INCORPORATION OF COMBINED RESISTANCE TO COMMON BACTERIAL BLIGHT AND COMMON BEAN VIRUSES INTO BEAN BRUCHID RESISTANT GENOTYPE

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

The study was undertaken to incorporate resistance to Common Bacterial Blight (CBB), Bean Common Mosaic Virus (BCMV) and Bean Common Mosaic Necrosis Virus (BCMNV) into a bean bruchid resistant genotype which have farmer preferred traits (Kablanketi type) to improve common beans yield and increase the storage time of the common beans in Tanzania. First, Arcelin-Phytohaemmagltinin-Alfa (APA) bruchid resistant bean genotypes were phenotypic screened against *Xanthomona axonopodis* pv. *Phaseoli* (Xap) (the causal agent of CBB disease) and BCMV diseases. Results showed 3 genotypes with resistance to disease pathogens i.e AO 29-3-3A, KT020, and 13A/59-98-3x3-3A while BR 59-63-10 had intermediate resistance to CBB but complete resistant to BCMV, while 'Kablanketi' was susceptible to both diseases. Selection based on phenotypic screening was done, at which BR 59-63-10 line having bruchid resistance and BCMV was selected and KT020 resistant line to CBB and BCMNV was used as non-recurrent parent to incorporate CBB and BCMNV resistance into BR 59-63-10 (recurrent parent). A single way cross was used between recurrent parent BR 59-63-10 and non-recurrent parent KT020. The F_1s were self-pollinated to produce the F_2 generation; F_2s were screened using Sequence Characterized Amplified Region (SCAR) markers for presence of resistance genes using SAP6, SW13 and ROC11 markers. Nine F_2 individuals had combination genes for CBB, BCMV and BCMNV, while 17 had combination of two genes for resistance and 10 had only one gene for resistance to either of the diseases. Forty plants were phenotypically validated with *Xap* in the screen house and 31 plants were resistant to *Xap*.

Moderate high narrow sense heritability of 61.1% and 66.8% for CBB, on leaf and pod respectively were obtained, which indicating selection can be done in early generation for CBB. Results showed that CBB resistance was conditioned by one major gene. Result also demonstrated a positive correlation between phenotype and marker score (r=0.41for SAP6) which implied that there high chance of obtaining resistance individual using marker assisted selection to cut down time spent on phenotypic selection. These lines carrying disease resistance need to be fixed for resistance and evaluated in bruchid feeding trials to validate presence of APA protein after which, they will need field evaluation prior to release.

DECLARATION

I, Nuhu Mbwebwe Aman, declare to senate of Postgraduate Studies Committee that this dissertation is my own original work and that it has neither been submitted nor being currently submitted in any other institution.

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Nuhu Mbwebwe Aman Date

(MSc. Crop Science Candidate)

The above declaration is confirmed

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Prof. Paul Mbogo Kusolwa Date

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DEDICATION

To my parents Mr. & Mrs. Aman Rubeba and beloved twins Jassiminah and Jassim Nuhu Aman

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LIST OF ABBREVIATIONS AND SYMBOLS

ALS	Angular Leaf Spot
ANOVA	Analysis of Variance
BCMNV	Bean Common Mosaic Necrotic Virus
BCMV	Bean Common Mosaic Virus
BGYMV	Bean Golden Yellow Mosaic Virus
BR	Bruchid Resistant
CBB	Common Bacterial Blight
CIAT	The International Center for Tropical Agriculture
CRD	Completely Randomized Design
CV%	Coefficient of Variation
DAI	Days After Inoculation
DAI-P	Days After Inoculation on Pods
DAP	Days After Planting
DCSH	Department of Crop Science and Horticulture
DNA	Deoxyribonucleic acid
EDTA	Ehtylene diamine tetra acetic acid
g	Grams
ha	Hectare
i.e	That is
kg	Kilograms
NaClO	Sodium hypochlorite
PBS	Phosphate Buffer Saline
PHR	Plant Host Resistance

QTL	Quantitative Trait Loci
RH	Relative Humidity
RNA	Ribonucleic Acid
SCAR	Sequence Characterized Amplified Region
SUA	Sokoine University of Agriculture
TOSCI	Tanzania Official Seed Certification Institution
USA	United State of America
Xap	*Xanthomonas axonopodis* pv. *Phaseoli.*
YDCA	Yeast Dextriose Carbonated Agar

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Economic Importance of Common Bean

Common bean (*Phaseolus vulgaris* L.), is an important edible legume for human consumption worldwide (Miklas *et al.,* 2005). Common bean is the staple food for more than 100 million people in Africa with per capital consumption of 60 kg/person/year (Chirwa, 2002). It is one of the principal crops in East Africa in terms of total area planted and number of farmers involved in production compared to other grain legumes (Alladasi *et al.,* 2017). On the other hand, the per capital consumption of common beans in Tanzania is 19.3 kg/person/year (Kilimo, 2012).

Also, common bean is being consumed throughout its crop growth cycles as leaves, green bean, and dry bean (Chirwa, 2002). Common beans are adapted to different environmental conditions and have short maturity period. Moreover, common bean is used as soil conditioning agent due to its ability to fix nitrogen where it contributes up to 40 Kg of nitrogen (N)/ha (Bänziger, 2004; Hillocks *et al.,* 2006). Common bean is said to be a near perfect food in which 60% of its dry weight is carbohydrates serving as an essential source of calories (Fivawo and Nchimbi-Msolla, 2011). According to Alladasi *et al.* (2018), common bean provides up to 25% of the calories in take and 45% of dietary protein in dry weight basis. The dietary fibre of these carbohydrates reduces cholesterol and prevents colon cancer (Fivawo and Nchimbi-Msolla, 2011). Common beans contain vitamin B and high content of minerals especially Iron (Fe) and Zinc (Zn) (Tryphone and Nchimbi-Msolla, 2010). It is

estimated over 75% of all households in Tanzania depend on common bean to meet their daily dietary requirement (Tryphone *et al*., 2012) and as a source of income and food nutrition to foster livelihood growth and at large contributing to national economy and food security (Bucheyeki *et al*., 2013).

In Tanzania the total area under common bean production, estimated to be 732 531 ha of which 732 495 ha (99.9%) is in mainland and 37 ha (0.1%) is in Zanzibar; with total production of 1.14 million metric tons and average yield of 0.9 tons/ha (Mkonda and He, 2017). About 90% of the production is being done by small scale farmers under farm size ranging 0.5-2.0 ha (Ndakidemi *et al.*, 2006). Tanzania ranks 6th among top 10 common bean producers in the world, and being the first among East African countries followed up with Uganda and Kenya. Although Tanzania is being a leading common bean producer, the total production has been decreasing, from 1.2 million metric tons in 2014 to 1.14 million metric tons in 2017 due to use of unimproved seeds, poor management, insect pests, diseases, and adversely climatic change (FAOSTAT, 2014; FAOSTAT, 2017).

1.2 Constraints to Common Bean Production

Most of people involving in bean production are cultivating the local landrace which combine high market value with good culinary characteristics. Regardless to its high market demand but these bean landraces have been constrained by both abiotic and biotic factors. Abiotic factors include lack of soil fertility, and weather conditions defined mostly by the amount of rainfall, and temperature (Miklas *et al.,* 2005). Biotic factors includes, insect pest such as bean weevils (Kusolwa, 2007; Kipato *et*

al., 2015; Kusolwa *et al.,* 2016), and diseases including fungal, bacterial and viral diseases (CIAT, 2014). A large portion of yield losses are reported to being caused by diseases (Haryeson and Schwartz, 2007; Tryphone *et al.*, 2012; Chilagane *et al.*, 2013). Major diseases constraints to common bean production in East and Central Africa include Common bacterial blight (CBB) caused by *Xanthomona axonopodis* pv *Phaseoli* (Tryphone *et al.,* 2012; Alladasi *et al.,* 2018; Mondo *et al.,* 2019), root rots caused by either *Pythium* spp, *Fusarium* spp., *Sclerotium rolfsii*, or *Rhizoctonia solani* (Nzungize *et al.,* 2011a; Obala *et al.,* 2012; Burachara *et al.,* 2015; Mukankusi *et al.,* 2018), Angular Leaf Spot (ALS) caused by *Pseudocercospora griseola* (Sacc.) (Chilagane *et al.,* 2013: Leitich *et al.,* 2016), Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) (Kiryowa *et al.,* 2016) and Bean common mosaic and bean common mosaic necrosis viruses (BCMV/BCMNV) caused by a group of Potyviruses (Chilagane *et al.,* 2013; Mwaipopo *et al.,* 2017). These diseases cause severe losses to both seed quality and yield (Alladasi *et al.,* 2018). The latter loss ranges from 20% to as high as 100% (Mondo *et al.,* 2019). Wortmann *et al.* (1998) estimated that in Eastern Africa the annual production losses caused by CBB to be 145 900 tons, BCMV 144 600 tons, root rot 179 800 tons, ALS 281 300 tons, and Anthracnose being 247 400 tons.

1.2.1 Economic Importance of Common Bacterial Blight (CBB)

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (syn.=*X. campestris* pv. *phaseoli*) is one of the major seed-borne diseases and generally endemic in bean growing regions with high temperature, rainfall, and relative humidity (Alladassi *et al.,* 2018). CBB pathogen belongs to genus *Xanthomonas* which are gram negative group of γ-proteobacteria which change rapid

its genetic diversity to host even in common beans. It was categorized into fuscous and non-fuscous strains which were grouped into single taxon, (*Xanthomonas campestris* pv. *phaseoli*) (Akhavan *et al.,* 2013), following the revision of taxonomical of the particular genus (*Xanthomonas*) pathovar phaseoli was transferred to *X. axonopodis* with fuscous strains forming a variant within this pathovar (Tugume *et al.,* 2018). CBB is considered as a common threat to all bean growers' worldwide (Alladasi *et al.,* 2018), which can be spread through seed and rain-splash in field, and its infections largely occurs through stomata, colonizing mesophyll cells (Tugume *et al.,* 2018), and causing water-soaked symptoms on leaf which developed into pin-point spots that then enlarge and become necrotic bordered by a chlorotic zone on both leaves and pods and chlorotic (Alladasi *et al.,* 2017). Shi *et al.* (2011) reported that, yield losses caused by CBB can exceed 40% in susceptible varieties under condition favoring the disease. Also, degraded of the seed quality due to staining and browning of the infected seeds (Vandemark *et al.,* 2008; Shi *et al.,* 2011; Yu *et al.,* 2012; Tugume *et al*., 2018)

Opio *et al.* (1992), reported a reduction of 11.5 kg per ha at growth stage R7 which corresponding to 1% increase in number of leaves infected with CBB. Infected seed constitutes significant source of inoculum, thus acting as the major factor in spread of the disease because viability of the pathogen can be maintained 30 years on the seed (Spence and Walkey, 1995) as well as conditions required by the seed are the same for pathogen survival (Erdinc *et al.,* 2018).

Different strategies have been suggested to control this disease, including the combination of both cultural and chemical means but it has been reported not to be

effective (Okii *et al.,* 2017: Mondo *et al.,* 2019). Also chemicals have negative effects to environments, associated with high cost that are not practical for low-input systems (Zanatta *et al.,* 2007); the use pathogen-free seeds, crop rotation, weed management, removal of plant debris (Singh and Munoz, 1999) and usage of resistant cultivars and ecofriendly practices are the most effective ways to control the disease (Erdinc *et al.,* 2018).

