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Study on *Phytophthora infestans* sensitivity against Fungicides in Indonesia

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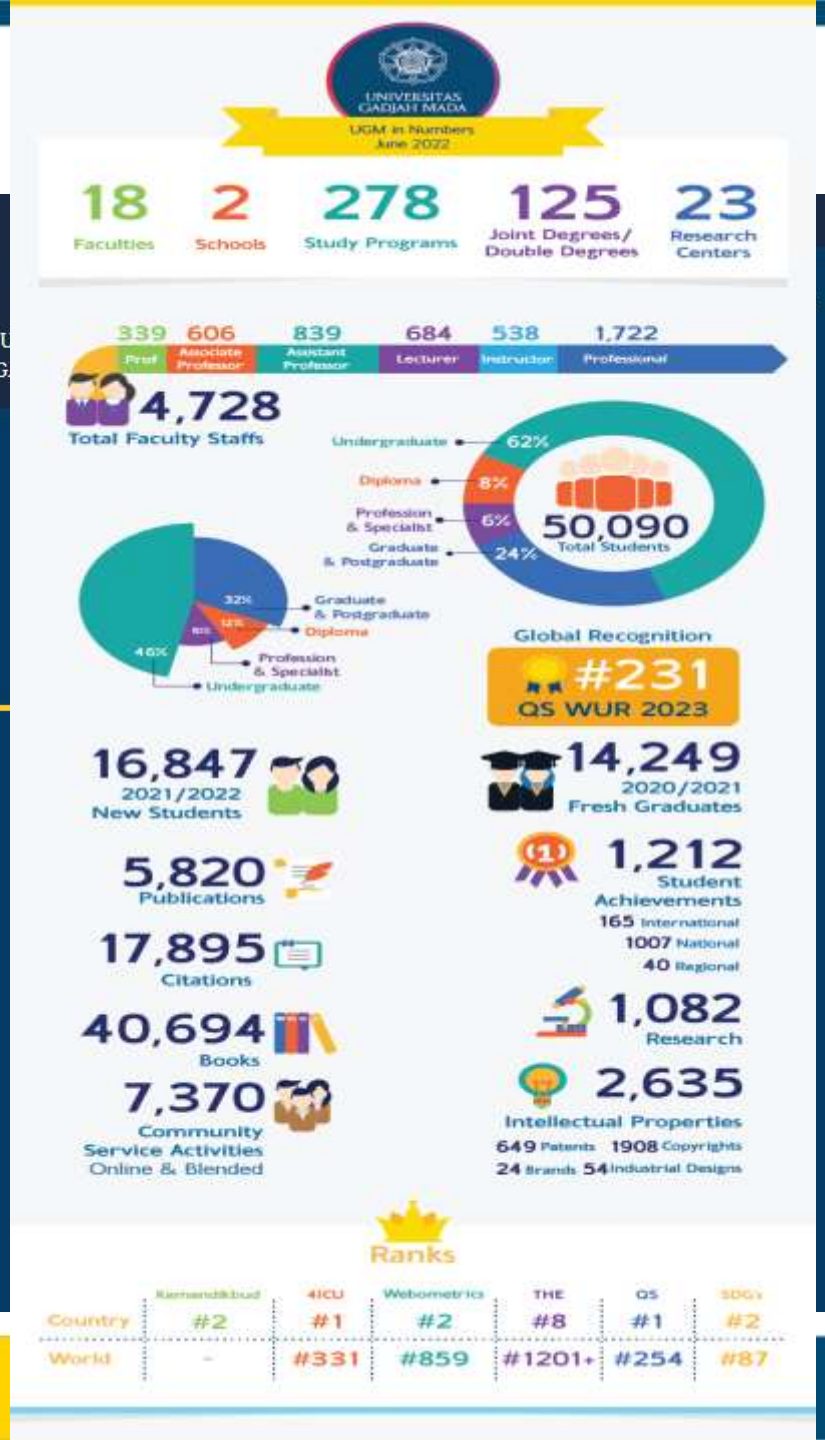

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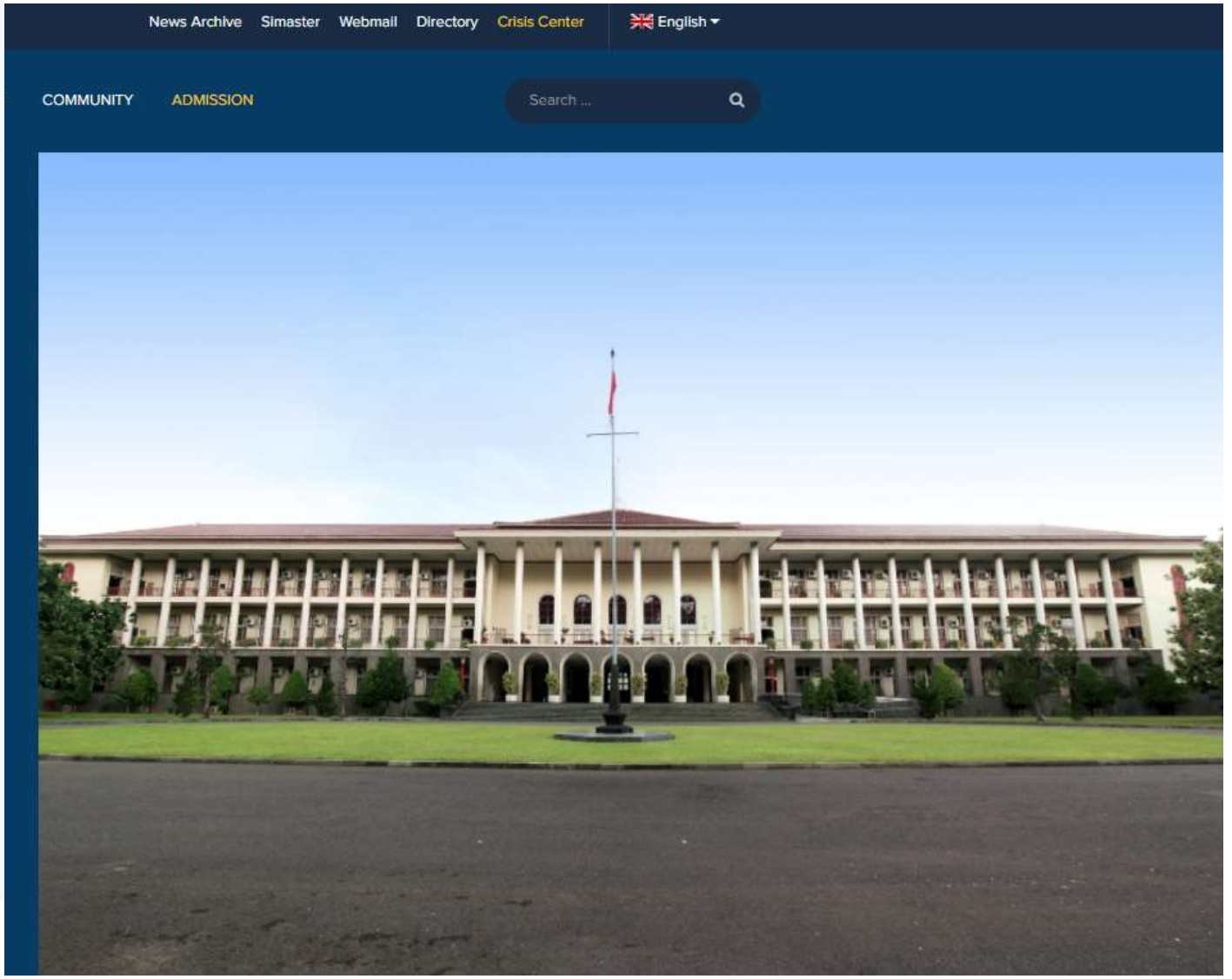

