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Development of species-specific primers for detection of *Xanthomonas campestris* pv. *campestris* causing black rot of crucifers

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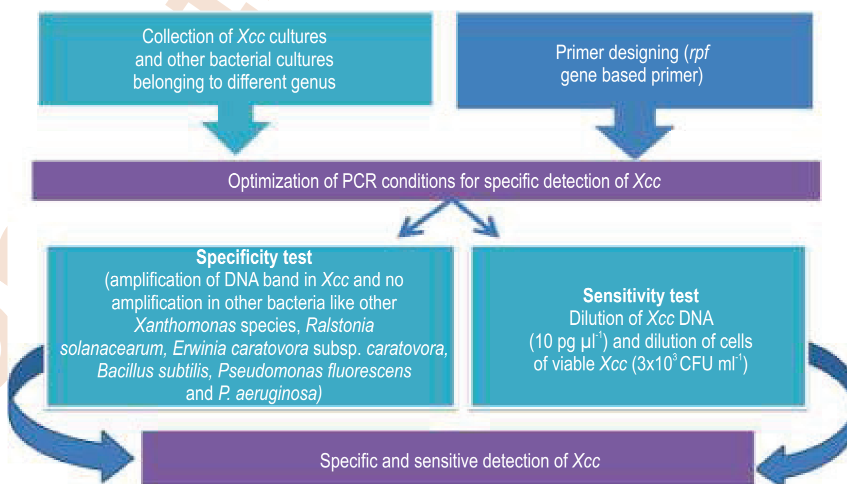
Abstract

Aim : Black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson *(Xcc)* is a major seed-borne disease. The present study aimed to develop a rapid diagnostic protocol for the specific and sensitive detection of this pathogen.

Methodology : A specific primer set was designed based on *rpf* gene and optimization of PCR condition was done for specific detection of *Xcc*. Sensitivity of PCR for primer set was then determined by diluting the *Xcc* DNA and cells.

Results : Specific primer set was able to amplify a specific band of 304 bp in all 11 isolates of *Xcc* but failed to amplify other *Xanthomonas* species and one each of *Ralstonia solanacearum, Erwinia caratovora* subsp. *caratovora, Bacillus subtilis, Pseudomonas fluorescens* and *P. aeruginosa*. The primer set was highly sensitive as it was able to detect 10 pg μl^{-1} bacterial DNA and up to $3x10^3$ CFU ml^{-1} corresponding to 12 viable cells of *Xcc* which were used as template for PCR reaction.



Interpretation : The results suggest that develope d PCR primers are highly specific and sensitive and it can be used to detect the pathogen at an early stage of infection for disease management.

Key words: Black rot, Diagnostic protocol, pv. *campestris, rpf* gene, *Xanthomonas campestris*

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Introduction

Xanthomonas campestris pv. *campestris* (Pammel) Dowson (*Xcc*) causing black rot of crucifers is a major seed-borne disease of crucifers worldwide (William, 1980). The pathogen initially produces marginal chlorotic spots on the leaves followed by darkening or blackening of mid rib and veins (Cook *et al.,* 1952). *Xcc* produces an extracellular polysaccharide (EPS) called xanthan, which obstructs the xylem and causes necrosis of tissues and severe leaf wilting (Williams, 1980). The disease reduces seed vigour, quality of curd and heads in cauliflower and cabbage, respectively, resulting in considerable yield loss of 10-50% (Dhar and Singh, 2014). Therefore, specific and rapid detection of *Xcc* in seeds and plants is essential to prevent introduction and spread of disease.

The classical methods for detection of *Xcc* in seed lots and plants are based on selective media, serological techniques and grow out test. However, these methods are time consuming and cumbersome as compared to PCR based detection methods. PCR based detection methods of plant pathogens have been shown to hold great promise in last two decades (Henson and French, 1993; Singh and Dhar, 2011; Singh *et al.,* 2014; Kumar *et al.,* 2017). It is well known that *rpf* (*regulation of pathogenicity factors*) gene cluster, which is responsible for regulation of pathogenicity factors as it co-ordinates regulation of extracellular enzymes, extracellular polysaccharide synthesis and diffusible signal factor (DSF) signal synthesis (Dow and Daniels, 1994; O'Cornell *et al.,* 2013). It has been demonstrated by transposon mutagenesis that at least eight genes (*rpf A* to *H*) exists, which co-ordinates reduction in the levels of all extracellular enzymes tested (Tang *et al.,* 1991).