1.2.2 Economic Importance of Bean Common Mosaic Virus (BCMV), and Bean Common Mosaic Necrotic Virus (BCMNV)

BCMV and BCMNV are positive-sense single-stranded RNA viruses belonging to the genus Potyvirus in family Potyviridae (King *et al.,* 2011; King *et al.,* 2018). The genomic RNA of BCMV and BCMNV translate into a single polyprotein that auto-catalytically cleaves into 10 mature proteins; The first protein (P1), helper component proteinase (HC-Pro), third protein (P3), first 6-kDa protein (6K1), cytoplasmic inclusion (CI), second 6-kDa protein (6K2), genome linked viral protein (VPg), nuclear inclusion a (NIa), Nuclear inclusion b (NIb), and coat protein (CP). An additional short open reading frame known as Pretty Interesting Potyviridae ORF (PIPO) has been described in the P3 cistron (Mwaipopo *et al*., 2017). Both BCMV and BCMNV affect common bean seeds causing economic yield losses of up to 80% (King *et al.,* 2011). The viruses are transmitted in common bean seeds (which contributes to long distance movement), and by several aphids in a non-persistent manner over short distances (Kabeja, 2020; Mwaipopo *et al*., 2017). It took 15 to 16 seconds for the aphids to acquire the virus and it could be transmitted within a minute (King *et al.,* 2018). It can also be transmitted from plant to plant by abrasion, via pollen, and from people and equipment used in the field (Worrall *et al.*, 2015;

Feng *et al*., 2017). The strains of the two viruses are distributed worldwide and considered as the major important diseases in common bean production (Morales and Bos, 1988). However, rates of transmission of BCMV and BCMNV strains depend on the common bean genotypes (Haggard and Myers, 2007). BCMV strains were shown to be transmitted through infected seeds (Drijfhout *et al.,* 1978). Transmission of BCMV and BCMNV is not possible in common bean plants having the dominant *I* gene from plant tissue because of a massive systemic necrosis reaction (black root symptom), preventing virus replication and resulting in plant death. There are also not seed transmission because black root symptomatic plants die before seeds for next season can be produced (Mwaipopo *et al.,* 2017). The virus strains induce mosaic mottle or darkening patches in the leaves, leaf deformations, blistering and stunting in susceptible bean cultivars (Worrall *et al.,* 2015) and may result in yield loss of up to 80% of production also crop failure can result from BCMNV spreading from a susceptible cultivar to a cultivar with unprotected *I* gene because of the black root reaction (Elsharkawy and Sawy, 2015).

Different methods have been used to reduce the transmission of the virus by using chemical spraying to control the vectors such as aphids, use of disease-free seed, removing weedy host species around the field, and cultivation of resistant cultivars (Haggard and Myers, 2007).

1.3 Breeding for Improved Combined Resistance to CBB and BCMV

Breeding for resistant genotypes to BCMV and BCMNV disease is an efficient control measure for the disease which cuts down on the operational costs of common bean production and long term one (Miklas *et al*., 2015). Different bean breeders

have developed common beans with improved resistance to different important diseases. Beaver *et al.* (2018) developed and registered the white seeded common bean cultivar (Bella) which has multiple resistances to BCMV, BCMNV, BGYMV, CBB and Web blight in Puerto Rico. But also, the team, developed germplasm with broad resistance to BGYMV, BCMV, BCMNV and rust with PR1572 and PR1572-26 pinto beans (Beaver *et al.*, 2019) while Prophete *et al.* (2014) in Puerto Rico develop and registered the PR0633-10 and PR0737 Red mottled dry bean lines with resistance to BGYMV, BCMV, BCMNV and CBB. Urrea *et al.* (2019), successfully improved the resistance to rust, CBB, and BCMV in 'Panhandle Pride' released in 2016 in Nebraska, USA. Wani *et al*. (2017) reported six improved genotypes having resistance to BCMV for production in Kashmir, India. Similarly, Osomo *et al.* (2020) developed the 'ND Whitetail' having resistance to white mold and BCMV with intermediate resistance to CBB in North Dakota, USA.

1.4.1 Correlation of phenotypic and genotypic inheritance in segregated population ($F_{2:3}$)

Understanding mode of inheritance and type of gene action is very important for successful breeding (Tryphone *et al*., 2012; Alladassi *et al*., 2017). Several inheritance studies have been conducted on CBB and BCMV/BCMNV where different results were reported depending on pathogenic variability and the genetic background of the parental lines (Alladassi *et al.*, 2017). Tryphone *et al.*, (2012), Muimui *et al.* (2011) and Zapata *et al.* (2011) reported that CBB resistance was governed by a major dominant gene in resistant lines Wilk-2 and VAX6, VAX4 and PR 0313-58, respectively. Tryphone *et al.* (2012), reported a narrow sense

heritability for CBB (0.32) with significant correlation between phenotypic reaction and molecular markers screening ($r=0.502$; $p\leq0.05$)

Chilagane *et al.,* (2013) reported that resistance to bean common mosaic and necrosis virus diseases are governed by single recessive gene. This is confirmed by phenotypic screening results using F_2 and $F_{2:3}$ population by showing good fit to phenotypic segregation ratios of 1:3 and genotypic segregation ratio of 1:2:1 respectively using marker for $F_{2:3}$ generation (Mukeshimana *et al*., 2005; Chilagane *et al.,* 2013). In same study using the molecular SCAR marker ROC 11, the *bc-3* gene was screened and the results show that the gene is a recessive gene and it segregated in a single gene inheritance pattern ($X^2 = 1.609$; $P\leq 0.05$).

1.4.2 Screening plant reaction to foliar diseases

Breeding for disease resistance in common bean is essential, and as a first step, screening the materials needed to be bred is necessary. Osdaghi *et al.* (2009) evaluated 29 lines and one cultivar of common beans for their reaction to *Xap* under screen house and field conditions, in which reaction to *Xap* was assessed as diseased leaf area (DLA), and number of spots on the leaves after inoculation. In similar study resistant lines to CBB were identified and used for cultivation or source of resistance (Osdaghi *et al*., 2009). Alladasi *et al.,* (2018) screened 139 genotypes found in Uganda to obtain the landrace which have resistance to CBB. Such genotypes can be used as breeding lines to develop resistant cultivars.

1.5 Incorporation of Combined Resistance to CBB and BCMV/BCMNV into Preferred Cultivars

Incorporating improved resistance to foliar disease into farmers preferred cultivars and release the improved common beans to enhance levels of disease resistance have been an important goal to all bean breeders worldwide (Miklas *et al*., 2005; Beaver *et al.,* 2018; Beaver *et al.,* 2019). Cultivars with improved resistance can reduce reliance of pesticides and risk of crop yield loss from pests in low and high input system which enables stable common bean production (Kabeja, 2020; Kusolwa *et al.,* 2016; Osomo *et al.,* 2020).

Breeding for the disease resistant cultivars is reported to be one of the most effective and long term strategy to control seed borne diseases in common beans. Seed is the primary source of inoculum (Alladasi *et al*., 2018), and in order for these to be efficiently achieved, biotechnology tools have to be incorporated, and different breeding lines have been developed in this regard (Mahuku, 2009; Chilagane *et al*., 2013). Chilagane *et al*. (2013), incorporated resistance to Angular Leaf Spot (ALS) and BCMV/BCMNV into the preferred cultivar (Kablanketi) using SCAR markers SNO2, ROC11 and SW13 linked to *Phg-2*, *bc-3* and *I gene*, respectively. The parents Mexico 54 and UBR (25) 95 were donors of *Phg-2* and *I/bc-3 genes*. Similarly, CBB resistance was introgressed into Kablanketi by crossing with Vax4, followed by marker assisted selection using SCAR marker SAP6 linked to a Quantitative Trait Loci (QTL) for CBB resistance (Tryphone *et al*., 2012; Mondo *et al*., 2019).

1.6 Bruchid Resistant Cultivars

In Tanzania, smallholder farmers employ different strategies to limit bruchid damage, which include; mixing and storing seed with dust and wood ashes, use plant extract sprayed on the seed and use of insecticides (Kusolwa, 2007; Kipato *et al.*, 2015). Host plant resistance has been observed to be effective in controlling bruchid damage (Kamfwa *et al.*, 2018). Resistance increases mortality, reduce adult emergence, and prolong larval development time (Kusolwa *et al.*, 2016). Cultivars and accessions with resistant to bruchids include the tepary bean accession G40199 from CIAT identified by Goosens *et al.* (2000) and AO 1021 29-3-3A developed by Kusolwa (2007) and registered in 2016 (Kusolwa *et al.*, 2016). Improving resistance to common beans it is feasible and helpful to smallholder farmers (Kusolwa *et al.*, 2016; Maro, 2017).

Currently, in Tanzania and elsewhere, there has been no study conducted to combine resistance to CBB, BCMV/BCMNV and bruchid resistance into single preferred cultivar. Therefore, this study aimed to incorporate combined resistance to CBB, BCMV/BCMNV and bruchid resistance into a single famers' preferred variety.

1.7 Objectives

1.7.1 Overall objective

The overall objective of this study was increasing yield and reduces postharvest losses through incorporating foliar disease resistance gene into bruchids resistant bean genotypes.

1.7.2 Specific objectives

(i) To screen for resistance of common bean genotypes to Common Bacterial Blight (CBB), and Bean Common Mosaic Virus (BCMV)/ Bean Common Mosaic Necrosis Virus (BCMNV)

(ii) To incorporate foliar disease (CBB and BCMV/BCMNV) resistance genes into bean bruchid resistant genotypes, and heritability studies at $F_{2:3}$

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CHAPTER TWO

2.0 SCREENING FOR RESISTANCE OF THE COMMON BEAN GENOTYPES TO COMMON BACTERIAL BLIGHT, AND BEAN COMMON MOSAIC AND NECROTIC VIRUSES

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2.1 Abstract

Common bacterial blight (CBB), bean common mosaic and bean common mosaic necrosis viruses (BCMV and BCMNV) limits common beans (*Phaseolus vulgaris* L.) production worldwide. This study was carried out to perform phenotypic screening and asses the leaf reaction of a resistant line to CBB and BCMV/BCMNV. The experiment was conducted using Completely Randomized design with three replications under screen-house conditions. Four improved bean genotypes for bruchid resistance were collected from bean improvement projects at Sokoine University of Agriculture and one commonly cultivated susceptible cultivar was collected from a local market. Bean seeds were sown in pot with sterilized soil and *Xap* inoculated by spraying with a bacterial suspension at 18 days after planting, while mechanical inoculation was performed for BCMV on 10 days old leaves. Disease severity of CBB was assessed three times at 14, 21, and 35 days after inoculation using a 1-9 CIAT scale, while for BCMV, symptoms were assessed at 15 days after inoculation. Results show significant differences ($p \leq 0.001$) on resistance to both diseases among the common beans genotypes tested. 13A/59-98-3x3-3A

(scored 1.3 for CBB; no infected plant with BCMV), AO 29-3-3A (scored 2.0 for CBB; no infected plant with BCMV) and KT020 (scored 1.3 for CBB; only 1 plant was infected with BCMV) had resistance to both diseases while BR59-63-10 was resistant to BCMV and intermediate resistance (scored 3.5) to CBB. Kablanketi was susceptible to both diseases (scored 8 for CBB; 2 plants infected with BCMV). This study verified the resistance against CBB and BCMV in three lines obtained from SUA used for breeding multiple disease resistance cultivars.

Keywords; *Phaseolus vulgaris* L., Phenotypic screening, resistance, susceptible

2.2 Introduction

Common bean (*Phaseolus vulguris* L.; 2n=2x=22), is the most preferred consumable legume and being distributed worldwide (Razvi *et al.,* 2018). It is an important and essential component of diets in most households of Tanzania (Letaa *et al.,* 2020). Common beans are cultivated as vegetable (Laizer *et al.*, 2019). Their grains which have high dietary protein content around 22% or even higher on a dry matter basis (Philipo *et al.,* 2020). It is the source of essential minerals, and vitamins (Mazengo *et al*., 2019). Its proteins and carbohydrates provide calories of up to 25% of the diet (Beebe *et al.,* 2013). Their nitrogen fixing ability contributes about 50N kg per ha to soil fertility (Bänziger, 2004; Hillocks *et al.,* 2006). Common bean is essential to smallholder farmers to meet their daily nutritional needs and for income generation (Mangeni *et al.,* 2020).

Tanzania ranks first in Africa and sixth in world top bean producers, with total production of 1.14 million metric tons and average of yield of 0.9 tons per hectare (Letaa *et al.,* 2020). However, its productivity is still low because the crop has been

stressed with both abiotic and biotic factors, including diseases and insect pest (Mishili *et al.,* 2011; Mazengo *et al.,* 2019). Pests are estimated to be the second biggest constraints to bean production after low soil fertility and its annual loss caused by pests vary from 20 to 100% (Oladzad *et al.,* 2019; Dramadri *et al.,* 2019). Reduction in yield has been attributed by the effect of disease and insect pest, specifically, Common Bacterial Blight (CBB), Bean Common Mosaic Virus (BCMV) and/or Bean Common Mosaic Necrotic Virus (BCMNV) (Tryphone *et al.*, 2012; Chilagane *et al.,* 2013; Alladasi *et al.,* 2018), and secondarily from bruchid (bean weevils) damage (Kipato *et al*., 2015; Kusolwa *et al*., 2016).

