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Screening of local and improved bean varieties for resistance to halo blight disease

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The study was conducted to screen local and improved bean varieties for resistance to halo blight disease caused by *Pseudomonas syringae* pv. *phaseolicola*. A total of eight improved (Sokoine University of Agriculture (SUA) 90, Rojo, Zawadi, Mshindi) and local (Mwasipenjele, Masusu, Kabhaya, Mabula) bean varieties were collected from breeders at SUA and farmers at Ileje district in Songwe region. Isolation of *P. syringae* pv *phaseolicola* from bean seeds was conducted using Liquid Assay method. Results indicated that, bacterial isolate L1 from local bean variety Mwikala produced similar characteristics as those obtained from reference strain of *P. syringae* pv *phaseolicola* in the biochemical and pathogenicity tests on host plants. Using bacterial isolate L1, there was significance difference ($P < 0.05$) on incidence and severity of halo blight disease. The highest disease incidence had (89%) was on local bean variety Mabula, while the lowest had (67%) was on improved varieties Zawadi and Mshindi. Disease severity of improved varieties zawadi and mshindi when compared with other varieties were at low severity (disease score 4). It was concluded that the Zawadi and Mshindi were less susceptible to halo blight disease. This study needs to be repeated if same results were obtained aside these two improved varieties (Zawadi and Mshindi) of bean which could be recommended to farmers.

Key words: Assay, incidence, inoculum, isolate, *Pseudomonas syringae* pv. *phaseolicola*, disease severity.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important leguminous crop and native to America (Gepts and Dpbouk, 1991). It is an important source of minerals, protein content (~22%) and vitamins for many developing countries where they cannot afford meat (Moraghan and Grafton, 2001; Hillocks et al., 2006; Graham et al., 2007;). Common bean complements cereals and other carbohydrate rich foods in providing near perfect nutrition to people of all ages and helps to lower cholesterol and cancer risks (Singh, 1999). The major producers of common bean worldwide are Brazil, Mexico, United State, Ethiopia, Turkey, Indonesia, Tanzania, Uganda and Angola (Beebe et al., 2013). In Africa, bean production is concentrated in densely populated Eastern and Southern Highlands of the continent (Beebe et al., 2000). In Tanzania, bean is grown in cool regions, particularly the Southern Highlands (Iringa, Songwe, Mbeya, Rukwa, Katavi, Njombe and Ruvuma regions), Northern Highlands (Arusha, Tanga, Kilimanjaro and Manyara), Western Highlands (Kagera and Kigoma)

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and in Central Morogoro especially in areas surrounding Uluguru Mountains (Mabagala et al., 2000). Over 75% of rural households in Tanzania depend on common beans for daily sustenance (CIAT, 2008). Consumption of common bean is high because it is relatively inexpensive as compared to meat (Pachico, 1989; Fivawo and Msolla, 2011). However, its production is constrained by a number of factors including diseases especially halo blight caused by *Pseudomonas syringae* pv *phaseolicola* (Tock et al., 2017). Halo blight is a bacterial disease caused by *P. syringae pv phaseolicola*. It is a serious seed-borne disease of common bean worldwide (Popovi et al., 2012; Boersma et al., 2014; Chatterton et al., 2016). Halo blight disease of beans cause low yield of about 37% in many places of the world especially in the developing countries (Taylor et al., 1996a, b; Gondwe, 1998; Chataika et al., 2011; Duncan et al., 2014). Halo blight is difficult to control. Pathogen free seeds can be used to control the spreading of the disease however; it does not guarantee disease control as other inoculum sources exist (Fourie, 2011). The disease can be controlled by using new improved resistant varieties (Tock et al., 2017). Cultural practices such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimizing movement within fields when foliage is wet, may reduce the disease (Mbega et al., 2012). However, these cultural practices do not eliminate the disease especially when environmental conditions are favourable for disease development. Chemical control is considered effective but most chemicals fail to treat disease due to resistant strains and improper application by farmers which may cause environmental pollution (Mbega et al., 2012). Host plant resistance is considered the most effective control strategy for halo blight (Fourie, 2011). Therefore, this study aimed to evaluate improved and local bean varieties for resistance to halo blight under greenhouse condition. The results can be used for breeding purposes against this disease in Morogoro, Tanzania.

