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Diversity of Type III Effectors in Bacterial Blight Pathogen *Xanthomonas citri* pv. *malvacearum* and Defence Gene Expression Analysis in Cotton using Biocontrol Agents

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Abstract

Virulence-associated genes allow plant pathogenic bacteria to adapt effectively to their hosts. Among them, the function of type III effectors (T3Es) has been well documented. T3Es are introduced directly into host cells via the type III secretion system (T3SS). The presence of five Type III effector genes was tested in thirty-four *X. citri* pv. *malvacearum* isolates from major cotton-growing regions of India. Thirteen isolates carried the *avrBs3* avirulence gene. Eight isolates were positive for the *xopK* gene, seven for the *HopG1* gene, six for the *xopAD* gene, and five for the *xopI* gene. Defense gene expression was studied in cotton using PR-1 (chitinase), PR-5 (ribonuclease-like protein), and polyubiquitin as an internal control in a susceptible cotton cultivar (LRA5166) by quantitative real-time PCR. The differential gene expression profiles were studied in cotton leaves by pre-treatment of cotton seedlings with effective biocontrol agents such as *Streptomyces mutabilis* (RAL2) and *Bacillus amyloliquefaciens* (ETL2), along with salicylic acid (1000 ppm), before *X. citri* pv. *malvacearum* infection. Plants treated with *S. mutabilis* (RAL2) showed numerically maximum expression of the defense gene *PR1* compared to those treated with *B. amyloliquefaciens* (ETL2). *PR5* gene expression was numerically higher in plants treated with *B. amyloliquefaciens* (ETL2) than in those treated with *S. mutabilis* (RAL2).

Keywords: Type III Effectors (T3Es), *xopAD, avrBs3, HopG1, xopI* and *xopK, Xanthomonas citri* pv. *malvacearum,* Cotton, Defense Genes (*PR1* and *PR5*)

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INTRODUCTION

Cotton, often called white gold, is a major economic crop grown in India. It is affected by several diseases from the seedling to maturity. Among them, bacterial blight caused by *Xanthomonas citri* pv. *malvacearum* (*Xcm*) is a significant disease that causes crop yield losses in numerous cotton-growing regions worldwide,[1,2] including India.[3] Effectors are bacterial proteins injected into plant cells via the type III secretion system. They suppress plant defence signalling pathways, enabling the pathogen to evade the plant's immunity. Type III effectors (T3E) in *Xanthomonas citri* pv. *malvacearum* (*Xcm*) are essential for its pathogenicity in cotton. Type III effectors are primary factors in *Xcm*-mediated disease development in cotton plants and in the pathogen's escape from host resistance. Cotton *Xcm* injects various effector proteins into plant cells via the type III secretion system, aiding the pathogen's nutrient absorption and disease development.[4] The AvrBs3/PthA (TAL) family effectors activate host genes, while Xops effectors influence host defence pathways to enhance pathogen virulence or induce plant resistance. The variation among these effectors shapes the host range and virulence races of *Xcm*.

The role of type III effectors (T3Es) is among the best documented of virulence-related genes, helping explain how plant pathogenic bacteria adapt to their hosts. T3Es are inoculated into host cells via the type III secretion system (T3SS). It is a highly conserved protein secretion system encoded by a cluster of hrp (hypersensitive response and pathogenicity) genes.[5] Avr proteins are a type of type III effectors that trigger disease resistance in hosts possessing specific resistance (R) genes, serving as determinants of race-cultivar compatibility specificity.[6] Effector proteins are crucial for the virulence and host specificity of all bacterial species that possess the T3 secretion system.[7] T3E repertoires vary both between species and among strains within a species, and are therefore considered key determinants of the range of host interactions.[8] Over twenty (20) effector genes have been identified within the genus *Xanthomonas.*[9] The effector genes determine the race of the pathogen and control the pathogen's aggressiveness.[10] Xanthomonads have evolved effectors that employ a variety of mechanisms to enhance virulence, including processes unique to them and to eukaryotes.[11] *X. citri* pv. *malvacearum* isolate race 18 contained 3-5 more effectors than other strains.[2] Different races of *X. citri* pv. *malvacearum* have various combinations of avirulence (*avr*) genes, which define races and influence cultivar specificity.[12]

