Characterization of isolates that cause black rot of crucifers in East Africa

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Abstract

A study was conducted in the East African countries of Kenya, Tanzania and Uganda in the months of July and August 2009 with the objectives of assessing the status of black rot and race structure of *Xanthomonas campestris* pv. *campestris* in the three countries. Samples infected with black rot were collected from farmers' fields mainly from *Brassica oleracea* crops (broccoli, cabbage, cauliflower and kales). A total of 399 farms were surveyed of which 260 were from Kenya, 91 from Tanzania and 48 from Uganda. Following successful isolations, a total of 249 isolates of the causal agent, *Xanthomonas campestris* pv. *campestris* were recovered. Pathogenicity of all isolates was confirmed on *B. oleracea* susceptible cultivars Copenhagen Market F1 and Wirosa F1. Sixty of the 250 isolates were race-typed using a differential set *Brassica* spp. Only two races, 1 (Kenya and Tanzania) and 4 (Kenya, Tanzania and Uganda) were observed however, another race (5) was observed from one isolate recovered from a *B. rapa* sample obtained from Tanzania in 2003. Genomic fingerprinting with repetitive-PCR revealed clusters that did not depict significant correlations between isolates and geographical location, isolates and host adaptation or isolates and race. However, it did demonstrate existence of genetic differences within the East African *X. campestris* pv. *campestris* population indicating that it is not a similar clonal population of the same genetic background.

Keywords: *Brassica oleracea*, genetic diversity, pathogenicity, physiological races *Xanthomonas campestris*

Introduction

Cabbage (*Brassica oleracea* ssp. *capitata*) and kales (*B. oleracea* ssp. *acephala*) are widely grown in East Africa (EA) by small-scale farmers as a supplement to their diet and a source of income (Mwasha, 2000; Wambani et al., 2007). Black rot caused by *Xanthomonas campestris* pv. *campestris* (thereafter *Xcc*) (Pammel) Downson has been reported as one of the main constraints to sustainable production resulting in 100% losses in susceptible varieties (Mgonja and Swai, 2000; Massomo et al., 2004). It is characterised by V-shaped, chlorotic to necrotic lesions at the margin of the leaf coupled with the darkening of leaf veins and vascular tissue in the stem (Cook et al., 1952). Management of this disease is mainly based on using pathogen-free planting material (seeds or transplants) and eliminating potential sources of inoculum especially crop debris and cruciferous weeds (Taylor et al., 2002). However, *Xcc* is a seed-borne pathogen that is difficult to eliminate from seeds (Roberts et al., 1999) and therefore pathogen-free planting material is not always available. This is due to the fact that *Xcc* epidemics can be initiated from a single infected seed within a batch of 10,000 seeds (Schaad et al., 1980). The seed-borne nature of this pathogen also complicates disease management as seed plays a significant role in the survival and long distance dissemination of the bacteria (Schaad and Dianese, 1981). Deployment of host resistance remains the most effective strategy. However, this strategy is complicated by the high variability of *Xcc* strains as reported in many studies (Kamoun et al., 1992; Vicente et al., 2001; Fargier and Manceau, 2007; Jensen et al., 2010).

Kamoun et al. (1992) described five physiological races (0-4) based on response of two cultivars of turnip (*Brassica rapa*) and two of mustard (*Brassica juncea*). Vicente et al. (2001) reported existence of six (1-6) races based on the reaction of eight *Brassica* differential genotypes including cultivars of *B. carinata*, *B. juncea*, *B. napus*, *B. oleracea* and *B. rapa*. Jensen et al. (2010) used the same differential genotypes as Vicente et al. (2001) and reported an additional race 7. Fargier and Manceau, 2007 reported three new races (7-9) in addition to the six reported by Vicente et al. (2001). Races 1 and 4 have been reported to predominate worldwide. Resistance to these races has not been reported in *B. oleracea* (C genome) but in *B. rapa* (A), *Brassica nigra* (B) and *Brassica carinata* (BC) (Taylor et al., 2002). Studies on the nature of resistance to race 1 and 4 in *B. rapa* ssp. *pekinensis* have revealed quantitative trait loci (QTL) that condition resistance on three linkage groups (A02, A06 and A09) of *B. rapa* (Soengas et al., 2007). This study was carried out to achieve four major objectives: (i) survey the East African countries of Kenya, Tanzania and Uganda and obtain a collection of *Xcc*-infected *Brassica* samples, (ii) isolate *Xcc* strains from infected *Brassica* samples and determine their pathogenicity on *B. oleracea* susceptible cultivars, (iii) determine the race-structure of pathogenic *Xcc* strains from EA and, (iv) assess the molecular (genetic) diversity within this collection of isolates. Race-typing was achieved through infection of a selected set of differential genotypes with *Xcc*. Information on race structure of *Xcc* in EA is essential for targeted breeding of *B. oleracea* cultivars that are beneficial to resource poor small-scale farmers in the developing world. Molecular variation was assessed using three PCR-based DNA fingerprinting methods based on repetitive sequences distributed in bacterial genomes. They include the extragenic palindromic (REP) sequences, enterobacterial repetitive intergenic consensus (ERIC) sequences, and BOX elements and are

collectively referred to as repetitive PCR (rep-PCR) (Versalovic et al., 1991; Versalovic et al., 1994). The approach has been used in previous surveys of diversity within and between bacterial species (Vicente et al., 2006; Vicente and Roberts, 2007; Jensen et al., 2010).

Materials and Methods

Collection of samples

A survey was conducted in Kenya, Tanzania and Uganda between the months of July and August 2009 to collect *Brassica* leaf samples displaying symptoms of black rot from farmers' fields. Only the major *Brassica* growing regions in the three countries were considered. Samples were collected from broccoli (*B. oleracea* spp. *italica*), cabbage (*B. oleracea* spp. *capitata*), cauliflower (*B. oleracea* spp. *botrytis*), and collard/kale (*B. oleracea* spp. *acephala*). *Brassica carinata* (African kale) commonly grown in Tanzania as a *Brassica* intercrop and locally known as *Loshuu* was also examined for black rot symptoms. Samples were collected from *Brassica* crops of all stages (seedlings and mature). No samples were obtained from weed relatives of *Brassicas*. All collected leaf samples were placed in paper bags in between newspapers and transported to the laboratory for further processing.

