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Full Length Article

Phenotypic Characterization of *Phytophthora infestans* Population in Southwestern China

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Abstract

Potato late blight, caused by the aggressive pathogen *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive potato diseases worldwide. A total of 688 isolates of *P. infestans* collected from five provinces in southwestern China during 2012 to 2018, were assessed with regard to mating type, response to metalaxyl, virulence race and mitochondrial (mt)DNA haplotype. In terms of mating type, 76% of 679 isolates were of the self-fertile mating type, 16.6% of the isolates were of the A1 mating type and only 7.8% were of the A2 mating type. Among the 299 isolates tested as representative samples, the frequency of resistant to metalaxyl reached 80% in these regions. The race structure of thestrains presented variety and complexity and 9.04 virulence factors per isolate and 92 virulence races were found among the 365 tested isolates. The most common pathotypes were 1.2.3.4.5.6.7.8.9.10.11 and 1.2.3.4.5.6.7.8.10.11, representing 46% of the tested strains. Three types of mtDNA haplotypes were found: 78.7% of 362 isolates were Ia, 16.6% were IIb and 4.7% were IIa. The results indicated that the population of *P. infestans* in southwestern China is diverse; andthe appearance of the self-fertile isolates with resistance to metalaxyl may contribute to the potential for sexual recombination and disease epidemics. © 2020 Friends Science Publishers

Keywords: Mating type; Metalaxyl sensitivity; mtDNA; *Phytophthora infestans*; Virulence phenotype

Introduction

Phytophthora infestans caused the famous Great Famine in Ireland in the 19th century, resulting in potato tuber yield loss and approximately one million people starved to death (Fry and Goodwin 1997a). *P. infestans* is known as a heterothallic oomycete. It is widely believed that the firstmigration of *P. infestans* from the highlands of central Mexico likely occurred in the1840s (Fry and Goodwin 1997b). Migration plays an important role in the population structure of *P. infestans*. Previous studies have suggested that the population structure of *P. infestans* changed in the past (Hohl and Iselin 1984; Fry *et al*. 1993; Drenth *et al*. 1994; Wharton *et al*. 2015). Oosporesmay be expected to beproducedin the populations of *P. infestans* consisting of the A1 and A2 mating types. The evidence that *P. infestans* reproduces sexually on a regular basis is increasing in northern Europe (Yuen and Andersson 2013). Sexual reproduction may occur, which would contribute to increase the genotypic diversity in the *P. infestans* population (Drenth *et al*. 1994; Pipe *et al*. 2000; Śliwka *et al*. 2006). The population characteristics of *P. infestans* are complex and variable in many countries and many different virulence races have been identified (Kiiker *et al*. 2018; Fukue *et al*. 2018). Potato late blight is considered to be a devastating disease for potato growers and causes more than $6 billion in losses and management costs every year (Haverkort *et al*. 2008). Due to the widespread use of fungicides, metalaxyl-resistant strains have been widely reported (Aav *et al*. 2015).

Mitochondrial (mt)DNA is uniparentally inherited (Whittaker *et al*. 1994), ideal for tracing lines of descent and easily detected. In addition, mtDNA polymorphisms of *P. infestans* are used to monitor pathogen populations (Griffith and Shaw 1998). Haplotype Ib was identified as the 'old' population of *P. infestans* only in the highlands of central Mexico, andhaplotypes Ia, IIb and IIa were classified as the 'new' population (Runno-Paurson *et al*. 2009). Previous studies of phenotypic diversity in China showed that haplotypes Ia, IIa and IIb exist in China. Haplotype Ia is the most common genotype in Fujian (Han *et al*. 2014), Sichuan (Li *et al*. 2013a) and Yunnan (Zhao *et al*. 2002) in China, whereas haplotype IIa is common in Qinghai (Lian *et al*. 2012).

