

Full Length Research Paper

Introgression of common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) resistance to common bean (*Phaseolus vulgaris* L.) adapted to Tanzania facilitated by marker assisted selection

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Received 22 May, 2012; Accepted 09 October, 2012

Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv *phaseoli* (*Xap*) is an important disease of common bean in Tanzania causing severe damage. This study was carried out to introgress resistance to CBB to the adapted common bean in Tanzania with the facilitation of molecular markers along with determining the inheritance and heritability of the disease. Crosses were made between the adapted parent Kablanketi and the resistant parent Vax 4 and their F₁, F₂ and the backcrosses to both parents generated. The phenotypic evaluation was carried out after inoculation with *Xap* and the molecular marker was applied on the F₂ generations using the SCAR marker SAP 6 linked to a QTL for CBB resistance. The result shows no significant deviation from the expected 3:1 ($\chi^2 = 0.47$; P>0.05) in the F₂ population and 1:1 for the backcross to the susceptible parent. These results that resistance in Vax 4 to *Xap* is conditioned by the presence of dominant genes. The moderate heritability of 0.32 was estimated implying that resistance is conditioned by one major gene which has effects of partial resistance. There were significant correlation between the phenotypic reaction and molecular marker screening (resistant QTL) ($r = 0.502$; p<0.05). This indicates there are greater chances of selecting resistant individuals using molecular markers which also exhibited resistance under field conditions.

Key words: Common bacterial blight, marker assisted selection, *Xanthomonas axonopodis* pv *phaseoli*, Tanzania.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important grain legume crop in Tanzania as a source of protein and other mineral contents for the rural and urban households (Wortmann et al., 1998; Hillocks et al., 2006; Tryphone and Nchimbi-Msolla, 2010). The regular intake of common beans has medicinal benefits which contribute to lower risks to cancer, diabetes, and heart diseases (Singh, 2000; Hangen and Bennink, 2003). It is a source of income where fresh pods and dry seeds

attract a higher market price (Wortmann et al., 1998; Broughton et al., 2003). Despite the importance of common beans in Tanzania and other developing countries, they are mostly produced by small scale farmers whose production is largely based on unimproved local cultivars that have been selected over many years in their localities (Chataika et al., 2011). Major seed classes grown in Tanzania include red mottled, large red kidney, small red, yellow, navy, purples, grey purplish mottled (Kablanketi type) and sugar bean. These bean classes are grown in different areas of the country depending on local preferences and market demand (Wortmann et al., 1998; Hillocks et al., 2006).

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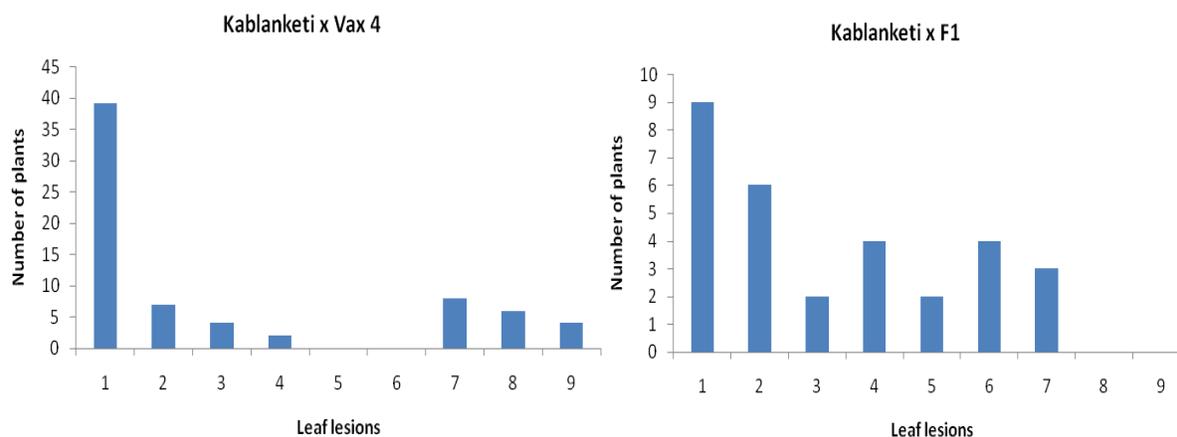


Figure 1. Distributions of F₂ (Kablanketi x Vax 4) and backcross to Kablanketi (Kablanketi x F₁) plants for the reaction to *Xap*.

