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Severe outbreaks of late blight on potato and tomato in South India caused by recent changes in the *Phytophthora infestans* population

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Late blight, caused by *Phytophthora infestans*, has emerged as the most destructive disease of potato and tomato in South India since 2008. One hundred and fifty-seven isolates of *Phytophthora infestans*, 63 from potato and 94 from tomato, were collected from major potato and tomato production areas of South India between 2010 and 2012. Their phenotypic and genotypic characteristics were determined and compared with reference isolates. Isolates were characterized based on mating type, *in vitro* metalaxyl sensitivity, mitochondrial DNA haplotype, RG57 DNA fingerprinting patterns, SSR markers and aggressiveness on potato and tomato, in order to monitor population changes in *P. infestans*. All isolates were A2 mating type, metalaxyl resistant, mtDNA haplotype Ia and had RG57 and SSR fingerprints almost identical to the 13_A2 clonal lineage reported in Europe. Variation at the D13 and SSR4 loci allowed discrimination of minor variants, designated as 13_A2_3, 13_A2_3b, 13_A2_3c and 13_A2_1. A comparison of the lesion diameters caused by 157 isolates on detached leaflets of three potato and tomato cultivars showed all isolates to be equally aggressive, confirming that the same clonal population is infecting both hosts. This study demonstrates that the 13_A2 lineage was responsible for severe late blight outbreaks on potato and tomato in South India and has replaced the prior population represented by the US-1 and other genotypes. Revised management strategies will be required to combat this destructive 13_A2 clonal lineage and monitoring of the population across other potato- and tomato-growing regions of India is warranted.

Keywords: 13_A2 blue lineage, late blight, *Phytophthora infestans*, tomato

Introduction

The stramenopile *Phytophthora infestans*, commonly known as the Irish potato famine pathogen, is responsible for yield losses of $6·7 billion annually in potato, and crop losses up to 100% in tomato (Nowicki *et al.*, 2012), and has been considered a threat to global food security (Cooke *et al.*, 2012). *Phytophthora infestans* is a heterothallic species with A1 and A2 mating types, and pairing between these two isolates results in the formation of sexually recombinant oospores (Goodwin & Drenth, 1997). Before the 1980s, the *P. infestans* population in many countries of the world, except central Mexico, was dominated by a clonal lineage, the 'old' population (Goodwin *et al.*, 1994). This 'old' population was thought to consist of only a single A1 mating type lineage termed US-1, defined by its mitochondrial DNA (mtDNA) haplotype Ib, allozymes Gpi 86⁄100 and Pep 92⁄100 and a characteristic RG57 pattern (Goodwin *et al.*, 1994). More recently, another lineage termed HERB-1 has also been discovered occurring prior to US-1 (Yoshida *et al.*, 2013). During the 1980s, a 'new' population from central Mexico, the centre of diversity of *P. infestans*, spread to other parts of the world through imported potatoes (Goodwin & Drenth, 1997). This 'new' population consisted of both A1 and A2 mating types, and Ia and IIa mtDNA haplotypes (Spielman *et al.*, 1991; Lebreton & Andrivon, 1998). New populations also carried resistance to metalaxyl, which had been widely used to control late blight, making management more difficult (Gisi & Cohen, 1996). The 'new' population rapidly replaced the US-1 population in many countries in Europe (Spielman *et al.*, 1991). Population displacement of the 'old' population or sexual recombination within the more aggressive 'new' population has resulted in the severe outbreaks of late blight caused by *P. infestans* worldwide in recent decades (Spielman *et al.*, 1991; Fry & Goodwin, 1997; Yuen & Andersson, 2013).

Several studies on characterization of the *P. infestans* population in Asian countries have shown a migration of the 'new' population to these areas. Nishimura *et al.* (1999) reported the presence of A2 mating type isolates and Asian-specific allozyme genotypes in the isolates collected from Korea, India, Taiwan, Indonesia, Thailand,

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Nepal and China between 1992 and 1997. A dominant clonal lineage, SIB-1, was found in Siberian populations sampled between 1997 and 1998 (Elansky *et al.*, 2001) and this clonal lineage subsequently moved to China and Japan (Akino *et al.*, 2004). In the Taiwanese population, sampled between 1991 and 2001, Deahl *et al.* (2002) reported population displacement. Ghimire *et al.* (2003) described 11 diverse genotypes and a new dominant population with mtDNA haplotype Ia in Nepal. Gotoh *et al.* (2005) found 20 genotypes in isolates collected in eight southeast Asian countries between 1992 and 2000.

In India, potato and tomato are the most important vegetable crops, grown on 1·83 and 0·53 million ha, with a total annual production of 3·73 and 9·36 million tonnes, respectively (Chowdappa *et al.*, 2011). Prior to 2006, late blight was an annual threat in the states of northern India, but it was not considered a major problem on potato or tomato production in South India (Chowdappa *et al.*, 2011). Since the 2008 growing season, severe late blight epidemics have occurred on both tomato and potato crops in Karnataka, Tamil Nadu and Andhra Pradesh and have often caused 100% crop loss (Chowdappa *et al.*, 2013). The disease incidence was very severe even on Kufri Jyoti, a highly popular potato cultivar known to be partially resistant to late blight. Although late blight has been known on potato in the Karnataka state of India since 1953, serious epidemics have only been observed on tomato since 2008 (Chowdappa *et al.*, 2013).

