



Severe outbreaks of *Phytophthora infestans* on potato in Turkey caused by recent changes in the pathogen population structure

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Abstract Potato late blight, caused by *Phytophthora infestans*, has been the most damaging disease in Turkey since 2010. In this study, 127 isolates of *P. infestans* were obtained from the main growing areas of Turkey between 2015 and 2017. Their phenotypic and genotypic features were revealed and presented with those of reference isolates. These isolates were categorized by their mating type, in vitro mefenoxam sensitivity, mtDNA haplotype, RG57 DNA fingerprinting patterns,

simple sequence repeat (SSR) markers, and aggressiveness on a set of potato differential lines. All isolates were of the A2 mating type and mtDNA haplotype Ia, were resistant to mefenoxam, and had RG57 and SSR fingerprints similar to the 13_A2 clonal lineage reported in Europe. This is the first report of 13_A2 in Turkey. Virulence abilities against potato resistance (*R*) genes *R1*, *R2*, *R3*, *R4*, *R6*, *R7*, *R10*, and *R11* were observed in most of the isolates. The mating type ratios and SSR marker analysis indicate that in Turkey, the sexual reproduction of *P. infestans* is limited. These results underline that the movement of asexual individuals and the generation of sub-clonal difference are the factors driving the population structure of *P. infestans* in Turkey.

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Introduction

Phytophthora infestans (Mont.) de Bary, an oomycete plant pathogen, is one of the most important diseases of tomato and potato throughout the world. The pathogen has a heterothallic reproductive system and needs the interaction of two opposite mating types, designated as A1 and A2 (Judelson 1997). The late blight disease caused by the pathogen appears to be very severe especially in areas where the temperate climate prevails and there is a need for intensive use of pesticides in its control (Fry 2008). Detailed information about the population structure,

diversity, and dynamics of *P. infestans* is important in terms of the development and updating of management strategies of the disease, as well as the breeding and improvement of resistant cultivars (Forbes 2012).

By the nineteenth century, the late blight caused the famous Great Famine in Ireland with several years of epidemics, which resulted in the deaths of more than one million people and the migration of more than one million people (Brurberg et al. 2011; Kröner et al. 2017). Since then, migration probably through exported seed tubers has enabled this pathogen to spread worldwide (Fry et al. 1993). As the latest result of these movements, in some Northern European countries in the 1980s, pathogen populations underwent quick changes. Many studies on the characterization of *P. infestans* have shown that new pathogen populations rapidly replace old populations of European populations, and most probably this population consists of individuals who have genetically diverse capabilities from Mexico (Fry et al. 1993). The geographical distribution of *P. infestans* populations in potato production areas is expressed by clonal lineages defined after an analysis of genotypic and phenotypic characterization (Cooke and Lees 2004). Virulence that originated in Europe, an effective A2 mating type, Ia mtDNA haplotype, and mefenoxam resistance with increased aggressiveness are the characteristics of clonal lineage 13_A2 (Li et al. 2012; Cooke et al. 2012; Kiiker et al. 2018), which was recorded for the first time in Germany and the Netherlands in 2004, followed by Poland in 2006, and affected 80% of the pathogen population in Great Britain in 2008 (Chmielarz et al. 2014; Rekad et al. 2017). Since its first report, this clonal lineage has maintained its presence throughout Europe, and new clonal lineages that have emerged over time have disappeared into the pathogen populations, while 13_A2 has remained. Because of its high sporulation ability and infection even at low temperatures, the 13_A2 clonal lineage replaced the populations of US-1 and other genotypes rather quickly. Indeed, such displacements have been identified in areas of potato production in China, Algeria, and Southern India (Chowdappa et al. 2015; Rekad et al. 2017; Tian et al. 2016).

Although the cultivation area and production of potato in Turkey varies from year to year, approximately 4.5 million tons of production is provided from 140,000 ha (TUIK 2018). Plant is mainly cultivated in

three regions, categorized by their weather condition and various types of agricultural practices. In central part of Turkey, plants are grown from May until October in large-acreage fields with average Turkey weather. In western Turkey, they are cultivated in intermediate sized fields and grown twice a year. In South-Eastern Turkey, they are planted in small areas in the winter. The control of late blight outbreak in Turkey has become more difficult in a manner similar to observations in Europe and the USA. In Europe and the USA, this situation is based on changes in the population structure of the pathogen (Cooke et al. 2009; Hu et al. 2012; Mariette et al. 2016). Therefore, this study was carried out in Afyon, Bolu, Bursa, Izmir, Nevşehir, Niğde, Tokat, and Trabzon provinces in order to represent the population of the pathogen in the potato production areas of the country.

Climate of major potato-growing regions in Turkey is suitable for *P. infestans*, and thus, pathogen constitutes a serious threat to production in the country (Gunacti et al. 2019; Tosun et al. 2007). It occurs intensely in cases where disease control measures are not taken and this limits the production of potato to a large extent. While the strategies used to control the disease were successful until 2010 and prevented the emergence of the epidemic character of the disease, after 2010, a period began in which the character of the epidemics of the pathogen was frequently seen. Today, disease control requires more intensive fungicide applications than earlier and possible failures in these applications cause yield losses up to 100%. All these issues mentioned above suggest that there is a change in the population of the pathogen after 2010 and that new clonal strains may have entered the potato production sites. As a matter of fact, the reports of the potato growers' association indicated that the epidemics and threats caused by the disease increased gradually during this period. *P. infestans* isolates were collected from potato fields during 2015–2017, and their clonal lineage, mating types, race diversity, and mefenoxam susceptibility were determined in order to better understand the epidemics and their ecology caused by the individuals forming the present pathogen populations. In order to provide timely information to the growers and to create and modify the management strategies of the disease, genotypic properties were tried to be shaped by fast-performing simple sequence repeat (SSR) analyses, and these results were supported by restriction fragment length polymorphism (RFLP) with the RG-57 probe, mtDNA haplotypes, and allozyme

assays. The results obtained from the study are expected to contribute to the short- and long-term disease management in Turkey.

