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Original Research Article

Genotype and Phenotype Characterization of Indonesian *Phytophthora infestans* Isolates Collected From Java and Outside Java Island

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ABSTRACT

Phytophthora infestans, the cause of late blight disease, is a worldwide problem in potato and tomato production. To understand the biology and ecology of *P. infestans* and the mechanism of spatial and temporal factors for the variation in *P. infestans*, the population diversity is required to be fully characterized. The objective of this research is to characterize the diversity of *P. infestans*. Surveys and collection of *P. infestans* isolates were performed on many locations of potato's production center in Indonesia, as in Java (West Java, Central Java, and East Java) and outside of Java islands (Medan, Jambi, and Makassar). The collected isolates were then analyzed for their virulence diversity via plant disease bioassays on differential varieties and genotype diversity based on fragment analysis genotypes profile using the multiplexing 20 simple sequence repeat markers. The virulence characterization showed that the isolates group from Makassar, South Sulawesi, have the broad spectrum virulence pathotype to R1, R2, R3, R4, and R5 differential plants. Simple sequence repeat genotype characterization showed that in general, the population structure of *P. infestans* grouping is accordance to the origin of the sampling locations. The diversity between populations is lower than diversity between isolates in one location population groups. The characters of *P. infestans* population showed that the population diversity of *P. infestans* more occurs on individual isolates in one location compared with the diversity between the population location sampling.

1. Introduction

Phytophthora infestans, the cause of late blight disease, is a worldwide problem in potato and tomato production (Cooke *et al.* 2003). In Indonesia, this disease could cause 80% loss in potato production, particularly in rainy seasons (Chujoy *et al.* 1999). Studies on genetic diversity of *P. infestans* population have revealed the presence of both clonal and genetically (Knapova *et al.* 2002; Cooke *et al.* 2003), but the mechanisms responsible for this diversity have not been studied in detail (Lees *et al.* 2006). To understand the biology and ecology of *P. infestans* and the mechanism of spatial and temporal factors for the variation in *P. infestans*, the population diversity is required to be fully characterized.

Introgression breeding approach has been used to introduce disease resistance in many crop species, also in potato. This has resulted in the cultivars that are resistant to different races of the late blight pathogen (Harbaoui and Harrabi 2011). Initially, 11 different recognition specificities were identified, all originating from *Solanum demissum* and named R1–R11. This was the basis for a differential set of potato cultivars used worldwide to identify the virulence races occurring in *P. infestans* population (Poppel *et al.* 2009).

The simple sequence repeat (SSR) or microsatellite markers are tandemly repeated motifs of one to six bases found in the nuclear genomes of all eukaryotes tested and are often abundant and evenly dispersed (Tautz and Renz 1984; Langercrantz *et al.* 1993). Microsatellite sequences are usually characterized by a high degree of length polymorphism and are ideal single-locus codominant markers for genetical studies. The SSR markers have been characterized for *P. infestans* population on potato and tomato in France (Knapova *et al.* 2002). A review of a range of markers used for the characterization of *P. infestans* is given by Cooke and Lees (2004). The objective of this research is to identify the genetic diversity of *P. infestans* population, which is collected from some areas of potato's production center in Indonesia, using SSR markers. This marker offers a much higher taxonomic resolution and thereby enable more accurate estimates of the population structure (Widmark *et al.* 2003). This type of marker system is considered well suited for estimating genealogical relationships (Blouin 2003).

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2. Materials and Methods

2.1. Sources of isolates

From September 2011 to April 2012, potato and tomato leaflets with symptoms of late blight were collected from commercial fields and research stations in five provinces in Indonesia, which is from East Java, Central Java, West Java, Sumatera, and Sulawesi.

2.2. Culture isolation and purification

Samples were collected from infected leaflets and placed on newspaper leaflet, directly lace on cool box as preparation for isolation in laboratory. *P. infestans* isolation was made by placing diseased leaf fragments on water agar medium after sterilizing using double distilled water (ddH_2O) and 90% of ethanol, then incubated at 28°C. After 5–7 days, the pure culture of *P. infestans* were grown on the surface of the medium, and then transferred the hyphal tip on a selective medium of V-8 juice agar (200 mL of V-8 juice, 4.5 g of calcium carbonate, 20 g of agar, 800 mL of distilled water) containing 500 mL/L of ampicillin, 200 mL/L of vancomycin, 50 mL/L of rifampicin, 100 mL/L of pimaricin, 35 mL/L of PCNB, and 10 mL/L of benomyl. Purified isolates were maintained at 28°C on V-8 juice rye agar medium.

2.3. Genetic materials

Six differential plants for Races 1–6 were used for analyzing the pathotype patterns of selected isolates. There were five isolates for each sampling location in different collection sites as shown in Table 1.

