Pak. J. Bot., 39(3): 897-902, 2007.

GENETIC VARIATION AMONG *PHYTOPHTHORA INFESTANS* (TOMATO BLIGHT) ISOLATES FROM WESTERN TURKEY REVEALED BY INTER SIMPLE SEQUENCE REPEAT (ISSR) AND RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

NECIP TOSUN[1] ARZU YILDIRIM[2], HUSEYIN TURKUSAY[1] AND BAHATTIN TANYOLAC[2]

[1]*Department of Plant Pathology, Agriculture College of Ege University, 35100 Bornova-Izmir, Turkey*
[2]*Department of Bioengineering, Ege University 35100 Bornova-Izmir, Turkey*
Corresponding Author: Bahattin Tanyolac e-mail: bahattin.tanyolac@ege.edu.tr

Abstract

Severe late blight disease epidemics in western Turkey during the 1997 tomato growing season prompted an investigation into the genotypes and mating types of *Phytophthora infestans* populations. A western Turkey-wide survey was conducted from 1999 to 2000 in which isolates were collected from 25 different fields. Twenty-five isolates were amplified with 21 RAPD and 18 ISSR primers. The isolates were analyzed for mating type. Genetic variation ranged between 0.002 (M. Kemalpasa (5) and M. Kemalpasa (12)) and 0.290 (Uluabat (46) and Uluabat (39)). Cluster analysis formed two main groups, one of which contained US-1 (A1 mating type) and the other US-8 (A2 mating type). Analysis of RAPD marker data indicated that in clade I, US-1 and Ulubat (46) were closest, while US-8 and Y. Sehir (26) were closest in clade II. This study shows that the A2 mating type of *Phytophthora infestans* exists in Turkey.

Introduction

Phytophthora infestans (Mont.) de Bary, is one of the most important causes of severe late blight, disease in potato and tomato worldwide. It is heterothallic and can reproduce sexually and asexually (McLoad *et al*., 2001) with rapid asexual reproduction under wet conditions. When weather conditions are favorable, an uncontrolled epidemic can result in 100% losses in some fields (Trout *et al*., 1997).

In recent years, late blight has become a significant problem in Turkey. Disease epidemics were experienced almost every year in the towns of in southern Turkey Fethiye and Kumluca, where fresh-market greenhouse tomatoes are extensively produced and in the cities Balıkesir, Bursa and Çanakkale in the northern Turkey, where primarily processing tomatoes are grown.

A range of methods has been used to study genetic structure of *P. infestans* populations; isozymes (Tooley *et al*., 1985), mitochondrial DNA restriction digest profiles (Carter *et al*., 1990) and restriction fragment length polymorphism (RFLP) with the RG57 probe (Goodwin *et al*., 1992). Amplified fragment Length Polymorphism (AFLP) (Vas *et al*., 1995) have proved useful in mapping studies (Van der Lee *et al*., 1997) and for population studies of *P. infestans* (Perez *et al*., 2001). RAPD and ISSR markers are simple to generate, requiring only PCR amplification and electrophoresis. RAPD and ISSR markers are appropriate for mapping, as well as DNA fingerprinting and population genetics studies. Also, Demeke *et al*., (1992) concluded that RAPD markers might be useful for taxonomic studies.

Table 1. Isolates collected from different farm in various locations in Turkey.