PR-1, one of the many PR gene families, has often served as a marker for systemic acquired resistance in numerous plant species.[13] Transgenic tobacco and *Arabidopsis* expressing the *PR1* gene are reported to confer a high level of resistance to bacterial diseases.[14] Patil et al.[15] identified the genes that were up-regulated in cotton IM216, resistant to bacterial blight, after inoculation with *X. citri* pv. *malvacearum*, using the microarray analysis technique. They found 98% of genes were significantly up-regulated at one or more sampling times, and 63% matched plant genes involved in defense responses, including those related to disease, protein synthesis, secondary metabolism, signalling, stress, programmed cell death, or encoding pathogenesis-related or retrotransposon-like proteins. Chandrasekaran and Chun[16] reported that *B. subtilis* induced PAL genes, thereby conferring soft-rot disease resistance. Yim et al.[17] observed higher accumulation of PR proteins in tomato plants inoculated with *Methylobacterium* when challenged with *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato.*

This study was conducted to understand how pathogen virulence relates to effector diversity and host defence gene expression with the following objectives.

1. Detection of various effector genes in the pathogen population and
2. Defence gene expression analysis in cotton using effective bacterial biocontrol agents challenged with the pathogen.

MATERIALS AND METHODS

Type III- effector gene diversity in *X. citri* pv. *malvacearum*

The presence of Type III effector genes in 34 *X. citri* pv. *malvacearum* (*Xcm*) isolates from India were assessed. Partial sequences of the

Table 1. Designed primer sequences used for PCR amplification

gene	Primer sequences	
	Forward	Reverse
xopAD	GAGCTAACGGAGGATCAGGC	CAGCAGAAGAACTGACGGGT
avrBs3	TCAACTCAAACGCCGGATCA	TCTTCGTTGAATGCCGGGAA
HopG1	GCTATCGCAAGAGCAAACCG	TGCGTTGAGTTTGAAGTGCG
xopI	CACTCTCGGTGCTGTCCAAT	GCTTGACGAATGACCTGGGA
xopK	GCGATCGATACCAACGCCTA	ACGACAAAGCCCTTGTTCCA

effector genes, namely *xopAD* (954 bp), *avrBs3* (374 bp), *HopG1* (232 bp), *xopI* (573 bp), and *xopK* (467 bp), were amplified using PCR with gene-specific primers. The primers were designed for the *avrBs3, xopI,* and *xopK* genes by retrieving the sequences of the products of the avirulence protein AvrBs3 and T3SS effector proteins XopI and XopK of *X. citri* pv. *malvacearum* strain AR81009, complete genome (accession number: CP023155.1) from NCBI. The primer for the *xopAD* gene was designed using the sequence of the avirulence protein XopAD from the complete genome of the *Xcm* strain MS14003 (accession number: CP023159.1). The primer for the *HopG1* gene was designed through the type III secretion system effector protein HopG1 of *Xcm* strain XcmN1003, complete genome (accession number: NZ_CP013006.1). The primers were designed using the Primer3 software available in NCBI (Table 1).

The PCR amplifications were performed with the following cycling conditions as described by Hajri et al.[18] with slight modifications. The initial denaturation at 94 °C for 2 minutes was succeeded by 35 cycles of 94 °C for one minute (denaturation) and 60 - 63 °C (depending on gene: *xopAD* - 63 °C*, avrBs3* - 60 °C, *HopG1* - 61 °C, *xopI* - 62 °C and *xopK* - 61 °C) for one minute (annealing) and 72 °C for one minute (extension). The final extension was performed at 72 °C for 10 minutes. The 10 µL PCR reaction mixture consisted of 2 µL of template DNA (50 ng/mL), 1 µL of each forward and reverse primer, 5 µL of PCR master mix (Smart Prime 2X master mix-Red), and 1 µL of sterile water. PCR amplifications were performed using a thermocycler (Eppendorf Master cycler Nexus Gradient S, Eppendorf AG, Hamburg, Germany). The presence or absence of a band of the expected size was used to determine whether a specific effector gene was present in the isolates.

