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Characterization, genetic diversity and distribution of *Xanthomonas campestris* pv. *campestris* races causing black rot disease in cruciferous crops of India

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Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Xcc) is a disease of crucifer crops. The objective of this study was to characterize races of Xcc, their distribution and genetic diversity in India. Two hundred and seventeen isolates of bacteria were obtained from 12 different black rot-infected crucifer crops from 19 states of India; these were identified as Xcc based on morphology, *hrp* gene and 16S rRNA gene based molecular markers and pathogenicity tests. Characterization of races was performed by using a set of seven differential crucifer hosts, comprising two cultivars of turnip (*Brassica rapa* var. *rapa*) and cultivars of Indian mustard (*B. juncea*), Ethiopian mustard (*B. carinata*), rapeseed mustard (*B. napus*), cauliflower (*B. oleracea*) and cabbage (*B. oleracea* var. *sabauda*). Races 1, 4 and 6 of Xcc were identified and, among these races, race 1 followed by race 4 dominated most of the states of India. Genetic diversity of the Indian isolates of Xcc was analysed using repetitive sequence-based PCR (rep-PCR) including primers for REP (repetitive extragenic palindromic), ERIC (enterobacterial repetitive intergenic consensus) and BOX (amplifying

with BOX A1 R primer) repetitive elements. This method of fingerprinting grouped the isolates into 56 different DNA types (clusters) with a 75% similarity coefficient. Among these clusters, DNA types 22 and 53 contained two different races 1 and 4, whereas DNA type 12 contained races 1, 4 and 6. However, no clear relationship was observed between fingerprints and races, hosts or geographical origin.

Keywords: Brassica, crucifers, genetic diversity, races, rep-PCR, *Xanthomonas campestris* pv. *campestris*

Introduction

Black rot disease of crucifers, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is an important disease across the world. Economically, the most important host species in the Brassicaceae family is *Brassica oleracea*; this species includes cole crops, e.g. cauliflower (*Brassica oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), kohlrabi (*B. oleracea* var. *gongylodes*), broccoli (*B. oleracea* var. *italica*), Brussels sprouts (*B. oleracea* var. *gemmifera*), kale (*B. oleracea* var. *acephala*), radish (*Raphanus sativus*), turnip (*B. rapa* var. *rapa*), Indian mustard (*B. juncea*), vegetable mustard (*B. juncea*) and black mustard (*B. nigra*), and other cruciferous crops such as weeds, and ornamentals can also be attacked. The bacterium enters the plant system through infected seeds, wounds and hydathodes. The disease is characterized by developing V-shaped yellow sectors with blackened veins along the margin of leaves; later these sectors enlarge, become brown, necrotic and papery, and may cover the whole leaf. For control of black rot disease, the best management strategy is planting disease resistant varieties. However, black rot resistance, particularly in cole crops (*B. oleracea*), is usually race-specific (Vicente *et al*., 2001), meaning that resistance breeding

programmes should be locally targeted and germplasm should be screened against locally prevalent races of Xcc.

For characterization of the bacteria at species and pathovar level, classical methods (Schaad *et al.*, 2001) and advanced molecular techniques, especially those based on PCR, are very powerful (Massimo *et al*., 2007; Singh & Dhar, 2011). In the PCR-based technique, a set of primers is developed from different conserved genes of bacteria, which is specific to a particular group of bacteria and identifies the bacteria at species or pathovar level (Vincell & Tisserat, 2008; Singh & Dhar, 2011). Previous studies have investigated the potential for specific amplification of the *hrp* (hypersensitive response and pathogenicity) genes to identify and detect *X. axonopodis* pv. *vesicatoria* in tomatoes and capsicums and *X. campestris* pathovars in crucifers (Berg *et al*., 2006; Singh & Dhar, 2011). The *hrp* gene cluster is crucial for the interaction between plant pathogenic bacteria and their hosts, resulting in disease in susceptible plants or the hypersensitive response in resistant plants (Walton, 1997). The *hrp* gene clusters are largely conserved among phytopathogenic bacteria, where they encode type III secretion systems that deliver pathogenicity factors, elicitors and avirulence proteins to the plant cell (Bonas, 1994; Hueck, 1998).

