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Genotypic characterization of *Phytophthora infestans* populations in Bangladesh

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Abstract

Late blight, caused by *Phytophthora infestans*, is an economically important disease of potato that causes significant yield losses, with severe outbreaks regularly occurring in Bangladesh. The objective of this study was to do a large-scale survey of potato fields in the main potato-growing divisions of Bangladesh examining genotypic diversity of *P. infestans* populations. A total of 160 samples were collected in 2018 from both potato ($n = 140$) and tomato ($n = 20$). Isolates were mainly collected on FTA cards ($n = 143$), but 17 were also collected and isolated into pure culture. Microsatellite analysis revealed high levels of subclonal diversity in *P. infestans* populations with 116 multilocus genotypes recorded from 160 samples. Comparisons with standards of European and US isolates showed that 74% of samples could be categorized as genotype EU_13_A2, 7% clustered near EU_6_A1 and EU_1_A1, and 19% were unique. Discriminant analysis of principal components showed that the *P. infestans* population clustered into four distinct groups: a main group that contained most of the samples from potato, two distinct tomato groups and one group of samples originating from the division of Mymensingh. Of 17 isolates from cultures, 16 were EU_13_A2 and one was EU_6_A1; 15 were insensitive to metalaxyl-M. Out of 160 samples, 158 were categorized as mating type A2 and two as mating type A1. These results indicate that Bangladesh populations of *P. infestans* from potato, like those from neighbouring countries, are dominated by genotype EU_13_A2. However, populations from tomato were distinct and appear to be specific to tomato.

KEYWORDS
FTA cards, genetic structure, late blight, microsatellite analysis, *Phytophthora infestans*, potato

1 | INTRODUCTION

Late blight, caused by *Phytophthora infestans*, is one of the most common and serious diseases of potato (*Solanum tuberosum*) wherever cool and humid environments prevail during crop production (Fry et al., 2015). Besides potato and tomato, the pathogen also infects other solanaceous weeds and crops. Late blight causes significant yield loss under conducive environmental conditions (high humidity and cool temperatures) both in the field and in storage and requires the use of chemical fungicides for control. Annual loss and management costs from late blight have been reported to be over $6.7 billion worldwide (Haverkort et al., 2008).

The origin of *P. infestans* has been strongly debated, with researchers suggesting both a central Mexico and South American Andean origin (Martin et al., 2015; Wang et al., 2017). *P. infestans* has historically been disseminated as a series of clonal lineages. A lineage containing the mtDNA haplotype HERB-1 and genotype FAM-1 was found in historic herbarium and late blight samples from Europe and is thought to have caused the Irish Potato Famine (Saville et al., 2016; Yoshida et al., 2014). This lineage was dominant

and caused widespread late blight outbreaks worldwide in the 19th and first half of the 20th century (Saville et al., 2016). The lineage was later displaced by the US-1 genotype that dominated late blight outbreaks worldwide until the early 1980s and was in turn displaced by other aggressive clonal lineages (Fry et al., 2015; Goodwin et al., 1994; Njoroge et al., 2016).

The US-1 lineage was reported from many potato-growing countries in Asia from the mid-20th century. With the emergence of A2 mating types first in Europe, then North America, and Asia in 1986, the *P. infestans* populations in Asia became much more complex (Goodwin, 1997). From the 1990s the "old" US-1 population was gradually replaced by the "new" A2 populations (Spielman et al., 1991). For the past 20 years, *P. infestans* populations in western Europe have been dominated by aggressive clonal lineages including EU_13_A2 and EU_6_A1 (Cooke et al., 2019). The EU_13_A2 genotype was first recorded in 2004 in the Netherlands and Germany and has subsequently been found in China and India (Chowdappa et al., 2013; Cooke et al., 2012; Li, Cooke, et al., 2013; Li, van der Lee, et al., 2013).

Bangladesh is the seventh largest potato producer in the world and third in Asia after China and India (FAO, 2020). Potatoes are grown in the winter months where winter temperatures in December and January range from 11 to 23°C, which are ideal for growing potatoes. Although the main potato-growing regions in Bangladesh have mostly dry weather from November to March, they tend to have cool and foggy days in January, which are ideal conditions for the spread of late blight. Yield losses of potato in Bangladesh in years when late blight occurs have been reported to be between 25% and 60% depending on the cultivar and the time of onset of disease (Kessel et al., 2019). The occurrence of late blight in the Indian region that subsequently became the country of Bangladesh was first reported in 1922 (Hossain et al., 2009), but relatively little information has been published on the *P. infestans* population (Islam et al., 2022).

There is no certified seed system in Bangladesh to provide growers with disease-free seed as there is in Europe and North America. Bangladeshi growers tend to save potatoes from one season to the next for use as seed or rely on local suppliers for seed. These seed sources may contain high levels of potato virus Y or other diseases such as late blight. Larger growers who supply commercial companies with potatoes rely on imports of certified disease-free seed from Europe. Although seed imports may be certified disease-free, *P. infestans* can remain dormant and asymptomatic in seed potatoes stored at 4°C (Johnson & Cummings, 2009). These asymptomatic tubers are easily overlooked during shipping and handling and can lead to the import of new European genotypes into the country. Forbes (2004) reported that both A1 and A2 mating types were present in Bangladesh, while more recently, Pronk et al. (2017) and Guha Roy et al. (2021) reported the presence of the EU_13_A2 genotype.

Although potatoes are grown in the winter months when the weather is drier and less conducive for late blight, growers are still heavily reliant on fungicides to manage the disease, applying 10–20 fungicide sprays in a single potato-growing season. Fungicides are often applied without any kind of personal protective equipment (authors' personal observations). The optimal way to overcome the problem of late blight in Bangladesh would be to develop a late blight-resistant potato cultivar. Breeding late blight resistance in potato cultivars would have significant benefits due to the reduced farmer exposure to fungicides and reduced fungicide residues in food, land and wastewater. Late blight-resistant cultivars would also reduce the cost of production through lower fungicide costs and lead to increased yields and yield stability, potentially increasing grower incomes (Kirk et al., 2001).

The USAID Feed the Future Biotechnology Potato Partnership was formed in 2015 to introduce 3 R-gene late blight resistance bioengineered potato products in farmer- and consumer-preferred cultivars into Bangladesh and Indonesia. These bio-engineered potatoes have been evaluated against *P. infestans* strains from Europe and the United States, and in confined field trials against endemic *P. infestans* populations in Indonesia (Wharton et al., 2022), but have not been evaluated against Bangladesh isolates. Thus, the objectives of this study were firstly to identify the genotypes causing late blight in the main potato-growing regions of Bangladesh and secondly to examine genotypic diversity in these populations. As little is known about these populations, this information is critical to ensure the effectiveness and durability of any 3 R-gene cultivars that are released in Bangladesh.