Table 1. List of the *P. infestans* isolates collected from several locations of potato field

Region	Sampling year	Collection sites	ID isolates
Central Java	2011	Kejajar 3	1-K-III
		Banjarnegara 2	
		Stieng Rajawali 1	
		Gembol Batur 3	
		Kejajar 5	
Makassar, South Sulawesi	2012	Tinggi Moncong 5-2	
		Tinggi Moncong 4-1	
		Tinggi Moncong 3	
		Tinggi Moncong 5-1	
		Tinggi Moncong 1	
East Java	2011	Wonorejo 2	
		Ngadirejo 3	
		Padakaya 1	
		Padakaya Tosari 4	
Garut, West Java	2011	Padakaya Tosari 3	
		Cisurupan Patrol	
		Cirandog Sampang 3	
		Cirandog Papandayan 1	
		Cirandog Papandayan 3	
		Cisurupan Patrol 2	
Berastagi, North Sumatera	2012	Jalan Udara, Gg Rukun	
		Tangkulen	
		Bulan Baru	
		Aji Julu	
Jambi, South Sumatera	2012	Dola Raya Tengah	
		Sangir Kerinci 1	
		Sangir Kerinci 2	
		Pangkalan Dua	
		Sumur Tujuh	
		Patok Empat Kayu Aro	
		Kuto Perang	

2.4. Preparation of inoculum

The first preparation, infected potato, was placed on the upper part of peeling potato tuber sliced on petri dish and incubated at 18°C, 90% humidity for 4 days. *P. infestans* mycelia was transplanted on V8 media for 7–9 days and collected for inoculation preparation. The isolates used in testing were grown at 18°C incubator for 2 weeks; it aims to grow sporangia from each isolate. For accelerate multiplication of sporangia-treated brushing with glass spatula, harvesting of sporangia performed by scraped use glass preparat + cover with distilled water. Cultures were stored in the refrigerator (4°C), for approximately 1–5 hours, to stimulate production of zoospores. To determine the release of zoospores, sporangia were observed microscopically every 1–2 hours. After seeing spores release from sporangia, cultures were placed at the room temperature for 1 hour. Next step for inoculum preparation is continue with calculations and dilution of zoospore on preparat glass using hemacytometer. The target of inoculum concentration for each isolate to be used is 1×10^4 zoospore/mL. Calculations of zoospore were done on four boxes preparat glass on hemacytometer. Each box was identical to the volume of 0.1 mL, so it can be determined how much volume of inoculum that should be taken for target volume that would be used for inoculation (usually 20–50 mL per pot).

2.5. Preparation of plant

Test plant is planted on sterile planting medium which contains manure, husk charcoal with a ratio of 1:1, and NPK fertilizer per pot used approximately 3 g. The inoculation of plants is done when the plant reaches the age of 3 weeks. Meanwhile, in parallel, the pure isolate on V8 juice is 1 week already. After a 1-month old, inoculated plants will be ready.

2.6. Inoculation

The process of each isolate should be performed separately, thus avoiding contamination between isolates. Before the process of inoculation, watering each test plant to create humid condition is necessary. Inoculation should be performed in the afternoon, so that the inoculum does not dries quickly. The inoculation process was conducted by spraying the whole plant. Each pot was inoculated with a total inoculum volume of approximately 3.5 mL (approximately 20 mL for 6 pots, in total). Scoring was done 6–7 days after inoculation. The scoring system is based on scale systems 1–5 (Said 1976), as shown in Table 2.

Attack rate was calculated using the following formula:

$$P = \frac{\sum (v \times n)}{n \times N} \times 100\%$$

where P is weight attack, v is the scale value of each attack category, n is the total no. of plants of each attack category, Z is the scale value of the highest attack category, and N is the total no. of sample plants.

As for comparison, another scoring system was used based on the percentage of infected leaves (Henfling 1979; Halterman *et al.* 2008), and the criteria were categorized based on Song *et al.* (2003), Colton *et al.* (2006), and Halterman *et al.* (2008), as shown in Table 3.

2.7. DNA extraction

The DNA extraction was done by using *P. infestans* small scale DNA extraction protocol (rev. 4/4/11). The first step is to grow mycelium in pea broth using two agar plugs per 90 mm petri plate and two petri plates per isolate. The mycelium is usually ready to harvest after 1 week. After that, harvest mycelium by filtering through a Buchner funnel containing filter paper (Whatman no. 1) and connected to a vacuum. Place mycelium in a 2.2 mL microfuge tube and cover the top with parafilm. Perforate the parafilm one or two times with a syringe needle or toothpick. Freeze the mycelium at −80°C, then lyophilize (freeze-dry) overnight or longer. The

Table 2. Scoring system based on scale systems 1–5 (Said 1976)

0 = no attack	3 = attack between 51% and 75%
1 = attack below 25%	4 = attack between 76% and 100%
2 = attack between 26% and 50%	5 = plants dead

Table 3. Scoring system based on the percentage of infected leaves

Score	% of infected leaf tissue	Criteria
0	100	Susceptible
1	>90	Susceptible
2	81–90	Susceptible
3	71–80	Susceptible
4	61–70	Susceptible
5	41–60	Susceptible
6	26–40	Moderate resistant
7	11–25	Moderate resistant
8	<10	Resistant
9	0	Resistant