Material and methods

Isolate collection and maintenance

Blighted material was obtained from the major producing areas represent 45% of the total cultivation area of the country during the vegetation period of 2015–2017 (Table 1; Fig. 1). The geographical location of the samples varied between growing seasons in relation to the occurrence and development of the disease. The leaves bearing a single lesion were washed with sterile distilled water and dried with the aid of filter paper. These leaves were then placed individually in Petri dishes containing 1.5% water agar for 48 h at 18 °C to stimulate sporulation. After observing sporulation with a dissecting microscope, an agar block of 3 to 4 mm² was contacted with several sporangia, allowing their passage onto the agar block.

In order to obtain pure culture, these agar blocks were individually placed in rye A agar amended with rifampicin and ampicillin (rye A + rif + amp) and allowed to grow in the dark at 18 °C (Caten and Jinks 1968). Following incubation under a 12-h light/dark cycle for several days, mycelium was moved to fresh medium. The short-term storage of the isolates was performed on rye A agar medium at 15 °C in the dark. For long-term storage, the isolates were incubated in pea agar medium for 2 weeks at 20 °C. For each isolate, 5 discs of 10 mm diameter from these cultures were transferred to cryotubes (2.2 mL) containing 1.5 mL of 15% dimethylsulfoxide (DMSO). The cryotube vials were allowed to pre-freeze at –80 °C for 24 h and finally transferred rapidly to –152 °C.

Mating type tests

A 10-mm-diameter agar disc from 2 weeks of culture of the isolate to be tested was placed to one side of a pea agar-containing Petri dish, and a mycelial disc of an A1 tester (US-17 genotype, US970001) or an A2 tester (US-8 genotype, US040009) was transferred on the other side. The isolates were maintained in the dark at 18 °C for 2–3 weeks. After the two mycelial colonies contacted each other, the contact site was regularly

checked for oospore formation for seven consecutive days using a microscope at ×100 magnification. If oospores were detected in a Petri dish using the A1 reference isolate, the tested isolate was classified as A2 mating type, and vice versa.

Mefenoxam sensitivity assay

Resistance to mefenoxam was tested on pea agar media amended with mefenoxam (Ridomil Gold EC, 49% active ingredient, Syngenta) at application concentrations of 5 and 100 ppm. During the experiments, isolates were maintained in the dark at 16 °C for 2 weeks. The isolates were grouped as susceptible in cases where the cultures of *P. infestans* on media with both 5 and 100 ppm mefenoxam were less than 40% of the diameter of the culture on the control Petri dishes without mefenoxam. The isolates were described as intermediate when the growth rate in cultures was > 40% of the control on the media with 5 ppm mefenoxam but less than 40% of the control on the media with 100 ppm mefenoxam. The isolates were defined as resistant when their cultures were bigger than 40% of the control on both types of mefenoxam Petri dishes (Bakonyi et al. 2002). *P. infestans* isolates used as references were as follows: resistant isolate US-8 (US040009), US-11 (US050007), and two susceptible isolates US-1 (2162 TW97-Pi-42) and US-23 (BL2009P4).

Allozyme analysis

For each isolate, allozyme genotypes were investigated in the glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) loci. Mycelial mass obtained from the pea broth culture or sporangia from sporulated leaves were placed in sterile 1.5-mL microcentrifuge tubes and extracted in accordance with the procedure of Goodwin et al. (1995). Allozyme genotypes were examined by cellulose-acetate electrophoresis for *Gpi* and *Pep* loci. Reference isolates of the genotypes US-1 (*Gpi* 86/100, *Pep* 92/100) and US-8 (*Gpi* 100/111/122, *Pep* 100/100) were included as controls in each acetate plate. The distance at which proteins from unknown isolates migrated was determined by comparison with reference isolates.

Table 1 The source and multi-locus genotypes of *Phytophthora infestans* isolates collected from different locations in Turkey from 2015 to 2017

Isolate	Province	County	Coordinate	Mating type	Allozyme genotype ^a		Metalaxyl sensitivity ^b	mtDNA ^c	RG-57 RFLP ^d	Genotype ^e
					Gpi	Pep				
1501#82	Afyon	Dinar	38 09 46/30 13 57	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#83			38 09 34/30 13 36	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#84			38 09 44/30 13 44	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#90		Sandıklı	38 11 58/30 11 22	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#97			38 22 29/30 12 05	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#113		Şuhut	38 27 51/30 34 45	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#114			38 27 54/30 35 15	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#116			38 28 31/30 35 04	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#104			38 32 34/30 37 36	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#105			38 32 23/30 37 23	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#106			38 32 12/30 37 09	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#109			38 31 49/30 36 59	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#110			38 32 03/30 36 22	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#112			38 32 36/30 37 56	A2	100/100	96/96	R	la	1100000101001101101111011	13_A2
1501#181	Bolu	Dörtdivan	40 41 55/32 03 29	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#172			40 43 46/32 04 08	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#173			40 43 38/32 04 04	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#167		Merkez	40 42 22/31 35 56	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#168			40 42 29/31 36 10	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#169			40 42 34/31 36 36	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#170			40 42 27/31 36 24	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#171			40 42 16/31 36 29	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#144			40 41 36/31 32 34	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#145			40 41 33/31 32 24	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#146			40 41 19/31 32 23	A2	100/100	96/96	R	la	1100000101001101101111011	13_A2
1501#147			40 41 43/31 32 38	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#148			40 41 39/31 32 54	A2	100/100	96/96	I	la	1100000101001100101111011	13_A2
1501#149			40 41 37/31 32 46	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#151			40 41 50/31 34 09	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2
1501#158			40 41 53/31 34 02	A2	100/100	96/96	R	la	1100000101001100101111011	13_A2

Table 1 (continued)

