



Pathogens identification and resistance evaluation on bacterial canker in *Actinidia arguta* germplasm

Hong Y. Qin¹ · Ying Zhao³ · Xiu L. Chen⁴ · Bao X. Zhang¹ · Xin Wen¹ · Chang Y. Li¹ · Shu T. Fan¹ · Yue Wang¹ · Yi M. Yang¹ · Pei L. Xu¹ · Ying X. Liu¹ · Jun Ai^{1,2}

Received: 25 March 2022 / Accepted: 16 October 2022 / Published online: 10 July 2023
© The Author(s) 2023

Abstract

Pathogen isolation and identification were performed on *Actinidia arguta* ‘Longcheng No. 2’ occurring bacterial canker from Liaoning Province, China. The pathogenic bacteria were identified as *Pseudomonas syringae* pv. *actinidiae* (Psa) by the analysis of morphology, 16S rRNA and *gyrB* sequence, which were identified as Psa biovar 2 by Psa-specific primer sequence analysis. The pathogenicity tests were carried out with the isolate ‘R12’ and type strain ‘M228’ (biovar 3) as a control; the results showed that the phloem of green stems in *A. arguta* ‘Kuiv’ could be infected rapidly by R12, and milky mucus flowed from wounds, then the phloem turned black-brown, but it had strong resistance to Psa M228. In order to evaluate the resistance on Psa R12, 54 *A. arguta* germplasm resources were infected by artificial inoculation of stems, with *A. deliciosa* cv. ‘Hongyang’ and *A. chinensis* cv. ‘Xuxiang’, as control plant material, and their resistance levels were classified according to the disease index. The 54 tested materials exhibited differences in resistance to Psa R12, but no immune materials were found. In general, the germplasms were divided into five disease resistance categories, including 2 accessions with high resistance ‘Jianfengkuiv’ and ‘TL20013’, accounted for 3.70% of all the inoculated accessions; there were 11 resistant accessions, 15 tolerant accessions, 21 susceptible accessions, 5 highly susceptible accessions among them, accounted for 20.37%, 27.78%, 38.89% and 9.26%, respectively. In this study, the screening of disease-resistant germplasms could lay a foundation for further research on gene mapping, resistance mechanisms and breeding-resistant varieties of *A. arguta* to Psa.

Keywords *Actinidia arguta* · Germplasm resources · Bacterial canker · Pathogen identification · Disease resistance evaluation

Introduction

Actinidia arguta (Sieb. et Zucc.) is an important deciduous fruit that belongs to *Actinidia* in Actinidiaceae, and is also known as hardy kiwifruit, kiwiberry, baby kiwi or mini kiwi. Although the fruit of *A. arguta* is smaller in size than *A. deliciosa* ‘Hayward’, its fruit is smooth, edible and rich in nutrients such as vitamin C, phenolics, carotenoids and various

minerals (Nishiyama et al. 2004; Okamoto and Goto 2005; Fisk et al. 2006; Latocha 2007; Latocha and Krupa 2008; Latocha et al. 2010). *A. arguta* has been widely planted as a promising species worldwide, especially in cold areas, because of its high cold hardiness (up to –30 °C in midwinter) and relatively short vegetation period (150 days) (Latocha 2008; Piotr et al. 2011). In recent years, many commercial orchards of *A. arguta* have been established in China, New Zealand, the USA, Japan, Chile and some European countries (Okamoto and Goto 2005; Williams et al. 2003).

With the continuous development of kiwifruit industry, related diseases began to occur. Among them, the bacterial canker is a common and devastating disease of kiwifruit, and its causal agent is *Pseudomonas syringae* pv. *actinidiae* (Psa). The pathogen was first discovered in the orchards of *A. chinensis* from California, USA (Opgenorth et al. 1983) and Shizuoka Prefecture, Japan (Serizawa et al. 1989), and subsequently also reported in Korea (Koh and Lee 1992; Koh et al. 1994), Italy (Scortichini 1994), Portugal (Balestra

✉ Jun Ai
aijun1005@163.com

¹ Institute of Special Animals and Plants Sciences, Chinese Academy of Agricultural Sciences, Changchun, China

² Jilin Agricultural University, Changchun, China

³ Pomology Institute, Jilin Academy of Agriculture Sciences, Gongzhuling, China

⁴ Applied Technology Research and Development Center for Sericulture and Special Local Products of Hebei Universities, Institute of Sericulture, Chengde Medical University, Chengde, China

et al. 2010) and New Zealand (Everett et al. 2011). In China, bacterial canker was first discovered in 1985. Then, large outbreaks occurred in areas of Sichuan, Shanxi, Anhui and others within a short period, resulting in numerous plant deaths and serious economic losses (Cheng et al. 1995; Liang et al. 2000). *Psa* can infect many species of Actinidia, among which *A. chinensis* and *A. deliciosa* are very susceptible, while *A. arguta* and *A. pubescens* are highly resistant to the pathogen (Song et al. 2020; Zhang et al. 2014; Shi et al. 2014). To date, *Psa* infection in *A. arguta* plants has only been reported in Japan (Ushiyama et al. 1992; McCann et al. 2013, 2017), New Zealand (Vanneste et al. 2014) and South Korea (Kim et al. 2016) accompanied by mild symptoms, such as brown spot with yalo on leaves, which did not lead to serious losses. However, we found that the plants of *A. arguta* ‘Longcheng No. 2’ were infected with bacterial canker in Liaoning Dandong of China (N 40°13'; E 124°30'), and spread rapidly with adjacent vines. Its infection speed is no less than that of *Psa* in *A. chinensis*. This is contrary to the previous studies that *A. arguta* is resistant to bacterial canker. Although bacterial canker has only affected few areas at present (only three gardens), it has potential to become the deadliest disease of *A. arguta*, causing great harm to the industry.

In this study, pathogens of bacterial canker in *A. arguta* Longcheng No. 2 plants were firstly identified by morphological identification, 16S rRNA and *gyrB* gene sequence analysis. Then, the species of the pathogen was further identified by specific primers, including *Psa* F1/R2, *Psa* F3/R4, *Tac* F/R, *PsaJ*-F/R and *PsaK*-F/R. Finally, the resistance of 54 *A. arguta* germplasm resources to *Psa* was evaluated by artificial inoculation on stems *in vitro*. The above related studies could provide a foundation for resistant breeding to bacterial canker in *A. arguta*.

Materials and methods

Plant materials

The soft green stems with semi-lignification of *A. arguta* cv. ‘Kuily’, *A. deliciosa* cv. ‘Hongyang’ and *A. chinensis* cv. ‘Xuxiang’, which were cut during the growing season and uniform thickness, were used as experimental materials in the pathogenicity tests of new isolates. The resistance of Xuxiang, Hongyang and 54 *A. arguta* germplasm resources to the new strain *Psa* ‘R12’ were evaluated by pathogenicity testing with healthy dormant woody stems collected from the *A. arguta* germplasm resource nursery (N 44°00'; E 126°01') locating in Institute of Special Animals and Plants Sciences of CAAS, Changchun. Among 54

A. arguta germplasm resources, 1 cultivar was from Russia, 12 were Chinese cultivars, and the remaining 41 were wild resources collected from three provinces (Heilongjiang, Jilin and Liaoning) in the northeast of China.

Isolation, purification and morphological characteristics of pathogenic bacteria

Stems were collected from the diseased plants (Fig. 2a). According to the method of Fang (1998), the pathogenic bacteria were isolated and cultured with NA medium at 24 °C. Then, single colonies were inoculated onto new plates for purification and cultured for 72 h at 24 °C in the dark. The morphology and growth of colonies were observed with a microscope, and the morphology of pathogens were observed under electron microscope, with the type strain *Psa* ‘M228’ (CP032631, biovar 3) as a control strain.