lyophilized mycelium should be stored at −20°C. Grind lyophilized mycelium in the microfuge tube using a tissue lyser machine to a fine powder. For DNA extraction, use 60–65 mg of the grounded lyophilized mycelium. This is the amount of tissue which fills a 2.2 mL microfuge tube to about 0.5 mL. The next step is to add 1.0 mL of extraction buffer, vortex and incubate at 65°C for about 1 hour and then add 333 μL of 5M potassium acetate, vortex and place on ice for 20 min. Spin in microfuge on high (14,000 rpm) for 10 min and pour supernatant into a clean 2.2 mL microfuge tube and add 800 μL isopropanol. Mix by inverting the tube several times, and incubate on ice for 30 min. Spin again in microfuge for 10 min on high speed (14,000 rpm), pour off and discard the supernatant, and dry the pellets in the speed vac for about 5 min. For RNase A treatment, resuspend DNA pellets in 500 μL TE buffer. For best results, incubate pellets in TE buffer at 4°C overnight first. If pellets do not resuspend easily, heat at 65°C for 10 min. Repeat heating if required. Add 10 μL Rnase A (10 mg/mL) to resuspended pellets and incubate for 10 min at 65°C, then on ice for 5 min continue adding an equal volume (500 μL) of phenol:-chloroform:isoamyl alcohol (25:24:1) pH = 8, vortex and microfuge on high speed for 5 min. Transfer the top aqueous layer (which contains your DNA in TE) to a new 2.2 mL microfuge tube. Further step, add an equal volume (500 μL) of chloroform:isoamyl alcohol (24:1), vortex and microfuge 5 min and transfer the top aqueous layer (which contains your DNA in TE) to a new 2.2 mL microfuge tube. Add 1/10 volume (50 μL) of 3M sodium acetate and 2 volumes (1 mL) of ethanol. Invert the tube several times to mix and incubate at −20°C for at least 2 hours and microfuge samples at high speed for 10 min and pour off supernatant. Continue with adding 200 μL 70% ethanol to wash the DNA pellet and microfuge at high speed for 2 min, pour off supernatant. Dry pellet in the speed vac for about 2 min and resuspend in ~50 μL dH_2O or 10 mM Tris pH = 8 (the DNA is most stable in Tris buffer). The last step is to determine DNA concentration using the Eppendorf BioPhotometer (dilute 2 μL DNA in 298 μL dH_2O). Also, check DNA sample on an agarose gel. DNA concentrations were determined by the nano drop machine. DNA extracted was stored at −20°C.

2.8. PCR amplification

Twenty SSR primers were used as previously research (Lees *et al.* 2006; Hadrami *et al.* 2007) with universal M-13 primers as fluorescent labels (Schuelke 2000) use in PCR reaction. The PCR amplification of the DNA was based on standards set condition: 5 min for initial denaturation at 94°C, continued with 30 cycles of: 30 s at 94°C, 45 s at 55°C, 45 s at 72°C, and 8 cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C, the last cycle for final elongation at 72°C, 10 min, in total reaction volume 20 μL using an MJ Research Thermal Cycle. The master mix contained the following components: 1× reaction buffer (16 mM $[NH_4]_2SO_4$, 67 mm Tris–HCL pH 8.8, 0.1% Tween, 25 mM $MgCl_2$; Roeche, UK), 5 mM each dNTPs (Invitrogen, USA), 5 μM each primer F and R; 5 μM primer M13 (Invitrogen); 1U/μL FastStar Taq polymerase (Roeche), and 10 ng/μL DNA template.

2.9. Fragment analysis

The diversity of SSR alleles were detected automatic sizing system by using the *genetic analyzer* CEQ8000 machine. PCR product from each primer was diluted on sample loading solution (SLS, Beckman Coulter, UK) and then mixed one with another PCR product to generate the *multiloading* set panel. Five primers were designed on one set panel *multiloading* with different allele size and fluorescent label, so they had not overlapped each other. The program used for *Genetic analyzer* (Beckman Coulter) running was "Frag-1," with the conditions at 35°C for capillary temperature, 2.0 kV, 30 s for injection, denaturation at 90°C in 120 s, and the next step is separation in 7.5 kV for 35 min (Table 4).

3. Results

3.1. Phenotype characterization

In *P. infestans*, there are effectors recognized by 11 *R genes*, named as R1–R11 and their combination (Lehtinen *et al.* 2008). Particularly in Indonesia, where the use of fungicides is not a rational strategy for the long-term; and in a long period, among 11 *R genes*, six of them (*R1–R6*) were relatively dominant found in several endemic locations of *late blight* disease in Indonesia (direct communication with Dra Euis Suryaningsih, MSc). Therefore, the dominant R1–R6 were used for analyzing the diversity of pathotype patterns of *P. infestans* isolates collected from a various potato centers in Indonesia.

The performance of the pathotype patterns evaluation was shown in Figure 1A and the results obtained of this evaluation were showed as the diseases progress (Figure 1B and 1C) and the pathotype spectrum (Figure 2).

Table 4. The SSR multiplexing setting up

No.	Primer	M13 labeled	Size range (bp)*
Panel 1			
1	Phi02-1	Blue (D4)	142–166
2	Phu-K1-17	Blue (D4)	400–500
3	Phi63-7	Green (D3)	148–170
4	Phi65-8	Black (D2)	145–151
5	Phi89-11	Black (D2)	179–185
Panel 2			
1	Phi66-9	Green (D3)	153–155
2	Va-Vd-20	Blue (D4)	250–452
3	Phi04-2	Blue (D4)	162–170
4	Phi16-3	Black (D2)	174–178
5	Phi33-5	Black (D2)	203–209
Panel 3			
1	G11-13	Blue (D4)	142–166
2	4B-12	Blue (D4)	205–217
3	D13-14	Black (D2)	108–142
4	Phi-ITS3-15	Black (D2)	300–456
5	Phi56-6	Green (D3)	174–176
Panel 4			
1	Phi70-10	Blue (D4)	189–195
2	Phe-ITS4-16	Blue (D4)	136
3	Phi26-4	Green (D3)	172
4	Fs-19	Green (D3)	315
5	Pha-ECA1-18	Black (D2)	200

* Allele size range based on Lees *et al.* (2006) and Hadrami *et al.* (2007).

