

Full Length Research Paper

# Incorporation of resistance to angular leaf spot and bean common mosaic necrosis virus diseases into adapted common bean (*Phaseolus vulgaris* L.) genotype in Tanzania

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Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* and Bean common mosaic and necrosis virus (BCMVB/BCMNV) are important diseases of common bean in Tanzania that can cause severe yield reduction when uncontrolled. This study was conducted to incorporate resistant genes for ALS and BCMVB/BCMNV diseases into adapted, market class and farmers and consumers preferred bean genotype using marker assisted selection. The parents Mexico 54 and UBR(25)95 donor of *Phg-2* and *I/bc-3* genes for ALS and BCMVB/BCMNV, respectively were used for the recipient being Kablanketi. In selection, SCAR markers SNO2, ROC11 and SW13 linked to *Phg-2*, *bc-3* and *I* gene, respectively were used. A parallel backcrossing (modified double cross) procedure was used. The F<sub>1</sub>, F<sub>2</sub> and backcrosses from single crosses were characterized. The Chi square values for ALS were 0.081 ( $P < 0.776$ ) and 0.017 ( $P < 0.896$ ) and for BCMNV were 1.609 ( $P < 0.205$ ) and 1.2 ( $P < 0.273$ ) for molecular and phenotypic screening, respectively. The resistance to ALS and BCMNV was found to be monogenic and the genes involved are dominant and recessive, respectively. The heritability of ALS was found to be high (0.772) implying that selection for ALS can be done early in segregating populations. High correlation values,  $r = 0.741$  and  $0.624$  for ALS and BCMNV, were obtained between phenotypic and molecular data, indicating high reliability for markers. In selection, it was possible to select lines with multiple disease resistances. This work signified the use of MAS for multiple gene screening.

**Key words:** Common bean, *Pseudocercospora griseola*, marker assisted selection, genotype, inheritance.

## INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) belong to the family fabaceae. It is an annual, predominantly self pollinated legume grown in all continents under extremely

variable environmental conditions (Melotto et al., 2005). It is the most commonly and widely cultivated crop in the tropics and sub tropical regions and is one of the most

important food legumes in the world because of its commercial value, extensive production, consumer use and nutrient value. Common bean is a source of carbohydrate, protein, minerals and vitamins; it is believed to be the only constant source of protein for some resource limited small holder farmers (Liebenberg and Pretorius, 1997). In several African countries, the dry bean has a consumption rate of as high as 50 kg per person per year (Voyses, 2000; CTA, 2010). The high nutritive value and the high intake rate observed make the common bean an important crop in many of the developing countries of Africa, Asia and Latin America (Mahuku, 2009). In Tanzania, common bean is the main grain legume crop. It is often intercropped with maize and the main areas of production are the mid to high altitude areas of the country. The most suitable areas for bean production in Tanzania are the northern zone particularly Arusha, Manyara, Kilimanjaro and Tanga regions; the lake regions and in the western zones in Kagera, and Kigoma and in the southern highlands in Mbeya, Rukwa and Iringa regions. Worldwide, Tanzania is among the top ten largest producers of dry beans and the largest producer in sub-Saharan Africa (FAOSTAT, 2010).

Despite being of economic value, common bean is constrained by several biotic and abiotic factors, most important of which are diseases, insects, low soil fertility, periodic water stress and poor crop management (Hillocks et al., 2006; Mwang'ombe et al., 2007). In Tanzania, average bean yield is around 500 kg/ha while the potential yields under reliable rainfall conditions and use of improved varieties and proper land husbandry is 1500 to 3000 kg/ha (FAO, 2007). Considering bean yields of 0.5 t/ha in developing countries as opposed to 1 to 2 t/ha commonly reported in experimental sites and even 4 t/ha reported in the USA, bean yields in developing countries are among the lowest worldwide (Mahuku, 2009).