2 | MATERIALS AND METHODS

2.1 | Collection of *P. infestans* samples

A total of 160 samples (from both potato and tomato) were collected from the five major potato-growing divisions of Bangladesh (Table 1). Isolates of *P. infestans* ($n = 143$) were collected on Whatman Flinders Technology Associates (FTA) cards using the protocol suggested by the manufacturer (Sigma-Aldrich; Table 1). Lesions on infected leaves were placed on top of the cellulose matrix on the FTA card, the paper flap was closed over the leaf and using the blunt end of a pen, pressure was applied by rubbing to force juice out of the leaf into the matrix as described by Dangi et al. (2021). In addition to FTA card isolates, five isolates were successfully isolated from these infected leaves shipped from Bangladesh using the tuber method described previously (Dangi et al., 2021). Twelve pure cultures of *P. infestans* isolates collected in Bangladesh were also sent to Aberdeen R&E Center on pea agar. Details about the location and number of samples collected are listed in Figure 1 and Table 1.

2.2 | DNA extraction/purification of FTA card samples

FTA cards were processed according to the protocol provided by the manufacturer (GE Healthcare UK) and as described by Dangi et al. (2021). Briefly, two 3-mm punches were taken from each FTA card using a Harris cutting pad and 3-mm puncher. Punches were

TABLE 1 Isolates collected in this study and their locations in Bangladesh.

Division/country	Location	No. of isolates	DNA sample or isolates
Dhaka	Kishorganj, Joyedebpur, Munshiganj, Gazipur	42	DNA samples = 42
Mymensingh	Sherpur, Nakla	21	DNA samples = 21
Rajshahi	Bogora Sadar, Rajshahi	41	DNA samples = 30 (potato), 11 (tomato)
Rangpur	Khaturia, Debiganj, Sadar, Burirhat, Rangpur, Bandarganj, Gaibandha, Panchgarh, Boda, Fultola, Najirerhat	43	DNA samples = 34 (potato), 9 (tomato)
Sylhet	Shibgonj, Madhabpur, North Bejura	13	DNA samples = 13
US genotypes	Michigan and J. R. Simplot	10	DNA samples =10
UK genotypes	NA	2	Genotypes = 2

Note: All samples were collected in Bangladesh in 2018 from grower fields. Samples were collected from potato cultivars Granola, Lady Rosetta, Cardinal, Asterix, BARI-ALU-37, 38, 41, 46, 53, 64, 66, 67, 70, 71, 72, 77, HZD06-1249, Alberta, Diamant, Destiny, Alverston Russet, Ottawa, Surjomukhi, Sayada, Flora, Rosa Gold, LB-6, Atlantic and Kalpakri. Tomato cultivars and UK locations were unknown.

stored in a 2-mL microcentrifuge tube. Punches were washed with the FTA purification reagent twice followed by EDTA or isopropanol depending on the colour of the FTA card. If the punches were green (contained chlorophyll) they were washed with isopropanol. If they were white (contained no chlorophyll) they were washed with EDTA. Washed FTA cards were dried and stored at either −20°C or 8°C for no more than 7 days before further testing was carried out.

2.3 | DNA extraction and purification from pure cultures

Mycelia were scraped from 14-day-old cultures and 0.5 g was transferred to a 2-mL microcentrifuge tube. A magnetic bead DNA purification kit (Promega) and Kingfisher ML robot (Thermo Fisher Scientific) was used to extract and purify DNA from pure cultures. Briefly, 0.5 g mycelia was added to 1 mL of cetyltrimethyl ammonium bromide (CTAB) in a 2-mL microcentrifuge tube and the mycelia were gently macerated using a rubber pestle. Lysis buffer B (250 µL) was added and the tube was vortexed to mix the buffer and macerated mycelia. This was followed by 750 µL precipitation buffer. Samples were then vortexed and centrifuged for 10 min at 17,530 *g*. Samples were then placed in the Kingfisher ML robot for further processing and purification of sample DNA. After extraction and purification, DNA was stored at −20°C until needed.

FIGURE 1 Map of Bangladesh showing the five (out of the eight) divisions where most potatoes are grown. Black dots indicate the approximate locations where samples of *Phytophthora infestans* were collected.

2.4 | Mitochondrial haplotype test

Mitochondrial (mtDNA) haplotypes were determined by PCR-RFLP (Griffith & Shaw, 1998). Mitochondrial haplotype was only determined for isolates in pure culture as FTA cards did not contain enough DNA for amplification. Isolates were analysed for mitochondrial haplotype using the primer pairs P2F/P2R and P4F/P4R for specific mitochondrial DNA regions P2 and P4, respectively (Table S1). PCR conditions were 1 cycle of 94°C for 90 s; 40 cycles of 94°C for 40 s, 55°C for 60 s and 72°C for 90 s; and final extension at 72°C for 15 min. (Griffith & Shaw, 1998). The amplified PCR product (4 µL) was digested with the restriction enzymes MspI for the P2 region and EcoRI for the P4 region in a 20-µL volume at 37°C in a heating block. For MspI, digestion was carried out overnight and for EcoRI, digestion was carried out for 1 h. The digested PCR products 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) in Tris-borate-EDTA buffer. The gel was run at 10 V/cm for 60–90 min. Restriction patterns were visualized with a UV transilluminator at 254 nm. Images were recorded using a gel documentation system.

2.5 | Mating type determination

Three sets of DNA markers were used to determine the mating type of samples collected on FTA cards and pairing tests were carried out on live cultures. The living isolates were also tested with the three DNA markers to identify any differences in the two methods.

Cleaved amplified polymorphism sequence (CAPS) markers developed by Judelson et al. (1995) were used to determine mating types of *P. infestans* FTA card samples as described by Dangi et al. (2021). Briefly, DNA fragments were amplified using PCR and the products were cleaved with restriction enzyme BsuR1 and run on a 2% agarose gel for 90 min. Samples with the 550 base pairs (bp) band and 600 bp band were determined to be A1 mating type whereas samples with only the 600 bp band were determined to be A2 mating type. Results were cross-checked with AFLP molecular markers developed by Kim and Lee (2002).

Primer set Phyb1/Phyb2 was used to amplify only the A2 mating type samples. Target DNA samples were amplified by PCR, and the products were run on an agarose gel and visualized as described above. Samples with a 347 bp band were determined to be A2 mating type.

Finally, primers S1b/S2b developed by Judelson (1996) were used to identify A1 mating types as described above. Samples with a 1250 bp band were determined to be A1 mating type.

Pairing tests were done for the 17 living isolates with known genotypes EU_6_A1 or EU_13_A2 as described by Forbes (1997).