Survey data used to characterize the population of *P. infestans* in India is fragmentary. An analysis of a small sample of *P. infestans* isolates from northern India in 1993 revealed A1 mating type isolates that were distinct from the US-1 genotype (Nishimura *et al.*, 1999; Gotoh *et al.*, 2005). Conversely, Chimote *et al.* (2010) examined 70 isolates, also primarily from the north of India, and reported the presence of A1 mating type, metalaxyl-sensitive isolates of the Ib mtDNA haplotype (presumed US-1 lineage) prior to 2002 and a progressive displacement by 'new' A1 and A2 lineages in the subsequent years up until 2005. Singh *et al.* (1994) reported the occurrence of the A2 mating type on potato in northern India in 1994. On the basis of analysis of tomato isolates collected from certain localities in Bangalore, Chikkaballapur and Kolar districts of Karnataka from 2009 onwards, Chowdappa *et al.* (2013) proposed that the 13_A2 clonal lineage was recently introduced into India from Europe. The objectives of this study were: (i) to determine the mating type distribution of *P. infestans*, (ii) to monitor the phenotypic and genotypic changes in the *P. infestans* population, and (iii) to relate these changes to the recent severe late blight outbreaks on potato and tomato in South India.

Materials and methods

Collection and isolation of *Phytophthora* cultures

Isolates of *P. infestans* were obtained from leaves and fruit of tomato, and leaves of potato plants in Bangalore Rural, Hassan, Chikkaballapur, Chikmagalur and Kolar districts of Karnataka, Udhagamandalam in Tamil Nadu and the Chittoor district of Andhra Pradesh (Table 1; Fig. 1) during severe late blight epidemics in the July–December crop seasons of 2010, 2011 and 2012. When late blight infection was widespread in the tomato and potato fields in these districts, leaflets with single lesions were sampled and one isolate was obtained from each field. Sampled fields were 10 to 15 km apart. From this collection, a representative set of 157 isolates were chosen that covered 157 fields in all of the important potato- and tomato-growing regions in South India (Table 1). Isolations were made directly from sporulating lesions by picking sporangia. For isolations from non-sporulating lesions on tomato, infected leaf tissue was placed in a 90 mm Petri plate on a moistened sterile filter paper, covered with Parafilm, and incubated at 18°C in darkness for 2–3 days to induce sporulation. In the case of potato, leaf pieces (5 × 5 mm) cut from the advancing margin of late blight lesions were placed between two slices of potato tuber and incubated in Petri plates on moistened filter paper for 4–5 days at 18°C in darkness for induction of mycelia growth and sporulation. The sporangia were picked with the tip of a sterile scalpel and transferred onto rye agar A plates amended with ampicillin (100 mg L^{-1}), nystatin (100 mg L^{-1}) and rifampicin (50 mg L^{-1}). Pure cultures were obtained by transferring hyphal tips to unamended rye agar A plates at 18°C for 10–15 days. Isolates were maintained on rye agar A at 18°C in darkness and were transferred every 4–5 weeks. For long-term maintenance,

Table 1 Origin and number of *Phytophthora infestans* isolates collected from potato and tomato at different locations in South India from 2010 to 2012

	Potato				Tomato			
State and district	2010	2011	2012	Total	2010	2011	2012	Total
Karnataka								
Bangalore rural	4	1	4	9	5	3	2	10
Chikkaballapur					8	5	4	17
Chikmagalur	2	2	5	9	3	2	4	9
Hassan	1	2	5	8	5	7	1	13
Kolar	5	2	6	13	7	9	6	22
Tamil Nadu								
Udhagamandalam	5	7	12	24	2	3	6	11
Andhra Pradesh								
Chittoor	–	–	–	–	8	2	2	12
Total	17	14	32	63	38	31	25	94