LOCALLY ROOTED, GLOBALLY RESPECTED

Introduction Review: Potatoes in Indonesia

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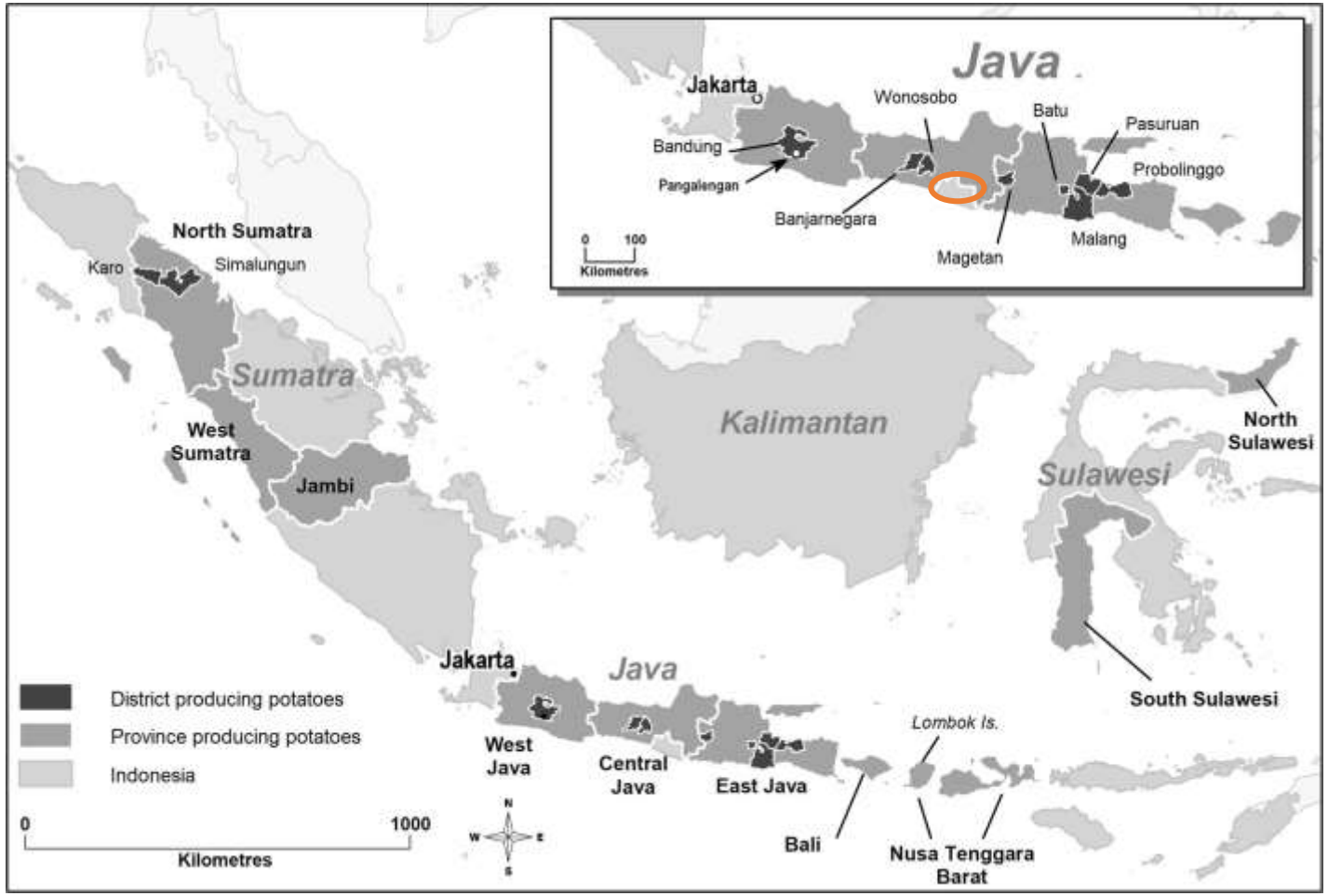
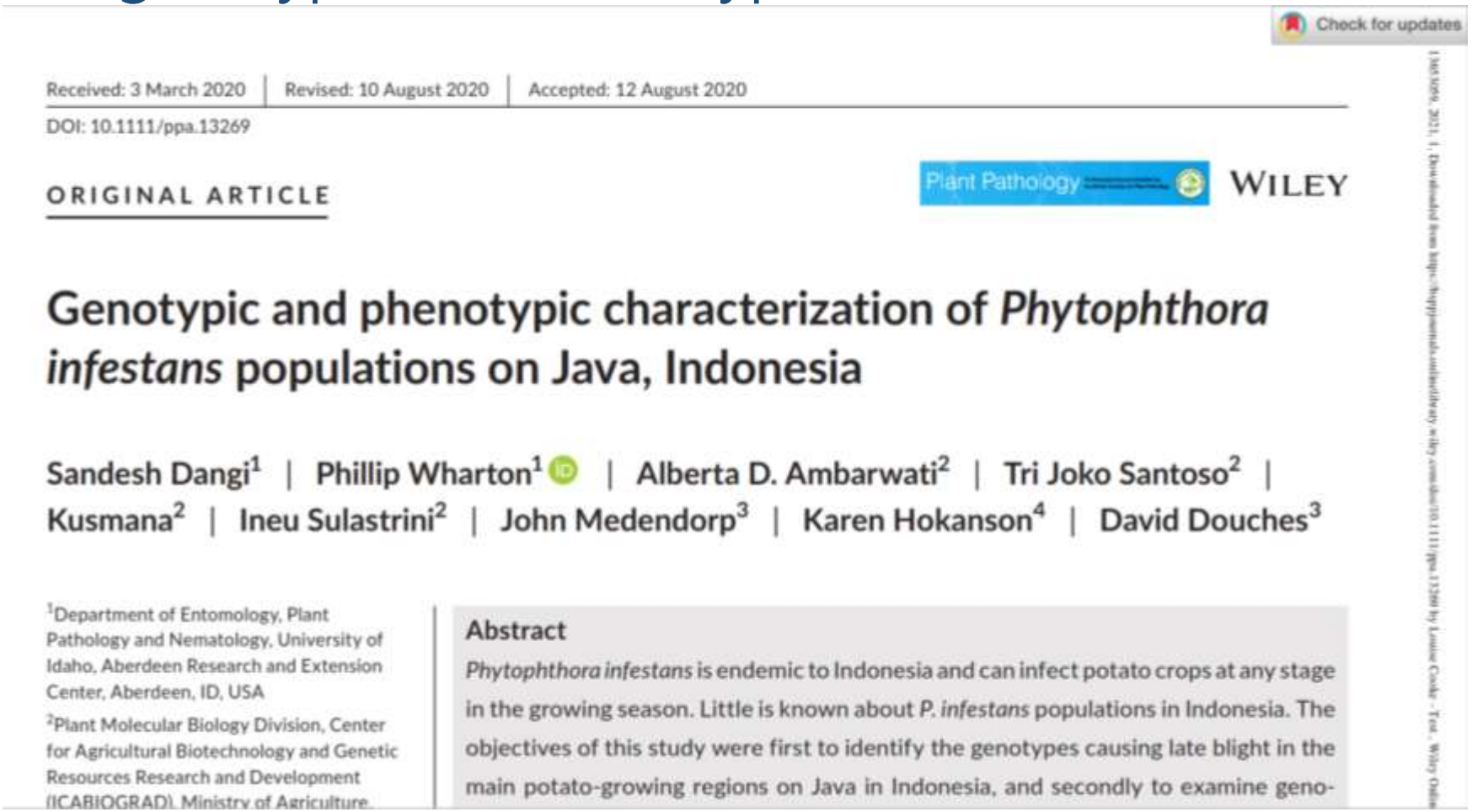
- Dutch East India Company first introduced potatoes to West Java around 1795 (FAO, 2008).
- Total cropping area for potatoes in Indonesia from 2012 to 2019 has fluctuated between 66,000–76,000 ha (FAO 2021).
- The early 1980's to mid-1990's : rapid growth in potato production from increases in planted area and large gains made in yields, rising from 7.2 t/ha to 15.9 t/ha – due to export opportunity
- Favorite variety: Granola dominated the Indonesian table potato market since 1989; However Granola is susceptible against late blight

