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Development of Real-time PCR Method for Detection of *Xanthomonas campestris* pv. *juglandis* Causing Walnut Bacterial Blight

(Pembangunan Kaedah PCR Masa Nyata untuk Pengesanan *Xanthomonas campestris* pv. *juglandis* Penyebab Hawar Bakteria Walnut)

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ABSTRACT

Walnut is one of the important economic tree species in the world. For the past few years, with the continuous expansion of walnut planting area, walnut bacterial blight caused by *Xanthomonas campestris* pv. *juglandis* has critically affected walnut production and caused economic losses. In this research, by comparing the gene sequences of *X. campestris* pv. *juglandis* and its closely related bacterial species, the 16S rDNA gene sequence was selected, and the specific primers were evaluated using PCR. Subsequently, the real-time fluorescence PCR specific detection method of *X. campestris* pv. *juglandis* was established and optimized. The specificity of real-time PCR was verified for 23 strains isolated from walnut and its surrounding soil, including eight target pathogens. The specific amplification was possible even when the cDNA template concentration was 7.0×10^{-5} μg/mL, hence, the detection sensitivity of the standard method is 7.0×10^{-5} ***μg/***mL. The reliability of the established real-time PCR detection method in field detection was verified by the identification of target bacteria in 8 randomly collected healthy leaves and leaves of suspected walnut blight. This method laid a foundation for the early diagnosis of *X. campestris* pv. *juglandis*.

Keywords: Rapid detection; real-time fluorescence PCR; walnut bacterial blight; *Xanthomonas campestris* pv. *juglandis*

ABSTRAK

Walnut adalah salah satu spesies pokok yang penting dalam ekonomi dunia. Sejak beberapa tahun kebelakangan ini, dengan pengembangan berterusan kawasan penanaman walnut, hawar bakteria walnut yang disebabkan oleh *Xanthomonas campestris* pv. *juglandis* telah menjejaskan pengeluaran walnut secara kritikal dan menyebabkan kerugian ekonomi. Dalam penyelidikan ini, dengan membandingkan jujukan gen antara *X. campestris* pv. *juglandis* dan spesies bakteria yang berkait rapat, jujukan gen 16S rDNA telah dipilih dan pencetus khusus telah dinilai menggunakan PCR. Seterusnya, kaedah pengesanan khusus PCR pendarfluor masa nyata bagi *X. campestris* pv. *juglandis* telah dibangunkan dan dioptimumkan. Kekhususan PCR masa nyata telah disahkan untuk 23 strain yang dipencilkan daripada walnut dan tanah di sekelilingnya, termasuk 8 patogen sasaran. Amplifikasi khusus masih berlaku walaupun apabila kepekatan templat cDNA ialah 7.0×10-5 μg/mL, oleh itu, kepekaan pengesanan kaedah piawai ialah 7.0×10-5 μg/mL. Kebolehpercayaan kaedah pengesanan PCR masa nyata yang telah dibangunkan dalam pengesanan di lapangan telah disahkan dengan pengenalpastian bakteria sasaran dalam 8 daun sihat yang dikumpul secara rawak dan daun yang disyaki menghidapi hawar walnut. Kaedah ini meletakkan asas untuk diagnosis awal *X. campestris* pv. *juglandis*.

Kata kunci: PCR pendarfluor masa nyata; pengesanan yang pantas; penyakit hawar bakteria walnut; *Xanthomonas campestris* pv. *juglandis*

INTRODUCTION

Walnut (*Juglans regia* L.) is an economically important tree and considered one of the healthiest foods in the world, rich in plant-based protein, fiber, magnesium, and α-linolenic acid (Martínez et al. 2010; Ozkan et al. 2005). The growing demand for walnut has led to the growing area of walnut cultivation, and walnut pests and diseases are becoming more frequent. Among them, walnut bacterial blight occurs continuously in many places which is widely distributed in walnut producing areas of many cities in China, and is also found in most walnut producing regions in the world (Fu et al. 2021; Kim et al. 2021; Sagawa et al. 2021). This disease is severely harmful to walnut, affecting the healthy development of the walnut industry.

