Received: 3 March 2020 | Revised: 10 August 2020 | Accepted: 12 August 2020

DOI: 10.1111/ppa.13269

ORIGINAL ARTICLE

Plant Pathology | WILEY

Genotypic and phenotypic characterization of *Phytophthora infestans* populations on Java, Indonesia

Sandesh Dangi[1] | Phillip Wharton[1] | Alberta D. Ambarwati[2] | Tri Joko Santoso[2] | Kusmana[2] | Ineu Sulastrini[2] | John Medendorp[3] | Karen Hokanson[4] | David Douches[3]

[1]Department of Entomology, Plant Pathology and Nematology, University of Idaho, Aberdeen Research and Extension Center, Aberdeen, ID, USA

[2]Plant Molecular Biology Division, Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Ministry of Agriculture, Bogor, Indonesia

[3]Department of Plant, Soil and Microbial Science, Michigan State University, East Lansing, MI, USA

[4]Department of Horticultural Sciences, University of Minnesota, St Paul, MN, USA

Correspondence
Phillip Wharton, Department of Entomology, Plant Pathology and Nematology, University of Idaho, Aberdeen Research and ExtensionCenter, Aberdeen, Idaho 83210, USA.
Email: pwharton@uidaho.edu

Funding information
United States Agency for International Development, Grant/Award Number: #AIDOAALA1500056

Abstract

Phytophthora infestans is endemic to Indonesia and can infect potato crops at any stage in the growing season. Little is known about *P. infestans* populations in Indonesia. The objectives of this study were first to identify the genotypes causing late blight in the main potato-growing regions on Java in Indonesia, and secondly to examine genotypic diversity in the *P. infestans* populations in those regions. Samples were collected on FTA cards (n = 140) or in tubers (n = 6) from 15 locations in nine regencies over four years (2016–2019). Microsatellite analysis revealed that late blight outbreaks in these regencies were caused by EU_2_A1 and other genotypes that are unique to Indonesia. Eighty percent of the samples that amplified with CAPS markers were the A1 mating type. Cultures of six isolates were determined to be the A1 mating type based on the pairing test, and of these, two isolates were intermediate and four were sensitive to metalaxyl-M (mefenoxam). The mode of reproduction of the *P. infestans* population on Java, Indonesia, was found to be clonal. However, as the sample size in this study was small, more isolates need to be tested to confirm this. Microsatellite analysis revealed that 90% of Indonesian samples had trisomic loci. A high number of multilocus genotypes (MLGs) were found in all nine regencies (131 MLGs out of 146 samples). Results indicate that there is ongoing polyploidization in these populations due to a high mutation rate and no selection pressure from the susceptible potato hosts that are being grown in Indonesia.

KEYWORDS
FTA cards, genetic structure, late blight, microsatellite analysis, pathogen, potato

1 | INTRODUCTION

Phytophthora infestans is recognized as one of the most destructive pathogens of potato, tomato, and other solanaceous crops (Fry, 2008). The pathogen causes late blight of potato, which is a major constraint of profitable potato production worldwide (Fry, 2008). Late blight management costs and losses from yield reductions are estimated at over $6 billion a year (Haverkort *et al*., 2008).

Populations of *P. infestans* are dynamic, with mutation, migration, sexual reproduction, and host having contributed to the evolution of new clonal lineages or genotypes with different epidemiological, genotypic, and phenotypic characteristics (Goodwin, 1997; Hu *et al*., 2012). Globalization has increased the trade in agricultural commodities such as potato between countries. Potato tubers intended for processing or for use as seed are imported or exported from one country to another based on supply and demand. *P. infestans* remains dormant (latent) in seed tubers at low temperatures (4°C). As such, symptomless seed tubers are easily overlooked in intercontinental shipments (Johnson and Cummings, 2009). The introduction of new genotypes in seed tubers has resulted in changes in local population compositions of

P. infestans (Goodwin, 1997). Because the evolution of *P. infestans* is rapid, it has been a challenge to manage the pathogen even in developed countries where modern technologies such as disease and weather forecasting systems, modern fungicide chemistries, and disease resistant cultivars are available. As such, in developing countries like Indonesia, late blight is a significant impediment to profitable potato production.

Indonesia is one of the largest potato producers in Southeast Asia (FAO, 2008). It is believed that the Dutch East India Company first introduced potatoes to West Java around 1795 (FAO, 2008). Since then, potatoes have been grown in Indonesia by small-scale farmers throughout the archipelago, mainly in the highlands (800–1,800 m a.s.l.; FAO, 2008). Potato production in Indonesia has increased significantly since 1969, mainly due to an increase in the total production area and to a lesser extent to increases in productivity (Adiyoga *et al*., 1999). In Indonesia, more than 50% of potatoes are grown on the island of Java, with 37% of those grown in West Java. In 2003, the average production per hectare was as high as 25–28 t/ha (Adiyoga, 2009). Potato cultivar Granola was bred and released in Germany in the late 1970s and later imported to Indonesia in the early 1980s (Adiyoga, 2009). In Indonesia this cultivar is prized for its medium to high yield potential, early maturity, and adaptation to the Indonesian climate. As a result, Granola is the most widely grown potato cultivar in Indonesia, accounting for 90% of production, and is grown mainly for the fresh market (Adiyoga *et al*., 1999; Adiyoga, 2009). However, Granola is susceptible to late blight (Adiyoga, 2009).

There are several factors that impact profitable potato production in Indonesia. Among them, late blight is one of the most important due to its impact on crop yield. Yield reductions may be as high as 90% depending on the growth stage of the crop when a disease outbreak occurs (Kusmana, 2003). Potato-growing regions in Indonesia, such as Sumatra and Java, have conducive environmental conditions for late blight year-round and *P. infestans* is endemic to these regions (Adiyoga, 2009). Infection can occur at any point in the potato crop growth cycle as soon as there is green plant tissue that can be infected. Thus, growers in Indonesia rely heavily on fungicides to control late blight, applying as many as 20–30 fungicide sprays in a single potato-growing season (Adiyoga, 2009). The best way to overcome the problem of late blight in Indonesia would be to develop a late blight-resistant potato cultivar. Breeding late blight resistance in potato cultivars has significant benefits due to the reduced cost of production through lower fungicide costs, reduced farmer exposure to fungicides, and reduced fungicide residues in food, land, and wastewater (Kirk *et al*., 2001).

Conventional breeding for resistance may take more than 10 years to produce a new resistant cultivar and by the time the new variety is released it may no longer be resistant, as local strains of the pathogen may have changed. The Feed the Future Biotechnology Potato Partnership was formed in 2015 as a 5-year, $6 million multi-institutional cooperative agreement with USAID to introduce bioengineered potato products in farmer- and consumer-preferred cultivars into Indonesia and Bangladesh. These bioengineered potatoes will have 3 *R*-gene resistance to late blight. These genes have been evaluated against *P. infestans* strains from Europe and the USA but have not been evaluated against Indonesian isolates. Thus, the objectives of this study were to identify the genotypes causing late blight in the main potato-growing regions of Java in Indonesia and to examine genotypic diversity in the *P. infestans* populations in the same regions. Because very little is known about the *P. infestans* populations in Indonesia, this information is critical to ensure the effectiveness and durability of any 3 *R*-gene cultivars that are released.