Isolation of bacteria

From each infected leaf sample, leaf tissue segments of 2 x 3-4 mm were excised from lesion margins. Each leaf segment was macerated in 500 µl of sterile tap water

and left to stand for 5 min in a laminar airflow cabinet. The suspension containing the bacteria was streaked on plates containing King's Medium B (KB) (King et al., 1954) and Yeast Dextrose Chalk (YDC) (Lelliot and Stead, 1987). Isolates were incubated for 24 hr at 28^{o}C and subsequently purified by repeated sub-culturing of single colony. All purified isolates were stored at -76^{o}C in liquid medium containing 8 g per ltr of nutrient broth (Difco) and 150 ml per ltr of glycerol (Vicente et al., 2001).

Determination of pathogenicity of isolates

Successfully purified isolates were tested for pathogenicity on *B. oleracea* ssp. *capitata* susceptible cultivars of Copenhagen Market F1 and Wirosa F1. Seeds were sown in 7 cm plastic pots filled with Levington M2 compost (The Scotts Company, Ipswich, UK) and grown in a glasshouse with a minimum temperature of 20/15^{o}C (day/night) with venting at 22/17°C (day/night). Isolates were recovered from stocks at -76^{o}C and grown on KB at 28^{o}C for 48 hr before inoculation. A sterile pin charged with growth from a KB plate was used to inoculate four-week-old plants. One plant was inoculated for every isolate. The presence of symptoms was recorded two and three weeks after inoculation.

Determination of race structure

Race-typing was carried out using a standard set of eight *Brassica* differential genotypes recommended by previous studies (Vicente et al., 2001; Jensen et al., 2010). This set is a collection of *B. oleracea* genotypes, Wirosa F1 (*B. oleracea* ssp. *Sabauda*), Miracle F1 (*B. oleracea* ssp. *Botrytis*) and SxDI (*B. oleracea*); *B. rapa*

genotypes, Just Right Turnip (*B. rapa* ssp. *rapifera*) and Seven Top Turnip (*B. rapa* ssp. *rapifera*); a *B. juncea* genotype, Florida Broad Leaf Mustard (*B. juncea*); a *B. napus* genotype, Cobra (*B. napus* ssp. *oleifera*) and a *B. carinata* genotype, PI 199947 (*B. carinata*). The inoculation method and experimental conditions described in Vicente et al. (2001) were used.

Extraction of DNA

Isolates were recovered from -76^{o}C and grown on KB and incubated at 28^{o}C for 24 h. Bacterial growth was scrapped from the surface of KB plates and suspended in 1 ml of sterile distilled water to produce turbid suspensions that corresponded visually to the McFarland turbidity standards of 3-4 and matched a concentration of 10^{8}-10^{9} cells per ml (Smibert and Krieg, 1994; Vicente et al., 2006). Cells from this suspension were harvested by centrifugation for 10 min at 13,000 rpm (12,600 x *g*) in a microfuge. Genomic DNA was extracted using the Qiagen DNeasy blood and tissue kit (Qiagen, Ltd, West Sussex, UK) according to the manufacturer's instructions for extracting DNA from gram-negative bacteria. Purity and quantity of extracted DNA was determined with a NanoDrop (NanoDrop Technologies, Wilmington, Delaware).

Genomic fingerprinting with rep-PCR

DNA from *Xcc* isolates obtained from EA in this study, other *Xcc* isolates previously obtained from Kenya, Tanzania and Zambia and some *Xcc* isolates used in previous studies (Vicente et al., 2001; Jensen et al., 2005) was subjected to three repetitive PCR fingerprinting protocols, BOX-PCR, ERIC-PCR and REP-PCR. Three

representative isolates of the pathovar X. *campestris* pv. *raphani* (thereafter *Xcr*) and one representative isolate of the species *X. vesicatoria* (thereafter *Xv*) were included for comparison. Primers for BOX-PCR (BOXA1R, 5′-CTACGGCAAGGCGACGCTGACG-3′), ERIC-PCR (ERIC1R, 5′-ATGTAAGCTCCTGGGGATTCAC-3′ and ERIC2, 5′-AAGTAAGTGACTGGGGTGAGCG-3′) and REP-PCR (REP1R, 5′-IIIICGICGICATCIGGC-3′) and REP2I (5′-ICGICTTATCIGGCCTAC-3′) (Versalovic et al., 1991; Versalovic et al., 1994) were synthesized by Invitrogen, Paisley, UK. PCR reactions were carried out in a total volume of 20 µl containing 1× Gitschier buffer (Kogan et al., 1987), 2 µl of dimethylsulfoxide (FULKA, Dorset, UK), 3.2 µg of bovine serum albumin (Roche, Mannheim, Germany), 50 pmol of each primer, 1.25 mM of each of four deoxynucleoside triphosphates, 1.6 unit of *Taq* DNA polymerase (Invitrogen, Paisley, UK), and approximately 80 ng of template genomic DNA. PCR amplifications were performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, CA). The rep-PCR programme consisted of one cycle at 95°C for 2 min; 30 cycles of denaturation at 94^{o}C, 3 sec, 92^{o}C for 30 sec, annealing at 53^{o}C (BOX-PCR), 52^{o}C (ERIC-PCR) and 40^{o}C (REP-PCR) for 1 min, extension at 65^{o}C for 8 min and final extension of 8 min at 65°C. A molecular mass marker (1-kilobase plus DNA ladder, Invitrogen) was loaded on both sides and middle of each gel. The negative control (water) was included in each gel while the control *Xcc* isolates (3811 and 1279A) were included in five different gels. Following PCR amplification, the PCR products were subjected to electrophoresis on a 1.0% agarose gel in 0.5X TBE stained with GelRed^{TM} nucleic acid gel stain (Biotium Inc. Hayward, CA) for 18 hr at 60 V. The gels were visualized under UV light and photographed using the Gel Logic 200 Imaging System (Kodak) to obtain digital

images for each gel. Each amplification band was treated as a unit character and was visually scored as either present (1) or absent (0). The BOX-PCR, ERIC-PCR and REP-PCR results were combined in a data matrix and subjected to cluster analysis using the dice similarity coefficient and unweighted pair group method using arithmetic averages (UPGMA) to calculate similarity of the DNA fingerprint patterns in PAST (Hammer et al., 2001) and the resulting dendogram visualized in MEGA5 (Tamura et al., 2007).

