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CHARACTERIZATION OF *PHYTOPHTHORA INFESTANS* ON THE BASIS OF PHENOTYPIC MARKER (MATING TYPE)

Ghulam Raza[1], J. I. Mirza[2], Iftikhar Ahmad[2], Shazia Iram[2] and S. A. Malik[1]

[1]*Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[2]*Institute of Plant Environmental Protection (IPEP), National Agricultural Research Centre, Islamabad, Pakistan*

ABSTRACT

Phytophthora infestans is one of the most destructive pathogens of potato and causal agent of notorious disease late blight in Pakistan. Late blight infected potato samples were collected from the different potato growing areas of Pakistan, where late blight is a problem and from diseased samples 168 isolates were isolated. *Phytophthora infestans* sexually reproduce by mating type A1 and A2 were investigated by standard A2 strain (US8). In mating type test, when *P. infestans* isolates mated with known A2 standard isolate, produced oospore and showed A1 mating type but when no oospore production then indicated A2 mating type. During this study population of A2 mating type was higher (70%) in the population of Punjab (zone 2) but in Swat valley (Zone 5 and 6b), the percentage of A2 mating type was lower (44.5% and10%). The changes in the percentage of mating types (A1 and A2) showed the variable nature of *P. infestans*. Present study proved that the pattern of mating type distribution has been changed as compared to previous studies.

Key-words: *Phytophthora infestans*, potato, late blight, sexual reproduction

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important vegetable crop of Pakistan with an area of 109.7 thousand hectares and annual gross production of 1938.1 thousand tones (Anonymous, 2003-04). It is ranked as 10th major crop on the basis of its cultivated area however; it is third major crop of country regarding its monthly consumption per capita (Anonymous, 2003-04). In 1845, a strange disease appeared on potato named late blight of potato caused by fungus *Phytophthora infestans* (Debary, 1876). Late blight of potato is remarkably explosive disease, which can destroy potato fields in a few days. *Phytophthora infesrtans* is a heterothallic fungus that reproduces sexually by means of two mating types, designated as A1 and A2. Until the mid-1980, A2 mating type strains had been considered to be restricted to central Mexico, while only A1 strains were distributed worldwide. Mating type A2 was first time reported outside of Mexico in Switzerland (Hohl and Iselin, 1984). Since then a number of reports revealed that A2 mating type strains occur in most parts of the world (Mosa *et al.*, 1989, Marshall-Farrar *et al.*, 1998 and Carlisle *et al.*, 2001). In Pakistan, the presence of the two mating types of the fungus was reported in 1995 (Ahmad and Mirza, 1995). In case of *P. infestans*, most important possible factor for the emergence of resistance is sexual reproduction. The population produced as a result of sexual reproduction is always pathogenically variable with the higher fitness than those reproducing strictly asexually. There is a certain magnitude of hazard to potato crop posed by isolates from sexual population. Even if the sexual and asexual population is equal in fitness and virulence, second mating type poses a threat because it introduces the ability to produce oospores, which can survive in soil for undetermined time period; this introduces a source of initial inoculums (Tooley *et al*, 1986). Further, the dormant nature of oospores can help the fungus to escape the fungicide used (Goodwin and Fry, 1994 and Matuszak *et al.*, 1994). Recent reports showed that both mating type distributed in all potato growing areas of Pakistan (Batool *et al.*, 1998 and Ahmad, 2000). The old population consisted exclusively of A1 mating type, where as the new population comprised isolates of both A1 and A2 mating types (Speilman *et al.*, 1991; Day and Shattock, 1997 and Lebreton and Andrivon, 1998).

The goal of this investigation was to determine whether phenotypic change (on the basis of phenotypic marker, mating type) have occurred among populations of *Phytophthora infestans*. The frequency and distribution of both mating types were of key interest to assess the likelihood of sexual recombination with its potential to impact on both practical control measures and in the longer term, affect the rate of genetic change in populations.

MATERIALS AND METHODS

The potato samples infected with late blight were collected from different potato growing agro-ecological zones/areas of Pakistan from 2003 to 2004. These samples were brought to Crop Diseases Research Programme (CDRP) laboratory for isolation of *P. infestans*.

