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Variation in Protein Profiles of *Phytophthora infestans* Isolates Collected from Potato Production Zones of Pakistan.

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Abstract

Forty-nine isolates of *Phytophthora infestans*, the causal organism of late blight in potato, studied to understand variations in protein profile using SDS-PAGE analysis. Of these fifty-five were from potato production zones of Pakistan and four isolates were acquired from Cornell University, U.S.A. Rye agar medium was used to grow isolates of *P. infestans*. Polyacrylamide gel was used to compare the protein profile of isolates. Ten bands were found polymorphic and scored to generate dendrogram. Two major groups (A & B) were observed with the help of cluster analysis. Group A is furthers divided into six and group B into three subgroups. All the isolates from Cornell University and 50% isolates from Pakistan were grouped in the same cluster indicating high level of homology among each other.

Introduction

Potato is an important vegetable all over the world. It ranks third among food crop after wheat and rice and fifth in total production in Pakistan (Mallik, 1995). More than 18 potato diseases are reported in the country, of which 13 are of common occurrence (Ahmad *et al.*, 1991). Most commonly occurring potato diseases in Pakistan are early and late blight, powdery and common scab, black scurf, stem rot, soft rot, brown rot, wilts, potato cyst nematode and root knot nematode (Ahmad, 1998).

Late blight of potato, caused by *Phytophthora infestans* (Mont.) de Bary is one of the oldest and most serious diseases of potato wherever this crop is grown. The developments of late blight epidemics depends greatly on the effect of humidity and temperature on the different stages of the life cycles of the fungus. The disease can destroy the whole crop within a week or two. Symptoms of late blight of potato appears both on above and under ground parts of the plant. Foliage symptoms appear as circular or irregular water soaked spots. In moist weather the spots enlarge rapidly and form brownish, blighted areas with indefinite borders, although frequently a pale yellowish green zone surrounds the rapidly expanding lesions. In dry weather the activities of the fungus are checked (Singh, 1973; Agrios, 1973). Affected tubers at first shows more or less irregular, purplish black or brownish blotches with a metallic dark dull color (Agrios, 1973; Rich, 1983).

Many scientists have reported that the identification of *Phytophthora* species is based on the morphology of sexual and asexual reproductive bodies and on cultural characteristics (Mircetich & Matheron, 1976; Jeffers *et al.*, 1982; Gallegly, 1983; Wilcox & Mircetich, 1985: Bielenin & Jones, 1988 and Jeffers & Aldwinckle, 1988). Although the isolates of *Phytophthora* in this study recovered from potato plants often can be identified using these criteria, problems of

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identification remain. For example, individual isolates may exhibit a typical morphological characteristics or may fail to form a sufficient number of reproductive structures for proper identification. Isolates exhibiting characteristics not conforming to the currently described species have been reported ((Mircetich & Matheron 1976; Wilcox *et al.*, 1985 and Wilcox & Mircetich, 1985). Technique that aid in identification could be important to improving our understanding of the ecology of *Phytophthora* and the epidemiology of late blights on potato crop.

The present study was initiated to compare genetic variation in isolates from Pakistan and Cornell University U.S.A. Genetic diversity in the population mainly affects the virulence of the fungus and new population is mostly more aggressive than the older one. The resistant and diverse inoculum is the main reason of the failure of efforts to manage the disease.

Material and Methods

Samples collection: The late blight affected samples of potato were collected from different potato production zones during October 1997-February 2000 (Fig 1). Four standard isolates of *Phytophthora infestans* of known lineage were also included in this study acquired from Cornell University, U.S.A.

***Phytophthora* Cultures:** Samples were collected on the basis of appearance of clear visual symptoms of disease on leaves and stem. The presence of white fungal mycelium around the borders of lesion, on the upper side of leaf was considered as ideal criterion for sample selection. However, minimum criterion for sample collection was the presence of brown, blighted lesions with yellow margins (early stage of infection). The twigs with at least 4-7 leaves were picked from the infected potato and placed in polyethylene bags. Individual infected leaves were also taken as a sample. Some of the infected leaves were sandwiched in the two halves of sliced tubers. The samples were kept in a mini, portable refrigerator and brought to laboratory for isolation purpose.

Isolation: For the isolation of *Phytophthora infestans*, rye agar medium amended with antibiotics was used (Tantius *et al.*, 1986). Pieces from the infected leaves along with some healthy portion were separated and washed thoroughly in running tap water for 10-15 min. Washed roots and foliar parts were cut into pieces, immersed in 0.1% colorox for 1 min, and rinsed three times in sterilized distilled water. Roots and foliar pieces were dried on sterile blotting paper and placed over rye agar medium. The plates were incubated at 27°C for 3-4 days.