Large portion of the losses is due to diseases caused by bacteria, fungi and viruses. However, among the important diseases that affect beans in Tanzania are angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* and Bean common mosaic virus disease (BCMV)/bean common mosaic necrosis virus (BCMNV) (Hillocks et al., 2006; Tryphone et al., 2012). Therefore, breeding for diseases resistance is of considerable importance in common bean breeding. To achieve a rapid progress in such activities, incorporating biotechnological tools in order to increase efficiency and effectiveness is mandatory (Mahuku, 2009). Developing bean cultivars with higher yields and with multiple disease resistance will enable farmers to increase bean productivity and achieve greater yield stability. Selection for ALS and BCMNV resistance in beans and the incorporating resistance genes in one or more adapted genotypes will offer a durable solution to the problem of

these two diseases and it is a way through more research to incorporate more resistances to other diseases and also offer germplasm for ALS and BCMNV resistance.

The work aimed at incorporating resistance genes to angular leaf spot and bean common mosaic and necrosis virus diseases into one adapted common bean genotype using molecular marker assisted selection technique and to determine the inheritance pattern of the diseases.

## MATERIALS AND METHODS

### Genotypes

The genotypes used in this study included two non recurrent genotypes that are resistant to angular leaf spot (Mexico 54) and to BCMV/BCMNV [UBR (95) 25] and one adapted recurrent variety (Kablankei) which is susceptible to both diseases and to which resistance is to be incorporated. Some of the important features and characteristics of these genotypes are described in Table 1.

### Generation of breeding lines

Planting was done in the screen house in pots filled with sterilized forest soil and maintained in the screen house. Marker validation was followed where all the genotypes used were screened by SCAR markers to be used in selection for the presence of the desired character on all the non recurrent parents and the absence of it on the recurrent parent. The breeding lines were developed through hybridization, where modified double cross procedure was performed. It involved the first cross where the ALS disease resistance gene was incorporated into the adapted cultivar Kablankei, (Kablankei × Mexico 54) and the cross to incorporate the BCMNV disease resistance to Kablankei [Kablankei × UBR(95) 25]. The resulting F<sub>1</sub> hybrids from these two crosses were then inter-crossed to one another to combine the genes from the two previous F<sub>1</sub> crosses. The resulting hybrids from the double cross were then characterized using markers for the two diseases. Backcrosses, F<sub>1</sub> and F<sub>2</sub> from each of the single crosses for ALS and BCMV/BCMNV diseases were developed also for the inheritance studies on ALS and BCMNV diseases. The crossing procedures used was both the emasculation method and the hook method as described by Bliss (1980) and Buishand (1956).

### Inoculum collection and preparation

The inocula that was used for both diseases was collected from different places; Morogoro town, Mgeta in Morogoro, Lushoto in Tanga, and from Uyole, Mbeya. Sample collection sheets were used where all important information was recorded. They were collected from naturally infected fields with typical symptoms of the respective disease. The inoculum for *P. griseola* was stored in paper envelopes and then labeled with the description of bean variety and the location where the inoculum was collected. For BCMV/BCMNV, leaf samples were stored in plastic clip bags in an ice container and likewise labeled with description of the inoculums. For ALS, infested part of the leaf indicating the presence of fungal spores were isolated and plated into V8 agar. For inoculum preparation, well established fungal colonies were sub-cultured onto fresh medium. The plates containing isolates of fungal spores

**Table 1.** Bean cultivars used and their characteristics.

Cultivar	Collected from	Seed size	Seed color	Flower color	Description
Kablanketi	SUA-Morogoro	Medium	Cream	White	Susceptible
Mexico 54	SUA-Morogoro	Medium	Pink	Purple	Resistant to ALS
UBR(95)25	CIAT-Uganda	Small	White	White	Resistant to BCMNV/BCMV

were incubated at 24°C for 10 to 15 days to allow for sporulation (Aparicio, 1998). Following sufficient sporulation, sterile distilled water containing 0.05% (v/v) Tween 20 was added to the plates and the medium surface scraped smoothly using a sterile toothbrush and the spore suspension was filtered using sterile gauze and the final spore concentration adjusted to  $2 \times 10^4$  spores/ml using the hemocytometer, ready for inoculation.