Isolation of *Phytophthora infestans*

Two types of methods were used for the isolation of *P. infestans* from infected leaf samples of potato i.e., potato disc method (Tantius *et al.*, 1986) and the other was leaf disc method (De Wolf *et al.*, 1998). The samples purified by rye agar medium containing rye broth 11, glucose (20g/l), agar (15g/l), rifampicin (0.02g/l), polymixin B (0.05g/l), ampicillin (0.10g/l), vancomycin (0.05g/l), benlate (50%) (0.1g/l), PCNB (75%) (0.67g/l).

Mating Type Analysis

For the determination of mating type V8 Rye agar medium containing rye broth (800ml), V8 juice (200ml), $CaCO_3$ (1g/l) and agar (15g/l) was used.

Mating Type Determination

The mating type of each isolate of *P. infestans* was determined *in-vitro* by mating with standard US-8 isolate of known A2 mating type (Shattock *et al.*, 1990). A small agar plug of approximately 5mm diam., from isolate to be tested was plated in the center of each plate. An agar plug with mycelium of a known A2 strain (US 8) was placed on either side of the sample. After 5-10 days incubation at 15-20°C, plugs from the interfacing region of the two cultures were observed under microscope for the presence or absence of oospores. If oospores were seen on the plate with the known A2, the tested isolate was rated as A1 and if no oospore was observed, it was rated as A2.

RESULTS

Collection of Isolates

Fifteen different locations of three potato-growing zones (2, 5 and 6b) were surveyed for potato late blight disease and a number of *P. infestans* isolates were obtained from infected samples.

Mating Types of *Phytophthora infestans* Isolates

Frequency of Two Mating Types (2003-04)

One hundred and sixty eight isolates from zone 2, 5 and 6b were isolated to examine the mating types. Out of 168 isolates 37.5% isolates belonged to A_1 mating type and 62.5% isolates belonged to A_2 mating type. The percentage of A_2 mating type isolates was higher as compared with A_1 (**Fig.1**).

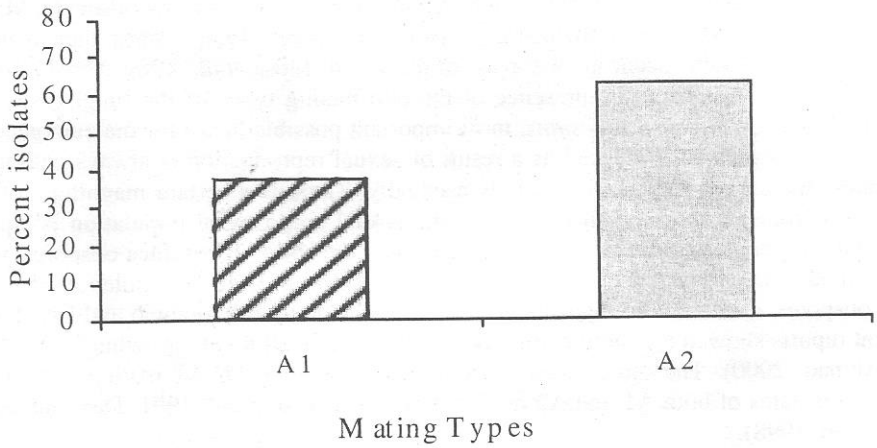

Fig. 1. Frequency of mating types A1 and A2 in agro ecological zones of potato.

In mating type analysis from zone 2, A2 mating type isolates were higher (70%) as compared with A_1 (30%) mating type but in zone 6b the frequency of A2 isolate was less (10%) as compared with A1 (90%) mating type isolates. Similarly in zone 5, the percentage of A_2 mating type (44.5%) was lower as compared with A_1 mating type. (**Fig. 2**).

From Zone 6b, locations Cani, Kalam, Sangum, Atrund, Batander only A1 mating type was obtained where as at location Sharan both of mating types were present (**Fig. 3**).

