 | BC3F1x BC3F1 | + | ± | - |
| 22 | BC3F1x BC3F1 | + | ± | ± |
| 23 | BC3F1x BC3F1 | - | ± | ± |
| 24 | BC3F1x BC3F1 | + | ± | + |
| 25 | BC3F1x BC3F1 | + | - | - |
| 26 | BC3F1x BC3F1 | + | + | + |

STK-1 = Co dominant marker linked to *Co-1*, SQ4 = A dominant marker linked to *Co-2*

ENM-CAPS = Co dominant marker linked to *bc-3, BCMV-48289723-CAPS* = Co dominant marker linked to *I*

+ = Presence of marker, - = Absence of marker, ± = Heterozygous for the marker

# CURRICULUM VITAE



**Charles RUHIMBANA**
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## Academic background and qualifications

| Qualification | University |
|---|---|
| Doctorate<br>2016-2019 | Akdeniz University<br>Graduate School of Natural and Applied sciences,<br>Department of Biotechnology, Antalya/Turkey. |
| Masters<br>2011-2013 | Jain University<br>Post Graduate of Life Sciences<br>Department of Microbiology, Bangalore/ India. |
| Bachelor<br>2006-2009 | University of Rwanda<br>College of Science and Technology<br>Department of Biology,Kigali/Rwanda. |

## Working experience

| Occupation | Institution |
|---|---|
| Lecturer<br>2011-Now | University of Rwanda<br>College of Agriculture,Animal Sciences and Veterinary Medicine,<br>Deparment of Animal Production, Nyagatare/Rwanda. |
| Teacher<br>2005-2010 | Nyagatare secondary School<br>Department of Biology and Chemistry, Nyagatare/Rwanda. |

## Publication

Shivakumar, S., Karmali, A.N. and Ruhimbana, C. 2014. Partial purification, characterization, and kinetic studies of a low-molecular-weight, alkali-tolerant chitinase enzyme from *Bacillus subtilis* JN032305, a potential biocontrol strain. *Preparative Biochemistry and Biotechnology*, 44(6): 617-632.

Ruhimbana, C., Mutlu, N. 2019 Marker-assisted pyramiding potyvirus resistance genes into Rwandan common bean (*Phaseolus vulgaris* L.) genotypes. *Mediterranean Agricultural Sciences*, *32*(3): 381-385.

## Conferences

Ruhimbana, C. and Mutlu, N. 2019. Utilizing CAPS markers to pyramid potyvirus resistance genes into Rwandan common bean (*Phaseolus vulgaris*, L) genotypes. 1st International Molecular Plant Protection congress 10-13 April,2019, Cukurova University , Adana, Turkey.

## Awards and Trainings

Ph.D Awardee of TÜBİTAK 2235 Graduate Scholarship Programme For Least Developed Countries, in 2015.

Training on DNA Extraction, gel electrophoresis and marker Analysis, Akdeniz University Molecular biology laboratory

Training on cross pollinations in peppers, tomatoes and common beans

MSc awardee of H.E Paul Kagame Scholarship Tenable at Jain University, 2011

Microbiology laboratory experiments, 2 years experience (2011-2013): India.

## Language proficiency

English: Excellent, Kinyarwanda: Mother tongue

## Computer skills

Ms Word ,Ms Excel, Ms pdf, Ms Power point and SPSS.

## Personal ethics

Brilliant, Humble, simple and Hardworking

## Referees

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I certify that the information contained in this CV is true and complete. I understand that false information may be grounds for immediate disqualification of the training at any point. I authorize the verification of any or all information listed above.

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