Defense gene expression analysis in cotton against *X. citri* pv. *malvacearum* using qRT-PCR

The present study was intended to investigate the differences in the mRNA abundance of defence genes in cotton, such as *PR-1* (chitinase) and *PR-5* (ribonuclease-like protein), using *polyubiquitin*[19] as an internal control, which is a non-regulated reference gene in a susceptible cotton cultivar, LRA5166, following infection with *X. citri* pv. *malvacearum* (MNSu) isolate. The differential gene expression profiles were studied in cotton leaves by pre-treatment of cotton seedlings with effective biocontrol agents such as *Streptomyces mutabilis* (RAL2) and *Bacillus amyloliquefaciens* (ETL2), along with salicylic acid (1000 ppm), before *X. citri* pv. *malvacearum* infection. The primers were designed for the *PR1* gene through retrieving the sequences of the product of pathogenesis-related protein 1 (*PR1*) gene in *Gossypium hirsutum* cultivar TM-1, chromosome 1 (accession number: NC_030074.1) and the *PR5* gene using the product of pathogenesis-related protein 5 (PR5) gene in *Gossypium hirsutum* cultivar TM-1, chromosome 4 (accession number: NC_030077.1). Primer design was carried out using the Primer3 software available at NCBI (Table 2).

Defence gene expression after biocontrol agent application was confirmed by quantitative real-time PCR (qRT-PCR) using the Step One Plus detection system (Applied Biosystems, USA). Treatments were imposed on 20 day old cotton seedlings of the susceptible cultivar LRA5166 raised in pots. The biocontrol agents were applied as a foliar spray at 10 ml/plant, containing 10^8 CFU/ml. Salicylic acid (1000 ppm) was used as a chemical control. The highly virulent *X. citri* pv. *malvacearum* isolate, 24 hours old, was spray-inoculated onto cotton seedling leaves 24 hours

Table 2. Designed primer sequences used for qRT-PCR amplification

gene	Primer sequences	
	Forward	Reverse
PR1	CCATAAGGTCCACCGGAGTG	ACTACCTAGCCTTGGGAGGG
PR5	TTCGGCTATGGAAACAGGCA	TGGCCTGCAATAATCACCGA
Polyubiquitin (internal control)	GAAGGCATCCCACCAGACCAG	AACAGGGCCAGACATCACAATCAT

Table 3. Molecular detection of effector genes of *X. citri* pv. *malvacearum* isolates using gene-specific primers

No.	Isolate Code	Effector genes				
		xopAD	*avrBs3*	*HopG1*	*xopI*	*xopK*
1	MNSu	+	+	-	+	-
2	MNSn	-	+	+	+	+
3	MND	+	+	-	-	-
4	MNR	-	+	-	-	-
5	MNN	+	+	-	-	-
6	MNAn	+	+	-	+	-
7	MAB	-	+	+	-	+
8	MAA	-	-	+	-	-
9	MRA	+	-	-	-	+
10	MRN	-	-	-	-	-
11	PBH	-	-	-	-	-
12	PFN	-	-	-	-	+
13	PFA	-	-	-	-	-
14	PFB	-	-	-	-	+
15	PFF	-	-	-	-	+
16	KDS	-	-	+	-	-
17	KDR	-	+	-	+	+
18	KDG	-	-	-	-	-
19	KDB	-	+	-	-	+
20	KDSp	-	-	-	-	-
21	TCSrb	-	-	-	-	-
22	TCT	-	-	-	-	-
23	TCD	-	+	-	-	-
24	TCS	-	+	-	-	-
25	TCB	-	-	-	-	-
26	TCL	-	-	-	-	-
27	TDR	-	-	-	-	-
28	TDJ	-	-	-	-	-
29	ANR	-	-	-	-	-
30	AGD	-	-	+	-	-
31	AGR	-	-	-	-	-
32	TWR	-	-	+	+	-
33	TWB	-	+	+	-	-
34	THV	-	-	-	-	-
35	PC-CICR	+	+	-	-	-

(+) - Presence of effector gene; (-) - Absence of effector gene

post-treatment at a concentration of 2 x 10^6 CFU/ml. Control plants, including both inoculated and uninoculated, were also maintained.