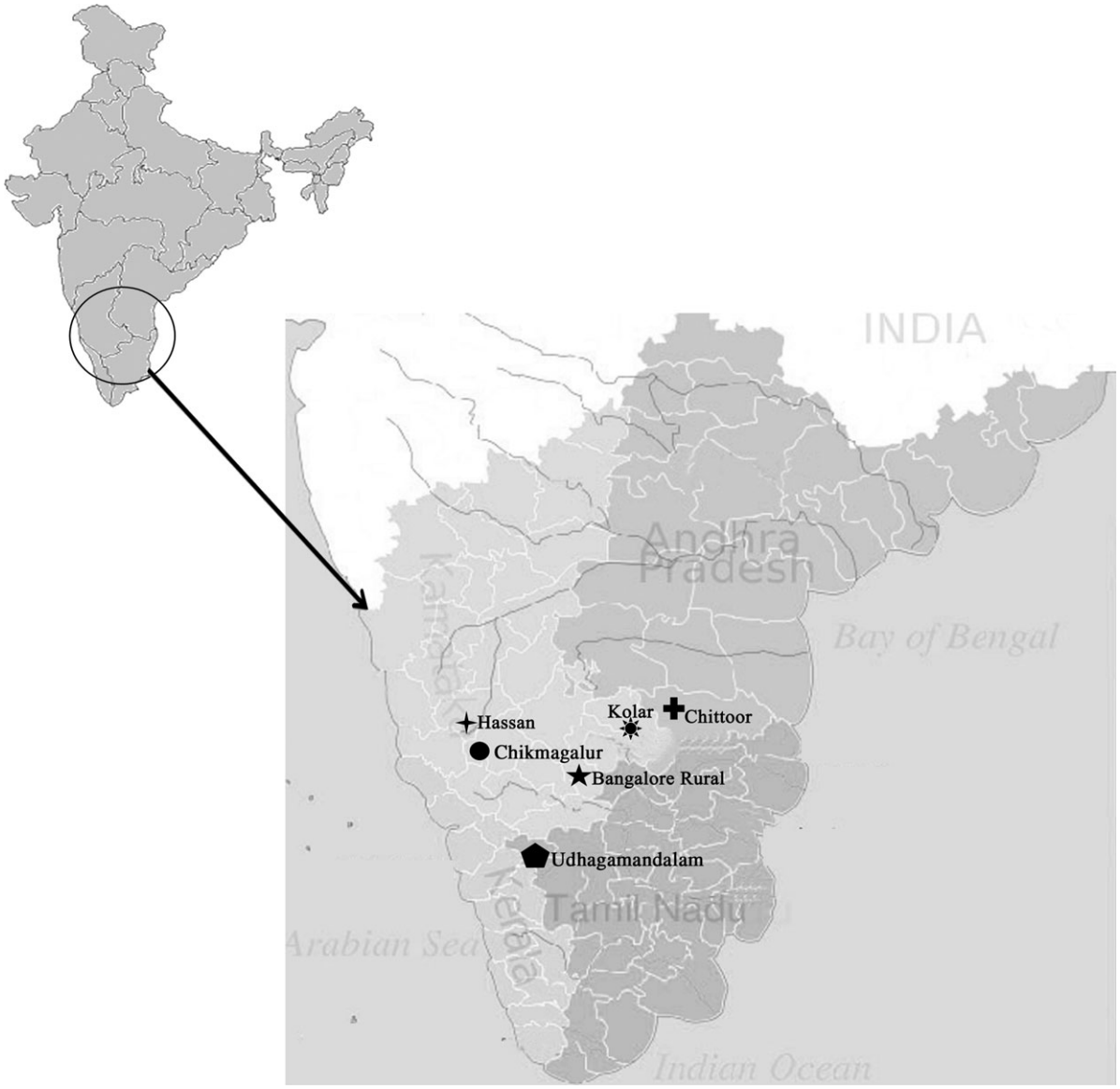

Figure 1 Map of South India where isolates of *Phytophthora infestans* were collected from 2010 to 2012. Most of the potato fields are in Hassan and Chikmagalur districts of Karnataka and Udhagamandalam districts of Tamil Nadu and tomato fields are in Bangalore, Chikkaballapur and Kolar districts of Karnataka and Chittoor district of Andhra Pradesh.

isolates were stored on rye agar A slopes at 10°C under sterile mineral oil.

Mating type determination

The mating type of all the isolates was determined by placing 4 mm diameter mycelial plugs of *P. infestans* isolates and reference isolates of the A1 (Jab10-23) or A2 (HP-10-85) mating types at opposite edges (approximately 20 mm apart) of Petri dishes containing rye agar A medium (Chowdappa *et al.*, 2013). The paired cultures were incubated at 15°C for 15–20 days and then microscopically examined for the presence of oospores at the contact zones between the unknown and the reference isolates. Closely linked molecular markers such as cleaved amplified polymorphic sequence (CAPS) assays using the primer pair W16-1 and W16-2 (Judelson *et al.*, 1995) and the PHYB-1 and PHYB-2 primer set (Kim & Lee, 2002) were also used to confirm the mating type.

Metalaxyl sensitivity

In vitro sensitivity to metalaxyl was determined by assessing growth on rye A agar plates amended with 5 or 100 μg mL^{-1} metalaxyl at 18°C after 7 days' inoculation. Isolates with <40% of control growth at 5 and 100 μg mL^{-1} were designated as metalaxyl sensitive; those with >40% of control growth at 5 μg mL^{-1} but <40% of control growth at 100 μg mL^{-1} were classified as intermediate, and those isolates with >40% of control growth at both 5 and 100 μg mL^{-1} were classified as metalaxyl resistant (Forbes *et al.*, 1997).

DNA isolation

DNA was extracted from 10–12-day-old mycelia cultured in rye A broth in the dark at 18°C according to the procedure described by Raeder & Broda (1985) and slightly modified by Chowdappa *et al.* (2003), and stored at −20°C.

Mitochondrial DNA haplotype

Mitochondrial DNA (mtDNA) haplotypes were identified using the PCR-based method of Griffith & Shaw (1998) in which the PCR products were digested with restriction enzymes *Cfo*I for the P1 region, *Msp*I for P2 and *Eco*RI for P3 and P4, followed by agarose gel electrophoresis.

Simple sequence repeat marker analysis

Polymorphic simple sequence repeat (SSR) regions of *P. infestans* isolates were amplified using primers for loci Pi02, Pi4B, G11, Pi04, Pi63, Pi70, D13, SSR2, SSR4, SSR6, SSR8 and SSR11. The Type-it Microsatellite PCR kit (QIAGEN) was used following previously published methods (Li *et al.*, 2013a). Samples were analysed using an ABI 3730 capillary DNA sequencer according to the manufacturer's instructions (Applied Biosystems). The peak size was determined against a GeneScan 500 LIZ standard and alleles were scored using GeneMapper v. 3.7 (Applied Biosystems). The SSR fingerprint patterns of Indian isolates of *P. infestans* were compared with those of two 13_A2 clonal lineage reference isolates (PCL_10_7850B and PCL_10_7922A).