Taylor and Dawson, 2021,
https://doi.org/10.1007/s12230-021-09831-6



Location of UGM

Review:
Latest report on genotypic and Phenotypic of *P. infestans* in Indonesia



Check for updates

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DOI: 10.1111/ppa.13269

ORIGINAL ARTICLE

Plant Pathology WILEY

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

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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 geno-

Dangi et al., 2020 DOI: 10.1111/ppa.13269

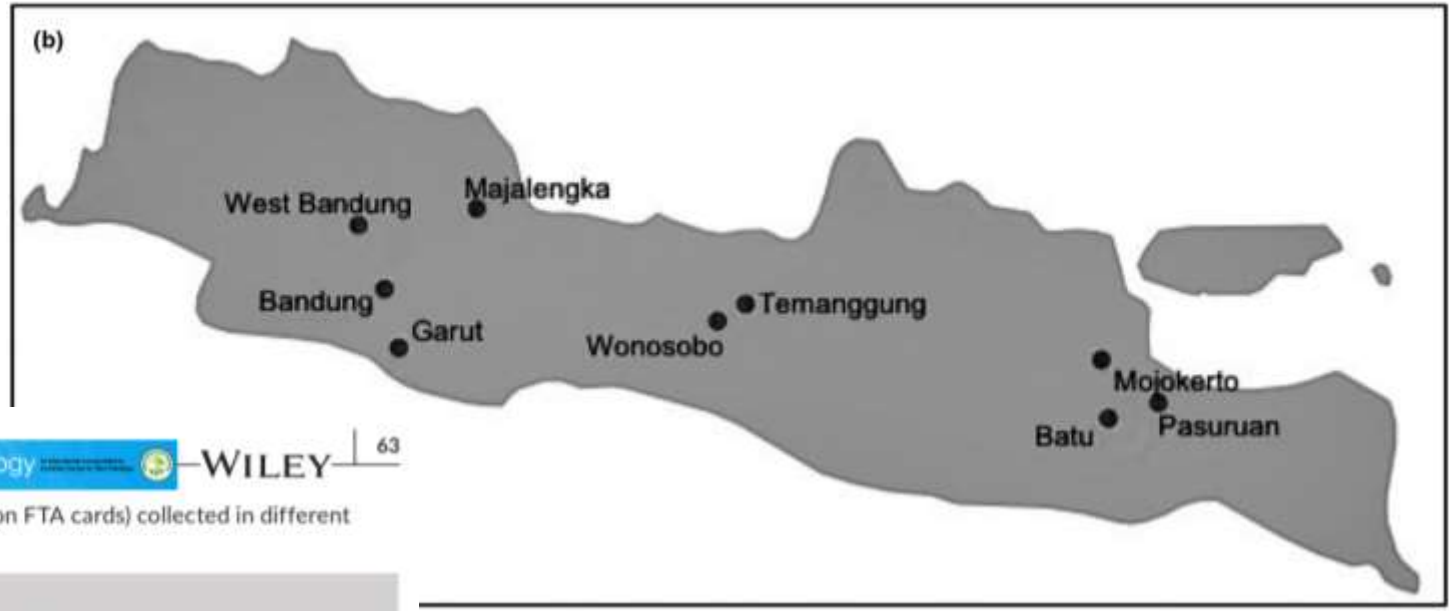


TABLE 1 Geographical information on *Phytophthora infestans* samples (isolates and DNA samples on FTA cards) collected in different years from Java, Indonesia, and standard isolates used in the study