Leaf samples were collected 6 days after inoculation with the pathogen for each treatment and immediately preserved in liquid nitrogen. The samples were ground to a fine powder using a sterile DEPC-treated pestle and mortar. The Spectrum Total Plant RNA Isolation Kit (Sigma-Aldrich, USA) was used to isolate total RNA according to the manufacturer's instructions. The RNA was treated with 1 Unit of *DNase* (Thermo Scientific, USA) according to the manufacturer's instructions, incubated at 37 °C for 30 minutes, and the reaction was halted by adding 1 µl of EDTA, then incubated at 65 °C for 10 minutes. Total RNA treated with *DNase* was reverse-transcribed into single-stranded DNA using the Transcriptor High Fidelity cDNA Synthesis

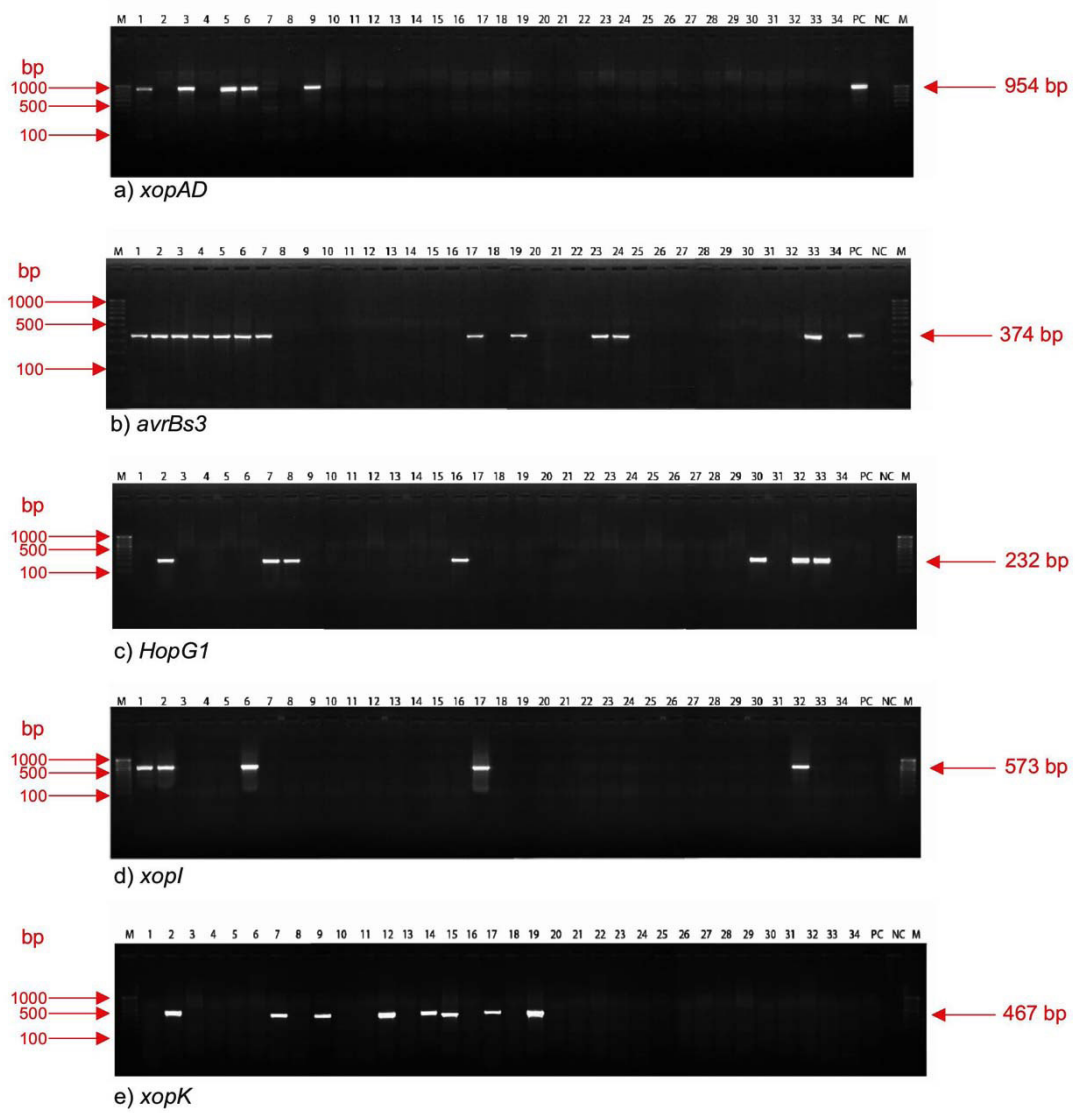


Figure 1. Molecular detection of effector genes of *X. citri* pv. *malvacearum* isolates using genes specific primers. a) *xopAD,* b) *avrBs3,* c) *Hop G1,* d) *xopI,* e) *xopK*. M: 100 bp ladder, Lanes 1 to 34: MNSu, MNSn, MND, MNR, MNN. MNAn, MAB, MAA, MRA, MRN, PBH, PFN, PFA, PFB, PFF, KDS, KDR, KDG, KDB, KDSP, TCSrb, TCT, TCD, TCS, TCB, TCL, TDR, TDJ, ANR, AGD, AGR, TWR, TWB and THV, respectively. Lane 35: positive control (PC-CICR)

Kit (Roche, Germany). The resulting cDNA was then used for qRT-PCR analysis.

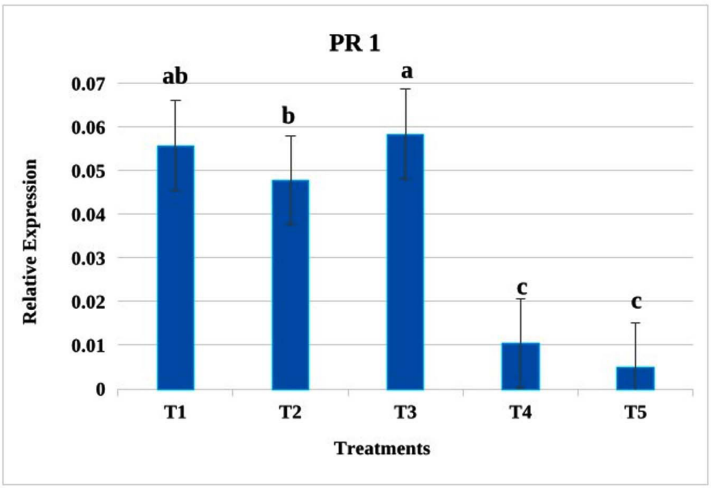
Quantitative RT-PCR was performed on single-stranded DNA from all five samples using standard cycling conditions (10 min at 95 °C, 40 cycles of 95 °C for 15 sec and 60 °C for 60 sec), followed by melt curve analysis to assess amplification specificity. The reaction mixture includes 7.0 µl of SYBR Green Master Mix (Roche Diagnostics), 0.5 µl of each forward and reverse primer specific to the gene, and 2 µl of cDNA at 50 ng/µl. 