Molecular identification

Pathogenic bacteria were inoculated in buffered peptone water (BP) (Gao et al. 2016) liquid medium and then incubated for 18 h on a shaking table at 24 °C and 180 r·min⁻¹. After centrifugation at 1200 rpm for 2 min, the bacterial pellet was collected. Finally, pathogen DNA was extracted by the Tianjin Lanrui Biotechnology DNA Extraction Kit. The pathogen gene sequences were obtained by PCR amplification using primers 16S rRNA and *gyrB* gene (Yamamoto and Harayama 1995) respectively, and the sequences were deposited to GenBank (16S rRNA: OP218747-OP218754; *gyrB*: OP598821-OP598822, OP586787). PCR products were detected by agarose gel electrophoresis, tapped and recovered, TA ligated and transformed, and then sent to Shanghai Bioengineering Co., Ltd. for sequencing. The sequences were compared with those of standard strains in the GenBank database by BLAST to determine their taxonomic status. For further genome-level analysis, specific sequences were obtained by PCR amplification with the extracted DNA as a template and *Psa*-specific primers *Psa*F1/R2, *Psa*F3/R4 (Rees-George et al. 2010), *Tac*-F/R (Koh et al. 2014), *PsaJ*-F/R and *PsaK*-F/R (Lee et al. 2016). The PCR of *gyrB* gene was performed by an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 1 min, extension at 72 °C for 2 min and final elongation at 72 °C for 8 min. The other PCR was performed by an initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, extension at 72 °C for 30 s and final elongation at 72 °C for 5 min. The nucleotide sequences, annealing temperatures and amplicon sizes of the primers used in this experiment are shown in Table 1.

Table 1 Primer sequence, genes utilized in design, expected product size and source

Primer pair	Sequence (5'-3')	Annealing temp. (°C)	Product size	Target	Reference
16F27	AGAGTTTGATCCTGGCTCAG	53	–	–	Lane (1991)
1492R	TACGGTTACCTTGTACGACTT				
gyrB-UP1	GAAGTCATCATGACCGTTCTGCAY	62		gyrB	Yamamoto and Harayama (1995)
	GCNCGNGGNAARTTYGA				
gyrB-UP2	AGCAGGGTACGGATGTGCGAGCCR				
	TCNACRTCNGCRTCNGTCAT				
PsaF1	TTTTGCTTTGCACACCCGATTTT	65	280 bp	ITS	Rees-George et al. (2010)
PsaR2	CACGCACCCTTCAATCAGGATG				
PsaF3	ACCTGGTGAAGTTGGTCAGAGC	65	175 bp	ITS	Rees-George et al. (2010)
PsaR4	CGCACCCCTTCAATCAGGATG				
PsaJ-F	GACGTCGACGACAAGGTGAT	65	481	biovar 1	Lee et al. (2016)
PsaJ-R	AGTAAACCGTGCCGTCATCTC				
PsaK-F	GACAAAGCCAAAAGGCCGA	65	413	biovar 2	Lee et al. (2016)
PsaK-R	TGCCAAGCCCAAGTATCCAAGC				
TAC-F	CGGGCTAGACAGTACGCTGT	65	545	–	Koh et al. (2014)
TAC-R	CAGGCCCTTCTACCGCTAC				

Pathogenicity tests of pathogenic bacteria

According to the method of Hoyte et al. (2015), pathogenicity tests of new strain Psa R12 were performed on the soft green stems *in vitro* from healthy *A. arguta* cv. Kuilv, *A. deliciosa* cv. Hongyang and *A. chinensis* cv. Xuxiang, with Psa M228, as a control. The soft green stems (15 cm long) *in vitro* were sterilized and then wounded, penetrating into the xylem, in the middle part of the stems with a sterile knife, and 20 µL of bacterial suspension (10^8 CFU·mL⁻¹) of Psa R12 and M228 was added to the wound respectively. Similarly, controls were inoculated using sterile bacteriological saline (0.85% NaCl). The upper and lower incisions of the stems were wrapped with absorbent cotton containing sterile water to keep wet, and the water has been sterilized by high temperature. Sterile water was sprayed into the plastic boxes in which the plant materials were held once a day to maintain humid conditions. Stems were placed in a Petri dish with moist filter paper and kept at 18 °C in the dark for 21 days. Finally, the pathogenesis of the materials was observed and recorded. Each treatment was conducted with 5 stems and 3 replicates. Finally, bacterial colonies reisolated from the diseased spots of Kuilv and Hongyang green stems inoculated with Psa R12 were identified by PCR tests using specific primers Psa F1/R2 and Psa F3/R4.

Evaluation on disease resistance of *A. arguta* germplasm resources

According to the method of Hoyte et al. (2015), the resistance was evaluated by artificial inoculation *in vitro* in 54 *A. arguta* germplasm resources, with Hongyang and Xuxiang

as control plant materials. The dormant woody stems of Hongyang, Xuxiang and 54 *A. arguta* germplasm resources were artificially inoculated with Psa R12 in 2019 and 2020. The dormant woody stems (15 cm long) *in vitro* were sterilized and then wounded, penetrating into the xylem, in the middle part of the stems with a sterile knife, and 20 µL of bacterial suspension (10^8 CFU·mL⁻¹) of Psa R12 was added to the wound. The upper and lower incisions of the stems were wrapped with sterile absorbent cotton. Sterile water was sprayed into the plastic boxes in which the plant materials were held once a day to maintain humid conditions. Stems were placed in a Petri dish with moist filter paper and kept at 18 °C in the dark for 21 days. Finally, the disease incidence was determined, and the resistance level was divided into 7 grades based on the standards shown in Table 2 and symptoms shown in Fig. 1.

A disease severity index (DSI) was calculated for each accession using the following formula:

$$DSI = \frac{\sum \text{Ratings for each plant}}{7 \times \text{Number of plants rated}} \times 100$$

Table 2 Grading standards for bacterial canker severity of *A. arguta*

Grade	Canker length
0	LD ≤ 10 mm
1	10 mm < LD ≤ 30 mm
3	30 mm < LD ≤ 60 mm
5	60 mm < LD ≤ 100 mm
7	LD > 100 mm

LD represented Lesion Diameter

Fig. 1 Grading standards for bacterial canker of *A. arguta*. The resistance level was divided into 7 grades based on the standards



According to the disease index results, a resistance evaluation standard was developed as follows: highly resistant: $DSI \leq 10$; resistant: $10 < DSI \leq 30$; moderately susceptible: $30 < DSI \leq 50$; susceptible: $50 < DSI \leq 80$; highly susceptible: $80 \leq DSI$.

Statistical analysis

All disease severity index data were analysed by SAS PROC ANOVA using Duncan's multiple range tests at $p \leq 0.05$ (SAS Version 9.2).

Results

Description of disease symptoms

Symptoms caused by Psa were first observed on *A. arguta* in Dandong, Liaoning Province, China, in June 2019. In 2020, similar diseases occurred in more than three Longcheng No.

2 plantings, but disease development was terminated due to eradication of the infected vines and subsequent periodic spraying with the proper bactericides in 2021. The disease mainly harmed the stems of plants, and reddish-brown mucus overflowed from the wounds and buds of the diseased stems (Fig. 2a, b). The diseased leaves had obvious polygonal brown spots surrounded by yellow halos (Fig. 2c). Finally, the twigs became wilted and scorched. When the stems were placed in a Petri dish with moist filter paper for 24 h, reddish-brown bacterial oozing was observed (Fig. 2d).

Morphological characteristics of colonies and pathogens

Eight representative round and convex colonies were isolated from the infected tissues of the vines. The colonies were white-creamy or light yellow, round, semiraised and smooth, with neat edges (R12 is shown as Fig. 3a) on NA medium as well as the type strain M228 (Fig. 3b). After culture for 72 h, the colony diameter was only 1–2 mm,



Fig. 2 Symptoms in the field showing red exudate from cankers at the base of a cane (**a**), brown staining of the diseased stems (**b**), dark angular leaf spots with yellow halo on *A. arguta* Longcheng No. 2 in

June 2019 in the field of Dan Dong, China (**c**) and red exudate from the diseased stems cultured in Petri dishes for 24 h (**d**)

reflecting a very slow growth rate. Electron microscopy showed that both R12 (Fig. 3c) and M228 (Fig. 3d) were rod-shaped, with blunt round ends and flagella. R12 was 2.3–3.9 μm long and 0.6–1.1 μm wide, with a length/width of 3.68. M228 was 2.6–4.9 μm long and 0.7–1.5 μm wide, with a length/width of 3.60.