The *rpfH* gene, which encodes elements of two-component sensory-transduction system (Tang *et al.,* 1991; Slater *et al.,* 2000; Dow *et al.,* 2000), is present in *Xcc* and *X. campestris.* pv. *euvasicatoria* (*Xcv*), while absent in *Xanthomonas axonopodis* pv. *citri* (*Xac*) and *X. oryzae* pv. *oryzae* (*Xoo*) (Potnis *et al.,* 2011). Therefore, specific primers were designed from *rpfH* region to prevent cross species amplification in PCR. The aim of the present study was to develop a sensitive and specific PCR based assay for the detection of *Xcc.*

Materials and Methods

Bacterial cultures : Ten isolates of *Xcc* used were isolated from infected cabbage and cauliflower leaves obtained from different regions of India and one isolate was obtained during post entry quarantine inspection of cabbage, which was imported from China (Table 1). Diseased portion of leaf was surface sterilized using 1% sodium hypochlorite for 30 sec followed by 70% ethanol for 30 sec and two times washing with distilled water for one minute each. Small diseased portion from diseased leaf was chopped with the help of sterilized blade on a clean glass slide having a drop of water and allowed to ooze out bacteria. Now, with the help of loop, this water was streaked on Nutrient Sucrose Agar (NSA) medium. Yellow mucoid, glistering and convex colonies were obtained. These colonies were further sub-cultured into fresh media for obtaining pure colonies of *Xcc*. These cultures were maintained on Yeast Dextrose Carbonate Agar (YDCA) medium (1% yeast extract, 2% dextrose, 2% calcium carbonate and 1.5% agar) at 4˚C for further use. Cultures of other bacteria *viz. X. campestris.* pv. *euvasicatoria, X. oryzae* pv. *oryzae, X. axonopodis* pv. *punicae, X. citri* subsp. *citri, Ralstonia solanacearum, Erwinia caratovora* subsp. *caratovora, Bacillus subtilis, Pseudomonas fluorescens* and *Pseudomonas aeruginosa* were procured from Bacteriology Laboratory, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, The Microbial Type Culture Collection and Gene Bank, Chandigarh and ICAR-National Bureau of Agriculturally Important Microorganisms, Mau, India (Table 1).

Extraction of Genomic DNA : Pure culture of eleven isolates of *Xcc* and other test bacteria (*X. campestris.* pv. *euvasicatoria, X. oryzae* pv. *oryzae, X. axonopodis* pv. *punicae, X. citri subsp. citri, Ralstonia solanacearum, Erwinia caratovora* subsp. *caratovora, Bacillus subtilis, Pseudomonas fluorescens* and *Pseudomonas aeruginosa*) were inoculated in pre-autoclaved 15 ml tube containing 5 ml nutrient sucrose broth media, incubated at 28˚C for 24 hrs at 140 rpm and DNA was extracted as described by Pitcher *et al.* (1989).

Primer design and PCR amplification : A set of forward and reverse primers, rpfH_F 5'AGTTGCAGCAGCTGTTCT3' and rpfH_R 5'ATAGCACGTATTGGCAGGG3', respectively, was designed from an *rpfH* region of *rpf* gene family of *X. campestris* pv. *campestris* strain ATCC 33913 chromosome (NC_003902.1), which predicted a PCR products of 304 bp. DNA sequences from *rpfH* gene were randomly selected from the draft genome and a standard nucleotide BLASTN search was performed on NCBI database (http://www.ncbi.nlm.nih.gov). Through BLASTN analysis, regions unique to *Xcc* were selected to provide specific target sequences for specific primer design. The primers were designed using PrimerQuest Tool of IDT (https://eu.idtdna.com/Primer Quest). PCR amplification was performed with a final volume of 25 μl containing 2.5 μl of 10X reaction buffer, 0.2 mM dNTPs mix, 1 U Taq DNA polymerase (New England BioLabs), 10 pM of each forward and reverse primer and 100 ng μl^{-1} template DNA. The PCR programme consisted of initial denaturation at 95˚C for 3 min followed by 33 cycles at 95˚C for 30 sec, 63˚C for 45 sec and 72˚C for 45 sec and a final extension at 72˚C for 5 min. PCR reactions were performed using a Bioer GenePro PCR thermocycler. The amplified PCR products were separated by gel electrophoresis using 1.2% agarose in 1X TBE buffer and run at 100 V for 60 min.