RG57 fingerprinting

DNA fingerprinting was carried out using the RG57 multilocus nuclear DNA probe (Goodwin *et al.*, 1992a). Total DNA was digested with *Eco*RI and subjected to electrophoresis on 0·8% agarose gels for 18 h at 40 V and transferred on to Hybond-N^+ nylon membrane (Amersham Biosciences). The membrane was neutralized in 1 M Tris, pH 7·5, 1·5 M NaCl for 2–3 min. DNA was fixed on the membrane by UV cross-linking. Hybridization with the RG57 probe was performed following the protocol of the AlkaPhos probe labelling system (GE Healthcare UK Ltd.) and bands were detected according to manufacturer's instructions. The DNA fingerprint patterns of Indian isolates of *P. infestans* were compared with those of two reference isolates belonging to US-1 (SA196008) and 13_A2 (06_3928A) clonal lineages.

Aggressiveness

The aggressiveness of the isolates was determined on detached leaflets of potato and tomato as per the method of Klarfeld *et al.* (2009). Potato cultivars Kufri Jyoti, Kufri Surya and Kufri Pukhraj, and tomato cultivars Arka Vikas, Arka Megali and Arka Sourabh, free of known genes for resistance, were used. Tomato and potato plants were grown in a glasshouse in plastic pots (18 cm diameter) filled with a mixture of equal proportions of red loamy soil, sand and composted farmyard manure. The plants were maintained at a mean daily temperature of 22–23°C under a 16-h day photoperiod. The uniform leaflets from six to nine leaves from the base of the stem of 6-week-old potato and tomato plants were detached and placed, lower side uppermost, on moist filter paper in 90 mm Petri dishes. As all isolates caused sporulating lesions on both hosts, 10 isolates from each host were chosen for aggressiveness assays. Sporangial suspensions of *P. infestans* were prepared by gently scraping the surface of 15-day-old cultures in rye agar B in sterile water and adjusting the concentration to 1×10^4 sporangia mL^{-1} with a haemocytometer. Each leaflet was inoculated by placing one 20 μL drop of a sporangial suspension near the midrib and incubated for 7 days at 18°C and 16 h daylength. To prevent petioles from drying and to enhance fungal development, a high relative humidity was maintained in the Petri dishes throughout the experiments by adding water as required to the filter paper. The lesion diameter was measured with a ruler parallel to the leaflet midrib (Oyarzun *et al.*, 1998) 7 days after inoculation. For each host and isolate combination, four leaflets were inoculated and these were placed in pairs in two replicate Petri dishes. All Petri dishes were laid out in a completely randomized design. The experiment was repeated three times. The average diameter of the two pseudoreplicate lesions in each Petri dish was taken as the experimental unit for analysis. The variance among pseudoreplicates was not used in tests of statistical significance. The statistical model, including appropriate *F*-tests, is described by Oyarzun *et al.* (1998).

Results

Mating types

A mating type assay based on pairing with A1 and A2 tester isolates on rye A agar showed that all 63 potato and 94 tomato isolates were of the A2 mating type (Table 2). Molecular assays with CAPS marker and PHYB primer provided additional supporting evidence. Amplification of DNA of potato and tomato isolates with primers W16 yielded a PCR product of 557 bp. Subsequent digestion with the *Hae*III restriction enzyme produced two fragments, 457 and 100 bp, indicative of the A2 mating type. The expected 347 bp PCR product was amplified from all isolates by the primers PHYB-1 and PHYB-2 and proved specific for the A2 mating type.

Table 2 Metalaxyl resistance, mating type, mtDNA haplotype and RG57 fingerprints of *Phytophthora infestans* isolates of potato and tomato collected at different locations in South India from 2010 to 2012

Isolate	Metalaxyl sensitivity	Mating type	mtDNA haplotype	RG57 fingerprint
Potato isolates (63)[a]	R	A2	Ia	1, 2, 8, 10, 13, 14, 17, 19, 20, 21, 22, 24, 25
Tomato isolates (94)	R	A2	Ia	1, 2, 8, 10, 13, 14, 17, 19, 20, 21, 22, 24, 25
13_A2 (06_3928A)	R	A2	Ia	1, 2, 8, 10, 13, 14, 17, 19, 20, 21, 22, 24, 25
US-1 (SA196008)	S	A1	Ib	1, 3, 4, 5, 7, 9, 10, 13, 14, 16, 20, 21, 24, 25

[a]Number of isolates in parentheses.

Metalaxyl sensitivity

All 63 isolates from potato and 94 isolates from tomato were resistant to metalaxyl (Table 2).

Mitochondrial DNA haplotypes

All 63 isolates of *P. infestans* from potato and 94 isolates from tomato were identified as haplotype Ia based on *Msp*I digestion of the P2 region of mtDNA, that produced two characteristic fragments of 720 and 350 bp (Table 2).

RG57 fingerprinting and SSR analysis

All 63 isolates from potato and 94 isolates from tomato were of the same phenotype and were of mtDNA haplotype Ia with identical RG57 fingerprints (Table 2), strongly indicating that all were of the same genotype. Thus, only 45 isolates (27 isolates from potato and 18 isolates from tomato) were SSR fingerprinted. All the potato and tomato isolates exhibited identical SSR patterns at all the loci except for minor variation at D13 and SSR4 loci (Table 3). The minor variants based on SSR analysis were named 13_A2_3, 13_A2_3b, 13_A2_3c and 13_A2_1. All potato isolates belonged to 13_A2_3. In tomato isolates, 13_A2_3, 13_A2_3b, 13_A2_3c and 13_A2_1 were detected. The RG57 and SSR patterns matched with the 13_A2 genotype of *P. infestans* reported earlier in European countries (Cooke *et al.*, 2012; Li *et al.*, 2013a).