Figure 1. (A) The performance of the pathotype patterns evaluation of isolates groups on differential varieties on 5 days after inoculation. (B) Differential *variety R3* inoculated with isolate from Berastagi-5. (C) Differential *variety R1* inoculated with isolate from Garut, named Cirandog Papandayan-1. White arrows indicated the lesion of *P. infestans* symptoms.

Pathotype diversity was low detected on R1 (A) and R6 (F). All the isolates were showed the virulence patterns in 1–10 days observation after inoculation. All the isolates were virulence to R1, because the plants were susceptible on 7 days after inoculation. In contrary condition in R6 (F), virulence to R6 was not found. Up to 10 days after inoculation, the diseases score still 6–5.

There was diversity among the isolates for pathogenicity to R2, R3, R4 and R5 (Figure 2). In R2, isolates from Central Java, South Sulawesi, West Java and Berastagi, North Sumatera were showed on the same patterns; whereas, isolates group from Jambi and East Java were showed in different patterns. In R3, two groups of pathotype patterns were showed in the total isolates. The most virulence to R3 is isolates from South Sulawesi. In R4, almost all isolates were same pathotype patterns, except isolates from Jambi, was not virulence found. In R5, there were two groups of pathotype patterns, Jambi and West Java's isolates in first group and others in different groups. The most virulence to R5 was isolates from Centre of Java.

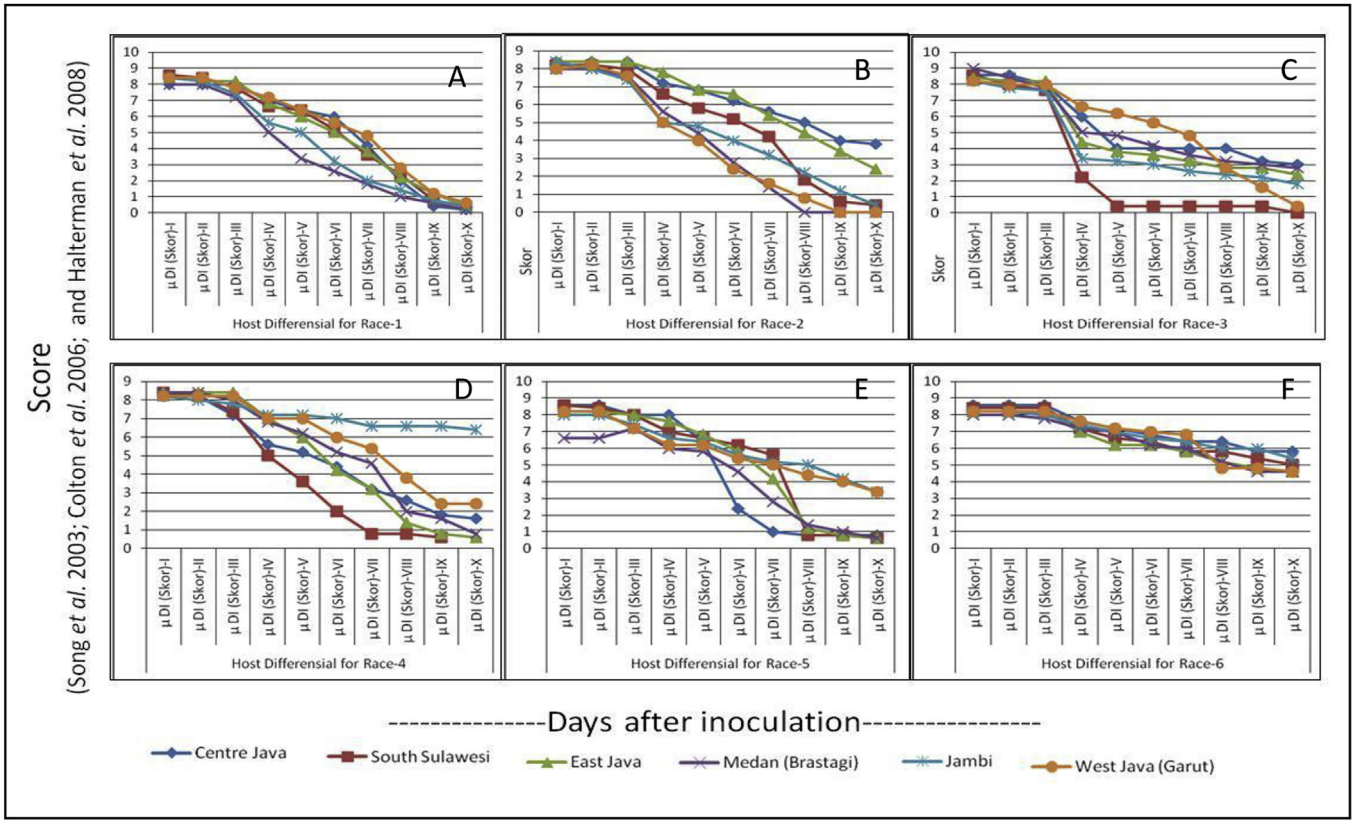


Figure 2. Disease progress of groups isolates collected from Java (West, Centre and East) and outside of Java island on host differential for Race 1 (R1), host differential for Race 2 (R2), host differential for Race 3 (R3), host differential for Race 4 (R4), host differential for Race 5 (R5) and host differential for Race 6 (R6). The diagram were plot between Disease Intensity (DI Score on Y axis) and days after inoculation (on X axis).