Specificity testing of PCR primers : The specificity of primers were assessed using DNA extracted from a collection of pure bacterial cultures. Eleven isolates of *Xcc* along with nine cultures of other bacteria like *X. campestris.* pv. *euvasicatoria, X. oryzae* pv. *oryzae, X. axonopodis* pv. *punicae, X. citri* subsp. *citri, Ralstonia solanacearum, Erwinia caratovora* subsp. *caratovora,*

Table 1 : Details of bacterial isolates used in this study

Bacterial species	Bacterial isolates	Host	Place/Source of collection
Xanthomonas campestris pv. *campestris*	Xcc-C218	Cauliflower	Himachal Pradesh, India
	Xcc- C219	Cauliflower	Himachal Pradesh,India
	Xcc- C220	Cauliflower	Himachal Pradesh,India
	Xcc- C221	Cauliflower	Himachal Pradesh, India
	Xcc- C222	Red cabbage	Himachal Pradesh, India
	Xcc- C223	Cauliflower	Himachal Pradesh, India
	Xcc- C224	Cabbage	Himachal Pradesh, India
	Xcc-C211	Cauliflower	Karnataka, India
	Xcc-C197	Cauliflower	West Bengal, India
	Xcc-C201	Cauliflower	West Bengal, India
	XccP1	Cabbage	China (During post entry quarantine from Syngenta India Limited Research Farm, Karnal)
X. campestris pv. *euvasicatoria*	*XCVT-1*	Tomato	Himachal Pradesh, India
X. oryzae pv. *oryzae*	*BB1*	Rice	Haryana, India
X. axonopodis pv. *punicae*	-	Pomegranate	ICAR-IARI, New Delhi, India
X. citri subsp. *citri*	-	Acid lime	ICAR-IARI, New Delhi, India
Ralstonia solanacearum	-	Tomato	Uttarakhand, India
Erwinia caratovora subsp. *caratovora*	-	-	ICAR- NBAIM, Mau, India
Bacillus subtilis	-	Tomato	ICAR-IARI, New Delhi, India
Pseudomonas fluorescens	-	-	MTCC, Chandigarh, India
Pseudomonas aeruginosa	-	-	ICAR-IARI, New Delhi, India

ICAR-NBAIM: National Bureau of Agriculturally Important Microorganisms; MTCC : Microbial Type Culture Collection and Gene Bank, ICAR-IARI: Indian Agricultural Research Institute

Bacillus subtilis, Pseudomonas fluorescens and *Pseudomonas aeruginosa* were used for specificity test.

Sensitivity testing of PCR primers : The sensitivity of PCR was tested by using dilution of DNA and bacterial cells separately. To analyse the sensitivity of PCR assay, 100 ng of DNA was diluted from 10^{-1} to 10^{-6} dilution and PCR amplification was done from each dilution of DNA as template with other PCR components as described earlier. In dilution of cell procedure, the *Xcc* was grown on YDCA medium for 48 hrs at 28°C, a single colony was picked using a sterile toothpick and re-suspended in 1 ml of sterile water making a bacterial suspension of approximately 10^{12} CFU ml^{-1}. The sample was thoroughly mixed to ensure uniform distribution of bacteria. The sample was serially diluted in tenfold increments using sterile double distilled water up to a dilution of 10^{-10} to give end concentration of cells ranging from 10^{12} to 10^{2} CFU ml^{-1}. From each of the diluted samples, 100 µl of the bacterial suspension was plated on YDCA media in duplicate. The samples were incubated at 28°C for 48 hrs. The colonies formed were manually counted and the CFU ml^{-1} were calculated using formulae CFU ml^{-1} = (no. of colonies x dilution factor)/ volume of culture plate. The remaining 800 µl portion of the suspension in each Eppendorf tube was kept at 95°C for 5 min and then 4 µl of it was used as a template in PCR.