2.6 | Metalaxyl-M sensitivity test

Seventeen isolates from Rangpur ($n=1$), Dhaka ($n=5$) and Mymensingh ($n=11$) were tested for sensitivity to metalaxyl-M using the spiral gradient dilution method (Fairchild et al., 2013) and the poison agar method (Forbes, 1997). Metalaxyl-M solutions (Ridomil Gold EC 479.31 g metalaxyl-M/L; Syngenta Ag) and plates were prepared as described by Dangi et al. (2021). Briefly, in the poison agar method a 5-mm circular disk of actively growing mycelia from a 7-day-old culture was placed in the centre of a pea agar plate amended with fungicide concentrations of 0, 5, 10 or 100 mg/L. Plates were incubated in the dark at 18°C. After 7 and 10 days, radial growth of mycelia was measured, compared with the control plate and sensitivity calculated. In the spiral gradient dilution method, a spiral gradient of metalaxyl-M was laid down on plates so that the highest concentration of the fungicide was 10 g/L in the centre of the plate and zero at the edge of the plate. After 4 h, lines of 10 μL of sporangial suspension (10^5 sporangia/mL) were laid down from the outside to the inside of the plate. After 7 days, plates were scanned, growth of mycelia was measured using Adobe Photoshop and the EC_{50} determined as described by Fairchild et al. (2013).

2.7 | Multiplex simple-sequence repeat genotyping

One-step multiplex simple-sequence repeat (SSR) genotyping was conducted using 12 microsatellite markers as described by Li, Cooke, et al. (2013). The Type-it microsatellite PCR kit was used for multiplex amplification (Qiagen). The manufacturer's protocol was modified slightly as the PCRs were run in 15 μL reaction volumes instead of 25 μL. The PCRs were run on a thermocycler (Eppendorf). Twelve genotypes of *P. infestans* with known SSR alleles were also included in the PCR (Table 1). All PCR products from FTA cards were diluted at 1:50 (PCR product:PCR-grade water) and from living isolates were diluted at 1:100. The PCR products were submitted to Molecular Research Core Facility of Idaho State University (Pocatello, Idaho) for fragment analysis. Fragment analysis was performed with an AB 3130xl Genetic Analyser (Applied Biosystems). Alleles were scored on GeneMapper v. 5 software (Applied Biosystems, release 5.0, build ID FC3, licensed by ISU Molecular Research Core Facility). Allele bins were set for each marker and alleles were scored.

2.8 | Data preparation and analysis

Microsatellite data was obtained from all samples. Ninety-eight percent of the samples were triploids ($n=157$) with only a few diploids ($n=3$). To make the data uniform, a zero was added for the missing alleles of the diploids. Samples were collected from multiple potato cultivars in each division but cultivar was not considered as a factor in the data analysis. Standard genotypes from the Wharton laboratory *P. infestans* collection and some previously published data (Dey et al., 2018; Li, Cooke, et al., 2013) were included in the frequency-based analysis, such as neighbour-joining tree and minimum spanning network, to see how our data correlated with these standards. Data were analysed in R package poppr v. 0.2.3.0, polysat and genodive software as described previously by Grünwald et al. (2017) and Kamvar et al. (2014, 2015).

2.9 | Multilocus genotype analysis, population diversity statistics and mode of reproduction

Population diversity was determined by the number of multilocus genotypes (MLG) as described previously by Dangi et al. (2021). Estimated multilocus genotypes (eMLGs) after rarefaction and diversity statistics for the 160 polyploid data for 12 microsatellite loci were calculated using the R package poppr v. 2.3.0. (Grünwald et al., 2017; Kamvar et al., 2014, 2015; R Core Team, 2016). MLG diversity was estimated using the Shannon–Weiner Index in R package poppr (Kamvar et al., 2014, 2015; Shannon & Weaver, 1949). The expected heterozygosity (H_{exp}) was calculated for all samples based on Nei's unbiased

gene diversity (Nei, 1978). The mode of reproduction in each population was estimated based on the index of association (I_A) and standardized index of association value ($\bar{r}_d$; Agapow & Burt, 2001). Allelic diversity was calculated based on Simpson's index and Nei's unbiased gene diversity in poppr (Nei, 1978; Simpson, 1949).

2.10 | Population structure and differentiation

The genetic distance between the individual DNA samples was computed using a stepwise mutation model as described by Bruvo et al. (2004). A minimum spanning network and neighbour-joining tree were constructed in poppr based on Bruvo's distance. A neighbour-joining tree was constructed with 1000 bootstraps, viewed and modified on Figtree v. 1.4.3 (Kamvar et al., 2014, 2015; http://tree.bio.ed.ac.uk/software/figtree/). Discriminant analysis of principal components (DAPC) was performed in R package adegenet v. 2.0.1 to examine how the individual samples cluster to a population (Jombart, 2008; Jombart et al., 2010). Pairwise fixation indices (F_{ST}) were calculated on clone-corrected data using Bruvo's genetic distance in R package strataG v. 1.0.5 (Archer et al., 2017). To estimate variance in individual samples within and between populations, analysis of molecular variance (AMOVA) was carried out on clone-corrected data based on Bruvo's genetic distance in R package ade4 v. 1.7.5 (Excoffier et al., 1992).

3 | RESULTS

3.1 | Mating type and metalaxyl-M sensitivity test

DNA from all the FTA card samples and pure culture isolates was amplified with CAPS markers and determined to be the A2 mating type (Table 2). Results from amplification with the Phyb1/Phyb2 and S1b/S2b markers corroborated the CAPS results. However, only two samples from Rangpur were found to be A1 mating type, which is at odds with the SSR genotyping data that showed 11 samples clustering more closely with A1 genotypes. Seventeen isolates were tested for mating type using both the pairing test and CAPS markers and determined to be A2 mating type. All the pure cultures tested for metalaxyl-M were resistant except for two that were categorized as intermediate (Table 2; Figure S1). Testing of the isolates for mitochondrial haplotype showed that all of them were Ia haplotype.

3.2 | SSR genotyping

SSR data were compared with standard data from previously published sources (Dey et al., 2018; Li, Cooke, et al., 2013) and US and European isolates in our *P. infestans* collection. In this study, we designated most of our samples (74%) as genotype EU_13_A2 and its subclones (Figure 2; Table S2). This was based on SSR genetic fingerprints of the standard EU_13_A2 from more than 40 samples published previously (Dangi et al., 2021; Dey et al., 2018; Li, Cooke, et al., 2013), the haplotype test and the mating type and metalaxyl-M sensitivity tests. When a neighbour-joining tree was constructed including standard isolates, 74% of our DNA samples clustered with the standard EU_13_A2, 7% clustered next to EU_1_A1 and EU_6_A1, and 19% clustered separately from all the others (Figure 2). The percentage detection of *P. infestans* based on host and divisions revealed the dominance of EU_13_A2 in Bangladesh with other unique genotypes (Figure 3).