Results

Sample collection

Farmers' fields in the East African countries of Kenya, Tanzania and Uganda (Fig. 1) were surveyed in July and August 2009 to obtain a collection of *Brassica* samples displaying symptoms of black rot. Six districts (Embu, Kiambu, Nairobi, Nakuru, Nyandarua and Taita/Taveta) were surveyed in Kenya, one district (Meru) in Tanzania and two districts (Kabale and Wakiso) in Uganda (Fig. 1). The altitude of the surveyed areas ranged from 1,190 - 2,534 meters above sea level while farm sizes ranged from less than half to one hectare. Black rot was observed in all *B. oleracea* subspecies and all surveyed farmer fields in the three countries. There was no black rot symptoms observed on *B. carinata* (*Loshuu*). On average, three to five samples were collected per farmer field visited however; the number collected was dependent on the size of the crop and quality of the samples. All collected samples were examined keeping only one sample from which feasible isolations could be made per field. In total, 399 farms were visited of which 260 were from Kenya, 91 from

Tanzania and 48 from Uganda. Of the 260 farms visited in Kenya, 62 were from Embu, 34 from Kiambu, 41 from Nairobi, 8 from Nakuru, 11 from Nyadarua and 104 from Taita-Taveta. All the 91 farms visited in Tanzania were from Meru district while the 48 farms visited in Uganda, 12 were from Kabale and 36 from Wakiso. Some of the samples were dropped because they were either too dry or rotten to achieve successful bacterial isolation. As a result, only 250 good quality samples were retained following examination. Of these, 141 were from Kenya, 79 from Tanzania and 30 from Uganda. All these samples were only obtained from *B. oleracea* subspecies of *acephala* (kales), *botrytis* (cauliflower), *capitata* (cabbage) and *italica* (broccoli). Black rot was observed on all farms surveyed. Disease incidences were low in reported tolerant varieties such as Pruktor F1 (Massomo et al., 2004) and high in susceptible varieties such as Gloria F1 (Massomo et al., 2004).

Bacterial isolation and pathogenicity tests

All samples from Kenya were processed and *Xcc* isolations made at National Agricultural Research Laboratories, Kenya Agricultural Research Institute, Nairobi, Kenya. Samples from Tanzania and Uganda were processed following collection and sent to Warwick-HRI (now School of Life Sciences), University of Warwick, Wellesbourne, United Kingdom for *Xcc* isolation. The causal bacterium was isolated based on the procedure described by Vicente *et al*. (2001). A total of 249 *Xcc* isolates were recovered from the 250 retained samples. *Xanthomonas campestris* pv. *campestris* was recovered from all the 141 and 79 samples from Kenya and Tanzania respectively but only 29 samples from Uganda. This resulted in the purification of 141, 79, and 29 isolates from Kenya, Tanzania and Uganda respectively. All these

isolates are available in the collection at the School of Life Sciences, University of Warwick, United Kingdom. Of the 141 isolates from Kenya, 95 were recovered from *B. oleracea* spp. *acephala*, five from *B. oleracea* spp. *botrytis*, 38 from *B. oleracea* spp. *capitata* and three from *B. oleracea* spp. *italica*. The 79 isolates from Tanzania were recovered from only two subspecies, *B. oleracea* spp. *acephala* (10) and *B. oleracea* spp. *capitata* (69). Like Tanzania, all isolates from Uganda were recovered from only two subspecies with the majority of isolates (21) from *B. oleracea* spp. *capitata* and the remaining (8) from *B. oleracea* spp. *acephala*. The causal pathogen was confirmed using two methods; nature of growth on YDC medium and pathogenicity tests on susceptible *B. oleracea* spp *capitata* genotypes. All isolates produced a yellow or cream mucoid growth on YDC, a characteristic observed with all *Xanthomonads* and also produced clear and distinct V-shaped chlorotic lesions associated with *Xcc* pathogenicity on the susceptible genotypes of Copenhagen Market F1 and Wirosa F1. No non-pathogenic *Xanthomonads* were isolated.

Race typing of Xcc isolates from East Africa

Of the 249 isolates recovered from infected *Brassica* leaf samples, only 60 isolates were selected for race-typing. Selection was initially based on the REP-PCR fingerprint profile from which isolates representing clusters displaying similar fingerprint profiles were obtained (Data not shown). This selection was followed by another level, which was based on the proportion of isolates collected per host. Selection based on distribution of isolates was important but not much considered. Using this criterion, the majority of isolates (14) race-typed for the Kenya collection were selected from *B. oleracea* spp. *acephala*. More isolates were recovered from this

than any other subspecies in Kenya. Representative isolates were also selected for other host genotypes. For instance, 5 were selected from *B. oleracea* spp. *capitata*, 3 from *B. oleracea* spp. *italica* and 1 from *B. oleracea* spp. *botrytis*. A total of 23 isolates were selected from Kenya. Similarly, the majority of isolates in Tanzania were recovered from *B. oleracea* spp. *capitata*; consequently 15 isolates were selected from this subspecies and eight from *B. oleracea* spp. *acephala* making a total of 23. In Uganda, 12 isolates were selected from *B. oleracea* spp. *capitata* and 2 from *B. oleracea* spp. *acephala* for the same reasons making a total of 14 isolates. Details of the selected and race-typed isolates are presented in Table 1. Only two races (1 and 4) were identified among all isolates selected and race-typed from this study (Table 1). Race 1 was identified in 11 and five isolates from Kenya and Tanzania respectively, but in none of the isolates in Uganda. Race 4 was identified in 12, 18 and all race-typed isolates from Kenya, Tanzania and Uganda, respectively. A race 5 was recorded from one isolate from Tanzania. This race 5 isolate was isolated in 2003 from a *B. rapa* sample collected from Tanzania hence was not part of this study. This isolate is also available in the *Xcc* collection at the School of Life Sciences, University of Warwick.

