Potato Research (2023) 66:255–273
https://doi.org/10.1007/s11540-022-09581-w

Phenotypic and Genotypic Analysis of the Population of *Phytophthora infestans* in Bangladesh Between 2014 and 2019

Md. Huzzatul Islam[1,2] · Sangee Sarkar Shanta[1] · Muhammad Iqbal Hossain[1] · Muhammed Ali Hossain[1] · Md. Monower Hossain[3] · Ebna Habib Md. Shofiur Rahaman[3], et al. ***[full author details at the end of the article]***

Received: 22 April 2022 / Accepted: 4 August 2022 / Published online: 1 September 2022

Abstract

In Bangladesh, the third largest producer of potatoes in Asia, late blight, caused by *Phytophthora infestans*, is the major constraint to production. Nevertheless, there is a lack of published information on the pathogen population. A collection of 69 isolates obtained from samples of infected potato foliage collected in 2018–2019 varied significantly in their aggressiveness to detached potato leaflets and tuber slices of cv. Diamant. On leaflets, most isolates were highly aggressive, colonizing more than half the surface in 7 days. On tuber slices, while some isolates were highly aggressive, those collected in 2019 were less so. There was a significant effect of the locations from which the isolates were obtained on aggressiveness to both leaflets and tuber slices, but aggressiveness to leaflets and to tuber slices was not significantly correlated. All isolates were A2 mating type and either intermediate (38%) or resistant (62%) in sensitivity to the fungicide metalaxyl. A subset of 24 isolates all had mitochondrial DNA haplotype Ia.

Samples of *P. infestans* DNA (124) from infected potato foliage obtained from Bangladesh (2014–2019) were genotyped by 12-plex SSR. The vast majority (95%) were assigned to EU_13_A2, which was present in every year in which samples were collected. Numerous EU_13_A2 variants were identified, many specific to Bangladesh. Six samples (from 2017 to 2019) had genotypes distinct from EU_13_A2 and with no close match to known European lineages. It is concluded that the current *P. infestans* population of Bangladesh is dominated by the aggressive lineage EU_13_A2, also dominant in India and Pakistan. The implications of this for control of late blight are discussed.

Keywords Aggressiveness · Clonal lineage · EU_13_A2 · Mating type · Metalaxyl · Oomycete · *Phytophthora infestans*

Introduction

Production of potatoes, the world's most important vegetable crop (Jansky et al. 2019), is now centred in Asia where nearly 40% of the crop is produced. Bangladesh is now the third largest producer of potatoes in Asia (after China and India); in 2020, it produced 9.6 million tonnes on 0.5 million ha, which represents 2.6% of world production (FAOSTAT 2022). Potato production has increased very greatly since Bangladesh gained independence in 1971: production in 1972 was only 0.75 million tonnes. During the period between 1972 and 2020, area, production and yield of potato increased by 4.6%, 6.6% and 2.0% per annum, respectively (Sabur et al. 2021), the increase was particularly marked in the period after 1997/1998. However, the increase in production of local varieties (viz. Lal Pakri, Sheel Bilati, Barma Shield, Romana Pakri) has been much less than that of high yielding varieties (viz. Diamant, Cardinal, Granola, Kufri Sindhuri, Asterix, Lady Rosetta, Courage); the latter now provide 91% of production in Bangladesh compared with 46% during 1979–1980 (Sabur et al. 2021). Bangladesh exported 45,000 tonnes of fresh potatoes in 2019–2020, as production exceeds local demand (Parvez 2020). The annual consumption of potatoes within Bangladesh has increased greatly since the late 1990s, when it was approx. 10 kg per capita, reaching 52.7 kg per capita in 2019 (HelgiLibrary 2022).

The average yield of potatoes in Bangladesh in 2020 as calculated by the FAO (FAOSTAT 2022) was slightly lower (20.8 t/ha) than that of India (23.8 t/ha) and Pakistan (24.6 t/ha), but greater than that of mainland China (18.7 t/ha). However, all these yields are low compared to those reported for the USA (50.8 t/ha in 2020) and European countries, such as Ireland and The Netherlands, where the average overall yield was 33.8 and 42.7 t/ha, respectively in 2020 (FAOSTAT 2022). Potato cultivation occurs in all regions of Bangladesh during the winter months; crops are mostly planted in December and harvested in March of the following year.

In Bangladesh, more than 50 diseases (both biotic and abiotic) of potato have been recorded, but late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the major constraint to cultivation and causes mean annual yield losses of up to 30% (Hossain et al. 2009). Weather conditions during the winter include predominantly northerly winds, bringing little rain, but often causing mist/fog events lasting several days. Mist is formed after contact between the cold and dry air (after passing over the Himalaya mountain range) in the higher atmospheric layers and the relatively warm and moist atmospheric surface layer in Bangladesh. The resultant frequent mist periods are highly conducive to potato late blight. Although late blight was first reported in 1922 in the region which later became Bangladesh (Hossain et al. 2009), relatively little information has been published on the *P. infestans* population in Bangladesh.

The global history of *P. infestans* is one of a series of migration events. Study of historic late blight samples from Europe and America collected since 1845 identified the presence of a specific multilocus genotype that Saville et al. (2016) named FAM-1; this was associated with the mtDNA haplotype HERB-1 (Saville and Ristaino 2021) identified by Yoshida et al. (2013, 2014) and is thought to

have caused the Irish Potato Famine of the 1840s. FAM-1 caused widespread outbreaks in the USA, Europe, Africa, Asia and Oceania during the nineteenth and the first half of the twentieth century (Saville et al. 2016; Ristaino 2020; Saville and Ristaino 2021). The oldest characterized herbarium late blight specimen from the Indian sub-continent was collected from Eastern India (Bhagalpur) in 1913 and belonged to the FAM-1 lineage (Saville and Ristaino 2021). Between the 1930s and 1950s, this lineage was displaced worldwide by US-1; specimens collected in India in 1968 and 1974 were of the US-1 genotype (Saville and Ristaino 2021). Late blight was first reported in 1922 in the region which is now Bangladesh (Hossain et al. 2009), but relatively little information has been published on the *P. infestans* population there. Hossain et al. (2009) showed that metalaxyl resistance was present in *P. infestans* isolates collected in 1995–1996 from Bangladesh, and the presence of both the A1 and A2 mating types was reported by Forbes (2004).

Ongoing changes in populations of *P. infestans* worldwide have major implications for the epidemiology of late blight and generally make it more difficult to control (Andersson et al. 2009; Fry et al. 2015; Govers et al. 1997; Smart and Fry 2001). One such major change has been the emergence of the aggressive, metalaxyl-resistant clonal lineage EU_13_A2 (Blue 13), first identified in Europe with the earliest findings being in isolates collected in 2004 from The Netherlands and Germany (Cooke et al. 2012) and subsequently identified in China amongst isolates collected in 2005 (Li et al. 2013b). In 2008–2010, severe epidemics of late blight on tomato in Southern India were shown to be associated with the EU_13_A2 clonal lineage (Chowdappa et al. 2013). Chowdappa et al. (2015) collected isolates from both potato and tomato in Southern India and showed that all were EU_13_A2 and equally aggressive to both potato and tomato. In 2014, a major late blight epidemic on potato in West Bengal in the east of India bordering Bangladesh resulted in crop losses so severe that they led to suicides amongst potato farmers (Fry 2016). Isolates collected from this area were all found to belong to the EU_13_A2 genotype; 24 sub-clonal variants within this genotype were identified of which 19 were unique to this region (Dey et al. 2018).

