Mycobiology 29(3): 164-169 (2001)


# Differential Growth Response of $A_1$ and $A_2$ Mating Types of *Phytophthora infestans* on Rye A and V-8 Juice Agar Media Supplemented with Rhizome Powder of *Cyperus rotundus*

**U. P. Singh[1]*, B. K. Sarma[1], Ruo Nishimura[2], Kiroku Kobayashi[2], Akira Ogoshi[2], Volker Zinkernagel[3], Alexendra Schlenzig[3], Barbel Schöber-Butin[4] and H. J. Aust[5]**

[1]*Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India*
[2]*Department of Plant Pathology, Faculty of Agriculture, Hokkaido University, Sapporo-060, Japan*
[3]*Institut für Phytopathologie der Technischen, Universität Munchen, Freising-Weihenstephan, Germany*
[4]*Institut für Planzenschutz in Ackerbau und Grünland, Biologische Bundesangestalt für Land-und Forstwirtschaft, D-38104 Braunschweig, Germany*
[5]*Institut für Mikrobiologie der Technischen Universität Braunschweig, Spielmann Str. 7, Braunschweig, Germany*

**A new medium for studies of diversity among populations of $A_1$ and $A_2$ mating types of *Phytophthora infestans* has been evolved. The rye A agar and V-8 juice agar media on which *P. infestans* grows well have been amended with rhizome powder of *Cyperus rotundus*. A total of 259 isolates of $A_1$ and $A_2$ mating types representing Japan, Korea, India, Taiwan, Indonesia, Thailand, China, Nepal, U.K. and Mexico were screened for their growth response on these two media. Most of the A1 isolates did not grow well on them except Thailand while growth of $A_2$ mating types differed as some grew on it whereas others did not. It is quite likely that the populations of $A_2$ mating types that did not grow well on rhizome-amended medium are of different clonal lineage. This suggests that this medium can be used for the study of diversification among the isolates of the same or both the mating types as well as to detect the newly introduced genetically different isolates of *P. infestans* in a locality where it was not reported earlier.**

**KEYWORDS:** ***Cyperus rotundus*, Mating types, *Phytophthora infestans*, Rhizome agar medium**

de Bary (1876) was the first to describe in detail the biology of *Phytophthora infestans* (Mont.) de Bary. Since then several workers were interested in observing the formation of sexual stage (Jones, 1909; Jones and Giddings, 1909; Pethybridge, 1912). Pethybridge and Murphy (1913) reported for the first time the oospores of this fungus. Scattered reports on the formation of oospores have, since then, appeared in literature (Tucker, 1931). However, it was only in 1958 that the two mating types, viz., $A_1$ and $A_2$, were identified among Mexican isolates (Gallegly and Galindo, 1958) demonstrating the existence of heterothallism in *P. infestans*. The recognition of heterothallic nature in this and other fungi of similar genetic constitution is usually based on crossing the two mating types for the formation of sexual stage. While collecting *Sclerotium rolfsii* from different plants in the field, it was observed that the fungus is growing on the rhizomes of *Cyperus rotundus*. The rhizomes were brought to the laboratory and *S. rolfsii* was cultured on potato dextrose agar (peeled potato 200 g, dextrose 20 g, agar 15 g, 1 *l* distilled water) medium supplemented with rhizome powder (RP) of *C. rotundus*. Interestingly, it was observed that the fungus formed athelial (sexual) stage in profusion which is rarely observed in culture. The powder was tried on some other fungi also where it induced sexual reproduction (Singh *et al.*, 1996). Keeping this in view that it may induce oospore formation in a mating type of *P. infestans*, experiments were conducted in two laboratories in Germany and also in Japan where instead of inducing sexual stage, the powder affected differentially the growth of $A_1$ and $A_2$ mating types of *P. infestans* and the results are presented here.

*Corresponding author <E-mail: ups@banaras.ernet.in>

## Materials and Methods

**Experiments done on rye A agar medium in Freising - Weihenstephan (Fr.), Germany.** Two hundred g rye was kept in 1 *l* distilled water and cooked for 1.5 h and then filtered. Ten g agar and 5.9 g glucose were added to the filtrate and supplemented with 1, 2, 3 and 5% RP separately and then autoclaved for 20 minutes at 121°C. The $A_1$ and $A_2$ mating types were inoculated in the same plates at equidistance and also separately and incubated at 16±1°C for growth. Both the mating types were also inoculated on rye A agar medium without RP which served as control. The experiments were done in triplicate.