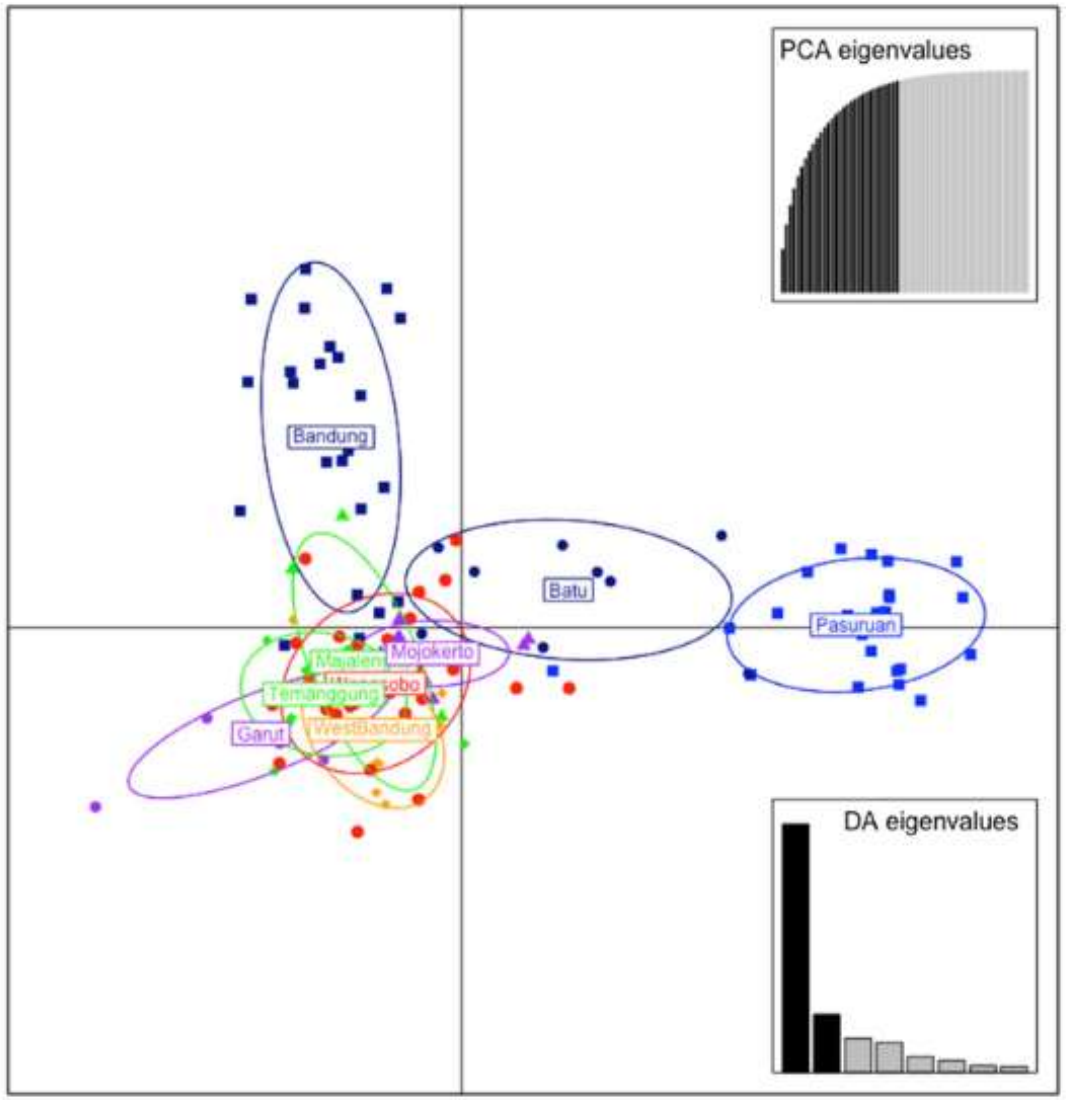
Region or country	Location	Total	DNA samples or isolates and collection year
Bandung	Pangalengan	32	DNA samples = 28, 2016; isolates = 4, 2019
Batu	Tutungrejo	9	DNA samples = 4, 2018; 5, 2019
Garut	Cisurupan	6	DNA samples, 2016
Majalengka	Argalingga	6	DNA samples, 2016
Mojokerto	Kebonaga	12	DNA samples = 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)

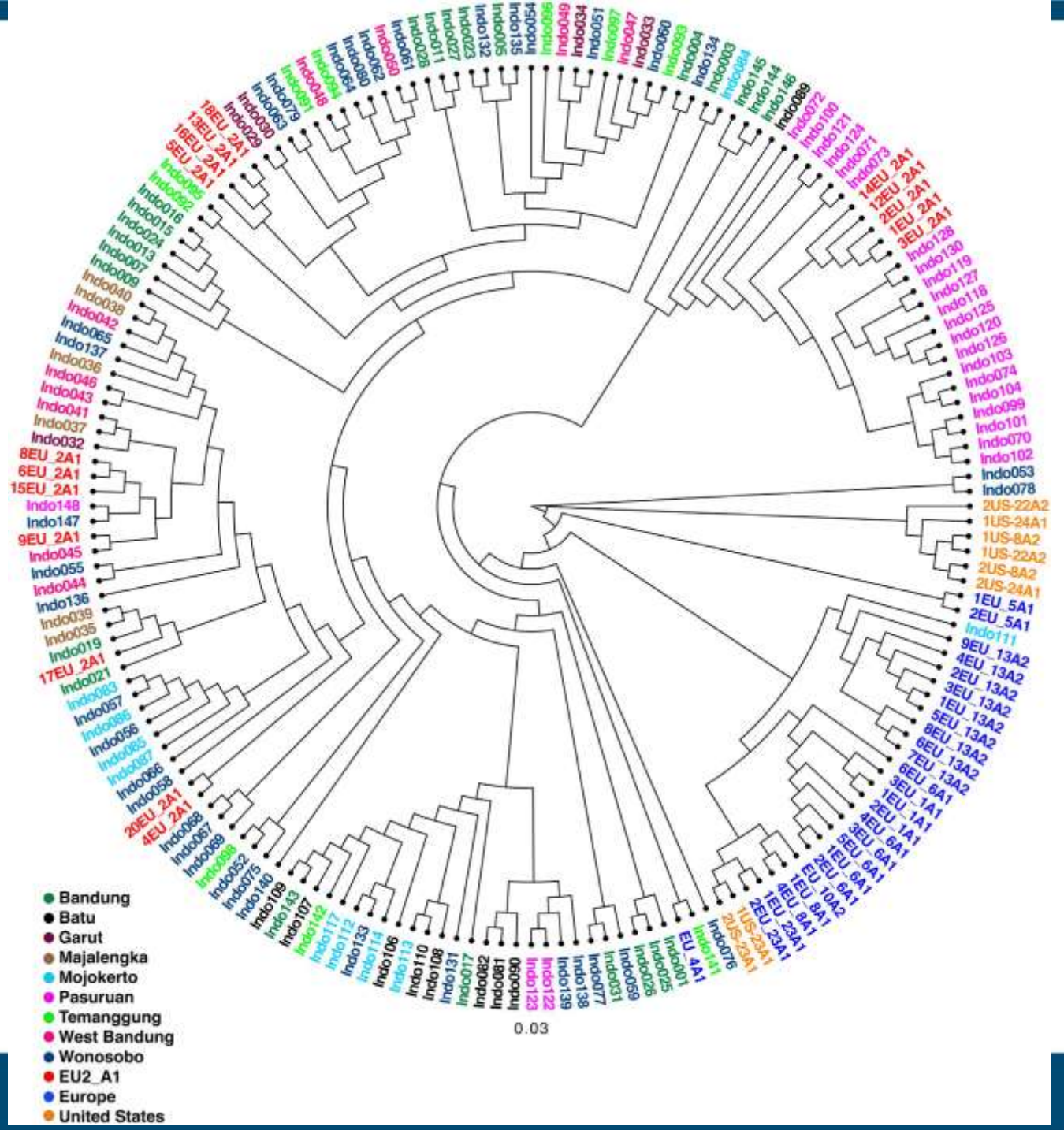
Note: All Indonesian DNA samples and isolates were collected from local subsistence farmers' fields (multiple fields). Potato cultivar is Granola (seed produced in Indonesia), and crop rotation is potato–carrot/cabbage; fungicide spray (if any) is chlorothalonil- and/or mancozeb-based fungicides two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