Pathovars of *X. campestris* can be subdivided into races on the basis of differential responses caused by pathogen on various crucifer hosts. A postulated gene-for-gene model to explain the relationship between races and cultivars of crucifer was previously described by Vicente *et al*. (2001) within Xcc. The model presented was the simplest hypothesis involving the smallest number of genes necessary to explain the observed interactions. However, nine races of Xcc have been identified based on the interaction between differentials with *R* genes and avirulence genes of the bacterial pathogen across the world. Races 1 and 4 predominate worldwide, whereas other races 2, 3 and 5 are rare, and race 6 has been reported only in *B. rapa* (Vicente *et al.*, 2006).

Assessment of the genetic variability of a bacterium can facilitate both its detection and the investigation of its taxonomy and epidemiology. Genomic fingerprinting by PCR amplification, with primers specific to highly conserved, repetitive elements such as the 35–40 bp repetitive extragenic palindromic (REP) sequence, the 124–127 bp enterobacterial repetitive intergenic consensus (ERIC) and the 154 bp BOX element, has been used successfully to characterize a large number of bacteria and also differentiates closely related strains of bacteria (Versalovic *et al*., 1991). Repetitive sequence-based PCR (rep-PCR) is a rapid, low-cost, and reliable method that has been extensively used to assess the genetic diversity of Xcc strains (Lema *et al*., 2012; Mulema *et al*., 2012).

Xanthomonas campestris pv. *campestris* is found widely in the tropics, subtropics and temperate climatic conditions in India (Singh *et al*., 2011), but no information is available on the races of Xcc and their distribution in different agroclimatic regions. Understanding local bacterial pathogen races and their genetic diversity is the first step to successful plant breeding and integrated disease management programmes. The purpose of this study was to characterize the races of Xcc isolates collected from different crucifer crops from different agroclimatic conditions and investigate their genetic diversity and geographic distribution. The resulting distribution and race profiling of the pathogen will be used to optimize a breeding programme for black rot resistance, particularly in cole crops, for Indian farmers.

Materials and methods

Isolation and characterization of Xcc

Leaf samples from one or two plants with black rot disease (V-shaped lesions with blackened veins) were collected per field from 12 crucifer crops: cauliflower, cabbage, kohlrabi,

broccoli, Brussels sprouts, kale, radish, turnip, Indian mustard, Ethiopian mustard, vegetable mustard, black mustard. Black rot disease samples were collected from 12 of the 15 agroclimatic regions of India, covering 19 major states of the country, where the majority of crucifer crops are grown (Table S1). The diseased samples were dried between sheets of paper at room temperature before isolation of Xcc. A loopful of suspension was streaked on nutrient sucrose agar medium containing 23 g nutrient agar, 20 g sucrose and 5 g agar powder per litre and incubated at 28 °C for 48 h (Schaad *et al*., 2001). A typical Xcc colony (pale yellow, raised, mucoid) from each plate was subcultured on YGCA slants containing 10 g yeast extract, 10 g D-glucose anhydrous, 20 g calcium carbonate and 20 g agar powder in 1 L water (Schaad *et al*., 2001). The cultures were routinely grown on YGCA medium and stored at –80 °C in a mixture of nutrient broth and glycerol (100%) in a ratio of 1:1 for further study. Representative isolates of races 1, 4 and 6 were deposited in the Indian Type Culture Collection (ITCC) New Delhi, India with accession numbers Xcc-C102 (ITCC-BH-0009) and Xcc-C12 (ITCC-BH-0010) for race 1, Xcc-C30 (ITCC-BH-0011) and Xcc-C106 (ITCC-BH-0013) for race 4 and Xcc-C278 (ITCC-BH-0012) for race 6.