Protein Profiling: Mycelium of *Phytophthora infestans* was prepared by placing agar disks of each isolate in Petri dishes containing rye agar medium. The mycelium was removed from the rye agar medium with the help of forceps after fourteen days. Samples were ground with the help of pestle and mortar in the presence of 100ul-extraction buffer (0.05M Tris + 0.2% SDS + 5M Urea, pH 8.0 with HCl) and placed in eppendrof tubes containing 200ul-extraction buffer. The tubes were centrifuged at 15000 rpm for five min and kept in refrigerator for future use. A 15ul sample solution was applied into the wall of gel.

Electrophoresis: The procedure for extracting buffer soluble proteins was a modification of Laemmli (1970). The concentration of polyacrylamide gel was

was performed for 2 hours at 100mA until the blue line of Bromo Phenol Blue (BPB) solution was passed the separation gel. Protein pattern was visualized by staining 30 minute with coomasie briliant blue and destaining in a 5% methanol and 20% acetic acid until the colour of background of gel was disappeared. Before drying on white filter paper, the gels were impregnated with distilled water. The gels were photographed and scanned for statistical analysis.

Results and Discussion

Considerable interest has been focused on the use of biochemical methods for identification and genetic diversity assessment. Forty-nine isolates of *Phytophthora infestans* were characterized by SDS-Polyacrylamide gel electrophoresis. A considerable amount of genetic diversity was observed among forty-nine isolates based on protein profile. Ten different types of electrophoregram were recognized among the isolates (Table 1).

A dissimilarity coefficient was computed and it was based on banding patterns. Dissimilarity coefficient ranged from 0-3.1. The isolates SL2005, SL2017, BN981 and DB2007 exhibited maximum dissimilarity coefficient indicating high level of variability (Table 2).

Cluster analysis placed forty-nine isolates into major groups (A & B). A critical evaluation of cluster A & B reveals six and three subgroups, respectively (Fig 3). Frequency distribution of A_1 group is higher than other groups and frequency distribution of A_4 group is lower. The frequency distribution of B_1 is higher and B_3 is lower.

The Sub-cluster A_1 consisted of the isolates originating from Sahiwal and Depalpur area. The four isolates (US-7, US-8, US-1 and US-6.4) were also included in this cluster. It indicates some sort of relationship with the most of isolates from Pakistan in term of polypeptide banding pattern. Cluster analysis placed isolate BT987, SR2002 and MD986 close to isolates from US showing a high level of relatedness (Fig 4). Cluster A_2 composed of the isolates from Depalpur and Deska whereas A_3 only consisted of two isolates, which were from Swat valley. Sub-group A_4 has only one isolate from Daska. The sub-group A_5 & A_6 have two and four isolate respectively. In A_6 all the isolates from Sahiwal with the exception of NR985 from Naran.

Similarly Major cluster B has three sub-groups (B1, B2 & B3). Majorities of isolates were originating from Sahiwal area. The isolates in this cluster are more diverse from rest of the isolates.

Similarly Hamm & Hansen in 1983 observed variations in the isolates of *Phytopthora pseudotsugae*. *Phytopthora pseudotsugae* causing root rot of Douglas-fir. Same work was done by Kaosiri & Zentmyer ,1980 ; Wilcox *et al.*, 1987; Wilcox & Mircetich, 1985 and Wilcox & Nevill, 1985. They confirmed variations in *Phytophthora spp.* So the result of these people are similar to our results because we observed a lot variations in our isolates of *Phytophthora infestans*. But Wilcox & Ellis, 1987 reported that the isolates of single species produced largely homogenous banding pattern. Based on our procedures and isolates analyzed it appears that electrophoresis banding pattern of SDS-protein provide a more conservative, less fragmentary criterion for indicating differences among *Phytopthora* species Fig 2. Electrophoresis of soluble proteins from mycelia has been useful (Erselius & de Vallavieille, 1984 and Gallegly, 1983) and is increasing in importance (de Vallavieille & Erselius, 1984 and Faris *et al.*, 1986) as an aid in the identification and classification of numerous species of *Phytophthora*. Gallegly (1983) critiqued the application of several physiological methods for the identification and classification of species

of *Phytophthora* and concluded that electrophoretic analysis of soluble proteins was the most promising method.

Table 1: Polymorphism detected by SDS-PAGE in forty-nine isolates of *Phytophthora infestans* (+ Present & - absent).