2 | MATERIALS AND METHODS

2.1 | Collection of *P. infestans* DNA samples and isolates

In this study, 146 samples were collected from 15 locations in nine regencies (second level administrative divisions equivalent to a municipality) in the major potato-growing areas of Java, Indonesia, from 2016 to 2019 (Table 1). DNA samples (140) were collected on Whatman Flinders Technology Associates (FTA) cards using the protocol suggested by the manufacturer (Sigma-Aldrich). Briefly, lesions on infected leaves were placed on top of the cellulose matrix on the FTA card, the paper flap was closed over the leaf, and using the blunt end of a pen, pressure was applied by rubbing to force juice out of the leaf, thus depositing DNA into the specialized matrix. The cards were then allowed to air dry for 30 min at room temperature, after which time they were suitable for long-term storage or shipment. Thirty tubers with infected leaves sandwiched between the two halves were shipped from Indonesia to Aberdeen, ID, USA. Unfortunately, due to delays in transit most of the tubers had started to decay by the time they were received, and only six separate isolates were obtained from the infected leaves. FTA cards were stored in a dry place at room temperature until processed for analysis. For pure cultures, actively growing hyphal tips or single spores were transferred to pea agar amended with RAN (rifamycin, 75 mg/L; ampicillin, 100 mg/L; and nystatin, 75 mg/L; Forbes, 1997). Details about the location and number of samples collected each year are listed in Figure 1 and Table 1.

2.2 | DNA extraction/purification of FTA card samples

FTA cards were processed in two different ways, either by purification of FTA cards using Whatman FTA purification reagent (GE Healthcare UK) or extraction of DNA from FTA cards using QIAmp DNA Investigator kit (Qiagen). The manufacturer's protocol was used to purify or extract the DNA from FTA cards. If insufficient DNA was extracted using QIAmp DNA Investigator kit, Whatman FTA purification reagent was used to purify the samples and a single FTA punch was used in PCR. DNA from isolates was extracted using DNeasy Plant Mini kit following the manufacturer's protocol (Qiagen).

TABLE 1 Geographical information on *Phytophthora infestans* samples (isolates and DNA samples on FTA cards) collected in different years from Java, Indonesia, and standard isolates used in the study

Region or country	Location	Total	DNA samples or isolates and collection year
Bandung	Pangalengan	32	DNA samples = 28, 2016; isolates = 4, 2019
Batu	Tutungrejo	9	DNA samples = 4, 2018; 5, 2019
Garut	Cisurupan	6	DNA samples, 2016
Majalengka	Argalingga	6	DNA samples, 2016
Mojokerto	Kebonaga	12	DNA samples = 5, 2018; 7, 2019
Pasuruan	Sedaeng, Tosari, Wonokitri, Ngadiwono	25	DNA samples = 11, 2018; 13, 2019; isolate = 1, 2019
Temanggung	Kledung	10	DNA samples = 8, 2018; 2, 2019
West Bandung	Lembang	10	DNA samples, 2016
Wonosobo	Serang, Parikesit, Tieng, Dieng	36	DNA samples = 25, 2018; 10, 2019; isolate = 1, 2019
USA	Michigan and Idaho	10	Isolates (year = 2008–2015; genotypes = US-8, US-22, US-23, and US-24)
UK	NA	2	Isolates from Michigan State University Kirk laboratory collection (year = 2004; genotypes = EU_6_A1, EU_13_A2)

Note: All Indonesian DNA samples and isolates were collected from local subsistence farmers' fields (multiple fields). Potato cultivar is Granola (seed produced in Indonesia), and crop rotation is potato–carrot/cabbage; fungicide spray (if any) is chlorothalonil- and/or mancozeb-based fungicides two to three times per week for 12 weeks. NA, not available

2.3 | Mating type determination

Cleaved amplified polymorphism sequence (CAPS) markers developed by Judelson *et al.* (1995) were used to determine mating types of *P. infestans* samples (n = 146) using standard PCR. The PCR products were cleaved using restriction enzyme *Hae*III and run on a 2% agarose gel for 90 min in a gel electrophoresis chamber (0.5 × TBE), stained with Gel Red (Biotium Inc.) and visualized with a Gel Doc EZ imager (Bio-rad). Samples with bands of 550 and 600 bp were determined to be A1 mating type, whereas samples with only a 600 bp band were determined to be A2 mating type. Results were cross-checked with AFLP molecular markers developed by Kim and Lee (2002).

Pairing tests were done for six isolates as described by Forbes (1997). Circular disks (5 mm) of actively growing mycelia from known isolates of the EU_6_A1 and EU_13_A2 genotypes (Table 1) and the unknown isolate were placed in the centre of the plate (pea medium) separated by 2–3 cm. The cultures were stored in the dark at 18°C for 18 days and formation of any oospores was checked under the microscope. If the known isolate was A1 and oospores were present, the mating type was determined to be A2, and conversely if the known isolate was A2 and oospores were formed, then the unknown isolate was determined to be A1.

2.4 | Metalaxyl-M sensitivity test

Six isolates from Pangalengan, Bandung (4), Parikesit, Wonosobo (1), and Wonokitri, Pasuruan (1) were used for metalaxyl-M sensitivity testing using a spiral gradient dilution method as described by Fairchild *et al.* (2013) and the poison agar method (Forbes, 1997). Pea agar medium was prepared as described above. In the spiral plating method, 50 ml pea agar was poured in each 100 mm Petri plate. After the medium was set, 10 g/L of metalaxyl-M was prepared from filtered Ridomil Gold EC (479.31 g metalaxyl-M/L; Syngenta Ag). Using a spiral plating robot, metalaxyl-M (54 μl) was placed in a spiral gradient on the plate so that the highest concentration of metalaxyl-M (10 g/L) was in the centre, with a gradient down to zero concentration at the edge of the plate. After 4 hr, lines were laid down on the medium from the outside to the inside of the plate with 10 μl of sporangia and zoosporangia (10^5 sporangia/ml). After 7 days, plates were scanned, and growth of mycelia was measured using Adobe Photoshop and the EC_{50} determined as described by Fairchild *et al.* (2013).

In the poison agar method, aqueous solutions of metalaxyl-M (prepared from Ridomil Gold EC as above) were added to flasks of pea agar (amended with RAN) to give final concentrations of either 0, 5, 10, or 100 mg/L, before 25 ml was poured into 50 mm plates. A plug of actively growing *P. infestans* mycelia from a 7-day-old culture was placed in the centre of the plate. After 7 and 10 days, radial growth of mycelia was measured, compared with the control culture plate, and sensitivity calculated as previously described by Forbes (1997).

2.5 | Multiplex simple sequence repeat genotyping

One-step multiplex simple sequence repeat (SSR) genotyping was performed using 12 microsatellite markers as described by Li *et al.* (2013). The Type-it microsatellite PCR kit was used for multiplex amplification (Qiagen). Twelve genotypes of *P. infestans* with known SSR alleles were also included in the PCR (Table 1). All PCR products from FTA cards were diluted at 1:50 (PCR product:PCR-grade water), and from genomic DNA were diluted at 1:100. PCR products were submitted to the Molecular Research Core Facility of Idaho State University (Pocatello, ID, USA) for fragment analysis. Fragment analysis was performed with an AB 3130xl Genetic Analyzer (Applied Biosystems). Each sample was processed in-line with a size standard GeneScan 500 LIZ (Life Technologies) for

FIGURE 1 (a) Map of Indonesia showing the three different regions, west, central and east Java. (b) Enlarged map of the island of Java showing the nine regencies of Java from where samples of *Phytophthora infestans* were collected (map developed in R)

fragment size determination. Raw data and sizing determination were analysed in GeneMapper software v. 5 (Applied Biosystems, Release 5.0, Build ID FC3, licensed by ISU Molecular Research Core Facility). Allele bins were set for each marker and alleles were scored.