'No template controls' (NTC) were kept to prevent cross-contamination of reagents and surfaces. The NTC contains all RT-PCR reagents except the cDNA template. Relative mRNA quantities were determined using the comparative Ct method. For each sample, three biological replicates and two technical replicates were maintained, with *polyubiquitin* serving as the endogenous reference gene for normalising Ct values. Relative fold-changes were determined using the $2^{-\Delta\Delta Ct}$ method, as outlined by Livak and Schmittgen.[20] Statistical analyses were performed using R software. Differences in gene expression among treatments were assessed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test. Data were presented as fold expression level. Differences for gene expression to different treatments were statistically compared at $P < 0.05$.

RESULTS

Type III- effector gene diversity in *X. citri* pv. *malvacearum*

Thirteen *X. citri* pv. *malvacearum* isolates out of 34, *viz.,* MNSu, MNSn, MND, MNN, MNAn, MNR, MAB, KDR, KDB, TCS, TCD, TWB, and PC-CICR, showed the presence of the *avrBs3* avirulence protein gene with an amplicon size of 374 bp. Of 10 Maharashtra isolates, 7 (70%) were positive for the *avrBs3* gene. The effector (type III secretion system) protein gene *xopK* (467 bp) was identified in eight isolates (MNSn, MAB, MRA, PFN, PFB, PFF, KDR, and KDB) (Table 3 and Figure 1).