Results and Discussion

The *Xcc* specific primers were designed from *rpfH* gene region of *rpf* family of *Xanthomonas campestris* pv. *campestris* str. ATCC 33913 chromosome (Fig.1). The specific primer sequences were tested in silico by BLASTN sequence database (http://www.blast.ncbi.nlm.nih.gov/) of National Center for Biotechnology Information (NCBI) and no significant matches were detected with other *Xanthomonas* spp. sequences. When genomic DNA sequence of *Xcc* was compared with other Xanthomonad (*Xanthomonas axonopodis* pv. *citri*), a significant difference was evident, and one of these differences is in a region contained genes that regulate the expression of pathogenicity factors, the *rpf* gene (da Silva, 2002). The *rpf H* gene from *rpf* cluster, present in *Xcc,* was not present in *Xac* and *Xoo* (Dow *et al.,* 1994; Potnis *et al.,* 2011). Hence, this difference promoted to develop a new specific diagnostic primer set for *Xcc.* As PCR assay have been used successfully for the identification and detection of important Xanthomonads such as *Xanthomonas campestris* pv. *musacearum* (Adikini *et al.,* 2011), *Xanthomonas campestris* pv. *vesicatoria* (Leite *et al.,* 1994; Park *et al.,* 2009), *Xanthomonas axonopodis* pv. *citri* (Park *et al.,* 2006), *Xanthomonas campestris* pv. *glycines* (Oh *et al.,* 1999) etc., and different specific gene based primers were used for the detection of *Xcc* like *hrpF* gene (Park *et al.,* 2004; Berg *et al.,* 2005; Singh *et al.,* 2014), hrcC (Zaccardelli *et al.,* 2007). To the best of our knowledge, *rpf* gene based primer set was first time explored for specific detection of *Xcc.*

When specificity test was done using two *Xcc* isolates (Xcc-C218 and Xcc-C219) and other test bacteria, amplification of 304 bp DNA band was observed in both the isolates of *Xcc,* whereas no amplification was observed in other *Xanthomonas*

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Fig. 1 : Design of specific primer set for *Xcc* based on *rpfH* gene. Underlined sequences represent nucleotide sequence of forward (rpfH_F) and reverse (rpfH_R) primers.