Similarly, both of types were also prevailing in locations (Aroop and Salara) of zone 2 while in Chiniot only A2 mating type was found. In Aroop, A1 and A2 mating types were counted to be as 83% and 17% respectively.

Where as at location Salara, A1 mating type was 14.5 % and A2 mating type was 85.5 %. In Cheniot, A 2 mating type was found with the value of 100% (**Fig. 4**).

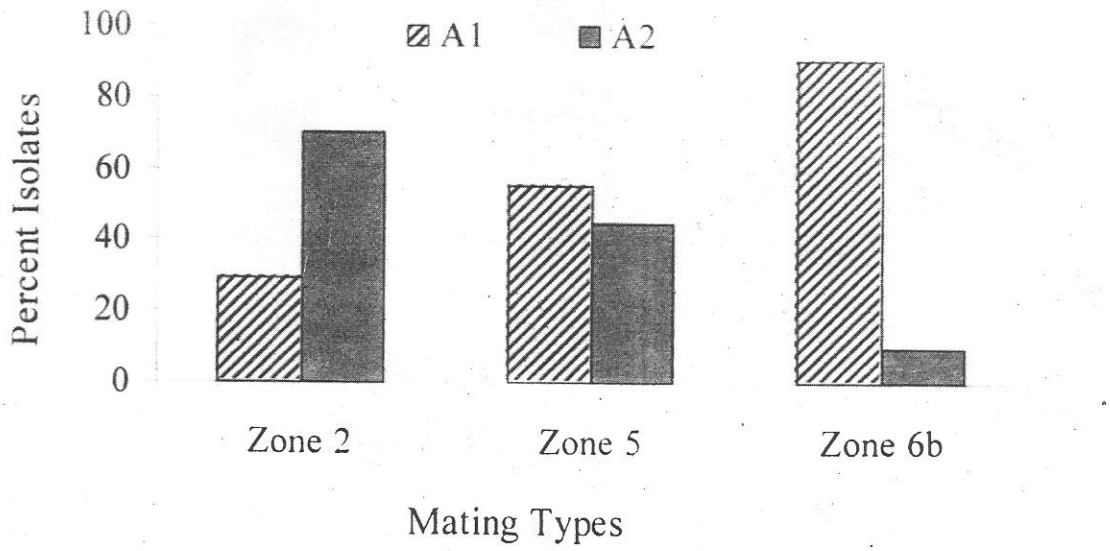

Fig. 2. Frequency of mating types A1 and A2 in zone 2, zone 5 and zone 6b.