Isolate	Province	County	Coordinate	Mating type	Allozyme genotype ^a		Metalaxyl sensitivity ^b	mtDNA ^c	RG-57 RFLP ^d	Genotype ^e
					Gpi	Pep				
1501#159			40 41 54/31 34 19	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#160			40 41 52/31 34 25	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#138			40 42 31/31 32 27	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#139			40 42 35/31 32 31	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#142			40 42 17/31 32 30	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#162			40 41 43/31 34 20	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#163			40 41 20/31 34 42	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#164			40 41 14/31 34 36	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#199	Izmir	Bozdağ	38 20 41/28 03 54	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#200			38 20 16/28 03 58	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#201			38 20 21/28 03 42	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#59		Kiraz	38 12 03/28 12 43	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#51			38 12 48/28 13 29	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#52			38 12 35/28 13 36	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#45		Ödemiş	38 08 44/28 07 58	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#35			38 12 49/28 02 56	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#38			38 12 41/28 02 54	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#4			38 11 24/28 02 26	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#57			38 07 55/28 05 06	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#53			38 10 53/28 00 23	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#56			38 10 46/28 00 07	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#40		Tire	38 07 50/27 53 48	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#312	Nevşehir	Acıgöl	38 31 47/34 33 47	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#305		Merkez	38 26 05/34 44 48	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#310			38 26 48/34 43 21	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#311			38 26 46/34 44 07	A2	100/100	96/96	R	Ia	1100000101001100101111111	13_A2
1501#266		Ürgüp	38 30 57/34 56 36	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#267			38 30 59/34 56 35	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#268			38 31 00/34 56 40	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#269			38 30 56/34 56 39	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2

Table 1 (continued)

Isolate	Province	County	Coordinate	Mating type	Allozyme genotype ^a		Metalaxyl sensitivity ^b	mtDNA ^c	RG-57 RFLP ^d	Genotype ^e
					Gpi	Pep				
1501#270			38 30 55/34 56 35	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#271			38 30 59/34 56 31	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#272			38 30 54/34 56 40	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#273			38 30 51/34 56 40	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#274			38 30 48/34 56 40	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#275			38 30 55/34 56 40	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#276			38 30 53/34 56 44	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#277			38 30 47/34 56 48	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#278			38 30 50/34 56 49	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#279			38 30 44/34 56 52	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#280			38 30 40/34 56 56	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#313			38 28 05/34 52 53	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#281			38 28 54/34 57 37	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#282			38 29 04/34 57 28	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#283			38 28 59/34 57 29	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#284			38 28 58/34 57 33	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#285			38 28 55/34 57 32	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#286			38 28 59/34 57 35	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#287			38 28 50/34 57 42	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#288			38 28 46/34 57 42	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#347	Niğde	Çiftlik	38 12 47/34 31 36	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#365			38 10 14/34 28 52	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#366			38 10 07/34 28 51	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#367			38 10 10/34 28 31	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#328		Merkez	38 17 45/34 41 08	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#329			38 17 50/34 41 43	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#330			38 17 41/34 41 49	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#331			38 17 33/34 41 34	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#332			38 17 07/34 41 30	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#333			38 17 11/34 41 29	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2

Table 1 (continued)

Isolate	Province	County	Coordinate	Mating type	Allozyme genotype ^a		Metalaxyl sensitivity ^b	mtDNA ^c	RG-57 RFLP ^d	Genotype ^e
					Gpi	Pep				
1501#186			38 12 29/34 52 35	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#224			38 08 25/34 48 24	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#248			38 08 28/34 46 22	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#252			38 08 15/34 46 01	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#253			38 08 36/34 45 51	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#230			38 08 59/34 47 03	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#231			38 08 01/34 47 17	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#232			38 08 08/34 47 04	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#238			38 07 38/34 46 55	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#239			38 07 48/34 46 48	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#240			38 07 34/34 47 04	A2	100/100	96/96	R	Ia	1100000101001100101111111	13_A2
1501#241			38 07 31/34 46 39	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#242			38 08 23/34 46 39	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#243			38 08 20/34 46 35	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#244			38 08 17/34 46 25	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#245			38 08 10/34 46 28	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#246			38 08 07/34 46 40	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#247			38 08 13/34 46 46	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#254			38 08 23/34 46 40	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#255			38 08 22/34 46 49	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#256			38 08 25/34 47 08	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#257			38 08 34/34 47 07	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#258			38 08 39/34 46 55	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#259			38 08 37/34 46 35	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2
1501#133	Tokat	Merkez	40 24 40/36 42 47	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#134			40 25 06/36 43 03	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#135			40 25 27/36 43 15	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#136			40 25 49/36 43 15	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#137			40 25 38/36 43 00	A2	100/100	96/96	R	Ia	1100000101001100101111011	13_A2
1501#121	Trabzon	Akçaabat	40 55 23/39 30 38	A2	100/100	96/96	I	Ia	1100000101001100101111011	13_A2

Table 1 (continued)

Isolate	Province	County	Coordinate	Mating type	Allozyme genotype ^a		Metalaxyl sensitivity ^b	mtDNA ^c	RG-57 RFLP ^d	Genotype ^e
					Gpi	Pep				
1501#122			40 55 21/39 30 36	A2	100/100	96/96	I	Ia	11000000101001100101111011	13_A2
1501#123			40 55 24/39 30 41	A2	100/100	96/96	R	Ia	11000000101001100101111011	13_A2
1501#119			40 58 05/39 29 53	A2	100/100	96/96	R	Ia	11000000101001100101111011	13_A2
1501#126		Vakfikebir	40 59 02/39 18 02	A2	100/100	96/96	R	Ia	11000000101001100101111011	13_A2
1501#127			40 58 56/39 18 00	A2	100/100	96/96	R	Ia	11000000101001100101111011	13_A2
1501#124			41 00 26/39 18 18	A2	100/100	96/96	R	Ia	11000000101001100101111011	13_A2
1501#125			40 00 33/39 18 33	A2	100/100	96/96	R	Ia	11000000101001100101111011	13_A2

^a Allozyme genotyping performed using the methods of Goodwin et al. (1995); Gpi, glucose-6-phosphate isomerase; Pep, peptidase

^b Sensitivity to mefenoxam. R, mefenoxam resistant; I, intermediate; S, sensitive by the methods of Bakonyi et al. (2002)