2.6 | Data preparation and analysis

Microsatellite data was obtained from 146 samples. Ninety percent of samples were triploids ($n = 131$), with a few diploids ($n = 11$) and tetraploids ($n = 4$). To make the data uniform and polyploid, zero was added in missing alleles of diploid and triploid. Data from the same location from multiple years were combined and analysed based on geographical locations. Standard isolates (Table 1), data kindly provided by Dr David Cooke (The James Hutton Institute, Invergowrie, Dundee, UK), and some previously published data by Li *et al.* (2013) were compared for SSR fingerprints to assign specific genotypes. These data were also included in frequency-based analysis such as neighbour-joining (NJ) tree and minimum spanning network to see how our data correlated to them. Data were analysed in R package poppr v. 2.3.0. as described by Grünwald *et al.* (2017), Kamvar *et al.* (2014, 2015), and Shakya *et al.* (2018).

2.7 | Multilocus genotype analysis, diversity statistics, and mode of reproduction

Multilocus genotype (MLG), estimated multilocus genotypes (eMLG) after rarefaction, and diversity statistics for 146 polyploid data for 12 microsatellite loci were calculated using the R package poppr v. 2.3.0 (Kamvar *et al*., 2014, 2015; R Core Team, 2016; Grünwald *et al*., 2017). MLG diversity of a population was estimated using Shannon–Weiner Index in R package poppr (Shannon and Weaver, 1949; Kamvar *et al*., 2014, 2015). The expected heterozygosity (H_{exp}) was computed for all samples based on Nei's unbiased gene diversity (Nei, 1978). The mode of reproduction in each population was estimated based on index of association (I_A) and standardized index of association value ($\bar{r}_d$; Agapow and Burt, 2001; Kamvar *et al*., 2014, 2015). Both clone-corrected and non-clone-corrected data were used to calculate $\bar{r}_d$ and the significance was tested with 999 permutations in R package poppr (Kamvar *et al*., 2014, 2015). Allelic diversity was calculated based on Simpson's index and Nei's unbiased gene diversity in R package poppr (Simpson,1949).

2.8 | Population structure and differentiation

The genetic distance between the individual DNA samples was computed using a stepwise mutation model as described by Bruvo *et al*. (2004). A neighbour-joining tree was constructed based on Bruvo's distance with 1,000 bootstraps in poppr and the NJ tree was viewed and modified on Figtree v. 1.4.3 (Kamvar *et al*., 2014, 2015; http://tree.bio.ed.ac.uk/software/figtree/). NJ trees are useful to visualize how samples cluster and group together compared to the standard samples. Discriminant analysis of principal components (DAPC) was performed in R-package adegenet v. 2.0.1 to examine how the individual samples cluster to a population (Jombart, 2008; Jombart *et al*., 2010). Minimum spanning network (MSN) was constructed in poppr.

Pairwise fixation indices (F_{ST}) were calculated on clone-corrected data using Bruvo's genetic distance using R package strataG v. 1.0.5 (Archer *et al*., 2017). The F_{ST} values were used to examine the differentiation between the populations separated by geographical locations. Analysis of molecular variance (AMOVA) was performed based on Bruvo's genetic distance on clone-corrected data in R package ade4 v. 1.7-5 (Excoffier *et al*., 1992; Dray and Dufour, 2007). AMOVA estimates variance due to individual samples within the population or among populations.

3 | RESULTS

3.1 | Mating type and metalaxyl-M sensitivity test

Eighty percent of DNA samples were positively amplified with CAPS markers and determined to be the A1 mating type (Table 2). One sample (Indo 111) was identified as A2 mating type. However, the mating type could only be determined using CAPS and could not be confirmed using the Phyb markers. Six isolates were also tested for mating type using both the pairing test and CAPS markers and determined to be A1 mating type. Out of the six isolates tested for metalaxyl-M sensitivity, two isolates, Pasuruan and Wonosobo, were intermediate and four, from Bandung, were sensitive. See Table 2 for definition of categories used in the metalaxyl sensitivity test.

3.2 | SSR fingerprints

SSR fingerprints of our DNA samples were compared with standard data (our data, data provided by Dr David Cooke, and data published by Li *et al*., 2013) and the specific genotypes were assigned based on the same or close fingerprints. The specific genotypes were EU_2_A1 (60%), EU_4_A1, and EU_13_A2 (1.5%). The rest of the DNA samples were unique but closer to European genotypes than US genotypes. An NJ tree was constructed including standard isolates; our assigned genotypes clustered together with EU_2_A1, EU_4_A1, EU_13_A2, or separately (Figure 2). Mating type results also supported the assigned genotypes.

3.3 | MLG analysis, diversity statistics, and mode of reproduction

Genotypic diversity of individuals in a population was evaluated and a total of 131 MLGs were determined out of 146 DNA samples. The DNA samples from Wonosobo had a greater number of MLGs (36) and were more diverse compared to other regencies, followed by Pasuruan (24) and Bandung (23). DNA samples from Garut (6) and Majalengka (6) were the least diverse. DNA sample diversity was even in many regencies, with a value of 1.0 (Table 3). All of the regencies had a standardized index of association value greater than zero except Majalengka ($\bar{r}_d = -0.0235$, $p < .724$; Figure S1), supporting the evidence of clonal reproduction in eight regencies. An index of association with a zero value indicates sexual reproduction, whereas any value other than zero suggests asexual reproduction. Wonosobo, Pasuruan, and Bandung had a greater Shannon–Weiner index value compared to the other regencies, indicating a high diversity, and these regencies had a higher number of MLGs.

Allelic diversity of the 146 DNA samples based on geographic distribution was calculated using poppr. A total of 75 different alleles were detected from 146 DNA samples ranging from 2 (SSR2) to 21 (D13) alleles, with a mean of 6.25 alleles per locus (Table 4). Gene diversity was estimated using Simpson's index and ranged from 0.48 (SSR2) to 0.70 (D13), indicating that the SSR2 locus was the least diverse and D13 was the most diverse locus (Table 4; Figure S2). Nei's unbiased estimation was correlated with Simpson's index. Evenness values ranged from 0.42 (D13) to 0.97 (SSR6 and SSR8). The D13 locus had the highest number of alleles (21) and high allelic diversity with the least even distribution, resulting in large number of MLGs (Table 4; Figure S2). PiG11 (12) and Pi4B (8) also had a high number

TABLE 2 Phenotypic characterization of *Phytophthora infestans* isolates from nine regencies of Java, Indonesia

Isolates	Location	No. of DNA samples or isolates	Mating type	Mating type determination method	Metalaxyl-M sensitivity[a]
Indo147	Wonosobo	1	A1	Pairing test/PCR	Intermediate
Indo 148	Pasuruan	1	A1	Pairing test/PCR	Intermediate
Indo 143–146	Bandung	4	A1	Pairing test/PCR	Sensitive
Indo 1–28	Bandung	28 (NAmp = 5)	A1	PCR	NA[c]
Indo 29–34	Garut	6	A1	PCR	NA
Indo 35–40	Majalengka	6 (NAmp = 1)	A1	PCR	NA
Indo 41–50	West Bandung	10 (NAmp = 1)	A1	PCR	NA
Indo 51–69, 75–80, 131–140	Wonosobo	35 (Namp = 10)	A1	PCR	NA
Indo 70–74, 99–104, 118–130	Pasuruan	24 (NAmp = 5)	A1	PCR	NA
Indo 83–87, 111–117	Mojokerto	12 (NAmp = 1)	A1	PCR	NA
Indo 91–98, 141–142	Temanggung	10 (NAmp = 2)	A1	PCR	NA
Indo 81–82, 89–90, 106–110	Batu	9 (NAmp = 2)	A1	PCR	NA

Note: NAmp, not amplified in PCR and mating type not known; NA, not available as only DNA of the isolate was collected on FTA cards.

