CHAPTER 5
DISCUSSION
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Late blight caused by *Phytophthora infestans* was, and still continues to be, the most dreaded disease of potato crop world over even after 150 years of in depth investigations. Most of the enigma associated with the disease have been de-mystified, yet absolute control of the disease is still beyond comprehension. Although, there are several plausible reasons for it but variability within the causal fungus like organism *P. infestans* and its fall out on the host crop is a major contributing factor. Several biological markers have been employed for grouping the pathogen population and the resulting population groups in turn have been evaluated in relation to host resistance. Mating types is one such biological marker which has been used extensively for categorizing pathogen populations and its implications on disease development. Uptill 1984, except Mexico, only A1 mating type was known to occur throughout the world including India (Tooley et al., 1985) as a result of series of migrations in early 1970 (Fry et al., 1991) A2 mating type was introduced into Europe (Hohl and Iselin, 1984; Malcolmson, 1985; Schober and Rullich, 1986; Tantius et al., 1986). Subsequently, it was recorded from Asia (Shaw et al., 1985; Grinberger et al., 1989, Mosa et al., 1989; Singh et al., 1994; Ahmad and Mirza, 1995; Zhiming et al., 1996), USA (Deahl et al., 1991; 1995), South America (Brommonschenkel, 1988; Nustez, 1999), Canada (Deahl et al., 1991) and Africa (Goodwin and Fry, 1991; Fry et al., 1992; Sengooba and Hakiza, 1999). However, there are still few pockets left where A2 mating type has not yet been recorded (Adler et al., 2002). In India, A2 mating type was detected during 1986 (CPRI, 1986) but systematic research on this aspect began since 1990 (Singh et al., 1994). Since then population of the two mating types has been closely monitored (Gupta, J., 2000). The new strain (A2 mating type)
has since been detected from most of the potato growing regions except few pockets (Singh, 2002).

Ever since introduction of the new strain (A₂ mating type) in different parts of the world, its further buildup vis a vis the old population (A₁ mating type) has not followed a definite trend. Marshall-Farar et al. (1998) observed that the new strain (A₂ mating type) recorded in Wisconsin region (USA) was more aggressive and was observed to be fast displacing the old population (A₁ mating type). This change in the population dynamics of the two mating type has also been reported from other parts of USA (Deahl et al., 1995) and Canada (Chycoski and Punja, 1996; Peters et al., 1998; Daayf and Platt, 1999). A₂ mating type was also found to be predominating in Israel (Grinberger et al., 1989) and Japan (Mosa et al., 1989; Koh et al., 1994). On the other hand, buildup of A₂ mating type had been slow in most of the European countries (Therrien et al., 1989; Gotz, 1991; O'Sullivan and Dowley, 1991; Andrivon et al., 1994; Day and Shattock, 1997). In fact recent reports tend to suggest that A₂ frequency has declined in most of the European countries (Hermansen et al., 2000).

In India, A₂ mating type is fast displacing the A₁ mating type, but the pace of displacement varied from region to region (Gupta, J., 2000), as was the case with Canada (Chycoski and Punja, 1996). Displacement of A₁ mating type was almost complete in North-eastern hills by 1998, whereas in North-western hills the pace of displacement was slightly slow (90.9% frequency of A₂ mating type in 1997). On the other hand, A₂ mating type was recorded only at few places in sub-tropical plains. Its frequency at these places was lower (36.8%) than the old strain. Differential buildup of A₂ mating type in different climatic zones in India can be ascribed to varying weather conditions. In North-eastern hills the weather remains congenial for late blight development almost throughout the season which might have been responsible
for fast multiplication of A2 mating type and its selection over A1 type within a short period of time. The weather in North-western hills is, however, moderate and it becomes congenial for the fungus for 2-3 months as against five months in North-eastern hills. This gives little less time to the fungus to multiply over time as compared to North-eastern hills which is reflected in the form of slow displacement of the A1 mating type. Sub-tropical plains normally do not favour late blight development. Its only in few years that the weather becomes congenial for late blight development, but for a very short period (1-2 weeks) (Singh and Shekhawat, 1999). Since the fungus gets little time to multiply the new strain (A2 mating type), expectedly, took longer time to multiply and manifest itself (Sharma and Singh, 1999; CPRI, 1999). Apparently for this reason, A2 mating type has been reported from three locations only in Indian sub-tropical plains (Modipuram, Patna and Jalandhar). It is believed that population of A2 mating type will continue to remain low in the years to come. Non-detection of A2 mating type at most of the places in sub-tropical plains does not mean that it has not been introduced there. In view of the free flow of seed material from one region to another, the A2 mating type might have reached all the nook and corner of the country. It might take little more time for its population to cross the threshold level.