Sequence analysis results of pathogenic bacteria

In 8 isolated strains, a 1368-bp fragment was amplified using the 16S rRNA primer set 16F27/1492R, while a 984-bp fragment was amplified using *gyrB* gene. The 16S rRNA and *gyrB* gene phylogenetic tree was constructed by comparison with published 16S rRNA and *gyrB* gene sequences of the relative bacterial species using the neighbour-joining method in MEGA 6.0 (Fig. 4). Combined with the results of morphological identification, the pathogen was identified as *P. syringae* pv. *actinidiae* (Psa).

There are differences in genetic structure of Psa prevalent in different regions or different periods. On the basis of physiological, biochemical, and genomic evidence, Psa can be divided into five separate clades (now popularly referred to as biovars 1–5) (McCann et al. 2013; Chapman et al. 2012;

Fujikawa and Sawada 2016). To further determine the clade, 5 pairs of specific primers were used to conduct PCR amplification on the 8 isolated strains and M228 (biovar 3). The amplified products were analysed by agarose gel electrophoresis. The expected fragments of 280 bp with primer pairs Psa F1/R2 and 175 bp with primer pairs Psa F3/R4 were amplified from all nine strains (Fig. 5a, b lanes 1 to 9), indicating that they corresponded to Psa. Specific primers PsaJ-F/R, PsaK-F/R and Tac F/R were used to distinguish biovars 1, 2 and 3, respectively. Primers PsaJ-F/R produced a 481-bp region with genomic DNA of biovar 1 strains, while primers PsaK F/R amplified a 413-bp region present only in the genome of biovar 2 strains, and primers Tac F/R amplified a 545 bp region present only in the genome of biovar 3 strains. No signal was obtained from these 9 bacterial strains when using the primers PsaJ F/R (Fig. 5c). The 8 isolated strains produced a 413-bp amplicon (Fig. 5d lanes 2 to 9), while M228 did not show amplification of this DNA segment with the primer pair PsaK-F/R (Fig. 5d lanes 1). In contrast, the expected fragments of 545 bp were amplified from M228 (Fig. 5e lane 1), but the 8 new strains did not produce any amplicons (Fig. 5e lanes 2 to 9) with the primer pair Tac F/R. Based on these results, it is suggested that these 8 strains belong to Psa biovar 2.

Pathogenicity tests

Pathogenicity tests of the isolate Psa R12 were conducted on the soft green stems of Kuilv, Hongyang and Xuxiang *in vitro*, with Psa M228 as a control. After inoculation with R12 for 21 days, obvious brown or black-brown diseased spots could be observed at the inoculation sites of Kuilv stems (Fig. 6a), Milky white mucus overflowed from the wounds, and the phloem turned black–brown or black, which was more serious than Hongyang (Fig. 6b) and Xuxiang (Fig. 6c). Inoculated with Psa M228, the incidence of Hongyang (Fig. 6e) was more serious than that of Xuxiang (Fig. 6f), while that of Kuilv was less serious (Fig. 6d). No disease symptoms developed on the control stems. These symptoms were similar to those caused by natural infections. Bacterial colonies reisolated from the diseased spots of Kuilv and Hongyang green stems inoculated with Psa R12 were identified as Psa by PCR tests using specific primers Psa F1/R2 and Psa F3/R4, fulfilling Koch's postulates.

Evaluation of resistance of *A. arguta* germplasm resources to Psa

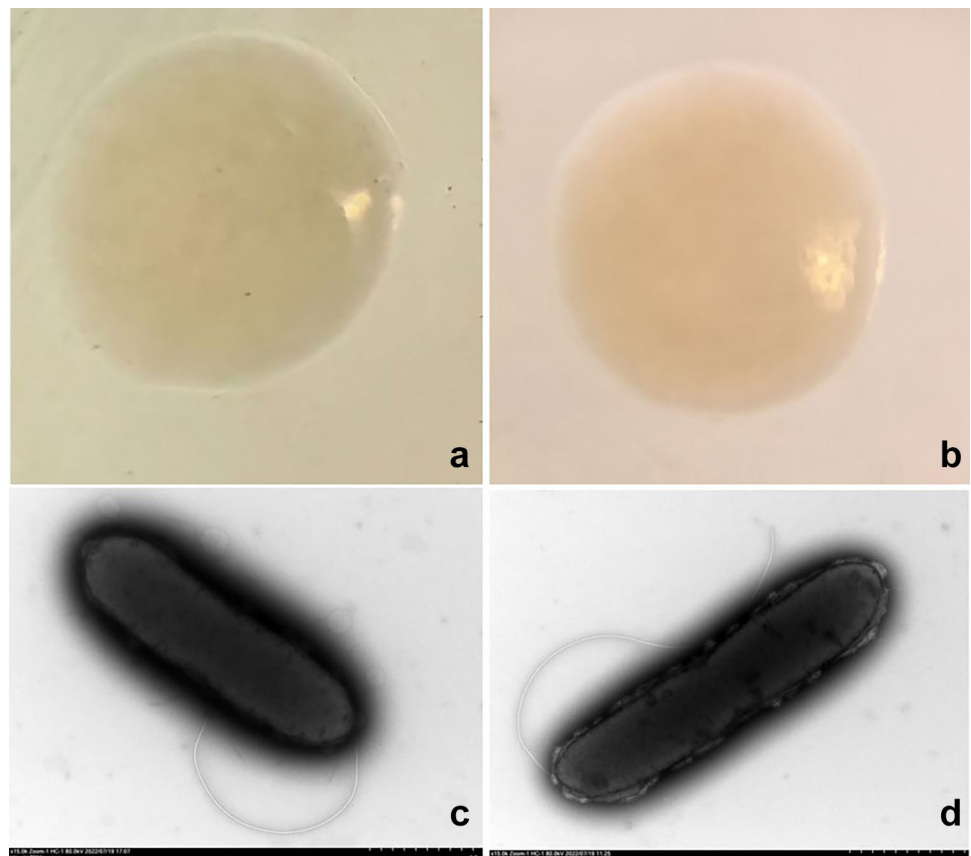
The dormant stems of 54 *A. arguta* germplasm resources, Hongyang and Xuxiang were inoculated with Psa R12 by artificial wounding. After inoculation for