In the GEOPOTATO project, funded by the Netherlands Space Office (NSO), Geodata for Agricultural and Water Program (G4AW), sampling starting in 2014 showed that the EU_13_A2 genotype was widespread in Bangladesh with multiple sub-clonal lineages (Kessel et al. 2017). In the present study, *P. infestans* DNA samples collected from Bangladesh between 2014 and 2019 were genotyped, and isolates of *P. infestans* from the 2017–2018 and 2018–2019 growing seasons were analysed for mating type, aggressiveness and fungicide resistance with the objective of determining the population structure of the pathogen in order to improve late blight management.

Materials and Methods

Sample Collection and Isolation of *Phytophthora infestans* for Phenotypic Analysis

A survey was conducted in the major potato-growing areas of Bangladesh in December and January in 2017–2018 and 2018–2019 to assess the occurrence

of late blight and to collect samples for phenotypic analysis. Potato leaf samples with late blight were collected from 16 potato-growing districts of Bangladesh viz. Bogura, Chandpur, Cumilla, Dinajpur, Gaibandha, Jamalpur, Joypurhat, Kurigram, Munshiganj, Mymensingh, Naogaon, Nilphamari, Panchagarh, Rangpur, Sherpur and Thakurgaon.

Five locations in each district and three farmers' fields in each location were considered during each survey. Details of locations, cultivars sampled and dates of sampling were recorded. For the isolation of *P. infestans* from fields of different growing areas, leaves with sporulating lesions were collected from ten plants, and these formed a composite sample from which representative material was taken to the laboratory in ballooned zipper bags to avoid any disturbance of the growth of *P. infestans*. The samples were then kept in a refrigerator at 4 °C, and *P. infestans* was isolated as soon as possible.

P. infestans was isolated as described in the International Potato Center (CIP) Laboratory Manual (Gamboa et al. 2019). Leaf samples were washed in tap water, blotted dry, then incubated on filter paper in Petri dishes at 18 °C for 24 h to allow fresh sporulation to develop. Small pieces (about 1 cm^2) of infected tissue from the sporulating margin of the lesion were cut and placed under 2×1 cm^2 size potato slices which were cut by a sterilized knife from surface sterilized potato tubers (cv. Diamant) in empty Petri dishes. These dishes were incubated at 18 °C for 1 week, until abundant sporulation had developed on the upper side of the tuber slices. Sporangia were then transferred onto pea agar (frozen green peas 120 g, $CaCO_3$ 2 g, agar 17 g for 1 L media including ampicillin (200 mg/L), rifampicin (20 mg/L) and PCNB (67 mg/L)) by picking them off with a pea agar block attached to the tip of an inoculating loop. Inoculated pea agar plates were then incubated for 5 to 7 days at 18 °C. Subsequently, individual young colonies of *P. infestans* were transferred to pea agar plates and sub-cultured.

For identification of the isolates as *P. infestans*, they were checked by microscopic examination of the cultures on agar and by PCR using the *P. infestans* specific primer PINF (Trout et al. 1997). The isolates were then maintained on rye B agar medium (Caten and Jinks 1968) at 18 °C. A total of 69 isolates was obtained.

Assessment of the Aggressiveness of *P. infestans* Isolates on Detached Potato Leaflets and Tuber Slices

A total of 69 *P. infestans* isolates were assessed for their aggressiveness to detached potato leaflets and tuber slices. Inoculum was prepared from Petri plate cultures of the *P. infestans* isolates on pea agar with β-sitosterol (50 mg/L) grown until the colonies completely covered the plates on the day before inoculation, the mycelia were rubbed with a sterile test tube and the plates were left at 18 °C overnight for production of sporangia. The sporangia were harvested by washing them off the plates with Sato's solution (Sato 1994). The sporangial concentration was determined by counting with a haemocytometer and adjusted to 10^4 sporangia/mL of Sato's solution.

To determine aggressiveness of *P. infestans* isolates on detached potato leaflets, six 10 µL drops of the sporangial suspension (10^4 sporangia/mL of Sato's solution) were spot inoculated on the abaxial surface of each detached fully expanded leaflet 3rd from the top of 8-week-old healthy potato plants (cv. Diamant, a susceptible cultivar). Inoculated leaflets were individually placed in sterile, otherwise empty 9-cm Petri dishes. The plates containing the inoculated leaflets were incubated in moist chambers created in plastic boxes. Three replications were used in a fully randomized design.

To determine aggressiveness to tubers, 60 µL of sporangial suspension (10^4 sporangia/mL of Sato's solution) were 'spot' inoculated at the centre of each tuber slice individually placed in sterile plastic Petri dishes. Three replicate slices were used for each isolate, and these were arranged in a fully randomized design.

The Petri plates in moist chambers (for both leaflets and tuber slices) were incubated at 18 °C in the dark for 24 h, then with a 14-h light period for 6 days. Aggressiveness was assessed 7 days after inoculation by estimating the percentage of the surface of the leaflet or tuber slice that had developed symptoms.

Determination of Mating Type of *P. infestans*

The mating types of all 69 *P. infestans* isolates were determined by pairing an A1 tester or an A2 tester isolate on pea agar medium with each test isolate (Lehtinen et al. 2008; Hu et al. 2012). A mycelial plug (5-mm diameter) of a known A1 or A2 isolate was placed on one side of a Petri dish (90-mm diameter) and a mycelial plug of the unknown isolate at the other side. Oospore formation was checked after 10 to 14 days of incubation at 18 °C in the dark, using a stereo-binocular microscope. Isolates that produced oospores with the known A1 tester isolate were designated as the A2 mating type, and isolates that produced oospores with the known A2 tester isolate were designated as the A1 mating type. Positive controls consisted of pairings between the opposite mating type of tester isolates, and negative controls consisted of pairings between isolates of the same mating type. The tester A1 and A2 *P. infestans* isolates were supplied by Professor William Fry's Laboratory (Cornell University, USA).

Assessment of Metalaxyl Sensitivity

Metalaxyl sensitivity was determined using a modification of the method described by Gamboa et al. (2019). Metataf 25WP (250 g metalaxyl/kg; Auto Crop Care Ltd. Bangladesh) was used to determine the metalaxyl sensitivity of the 69 isolates of *P. infestans*. Metataf 25WP (400 and 20 mg, respectively) was added to 1 L pea agar medium just before plating (90-mm diameter Petri dishes) to give final concentrations of 100 and 5 mg metalaxyl/L, respectively. After solidification of the medium, a single 5-mm mycelial plug of the *P. infestans* isolate to be tested was placed at the centre of a pea agar plate; three replicate plates were inoculated with each isolate. Plates without metalaxyl were used as controls. Plates were incubated at 18 °C for 2 weeks, then the colony diameters were measured using a 15-cm ruler, and the

percentage growth compared with the control plates was calculated. Isolates were classified as metalaxyl-resistant if they exhibited growth greater than 40% relative to the untreated control on both metalaxyl concentrations, as intermediate if they exhibited growth greater than 40% relative to the control on 5 mg metalaxyl/L, but less than 40% relative to the control on 100 mg metalaxyl/L and sensitive if they exhibited growth less than 40% on both metalaxyl concentrations (Daggett et al. 1993).