Present studies were designed to elicit information on population dynamics of A1 and A2 mating types under protected controlled environment by mix inoculating the isolates of two mating types in equal proportion. Results revealed that multiplication of A2 mating type was faster, to the extent that, at 70% DI, entire A1 population was displaced by A2 mating type, and subsequently, only A2 mating type was recorded till entire crop was killed (Fig. 34) Even tuber infection at harvest carried A2 mating type only. Earlier studies (Gupta, J., 2000) also showed that
population of A₂ mating type increased with increasing clonal generation on detached leaves. By 27th clonal generation, A₁ mating type was completely displaced by A₂ mating type suggesting that A₂ mating type is definitely more fit and is comparatively more competitive than the old strain (A₁ mating type). Similar views were expressed by Grinberger et al. (1989), Mosa et al. (1989), Fry et al. (1993), Dehal et al. (1995), Kato and Fry (1995), Fry and Goodwin (1997b), Kato et al. (1997), Forbes et al. (1998), Marshall-Farrar et al. (1998), Miller et al. (1998), Nishimura et al. (1999), Sedegui et al. (2000), Bakonyi et al. (2002b) and Hammi et al. (2002). Chycoski and Punja (1996) also observed displacement of A₁ mating type from mixed colony of A₁ and A₂ mating type after 14 to 16 month. Nevertheless, A₁ population is still dominating over A₂ mating type in Poland (Zarzycka and Sobkowiak, 1997; Bartkowiak and Weber, 2000), China (Zhiming et al., 1996; Jie Hua et al., 2000, Weng-Wen Qiao et al., 2002), Italy (Cristinzio and Testa, 1997), Finland and Norway (Hermansen et al., 2000), Nepal (Ghimire et al., 2001b), Belgium (Bakonyi et al., 2002a), Switzerland and France (Knapova and Gisi, 2002) and Egypt (Shaat, 2002). So far no plausible reasons have been ascribed for this behavior but this needs through investigation.

Polyploidy in *P. infestans* was first reported among some British isolates by Sansome (1977). In that report it was speculated that polyploidy is widespread in temperate isolates of this species, since tetraploids may be better adapted to the cooler climates and, therefore favored by the selection pressure. Sansome also reported that tetraploid nuclei formed bivalent rather than quadrivalents during meiosis, which may therefore behave as functional diploids. Recent evidence presented by Tooley et al., (1985) in which tetraploids displayed isoenzyme banding patterns characteristics of diploid supports Sansome’s (1977) hypothesis that some tetraploids are functional.
diploids. In another report (Tooley and Therrien, 1987) evidence was presented which confirmed Sansome’s prediction that polyploidy is wide spread in temperate isolates, whereas diploidy is more prevalent in the sexually reproducing Mexican isolates of *P. infestans*.