The pathogen of walnut bacterial blight is *Xanthomonas campestris* pv. *juglandis*, which is an exclusive parasitic bacterium that can only infect walnut species (Ramos 1998, 1985). This bacterium has been named from *X. campestris* pv. *juglandis* (Pierce) Dye to *X. arboricola* pv. *juglandis* (Pierce) Vauterin, and then the development of changing *X. arboricola* into *X. juglandis* (Pierce) Dowson (Dye et al. 1980; Schaad et al. 2000; Vauterin et al. 1995). The bacterium has the common microbial characteristics of *Xanthomonas*. It is a Gram-negative bacterium, which can normally move due to its unipolar flagella. It is strictly aerobic, and the colonies are pale yellow and glossy (Kałużna et al. 2021). In addition, it is a vascular pathogen that infects cruciferous plants through wounds and migrates to xylem ducts where it multiplies and produces v-shaped necrotic lesions where parts of the leaves turn yellow (Vicente & Holub 2013).

Early diagnosis and identification of walnut bacterial blight is based on biochemical, physiological and pathogenicity tests, which are time-consuming and often ambiguous (Lee, Lo & Yu 1999). Subsequently, the detection method of walnut bacterial blight based on PCR molecular detection technology has greatly improved the detection efficiency of this pathogen compared with the traditional isolation and culture method. The multiplex polymerase chain reaction, Amplified Fragment Length Polymorphism (AFLP) technology and other molecular methods have been established for *X. campestris* detection and identification (Inoue, Fujikawa & Takikawa 2021; Loreti et al. 2001), but there are still some problems of low specificity and sensitivity. Among all molecular technologies, real-time fluorescent PCR has been widely used for the rapid detection of plant diseases due to its high sensitivity, reliability and ability to monitor the entire PCR process in real-time.

In this study, *X. campestris* pv. *juglandis* was used as the research focus, and specific primers were used to establish an amplification system and a real-time fluorescence PCR detection system, which laid a certain foundation for the early diagnosis and advanced prevention and control of walnut bacterial blight.

MATERIALS AND METHODS

BACTERIAL STRAINS

The tested strains in this study were isolated from walnut plants collected from different areas, except *X. campestris* pv. *juglandis* which was isolated from the Forest Pathology Laboratory, College of Forestry, Sichuan Agricultural University. The tested strains were preliminarily identified at genus level through molecular biology technology, and some strains were identified at species level, as shown in Table 1. The isolates were cultured in NA medium at 28 °C until saturated and DNA was extracted.

DNA EXTRACTION AND CONVENTIONAL PCR TEST

Total DNA was extracted from the strains in Table 1 according to the instructions of the TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China). The quality and concentration of DNA were analyzed using 1% agarose gel electrophoresis and UV absorption method, and stored at -20 °C for later use. Using the genomic DNA as a template, bacterial universal primers (27F: 5'-GAGAG T T T G A T C C T G G C T C A G - 3 ' , 4 9 2 R : 5'-TACGGCTACCTTGTTACGAC-3' (Ortiz-Pallardó et al. 2000)) were used for PCR amplification of the tested strains. PCR conditions were as follows: 1 cycle for 4 min at 95 °C, 1 min at 94 °C, 1 min at 55 °C, 35 cycles at 72 °C, and final extension for 10 min at 72 °C. PCR products were observed using electrophoresis in 1.0% agarose gel and sequenced by Tsingke Biotechnology.

PRIMER DESIGN

Primers (Table 2) were designed using the Oligo 6.0 and Primer Premier 5.0 softwares according to the 16S rDNA gene sequence of *X. campestris* pv. *juglandis*. Total RNA was extracted from the *X. campestris* pv. *juglandis* using the Bacterial Total RNA Extraction Kit (Tiangen Biotech, Beijing, China) and reverse-transcribed into cDNA. The primers were used for PCR amplification of *X. campestris* pv. *juglandis,* and the product was purified and sequenced to determine the primer specificity.

OPTIMIZATION OF REAL-TIME PCR SPECIFIC DETECTION SYSTEM FOR *X. campestris* pv. *juglandis*

The cDNA of tested strains was used as a template in the specific real-time PCR together with the designed specific primers for the real-time PCR. RNase-Free H_2O was used as a negative control and the real-time PCR was performed with three replicates per sample. The specific real-time PCR detection system consisted of 5.0 μL of SYBR® Premix Ex Taq II, 0.2 μL of each of the forward and reverse primers, 0.5 μL of cDNA and 4.1 μL of RNase Free H_2O. The gradient annealing temperature was set from 55 °C to 65 °C, and the cDNA template was amplified using real-time PCR to find the optimal annealing temperature and optimize the reaction parameters of the specific detection system. A cycle threshold (Ct) value below 30 was scored as a positive result.