[a]Resistant: growth on both 5 and 100 mg/L poison agar plates with >40% growth compared to control (0 mg/L); intermediate: growth on 5 mg/L plate with >40% growth compared to control (0 mg/L); sensitive: <40% growth on both 5 and 100 mg/L plates compared to control (0 mg/L).

of alleles compared to the rest of the loci, with higher allelic diversity and less evenness, contributing to a larger number of MLGs.

3.4 | Population differentiation and structure

AMOVA was computed based on Bruvo's genetic distance on clone-corrected data to determine whether variation was due to individual DNA samples within a population or among populations (Table 5). Results showed that variation between populations (regencies) was only about 20% (Table 5). However, 80% of the variation was explained by individual DNA samples within the population, which was supported by the F_{ST} values. The F_{ST} calculated on clone-corrected data using Bruvo's genetic distance revealed low to modest differentiation between populations (Table 6), with values ranging from 0.02 to 0.12. The highest population differences were found between Garut and Majalengka (0.12), indicating that there is limited gene flow or limited migration of isolates between these regencies. Despite having the highest differentiation, these two regencies are geographically close. The lowest population differences were observed between Bandung and Wonosobo (0.02), even though these two regencies are not geographically close. Furthermore, the F_{ST} value indicates ongoing migration events between these two regencies. The least differentiation was between Wonosobo and the other regencies, even Majalengka, revealing two-way migration from Wonosobo, whilst the highest differentiation was between Majalengka and the other regencies (Table 6). The regencies of Wonosobo and Temanggung are geographically close and had modest differences between their populations (0.04). Likewise, Pasuruan, Mojokerto, and Batu are also geographically close, and had modest differentiation between their populations (0.07).

The discriminant analysis of principal components (DAPC) results support the small amount of variation among the regencies, because most of the populations (6 out of 9) were clustered together with the exception of populations from three regencies (Pasuruan, Bandung, and Batu; Figure 3). All 146 DNA samples from the nine regencies grouped together in four clusters. DNA samples from Bandung, Batu, and Pasuruan clustered separately, while the rest of the DNA samples clustered together to make the "mixed" fourth cluster. However, a few of the DNA samples from Wonosobo overlapped with other regencies, except Pasuruan (Figures 3 and 4). Most of our samples were also grouped together with standard data of EU_2_A1 (60%) EU_4_A1, and EU_13_A2 (1.5%), in an NJ tree based on Bruvo's distance (Figure 2). Standard US and other European genotypes clustered separately in the NJ tree.

4 | DISCUSSION

This study provides the first in-depth investigation into the genotypes that make up the *P. infestans* populations in the main potato-growing regions on the island of Java, Indonesia. Although in the past a few studies have been conducted with limited isolates, populations of *P. infestans* in Indonesia have not been thoroughly studied until now. Nishimura *et al.* (1999) included four samples obtained from an unknown location in Indonesia in 1993 while characterizing the *P. infestans* population in seven Asian countries and found that all four were A2 mating type and resistant to metalaxyl-M. In our study we only found one DNA sample, Indo 111, that was identified as A2 using CAPS markers. However, this mating type could not be confirmed using the Phyb markers. All of our DNA samples that were successfully amplified were determined to be the A1 mating type based on the CAPS marker results, and these results were

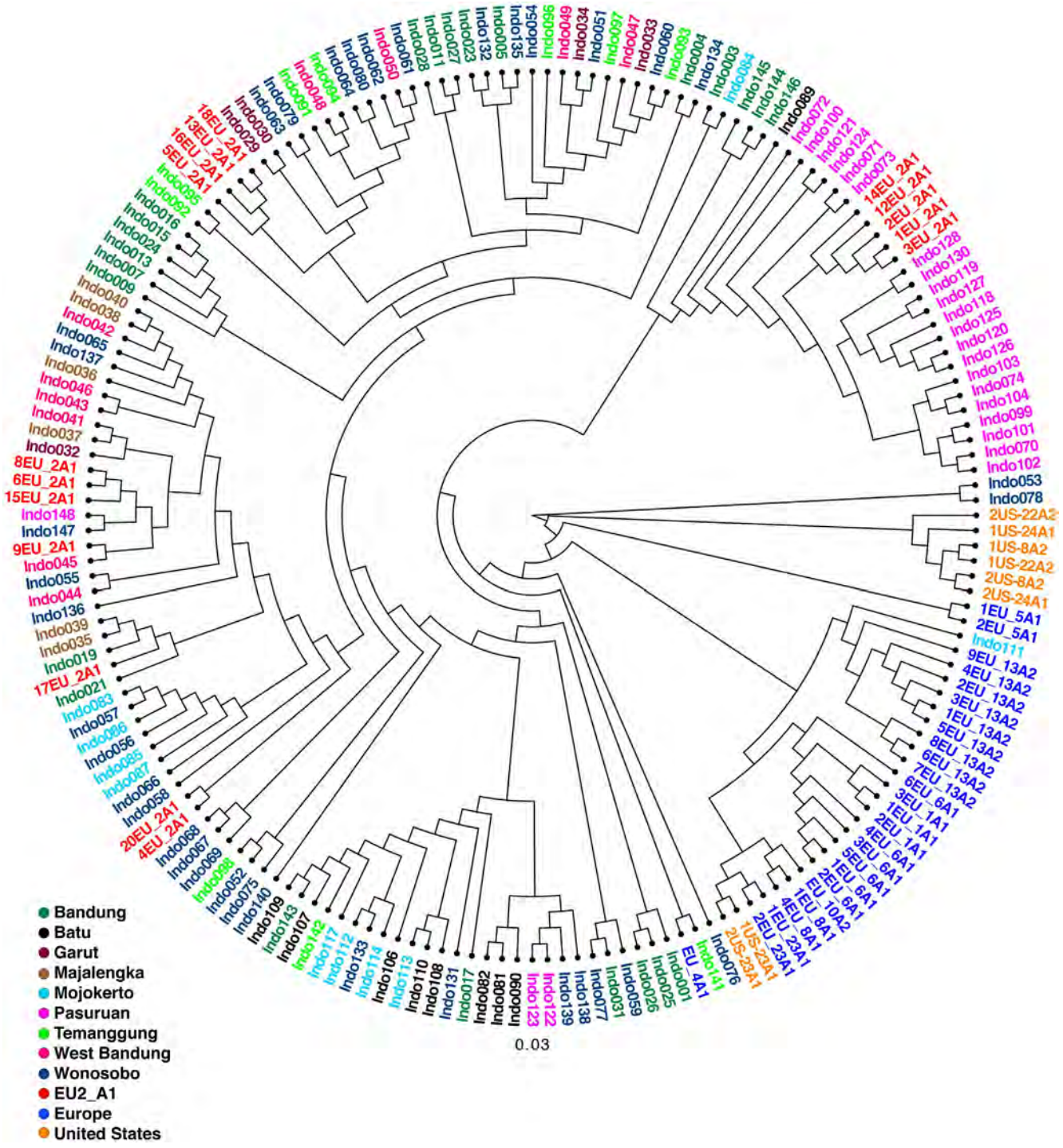

FIGURE 2 Neighbour-joining tree based on Bruvo's distance for *Phytophthora infestans* populations from nine regencies in Java, Indonesia, European and US standard isolates with 1,000 bootstrap replicates (data analysed in the R package poppr v. 2.3.0, results viewed and modified on Figtree v. 1.4.3). The colour of the isolate names indicates which location they are from

corroborated with the pairing test carried out with six isolates from Indonesia. Brylińska *et al*. (2018) have reported that isolates were not completely (100%) assigned to specific mating types using these molecular markers (CAPS and Phyb assigning isolates correctly by 96% and 84%, respectively). As such, Brylińska *et al*. (2018) recommended that local populations such as these need to be validated with the pairing test method before these markers are used for DNA samples.