Existence of polyploids both in temperate regions and sub-tropics tend to suggest that they have a definite role to play in *P. infestans* population biology. Therrien *et al.* (1990) had hypothesized that polyploids may act as a barrier to sexual reproduction in nature and asexual reproduction might only be possible. Although, it has been experimentally demonstrated that *P. infestans* isolates with different ploidy status (2C x 4C) can be crossed and produce germinating progeny but it is still not understood what impact it will have on the population biology, especially its fitness/aggressiveness. Since European population is predominantly tetraploid, it was assumed that tetraploids are more fit under temperate condition (Sansome, 1977). However, there is no experimental proof for this. In fact, there is hardly any report on comparative fitness/aggressiveness of different polyploids, except that of Gupta, H. (2000). This was only preliminary study and needed further confirmation. It was with this view that aggressiveness of different polyploids was studied under protected condition which simulated field conditions. Results in the present studies revealed that in the beginning when the disease severity was low (10-20%), frequency of triploids was highest (75%) which was followed by diploids (25%). Tetraploids were altogether absent (below detectable limits). At 30-35% DI, population of diploids increased to 50% whereas triploid and tetraploids were 25% each. Thereafter, tetraploids were not recorded till crop maturity. Up to 75-80% DI, population of diploids and triploids were equal (50:50), however, at 100% DI, population of diploids increased to 80%. Based on these results it is concluded that under Indian
conditions diploids are more aggressive and fit than the other polyploids. Tetraploids seem to be the least fit which supports the earlier view that tetraploids are more suited to temperate conditions (Sansome, 1977). Gupta, H. (2000) also reported that in Indian population diploids were most aggressive followed by triploids and tetraploids. She also reported that in Shimla hills, triploids marginally out-numbered diploids, this might be transitional phase as has happened in the case of the Netherlands (Therrien et al., 1989), and it is expected that diploids ultimately would become predominant in the years to come.

Development of new physiologic races from a single, or a mixture of races, in the presence/absence of host has been investigated in detail in the past (Black et al., 1953; Gallegly and Eichenmuller 1959; Malcolmson, 1970; Shattock, 1976). When two races are mixed, the resultant recombinant race possesses the virulence genes of the parents (Leach and Rich, 1969; Shattock, 1976, CPRI, 1985).

Vegetative hybridity in *P. infestans* was also demonstrated by Malcolmson (1970) who recorded development of new complex races from mixed inoculations. Black (1952) inoculated Craigs royal with a mixture of equal quantities of sporangia of races 0.4; 1.4; 2.4; 1.3 and 1.3.4. The resultant sporangia were collected and reinoculated. Results revealed that the population changed with time, several races were lost and race 0.4 and 2.4 continued to sporulate freely indicating that race 0.4 and 2.4 were more fit than others. Gallegly and Eichenmuller (1959) demonstrated that race 4 could arise from every race, which they develop from single zoospore isolations. They postulated that the occurrence of race 4 character was as a result of mutations.

This aspect of development of new races was further investigated in the present study by mix inoculation of race 1.7, 1.3.7, 1.2.3.4.11 and 1.2.3.4.7.11 under
protected conditions. Results revealed that out of four races, race 1.2.3.4.7.11 was

detected from the very beginning till 10-40% DI. Race 1.7 and 1.3.7 could not be
detected at any stage whereas race 1.2.3.4.11 was detected up to 45% DI. The new
virulences viz. 5 and 10 which were not present in any of the physiological races
mixed inoculated in the beginning of the experiment were detected for the first time at
30% and 90-95% DI, respectively. They continued to be part of the race flora till crop
maturity (Table 4). Similar results were obtained when mixture of races were
inoculated on detached leaves. This showed that the new physiological races arose
during the course of disease development/clonal multiplication both by addition and
deletion of new virulence factors. These results supports the earlier findings that new
races may arise from a single or a mixture of races without exerting any selection
pressure (Black, 1952; Malcolmson, 1970; Shattock, 1976).