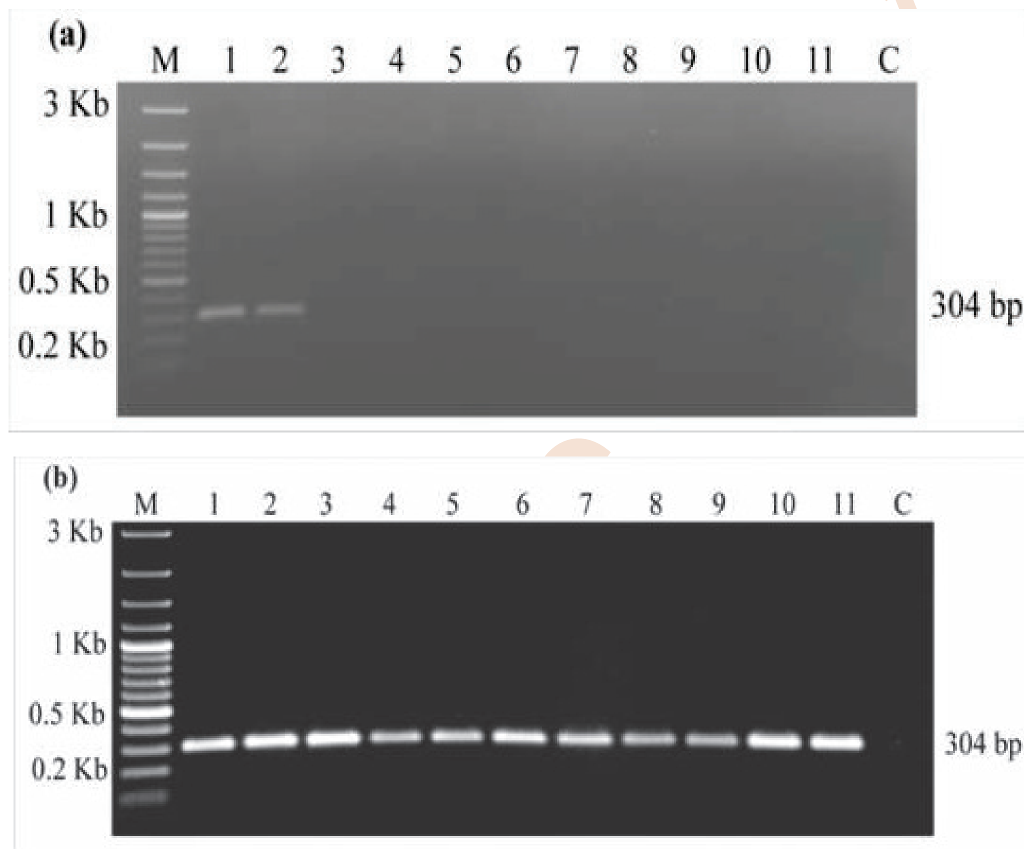

Fig. 2 : Specific amplification of *X. campestris* pv. *campestris* targets by PCR using primer set rpfH_F and rpfH_R. Lane M-100 bp plus DNA ladder; (a) Lanes 1 and 2- *Xcc isolates* (Xcc-C218 and Xcc-C219), Lane 3- *X. campestris.* pv. *euvasicatoria,* Lane 4- *X. oryzae* pv. *oryzae,* Lane 5- *X. axonopodis* pv. *punicae,* Lane 6- *X. citri* subsp. *citri,* Lane 7- *Ralstonia solanacearum,* Lane 8- *Erwinia caratovora* subsp. *caratovora,* Lane 9- *Bacillus subtilis,* Lane 10- *Pseudomonas fluorescens,* Lane 11- *P. aeruginosa* and Lane C- Negative control; (b) Lanes 1 to 11- *X. campestris* pv. *campestris* isolates viz. Xcc-C218, Xcc- C219, Xcc- C220, Xcc- C221, Xcc- C222, Xcc- C223, Xcc- C224, Xcc-C211, Xcc-C197, Xcc-C201, XccP1, Lane C- Negative control.

spp. and other test bacteria (Fig. 2a). PCR was performed at different annealing temperature (48 to 69˚C) and optimum time (30 sec and 45 sec) using this primer set, with appropriate negative control. It was identified that the best temperature and time for annealing of this primer set was 63˚C for 45 sec. All the eleven *Xcc* isolates used in this study showed positive result by this primer set (Fig. 2b). Similar results were obtained when *rpf* gene based primers were designed for the detection of *Xanthomonas axonopodis* pv. *citri* in infected citrus plants (Coletta-Filho *et al.,* 2006).

Primers sets should not only be specific but also sensitive to detect small quantity of pathogen as these can be used as a diagnostics in quarantine and inspection. These primers were