Seven isolates (MNSn, MAB, MAA, KDS, AGD, TWR, and TWB) showed positive amplification of the type III secretion system effector protein gene *HopG1* (232 bp). Avirulence protein gene *xopAD* (954 bp) was recorded in six isolates (MNSu, MND, MNN, MNAn, MRA and PC-CICR). The other effector protein gene, *xopI* (573



Treatment details
T1- *Streptomyces luteus* (RAL2)
T2- *Bacillus amyloliquefaciens* (ETL2)
T3- Salicylic acid (1000 ppm)
T4- Pathogen inoculated control
T5- Pathogen uninoculated control

	PR1	CRD analysis
T1	0.055713	ab
T2	0.04766	b
T3	0.058342	a
T4	0.010349	c
T5	0.004839	c

Figure 2. Expression analysis of defense gene PR1 in cotton seedlings (LRA5166) treated with biocontrol agents and chemical inducer challenge inoculated with *X. citri* pv. *malvacearum* (MNSu) using qRT-PCR. *Polyubiquitin* gene was used as internal control. Statistical analyses were performed using R software. Differences in gene expression among treatments were assessed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test. Data is presented as fold expression level. Error bars indicated standard error obtained from three replicates per treatment. Means followed by different letters are significantly different for gene expression at $P < 0.05$

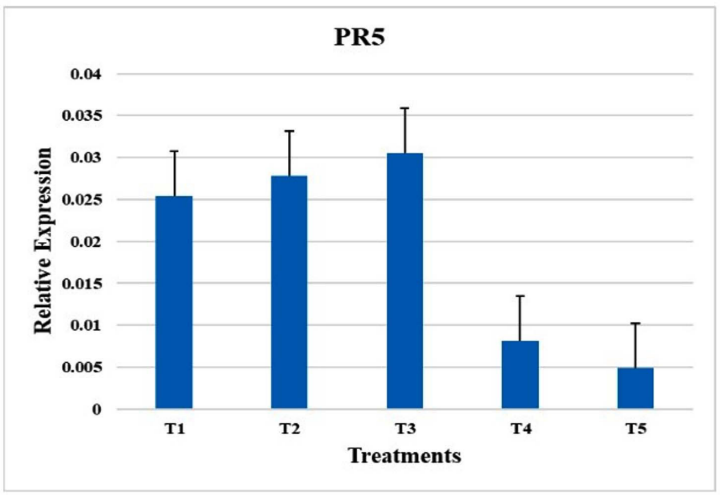
bp), also involved in the type III secretion system, was detected in five isolates, including MNSu, MNSn, MNAn, KDR and TWR (Table 3 and Figure 1).

Defense gene expression analysis in cotton against *X. citri* pv. *malvacearum* using qRT-PCR

Plants treated with *S. mutabilis* (RAL2) showed maximum expression of the defence gene *PR1* compared to those treated with *B. amyloliquefaciens* (ETL2). The *PR5* gene expression was higher in *B. amyloliquefaciens* (ETL2) treated plants, followed by *S. mutabilis* (RAL2). However, the salicylic acid (1000 ppm)- treated plants showed much higher expression of both genes than the biocontrol agents. The pathogen-inoculated and uninoculated controls expressed low levels of *PR1* and *PR5* genes (Figures 2 and 3).