CBB and BCMV/BCMNV are both seed borne diseases in which the infected seeds play a great role as the primary source of inoculum for the diseases. In addition, BCMV/BCMNV can be transferred over short distances from the infected plants to healthy ones through vectors such as aphids in a non-persistent manner (Mwaipopo *et al.,* 2017). Breeding for host plant resistance is most reported to be a more effective and long term solution to control these diseases, and many. CBB and BCMV/BCMNV resistant lines have been developed in this regard. Resistance of CBB has been reported being governed by quantitative trait loci (QTL), while BCMV/BCMNV is being controlled by qualitative gene (Tryphone *et al.,* 2012; Alladasi *et al.,* 2018). Screening of the breeding material is very essential in order to be sure of the plant reactions to the disease races. It has been reported that, there is differential expression of resistance to CBB in different plant parts (Alladasi *et al.*, 2018). Infection in leaves and pods is reported as a major challenge in controlling CBB disease in common bean and therefore past studies have focused on the

association between leaf and pods to *Xap*/*Xapf* (Alladasi *et al.,* 2018). Armaud-Santana *et al.,* (1994) reported lower genetic correlation between leaf and pod reactions and leaf and seed reaction to CBB disease. Similarly, Part *et al.* (1998) found low to intermediate correlation between leaf and pod reactions to CBB in common beans. Jung *et al.* (1997) also reported different genes controlling CBB resistance in leaf, pod and seed in common beans. All findings have shown that some CBB resistant genotypes possess resistance to CBB in only one organ; thus, screening of multiple organs is important in order to obtain the resistant line with combined resistance. According to Belarmino, (2015) screening of genetic resources against the specific pathogens is significant in developing resistant cultivar. Therefore, the objective of this study was to screen and assess the plant reaction of the provided resistant lines using inoculum for Common Bacterial Blight (CBB), and Bean Common Mosaic Virus (BCMV)/ Bean Common Mosaic Necrotic Virus (BCMNV).

2.3 Materials and Methods

2.3.1 Description of the Study site

The study was conducted in the screen house of Horticulture Section at Sokoine University of Agriculture (SUA). The University is located at latitude 6º5’ South and Longitude 37º39’ East and 549 meters above sea level on the foot of Uluguru Mountains.

2.3.2 Experimental Plant Materials

The experimental material used were seed of locally adopted bean cultivar ‘Kablanketi’, which is susceptible to CBB, BCMV/BCMNV but fetches high market

price in local markets, used as a check in this study, KT020 (Improved genotypes from Bean Improvement Project). KT020 is derivate of Mexico54, Vax3 and Mshindi following four backcross to Kablanketi; an indeterminate climbing (Type IV) having medium sized seeds, grayish in color, and have resistance to CBB and BCMNV; 59-63-10 derived from crossing black seeded (APA-ICA Pijao x G40199) x Kablanketi followed three backcross to Kablanketi, indeterminate vine, but lacking climbing ability (Type IIIB) and have medium sized seeds, grayish in color, and have resistance to Bruchid damage and BCMV/ BCMNV; AO 29-3-3A which is resistant to bean Bruchid and , it is indeterminate bush (Type II) having medium sized seeds with kidney red color also having resistance to BCMV/BCMNV and was used as a check; 13A/59-93-9 x3-3A, a successful cross of APA lines and AO 29-3-3A having large cream sized seeds, resistant to bruchid damage and BCMV/BCMNV, and it is indeterminate bush (Type II).

Each of these genotypes were planted per pot using Completely Randomized Design (CRD) with three replicates where pot was treated as replicates under screen house condition and germinated seedlings were inoculated with respective pathogens when they were 18 days old for CBB and 10 days after planting (DAP) for BCMNV pathogens.

2.3.3 Inocula Collection

In order to obtain inoculum for each pathogen, diseased leaves with typical disease symptoms were collected from naturally infected fields or farms from different area around Morogoro where beans are grown i.e the SUA-crop museum, Mgeta, and Kilosa. For CBB infected plants, leaves were detached from the plant and transferred

into labeled plastic bags with name of bean variety, date, and location from where the sample was collected, and placed in the ice cool box for transportation to the laboratory. For BCMV specimens, fresh samples were placed on ice in plastic bags ready for inoculum preparations. The samples were then brought to the pathology laboratory in the TOSCI laboratory for isolation and characterization of the *Xap* pathogen.

2.3.4 Pathogen Isolation, Preparations of Inoculum and Inoculation

2.3.4.1 Common Bacterial Blight

Isolation of *Xap*

Differential media was prepared following the procedures described by Mortensen (2005). Infected leaves were taken to the laminar air flow chamber and a section from the margin of healthy and disease leaf tissue were sterilized by immersing the materials into 2% sodium hypochlorite (NaClO) for two minutes, then excess NaClO was rinsed three times using distilled water. The materials were macerated using a sterile blade and forceps, then macerated leaf were placed into a 30ml bottle with addition of 2 ml/g of Phosphate buffer saline (PBS) and left overnight. Then serial dilutions of the homogenate were made; each serial bottle contains 4.5 ml of PBS and 500µl of the leaf homogenate were pippeted for each dilution and the final the homogenate was streaked on the petri dish contains Yeast dextrose carbonate agar (YDCA) media labeled with the specific dilution, name of the pathogen and date. Plates were incubated at room temperature (28ºC) for three days (72 hours). After three days, yellow mucoid colonies were observed (Figure 2.1.A). Cell suspensions were made using sterile distilled water and its concentration was adjusted to 10^6 cfu ml^{-1} using haemocytometer (Figure 2.1.B).

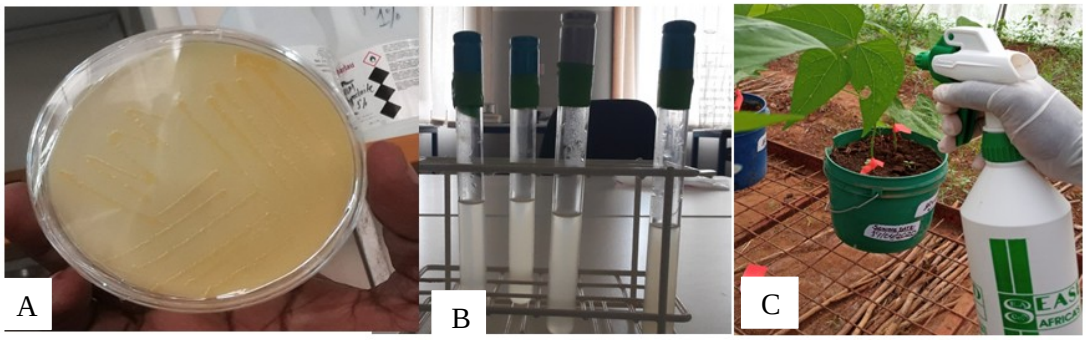

Figure 2.1: Isolation and preparation of common bacterial blight for inoculation of common bean genotypes. A; *Xap* colonies grown on the YDCA in a petri dish: B; Cell suspension after being diluted by sterile distilled water and adjusted: C; Inoculation process by spraying method

Inoculation

Plants were inoculated at 18 days after planting when they have fully expanded trifoliolate leaves by spraying the inoculum on both side of the leaves using hand pump sprayer (Figure 2.1.C) and covered by plastic sheets to increase relative humidity (RH) for 72 hours. After 72 hours the plastic sheets were removed and the plant pots were transferred and placed to the screen-house benches made of meshed steel, one meter high for symptoms development, while the floor was kept wet for 24 hours.

Disease scoring

The disease severity was assessed on all leaves weekly from seven days after inoculation (DAI), then 14 DAI and 21DAI. The disease severity rating was estimated following CIAT 1-9 (van-Schoonhoven and Pastor-Corrales, 1987)

Table 2.1: General scale used to evaluate the reaction of bean germplasm to common bacterial blight (van Schoonhoven and Pastor-Corrales, 1987)

Rating	category	Description	Comments
1-3	Resistant	No visible to very light symptoms resulting in little or no economic damage	Germplasm useful as parent or commercial variety
4-6	Tolerant or Intermediate	Visible and noticeable symptoms resulting only in limited economic damage	Germplasm can be used as commercial varieties or sources of resistance to certain diseases
7-9	susceptible	Severe to very severe symptoms causing useful yield losses or plant death	Not useful to be used as parent or commercial variety

2.3.4.2 Bean Common Mosaic and Necrosis Virus

Inoculum preparation and inoculation for BCMNV

The fresh infected leaves with typical symptoms of disease were collected from the field, one gram (1.0gm) of infected leaf was grounded using mortar and pestle in cold 5 ml of cold 0.01 M Potassium phosphate buffer containing 0.1% Tween 20. The mixture was sieved to eliminate the plant debris, then the sieved one were used for inoculation after adding 10 g of carborundum powder (300 mesh) and sterile PBS, and the mixture were stirred.

Inoculation

Mechanical inoculation was performed; where by the index finger was dipped into the inoculum and then sap was slightly rubbed on both surfaces of the primary leaves of 10 days old plants. Control seedlings were not inoculated but simply sprayed with distilled water.

Disease severity rating

Disease was assessed at 15 days after inoculation (DAI) at which plant showing reaction or symptoms such as mosaic mottle, systemic necrosis or vein banding were counted and recorded and removed from the pots leaving the healthy plants.

2.3.5 Disease resistance rating

Disease was assessed at three phases which are; 14, 21 and 35 days after inoculation on trifoliate leaves. The disease scoring was done based on phenotypic observation and appearance of the leaves due to absence or presence of the typical symptoms of the CBB, using the CIAT scale of 1-9 with some modification at which the plant with score of 1-3.3 were considered as the resistant, 3.4-6.4 were considered as Intermediate resistant, and 6.5-9 were considered as susceptible genotypes as shown in Table 2.1 (Van Schoonhoven and Pastor-Corrales, 1987). For BCMV, assessment was done once at 15 days of inoculation where by number of plants with typical symptoms were counted, removed from the experiment and recorded its symptom.

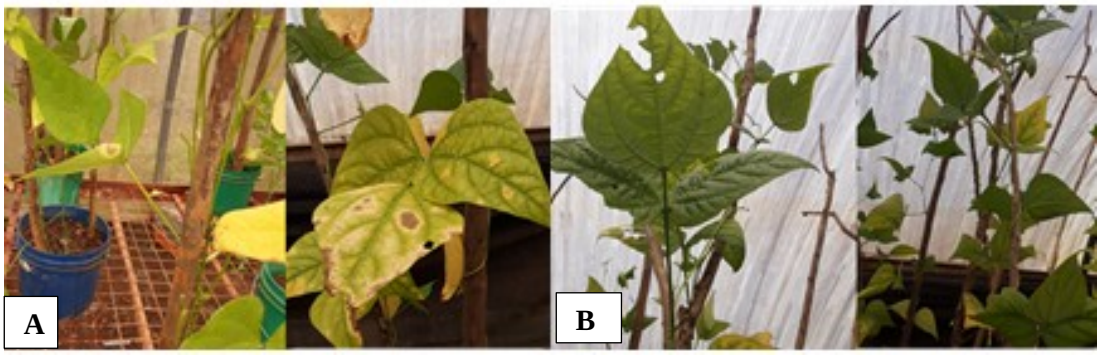

Figure 2.2: Showing typical leaf symptoms of the diseases after inoculation; A= CBB symptoms; B=Mosaic symptoms (BCMV)

2.3.6 Data collection and analysis

Data were collected on the disease severity for CBB on each genotype and for BCM/NV, counted number of plants with virus symptoms, were then subjected to the GENSTAT-16th edition (VSN INTERNATIONAL, 2013) to generate variance, standard errors and the means of disease severity on leaves were separated using Tukey's Test at probability level of 5 percent. Microsoft excel was used to construct graphs of the disease reaction.

2.4 Results and Discussion

2.4.1 Results

2.4.1.1 Reaction of common bean genotypes to CBB (Xap) disease

Results showed significant differences ($p \leq 0.001$) among 5 genotypes tested (Table 2.2). At 14 DAI all genotypes were observed to be resistant to Xap with average visual score ranging from 1.00 to 3.33 which were considered as resistance in this study (Table 2.2). Leaf severity scored at 21 DAI showed a significant reaction among the tested genotypes in which KT020, 13A/59-98-3X3-3A and AO 29-3-3A had visual scores of 1.00, 1.33 and 1.33 respectively (resistant). BR 59-63-10 had a score of 4.33 and Kablanketi scored 4.67 (intermediate). There were significance differences for observed reaction of the genotypes at 35 DAI to *Xap* in which KT020, 13A/59-98-3x3-3A and AO29-3-3A had visual scores of 1.33, 1.33 and 1.67, respectively and were categorized as resistant to *Xap* reaction while BR 59-63-10 was observed to have lesions on the leaves having a visual score of 4.67 (intermediate resistance) and Kablanketi had typical and large lesion on leaves with visual score of 8.00 which categorized as susceptible (Figure 2.1 and Table 2.2). Results show that there were development of the CBB symptoms over time as shown

in Table 2.2, in which KT020 and 13A/59-98-3X3-3A did not develop any disease symptoms, while AO 29-3-3A had a few leaves with water-soaked symptoms. Kablanketi shown tremendous development of disease on leaves per time as well as BR 59-63-10.