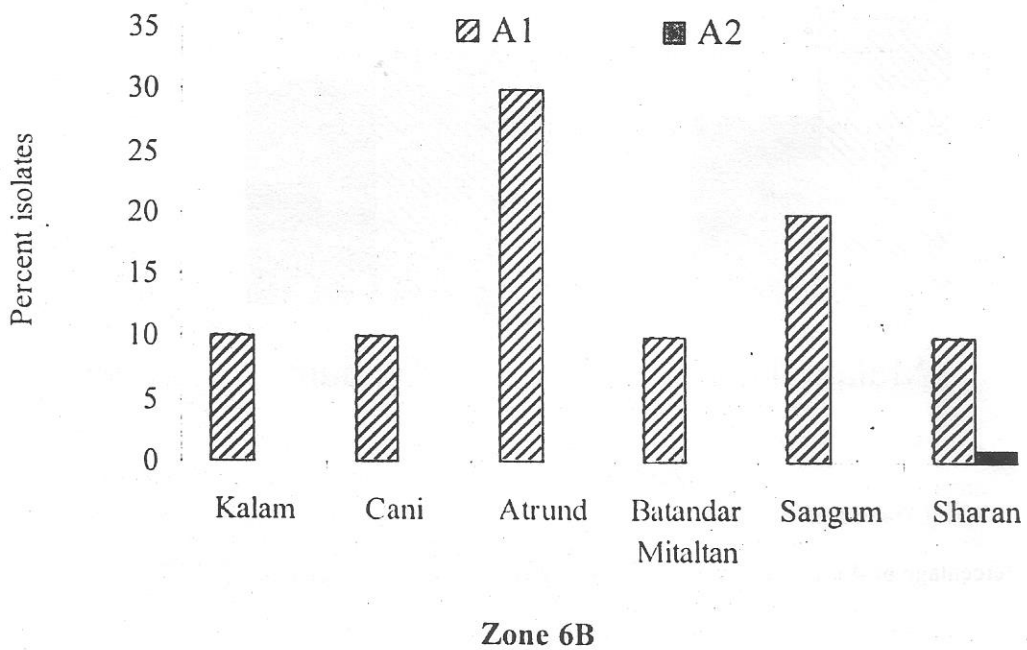

Fig. 3. Frequency of A1 and A2 mating types in different locations of Zone 6b.

At location Malam Jaba and Miandam of Zone 5 both of population i.e. A1 and A2 mating types were existing with an intensity of 60 and 40% respectively in Malam Jaba and 53 and 47% in Miandam (**Fig. 5**).

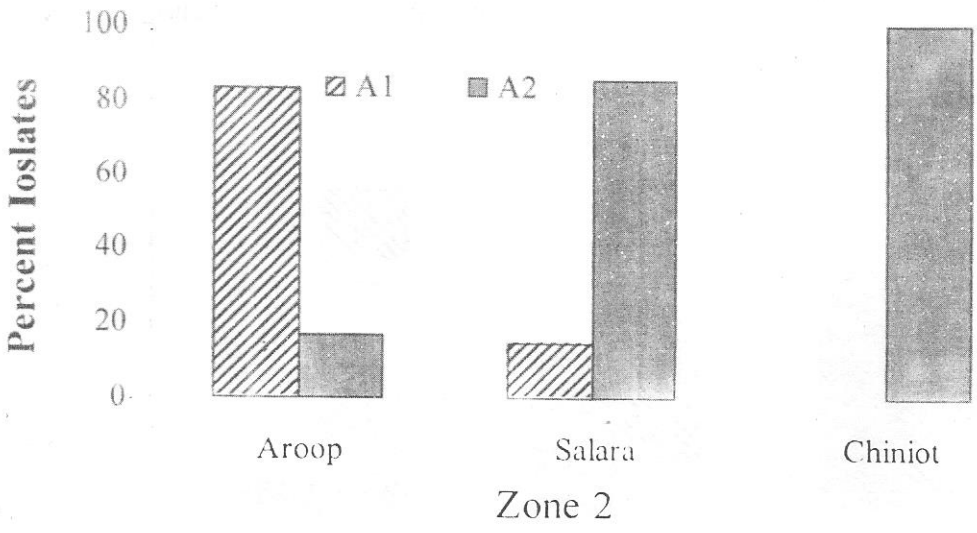

Fig. 4. Frequency of A1 and A2 mating types in different locations of Zone 2.