Pathogenicity test

The suspected isolates of Xcc were tested for their pathogenicity on susceptible cauliflower cv. Pusa Sharad seedlings, which had been grown in the field at the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. The three youngest leaves of 30-day-old plants were inoculated using small scissors that had been dipped in a suspension from a 48-hour-old culture of Xcc grown on NSA medium at 28°C (Singh *et al*., 2011). The black rot disease reaction was recorded 15 days after pathogen inoculation.

Race characterization

Race characterization of 217 isolates of Xcc isolated from different agroclimatic regions of India was performed using a set of seven cultivars of *Brassica* species: turnip (*B. rapa* var. *rapa*) 'Just Right' F_1 and 'Seven Top' turnip, Indian mustard (*B. juncea*) 'Florida Broad Leaf', Ethiopian mustard (*B. carinata*) PI 199947, rapeseed mustard (*B. napus*) 'Cobra' line 14R, cauliflower (*B. oleracea*) 'Miracle' F_1 and cabbage (*B. oleracea* var. *sabauda*) 'Wirosa' F_1. The cultivars and accessions of *Brassica* spp. used for race typing were as described by Vicente *et al*. (2001, 2006). The seeds of these differential lines were obtained from University of Warwick, UK and from Otis S. Twilley Seed Co. Inc. (*B. juncea*, *B. rapa* var. *rapa* (EC732033 to EC732035)). A 48-h-old culture of each isolate of Xcc was pelleted and resuspended in sterilized distilled water to give an OD of 0.1 at 600 nm using a spectrophotometer. The suspensions were then used to inoculate 35-day-old plants of Just Right turnip F_1, Seven Top turnip, Florida Broad Leaf, PI 199947 and Cobra 14R and 30-day-old plants of Miracle F_1 and Wirosa F_1 plants. Leaves were inoculated by clipping secondary veins, near the margins, with small scissors dipped in the bacterial suspension. Ten points of inoculation were made in the youngest leaves on each plant, with three replications. The number of infections per leaf and the severity of symptoms were assessed 15 and 30 days after inoculation. Symptoms were rated on a scale of 0–9, based on the relative size of the largest lesion on the leaf, as described by Vicente *et al*. (2001).

DNA extraction and molecular characterization by PCR of a *hrp* gene

The isolates of Xcc were grown in nutrient broth for 24 h at 28 °C. The total genomic DNA of the bacteria was extracted by the CTAB method (Murray & Thompson, 1980). Molecular characterization of 217 isolates of Xcc was performed using PCR of a *hrp* gene, as described

by Singh *et al*. (2014). Two primers, designed to amplify the 3′ end region of the *hrpF* gene locus 3521496 to 3522264 with product size 769 bp were used: Dhrp_Xcc_F 5′-GTGGCCATGTCGTCGACTC-3′ and Dhrp_Xcc_R 5′-GAATAAACTGTTTCCCCAATG-3′. Twenty-five microlitres of PCR reaction mixture containing 1 x *Taq* buffer, 0.2 mM dNTPs (0.5 µL), 1.5mM $MgCl_2$, 200 nM forward and reverse primers, 1.2 U GoTaq Flexi DNA polymerase (Promega) and 100ng DNA was used for PCR amplification. The PCR was carried out under the following conditions using a C-1000 gradient thermocycler (Bio-Rad): 94 °C for 3 min; 40 cycles of 95 °C for 40 s, 60 °C for 40 s and 72 °C for 40 s; and 72 °C for 5 min. Electrophoresis was carried out using 1.2% agarose gel containing ethidium bromide at 60 V for 1.5 h. Products were visualized on a Gel Doc XR+ gel documentation system (Bio-Rad) under UV light (300 nm) and photographed using IMAGELAB v. 2.0.1 software (Bio-Rad) for gel analysis.