Table 2.2: Leaves severity visual score rating of the tested common bean genotypes to CBB inoculum (Xap) at specified time interval

Genotype	Leaf severity score		
	14 DAI	**21 DAI**	**35 DAI**
BR 59-63-10	3.00 b	4.33 b	4.67 b
13A/59-98-3X3-3A	1.00 a	1.33 a	1.33 a
KT020	1.00 a	1.00 a	1.33 a
AO 29-3-3A	1.00 a	1.33 a	1.67 a
Kablanketi	3.33 b	4.67 b	8.00 c
Grand mean	**1.87**	**2.6**	**3.4**
s.e.d	**0.2981**	**0.422**	**0.558**
CV%	**17.7**	**19.9**	**20.1**
F pro.	**<.001**	**<.001**	**<.001**

*Values with same letter in the same column are not significant different (Tukey's Test, p≤0.05); DAI=Days after inoculation, CV%=coefficient of variation, s. e. d=Standard error of difference of means, F pro.=F probability at p≤0.05.

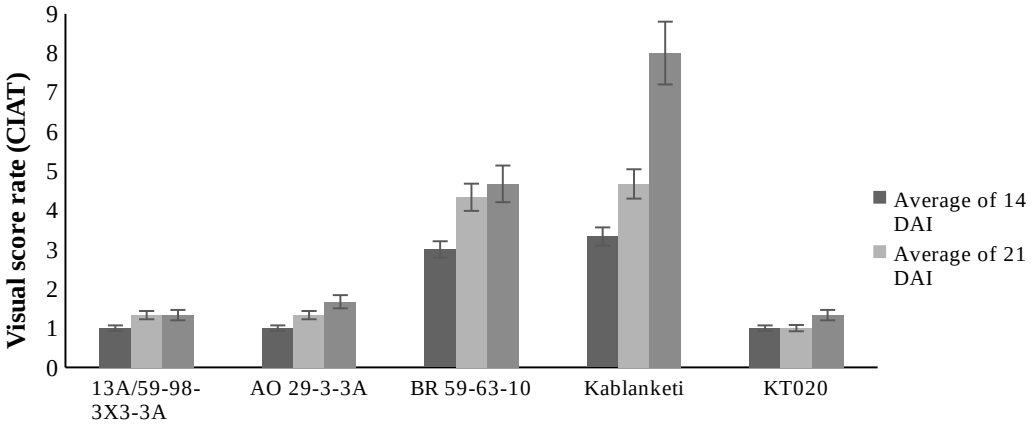

Figure 2.3: Average of common bean leaves severity visual score of the tested genotypes to *Xap* inoculum at different time intervals

2.4.1.2 Plant reaction to BCMV

There was significant difference ($p \leq 0.05$) on plant reaction to BCMV inocula whereby Kablanketi (control), observed to have an average of two plants affected and showing the typical mosaic symptoms of the BCMV while 13A/59-98-3X3-3A observed to have some mosaic symptoms with no development. In this study, A0 29-3-3A and KT020 observed with no any plant having the disease symptoms while BR 59-63-10 genotype only one plants observed to have mosaic symptoms on the leave (Table 2.3 and Figure 2.2).

Table 2.3: Numbers of common bean plants with typical mosaic symptoms discarded from trial after inoculated with BCMV inoculum.

Genotypes tested	Number of plant infected
AO 29-3-3A	0.00 a
BR 59-63-10	1.00 b
13A/59-93-9X3A	0.00 a
KT020	0.00 a
Kablanketi	1.67 b
Grand mean	**0.533**
s. e. d	**0.211**
CV%	**48.4**
F prob.	**<.001**

*No significant difference to the values with same letter in the same column according to Tukey's Test at $p \leq 0.05$; s.e.d=standard error of difference of means, CV %=coefficient of variance, F prob.=F probability at $p \leq 0.05$.

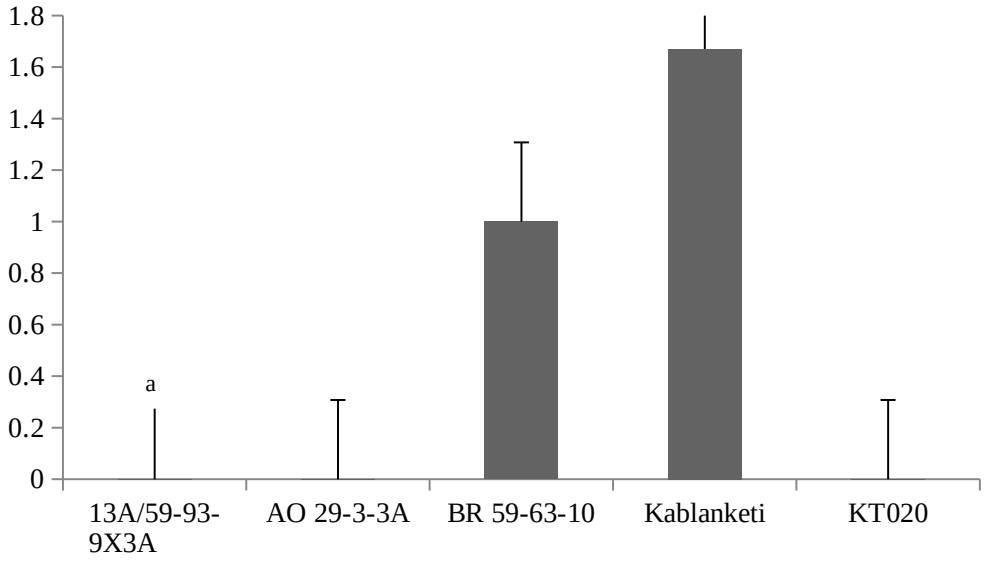

Figure 2.4: Number of common bean plants observed having typical mosaic symptoms after being inoculated with BCMV inoculum

2.4.3 Response of the genotype to both Diseases

Result showed differences among the genotypes tested to both diseases i.e CBB and BCMV (Table 2.4). BR 59-63-10 has intermediate resistance to CBB (4.67 visual score) and one plant showed mosaic symptoms of BCMV reaction. KT020 results on CBB severity was resistant with 1.33 visual score to *Xap* but no plant among those tested showed symptoms of the BCMV (Table 2.4). AO 29-3-3A showed resistance to both diseases tested (had visual score of 1.67 for CBB and no plant have mosaic symptoms for BCMV) as shown in Table 2.4 while Kablanketi showed susceptibility to both diseases in which both necrotic and typical symptoms of CBB were observed (had high visual score of 8.0); as well an average of two plants had typical BCMV mosaic symptoms (Table 2.4).

Table 2.1: Response of the common bean genotypes tested to Common Bacterial blight severity and number of plants showing symptoms of BCMV

Bean genotypes	CBB severity	BCMV reaction
BR 59-63-10	4.67 b	1.00 a
13A/59-98-3X3-3A	1.33 a	0.00 a
KT020	1.33 a	0.00 b
AO 29-3-3A	1.67 a	0.00 a
Kablanketi	8.0 c	1.67 c
Grand mean	**3.4**	**0.53**
CV%	**20.1**	**0.211**
s.e.d	**0.558**	**48.4**
F pro.	**<.001**	**<.001**

*No significant difference to the values with same letter in the same column according to Tukey's Test at p≤0.05; s.e.d=standard error of difference of means, CV %=coefficient of variance, F prob.=F probability at p≤0.05

2.4.2 Discussion

Phenotypic screening of the germplasm used for disease resistance incorporation is important (Alladasi *et al.,* 2018). The results in this study have revealed that, there were significant differences on visual score to *Xap* reaction observed on leaves, implying that all genotypes have different levels of resistance to *Xap*. These results are in agreement with those obtained by, Tryphone *et al.* (2012), Alladasi *et al.* (2018) and Beaver *et al.* (2018) as well as in the similar study showed continues development of the disease symptoms as observed in this study particular in *Xap* reactions.

Results obtained from this study, observed three range of disease score severity on leaf reaction which suggested three categories of resistance with score of 1 to 3, intermediate with score of 4 to 6 and susceptible with score of 7 to 9 as obtained in this study. Alladasi *et al.* (2018) and Kabeja, (2020) reported similar results which

further confirmed the high genetic diversity of the common bean genotypes tested to CBB.

Based on results obtained on 35 DAI, KT020 and 13A/59-98-3X3-3A observed to have low scores indicating presence of resistance gene for CBB. Also, AO 29-3-3A showed some resistance to *Xap* reactions while BR 59-63-10 was observed to have intermediate resistance to CBB. Kablanketi observed to be susceptible to the disease which was an indication of lack of resistance gene to CBB. Kablanketi cultivar was also reported by, Tryphone *et al.* (2012), being susceptible to CBB and BCMV/BCMNV while Chilagane *et al.* (2013) reported Kablanketi cultivar to be susceptible to ALS and BCMNV.

Results on plant reactions to BCMV showed BR 59-63-10 to possess resistance gene to the virus. AO 29-3-3A line showed resistance to BCMV pathogen used similar to results found by Kusolwa *et al.* (2016) who reported the same line having resistance to both bean bruchids and BCMV/BCMNV.

In this study, KT020 observed to have low infection reaction to both diseases followed up by 13A/59-98-3X3-3A which had few mosaic symptoms of BCMV and low scale to CBB reaction (ranged 1.0 to 1.3) considered as resistance to CBB. The genotypes showed positive response to both diseases. Tugume *et al.* (2019), reported that gene-to-gene interaction is not involved in resistance to CBB, and our study was in agreement. There was a slight increase in CBB symptoms on BR 59-63-10 which can be considered as a negative response to CBB. Tugume *et al.* (2019), Kiryowa *et al.* (2016) and Tryphone *et al.* (2012) reported that, infection can be modulated by

environment factors and amount or concentration of the inoculum which suggests that the BR 59-63-10 genotype might respond more negatively if the amount of *Xap* were in greater abundance. Kablanketi genotype was susceptible to both diseases in this trial.

2.5 Conclusion and recommendation

2.5.1 Conclusion

Among five genotypes tested in this study, three genotypes had resistance to CBB and BCMV/BCMNV (AO 29-3-3A, KT020, and 13A/59-98-3X3-3A), and one was resistant to BCMV/BCMNV but had intermediate resistance to CBB (BR 59-63-10). This foliar disease screening trial helped to select a genotype that can be used to improve common bean without changing the market class trait especially the background color of the seed.

2.5.2 Recommendation

Based on results obtained from this study it is recommended that the germplasm tested should be screened again under greenhouse conditions. Following intensive field evaluation of the similar germplasm for resistance to common bacterial blight and bean common mosaic and necrosis viruses. However, Kipato *et al.* (2015) reported BR 59-63-10 being among the genotypes with good market class traits and having resistance to bean bruchids, and will be involved to be improved by incorporating the resistance of CBB and BCMNV from KT020.

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CHAPTER THREE

3.0 INCORPORATION OF FOLIAR DISEASE RESISTANCE INTO BEAN BRUCHID RESISTANT GENOTYPES AND HERITABILITY STUDIES AT $F_{2:3}$

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3.1 Abstract

Common bacterial blight (CBB), Bean common mosaic virus (BCMV) and Bean common Mosaic Necrosis Virus (BCMNV) are the most common foliar diseases affecting common bean worldwide. CBB and BCMV/BCMNV are caused by *Xanthomona axonopodis* pv. *Phaseoli* and group of *Potyviruses* respectively, contributing to high yield and quality losses in Tanzania. Chemical control has not been effective or economical on both of these seed borne diseases. Elsewhere, breeding for resistant cultivars have been reported to be effective and a long-term control measure. The objective of this study was to incorporate foliar disease resistance to CBB, BCMV and BCMNV from KT020 into existing bruchid resistant genotype BR 59-63-10. One way cross was performed under screen-house condition followed F_1 advancement to F_2 at which $F_{2:3}$ was screened using SCAR markers i.e SAP6 for *QTL*-CBB, SW13 for *I gene*-BCMV and ROC11 for *bc-3 gene*-BCMNV resistance. Among forty individuals screened, nine derivatives had resistance to all diseases; seventeen had two resistance genes to either of the disease while ten derivatives of APAx KT020 had one resistance gene to either of the diseases.