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

Dangi et al., 2020 DOI: 10.1111/ppa.13269





Dangi et al., 2020 DOI: 10.1111/ppa.13269

Sensitivity study of *P. infestans* against Oxathiapiproline

- UGM team started in 2019
- Try to isolate *P. infestans* using some methods in literature: soil baiting, direct isolation, etc
- Instead of gaining *P. infestans* culture, we found some pathogens associated with potato late blight

Our Latest study:
Pathogens associated Potato Late Blight Symptom in Indonesia

Jurnal Perlindungan Tanaman Indonesia, Vol. 25, No. 2, 2021: 173–181
DOI: 10.22146/jpti.67556
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Short Note

First Report of *Phytopythium vexans* (de Barry) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque Causing Potato Tuber Rot in Indonesia

Islaminati Anna Santika[1)], Ani Widiastuti[1)], & Arif Wibowo[1)*]

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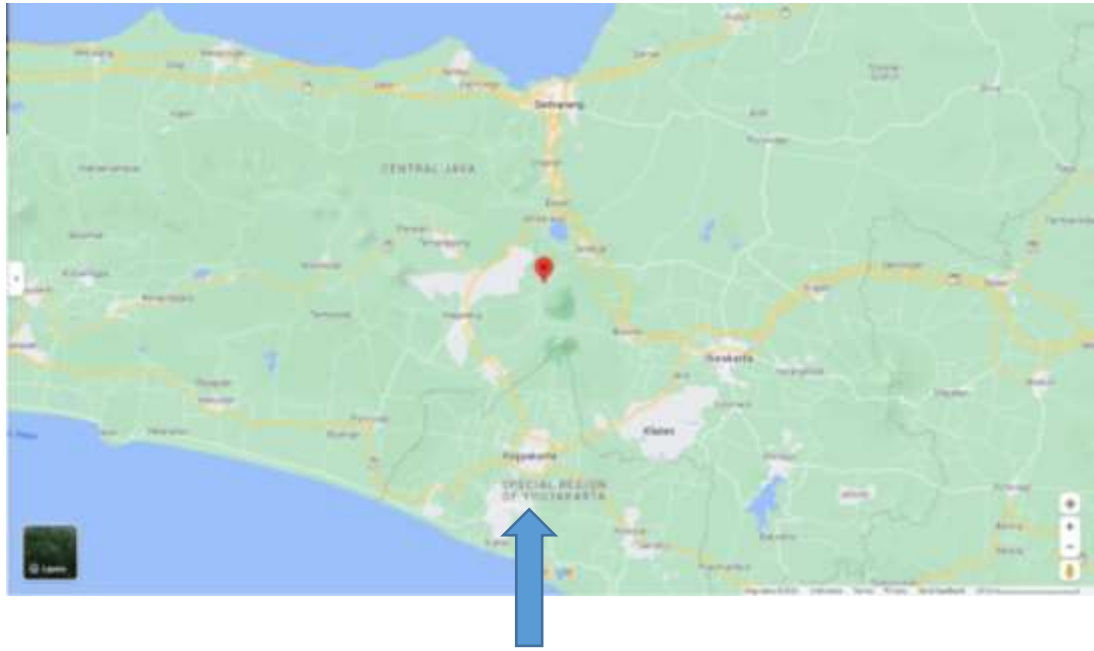
ABSTRACT

Phytopythium vexans (de Barry) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque was successfully isolated from soil of potato fields in Ngablak, Magelang. This research aimed to obtain knowledge of *P. vexans* potency as a pathogen on potatoes, and also morphologically and molecularly identify *P. vexans* compared to Oomycetes,



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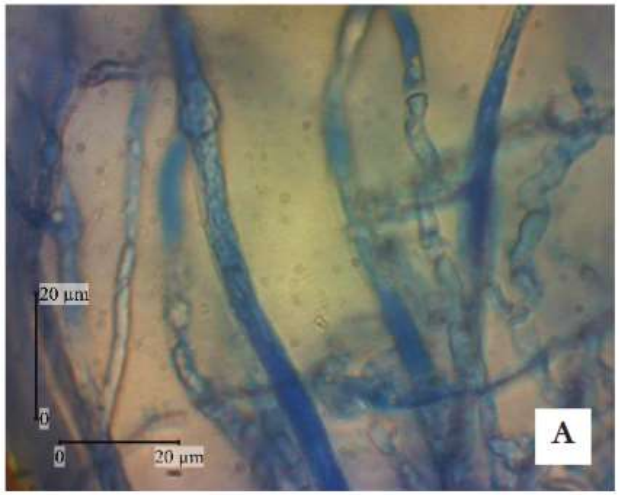

