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PATHOGENICITY AND VIRULENCE OF UGANDAN ISOLATES OF COMMON BACTERIAL BLIGHT DISEASE PATHOGEN (*Xanthomonas* spp.)

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ABSTRACT

Breeding for resistance is a major component in the integrated management of common bacterial disease of beans (*Phaseolus vulgaris* L.). Use of less virulent strains or strains with attenuated virulence may lead to selection of resistant genotypes with intermediate response, when exposed to more virulent strains of the pathogen. The objective of this study to identify and characterise Ugandan isolates of common bacterial blight disease-causing pathogens for virulence. Bacteria were isolated from leaf samples collected from districts of Kabale, Masaka, Bukomansimbi, Mubende, Mbale, Bulambuli and Apac, all in Uganda, during the first season of 2016. The bacteria were tested for pathogenicity, as well as virulence on both breeding and local varieties. The study identified three most virulent isolates, namely MBL020, KAB-3 and BUL-14, all belonging to *Xanthomonas citri* pv *fuscans*. These isolates are very similar to those previously identified from Uganda (NCPB 670 and NCCPB 1402) more than 50 years ago. The study further revealed that NAROBEAN1, NAROBEAN 2, NAROBEAN 4, VAX 3, VAX5 and NE 2- 14- 8 had better resistance compared to other tested genotypes.

Key Words: *Phaseolus vulgaris*, Uganda, virulent strains

RÉSUMÉ

La sélection pour la résistance est un élément majeur de la gestion intégrée des maladies bactériennes courantes des haricots (*Phaseolus vulgaris* L.). L'utilisation de souches moins virulentes ou de souches à virulence atténuée peut conduire à la sélection de génotypes résistants à réponse intermédiaire, lorsqu'ils sont exposés à des souches plus virulentes du pathogène. L'objectif de cette étude était d'identifier et de caractériser les isolats ougandais de pathogènes causant la maladie bactérienne commune pour la virulence. Des bactéries ont été isolées à partir d'échantillons de feuilles prélevés dans les districts de Kabale, Masaka, Bukomansimbi, Mubende, Mbale, Bulambuli et Apac, tous en Ouganda, au cours de la première saison de 2016. Les bactéries ont été testées pour leur pathogénicité, ainsi que leur virulence à la fois pour la reproduction et variétés locales. L'étude a

identifié les trois isolats les plus virulents, à savoir MBL020, KAB-3 et BUL-14, tous appartenant à *Xanthomonas citri* pv *fuscans*. Ces isolats sont très similaires à ceux précédemment identifiés en Ouganda (NCPB 670 et NCCPB 1402), il y a plus de 50 ans. L'étude a en outre révélé que NAROBAN1, NAROBAN 2, NAROBAN 4, VAX 3, VAX5 et NE 2- 14-8 avaient une meilleure résistance par rapport aux autres génotypes testés.

Mots Clés: *Phaseolus vulgaris*, Ouganda, souches virulentes

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a staple for millions of people around the world, and is one of the most traded commodity in eastern Africa (OEC, 2017, MFPED, 2018). However, the productivity of beans in the region, and Uganda in particular remains low due to devastating diseases, among which is common bacterial blight disease. Common bacterial blight (CBB) disease, caused by *Xanthomonas* spp., has been reported in Uganda for more than 60 years (Aritua *et al.*, 2015); reducing yield by more than 40% under conditions of high disease pressure (Opio *et al.*, 1993). CBB disease is mainly spread in eastern Africa through seed (Opio *et al.*, 1996; Karavina *et al.*, 2011).

Management of common bacterial blight is mainly through use of resistant varieties, in combination with other measures such as disease-free seed, field sanitation and crop rotation (Opio *et al.*, 1996; Karavina *et al.*, 2011). Host resistance is the most practical solution in the management of common bacterial blight disease. However, the development of disease-resistant varieties, with improved yields that are stable, is a challenge that is compounded by climate change and global trade that introduces new pathogen strains. The new bacterial strains resulting from introduction and/or pathogen evolution may compromise the existing resistance and breeding efforts. Bean grain trade may aid the movement and distribution of common bacterial blight pathogen strains to new areas, changing the diversity and disease pressure due to introduction of more virulent strains or pathotypes.

Opio *et al.* (1996) reported pathogenic variation in Ugandan populations of *Xanthomonas* spp. infecting beans. Variation in virulence has been observed in both *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans sub fuscans*. Use of less virulent strains or strains with attenuated virulence may lead to selection of resistant genotypes with intermediate response when exposed to more virulent strains (Duncan *et al.*, 2011). This has already been observed on USDK CBB-15 and USWK-CBB-17 when exposed to a more aggressive Xcp25 (Viteri *et al.*, 2014).

In Uganda, Alladassi *et al.* (2018) noted that some genotypes like ADP660 that had high resistance in the screen house, became moderately resistant when exposed to field conditions; which points to existence of more aggressive and virulent strains in Uganda's fields. The East African strains of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans sub fuscans* have been reported to be more virulent in some studies and less in others (Schuster *et al.*, 1973; Gilbertson *et al.*, 2008; Gilbertson, 2011) The purpose of this study was to identify more virulent Ugandan isolates of *Xanthomonas* that could be used in selection of bean genotypes with high level of resistance to CBB.