MATERIALS AND METHODS

Location of the study area

The research was conducted at the AfSHC Laboratory at SUA in 2016/2017. The university is located at 3 km from the Centre of Morogoro Municipality, which is about 200 km west of Dar es Salaam. It has an altitude of 526 m and lays at latitude 6°19'S and longitude 37°40'E.

Survey and seed collection

The survey was conducted in Morogoro and Songwe regions. Improved bean seed sample varieties (Mshindi, Zawadi, SUA 90 and Rojo) were obtained from plant breeders at SUA in Morogoro region. Four local bean sample varieties (Masusu, Mwasipenjele, Kabhaya and Mwasipenjele) were collected from randomly selected farmers at Ileje-Songwe. Seed samples (1 kg) were packed in paper bags and labelled appropriately: collection date, date of harvest, name of collector, variety and location. The samples were transported to the AfSHC Laboratory for further analysis.

Working seed samples

The working seed samples were obtained by the following International Seed Testing Association rules (ISTA, 2005). The conical divider was cleaned with compressed air before seeds were divided. The seed sample was poured over the hopper then valve was released and locked. In this procedure, the sample was divided into two halves and was collected in the two collecting pans. The seeds were removed from one of the pans into a container and poured back into the original seed bag and the seeds from the other pan were poured again into the hopper and the procedure was repeated until a working sample (700 g) was obtained as described by Mathur and Kongsdal (2003).

Determination of purity, moisture content and seed germination

Purity analysis

The purity of seed samples was determined using ISTA rules (ISTA, 2005). Each sub sample was analysed into the following categories: pure seed, inert matter and seed of the other crops and the results were expressed in percentages.

Determination of moisture content (MC)

The lower constant temperature oven method was used to determine the moisture content of bean seeds (Anonymous, 1999). In this method, 10 g of seed was randomly picked from the original submitted sample and were evenly distributed over the surface of 8.5 cm diameter container (Petri dish). The container and its cover were weighed before and after filling it with the seeds. The containers were placed in an oven set at 103±2°C and dried for 17 h. The moisture content determination was carried out using two independently drawn working samples. After drying the seed in the oven, the container was covered and cooled for 30 to 40 min. The moisture content percentage was calculated using the formula: $MC= (M_2-M_3) \times 100/M_2-M_3$; where M_1 was weight in grams of the container with its cover; M_2 was weight in grams of the container, cover and the content before drying. M_3 was weight in grams of the container, cover and the content after drying.

Isolation of bacteria from bean seed samples

Liquid assay was used to detect *P. syringae* pv. *phaseolicola* from bean samples. About 300 g of bean were tested using procedures described by Mortensen (2005). Seeds were washed three times in sterile distilled water put in transparent plastic bags containing 620 ml of sterile distilled water based on the formula (1.9 × weight of the seeds) + 50 described by Mortensen (2005). The samples were incubated at room temperature for 3 h. The 1 ml of the suspension was drawn from the sample and serially diluted to 10^{-3}. The samples were plated on Nutrient Agar (NA) plates at 28°C for 24 to 48 h. The colonies with morphology similar to *P. syringae* pv. *phaseolicola* reference strains were purified on NA and further tested as described under biological test.

Biochemical tests

The isolates from bean samples were tested for Gram reaction

using Potassium Hydroxide solution as described by Mortensen (2005). Only Gram-negative isolates that had colony morphology and colour similar to the reference bacterial strain of *P. syringae* pv. *phaseolicola* were selected for further tests as described under pathogenicity test.

Pathogenicity tests

Gram-negative isolates as previously described were tested for their pathogenicity on host bean variety Kabhaya. Pathogenicity test was conducted as described by Mortensen (2005). Bean seedlings grown in plastic pots (16 cm diameter × 13 cm height) containing forest soil at 2 leaf stage were spray-inoculated with a bacterial suspension of ca.ca. 10^8 cfu/ml from bacterial isolates and reference bacteria strain (*P. syringae* pv. *phaseolicola*). Plants sprayed with sterile distilled water alone served as a negative control. Isolates which produced water-soaked symptoms as those produced on plants sprayed with a *P. syringae* pv. *phaseolicola* were selected for further tests.