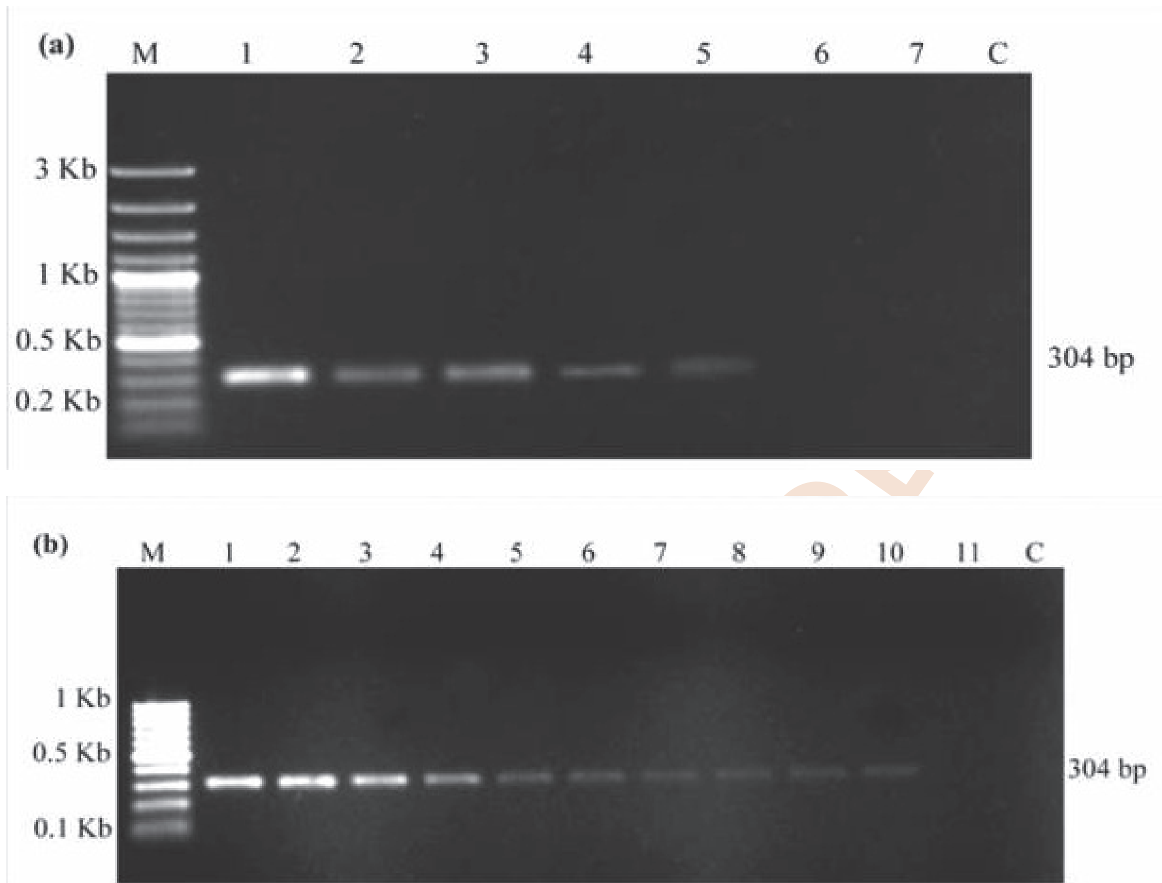

Fig. 3 : Sensitivity of PCR amplification for *X. campestris* pv. *campestris* using of primer set rpfH_F and rpfH_R; (a) tenfold dilution of DNA. Lane M- 10 0 bp plus DNA ladder; Lane 1- 100 ng μl^{-1}, Lane 2- 10 ng μl^{-1}, Lane 3- 1 ng μl^{-1}, Lane 4- 100 pg μl^{-1}, Lane 5- 10 pg μl^{-1}, Lane 6-1 pg μl^{-1}, Lane 7- 0.1 pg μl^{-1}, Lane C- Negative control; (b) tenfold-diluted cells (10^{12} CFU ml^{-1}). Lane M- 100 bp DNA ladder; Lane 1- 2.3×10^{12} CFU ml^{-1}; Lanes 2 to 11- 10^{-1} to 10^{-10} dilution.

found highly sensitive as it could detect DNA upto 10 pg. The sensitivity of PCR assay was tested by serial dilution of bacterial DNA. A 100 ng concentration of DNA was taken and diluted up to 10^{-6} dilution. A single 304 bp amplification was seen in 100 ng, 10 ng, 1 ng and faint bands were seen in 100 pg and 10 pg concentration of DNA, no amplification was obtained when DNA was further diluted (Fig. 3a). Similar results were reported by Back *et al.* (2015) where detection limit was 10 pg μl^{-1} for XPN primer used for detection of *X. axonopodis* pv. *poinsettiicola* and XHG for the detection of *X. hyacinthi.* Whereas, detection limit was 100 pg μl^{-1} DNA of *Xcc* was reported by Singh *et al.* (2014) using *hrp*F gene based primers.