Introduction of new strains (A₂ mating type) to rest of the world, outside
Mexico through a series of migrations have opened up possibilities of sexual
reproduction in *P. infestans* in nature. This has since has been demonstrated by
several workers across the globe (Fry *et al.*, 1992; Drenth *et al.*, 1993a; 1995;
Andrivion 1995; Erwin and Riberio 1996; Grinberger *et al.*, 1989). The A₂ mating
type as reported earlier was introduced in India during 1986 and since then it has
spread far and wide across the country. Presence of thick walled oospores in nature
both in temperate in sub tropical plains in India have shown that *P. infestans* has
started reproducing sexually. Expectedly, this is going to have far reaching
implications on *P. infestans* structure, biology and its epidemiology. It was with this
background that crosses were performed between A₁ and A₂ mating types for studying
the effect of sexual reproduction on variability in *P. infestans* with regard to mating
type, ploidy, races and RAPDs.
It has been established that mating type is a simple character to determine but its genetic basis is unclear. Varying ratios in A1 and A2 mating type in F1 progenies have been obtained by various workers which include (i) 1:1 (Earnshaw and Shatlock, 2002) (ii) 2:1 (Castro and Zentmyer, 1968; Romero and Erwin, 1969; Shatlock et al., 1986; Mosa et al., 1993; Mayton et al., 2000) (iii) 1:3 (Shatlock et al., 1986) (iv) 1:2 (Romero and Erwin, 1969) (v) 4:1 (Earnshaw and Shatlock, 2002) and (vi) all A1 mating type (Romero and Erwin, 1969).

In the present studies four crosses were attempted using four different A1 and A2 mating type isolates which yielded four different ratios viz. 1:2, 2:1, 3:1 of A1 and A2 mating type and only A2 mating types in F1 progeny. These results are in the conformity with earlier findings who have reported almost similar ratios in F1 progeny. Reasons for throwing out varying ratios of A1 and A2 progenies have been discussed by Shaw (1983b), Sansome (1980), Shatlock et al. (1986; 1987) and Shatlock (1988) and this may hold true for present findings as well.

Development of new physiological races as a consequence of sexual reproduction has been demonstrated by several workers. Romerio and Erwin (1969) detected recombination of factors for virulence in F1 progeny. Similar results were obtained by Gallegly (1968, 1970) and Laviola and Gallegly(1983).

New races arose either by rearrangements of virulence factors (Romerio and Erwin, 1969), or some times, new factors were also detected in F1 progeny (Smoot et al., 1958; Niederhauser, 1959). In the present studies only one out of two parent races could be recovered in F1 progeny. A total of 13 new races comprising 2-6 genes were recorded. These results are in conformity with above reports where new physiological races were detected in F1 progeny. Several studies on genetic control of virulence have demonstrated that segregation of virulence phenotype in F1 progeny is controlled
by both single locus and/more than one locus (Spielman et al., 1989; 1990). This might hold true for present studies as well.

Impact of sexual reproduction on ploidy status of the progeny isolates has been studied by Whittaker et al. (1991b) in detail. They observed that most of progeny isolates were hybrid in nature. Crosses involving 2C x 2C parents produced 2C F1 progeny only whereas crosses involving 2C x 4C produced F1 progeny comprising of 2C to 3C. In the present study progeny of cross involving 2C x 2C consisted of diploids (75%) and triploids (25%) which is at variance with the result of Whittaker et al. (1991b). Shaw (1983a) has extended plausible reasons based on bisexual nature of Phytophthora isolates for varying ratios of polyploids in F1 progeny. This might hold true for present results as well.

Although there are no specific report on use of RAPDs as markers for studying genetic variability in sexual population of P. infestans, however, RAPDs have been used to established hybrid nature of F1 progeny of P. sojoe and P. cinnamomi (Whisson et al., 1994; Tyler et al., 1995; Linde et al., 2001). It was proved conclusively that RAPDs can be employed for determining hybrid nature of F1 progeny in both the species. In P. cinnamomi all but one of the F1 progeny isolates contained one or more RAPD band from each parent indicating that they were likely to be hybrid. Present findings are not in total agreement with that of Linde et al. (2001). All the 12 progeny isolates possessed one of the bands of A2 mating type parent whereas only four out of 12 progeny isolates had common band with A1 mating type parent indicating that only part of the progeny has received genetic material from A1 parent. This is also evident from the phenogram showing relationship between parent isolate and F1 progeny using RAPDs as marker (Fig. 36). A1 mating type parent was clustered on a solitary branch of the phenogram with an average similarity
of 0.68 with other isolates. On the other hand, \( \text{A}_2 \) mating type parent and other 12 progeny isolates were grouped together in the phenogram with an average similarity of 0.74; SD ±0.07. The 12 progeny isolates were grouped into 3 different sub-clusters with an average similarity ranging from 0.56 to 0.93. The high level of genetic relatedness among the progeny isolates is expected because all of them belonged to \( \text{A}_2 \) mating type.