MATERIALS AND METHODS

Isolation of bacterial pathogens. Bacteria were isolated from leaf samples collected from districts of Kabale, Masaka, Bukomansimbi, Mubende, Mbale, Bulambuli and Apac in Uganda during a disease survey of farmers' fields, conducted in the first cropping season

of 2016. Isolation and identification of the pathogen were done at the National Biotechnology Laboratory at Kawanda, using established CIAT protocol (Guillermo *et al.*, n.d). The infected materials were rinsed under running tap water for 10 min, surfaced sterilised for 3 min in 3.5% sodium hypochlorite, and then rinsed twice in sterile water for 1 min each.

Leaf materials were macerated and incubated overnight at 5 °C in a 1 ml Saline solution, for 16 hours. Using an inoculation loop, streaking was done on Nutrient agar (NA). Plates were incubated at 28 °C for 48 hours. Following the 48 hours incubation period, yellow-pigmented colonies typical of *Xanthomonas* spp., were purified on YDC, agar by a series of single colony transfers. Pure colonies were then suspended in 80% glycerol for long term storage at -80 °C.

Identification of bacteria. The isolated bacteria were identified using a combination of phenotypic and Molecular methods. Phenotypically, bacterial colonies were identified on YCDA based on yellow-pigmentation and mucoid nature, which is typical of *Xanthomonas* spp. After 48 hr of growth, genomic DNA was extracted from yellow pigmented colonies using a quick fungal/bacterial kit (Zymo research, Irvine, California).

The colonies were further confirmed through PCR, using a set of two specific primers (Gyr-R1 5' cttgaagcgtgtacctg 3', Gyr-L1 5' taccctgctgtgacatt 3' targeting the gyraseB gene) and R1 5' gttgacgccgaactggta 3' and Fyu-L15' gacgtcaacgcaccctac 3' targeting Fyugene of *Xanthomonas*. The PCR reaction contained 0.5 ul each primer, 1ul of DNA and 22 µl water of to make the final volume of 25 µl. The cycling conditions were as follows: (95'1 min, 95'30 s, 60'1 min, 72'30 s, 72's for 35 cycles). The only isolates that tested positive for PCR were used in the pathogenicity and virulence tests.

Pathogenicity tests. The 22 bacterial isolates that were PCR positive for gyrase and fyu gene were functionally characterised for their pathogenicity and virulence on susceptible genotype Kanyebwa, and later on other bean genotypes that included VAX 1, VAX 2, VAX 3, VAX 4, VAX 5, VAX 6, MCM 2001, Montcalm and VTT923. Three bean plants were planted in 5-litre buckets containing sterilised soil in screen house at ambient temperatures for 18 days (Guillermo *et al.*, n.d).

Three days prior to inoculation of plants, the bacterial inocula was prepared by first re-activating frozen bacterial stocks on YCDA medium for 48 hours. Bacterial culture for each isolate was then prepared by scooping the bacterial cells from the plate using a spatula and suspending them in 15 ml of 0.5X PBS buffer. The bacterial concentration was then adjusted to a working concentration of ~0.05OD₆₀₀, after using the Nano drop to measure absorbances.

Inoculation was done using the razor blade method. The inoculations were mainly done between 5 and 7 pm, to avoid high temperatures that could kill the bacteria. Inoculated plants were scored for disease severity on day 14 after inoculation for foliar disease rating. The CIAT disease rating scale of 1-9 (1 to 3 indicate a resistant plant, 4 to 6 indicate a plant with an intermediate r, and 7 to 9 indicate a susceptible plant eaction (Guillermo *et al.*, n.d.).

Phylogenetic analysis. Bacterial strains of the three most virulent isolates, namely MBL020, BUL14 and KAB-3, were re-activated from freeze stocks by streaking onto YCDA plates and incubating at 28 °C. Individual colonies were then re-streaked twice to limit possible contamination. After 48 hr of growth, genomic DNA was extracted using quick fungal/bacterial kit (Zymo research, Irvine, California).

The DNA from the three isolates was amplified using 16s ribosomal RNA Primer. The PCR reaction contained 0.5 µl each primer, 1ul of DNA and 22 µl water of to make the final volume of 25 µl. Amplification of the DNA sequences was conducted in a thermocycler (appendof) for 95'1 min, 95'30 s, 60'1 min, 72'30 s, 72's for 35 cycles. To determine the phylogenetic relationships of the virulent isolates with other *Xanthomonas* spp., a total of 11 nucleotide sequences were used in the analysis (Table 1). Raw sequence data of newly isolated bacteria were trimmed, and assembled using MEGAX alignment explorer.

Additional 16s ribosomal sequences of *Xanthomonas* were mined from online databases (NCBI). Sequences were aligned using CLUSTALW, implemented in MEGAX (Tamura *et al.*, 2018). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in units of the number of base substitutions per site. A Phylogenetic tree was constructed using UPGMA.

