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RESEARCH ARTICLE

Introduction of foliar disease resistance into bean bruchid resistant genotypes and heritability studies at $F_{2:3}$

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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 *Xanthomonas 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, 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. 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

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) 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; Mukankusi et al., 2018).

Biotic factors includes, insect pest such as bean weevils (Kusolwa, 2007; Kipato et al*.,* 2015; Kusolwa et al., 2016), and major diseases constraints to common bean production in East and Central Africa include Common bacterial blight (CBB) caused by *Xanthomonas 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).

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.

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-1²*, *bc-2, bc-2²* 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-2²* 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.

MATERIALS AND METHODS

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 (F_5) has resistance to CBB (average phenotypic score of 1.3 based on CIAT 1-9 scale) (van-Schoonhoven and Pastor-Corrales, 1987) and BCMNV.

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).

Hybridization

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.

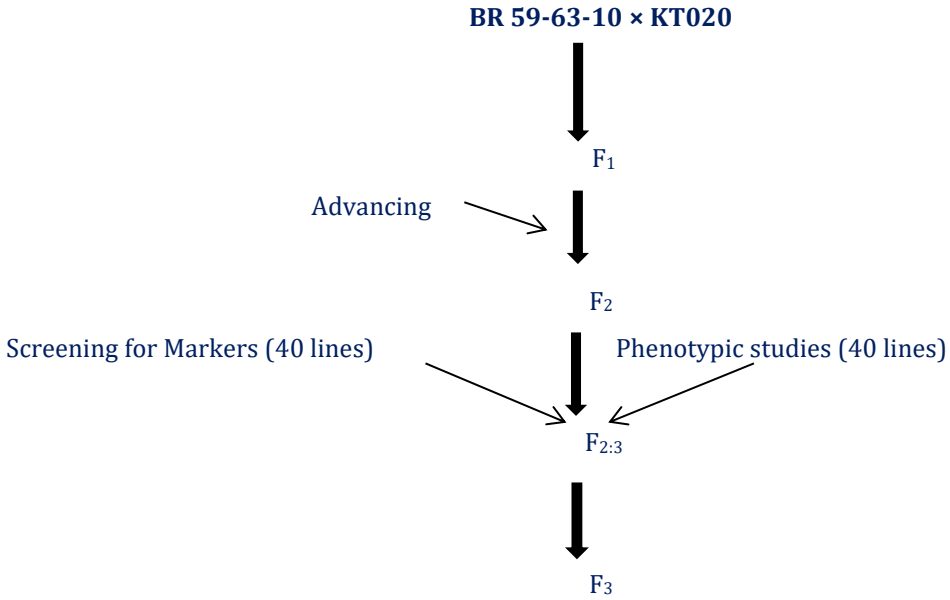

Figure 1. Crossing scheme to incorporate the resistance to disease into bean bruchid resistant genotype

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.

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 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

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.

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.

Inoculum preparation and inoculation of 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 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.

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.

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 developed by van-Schoonhoven and Pastor-Corrales (1987).

Data collection

Leaf disease severity

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

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)

Data analysis

Data collected were subjected to analysis of variance (ANOVA) at p≤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≤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). Inheritance was calculated based on the crosses generated. MS Excel 2010 was used to generate disease severity graphs.

RESULTS AND DISCUSSION

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 2). 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.

Tables 2. 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
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. 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