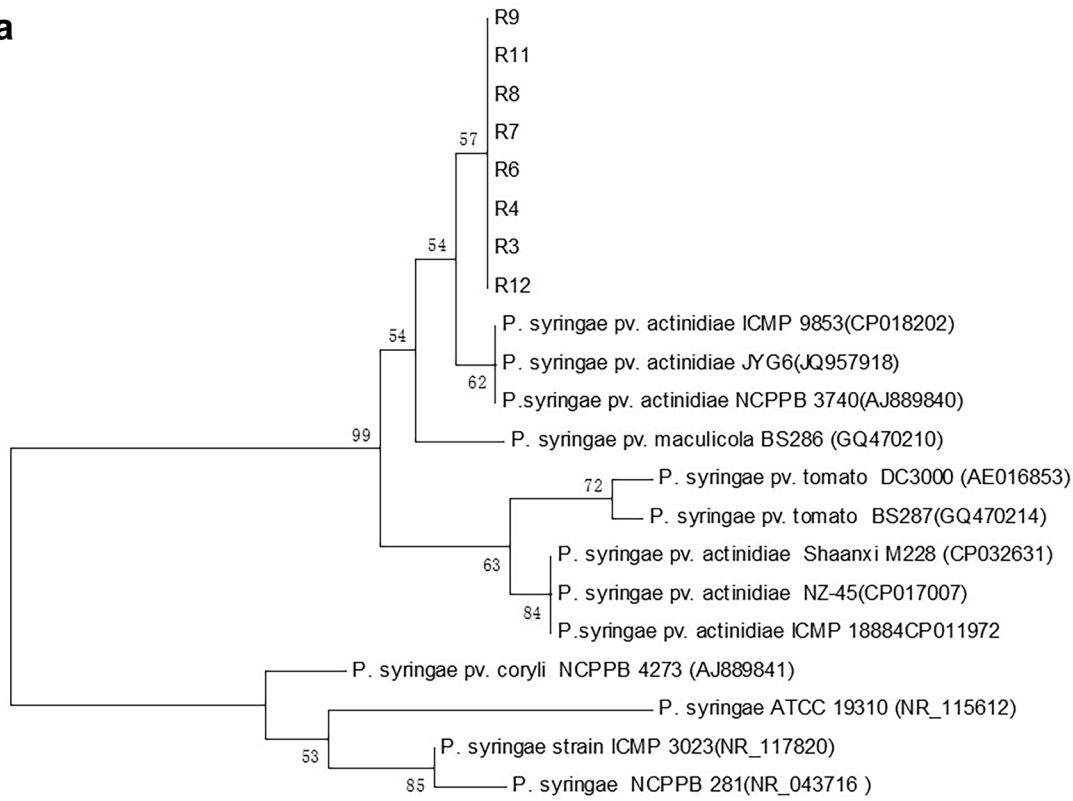
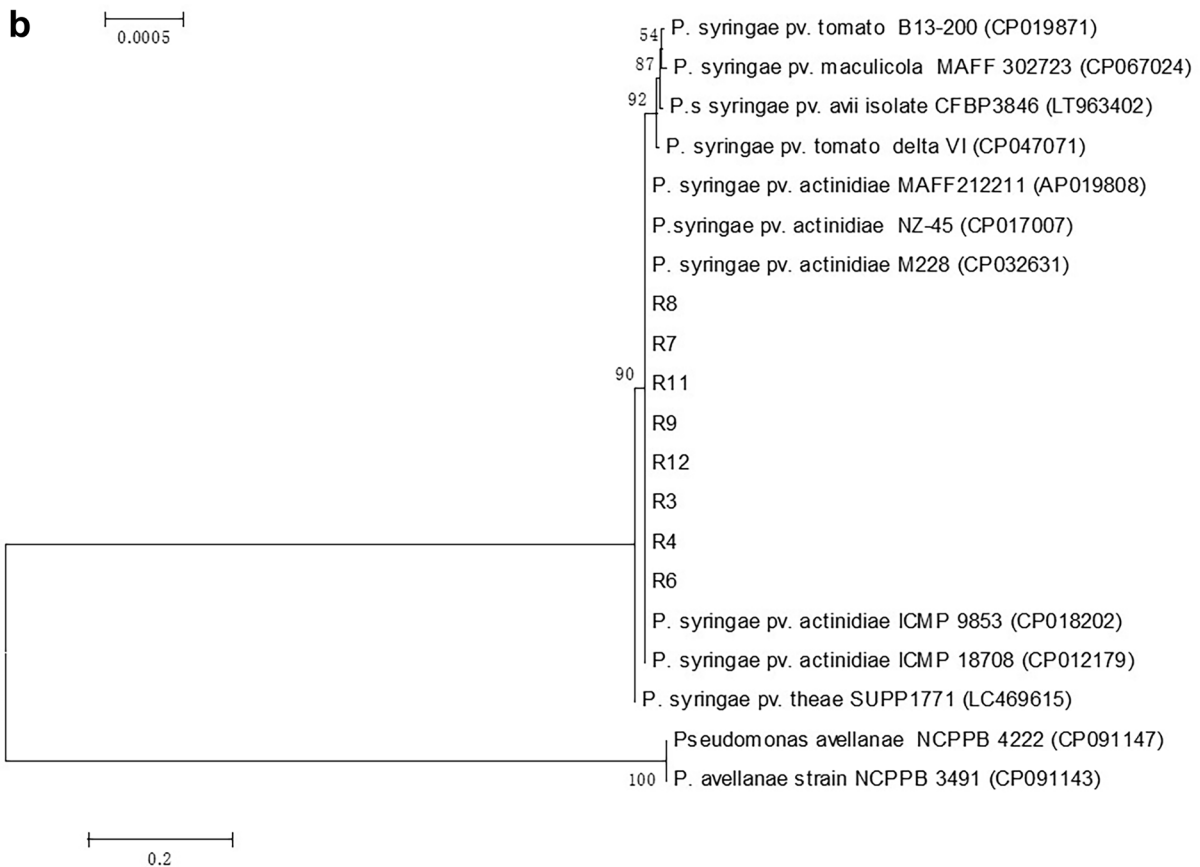
Fig. 4 Phylogenetic analysis of the new isolated strains and the relative bacterial species published in GenBank based on sequences of 16S rRNA (a) and *gyrB* gene (b). Confidence values on the stems were obtained for 1,000 bootstrap replicates with Mega version 6.0 software

10 days, milky white mucus could be observed from some germplasms. After inoculation for 21 days, milky white, milky yellow or rust-red mucus overflowed conspicuously from the wounds of stems (Fig. 7a–d). Moreover, the mucus colour varied among germplasms. The phloem and xylem also turned brown or dark brown (Fig. 7e, f).

The 54 *A. arguta* germplasm resources showed significant differences in their degree of resistance to Psa R12 (Table 3), and there were no immune materials. Germplasm resources with a disease index below 30% accounted for 24.07%, indicating very high resistance to Psa R12. Twenty-five accessions, accounting for 55.56%, had a disease index greater than 50%. According to the 2-year comprehensive disease index, the 54 *A. arguta* germplasm resources were divided into five disease-resistance grades. Among them, 2 accessions, 'Jianfengkulv' and 'TL20013', were highly resistant with a disease index less than 10%, accounting for 3.70% of all inoculated accessions; 11 accessions, 'Jiayuan 5', were resistant, accounting for 20.37%; 15 tolerant accessions accounted for 27.78%; 21 accessions were susceptible, accounting

Fig. 3 The colonies of Psa 'R12' were white-creamy or light yellow, round, semiraised and smooth, with neat edges on NA medium (a) as well as the type strain 'M228' (b). Electron microscopy showed that there was no difference in morphology between R12 (c) and M228 (d), and they were rod-shaped, with blunt round ends and flagella with a length of 0.6–3.9 μm , 0.7–4.9 μm respectively



a**b**

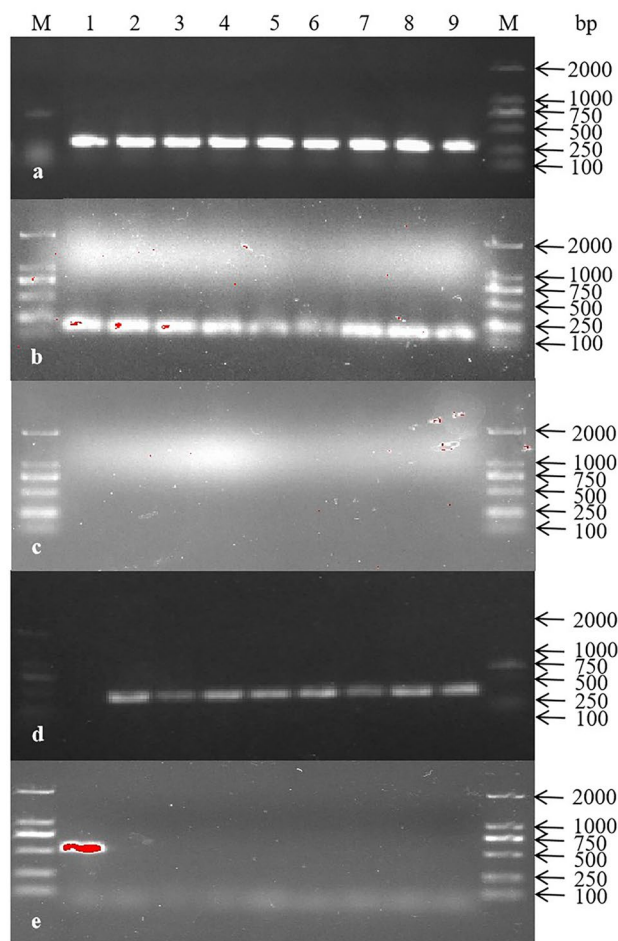


Fig. 5 PCRs using five sets of primers to identify *Pseudomonas syringae* pv. *actinidiae*. **a** Psa F1/R2, **b** Psa F3/R4, **c** PsaJ-F/R, **d** PsaK-F/R, **e** Tac F/R. Bacterial DNA was extracted from cultures described in full in Table 1; Lane 1: *Pseudomonas syringae* pv. *actinidiae* M228; lanes 2–9: isolated strains