**Experiments done on V-8 juice agar medium in Braunschweig (Br.), Germany.** $A_1$ and $A_2$ mating types were grown on V-8 juice agar medium supplemented with

**Table 1.** Origin and number of $A_1$ and $A_2$ isolates of *Phytophthora infestans*

| Country | Number of isolates | | |
|---|---|---|---|
| | $A_1$ | $A_2$ | Total |
| Japan | 5 | 11 | 16 |
| Korea | 0 | 13 | 13 |
| India | 9 | 0 | 9 |
| Taiwan | 26 | 0 | 26 |
| Indonesia | 0 | 4 | 4 |
| Thailand | 21 | 25 | 46 |
| China | 92 | 1 | 93 |
| Nepal | 47 | 3 | 50 |
| U.K. | 0 | 1 | 1 |
| Mexico | 0 | 1 | 1 |
| Total | 200 | 59 | 259 |

1, 2, 3 and 5% RP before autoclaving. Control experiments were run on the same medium without RP. All the experiments were done in triplicate.

**Experiments done on rye A agar medium in Sapporo, Japan.** A total of 259 isolates of $A_1$ and $A_2$ mating types collected from Japan, Korea, India, Taiwan, Indonesia, Thailand, China, Nepal, U.K. and Mexico were used in the experiment (Table 1). The rye A agar medium was

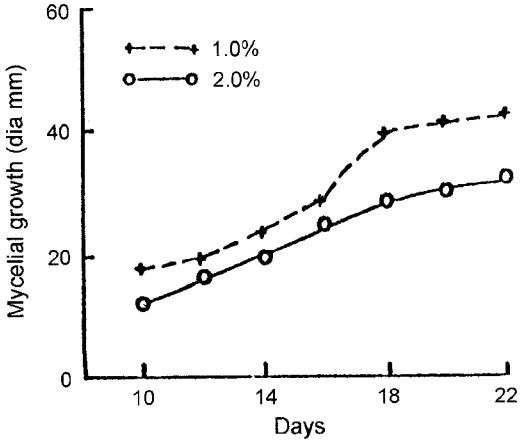
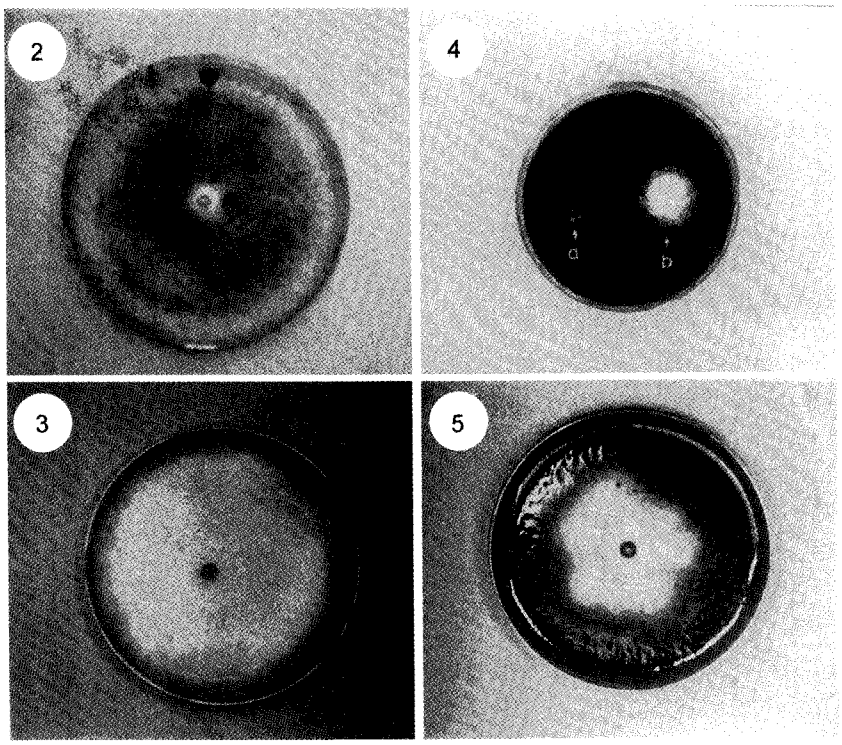