Mitochondrial DNA Haplotyping

The mitochondrial DNA (mtDNA) haplotype was determined for a subset of 24 isolates. DNA was isolated from small slabs (1 cm^2) of agar containing mycelium from cultures grown on pea agar and mtDNA haplotyping carried out using the method of Griffith and Shaw (1998) with slight modifications as described by Blandón-Diaz et al. (2012). Briefly, PCR amplification was performed in reaction mixtures containing: deoxynucleoside triphosphates, 200 µM each; $MgCl_2$, 2.75 mM; oligonucleotide primers 0.325 mM each; ethidium bromide, 0.2 µg mL^{-1}; bovine serum albumin, 160 µg mL^{-1}; 1×PCR buffer (Thermo Scientific); *Taq* DNA polymerase, 0.2 µL (1 U). One to 10 ng of total DNA was mixed with 20 µL of a master mix of the other PCR reactants in 0.5-mL microcentrifuge tubes (final volume, 25 µL). The PCR conditions were as follows: 1 cycle of 94 °C for 90 s and 40 cycles of 94 °C for 40 s, 55 °C for 60 s and 72 °C for 90 s. Then, 3 to 4 µL of the amplified DNA was digested with the following restriction enzymes in a 20-µL volume restriction digest at 37 °C for a period lasting between 1 h and overnight with the mtDNA regions, P1 with *Cfo*I; P2 with *Msp*I; P3 and P4 with *Eco*RI. The digested DNA samples were then mixed with 5 µL of gel-loading buffer, and 15 µL was loaded into a slot on a 2% agarose gel (Gibco BRL Ltd.) in 1×Tris–borate-EDTA (TBE) buffer (containing 0.1 µg of ethidium bromide mL^{-1}). The gel was then run at 10 V cm^{-1} for 60 to 90 min. Restriction patterns were visualized with a UV transilluminator, and the images were recorded by a gel documentation system.

Genotyping

Pathogen DNA for genotyping was collected from lesions onto FTA cards (Whatman FTA Classic cards). Thirty-eight samples were collected in 2018 and 2019 at the same time as the samples for isolation detailed above. In addition, a further 86 samples from Bangladesh were included in the genotyping analyses; of these, 51 were collected between 2016 and 2019 as part of the GEOPOTATO project, 24 were collected in 2017 as part of an AsiaBlight population mapping project and a further 11 collected between 2014 and 2017 were submitted directly to the James Hutton Laboratory. All FTA card samples were collected from commercial crops in January or February (122 from potato and two from tomato) and across the same regions as the main survey (see Supplementary Table 1). Briefly, the abaxial sides of sporulating foliar lesions were pressed against FTA cards. All samples were genotyped using a 12-plex Simple Sequence Repeat (SSR) assay according to published methods (Li et al. 2013a). A 2-mm disk was cut from the 'print' on the FTA card at the

interface of green leaf tissue and brown lesion. This disk was washed according to the manufacturer's protocols and subjected to PCR amplifying 12 SSR loci (Li et al. 2013a). After running on an ABI3730 capillary sequencer, the peak profiles were edited in GeneMapper (Applied Biosystems) version 5.0 and the profiles exported. A file of SSR data from 238 samples from Bangladesh (124) plus reference samples of EU_13_A2 from Pakistan (6), India (89) and Europe (11) and 2 samples each of another four European clonal lineages (EU_5_A1, EU_6_A1, EU_8_A1 and EU_2_A1) was compiled. Unique Multi Locus Genotypes (MLG) and Bruvo's distances were calculated and used to generate a minimum spanning network (MSN) in *poppr* 2.8.5 (Kamvar et al. 2015) in R 3.6.3 (R Core Team 2020) .

Results

Isolation and Identification of the Late Blight Pathogen, *P. infestans*

All 69 isolates were confirmed as *P. infestans* by morphology of the cultures on agar (fluffy white mycelial growth bearing characteristic lemon-shaped sporangia visible under the microscope) and PCR (production of a 600 base pair amplicon with *P. infestans* specific primer PINF; Trout et al. 1997). Isolates were obtained from all 16 potato-growing districts sampled and from three named cultivars, Asterix (24 isolates), Diamant (31 isolates), 747 (3 isolates) plus three local varieties, Challisha (4 isolates), Lal Pakri (4 isolates) and Romana Pakri (3 isolates).

Assessment of the Aggressiveness of *P. infestans* Isolates on Detached Potato Leaflets and Tuber Slices

The 69 *P. infestans* isolates differed significantly ($P<0.001$) in their aggressiveness to detached potato leaflets cv. Diamant as assessed by the area colonized 7 days after inoculation, which ranged from 15 to 98% (Fig. 1). Isolates also differed significantly ($P<0.001$) in aggressiveness to tuber slices; the areas of tuber slices colonized after 7 days ranged from 4 to 95% (Fig. 2). Isolates obtained in 2018 were markedly more aggressive to tuber slices than those collected in 2019 (75.0% and 19.3% areas infected for 2018 and 2019 isolates, respectively), but there was only a slight difference between both years with respect to their aggressiveness to detached leaflets (70.6% and 61.7% areas infected in 2018 and 2019, respectively). There was no significant correlation ($r=0.325$) between aggressiveness to leaflets and to tubers (Fig. 3).

A significant ($P<0.001$) effect was observed between the location from which the isolates were obtained and their aggressiveness to both detached leaflets and tuber slices (Table 1). Overall, isolates obtained from Nilphamari were the most aggressive and those from Dinajpur the least aggressive to both leaflets and tuber slices. There was also a significant ($P<0.05$) effect of the cultivar from which the isolates were obtained on their aggressiveness to tuber slices, but not to leaflets (Table 2). However, these results should be interpreted with caution as only a few isolates were obtained from some cultivars, notably cv. 747 (which was only sampled in 2019) and Romana Pakri (which was only sampled in 2018).

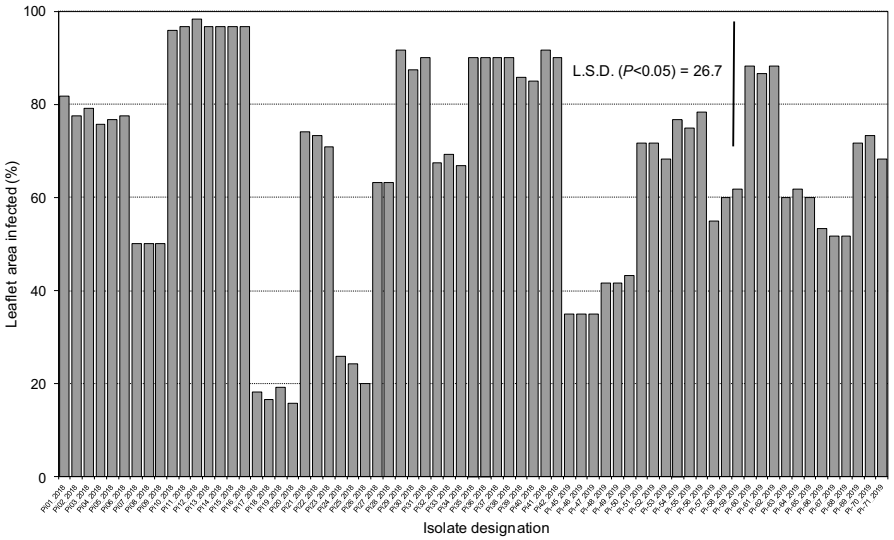

Fig. 1 Aggressiveness to detached potato leaflets cv. Diamant of isolates of *Phytophthora infestans* obtained from potato crops in Bangladesh, 2018–2019, as assessed by the area colonized 7 days after inoculation