Isolates	Locations/Origin	Genotypes									
		1	2	3	4	5	6	7	8	9	10
DB2002	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2003	Punjab/Depalpur	+	-	-	+	-	+	+	+	+	+
DB2005	Punjab/Depalpur	+	+	-	+	+	+	+	+	+	+
DB2006	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2010	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2016	Punjab/Depalpur	+	+	+	+	-	+	+	+	+	+
DB2013	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2014	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2015	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2017	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
SL2001	Punjab/Sahiwal	+	+	+	+	+	+	+	+	+	+
SL2006	Punjab/Sahiwal	+	+	+	+	+	+	+	+	+	+
SL2008	Punjab/Sahiwal	+	+	+	+	+	+	+	+	+	+
SL2012	Punjab/Sahiwal	+	+	+	+	+	+	+	+	+	+
SL2013	Punjab/Sahiwal	+	+	+	+	+	+	+	+	+	+
SL2016	Punjab/Sahiwal	+	+	+	+	-	+	+	+	+	+
SL2021	Punjab/Sahiwal	+	+	+	+	+	+	+	+	+	+
SL2002	Punjab/Sahiwal	-	-	+	+	+	+	+	+	+	+
SL2004	Punjab/Sahiwal	-	-	-	-	+	-	+	+	-	+
SL2005	Punjab/Sahiwal	-	-	-	-	-	-	-	-	-	+
SL2007	Punjab/Sahiwal	-	-	-	-	-	+	-	+	-	+
SL2009	Punjab/Sahiwal	-	-	-	-	-	-	+	+	+	+
SL2017	Punjab/Sahiwal	-	-	-	-	-	-	-	-	-	-
SL2015	Punjab/Sahiwal	-	-	-	+	+	+	+	+	+	+
SL2022	Punjab/Sahiwal	-	-	-	+	-	+	+	+	+	+
DA2001	Punjab/Daska	-	-	-	-	-	-	-	-	+	+
DA2002	Punjab/Daska	-	+	+	+	-	-	-	-	+	+
DA2003	Punjab/Daska	+	+	+	+	+	+	+	+	+	+
DA2004	Punjab/Daska	-	+	+	+	+	-	+	+	+	+
CH2001	Punjab/Cheniot	+	+	+	+	+	+	+	+	+	+
SR2002	Punjab/Salar	+	+	+	+	+	+	+	+	+	+
MD981	Swat Valley/Miandam	+	+	+	+	+	+	-	+	-	+
MD986	Swat Valley/Miandam	+	+	+	+	+	+	+	+	+	+
KN973	Kaghan Valley/Kaghan	+	-	+	+	+	-	+	+	+	-
BN981	Kaghan Valley/Ballan	-	-	-	-	-	-	-	-	-	+
NR985	Kaghan Valley/Naran	+	-	+	+	+	+	+	+	+	+
BT987	Kaghan Valley/Batal	+	+	+	+	+	+	+	+	+	+
PA982	Swat Valley/Peshmal	+	+	+	+	+	+	-	-	+	+
US-1	Cornell University, America	+	+	+	+	+	+	+	+	+	+
US-6	Cornell University, America	+	+	+	+	+	+	+	+	+	-
US-7	Cornell University, America	+	+	+	+	+	+	+	+	+	+
US-8	Cornell University, America	+	+	+	+	+	+	+	+	+	+
DB2001	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2004	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2007	Punjab/Depalpur	-	-	-	-	-	-	-	-	-	+
DB2008	Punjab/Depalpur	-	+	+	+	+	+	+	+	+	+
DB2009	Punjab/Depalpur	-	+	+	+	+	+	+	+	+	+
DB2011	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+
DB2013	Punjab/Depalpur	+	+	+	+	+	+	+	+	+	+

Table 2. Dissimilarity index for protein patterns of forty-nine isolates of *Phytophthora infestans* as determined by SDS-PAGE.