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Although China has become the largest potato producer in the world (Alva *et al*. 2011), the further development of the potato industry has been seriously threatened by potato late blight, especially in southwestern China. Potato production has decreased by 30% every year due to this disease. Unfortunately, very little is known about the population characteristicsof this pathogen in southwestern China. To better understand the characteristics of this pathogen in southwestern China, phenotypic and mtDNA haplotypes were analysed in this study, and the sampling area covered the region with the largest potato planting area in China. The results will provide a guideline for future potato breeding and effective prevention and control measures for potato late blight.

Materials and Methods

Collection and isolations

Potato leaves with single lesions showing classical typical late blight symptoms were obtained from 33 locations in southwestern China from April to October. The distance between each sampling point is more than 50 kilometres. The elevations ranged from 189 m to 3430 m. In those areas, farmers plant high-quality virus-free seed potato and apply fungicide three to five times to control potato late blight. The infected leaves were cut into small pieces (5 mm^2) and placed under potato tuber slices from the susceptible cv. Favorita. Small tufts of mycelia grew on the potato slices after being incubated for five to seven days at 18°C in darkness, andthe hyphae were transferred to culture medium plates with rye B agar and incubated at 18°C (Li *et al*. 2013a).

During 2012–18, 688 isolates of *P. infestans* were collected (Table 1). Some isolates from each site were chosen for the analysis of the population diversity of *P. infestans* in terms of mating type, sensitivity to metalaxyl, virulence race and mtDNA haplotype.

Table 1: Origin of *P. infestans* isolates collected from southwestern China (2012–2018)

Region	Location	Number of isolates	Latitude	Longitude	Altitude (m)
Guizhou	Weining	28	26°83'	104°24'	2169
	Chishui	8	28°47'	105°76'	299
Hubei	Lichuan	10	30°23'	108°99'	1129
Chongqing	Kaizhou	23	31°20'	108°34'	529
	Shizhu	4	29°99'	108°11'	553
	Wuxi	20	31°41'	109°56'	256
	Yunyang	3	31°36'	108°91'	1280
	Zhongxian	2	30°29'	108°03'	189
Sichuan	Beichuan	6	31°94'	104°41'	996
	Chaotian	11	32°62'	106°10'	1412
	Chongzhou	37	30°54'	103°65'	508
	Danling	6	29°98'	103°36'	485
	Daofu	31	30°48'	101°48'	3430
	Ebian	10	29°24'	103°18'	1197
	Jiangyou	9	31°97'	104°78'	588
	Leibo	7	28°39'	103°77'	1118
	Luding	59	29°64'	102°12'	1562
	Mianning	9	28°74'	102°25'	2076
	Pengzhou	193	31°21'	103°78'	1050
	Puge	1	27°48'	102°48'	1417
	Shunqing	24	31°05'	106°13'	324
	Tongjiang	10	32°47'	107°37'	1200
	Wanyuan	10	32°11'	108°10'	1044
	Xiaojin	43	31°08'	102°30'	3162
	Xide	16	28°30'	102°45'	2410
	Xindu	8	30°77'	104°21'	478
	Xingwen	8	28°27'	105°29'	399
	Xuanhan	27	31°35'	107°72'	297
	Yilong	10	30°87'	106°08'	313
	Zhaojue	26	28°01'	102°51'	2600
Yunnan	Daguan	6	27°74'	103°89'	1135
	Huize	11	26°43'	103°32'	2129
	Ludian	12	27°17'	103°58'	1908
Total		688			

Mating type detection

Isolates of *P. infestans* were evaluated to identify their mating type on rye B agar by pairing themwith the *P. infestans* reference isolates A1 (VK98014) and A2 (90128). The confrontational strains were stored at 18°C in the dark for 5 to 7 days and were observed microscopically for oospores in the hyphae interaction area. Mating types were identified according to the method described by Runno-Paurson *et al*. (2009). The isolates generating oospores with the A1 were classified as A2; the isolates that generating oospores with A2 were classified as A1. The isolates forming oospores with or without A1 and A2were designated as self-fertile.