Results also showed positive correlation between phenotypic score and Markers (r=0.410 for SAP6 marker), while in phenotypic studies all individuals had resistance ranging from 1.40 to 3.29 on leaf lesions and 2.14 to 3.30 on pod severity for CBB based on 1-9 CIAT scale. High heritability of reduced infestation explained by 61.1% and 66.8% on leaf and pod symptoms respectively was obtained. There was significant positive correlation between phenotypic reaction and marker screening indicating a reliable procedure for selecting resistant individual using marker assisted selection (MAS).

Keywords; Common beans, marker assisted selection, Tanzania, phenotypic score, heritability.

3.2 Introduction

Common bean is one of the important consumable legumes around the world. Due to its essential dietary protein, it sometimes is sometimes named the poor man's meat (Muthoni *et al*., 2017). It also provides calories in the form of carbohydrates and minerals (Mulambu *et al*., 2017). Common bean can be consumed as green vegetable, fresh, and dry. In Western countries common bean is mostly eaten as a vegetable while in Africa dry beans are most preferred (Musimu, 2018).

In Tanzania, common bean is considered as the source of income to small holder farmers where it grown on about 1.4 million hectares per year (Nassary *et al.,* 2020). Tanzania is the major producer of common bean in Africa with average yield of 984 kg per hectare which is very low compared to an estimated yield of 1500 to 2000 kg per hectare under good management with use of improved seeds (FAOSTAT, 2014).

The low yield has been associated with different constraints from both abiotic and biotic factors such as drought, temperature, diseases and insects which altogether can cause total failure of the crop (Mongi *et al.*, 2018). In Africa, diseases are the second most important constraint to common bean production after abiotic factors. Diseases may cause yield loss of up to 80 to 100%, as well as degraded quality of seeds (Mukankusi *et al*., 2018). Farmers have been tried to use different chemicals to reduce the effect of diseases and insect which turn out to have an impact to environment and health of the farmer as well consumers (Kusolwa 2007; Mwamahonje *et al.,* 2018).

In many regions there are several production seasons per year associated with minimal rotation and fallow periods, which has led to an increase in insect and disease pressure. These have resulted in annual losses varying from 20 to 100% in both yields and income of the growers (Miklas *et al.,* 2020). Reduction in yield have been attributed to the effects of insect and disease especially bean weevils (bruchids) CBB, ALS, BCMNV and BCMV (Mwaipopo *et al.,* 2018). These constraints have been accelerated by the use of unimproved cultivars which are susceptible to abiotic and biotic factors (Tryphone *et al.,* 2012; Chilagane *et al.,* 2013). Using of improved cultivars with resistance to biotic factors will increase yield, reduce production costs, and stabilize food security and benefit both smallholder farmers and the environment (Wortmann *et al*., 1998; Mahuku *et al.,* 2007)

Either use of plant host resistance (PHR) or use of eco-friendly practices has been suggested to be the best option to control the diseases (Conner *et al.,* 2020).

Common bacterial blight (CBB) resistance is conditioned by polygenic genes and 24 QTL have been identified across 11 linkage chromosomes (Sultana *et al*., 2018).

Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV), are wide spread and important viral diseases that affect bean production in Africa causing yield loss of up to 80%. Number of resistance gene to BCMV/BCMNV have been identified and tagged (Miklas and Kelly, 2002). They include the single dominant *I* gene and the recessive genes bc-u, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2} and *bc-3* (Drijfhout, 1978; Melotto *et al*., 1996). The dominant *I* gene inhibits all known strains of the BCMV (Drijfhout, 1978). When a germplasm with *I* gene is infected by BCMNV at any growing temperature, or BCMV at temperatures >30ºC, plants show black root symptoms. The interaction of *I* gene and BCMNV can be protected by combining *I* gene with race-interspecific resistance recessive gene i.e *bc-3* or *bc-2*^{2} can provide broad and stable based resistance (Melotto *et al*., 1996).

To achieve high level of cultivar resistance with multiple disease resistance, different genotypes have been developed by CIAT, which are resistant to pathogens causing CBB, and BCMV and BCMNV diseases (Tryphone *et al*., 2012; Chilagane *et al*., 2013). Those genotypes include VAX3 and VAX4 lines, and MCM 5001 (line with bc-3 gene confers resistance to BCMNV) (Miklas and Kelly, 2002). Also, AO-29-3-3A developed by Kusolwa (2007) has been confirmed to have resistance to bruchids and BCMV/BCMNV (Kusolwa *et al*., 2016).

According to TOSCI (2020) 40 improved common bean varieties have been released since 1990 to 2019 with different resistance to both biotic and abiotic constrains.

Most of the varieties released have resistance to Ascochyta blight, halo blight, angular leaf spot, anthracnose, Nematodes, rust and bean common mosaic virus and one variety (Rojo) has moderate resistance to common bacterial blight. The report documented that there is still no variety with combined resistance to CBB, BCMV/BCMNV and bruchids in the same background as the preferred landrace 'Kablanketi'.

Incorporation of resistance into a preferred cultivar is possible using the traditional breeding methods, but to hasten the process, efficient biotechnological tools and techniques have to be employed (Mahuku *et al*., 2002; Mahuku *et al*., 2007). The molecular markers linked to the genes include SAP6 for a *QTL* of CBB, ROC11 for the *bc-3* gene effective against all pathogroups of BCMNV and SW13 for *I* gene that provides resistance to BCMV. Pyramided lines can be obtained with resistance alleles to several pathogens by means of marker assisted selection (Nchimbi-Msolla *et al.,* 2020). Selection assisted by molecular markers can help to identify plants with desirable traits and prevent keeping the promising plant from being submitted to later stages of selection (Miklas *et al.,* 2020). Therefore, the objective of this study was to incorporate combined resistance from a CBB and BCMV/BCMNV containing genotype into a bruchid resistant genetic background and perform phenotypic and heritability studies using SCAR markers in the segregated population.

3.3 Materials and Methods

3.3.1 Description of the parental genotypes used

The bean lines used were collected from Department of Crop Science and Horticulture (DCSH) which were developed under the Bean improvement project at

SUA. Two bean lines were selected based on the results obtain from phenotypic screening using the inoculum and their testa color which are; BR 59-63-10 which was reported by Kipato *et al*. (2015) having resistance to bean weevils and have an average visual score of 3.5 based on CIAT 1-9 scale (van-Schoonhoven and Pastor-Corrales, 1987). This genotype is Type IV indeterminate climber, with pink background with a purplish grey fine flecking and medium-sized seeds. It is the progeny of Kablanketi and was either of F_5 or F_6. KT020 is the non-recurrent parent. It is a Type IV intermediate climbing medium seed having pink background with a purplish grey testa color. KT020 (F5) has resistance to CBB (average phenotypic score of 1.3 based on CIAT 1-9 scale) (van-Schoonhoven and Pastor-Corrales, 1987) and BCMNV.

3.3.2 Planting condition

Plants was grown in pots under screen house conditions. The pots were filled with sterilized loam soil mixed with rice husks and cow dung manure at ratio of 2:1:1. Each pot was planted with two seed and thinned to one after germination. Plants were irrigated using rose cane at intervals of one day to maintain the required moisture. Urea (20kg N per hectare approx. 0.04g N per pot) was applied at flowering in order to improve plant vigor. For the crossing block establishment, the recipient and donor plants were staggered to ensure that there were constant flowers for both parents. Control of insect pests' especially spider mites and white flies was done by spraying Thionex 35 EC (40mls/20 litres of water).

3.3.3 Hybridization

3.3.3.1 Incorporation of CBB and BCMNV into Bruchid resistant genotype

One-way crosses were conducted in Horticulture screen house at SUA to incorporate disease resistance genes into the bruchid resistant genotype. The crossing procedure involved emasculation of female flowers (BR 59-63-10) and transfer of pollen from just opened flowers (KT020) to the stigma of emasculated plants. The crossing of BR 59-63-10 and KT020 was performed during morning and evening when the temperature was between 18º and 27º C, because higher temperature cause flower abortion (Bliss, 1980). During the first month mean temperature ranged between 25º and 28º C with daily mean minimum of 26º C and mean maximum temperature of 30º C. In the middle of the second month, the experiment was challenged with a drought period where screen house temperatures rose to 28º to 35º C, and caused high rates of abortion. Pots with successful crosses were shifted from the iron bench and arranged on the ground. The ground was kept wet to maintain the moisture and also black net shade was installed in the screen house to minimize the temperature. The resultant F_1 plants (five lines) were advanced by self-pollination to obtain the F_2 population. The F_2 population (40 plants) was planted for phenotypic screening, heritability studies and a marker screen to identify the plants with resistance to CBB, BCMV and BCMNV.

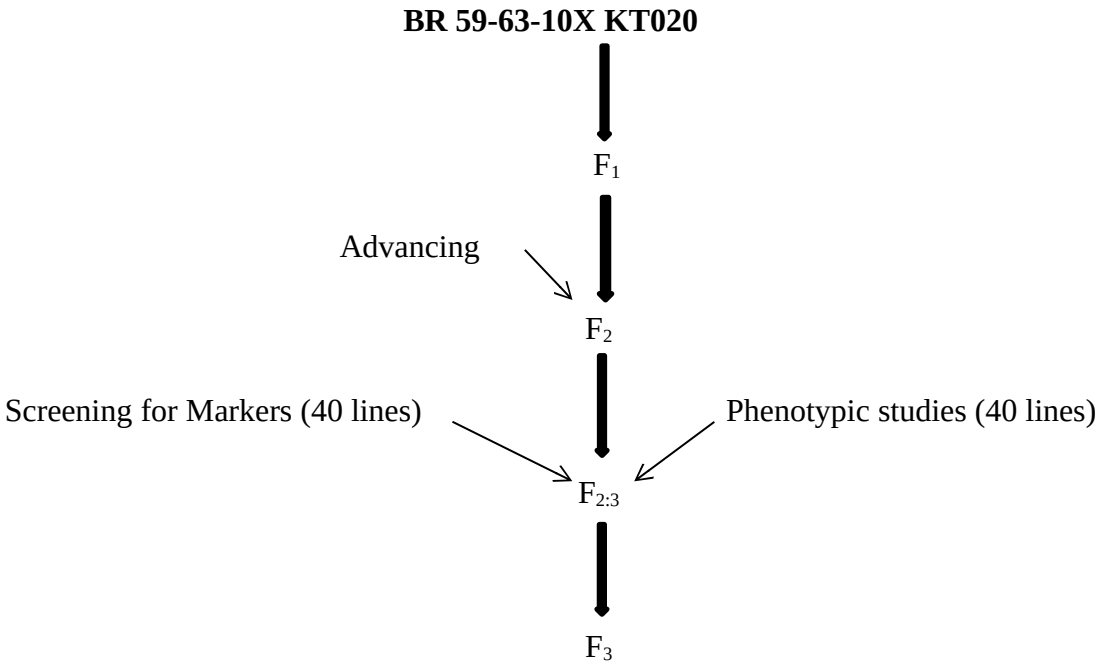

Figure 3.1: Crossing scheme to incorporate the resistance to disease into bean bruchid resistant genotype

3.3.3.2 Extraction of the DNA

Total genomic DNA was extracted from young trifoliolate leaves collected from $F_{2:3}$ plants and their parents in the screen house using two-disc punches into eppendorf tubes. The DNA extraction was carried out in Molecular biology laboratory of DCSH at SUA, using Mahuku (2004) protocol in which leaf samples were ground using a micro-pestle, followed with the addition of 300µl of TES extraction buffer to into a 1.5µl tube. Then 200µl of TES containing proteinase K was added, vortexed to mix the sample and incubated in a water bathe at 65ºC for 30 minutes. Half of the volume (250µl) of 7.5 ammonium acetate was added, vortexed to mix the sample and incubated at 5ºC in the refrigerator for 10 minutes. It was then centrifuged for 10 minutes at 14700rpm. 500µl of the supernatant was transferred into a new tube and equal volume of cold isopropanol was added, and precipitated at -20ºC for 2 hours.

The samples were then centrifuged for 10minutes at 14700rpm, the supernatant was decanted and DNA pellets were washed with 800µl of cold 70% ethanol. The mixture was centrifuged at 13000 rpm for 5 minutes, the supernatant was discarded by inverting the tube. The tubes were placed upside down on clean sterile paper towel for 15 minutes to dry and finally the DNA were resuspended in 60µl of 1X TE to elute the DNA.