Isolate no.	Locations	Plant organ	Species	Host	Mating type
2	Karacabey	Fruit	*P. infestans*	Tomato	A1
5	M. Kemalpasa	Fruit	*P. infestans*	Tomato	A1
7	Biga	Fruit	*P. infestans*	Tomato	A1
12	M. Kemalpasa	Fruit	*P. infestans*	Tomato	A1
21	Y. Sehir	Fruit	*P. infestans*	Tomato	A1
22	Y. Sehir	Fruit	*P. infestans*	Tomato	A1
27	Biga	Fruit	*P. infestans*	Tomato	A1
34	Y. Sehir	Leave	*P. infestans*	Tomato	A1
28	Y. Sehir	Fruit	*P. infestans*	Tomato	A1
24	US1	-	*P. infestans*	Tomato	A1
46	Uluabat	Fruit	*P. infestans*	Tomato	A1
25	Karacabey	Fruit	*P. infestans*	Tomato	A1
25	Karacabey	Fruit	*P. infestans*	Tomato	A2
3	Fethiye	Fruit	*P. infestans*	Tomato	A2
29	Karacabey	Fruit	*P. infestans*	Tomato	A2
8	Fethiye	Fruit	*P. infestans*	Tomato	A2
38	Y. Sehir	Fruit	*P. infestans*	Tomato	A2
39	Uluabat	Fruit	*P. infestans*	Tomato	A2
41	Bilecik	Fruit	*P. infestans*	Tomato	A2
20	US8	-	*P. infestans*	Tomato	A2
26	Y. Sehir	Fruit	*P. infestans*	Tomato	A2
4	Fethiye	Stem	*P. infestans*	Tomato	A2
1	Karacabey	Fruit	*P. infestans*	Tomato	A2
6	M. Kemalpasa	Fruit	*P. infestans*	Tomato	A2
18	Biga	Fruit	*P. infestans*	Tomato	A2

The main purpose of the study was to characterize the populations of *P. infestans* present in the West Turkey. The distribution of both mating types were key interest with its potential impact on both practical control measures and, in the longer term, affect the range of genetic change in populations.

Materials and Methods

Sample collection and culture of isolates: Twenty-five *P. infestans* isolates were collected from diseased tomato plants (from leaves, stem and fruits) in western Turkey during the 1999 and 2000 tomato growing seasons. These isolates are maintained in a collection at Ege University, Department of Plant Pathology. A list of *P. infestans* isolates is shown in Table 1. Culturing of *P. infestans* isolates were carried out as described by Caten & Jinks (1968). Infected leaves were placed in glass Petri dishes lined with moist filter paper and incubated in the dark at 20^{o}C for 2 days. Once sporulation was evident, a small piece of agar was wiped over the sporongia and was transferred to a plate of pea agar. *P. infestans* grown on pea agar plates was incubated in the dark at 20^{o}C for growth and culture maintenance. US1 (A1 mating type) and US8 (A2 mating Type) were kindly provided by Dr. William E. Fry.

Mating type: Mating type test was performed as described by Peters *et al*., (1998). To determine the mating type of the isolates, the isolates were placed as an unknown isolate and known A1 and A2 mating types in a equidistant triangular pattern on pea agar plate.

Abundant oospores appeared between the thalli of compatible mating types as a distinct thickened band. If oospor formed between the unknown an A1 mating type but not between the unknown and A1 mating type, the unknown isolate was designated as an A1 mating type.

DNA isolation and PCR assay: DNA was isolated as described Goodwin *et al*., (1992) and used as template for PCR amplification. Amplification was done with 21 10-mer RAPD primers (kits AB, AC and AD, Operon Technology Inc. Alemada CA, US) and 18 ISSR primers (British Columbia Set 800-900) using a PTC-100 Programmable Thermal Controller (MJ Research, Inc. Nevada, US) as described by McLoad *et al*., (2001). Amplification products were resolved by electrophoresis (100 V for 4 h in a 2% agarose gel), and photographed after EtBr staining. Each RAPD and ISSR assay was repeated five times to confirm reproducibility of the results.

Statistical analysis: Bands on agarose gels from RAPD methods were scored for the presence (1) or absence (0) of amplified fragments in the 25 DNA samples. The data was combined to generate a pairwise distance matrix (data not shown). Pairwise comparisons of the PCR phenotypes were used to calculate genetic distance (GD), as described by Goodwin *et al*., (1995).

GD= $1-N_{xy}/(N_{XY}+N_X+N_y)$
Where:
GD: Genetic distance.
$\mathbf{N_{XY}}$**:** number of amplified products common to line x and y.
$\mathbf{N_X}$ **:** number of amplified products present in line x and absent in line y.
$\mathbf{N_Y}$ **:** number of amplified products present in line y and absent in line x.