The sensitivity of PCR assay was determined using a ten-fold dilution series of cells of *Xcc,* the expected PCR fragment product was amplified in sample up to 3×10^{3} CFU ml^{-1}. The initial sample was having 2.3×10^{12} CFU ml^{-1} and this primer set was able to detect upto 10^{-9} dilution *i.e.,* about 12 cells per reaction of PCR (Fig. 3b). Unlike other primers based on *hrp* region of the *Xcc, rpf* gene based primer set was more sensitive as it has detected upto 3×10^{3} CFU ml^{-1} (approximately 12 cells per PCR reaction), whereas *hrp* gene based primers detected 1.3×10^{4} CFU ml^{-1} of *Xcc* (Park *et al.,* 2004). Similarly, a detection limit of 10^{3} CFU ml^{-1} *i.e.,* about 4 cells per reaction was observed in *Xanthomonas campestris* pv. *musacearum* using seven primer sets (Adikini *et al.,* 2011).

Black rot of cabbage and cauliflower caused by *Xcc* is very important regulated non-quarantine pathogen (PQ Order 2003, amended till May 2017). The results of this study revealed that this *rpf* gene based primers were useful for the sensitive, rapid and routine detection of *Xcc* in quarantine laboratories and probably used in detection of field infected samples and for certification. The fast detection of causal agent of black rot disease also permits timely deployment of control strategies to prevent spread of the disease.

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References

Adikini, S., L. Tripathi, F. Beed, G. Tusiime, E.M. Magembe and D.J. Kim: Development of a specific molecular tool for detecting *Xanthomonas campestris* pv. *musacearum*. *Plant Pathol.,* **60**, 443-452 (2011).

Back, C.G., S.Y. Lee, B.J. Lee, M.C. Yea, S.M. Kim, I.K. Kang, J.S. Cha and H.Y. Jung: Development of a species-specific PCR assay for three *Xanthomonas species,* causing bulb and flower diseases, based on their genome sequences. *The Plant Pathol. J.,* **31**, 212-218 (2015).

Berg, T., L. Tesoriero and D.L. Hailstones: PCR-based detection of *Xanthomonas campestris* pathovars in *Brassica* seed. *Plant Pathol.,* **54**, 416-427 (2005).

Coletta-Filho, H.D., M. A. Takita, A.A. de Souza, J.R. Neto, S.A.L. Destefano, J.S. Hartung and M.A. Machado: Primers based on the *rpf* gene region provide improved detection of *Xanthomonas axonopodis* pv. *citri* in naturally and artificially infected citrus plants. *J. Appl. Microbiol.,* **100**, 279-285 (2006).

O'Connell, A., S.-Q. An, Y. McCarthy, F. Schulte, K. Niehaus, Y.Q. He, J.L. Tang, R.P. Ryan and J.M. Dow: Proteomics analysis of the regulatory role of Rpf/DSF cell-to-cell signaling system in the virulence of *Xanthomonas campestris. Mol. Plant Microbe Interact.,* **26**, 1131–1137 (2013).

Cook, A.A., R.H. Larson and J.C. Walker: Relation of the black rot pathogen to cabbage seed. *Phytopathology,* **42**, 316-320 (1952).

da Silva, A.C. Rasera, J.A. Ferro, F.C. Reinach, C.S. Farah, L.R. Furlan, R.B. Quaggio and C.B. Monteiro-Vitorello: Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature,* **417**, 459-463 (2002).

Dhar, S. and D. Singh: Performance of cauliflower genotypes for yield and resistance against black rot (*Xanthomonas campestris* pv. *campestris*). *Indian J. Hort.,* **71**, 197-201 (2014).

Dow, J.M. and M.J. Daniels: Pathogenicity determinants and global regulation of pathogenicity of *Xanthomonas campestris* pv. *campestris.* In: Bacterial Pathogenesis of Plants and Animals (Ed.: J.L. Dangl). Springer Berlin Heidelberg, pp. 29-41 (1994).

Dow, J.M., J. Feng, C.E. Barber, J. Tang and M.J. Daniels: Novel genes involved in the regulation of pathogenicity factor production within the *rpf* gene cluster of *Xanthomonas campestris. Microbiology,* **146**, 885-891(2000).

Henson, J.M. and R. French: The polymerase chain reaction and plant disease diagnosis. *Annu. Rev. Phytopathol.,* **31**, 81-109 (1993).