For BCMNV, the collected leaf samples were ground in cold 0.01 M phosphate buffer solution containing 0.1% Tween 20 at a pH 7 in a ratio of 1 g young BCMNV infected leaves in 5 ml of cold 0.01 M phosphate buffer. The supernatant was sieved to eliminate plant debris and the resulting solution was used for inoculation.

### Inoculation

For ALS, a hand sprayer was used where a mist of inoculum was sprayed onto both surfaces of the plant leaves with at least two trifoliolate leaves (15 to 18 days old). The plants were maintained in the mist chamber with high relative humidity for 72 h, then the plants were removed from the chamber and maintained in the screened house and observations were performed daily after one week for disease reaction. In the case of BCMNV, the primary leaves were dusted with carborundum powder and the inoculum was gently rubbed on the entire surface of the leaves and observation for disease reaction followed starting one week after inoculation.

### DNA extraction, PCR and gel electrophoresis

The  $F_1$ ,  $F_2$  and backcrosses were planted in pots in the screen house and leaf samples for DNA extraction were collected from young leaves of seedlings at the second trifoliolate stage. The DNA was extracted using the Whatman FTA card technology where the leaf sample was placed over the marked area of the FTA Plant Saver card and the leaf was overlaid with parafilm. Using a pestle, the leaf was pound making sure that the leaf material was transferred to the paper by checking the back of the FTA card. The samples were left to air dry. Using the Harris 2 mm unicore punch, the samples from the FTA cards were cut with the assistance of the cutting mat and placed into the PCR tube and then washed twice using both 200 ml of FTA purification reagent followed by 200 ml of 1X TE buffer (10 mM Tris HCl, 0.1 mM EDTA, pH 8.0) leaving the sample incubated at room temperature for about 5 min in each wash, then the leaf disks were left to dry ready for PCR reaction.

### DNA amplification

The PCR reaction was prepared by adding 1  $\mu$ l of each forward and reverse primers, 18  $\mu$ l of PCR water in the PCR premix which contained 1 U of Taq polymerase, 250  $\mu$ M of dNTPs, 10 mM Tris-HCl (pH 9.0), 30 mM KCl, 1.5 mM MgCl<sub>2</sub> and stabilized and tracking dye. To 20  $\mu$ l of the reaction mixture, the 2 mm disc of the washed

FTA card was added ready for PCR using BIO RAD "MyCycler" thermal cycler machine. The PCR conditions were set to correspond to each primer requirements in terms of number of cycles and the temperatures as per Miklas (2009). Samples for ALS were amplified using the primer SN 02 and for BCMNV; ROC 11 and for BCMV; SW 13, the PCR conditions for these primers were as per Miklas (2009).

### Gel electrophoresis and documentation

A 1.2% agarose gel was prepared by mixing 1XTBE (Tris-Borate EDTA) buffer, 10  $\mu$ l of the PCR products were loaded into the gel for electrophoretic separation of DNA fragments in a solution of 1xTBE buffer with electric potential maintained at 100 V for 1 h run time. The gel was then stained with 0.5  $\mu$ g/ml ethidium bromide (EtBr) for 30 min, destained in distilled water for 20 min and then visualized using UV trans-illuminator. For documentation, a mounted digital camera was used to capture the amplified fragments from the gel on the UV trans-illuminator.