3.3.3.3 Amplification of DNA

The PCR reaction mixture of 25µl was prepared, containing 1µl of each forward and reverse primers, 12.5µl of 2 Taq-master mix, 9µl of PCR water, and 1.5µl of DNA sample. PCR conditions were set corresponding to particular primers requirement in term of number of cycles and temperature. Samples for CBB, BCMV and BCMNV were amplified using the SCAR markers obtained from Eurofins genomics namely SAP6, SW13 and ROC11 respectively with their specific PCR conditions as shown in Table 3.1.

Tables 3.1: Polymerase chain reaction conditions of different SCAR markers used for amplification (Miklas, 2009)

Primer	Primer sequences	PCR conditions
SAP6	F-5'-GTCACGTCTCCTTAATAGTA-3' R-5'-GTCACGTCTCAATAGGCAAA-3'	34 cycles of 1min at 94ºC, 10s at 94ºC, 40s at 56ºC and 2min at 72ºC; followed by one cycle of 5min at 72ºC
SW13	F-5'-CACAGCGACATTAATTTTCTTTC-3': R-5'CACAGCGACAGGAGGAGCTTATTA-3'	34 cycles of 1min at 94ºC, 40s at 67ºC and 2min at 72ºC; followed by one cycle of 5min at 72ºC
ROC11	F-5'-CCAATTCTTTCACTTGTAA-3' R-5'-GCATGTTCCAGCAAACC-3'	34 cycles of 1min at 94ºC, 40s at 58ºC and 2min at 72ºC; followed by one cycle of 10min at 72ºC

3.3.3.4 Electrophoresis and gel documentation

Amplification products were separated through electrophoresis in a 1.5% agarose gel with 6.0 µL DNA ladder in 0.5X TBE (Tris-Borate EDTA) buffer under a voltage of 100 V for 80 min. The gel was stained in ethidium bromide (EtBr) with concentration of 0.5µl/ml for 30 minutes, de-stained for 30 minutes by distilled water.

The stained gel was illuminated with ultraviolet light, the bands present on the gel were observed and the digital camera was used to capture the amplified fragments for documentation and scoring according to specific base pair of SAP6-820bp, SW13-690bp, and ROC11-460bp by comparing with a reference molecular weight of the 100bp DNA ladder.

3.3.6 Marker scoring

Gel products were scored by observing the presence (+) and absence (-) of bands. Presence of the band means there gene corresponding to resistance to diseases in question and absent band means no gene corresponding to resistance to diseases in question. With exception to ROC11 marker, where absence of the band means there gene corresponding to resistance to disease in question while presence of the band means the no gene corresponding to resistance to disease in question.

3.3.4 Inoculum preparation and inoculation of Common Bacterial Blight

3.3.4.1 Isolation of *Xap*

Differential media was prepared following the procedures described by Mortensen (2005). Infected leaves were taken to the laminar air flow chamber and a section from the margin of healthy and diseased leaf tissue were sterilized by immersing the materials in 2% sodium hypochlorite (NaClO) for 2 minutes, then rinsing off the excess NaClO three times using distilled water. The materials were chopped using sterile blade and forceps, then macerated leaves were placed into a 30 ml bottle following addition of 2 ml/g of Phosphate buffer saline (PBS) and left overnight for the materials to soak into PBS. Thereafter, the homogenate was serially diluted where each serial dilution bottle contained 4.5 ml of PBS and 500µl of the leaf homogenate and was pippeted at each dilution. The dilutions of the homogenates were streaked on petri dishes containing Yeast dextrose carbonate agar (YDCA) media and were labeled with the specific dilution, name of the pathogen and date. Plates were incubated at room temperature (28ºC) for three days. After 3 days (72h) yellow mucoid colonies were observed. Colonies of cells were suspended in sterile

distilled water and the concentration was adjusted to 10^6 cfu ml^{-1} using a haemocytometer.

3.3.4.2 Inoculation

Leaf inoculation

Plants were inoculated at 18 DAP when they had fully expanded trifoliolate leaves by spraying the inoculum on both side of the leaves using hand pump sprayer. They were then covered by plastic sheets to increase relative humidity (RH) for 72h while the floor was kept wet for 24h. After 72h the plastic sheets were removed and the plant pots were transferred and placed in the screen-house on benches made of meshed steel, one meter high for symptoms development.

Pod inoculation

Plants was inoculated at pod filling stage in which two pods of each plant were injected with 0.5ml of *Xap* using 2ml syringe.

3.3.4.3 Disease scoring

The disease severity was assessed on all leaves beginning seven days after inoculation (DAI), then 14 DAI, 21DAI, and 35DAI. For pods, disease severity was assessed once at 10 DAI. The disease severity rating was estimated following CIAT 1-9 (Table 3.2) (van-Schoonhoven and Pastor-Corrales, 1987).

Tables 3.2: General scale used to evaluate the reaction of bean germplasm to common bacterial blight (van Schoonhoven and Pastor-Corrales, 1987)

Rating	category	Description	Comments
1-3	Resistant	No visible to very light symptoms resulting in little or no economic damage	Germplasm useful as parent or commercial variety
4-6	Tolerant or Intermediate	Visible and noticeable symptoms resulting only in limited economic damage	Germplasm can be used as commercial varieties or sources of resistance to certain diseases
7-9	susceptible	Severe to very severe symptoms causing useful yield losses or plant death	Not useful to be used as parent or commercial variety

3.3.5 Data collection

3.3.5.1 Leaf disease severity

Disease severity was scored using visual score rating scale of 1 to 9 with little modification (Table 3.2) (van Schoonhoven and Pastor-Corrales, 1987). The disease score were done at 14 DAI, 21 DAI, and 35 DAI.

3.3.5.2 Pod reaction severity

Pod severity score was performed once at 10 DAI following the disease scale rating of 1-9 by van Schoonhoven and Pastor-Corrales (1987)

3.3.7 Data analysis

Data collected were subjected to analysis of variance (ANOVA) at $p \leq 0.05$ using GenStat 16th Edition statistical package. Treatment means were separated using Duncan Multiple Range Test (DMRT). Correlation coefficient between phenotype score and marker score were calculated using Microsoft Excel 2010. The p-value of the correlation was calculated by subject the Correlation coefficient (*r)* online Pearson's Correlation Coefficient Calculator at ($p \leq 0.05$). the variances of Parents, F_1 and F_2 were generated and used to estimate the narrow sense heritability based on scaling test as described by Hill and Mackay (2004) at which the following formulas were used;

$(h^2) = {}^{1/2}D/VF_2$…… (i) Where VF_2 is the total variance of F_2 and 1/2D is the additive genetic component of variance of F_2 which calculated as;

$1/2D = 2VF2 - (VP_1+VP_2+VF_1)$ ……(ii). Inheritance was calculated based on the crosses generated. MS Excel 2010 was used to generate disease severity graphs.

3.4 Results and Discussion

3.4.1 Incorporation of CBB, BCMV and BCMNV into bruchid resistant genotypes

Total of 40 $F_{2:3}$ plants were screened using SCAR markers (SAP6 linked to *QTL*-CBB, SW13 for *I* gene-BCMV and ROC11 for *bc-3* gene-BCMNV) for the three genes targeted to be incorporated into bruchid resistant genotype (Table 3.3). Results showed, that there was success in incorporation of disease resistance genes to common bacterial blight, bean common mosaic virus and bean common mosaic

necrosis virus in which among the 40 $F_{2:3}$ screened using Marker assisted selection (MAS), 9 plants had all three genes, 17 plants had two gene combination, 10 plants with only one gene of resistance and 4 plants which have no any of the resistance gene tested as shown in Table 3. 4.

Tables 3.3: SCAR Marker screening for combined gene present in the $F_{2:3}$ bruchid resistant plants and percentage of gene combination in each plant screened.

CROSSES	SAP6-QTL	SW13-*I* gene	ROC11-*bc-3*	%gene present
BR 59-63-10X KT020-1-1	+	-	-	**66.67**
BR 59-63-10X KT020-1-2	+	+	+	**66.67**
BR 59-63-10X KT020-1-3	+	+	+	**66.67**
BR 59-63-10X KT020-1-4	-	-	+	**0**
BR 59-63-10X KT020-1-5	+	-	-	**33.33**
BR 59-63-10X KT020-1-6	-	+	-	**33.33**
BR 59-63-10X KT020-1-7	-	-	-	**0**
BR 59-63-10X KT020-1-8	+	+	-	**66.67**
BR 59-63-10X KT020-1-9	-	-	+	**33.33**
BR 59-63-10X KT020-1-10	+	+	+	**100**
BR 59-63-10X KT020-2-1	+	+	-	**66.67**
BR 59-63-10X KT020-2-2	+	+	+	**100**
BR 59-63-10X KT020-2-3	+	+	-	**66.67**
BR 59-63-10X KT020-2-4	+	-	-	**33.33**
BR 59-63-10X KT020-2-5	+	+	-	**66.67**
BR 59-63-10X KT020-2-6	-	-	+	**33.33**
BR 59-63-10X KT020-2-7	+	+	-	**100**
BR 59-63-10X KT020-2-8	+	+	-	**66.67**
BR 59-63-10X KT020-3-1	+	-	+	**66.67**
BR 59-63-10X KT020-3-2	-	-	+	**33.33**
BR 59-63-10X KT020-3-3	+	+	-	**66.67**
BR 59-63-10X KT020-3-4	-	-	-	**0**
BR 59-63-10X KT020-3-5	+	+	+	**100**
BR 59-63-10X KT020-3-6	+	-	-	**33.33**
BR 59-63-10X KT020-3-7	+	+	-	**66.67**
BR 59-63-10X KT020-4-1	+	-	-	**66.67**
BR 59-63-10X KT020-4-2	+	-	-	**66.67**
BR 59-63-10X KT020-4-3	+	-	-	**66.67**
BR 59-63-10X KT020-4-4	+	+	-	**100**
BR 59-63-10X KT020-4-5	+	+	-	**100**
BR 59-63-10X KT020-4-6	+	+	-	**100**
BR 59-63-10X KT020-4-7	+	-	+	**33.33**
BR 59-63-10X KT020-4-8	-	+	-	**66.67**

Tables 3.3: SCAR Marker screening for combined gene present in the $F_{2:3}$ bruchid resistant plants and percentage of gene combination in each plant screened.

	-			
BR 59-63-10X KT020-5-1		-	+	**0**
BR 59-63-10X KT020-5-2	-	+	+	**33.33**
BR 59-63-10X KT020-5-3	+	+	-	**100**
BR 59-63-10X KT020-5-4	+	-	+	**33.33**
BR 59-63-10X KT020-5-5	+	+	+	**66.67**
BR 59-63-10X KT020-5-6	+	+	-	**100**
BR 59-63-10X KT020-5-7	+	-	-	**66.67**

Key: += presence of resistance marker-gene; and -=absence of resistance marker-gene with respect to disease in question for SW13 and SAP6: ROC11: -= presence of resistance marker-gene and += absence of resistance marker-gene.