As indicated earlier, \( \text{P. infestans} \) had started reproducing sexually small measures at least in some pockets, especially in Europe. Beside, enhanced variability in the resultant sexual progeny, there are apprehensions that the sexual population would be more fit and aggressive that the asexual population. This would have a far reaching implications on epidemiology of the pathogen, and consequently, on the management of the disease. Tooley \textit{et al.} (1986) in a comprehensive study analyzed fitness of sexual population vs. asexual population using detached leaves. They observed that the lesions were larger for isolates from sexual than asexual population. However, no significant difference in sporulation capacity were observed, nor were isolates from one population were more fit on the basis of composite fitness index. Results obtained by Mayton \textit{et al.} (2000) on pathogenicity of recombinant progeny tend to suggest that sexual population is likely to be less pathogenic than either parent. They further opined that most of the progeny will therefore, not be selected and will not survive.

In the present studies, five isolates each of sexual and asexual population were evaluated for their aggressiveness using whole plant and detached leaves of two potato cultivars of Kufri Chandramukhi and Kufri Jyoti. The aggressiveness was assessed by determining composite fitness index and its components. Results revealed that sexual population was comparatively more aggressive than asexual population.
when tested on whole plant and detached leaves (Table 10 and 14). Differences within sexual population was narrow when tested on whole plant. No significant difference were recorded in sexual population with regard to composite fitness index and lesion area although they differed significantly in respect of their sporulation count. However, significant differences were detected with respect to composite fitness index and lesion area when tested on detached leaves. Further, it was also observed that, although on overall basis, sexual population was more aggressive than asexual population but there were isolates from asexual population which were comparatively more aggressive than some of isolates from sexual population. These results tend to suggest that some of the isolates in sexual population are more aggressive than asexual population which may get selected by their rapid multiplication over seasons thereby displacing the less fit asexual population in the years to come as suggested by Mayton et al. (2000).

Genetic changes in *P. infestans* population over time have been documented in Europe, USA and Asia (Drenth et al., 1994; Sujkowski et al., 1994; Shattock and Day, 1996; Day and Shattock, 1997; Miller et al., 1997; Marshall-Farar et al., 1998). It has been demonstrated that the old population which was predominantly A1 mating type has been gradually displaced with new population consisting primarily of A2 mating type. Goodwin et al. (1998) reported that increased late blight epidemic in United States during 1994 through 1996 had been primarily due to development of new genotype viz. US7 and US8 which almost displaced old population (US1 type) during 1993-1996. Rapid displacement of US1 by US8 also has been documented locally in Wisconsin (Marshall- Farrar, 1998). Similar displacement of old population by the new population has been observed at many places although structure of new population varied from place to place (Goodwin et al., 1996; Forbes et al., 1997). In
the present studies three isolates each collected during 1992, 1996 and 1999 were compared for their aggressiveness on cv. Kufri Chandramukhi (susceptible) and cv. Kufri Jyoti (moderately resistant) using whole plant and detached leaf method. Result revealed that isolates collected during 1999 were significantly more aggressive than isolates collected during 1992 and 1996 when tested on whole plant (CFI 49908.44, 40713.99 and 38471.26, respectively; LSD0.05 2058.26). Similar results were obtained when isolates were tested by detached leaf method (Table 20). Fitness component \textit{viz.} lesion area, sporulation count also followed similar trend (Tables 17 and 20). Perusal of the status of the isolates collected during three years revealed that during 1992, \textit{P. infestans} population primarily consisted of \textit{A}1 mating type, ploidy ranging from triploid to tetraploid and race consisting of 5-6 genes. In the subsequent years (1996, 1999) the isolates were predominantly \textit{A}2 mating type, diploid in nature and consisting 5-7 gene races. This indicated that population had undergone a significant change during 1992-1999 with regard to mating type and ploidy. As is evident from above, the new population is predominantly \textit{A}2 mating type and diploid in nature, the traits which makes \textit{P. infestans} more aggressive as demonstrated by Kato and Fry (1995); Fry (1999); Peters \textit{et al.} (1999); Gupta, J. (2000) and Singh (2002).