^c mtDNA haplotype determined by the methods of Griffith and Shaw (1998)

^d The restriction fragment length polymorphic (RFLP) pattern produced using Eco RI and RG57

^e Includes variants of the common clonal lineages detected in Turkey. Variants within a lineage are indicated by adding a period and a number after the genotype name and are numbered sequentially. For example, 13_A2-1.3 is the third variant identified within the 13_A2 clonal lineage. These variants are identical to the “parent” clone except for one or two changes at DNA fingerprint loci. They are assumed to have arisen from asexual (somatic) variation within lineages

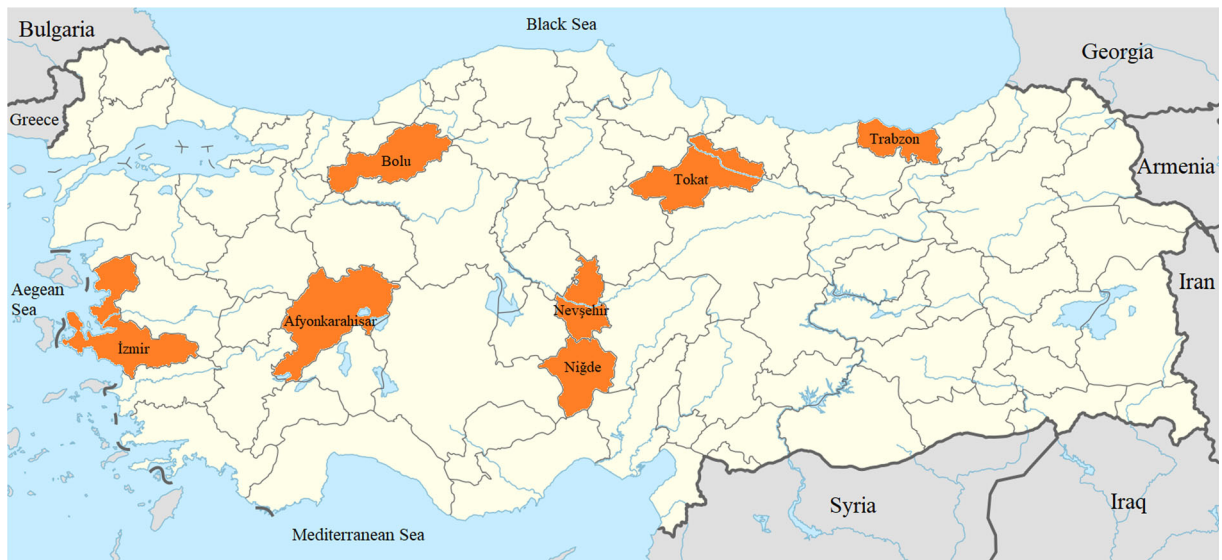


Fig. 1 Sampling provinces of *Phytophthora infestans* in major potato production areas in Turkey

mtDNA haplotype

The mtDNA haplotype analyses were performed with small modifications in the method of Griffith and Shaw (1998). Two primer sets derived from the genomic DNA library of *P. infestans* were used: P2 (5'-TTCC CTTTGTCTCTACCGAT-3' and 5'-TTAC GGCGGTTTAGCACATACA-3') and P4 (5'-TGGT CATCCAGAGGTTTATGTT-3' and 5'-CCGA TACCGATACCAGCACCAA-3'). The PCR reaction of P2 was performed in a 25- μ L reaction mixture containing 2.5 μ L of 10 \times buffer, 250 μ M dNTP, 1.875 μ M MgCl₂, 2.5 μ M of each primer, 1 U *Taq* DNA polymerase, and 10–50 ng of template DNA. The amplification was conducted under the following cycling conditions: after 30 s of initial denaturation at 94 °C, PCR amplification was carried out for 40 cycles (94 °C for 30 s, 64 °C for 60 s, and 72 °C for 60 s and a final extension of 5 min at 72 °C). The products were digested using restriction enzyme *MspI*, resolved on a 1.5% agarose gel stained with ethidium bromide (1 mg mL⁻¹), and visualized on a UV transilluminator. The reaction of P4 was performed similarly to the P2 reaction, but with 0.7 μ M MgCl₂. The conditions of the reactions were also similar, but the initial denaturation time was 90 s, primer annealing temperature 60 °C, and extension time 90 s. The PCR products were digested using the restriction enzyme *EcoRI* and visualized as described for the P2 reaction.

RFLP analysis with probe RG-57

Isolates were incubated in pea broth at 18 °C for 2 weeks. The mycelia were vacuum-filtered with Whatman No. 1 filter papers and lyophilized overnight at -5 °C. Genomic DNA was individually isolated using Sigma's GenElute Plant Genomic DNA Extraction Kit, and a RFLP analysis with probe RG-57 was undertaken on 127 isolates using the method specified by Goodwin et al. (1992). Transfer to a positively charged nylon membrane, hybridization with a non-radioactive RG-57 probe, and autoradiography were used in accordance with the manufacturer's instructions (Roche Applied Science DIG High Prime Labeling and Detection Kit II). The genotype of the isolates was identified using the band patterns of the reference isolates belonging to the clonal lineages US-1, US-8, and US-18 (Forbes et al. 1998). After comparing the obtained data with the profiles of known clonal lineages, genotypic discrimination of the tested isolates was performed (Chowdappa et al. 2013; Danies et al. 2013; Runno-Paurson et al. 2009).