Aggressiveness

The US-1 reference isolate was significantly less aggressive than other isolates on potato and failed to infect tomato, but all other isolates of *P. infestans*, irrespective of origin, were equally aggressive on both potato and tomato detached leaflets based on lesion size (Table 4). As a result, no statistically significant interaction occurred between origin of isolate (tomato or potato) and host or cultivar (Table 5).

Discussion

Effective management of late blight on potato and tomato is influenced by the characteristics of the current pathogen population. Several studies have demonstrated that severe epidemics of late blight were associated with the emergence of 'new' populations of *P. infestans* in which fungicide resistance or an ability to overcome host resistance had evolved (Lees *et al.*, 2012), for example the significant potato crop losses in the USA from 1985 to 1995 due to the replacement of the US-1 clonal lineage by the aggressive and metalaxyl-resistant A2 mating type US-8 lineage (Fry & Goodwin, 1997). In this study, the *P. infestans* population infecting tomato and potato collected from different commercial fields in South India were A2 mating type, mitochondrial DNA haplotype Ia, resistant to metalaxyl and highly aggressive on both

Table 3 SSR alleles at 12 loci used for characterization of isolates of *Phytophthora infestans* from potato and tomato collected at different locations in South India from 2010 to 2012

Isolate source	No. isolates	Pi02	Pi4B	G11	Pi04	Pi63	Pi70	D13	SSR11	SSR2	SSR4	SSR6	SSR8	Genotype
Potato	27	266/268	205/213	154/160	166/170	273/279	192/192	136/158	341/341	173/173	284/294	240/244	260/266	13_A2_3
Tomato	2	266/268	205/213	154/160	166/170	273/279	192/192	136/154	341/341	173/173	284/294	240/244	260/266	13_A2_1
Tomato	9	266/268	205/213	154/160	166/170	273/279	192/192	136/158	341/341	173/173	284/292/294	240/244	260/266	13_A2_3b
Tomato	1	266/268	205/213	154/160	166/170	273/279	192/192	136/158	341/341	173/173	284/294/296	240/244	260/266	13_A2_3c
Tomato	6	266/268	205/213	154/160	166/170	273/279	192/192	136/158	341/341	173/173	284/294	240/244	260/266	13_A2_3
13_A2 (PCL_10-7850B)	1	266/268	205/213	154/160	166/170	273/279	192/192	136/154	341/341	173/173	284/294	240/244	260/266	13_A2_1
13_A2 (PCL_10-7922A)	1	266/268	205/213	154/160	166/170	273/279	192/192	136/158	341/341	173/173	284/294	240/244	260/266	13_A2_3

Table 4 Foliar aggressiveness of isolates of *Phytophthora infestans* collected at different locations in South India from 2010 to 2012 on potato and tomato cultivars

	Lesion area (cm^2)					
	Potato cultivar			Tomato cultivar		
Isolate	Kufri Jyoti	Kufri Surya	Kufri Pukhraj	Arka Vikas	Arka Megali	Arka Sourabh
Potato isolates						
PIP2	6·43 ± 0·32	6·96 ± 0·31	6·95 ± 0·26	6·91 ± 0·21	6·82 ± 0·29	6·89 ± 0·23
PIP4	7·23 ± 022	6·46 ± 0·24	6·59 ± 0·35	7·31 ± 0·29	6·35 ± 0·32	7·29 ± 0·29
PIP12	7·10 ± 0·29	7·20 ± 0·28	6·37 ± 0·29	6·20 ± 0·31	6·29 ± 0·42	6·23 ± 0·33
PIP28	6·99 ± 0·37	6·34 ± 0·44	6·29 ± 0·33	6·78 ± 0·35	6·80 ± 0·35	6·19 ± 0·43
PIP30	7·57 ± 0·24	6·26 ± 0·53	6·78 ± 0·32	6·11 ± 0·29	6·67 ± 0·24	6·20 ± 0·29
PIP34	6·33 ± 0·22	6·75 ± 0·36	6·19 ± 0·26	6·25 ± 0·24	6·35 ± 0·21	6·19 ± 0·27
PIP44	6·87 ± 0·36	6·23 ± 0·29	6·35 ± 0·17	6·33 ± 0·32	6·72 ± 0·22	6·26 ± 0·26
PIP49	6·56 ± 0·40	6·96 ± 0·25	6·29 ± 0·32	6·26 ± 0·41	6·69 ± 0·25	6·33 ± 0·22
PIP52	6·29 ± 0·23	7·16 ± 0·30	7·10 ± 0·30	6·22 ± 0·33	6·80 ± 0·22	6·25 ± 0·28
PIP56	6·75 ± 0·28	6·34 ± 0·36	6·15 ± 0·38	6·11 ± 0·36	6·88 ± 0·24	6·19 ± 0·23
Tomato isolates						
PIT20	6·28 ± 0·41	6·77 ± 0·28	6·86 ± 0·18	7·12 ± 0·25	7·03 ± 0·29	6·95 ± 0·16
PIT31	6·19 ± 0·27	7·30 ± 0·29	6·26 ± 0·73	7·89 ± 0·38	7·89 ± 0·72	6·35 ± 0·29
PIT37	6·37 ± 0·35	6·27 ± 0·24	6·89 ± 0·39	6·42 ± 0·33	6·78 ± 0·56	6·92 ± 0·37
PIT40	6·56 ± 0·29	6·70 ± 0·50	6·78 ± 0·38	6·83 ± 0·55	7·12 ± 0·34	6·75 ± 0·39
PIT42	6·75 ± 0·67	6·68 ± 0·77	6·47 ± 0·70	7·11 ± 0·49	7·45 ± 0·45	6·43 ± 0·67
PIT48	6·90 ± 0·56	6·52 ± 0·54	6·43 ± 0·80	6·96 ± 0·63	7·23 ± 0·67	6·78 ± 0·89
PIT52	6·72 ± 0·34	6·65 ± 0·82	6·34 ± 0·79	6·56 ± 0·36	7·12 ± 0·77	6·56 ± 0·69
PIT55	6·59 ± 0·44	6·39 ± 0·79	6·28 ± 0·73	6·90 ± 0·37	6·95 ± 0·58	6·45 ± 0·76
PIT57	6·32 ± 0·49	6·27 ± 0·56	6·49 ± 0·58	7·12 ± 0·35	7·10 ± 0·47	6·29 ± 0·95
PIT59	6·98 ± 0·61	6·37 ± 0·93	6·29 ± 0·26	6·82 ± 0·29	6·86 ± 0·65	6·82 ± 0·78
US-1 (Jab10-23)	2·13 ± 0·80	1·98 ± 0·65	2·04 ± 0·54	0·00	0·00	0·00
CD%	0·92	0·85	0·91	0·88	0·85	0·90