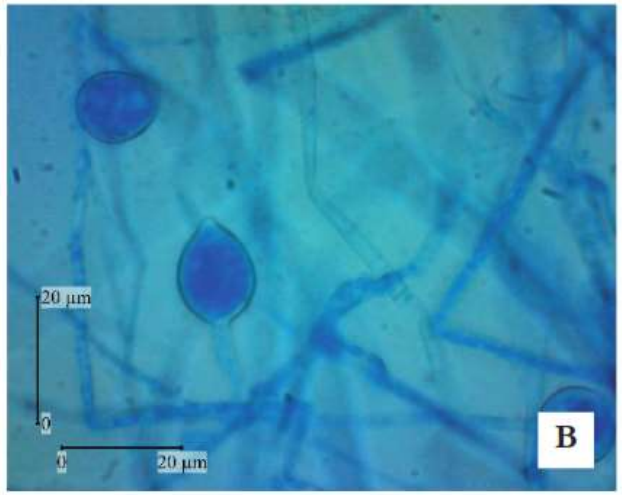

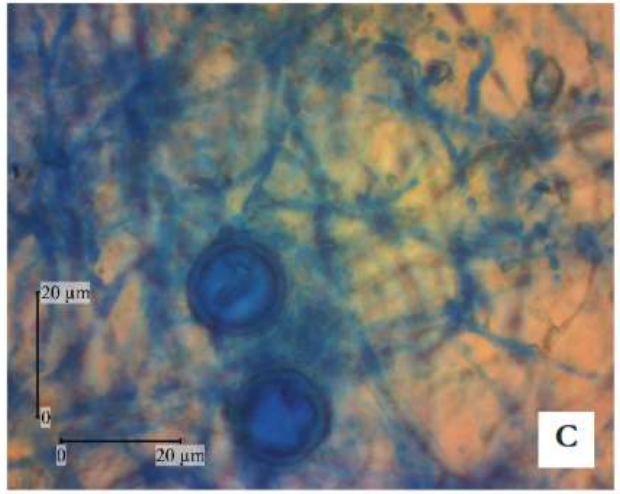

Santika *et al.,* 2021; https://doi.org/10.22146/jpti.67556

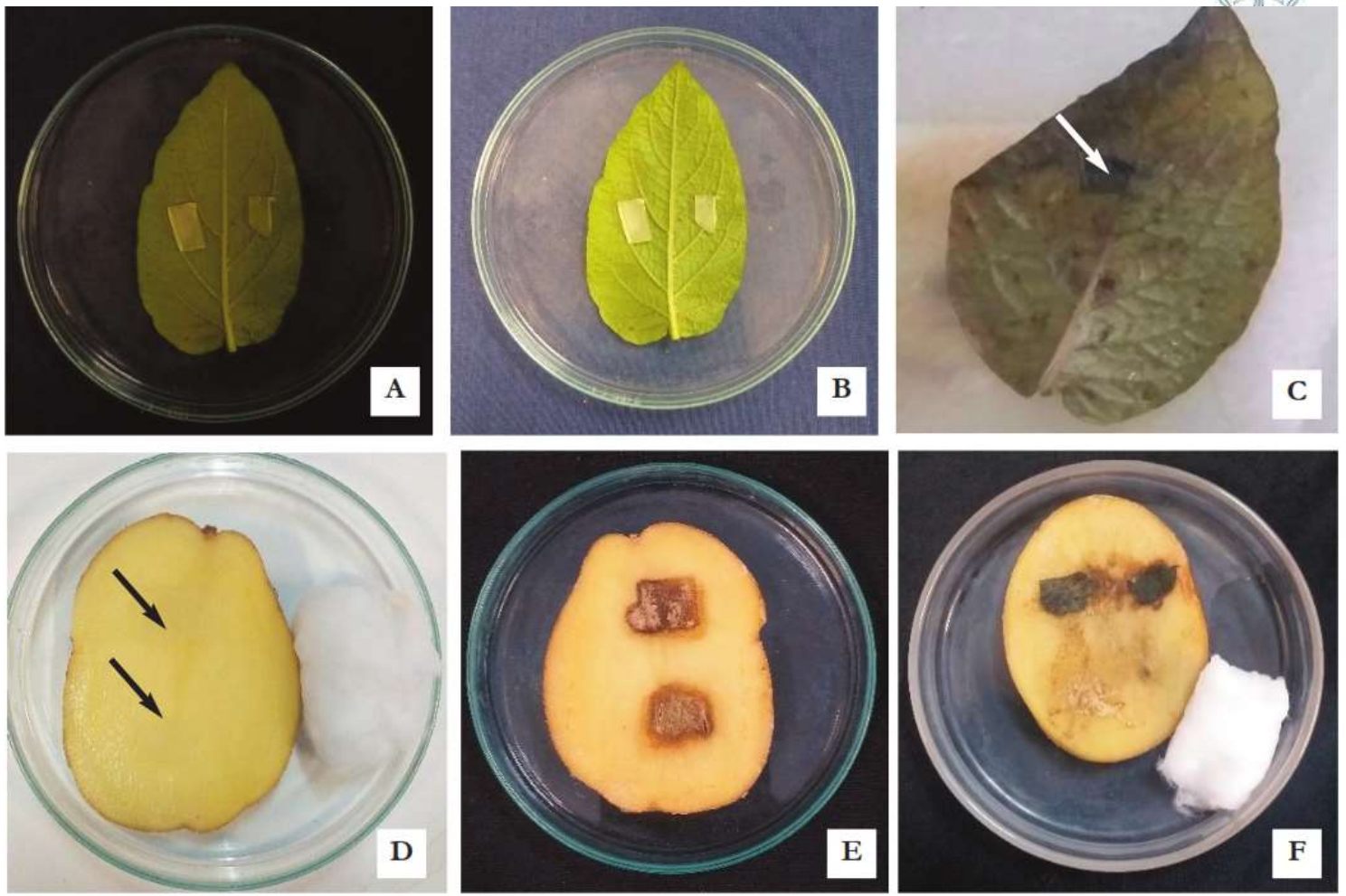

Figure 3. *In vitro* pathogenicity test on healthy potato leaves (A–C) and tubers (D–F); the materials were inoculated with water agar, negative control (A, D); *Phytopythium vexans* (B, E); and small cut of potato leaves infected by *Phytophthora infestans*, positive control (C, F) (→ : inoculum) Santika *et al.*, 2021; https://doi.org/10.22146/jpti.67556