Multiplex microsatellite marker analysis

For the analysis of SSR marker, a total of 12 SSR primers (Pi02, Pi04, Pi4B, Pi63, Pi70, D13, G11, PinfSSR2, PinfSSR4, PinfSSR6, PinfSSR8, and PinfSSR11) were used in this study (Knapova and

Gisi 2002; Lees et al. 2006; Li et al. 2010). Three separate multiplex reactions were conducted using three panels of primers. For automated fragment analysis, one primer of each locus was labeled with PET, FAM, NED, and VIC fluorescent dye according to Applied Biosystems™ (ABI). The dyes were assigned to loci in such a way that loci with the same dye had non-overlapping ranges of allele sizes. The PCR reactions were performed in a 10- μ L reaction mixture consisting of 1 μ L of 10 \times AmpliTaq (Applied Biosystems), 200 μ M dNTP, 1.5 mM MgCl₂, 10 pmol of each primer, and 1 U Taq DNA polymerase (Applied Biosystems). PCR amplifications were performed using a T-100 thermocycler (Bio-Rad) under the following conditions: 10 min at 95 °C; 28 cycles of 20 s at 95 °C, 25 s at 58 °C, 60 s at 72 °C; and a final extension of 20 min at 72 °C. Post-PCR processing was undertaken by dispensing 9.5 μ L of size standards in high-deionized formamide mix, 0.05 μ L of Genescan-500 LIZ size standard (Applied Biosystems [ABI], PN 4322682), 9.45 μ L of Hi-Di formamide (ABI, PN 4311320), and 0.5 μ L of each PCR product from panel 1–3 reactions into each of the 96-well ABI 3730xl plates. The PCR products were analyzed on an ABI 3730xl capillary system with POP-7 Polymer (ABI, PN 4335615). The PCR amplicons were compared with a set of size standards, and the alleles were scored accordingly (Lees et al. 2006).

Virulence assays

Virulence phenotypes of each of the 127 isolates were evaluated using a detached-leaf assay employing a combination of Black and Mastenbroek's differential sets for *S. demissum* R-genes *R1–R11* (*R1* (Mastenbroek 43154-5), *R2* (Black 1512c), *R3* (Mastenbroek 4642-1), *R4* (Mastenbroek 4431-5), *R5* (Black 3053-18), *R6* (Black XD2-21), *R7* (Black 2182ef(7)), *R8* (Black 2424a(5)), *R9* (Black 2573), *R10* (Black 3618ad(1)), and *R11* (Black 5008ab(6))), and potato cv. Bintje as a susceptible control (provided by the Scottish Agricultural Science Agency) (Black et al. 1953; Malcolmson and Black 1966). The leaves were obtained by cultivating the tubers having each resistance gene of the differential set under greenhouse or controlled climate room conditions. The young leaves of which the growth process was completed were collected from the middle part of the canopy of 6–8-week-old plants and used in

inoculations. Inoculations were performed in 150-mm-diameter Petri dishes containing 75 mL of water agar (1.2%). After solidification of water agar, Petri dishes were inverted to form a moisture cycle and 5 leaflets were placed (abaxial side up) on the lid of Petri dishes. Sporangial suspensions at 4.0×10^4 sporangia mL⁻¹ concentration were prepared from 10-day-old cultures on rye B agar, and inoculation to all leaflets was performed by giving 20- μ L sporangial suspension to both sides of the main vein of the leaflet. After inoculation, Petri dishes were sealed with double-layer parafilm and incubated in a 16-h-light/8-h-dark cycle at 16 °C. The virulence assays were repeated at least twice for each isolate. The effect of pathogen isolates on the potato genotypes constituting the differential set was evaluated using the following scale 7 days after inoculation: 0, no symptoms; 1, small necrotic lesion; 2, < 10% area covered; 3, 10–50% area covered; 4, 50–75% area covered; and 5, > 75% area covered. The reaction was compatible if sporulation was detected in at least six leaflets out of ten, and the cumulative score was at least 15. Compatible interactions were usually indicated by large, sporulating lesions (Runno-Paurson et al. 2009).

Data analysis

Calculation of genetic similarity of SSR patterns was performed using the statistical software R v. 3.6.1 (R Development Core Team 2019), using Jaccard's index coefficient based on the proportion of shared alleles for all SSR primers. The cluster analysis was evaluated with unweighted pair group method algorithms (UPGMA) using R and Mega X (Kumar et al. 2018).

Results

A total of 127 *P. infestans* isolates from 35 locations in 17 counties in Turkey during 2015–2017 were obtained from diseased plants (Table 1). Isolates derived from the single sporangium were identified as the allozyme genotypes *Gpi* 100/100 and *Pep* 96/96, mating type A2, and mtDNA haplotype Ia. DNA fingerprint analyses using probe RG57 also found that all of the isolates overlapped with fingerprint characteristic of the Blue 13 clonal lineage.

SSR genotyping analysis using the set of 12 primers (Pi02, Pi4B, PiG11, Pi04, Pi63, Pi70, D13, PinfSSR11,

PinfSSR2, PinfSSR4, PinfSSR6, and PinfSSR8) showed no polymorphism in 81 of the 127 isolates of *P. infestans* from Turkey. The notable exception to this was two uncommon genotypes that exhibited difference in one step at loci Pi02 and PiG11 when compared with the most commonly found genotypes. Minor variations at Pi02 and PiG11 loci were determined in 26 and 46 isolates, respectively, including 20.5% for Pi02 and 36.2% for PiG11 of the entire isolates tested. These 72 isolates were collected from four different provinces (Afyon, Bolu, İzmir, and Niğde). All of the remaining isolates belonged to a single multi-locus genotype. This dominant genotype was heterozygous for almost all of the analyzed loci (Pi02, 266/268/270; Pi4B, 205/213; PiG11, 154/160; Pi04, 166/170; Pi63, 273/279; D13, 134/156; PinfSSR4, 284/294; PinfSSR6, 240/244; and PinfSSR8, 260/266), except for the loci PinfSSR2 (173/173), Pi70 (192/192), and PinfSSR11 (341/341). Minor variants of this genotype were found as 268/270 at Pi02 (26 isolate) and 154/162 at PiG11 (46 isolates) (Table 2). A dendrogram based on cluster analysis of 127 isolates that have the similar simple sequence repeat genotype 13_A2 and 17 reference isolates is shown in Fig. 2. In total, 84 isolates were resistant to mefenoxam (66.1%), and 43 showed an intermediate reaction (33.9%) (Table 1).