DISCUSSION

This study aimed to identify five different effector genes in 34 *Xcm* isolates collected from key cotton-growing regions across India. Previous researchers also studied the diversity of effectors in the cotton bacterial blight pathogen. Zhai et al.[21] identified 181 and 178 potential virulence-related effectors through whole-genome sequencing of the highly virulent strain GSPB2388 of *X. citri* pv. *malvacearum* from Sudan and race 18 of GSPB1386 from Nicaragua, including type I to IV secretion systems. Type III secretion system effectors, called *Xanthomonas* outer proteins (Xops), are essential for bacterial growth and colonisation in various eukaryotic hosts.[22] Genes responsible for producing effector proteins in plant pathogens are functionally identified as avirulence (*avr*) genes.[23] In the present study, a maximum of thirteen isolates, including the positive control (PC-CICR), recorded positive for the *avrBs3* gene. Of 10 Maharashtra isolates, 7 (70%) were positive for the *avrBs3* gene. The result was consistent with Park et al.,[23] who studied the diversity of the *avrBs3* avirulence gene in 155 strains of *X. axonopodis* pv. *glycines*. They found that *avrBs3* was the primary pathogenic factor in soybean. The *avrBs3* mutant strain, or strains lacking *avrBs3*, lost pathogenicity or induced only a few pustules on highly susceptible cultivars. The *avrBs3* gene belongs to the transcription activator-like (TAL) effector family, also called



Treatment details
T1- *Streptomyces luteus* (RAL2)
T2- *Bacillus amyloliquefaciens* (ETL2)
T3- Salicylic acid (1000 ppm)
T4- Pathogen inoculated control
T5- Pathogen uninoculated control

	PR5	CRD analysis
T1	0.025368	b
T2	0.027774	ab
T3	0.030511	a
T4	0.008116	c
T5	0.00485	c

Figure 3. Expression analysis of defense gene PR5 in cotton seedlings (LRA5166) treated with biocontrol agents and chemical inducer challenge inoculated with *X. citri* pv. *malvacearum* (MNSu) using qRT-PCR. *Polyubiquitin* gene was used as internal control. Statistical analyses were performed using R software. Differences in gene expression among treatments were assessed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test. Data is presented as fold expression level. Error bars indicated standard error obtained from three replicates per treatment. Means followed by different letters are significantly different for gene expression at $P < 0.05$

the *AvrBs3/PthA* family, which is found in most *Xanthomonas* strain genomes.[18] Mokryakova et al.[24] also studied effector gene diversity in 53 strains of the genus *Xanthomonas*, including eight completely sequenced genomes representing four different species, using 19 effector gene-specific primers. They found that *xopD* was the most frequent gene (85% of strains) and indicated its importance for pathogenesis. 30% of the strains carried the *avrBs3* group. They also examined how the content and number of effector genes relate to different disease symptom types and the main pathogen race.

The Type III secretion system effector protein gene *xopK* was detected in eight isolates, and the *HopG1* gene in seven isolates. Likewise, Schwartz et al.[25] identified *XopE2* in all field strains of *Xanthomonas perforans* and considered it a commonly shared effector among *Xanthomonas euvesicatoria* and *Xanthomonas gardneri* strains. They also detected *AvrBsT* in most *X. perforans* field strains and an identical version of *X. euvesicatoria XopJ1* in *X. gardneri* field strains. This suggests a more broadly defined YopJ family within the shared effector list. *Xanthomonas axonopodis* pv. *punicae* contains six Xop-effectors, including XopC2, XopE1, XopL, XopN, XopQ and XopZ.[22] The current study results showed that the genes xopAD and xopI recorded the minimum number of isolates, namely six and five, respectively. Generally, the functions of each effector differ and play a crucial role in symptom expression in cotton plants. The *AvrBs3* activates host susceptibility genes, thereby inducing hypertrophy and water-soaking symptoms in plants. XopK effector prevents stomatal closure through suppressing ABA/MeJA signal suppression. The variable effector *XopAD* may be involved in host identification. XopI aids the pathogen's virulence, while HopG1 suppresses plant defence responses.

The highly virulent and race 18 MNSn isolate had the most effector genes, with a maximum of 4, followed by MAB and KDR, each with 3. The positive control (PC-CICR) recorded with two genes, namely *avrBs3* and *xopAD.* Nine of 10 isolates from Maharashtra carried at least one effector gene. Most of the isolates from the central zone were highly virulent and belonged to race 18. The south zone isolates recorded fewer effector genes, which are moderately to less virulent. The analysis of draft genomes of thirteen *X. citri* pv. *malvacearum* isolates, along with four previously published genomes, revealed the presence of 24 conserved and 9 variable type three effectors.[2] They also found that race 18 contains 3-5 more effectors than other strains. Ochiai et al.[6] noted that a variety of effector genes and mobile elements contribute to the significant race differentiation observed in *Xanthomonas* strains. The *AvrBs3* effector is used as a marker for the identification of *Xanthomonas* strains. Further study of effectors will help understand how *Xanthomonas* manipulates host cells and develop new pathogen-resistance genes in plants and disease management strategies.