Metalaxyl resistance assessment

The responses of different isolates to metalaxyl were tested on rye B agar plates (90 mm) with the corresponding metalaxyl concentration. The fungicide was dissolved in 0.1% acetone and prepared in a stock of 100 gL^{-1}. Three different metalaxyl concentrations (0, 5 and 100 mg L^{-1}) were tested. Uniformly-sized agar plugs (5 mm) were taken from actively growing colonies of *P. infestans*, and a plug was placedin the middle of each plate. After maintaining the cultures for 7 days at 18°C in the dark, the diameters of the fungal colonies were evaluated in two perpendicular directions through the centre of each plate. Three replicates were used for each isolate. Metalaxyl resistance was determined according to the following scale: those with growth on both the 5 and 100 mg L^{-1} plates ≥40% of that on the 0 mg L^{-1} plate were regarded as resistant (R), those with growth on the 5 mg L^{-1} plate ≥40% of that on the 0 mg L^{-1} plate were regarded as intermediate (I), and those with growth on both the 5 and 100 mg L^{-1} plates ≤40% of that on the 0 mg L^{-1} plate were regarded as sensitive (S) (Forbes 1997).

Virulence tests

The virulence pathotype was assessed by testing interactions on a subset of different potato genotypes with resistance genes *R*1-*R*11 (Malcolmson and Black 1966). The sterile

differential potato clones were preserved on Murashige and Skoog's medium and were maintained at 23°C with a 16 h light period. Four fully expanded leaves were selected from the potato plants for each isolate andplaced abaxial-side up on a wet filter paper in aplastic Petri dish. A 10 μL drop of sporangial suspension (2×10^4 sporangia mL^{-1}), prepared after 5–7 days on tuber slices from susceptible cv. Favorita was placed on each leaflet. After inoculation, the Petri dishes were maintained at 18°C under a16h light period. The leaves were checked with a stereomicroscope for sporulation 7 days later. If sporulation was detected, the interaction was evaluated as compatible; if a hypersensitive or no symptom response was detected, the interaction was evaluated as incompatible (Runno-Paurson *et al*. 2009). The results were credible when sporulation occurred on the leaves of impressionable potato clone *r* without any Rgenes.

DNA extraction

Isolates of *P. infestans* were incubated at 18°C in the dark for 7 days individually on potato tuber slices of susceptible cv. Favorita, and mycelia were carefullyharvested by removing them with an inoculating needle and stored at -20°C in centrifuge tubes for DNA extraction. Genomic DNA from 30 mg of dry mycelium was purified using anE.Z.N.A.® Fungal DNA Kit (OMEGA Bio-Tek, USA). The quantity of the purified DNA samples was measured using a NanoDrop ND-1000 (NanoDrop Technologies, Inc., USA). The DNA samples were diluted to 20 ng μL^{-1} and stored at -20°C for further analysis.

mtDNA haplotypes

The mtDNA haplotypes of the *P. infestans* isolates were described by Griffith and Shaw (1998). The mtDNA regions of *P. infestans* were amplified using primer pair 1 (F2 - TTCCCTTTGTCCTCTACCGAT; R2 - TTACGGCGGTTTAGCACATACA) and primer pair 2 (F4 - TGGTCATCCAGAGGTTTATGTT; R4 - CCGATACCGATACCAGCACCAA). Each PCR (25 μL) mixture consisted of 12.5 μL of 2×*Taq* PCR Master Mix (Sangon Biotech Co., Ltd, China), 10 μM of each primer, 30–50 ng of DNA sample and 8.5 μL of ddH_2O. The thermal cycling procedure was as follows: initial denaturation for 2 min at 94°C, 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 30s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

The PCR product P2 was digested with the restriction enzymes *Msp*I and the product P4 was digested with the restriction enzymes *Eco*RI at 37°C for 4 h. Digestion mix P2 (20 μL) consisted of 1 μL of *Msp*I (10 U μL^{-1}), 2 μL of buffer, 9 μL of ddH_2O and 8 μL of the PCR product P2. Digestion mix P4 (20 μL) consisted of 0.5 μL of *Eco*RI (20 U μL^{-1}), 2 μL of buffer, 9.5 μL of ddH_2O and 8 μL of the PCR product P4. Thetypes of mtDNA haplotypes were identified according to the sizes of the digested PCR products (Griffith and Shaw 1998).