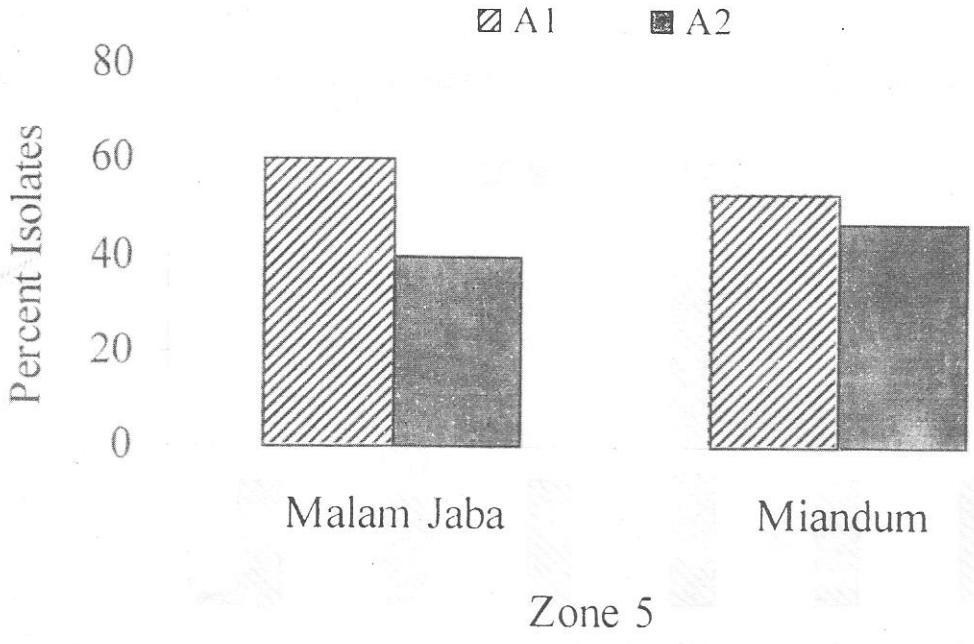

Fig. 5. Percentage of A1 and A2 mating types in different locations of Zone 5.

DISCUSSION

In the present study, 168 isolates of *P. infestans* were isolated and analyzed for mating type analysis which showed that 63 isolates were A1 mating types and 105 were A2 mating types. This study revealed that both A1 and A2 mating types of *P.infestans* were present in all zones/areas. In Pakistan, an interesting situation was in *P.infestans* population structure (mating types) as, A1 mating type was dominant over A2 mating type during 1998 and 2000 (Batool, 1998 and Ahmad, 2000) but the present study showed the dominance of A2 mating type over A1 mating type. It means that there was a replacement of old population (A1 mating type) with new one, which is according to the reports of Carter *et al.*, 1991 and Drenth *et al.*, 1994. The higher percentage of A2 mating type might be due to the greater fitness of this strain and virulence (Day and Shattock, 1997). Population of both mating

type (A1 and A2) changed from zone to zone. A2 mating type was dominated in zone 2, which contradicted earlier reports that A1 population dominated over A2 mating type. In zone 5, A1 mating type dominated over A2 which contrary to earlier reports however; comparatively higher percentage of A2 type was also present. The reason for returning from A2 to A1 mating is not clear. In zone 6b, A1 mating type completely dominated over A2 mating type. Although A2 mating type was present but in very lower percentage, which is similar to previous reports (Batool, *et al.*, 1998; Ahmad, 2000). The dominance of A1 might have been created again due to its greater fitness of either of the two mating types in respective zones.

A number of possible factors may be responsible for different level of displacement in different zones, which could be use of different varieties used in the zone, environmental conditions conducive to different strains and fungicide used (Ahmad, 2000). Slow and fast displacement of *P.infestans* in different zone, may be due to the use of those varieties, which have provided better host-pathogenic interaction for either of mating types. Environmental condition and different levels of selection pressure may also play a role in the proliferation of particular strain in particular areas (Chang and Ko, 1990; Ko, 1994). The above discussion indicated the displacement of old population (A1 mating) with new population (A2 mating) and has opened the possibility of sexual reproduction. Far apart from the fitness and virulence of the sexual and asexual reproduction, the presence of A2 mating type in particular areas posed a threat to plant pathogen to produce oospores (Tooley *et al.*, 1986). Oospores when present in field can dramatically change the epidemiology of the disease. (Drenth, *et al.*, 1995). This higher surviving ability of oospores and their increased rate of germination make it potential threat for potato production in a country like Pakistan where we have potato crops round the year in a diverse climatic condition.

REFERENCES

Ahmad, I. (2000). *Mating types Races and Genetic Variability in Phytophthora infestans, The Cause of Late Blight of Potato*: Final Technical Report Submitted to Pakistan Science Foundation for the Project C-NARC/Agr (216). Pp. 96.

Ahmad, I. and J.I. Mirza (1995). Occurrence of A2 mating type of *Phytophthora infestans* in Pakistan. In: *Proceedings of the National Seminar on Research and Development of Potatoes in Pakistan.* Organized by Pak-Swiss Potato Development Project, PARC, Islamabad, Pakistan. April 23-25, 1995: pp. 93-94.

Anonymous, (2003-2004). *Agriculture Statistics of Pakistan.* Ministry of Food and Agriculture Pakistan.