Molecular characterization by PCR of the 16S rRNA gene

Molecular characterization of Xcc isolates by PCR of the 16S rRNA gene was also performed. Two primers were designed to amplify the 3′ end region of the 16S rRNA gene locus 4561337 to 4562295 of Xcc B100 (accession no. AM920689) to give a product size of 959 bp: Xcc 16S_ F: 5′-GCAAGCGTTACTCGGAATTA-3′ and Xcc16S_R: 5′-TACGACTTCACCCCAGTCAT-3′. The primers were designed using PRIMER3 (www.frodo.wi.nit.edu) and checked for specificity *in silico* using www.insilico.ehu.es. The primers were validated for their universality across *Xanthomonas* and related bacteria by primer BLAST using www.ncbi.nlm.nih.gov. Twenty-five microlitres of PCR reaction mixture containing 5× *Taq* buffer (5 µL), 10 mM dNTPs (0.5 µL), 25 mM $MgCl_2$ (1.5 µL), 10 µM forward and reverse primers (0.5 µL each), *Taq* DNA polymerase (0.24 µL), molecular grade

water and 1 μL DNA (100 ng) was used for PCR amplification. PCR was carried out under the following conditions using a C-1000 gradient thermocycler: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min; and 72 °C for 5 min. The PCR product was mixed with 1 μL loading dye. A 1.2% agarose gel, prepared in 1 × Tris-acetate-EDTA (TAE) buffer containing ethidium bromide (0.5 μg mL^{-1}), was used for electrophoresis, which was carried out at 60 V for 1.5 h. Products were visualized on a Gel Doc XR+ gel documentation system under UV light (300 nm) and photographed using IMAGELAB v. 2.0.1 for gel analysis. Out of 217 isolates, the PCR products of five isolates, representing different races, were purified: Xcc-C102 (race 1, cabbage, Laxminagar, Delhi), Xcc-C12 (race 1, black mustard, Laxminagar, Delhi), Xcc-C30 (race 4, cabbage, Karnataka), Xcc-C 106 (race 4, vegetable mustard, Laxminagar, Delhi) and Xcc-C278 (race 6, cabbage, Laxminagar, Delhi). Purification was performed using a Gel and PCR Clean-Up system (Promega) kit. Sequencing of these purified products was performed by Sanger's method (Applied Biosystem Machine-3130; Chromas Biotech, Bangalore, India). The 16S rRNA gene sequences of these isolates were submitted to NCBI GenBank. The accession number of isolates Xcc-C102, Xcc-C12, Xcc-C30, Xcc-C106 and Xcc-C278 were KM458092, KM458093, KM458094, KM458095 and KR061873, respectively. The sequences obtained were aligned pairwise. Multiple alignments compared to those of the type/reference strains were performed with CLUSTALW v. 1.7 software (Thompson *et al.*, 1997). Phylogenetic trees were generated using MEGA v. 6.0 (Tamura *et al.*, 2013) with default parameters, K2P distance model (Kimura, 1980) and the neighbour-joining algorithm (Saitou & Nei, 1987). Statistical support for tree nodes was evaluated by bootstrap (Felsenstein, 1985) analyses with 1000 samplings.

Genetic diversity by rep-PCR

Genetic diversity of 217 Xcc isolates was assessed by repetitive sequence-based PCR (rep-PCR) with the BOX-PCR (BOXA1R: 5′-CTACGGCAAGGCGACGCTGACG-3′), ERIC-PCR (ERIC-1R: 5′-ATGTAAGCTCCTGGGGATTCAC-3′ and ERIC-2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′), and REP-PCR (REP1R-I 5′-IIIICGICGICATCIGGC-3′ and REP2-I 5′-ICGICTTATCIGGCCTAC-3′) using conditions described by Schaad *et al*. (2001). For REP-, ERIC- and BOX-PCR amplifications were carried out in a final volume of 25 µL. PCR products were analysed by gel electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, documented in Gel Doc XR+ gel documentation system under UV light (300 nm) and photographed using IMAGELAB v. 2.0.1 as described by Singh *et al*. (2011). The positions of bands were assessed visually. The normalized data generated from PCR (BOX-, ERIC- and REP-PCR) fingerprinting profiles were used either separately or combined together for generating a similarity matrix by using SIMQUAL module for the NTSYSPC v. 2.02e. The similarity matrix thus generated was used for cluster analysis by the unweighted pair group method of arithmetic average (UPGMA) using sequential, agglomerative, hierarchical, nested clustering module of NTSYSPC v. 2.02e. The output data were graphically presented as a phylogenetic tree (Rohlf, 1998).