3.3 | MLG analysis population diversity statistics

Genotypic diversity of individuals in the Bangladeshi *P. infestans* population was evaluated and a total of 116 MLGs were determined from 160 DNA samples (Table 3; Table S2). The DNA samples from

TABLE 2 Phenotypic characterization of *Phytophthora infestans* samples from five divisions of Bangladesh.

Isolate	Location (host)	No. of DNA samples or isolates	Mating type[a]	Metalaxyl-M sensitivity test[b]
BN 16-18, 42-54, 56, 77-88, 116-123, 145-149	Dhaka (potato)	Isolates = 5, DNA = 37	A2	Resistant = 5
BN 3-7, 20, 21, 89-91, 150, 152-161	Mymensingh (potato)	Isolates = 11, DNA = 10	A2	Resistant = 9, intermediate = 2
BN 38-41, 57, 65-69, 96-115	Rajshahi (potato)	DNA = 30	A2	
BN 133-140, 142-144	Rajshahi (tomato)	DNA = 11	A2	
BN 8-13, 19, 22, 26-30, 32-37, 55, 58-61, 71-76, 92, 93, 95, 151	Rangpur (potato)	Isolates = 1, DNA = 33	A1 = 2, A2 = 32	Resistant = 1
BN 124-132	Rangpur (tomato)	DNA = 9	A2	
BN 1, 2, 14-15, 23-25,31,62-64,70,94	Syllhet (potato)	DNA = 13	A2	

[a]Isolates were tested for mating type using both the pairing test and PCR method. DNA samples were tested using PCR.

[b]Only living isolates were tested for metalaxyl-M sensitivity. Isolates were considered resistant if they had growth on both 5 and 100 mg/L poison agar plates with greater than 40% growth compared to control (0 mg/L), intermediate if they had growth on 5 mg/L plate with greater than 40% growth compared to control (0 mg/L) and sensitive if they had less than 40% growth on both 5 and 100 mg/L plates compared to control (0 mg/L).

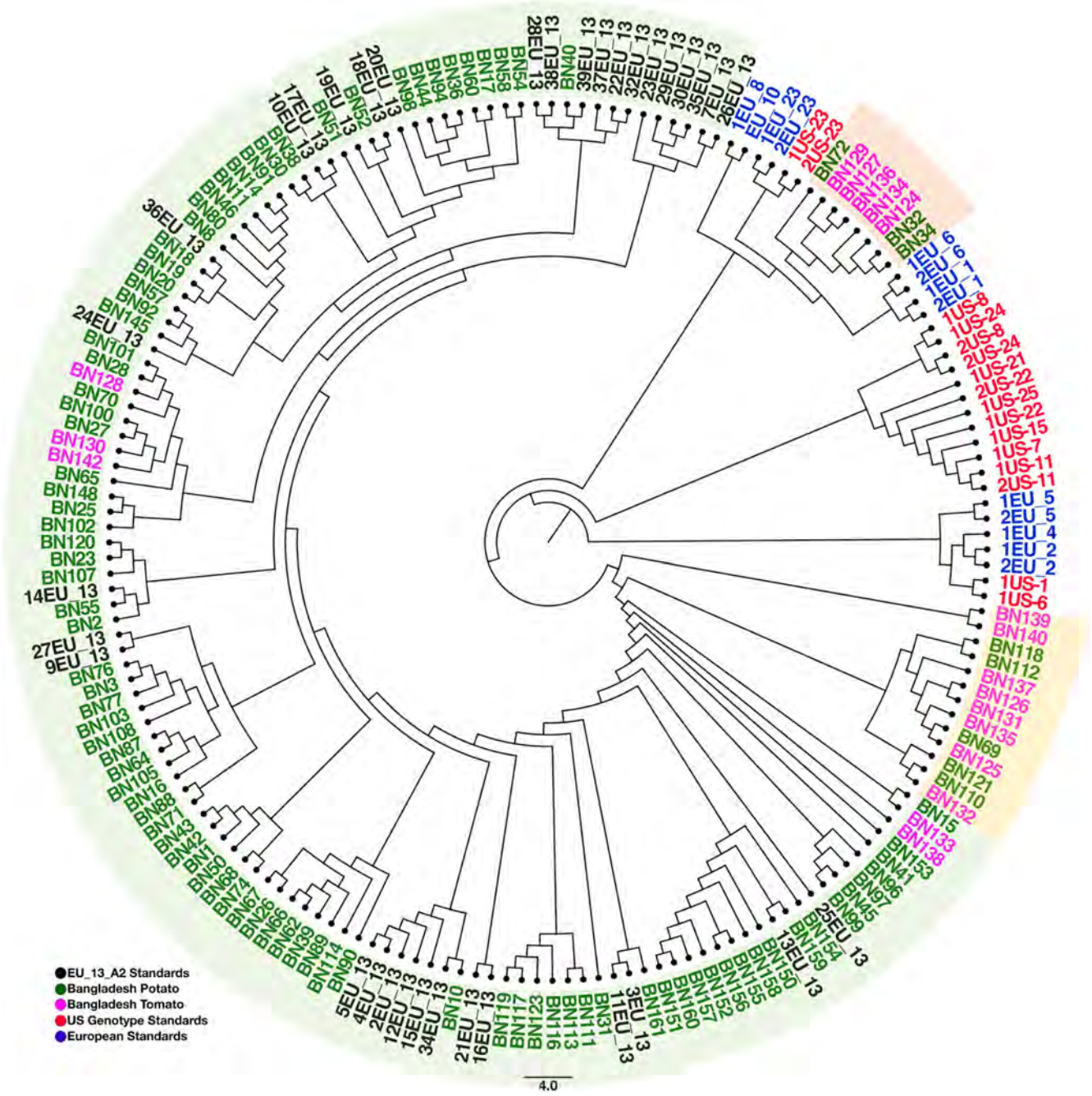

FIGURE 2 Neighbour-joining tree based on Bruvo's genetic distance for *Phytophthora infestans* populations from potato and tomato collected in five divisions of Bangladesh along with European and US standard isolates (with 1000 bootstraps replicates). Data was analysed in the R package poppr v. 2.3.0, results viewed and modified using Figtree v. 1.4.3. The colour of the sample name indicates their geographic location. EU_13_A2 standards and variants are highlighted in light green shading; A1 mating type variants are shaded in dusky pink and other unique MLGs are shaded in yellow.

potato in Rajshahi had the greatest number of MLGs ($n = 26$) and based on the Shannon–Weiner index of diversity, H (3.22), were the most diverse (Table 3). However, *P. infestans* populations from potato in Rangpur and Dhaka also had high H values. Among the divisions, the Rajshahi population from potato also had the highest genetic richness (eMLG) at 9.59 (Table 3). Of the 20 DNA samples collected from tomato in Rajshahi and Rangpur, 18 were distinct MLGs. However, based on the Shannon–Weiner index, they had a lower population diversity than those from potato, with H values of 2.1 and 2.2, respectively (Table 3). All the divisions had a standardized index of association (I_A) value greater than zero, supporting the evidence of clonal reproduction in all divisions (Table 3). An I_A of zero indicates sexual reproductionwhereas any value other than zero suggest asexual or clonal reproduction.