Data analysis

The normalised Shannon index (Sheldon 1969; Runno-Paurson 2009) was used to summarize the pathotype diversity and the formula was as follows: $Hs = -\Sigma g_i \ln g_i / \ln N$, where g_i is the frequency of races *i* and N is the number of isolates. The index was normalized to a scale of0 (no diversity) to 1 (a unique pathotype per isolate). SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. To identify the differences in terms of specific virulence among the different potato R genes or sampling years, ANOVA was performed.

Results

Mating type

In total, 679 isolates were randomly chosen for each mating type. The results showed that 16.6% of the isolates were of the A1 mating type, 7.8% of the isolates were of the A2 mating type and 75.6% of the isolates were self-fertile (Fig. 1A). Many self-fertile isolates were detected in the five regions.

A1 mating type isolates were detected every year, and their frequency ranged from 26.7 to 4.2% throughout the survey. However, the A2 mating type was not found in 2015 and 2017, with frequencies ranging from 0 to 33.3%. Conversely, the self-fertile mating type prevailed among theisolates, and the frequency during the sampling years was in the range of 60.0–87.7% (Fig. 1B). Interestingly, most isolates collected in the fall in Sichuan in 2016–2018 were of the A1 mating types. The A1, A2 and self-fertile mating types were sometimesfound in the same field in Sichuan. The isolates collected from Hubei and Chongqing were allof theself-fertile mating type. Furthermore, the self-fertile mating type frequencies in Guizhou and Yunnan provinces were 97.2 and 93.1%, respectively (Fig. 1C).

Metalaxyl resistance

The metalaxyl resistance of 299 isolates was determined on rye B agar plates. Of these isolates, 79.6% were classified as resistant, 17.4% had intermediate sensitivity, and 3.0% were sensitive to metalaxyl (Fig. 2A). Metalaxyl-resistant isolates were detected among the three mating types (A1, A2 and self-fertile mating types), and no sensitive isolates werefound in the A2 mating type (Fig 2B). The proportion of metalaxyl-resistant isolates reached from 55.0 to 91.4% in southwestern China during the period of 2012–18. The metalaxyl-sensitive phenotype was not found in 2013, 2014, 2015 or 2017; only 10.0% (n = 2), 4.5% (n = 3) and 16.0% (n = 4) of the isolates were sensitive in 2012, 2016 and 2018, respectively (Fig. 2C). All isolates collected from Guizhou, Hubei and Yunnan were resistant to metalaxyl. Additionally, 76.6% (n = 183) of the isolates collected from Sichuan and 80.0% (n=20) of the isolates collected from Chongqing showed resistance to metalaxyl (Fig. 2D).

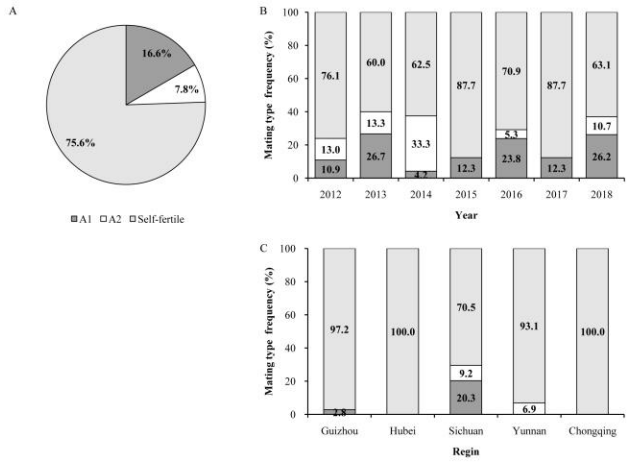

Fig. 1: Frequency of mating types of *P. infestans* isolates from different regions in southwestern China during the 2012–2018 period. (A) all isolates; (B) different sampling years; (C) different sampling regions