The Tanzanian common bean industry is affected by many diseases that limit crop production in Tanzania. Among the diseases is the common bacterial blight (CBB) caused by *X. axonopodis* pv *phaseoli* (*Xap*) (Wortmann et al., 1998; Mkandawire et al., 2004; Hillocks et al., 2006). The disease is endemic in almost all bean growing areas especially in hot lowland and mid altitude areas (Mkandawire et al., 2004). It causes severe damage under warm temperatures, high rainfall and high humidity with maximum disease development at around 28°C (Wortmann et al., 1998). Infected seeds many fail to grow abort or shrivel and discoloured as they mature (Allen and Lenne, 1998). The losses due to CBB disease are estimated to be 20 - 75% (Opio et al., 1996; Mahuku et al., 2003; Lema-Marquez et al., 2007; Mutlu et al., 2008). It was estimated that each 1% increase in blight severity causes yield loss of about 10.5 - 78 kg ha⁻¹, depending on the season and crop growth stage (Allen and Lenne, 1998). The extent of yield loss and quality is determined by weather conditions, susceptibility of the cultivars in use and disease pressure (Allen and Lenne, 1998; Lema-Marquez et al., 2007).

The control of this disease is challenging due to its complexity and seed borne nature. Breeding for CBB resistance is complicated by pathogen genetic diversity (Mkandawire et al., 2004). There are different genes conditioning resistance separately in leaves, pods and seeds (Arnaud- Santana et al., 1994; Miklas et al., 1996; Zapata, 1996; Yu et al., 2004; Mkandawire et al., 2004; Crous et al., 2006) and linkage of resistance with undesirable traits (Yu et al., 2004; Crous et al., 2006). Resistance to CBB is quantitatively and qualitatively controlled depending on the source of germplasm with pod and leaf resistance being controlled by different genes (Park et al., 1998; Singh and Muñoz, 1999). Breeding for resistance to CBB has become successful and reliable for the disease control (Opio et al., 1996;

Chataika et al., 2011). The number of genes involved in resistance to *Xap* range from one to several genes with varying degrees of expression and interactions (Beebe and Pastor-Corrales, 1991; Zapata et al., 2010). Therefore, molecular marker linked to genes, can assist in speeding up selection and offer opportunities to incorporate both quantitative and qualitative resistance in the common bean. The SCAR markers available include SU91, R7313 and R4865 which are linked to a QTL on linkage group B8, SAP 6 and BAC 6 linked to group B10 and BC420 linked to QTL on linkage group B6 (Miklas et al., 2000; Yu et al., 2004).

Sources of resistance to *Xap* which can be used to introgression of resistant QTL have been developed and identified (Zapata et al., 2004; Mutlu et al., 2008). However, an effective breeding for resistance to CBB requires an understanding of the mode of inheritance and resistance gene expression in the developed materials (Namayanja et al., 2006; Chataika et al., 2011) with both characters being subjected to the background used for introgression. Therefore, the objective of this study was to introgress resistance to CBB in the locally adapted cultivar Kablanketi and determine its inheritance.

MATERIALS AND METHODS

Experimental plant material

The experimental plant material consisted of local adapted cultivar Kablanketi which is susceptible to CBB, the resistant line Vax 4 and their progenies F₁, F₂ and backcrosses to both parents. The progeny derived from backcrossing the F₁ to the female parent was designated BC1F1-P1 and those from the backcrossing to the male parent as BC1 F1-P2. Kablanketi was used as a female parent. It is medium seeded, gray in colour and semi

climber. It fetches high prices because of its colour, short cooking time and good palatability (Wortmann et al., 1998; Hillocks et al., 2006). Vax 4 line was used as male parent due to its high level of resistance to CBB and was obtained from CIAT – Uganda (Singh et al., 2001).