**Fig. 1.** Mycelial growth of $A_2$ mating type (Fr.) of *Phytophthora infestans* on V-8 juice agar medium supplemented with 1 and 2% *Cyperus rotundus* rhizome powder.

supplemented with 1, 2 and 3% RP separately. Such RP amended medium was again supplemented with metalaxyl (0.05 and 0.1 ppm separately) before autoclaving. Both the isolates were inoculated individually on such plates. Control plates received only rye A agar medium without RP. All the plates were incubated at 18°C and the




**Figs. 2-5.** Mycelial growth of $A_1$ and $A_2$ mating types (Fr.) of *Phytophthora infestans* on V-8 juice agar alone and also on *Cyperus rotundus* rhizome powder supplemented V-8 juice agar medium. Figs. 2 and 3. $A_1$ and $A_2$ mating types respectively on V-8 juice agar only (almost natural size). Fig. 4. No growth of $A_1$ (arrow a) and slow growth of $A_2$ (arrow b) mating types on V-8 juice agar supplemented with 1% powder of *C. rotundus* rhizomes (observed after 10 days). Fig. 5. Mycelial growth of A2 mating type on V-8 juice agar supplemented with 2% powder of *C. rotundus* rhizomes (observed after 20 days) (4 and 5 1/2 natural size).

growth diameter was measured after every second day, upto 14 days after inoculation. All the experiments were done in triplicate.

## Results

**Results of Freising - Weihenstephan (Fr.) and Braunschweig (Br.) Experiments.** The growth pattern of both the Fr. mating types indicates that $A_2$ isolates grew on V-8 juice agar medium supplemented with RP, although slowly (Figs. 1, 4) as compared to control (Figs. 2, 3). However, there was no growth of A1 isolates on any concentrations of the RP (Fig. 4 arrow a) but the growth was also observed at higher concentration (2, 3 and 5%) after prolonged incubation, for instance, after 20 days some growth was observed on 2% (Fig. 5) and after 25 to 30 days on 3 and 5%. In one plate there was segregation in the fungal growth into more compact (Fig. 6a) and thin colony types (Fig. 6b) whose microscopic observations revealed that the mycelia of compact growth was sporulating forming sporangia while the thin mycelial growth was nonsporulating. Figure 6c shows no growth of A1 mating type even after prolonged incubation.

The growth behaviour of 10 different isolates of $A_1$ and $A_2$ mating types (Br.) shows that maximum growth was discerned in Scotaland isolate while other isolates exhibited variable response in growth intensity. There was no growth of $A_1$ isolates on this medium (Table 2).

**Results of Sapporo experiments.** The growth of $A_1$ isolates of Japan, India, Taiwan, Thailand, China and Nepal on rye A agar medium indicates that Chinese isolates showed maximum growth (70 mm dia) as compared to other isolates whereas Taiwanese were slowest (65 mm dia) in two weeks of incubation (Fig. 7). The growth was

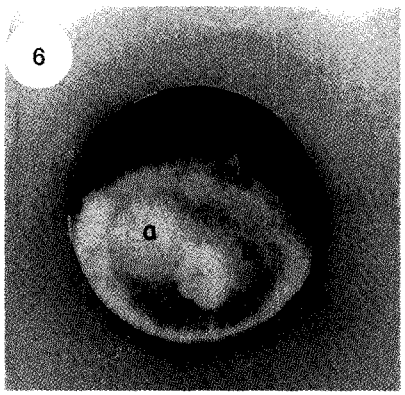



**Fig. 6.** Mycelial growth and segregation into compact (a) and thin mycelial types (b) of $A_2$ mating types and no growth of $A_1$ mating type (c) of *Phytophthora infestans* on V-8 juice agar supplemented with 5% powder of *C. rotundus* rhizomes (observed after 30 days) (1/2 natural size).