GD values (data not shown) were used to construct a dendrogram by neighbor-joining analysis performed in the PAUP* 4.0 software (Swofford, 2001).

Results

Twenty-one RAPD primers and 18 ISSR primers were used to detect variation among the populations sampled from western Turkey. These primers amplified 252 loci in the genome, with an average of 12 loci per primer. A representative gel of the amplification products from RAPD primer AD05 is shown in Fig. 1.

Figure 2 shows a dendrogram of the relations among western Turkey strains of *P. infestans* as revealed by RAPD and ISSR analysis. According to genetic distance value calculated, the genetic variation among isolates ranged between 0.002 (M. Kemalpasa (5) and M. Kemalpasa (12)) and 0.290 (Uluabat (46) and Uluabat (39)). The dendrogram (Fig. 2) shows the isolates forming two distinct clades. Clade I consisted of 11 member with 3 isolates from Karacabey, 3 isolates from M. Kemalpasa, 2 from Yenisehir, and one from Biga, Ulubat, US1 (control). Clade II contained 14 members with 2 from Karacabey, 3 from Fethiye, 2 from Biga, 4 from Y.Sehir, one from Ulubat, Bilecik and US8 (control). In clade I, the control (US1) was most closely related to Ulubat (46) while the control for clade II (US8) was most closely related to Y. Sehir (26). The total genetic variation in clade I was lower than in clade II (Fig. 2). When compared with the mating type results, it was clear that the division between clades corresponded to the difference in the mating type. Twelve members of clade I were mating type A1, 12 members of clade II were mating type A2. The only exception was Biga (7) which was mating type A1.

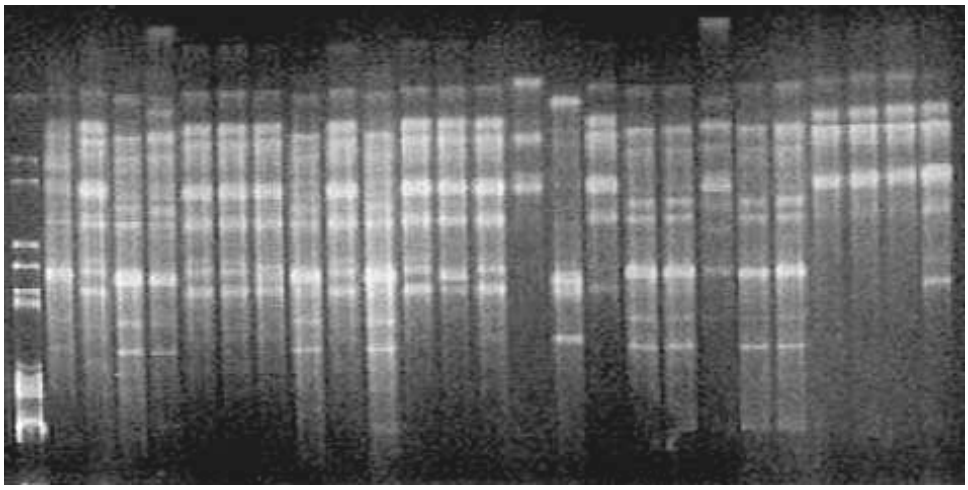

Fig. 1. An amplification pattern of primer AD05 of *Phytophthora infestans*. M: Lambda DNA Eco RI and Hind III. 1. Karacabey (1), 2. Karacabey (2), 3. Fethiye (3), 4.Fethiye (4), 5. M. Kemalpasa (5), 6. M. Kemalpasa (6), 7. Biga (7), 8. Fethiye (8), 9. M. Kemalpasa (12), 10. Biga (18), 11. Y. Sehir (21), 12. Y. Sehir (22), 13. Karacabey (25), 14. Biga (27), 15. Karacabey (29), 16. Y. Sehir (34), 17. Y. Sehir (38), 18. Uluabat (39), 19. Bilecik (41), 20. US8, 21. Y. Sehir (26), 22. Y. Sehir (28), 23. Ulubat (46), 24. US1, 25. Karacabey (25). The isolate numbers for each sample is given in ( ).