Results

Characterization of bacteria

Two hundred and seventeen isolates of Xcc were isolated from 12 crucifer crops from 12 agroclimatic regions ranging from temperate to subtropical climates, in 19 states of India (Table S1). These isolates produced yellow, translucent, raised, mucoid colonies on NSA

medium. They were Gram-negative, rod shaped, aerobic and with a monotrichous flagellum. All the isolates were pathogenic on cauliflower cv. Pusa Sharad and produced typical black rot symptoms including blackening of veins within 15 days after inoculation. These isolates were considered to be Xcc on the basis of morphological and pathogenicity tests. Amplification of DNA from the isolates using primers Dhrp_Xcc_F and Dhrp_Xcc_R, which are specific to Xcc, confirmed the identification. Amplification was not observed from any isolates belonging to other *Xanthomonas* species such as *X. oryzae* pv. *oryzae* or *X. axonopodis* pv. *punicae* (data not shown).

The nucleotide sequences of the 16S rRNA gene of three races, race 1 (Xcc-C102, Xcc-C12), race 4 (Xcc-C30, Xcc-C106) and race 6 (Xcc-C278) of Xcc were analysed. Four isolates had a more than 99% similarity index to each other and also to Xcc isolates B100 (race 1, China) and 8004 (race 9, cauliflower, UK; Fig. 1). In contrast, Xcc-C106 (race 4), isolated from vegetable mustard cv. Pusa Sag 1 from Delhi, showed a 99% similarity index with Xcc ATCC 33913 (race 3, Brussels sprout, UK), *X. arboricola*, Xcc DBRU and *X. campestris* LMG 5793, *Pseudomonas* sp. DTPF-3 and *Xylella fastidiosa* were used as the out-groups and these showed an 85% similarity index with *Xanthomonas* spp.

Race characterization of Xcc

The Indian isolates belong to three races, i e. races 1, 4 and 6. *Xanthomonas campestris* pv. *campestris* isolates that showed a positive disease reaction in 5 differential cultivars (Wirosa F_1, Just Right turnip, line 14R of Cobra, PI 199947, Florida Broad Leaf Mustard) were designated as race 1 isolates. Race 4 showed positive disease reactions only in 2 cultivars i.e. Wirosa F_1 and Miracle F_1, whereas race 6 showed positive reactions in all seven cultivars (Table 1). Out of 217 isolates of Xcc, 119 isolates belonged to race 1 (54.83%), 97 isolates

belonged to race 4 (44.70%) and a single isolate, Xcc-C278 (from Cabbage, Delhi), belonged to race 6 (0.47%). All three races of Xcc were isolated from cabbage, whereas only race 1 and race 4 infected cauliflower, turnip, broccoli, kohlrabi, Indian mustard, radish, vegetable mustard and black mustard. It was also noted that race 1 also infected kale and race 4 infected Brussels sprouts. Race 1 dominated in all 12 agroclimatic regions of India compared to race 4 and 6. Race 1 was found in most of the states of India except Andhra Pradesh and Orissa, whereas race 4 was found in all the states of India except Meghalaya, Manipur, Goa (Fig. 2); race 6 was rare and found only in cabbage from Delhi.