There were no significant differences among all isolates on potato and tomato except from the control (US-1). The values between potato and tomato isolates and the control (US-1) are significantly different at $P < 0·01$ according to Fisher's LSD test.

tomato and potato. This clonal lineage has a characteristic RG57 pattern and SSR loci matching with the multilocus genotype (MLG) 13_A2 lineage reported in European countries and China (Cooke *et al.*, 2012; Li *et al.*, 2013b). A novel A2 mating type and metalaxyl-resistant clonal lineage, termed 13_A2, was first reported from the Netherlands and Germany in 2004 and migrated to Great Britain in 2005 and displaced the other clonal lineages in less than 3 years (Cooke *et al.*, 2012). Recently, this 13_A2 clonal lineage was identified as responsible for severe outbreaks of late blight on tomato in Bangalore, Chikkaballapur and Kolar districts of Karnataka state in India (Chowdappa *et al.*, 2013). This is the first report of the 13_A2 MLG of *P. infestans* on potato in India and over a wider geographic region than the previous report (Fig. 1).

Previous studies have used pathogenic aggressiveness assays to measure host adaptation (Oyarzun *et al.*, 1998; Vega-Sánchez *et al.*, 2000). In this study, all isolates of the 13_A2 lineage studied, regardless of their host or geographic origin, were equally pathogenic on both tomato and potato and caused severe field epidemics on both hosts. There is some precedent for this; for example the US-22 and US-23 lineages were reported on tomato and potato (Fry *et al.*, 2013). In contrast, many other reports demonstrate that genotypes are more aggressive on their host of origin (tomato or potato) than on other hosts and that distinct clonal lineages of *P. infestans* are associated with potato and tomato within the same region (Goodwin *et al.*, 1992b; Legard *et al.*, 1995; Lebreton & Andrivon, 1998; Oyarzun *et al.*, 1998). No such specialization is apparent in the 13_A2 lineage in South India. Aggressiveness, a key component of pathogen fitness, is determined by measuring traits such as lesion size and latent period (Andrivon, 1993). These are important parameters for successful establishment and spread of the pathogen in the field and closely correlate with spore production and infection frequency (Carlisle *et al.*, 2002). The 13_A2 isolates have been reported to produce larger lesions with a shorter latent period on potato cultivars compared to other genotypes (Cooke *et al.*, 2012). Furthermore, 13_A2 isolates have been reported to be the most aggressive on all potato cultivars at 13°C, suggesting that they were better adapted to cooler conditions (Cooke *et al.*, 2012). In contrast, the severe disease epidemics on potato and tomato that occured during the August–December crop season in South India, in which the average temperature varied from 20·8 to 22·5°C, showed that the European 13_A2 lineage (Chowdappa *et al.*, 2013) was also highly adapted to local temperatures and highly aggressive on potato and tomato, enabling it to displace the existing

Table 5 Results of an analysis of variance that tested effects of origin (tomato or potato) of isolates of *Phytophthora infestans* collected at different locations in South India from 2010 to 2012 and inoculated host species (tomato or potato) and cultivar on diameter of lesions (cm) in a detached-leaf inoculation assay

Source	d.f.	MS[a]	*F*	*P*
Isolate origin (O)	1	0·021934	0·7093	0·4005
Host species (H)	1	0·038422	2·9755	0·1300
Cultivar (C)	2	0·061908	2·0020	0·1373
Isolate (I)	9	0·075873	2·4537	0·0109
O*H	1	0·038647	1·2498	0·2647
O*C	2	0·037368	1·2084	0·3005
O*I	9	0·062379	2·0173	0·0382
H*C	2	0·062530	2·0222	0·1346
H*I	9	0·041196	1·3322	0·2208
V*I	18	0·008191	0·2649	0·9990
O*H*C	2	0·016768	0·5423	0·5822
O*H*I	9	0·076243	2·4656	0·0105
O*V*I	18	0·009793	0·3167	0·9969
H*V*I	18	0·012162	0·3933	0·9882
O*H*C*I	18	0·006646	0·2149	0·9998
Total	359	0·036376	1·1764	
Treatment	119	0·047896	1·5489	
Residual error[b]	238	0·030922		

[a]Main effects of isolate origin and host species were tested. This interaction was tested by using the mean square for the interaction between individual isolates and hosts, designated isolate and host.
[b]Based on variance among Petri dishes, which are pseudoreplications of the experiment.

lineages. Genome analysis of the 13_A2 isolate 06_3928A revealed a high rate of sequence variation, changes in expression patterns of effector genes and a remarkable pattern of extended biotrophic growth, probably contributing to enhanced virulence and aggressiveness to drive population displacement and an ability to cause diseases on previously resistant potato cultivars (Cooke *et al.*, 2012).