**Table 2.** Growth of $A_1$ and $A_2$ mating types of *Phytophthora infestans* on rye A agar and V-8 juice agar media supplemented with 1%, 2%, 3% and 5% powder of *Cyperus rotundus* rhizomes

| Mating types | Isolate number | Growth on rye A agar | | | | |
|---|---|---|---|---|---|---|
| | | 0% | 1% | 2% | 3% | 5% |
| $A_1$ | 1-4a | +++++ | - | - | - | - |
| $A_1$ | 1-11a | +++++ | - | - | - | - |
| $A_1$ | 1-4b | +++++ | - | - | - | - |
| $A_1$ | 1-11b | +++++ | - | - | - | - |
| $A_1$ | 1-4c | +++++ | - | - | - | - |
| $A_2$ | Scotland | +++++ | ++++ | ++++ | +++ | +++ |
| $A_2$ | 13/94 | +++++ | ++ | ++ | + | + |
| $A_2$ | 46/94 | +++++ | +++ | +++ | ++ | ++ |
| | | Growth on V-8 juice agar | | | | |
| $A_1$ | Freising | +++++ | - | - | - | - |
| $A_2$ | Freising | +++++ | ++ | + | +* | +** |

+ = Very poor growth, ++ = Poor growth, +++ = Good growth, ++++ = Very good growth, +++++ = Excellent growth, - = No growth, * = Grows after about 20 days of incubation, ** = Grows after 25~30 days after incubation.

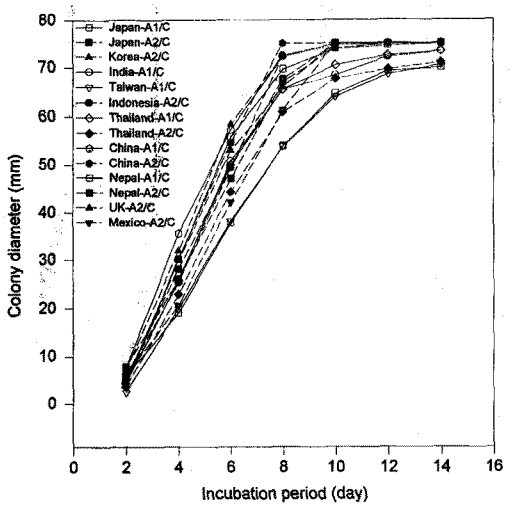



**Fig. 7.** Growth of $A_1$ and $A_2$ isolates of *Phytophthora infestans* from Japan, India, Taiwan, Thailand, China and Nepal on rye A agar medium.

inhibited on 1% RP supplemented medium as compared to control. Thailand $A_1$ isolates showed maximum growth reaching about 55 mm dia in two weeks while Nepalese isolates attained about 20 mm in the same incubation period. The growth of other isolates was between the above two maximum and minimum growth parameters (Fig. 8). The growth of all $A_1$ isolates was further inhibited on 2% RP supplemented medium. The initiation of growth also started after 3~4 days of incubation. Chinese and Nepalese isolates showed minimum growth while others being very slow reached a maximum of 20 mm dia (Fig. 9). Interestingly, on 3% the growth commenced after 5~6 days in some while in others there was no growth at

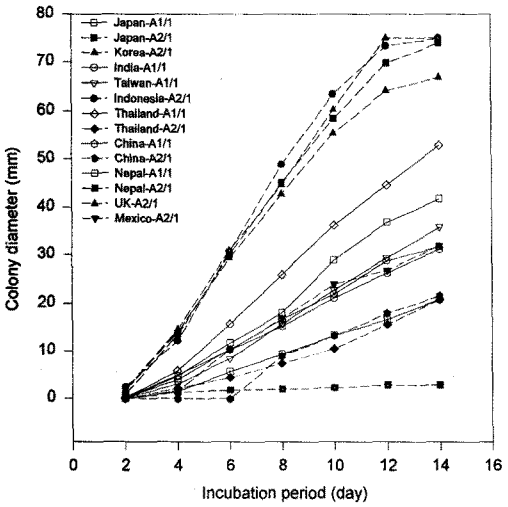



**Fig. 8.** Growth of $A_1$ and $A_2$ isolates of *Phytophthora infestans* from Japan, India, Taiwan, Thailand, China and Nepal on rye A agar medium supplemented with 1% *Cyperus rotundus* rhizome powder.