Virulence results obtained from tests showed that there were significant differences among the isolates of *P. infestans* in Turkey, and this collection of isolates was found to consist of 16 races. The most common race identified in the isolate population was R1.2.3.4.6.7.10.11 (54 isolates), followed by R1.2.3.4.6.7.8.10.11 (34 isolates). R1.3.4.6.7.8.10.11, R1.2.3.4.5.6.7.10.11, and R1.2.3.4.6.7.11 were represented by five isolates each. Four races were represented by a single isolate, and the remaining races by two or four isolates (Table 3). All isolates overcame resistance genes *RI*, *R4*, and *R7* in the same proportion (88%). Only six isolates collected in 2015 were able to overcome resistance genes *R5* and *R9*, and one isolate was able to overcome ten resistance genes, with the exception of *R9*. The number of virulence factors in each isolate was 6 to 10 in potato isolates. Of the isolates, 46 (grouped in four races) were found to have nine virulence factors. The mean numbers of virulence factors per isolate (*Ci*) and race (*Cp*) for isolates were calculated (Blandón-Díaz et al. 2012) as 8.2 and 7.8,

respectively. *Ci* being higher than *Cp* in isolates indicates that complex races predominate within potato populations of *P. infestans* in Turkey.

Discussion

Knowledge about the degree of diversity that can be identified across a population is extremely dependent on the type of molecular marker used. Of the three types of genotypic response models used in this study, mtDNA haplotype analysis is the less descriptive for the characterization of individuals within a pathogen population; in *P. infestans*, it permits significant differentiation among only four genotypes. mtDNA is, however, a proper tool for determination of the different clonal lineages within the population, as clearly indicated by Ristaino et al. (2001). On the other hand, SSR and RFLP molecular techniques give a high degree of polymorphism and quickly and easily determine the frequency of genes or alleles in populations. With reducing cost of DNA sequencing and increasing availability of large sequence data sets permit the mining of this data for large numbers of SSRs. Thus, SSR genotyping has been widely used in genetic diversity analysis of *P. infestans* populations (Kiiker et al. 2018; Knapova and Gisi 2002; Tian et al. 2016). In this study, using as few as two SSR markers among the most polymorphic, we were able to differentiate almost all isolates. On the other hand, since all isolates from Turkey exhibit the single SSR genotype, even though we increased the number of SSR markers from 2 to 12, the SSR genotype in all 127 isolates remained the same. These data indicate that the population of *P. infestans* in Turkey has revealed that consists of a single clonal lineage, although RFLP analysis showed only minor variations in a few isolates.

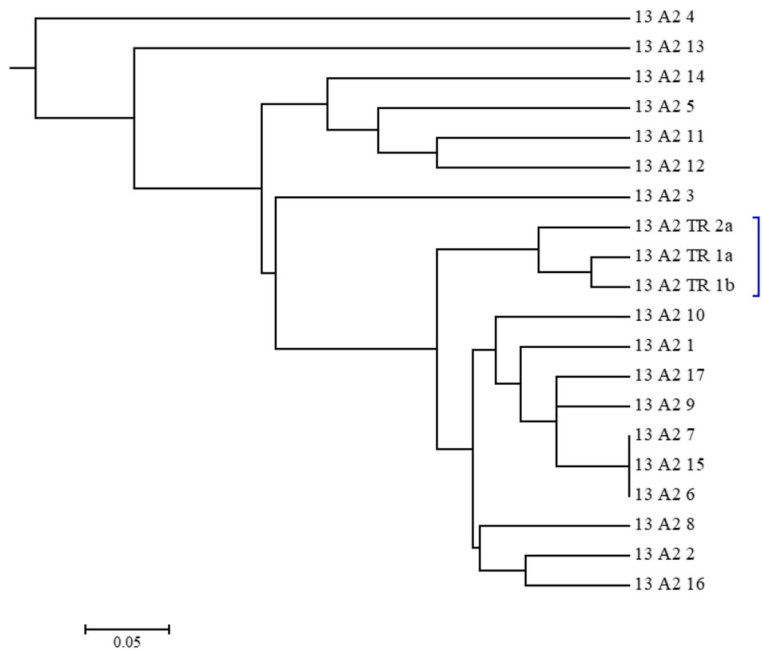
The late blight epidemics in Turkey during 2015–2017 were important, being started by one new clonal lineage of *P. infestans*, 13_A2. This led to occurrence of the disease that had not been seen in the country in previous years. US-8 and US-1 genotypes detected in tomato in previous years (Tosun et al. 2007) were not found in these epidemics in Turkey. The 13_A2 lineage was first reported in Germany and the Netherlands in 2004, followed by Poland in 2006, then in Great Britain in 2008, as well as in China and was also recently reported in India, where it was most likely to have entered from Europe (Cooke et al. 2012; Dey et al. 2018; Li et al.

Table 2 Allele sizes (bp) for *Phytophthora infestans* isolates amplified with each of the 12 SSR markers