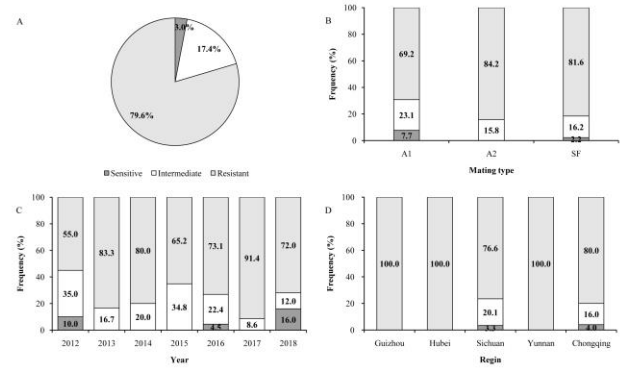

Fig. 2: Metalaxyl resistance of *P. infestans* isolates from different regions in southwestern China during the 2012–2018 period. (A) For all isolates; (B) with respect to mating type; (C) for different sampling years; (D) for different sampling regions. S: metalaxyl sensitive, I: intermediate metalaxyl sensitivity, R: metalaxyl resistant

Virulence phenotype

A total of 365 isolates as representative samples purified from the five provinces were tested for pathogenicity. The results showed that virulence factors that could overcome all known resistance genes (R1-R11) were detected. The most common physiological races were 1.2.3.4.5.6.7.8.9.10.11 (26.58%) and 1.2.3.4.5.6.7.8.10.11 (18.90%). The frequency of virulence genes was in the range of 67–89%; the lowest frequency was found for virulence against R9 (67% ± 4.6 SE) and the highest frequency was found forvirulence against R6 (89% ± 4.4 SE) (Fig. 3). There were no differences in specific virulence ($P \geq 0.05$), but there were significant differences among the seven years ($P \leq 0.01$). Ninety-nine percent of the tested strains were able to overcome five or more R genes. Moreover, 92 virulence

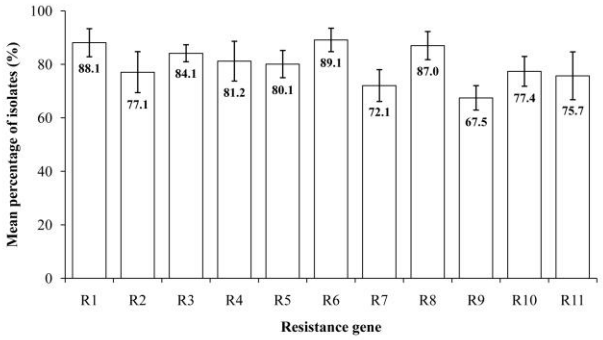

Fig. 3: Frequency of virulence to resistance genes in *P. infestans* isolates from different regions in southwestern China during the 2012–2018 period

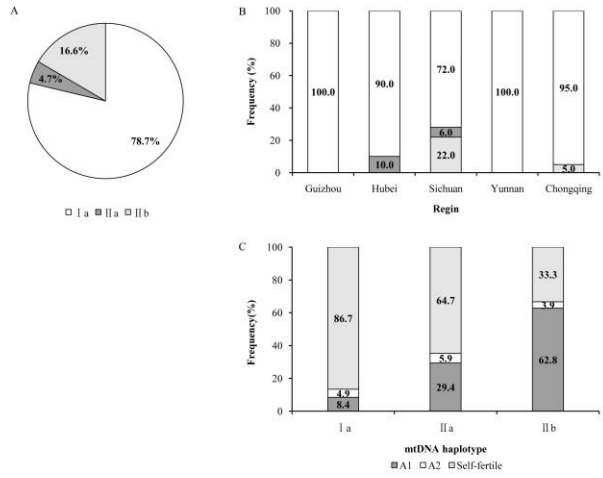

Fig. 4: Composition and frequency of mitochondrial haplotypes of isolates from different regions in southwestern China during the 2012–18 period. (A) For all isolates (n=362); (B) for different sampling regions; (C) with respect to mating type

races were detected. The mean number of virulence factors was 9.04 (Table 2) and ranged from 7.2 to 10.4 among the sampling years (Table 3). The normalized Shannon diversity index of the *P. infestans* population in southwest China was 0.55.