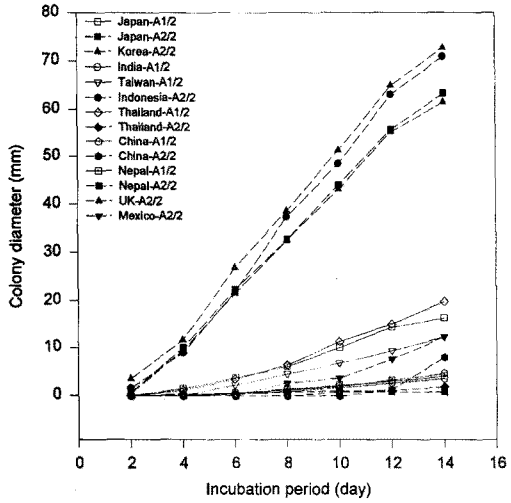



**Fig. 9.** Growth of $A_1$ and $A_2$ isolates of *Phytophthora infestans* from Japan, India, Taiwan, Thailand, China and Nepal on rye A agar medium supplemented with 2% *Cyperus rotundus* rhizome powder.

all even after 14 days of incubation (Fig. 10).

Growth of all $A_2$ isolates of Japan, Korea, Indonesia, Thailand, China, Nepal, U.K. and Mexico on rye A agar medium (control) commenced soon after inoculation and reached 70~75 mm dia in 14 days. Most of the isolates showed almost similar growth pattern on this medium (Fig. 7). On 1% rye A agar medium some isolates, for instance, Japanese, Korean, Indonesian and U.K., grew faster than the isolates of Thailand, China, Nepal and Mexico. The slowest growth was of Nepalese isolates (Fig. 8). While some of the isolates as mentioned in Fig. 12 showed similar growth but in descending order on 2% RP (Fig. 9), there was no growth of Thailand, Chinese,

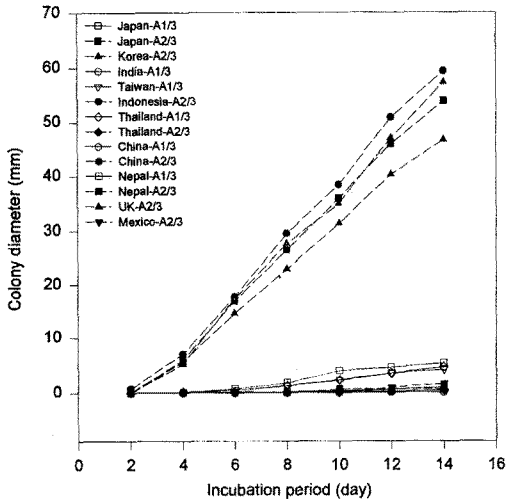



**Fig. 10.** Growth of $A_1$ and $A_2$ isolates of *Phytophthora infestans* from Japan, India, Taiwan, Thailand, China and Nepal on rye A agar medium supplemented with 3% *Cyperus rotundus* rhizome powder.

Nepalese and Mexican isolates on 3% RP. The other isolates grew slowly during 2 weeks incubation period (Fig. 10). A comparison of all the isolates of $A_1$ mating type on rye A agar medium with and without RP indicates that the growth was considerably inhibited on supplemented medium. Similarly, growth of $A_2$ was also suppressed as compared to control but relatively less as compared to $A_1$ isolates. When both the isolates were compared together on both types of media (control and supplemented ones), it is evident that $A_1$ isolates grew at par on 1% compared to $A_2$ on 2% (Fig. 11).

In another experiment both the mating types were grown on two different doses of metalaxyl at 0.05 and 0.1 ppm. There was no growth of $A_1$ on 3% rhizome + 0.05 ppm metalaxyl on rye A agar medium (Fig. 12). The growth pattern was almost same even on 0.1 ppm metal-

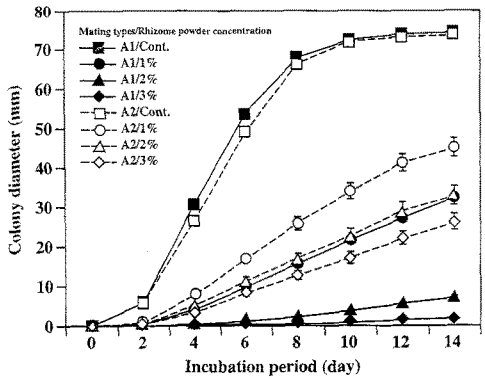



**Fig. 11.** Growth of all $A_1$ and $A_2$ mating types of *Phytophthora infestans* on rye A agar medium and on rye A agar medium supplemented with 1, 2 and 3% *C. rotundus rhizome* powder.