EVALUATION OF SENSITIVITY

After specificity detection of all tested strains according to the optimized reaction system, the cDNA of *X. campestris* pv. *juglandis* was diluted. The real-time PCR specific amplification was performed using 8 cDNA

TABLE 1. Bacterial strains and sources

Number	Species	Host	Source
1	*Xanthomonas campestris* pv. *juglandis*	Walnut	Forest Pathology Laboratory, College of Forestry, Sichuan Agricultural University
2	*Xanthomonas campestris* pv. *juglandis*	Walnut	Isolated from walnut disease plant in Chengdu
3	*Xanthomonas campestris* pv. *juglandis*	Walnut	Isolated from walnut disease plant in Yaan
4	*Xanthomonas campestris* pv. *juglandis*	Walnut	Isolated from walnut disease plant in Guangyuan
5	*Xanthomonas campestris* pv. *juglandis*	Walnut	Isolated from walnut disease plant in Bazhong
6	*Achromobacter xylosoxidans*	Soil	Isolated from soils around walnut in Chengdu
7	*Bacillus anthracis*	Soil	Isolated from soils around walnut in Chengdu
8	*Enterobacter* sp.	Walnut	Isolated from walnut disease plant in Chengdu
9	*Enterobacter* sp.	Walnut	Isolated from walnut disease plant in Guangyuan
10	*Kluyvera* sp.	Walnut	Isolated from walnut disease plant in azhong
11	*Kluyvera* sp.	Walnut	Isolated from walnut disease plant in Yaan
12	*Pantoea*	Walnut	Isolated from walnut disease plant in Chengdu
13	*Pantoea*	Walnut	Isolated from walnut disease plant in Yaan
14	*Pantoea*	Walnut	Isolated from walnut disease plant in Guangyuan
15	*Pantoea*	Walnut	Isolated from walnut disease plant in Bazhong
16	*Bacillus* sp.	Walnut	Isolated from walnut disease plant in Chengdu
17	*Bacillus* sp.	Walnut	Isolated from walnut disease plant in Yaan
18	*Bacillus* sp.	Walnut	Isolated from walnut disease plant in Guangyuan
19	*Bacillus* sp.	Walnut	Isolated from walnut disease plant in Bazhong
20	*Leclercia* sp.	Walnut	Isolated from walnut disease plant in Chengdu
21	*Leclercia* sp.	Walnut	Isolated from walnut disease plant in Bazhong
22	*Serratia* sp.	Walnut	Isolated from walnut disease plant in Chengdu
23	*Serratia* sp.	Walnut	Isolated from walnut disease plant in Yaan

TABLE 2. Specific primers for real-time PCR

Primer	Sequence (5'-3')	Amplicon size (bp)
XC-F	TACCCGATTGTTCTGACG	211
XC-R	ATTCCGCTACCCTCTACCAC	

templates with concentrations ranging from 7.0×10^{-7} to 7.0 μg/mL. The amplification results were used to verify the sensitivity of the real-time PCR reaction system, and the RNase-Free H_2O was used as a blank control.

FIELD EFFECTIVENESS TESTING

Random sampling was conducted on leaves of suspected walnut bacterial blight and healthy leaves in walnut gardens in Sichuan Province. RNA was extracted from diseased leaves and healthy leaves, which were reversed-transcribed into cDNA and amplified by ordinary PCR and real-time fluorescence PCR. At the same time, the same amount of RNase-Free H_2O was used as a negative control, and the amplification results were observed.

RESULTS

IDENTIFICATION AND SEQUENCE COMPARISON OF *X. campestris* pv. *juglandis*

The bacterial samples were collected in the field during the onset of walnut bacterial blight, isolated and cultured in the laboratory. The walnut bacterial blight pathogen was purified, and its bacterial suspension was cultured. After total DNA was extracted, 5.0 μL total DNA was analyzed using 1% agarose gel electrophoresis to detect the quality of total DNA. The PCR products were purified and sequenced. BLAST analysis (http://www.ncbi.nlm.nih.gov) was used for sequence identification, and the results were shown in Table 3, confirming that the bacterium was *X. campestris* pv. *juglandis*.

SPECIFICITY ANALYSIS OF PRIMERS FOR *X. campestris* pv. *juglandis*

Specific primers, XC-F/XC-R, designed from the 16S rDNA sequence were used to amplify *X. campestris* pv. *juglandis,* and the PCR products were purified and sequenced. The amplified gene sequence was consistent with the sequence obtained by identification sequencing, and the length was 211 bp, as shown in Figure 1, which was absolutely the same as the expected result, demonstrating that the primer specificity was accurate.