Data analysis. Virulence of the pathogen isolates was analysed by computing average disease scores of each pathogen isolate on each genotype tested. Based on the disease

scores, the isolates with high disease scores were categorised as more virulent; while those with low disease scores were considered to be less virulent. The number of genotyped infected by each genotype was used as a measure of pathogenicity of the isolate.

Isolates with high disease scores infecting more genotypes were considered more pathogenic. The relationship between disease severity in the field and screen house was done graphically by plotting both disease scores of each isolate on the same graph.

Evaluation for CBB resistance. One of the most virulent and adapted isolates (MBL020) was used to evaluate 34 genotypes comprising of breeding germplasm (9) and local varieties (25), along side resistant checks (2) and susceptible (2) checks. The plants and inoculum were prepared as already described under the pathogenicity test section. Inoculation was done using the razor blade method for foliar; while the needle method was used for pods (Alladasi *et al.*, 2018).

Inoculated plants were scored for disease severity on day 10 and 14 after inoculation for pod and foliar disease rating, respectively. The CIAT disease rating scale of 1-9 (Guillermo *et al.*, n.d.). Virulence of MBL020 was further

TABLE 1. Bacterial isolates used in phylogenetic analysis

Bacterial ID	Taxonomic ID	Host	Year of isolation	Country
KAB-3	UNKNOWN	<i>P. vulgaris</i>	2016	Uganda
MBL020	UNKNOWN	<i>P. vulgaris</i>	2016	Uganda
BUL014	UNKNOWN	<i>P. vulgaris</i>	2016	Uganda
LMG 832 (NCPBP 670)	<i>X. citripv. fuscans</i>	<i>P. vulgaris</i>	1958	Uganda
LMG7479 (NCPBP 1402)	<i>X. citripv. fuscans</i>	<i>P. vulgaris</i>	1962	Uganda
LMG8012 (NCPBP1680)	<i>X. axonopodis</i>	<i>P. vulgaris</i>	1964	Tanzania
KNUC393	<i>X. axonopodis</i>	Paper rhizosphere	2006	Korea
LMG 538	<i>X. axonopodis</i>			
LMG 826	<i>X. fuscans</i>	<i>P. vulgaris</i>	2014	Belgium:
R-22578	<i>X. axonopodispoinsetticola</i>	<i>Poinsettia</i>		China
XAP-1	<i>X. axonopodispoinsetticola</i>	<i>Poinsettia</i>		Taiwan

ID = Identification

tested on genotypes containing QTL markers SU91 + SU91 (VAX 3, VAX 5, RWR 2154, ADP660) and SAP6 (Montcalm, VAX 1, VAX 2, Montcalm and MCM 2001).

Data analysis. Virulence of MBL020 isolate on breeding germplasm and local cultivars was analysed by comparing mean disease ratings on each genotype, and also using box plot analysis (Thirumalai and Manickam, 2017). Foliar disease rating was compared with pod disease rating through a correlation analysis. Based on both foliar and pod disease ratings, genotypes were classified as susceptible (6-9), moderately resistant (4-5) and resistant (1-3.) (Duncan *et al.*, 2011; Castellanos *et al.*, n.d.)

RESULTS

Pathogenicity and virulence of isolates. The study identified 22 bacterial isolates as *Xanthomonas* spp. based on phenotypic and PCR categorisation (Table 2). The isolated bacteria comprised of fuscous type 54.5 and 45.5% phaseoli. The bacteria were isolated from fields varying in incidence and severity, in the different agro-ecologies (Table 2). The bacteria were isolated from different varieties which included; Black (s), Kanye bwa, Nambale (s), Mugagawale, NABE 12C, K20, white (s) and unknown varieties. The highest foliar disease severities in the field were recorded on varieties Mugagawale, 7.6, White small (NABE 6) 7.4 and Nambale small (NABE 16).

The study revealed that 95% of the isolates confirmed through PCR, were pathogenic on the universally susceptible Kanye bwa. At least each agro-ecology had an isolate with disease score (disease score >7). Isolates BUL14, BUL 15 and KAB-3 induced the highest disease scores when compared with other isolates (Table 2). The virulence of the isolates ranged from low to high, with disease scores ranging from 3-9 on the universally susceptible genotype, Kanye bwa.

Two isolates, MBL020 and KAB-3, were universally pathogenic infecting 9/9 of the tested genotypes, most of which considered resistant to CBB. Isolate MSK005 was also highly pathogenic, infecting 7/9 tested genotypes. Most isolates (87.0%) of the isolates tested were only pathogenic on two genotypes (Kanye bwa and VTT923), of the 9 tested genotypes (Table 3). Isolates from Virulence of isolates in screen house were not correlated with virulence in the field where the pathogens were isolated (Fig. 1). Screen house disease severity scores were slightly higher (5.8) than field disease severity scores.

Phylogeny of most virulent isolates. The isolates analyses were grouped into two clusters; one containing mainly isolates from Uganda, and the other cluster contained isolates from other countries. The study revealed that the isolates MBL020, KAB-3 and BUL14 clustered together with isolates NCPPB 1402 and NCPPB 670. However, MBL020, KAB-3 and BUL14 were more similar when compared to 1402 and NCPPB 670 (Fig. 2). Surprisingly, isolate LMG 826 *Xanthomonas fuscans* from Belgium, clustered closer to *Xanthomonas axonopodis* pv. *Poinsettiicola* isolated from *Poinsettia* than to the Ugandan cluster of fuscans isolates from *Phaseolus vulgaris*.