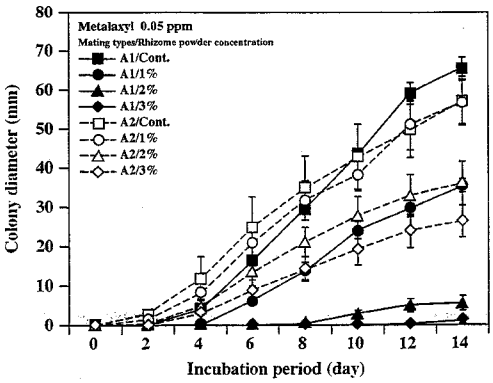



**Fig. 12.** Growth of all $A_1$ and $A_2$ mating types of *Phytophthora infestans* on rye A agar medium without and with 1, 2 and 3% *Cyperus rotundus* rhizome powder and 0.05 ppm metalaxyl.

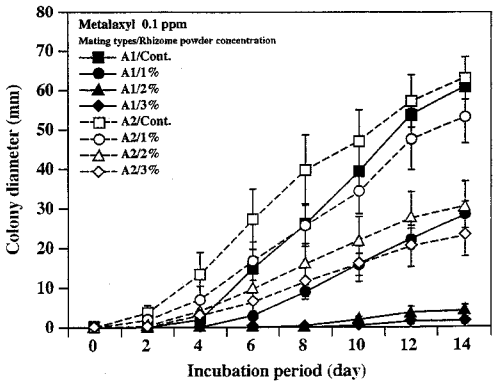



**Fig. 13.** Growth of all $A_1$ and $A_2$ mating types of *Phytophthora infestans* on rye A agar medium without and with 1, 2 and 3% *Cyperus rotundus* rhizome powder and 0.1 ppm metalaxyl.

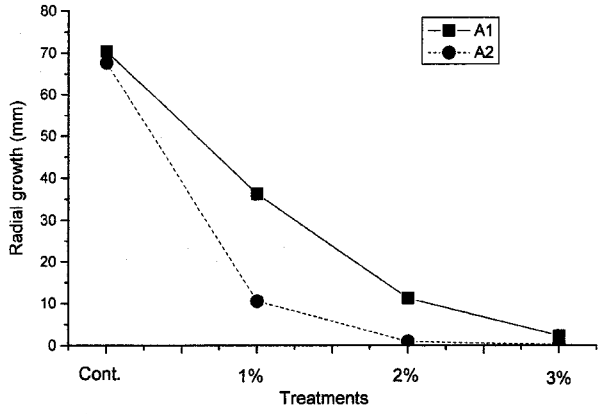



**Fig. 14.** Growth of all $A_1$ and $A_2$ mating types of Thailand isolates of *Phytophthora infestans* on rye A agar medium without and with 1, 2 and 3% *Cyperus rotundus* rhizome powder.

axyl-supplemented rye A agar medium (Fig. 13). Interestingly, growth of the Thailand A2 isolates (25) reduced significantly on rye A agar medium supplemented with rhizome powder (1, 2 and 3%) in comparison to A1 isolates (Fig. 14).