OPTIMIZATION OF REAL-TIME FLUORESCENCE PCR SYSTEM

The amplification results of the primer pair, XC-F/XC-R, at different annealing temperatures for the cDNA template of *X. campestris* pv. *juglandis* were shown in Figure 2. The results indicated that the two primers were able to produce very good specific curves at the annealing temperature from 55 ℃ to 65 ℃. In order to ensure the best amplification effect and the highest specificity of the primer, 59.0 ℃ corresponding to the specificity curve with the best amplification effect was selected as the annealing temperature of the specific primer.

SPECIFICITY OF REAL-TIME FLUORESCENCE PCR SYSTEM

The specific real-time fluorescence PCR detection system of *X. campestris* pv. *juglandis* was used to amplify the relevant bacterial samples from walnut plants. The cDNA template was amplified by real-time fluorescence PCR according to the optimized reaction parameters, and the analysis showed that only a single peak appeared in the dissolution curve (Figure 3(a)). Therefore, the specific amplification curve generated within 30 cycles was for *X. campestris* pv. *juglandis*, but not for other test samples with or without amplification curve after 30 cycles (Figure 3(b)). The PCR products were verified using agarose gel electrophoresis. At the same time, the above cDNA was used as a template, and the specific primers designed and synthesized were used for ordinary PCR amplification. As shown in Figure 4, only the cDNA template of *X. campestris* pv. *juglandis* could amplify the target band with a size of 211 bp. No bands were produced using cDNA of other tested strains and blank control (RNase-Free H_2O). The results were consistent with those of fluorescence quantitative PCR.

SENSITIVITY OF REAL-TIME FLUORESCENCE PCR SYSTEM

The concentration of RNA template was detected using Ultraviolet spectrophotometer ND2000C as 7.0 *μg/m*L, and the reverse-transcribed cDNA template was diluted by an equal concentration gradient. The sensitivity of the cDNA template was verified by general PCR and real-time fluorescence PCR, respectively. For general PCR, when the concentration was lower than 7.0×10^{-3} μg/mL, the amplification results were poor (Figure 5(a)). For real-time fluorescence PCR, the specific amplification curve was *X. campestris* pv. *juglandis*, which could be specifically amplified when the concentration was greater than 7.0×10^{-5} μg/mL (Figure 5(b)). Hence, it was shown that the sensitivity of the standard method was 7.0×10^{-5} μg/mL.

FIELD TIMELINESS OF REAL-TIME FLUORESCENCE PCR SYSTEM

Samples were collected from leaves of suspected walnut bacterial blight and healthy leaves in walnut gardens in Chengdu, Yaan, Guangyuan, Bazhong and other places in Sichuan Province, China. RNA was extracted from

TABLE 3. BLAST analysis of PCR product sequence

Description	Max score	Total score	Query cover	E value	Ident	Accession
Xanthomonas campestris strain 7GY-2 16S ribosomal RNA gene, partial sequence	1738	1738	99%	0.0	99%	KR708824.1
Xanthomonas campestris strain XJPY3 16S ribosomal RNA gene, partial sequence	1733	1733	99%	0.0	99%	KR708889.1
Xanthomonas sp. SAM4215 16S ribosomal RNA gene, partial sequence	1733	1733	99%	0.0	99%	KP419710.1
Xanthomonas arboricola strain QH 16S ribosomal RNA gene, partial sequence	1731	1731	99%	0.0	99%	KP340804.1
Xanthomonas sp. SAM0302 16S ribosomal RNA gene, partial sequence	1731	1731	98%	0.0	100%	KP419708.1
Xanthomonas campestris strain XJGY7 16S ribosomal RNA gene, partial sequence	1729	1729	99%	0.0	99%	KR708882.1
Xanthomonas axonopodis strain UF-CrpMy_8 16S ribosomal RNA gene, partial sequence;	1729	1729	99%	0.0	99%	KF926682.1
Xanthomonas arboricola strain lxj1 16S ribosomal RNA gene, partial sequence	1729	1729	98%	0.0	99%	JF835909.1
Xanthomonas campestris isolate PDD-33b-3 16S ribosomal RNA gene, partial sequence	1729	1729	98%	0.0	100%	HQ256868.1
Xanthomonas campestris strain 7GY-4 16S ribosomal RNA gene, partial sequence	1727	1727	99%	0.0	99%	KR708838.1
Xanthomonas campestris strain 7PY-6 16S ribosomal RNA gene, partial sequence	1727	1727	99%	0.0	99%	KR708826.1
Xanthomonas sp. SAM3302 16S ribosomal RNA gene, partial sequence	1727	1727	98%	0.0	100%	KP419713.1
Xanthomonas arboricola strain lxj2 16S ribosomal RNA gene, partial sequence	1727	1727	98%	0.0	99%	JF835910.1
Xanthomonas arboricola pv. *poinstticola* strain LMG 8676 16S ribosomal RNA gene, partial sequence	1727	1727	99%	0.0	99%	GU144260.1
Xanthomonas campestris strain XJGY222 16S ribosomal RNA gene, partial sequence	1725	1725	99%	0.0	99%	KR708907.1
Xanthomonas campestris strain XJPY9 16S ribosomal RNA gene, partial sequence	1725	1725	98%	0.0	99%	KR708892.1
Xanthomonas sp. CR 7-08 16S ribosomal RNA gene, partial sequence	1725	1725	98%	0.0	99%	KM252981.1
Xanthomonas axonopodis strain UF-CrpMy_ 5 16S ribosomal RNA gene, partial sequence;	1725	1725	100%	0.0	99%	KF926680.1
Xanthomonas campestris pv. *campestris* strain 3811 chromosome, complete genome	1722	3444	98%	0.0	99%	CP025750.1