Reactions to MBL020 isolate of *Xanthomonas citri* pv. *fuscans*. The isolate was less aggressive on germplasm compared to local varieties (Fig. 3). Intermediate resistance with foliar disease rating varied from 4-6.5 on leaves and 4.8-6 on pods at 14 days after inoculation (DAI) (Fig. 3). The disease rating for local varieties was higher than that of breeding materials, ranging from 3-9 and 3-8 for leaves and pods, respectively (Fig. 3).

The isolate was less aggressive on genotype NE 2-14-8, which had the lowest disease rating on foliage of all the tested breeding material (Table 4). This isolate was also less

TABLE 2. Identity, pathogenicity and virulence of bacterial isolated in this study

Agro-ecology	Isolate	Bean variety	Isolate identity	Field incidence	Field severity	Screen house severity	No. of infected genotypes
SWGf	BMS -TC	Unknown	Fuscan	15	4.5	4.5	O2
NMF	APA10-1	Black (s)	Phaseoli	40	5.9	7.0	O2
	APA10-2	Black (s)	Fuscan	40	5.9	4.0	O2
	APA005	Unknown	Fuscans	65	6.3	4.0	O2
	AP004	White (s)	Fuscans	80	7.4	4.0	O2
CV&MF	MSK002	Kanyebwa	Phaseoli	30	5.6	4.5	O2
	MSK005	Kanyebwa	Phaseoli	10	3.4	6.0	O5
	MSK009	Nambale (s)	Phaseoli	60	7.0	4.0	O2
SW	KBL005	Unknown	Phaseoli	1	2.7	6.0	O2
	KAB-3	Nambale (s)	Fuscans	30	6.1	8.3	O6
EF	MBL020	Mugagawale	Fuscans	30	4.5	7.0	O9
	BUL009	Unknown	Fuscans	15	6.8	4.0	O2
	MBL013	Kanyebwa	Phaseoli	15	4.5	4.3	O2
	MBL005	Mugagawale	Fuscans	3	5.6	7.0	O2
	BUL008	NABE 12C	Phaseoli	20	5.0	4.0	O2
	BUL014	NABE 12C	Fuscans	5	3.4	9.0	O2
	BUL15-1	NABE 7	Fuscans	1	2.3	8.0	O2
	MBL006	Mugagawale	Fuscans	100	7.6	9.0	O2
	MBL024	Mugagawale	Fuscans	2	2.2	3.0	O2
CWS	MBE004	K20	Phaseoli	60	5.4	5.0	O3
	MBE003	White (s)	Phaseoli	10	5.0	7.0	O2
	MBE002-1	Nambale (s)	Phaseoli	50	4.5	7.0	O2

TABLE 3. Reaction of Ugandan isolates of CBB on selected bean genotypes

Isolate ID	Agro-ecology	CBB foliar rating (1-9)									
		VAX1	VAX2	VAX3	VAX4	VAX5	VAX6	Montcalm	MCM2001	VTT923	*Kanyebwa
BMS -TC	SWGF	1	1	1	1	1	1	4	1	5	4.5
APA010	NMF	1	1	1	1	1	1	1	1	4	4.0
MSK002	CV&MF	1	1	1	1	1	1	1	1	3	4.5
MBL020	EF	7	7	4	5.6	5.0	4.7	6	7	6	7.0
MBE004	CWS	1	1	1	1	1	3.0	1	1	5	5.0
MSK005	CV&MF	4	5	1	1	4	5.0	5	5	6	6.0
MSK009	CV&MF	1	1	1	1	1	1	1	1	4	4.0
KBL005	EF	1	1	1	1	1	1	1	1	4	6.0
BUL009	EF	1	1	1	1	1	1	1	1	4	4.0
APA005	EF	1	1	1	1	1	1	1	1	4	4.0
MBE003	CWS	1	1	1	1	1	1	1	1	4	7.0
MBL013	EF	1	1	1	1	1	1	1	1	4	4.3
MBL005	EF	1	1	1	1	1	1	1	1	4	7.0
BUL008	EF	1	1	1	1	1	1	1	1	4	4.0
XAP1680	TZ	1	1	1	1	1	1	1	1	3	8.0
BUL014	EF	1	1	1	1	1	1	1	1	3	9.0
BUL15-1	EF	1	1	1	1	1	1	1	1	4	8.0
APA10-1	NMF	1	1	1	1	1	1	1	1	4	7.0
MBL006	EF	1	1	1	1	1	1	1	1	4	9.0
MBE002-1	CWS	1	1	1	1	1	1	1	1	4	7.0
APA004	NMF	1	1	1	1	1	1	1	1	4	4.0
MBL024	EF	1	1	1	1	1	1	1	1	4	3.0
KAB-3	SW	4.8	4.1	3.2	4.5	3.1	-	-	6.2	-	8.3

Pathogenicity and virulence of Ugandan isolates

*The isolates were tested on two separate occasions on genotype Kanyebwa before being tested on other genotype