A large number of markers have been used over the years for cataloging variability in plant pathogens. They include (1) Biological markers (mating types, fungicide resistance, virulence), (2) Cytoplasmic markers (mitochondrial DNA based markers), (3) Neutral markers (Allozyme markers, polymorphic DNA markers). All the above markers have been used extensively in characterizing \textit{Phytophthora} species including \textit{Phytophthora infestans}. Amongst polymorphic DNA markers, single locus co-dominant markers (micro-satellite, SNP, SSCP) (Wattier \textit{et al.}, 2002) are better over dominant multilocus markers (RFLP and AFLP), since each allele of markers
can be detected and data on frequency of various homozygote and heterozygote can be used to characterize the breeding system.

RAPDs are another set of molecular marker, which require no cloning, they are easier to carry out and require very small amount of crude DNA. RAPD fingerprinting can be faster than RFLP analysis (Williams et al., 1990). However, genetic interpretation is more difficult than with RFLP since non-amplification can result from base pair substitution at any of 20 different nucleotides complimentary to the primers at the end of amplified fragment. In this case, all the possible base pair substitutions are lumped into the same category (which is treated as an allele) as a non-amplified fragment. However, despite above deficiency, RAPDs have been used extensively to (i) establish hybrid nature of F1 progeny (Tyler et al., 1995), (ii) study gene flow between Phytophthora species (Goodwin et al., 1999), (iii) study taxonomy/intraspecific variation (Cooke et al., 1996; Zheng and Ward, 1998; Elena and Paplomatas, 1999) (iv) to establish relatedness and catalogue variability within the Phytophthora species (Liew and Erwin, 1994; Chang et al., 1996; Darmono, 1997; Meng et al., 1999; Nyasse et al., 1999). RAPDs have also been used in recent years to study P. infestans population structure by various workers (Punja et al., 1998; Mahuku et al., 2000; Abu-El-Samen et al., 2003; Daayf et al., 2001; Paez et al., 2002). Mahuku et al. (2000) employed RAPD analysis to study P. infestans structure in Canada. High level of genetic diversity was detected by them within population indicating that migration and sexual recombination probably played an important role in the population biology of P. infestans in Canada. RAPDs could also be employed to distinguish Costa Rica P. infestans population from that of Northern Cartago and Zarcero. Paez et al. (2002), on the other hand, used RAPDs successfully to establish relationship between pathotypes and other markers such as mating types, metalaxyl
resistance and Gpi allozyme. RAPD data revealed a good distinction between A1 and A2 mating types groups (Daayf et al., 2001).

In India, P. infestans has been catalogued extensively by using biological markers. They include physiological races, mating types and fungicide resistance (CPRI 1986; Singh and Shekhawat 1999; Gupta, H., 2000; Gupta, J., 2000; Singh, 2002). Amongst neutral markers, allozyme markers have also been used for cataloging variability in P. infestans (Gupta, J., 2000). Since above markers cannot resolve population structure beyond a point, polymorphic DNA marker (RAPD) were employed in the present study to characterize P. infestans population. A total of 67 isolates belonging to different geographical locations (Table 1) and representing various marker groups viz. mating types physiological races, ploidy and Gpi and pep isozyme genotypes were analyzed for their RAPD profile using ten random primers. The results revealed that RAPDs were able to resolve all the 67 isolates individually. Analysis of the phenogram also revealed that RAPDs could separate out isolates based on their geographical locations. Grouping of all the isolates from one region into one group tend to suggest that geographical barriers have played an important role in developing P. infestans population specific to those regions and that there has been very little gene flow between different geographical populations.