diseased leaf tissues and healthy leaf tissues, which were reversely transcribed into cDNA. The specific primers XC-F/XC-R designed and synthesized in this study were respectively used for general PCR amplification and real-time fluorescence PCR amplification. As shown in Figure 6, the results of general PCR (Figure 6(a)) and real time-PCR (Figure 6(b)) were consistent, indicating that this specific real time-PCR rapid detection system could differentiate walnut leaf tissue containing *X. campestris* pv. *juglandis* from healthy walnut leaf tissue in field effectiveness detection. The results demonstrated that the system could be used for field detection of *X. campestris* pv. *juglandis*.

DISCUSSION

X. campestris pv. *juglandis* is the main pathogenic factor of walnut bacterial blight (Kałużna et al. 2014). Walnut bacterial blight has been found in all the major walnut-growing regions of the world, causing great damage to the walnut industry (Belisario et al. 2002; Moragrega et al. 2011; Scortichini et al. 2010). Nonetheless, the traditional diagnosis and identification of walnut bacterial blight is based on the main symptoms of plant disease and the characteristics of pathogen isolated and cultured. It is easy to lead to wrong judgement, and the isolation and cultivation operation of pathogenic bacteria is tedious and requires a long period, which often affects the timely and accurate prevention and control. In order to overcome these

TACCCGATTGTTCTGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTG
CCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGG
GCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGTTGTGAAAGCCCTGGGC
TCAACCTGGGAATTGCAGTGGATACTGGGTCACTAGAGTGTGGTAGAGGGT
AGCGGAATA

FIGURE 1. Nucleotide sequence amplified using primer XC-F/XC-R. The underlined gene is specific primer sequence or its reverse complementary sequence