Repetitive-PCR genomic fingerprinting

All the 249 isolates recovered from infected *Brassica* leaf samples collected from EA were initially subjected to REP-PCR. This criterion was used to select reasonable isolate numbers for race -typing as explained above and detailed rep-PCR analysis with BOX-PCR and ERIC-PCR. Based on the REP-PCR fingerprint profiles, 60 isolates were selected for race-typing while 40 isolates (10 from Kenya, 16 from

Tanzania and 14 from Uganda) were selected for BOX-PCR and ERIC-PCR analysis. More informative polymorphic fingerprint patterns were obtained from ERIC-PCR profiles (Fig. 3). REP-PCR resulted in good but few polymorphic bands while BOX-PCR generated more bands than REP-PCR but not as informative as ERIC-PCR (Fig 2.). The number of comprehensible polymorphic bands scored was 14 for REP-PCR, 19 for ERIC-PCR and 3 for BOX-PCR. Cluster analysis of the combined fingerprint profiles for the three rep-PCR protocols resulted in a dendogram with a cophenetic correlation coefficient of 0.871. The individual dendograms (data not shown) resulting from each primer set also had cophenetic correlation coefficients above 0.8 with BOX-PCR recording 0.882 while ERIC-PCR recorded 0.841 and REP-PCR recorded 0.855. The profiles of control isolates (3811 and 1279A) that were repeated in five different gels had similarities ranging from 82% to 94%. The combined dendogram demonstrated a clear distinction between the two species (*X. campestris* and *X. vesicatoria*) and pathovars (*Xcc* and *Xcr*) (Fig. 3). The *Xcr* isolates (6518, 7803 and 8299) formed a clear and distinct clade, which was connected to the bigger clade comprising *Xcc* isolates. Four clades of *Xcc* were observed, the first clade comprising isolates 8619 and 8021, and was distinct from the second clade, which comprised of isolates 8544, 8420A and 8432A. The third and fourth clades, which were connected to the first and second clades comprised of 32 isolates (8653-8649) and 31 isolates (8650-7804) respectively. There was no significant correlation between the observed *Xcc* clade structure with host subspecies, county of origin and race-type of the isolate. Isolates recovered from samples collected from the same host subspecies, country, and race did not fall into one macro cluster, but into micro clusters spread within the main clades.

Discussion

This study was conducted to establish the importance of black rot in EA and also determine the race structure of the causal pathogen, *Xcc* in the same region. Black rot was observed on all farmers' fields and the causal bacterium was isolated from all *Brassica* leaf samples except for one demonstrating the importance of black rot to *Brassica* production in EA. This is consistent with previous studies, which reported the importance of this disease in Tanzania (Massomo et al., 2003; Massomo et al., 2004; Jensen et al., 2005). There was no black rot symptoms observed on *B. carinata* even though it is widely grown in Tanzania and in close proximity of the black rot infested *B. oleracea* crop. Race-typing revealed that only two races (1 and 4) predominate the East African population. Previous studies demonstrated Race 1 to be the most common in the United Kingdom (Vicente et al., 2001) and race 4 the most common in Nepal (Jensen et al., 2010) and Portugal (Vicente et al., 2001). The predominance of races 1 and 4 can be attributed to wide cultivation of *B. oleracea* cultivars that are susceptible to these races. This is also supported by the lack of observation of symptoms on *B. carinata* which has been reported to display resistance to races 1 and 4 (Taylor et al., 2002). The limited observation of race 1 in Tanzania and absence in Uganda could be attributed to the small sampling population. Race 4 was identified in all the three countries, which indicates that it could be most important *Xcc* race in EA. Races 2, 3, 5, 6 and 7 were not observed amongst the isolates obtained in this survey although one isolate previously obtained from Tanzania and recovered from *B. rapa* in 2003. All these races have been in previous studies shown to be very limited (Vicente et al., 2001; Jensen et al., 2010). The observation of a race 5 demonstrates that it could be available in EA however, the

predominance of *B. oleracea* subspecies could have limited its dissemination as *B. oleracea* displays some resistance to this race (Vicente et al., 2001). The occurrence of a race 5 suggests that more races could be present in EA. Information from this study is relevant for *Brassica* breeding in EA. Previous studies have demonstrated that *B. oleracea* subspecies which are widely grown in EA (cabbage and kales) display high susceptibility to races 1 and 4 (Taylor et al., 2002) which have been shown to be the most important in EA. Sources of resistance to race 1 and 4 have been reported in *B. rapa* and localised on linkage groups 1 and 4 of the *B. rapa* B162 and R-o-18 map (Soengas et al., 2007).

Representative isolates from this study some of which were race-typed were subjected to repetitive PCR along with some published (Vicente et al., 2001; Jensen et al., 2010) and unpublished isolates. This protocol demonstrated differences between isolates belonging to the two different species (*Xanthomonas campestris* and *X. vesicatoria*) and two different pathovars (*Xcc* and *Xcr*). This shows that polymorphisms resulting from repetitive sequences in bacterial genomes may be used to define differences between species and pathovars. Similar level of separation was observed with previous rep-PCR studies of *Xanthomonas* (Vicente et al., 2006; Vicente and Roberts, 2007; Jensen et al., 2010). The *Xcc* and *Xcr* clades were connected together demonstrating a close evolutionary relationship. The strains within the *Xcc* clade revealed complex but polymorphic bands resulting in groups of closely related strains based on fingerprint pattern. However, isolates recovered from samples from the same host and country with the same race did not fall into one macro but micro clusters spread over the dendogram yet previous studies had demonstrated a correlation with race (Vicente et al., 2006; Jensen et al., 2010) or geographical origin (Shakya et al.,

2000; Massomo et al., 2003). Races of the isolated strains were not determined in the studies of Massomo et al. (2003) and Shakya et al. (2000). The lack of such a correlation in the East African *Xcc* population may be attributed partially to cross boarder movement of seed and *Brassica* commodities within EA all of which could spread of *Xcc* resulting in a more heterogeneous population. On the other hand, a relationship between rep-PCR and races of a given group of strains may also not occur in all analyses because rep-PCR fingerprints result from repetitive sequences within bacterial genomes (Lupski and Weinstock, 1992) yet races are determined based on the gene-for-gene hypothesis (Flor, 1971; Flor and Comstock, 1972) in which different combinations of *avirulence* genes in the pathogen population will be recognized by different combinations of resistance genes in the host (differential genotypes). This could also explain why rep-PCR fingerprints could not relate to isolates infecting the same host. This study has revealed the existence of variations within the East African *Xcc* population indicating that it is not a similar clonal population of the same genetic background. It has shown that breeding for resistance to *Xcc* in EA should at least focus on two major races. The isolates characterised here can be used for screening of *Brassica* cultivars to be used in breeding programmes. The information generated forms the foundation and opens avenues for more studies that will improve the understanding of the population dynamics of this pathogen in EA and else-where.