for 38.89%; and 5 accessions were highly susceptible, accounting for 9.26%. Hongyang, which is extremely susceptible to Psa biovar 3, showed disease resistance, and Xuxiang, which has strong resistance to Psa biovar 3, showed high resistance to Psa R12.

Pearson's correlation analysis was carried out on the 2-year disease index results of the tested germplasms, and the results showed that there was a very significant positive correlation between the 2-year disease index ($p < 0.0001$, $r = 0.83957$). The results showed that the evaluation method was stable, reliable and accurate and could be used to identify the canker resistance of *A. arguta*.

Discussion

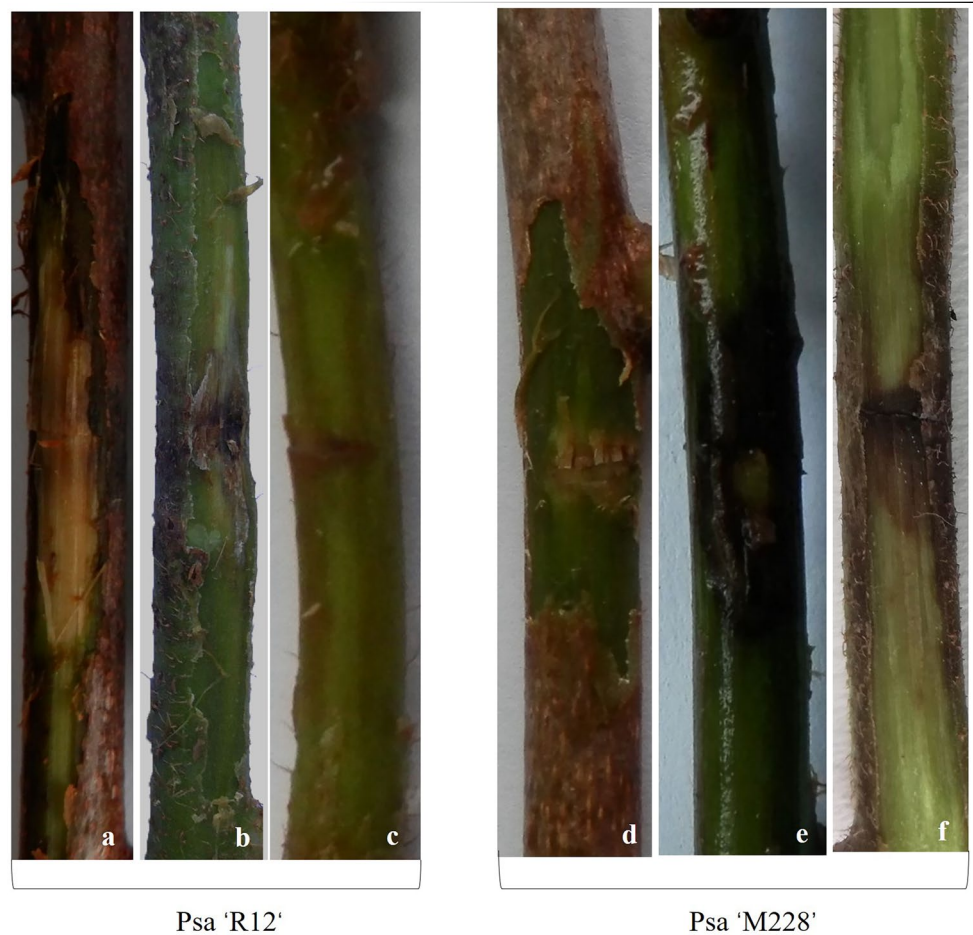
The pathogen of bacterial canker in kiwifruit is *Pseudomonas syringae* pv. *actinidiae* (Psa), which can quickly infect kiwifruit plants and cause outbreaks in a short time

period. It is considered a fatal threat to the kiwifruit industry worldwide. At present, it had a serious impact on the kiwifruit industry in Europe, Asia, New Zealand, Chile and other regions (Balestra et al. 2010; Abelleira et al. 2011; Everett et al. 2011; Vanneste et al. 2011; Koh et al. 2012; Zhao et al. 2013; Sawada et al. 2015).

Although many species of *Actinidia* can be infected by Psa, there are great differences in disease resistance among the species. The disease sensitivity of *A. chinensis* has been found in Italy, New Zealand and South Korea. According to the species of *Actinidia*, the disease incidence of *A. chinensis* cv. 'Hort16A' was significantly higher than that of *A. deliciosa* cv. Hayward. And the disease incidence of *A. deliciosa* cv. Hayward, 'Yate' and 'Qinmei' decreased in turn. In China, Shen et al. (2008) and Gao et al. (2011) also found that the incidence in Hongyang was the highest and that in Xuxiang was the lowest. The similar result was found in New Zealand that the susceptibility of Hongyang was the highest, while the susceptibility levels of Hort16A, 'Gold 3', 'Gold 9' and Hayward gradually decreased. After the screening of disease-resistant species, a number of disease-resistant varieties were obtained by crossing these disease-resistant varieties of *A. deliciosa* with *A. chinensis*. However, it is rare for *A. arguta* plants to be infected by Psa, and was only reported in Japan (Ushiyama et al. 1992; McCann et al. 2013, 2017), New Zealand (Vanneste et al. 2014) and South Korea (Kim et al. 2016) with mild symptoms and no serious losses. Psa was isolated and reported to cause disease symptoms on *A. arguta* for the first time in China; its symptoms are similar to those of kiwifruit canker (Koh et al. 2010; Scortichini et al. 2012; Everett et al. 2011) and include reddish-brown mucus overflowing from the wounds and buds of diseased stems and obvious polygonal brown spots surrounded by yellow halos on diseased leaves.