Planting, inoculum preparation, inoculation and disease resistance rating

One bean seed was planted in each pot in a screen house. The progenies evaluated were 60 F₁, 70 F₂, 40 BCF₁-P₁ and 53 BCF₁-P₂. Bacterial blight differential media was prepared following the procedures described by Mortensen (2005). The storage culture of *Xap* was revived by growing it on Y east Dextrose Carbonate Agar (YDCA) media plates at 28°C for 48 h. Cell suspensions were made using distilled water and concentration was adjusted to 10⁶cfu ml⁻¹ using haemocytometer. Plants were inoculated at the age of 17 days from sowing when they had 2 to 3 sets of fully expanded trifoliolate leaves by spraying the inoculums on the leaves. The inoculated plants were covered with plastic sheets for 72 h to create humidity. After 72 h, the plastic sheets were removed and plants put on tables in screenhouse and monitored daily till the appearance of first symptoms. Starting from seven days after inoculation disease severity was scored weekly for a total of four ratings, using the CIAT 1–9 visual scale (van Schoonhoven and Pastor-Corrales, 1987). Plants that had a score of 1 - 3 were Considered resistant, 4 - 6 intermediate and scores greater than 6 were considered susceptible.

Leaf sample preparation and deoxyribonucleic acid (DNA) extraction

Total genomic DNA was extracted from young trifoliolate leaves collected from two week old F₂ plants grown in the screen house. The leaves were plucked from the plants and put in plastic bags labeled with the right identification number. The bags were then put on ice and transferred to the laboratory for DNA extraction using the FTA card technology. The leaves were crushed on the FTA plant saver card and the DNA binds to the matrix of the card. The chemical coating on the FTA card can inactivate pathogens, protect the DNA from degradation and allows the cards to be stored at room temperature for extended period of time. To prepare sample for polymerase chain reaction (PCR), a 2 mm disc of the matrix was punched using a Harris Unicore and put in the 0.2 ml PCR tube. Then discs were washed with FTA purification reagent and Iso-propanol. The washed leaf disc in the PCR tubes were left to dry at room temperature for 5 min.

The DNA remains bound to the matrix throughout purification process, thus the matrix provides enough templates for PCR analysis.

Deoxyribonucleic acid (DNA) amplification

The quantitative trait loci (QTL) of common bacterial blight sequenced characterized amplified region (SCAR) marker SAP 6 was used. The PCR premix consisted of 1 U of Taq polymerase, 250 µM of dNTPs, 10 mM Tris-HCl (pH 9.0), 30 mM KCl, 1.5 mM MgCl₂, stabilized and tracking dye and 0.5 µM of each primer (forward and reverse) to make a 20 µl reaction volume. The 20 µl PCR reaction mix was subjected to 34 amplification cycles using a BIO RAD “MyCycler” thermal cycler machine consisting of 1 cycle 94 °C for 2 min, and 34 cycles including the steps of denaturation at 94 °C for 20s, annealing at 55.5 °C for 30 s, and extension at 72 °C for 2 min. These cycles were followed by a final extension at 72 °C for 5 min and a holding temperature of 4 °C.

Electrophoresis and gel documentation

Amplification products were separated through electrophoresis migration in a 1.5% agarose gel with 6.0 µL DNA ladder in 0.5X TBE buffer under a voltage of 100 V for 80 min. The gel was stained in ethidium bromide with concentration of 0.5µg ml⁻¹ for 30 min, de-stained for 20 min by using distilled water. The stained gel were lighted with ultraviolet light, the bands present on the gel were observed and photographed for documentation and scoring according to specific base pair of SAP 6 – 820bp by comparing with a reference molecular weight marker (100bp DNA ladder).