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Table 1 Represents isolates of two *X. campestris* pathovars, *X. campestris* pv. *campestris* and *X. campestris* pv. *raphani* and a *Xanthomonas* species, *X. vesicatoria* used in the study. Isolates collected from East Africa and race-typed are represented with "This study" in the source column; unpublished isolates are represented with "Warwick HRI" while published isolates are represented with respective references. Isolates with an asterisk represent the standard physiological races.

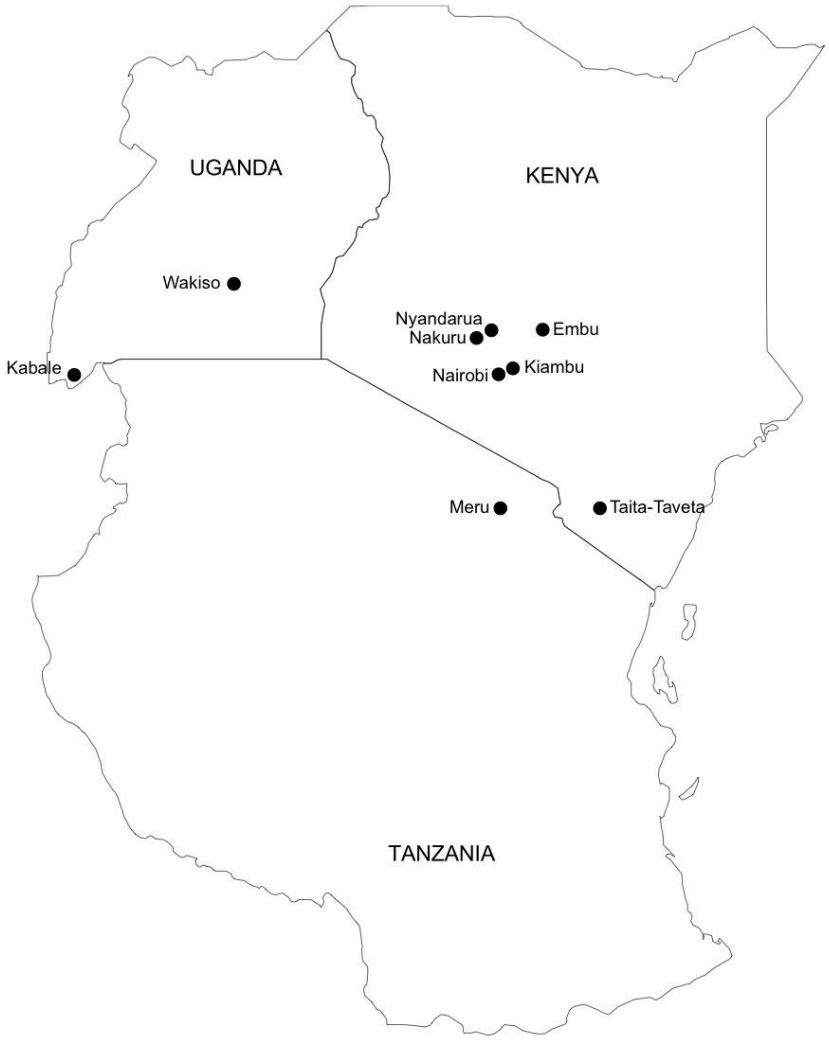
Isolate	Host of origin	Country	Region	Year isolated	Source
X. campestris* pv. *campestris					
Race 1					
3877	*B. oleracea* ssp. *acephala*	Kenya			Vicente et al. (2001)
8409	*B. oleracea* ssp. *acephala*	Kenya	Nairobi	2003	Warwick HRI
8416A, 8420A	*B. oleracea* ssp. *capitata*	Kenya		2003	Warwick HRI
8649, 8697, 8700, 8761	*B. oleracea* ssp. *acephala*	Kenya	Taita/Taveta	2009	This study
8662	*B. oleracea* ssp. *italica*	Kenya	Nairobi	2009	This study
8730	*B. oleracea* ssp. *acephala*	Kenya	Kiambu	2009	This study
8733	*B. oleracea* ssp. *acephala*	Kenya	Embu	2009	This study
8788	*B. oleracea* ssp. *acephala*	Kenya	Nairobi	2009	This study
8759	*B. oleracea* ssp. *capitata*	Kenya	Nairobi	2009	This study
8737	*B. oleracea* ssp. *capitata*	Kenya	Embu	2009	This study
8708	*B. oleracea* ssp. *italica*	Kenya	Taita/Taveta	2009	This study
7809	*B. oleracea* ssp. *capitata*	Namibia	Caprivi Strip	1999	Vicente et al. (2001)
8541, 8542	*B. oleracea* ssp. *acephala*	Tanzania	Meru	2009	This study
8572, 8577, 8578	*B. oleracea* ssp. *capitata*	Tanzania	Meru	2009	This study
7804	*B. oleracea* ssp. *capitata*	South Africa	Brits	1997	Vicente et al. (2001)
7805	*B. oleracea* ssp. *botrytis*	South Africa	Marble Hall	1998	Vicente et al. (2001)
7807	*B. oleracea* ssp. *capitata*	South Africa		1998	Vicente et al. (2001)
7810	*B. oleracea* ssp. *botrytis*	South Africa	Marble Hall	1999	Vicente et al. (2001)
8022D	*B. oleracea* ssp. *capitata*	Zambia	Lusaka	2000	Warwick HRI
3811*	*B. oleracea*	USA			Vicente et al. (2001)
Race 2					
3849A*	*B. oleracea* ssp. *botrytis*	USA			Vicente et al. (2001)
Race 3					
5212*	*B. oleracea* ssp. *gemmifera*	UK		1957	Vicente et al. (2001)
Race 4					
2243A	*Brassica* spp.				Vicente et al. (2001)
8020D	*B. oleracea* ssp. *capitata*	Kenya	Nairobi	2000	Warwick HRI
8021	*B. oleracea* ssp. *capitata*	Kenya	Laikipia	2000	Warwick HRI
8414A	*B. oleracea* ssp. *capitata*	Kenya		2003	Warwick HRI
8423A	*B. oleracea* ssp. *acephala*	Kenya		2003	Warwick HRI
8648, 8779	*B. oleracea* ssp. *acephala*	Kenya	Embu	2009	This study
8653, 8723, 8760	*B. oleracea* ssp. *acephala*	Kenya	Nairobi	2009	This study
8758	*B. oleracea* ssp. *acephala*	Kenya	Nakuru	2009	This study
8650	*B. oleracea* ssp. *acephala*	Kenya	Embu	2009	This study
8713	*B. oleracea* ssp. *botrytis*	Kenya	Taita/Taveta	2009	This study
8746, 8748	*B. oleracea* ssp. *capitata*	Kenya	Kiambu	2009	This study
8762	*B. oleracea* ssp. *capitata*	Kenya	Nairobi	2009	This study
8661	*B. oleracea* ssp. *italica*	Kenya	Nairobi	2009	This study
8401	*B. oleracea* ssp. *capitata*	Tanzania	Meru	2003	Warwick HRI
8539, 8540, 8543	*B. oleracea* ssp. *acephala*	Tanzania	Meru	2009	This study
8544, 8607, 8616	*B. oleracea* ssp. *acephala*	Tanzania	Meru	2009	This study
8549, 8553, 8573	*B. oleracea* ssp. *capitata*	Tanzania	Meru	2009	This study
8604, 8588, 8592	*B. oleracea* ssp. *capitata*	Tanzania	Meru	2009	This study
8596, 8601, 8611	*B. oleracea* ssp. *capitata*	Tanzania	Meru	2009	This study
8555, 8556, 8559	*B. oleracea* ssp. *capitata*	Tanzania	Meru	2009	This study
8646, 8647	*B. oleracea* ssp. *acephala*	Uganda	Wakiso	2009	This study
8618, 8619, 8620,	*B. oleracea* ssp. *capitata*	Uganda	Kabale	2009	This study
8637, 8642, 8643	*B. oleracea* ssp. *capitata*	Uganda	Kabale	2009	This study
8622, 8623, 8626	*B. oleracea* ssp. *capitata*	Uganda	Wakiso	2009	This study
8627, 8628, 8629	*B. oleracea* ssp. *capitata*	Uganda	Wakiso	2009	This study
7806	*B. oleracea* ssp. *capitata*	South Africa	Northam	1998	Vicente et al. (2001)
7808	*B. oleracea* ssp. *capitata*	South Africa		1999	Vicente et al. (2001)
8023A	*B. oleracea* ssp. *capitata*	Zambia	Lusaka	2000	Warwick HRI
1279A*	*B. oleracea* ssp. *capitata*	UK	Cornwall	1984	Vicente et al. (2001)
Race 5					
8403	*B. rapa* ssp. *chinensis*	Tanzania	Meru	2003	Warwick HRI
3880*	*B. oleracea* ssp. *capitata*	Australia		1975	Vicente et al. (2001)
Race 6					
6181*	*B. rapa*	Portugal	Sardoal	1996	Vicente et al. (2001)
Race 7					
8450A*	*B. oleracea* ssp. *capitata*	Nepal		2003	Jensen et al. (2010)
X. campestris* pv. *raphani					
6519	*Raphanus sativus*	Japan		1985	Vicente et al. (2006)
7803	*B. oleracea* ssp. *capitata*	South Africa	Brits	1997	Vicente et al. (2001)
8299	*Lycopersicon esculentum*	Canada			Vicente et al. (2006)
X. campestris* pv. *vescatoria					
5235	*Lycopersicon esculentum*	New Zealand		1955	Vicente et al. (2006)