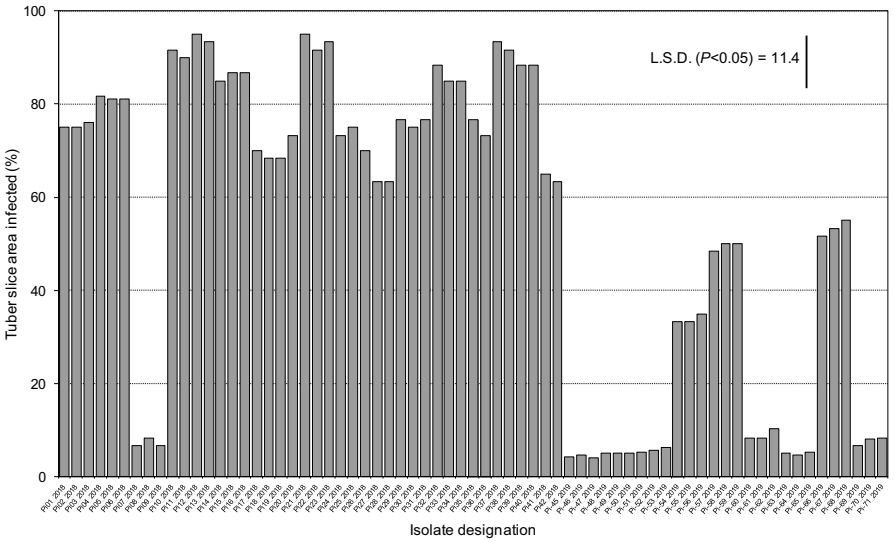

Fig. 2 Aggressiveness to potato tuber slices cv. Diamant of isolates of *Phytophthora infestans* isolates obtained from potato crops in Bangladesh, 2018–2019, as assessed by the area colonized 7 days after inoculation

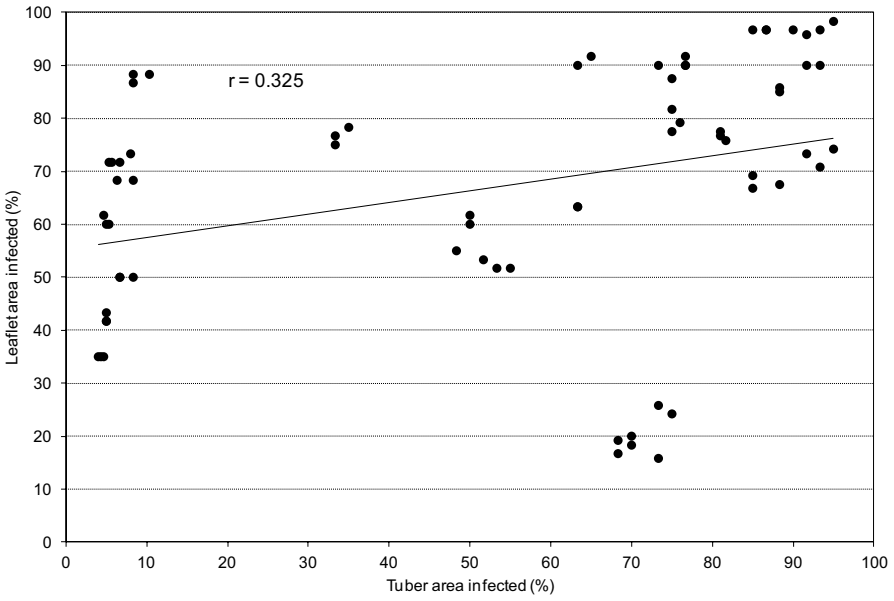

Fig. 3 Relationship between aggressiveness to detached potato leaflets and to tuber slices cv. Diamant of isolates of *Phytophthora infestans* obtained from potato crops in Bangladesh, 2018–2019, as assessed by the areas colonized 7 days after inoculation

Determination of Mating Type of *P. infestans*

All the 69 isolates proved to be of the A2 mating type forming oospores when paired with the known A1 tester, but not with the A2 tester on pea agar plates. Characteristic thick-walled amphigynous oospores of *P. infestans* were photographed.

Assessment of Metalaxyl Resistance/Sensitivity

None of the 69 isolates was categorized as sensitive to metalaxyl, all having growth on 5 mg metalaxyl/L of at least 40% of the growth on the untreated control. Twenty-six isolates (38%) were classed as intermediate in sensitivity to metalaxyl, while the remaining 43 isolates (62%) were metalaxyl-resistant (full data not presented).

Mitochondrial DNA Haplotyping

The mtDNA haplotype was determined for a subset of 24 isolates (15 from 2018 and nine from 2019). On the basis of the bands produced by digestion with *Cfo*I (P1), *Msp*I (P2) and *Eco*RI (P3 and P4), all isolates were classified as having mtDNA Ia. Table 3 shows the mtDNA haplotype, mating type and metalaxyl sensitivity for this subset of isolates.

Table 1 Aggressiveness of *Phytophthora infestans* isolates from Bangladesh on detached potato leaflets and tuber slices (cv. Diamant) comparing locations from which isolates were obtained

Location	Cultivar	Year sampled	Number of isolates	Leaf area diseased (%)	Tuber area (%)
Bogura	Romana Pakri	2018	3	72.8	93.3
Chandpur	Diamant	2018, 2019	5	79.0	30.3
Cumilla	Diamant	2018, 2019	5	67.3	69.0
Dinajpur	Diamant and 747	2018, 2019	6	42.5	5.8
Gaibandha	Asterix	2019	3	76.7	33.9
Jamalpur	Challisha	2018	3	89.7	76.1
Joypurhat	Diamant	2018	3	23.3	72.8
Kurigram	Asterix and Diamant	2018, 2019	7	40.2	42.5
Munshiganj	Diamant	2018, 2019	5	70.5	38.3
Mymensingh	Diamant and Lal Pakri	2018, 2019	5	71.3	59.7
Naogoan	Lal Pakri	2018	2	63.3	63.3
Nilphamari	Asterix	2018	4	96.9	92.5
Panchagarh	Diamant	2018	3	79.4	75.3
Rangpur	Asterix and Diamant	2018, 2019	6	69.4	45.6
Sherpur	Asterix and Challisha	2018, 2019	6	77.8	47.6
Thakurgoan	Asterix	2018	3	76.7	81.2
S.E. (53 D.F.) min. rep.				11.66	19.61
S.E. (53 D.F.) max. rep.				6.23	10.48
Significance				<0.001	<0.001
L.S.D. ($P<0.05$) min. rep.				33.07	55.61
L.S.D. ($P<0.05$) max. rep.				17.67	29.72