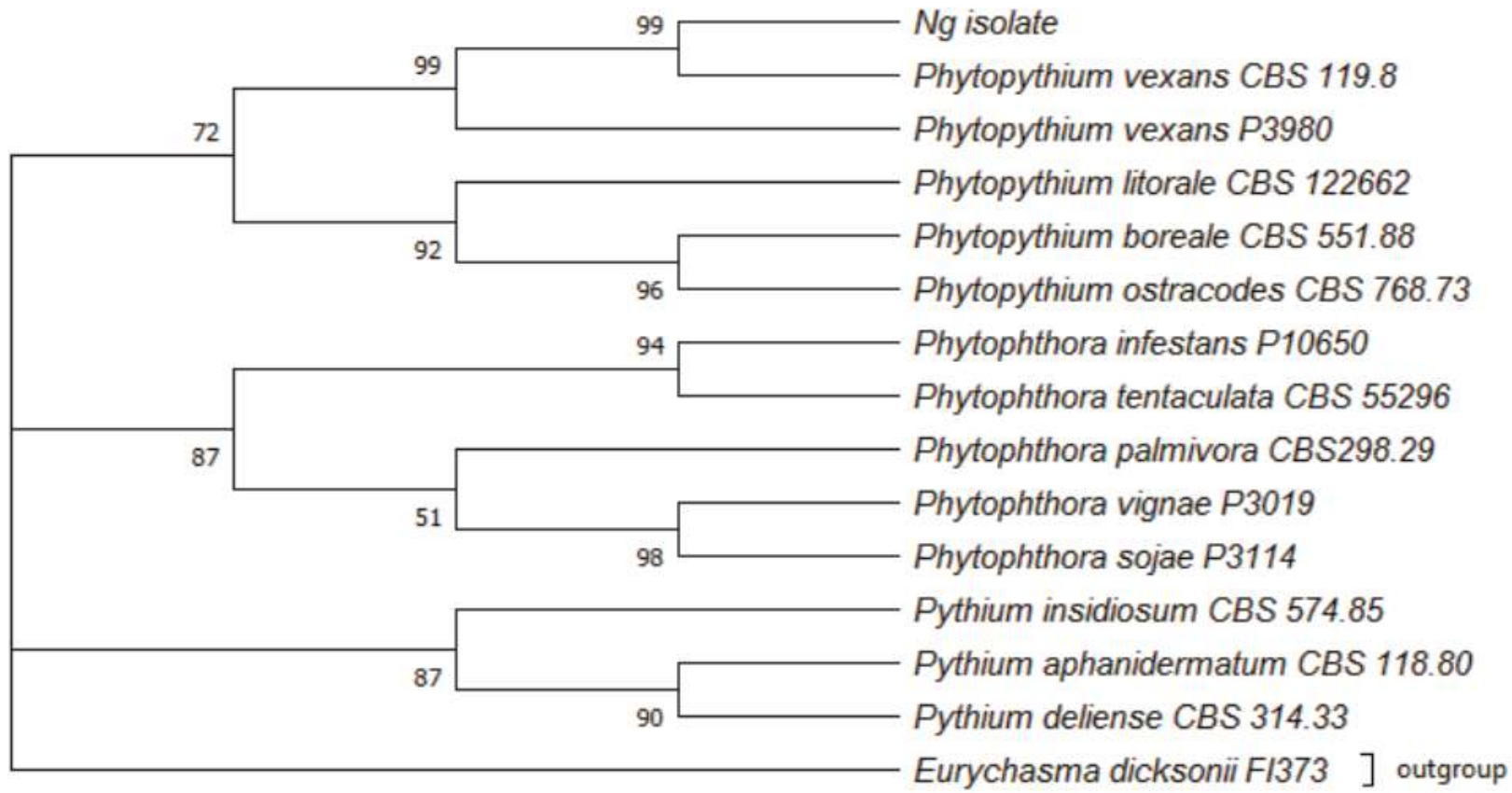

Figure 4. Phylogenetic analysis of NG Isolate Maximum likelihood phylogeny from multigene analysis ITS and LSU sequence alignment with 1000× bootstrap; *Eurychasma dicksonii* F1373 is used as an outgroup

Santika *et al.*, 2021; https://doi.org/10.22146/jpti.67556

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Pythium ultimum and *Phytopythium vexans*, the Potential Pathogen Isolated from Potato Rhizosphere in Central Java, Indonesia

Pythium ultimum dan *Phytoppythium vexans*, Patogen Potensial yang Diisolasi dari Risosfer Kentang di Jawa Tengah Indonesia

Miratun Karmila, Ani Widiastuti, Arif Wibowo, Suryanti*
Universitas Gadjah Mada, Yogyakarta 55281

ABSTRAK

Table 1 Primers used for PCR amplification and DNA sequencing

Gene	Primer	Primer Sequence (5'-3')	Reference
ITS	ITS 1	TCCGTAGGTGAACCTGCGG	Ochoa *et al.* (2012)
	ITS 4	TCCTCCGCTTATTGATATGC	
LSU	UN-up28S40	5-GCATATCAATAAGCGGAGGAAAAG-3	Schurko *et al.* (2003)
	UN-LO28S576B	5-CTCCTTGGTCCGTGTTTCAAGACG-3	Bakkeren (2000)
COX1	OomCoxILevup	5-TCAWCWMGATGGCTTTTTTCAAC-3	Martin and Tooley (2003)
	Fm85mod	5-RRHWACKTGACTDATRATACCAAA-3	

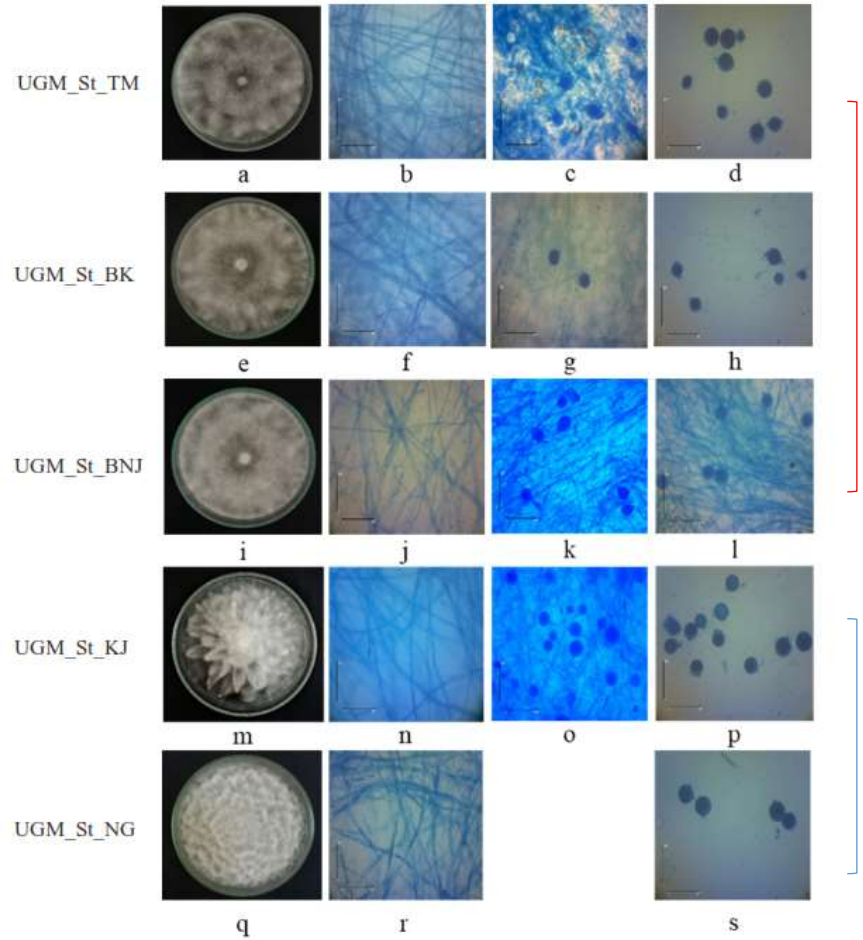

Figure 1 Morphology of fungal isolates. a, e, i, m, q are colony patterns; b, f, j, n, and r are aseptate hyphae; c, g, k, o are sporangium; d, h, l, p are terminal and intercalary chlamydospores with thick walls. Bar scale 50 µm.