In the present study, the effective biocontrol agents, namely *Streptomyces mutabilis* (RAL2) and *Bacillus amyloliquefaciens* (ETL2), treated plants showed increased expression of defence genes, such as *PR1* and *PR5*, in cotton seedlings challenged with *X. citri* pv. *malvacearum* in cotton compared to the control. Both the biocontrol agents did not show any statistically different gene expression for both the genes. *PR1* and *PR5* genes are the key pathogenesis-related genes in cotton, signalling the salicylic acid (SA) pathway during plant defence against pathogens and stress. Several workers have observed the activation of defence genes in crop plants when using biocontrol agents and chemicals to protect against bacterial and fungal pathogens. Gupta et al.[26] described how the *B. subtilis* strain FZB-G activates defence-related compounds in tomato plants. In the present study, salicylic acid-treated plants showed higher expression of defence genes, indicating the effectiveness of chemical inducers. In pot and field experiments, the biocontrol agents significantly reduced the incidence of bacterial blight and promoted plant growth and yield. This shows that chemical inducers can rapidly activate the plant defence system. Biocontrol agents have the added advantage of promoting plant growth compared to chemical inducers. Similarly, Marmey et al.[27] found that the hypersensitive reaction (HR) of cotton in response to inoculation of *X. citri* pv. *malvacearum* race 18 was associated with 9S-lipoxygenase activity (LOX) responsible for lipid peroxidation. *GhLOX1* was highly expressed during pathogen-induced HR. Sequence analysis

showed that *GhLOX1* is a putative 9-LOX, and the *GhLOX1* promoter contains SA- and JA-responsive elements, suggesting that *GhLOX1* encodes *9-LOX* activity and is involved in cell death during cotton HR. Zambounis et al.[19] examined the relative expression levels of two pathogenesis-related (PR) genes (*PR-3* and *PR-10*) and a detoxification gene (*GST18*) in fully susceptible (Lacta) and partially field-resistant (Emerald) cotton cultivars challenged with an Australian isolate of *Fusarium oxysporum* f. sp. *oxysporum*. Chemical inducers, such as BION® (a chemical analogue of salicylic acid) and methyl jasmonate (MeJA), were applied as a pretreatment before pathogen inoculation. They found that all PR genes were overexpressed in both hypocotyls and roots after pathogen inoculation in the Emerald cultivar, but were not affected in cultivar Lacta. Likewise, Khan et al.[28] investigated how *G. hirsutum* expresses various pathogenesis-related protein genes, including *PR1, PR4, PR5,* β-1,3-glucanase, and *chitinase*, in response to cotton leaf curl virus disease. They found a significant interaction between the virus coat protein gene and the *PR1, PR5*, and *Chitinase* genes in plants. They concluded that the expression of *PR1, PR5*, and *Chitinase* genes can be exploited to confer resistance to leaf curl virus in cotton.

CONCLUSION

Of thirty-four *X. citri* pv. *malvacearum* isolates, 13 harboured the *avrBs3* avirulence gene, as evidenced by 374 bp amplicons. The effector protein gene *xopK* (467 bp) was identified in eight isolates. Seven isolates showed amplification of the type III secretion system effector protein gene *HopG1* (232 bp). Avirulence protein gene *xopAD* (954 bp) was recorded in six isolates. The gene *xopI,* coding for the type III secretion system effector protein (573 bp), was found in five isolates. Plants treated with *S. luteus* (RAL2) showed numerically maximum expression of the defence gene *PR1* compared to those treated with *B. amyloliquefaciens* (ETL2). The *PR5* gene expression was numerically higher in *B. amyloliquefaciens* (ETL2) treated plants, followed by *S. mutabilis* (RAL2).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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