Allelic diversity of the 160 DNA samples based on geographic distribution was calculated using poppr. A total of 49 different

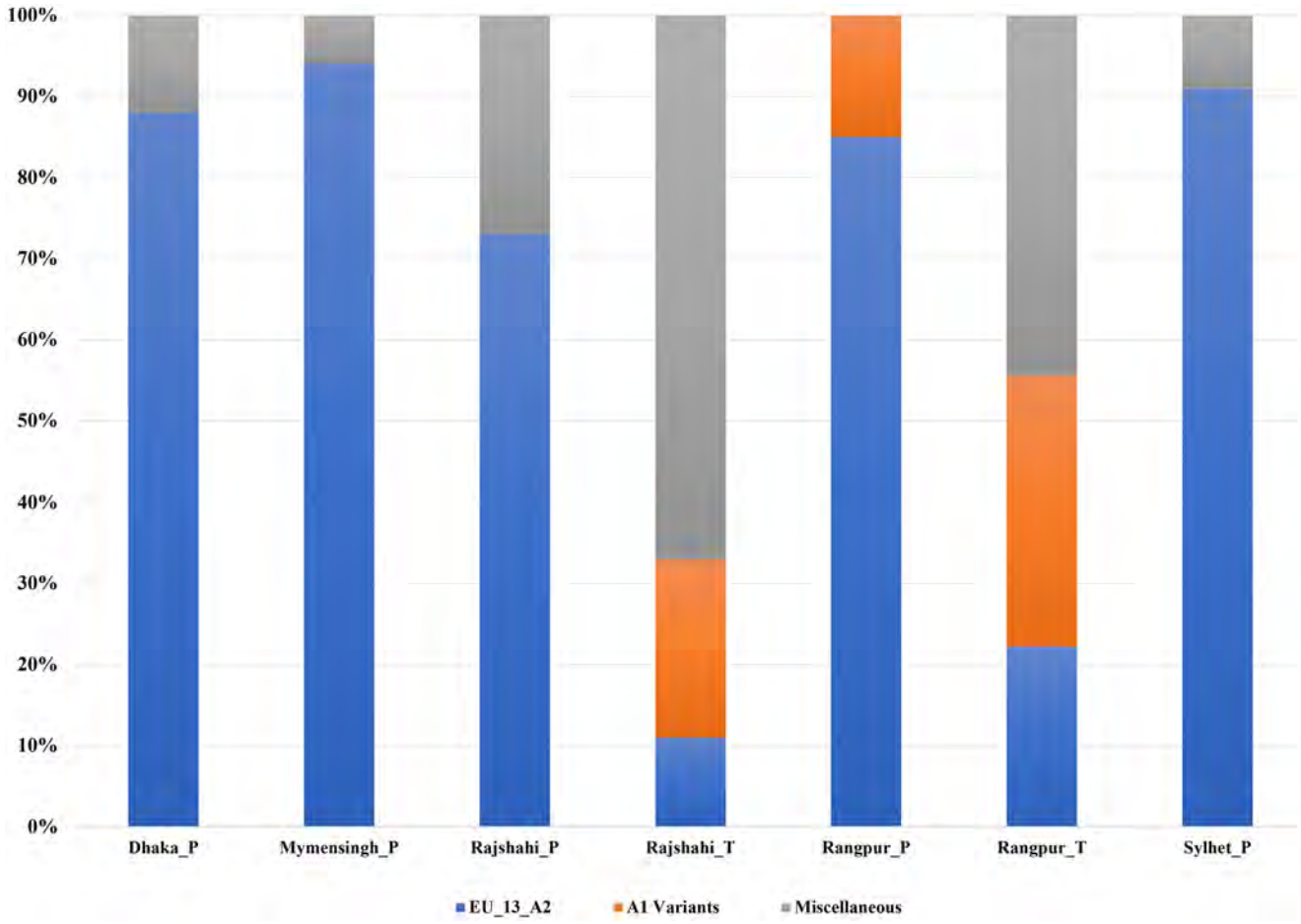

FIGURE 3 Percentage detection of genotypes of *Phytophthora infestans* by Bangladesh division. Genotypes isolated from potato are indicated by a P following the division name and those from tomato are indicated by a T.

alleles were detected from 160 DNA samples with a mean of 4.083 alleles per locus (Table 4). Among these, D13 had the most with 13 followed by PiG11 with seven, and Pi04, Pi70 and SSR2 were the least diverse with two (Table 4). Gene diversity was estimated using Simpson's index (1–*D*) and ranged from 0.043 for SSR2 to 0.798 for D13, indicating that the SSR2 locus was the least diverse and D13 was the most diverse. Of the loci, Pi04 and SSR6 were found to be the most evenly distributed alleles, with evenness values of 1.0 and 0.965, respectively (Table 4). With only two alleles and an evenness value of 0.403, locus SSR2 was the least distributed allele. Although the D13 locus had the highest number of alleles and a high allelic diversity it only had a moderate evenness distribution of 0.744 (Table 4).

3.4 | Population differentiation and structure

AMOVA was computed based on Bruvo's genetic distance on clone-corrected data to determine whether variation was due to individual DNA samples within a population or among populations. Results showed that variation between populations (divisions) was 9%, whereas 91% of variation was accounted for within populations in each division (Table 5). Pairwise F_{ST} values were calculated on clone-corrected data using Bruvo's genetic distance and supported the AMOVA data (Table 6). The pairwise F_{ST} values for the different populations ranged between 0.0024 and 0.3174, revealing low to modest differentiation between the populations in each division (Table 6). The highest differences were found between populations from tomato in Rangpur and populations from potato in Mymensingh (0.3174). Populations from potato in Rajshahi and Rangpur had the lowest F_{ST} values (0.0024). The low F_{ST} values between most of the divisions indicates ongoing migration events between all divisions except Mymensingh. Even populations in Sylhet, which is geographically separated from Rangpur and Rajshahi, had low F_{ST} values indicating very little differences between their *P. infestans* populations (Table 6).

The DAPC result supports the small amount of variation among the divisions as most of the populations (four out of seven) were clustered together. Only DNA samples from Mymensingh and tomato DNA samples from Rangpur and Rajshahi clustered separately (Figure 4). All 160 samples from five divisions grouped together in four clusters. The main cluster consisted of DNA samples from Dhaka, Rangpur, Rajshahi and Sylhet with separate clusters of DNA

TABLE 3 Multilocus genotype and diversity statistics for 12 microsatellite loci in populations of *Phytophthora infestans* from Bangladesh by division and host collected in 2018.