Table 2 Aggressiveness of *Phytophthora infestans* isolates from Bangladesh on detached potato leaflets and tuber slices (cv. Diamant) comparing cultivars from which isolates were obtained

Cultivar	Number of isolates	Leaf area diseased (%)	Tuber area (%)
747	3	35.0	4.3
Asterix	24	66.0	50.9
Challisha	4	84.0	78.3
Diamant	31	67.1	50.5
Lal Pakri	4	76.7	69.2
Romana Pakri	3	72.8	93.3
S.E. (63 D.F.) min. rep.		12.99	18.37
S.E. (63 D.F.) max. rep.		4.04	5.71
Significance		n.s	<0.05
L.S.D. ($P<0.05$) min. rep.		n/a	51.91
L.S.D. ($P<0.05$) max. rep.		n/a	16.15

Genotyping

Microsatellite genotyping of 124 pathogen DNA samples collected from Bangladesh in 2014 (5), 2015 (1), 2016 (2), 2017 (35), 2018 (66) and 2019 (15) revealed that the vast majority (118 samples; 95%) belonged to the EU_13_A2 lineage, while six were assigned to a genotype 'other' that did not correspond to any previously named genotype (Supplementary Table 1). The two samples obtained from tomato crops (one in 2014 and one in 2018) were both EU_13_A2. Figure 4 shows a MSN generated in *poppr* 2.8.5 which includes all the genotyped samples from Bangladesh plus representative samples of EU_13_A2 from Pakistan, India and Europe and samples of four other clonal lineages sampled in Europe (EU_5_A1, EU_6_A1, EU_8_A1 and EU_2_A1).

Amongst the 118 EU_13_A2 samples from Bangladesh, 41 distinct MLGs were determined with no single MLG dominating. The largest three groups comprised 25,

Table 3 Metalaxyl sensitivity, mating type, mtDNA haplotype and genotype of a subset of 24 isolates of *Phytophthora infestans* from Bangladesh

Isolate ID	Cultivar	Location	Metalaxyl sensitivity	Mating type	mtDNA haplotype	Genotype
Pi01_2018	Diamant	Panchagarh	I	A2	Ia	EU_13_A2
Pi04_2018	Asterix	Thakurgoan	I	A2	Ia	EU_13_A2
Pi07_2018	Diamant	Dinajpur	I	A2	Ia	EU_13_A2
Pi12_2018	Asterix	Nilphamari	I	A2	Ia	nd
Pi16_2018	Diamant	Rangpur	I	A2	Ia	EU_13_A2
Pi20_2018	Asterix	Kurigram	R	A2	Ia	nd
Pi23_2018	Romana Pakri	Bogura	R	A2	Ia	nd
Pi24_2018	Diamant	Joypurhat	R	A2	Ia	nd
Pi27_2018	Lal Pakri	Naogoan	R	A2	Ia	EU_13_A2
Pi29_2018	Challisha	Jamalpur	I	A2	Ia	EU_13_A2
Pi32_2018	Asterix	Sherpur	R	A2	Ia	EU_13_A2
Pi35_2018	Lal Pakri	Mymensingh	I	A2	Ia	nd
Pi37_2018	Diamant	Cumilla	I	A2	Ia	nd
Pi40_2018	Diamant	Munshiganj	I	A2	Ia	nd
Pi41_2018	Diamant	Chandpur	R	A2	Ia	EU_13_A2
Pi-45_2019	747	Dinajpur	R	A2	Ia	EU_13_A2
Pi-48_2019	Asterix	Rangpur	R	A2	Ia	EU_13_A2
Pi-52_2019	Asterix	Kurigram	R	A2	Ia	nd
Pi-54_2019	Asterix	Gaibandha	R	A2	Ia	Other
Pi-58_2019	Diamant	Mymensingh	R	A2	Ia	nd
Pi-61_2019	Asterix	Sherpur	I	A2	Ia	nd
Pi-65_2019	Diamant	Munshiganj	R	A2	Ia	nd
Pi-68_2019	Diamant	Cumilla	R	A2	Ia	nd
Pi-70_2019	Diamant	Chandpur	R	A2	Ia	nd

nd not determined

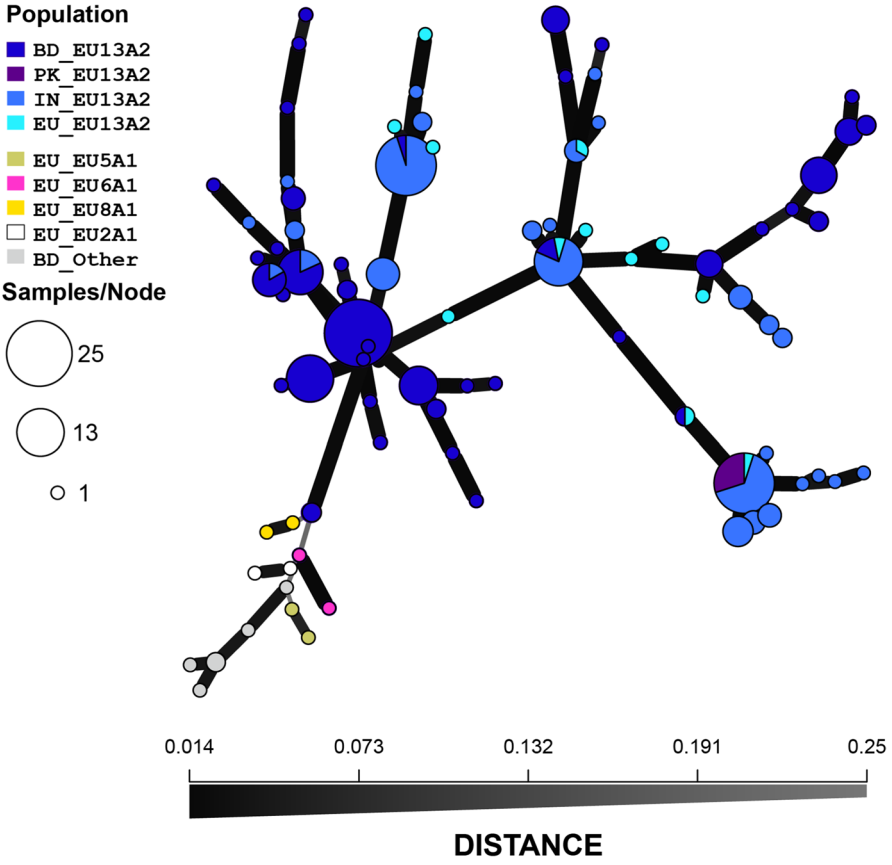

Fig. 4 Minimum spanning network (MSN) of samples of *Phytophthora infestans* DNA collected from Bangladesh, India, Pakistan and Europe. Each node represents an MLG with edge width and shading reflecting genetic relatedness based on Bruvo's genetic distance. This MSN indicates a cluster of clonal MLG variants of EU_13_A2 that are genetically closely related in comparison to the more genetically distinct pairs of isolates of other clonal lineages and the 'other' genotype from Bangladesh

12 and 9 samples, and more than half of the MLGs (25) were detected only once. Amongst the wider set of 224 EU_13_A2 samples from Europe, Pakistan, India and Bangladesh, 72 distinct MLGs were determined. However, only five of the 72 MLGs were common to Bangladesh and those in other countries; four were shared with MLGs from India and one matched a sample from Europe. Bangladesh variants of EU_13_A2 were observed across most of the EU_13_A2 minimum spanning network (Fig. 4). However, a Bangladesh-specific and Bangladesh-India specific cluster of at least 20 nodes centred around the largest 25-sample node on the left hand side of the network was apparent. Also on the top right hand side a cluster of eight connected Bangladesh only nodes was noted (Fig. 4). The single EU_13_A2 MLG present in Pakistan was identical to one found in India and Europe, but was not found in Bangladesh. The five samples from Bangladesh assigned to the genotype 'other'

were genetically similar to each other and likely represent variants of a single genotype, but none of these is related to either the four representative known clonal genotypes from Europe or to many others in the database (data not shown).