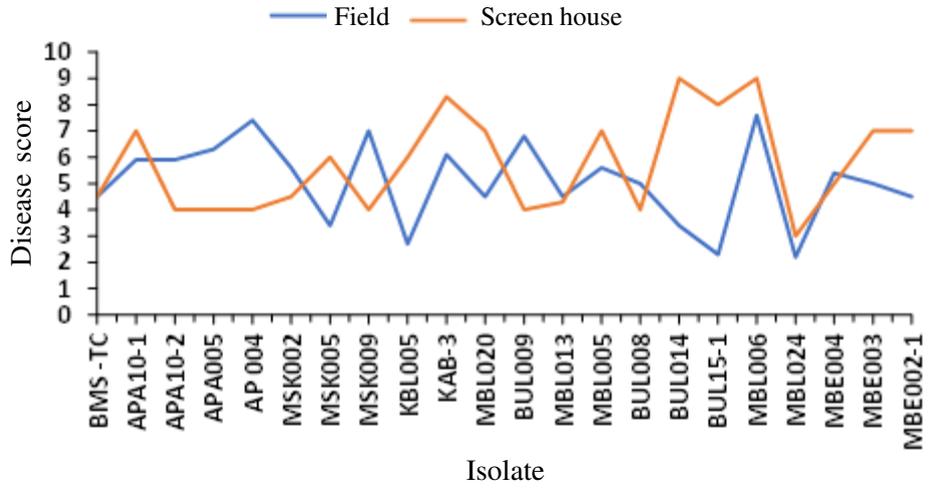


Figure 1. Relationship between field disease severity and screen house severity scores of isolates.

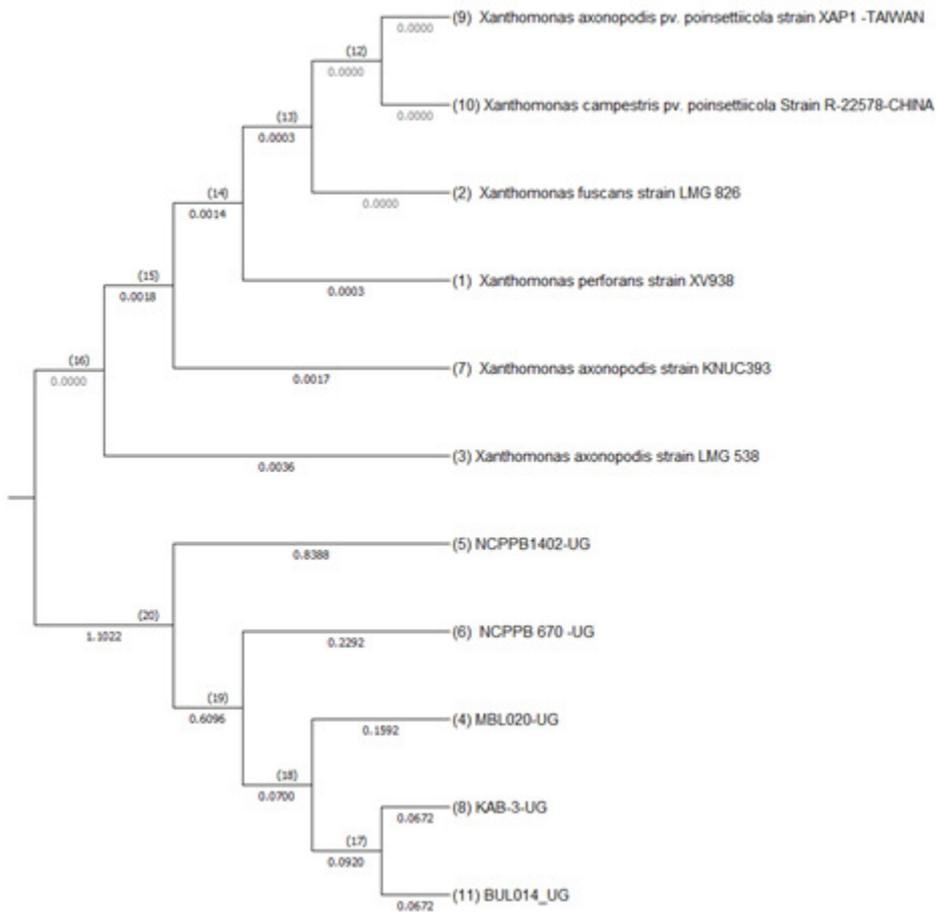


Figure 2. Comparison of 3 most virulent isolates of *Xanthomonas* from Uganda and those isolated previously from beans and other species based on 16s ribosomal gene.