### Plant evaluation for diseases

For disease reaction, the  $F_1$ ,  $F_2$  and backcross ( $BC_{1-1}F_1$  and  $BC_{1-2}F_1$ ) generations were evaluated phenotypically in the screen house and genotyping for disease resistance was done in the laboratory. For ALS disease, the data were taken as scores on a 0 to 9 CIAT scale, whereby plants with disease score of 1 to 3 are considered to be resistant, 4 to 6; intermediate resistant and 6 to 9; susceptible (CIAT, 1987). For the BCMNV, the disease rating was based on the presence and absence of disease symptoms (necrosis). The score of 1 was for the presence of the disease and the score of 0 was for the absence of the disease symptoms. In genotyping, the evaluation was based on the presence of the gene or absence of the gene as revealed by banding pattern in the gel and this was done by using molecular markers to predict the presence or absence of the gene. A score of 1 is for the presence and a score of 0 is for the absence of the named gene.

### Data collection

Data were collected from the screen house as disease score, observation were made to the first appearance of the disease symptoms and scoring was performed starting from the third week at one week interval for a total of three weeks consecutively. The molecular data was taken in the lab as scores from the gel. A score of 1 was for the presence of gene and a score of 0 was for the absence as revealed by the banding pattern in the agarose gel.

### Data analysis

The disease score data were subjected to the Gen Stat statistical

**Table 2.** Frequencies of phenotypic and genotypic/marker classes for ALS resistance.

Parent/Cross/Marker	Generation	Number of plant		Expected ratio	$\chi^2$	P
		Resistant	Susceptible			
Kablanketi (P <sub>1</sub> )	P <sub>1</sub>	0	20			
Mexico 54(P <sub>2</sub> )	P <sub>2</sub>	20	0			
P <sub>1</sub> × P <sub>2</sub>	F <sub>1</sub>	39	1			
P <sub>1</sub> × P <sub>2</sub>	F <sub>2</sub>	58	20	3:1	0.017	0.896
P <sub>1</sub> × F <sub>1</sub>	BC <sub>1-1</sub> F <sub>1</sub>	11	9	1:1	0.2	0.655
P <sub>2</sub> × F <sub>1</sub>	BC <sub>1-2</sub> F <sub>1</sub>	18	2	1:0	-	-
SN02 ( <i>Phg-2</i> )	F <sub>2</sub>	27	10	3:1	0.081	0.776

P<sub>1</sub> = Parent susceptible (Kablanketi); P<sub>2</sub> = Parent resistant (Mexico 54); BC<sub>1-1</sub>F<sub>1</sub> = Backcross to susceptible parent; BC<sub>1-2</sub>F<sub>1</sub> = Backcross to resistant parent.

package for analysis. The *chi*-square test was used to test the phenotypic segregation of the populations from the crosses between Kablanketi × Mexico 54 and Kablanketi × UBR (25) 95 in the inheritance study. Molecular data were also subjected to the *chi*-square test to assess the gene segregation pattern of the populations derived from the two crosses. A correlation study was done to compare the molecular marker data with the phenotypic expression of the diseases. The heritability was also studied from the variances from the distribution of the score data for ALS disease.

## RESULTS AND DISCUSSION

### Phenotypic and molecular marker evaluation for ALS disease

The disease symptom development started during the third week after inoculation. The symptoms were observed to be skewed on different ends among the parents while in Mexico 54, the score were in the resistant side (1 to 3) and in Kablanketi, on the susceptible side (4 to 9). In the F<sub>1</sub> populations, the score were also in the resistant side with few susceptible plants as it was expected and in the F<sub>2</sub> populations, the scores were distributed along the scale but it skewed to the left side showing more of the resistant plants than the susceptible ones. In the backcrosses, the distribution was almost normal in BC<sub>1-1</sub>F<sub>1</sub> showing equal distribution of plants among the susceptible and resistant ones and in BC<sub>1-2</sub>F<sub>1</sub>, the scores concentrated to the resistant sides. The frequency of phenotypic classes in the disease score for resistance and susceptibility reaction to the pathogen in F<sub>1</sub>, F<sub>2</sub> and backcross populations obtained from the cross between Kablanketi with Mexico 54 and the segregation classes as per molecular marker SN 02 for *Phg-2* gene for ALS resistance are shown in Table 2. Segregation ratios were 3:1 (resistant: susceptible) for the F<sub>2</sub> populations in both the phenotypic evaluation and genotypic evaluation, 1:1 for the BC<sub>1-1</sub>F<sub>1</sub> and 1:0 (all resistant) for the BC<sub>1-2</sub>F<sub>1</sub>. These results show that the