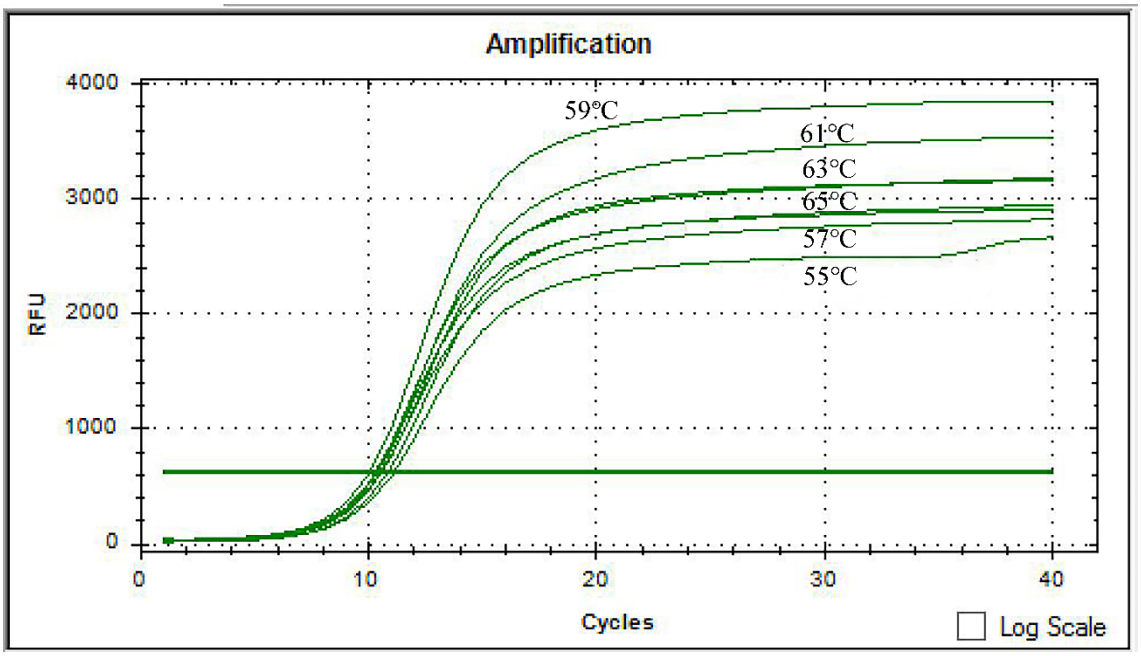

FIGURE 2. The amplification curves at temperature gradient of 55 °C to 65 °C

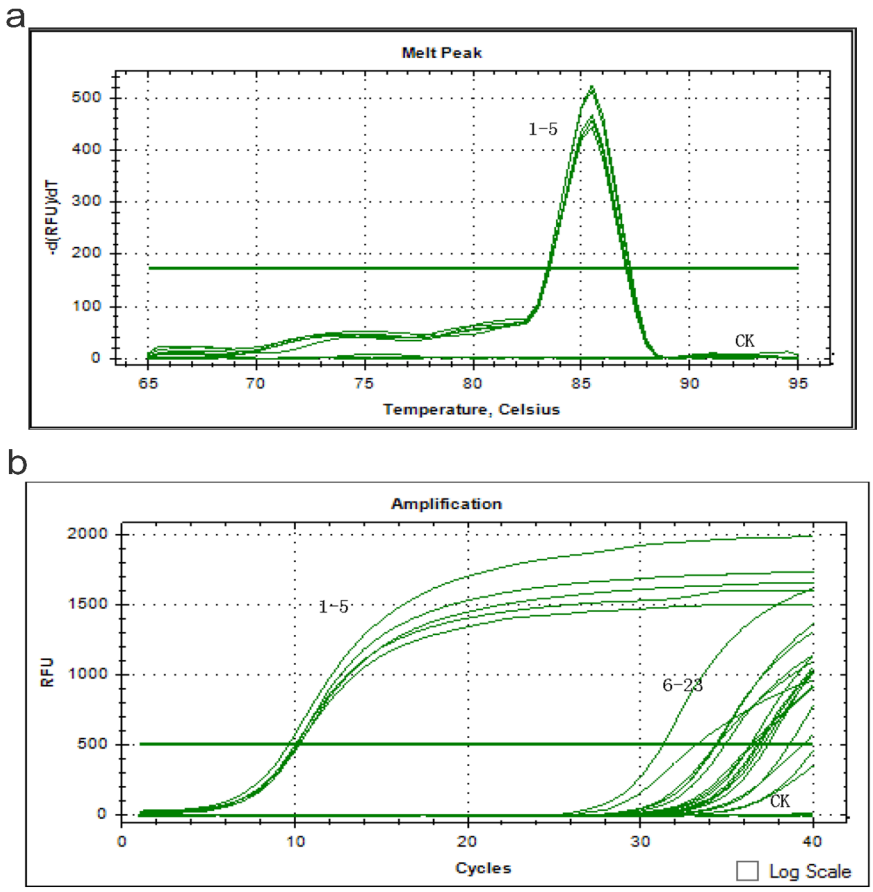

FIGURE 3. The real-time fluorescence PCR specific detection (a) Dissolving curve analysis (b) Schematic diagram of real-time fluorescence PCR specificity detection results. Amplification curves 1-5: Walnut bacterial blight pathogen; Amplification curves 6-23: Other strains in Table 1; Amplification curve CK: Negative control