Discussion

The present study of the *P. infestans* population in Bangladesh examined isolates and DNA samples collected from potatoes between 2014 and 2019. Genotyping of 124 samples showed that the aggressive European lineage EU_13_A2 (Blue 13) has been present across the potato-producing areas of the country since at least 2014 and dominated the population sample in this study (95% of samples genotyped were EU_13_A2). The six genotyped samples that were assigned to 'other' were closely related to each other, but not to EU_13_A2 (variation at alleles G11, D13, SSR4 only). They were sampled from several different locations in 2017 (1), 2018 (1) and 2019 (4) suggesting this is a locally spread clonal lineage.

All isolates belonged to the A2 mating type, were either resistant or intermediate in sensitivity to metalaxyl and those haplotyped were all mtDNA Ia. This is in agreement with the multilocus characters of the EU_13_A2 lineage found in India (Chowdappa et al. 2015; Dey et al. 2018). The considerable diversity of EU_13_A2 within Bangladesh also mirrors the high diversity of this lineage in India (Dey et al. 2018) but contrasts with the situation in Pakistan where only a single variant of EU_13_A2 was identified in *P. infestans* DNA samples from 2019 and 2020 (Raza et al. 2021). The data reveal a collection of Bangladesh-specific variants of the EU_13_A2 lineage which suggests they are locally evolved from a population that has been present in Bangladesh for a considerable time since many of the variants found were specific to Bangladesh, which would suggest that these arose within the country after the initial introduction.

EU_13_A2 appears to have originated in Europe where its earliest identification was in isolates obtained from Germany and The Netherlands in 2004 (Cooke et al. 2012); it is unrelated to *P. infestans* genotypes prevalent in Asia before the year 2000 (Guha Roy et al. 2021) . As far as can be ascertained, the first finding of EU_13_A2 in Asia was in an isolate from Yunnan, China in 2005 (Li et al. 2013b; Guha Roy et al. 2021) . The EU_13_A2 lineage was subsequently identified in southern India causing severe late blight outbreaks on tomato in 2008 and 2009 (Chowdappa et al. 2013) and on both potato and tomato in 2010–2012 (Chowdappa et al. 2015). In 2014, there was a major late blight epidemic on potato in north-eastern India which caused major crop losses; detailed study of isolates obtained in 2013 and 2014 suggested either that EU_13_A2 had been imported into different regions of India on several separate occasions or that local mutations resulted in highly diverse EU_13_A2 populations (Dey et al. 2018). Bangladesh, like India, has imported potatoes from The Netherlands in the past so that the introduction of EU_13_A2 into Bangladesh may have been a result of a separate importation in seed potatoes from Europe or occurred via migration from neighbouring potato growing regions such as West Bengal during the 2014 epidemic (Fry 2016). EU_13_A2 could also have been introduced from India via formal trade between India and Bangladesh or informal

movement of potatoes or tomatoes across the border as well as by aerial movement of sporangia from fields in Eastern India. Although EU_13_A2 was present in China as early as 2005, potatoes from China are not imported into Bangladesh or India so this is not a likely route of its introduction (Guha Roy, personal communication). Variants of EU_13_A2 in Bangladesh that are common to samples from India and clusters of related India- and Bangladesh-specific MLGs in the MSN suggest a shared population in the region (Fig. 4). The majority of the sub-clonal variation is due to three highly variable markers (G11, D13 and SSR4), and homoplasy at these loci is also possible.

The *P. infestans* lineage EU_13_A2 has thus been responsible for severe late blight epidemics on potato in Bangladesh. Surveys of the incidence and severity of late blight in selected potato-growing areas of Bangladesh in 2018 and 2019 found that the disease was very widespread in 2018 with a severity of up to 98% in farmers' field in some areas; in 2019, the disease was less widespread and severe, but was still present in most areas (Md. R. Islam, unpublished data). These differences between years in late blight severity are most probably related to the weather during the winter growing season.

The *P. infestans* isolates tested varied significantly in their aggressiveness to detached potato leaflets cv. Diamant, but the majority had colonized more than 70% of the leaflet area within 7 days of inoculation. This high level of foliar aggressiveness is in agreement with previously published research on the aggressiveness of EU_13_A2 (e.g. Cooke et al. 2012; Chowdappa et al. 2015) and helps to explain the severity of recent late blight epidemics in Bangladesh. Aggressiveness to potato tuber slices varied even more than that to leaflets; whereas all but three of the isolates collected in 2018 had colonized more than 60% of the tuber slice area within 7 days of inoculation, although all those collected in 2019 successfully infected the tuber slices, none had achieved 70% colonization within 7 days. Host resistance in different parts of the potato plant is often not related, and indeed, the aggressiveness of isolates to leaflets and tubers was not correlated. Variation in aggressiveness was related to sampling locations rather than the potato cultivars from which isolates were obtained. However, as some cultivars were sampled in only one of the two years (2018 and 2019), the effect of cultivar was confounded with differences between isolates from the two sampling years. Of the 69 *P. infestans* isolates tested for aggressiveness, 32 were genotyped; 30 were EU_13_A2 while two were classified as 'other'. The aggressiveness in the genotyped EU_13_A2 isolates ranged from 18 to 97% of leaflet area colonized and from 4 to 95% of tuber slice area colonized. Considerable variation in aggressiveness within clonal lineage EU_13_A2 was also reported on isolates from India tested on tomato leaves (Chowdappa et al. 2013). The two isolates classified as genotype 'other' exhibited aggressiveness to potato leaflets and tuber slices within the same range as the EU_13_A2 isolates.