Population	Host	*N*	MLG	eMLG (*SE*)	*H*	H_{exp}	Evenness	I_A	$\bar{r}_d$
Division									
Dhaka	Potato	42	25	8.11 (1.13)	2.91	0.44	0.68	1.45	0.216
Mymensingh	Potato	21	16	8.13 (1.04)	2.53	0.44	0.66	0.41	0.076
Rajshahi	Potato	30	26	9.59 (0.58)	3.22	0.46	0.95	0.67	0.099
Rajshahi	Tomato	11	9	8.27 (0.45)	2.10	0.52	0.86	0.79	0.082
Rangpur	Potato	34	20	7.95 (1.12)	2.73	0.47	0.74	3.94	0.451
Rangpur	Tomato	9	9	9.00 (0.00)	2.20	0.54	1.00	1.14	0.115
Sylhet	Potato	13	11	8.85 (0.64)	2.35	0.44	0.94	0.20	0.069
Total		160	116	9.05 (0.94)	4.09	0.46	0.52	2.05	0.223
Host									
Potato		140	98	16.30 (1.68)	3.92	0.45	0.53	1.69	0.204
Tomato		20	18	17.00 (0.00)	2.72	0.52	0.81	0.99	0.101
Total		160	116	16.70 (1.64)	4.09	0.46	0.53	2.05	0.223

Abbreviations: eMLG, expected number of MLG; *H*, Shannon–Weiner index of MLG diversity; H_{exp}, Nei's unbiased gene diversity; I_A, index of association; MLG, number of multilocus genotypes; *N*, number of individuals; $\bar{r}_d$, standardized index of association.

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

Locus	Allele	1–*D*	H_{exp}	Evenness
D13	13.0	0.798	0.800	0.744
Pi4B	3.0	0.532	0.533	0.900
PiG11	7.0	0.694	0.696	0.815
Pi04	2.0	0.500	0.502	1.000
Pi63	3.0	0.532	0.533	0.900
Pi70	2.0	0.123	0.123	0.510
SSR2	2.0	0.043	0.043	0.403
SSR3	4.0	0.567	0.569	0.868
SSR4	4.0	0.637	0.639	0.921
SSR6	3.0	0.498	0.499	0.965
SSR8	3.0	0.527	0.529	0.888
SSR11	3.0	0.072	0.073	0.419
Mean	4.083	0.460	0.461	0.778

Abbreviations: 1–*D*, Simpson index; $H_{exp,}$ Nei's unbiased gene diversity.

TABLE 5 Analysis of molecular variance for clone-corrected *Phytophthora infestans* populations in Bangladesh based on Bruvo's genetic distance.

Source	*df*	SS	MSS	% variance
Between divisions	6	0.009	0.002	9.10
Within divisions	109	0.065	0.001	90.90
Total	115	0.074	0.0006	100

samples from Mymensingh and tomato DNA samples from Rangpur and Rajshahi. However, a few DNA samples from Rangpur clustered with Mymensingh and tomato DNA samples from Rangpur (Figure 4). The minimum spanning networkrevealed that most of the DNA samples clustered with the EU_13_A2 standards, whereas the US genotypes and other EU genotypes were distinct and separate (Figure 5).

In a neighbour-joining tree constructed based on Bruvo's distance with 1000 bootstraps, 86 MLGs grouped together with the EU_13_A2 standards (Figure 2). Of the 18 MLGs from tomato, only three grouped together with EU_13_A2. Most of them (10 MLGs) grouped separately from the standards. However, five of the tomato MLGs grouped next to EU_6_A1 and EU_1_A1 (Figure 2), and these tended to be characterized by the possession of allele 190 at Pi70 (Table S2). Only 12 MLGs from potato grouped separately with the tomato DNA samples and three grouped next to EU_6_A1 and EU_1_A1. These potato MLGs also possessed allele 190 at Pi70.

4 | DISCUSSION

This study provides an in-depth investigation into the genotypes that make up populations of *P. infestans* in the main potato-growing regions of Bangladesh. Of 160 DNA samples that were collected, all but two were determined to be the A2 mating type, all were Ia haplotype and 15 of the 17 pure cultures tested were resistant to metalaxyl-M. These results correlate well with those from the more limited previous studies where all isolates were identified as EU_13_A2 (Guha Roy et al., 2021; Pronk et al., 2017).

Microsatellite analysis revealed that about 74% of the DNA samples were EU_13_A2 with a large amount of subclonal variation. Similar levels of subclonal variation were found in EU_13_A2 populations in India (Dey et al., 2018) and EC-1 populations in Ecuador (Delgado et al., 2013). There may be many reasons for the large subclonal variation that is seen in clonal lineages. One is polyploidization,

TABLE 6 Pairwise F_{ST} values for clone-corrected *Phytophthora infestans* populations from two hosts and five divisions in Bangladesh.

	Sylhet	Mymensingh	Rangpur	Dhaka	Rajshahi	Rangpur (T)
Mymensingh	0.0151					
Rangpur	0.0059	0.1399				
Dhaka	0.0071	0.1597	0.0028			
Rajshahi	0.0092	0.1869	0.0024	0.0049		
Rangpur (T)	0.0264	0.3174	0.0169	0.0202	0.0204	
Rajshahi (T)	0.0245	0.3144	0.0188	0.0230	0.0228	0.0144

Note: The lower the F_{ST} value, the greater the similarity between two populations. Divisions followed by (T) indicate DNA samples collected from tomato.