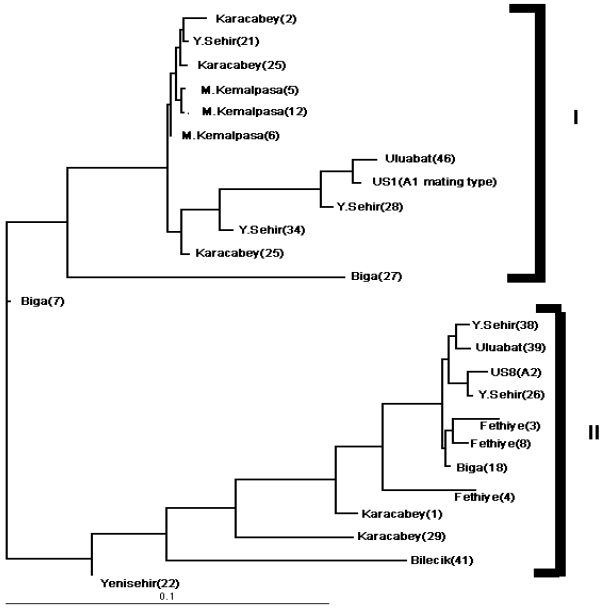

Fig. 2. Dendrogram of western Turkey strains of *P. infestans* revealed by RAPD and ISSR studies. Dendrogram were constructed using the Neighbor-joining option of PAUP software. The number in parentheses indicates isolate # in Table 1.

Discussion

To our knowledge this is the first report that the A2 mating type of *P. infestans* exists in Turkey. The RAPD and ISSR studies reported here were initiated to gain a better understanding of the genetic variation of *P. infestans* in Turkey. Results obtained with RAPD and ISSR primers indicate that isolates 23 of the 25 were clustered according to mating type. The only exceptions were Biga (7) & Y. Sehir (22) which were not closely related to any of the other isolates within their mating types. Due to recombination, the DNA genotype and phenotypes do not always coincide (Drenth *et al*., 1994). These isolates could carry heterozygous alleles for mating type. But, in order to conclude whether the isolates carry the alleles or not, we need to do further experiments by crossing the two mating types. Mating type studies have not been reported previously for Turkey.

Genetic variation in the first cluster was lower than in the second cluster. The first cluster formed two clades. Ulubat (46) was the closest isolate to US-1. In the second cluster, Y. Sehir (26) was the closest isolate to US-8 (Fig. 1). In a previous study (Drenth *et al*., 1993), 17 genotypes were identified among 205 isolates using the RG57 DNA marker. RAPD and ISSR genotyping indicated that high levels of genetic variation exist among isolates sampled from western Turkey. It is possible that variation may arise from recombination during sexual reproduction. Both A1 and A2 mating types were recovered from the same field (Peters *et al*., 1998). Although isolates formed two main clades, genetic variation within subclusters (clade-I and II) was also present. This might represent allelic variation for each mating type. In the dendrogram (Fig. 2), it was apparent that isolates tended to cluster within clades by geographical locations. Similar results were seen for the isolates of *Xathomonas oryzae* pv. Oryzae in India (Yashitola *et al*., 1997).

RAPD and ISSR results showed that isolates were grouped into mating types except Biga. This result are not agreeable with Mahuku *et al*., (2000). Mahuku *et al*., (2000) used 6 primers to detect genetic variation among *Phytophthora infestans* isolates. Six primers are not enough to cover entire genome to detect genetic variation. RAPD and ISSR detect variation that distributed throughout the genome and these markers provide useful information because they detect length polymorphisms arising from base sequence changes, insertions, deletions and substitutions either at or between the priming sites (Paran *et al*., 1997). High level of genetic diversity among isolates indicates that sexual recombination probably play important roles in the population dynamics of *Phytophthora infestans* in Western Turkey. The methods (RAPD and ISSR) used in this study is a simple, rapid, applicable and accurate way to distinguishing *P. infestans* isolates and grouping them according to mating type.