Many studies have shown that there are differences in the genetic structure of Psa prevalent in different regions and even in different periods. At least five clades of Psa are distributed in different regions of the world (McCann et al. 2013; Chapman et al. 2012; Fujikawa and Sawada 2016), and they also show certain differences at the physiological, biochemical and genetic levels. Biovar 1 comprises strains isolated from Japan in 1984 and Italy in 1992, which contain an *argK-tox* gene cluster (Chapman et al. 2012). The occurrence of this biovar is high in Japan but is only sporadic in Italy. Biovar 2 is represented by strains isolated from South Korea in 1988, and it can produce coronatine (Shim et al. 2003), but the *argK-tox* gene cluster is deleted in this group. Biovar 3 was discovered in Italy in 2008, has high virulence and is the causative agent of the current outbreaks of kiwifruit canker disease in Europe, New Zealand, Chile and Asia (Chapman et al. 2012; Ferrante and Scortichini 2010; Marcelletti et al. 2011). Recently, biovar 4 was identified as the de novo pathovar *P. syringae* pv. *actinidifoliorum* (Pfm)

Fig. 6 After inoculation with R12 for 21 days, obvious brown or black-brown diseased spots could be observed at the inoculation sites of ‘Kuilv’ stems (a), which was more serious than ‘Hongyang’ (b) and ‘Xuxiang’ (c). After inoculation with M228, the incidence of Kuilv was mild, while the incidence of Hongyang was extremely severe (e), significantly more serious than that of Xuxiang (f)



(Cunty et al. 2015), and biovar 5 is currently only found in a localized area of Japan (Fujikawa and Sawada 2016). Based on morphological methods and 16S rRNA and *gyrB* gene

amplification fragment alignment, the isolated strains were identified as *P. syringae*. The isolated strains were further characterised as Psa biovar 2 by amplified specific primer

Fig. 7 Milky white, milky yellow, pink or rust-red mucus overflowed (a–d) from the inoculation wound of dormant stems, and the phloem turned brown or dark brown by 21 days after inoculation (e, f)



Table 3 Disease index of different germplasm resources

Cultivar or biotype	2019		2020		Average disease index of 2 years	Comprehensive evaluation
	Disease severity index	Evaluation	Disease severity index	Evaluation		
Hongyang- $\alpha\beta\gamma\delta$	18.26 $xyz\alpha\beta$	R	12.70 xyz	R	15.48	R
Xuxiang	4.76 γ	HR	7.14 yz	HR	5.95	HR
Jialv	80.95 $cdefg$	HS	58.73 $ghigk$	S	69.84	S
Kuilv	73.81 $fghi$	S	44.45 $mnpq$	M	59.13	S
Fenglv	87.30 bcd	HS	90.48 a	HS	88.89	HS
Tianxinbao	28.57 vwx	R	33.33 $qrstuv$	M	30.95	T
Wanlv	82.54 $cdefg$	HS	76.19 bcd	S	79.37	S
Xinlv	64.29 $ijkl$	S	47.62 $klmno$	M	55.95	S
Pinglv	54.76 $lmnopq$	S	34.92 $qrst$	M	44.84	T
Ruilv	14.29 $\alpha\beta\gamma$	R	7.14 yz	HR	10.32	R
Lvbao	46.82 $pqrs$	M	26.19 $tuvw$	R	36.51	T
Longcheng 2	76.19 $defgh$	S	53.97 $ijklm$	S	65.08	S
Huanyou1	15.87 $\alpha\beta\gamma$	R	22.22 vwx	M	19.05	R
lvwang	47.62 $pqrs$	M	29.37 $stuv$	R	39.29	T
RSZ-06	50.79 $nopqr$	M	42.07 $nopqr$	M	46.43	T
Jiayuan 12	33.33 $tuvw$	M	57.14 $ghijkl$	S	45.24	T
Jiayuan 5	18.26 $xyz\alpha\beta$	R	11.90 xyz	R	15.08	R
Jiayuan	73.02 $fghij$	S	49.21 $klmno$	M	61.11	S
TL21011	100.00 a	HS	95.24 a	HS	97.62	HS
Jianfengkuilv	9.52 $\beta\gamma$	HR	4.76 z	HR	7.14	HR
Jianfengmeigui	66.67 $higk$	S	46.82 $lmnop$	M	56.75	S
Liaofeng1	85.71 $bcde$	HS	94.44 a	HS	90.08	HS
Liangzao808	63.49 $ijklm$	S	66.67 $cdefgh$	S	65.08	S
Danfeng1	43.65 $qrstu$	M	68.26 $bcdefg$	S	55.95	S
TG07011	71.43 $ghij$	S	71.43 $bcdef$	S	71.43	S
TG09062	19.05 $xyz\alpha\beta$	R	16.67 wxy	R	17.86	R
TT01043	87.30 bcd	HS	61.90 $efghij$	S	74.60	S
TT02011	27.78 vwx	R	39.69 $opqrs$	M	33.73	T
TT04011	59.52 $klmno$	S	54.76 $ijklm$	S	57.14	S
TT04023	50.00 $opqr$	M	48.41 $klmno$	M	49.21	T
TT04043	17.46 $yz\alpha\beta$	R	7.93 yz	HR	12.70	R
TT04051	52.38 $nopqr$	S	38.10 $opqrs$	M	45.24	T
TT05053	29.36 vwx	R	34.13 $qrstu$	M	31.75	T
TT06031	17.46 $yz\alpha\beta$	R	35.71 $pqrst$	M	25.79	T
TT10103	44.45 $pqrst$	M	73.02 $bcde$	S	58.73	S
TT09093	15.08 $\alpha\beta\gamma$	R	8.33 yz	HR	11.71	R
TL11102	47.62 $pqrs$	M	77.78 bc	S	62.70	S
TL12011	74.60 $efghi$	S	76.19 bcd	S	75.40	S
TL13081	11.90 $\beta\gamma$	R	28.57 $stuv$	R	20.24	R
TL14112	26.19 $wxyz$	R	30.95 $rstuv$	M	28.57	R
TL14042	19.05 $xyz\alpha\beta$	R	14.29 xyz	R	16.67	R
TL14061	77.78 $defgh$	S	56.35 $hijkl$	M	67.06	S
TL16071	42.86 $rstu$	M	44.45 $mnpq$	M	43.65	T
TL16091	32.54 uvw	M	23.02 uvw	R	27.78	R
TL17011	32.50 uvw	M	32.54 $rstuv$	M	32.52	T
TL17031	90.48 abc	HS	65.08 $defghi$	S	77.78	S

Table 3 (continued)

Cultivar or biotype	2019		2020		Average disease index of 2 years	Comprehensive evaluation
	Disease severity index	Evaluation	Disease severity index	Evaluation		
TL20013	10.58 $\alpha\beta$	R	3.97 z	HR	7.28	HR
TL02013	38.10 stuv	M	56.35 hijkl	S	47.22	T
TL02062	95.24 ab	HS	78.57 b	S	86.90	HS
TL04091	53.97 lmnpq	S	61.11 fghij	S	57.54	S
TT06053	74.60 efghi	S	51.59 jklmn	S	63.10	S
TG03012	61.90 jklmn	S	55.55 hijklm	S	58.73	S
TG05052	84.12 bcdef	HS	100.00 a	HS	92.06	HS
TG06052	24.52 wxyz α	R	5.55 yz	HR	15.04	R
TG08062	46.03 pqrs	M	32.54 rstuv	M	39.29	T
TG09021	55.55 klmnop	S	58.73 ghijk	S	57.14	S

HS, S, T, R, and HR respectively represented highly susceptible, susceptible, tolerant, resistant, and highly resistant. Different lowercase letters indicate significant differences at $p < 0.05$

fragment analysis. In Korea, biovar 2 strains have been isolated since the late 1980s, causing severe economic losses to *A. deliciosa* cv. Hayward as well as *A. chinensis* cv. Hort16A (Koh et al. 1994, 2010).