## Discussion

The usual procedure for characterizing $A_1$ and $A_2$ mating types of a fungus, in general and also of *P. infestans* in particular, is based on crossing the isolates with a known mating type. Enough literature is available on the presence of only one mating type of *P. infestans* in some parts of the world (Fyfe and Shaw, 1992) and its characterization needs import of a know mating type which does not eliminate the possibility of spread of that mating type in a locality where it did not exist earlier. Similarly, the studies related to diversity of the existing population depends on DNA finger printing, allozyme studies for different loci and sensitivity to metalaxyl. Depending on these techniques genetically diversified populations of $A_1$ and $A_2$ mating types of *P. infestans* have now been reported from many countries where they were not discovered prior to 1980 (Deahl *et al.*, 1991; Koh *et al.*, 1994; Malcomson, 1985; Mosa *et al.*, 1989; Nishimura *et al.*, 1999; Shaw *et al.*, 1995; Tartius *et al.*, 1986). In most of the cases the diversification is due to migration and sexual recombination of both $A_1$ and $A_2$ mating types as well as mutation *de novo*. The populations that show similar genotypes and allozyme pattern with the old Mexican population of *P. infestans* are grouped in "old" clonal lineage or US-1 clonal lineage whereas those that differ genotypically or in allozyme pattern with the old Mexican populations belong to the "new" clonal lineage which might have evolved due to genetic recombination, mutation *de novo* or through migration.

In the present study the inhibition of growth of all $A_1$ and $A_2$ isolates from China, Nepal, Thailand and Mexico on Rye-A agar medium supplemented with RP indicates that these isolates probably are of the same clonal lineage and clones of the Mixican isolates as they show similar trend as that of the Mexican isolates on the same medium. However, the growth of $A_2$ isolates from Japan, Korea, Indonesia and U.K. on the same medium indicates that they are of different colonal lineage showing diversity among $A_2$ mating types of these countries. The good growth of Thailand $A_1$ isolates on the RP supplemented rye A agar medium and less growth of $A_2$ isolates on the same medium is due to the different clonal lineage of the $A_2$ isolates from Thailand which was confirmed earlier by Ogoshi's group in Japan (personal communication). The reason of this variability may also be due to *de novo* evolution of the Thailand isolates. The genetic and allozyme variability in the isolates from some Asian countries are already reported (Koh *et al.*, 1994; Nishimura *et al.*, 1999). Thus, such diversifications are presumed to be the result of migration of new genotypes of *P. infestans*

through various paths. The second genotype detected by Nishimura *et al.* (1999) in the $A_1$ mating type of *P. infestans* isolates of some Asian countries has also been reported from the United States for the first time outside central Mexico. Similarly, some $A_2$ isolates belonging to the new population have also been detected in the United States, Canada, the Netherlands, Poland, Germany, Israel and Japan (Nishimura *et al.*, 1999). Therefore, the most accurate pathway of migrations of these isolates or diversification due to *de novo* evolution is difficult to describe.

The segregation of $A_2$ mating type into sporulating and nonsporulating colonies on prolonged incubation on rye A agar medium supplemented with RP further indicates that the phenomenon of segregation is either time-dependent action of the chemical present in the RP which may be slow acting on the genomic/cytoplasmic materials of the fungal thallus or the expression of segregation is an aging process. It may also be possible that the diploid thallus which ought to be segregated through gametangial copulation is hastened much earlier with the help of RP during mitosis of nuclei. Interestingly, this phenomenon may account for the evolution of new genotypes in *P. infestans*.

It is know that $A_1$ type is more sensitive to metalaxyl than $A_2$ (Goodwin *et al.*, 1996; Matuszak *et al.*, 1994; Sujkowski *et al.*, 1995; Therrien *et al.*, 1993). Inhibition of growth of $A_1$ mating type was observed on rye A agar medium supplemented with either 0.05 ppm or 0.1 ppm metalaxyl or on RP plus metalaxyl supplemented medium. This sensitivity indicates that RP may be acting in a similar way as metalaxyl and thr RP supplemented medium can be used for study of the growth of mating types of *P. infestans* which may serve as a selective medium for identifying $A_1$ and $A_2$ mating types of *P. infestans* of some countries. Further study is needed in order to identify the active component of RP which may make the process of differentiating mating types of *P. infestans* easier in new localities where it was not reported earlier.

## Acknowledgments

U. P. Singh is grateful to H. J. Aust, German Academic Exchange Service and Japan Society for the promotion of Science (JSPS) for financial support during the study.

## References

de Bary, A. 1876. Researches into the nature of the potato fungus *Phytophthora infestans*. *J. Royal Agri. Soc. England* **12**: 239-269.

Deahl, K. L., Goth, R. W., Young, R., Sinden, S. L. and Gallegly, M. E. 1991. Occurrence of the $A_2$ mating type of *Phytophthora infestans* in the United States and Canada. *American Potato J.* **68**: 717-725.