mtDNA haplotypes

Three types of mtDNA haplotypes (Ia, IIa and IIb) were identified in 362 tested isolates. In total, 78.7% (n = 285) of the isolates were Ia. The frequencies of IIa and IIb were 4.7% (n = 17) and 16.6% (n = 60), respectively (Fig. 4A). Haplotype Ia was the common type in all regions, with a frequency of 100% among the tested isolates from Guizhou and Yunnan and 90.0, 72.0 and 95.0% in Hubei, Sichuan and Chongqing, respectively. Haplotype IIa was detected in Hubei and Sichuan. Haplotype IIb was found only in Sichuan and Chongqing (Fig. 4B). Among the five regions, three mtDNA haplotypes were detected in Sichuan. Each

Table 2: Number and frequency of isolates of different races among *P. infestans* isolates from southwestern China (2012–2018)

Race	Isolates (*n*)	Frequency (%)	Race	Isolates (*n*)	Frequency (%)
1.2.3.4.5.6.7.8.9.10.11	97	26.58	1.2.3.4.6.7.9.10.11	1	0.27
1.2.3.4.5.6.7.8.10.11	69	18.90	1.2.3.4.6.9.10.11	1	0.27
1.3.4.6.7.8.10	16	4.38	1.2.3.4.7.8.10	1	0.27
1.3.4.5.6.7.8.9.11	8	2.19	1.2.3.5.6.8	1	0.27
1.3.4.5.8.9.10	8	2.19	1.2.3.5.6.8.10	1	0.27
2.3.5.6.7.8.9.11	8	2.19	1.2.3.6	1	0.27
1.2.3.4.5.6.7.8.9.11	7	1.92	1.2.4.5.6.7.8.10	1	0.27
1.2.3.4.6.7.8.10	7	1.92	1.2.4.5.6.8.11	1	0.27
1.2.4.5.6.8.9.10	6	1.64	1.2.4.5.6.8.9	1	0.27
1.3.4.5.6.7.8.9.10.11	6	1.64	1.2.4.5.6.9.11	1	0.27
1.2.3.5.6.8.9.11	5	1.37	1.2.4.6.7.9.11	1	0.27
1.2.4.6.7.8.9.10.11	4	1.10	1.2.4.7.8.9.10.11	1	0.27
1.2.4.6.8.9.10.11	4	1.10	1.2.5.6.7.9.10.11	1	0.27
1.2.3.4.5.6.7.9.11	3	0.82	1.3.4.5.6.7.10.11	1	0.27
1.2.3.4.5.6.8.9.11	3	0.82	1.3.4.5.6.7.8.10.11	1	0.27
1.2.3.4.5.8.9.10.11	3	0.82	1.3.4.5.6.7.8.9.10	1	0.27
1.2.3.4.5.8.9.11	3	0.82	1.3.4.5.6.7.9	1	0.27
1.2.3.4.6.10.11	3	0.82	1.3.4.5.6.9.10.11	1	0.27
1.2.3.4.6.8.10.11	3	0.82	1.3.4.5.7.9.11	1	0.27
1.2.4.5.6.7.9.10.11	3	0.82	1.3.4.5.8.10	1	0.27
1.2.5.6.7.9	3	0.82	1.3.4.5.8.9.10.11	1	0.27
1.2.5.6.7.9.11	3	0.82	1.3.4.5.9.10	1	0.27
1.2.5.6.8.9.10.11	3	0.82	1.3.4.6.8.9.10.11	1	0.27
1.3.4.5.6.10	3	0.82	1.3.4.7.8.10.11	1	0.27
3.4.5.6.7.8.9.10.11	3	0.82	1.3.5.6.7.9.11	1	0.27
1.2.3.4.5.6.10.11	2	0.55	1.3.5.6.8.9.11	1	0.27
1.2.3.4.5.6.9.10	2	0.55	1.3.5.8.10	1	0.27
1.2.3.4.5.9.10	2	0.55	1.3.6.8.10	1	0.27
1.2.3.5.6.7.8.9.10.11	2	0.55	1.4.5.6.7.9.10.11	1	0.27
1.2.4.5.6.7.8.10.11	2	0.55	1.4.5.6.7.9.11	1	0.27
1.2.4.5.6.7.8.9.10	2	0.55	1.4.5.6.8.9.10.11	1	0.27
1.2.4.5.6.8.10.11	2	0.55	1.4.5.6.9.10.11	1	0.27
1.2.4.6.7.8.10.11	2	0.55	1.4.6.7.8.10.11	1	0.27
1.2.5.6.7.9.10	2	0.55	1.4.6.9.10.11	1	0.27
1.2.5.6.8.9.10	2	0.55	2.3.4.5.6.8.10	1	0.27
1.3.4.5.6.9.11	2	0.55	2.3.4.7.8.9.10.11	1	0.27
1.3.4.6.7.8.9.10.11	2	0.55	2.3.4.9.11	1	0.27
1.3.4.6.7.9.10	2	0.55	2.3.5.8.9.10	1	0.27
1.3.5.8.9.10	2	0.55	2.3.7	1	0.27
1.4.5.6.7.8.9.11	2	0.55	2.4.5.6.8	1	0.27
2.3.4.5.8.9.10	2	0.55	2.4.5.7.8	1	0.27
2.3.5.6.8.9.11	2	0.55	2.4.6.7.9.10.11	1	0.27
1.2.3.4.5.6.7.10.11	1	0.27	2.5.6.8.9.10	1	0.27
1.2.3.4.5.6.8.10.11	1	0.27	2.6.8.9.10	1	0.27
1.2.3.4.5.6.8.9.10.11	1	0.27	3.4.7.8	1	0.27
1.2.3.4.6.7.8.9.10.11	1	0.27	5.8.10.11	1	0.27
Total number of isolates	365				
Total number of races	92				
Virulence complexity	9.04				