Screening bean varieties for resistance to halo blight

Seed inoculation with bacteria

Eighteen seeds from each bean variety previously described were pre-germinated using between paper method (ISTA, 2005) and after three days, the germinating seedlings were inoculated by stabbing the cotyledons with a needle smeared with bacteria suspension of ca. 10^8 cfu/mL isolated from variety Mwikala collected from Ileje, Songwe. In addition, seeds inoculated with a reference bacterial strain *P. syringae* pv. *phaseolicola* (obtained from the AfSHC) and sterile distilled water were used as controls (Mortensen, 2005). The seedlings were further evaluated as described under greenhouse experiment.

Greenhouse experiment

Complete randomized design (CRD) with three replications was used in the greenhouse to test eight bean varieties for their resistance to Halo blight disease. Three seedlings previously inoculated with the bacteria and controls (in three replications) were planted in sterile forest soil in a screen house at 25 to 28°C with relative humidity of 70 to 80% for 56 days. Data were recorded in one week interval for six weeks.

Determination of disease incidence

The disease incidence was calculated by dividing total number of infected plants by total number bean plants by 100%.

Disease severity

Halo blight disease was evaluated by visual observation using a 1 to 9 scale (Schoonhoven and Pastor-Corrales, 1987), where 1= no disease symptoms, >1- 3= slightly disease symptoms, >3-6= moderate disease symptoms and >6- 9= severe symptoms to death of plants.

Data analysis

The data was analysed at the significance level of 5% using GEN START-software. The significance difference between bean varieties was computed using Analysis of Variance (ANOVA) and the difference between means was established using Duncan's Multiple Range Test (Kothari, 2004).

RESULTS

Purity of bean seed samples used in this study

The results for purity analysis indicated that bean seed sample number 3 variety Rojo collected from SUA had the highest (99.98%) proportion of pure seed followed by sample number 2 (99.97%), 1 (99.93%), 6 (99.90%), 4 (99.89%), 7 (99.67%), and 5 (99.16%) (Table 1). Bean seed sample number 8 variety Mabula had the lowest (96.37%) proportion of pure seed.

Moisture content of bean seed samples used in this study

The results showed that the moisture content determined using lower constant temperature oven method of bean seed samples ranged from 10 to 14.5% (Table 1). Bean variety Kabhaya collected from Ileje had the highest moisture content (14.5%). The lowest moisture content (10%) was obtained from bean variety Mwasipenjele collected from Ileje, Songwe (Table 1).

Seed germination

The results of germination test conducted using blotter paper method indicated that all seed samples had 100% of germination (Table 1). Such results demonstrated that, bean seed samples whether local or improved used in this study had a good germination.

Bacterial isolates obtained from bean seed samples

Using liquid assays to isolate bacteria from bean seed samples, a total of three isolates (Table 2) with similar morphological characteristics like the reference *P. syringae* pv. *phaseolicola* were obtained from variety Mwikala. No bacterial isolates resembling the reference strain (*P. syringae* pv. *phaseolicola*) was obtained from other bean seed samples used in this study.

Gram reaction of the isolates

The gram reaction test using 3% potassium hydroxide solution showed that, all three isolates resembled the colon colour and morphology of reference bacterial strain of *P. syringae* pv. *phaseolicola* were Gram-negative (Table 2). The results of pathogenicity on host bean plants as described by Mortensen (2005) indicated that

Table 1. Purity, moisture content and germination of bean seeds used in this study.

Sample No.	Variety	SG	Location	PS	IS	OSC	MC (%)	GM (%)
1	SUA 90	Improved	SUA	0.01	0.01	0	12.5	100
2	Mshindi	Improved	SUA	99.97	0.01	0	13	100
3	Rojo	Improved	SUA	99.98	0.03	0	14	100
4	Zawadi	Improved	SUA	99.89	0.01	0.03	11.5	100
5	Mwikala	Local	Ileje	99.16	0.4	0	12.4	100
6	Kabhaya	Local	Ileje	99.9	0.9	0	14.5	100
7	Mwasipenjele	Local	Ileje	99.67	0.03	0	10	100
8	Mabula	Local	Ileje	96.37	3.34	0	12	100

SG: Seed grade, PS: pure seed, IS: inert seed, OSC: other seed, MC: moisture content, GM: germination.