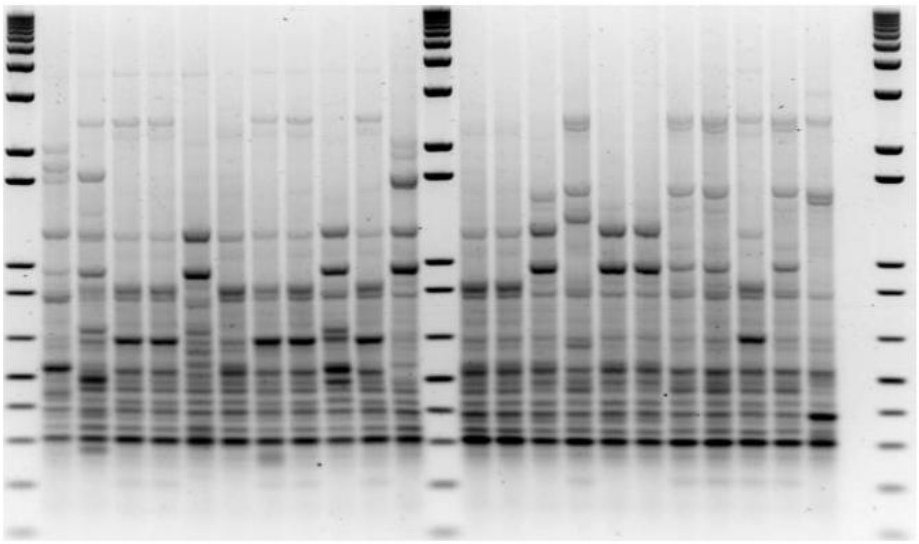
Captions

Fig. 1 *Brassica* growing areas in the three East African countries of Kenya, Tanzania and Uganda from which leaves displaying typical black rot symptoms were collected in July and August 2009.