Fyfe, A. M. and Shaw, D. S. 1992. An analysis of self-fertility in field isolates of *Phytophthora infestans*. *Mycol. Res.* **96**: 390-394.

Gallegly, M. E. and Galindo, A. J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. *Phytopathology* **48**: 274-277.

Goodwin, S. G., Sujkowski, L. S. and Fry, W. E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and Western Canada. *Phytopathology* **86**: 793-800.

Jones, L. R. 1909. Resting spores of potato fungus, *Phytophthora infestans*. *Science* **30**: 813.

Johnes, L. R. and Griddings, N. G. 1909. Studies of the potato fungus *Phytophthora infestans*. *Science* **29**: 271.

Koh, Y. J., Goodwin, S. B. Dyer, A. T., Cohen, B. A., Ogoshi, A., Sato, N. and Fry, W. E. 1994. Migrationss and displacements of *Phytophthora infestans* populations in east asian countries. *Phytopathology* **84**: 922-927.

Malcolmson, J. P *Phytophthora infestans* $A_2$ compatibility type recorded in Great Britain. *Trans. Brit. Mycol. Soc.* **85**: 531.

Matsuzak, J. M., Fernandez-Elquezabal, J., Gu, W. K., Villarreal-Gonzalez, M. and Fry, W. E. 1994. Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico : Distribution and dynamics. *Pl. Dis.* **78**: 911-916.

Mosa, A. A. Kato, M., Sato, K., Kobayashi, K. and Ogoshi, A. 1989. Occurrence of the $A_2$ mating type of *Phytophthora infestans* on potato in Japan. *Ann. Phytopathol. Soc. Japan* **55**: 516-620.

Nishimura, R., Sato, K., Lee, W. H., Singh, U. P., Chang, T., Suryaningsih, E., Suwonakenee, S., Lunyong, P., Chamswarng, C., Tang, W., Shreshtha, S. K., Kato, m., Fujii, N., Akino, S., Kondo, N., Kobayashi, K. and Ogoshi, A. 1999. Distribution of *Phytophthora infestans* populations in Seven Asian Countries. *Ann. Phytopathol. Soc. Japan* **65**: 163-170.

Pethybridge, G. H. 1992. Investigations on potato diseases (3rd report). *Dept. Agric. Tech. Indut. J.* **12**: 334-359.

_____ and Murphy, P. A. 1913. On pure culture of *Phytophthora infestans* de Bary and the development of oospores. *Sci. Proc. Royal Soc. Dublin* **13**: 556-588.

Shaw, D. S., Fyfe, A. M., Hibberd, P. G. and Abdel-Sattar, M. A. 1985. Occurrence of the rare $A_2$ mating type of *Phytophthora infestans* on imported Egyptian potatoes and the production of sexual progeny with $A_1$ mating types from the U.K. *Pl. Pathol.* **34**: 552-556.

Singh, U. P., Prithiviraj, B., Khiste, S., Dalai, S. and Amitabh Singh. 1996. Induction of sexual reproduction in *Sclerotium rolfsii*, *Ustilago cynodentis* and *Cintractia limitata* by *Cyperus rotundus* rhizomes. *Can. J. Bot.* **74**: 803-806.

Sujkowski, L. S.. Fry, B. A., Power, R. J., Goodwin, S. B., Hamlen, R. A. and Fry, W. E. 1995. Sensitivity of Mexican isolates of *Phytophthora infestans* to chlorathalonil, cymoxanil and metalaxyl. *Pl. Dis.* **79**: 1117-1120.

Tartius, P. H., Fyfe, A. M., Shaw, D. S. and Shattock, R. C. 1986. Occurrence of the $A_2$ mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. *Pl. Pathol.* **35**: 578-581.

Therrien, C. D., Rooley, P. W., Spielman, L. J., Fry, W. E., Ritch, D. L. and Shelly, S. E. 1993. Nuclear DNA content, allozyme phenotypes and metalsxyl sensitivity of *Phytophthora infestans* from Japan. *Mycol. Res.* **97**: 945-950.

Tucker, C. M. 1931. The taxonomy of *Phytophthora* de Bare. *Res. Bull. Minn. Agri. Res. St. No.* 153.