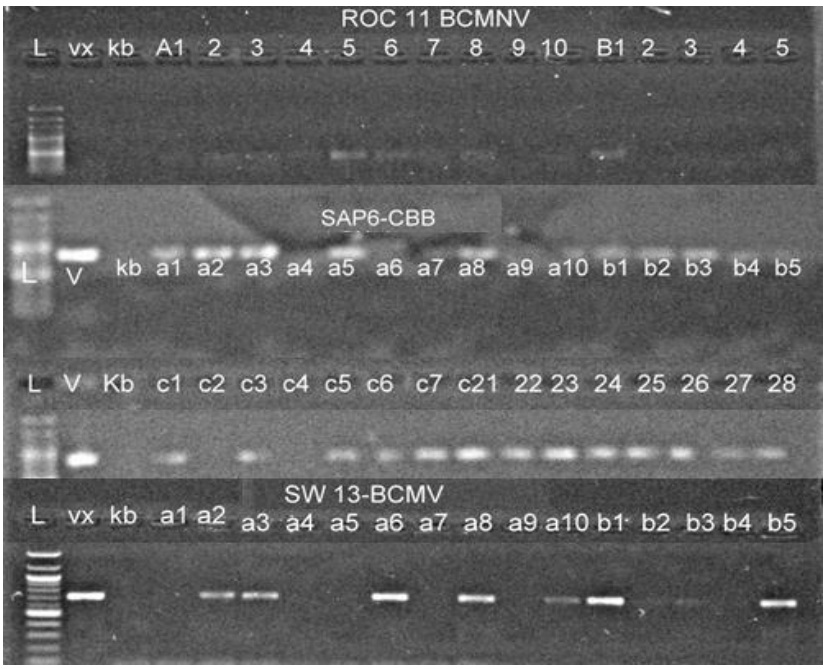

Figure 2. *PCR products of 15 $F_{2:3}$ common bean lines scored at different SCAR markers.* ROC11-BCMNV; 460bp, SAP6-QTL (CBB); 820bp, and SW13-BCMV; 690bp as observed at 1.5% Agarose gel. Presence of the band corresponding to the presence of gene of interest with exception to ROC11-BCMNV where absence 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. Different seeds of the common bean segregating population (F_2) harvested from selfed plants for generation advance

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.

$F_{2:3}$ 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 5).

Tables 4. $F_{2:3}$ 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

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 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 7;

Table 2; Figure 2). 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)

Tables 5. 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
SED	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.

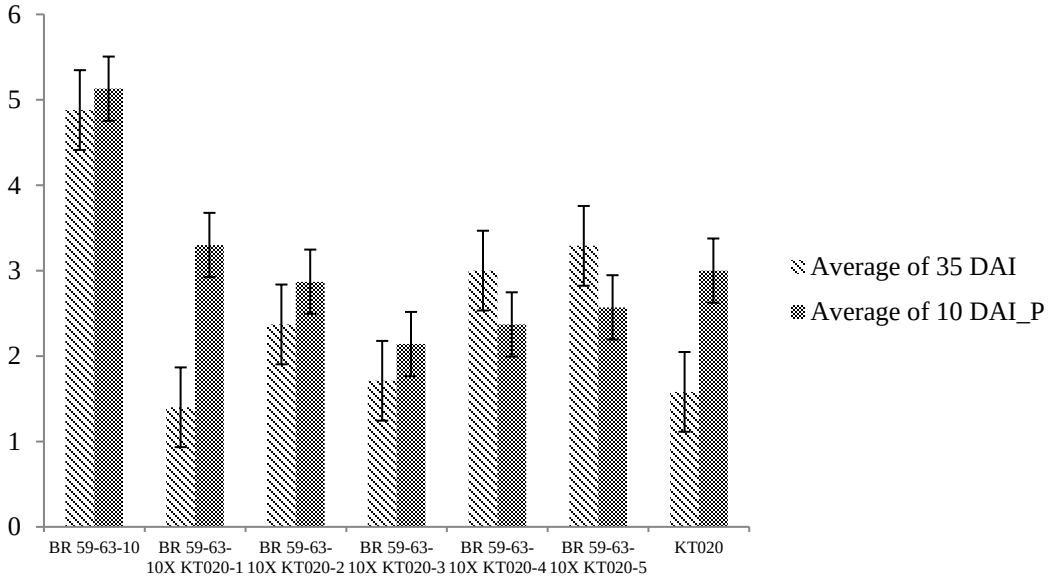

Figure 3. Average disease severity score using visual score rating (1-9) on both leaf lesion and pod lesion of the F_3 crosses (BR 59-63-10 xKT020)

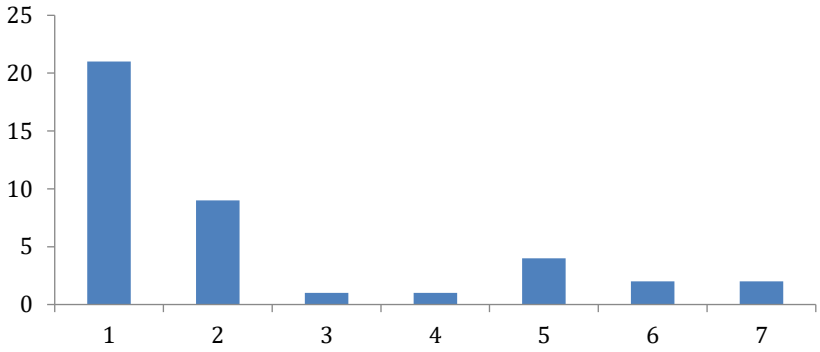

Figure 4. Distribution of the BR 59-63-10 X KT020 (F2) common bean plants for the reaction to Xap using scale of 1-9 CIAT

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.

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.

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 6. 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

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 was 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.

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. 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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AUTHOR CONTRIBUTIONS

Nuhu M. A performed the conception or design of work, data collection, data analysis, interpretation and was a major contributor in writing the manuscript. Paul M. K and Newton L.K performed a critical revision of the manuscript providing critical comment on discussion of results, conclusion and recommendations. All authors read and approved the final manuscript to be published.

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DISCLOSURE STATEMENT

The authors declared that they have no competing interests.

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