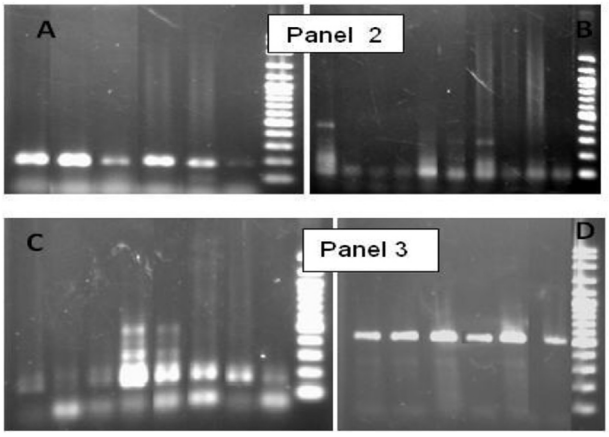


Figure 3. Parts of PCR product using primers on Panel 2 ([A] Phi04 and [B] VaVd) and Panel 3 ([C] G11 and [D] Phi-ITS3), viewing on agarose gel 2%.

3.2. PCR amplification of genotype characterization

A total of 48 isolates of *P. infestans* collected in five locations in three provinces in Java Island were amplified using 20 SSR primers as setting up as a four multiplex panels on PCR analysis. Part of the PCR products were viewed at 2% agarose gel as shown in Figure 4.

3.3. Singleplexing on fragment analysis

For optimization purpose, it was needed to confirm the peak performance of each color label and primers, so it could be used for predicting how much the volume of PCR products were needed for multiplexing. The singleplexing optimization results as shown in Figure 5.

3.4. Multiplexing fragment analysis by CEQ8000

The multiplexing fragment analyses for all samples were performed for a total of 20 SSR markers. Some of the results are illustrated in Figure 3. Among the SSR markers used, there are SSR that detects the multiple allelic and heterozygous performance on some samples analyzed. These markers are: VaVd and G11, which detected multiple allelic and heterozygous performance on one of the samples from Pengalengan location (Figure 6). The total data of allele size were collected on database (as attached Table of this report).

Heterozygote and multiallelic performances were detected in VaVd and G11 locus, as shown in Figure 7.

3.5. Genetic diversity based on SSR allele size and structure population

Fluorescent SSR genotyping by capillary electrophoresis was performed on the 66 total DNA samples of *P. infestans* across 20 SSR markers. The mean number of alleles detected at the 20 locus of SSR across 66 total samples of DNA *P. infestans* is 32. The number of alleles in each locus was ranging from 10 (in locus Phi70-10) to 53 (in locus VaVd-20). The gene diversity for each locus was ranging from 0.762 (in locus Phi70-10) to 0.9765 (in locus VaVd-20). The polymorphism information content (PIC) values ranged from 0.7376 (Phi70-10) to 0.9760 (Vavd-20), with an average polymorphism information content of 0.9327. The biggest major allele frequency was detected in locus Phi70-10, reached around 42%. It means, the allele in this locus was shared as a major allele in total 66 samples of *P. infestans* collected from many sites in Indonesia. The summary of genotype statistical is shown in Table 5.

4. Discussions

4.1. Phenotype characterization

The Figure 3 showed that isolates from Makassar, South Sulawesi have a broad spectrum pathotype (virulence to R1, R2, R3, R4 and R5) because they give score 0–1 in host differential. The next isolates group which also has a broad spectrum pathotype is isolates from Berastagi, Medan, North Sumatera. This group had virulence to R1, R2, R4 and R5. The group isolates on the third rank is isolates from West Java and East Java, which had virulence to R1, R2, R3 and R1, R4, R5, respectively. The isolates group from Central Java and Jambi have the narrowest spectrum pathotype because they are only virulence to R1, R5 and R1, R2, respectively.

Further analysis is the population structure from a total sample of isolates, both originating from the Java and outside of Java Island. In the analysis of the population structure at once also carried out a robustness analysis with repetition in permutations (1000×) to see the level of robustness of each group (cluster) that has been obtained. Robustness analysis for each groups has been performed using probability approaches algorithms with program STRUCTURE 2.3.2. Based on this analysis, the value of the robustness of each