Study on sensitivity *P. infestans* against Oxathiapiproline (2019 – 2021)

Sensitivity Tests

The following test concentrations of oxathiapiprolin are prepared with a 0.05 % Uniperol-solution: 0, 0.000064, 0.00032, 0.0016, 0.008, 0.040, 0.200, 1 ppm, in order to obtain an EC_{50} evaluation. For the test, whole potato plants cv. Bintje are sprayed with the respective fungicide solutions to run-off conditions. One day after treatment, leaf disc tests are prepared and inoculated. Separate disposable Petri dishes of 6 cm diameter are used for each concentration. Each Petri dish contains four leaf discs (14 mm diameter) considered as replicates. They are originating from different leaves from plants treated with the same fungicide concentration. A test set for one isolate with eight fungicide concentrations (including untreated control) consists therefore of eight Petri dishes. Each test set is inoculated with sporangia suspensions by equally spraying the suspension onto the leaf discs. After a dark and cold (2-4°C) period of 2 h, the test is incubated in the climate chamber for 6 days (18 °C, 100 µmol/m²s, 16/8 h light/darkness period, 80 % RH).

Source: FRAC, 2017

However the result showed

- Up to the highest concentration of the protocol (1ppm); leaf blight symptom still appeared, even when the concentration was increased up to 1000ppm

- Blight symptom emerged very fast in leaf surface (2 – 4 days after inoculation)

- Inconsistency symptom: K- (without any inoculation) also showed blight symptom in several experiments.

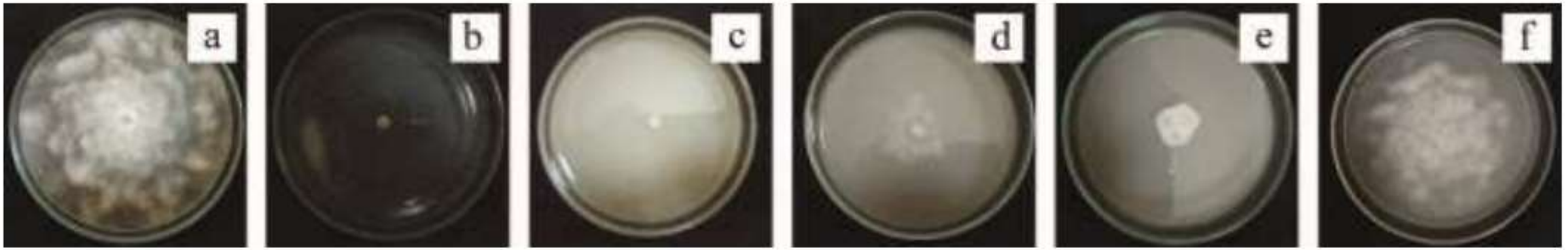

Figure 2. *P. infestans* grown on BSEA media containing fungicides. (a) BSEA media without fungicide. (b) Metalaxyl 2000 ppm. (c) Mancozeb 2,000 ppm. (d) Dimetomorph 1,000 ppm. (e) Chlorothalonil 2,000 ppm. (f) Oxathiapiprolin 3,000 ppm

Widiantini et al., 2022 DOI: 10.24198/agrikultura.v33i2.40357

Aims of our Recent Study

- To get primers or restriction enzyme for monitoring sensitive *P. infestans* against some fungicide: Oxathiapiproline as one of them

OSBPI Working Group

FRAC

Minutes of the FRAC OSBPI Working Group Meeting

4 April 2022 – 13:00 to 17:00

Virtual meeting

Potato/tomato late blight (*Phytophthora infestans*)

Data presented by Bayer, Corteva and Syngenta

In 2021, sensitivity data have been generated for samples originating from potato and tomato crops in Belgium, China, Czech Republic, Denmark, France, Germany, Greece, Hungary, Indonesia, Ireland, Italy, Netherlands, Poland, Portugal, Spain and Sweden.

All 2021 samples in monitored areas of Europe were sensitive. Molecular characterization of samples from Indonesia revealed the presence of mutations N837I and G770V at the OSBPI target site (based on *P. infestans* homology numbering).

Reduction of sensitivity has been recorded since 2019

A FRAC OSBPI Working Group was formed in 2015 to generate common resistance management recommendations for the fungicides oxathiapiprolin and fluoxapiprolin. OSBP fungicides are active against oomycete fungi and used for the control of Phytophthora and downy mildews of numerous crops. OSBPIs inhibit an oxysterol binding protein (OSBP) homologue. Oxysterol binding proteins are implicated in the movement of lipids between membranes, among other processes. Inhibiting OSBP may disrupt other processes in the fungal cell, such as signaling, maintaining cell membranes, and the formation of more complex lipids that are essential for the cell to survive.

Oxathiapiprolin and fluoxapiprolin are cross-resistant.
OSBPIs have been classified under the FRAC Code 49. The resistance risk is medium to high.

FRAC Code	Target site and code	Group name	Chemical group	Common name	Comments
49	F9 lipid homeostasis and transfer/ storage	OSBPI oxysterol binding protein homologue inhibition	piperidinyl-thiazole-isoxazolines	Oxathiapiprolin Fluoxapiprolin	Resistance risk assumed to be medium to high (single site inhibitor). Resistance management required. .

https://www.frac.info/frac-teams/working-groups/osbpi-fungicides/recommendations-for-osbpi

What we are doing now

- Samples collection: Potato centers in Java island; with different farmer practice in fungicides: 93 DNA extract and 12 isolates confirmed as Phytin

- Phytin confirmation using primers developed by: Hussain et al., (2015) and Judelson & TooleY (2000).

- Mating Type confirmation: RFLP restriction enzyme (HaeIII or BsuRI) –
- Genotyping profile using SSR markers – optimization (Lee et al., 2013)

What we are doing now? (Ongoing)

- Screening for resistance of some fungicide : Oxtp (mixed Active Ingredienst of Oxtp and Famoxadone); Dimetomorph, Metalaxyl as reference
- Against Metalaxyl: found some resistant isolates
- Sequence for the gene on fungicide targeted and multiple alignment analysis
- Molecular markers – primer designing – based on the protein target of fungicide

Acknowledgement

- Prof. Christanti Sumardiyono
- Dr. Suryanti
- Dr. Arif Wibowo
- Islaminati Anna Santika, M.Sc.
- Ade Mahendra, M.P. (Ph.D. student)

Discussions are welcome
Thank you