observed ratios and the calculated ratios are not significantly different at the 0.05 level of significance; where P < 0.896 for F<sub>2</sub> and P < 0.655 for the backcross in phenotypic screening and P < 0.776 for F<sub>2</sub> in molecular marker screening. These ratios and the results are in agreement with the previous work on ALS using Mexico 54 as the donor parent that suggested that the gene for ALS follows the single gene inheritance pattern (Namayanja et al., 2006; Chataika et al., 2010; Sartorato et al., 2000).

The study by Tryphone et al. (2012) working on ALS using Mexico 54 as resistant parent, confirmed the single gene inheritance. However, previous studies showed that the resistance to different *P. griseola* pathotypes may be controlled by one, two or even three dominant or recessive genes (Carvalho et al., 1998). More recently, studies have also demonstrated that the resistance of the cultivars AND 277, Cornell 49242, MAR 1, G 10474 and MAR 2 to some pathotypes were each conditioned by a single dominant gene (Nietsche et al., 2000; Ferreira et al., 2000; Mahuku et al., 2002).

Jara (2003) reported that, in addition to major genes, there are some minor genes that are also found to condition resistance of common bean to *P. griseola*. These findings support the results of this work, which show that the background in our case Kablanketi also determine the inheritance pattern of resistance to the disease (Sartorato et al., 1999; Mahuku et al., 2002). Namayanja et al. (2006) reported that the resistance is conferred by single dominant gene regardless of using different backgrounds. The results were obtained using marker SN 02 for the gene *Phg-2* for ALS resistance.

Table 2 shows that among 37 DNA samples from individual F<sub>2</sub> plants, the ratio of 27:10 for resistant (R) and susceptible (S) was observed. The Chi square value from these findings,  $\chi^2 = 0.081$  shows that there is no significant difference between the observed and expected ratios at probability of 0.776 (that is, P < 0.776) as shown in

**Table 3.** Phenotypic and genotypic screening for BCMNV resistance.

Parent/Cross/Marker	Generation	Number of plant		Expected ratio	$\chi^2$	P
		Resistant	Susceptible			
Kablanketi (P <sub>1</sub> )	P <sub>1</sub>	0	20			
UBR(95)25 (P <sub>2</sub> )	P <sub>2</sub>	20	0			
P <sub>1</sub> × P <sub>2</sub>	F <sub>1</sub>	1	29			
P <sub>1</sub> × P <sub>2</sub>	F <sub>2</sub>	13	27	1:3	1.2	0.273
P <sub>1</sub> × F <sub>1</sub>	BC <sub>1-1</sub> F <sub>1</sub>	3	17	0:1	-	-
P <sub>2</sub> × F <sub>1</sub>	BC <sub>1-2</sub> F <sub>1</sub>	12	8	1:1	0.8	0.371
ROC 11 ( <i>bc-3</i> )	F <sub>2</sub>	12	23	1:3	1.609	0.205

P<sub>1</sub> = Parent susceptible (Kablanketi); P<sub>2</sub> = Parent resistant [UBR(95)25]; BC<sub>1-1</sub>F<sub>1</sub> = Backcross to susceptible parent; BC<sub>1-2</sub>F<sub>1</sub> = Backcross to resistant parent. For ROC 11, Resistant: rr = (*bc-3*, *bc-3*); Susceptible: R- = (*Bc-3*, *bc-3*), (*Bc-3*, *Bc-3*).

in Table 2. This analysis revealed a segregation ratio of 3:1 for SN 02 as a dominant marker associated to Phg-2 gene linked to ALS resistance.