Table 2. Gram reaction and pathogenicity of bacteria isolates obtained from variety Mwikala.

Isolate	Bean variety	Gram reaction	Pathogenicity tests of isolates on variety Kabhaya
L1	Mwikala	−ve	+
L2	Mwikala	−ve	+
L3	Mwikala	−ve	+
*P.s.*pv. *phaseolicola*	AfSHC	−ve	+
Steriledistilled water	N/A	N/A	N/A

N/A: Not applicable, -ve Gram negative reaction, + reaction for pathogenicity test and - negative reaction for pathogenicity test.

bean seedlings sprayed with the Gram negative bacterial isolated from bean sample variety Mwikala caused water-soaked symptoms as those produced in seedlings sprayed with the reference bacteria strain (*P. syringae* pv. *phaseolicola*) (Table 2). Thus, since these isolates were obtained from one bean seed sample, only one isolate (isolate L1) was selected and used in the screening of bean varieties for their resistance to halo blight.

Halo blight disease incidence on bean cotyledons and leaves inoculated with bacteria

In the greenhouse experiment, the results showed that bean cotyledon inoculated with bacterial isolate L1 and the reference bacterial strain *P. syringae* pv. *phaseolicola* produced disease symptoms and had higher disease incidence than bean cotyledons inoculated with sterile distilled water (Table 3). Disease symptoms were not observed in bean cotyledons inoculated with sterile distilled water control. Using isolate L1, bean sample number 8 (variety Mabula) collected from Ileje had the highest (89%) disease incidence on cotyledon when compared with other bean samples (Table 3). Bean sample numbers 4 (variety Zawadi) and 7 (variety Mwasipenjele) had the lowest (67%) disease incidence (Table 3). The disease incidence of other samples inoculated with the bacteria cultures is shown in Table 3. These results indicated that these two bean varieties were less susceptible to halo blight disease than other bean varieties screened. Such results also demonstrated that bean cultivars Zawadi and Mwasipenjele were resistant to halo blight disease compared with other bean samples screened.

Disease severity on cotyledons and leaves of bean inoculated with bacterial isolates

Using a scale of 1 to 9 to determine the severity of halo blight on bean cotyledon and leaves, the results showed that, there was significance difference ($P<0.05$) among bean varieties assessed. Bean samples inoculated with isolate L1 and the reference strain *P. syringae* pv. *phaseolicola* had high disease severity of 4 up to 7 in different varieties (Table 4). All plants inoculated with sterile distilled water had a disease score of 1, indicating no disease (Table 4). Of the plant tested, bean sample variety Mabula together with bean varieties SUA 90, Rojo and Mwikala had the highest (disease score of 7) disease severity both in the test isolate L1 and reference strain *P. syringae* pv. *phaseolicola*. Bean varieties Zawadi and Mshindi had the lowest (disease score of 4) on cotyledons inoculated with either isolate L1 or reference strain *P. syringae* pv. *phaseolicola* (Table 4). These results demonstrated that, bean cultivars Zawadi and Mshindi were less susceptible to halo blight when

Table 3. Halo blight disease incidence on cotyledons and leaves of bean varieties.

Bean variety	Incidence of halo blight			Bean variety	Incidence of halo blight		
	Cotyledons (%)		SDW		Leaves (%)		SDW
	L1	P.s.pv.p			L1	P.s.pv.p	
Zawadi	67^{a}	65^{a}	1^{a}	Zawadi	1^{a}	0^{a}	1^{a}
Mshindi	67^{a}	73^{ab}	1^{a}	Mshindi	1^{a}	0^{a}	1^{a}
Mwasipenjele	67^{a}	76^{ab}	1^{a}	Mwasipenjele	1^{a}	0^{a}	1^{a}
Kabhaya	78^{ab}	74^{ab}	1^{a}	Kabhaya	1^{a}	0^{a}	1^{a}
Mwikala	78^{ab}	77^{ab}	1^{a}	Mwikala	1^{a}	0^{a}	1^{a}
Rojo	78^{ab}	78^{ab}	1^{a}	Rojo	1^{a}	3^{ab}	1^{a}
SUA 90	78^{ab}	83^{b}	1^{a}	Mabula	3^{ab}	12^{b}	1^{a}
Mabula	89^{b}	91^{b}	1^{a}	SUA 90	3^{ab}	13^{b}	1^{a}