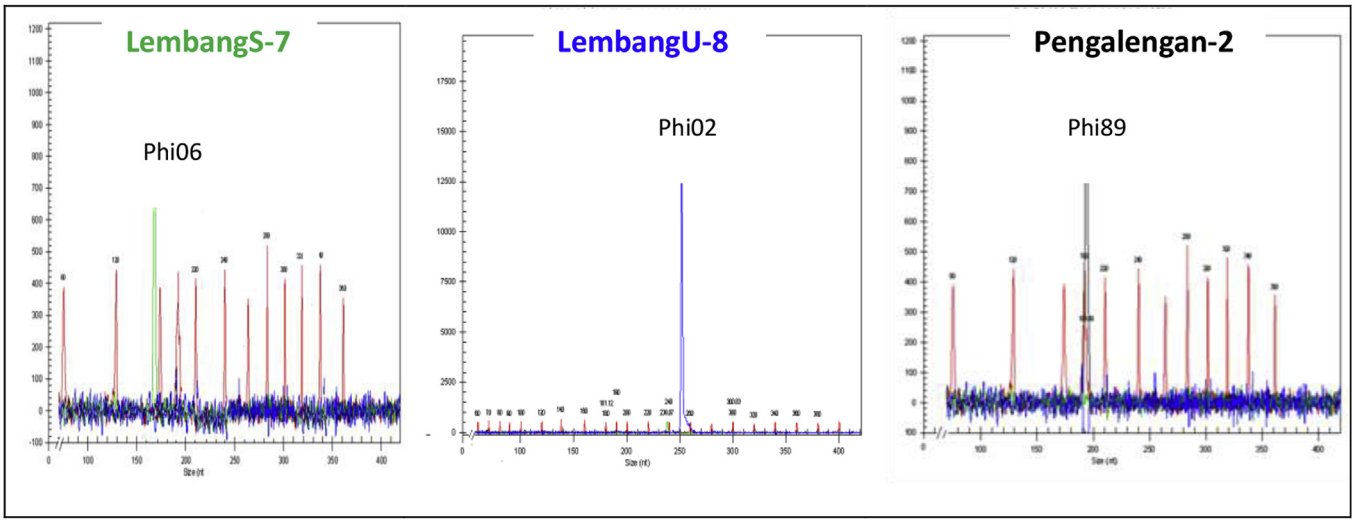


Figure 4. Singleplexing optimization of M13 fluorescent label for primer Phi06 (green), Phi02 (blue), and Phi89 (black), with Lembang-S7, Lembang-U8, and Pengalengan-2 previously sampled as a DNA template. The allele size of each locus is shown: 168 for Phi06, 252 for Phi02, and 194 for Phi89. The red peaks were a standard size.

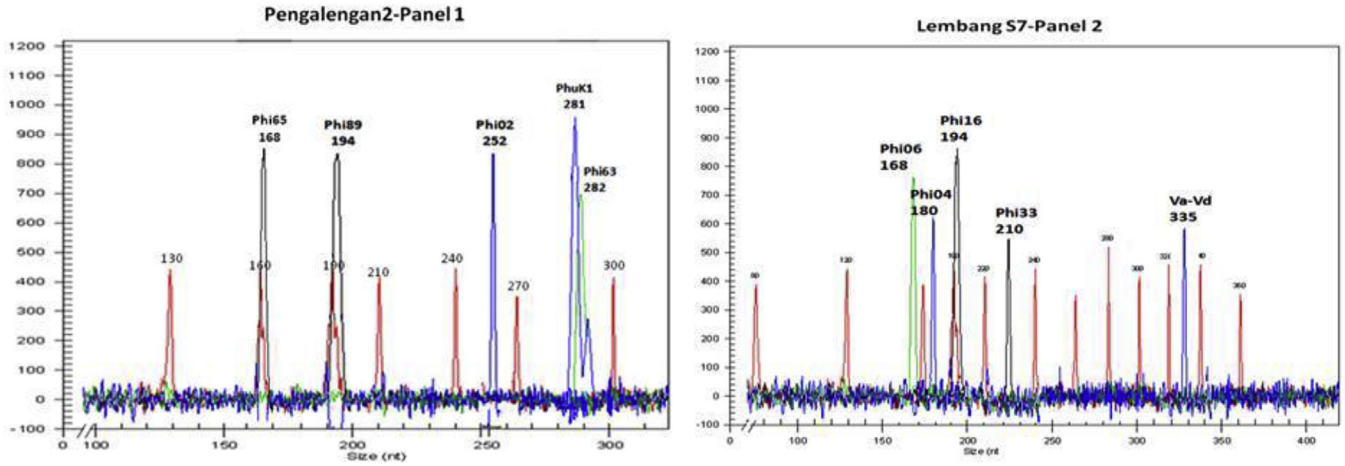


Figure 5. Parts of multiplexing fragment analyses of Panels 1 and 2 on sample Pengalengan-1 and Lembang-S7. Each panel was contained of five primers with different color label and allele size.