Isolate sources	Number of isolates	Allele sizes												Genotypes
		Pf02	Pf4B	PfG11	Pf04	Pf63	Pf70	Df13	PmfSSR11	PmfSSR2	PmfSSR4	PmfSSR6	PmfSSR8	
Potato	26	268/270	205/213	154/162	166/170	273/279	192/192	136/156	341/341	173/173	284/294	240/244	260/266	13_A2_TR_1a
Potato	20	266/268/270	205/213	154/162	166/170	273/279	192/192	136/156	341/341	173/173	284/294	240/244	260/266	13_A2_TR_1b
Potato	81	266/268/270	205/213	154/160	166/170	273/279	192/192	136/156	341/341	173/173	284/294	240/244	260/266	13_A2_TR_2a
Chowdappa et al. 2015	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_2
Dey et al. 2018	1	266/268	205/213	154/160/164	166/170	273/279	192/192	136/156	341/341	173/173	284/294/296	240/244	260/266	13_A2_1
Kanetis et al. 2013	1	266/268	205/213	154/160	166/170	273/279	192/192	135/155	341/341	174/174	285/295	240/244	260/266	13_A2_15
Kanetis et al. 2013	1	266/268	213/217	141/156/204	170/170	270/279	192/192	135/210	331/341	174/176	289/297	244/244	244/260/266	13_A2_16
Li et al. 2013a	1	266/268	205/213	nd*	166/170	273/279	192/192	nd	341/341	173/173	285/295	240/244	260/266	13_A2_8
Li et al. 2013a	1	266/268	205/213	154/160	166/170	273/279	192/192	136/156	341/341	173/173	284/294	240/244	260/266	13_A2_10
Li et al. 2013a	1	266/268	205/213	154/160/164	166/170	273/279	192/192	136/154	341/341	173/173	284/294	240/244	260/266	13_A2_11
Li et al. 2013a	1	266/268	205/213	154/160	166/170	273/279	192/192	132/136/148	341/341	173/173	284/294/296	240/244	260/266	13_A2_12
Li et al. 2013a	1	266/268	205/213	154/160	166/170	273/279	192/192	136/144/154	341/341	173/173	284/294	240/244	260/266	13_A2_13
Li et al. 2013a	1	266/268	205/213	154/160	166/170	273/279	192/192	136/140/152	341/341	173/173	284/294	240/244	260/266	13_A2_14
Montes et al. 2016	1	268/268	205/213	nd	166/170	270/279	192/195	nd	341/341	173/173	289/295	242/244	260/266	13_A2_4
Montes et al. 2016	1	268/272	205/213	nd	166/170	273/279	192/192	nd	341/356	173/175	289/295	242/244	260/266	13_A2_5
Montes et al. 2016	1	268/268	213/213	nd	168/168	279/279	192/192	nd	331/331	173/173	289/293	244/244	260/260	13_A2_6
Montes et al. 2016	1	268/268	213/217	nd	170/170	279/279	192/195	nd	341/356	173/175	285/285	242/244	260/266	13_A2_7
Stroud et al. 2016	1	266/268	205/213	154/160/164	166/170	273/279	192/192	136/154	341/341	173/173	284/294	240/244	260/266	13_A2_3
Stroud et al. 2016	1	266/268	205/213	154/160/164	166/170	273/279	192/192	136/150	341/341	173/173	284/294/296	244/244	260/266	13_A2_17
Stroud et al. 2016	1	266/268	205/213	154/160	166/170	273/279	192/192	136/142/154	341/341	173/173	284/294	240/244	260/266	13_A2_18

*nd, not determined

Fig. 2 The UPGMA dendrogram of 127 *Phytophthora infestans* that have the same simple sequence repeat genotype 13_A2 and 17 reference isolates



2013b; Rekad et al. 2017). In Southern India, the 13_A2 lineage was responsible for severe late blight epidemics in potato and tomato and displaced the previous population consisting of US-1 and other genotypes (Chowdappa et al. 2015). Due to the import of potato seeds from the Netherlands, France, Germany, and the UK, genotypic variation in *P. infestans* populations from Turkey was expected. In parallel with this hypothesis, it is seen that the pathogen population in Turkey A2 mating type and Ia with mtDNA haplotype consists of a single clonal lineage. The results of the study clearly revealed that the population of the pathogen consisting of a single clonal lineage was of European origin because in the analyses using the SSR marker, the allele sizes commonly obtained in Europe were determined (Cooke et al. 2012; Dey et al. 2018; Li et al. 2013a; Stroud et al. 2016).

Mefenoxam, which has the ability to cure the plant against the attacks of sensitive pathogen populations, is one of the best fungicides used successfully against *P. infestans* (Cohen et al. 1979). However, it is known that certain clonal lines such as US-8 and US-11 do not have sensitivity to mefenoxam. The findings of this and previous studies have shown that the reaction against mefenoxam can still be used as a reliable character in determining any clonal lineage. The use of this test

combines with allozyme assays that are rapid and can be helpful in predicting mefenoxam susceptibility. Although the products containing mefenoxam have been replaced by new active substances in the last 10 years, the isolates tested in this study were found to be resistant to mefenoxam (Chmielarz et al. 2014; Cooke et al. 2011; Kiiker et al. 2018). In contrast, recent studies conducted in the USA showed that the sensitivity to mefenoxam increased in the new clonal lineages of the isolates compared with the 2000s (Danies et al. 2013). This increased sensitivity is attributed to less use of fungicide than in the past. As a result of random mutations, resistance to phenylamide group fungicides, including mefenoxam, can occur naturally among pathogen populations. However, the use of intense fungicide causes an increase in populations by creating a selection pressure in favor of these resistant individuals (Stellingwerf et al. 2018). In the period from the mid-990s until the early 2000s, the frequent and unconscious use of mefenoxam in Turkey caused the increase in the amount of resistant individuals in the total pathogen population. This has made selective pressure for the present clonal lineages of *P. infestans* to consist solely of resistant individuals and caused the decrease in genotypic diversity in the population as determined in previous studies (Grünwald et al. 2006). On the other hand, a high phenotypic diversity was maintained in the

Table 3 The race structure and metalaxyl-M response of *Phytophthora infestans* isolates collected from potato plants during 2015–2017 in the main potato-growing areas of Turkey

Locations ^a	Races	RM ^b	N of I ^c
BDM	1.2.3.4.6.7	I (2)	2
NMKO	1.2.4.6.7.11	R (1)	1
İÖM, İÖO	1.2.3.4.7.10	R (1) I (1)	2
İÖG, NÇÇ	1.2.3.4.6.7.11	R (3) I (2)	5
İKA, İKŞ, İÖO	1.2.3.4.7.10.11	R (3) I (1)	4
NÜC	1.2.4.6.7.10.11	R (3)	3
ASÖ	1.2.3.4.6.7.10	R (2)	2
ADB, AŞA, AŞY, İBE, İÖÇ, NÜC, NÜT, NÇB, NMK, NMP, NMY, TMK, TAB	1.2.3.4.6.7.10.11	R (36) I (18)	54
NMP	1.2.3.4.6.7.8.10	I (1)	1
BDA	1.2.3.4.6.7.8.11	R (1)	1
BMD, NÜC	1.3.4.6.7.8.10.11	R (4) I (1)	5
ADB, NÜT, NMY	1.2.3.4.5.6.7.10.11	R (1) I (5)	6
BMB, BMD, BMK, BMS, İÖGE, NAK, NMK, NÜM, NMKO, NMY, TAA, TVR, TVS	1.2.3.4.6.7.8.10.11	R (24) I (10)	34
BMB, BMD	1.2.3.4.6.7.8.9.11	R (4)	4
BMK	1.2.3.4.6.7.9.10.11	I (2)	2
İTK	1.2.3.4.5.6.7.8.10.11	R (1)	1
Total		127	127