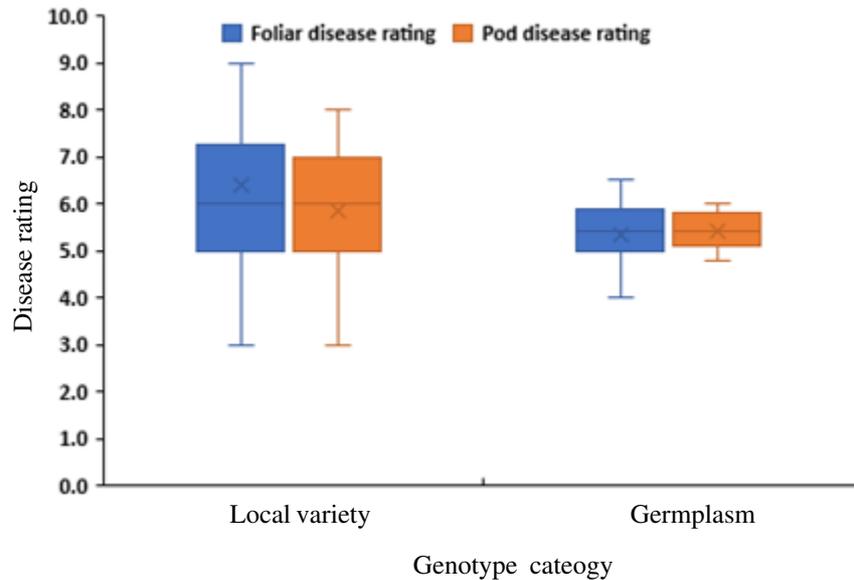


Figure 3. Foliar disease score on breeding germplasm and adopted bean genotypes in Uganda.

TABLE 4. Shows Reaction of bean breeding germplasm to MBL020 isolate of *Xanthomonas citri pv fuscans*

Genotype	Foliar disease rating (14 DAI)	Pod disease rating (10 DAI)	Resistance rating
A 197	5.7	5.1	Moderate
ACC 10	5.9	5.8	Moderate
ACC 4	5.2	5.9	Moderate
ACC 5	6	6	Susceptible
NE2- 14- 8	4	5.3	Moderate
NE14-09-78	5.2	5.1	Moderate
NE17-14-29	5.5	5.1	Moderate
MIB 465	5.4	5.4	Moderate
NUA 45	6.5	5.5	susceptible
VAX 3	4.3	5.4	Moderate
VAX 5	5	4.8	Moderate

aggressive on some local varieties, which included NARONEAN1, NOROBEAN2 NAROBEAN 4, NABE15 and NABE 17; and unknown landrace, which had low foliar disease rating in comparison with other genotypes (Table 5). Pod resistance weakly correlated positively with foliar resistance, with 19% of the pod resistance explained by resistance in the leaves (Fig. 4). The exception

to this relationship was that K20, NAROBEAN2, NABE 15 and 17 had low disease rating on leaves, but high on pods. The frequency of bean genotypes with moderate resistance was higher (69.1%) compared to that of susceptible genotypes (30.9%), in the tested breeding germplasm and local cultivar population.

TABLE 5. Shows reaction of adopted bean cultivars to MBL020 isolate of *Xanthomonas citri pv fuscans*

Cultivar	Foliar disease rating (14 DAI)	Pod disease rating (10 DAI)	Resistance rating
NABE 1 (OBA1)	7.2	7.0	Susceptible
Masindi Yellow	8.0	6.0	Susceptible
Kanyebwa	8.3	6.0	Susceptible
NABE 3 (MCM 2001)	6	5.7	susceptible
K131 (MCM 5001)	6.2	5.3	Susceptible
K132 (CAL 96)	5.7	5.3	Moderate
K20 (CAL 143)	5.3	4.7	Moderate
BUMUFU	6.0	6.0	susceptible
NABE 26	6.0	5.3	Moderate
BLACK	5.9	5.7	Moderate
Unknown	3.8	4.3	Moderate
NABE 14	6.0	4.3	Susceptible
NABE 6 (UBR92)	8.0	7.3	Susceptible
Rushoga	8.6	7.7	Susceptible
NABE 13	8.0	6.0	Susceptible
NABE 4 (POA2)	5.3	7.0	Pod susceptible
NABE 16	7.0	6.1	Susceptible
NABE 2 (MCM1015)	7.0	4.3	Pod resistant
NABE 17	5.6	7.0	Pod susceptible
NABE 15	5.0	6.0	Pod susceptible
K20	5.3	6.7	Pod Susceptible
NAROBAN 1	4.2	5.3	Moderate
NAROBAN 2	4.3	6.5	Pod susceptible
NAROBAN 3	7.3	4.5	Susceptible
NAROBAN 4	5.0	3.7	Moderate
NAROBAN 5	8.3	6.7	Susceptible
NABE 12 C	9	7.3	Susceptible

Virulence of MBL020 isolate. The virulence of Ugandan isolate (MBL020), like any other common bacterial blight pathogens isolated in other regions of the world, depended on the genotype and associated resistance sources. MBL020 was less virulent on infected genotypes containing SU91 and SAP6 as sources of resistance. Genotypes VAX3, VAX5 and ADP660 had foliar disease ratings, ranging between 3 to 4.5. MBL020 had a high foliar disease rating on genotypes containing SAP6 linked QTL as the source of resistance (Montcalm and MCM2001), with disease rating ratings of 5.5 to 6, at 14 DAI (Fig. 5).