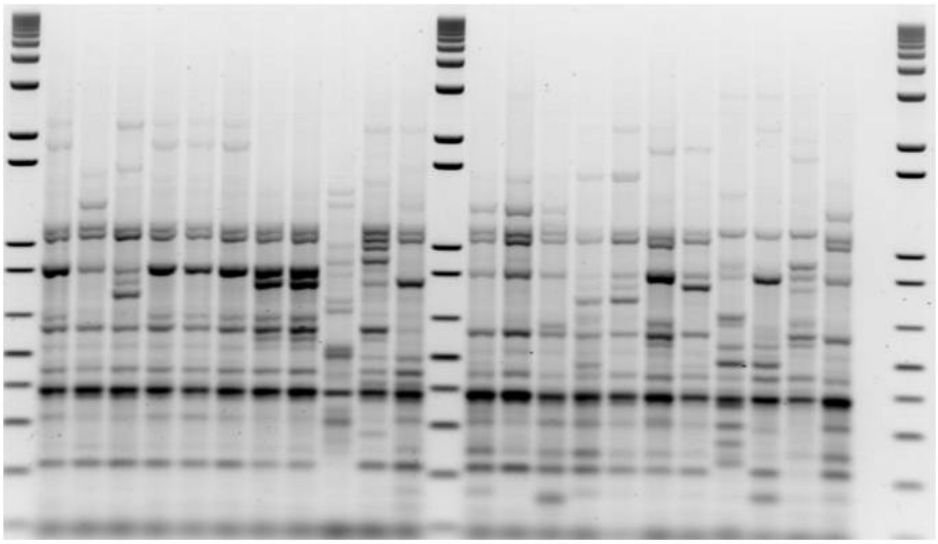
Fig. 2 Repetitive-PCR genomic fingerprint pattern for selected *Xanthomonas campestris* pv. *campestris* isolates. Representative gels for each rep-PCR fingerprint type is presented with images A, B and C representing BOX-PCR, ERIC-PCR and REP-PCR respectively. Lanes 1, 13, and 26 represent the 1-kilobase plus DNA ladder; lane 24 (1279A), the control isolate; lane 25, the negative control (water) respectively, and lanes 2-12 (8540, 8542, 8611, 8616, 8637, 8643, 8650, 8625, 8629, 8401, and 8022D) and 14-23 (8555, 8577, 8556, 8572, 8549, 8553, 8700, 8628, 8403, 8642), the test isolates.

Fig. 3 Dendrogram of genetic similarity of 72 *Xanthomonas* isolates (one *X. vesicatoria* (*Xv*), three *X. campestris* pv. *raphani* (*Xcr*) and 68 *X. campestris* pv. *campestris* (*Xcc*). The similarity is the result of the combined data set of rep-PCR fingerprint profiles using UPGMA analysis and dice's coefficient. The order represents the isolate code, the *X. campestris* pathovar, host from which the isolate was recovered, country of origin and race-type of isolate. The abbreviations *Xcc*, *Xcr* and *Xv* represent *X. campestris* pv. *campestris*, *X. campestris* pv. *raphani* and *X. vesicatoria* respectively, *Bo*, *Boa*, *Bob*, *Boc*, *Bog*, *Br*, *Le*, *Rs* represent *Brassica oleracea*, *B. oleracea* ssp. *acephala*, *B. oleracea* ssp. *botrytis*, *B. oleracea* ssp. *capitata*, *B. oleracea* ssp. *gemmifera*, *B. rapa*, *Lycospersicon esculentum* and *Raphanus sativus* respectively and S. Africa, UK and USA represent South Africa, the United Kingdom and the United States of America respectively.

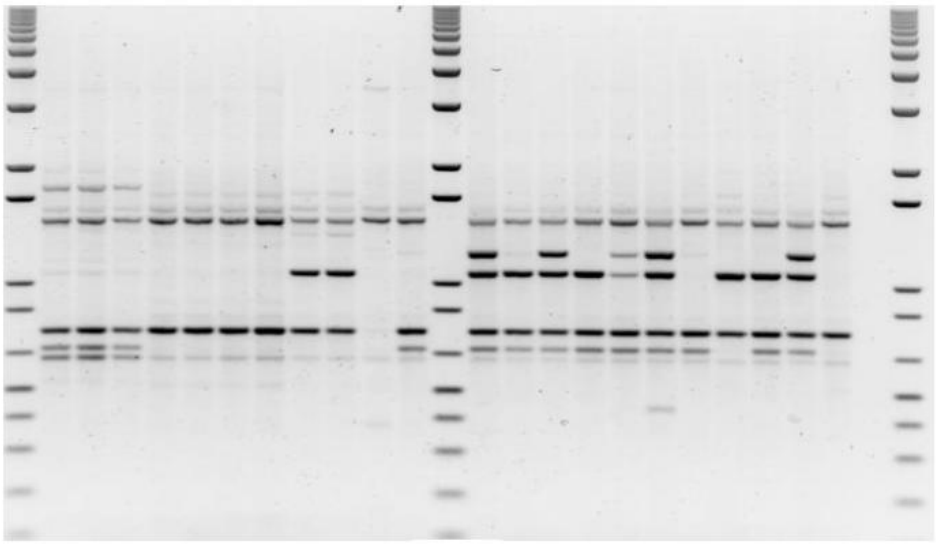

UGANDA
KENYA
Wakiso
Nyandarua
Nakuru
Embu
Kabale
Nairobi
Kiambu
Meru
Taita-Taveta
TANZANIA