Populations of *P. infestans* in Indonesia were reported to be resistant to metalaxyl-M (Nishimura *et al*., 1999; Adiyoga, 2009). However, of the six isolates we tested for metalaxyl-M sensitivity, two were found to be intermediate and four were found to be

TABLE 3 Multilocus genotype and diversity statistics for microsatellite data for 12 microsatellite loci in populations of *Phytophthora infestans* from Indonesia by regency, collected from 2016 to 2019

Population	*N* [a]	MLG[b]	eMLG (*SE*) [c]	*H* [d]	H_{exp} [e]	Evenness	I_A [f]	$\bar{r}_d$ [g]
Bandung	32	23	9.18 (0.75)	3.08	0.597	0.943	0.781	0.0913
Batu	9	9	9.00 (0.00)	2.20	0.589	1.000	1.245	0.1989
Garut	6	6	6.00 (0.00)	1.79	0.587	1.000	1.261	0.1977
Majalengka	6	6	6.00 (0.00)	1.79	0.557	1.000	−0.111	−0.0235
Mojokerto	12	10	8.50 (0.58)	2.21	0.594	0.862	1.249	0.1479
Pasuruan	25	24	9.85 (0.00)	3.16	0.517	0.978	0.436	0.0596
Temanggung	10	10	10.00 (0.00)	2.30	0.565	1.000	1.917	0.2078
West Bandung	10	10	10.00 (0.00)	2.30	0.544	1.000	0.210	0.0451
Wonosobo	36	36	10.00 (0.00)	3.58	0.606	1.000	1.092	0.1162
Total	146	131	9.93 (0.26)	3.97	0.601	0.949	0.656	0.0662

[a]*N*, number of individuals per regency.
[b]MLG, number of multilocus genotypes.
[c]eMLG, expected number of MLGs for each regency.
[d]*H*, Shannon–Weiner index of MLG diversity.
[e]H_{exp}, Nei's unbiased gene diversity.
[f]I_A, index of association.
[g]$\bar{r}_d$, standardized index of association.

TABLE 4 Population statistics for clone-corrected microsatellite data for 12 microsatellite loci in populations of *Phytophthora infestans* from Indonesia

Locus	Allele	1 − *D*[a]	H_{exp} [b]	Evenness
D13	21	0.70	0.70	0.42
Pi4B	8	0.59	0.59	0.74
PiG11	12	0.61	0.61	0.62
Pi04	3	0.51	0.51	0.94
Pi63	5	0.66	0.66	0.89
Pi70	5	0.65	0.65	0.91
SSR2	2	0.48	0.48	0.95
SSR3	4	0.66	0.67	0.93
SSR4	6	0.66	0.67	0.83
SSR6	3	0.50	0.50	0.97
SSR8	3	0.65	0.65	0.97
SSR11	3	0.52	0.52	0.93
Mean	6.25	0.60	0.60	0.84

[a]1 − *D*, Simpson index.
[b]H_{exp}, Nei's, 1978 gene diversity.

TABLE 5 Analysis of molecular variance (AMOVA) for clone-corrected *Phytophthora infestans* populations based on Bruvo's genetic distance

Source	*df*	SS	MSS	% variance
Between regencies	8	3.095	0.387	19.870
Within regencies	125	10.763	0.086	80.130
Total	133	13.858	0.104	100

sensitive to metalaxyl-M (see Table 2). With our small sample size, it is impossible to determine how widespread resistance to metalaxyl-M is on Java. However, due to the genetic diversity of *P. infestans* isolates from Java, it is probable that there are isolates with a range of metalaxyl-M sensitivities on the island, depending on the local use of metalaxyl-M.

Microsatellite analysis revealed that most of our samples were EU_2_A1 or subclonal variants of EU_2_A1 based on the SSR fingerprints compared with already published data, our standards, and data kindly provided by Dr David E. L. Cooke (Li *et al*., 2013; David Cooke, The James Hutton Institute, Invergowrie, Dundee, UK, personal communication). Dr Louise Cooke also reported that the limited number of samples obtained from West Java as part of an AsiaBlight study were all EU_2_A1 (Queen's University, Belfast, UK, personal communication). However, we found large subclonal variation in the Indonesian *P. infestans* population.

Based on the SSR fingerprints and NJ tree analysis we found that about 60% of the population were EU_2_A1 with large subclonal variation. There are many reasons behind the large variation within clonal lineages. One is polyploidization, which is an increase in the number of alleles in a locus (Li *et al*., 2017). In our analysis, there were DNA samples that were diploid, triploid, and tetraploid. Most of our DNA samples were triploid ($n = 131$). Out of 12 loci, six were diploid, five were triploid, and D13 had a few DNA samples that were tetraploid (Figure S3). In addition to that, some loci had a higher number of alleles with high genetic diversity and less evenness, such as D13 and PiG11. The number of MLGs we found was increased due to polyploidization in alleles in six loci. It has been reported that clonal lineages

TABLE 6 Pairwise F_{ST} values for clone-corrected *Phytophthora infestans* populations from nine regencies in Indonesia

	Bandung	West Bandung	Batu	Pasuruan	Mojokerto	Wonosobo	Garut	Majalengka	Temanggung
Bandung	–								
West Bandung	0.05	–							
Batu	0.06	0.09	–						
Pasuruan	0.05	0.07	0.07	–					
Mojokerto	0.05	0.08	0.07	0.07	–				
Wonosobo	0.02	0.04	0.05	0.04	0.05	–			
Garut	0.07	0.09	0.11	0.10	0.10	0.06	–		
Majalengka	0.07	0.09	0.11	0.09	0.10	0.06	0.12	–	
Temanggung	0.05	0.07	0.09	0.08	0.08	0.04	0.09	0.10	–

Note: The lower the value, the greater the similarity between two populations.