Severe late blight epidemics in the temperate regions of north Indian hills occur every year, causing 40–85% yield losses (Chimote *et al.*, 2010) and mild to moderate infections with epiphytotics once in every 2 to 3 years, resulting in 15–75% losses in the subtropical Indo-Gangetic plains (Bhattacharyya *et al.*, 1990). However, the late blight epidemics on both potato and tomato in Western Ghat hills (Udhagamandalam) and Malnad hills (Hassan) and plains of South India (Andhra Pradesh, Karnataka and Tamil Nadu) were not considered a serious threat until 2006, even though the disease has been reported in the region since 1953 (Chowdappa *et al.*, 2013). As a result of severe outbreaks of late blight, the area under potato cultivation has drastically reduced from 51 475 to 17 000 ha during 2007 and 2008 in Hassan district, a major potato-producing area of Karnataka state (Chowdappa *et al.*, 2011), affecting the livelihood of farmers in this region. Many samples of *P. infestans* from potato in northern India in the period 1998–2005 were of the A1 mating type, Ib mtDNA haplotype and metalaxyl sensitive (Chimote *et al.*, 2010). The disease was managed to a certain extent by using earlier Indian varieties (SLB series) in which *R* genes were introgressed from *Solanum demissum* (Singh & Singh, 2007). Singh *et al.* (1994) reported the presence of the A2 mating type in 1994 in India followed by an increase in metalaxyl resistance at most of the locations in India including South Indian Udhagamandalam hills. These changes are also associated with a shift from mtDNA haplotype Ib to Ia from 2004 to 2006, coinciding with emergence of late blight epidemics in South India (Chimote *et al.*, 2010). Since then, monogenic host resistance has broken down in Indian varieties (Singh & Singh, 2007) coupled with failure of metalaxyl in managing late blight during 2006–2007 across the country (Chimote *et al.*, 2010). Studies in Europe have also shown that 13_A2 has overcome some resistance previously considered as durable, such as that in cultivar Stirling (Cooke *et al.*, 2012; Lees *et al.*, 2012). Evidence has been provided in this study to show that 13_A2 was responsible for such severe epidemics both on potato and tomato in Hassan (Malnad hills), Udhagamandalam hills and other plains in South India and breakdown of resistance in Kufri Jyothi, a popular tolerant potato variety grown in these areas. The results of the present study also show that farmers should no longer rely on metalaxyl for management of late blight on potato and tomato in India.

SSR markers have been proved as an effective tool to discriminate isolates within the populations of *P. infestans* (Cooke *et al.*, 2012; Li *et al.*, 2013a). In this study, variation at highly mutable loci (SSR4 and D13) has allowed discrimination of minor variants amongst the 45 isolates of 13_A2. The D13 locus has previously been reported to be one of the most variable of the twelve (Li *et al.*, 2013a), and such differences are consistent with observations of subclonal population structure in the 13_A2 clonal lineage in the UK (Cooke *et al.*, 2012). The lack of host-specific substructure in the population in this study, in combination with aggressiveness data, indicates that this lineage is equally adapted to both hosts. In contrast to the clonality shown in this study, in some regions of Europe almost every isolate of *P. infestans* is genetically distinct (Yuen & Andersson, 2013). The migration of the 13_A2 lineage (Chowdappa *et al.*, 2013) and subsequent successful establishment on potato and tomato indicates that the pathogen population in India is changing quickly (Table 3). It is unclear whether the minor within-clone variants reported here are as a result of recent mutations in the local population or were present on the imported population.

The knowledge generated in the present study on population structure of *P. infestans* on potato and tomato will help in devising integrated management strategies. These techniques include the use of pathogen-free seed potatoes and tomato transplants, use of PCR for production and supply of disease-free seed and tubers, elimination of sources of inoculum by treating tubers and seed with fungicides, removal of metalaxyl from the fungicide

schedule, use of appropriate fungicides on foliage, host resistance and decision support systems that will help growers to minimize losses caused by this devastating pathogen.

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References

Akino S, Gotoh K, Nishimura R, Maeda A, Naito S, Ogoshi A, 2004. Comparison of Chinese and Japanese A1 isolates of *Phytophthora infestans*. *Journal of General Plant Pathology* **70**, 212–4.

Andrivon D, 1993. Nomenclature for pathogenicity and virulence: the need for precision. *Phytopathology* **83**, 889–90.

Bhattacharyya SK, Shekhawat GS, Singh BP, 1990. *Potato Late Blight. Technical Bulletin No. 27*. Shimla, India: CPRI.

Carlisle DJ, Cooke LR, Watson S, Brown AE, 2002. Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. *Plant Pathology* **51**, 424–34.

Chimote VP, Kumar M, Sharma PK, Singh PH, Singh BP, 2010. Characterization of changes in phenotype and genotype of *Phytophthora infestans* isolates from India. *Journal of Plant Pathology* **92**, 669–77.