mtDNA haplotype contained A1, A2 and self-fertile isolate clones. Most of haplotype Ia isolates were self-fertile and most of haplotype IIb isolateswere A1 (Fig. 4C).

Discussion

In this study, A1 and A2 mating types of *P. infestans* isolates were found in southwestern China, but most of the isolates were self-fertile. A2 isolates were only detected in Sichuan and Yunnan, and the isolates from Hubei and Chongqing were all self-fertile. The A2 mating type has been subsequently reported in many countries since the discovery of oospores produced by *P. infestans* under natural conditions in Mexico in 1956 (Niederhauser 1956). Since the first reportofA2 mating type isolates in northern

Table 3: Frequency of virulence to potato R genes among isolates across seven sampling years in southwestern China

Resistance gene	Sampling years						
	2012	2013	2014	2015	2016	2017	2018
R1	87	62	87	81	100	100	100
R2	93	55	57	55	88	91	100
R3	87	86	68	81	82	92	93
R4	53	62	70	84	100	99	100
R5	87	72	70	58	89	92	93
R6	100	72	86	77	88	100	100
R7	67	52	59	68	73	93	93
R8	100	86	63	74	89	96	100
R9	93	69	62	55	66	63	64
R10	60	62	79	71	78	92	100
R11	93	45	48	61	87	96	100
Mean number of virulence isolates	9.2	7.2	7.5	7.6	9.4	10.2	10.4
Number of isolates tested	15	29	63	31	94	119	14