Tables 3.4: Summary of crosses ($F_{2:3}$) with combination of different resistance gene per screened $F_{2:3}$ plant

CROSS	**no. of plant with 3 genes**	**no. of plant with 2 genes**	**no. of plant with 1 gene**	**no. of plant with 0 gene**
BR 59-63-10 X KT020-1	1	4	3	2
BR 59-63-10 X KT020-2	2	4	2	0
BR 59-63-10 X KT020-3	1	3	2	1
BR 59-63-10 X KT020-4	3	4	1	0
BR 59-63-10 X KT020-5	2	2	2	1
Total	**9**	**17**	**10**	**4**

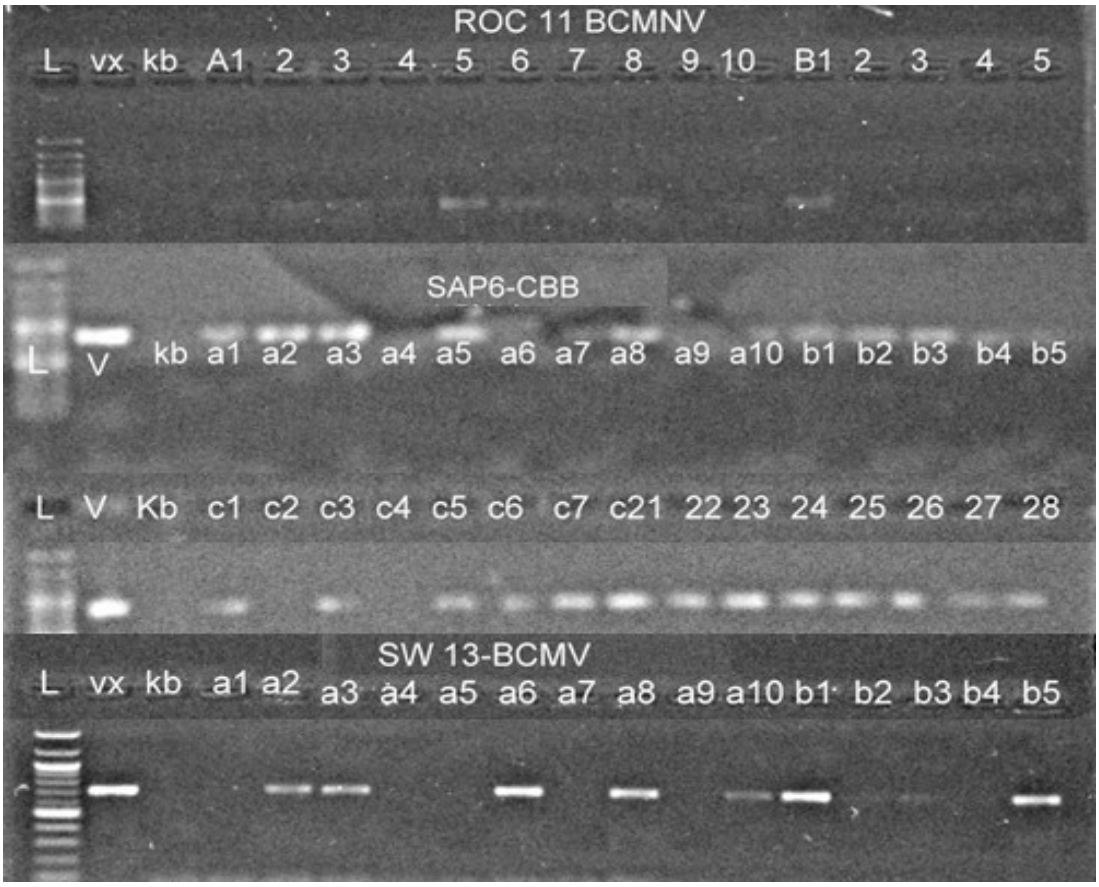

Figure 3.2: PCR products of 15 $F_{2:3}$ common bean lines scored at different SCAR markers: ROC11-BCMNV; 460bp, SAP6-*QTL* (CBB); 820bp, and SW13-BCMV; 690bp as observed at 1.5% Agarose gel. Presence of the band corresponding to the presence of gene of interest with exception to ROC11-BCMNV where absence of the band corresponds to presence of gene of interest (resistance gene is controlled by recessive gene): vx=Vax 3, kb= 'Kablanketi', a1 to c28= progenies (F_2).

Figure 3.3: Different seeds of the common bean segregating population (F_2) harvested from selfed plants for generation advance.

3.4.2 Percentage Inheritance of Resistance per Screened Markers

The results show that among 40 $F_{2:3}$ common bean lines screened, 75% of the lines derived from cross of BR 59-63-10 XKT020 have *QTL* which corresponding to CBB resistance, 47.5% of the lines screened with ROC11 marker had *bc-3* gene which corresponding to BCMNV resistance while 55% of the lines had *I gene* which corresponds to BCMV resistance.

3.4.3 $F_{2:3}$ plants with three resistant genes

Among 40 $F_{2:3}$ plants screened with SCAR markers; SAP6 (*QTL* for CBB), SW13 (*I gene* for BCMV) and ROC11 (*bc-3* gene for BCMNV), only 9 $F_{2:3}$ plants had all three resistance genes (Table 3.6).

Tables 3.5: $F_{2:3}$ plants screened with three resistance gene in combination

CROSSES	Marker-gene present		
	SAP6-QTL	SW13-*I* gene	ROC11-*bc-3*
BR 59-63-10 X KT020-1-10	+	+	+
BR 59-63-10 X KT020-2-2	+	+	+
BR 59-63-10 X KT020-2-7	+	+	+
BR 59-63-10 X KT020-3-5	+	+	+
BR 59-63-10 X KT020-4-4	+	+	+
BR 59-63-10 X KT020-4-5	+	+	+
BR 59-63-10 X KT020-4-6	+	+	+
BR 59-63-10 X KT020-5-3	+	+	+
BR 59-63-10 X KT020-5-6	+	+	+

Key: += presence of resistance marker-gene; and -=absence of resistance marker-gene with respect to disease in question

3.4.4 Phenotypic evaluation of the $F_{2:3}$ populations

Based on phenotypic evaluation of the $F_{2:3}$ populations, there was significant differences ($p \leq 0.001$) on leaf lesion between the crosses and their parent to *Xap* at 14, 21 and 35 DAI at which all $F_{2:3}$ populations observed no visible lesions on the leaf, BR 59-63-10 X KT020-1 population scored 1 at 14 DAI, 1.27 and 1.38 leaf lesion severity were observed on BR 59-63-10 X KT020-3 and BR 59-63-10 X KT020-2, and population respectively and BR 59-63-10 X KT020-5 and BR 59-63-10 X KT020-4 respectively both had leaf lesion severity score of 2.00 while BR 59-63-10 was scored 3.87 (Table 3.7). Also, on the 35 DAI all $F_{2:3}$ populations were observed to resistance to CBB (Table 3.2). There was significance difference ($p \leq 0.001$) on leaf lesion severity score at 35 DAI among the means of each $F_{2:3}$ populations.

Based on the pod severity score, result showed significance differences (p=<0.001) among the $F_{2:3}$ populations on pod reaction to *Xap* (CBB) and their parents, at 10 DAI where the means values of the populations ranged from 2.14 to 3.30 which categorized as resistant to CBB while BR 59-63-10 was scored 5.13 and KT020 scored 3.0 (Table 3.7; Table 3.2; Figure 3.2)

Tables 3.6: Visual disease score of the BR 59-63-10 XKT020 common bean derivatives to Common bacterial blight on both leaf and pod lesions (van Schoonhoven and Pastor-Corrales, 1987)

	Leaf lesion			
Genotypes	**14 DAI**	**21 DAI**	**35 DAI**	**10 DAI_P**
BR 59-63-10X KT020-1	1.00 a	1.10 a	1.40 a	3.30 a
BR 59-63-10X KT020-2	1.38 ab	1.63 abc	2.37 a	2.87 a
BR 59-63-10X KT020-3	1.29 ab	1.43 abc	1.71 a	2.14 a
BR 59-63-10X KT020-4	2.00 b	2.63 bc	3.00 a	2.37 a
BR 59-63-10X KT020-5	2.00 b	2.86 c	3.29 ab	2.57 a
BR 59-63-10	3.87 c	4.63 d	4.88 b	5.13 b
KT020	1.17 ab	1.33 ab	1.58 a	3.00 a
Grand mean	**1.75**	**2.13**	**2.50**	**3.08**
s. e. d	**0.845**	**1.399**	**1.742**	**1.305**
cv%	**48.30**	**65.60**	**69.70**	**42.30**
p value	**<.001**	**<.001**	**0.001**	**<.001**

*Means with same letter in each column have no significant different at p≤0.05; s.e.d= standard error of differences, cv%= Coefficient of variance, DAI= Days After Inoculation, DAI_P=Days After Inoculation on Pods,

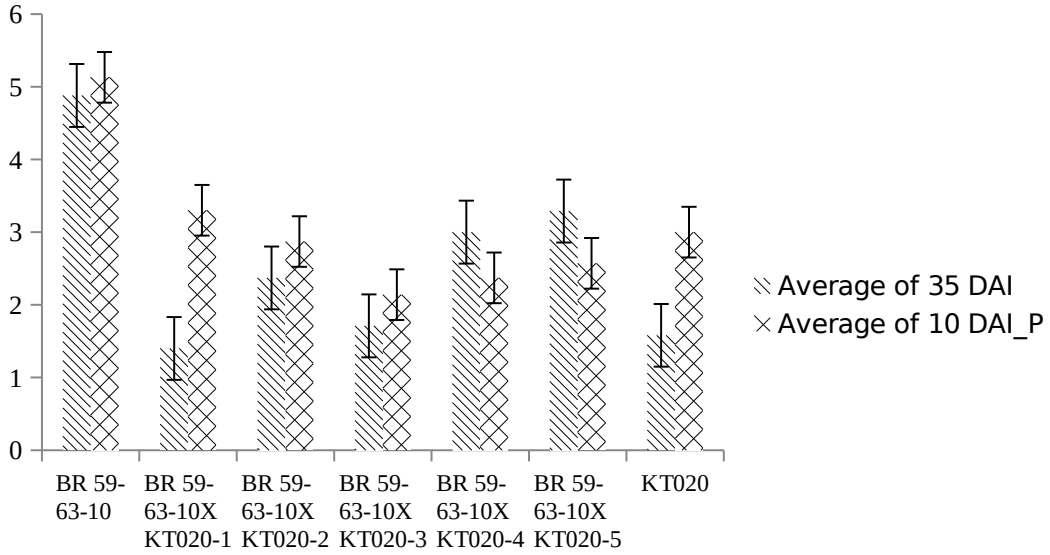

Figure 3.4: Average disease severity score using visual score rating (1-9) on both leaf lesion and pod lesion of the F_3 crosses (BR 59-63-10 xKT020)

However, most of the BR 59-63-10 x KT020 derivatives were observed with no any symptom of infection when phenotypically screened to Xap (Figure 3.3)

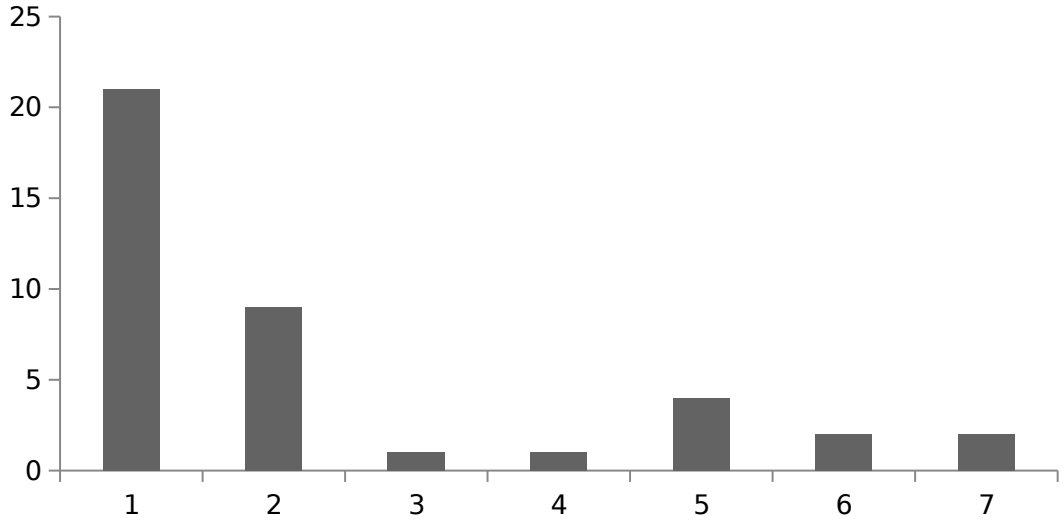

Figure 3.5: Distribution of the BR 59-63-10 X KT020 (F2) common bean plants for the reaction to *Xap* using scale of 1-9 CIAT

3.4.5 Correlation of leaf lesion against pod severity score

There were no significance differences (p = 0.706) and a very low correlation (r=0.062) between phenotypic disease score on leaves and the SAP6 for QTL marker score corresponding to CBB resistance gene for the $F_{2:3}$ populations (Appendix 1)

3.4.6 Correlation of phenotypic against SCAR marker

There were no significance differences (r) =0.706) between phenotypic disease score and the SAP6 for *QTL* marker score corresponding to CBB resistance gene, with very low correlation (r=0.062) of phenotypic scores against SAP6 marker scores of the $F_{2:3}$ populations (Appendix 1)

3.4.7 Heritability for disease resistance

The estimated narrow heritability of common bacterial blight was 61.1% and 66.8% for leaves and pods respectively (Table 3.8) for the progenies from the cross of BR 59-63-10 x KT020 which implies additive effect for the genes controlling disease resistance exists in F2 populations.