L1: Bacterial isolated from bean variety Mwikala, *P.s*.pv.*p*: *Pseudomonas syringae* pv *phaseolicola*, SDW: sterile distilled water. Means followed by the same letters in a column are not significantly different.

Table 4. Halo blight disease (1-9 scale) severity of cotyledons and leaves of bean varieties.

Bean variety	Severity of halo blight on			Bean variety	Severity of halo blight		
	Cotyledons (%)		SDW		Leaves (%)		SDW
	L1	P.s.pv.p			L1	P.s.pv.p	
Zawadi	4^{a}	4^{a}	1^{a}	Kabhaya	1^{a}	1^{a}	1^{a}
Mshindi	4^{a}	4^{a}	1^{a}	Mshindi	1^{a}	1^{a}	1^{a}
Mwasipenjele	4^{a}	4^{a}	1^{a}	Mwasipenjele	1^{a}	1^{a}	1^{a}
Kabhaya	7^{ab}	7^{ab}	1^{a}	Zawadi	1^{a}	1^{a}	1^{a}
Mwikala	7^{ab}	7^{ab}	1^{a}	Mwikala	1^{a}	1^{a}	1^{a}
Rojo	7^{ab}	7^{ab}	1^{a}	Rojo	1^{a}	1^{a}	1^{a}
SUA 90	7^{ab}	7^{ab}	1^{a}	SUA 90	3^{ab}	3^{ab}	1^{a}
Mabula	7^{ab}	7^{ab}	1^{a}	Mabula	3^{ab}	3^{ab}	1^{a}

L1: Bacterial isolated from bean variety Mwikala, *P.s*.pv.*p*: *Pseudomonas syringae* pv *phaseolicola*, SDW: sterile distilled water. Means followed by the same letters in a column are not significantly different.

compared with other bean varieties used in this study (Table 4).

DISCUSSION

Based on the results of this study the low proportion of pure seed was attributed to by contamination by inert matter as indicated by the proportion of as high as 3.34% (Table 1). Presence of high proportion of inert matter in bean samples may be caused by poor threshing and winnowing which lower the quality of seed. Purity of bean seed samples show that these bean samples had purity that met minimum requirements (98%) recommended by Food and Agriculture Organization (FAO) (FAO, 2011). On the other hand, variation in moisture contents of the seeds observed is accelerated by improper drying of the seed and/or poor storage rooms (Nahar et al., 2009). The results indicated significance differences of disease incidence and severity among bean varieties tested which is in agreement with previous studies which report different races of halo blight affecting common bean (Félix-Gastélum et al., 2016; Tock et al., 2017). The resistance common bean varieties for race 6 are available in East Africa. Resistance varieties may be introgressed into susceptible varieties using marker assisted backcrossing for production of new resistance varieties (Chataika et al., 2011). The absence of disease severity on plants inoculated with sterile distilled water was due to unavailability of causal pathogen (*P. syringae* pv. *phaseolicola*). For effective screening for resistance to halo blight, *P. syringae* pv. *phaseolicola* isolates should be originated from one among varieties tested, this improve effectiveness of screening and correct selection of resistance varieties to halo blight (Murillo et al., 2010). Application of bio-control concentration of *P. syringae* pv. *phaseolicola* isolates show variation of susceptibility to common bean varieties, this is an important approach for resistance common bean to the disease (Eman and Afafa, 2014). High disease incidence and severity on cotyledons for varieties (SUA 90, Rojo, Kabhaya, Mabula) and leaves for varieties (SUA 90, Rojo