Other previous studies using molecular markers reported the same observations that resistance to specific isolates of *P. griseola* is simply inherited (Mahuku et al., 2004; Ferreira et al., 2000; Carvahlo et al., 1998; Larsen et al., 2005). It was also found in the genotype AND 277 that its resistance to ALS is being conferred by single dominant gene *phg-1* (Ragagnin, 2005). When the genes are simply inherited then one can easily pyramid genes for resistance to ALS thus overcoming the issue of high degree of pathogenic variability as reported by Sartorato (2002).

### Heritability of ALS

The estimated heritability using the F<sub>2</sub> and backcross generations for the cross Kablanketi × Mexico 54 was found to be 0.772. This indicates that almost 77% of the trait from the parents has been transferred to the offspring and the contribution of genes is higher than the environmental effects. These results agree with what is reported by Amaro et al. (2007) that the resistance to angular leaf spot normally has a relatively high heritability values and plants can be phenotypically selected for recombination in the F<sub>2</sub> generation.

Some other studies revealed that the heritability for reaction to angular leaf spot was varied from 44.44 to 58.86% and it was suggested that in the case of low heritability, marker assisted selection is more advantageous (Melo et al., 2002 cited by Teixeira et al., 2005). It has also been reported that there is a complex interaction between genotype and environment which also gives the basis in deciding on the adoption of indirect selection based on molecular markers (Teixeira et al., 2005).

### Phenotypic and molecular marker evaluation for BCMNV

The summary statistics of the ratios between the susceptible and resistant plants as per phenotypic and molecular marker screening are as indicated in Table 3. These results (Table 3) suggests that the gene responsible for the disease resistance is recessive and the inheritance pattern is that of a single gene since the observed ratios do not differ significantly from the expected ratios based on Mendel's theory for a single gene inheritance. The probability of P > 0.05 (P = 0.273) in F<sub>2</sub> generation shows that the ratio of 13 resistant to 27 susceptible genotypes does not differ significantly from the expected ratio of 1:3. Likewise, the backcrosses showed ratios of 1:1 and 0:1 for resistant to susceptible in BC<sub>1-2</sub>F<sub>1</sub> and BC<sub>1-1</sub>F<sub>1</sub>, respectively at P > 0.05 in the former (that is, P = 0.371). Previous studies by Mukeshimana et al. (2005) also confirmed the results from the phenotypic screening using the F<sub>2</sub> and F<sub>2:3</sub> populations that also support the presence of the single recessive gene by showing a good fit to phenotypic segregation ratios of 1:3 for F<sub>2</sub> and genotypic segregation ratio of 1:2:1 using markers for F<sub>2:3</sub> generation. 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. It has been observed (Table 3) that the segregation ratios scored for the presence of the *bc-3* gene are not significantly different from those of the inheritance of a single recessive gene; the calculated chi-square was 1.609 and the probability was 0.205 (that is,  $\chi^2 = 1.609$ ; P > 0.05).

The chi square statistics confirms that there is no significant difference between the ratios observed and those for the inheritance of a single recessive gene. Previous studies using other types of markers also showed that the gene *bc-3* is a recessive gene; it was identified

that the RAPD marker OG6<sub>595</sub> was linked from the *bc-3* gene at 3.7 cM and the marker was used to confirm the location of *bc-3* gene on the linkage group B6. Another codominant AFLP marker EACAMCGG-169/172 was identified and linked at 3.5 cM from the *bc-3* gene. The absence of a band from the gel verifies that there is a recessive gene *bc-3* and the presence of the band shows that there is a dominant gene counterpart to the recessive *bc-3* gene (Mukeshimana et al., 2005).