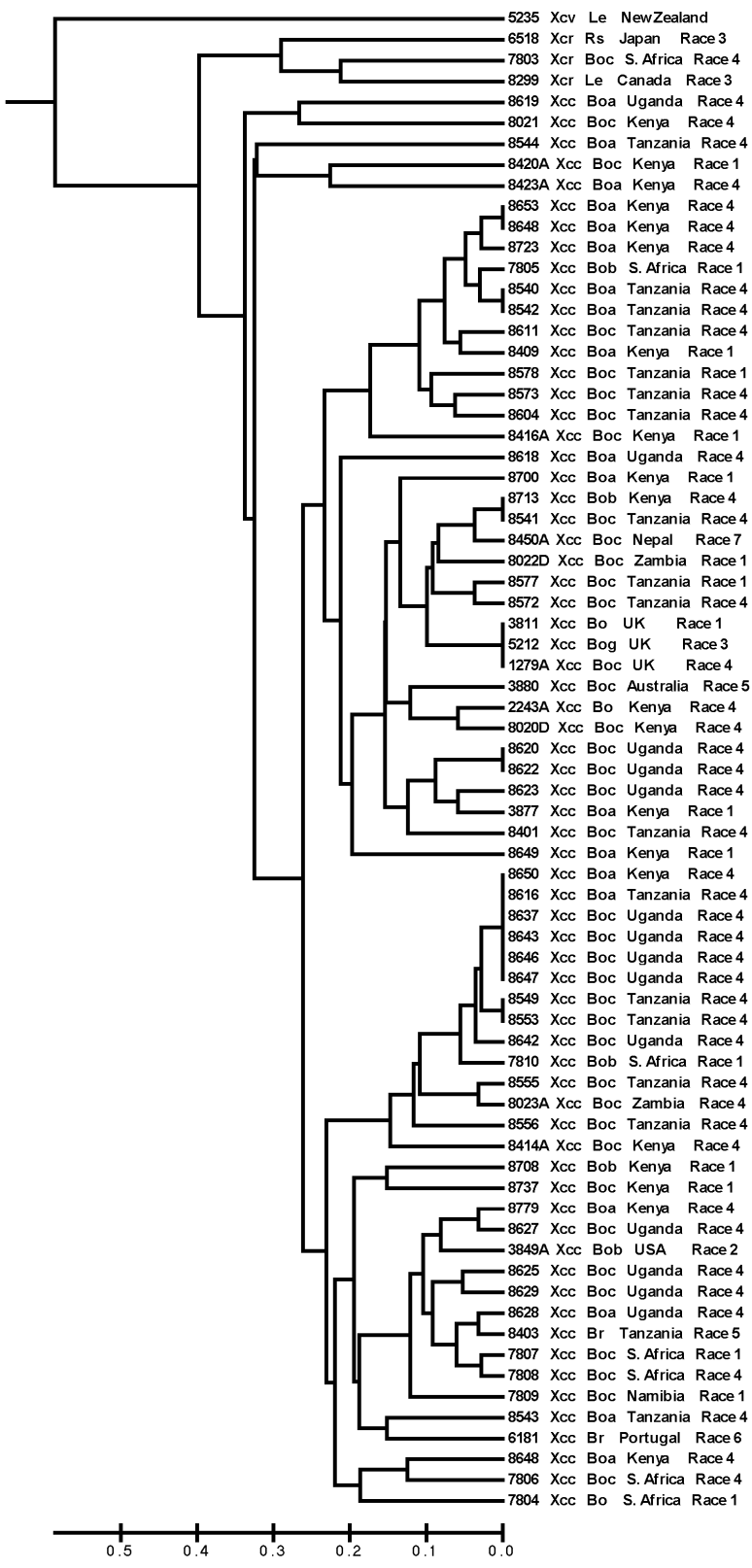
A



B



C


5235 Xcv Le NewZealand
6518 Xcr Rs Japan Race 3
7803 Xcr Boc S. Africa Race 4
8299 Xcr Le Canada Race 3
8619 Xcc Boa Uganda Race 4
8021 Xcc Boc Kenya Race 4
8544 Xcc Boa Tanzania Race 4
8420A Xcc Boc Kenya Race 1
8423A Xcc Boa Kenya Race 4
8653 Xcc Boa Kenya Race 4
8648 Xcc Boa Kenya Race 4
8723 Xcc Boa Kenya Race 4
7805 Xcc Bob S. Africa Race 1
8540 Xcc Boa Tanzania Race 4
8542 Xcc Boa Tanzania Race 4
8611 Xcc Boc Tanzania Race 4
8409 Xcc Boa Kenya Race 1
8578 Xcc Boc Tanzania Race 1
8573 Xcc Boc Tanzania Race 4
8604 Xcc Boc Tanzania Race 4
8416A Xcc Boc Kenya Race 1
8618 Xcc Boa Uganda Race 4
8700 Xcc Boa Kenya Race 1
8713 Xcc Bob Kenya Race 4
8541 Xcc Boc Tanzania Race 4
8450A Xcc Boc Nepal Race 7
8022D Xcc Boc Zambia Race 1
8577 Xcc Boc Tanzania Race 1
8572 Xcc Boc Tanzania Race 4
3811 Xcc Bo UK Race 1
5212 Xcc Bog UK Race 3
1279A Xcc Boc UK Race 4
3880 Xcc Boc Australia Race 5
2243A Xcc Bo Kenya Race 4
8020D Xcc Boc Kenya Race 4
8620 Xcc Boc Uganda Race 4
8622 Xcc Boc Uganda Race 4
8623 Xcc Boc Uganda Race 4
3877 Xcc Boa Kenya Race 1
8401 Xcc Boc Tanzania Race 4
8649 Xcc Boa Kenya Race 1
8650 Xcc Boa Kenya Race 4
8616 Xcc Boa Tanzania Race 4
8637 Xcc Boc Uganda Race 4
8643 Xcc Boc Uganda Race 4
8646 Xcc Boc Uganda Race 4
8647 Xcc Boc Uganda Race 4
8549 Xcc Boc Tanzania Race 4
8553 Xcc Boc Tanzania Race 4
8642 Xcc Boc Uganda Race 4
7810 Xcc Bob S. Africa Race 1
8555 Xcc Boc Tanzania Race 4
8023A Xcc Boc Zambia Race 4
8556 Xcc Boc Tanzania Race 4
8414A Xcc Boc Kenya Race 4
8708 Xcc Bob Kenya Race 1
8737 Xcc Boc Kenya Race 1
8779 Xcc Boa Kenya Race 4
8627 Xcc Boc Uganda Race 4
3849A Xcc Bob USA Race 2
8625 Xcc Boc Uganda Race 4
8629 Xcc Boc Uganda Race 4
8628 Xcc Boa Uganda Race 4
8403 Xcc Br Tanzania Race 5
7807 Xcc Boc S. Africa Race 1
7808 Xcc Boc S. Africa Race 4
7809 Xcc Boc Namibia Race 1
8543 Xcc Boa Tanzania Race 4
6181 Xcc Br Portugal Race 6
8648 Xcc Boa Kenya Race 4
7806 Xcc Boc S. Africa Race 4
7804 Xcc Bo S. Africa Race 1
0.5
0.4
0.3
0.2
0.1
0.0