Kumar, P., R. Kiran, A. Kandan, J. Akhtar, B. Singh, K. Nair and S.C. Dubey: Detection of *Xanthomonas campestris* pv. *campestris* in imported germplasm of cabbage during post entry quarantine. *Indian Phytopathol.,* **70**, 413-417 (2017).

Leite, R.P., G.V. Minsavage, U. Bonas and R.E. Stall: Detection and identification of phytopathogenic *Xanthomonas* isolates by amplification of DNA sequences related to the *hrp* genes of *Xanthomonas campestris* pv. *vesicatoria. Appl. Environ. Microbiol.,* **60**, 1068-1077 (1994).

Oh, C., S. Heu and Y. Choi: Sensitive and pathovar-specific detection of *Xanthomonas campestris* pv. *glycines* by DNA hybridization and polymerase chain reaction analysis. *Plant Pathol. J.,* **15**, 57-61 (1999).

Park, D.S., J.W. Hyun, Y.J. Park, J.S. Kim, H.W. Kang, J.H. Hahn and S.J. Go: Sensitive and specific detection of *Xanthomonas axonopodis* pv. *citri* by PCR using pathovar specific primers based on *hrpW* gene sequences. *Microbiol. Res.,* **161**, 145-149 (2006).

Park, D.S., J.K. Shim, J.S. Kim, C.K. Lim, R. Shrestha, J.H. Hahn and H.G. Kim: Sensitive and specific detection of *Xanthomonas campestris* pv. *vesicatoria* by PCR using pathovar-specific primers based on rhs family gene sequences. *Microbiol. Res.,* **164**, 36-42 (2009).

Park, Y.J., B.M. Lee, J. Ho-Hahn, G.B. Lee and D.S. Park: Sensitive and specific detection of *Xanthomonas campestris* pv. *campestris* by PCR using species-specific primers based on *hrpF* gene sequences. *Microbiol. Res.,* **159**, 419-423 (2004).

Pitcher, D.G., N.A. Saunders and R.J. Owen: Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.,* **8**, 151-156 (1989).

Plant Quarantine (Regulation of Import into India) Order 2003: The Gazette of India Part II Section-3-Sub-section (ii) published by Ministry of Agriculture (Department of Agriculture & Cooperation) Notification, New Delhi, p. 104 (2003).

Potnis, N., K. Krasileva, V. Chow, N.F. Almeida and P.B. Patil: Comparative genomics reveals diversity among Xanthomonads infecting tomato and pepper. *BMC Genomics,* **12**, 146 (2011).

Singh, D. and S. Dhar: Bio-PCR based diagnosis of *Xanthomonas campestris* pv. *campestris* in black rot of infected leaves of crucifers. *Indian Phytopathol.,* **64**, 7–11 (2011).

Singh, D., B.T. Raghavendra, P. S. Rathaur, H. Singh, R. Raghuwanshi and R.P. Singh: Detection of black rot disease causing pathogen *Xanthomonas campestris* pv. *campestris* by bio-PCR from seeds and plant parts of cole crops. *Seed Sci. Technol.,* **42**, 36-46 (2014).

Slater, H., A. Alvarez-Morales, C.E. Barber, M.J. Daniels and J.M. Dow: A two-component system involving an HD-GYP domain protein links cell–cell signalling to pathogenicity gene expression in *Xanthomonas campestris. Mol. Microbiol.,* **38**, 986-1003 (2000).

Tang, J.L., Y.N. Liu, C.E. Barber, J.M. Dow, J.C. Wootton and M.J. Daniels: Genetic and molecular analysis of a cluster of *rpf* genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pv. *campestris. Mol. Gen. Genet.,* **226**, 409-417 (1991).

Williams, P.H.: Black rot: A continuing threat to world crucifers. *Plant Dis.,* **64**, 736-742 (1980).

Zaccardelli, M., F. Campanile, A. Spasiano and M. Merighi: Detection and identification of the crucifer pathogen, *Xanthomonas campestris* pv. *campestris,* by PCR amplification of the conserved Hrp/ type III secretion system gene hrcC. *Eur. J. Plant Pathol.,* **118**, 299-306 (2007).

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