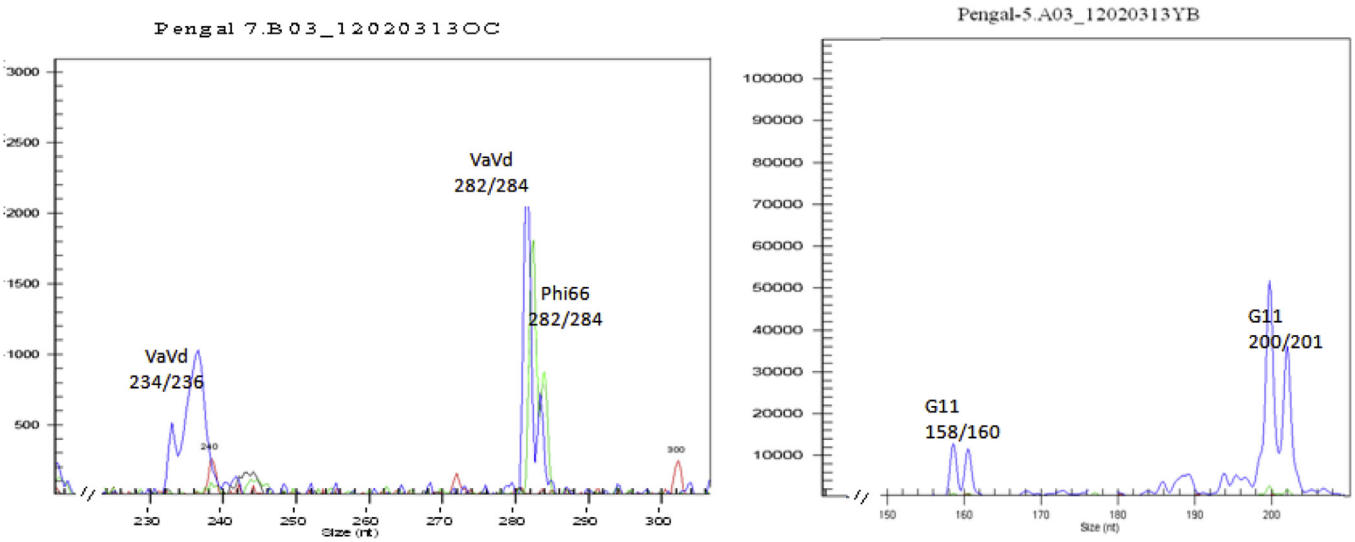


Figure 6. Heterozygote and multiallelic performances in locus VaVd (size: 234/236 and 282/284) and locus G11 (size: 158/160 and 200/201) in one of the samples from Pengalengan, West Java.

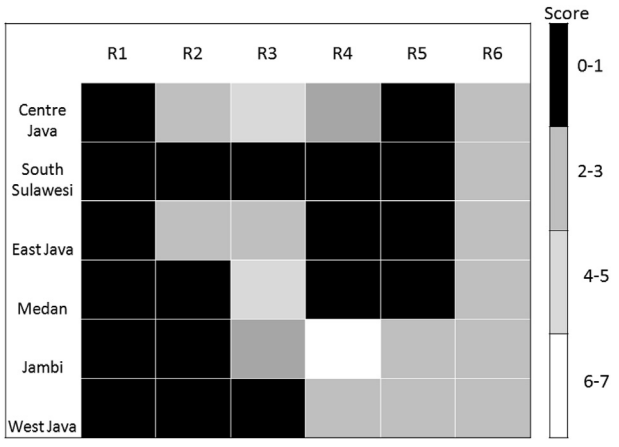


Figure 7. Pathotype spectrum of the isolates group collected from Java and outside Java Island.

group ranged between 0.643 and 0.998. (The overall data were not shown.) This suggests that the grouping analysis of a *P. infestans* sample is relatively robust.