Data collection and analysis

Disease severity scores were recorded for the populations used and molecular marker bands (present and absence of band) were recorded in the F₂ plants. Chi-squared tests were used for inheritance studies to determine the goodness of fit and narrow sense heritability for the reaction to *Xap* was calculated using the components of variance method (Fehr, 1987). The disease severity scores of F₂ and their associated marker scores were correlated. The data were processed using the 14th Edition GenStat

RESULTS AND DISCUSSION

Inheritance of common bacterial blight (CBB) resistance

Results obtained with F₁ show that 54 plants were

Table 1. Analysis of segregation ratios for resistance to susceptible in parental genotypes Kablanketi, Vax 4 and their crosses to artificial inoculation with *Xap*.

Parent/cross	Generation	Number of plants		Expected ratio	χ^2
		R	S		
Kablanketi	P1	0	70		
Vax 4	P2	80	0		
Kablanketi x Vax 4	F ₁	54	6		
Kablanketi x Vax 4	F ₂	50	20	3:1	0.47
Kablanketi x F ₁	BC ₁ F ₁ -P ₁	17	13	1:1	0.53
Vax 4 x F ₁	BC ₁ F ₁ -P ₂	44	9	1:0	

resistant while 6 of them were susceptible suggesting that resistance is rather dominant.

The hypothesis that only one dominant gene is segregating was confirmed by the F₂ and BC populations show the single dominant gene inheritance with F₂ segregation of 3:1 and the backcross segregation of 1:1 (Figure 1 and Table 1). The plants of Kablanketi were susceptible and all the plants of Vax 4 were resistant as expected. The backcrosses to susceptible parent, showed the segregation of 1:1 and F₂ segregation of 3:1 resistant to susceptible. However, the backcross to Vax 4 had few susceptible individuals. The F₂ progenies showed segregation patterns ranging from complete resistance to susceptibility (Table 1). The phenotypic segregation of F₂ progenies for the reaction to *Xap* largely segregated in the ratio of 3:1 ($\chi^2 = 0.47$; $P > 0.05$) suggesting the presence of dominant genes controlling resistance *Xap* in Vax 4. This result is in conformity with the results of other authors. For example, Muimui et al. (2011), indicated that resistance to *Xap* is controlled by dominant genes in Vax 4. The Vax 4 has been reported to have good level of resistance to common bacterial blight (Singh and Muñoz, 1999). The results by Miklas et al. (2006) and Chataika et al. (2011) showed that the resistance to CBB is quantitatively inherited with major gene effect. Resistance to CBB, quantitative patterns of inheritance, differential leaf and pod reaction has been reported (Jung et al., 1996). The complex inheritance to *Xap* makes the transfer of quantitatively inherited disease resistance genes into elite cultivars difficult (Jung et al., 1996). The nature of inheritance greatly depends on the genotype used as the susceptible parent among other factors (Pastor-Corrales et al., 1998). For example, it has been established that inheritance and gene action to *Xap* is influenced by plant architecture which includes growth habit influencing disease severity (Beebe and Pastor-Corrales, 1991). In addition, Silva et al. (1989) reported that inheritance of resistance to common bacterial blight in trifoliolate leaf and plant canopy was controlled by a single major gene. Therefore, resistance to common bacterial blight is different depending on the source of resistance and may be determined by both major and

minor genes (Singh, 1991). Since Kablanketi is semi determine it could have effect on the inheritance and gene action of *Xap*.