P. infestans population studied primarily belonged to two different geographical regions viz. sub-tropical plains and temperate highlands. Analysis of isolates collected from sub-tropical plains revealed that most of the isolates were clustered regionwise, with few exceptions. Nineteen isolates from Uttar Pradesh (Modipuram) were clustered into two distinct groups indicating that P. infestans population in Uttar Pradesh has multiplied clonally and it has undergone change in its genome by mechanisms involved in throwing out new genotypes during clonal
propagation. Similar trend was observed in respect of isolates collected from Punjab and Rajasthan (Fig. 41). Two isolates each from Rajasthan (R1, R2) and Bihar (B4, B5) and one from Punjab (P4) showed relatedness with Uttar Pradesh isolates belonging to cluster 2. This showed that *P. infestans* population has moved, although, in a small measure, to other States like Bihar, Rajasthan and Punjab. It seems possible since seed potatoes from Uttar Pradesh are carried over far and wide including the above States.

Twenty-four isolates collected from Khasi hills and Himachal Pradesh hills were analyzed for their relatedness (Figs. 38 and 39). Results revealed that hill isolates could not be separated out on regional basis indicating that *P. infestans* population in temperate highlands (hills) has evolved on similar pattern despite wide geographical separation. Comparison of isolates collected from Shimla hills revealed that except two isolates (HP5 and HP6) all were grouped into a single cluster. The two isolates which were distantly placed were collected, during 1996 and 1999, are part of new population (A2 mating type) introduced into India recently.

RAPDs failed to distinguish between A1 and A2 mating types isolates collected from diverse geographical background. Since A1 and A2 mating types isolates did not form distinct clusters in the phenogram (Fig. 37; for mating types for isolates details please see Table 1). Some of the A1 isolates (UP1) were closely related with A2 mating type (UP2). Whereas there were isolates within the two mating type populations which were distantly related (UP17 A1, HP6 A2 mating type). This indicates that the RAPDs can be employed used for resolving differences even within and/or between different *P. infestans* populations. As mentioned earlier, RAPDs also proved useful in differentiating hill isolates of A1 and A2 mating types from that of the plains except two isolates (Figs. 45 and 46). This supports our earlier finding that
RAPDs are useful markers for distinguishing isolates on the basis of geographical boundaries/regions.

RAPD analysis of 33 *P. infestans* isolates belonging to seven *Gpi* genotypes revealed that no distinct groups were formed based on *Gpi* genotypes. This indicated that the isolates within each *Gpi* genotypes differed at genomic level. RAPDs also resolved isolates from same genotypes into different subgroups (Fig. 48) suggesting that RAPDs are better marker than isozymes. This has also been opined by earlier workers as well (Nyasse *et al.*, 1999). In addition, some of the isolates belonging to two different *Gpi* genotypes were more related than some of the isolates from the same genotype. This further suggests that isolates of same genotype may not be having same genomic arrangement. Similar results were obtained when 35 isolates belonging to three *pep* genotypes were analyzed using RAPDs (Fig. 49). It is now well established that *P. infestans* population is polyploid in nature, although frequency of different polyploids varies from region to region (Tooley and Therrien, 1987; Gupta, H., 2000). By virtue of presence of different copies of chromosomes, genetic materials in polyploids, is expected to differ in their makeup. But even isolates belonged to the same ploidy showed marked differences (Fig. 52). This indicates that ploidy cannot be used to characterize *P. infestans* population. In present studies triploid isolates showed highest relatedness among themselves (average similarity 0.78, SD ±0.05) followed by diploids (average similarity 0.75; SD ±0.05) and tetraploids (average similarity 0.73; SD ±0.07) indicating involvement of outcrossing between different polyploids in nature.

Data on RAPDs profile of ten different physiological races revealed that although physiological races having different virulence factors are unique but are related to each other, since they were clustered into two main and three sub-clusters
(average similarity 0.52; SD ±0.14) (Table 42). This indicates that different physiological races are related to each other in their genomic constitution and therefore are not reliable in cataloging variability in plant pathogen.