The cultivars most frequently sampled in the present study, Asterix and Diamant, were recommended as suitable for cultivation in Bangladesh by Eaton et al. (2017), who reported that Asterix was more disease resistant than Diamant. However, the present authors have not found any published information to indicate that R-genes are present in either cultivar (they are not listed as containing resistance genes in a recent review by Paluchowska et al. 2022). R-gene characterization (virulence testing) was

therefore not included in the present study. However, virulence testing of EU_13_A2 isolates from elsewhere has shown that they are generally highly complex and overcome many of the R1-R11 R-genes in the differential set (Cooke et al. 2012; Göre et al. 2021) and also other sources of resistance (Lees et al. 2012). None of the isolates tested in the present study proved sensitive to metalaxyl, all being either metalaxyl-resistant (62%) or -intermediate (38%). This is in agreement with other studies that found EU_13_A2 to be metalaxyl-resistant (e.g. Cooke et al. 2012; Chowdappa et al. 2015) or metalaxyl-resistant and -intermediate and only extremely rarely metalaxyl-sensitive (Dey et al. 2018). EU_13_A2 was most probably introduced into the sub-continent as a metalaxyl-resistant and/or -intermediate lineage; usage of fungicide formulations containing metalaxyl or metalaxyl-M may have contributed to its spread, and further selection may have occurred after its arrival. Metalaxyl was introduced for late blight control in India in the late 1980s and by 1989 metalaxyl-resistant strains had been identified in India (Arora 1991; Singh and Pundhir 2013). Across Bangladesh, products containing metalaxyl have been used extensively for the control of late blight (Islam et al. 2002; Dey et al. 2010); however, metalaxyl resistance was identified in isolates from Bangladesh collected in 1995–1996 by Hossain et al. (2009). Now, the prevalence of metalaxyl-resistant strains in the *P. infestans* population in Bangladesh makes the value of applying products containing metalaxyl questionable. From field trials conducted in Rangpur, Bangladesh in 2010–2011, Anwar et al. (2015) concluded that of one of the most effective products for controlling potato late blight contained mancozeb and metalaxyl, but their results indicated that the reduction in foliar blight and increase in yield did not differ significantly from that achieved by mancozeb alone. Thus, metalaxyl may not be making a significant contribution to late blight control, and alternative active ingredients should be used for improved control of late blight in Bangladesh.

The most important factor driving population change in *P. infestans* in Asia and consequent severe late blight epidemics is migration of pathogen genotypes from Europe and also the Americas (Guha Roy et al. 2021) . Trade in potato tubers plays a key role in this process of migration (Fry 2020). The present study clearly indicates that the introduced EU_13_A2 lineage of *P. infestans* was primarily responsible for the destructive late blight epidemics in Bangladesh since 2014. The importance of bio-security in agricultural trade must be stressed. Mitigation of the impact of aggressive pathogen genotypes should involve deploying genetic host resistance in an integrated disease management strategy. This should be complemented by pathogen population monitoring, combined with genome analysis, and regional and global data sharing using networks such as AsiaBlight.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11540-022-09581-w.

Funding This research work was carried out with the financial support from Bangladesh Academy of Sciences (BAS)-USDA to Md. Rashidul Islam (Grant no.: BAS USDA PALS CR-38). David Cooke and Louise Sullivan received funding from the Rural and Environment Science and Analytical Services Division of the Scottish Government. The collection of the *P. infestans* samples contributed by Geert Kessel was funded by the GEOPOTATO project, part of the Geodata for Agriculture and Water programme commissioned by the Dutch Ministry of Foreign Affairs to improve food security in developing countries using satellite data from the Netherlands Space Office.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

Andersson B, Widmark AK, Yuen JE, Nielsen B, Ravnskov S, Kessel GJT, Evenhuis A, Turkensteen LJ, Hansen JG, Lehtinen A, Hermansen A, Brurberg MB, Nordskog B (2009) The role of oospores in the epidemiology of potato late blight. Acta Hort 834:61–68

Anwar MM, Parveen A, Hossain MM, Mahamud NU, Roy RK (2015) Efficacy of fungicides in controlling late blight of potato. Progress Agric 26:103–108

Arora RK (1991) Occurrence and frequency of metalaxyl resistant isolates of *Phytophthora infestans* in South Indian Hills. Indian Phytopathol 45(supplement):90

Blandón-Díaz JU, Widmark A-K, Hannukkala A, Andersson B, Högberg N, Yuen JE (2012) Phenotypic variation within a clonal lineage of *Phytophthora infestans* infecting both tomato and potato in Nicaragua. Phytopathology 102:323–330

Caten CE, Jinks JL (1968) Spontaneous variability of single isolates of *Phytophthora infestans* I: Cultural variation. Can J Bot 46:329–348

Chowdappa P, Kumar NBJ, Madhura S, Mohan Kumar SP, Meyers KL, Fry WE et al (2013) Emergence of 13_A2 blue lineage of *Phytophthora infestans* was responsible for severe outbreaks of late blight on tomato in south-west India. J Phytopathol 161:49–58

Chowdappa P, Nirmal Kumar BJ, Madhura S, Mohan Kumar SP, Myers KL, Fry WE, Cooke DEL (2015) Severe outbreaks of late blight on potato and tomato in South India caused by recent changes in the *Phytophthora infestans* population. Plant Pathol 64:191–199

Cooke DEL, Cano LM, Raffaele S et al (2012) Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. PLoS Pathog 8:e1002940

Daggett SS, Götz E, Therrien CD (1993) Phenotypic changes in populations of *Phytophthora infestans* populations from Eastern Germany. Phytopathology 83:319–323

Dey TK, Hossain M, Kadian MS, Hossain S, Bonierbale M, Mahmud AA (2010) Prevalence, epidemiology and management of potato late blight in Bangladesh. Potato Journal 37:99–102

Dey T, Saville A, Myers K, Tewari S, Cooke DEL, Tripathy S, Fry WE, Ristaino JB, Guha Roy S (2018) Large sub-clonal variation in *Phytophthora infestans* from recent severe late blight epidemics in India. Sci Rep 8:4429

Eaton TE, Azad AK, Kabir H, Siddiq AB (2017) Evaluation of six modern varieties of potatoes for yield, plant growth parameters and resistance to insects and diseases. Agric Sci 8:1315–1326. https://doi.org/10.4236/as.2017.811095

FAOSTAT (2022) Food and Agriculture Organization of the United Nations. [http://www.fao.org/faostat/en/#data/QC]. Accessed 14 February 2022

Forbes GA (2004) Global overview of late blight. Proceedings Regional Workshop on Potato Late Blight for East and Southeast Asia and the Pacific. Yezin, Myanmar 24–25 August 2004. GILB, International Potato Center (CIP), Lima, Peru, pp. 3–10

Fry WE (2016) *Phytophthora infestans*: new tools (and old ones) lead to new understanding and precision management. Annu Rev Phytopathol 54:529–547

Fry WE (2020) *Phytophthora infestans*: the itinerant invader; "late blight": the persistent disease. Phytoparasitica 48:87–94

Fry WE, Birch PRJ, Judelson HS, Grünwald NJ, Danies G, Everts KL, Gevens AJ, Gugino BK, Johnson DA, Johnson SB, McGrath MT, Myers KL, Ristaino JB, Roberts PD, Secor G, Smart CD (2015) Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology 105:966–981

Gamboa S, Perez W, Andrade-Piedra JL, Forbes G (2019) Laboratory manual for *Phytophthora infestans* work at CIP. International Potato Center, Lima, Peru, p 78. https://cipotato.org/publications/laboratory-manual-for-phytophthora-infestans-work-at-cip/

Göre ME, Altın N, Myers K, Cooke DEL, Fry WE, Özer G (2021) Population structure of *Phytophthora infestans* in Turkey reveals expansion and spread of dominant clonal lineages and virulence. Plant Pathol 70:898–911