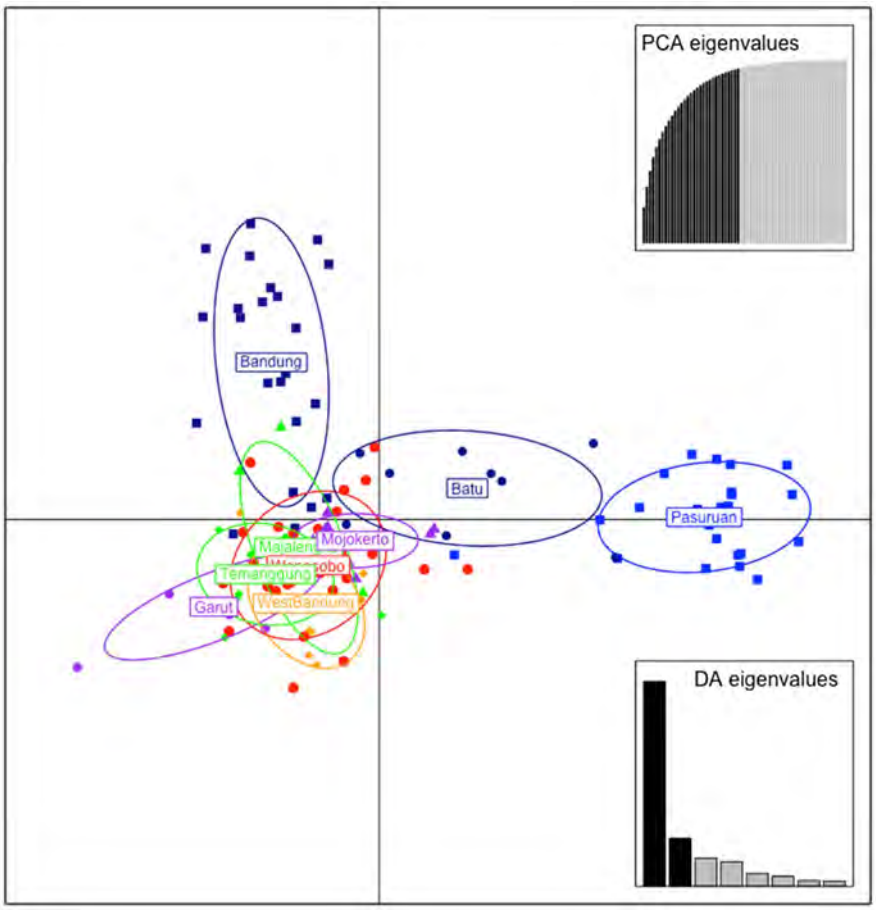

FIGURE 3 Discriminant analysis of principal components (DAPC) of *Phytophthora infestans* populations collected from 2016 to 2019 in nine regencies of Indonesia (data analysed in R package poppr v. 2.3.0)

tend to have more alleles (triploids) in multiple loci compared to progeny of sexual recombinants (Li *et al*., 2013). The occurrence of polyploidization may mask accumulated deleterious alleles in asexual populations (Li *et al*., 2017) that otherwise may lead to extinction during the course of evolution, according to Muller's law (Muller, 1964). That is why there is a prevalence of clonal lineages worldwide, as they are so successful due to their adaptability and fitness under many environmental conditions, even without sexual recombination (Li *et al*., 2017). We also found clonal reproduction based on the linkage disequilibrium analysis (conducted with both raw and clone-corrected data) except in Majalengka. However, we had a very small number of samples from this regency and we need to include more samples to get a more precise result. Shakya *et al*. (2018) found a different trend; most of the *P. infestans* isolates in Mexico had diploid alleles in both sexual and clonal populations. However, Li *et al*. (2017) reported that most of the successful clonal lineages tend to be triploid because they have more heterozygous single nucleotide polymorphisms (SNPs) and a higher level of functional variations compared to diploids.

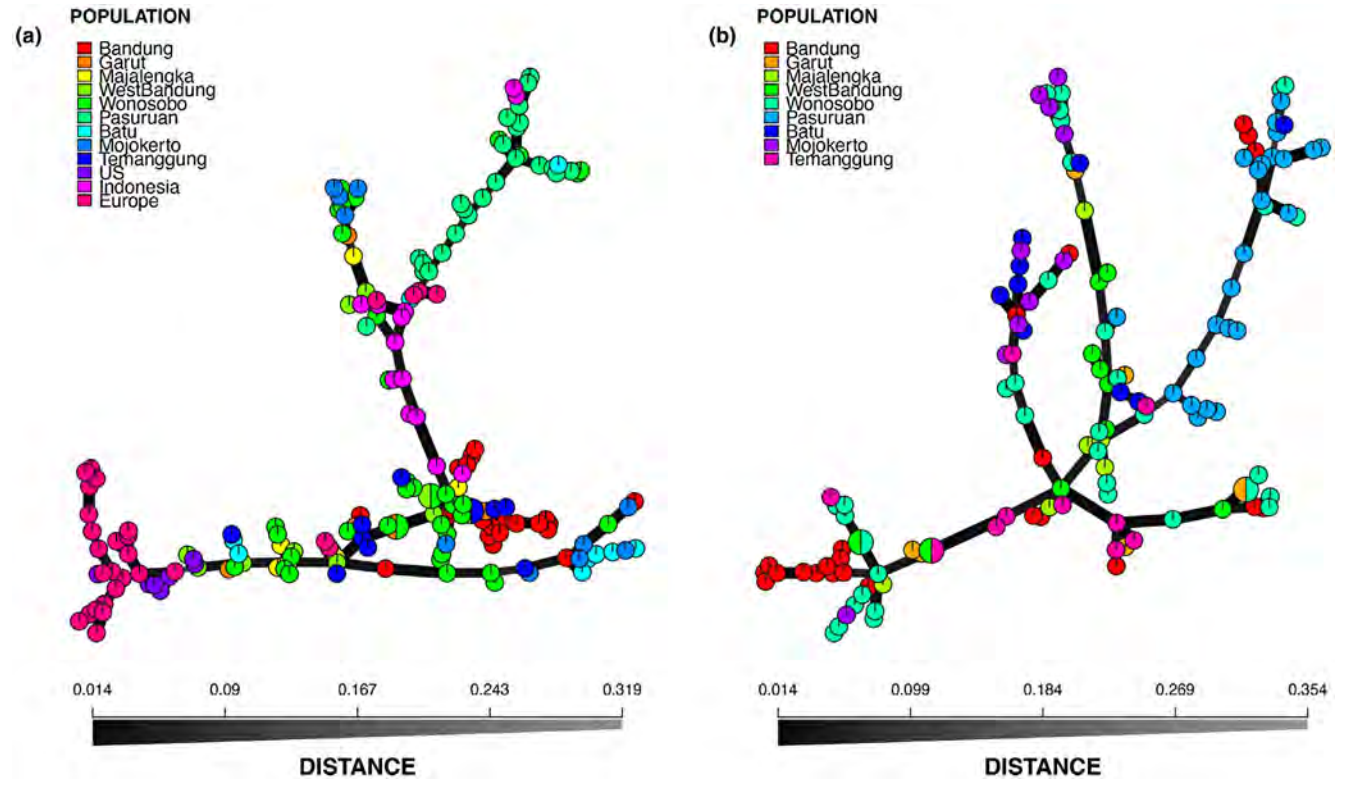

FIGURE 4 Minimum spanning network (MSN) of *Phytophthora infestans* isolates from Indonesia collected from 2016 to 2019 compared with some representative European and US isolates. (a) Samples from Indonesia and other standard samples from Indonesia, Europe, and the USA; (b) samples from Indonesia only

Another reason for subclonal variation may be mutation. Indonesia is right under the equatorial line and is subjected to high UV levels that may result in a higher mutation rate, as described in Ecuador (Delgado *et al*., 2013). Similar large subclonal variations of single clonal lineages were observed in Ecuador and India (Delgado *et al*., 2013; Dey *et al*., 2018). Large variations have also been reported in Nordic European countries (Brurberg *et al*., 2011). However, the variation in Nordic European countries is largely due to sexual recombination (Brurberg *et al*., 2011). Due to the subtropical climate, multiple potato crops can be grown throughout the year in Indonesia, India, and Ecuador so the disease cycle is maintained year-round. Li *et al*. (2017) stated that *P. infestans* isolates tend to decrease their number of alleles under adverse climatic conditions such as low carbon or exposure to sublethal levels of metalaxyl-M fungicide. In Indonesia, growers use fungicide sprays extensively, with up to 30 sprays in a single season (Adiyoga, 2009). This may have a negative impact on polyploidization (Li *et al*., 2017). However, factors such as high UV radiation, a continuous year-round disease cycle, small-scale growers who may not spray fungicides extensively, continuous use of susceptible cultivars, and very conducive environmental conditions for late blight in Java might lead to different scenarios where polyploidization levels are not impacted or are even increased. From our results we can speculate that the populations of *P. infestans* on the island of Java, Indonesia, are undergoing mutation through allele loss or gain.