Genetic diversity by rep-PCR

The genetic diversity of races of *X. campestris* pv. *campestris* was assessed by cluster analysis of the genomic fingerprint patterns obtained from rep-PCR. Three different sets of primers were used and DNA fragments of 300 bp to 6.0 kb were amplified by BOX-PCR, 400 bp to 8.0 kb by ERIC-PCR and 300 bp to 6.0 kb by REP-PCR. The highest number of amplicons was obtained by BOX-PCR (29 amplicons), while 27 amplicons were obtained with ERIC-PCR and 18 amplicons with REP-PCR. The amplicons obtained varied between isolates with each method of PCR (Fig. 3) and all 217 isolates of Xcc clustered into 56 DNA types (clusters) at 75% similarity coefficient. Representative isolates of each cluster are given in Figure 4; among them, DNA type 22 contained the maximum of 57 isolates that were obtained from different agroclimatic conditions and different crucifer hosts such as cauliflower, cabbage, broccoli Indian mustard and black mustard cultivars. The second largest cluster was DNA type 53 (21 isolates), followed by DNA type 12 (16 isolates). DNA types 22 and 53 both consisted of two different races, 1 and 4, whereas DNA type 12 contained races 1, 4 and 6 and was the most genetically diverse group of Xcc isolates (Fig. 4).

Discussion

Black rot disease is widely distributed in all agroclimatic regions of India. Until now, no exhaustive collection, isolation, identification and race characterization of Indian Xcc isolates has been accomplished. In the present study, isolates of Xcc causing black rot were characterized by classical methods (cultural and morphological), pathogenicity tests and molecular techniques including PCR of a *hrp* gene (Berg *et al*., 2006; Singh & Dhar, 2011), which is highly conserved and enables differentiation of pathovars (Singh *et al*., 2014). However, the PCR primers Dhrp_Xcc_F and Dhrp_Xcc_R, based on *hrp* gene sequences, could not differentiate races of Xcc. In addition, nucleotide sequence analysis of the 16S rRNA gene of representative isolates of three races i.e. race 1 (Xcc-C102, Xcc-C12), race 4 (Xcc-C30, Xcc-C106) and race 6 (Xcc-C278) of Xcc, using MEGA 6 software did not distinguish between races. Based on rRNA gene sequence analysis, the isolates of Xcc in this study were very close to isolate B100 from Italy, and Xcc8004 from the UK, apart from isolate Xcc-C106, which was very close to ATCC33913 from China, as reported earlier by Popovic *et al.* (2013). It might be speculated that the Indian strains may have migrated from these countries through seeds. This finding indicates that 16S rRNA gene sequence analysis is a useful tool to characterize the bacteria and also show diversity within the isolates, but cannot be used to identify races.

Worldwide, nine races of Xcc have been identified, based on their interaction with differential cultivars (Kamoun *et al.*, 1992; Ignatov *et al.*, 1999; Vicente *et al.*, 2001; Taylor *et al*., 2002; Fargier & Manceau, 2007; Jensen *et al*., 2010). In this study, the three races 1, 4 and 6, were found in *B. oleracea* crops in India . However races 1 and 4 dominated in most of the agroclimatic regions of India having tropical, subtropical and temperate climates,

indicating that distribution of races is not affected by climatic conditions. This is in agreement with the study of Vicente *et al.* (2001), which reported that races 1 and 4 were predominant worldwide and that the other races, 2, 3, 5 and 6 were rare. Although, in this study, 217 isolates of Xcc were taken from diverse climatic conditions and hosts, races 2, 3 and 5 were not found. However, race 6 was found on cabbage from Delhi, which is in the Trans-Gangatic plains region. Previously, race 6 has only been reported in *B. rapa* and found rarely (Vicente *et al.*, 2006). Jensen *et al*. (2010) reported that races 1, 4 and 6 are the most common in cabbage; blight-like symptoms were produced by a race 7 isolate and by some isolates of races 1, 5 and 6 but no blight symptoms were produced by isolates of race 4. This was contrary to the result of the present study, where all isolates of Xcc produced typical V-shaped black rot disease symptoms on their respective hosts. Hence, for development of varieties resistant to black rot disease, particularly in *B. oleracea* (cole crops), testing with both races 1 and 4, is a minimum requirement to evaluate the germplasm of crops (Soengas *et al*., 2007).