Tables 3.7: Estimation of narrow sense heritability for the reaction to Common Bacterial Blight in common beans leaves and pods

Cross	Organ assessed	Estimated heritability (h^2)
BR 59-63-10 x KT020	Leaves	0.611
	Pods	0.668

3.5 Discussion

Incorporation of resistance to seed borne disease namely, CBB, BCMV and/ or BCMNV is among the effective and long-term control measure. In this study resistance were incorporated to bruchid resistant genotypes from KT020 using one way cross. Bruchid resistant genotypes were developed at SUA having the market class background as the Kablanketi cultivar, regardless of these genotypes having resistance to bruchid damaged but are susceptible to CBB with intermediate resistance to BCMV/BCMNV diseases. Resistances to CBB, BCMV and/ or BCMNV were successful incorporated to 9 plants. All the plants were found to have all resistance genes incorporated while 27 plants found to have either one gene or two genes conferring resistance to diseases. Many resistant lines to CBB, BCMV/BCMNV and other foliar diseases have been developed. Chilagane *et al.* (2013) introgressed resistance to ALS and BCMNV into Kablanketi cultivar, similarly Tryphone *et al.* (2012) introgressed resistance to CBB and BCMV/BCMNV into preferred Kablanketi cultivar. While Kusolwa *et al.* (2016) developed AO 29-3-3A line (red seeds) which had resistance to bruchid damage and BCMV/BCMNV. However, common bean breeders have been using interspecific crosses to combine resistance gene to CBB into common beans to obtain lines and cultivar with resistance (Alladasi *et al*., 2018). Since CBB resistance is quantitative trait efforts on developing lines with pyramided resistance genes/ QTL have been done, such lines are; VAX 3, VAX 4, VAX 5, VAX 6, Wilk 2, XAN 307, and USPT-CBB 5 and have been widely used in various breeding programs (Singh and Miklas, 2015: Alladasi *et al.,* 2018). Also, lines with resistance to BCMV have been developed such as MCM 5001 and etc.

The current finding reveals that, selection of the resistant crosses in early generation can be efficient using Marker assisted selection (MAS) where by findings showed positive correlation with no significant differences between phenotypic score and marker scores which implies selection of the plants with presence of particular gene of resistance signifies the plant reaction to the pathogen. Chilagane *et al.* (2013) and Tryphone *et al.* (2012) also reported positive correlation of the phenotypic score against Marker. Also, MAS were used to validate the QTL and *bc-3/I* gene for CBB and BCMNV or BCMV respectively present in the resistant lines selected by phenotypic selection. Similar study were done by Miklas *et al.* (2000) to expedite MAS for combined resistance to CBB while Drijfhout (1987) and Mwaipopo *et al.* (2018) used marker to validate resistance for BCMV/BCMNV in common beans. Combining MAS and phenotypic selection is important and makes the development of breeding line more effective at which phenotypic selection retains the minor effect QTL and select for epistatic interactions that contributes to improved resistance (Miklas *et al.*, 2005).

Results from this study showed low correlation coefficient (r=0.140) between leaf and pod reactions to the *Xap*, suggesting that there is differential expression of resistance to CBB in different plant organs/ parts. Low genetic correlation between leaf and pod reactions and leaf and seed reactions to CBB have been reported by Alladasi *et al.* (2018) Arnaud-Santana *et al*., (1994), Park *et al.* (1998) and Jung *et al. (*1997) in similar studies. This low correlation between leaf and pods suggests that significant number of plants tested did not have consistent response to CBB. These results are in agreement with those reported by Adam *et al.* (1988) for mutants derived from *P. vulgaris* snap beans cultivar and by Drijfhout and Blok (1987) in

tepary beans. While Silva *et al.* (1989) reported different genes found to control disease reactions to different plant parts.

Narrow-sense heritability estimates for reactions on different plant parts (leaves and pod) were 61.1% and 66.8% on leaves and pods respectively. This heritability is termed as moderate high according to Hill and Mackay (2004). Similar results were reports by Silva *et al*. (1989), Coyne *et al.* (1965), Rava *et al.* (1987) and Fourie *et al.* (2011). Low to moderate heritability has been reported by other authors for leaf reaction to *Xap* in dry beans (Arnaud-Santana *et al.*, 1994; Ariyarathne *et al*., 1999; Tryphone *et al.*, 2012). Usually, the heritability values depend on population, environmental condition, experimental design precision on data collection and genetic complexity of the trait under study (Okii *et al.*, 2018). The former should have reduced the environmental effects on disease development and interaction between pathogen and environment, thus causing higher heritability as found in this study.

3.6 Conclusion and Recommendations

3.6.1 Conclusion

The objective of this study was to incorporate the resistance of CBB, BCMV and BCMNV into bruchid resistant genotypes and validate inheritance of resistance gene to the mentioned diseases using the MAS for resistance and eventually identify the genotypes with combined resistance to all diseases in question. Results demonstrated that there were nine lines with genes for resistance to CBB, BCMV and BCMNV namely; BR 59-63-10X KT020-1-10; BR 59-63-10X KT020-2-2; BR 59-63-10X KT020-2-

7; BR 59-63-10X KT020-3-5; BR 59-63-10X KT020-4-4; BR 59-63-10X KT020-4-5; BR 59-63-10X KT020-4-6; BR 59-63-10X KT020-5-3, and BR 59-63-10X KT020-5-6, which indicates the successfully transfer of the resistance genes (*QTL*, *bc*, and *I*) to bruchid resistant genotypes.

Positive correlation obtained between phenotypic selection and marker indicating the great chances of selecting resistant individuals using molecular markers which exhibit resistance by inoculation in the screen-house or in the field.

Also, the heritability for CBB disease in this study is moderate high which indicating that transferring of the traits from parents to offspring was successfully and selection can be performed on early generations.

3.6.2 Recommendations

Genotypes identified to have combined resistance are recommended for several advancement and evaluation for variety release as the multiple diseases and insect resistant. However, more research should be done on evaluating the genotypes with bruchid resistant using both bruchid feeding trials and protein extraction to identify the genotypes with good resistance to bruchid.

Several backcrosses must be considered, since the tested genotypes were in early generation and hence may lose some qualities in resistance and also retaining the seed quality differs widely from genotypes to genotype for variety release. Advancement of these genotypes to release stage could be important contribution to smallholder farmer on income generation, food quality and nutrition security.

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CHAPTER FOUR

4.0 GENERAL CONCLUSION AND RECOMMENDATION

4.1 General conclusion

This work has successfully incorporated three resistance factors into the farmers' preferred bean variety Kablanketi. The finding in this work demonstrates the significance of integrating classical breeding with marker assisted selection (MAS) which reduces the time spent on phenotypic selection by selecting the individuals with multiple resistance to diseases at an early generation as well as it allows a breeder to carry fewer materials which have promising traits to subjects on question. However, this work demonstrated a reliable procedure for selecting lines with resistance to CBB, BCMV and BCMNV by using both phenotypic reaction and marker screening. Breeding for host plant resistance is very important as a sustainable method for controlling pests leading to increased productivity which has a direct effect on smallholder farmers' income, health, living standards, food security and ecological well-being.

4.2 General recommendation

Therefore, the following have to be done;

i. Several generations of selfing should be done to reach the homozygosity at most of the loci. Following with bean bruchid feeding trials to assessing resistance of these lines to bruchid infestation
ii. Further field evaluations should be done to determine yield performance of these lines during season and off season so as to select individuals with high yielding prior to release.

iii. However, screening the BR 59-63-10 x KT020 using co dominant marker can be very suitable to ensure resistance factors have been fit to more loci.

APPENDICES

Appendix 1: Correlation coefficient of Leaf lesion severity and pod severity of the F2:3 populations

Beanlines	Pod lesion severity	Leaf lesion severity	Marker score-SAP6*
BR 59-63-10X KT020-5-5	1.7	2.0	**1**
BR 59-63-10X KT020-2-1	2.0	1.7	**1**
BR 59-63-10X KT020-2-7	2.0	1.3	**1**
BR 59-63-10X KT020-3-3	2.0	2.7	**1**
BR 59-63-10X KT020-1-1	2.3	2.0	**1**
BR 59-63-10X KT020-1-10	2.3	2.0	**1**
BR 59-63-10X KT020-2-2	2.3	1.3	**0**
BR 59-63-10X KT020-3-1	2.3	2.7	**1**
BR 59-63-10X KT020-3-2	2.3	2.0	**1**
BR 59-63-10X KT020-3-5	2.3	1.7	**1**
BR 59-63-10X KT020-3-6	2.3	1.7	**1**
BR 59-63-10X KT020-4-1	2.3	1.3	**1**
BR 59-63-10X KT020-4-3	2.3	3.7	**1**
BR 59-63-10X KT020-5-4	2.3	2.0	**1**
BR 59-63-10X KT020-5-7	2.3	2.3	**1**
BR 59-63-10X KT020-1-6	2.3	1.3	**0**
BR 59-63-10X KT020-1-7	2.3	1.3	**0**
BR 59-63-10X KT020-1-8	2.3	1.7	**1**
BR 59-63-10X KT020-2-3	2.7	5.0	**1**
BR 59-63-10X KT020-2-8	2.7	2.3	**1**
BR 59-63-10X KT020-3-4	2.7	1.7	**0**
BR 59-63-10X KT020-3-7	2.7	1.7	**1**
BR 59-63-10X KT020-4-2	2.7	2.0	**1**
BR 59-63-10X KT020-4-5	2.7	2.0	**1**
BR 59-63-10X KT020-4-7	2.7	3.7	**1**
BR 59-63-10X KT020-4-8	2.7	2.3	**0**
BR 59-63-10X KT020-5-6	2.7	2.3	**1**
BR 59-63-10X KT020-1-4	2.7	1.0	**0**
BR 59-63-10X KT020-4-6	3.0	2.3	**1**
BR 59-63-10X KT020-5-1	3.0	4.0	**0**
BR 59-63-10X KT020-5-2	3.0	5.0	**0**
KT020	3.0	1.6	**1**
BR 59-63-10X KT020-2-4	3.3	3.7	**1**
BR 59-63-10X KT020-2-5	3.3	1.7	**1**
BR 59-63-10X KT020-1-5	3.3	2.0	**1**

Appendix 2: Correlation coefficient of Leaf lesion severity and pod severity of the F2:3 populations

BR 59-63-10X KT020-2-6	4.0	2.7	**0**
BR 59-63-10X KT020-1-3	4.0	2.0	1
BR 59-63-10X KT020-5-3	4.0	5.0	**1**
BR 59-63-10X KT020-1-9	4.7	1.3	**0**
BR 59-63-10X KT020-4-4	5.0	5.7	**1**
BR 59-63-10	5.1	4.9	**0**
BR 59-63-10X KT020-1-2	5.7	1.3	**1**
Grand mean	**3.0**	**2.5**	
s. e. d	**1.0**	**1.1**	
cv%	**31.4**	**42.1**	
p value	**<.001**	**<.001**	
r(phenotype against marker)			**0.062**
p value (p≤0.05)			**0.706**
r (leaf severity against pod severity)		**0.41**	
p value(r) (p≤0.05)		**0.387**	

*bolded numerical represent the gel score at which 1=gene present and 0= gene absent, r= correlation coefficient, p value (r)=Pearson's probability value.

Appendix 3: Analysis of variance of the common bean genotypes reactions to Xap at 14 days after inoculation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	4	11.6	2.9	21.75	<.001
Residual	10	1.3333	0.1333		
Total	14	12.9333			

Appendix 4: Analysis of variance of the common bean genotypes reactions to Xap at 21 days after inoculation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	4	26.2667	6.5667	12.31	<.001
Residual	10	5.3333	0.5333		
Total	14	31.6			

Appendix 5: Analysis of variance of the common bean genotypes reactions to Xap at 35 days after inoculation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	4	94.4	23.6	23.6	<.001
Residual	10	10	1		
Total	14	104.4			

Appendix 6: Analysis of variance of the common bean genotypes reactions to BCMV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	4	6.2667	1.5667	11.75	<.001
Residual	10	1.3333	0.1333		
Total	14	7.6			

Appendix 7: Analysis of variance of the common bean derivatives reactions to Xap at 14 days after inoculation on leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	49.4048	8.2341	11.53	<.001
Residual	53	37.8452	0.7141		
Total	59	87.25			

Appendix 8: Analysis of variance of the common bean derivatives reactions to Xap at 21 days after inoculation on leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	79.17	13.195	6.74	<.001
Residual	53	103.763	1.958		
Total	59	182.933			

Appendix 9: Analysis of variance of the common bean derivatives reactions to Xap at 35 days after inoculation on leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	78.076	13.013	4.29	0.001
Residual	53	160.924	3.036		
Total	59	239			

Appendix 10: Analysis of variance of the common bean derivatives reactions to Xap at 10 days after inoculation on pods

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	46.287	7.714	4.53	<.001
Residual	53	90.296	1.704		
Total	59	136.583			