and Mabula) to halo blight could be influenced by poor genetic resistance of beans to halo blight races. Similar results were reported by Asensio et al. (2010) who conducted field evaluations under inoculated conditions and identified two accessions (BGE 002189 and BGE 029592) out of 199 from a Spanish core collection that had immune reaction to two races of *P syringae* pv. *phaseolicola* (races 6 and 7). Previous study by Küçük et al. (2016) reported the same trend of common bean susceptibility to halo blight disease. Nevertheless, field evaluation is recommended to confirm greenhouse results. Legume species have been infected by at least nine races of halo blight of which the races number 1, 2 and 6 are common worldwide (Taylor et al., 1996a, b), while races number 3, 4, 5 and 8 are common in the Eastern and Southern of Africa. Furthermore, races 5 and 8 are dominant in Africa (Teverson and Taylor, 1994). Up to 43% reductions in total yield have been reported and further losses occur owing to the poor quality and high incidence and severity of halo blight which infected pods, seeds and leaves (Dawn et al., 2010). In Tanzania, races 1 (45%), 2 (52%) and 3 (3%) have been reported, therefore, Tanzania is among the African countries which are affected by the disease; this correspond to our results of which most varieties were susceptible to the disease (Mabagala and Saettler, 1992). The varieties Zawadi and Mshindi which were resistant to halo blight probably could have genes cv or Quantitative Trait Loci (QTLs) associated with gene cv. QTLs have significance contribution to the expression of target trait under phenotypic evaluation. Therefore, QTL mapping for halo blight resistance in common bean is essential to identify the superior varieties (González et al., 2017). Red Mexican which is resistant to race 1 and PI 150414 for races 1 and 2 that have been useful in breeding for halo blight resistance (Hillocks et al., 2006). Lines with I-gene were also reported to be highly resistant to race 3 of halo blight in Burundi (Schmit, 1994). Our results indicated variation of bean varieties for susceptibility to halo blight where Zawadi and Mshindi had the lowest compared to other bean varieties. Previous study by Fivawo and Msolla (2011) has reported comparable results of which variation of susceptibility of halo blight disease on bean varieties accounted to exist. The variation of susceptibility can be influenced by several factors such as gene sequence, strength of leaves to resist and source of origin. By introducing new genes with resistance to a wide range of halo blight races infecting legumes will contribute to enhance high production of beans (Fourie, 2011). Based on our study, the varieties which showed high susceptibility to halo blight would be infected by the races which have been reported before. The genes sequence for resistance need be identified by sequencing technology for breeding improvement programmes (Fourie et al., 2004; Teverson, 1991; Taylor et al., 1996b). The varieties with less susceptible to halo blight therefore, is important in plant breeding and should be promoted to farmers for cultivation (Allen et al., 1998; Fourie, 2011).

Conclusion

It was concluded from this study that, bacterial isolate (LI) obtained from local bean variety Mwikala produced similar characteristics as those obtained with the reference strain of *P. syringae* pv. *phaseolicola* on the biochemical and pathogenicity tests on host plants. Such results indicated that his bean sample was infected by *P. syringae* pv. *phaseolicola*. By inoculating bacterial isolate L1 on different bean samples used in this study, the incidence of halo blight disease was the highest (89%) on the cotyledons of local bean variety Mabula collected from Ileje, Songwe followed by Mwikala (78%), SUA 90 (78%), Rojo (78%), Kabhaya (78%), Zawadi (67%) and Mshindi (67%). Disease symptoms were not observed in bean seedlings inoculated sterile distilled water. Such results indicated, the improved varieties Zawadi and Mshindi were less susceptible to halo blight disease as compared to other bean varieties used in this study. Further evaluation of disease severity indicated low severity (disease score of 4) on improved varieties Zawadi and Mshindi when compared with other bean varieties. Therefore, further evaluation was recommended (using large number of samples and in different environmental conditions) of bean varieties Zawadi and Mshindi so that if similar results will be obtained, then the two varieties can be promoted to farmers in Morogoro and Songwe regions as well as the source of breeding materials for improvement of bean in Tanzania.

CONFLICT OF INTERESTS

The author declared no conflict of interest in this paper.

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