^a ADB, Afyon-Dinar-Beyköy; ASÖ, Afyon-Sandıklı-Örenkaya; AŞA, Afyon-Şuhut-Anayurt; AŞY, Afyon-Şuhut-Yarıklı; BDA, Bolu-Dörtdivan-Aşağısayık; BDM, Bolu-Dörtdivan-Merkez; BMB, Bolu-Merkez-Berk; BMD, Bolu-Merkez-Doğancı; BMK, Bolu-Merkez-Karaköy; BMS, Bolu-Merkez-Sultanbey; İBE, İzmir-Bozdağ-Elmabağ; İKA, İzmir-Kiraz-Arkacılar; İKŞ, İzmir-Kiraz-Şemsiler; İÖÇ, İzmir-Ödemiş-Çaylı; İÖG, İzmir-Ödemiş-Gerçekli; İÖGE, İzmir-Ödemiş-Gereli; İÖM, İzmir-Ödemiş-Mescitli; İÖO, İzmir-Ödemiş-Ovakentyolu; İTK, İzmir-Tire-Kırtepe; NAK, Nevşehir-Acıgöl-Karapınar; NMK, Nevşehir-Merkez-Kaymaklı; NÜC, Nevşehir-Ürgüp-Cemil; NÜM, Nevşehir-Ürgüp-Mazı; NÜT, Nevşehir-Ürgüp-Taşkınpaşa; NÇB, Niğde-Çiftlik-Bozköy; NÇÇ, Niğde-Çiftlik-Çarşı; NMK, Niğde-Merkez-Kiledere; NMKO, Niğde-Merkez-Konaklı; NMP, Niğde-Merkez-Pıncık; NMY, Niğde-Merkez-Yaylayolu; TMK, Tokat-Merkez-Karakaya; TAA, Trabzon-Akçabat-Akpınar; TAB, Trabzon-Akçabat-Bozdoğan; TVR, Trabzon-Vakfikebir-Rıdvanlı; TVS, Trabzon-Vakfikebir-Sekmenli

^b RM, response to metalaxyl-M; R, resistant; I, intermediate resistant. Numbers in parenthesis indicate the number of isolates in each category

^c N of I, number of potato isolates employed in fungicide sensitivity tests and virulence testing using the potato differential set of resistance (R) genes (1 to R11)

Turkish population of *P. infestans* resistant to mefenoxam.

Despite low genotypic differences, a wide difference was found in response to virulence and fungicide resistance in the pathogen population. These results are consistent with the results of a study conducted in North China. In this study, virulence showed a wide variation on the basis of isolates and some of these isolates showed virulence to all resistance (R) genes (Guo et al. 2009). However, unlike the population in China, *P. infestans* population in Turkey did not succeed to infect all the R genes located in potato differential sets, and racial diversity and common structure consisted of groups that generally infect 8–9 potato clones. In other

parts of the world, a complex race structure and high virulence diversity have been identified (Deahl et al. 2003; Lehtinen et al. 2008; Pérez et al. 2001). Similar to other countries in the world, this complex race diversity and high virulence differences have been identified (Deahl et al. 2003; Lehtinen et al. 2008; Pérez et al. 2001). In the study conducted, all of the isolates in the culture stocks showed high virulence to potato clones bearing the resistance genes R1, R2, R4, and R7. These results are consistent with the general characteristics of the isolates in the EuroBlight database. As a matter of fact, isolates in this database are reported to show high virulence to R1, R2, R4, R7, and R11 (www.euroblight.net). A selection pressure created by the R genes found

in the most commonly cultivated cultivars may have played a role in this complex racial diversity determined in *P. infestans* isolates. For example, the ‘Sante’ variety found among the grower preferences is known to have the resistance genes *R1*, *R2*, and *R10*, and these *R* genes may, to some extent, be responsible for the high frequency of virulence (Flier et al. 2007). The occurrence of new blight races is associated with a mutation in the non-pathogenic (*Avr*) gene encoding the effector protein, so that the effector can no longer be recognized by the *R* protein. The high virulence diversity in isolates with the same SSR multi-locus genotype can be explained by the localization of the *Avr* genes in a hypervariable region of the genome (Jiang et al. 2008).

In conclusion, the present *P. infestans* population consists of a single clonal lineage (13_A2), belonging to the A2 mating type and the Ia mtDNA haplotype. Furthermore, based on SSR profiles, this population resembles the *P. infestans* population of European countries that provide the seed tubers that are planted in Turkey. However, the Turkish *P. infestans* population exhibited a large variation in race diversity and its composition, and the individuals resistant to mefenoxam remained high in the population. This clonal lineage, as mentioned above, has been common in potato production areas of European countries since 2006. In addition, critical issues, such as pathogen survival from year to year, sources of the primary inoculum that triggered epidemic outbreaks at the beginning of the cultivation period and analysis of their competition, and the performance of the wild solanaceous species as a source of inoculum remain largely unknown and could explain the details behind the survival and persistence of this clonal lineage notwithstanding possible attacks by other genotypes.

Authors’ contributions MEG and WEF designed the study. MEG, NA, GÖ, KM, AÇ, WEF, UP, TY, MA, HK, DELC, and NZ carried out the experiments. MEG, WEF, NA, GÖ, and DELC contributed to the interpretation of the results. MEG took the lead in writing the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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