### Correlation between molecular and phenotypic screening data

The data from phenotyping screening was compared with the data from the molecular marker screening to assess the reliability of MAS as a tool in breeding for disease resistance. The correlation coefficient between phenotypic and molecular screening for ALS was found to be 0.741. This high and positive correlation coefficient indicates that one has high chance of success in using molecular markers for screening for disease resistance. The probability of this occurring by chance is  $P < 0.001$  which indicates high correlation thus in early generations when number of segregating plants is high; one can rely on molecular marker data to equally determine the phenotype. For the BCMNV data, the same observation was made though the correlation coefficient was lower ( $r = 0.624$ ) with the probability of chance being less than 0.001 ( $r = 0.624$ ;  $P < 0.001$ ). This also shows that one can rely on molecular markers for selection of BCMNV resistant plants in the field. This correlation is also supported by the chi square test that both phenotypic and genotypic data presented single gene inheritance. This has also been observed in previous studies using marker assisted selection where both ratios from the molecular marker analysis and that from the field screening were not significantly different from those of Mendelian inheritance (Mukeshimana et al., 2005; Namayanja et al., 2006). It has been reported that the recovery of superior individuals based solely on phenotyping is insufficient because some traits are of low heritability or it is difficult to create favourable conditions for selection as the case for disease resistance. In contrast to this, the molecular markers are highly heritable and unaffected by the environment.

Due to this, then, there is a need to incorporate the molecular marker techniques in selection process in breeding programs in order to hasten the breeding work especially when the trait that is to be selected is of low heritability or its conditions for selection can hardly be set as the case for disease resistance. Apart from all that, there is a need to prove that there is high and positive correlation between the molecular marker and the trait to be selected, then, one can be confident and sure of using MAS in the following generations of selection. It has also

been cautioned that one should never assume that MAS is necessarily superior to phenotypic selection which for some trait can be as effective and efficient as the use of molecular markers (Blair et al., 2007). Apart from these correlations being useful in selection, also since there is no one to one correlation, then, the possibilities that there exist some variability among the isolates used that reacts differently to the genotype irrespective of the presence of the genes for resistance to the disease in question.

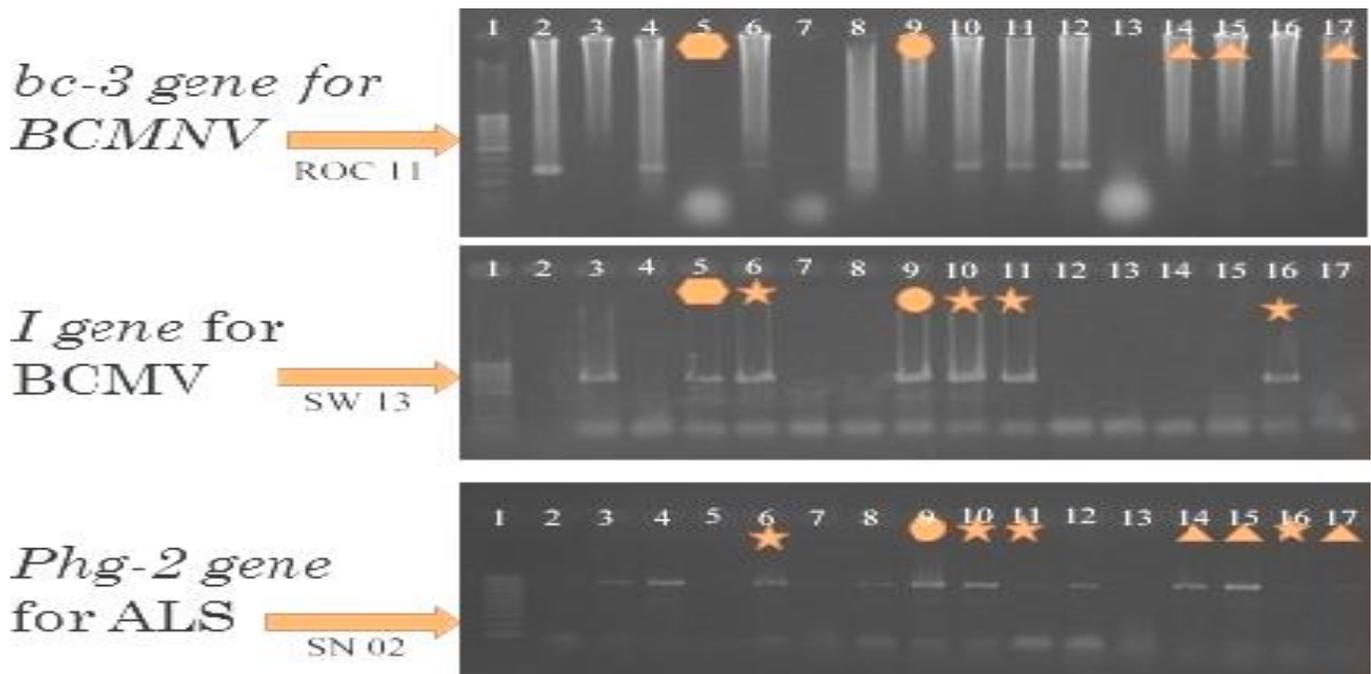
This necessitates the study of the pathogen variability in order to come up with an integrated management that will consider pathogen variability as part of the breeding strategy for disease resistance.