The distribution of genetic variation in Javan populations was examined using AMOVA. Individual samples within the regencies contributed 80% of the variation compared to the populations between regencies that explained only 20% of the variation. We determined a similar trend in population differentiation. F_{ST} values, which ranged from 0.02 to 0.12, indicated that the differentiation between populations was fairly low to modest. In recent years, potato seed tubers have been locally produced in West Java, with limited seed imported from Germany and the Netherlands (Fugile *et al*., 2006; Adiyoga, 2009). The population differentiation analysis revealed evidence of higher migration between regencies of West Java and Central Java, such as between Wonosobo/Bandung, Wonosobo/West Bandung, and Wonosobo/Temanggung, than between West Java and East Java. However, we did observe similar levels of population differentiation between Wonosobo in Central Java and Pasuruan in East Java, suggesting there has been migration between populations in Central and East Java.

Genetic diversity was found to be highest in Garut, but in Majalengka it was found to be lower compared to other regencies. The discrepancy in genetic diversity from these regencies is most probably due to the limited number of samples collected, but could also be due to lack of migration or gene flow between these regions. The population differentiation based on F_{ST} values also supports low or lack of gene flow in these two regencies. Linkage disequilibrium analysis of

our DNA samples suggests that the mode of reproduction in *P. infestans* populations from Majalengka is sexual. However, out of six DNA samples, five were determined to be A1 mating type from this regency. So, the question arises as to how it can be possible to have sexual recombination without the A2 mating type? Because the number of DNA samples we collected from this region was so small it is most likely that there are A2 mating types in the region. Nishimura *et al.* (1999) reported the presence of A2 mating type isolates from Java so it is probable that there are A2 mating types in the population that were not collected in our samples. Majalengka was the only regency in Java with a population where we detected indications of sexual reproduction. More DNA samples and isolates need to be collected over multiple years to get a clearer view of the main mode of reproduction in this regency.

In the NJ tree based on Bruvo's genetic distance, we found that 62% of our DNA samples clustered with European isolates. However, 38% of DNA samples clustered separately from the European isolates, whilst one sample clustered with the US isolates. Some of the *P. infestans* populations in regencies such as Batu and Bandung are dominated by unique MLGs. The Batu population was composed of all unique MLGs. The Bandung population was mixed with a few EU_2_A1 (based on SSR fingerprints matched with published data) and unique MLGs, whereas the Wonosobo population was found to have a mixture of subclonal variants of EU_2_A1 and a few unique MLGs. The Pasuruan population was mainly dominated by EU_2_A1, which was also supported by DAPC analysis. We also found 1.5% of the samples recovered were EU_4_A1 or EU_13_A2. EU_2_A1 and EU_4_A1 are older European genotypes that were dominant in Europe over 20 years ago and EU_13_A2 was first identified in isolates obtained from the Netherlands in 2004. Martin *et al.* (2019) classified worldwide haplotypes of *P. infestans* and discovered these European genotypes all had the same haplotype (I-15 of Ia), and contributed this as maternal parents for other contemporary genotypes. Because Indonesia used to import Granola seed tubers from Europe, these genotypes may have been introduced to Java in infected symptomless seed tubers. The European genotypes most probably served as maternal parents on Java from which unique genotypes could have evolved. With the history of occurrence of the A2 mating type and resistance to metalaxyl-M, the presence of the EU_13_A2 clonal lineage in Mojekerto (in one DNA sample from the current study) and potential sexual reproduction in Majalengka, close monitoring and intensive sampling are necessary in these regencies.

This study provides insights into the structure and diversity of *P. infestans* populations on Java, Indonesia. In recent years, there have been many reports of late blight epidemics caused by older lineages of *P. infestans* and their increasing prevalence in different parts of the world (Njoroge *et al.*, 2019a). In east Africa, the US-1 clonal lineage seemingly dominated *P. infestans* populations since its introduction in Kenya in 1941 (Njoroge *et al.*, 2019a). However, it has recently been found that the US-1 lineage has been completely displaced in Kenya by EU_2_A1and almost completely displaced in other east African countries (Njoroge *et al.*, 2019a). The increased dominance of EU_2_A1 in east Africa has been attributed to its increased aggressiveness on potato (Njoroge *et al.*, 2019b). We discovered EU_2_A1 dominating *P. infestans* populations on Java, particularly in the East Javan regency of Pasuruan, which clustered separately from West and Central Javan regencies in the DAPC analysis. It is possible that EU_2_A1 is the original genotype that was introduced to Indonesia and it is now being displaced in Central and West Java by variants of EU_2_A1 and unique MLGs that we identified in these regions.

In Europe, programmes to monitor and track changes in populations of *P. infestans* over time have been used to enhance the effectiveness of current management strategies. These strategies have included host resistance management, fungicide programme optimization, the use of integrated pest management tools, and sophisticated decision support systems (Cooke *et al.*, 2011). In Indonesia, these systems do not exist and growers rely almost exclusively on the use of fungicides to produce viable yields of potatoes. With our studies showing that there are isolates of *P. infestans* with intermediate sensitivity to metalaxyl-M, a total reliance on fungicides for management of late blight is not the best option. Alternative sources of disease management such as resistant cultivars should be given consideration. One such alternative would be the development of bioengineered local varieties with 3 *R*-gene resistance to late blight such as the ones being developed by the USAID Feed the Future Biotechnology Potato Partnership (FtFBPP). This would be of significant benefit to potato growers in Indonesia because it would reduce the cost of production through less fungicide costs, reduce farmer exposure to fungicides, and reduce fungicide residues in food, land, and wastewater.

Before and after release of resistant cultivars in late blight-prone areas, it is very important to track the diversity of *P. infestans* genotypes over time, monitoring for factors or new isolates that may overcome resistance. This is critical to ensure the effectiveness and durability of any 3 *R*-gene cultivars that are released in Indonesia. The data from this study will serve as a baseline to inform development of integrated strategies to extend the efficacy and durability of the USAID FtFBPP 3 *R*-gene potato cultivars that are being developed for release in Indonesia.

ACKNOWLEDGEMENTS

This work was funded in part by the United States Agency for International Development (USAID) under Agreement #AIDOAALA1500056 as part of the Feed the Future Biotechnology Potato Partnership. Any opinions, findings, conclusions, or recommendations expressed here are those of the authors alone. The authors have no conflicts of interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Phillip Wharton https://orcid.org/0000-0003-4362-3817

REFERENCES

Adiyoga, W. (2009). Cost and benefits of late blight resistant potato in Indonesia. In: Norton, G.W. and Hautea, D.M. (Eds). *Projected Impacts of Agricultural Biotechnologies for Fruits and Vegetables in the Philippines and Indonesia*. Laguna: ISAAA, pp. 86–104.