DISCUSSION

Virulence of Ugandan isolates. In contrast with Opio *et al.* (1996) who found fuscous phenotype being more dominant than the non-fuscous type, in the present study they existed almost in equal proportions (Table 2). However, isolates that gave high foliar disease severity score were of fuscous type, implying that they were more pathogenic than the non-fuscous type (*Phaseoli*).

The highest foliar disease severities in the field that occurred on varieties Mugagawale, 7.6, White small (NABE 6) 7.4 Nambale small

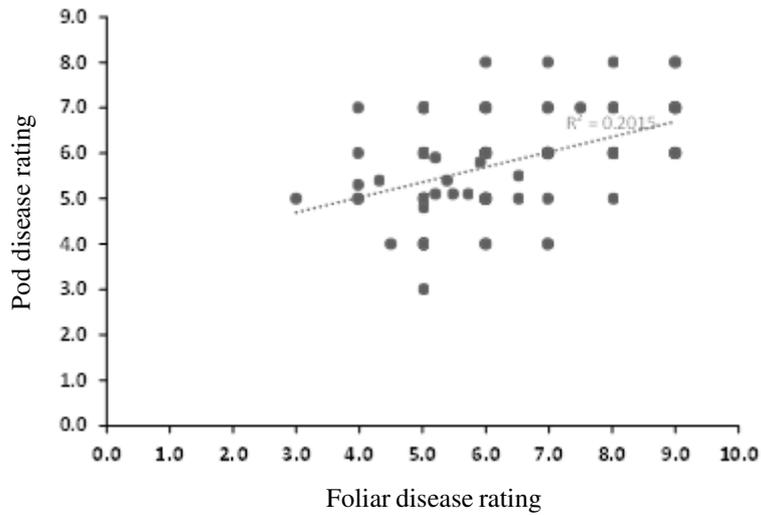


Figure 4. Relationship between foliar disease rating and pod disease rating among breeding and local varieties tested in screen house in Uganda.

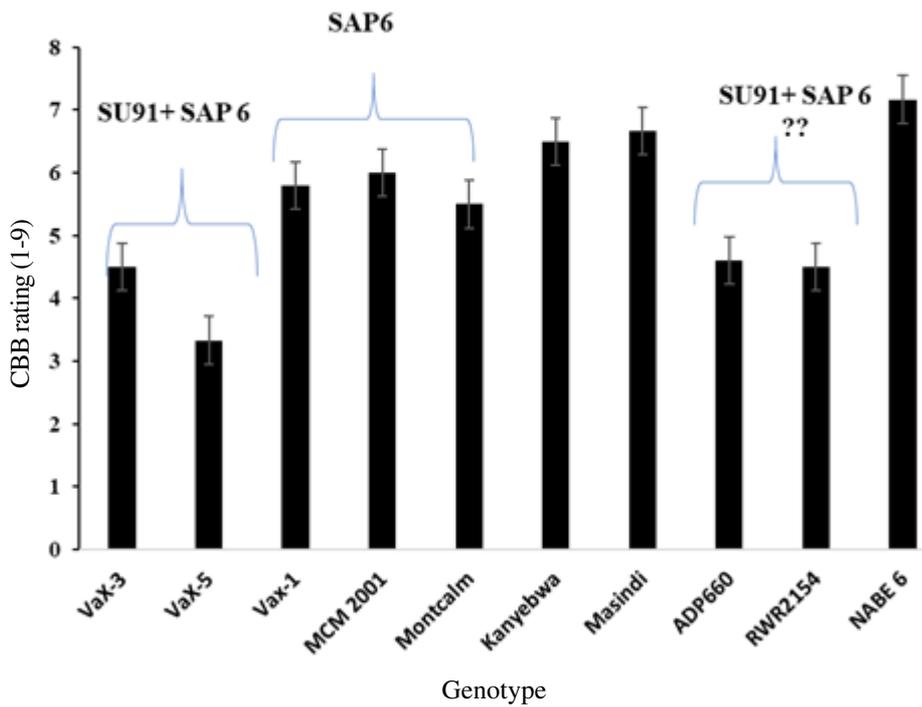


Figure 5. Response of Selected resistance sources to the Ugandan isolate of common bacterial blight pathogen 14 days after inoculation

(NABE 16) imply that these varieties were very susceptible. Continuous adoption of these varieties, despite their CBB adoption, remains a paradox but may be related to other attributes like cooking time and other culinary characteristics.

The occurrence of an isolate with disease score >7 in at least each agro-ecology when, tested under uniform screen house conditions, implies that variation in disease severity in different agro-ecologies could be a result of differences in varieties grown and local climatic conditions. On the other hand, two isolates (MBL020, KAB-3 and MSK005) which infected the most genotypes may reflect adaptation of these pathogens to both host and environment. Virulence of isolates in screen house was not correlated with virulence in the field where the pathogens were isolated (Fig. 1). This is not surprising considering that screen house conditions are uniform; while field conditions are different in terms of variety and ecology. This research has, therefore, confirmed the hypothesis of the existence of more virulent strains of *Xanthomonas* on beans in Ugandan fields.

Phylogeny of most virulent isolates.