Field screening is the most intuitive method for the determination of disease resistance, but this method is easily affected by various factors, such as climate and the environment, causing the easy spread of pathogenic bacteria and inconsistent identification results. Many studies have shown that artificial inoculation *in vitro* is an effective method to evaluate the resistance to Psa of kiwifruit varieties (Hoyte et al. 2015; Zhang et al. 2014; Shi et al. 2014); the results of lesion length bioassays correlate positively with actual differences in Psa resistance observed in the field and are used by the New Zealand kiwifruit industry to screen for Psa resistance in their breeding programs (Datson et al. 2015; Hoyte et al. 2015; Nardoza et al. 2015). In this experiment, inoculation of dormant stems *in vitro* was used to evaluate the disease resistance through 2 consecutive years. And it presented a highly positive correlation between the 2-year disease indexes, which further suggested that the evaluation method was stable, reliable and accurate. The evaluation results showed that milky mucus overflowed in some germplasms 10 days after inoculation, and all materials were infected with bacterial canker 21 days after inoculation. The severity of symptoms of many germplasms was no milder than that of Psa on *Actinidia chinensis* according to Song et al. (2020). However, as mentioned above, many previous studies showed that *A. arguta* has strong resistance to Psa (Zhang et al. 2014; Song et al. 2020; Shi et al. 2014), and in our previous studies (Wen et al. 2020), it was also found that *A. arguta* germplasm resources showed strong resistance to biovar 3 M228. In this experiment, *A. arguta* was generally susceptible to Psa R12, which belongs to biovar 2,

while Hongyang was extremely susceptible to biovar 3, and Xuxiang, which was highly resistant, showed resistance or high resistance to this strain, indicating that there are differences in the pathogenicity of different groups of Psa to different kiwifruit species. These differences could be evidence for the physiological race differentiation observed in Psa. According to their disease index values, 54 germplasm resources of *A. arguta* were divided into five levels of disease resistance, and the resistant or highly resistant resources only accounted for 24.07% of the total resources. This pathogen has strong pathogenicity to *A. arguta* and poses a potential threat to this industry. In this study, the screening of germplasms resistance lays a foundation for further research on gene mapping, resistance mechanisms and resistance breeding for *A. arguta*.

Funding This research was supported by the Central Public-interest Scientific Institution Basal Research Fund (#1610342020011) and the Project of Science and Technology Department of Changchun City (#21ZGN09).

Data availability The sequences data generated in this study were deposited to GenBank and freely available to any researcher.

Declarations

Ethics approval The authors declare that the research complies with ethical standards.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes

were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abeleira A, López MM, Peñalver J, Aguín O, Mansilla JP, Picoaga A, García MJ (2011) First report of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* in Spain. *Plant Dis* 95:1583–1583. <https://doi.org/10.1094/PDIS-06-11-0537>
- Balestra GM, Renzi M, Mazzaglia A (2010) First report of bacterial canker of *Actinidia deliciosa* caused by *Pseudomonas syringae* pv. *actinidiae* in Portugal. *New Dis Rep* 22:10
- Chapman JR, Taylor RK, Weir BS, Romberg MK, Vanneste JL, Luck J, Alexander BJR (2012) Phylogenetic relationships among global populations of *Pseudomonas syringae* pv. *actinidiae*. *Phytopathology* 102:1034–1044. <https://doi.org/10.1094/PHYTO-03-12-0064-R>
- Cheng HY, Li Y, Wang S, Zhang J, Pang Q, Li G, Xing JH (1995) Pathogenic identification of kiwifruit bacterial canker in Anhui. *J Anhui Agric Univ* 22:219–223
- Cunty A, Poliakoff F, Rivoal C, Cesbron S, Saux MF, Lemaire C, Jacques MA, Manceau C, Vanneste JL (2015) Characterization of *Pseudomonas syringae* pv. *actinidiae* (Psa) isolated from France and assignment of Psa biovar 4 to a *de novo* pathovar: *Pseudomonas syringae* pv. *actinidifoliorum* pv. nov. *Plant Pathol* 64:582–596. <https://doi.org/10.1111/ppa.12297>
- Datson P, Nardoza S, Manak K, Herrick J, Martinez-Sanchez M, Curtis C, Montefiori M (2015) Monitoring the *Actinidia* germplasm for resistance to *Pseudomonas syringae* pv. *actinidiae*. *Acta Horti* 1095:181–184. <https://doi.org/10.17660/ActaHortic.2015.1095.22>
- Everett KR, Taylor RK, Romberg MK, Rees-George J, Fullerton RA, Vanneste JL, Manning MA (2011) First report of *Pseudomonas syringae* pv. *actinidiae* causing kiwifruit bacterial canker in New Zealand. *Aust Plant Dis Notes* 6:67–71
- Fang ZD (1998) *Plant disease research methods*. China Agricultural Press, Beijing
- Ferrante P, Scortichini M (2010) Molecular and phenotypic features of *Pseudomonas syringae* pv. *actinidiae* isolated during recent epidemics of bacterial canker on kiwifruit (*Actinidia chinensis*) in central Italy. *Plant Pathol* 59:954–962. <https://doi.org/10.1111/j.1365-3059.2010.02304.x>
- Fisk CL, McDaniel MR, Strik BC, Zhao Y (2006) Physicochemical, sensory, and nutritive qualities of hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') as affected by harvest maturity and storage. *J Food Sci* 71:204–210. <https://doi.org/10.1111/j.1365-2621.2006.tb15642.x>
- Fujikawa T, Sawada H (2016) Genome analysis of the kiwifruit canker pathogen *Pseudomonas syringae* pv. *actinidiae* biovar 5. *Sci Rep* 6:21399
- Gao XN, Huang QL, Zhao ZB, Han QM, Ke XW, Qin HQ, Huang LL (2016) Studies on the infection, colonization, and movement of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit tissues using a GFPuv-labeled strain. *PLoS One* 11:e0151169. <https://doi.org/10.1371/journal.pone.0151169>
- Gao XN, Qin HQ, Zhao ZB, Huang QL, Huang LL (2011) Occurrence and pathogen identification of kiwifruit bacterial canker in Shaanxi Province. *Papers of Chinese Society of Botany Pathology 2011 Annual Conference*. PP255
- Hoyte S, Reglinski T, Elmer P, Mauchline N, Stannard K, Casonato S, Ah Chee A, Parry F, Taylor J, Wurms K, Yu J, Cornish D, Parry J (2015) Developing and using bioassays to screen for Psa resistance in New Zealand kiwifruit. *ISHS Acta Horti* 1095:171–180. <https://doi.org/10.17660/ActaHortic.2015.1095.21>
- Kim GH, Kim KH, Son KI, Choi ED, Lee YS, Jung JS, Koh YJ (2016) Outbreak and spread of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* biovar 3 in Korea. *Plant Pathol J* 32:545–551. <https://doi.org/10.5423/PPJ.OA.05.2016.0122>
- Koh HS, Kim GH, Lee YS, Koh YJ, Jung JS (2014) Molecular characteristics of *Pseudomonas syringae* pv. *actinidiae* strains isolated in Korea and a multiplex PCR assay for haplotype differentiation. *Plant Pathol J* 30:96–101. <https://doi.org/10.5423/PPJ.NT.09.2013.0095>
- Koh YJ, Cha BJ, Chung HJ, Lee DH (1994) Outbreak and spread of bacterial canker in kiwifruit. *J Plant Pathol* 10:68–72
- Koh YJ, Kim GH, Jung JS, Lee YS, Hur JS (2010) Outbreak of bacterial canker on Hort16A (*Actinidia chinensis* Planchon) caused by *Pseudomonas syringae* pv. *actinidiae* in Korea. *N Z J Crop Hortic Sci* 38:275–282. <https://doi.org/10.1080/01140671.2010.512624>
- Koh YJ, Kim GH, Koh HS, Lee YS, Kim SC, Jung JS (2012) Occurrence of a new type of *Pseudomonas syringae* pv. *actinidiae* strain of bacterial canker on kiwifruit in Korea. *Plant Pathol J* 28:423–427. <https://doi.org/10.5423/PPJ.NT.05.2012.0061>
- Koh Y, Lee D (1992) Canker of kiwifruit by *Pseudomonas syringae* pv. *Morsprunorum*. *Korean J Plant Pathol* 8:119–122
- Lane DJ (1991) 16S/23S rRNA sequencing. *Nucleic Acid Tech Bacterial Syst*, Eds. by E. Stackebrandt and M. Goodfellow. Wiley, Chichester, UK 15–175
- Latocha P (2007) The comparison of some biological features of *Actinidia arguta* cultivars fruit. *Annals of Warsaw University of Life Sciences—SGGW. Horti Landscape Architect* 28:105–109
- Latocha P (2008) Frost resistance and spring frost sensibility of a few cultivars of *Actinidia* grown in Central Poland. *Annals of Warsaw University of Life Sciences—SGGW. Horti Landscape Architect* 29:111–120
- Latocha P, Krupa T (2008) The mineral composition of new genotypes of hardy kiwifruit (*Actinidia* Lindl.) bred at SGGW. *Annals of Warsaw University of Life Sciences—SGGW. Horti Landscape Architect* 29:105–110
- Latocha P, Krupa T, Wołosia R, Worobie E, Wilczak J (2010) Antioxidant activity and chemical difference in fruit of different *Actinidia* sp. *Int J Food Sci Nutr* 61:381–394
- Lee YS, Kim GH, Koh YJ, Zhuang QG, Jung JS (2016) Development of specific markers for identification of biovars 1 and 2 strains of *Pseudomonas syringae* pv. *actinidiae*. *Plant Pathol J* 32:162–167. <https://doi.org/10.5423/PPJ.NT.10.2015.0224>
- Liang YM, Zhang XY, Tian CM, Gao AQ, Wang PX (2000) Pathogenic identification of kiwifruit bacterial canker in Shaanxi. *J Northwest For Univ* 15:37–39
- Marcelletti S, Ferrante P, Petriccione M, Firrao G, Scortichini M (2011) *Pseudomonas syringae* pv. *actinidiae* draft genomes comparison reveal strain-specific features involved in adaptation and virulence to *Actinidia* species. *PLoS One* 6:e27297. <https://doi.org/10.1371/journal.pone.0027297>
- McCann HC, Li L, Liu YF, Li DW, Pan H, Zhong CH, Rikkerink EHA, Templeton MD, Straub C, Colombi E, Rainey PB, Huang HW (2017a) Origin and evolution of the kiwifruit canker pandemic. *Genome Biol Evol* 9:932–944
- McCann HC, Rikkerink EHA, Bertels F, Fiers M, Lu A, Rees-George J, Andersen MT, Gleave AP, Haubold B, Wohlers MW, Guttman DS, Wang PW, Straub C, Templeton MD (2013) Genomic analysis of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* provides insight into the origins of an emergent plant disease. *PLoS Pathogens* 9:e1003503. <https://doi.org/10.1371/journal.ppat.1003503>
- Nardoza S, Martinez-Sanchez M, Curtis C, Datson PM, Montefiori M (2015) Screening *Actinidia* germplasm for different levels