Adiyoga, W., Suherman, R., Asgar, A. Irfansyah. (1999) *The Potato System in West Java, Indonesia: Production, Marketing. Processing, and Consumer Preferences for Potato Products*. Lima, PeruInternational Potato Center.

Agapow, P.M. and Burt, A. (2001) Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes*, 1, 101–102.

Archer, F.I., Adams, P.E. and Schneiders, B.B. (2017) STRATAG: An R package for manipulating, summarizing and analysing population genetic data. *Molecular Ecology Resources*, 17, 5–11.

Brurberg, M.B., Elameen, A., Le, V.H., Naerstad, R., Hermansen, A., Lehtinen, A. *et al*. (2011) Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology*, 115, 335–342.

Bruvo, R., Michiels, N.K., D'Souza, T.G. and Schulenburg, H. (2004) A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology*, 13, 2101–2106.

Brylińska, M., Sobkowiak, S., Stefańczyk, E. and Śliwka, J. (2018) Evaluation of PCR markers for *Phytophthora infestans* mating type determination. *European Journal of Plant Pathology*, 152, 33–44.

Cooke, L.R., Schepers, H.T.A.M., Hermansen, A., Bain, R.A., Bradshaw, N.J., Ritchie, F. *et al*. (2011) Epidemiology and integrated control of potato late blight in Europe. *Potato Research*, 54, 183–222.

Delgado, R.A., Monteros-Altamirano, A.R., Li, Y., Visser, R.G., van der Lee, T.A. and Vosman, B. (2013) Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces. *Plant Pathology*, 62, 1081–1088.

Dey, T., Saville, A., Myers, K., Tewari, S., Cooke, D., Tripaty, S. *et al*. (2018) Large sub-clonal variation in *Phytophthora infestans* from recent severe late blight epidemics in India. *Nature Scientific Reports*, 8, 4429.

Dray, S. and Dufour, A.B. (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20.

Excoffier, L., Smouse, P.E. and Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, 479–491.

Fairchild, K.L., Miles, T.D. and Wharton, P.S. (2013) Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. *Crop Protection*, 49, 31–39.

FAO (2008) *International Year of the Potato, 2008*. Available at: http://www.fao.org/potato-2008/en/world/asia.html [Accessed 3 July 2019].

Forbes, G.A. (1997) *Manual for Laboratory Work on* Phytophthora infestans. Quito, EcuadorCentro Internacional de la Papa.

Fry, W. (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. *Molecular Plant Pathology*, 9, 385–402.

Fugile, K.O., Adiyoga, W., Asmunati, R., Mahalaya, S. and Suherman, R. (2006) Farm demand for quality potato seed in Indonesia. *Agricultural Economics*, 35, 257–266.

Goodwin, S.B. (1997) The population genetics of *Phytophthora*. *Phytopathology*, 87, 462–473.

Grünwald, N.J., Kamvar, Z.N., Everhart, S.E., Tabima, J.F. and Knaus, B.J. (2017) *Population Genetics and Genomics in R*. Available at: https://grunwaldlab.github.io/Population_Genetics_in_R/ [Accessed 25 August 2020].

Haverkort, A., Boonekamp, P., Hutten, R., Jacobsen, E., Lotz, L., Kessel, G. *et al*. (2008) Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Research*, 51, 47–57.

Hu, C.H., Perez, F.G., Donahoo, R., McLeod, A., Myers, K., Ivors, K. *et al*. (2012) Recent genotypes of *Phytophthora infestans* in the eastern United States reveal clonal populations and reappearance of mefenoxam sensitivity. *Plant Disease*, 96, 1323–1330.

Johnson, D.A. and Cummings, T.F. (2009) Latent infection of potato seed tubers by *Phytophthora infestans* during long term cold storage. *Plant Disease*, 93, 940–946.

Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405.

Jombart, T., Devillard, S. and Balloux, F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, 11, 94.

Judelson, H.S., Spielman, L.J. and Shattock, R.C. (1995) Genetic mapping and non-Mendelian segregation of mating type loci in the oomycete, *Phytophthora infestans*. *Genetics*, 141, 503–512.

Kamvar, Z.N., Tabima, J.F. and Grünwald, N.J. (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281.

Kamvar, Z.N., Brooks, J.C. and Grünwald, N.J. (2015) Novel R tools for analysis of genome wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, 6, 208.

Kim, K.J. and Lee, Y.S. (2002) Genetic DNA marker for A2 mating type in *Phytophthora infestans*. *Journal of Microbiology*, 40, 254–259.

Kirk, W.W., Felcher, K.J., Douches, D.S., Coombs, J., Stein, J.M., Baker, K.M. *et al*. (2001) Effect of host plant resistance and reduced rates and frequencies of fungicide application to control potato late blight. *Plant Disease*, 85, 1113–1118.

Kusmana, K. (2003) Evaluation of some potato clones from stem cutting for resistance test to *Phytophthora infestans*. *Journal of Horticulture*, 13, 220–228.

Li, Y., Cooke, D.E.L., van der Lee, T. and Jacobsen, E. (2013) Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. *Journal of Microbiological Methods*, 92, 316–322.

Li, Y., Shen, H., Zhou, Q., Qian, K., van der Lee, T. and Huang, S. (2017) Changing ploidy as a strategy: The Irish potato famine pathogen shifts ploidy in relation to its sexuality. *Molecular Plant-Microbe Interactions*, 30, 45–52.

Martin, F.N., Zhang, Y., Cooke, D.E.L., Coffey, M.D., Grünwald, N.J. and Fry, W.E. (2019) Insights into evolving global populations of *Phytophthora infestans* via new complementary mtDNA haplotype markers and nuclear SSRs. *PLoS One*, 14, e0208606.

Muller, H.J. (1964) The relation of recombination to mutational advance. *Mutation Research*, 1, 2–9.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89, 583–590.

Nishimura, R., Sato, K., Lee, W.H., Singh, U.P. and Chang, T.T. (1999) Distribution of *Phytophthora infestans* in seven Asian countries. *Annals of the Phytopathological Society of Japan*, 65, 163–170.

Njoroge, A.W., Andersson, B. and Lees, A.K. (2019a) Genotyping of *Phytophthora infestans* in eastern-Africa reveals a dominating invasive European lineage. *Phytopathology*, 109, 670–680.

Njoroge, A.W., Andersson, B., Yuen, J.E. and Forbes, G.A. (2019b) Greater aggressiveness in the 2_A1 lineage of *Phytophthora infestans* may partially explain its rapid displacement of the US-1 lineage in east Africa. *Plant Pathology*, 68, 566–575.

R Core Team(2016). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. Available at: https://www.r-project.org/ [Accessed 25 August 2020].

Shakya, S.K., Larsen, M.M., Cuenca-Condoy, M.M., Lozoya-Saldaña, H. and Grünwald, N.J. (2018) Variation in genetic diversity of *Phytophthora infestans* populations in Mexico from the center of origin outwards. *Plant Disease*, 102, 1534–1540.

Shannon, C.E. and Weaver, W. (1949) *The Mathematical Theory of Communication*. UrbanaUniversity of Illinois Press.
Simpson, E.H. (1949) Measurement of diversity. *Nature*, 163, 688.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Dangi S, Wharton P, Ambarwati AD, et al. Genotypic and phenotypic characterization of *Phytophthora infestans* populations on Java, Indonesia. *Plant Pathol*. 2020;00:1–13. https://doi.org/10.1111/ppa.13269