Molecular comparison of these bacterial isolates with those previously isolated from Uganda, revealed the isolates to belong to *Xanthomonas citri pv fuscans* (current classification), formally *Xanthomonas fuscans subs. fuscans*. Isolate KAB-3 obtained from Kabarole in Uganda in the present study was highly similar to BUL014 obtained from Bulambuli Uganda (Fig. 2). This is not surprising considering the level of trade in bean grain, which in most cases doubles as seed in many farming communities in Uganda (Tugume *et al.*, 2019).

Although KAB-3 and BUL014 were very closely related based on sequence similarity, MBL020 was slightly different from the two isolates. Phylogenomic analysis further revealed that the 3 isolates were closely related to bacteria, previously isolated from beans in

Uganda more than five decades ago (NCPBP1402 and NCPBP670).

This study, therefore, has shown that the occurrence of more virulent isolates in Uganda is endemic, dating back 1958 when the first Ugandan isolate of CBB was reported (Aritua *et al.*, 2015). Comparative virulence tests of four strains of *Xanthomonas phaseoli* revealed that the Ugandan strain (U-2) was equally virulent as the two Columbian isolates C-6 and C-7, but more virulent than the Nabrasaka isolate XP-S (Schuster and Coyne, 1971; Schuster *et al.*, 1973). However, these isolates may have been re-named, lost virulence or lost completely over the years.

Aritua *et al.* (2015) observed three distinct sub-lineages within the *fuscans* lineage including the European sub-lineage, American sub-lineage and African sub-lineage. The American and African sub-lineages are largely tropical isolates. The recently isolated pathogens belong to the African sub-lineage that includes isolates largely from Uganda and Ethiopia (Aritua *et al.*, 2015). However, Aritua *et al.* (2015), observed *fuscans* isolates of African descent in Europe, which could be attributed to the work of Leakey during that period (1961-1973), where there were frequent bean germplasm exchanges between Uganda and UK. The differences among the NCPBP 1402 (1962) and NCPBP 670 (1958), and MBL020, KAB-3 and BUL014 imply that the divergence happened way back before the first isolation. Generally, bacterial isolates in this sub-lineage of *fuscans* lack XopG effector or that has been observed to elicit cell death, evading plant defense, thus contributing to more virulence. The continuous growing of susceptible varieties like Kanyebe, black dwarf, NABE 6, K20 and NABE 16 could have helped preserve the status of these pathogens over the years.

Virulence of MBL020 isolate. Testing of the most aggressive Uganda isolate of *Xanthomonas* on widely used sources of resistance (those linked to SU91 and SAP6

QTL markers), revealed moderate protection, and in some cases, susceptibility to this isolate. No genotypes could be categorised as resistant for both leaves and pods. Although SAP6 containing bean genotypes have previously shown resistance to some of the virulent tropical isolates like XCP123, they cannot be utilised as sole sources of resistance in the Ugandan bean breeding programme (Mutlu *et al.*, 2008; Tugume *et al.*, 2018). A combination of SU91 and SAP6, however, provides intermediate protection to this aggressive isolate. Since the aggressiveness of this isolate on NE 2-14-8 was comparable to that observed on VAX 3 and VAX 5 that are known to contain SAP6 and SU91, it may contain genes linked to these QTLs or other uncharacterised QTLs. Similarly, the higher frequency of bean genotypes with moderate resistance in breeding genotypes and local cultivars, implies that these genotypes may contain SU91 and SAP6 linked genes or other uncharacterised QTLs.

The existence of moderate resistance in some of Ugandan adopted varieties like NARONEAN1, NOROBEAN2, NABE14, NABE15 and NABE17 suggests that these genotypes may contain SU91 and SAP6 linked genes or other uncharacterised QTLs. Even though wide adoption of NAROBEAN varieties is still low due to their recent registration, they are promising for the future management of CBB in the country (National Variety List 2019). The observed association between foliar and pod resistance in breeding material and local varieties, though weak, reflect breeding effort towards dual CBB resistance.

The existence of moderate resistance among Andean bean genotypes, widely grown in Uganda, seems to be a common phenomenon. Leakey (1973) while working in Uganda also reported tolerance as opposed to immunity in genetic stocks at the time. Tolerance as opposed to complete resistance to Ugandan and Colombian isolates was also observed on PI207262, an important ancestor in the pedigree of CBB resistant genotypes (Schuster *et al.*, 1973).

Isolate MBL020 which was identified in this study may be more virulent than the Xcp25, which has been widely used for screening and developing CBB-breeding lines in the USA (Ducan *et al.*, 2011; Viteri *et al.*, 2014); and also Kawempe 1 previously used by Alladasi *et al.* (2018). This is because a high resistance that was observed on genotypes VAX3 and VAX 5 when using XCP25 isolate, had moderate resistance when exposed to the Ugandan isolate MBL020 (Table 3).

CONCLUSION

The study identified 3 most virulent isolates, MBL020, KAB-3 and BUL-14, all belonging to *Xanthomonas citri* pv *fuscans*. These isolates were very similar to those previously identified from Uganda (NCPB 670 and NCCPB 1402) more than 50 years ago. The study further revealed that NAROBEAN1, NAROBEAN 2, NAROBEAN 4, VAX 3, VAX5 and NE 2- 14- 8 had better resistance compared to other tested genotypes.

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