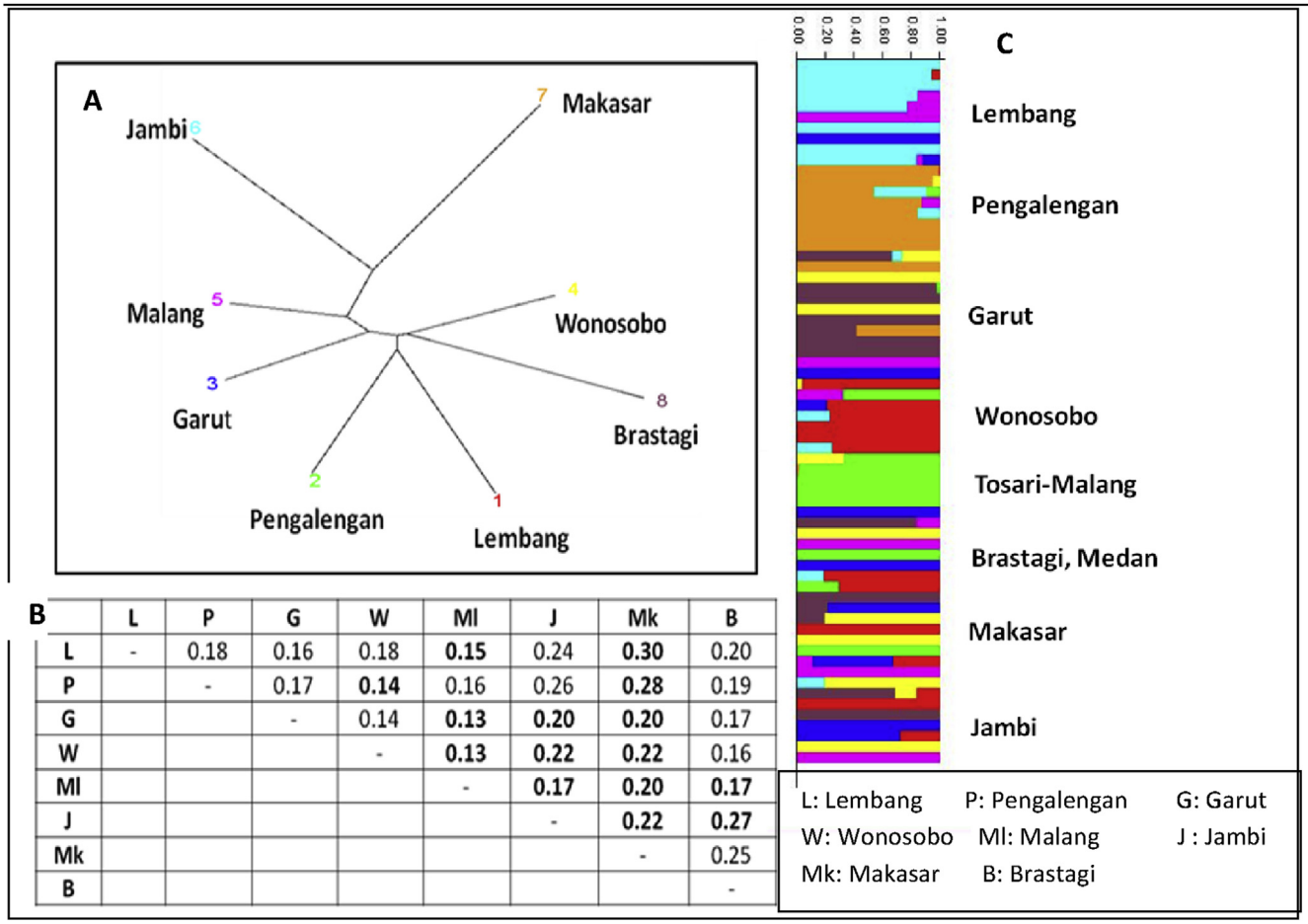
4.2. Genotype characterization

Genetic diversity based on genotype characters which showed in Figure 8 indicated that in sketch out, the variations in genetic profiles of the population *P. infestans* were according to the location sampling. Figure 8A shows the dendrogram of diversity, which is appropriate with location by genetic distance varying for each group populations (Figure 8B). The difference of any isolates in one group was showed on some isolate samples. Figure 8C shows the genetic profile of each isolates showed on different colors in one group, but were similar color to another location. This performance indicated that perhaps there was any migration in individual samples that caused changes in the frequency of alleles. This can occur because of there was an exchange a narrow segment of genetic materials the members of the population (genetic drift factors) (Husband and Schemske 1996).

Table 5. The summary statistic of allele diversity in 66 samples of *P. infestans* using 20 SSR markers

Marker	Major allele frequency	Genotype no.	Sample size	Allele no.	Gene diversity	Heterozygosity	PIC
Phi02-1	0.0833	27	66	25	0.9467	0.0606	0.9441
Phu-K1-17	0.0952	34	66	34	0.9534	0.0000	0.9515
Phi63-7	0.1136	27	66	25	0.9392	0.0758	0.9359
Phi65-8	0.1970	25	66	22	0.9101	0.0455	0.9040
Phi89-11	0.1846	17	66	16	0.9004	0.0308	0.8923
Phi66-9	0.0873	44	66	47	0.9646	0.2222	0.9635
Va-Vd-20	0.0410	52	66	53	0.9765	0.1803	0.9760
Phi04-2	0.1186	36	66	36	0.9520	0.1186	0.9500
Phi16-3	0.0714	41	66	42	0.9620	0.1270	0.9606
Phi33-5	0.1406	35	66	35	0.9479	0.0781	0.9456
G11-13	0.1111	36	66	32	0.9506	0.0952	0.9485
4B-12	0.2083	29	66	28	0.9161	0.0500	0.9113
D13-14	0.1270	29	66	24	0.9282	0.1270	0.9237
Phi-ITS3-15	0.0667	43	66	43	0.9676	0.1167	0.9667
Phi56-6	0.0820	39	66	38	0.9614	0.0656	0.9601
Phi70-10	0.4180	13	66	10	0.7624	0.0984	0.7376
Phe-ITS4-16	0.0982	30	66	28	0.9479	0.0893	0.9454
Phi26-4	0.0887	40	66	36	0.9577	0.1129	0.9561
Fs-19	0.1077	41	66	33	0.9517	0.1692	0.9496
Pha-ECA1-18	0.0984	30	66	23	0.9359	0.1475	0.9322
Mean	0.1269	33	66	32	0.9366	0.1005	0.9327

PIC = polymorphism information content.



B	L	P	G	W	Ml	J	Mk	B
L	-	0.18	0.16	0.18	**0.15**	0.24	**0.30**	0.20
P		-	0.17	**0.14**	0.16	0.26	**0.28**	0.19
G			-	0.14	**0.13**	**0.20**	**0.20**	0.17
W				-	**0.13**	**0.22**	**0.22**	0.16
Ml					-	**0.17**	**0.20**	**0.17**
J						-	**0.22**	**0.27**
Mk							-	0.25
B								-

Figure 8. Population structure of a total 66 isolates of *P. infestans* collected from multiple locations in Java and outside Java Island, based on multiplexing fragment analysis using 20 SSR markers. (A) Dendrogram of genetic diversity; (B) genetic distance among population; and (C) population structure in each sampling locations.

In addition, the difference of genetic diversity between populations also showed the ability to survive (fitness factors) that vary from each sample of isolates on one group of the population.

Genetic diversity analyses of *P. infestans* population were indicated that the diversity between populations is lower than the diversity between isolates in one sampling location groups. The level difference between populations is shown by the mean value of *Fst*, which is 0.06. While the average value of diversity (*expected heterozygosity*) between individuals in a group that reached a high of 0.93, with the most diverse is in samples from

West Java (0.94) and the lowest is in populations from East Java (0.92).

The SSR genotype data is a useful tool to study the distribution of oospores of *P. infestans* as primary inoculum on both intra or inter potato production center in Indonesia. Based on genetic relationship, enabled the identification of the origin of oospore-derived isolates. The genetic diversity information could be a reference for cultivating the resistance of potato varieties to late blight disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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