### Selection of plants with combined resistance to ALS, BCMV and BCMNV

The aim of selection was to find plants with combined markers or multiple genes for resistance for the three diseases, ALS, BCMV and BCMNV. Selection was done among the segregating  $F_1$  populations of the double cross plants using molecular markers. Having found that the genes for ALS and BCMV are dominant and have simple inheritance pattern and that of BCMNV being recessive and also having simple inheritance pattern, then, there is a probability of selecting plants with: a) all the three desirable genes, b) two of the genes, c) single desirable gene for each disease, and d) none of the genes (Figure 1). During the selection process, the SW 13 marker was also used to screen for *I* gene that confers resistance to BCMV. Among the plants screened, some showed the presence of markers or resistance to ALS, BCMV and BCMNV (lane 9); some to ALS and BCMNV (lanes 14, 15 and 17); some to ALS and BCMV (lanes 6, 10, 11 and 16); some to ALS (4, 8 and 12); some to BCMNV (lanes 7 and 13) and some to BCMV and BCMNV (lane 5) (Figure 1).

### Conclusion and recommendation

This work has substantiated the use of MAS in crop improvement and validated that it is possible to use these improved technologies and developed tools in developing countries in order to increase the pace of our programs and improve our food security by improving productivity and sustainability of our cropping systems. From the inheritance studies, it was found that, inheritance for these diseases is simple and stable utilizing single dominant gene for ALS and single recessive gene for BCMNV. These results provides strong basis for their use in improvement of common bean in Tanzania. The correlation between the phenotypic and genotypic data showed strong correlation which makes the use of molecular marker more valid. Apart from that, it was



**Figure 1.** Sample gel for multiple gene selection BCMNV (*bc-3 gene*), ALS (*Phg-2 gene*) and BCMV (*I-gene*). Lane 1, 100 bp ladder; Lane 2, susceptible parent (Kablanketi); Lane 3, resistant parent [Mexico 54 and UBR (25)95] and Lane 4 to 17 are the segregating F<sub>1</sub> double crosses.

found that there was no one to one relationship (that is,  $r < 1$ ) suggesting that MAS should not be used alone rather being integrated with phenotypic screening in the field/strong classical breeding at some points to validate the continuing molecular marker use, this will aim at breeding for more adapted common bean lines. Due to this, more studies should be done to check with the pathogen variability and validate the stability of these markers with time as the pathogen as well as the genotypes changes; this will promote improvement of the existing tools to suit the growing conditions, present genotypes and possibly new pathogen races.

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