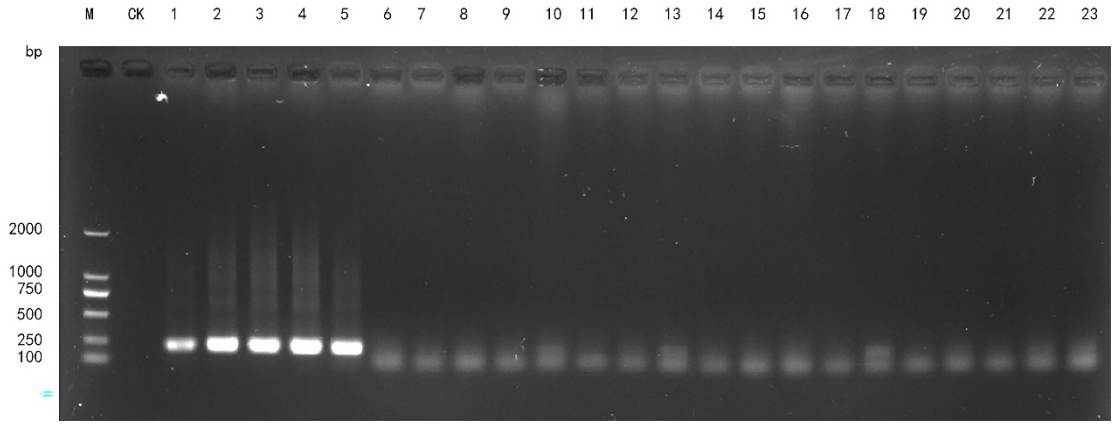

FIGURE 4. A schematic diagram of the result of general PCR amplification using specific primers. Lane M: DL2000 DNA marker; Lane CK: Negative control; Lane1-5: Walnut bacterial blight pathogen; Lane 6-23: Other strains in Table 1

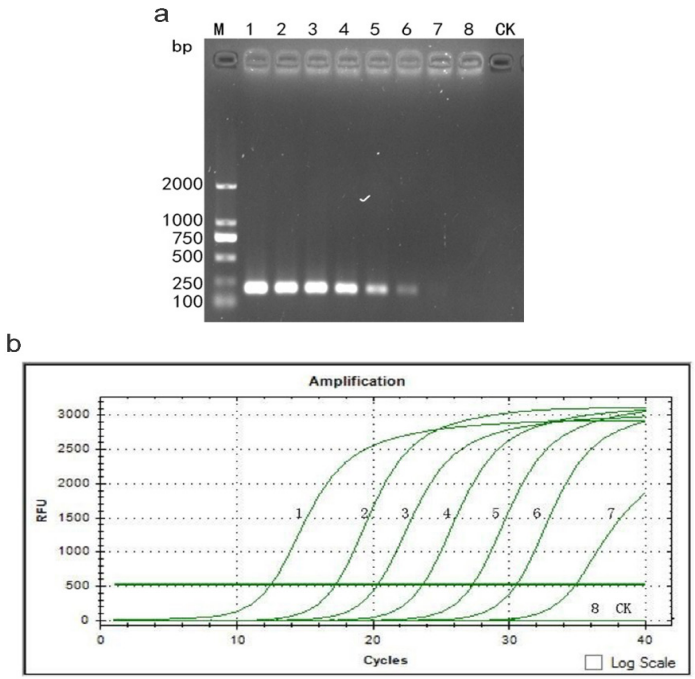

FIGURE 5. Schematic diagram of sensitivity results (a) General PCR, (b) real-time fluorescence PCR. LANES 1-8: CDNA AMPLIFICAtion results of different concentrations of template at 7.0, 7.0×10^{-1}, 7.0×10^{-2}, 7.0×10^{-3}, 7.0×10^{-4}, 7.0×10^{-5}, 7.0×10^{-6}, and 7.0×10^{-7} μg/mL, respectively; CK: Negative control