China in 1996 (Zhang *et al*. 1996), several A2 mating type isolates have been detected in many regions of China. Previous reports documented that the frequency of the A2 mating type was 10% in Yunnan, but it was 91% in Sichuan Province (Li *et al*. 2013b). The self-fertile isolate was first reported in China in 2002 (Huang 2002). Many self-fertile isolates have been found in China (Li *et al*. 2009; Li *et al*. 2013a; Zhu *et al*. 2015; Tian *et al*. 2016) and other countries (Aav *et al*. 2015; Casa-Coila *et al*. 2017). The probability of theoccurrence of self-fertile isolates isrelated to the survival, environment and genetic structure of *P. infestans*, and there are many cultural practices in which the age of the culture, presence of other organisms, wounding and the addition of fungicide to the culture medium can override heterothallism-inducing oospore production in *P. infestans* (Smart *et al*. 2000). Self-fertile isolates that replaced A1 and A2 mating type isolates were the dominant clones in southwestern China, indicating that sexual reproduction can occur in all studied potato fields. Early epidemics of potato late blight can be caused by oospores in the field (Hannukkala *et al*. 2007; Brylińska *et al*. 2016). In contrast to sporangia, oospores can withstand adverse conditions or circumstances and survive into the next growing season in the soil without a host (Andersson *et al*. 1998; Fernández-Pavía *et al*. 2004; Lehtinen and Hannukkala 2004). Furthermore, sexual reproduction not only increases the genotypic variability in *P. infestans* populations, but may also cause increased pathogenicity and/or antimicrobial resistance (Fry *et al*. 1993; Harutyunyan *et al*. 2008; Lozoya-Saldaña 2011).

Fungicide-resistance testing indicated that metalaxyl-resistant isolates arewidespread in southwestern China. The occurrence of metalaxyl-resistant isolates was not associated with the mating types. Many resistant isolates were detected in Yunnan (Zhao *et al*. 2007) in China. Resistance to metalaxyl was found in three regions, Guizhou, Hubei and Yunnan, with the highest proportion among the self-fertile isolates in our study. Metalaxyl-based fungicides are recommended for usein many regions because of their low price and high quality. It is possible that extensive use of fungicides caused the increase in the metalaxyl-resistant population. These studies suggest that it is necessary to change the strategy of using metalaxyl to control potato late blight. With the application of potato-resistant varieties and crop rotation (Bimšteine 2008), the amount of fungicides used can be reduced.

In our study, 92 different pathogenic types were identified among365 isolates and the virulence complexity was 9.04. The most common race was 1.2.3.4.5.6.7.8.9.10.11, which was virulent to 11 known R genes. This means that the *P. infestans* population in southwestern China is highly diverse and complex. The proportion of virulence against R genes was also different in other countries (Chmielarz *et al*. 2014), and the sexual reproduction in the *P. infestans* populations has been detected in some countries (Kiiker *et al*. 2018). Planting resistant cultivars is an economical way to control potato late blight, but susceptible cultivars, such as cv. Favorita, are widely grown due to the short growth period, such as cv. Favorita. In some poor areas in southwestern China, cheap fungicides, such as metalaxyl-based fungicides, areapplied for potato late blight control, or nothing is done about such infestations. As a result, potato late blight is common in southwestern China. There is a high level of pathotype diversity in southern China. This finding might be related to the specific conditions of year-round potato cultivation.

Overall, mtDNA haplotype tests revealed that all isolates in southwestern China were the 'new' population of *P. infestans*. The most common mtDNA haplotype was Ia, followed by haplotypes IIb and IIa; Ib is not detected in this study. Many researchers have reported that haplotype Ia is dominant in most of the populations of *P. infestans* studied (Shimelash *et al*. 2016; Fry *et al*. 1993). The proportion of each mtDNA haplotype differed in thedifferent regions in China. Haplotype IIa dominated the population of *P. infestans* in north-eastern China, growing faster than the other two haplotypes (IIa and IIb) on rye B agar medium (Tian *et al*. 2018).

Conclusion

The population of *P. infestans* in southern China is diverse and complex. Seventy-six percent of the tested isolates were self-fertile, 80% of the tested isolates were metalaxyl-resistant, and 26.58% of the tested isolates were members of the most common physiological race1.2.3.4.5.6.7.8.9.10.11. This suggests that metalaxyl-based fungicides should be carefully used and that resistance to metalaxyl should be monitored in southern China. The control strategies for potato late blight should be organically combined with the different cultivation models, which contribute to maintaining biodiversity and suppressing the occurrence of late blight.

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