Acknowledgements

The authors gratefully acknowledge the support of the Turkish Governmental Planning Organization (Project # 2000 DPT 02) and partial funding by the Research Foundation of Ege University (Project #: 2000/BIL/005) in Turkey.

References

Carter, D.A., S.A. Archer, K.W. Buck, D.S. Shaw and R.C. Shattock. 1990. Restriction Fragment Polymorphism of Mitochondiral DNA of *P. infestanse*. *Mycological Research*, 94: 1123-1128.

Caten, C.E. and J.L. Jinks. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can. J. Bot*., 46: 329-348.

Demeke, T., R.P. Adams and R. Chibbar. 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in Brassica. *Theor. Appl. Genet*., 84: 990-994.

Drenth, A., S.B. Goodwin, W.E. Fry and L.C. Davidse. 1993. Genotypic diversity of *Phytophthora infestans* in the Netherlands revealed by DNA polymorphisms. *Phytopathology*, 83: 1087-1092.

Goodwin, S.B., A. Drenth and W.E. Fry. 1992. Cloning and genetic analysis of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics*, 22: 107-115.

Goodwin, S.B., A.C. Barak and W.E. Fry. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proc. Natl. Acad. Sci. USA*., 91: 11591-11595.

Goodwin, S.B., R.E. Schineider and W.E. Fry. 1995. Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Dis*., 79: 1181-1185.

Jaccard, P. 1908. Nouvelles recherchers sur la distribution florale. *Bull. Vaud. Soc. Nat*., 44: 233-270.

Mahuku G., R.D. Peters, H.W. Platt and F. Daay. 2000. Random Amplified polymorphic DNA (RAPD) analysis of *P. infestanse* isolates collected in Canada during 1994 to 1996. *Plant Pathology*, 49: 252-260.

McLoad, A., S. Denman, A. Sadie and F.D.N. Denner. 2001. Characterization of South African Isolates of *Phytophthora infestans. Plant Dis*., 85: 287-291.

Paran, I., D. Gidoni and R. Jacobsohn. 1997. Variation between and within broomrape (Orobanche) species revealed by RAPD markers. *Heredity*, 78: 68-74.

Perez, W.G., J.S. Gamboa, Y.V. Falcon, M. Coca, R.M. Raymunda and R.J. Nelson. 2001. Genetic Structure of Peruvian populations of *P. infestanse*. *Phytopathology*, 91: 956-965.

Peters, R.D., H.W. Plant and R. Hall. 1998. Characterization of changes in populations of *Phytophthora infestans* in Canada during mating types and metalaxyl sensitivity markers. *Can J. of Plant Pathology*, 20: 259-273.

Peters, R.D., H.W. Plat and R. Hall. 1998. Use of allozyme markers to determine genotypes of *Phytophthora infestans* in Canada. *Can. J. Plant Pathol*., 21: 144-153.

Swofford, D.L. 2001. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tooley, P.W., W.E. Fry and M.J. Villereal Gonzales. 1985. Isozyme characterization of sexual and asexual *P. infestanse* populations. *Journal of Heredity*, 76: 431-435.

Trout, C.L., J.B. Ristaino, M. Madritch and T. Wangsomboondee. 1997. Rapid detection of *Phytophthora infestans* in late blight-infected potato and tomato using PCR. *Plant Dis*., 81: 1042-1048.

Van der Lee T., I. De Witte, A. Drenth, C. Alfonso and F. Govers. 1997. AFLP linkage map of the oomycette *P. infestanse*. *Fungal Genetics and Biology*, 21: 278-291.

Vos, P., R. Hogers, M. Bleeker, M. Van Reijans, T. van der Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zebeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nuc. Acid Res*., 23: 4407-4414.

Williams, J.G.K, A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nuc. Acid. Res*., 18: 7213-7218.

Yashitola, J., D. Krishnaveni, A.P.K. Reddy and R.V. Sonti. 1997. Genetic diversity with the population of *xanthomonas oryzae* pv. *Oryzae* in India. *Phytopathology*, 87: 760-765.

(Received for publication 8 November 2006)