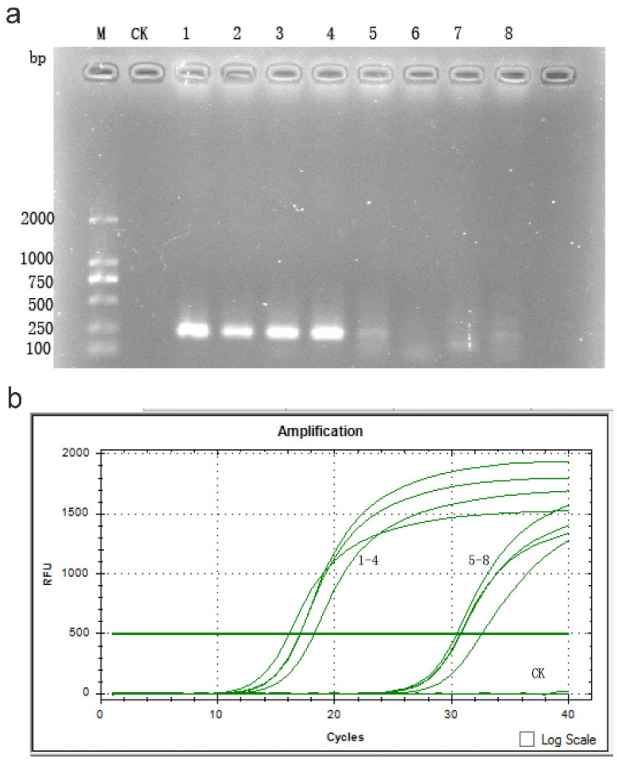

FIGURE 6. Field effectiveness testing (a) The result of general PCR amplification, (b) The results of real-time fluorescence PCR. Lanes and curves 1-4: cDNA amplification results of different morbidity leaf tissue; Lanes and curves 5-8: cDNA amplification results of different healthy leaf tissue; CK: Negative control

problems, in this study, a real-time PCR specific reaction system was established by using the fluorescence characteristics of double stranded DNA and fluorescent dye combination. The fluorescence of the DNA template in the reaction system was detected after binding with SYBR® Premix Ex Taq II, and the amplification of specific PCR products amplified using specific primers was detected. The specific amplification curve was obtained by collecting and processing the fluorescence signal.

Due to the high detection efficiency of various PCR detection techniques, it has become an important means for the detection of various pathogenic bacteria. For instance, multiplex real-time PCR detection of *Xanthomonas* (Penazova et al. 2020), LAMP detection of *Xylella fastidiosa* (Elbeaino et al. 2020), and real-time fluorescence (TaqMan) PCR detection of *Leifsonia xyli* subsp. *xyli* (Fu et al. 2016). However, most PCR detection techniques are prone to produce individual false-negative and false-positive reactions. To eliminate these interference phenomena, restriction enzyme fragment reversal pulse electric field electrophoresis is also required, which has high requirements on detection equipment and technology. On the other hand, real-time fluorescence PCR technology is to carry out the amplification reaction in an entirely closed space. The real-time situation of the reaction can be monitored on the computer linked to the PCR instrument, and only the dissolution curve analysis of the PCR product (a single peak indicates the singularity of the PCR amplification product) can determine whether the reaction produces false positive. Due to the real-time fluorescence PCR characteristics, this method is better than the general PCR method because of the higher specificity and sensitivity.

In this study, the minimum detection limit of real-time PCR was 7.0×10^{-5} μg/mL, and the sensitivity was 100 times that of general PCR. In addition, to ensure the best amplification effect and the highest specificity of the primers, gradient annealing temperature was set to optimize the real-time PCR system, and 59.0 ℃ corresponding to the specific curve with the best amplification effect was selected as the annealing temperature of the specific primer, which further improved the sensitivity of the detection system. When the specific primers, XC-F/XC-R, were used for real-time PCR amplification of the tested strains, the results indicated that only a single band with a length of 211 bp could be amplified by real-time PCR in the cDNA of the samples containing *X. campestris* pv. *juglandis*, but the specific band could not be amplified for other tested strains or negative controls. Therefore, the real-time PCR specific primers designed in this study have high specificity for the walnut bacterial blight pathogen *X. campestris* pv. *juglandis*, and can distinguish it from other bacteria on the walnut plant and from the soil bacteria around the walnut plants. The results of field disease detection showed that the specific real-time PCR rapid detection system could also distinguish walnut leaf tissue containing *X. campestris* pv. *juglandis* from healthy walnut leaf tissue in field effectiveness detection. In general, this study has successfully developed a real-time PCR detection system with high sensitivity and strong specificity for *X. campestris* pv. *juglandis*, providing supports for the early diagnosis of *X. campestris* pv. *juglandis*.

CONCLUSIONS

This study has developed specific primers for *X. campestris* pv. *juglandis* detection, to detect the pathogen at the early stage of walnut bacterial blight, which would prevent and control it as soon as possible, and hence reduce the loss. Through sequence comparison, specific primers were screened, and a real-time fluorescence PCR detection system for pathogens was established and optimized. Under our determination conditions, the detection limit of real time-PCR was 7.0×10^{-5} μg/mL, which was 100 times more sensitive than for the general PCR, and it could successfully differentiate the infected walnut tissues from healthy walnut tissues in the wild. The developed primer and fluorescence PCR detection method provides an accurate and rapid detection method for the *X. campestris* pv. *juglandis*, which can be used for the early prevention and control of walnut bacterial blight and prevent the disease from causing excessive losses.

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