Heritability to common bacterial blight resistance

The estimated heritability in narrow sense for common bacterial blight resistance trait was 0.32. The estimated heritability is classified as moderate according to Falconer and Mackay (1996). The low to moderate heritability has been reported for leaf reaction to *Xap* in dry bean by other authors (Arnaud-Santana et al., 1994; Ariyaratne et al., 1999). Breeding for resistance using quantitative genes involves shifting the population mean towards resistance (Bonos, 2006). Breeding programs relying on additive genetic variation for successful population improvement towards more resistant phenotypes could be advantageous. The moderate heritability implies that resistance is conditioned by few major genes with mean effects of partial dominance (Singh, 1991; Falconer and Mackay, 1996; Fourie et al., 2011). It was found that additive gene action was significant for leaf reaction with heritability in narrow sense ranging from 0.18 to 0.87 (Silva et al., 1989), 0.30-0.60 (Ariyaratne et al., 1999), 0.52-0.60 (Arnaud-Santana et al., 1994) and from 0.09 to 0.93 (Singh, 1991). Low heritability to CBB in leaf and pod reactions means the gene is inherited quantitatively (Arnaud-Santana et al., 1994). Usually heritability values depend on different aspects such as the population in consideration, environmental conditions and experimental design, precision of data collection and genetic complexity of the trait under study. Therefore, differences in heritability results for the same trait are quite common (Jung et al., 1996). Selection efficiency for the resistance to *Xap* may be increased using molecular markers such as SCAR markers in early generations (Ariyaratne et al., 1999). However, Ferreira et al. (2004) reported high heritability of 80% for F₆ and 88.3% for F₇ population. This demonstrates that evaluations being carried out with advanced materials contributed to its increase, enabling a

more accurate selection of superior genotypes. This calls for concerted efforts to explore more on the behavior of the pathogen. It also, demonstrates potential of existence and possibility to discover promising genotypes within the advanced populations based on their genetic variability. Singh and Munoz (1999) reported that low to moderate heritability was accompanied by the complex nature of resistance and the environmental effect on symptom development which makes screening for CBB resistance difficult.

Phenotypic against marker scores

Result shows that there was significant correlation between CBB phenotypic reaction and resistant QTL (SAP 6 marker) score ($r = 0.502$; $p < 0.05$). Positive and significant correlation between phenotypic data and molecular marker indicates the greater chance of selecting individuals by molecular markers and still attain resistance with artificial inoculation and/or with field screening. The breeding strategies that combine markers and phenotypic selection has been the most effective in developing lines with improved resistance to CBB (Miklas et al., 2006). Therefore, in case of many segregating plants, use of markers can be appropriate to select individuals with target allele. Studies by Mukeshimana et al. (2005) and Namayanja et al. (2006) established that both molecular marker score and field screening were not significantly from Mendelian inheritance. Depending on phenotypic data alone is not sufficient to some traits especially the one with low heritability like CBB. The breeding for CBB resistance requires markers that with high level of correlation and/or linkage should exist between CBB resistance and molecular markers (Yu et al., 2004). This marker however, must be stable, reproducible and easy to assay like SAP 6 (Miklas, 2006). However, the use of MAS will not completely eliminate the need for direct phenotypic selection for CBB resistance, MAS can be used to reduce the number of lines that require direct screening. Since the inheritance of CBB is affected by environmental factors and has low heritability, molecular markers can be used to select the promising genotypes within the existing segregating population at early stage with fixed resistant gene. This will play significant contribution to improving the selection and save resources which could have been used in advancing the generation to gain gene fixation.

Conclusion

The genetic resistance to common bacterial blight in the common bean was investigated for the disease reactions. The transfer of resistance to Xap from Vax 4 to Kabla nketi has been successful. The results suggest that the

control of resistance to Xap in Vax 4 is being conditioned by presence of dominant genes although moderate heritability indicates that resistance is conditioned by few major genes with mean effects of partial dominance. There is greater possibility of selection of individuals using molecular markers and still attains resistance in the field conditions due to association of phenotypic reaction and molecular marker scores. The use of MAS then in this instance is very efficient as it is helpful in selection of individuals in the early generations and as the heritability is not that much to be able to do phenotypic selection during the early segregating population. Apart from this, the use of MAS should not be final in selection of resistant genotypes rather there should be associated field screening to make sure that the selected materials express the trait phenotypically.

Furthermore, the use of co-dominant markers where possible could facilitate the selection for such a trait as it is quantitatively controlled.

ACKNOWLEDGEMENT

This paper is part of a PhD research thesis to be submitted at Sokoine University of Agriculture, Morogoro. Financial support for this work was provided by Kirkhouse Trust.

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