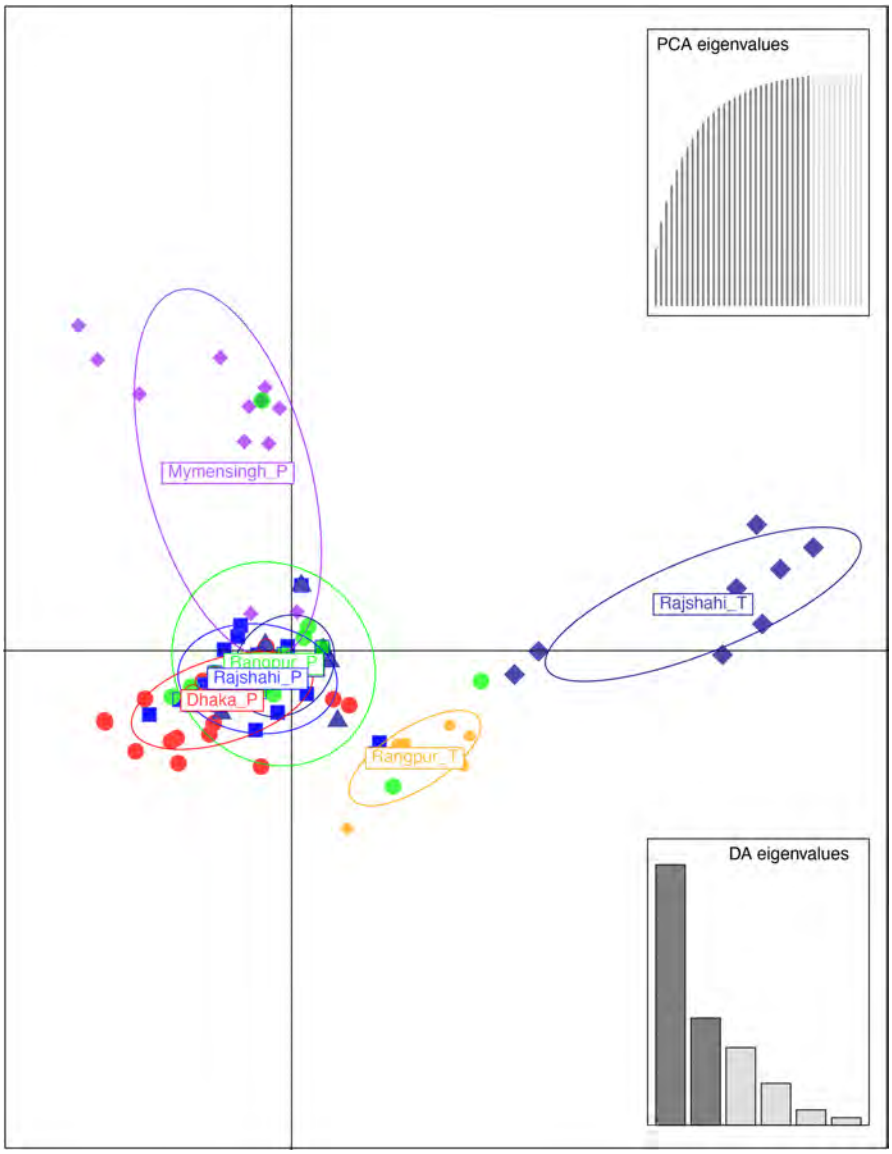

FIGURE 4 Discriminant analysis of principal components of *Phytophthora infestans* populations collected from five divisions of Bangladesh in 2018. Potato populations from each division are denoted by a P following the division name and tomato populations by a T (data were analysed in R package poppr v. 2.3.0).

which will potentially increase the number of alleles at a locus (Li et al., 2017). In our analysis, most of the DNA samples collected in this study were presumed to be triploid ($n = 157$) based on the presence of three alleles. It has been reported previously that clonal isolates tend to be triploid and most of the successful clonal lineages are triploid because they have more heterozygous single-nucleotide polymorphisms and a higher level of functional variations compared to diploids (Li et al., 2017). The occurrence of polyploidization may mask accumulated deleterious alleles in asexual populations that otherwise may lead to extinction during evolution (Li et al., 2017). In this study, the D13 and PiG11 loci had high allelic diversity compared to other loci, at 13 and 7, respectively. Li, van der Lee et al. (2013) previously reported a similar trend for high diversity in D13 and PiG11 loci in EU_13_A2 isolates. The EU_13_A2 genotype has been a very successful lineage in Europe and has been reported to be the dominant genotype in many Asian countries including China, India,

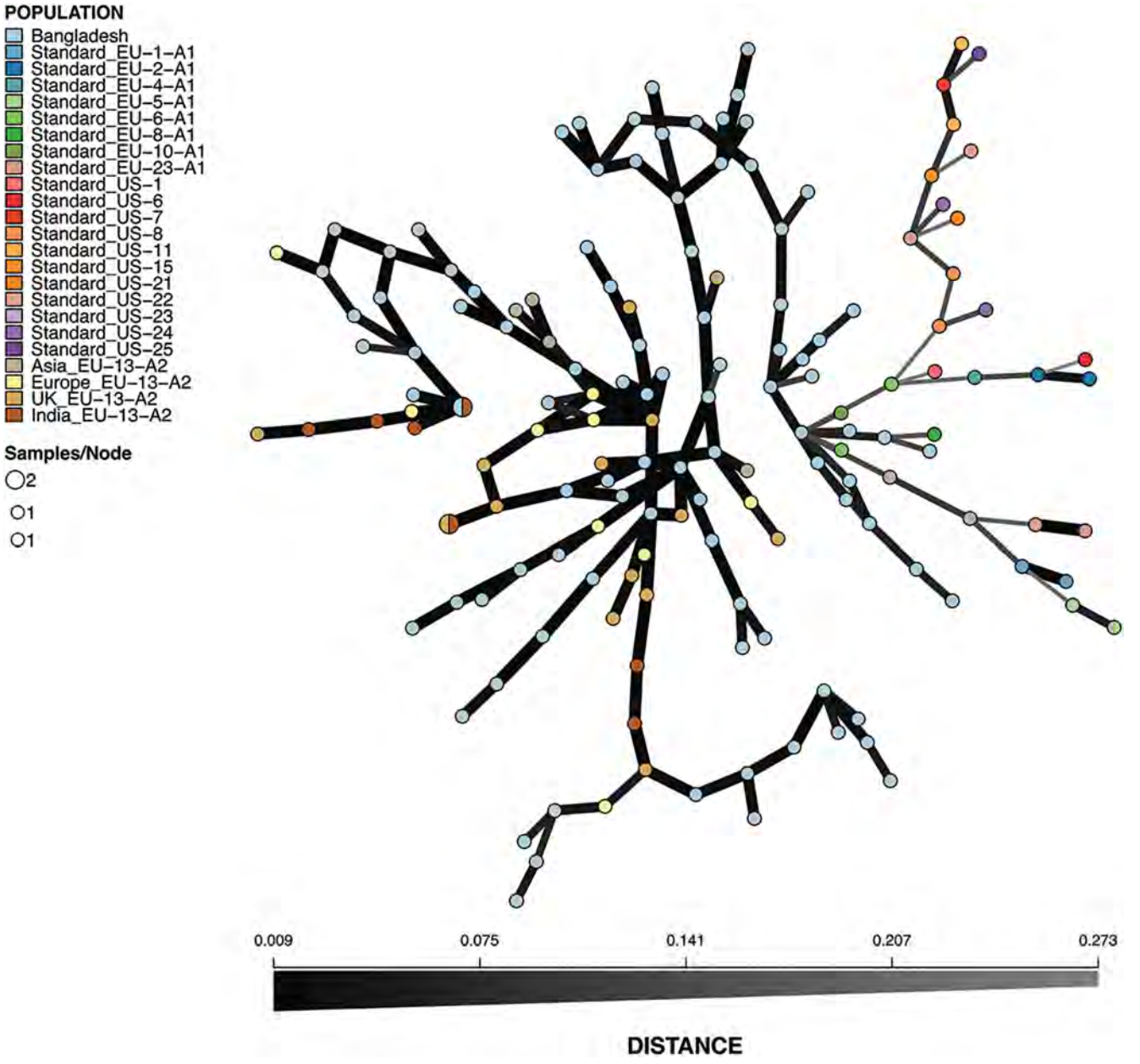

FIGURE 5 Minimum spanning network of *Phytophthora infestans* isolates/DNA samples from Bangladesh collected from potato and tomato in 2018. These are compared with representative European, Indian and US isolates. The size of the node represents the number of multilocus genotypes. Most samples from Bangladesh clustered with EU_13_A2 standards from Europe, the UK and India, whereas the standards from the United States clustered separately.