Govers F, Drenth A, Pieterse CMJ (1997) The potato late blight pathogen *Phytophthora infestans* and other pathogenic Oomycota. In: Carroll GC, Tudzynski P (eds) The Mycota V part B. Springer, Berlin Heidelberg, pp 17–36

Griffith GW, Shaw DS (1998) Polymorphisms in *Phytophthora infestans*: four mitochondrial DNA haplotypes are detected after PCR amplification from pure cultures or from host lesions. Appl Environ Microbiol 64:4007–4014

Guha Roy S, Dey T, Cooke DEL, Cooke LR (2021) The dynamics of *Phytophthora infestans* populations in the major potato-growing regions of Asia—a review. Plant Pathol 70:1015–1031

HelgiLibrary (2022) Potato consumption Per Capita in Bangladesh. https://www.helgilibrary.com/indicators/potato-consumption-per-capita/bangladesh/. Accessed 18 Aug 2022

Hossain MT, Dey TK, Iqbal HM, Begum SN, Kadian MS (2009) Research experience on potato late blight disease management in Bangladesh. Acta Hort 834:175–186

Hu C-H, Perez FG, Donahoo R, McLeod A, Myers K, Ivors K, Secor G, Roberts PD, Deahl KL, Fry WE, Ristaino JB (2012) Recent genotypes of *Phytophthora infestans* in eastern United States reveal clonal populations and reappearance of mefenoxam sensitivity. Plant Dis 96:1323–1330

Islam MR, Dey TK, Rahman MM, Hossain MA, Ali MA (2002) Efficacy of some fungicides in controlling late blight of potato. Bangladesh J Agril Res 27:257–261

Jansky S, Navarre R, Bamberg J (2019) Introduction to the special issue on the Nutritional Value of Potato. Am J Potato Res 96:95–97

Kamvar ZN, Brooks JC, Grunwald NJ (2015) Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Front Genet 6:208

Kessel G, Moene A, van Valkengoed E, van der Voet P, Michielsen JM, Ahsan H et al (2017) Geodata to control potato late blight in Bangladesh. PAGV—Special Report No 18:59–60

Lees AK, Stewart JA, Lynott JS, Carnegie SF, Campbell H, Roberts AMI (2012) The effect of a dominant *Phytophthora infestans* genotype (13_A2) in Great Britain on host resistance to foliar late blight in commercial potato cultivars. Potato Res 55:125–134

Lehtinen A, Hannukkala A, Andersson B, Hermansen A, Le VH, Nærstad R, Brurberg MB, Nielsen BJ, Hansen JG, Yuen J (2008) Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. Plant Pathol 57:227–234

Li Y, Cooke DEL, Jacobsen E, van der Lee T (2013a) Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. J Microbiol Methods 92:316–322

Li Y, van der Lee T, Zhu JH, Jin GH, Lan CZ, Zhu SX et al (2013b) Population structure of *Phytophthora infestans* in China—geographic clusters and presence of the EU genotype Blue_13. Plant Pathol 62:932–942

Paluchowska P, Śliwka J, Yin Z (2022) Late blight resistance genes in potato breeding. Planta 255:127. https://doi.org/10.1007/s00425-022-03910-6

Parvez S (2020) Potato exports on the rebound but remain far below previous peak. The Daily Star, 19 July 2020. https://www.thedailystar.net/business/news/potato-exports-the-rebound-remain-far-below-previous-peak-1932637. Accessed 9 Aug 2022

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 10 June 2020

Raza W, Ghazanfar MU, Sullivan L, Cooke DEL, Cooke LR (2021) Mating type and aggressiveness of *Phytophthora infestans* (Mont.) de Bary in potato-growing areas of Punjab, Pakistan, 2017–2018 and identification of genotype 13_A2 in 2019–2020. Potato Res 64:115–129

Ristaino JB (2020) The importance of mycological and plant herbaria in tracking plant killers. Front Ecol Evol 7. https://doi.org/10.3389/fevo.2019.00521

Sabur SA, Monayem Miah MA, Esmat Ara Begum M, Shahrukh Rahman M, Uddin Molla MM (2021) Demand, supply and reasons for price hike of potato in Bangladesh. Bangladesh J Agricult Econ 42:93–108

Sato N (1994) Effect of some inorganic salts and hydrogen ion concentration on indirect germination of the sporangia of *Phytophthora infestans*. Ann Phytopath Soc Japan 60:441–447

Saville AC, Ristaino JB (2021) Global historic pandemics caused by the FAM-1 genotype of the Irish potato pathogen *Phytophthora infestans*. Sci Reports Sci Reports 11:12335. https://doi.org/10.1038/s41598-021-90937-6

Saville AC, Martin MD, Ristaino JB (2016) Historic late blight outbreaks caused by a widespread dominant lineage of *Phytophthora infestans* (Mont) de Bary. PLoS ONE 11:e0168381

Singh VK, Pundhir VS (2013) Population structure and resistance to metalaxyl in isolates of *Phytophthora infestans*. Indian Phytopathol 66:53–56

Smart CD, Fry WE (2001) Invasions by the late blight pathogen: renewed sex and enhanced fitness. Biol Invasions 3:235–243

Trout CL, Ristaino JB, Madritch M, Wangsomboondee T (1997) Rapid detection of *Phytophthora infestans* in late blight-infected potato and tomato using PCR. Plant Dis 81:1042–1048

Yoshida K, Schuenemann VJ, Cano LM, Pais M, Mishra B, Sharma R et al (2013) The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. elife 2:e00731

Yoshida K, Burbano HA, Krause J, Thines M, Weigel D, Kamoun S (2014) Mining herbaria for plant pathogen genomes: back to the future. PLoS Pathog 10:e1004028

Authors and Affiliations

Md. Huzzatul Islam[1,2] · Sangee Sarkar Shanta[1] · Muhammad Iqbal Hossain[1] · Muhammed Ali Hossain[1] · Md. Monower Hossain[3] · Ebna Habib Md. Shofiur Rahaman[3] · Abdullah Al Mahmud[4] · Md. Abdullah Yousuf Akhond[5] · Louise Sullivan[6] · David E. L. Cooke[6] · Geert J. T. Kessel[7] · Louise R. Cooke[8] · Md. Rashidul Islam[1]

✉ Louise R. Cooke
l.cooke@qub.ac.uk; louiserenecooke@gmail.com

Abdullah Al Mahmud
http://www.bari.gov.bd

David E. L. Cooke
https://www.hutton.ac.uk/staff/david-cooke

Geert J. T. Kessel
https://www.wur.nl/en/Persons/Geert-Jan-dr.ir.-GJT-Geert-Jan-Kessel.htm

1 Plant Bacteriology and Biotechnology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

2 Department of Agricultural Extension (DAE), Khamarbari, Dhaka, Bangladesh

3 International Potato Center, House-25, Road-04, Block-F, Banani, Dhaka 1213, Bangladesh

4 On-Farm Research Division (OFRD), Bangladesh Agricultural Research Institute (BARI), 1701, Gazipur, Bangladesh

5 Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), 1701, Gazipur, Bangladesh

6 Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

7 Field Crops, Wageningen University and Research, Edelhertweg 1, 8219 PH Lelystad, The Netherlands

8 School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland, UK