Batool, S., I. Ahmad, S.A. Malik and J.I. Mirza (1999). Distribution of mating types of *Phytophthora infestans* in Pakistan during 1997-99. In: *Abstracts of Second National Conference of Plant Pathology*. September 27-29, 1999. University of Agriculture Faisalabad, Pakistan pp. 22.

Carlisle, D.J., L.R. Cooke and A.E. Brown (2001). Phenotypic and genotypic characterization of Northern Ireland isolates of *P. infestans. European J. plant pathology*, 107: 291-303.

Carter, D. A., S. A. Archer, K. W. Buck, D. S. Shaw and R. C. Shattock (1991). DNA polymorphism in *Phytophthora infestans*: the UK experience. In: *Phytophthora infestans*. (Lucas JA. Shattock RC, Shaw DS, Cooke LR, eds). Cambridge, UK: Cambridge University Press, 272-94.

Chang, T. T. and W. H. Ko (1990). Effect of metalaxyl on mating type of *Phytophthora infestans* and *P. paractica. Annuals Phytopathology Society Japan*, 56: 194-198.

Day, J. P and R.C. Shattock (1997). Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. *European J. Plant Pathology*, 103: 379-391.

De Bary, A. (1876). Researches into the nature of the potato fungus *Phytophthora infestans. J. the Royal Agriculture Society of England*, 12: 239-269.

De wolf, E.D., R.J. Effertz, S. Ali and I.L.J. Franc (1998). *Vistas of tan spot Research. Canadian J.Plant Pathology*, 20: 349-444.

Drenth, A., E. M. Ganssen and F. Gover (1995). Formation and survival of oospore of *Phytophthora infestans* under natural conditions. *Plant Pathology*, 44: 86-96.

Drenth, A., I. C. Q. Tas and F. Govers (1994). DNA finger printing uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European J. Plant Pathology*, 100: 97-107.

Goodwin, S. B. and W. E. Fry (1994). Continuous migration of A_2 mating types, metalaxyl resistant genotypes of *Phytophthora infestans* in the eastern United States and Canada. *Phytopathology*, 84: 1271.

Hohl, H. R. and K. Iselin. (1984). Strains of *Phytophthora infestans* from Switzerland and with A2 mating type behaviour. *Trans. British Mycological Society*, 83:529-31.

Ko, W. H. (1994). An alternative possible origin of the A2 mating type of *Phytophthora infestans* out side Mexico. *Phytopathalogy*, 84: 1224-1227.

Lebreton, L., D. Andrivon (1998). French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European J. Plant Pathology*, 104: 583-94.

Marshall-Farrer, K, D., M. Mchgrath, R. V. Janes and W. R. Stevenson (1998). Characterization of *Phytophthora infestans* in Wisconsin from 1993 to 1995. *Plant Disease*, 82: 434-436.

Matuszak, J. N., J. Frnanddez-Elquezsabal, W. K. GU, M. Villarealj-Gonzalez and W.K. Fry (1994). Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: Distribution and dynamics. *Plant Disease*, 78: 911-16.

Mosa, A. A., M. Kato, N. Sato, K. Kobayashi and A. Ogoshi (1989). Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Japan. *Annual Phytopathological Society of Japan*, 55: 615-20.

Shattock, R. C., D. S. Shaw, A. M. Fyfe, J. R. Dunn, K. H. Loney and J. A. Shattock (1990). Phenotypes of *Phytophthora infestans* collected in England and Wales from 1985 to 1988: mating type, response to metalaxyl and isoenzyme analysis. *Plant Pathology*, 39: 242-48.

Speilman, L. J., A. Drenth, L. J. Sujkowski, W. k. Gu, P.W. Tooley and W. E. Fry (1991). A second worldwide migration and population displacement of *Phytopathora infestans. Plant Pathology*, 40: 422-30.

Tantius, P.H., A.M. Fyfe, D.S. Shaw, and R.C. Shattock (1986). Occurrence of the A_2 mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. *Plant Pathology*, 35: 578-81.

Tooley, P. W., J. A. Sweighard and W. E. Fry (1986). Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual population. *The Annual Phytopathological Society*, 76: 1209-12.

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