Chowdappa P, Brayford D, Smith J, Flood J, 2003. Molecular discrimination of *Phytophthora* isolates on cocoa and their relationship with coconut, black pepper and bell pepper isolates based on rDNA repeat and AFLP fingerprints. *Current Science* **84**, 1235–8.

Chowdappa P, Mohan Kumar SP, Sanjeev S, Singh BP, 2011. Integrated management of early and late blight of potato and tomato. *ORP on Leaf Spot Diseases Series 17*. Bangalore, India: Indian Institute of Horticultural Research.

Chowdappa P, Nirmal Kumar BJ, Madhura S *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. *Journal of Phytopathology* **161**, 49–58.

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

Deahl KL, Cooke LR, Black LL *et al.*, 2002. Population changes in *Phytophthora infestans* in Taiwan associated with the appearance of resistance to metalaxyl. *Pest Management Science* **58**, 951–8.

Elansky S, Smirnov A, Dyakov Y *et al.*, 2001. Genotypic analysis of Russian isolates of *Phytophthora infestans* from the Moscow region, Siberia and the Far East. *Phytopathology* **149**, 605–11.

Forbes GA, Escobar XC, Ayala CC *et al.*, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* **87**, 375–80.

Fry WE, Goodwin SB, 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* **81**, 1349–57.

Fry WE, McGrath MT, Seaman A *et al.*, 2013. The 2009 late blight pandemic in the eastern United States – causes and results. *Plant Disease* **97**, 296–306.

Ghimire SR, Hyde KD, Hodgkiss IJ, Shaw DS, Liew ECY, 2003. Variations in the *Phytophthora infestans* population in Nepal as revealed by nuclear and mitochondrial DNA polymorphisms. *Phytopathology* **93**, 236–43.

Gisi U, Cohen Y, 1996. Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. *Annual Review of Phytopathology* **34**, 549–72.

Goodwin SB, Drenth A, 1997. Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. *Phytopathology* **87**, 992–9.

Goodwin SB, Drenth A, Fry WE, 1992a. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics* **22**, 107–15.

Goodwin SB, Spielman LJ, Matusak JM, Bergeron SN, Fry WE, 1992b. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology* **82**, 955–61.

Goodwin SB, Cohen BA, Deahl KL, Fry WE, 1994. Migration from northern Mexico was the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. *Phytopathology* **84**, 553–8.

Gotoh K, Akino S, Maeda A *et al.*, 2005. Characterization of some Asian isolates of *Phytophthora infestans*. *Plant Pathology* **54**, 733–9.

Griffith GW, Shaw DS, 1998. Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure culture or from host lesions. *Applied and Environmental Microbiology* **64**, 4007–14.

Judelson HS, Spielman LJ, Shattock RC, 1995. Genetic mapping and non-Mendelian segregation of mating type loci in oomycete, *Phytophthora infestans*. *Genetics* **141**, 503–12.

Kim KJ, Lee YS, 2002. Genetic DNA marker for A2 mating type in *Phytophthora infestans*. *Journal of Microbiology* **40**, 254–9.

Klarfeld S, Rubin AE, Cohen Y, 2009. Pathogenic fitness of oosporic progeny isolates of *Phytophthora infestans* on late-blight-resistant tomato lines. *Plant Disease* **93**, 947–53.

Lebreton L, Andrivon D, 1998. French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology* **104**, 583–94.

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 Research* **55**, 125–34.

Legard DE, Lee TY, Fry WE, 1995. Pathogenic specialization in *Phytophthora infestans*: aggressiveness on tomato. *Phytopathology* **85**, 1356–61.

Li Y, Cooke DEL, van der Lee TAJ, Jacobsen E, 2013a. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. *Journal of Microbiological Methods* **92**, 316–22.

Li Y, van der Lee T, Zhu JH *et al.*, 2013b. Population structure of *Phytophthora infestans* in China – geographic clusters and presence of the EU genotype Blue_13. *Plant Pathology* **62**, 932–42.

Nishimura R, Sato K, Lee WH, 1999. Distribution of *Phytophthora infestans* populations in seven Asian countries. *Annals of the Phytopathological Society of Japan* **65**, 163–70.

Nowicki M, Foolad MR, Nowakowska M, Kozik EU, 2012. Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. *Plant Disease* **96**, 1–17.

Oyarzun PJ, Pozo A, Ordonez ME, Doucett K, Forbes GA, 1998. Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology* **88**, 265–71.

Raeder U, Broda P, 1985. Rapid preparation of DNA from filamentous fungi. *Applied Microbiology* **1**, 17–20.

Singh PH, Singh BP, 2007. Races of *Phytophthora infestans* in India – present scenario. *Potato Journal* **34**, 75–6.

Singh BP, Roy S, Bhattacharyya SK, 1994. Occurrence of the A2 mating type of *Phytophthora infestans* in India. *Potato Research* **37**, 227–31.

Spielman LJ, Drenth A, Davidse LC *et al.*, 1991. A second world-wide migration and population displacement of *Phytophthora infestans*. *Plant Pathology* **40**, 422–30.

Vega-Sánchez ME, Erselius LJ, Rodriguez AM *et al.*, 2000. Host adaptation to potato and tomato within the US-1 clonal lineage of *Phytophthora infestans* in Uganda and Kenya. *Plant Pathology* **49**, 531–9.

Yoshida K, Schuenemann VJ, Cano LM *et al.*, 2013. The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. *eLife* **2**, e00731.

Yuen JE, Andersson B, 2013. What is the evidence for sexual reproduction of *Phytophthora infestans* in Europe? *Plant Pathology* **62**, 485–91.