Analysis of the genetic diversity of Xcc clearly indicates that the different DNA types did not originate from the same host and agroclimatic conditions. In addition, polymorphisms resulting from repetitive sequences in bacterial genomes may be used to define differences between species and pathovars. A similar level of separation was observed in previous rep-PCR studies of *Xanthomonas* (Vicente *et al*., 2006; Jensen *et al*., 2010; Singh *et al*., 2011). The data obtained from pathogenicity and genetic variability analysis in this study confirm previous findings describing the heterogeneity within Xcc (Ignatov *et al*., 1999). The isolates within the DNA types revealed complex but polymorphic bands resulting in groups of closely related isolates based on their fingerprint pattern. Isolates recovered from leaf samples from the same host and states/agroclimatic regions with the same race did not fall into one macro- but microclusters spread over the dendrogram, although previous studies had demonstrated a

correlation with races (Jensen *et al*., 2010) or geographic origin (Massimo *et al.*, 2007). In the present study, no correlation was obtained between races and genetic grouping. Thus, a relationship between rep-PCR and races of a given group of isolates may not occur in all investigations, and may depend on the repetitive sequences chosen for the analysis. This study revealed the existence of variability within the Indian isolates of the Xcc population and, among the three races, races 1 and 4 were distributed in most of the agroclimatic conditions in different states of India. This information will be used in future breeding programmes to develop varieties resistant to black rot disease, particularly in cole crops.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1 List of *Xanthomonas campestris* pv. *campestris* isolates obtained from crucifer crops grown in different states of India, year of collection, races, *hrp* gene-based PCR reaction and DNA typing.

Figure legends

Figure 1 Phylogenetic tree of 16S rRNA gene sequences of five Indian isolates of *Xanthomonas campestris* pv. *campestris* (indicated with *) and 24 nucleotide sequences obtained from the NCBI database. The evolutionary history was inferred using the maximum likelihood phylogenetic tree method using MEGA v. 6.0.

Figure 2 Distribution of races of *Xanthomonas campestris* pv. *campestris* in different states of India.

Figure 3 Fingerprinting of isolates of *Xanthomonas campestris* pv. *campestris* collected from different hosts of crucifer by using BOX- (a), ERIC- (b) and REP- (c) PCRs. Lane M: 1.0 kb DNA ladder, lanes 1-56 Xcc-C3, Xcc-C7, Xcc-C20, Xcc-C23, Xcc-C26, Xcc-C98, Xcc-C99, Xcc-C100, Xcc-C105, Xcc-C113, Xcc-C114, Xcc-C118, Xcc-C128, Xcc-C130, Xcc-C132, Xcc-C133, Xcc-C135, Xcc-C137, Xcc-C138, Xcc-C140, Xcc-C141, Xcc-C142, Xcc-C143, Xcc-C144, Xcc-C147, Xcc-C148, Xcc-C150, Xcc-C155, Xcc-C157, Xcc-C158, Xcc-C160, Xcc-C163, Xcc-C167, Xcc-C169, Xcc-C170, Xcc-C173, Xcc-C181, Xcc-C188, Xcc-C196, Xcc-C215, Xcc-C222, Xcc-C230, Xcc-C261, Xcc-C266, Xcc-C268, Xcc-C270, Xcc-C271, Xcc-C272, Xcc-C274, Xcc-C276, Xcc-C279, Xcc-C281, Xcc-C285, Xcc-C286.

Figure 4 Phylogenetic analysis of 217 isolates of *Xanthomonas campestris* pv. *campestris* collected from different hosts of *Brassica* spp. The normalized data from BOX-, REP- and ERIC-PCR of the isolates were used to generate a similarity matrix used for cluster analysis by the unweighted pair group method of arithmetic average (UPGMA), and the results are presented as a phylogenetic tree. The numbers in parentheses indicate the number of isolates in each DNA type. Details of DNA types are given in Table S1.