- of tolerance, or resistance, to *Psa* (*Pseudomonas syringae* pv. *actinidiae*). *Acta Horti* 1096:35–355. <https://doi.org/10.17660/ActaHortic.2015.1096.40>
- Nishiyama I, Yamashita Y, Yamanaka M, Shimohashi A, Fukuda T, Oota T (2004) Varietal difference in vitamin C content in the fruit of kiwifruit and other *Actinidia* species. *J Agric Food Chem* 52:5473–5475. <https://doi.org/10.1021/jf049398z>
- Okamoto G, Goto S (2005) Juice constituents in *Actinidia arguta* fruits produced in Shinjo, Okayama. *Scientific Reports of the Faculty of Agriculture Okayama University* 94:9–13
- Opgenorth DC, Lai M, Sorrell M, White JB (1983) *Pseudomonas* canker of kiwifruit. *Plant Dis* 67:1283–1284
- Piotr L, Paweł J, Jadwiga R (2011) Genotypic difference in postharvest characteristics of hardy kiwifruit (*Actinidia arguta* and its hybrids), as a new commercial crop Part I. Sensory profiling and physicochemical differences. *Food Res Int* 44:1936–1945. <https://doi.org/10.1016/j.foodres.2011.01.033>
- Rees-George J, Vanneste JL, Cornish DA, Pushparajah IPS, Yu J, Templeton MD, Everett KR (2010) Detection of *Pseudomonas syringae* pv. *actinidiae* using polymerase chain reaction (PCR) primers based on the 16S–23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions. *Plant Pathol* 59:453–464. <https://doi.org/10.1111/j.1365-3059.2010.02259.x>
- Sawada H, Shimizu S, Miyoshi T, Shinozaki T, Kusumoto S, Noguchi M, Naridomi T, Kikuhara K, Kansako M, Fujikawa T, Nakaune R (2015) Characterization of biovar 3 strains of *Pseudomonas syringae* pv. *actinidiae* isolated in Japan. *Ann Phytopathol Soc Jpn* 81:111–126
- Scortichini M (1994) Occurrence of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit in Italy. *Plant Pathol* 43:1035–1038. <https://doi.org/10.1111/j.1365-3059.1994.tb01654.x>
- Scortichini M, Marcelletti S, Ferrante P, Petriccione M, Firrao G (2012) *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Mol Plant Pathol* 13:631–640. <https://doi.org/10.1111/j.1364-3703.2012.00788.x>
- Serizawa S, Ichikawa T, Takikawa Y, Tsuyumu S, Goto M (1989) Occurrence of bacterial canker of kiwifruit in Japan: description of symptoms, isolation of the pathogen and screening of bactericides. *Ann Phytopathol Soc Jpn* 55:427–436
- Shen Z, Huang LL, Tu X, Cheng JJ, Kang ZS (2008) Control effect of kiwifruit bacterial canker by active metabolites from plant endophytic actinomycetes. *Chin J Biol Control* 24:329–334
- Shi ZJ, Zhang HQ, Xiao JP, Yang LQ, Sun ZW, Xie M, Ma Y (2014) The resistance evaluation of different kiwifruit varieties to canker. *Acta Agric Zhejiangensis* 26:752–759
- Shim HH, Koh YJ, Hur JS, Jung JS (2003) Identification and characterization of coronatine-producing *Pseudomonas syringae* pv. *actinidiae*. *J Microbiol Biotechnol* 13:110–118
- Song YL, Lin MM, Zhong YP, Chen JY, Qi XJ, Sun LM, Fang JB (2020) Evaluation of resistance of kiwifruit varieties (strains) against bacterial canker disease and correlation analysis among evaluation indexes. *J Fruit Sci* 37:900–908
- Ushiyama K, Suyama K, Kita N, Aono N, Fujii H (1992) Isolation of kiwifruit canker pathogen, *Pseudomonas syringae* pv. *actinidiae* from leaf spot of tara vine (*Actinidia arguta* Planch.). *Ann Phytopath Soc Japan* 58:476–479
- Vanneste JL, Cornish DA, Yu J, Stokes CA (2014) First report of *Pseudomonas syringae* pv. *actinidiae* the causal agent of bacterial canker of kiwifruit on *Actinidia arguta* vines in New Zealand. *Plant Dis* 98:418. <https://doi.org/10.1094/PDIS-06-13-0667-PDN>
- Vanneste JL, Poliakoff F, Audusseau C, Cornish DA, Paillard S, Rivoal C, Yu J (2011) First report of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit in France. *Plant Dis* 95:1311–1311
- Wen X, Qin HY, Ai J, Wang Y, Han XY, Li CY (2020) Establishment and evaluation of resistance identification method for *Pseudomonas syringae* pv. *actinidiae* disease in *Actinidia arguta* germplasm resources. *Plant Prot* 47:193–199
- Williams MH, Boyd LM, Mc Neilage MA, Macrae EA, Ferguson AR (2003) Development and commercialization of “Baby Kiwi” (*Actinidia arguta* Planch.). *Acta Horti* 610:81–86. <https://doi.org/10.17660/ActaHortic.2003.610.8>
- Yamamoto S, Harayama S (1995) PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* 61:1104–1109
- Zhang HQ, Mao XQ, Xiao JP, Zhang Z, Xie M (2014) Rapid molecular identification of *Actinidia* Bacterial canker and preliminary screening of resistant materials in kiwifruit. *J Nuclear Agric Sci* 28:1181–1187
- Zhao ZB, Gao XN, Huang QL, Huang LL, Qin HQ (2013) Identification and characterization of the causal agent of bacterial canker of kiwifruit in the Shaanxi province of China. *J Plant Pathol* 95:155–162

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.