Nepal and Pakistan (Guha Roy et al., 2021). It was also reported to be the main clonal lineage found in the states of India that neighbour Bangladesh, and responsible for a severe late blight epidemic in eastern India in 2014 (Dey et al., 2018).

A total of 17 MLGs were found out of 20 samples obtained from tomato. Furthermore, the MLGs from tomato were different to those found on potato with most of them grouping together in the neighbour-joining tree. Only three of the tomato MLGs clustered together with the standard EU_13_A2, five of them closer to EU_6_A1 and EU_1_A1, and 10 of them clustered separately from all other samples. Chowdappa et al. (2013) reported that EU_13_A2 is pathogenic on tomato and some subclonal isolates of EU_13_A2 have been reported to only infect tomato or be less aggressive on potato compared to tomato (Dey et al., 2018). Based on the neighbour-joining tree data, in this study it appears that the *P. infestans* populations found on tomatoes and potatoes were much more species specific. The subclonal variation in *P. infestans* populations from tomato was also higher than in populations from potato. Six out of the eight samples that clustered closer to EU_6_A1 and EU_1_A1 were from the Rangpur division with three from potato and three from tomato. Samples from Rajshahi had the highest diversity with 26 out of the 30 DNA samples from potato being unique MLGs and nine out of 11 DNA samples from tomato being unique MLGs. One explanation for this level of diversity in Rajshahi could be a lack of migration or gene

flow between regions. However, when the distribution of genetic variation in Bangladesh *P. infestans* populations was examined using AMOVA it was found that there was very little variation between populations in the different divisions. Individual samples within the divisions contributed 91% of the variation compared to the populations between divisions, which provided only 11% of the variation. This indicates that migration and gene flow are common between regions.

Looking at population differentiation we observed a similar trend. Pairwise F_{ST} values, which ranged from 0.0024 to 0.3174, indicate that the differentiation between populations was very low. In Bangladesh, only about 5%–10% of potato seed is brought to the farm each year and the remainder is graded from last year's harvest and cold stored until the next potato season starts in November. Only the larger growers can afford to purchase seed that has been imported from abroad, with the main seed imports coming from the Netherlands (Pronk et al., 2017). This reuse of seed would explain the high levels of variation found within the divisions. Interestingly, *P. infestans* populations from Mymensingh, which sits between the divisions of Rajashahi and Rangpur to the west and Sylhet to the east, had the highest level of differentiation from all the other divisions. The high F_{ST} values indicate that there is limited gene flow between Mymensingh and the other divisions. The locations of the sites where the DNA samples were collected in Mymensingh were all close to the border with India and the Indian state of Meghalaya. It may be that *P. infestans* in this region is more closely related to populations from Meghalaya that were introduced to Bangladesh through informal movement of potatoes and tomatoes across the border as well as aerial movement of sporangia from field to field. Dey et al. (2018) previously reported that isolates of EU_13_A2 from Meghalaya were distinct from the isolates of West Bengal. However, populations from Mymensingh and Meghalaya have not been compared so this remains speculation.

The DAPC separated the DNA samples into four distinct groups that corroborated the results from the other analyses. Results showed that *P. infestans* populations from Mymensingh clustered separately from the other potato populations, which clustered together. The *P. infestans* populations from tomato clustered into two separate groups, one group containing DNA samples from Rajshahi and the other samples from Rangpur and these were distinct from the potato populations. This is not surprising given that most of the tomato MLGs were not EU_13_A2 and supports the data from the neighbour-joining tree.

This study provides insights into the structure and diversity of *P. infestans* populations in Bangladesh. In recent years, EU_13_A2 has been reported to be the dominant *P. infestans* genotype in most south-east Asian countries such as India, Pakistan, Nepal, China, Myanmar and Bangladesh (Chowdappa et al., 2013, 2015; Cooke et al., 2019; Dey et al., 2018; Guha Roy et al., 2021). Chowdappa et al. (2013) reported the introduction of EU_13_A2 to India causing a severe outbreak of late blight on tomato in 2009. These authors speculated that EU_13_A2 was introduced to India on seed potatoes imported from the UK or the Netherlands. EU_13_A2 continues to dominate *P. infestans* populations in India and continental Asia so it was not surprising to find that EU_13_A2 is the dominant *P. infestans* genotype in Bangladesh. Trade in seed and ware potatoes plays an important role in the process of migration of *P. infestans* genotypes (Fry, 2020). Although officially there may be no trade in seed potatoes between India and Bangladesh, informal movement of potatoes, tomatoes and other vegetables across the border occurs frequently and represents an opportunity for the introduction of new genotypes into Bangladesh.

In Europe, programmes that monitor and track changes in populations of *P. infestans* over time have been extensively used to enhance the effectiveness of current management strategies. These strategies have included host resistance management, fungicide resistance management, the use of integrated pest management tools and sophisticated decision support systems (Cooke et al., 2011). For the most part these systems do not exist in Bangladesh and growers rely almost exclusively on the use of fungicides to produce viable potato yields. However, a total reliance on fungicides for the management of late blight is not the best option, especially when information on effective fungicides is limited. Decision support systems for the control of late blight in Bangladesh would be much more useful. In 2015, the GEOPOTATO project began developing a decision support system in Bangladesh to help smallholder growers manage late blight. To overcome the lack of a network of weather stations in the main potato-growing regions of Bangladesh the system uses satellite data in the disease risk models (Pronk et al., 2017). Another alternative method of disease management is the use of resistant cultivars. The USAID Feed the Future Biotechnology Potato Partnership (FtFBPP) was established to develop and introduce 3 R-gene late blight-resistant bioengineered potatoes of cultivars preferred by local farmers and consumers into Bangladesh. This will be of significant benefit to potato growers in Bangladesh because it will reduce the cost of production through less fungicide costs, reduced famer exposure to fungicides and reduced fungicide residues in food, land and wastewater.

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

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DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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