	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
2	1.7																																																
3	1.0	1.4																																														
4	.00	1.7	1.0																																													
5	.00	1.7	1.0	.00																																												
6	1.0	1.4	1.4	1.0	1.0																																										
7	.00	1.7	1.0	.00	.00	1.0																																									
8	.00	1.7	1.0	.00	.00	1.0	.00																																								
9	.00	1.7	1.0	.00	.00	1.0	.00	.00																																							
10	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00																																						
11	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00																																					
12	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00																																				
13	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00																																			
14	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00																																		
15	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00																																		
16	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00																																	
17	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00																																
18	1.4	1.7	1.7	1.4	1.4	1.7	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4																															
19	2.4	1.7	2.2	2.4	2.4	2.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4																															
20	3.0	2.4	2.8	3.0	3.0	2.8	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0																														
21	2.6	2.4	2.4	2.6	2.6	2.8	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6																													
22	2.4	1.7	2.2	2.4	2.4	2.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.0	1.4	1.7	1.7																									
23	3.1	2.6	3.0	3.1	3.1	3.0	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	2.8	2.0	1.0	1.7	2.0																									
24	1.7	1.4	1.4	1.7	1.7	2.0	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.0	1.7	2.4	2.0	1.7	2.6																								
25	2.0	1.7	1.7	2.0	2.0	2.2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.4	2.0	2.2	1.7	1.4	2.4	1.0																								
26	2.8	2.2	2.6	2.8	2.8	2.6	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.4	2.0	1.0	1.7	1.4	1.4	2.2	2.0																							
27	2.2	2.4	2.4	2.2	2.2	2.0	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.6	2.0	2.4	2.2	2.2	2.4	2.2	1.7																			
28	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	0.0	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2																			
29	1.4	1.7	1.7	1.4	1.4	1.0	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	2.0	2.6	2.6	2.0	2.8	1.7	2.0	2.4	1.7	1.4																			
30	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4																	
31	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00																
32	1.4	2.2	1.7	1.4	1.4	1.7	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	2.0	2.4	2.6	2.2	2.8	2.8	2.2	3.2	2.4	2.8	2.2	1.4	2.0	1.4																
33	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.6	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4															
34	1.7	1.4	2.0	1.7	1.7	1.4	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	2.2	2.8	2.8	2.2	2.6	2.0	2.2	2.6	2.4	1.7	1.7	1.7	1.7	2.2	1.7													
35	3.0	2.4	2.8	3.0	3.0	2.8	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.0	3.0	3.0	2.6	1.7	00	1.4	1.7	1.0	2.4	2.2	1.0	2.0	3.0	2.6	3.0	3.0	2.6	3.0	2.8											
36	1.0	1.4	1.4	1.0	1.0	1.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.2	2.8	2.4	2.2	3.0	1.4	1.7	2.6	2.4	1.0	1.7	1.0	1.0	1.7	1.0	1.4	2.8										
37	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.2	.00	1.7	3.0	1.0									
38	1.4	2.2	1.7	1.4	1.4	1.7	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	2.0	2.8	2.6	2.6	2.8	2.8	2.2	2.4	2.4	1.7	1.4	2.0	1.4	1.4	1.4	1.4	2.2	2.6	1.7	1.4									
39	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4								
40	1.0	2.0	1.4	1.0	1.0	1.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.7	2.6	3.1	2.8	2.6	3.0	2.0	2.2	3.0	2.4	1.0	1.7	1.0	1.0	1.7	1.0	1.4	3.1	1.4	1.0	1.7	1.0							
41	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4	.00	1.0						
42	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4	.00	1.0	.00					
43	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4	.00	1.0	.00	.00				
44	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4	.00	1.0	.00	.00	.00			
45	3.0	2.4	2.8	3.0	3.0	2.8	3.0	3.0	3.0	3.0	3.0	3.0	3	0	3.0	3.0	3.0	3.0	2.6	1.7	.00	1.4	1.7	1.0	2.4	2.2	1.0	2.0	3.0	2.6	3.0	3.0	2.6	3.0	2.8	00	2.8	3.0	2.6	3.0	3.1	3.0	3.0	3.0	3.0		
46	1.0	2.0	1.4	1.0	1.0	1.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.2	2.8	2.4	2.2	3.0	1.4	1.7	2.6	2.0	1.0	1.0	1.0	1.0	1.7	1.0	2.0	2.8	1.4	10	1.7	1.0	1.4	1.0	1.0	1.0	1.0	2.8	
47	1.0	2.0	1.4	1.0	1.0	1.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.2	2.8	2.4	2.2	3.0	1.4	1.7	2.6	2.0	1.0	1.0	1.0	1.0	1.7	1.0	2.0	2.8	1.4	10	10	10	10	2.8	00				
48	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4	.00	1.0	.00	.00	.00	.00	3.0	1.0	1.0
49	.00	1.7	1.0	.00	.00	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.4	2.4	3.0	2.6	2.4	3.1	1.7	2.0	2.8	2.2	.00	1.4	.00	.00	1.4	.00	1.7	3.0	1.0	.00	1.4	.00	1.0	.00	.00	.00	.00	3.0	1.0	1.0

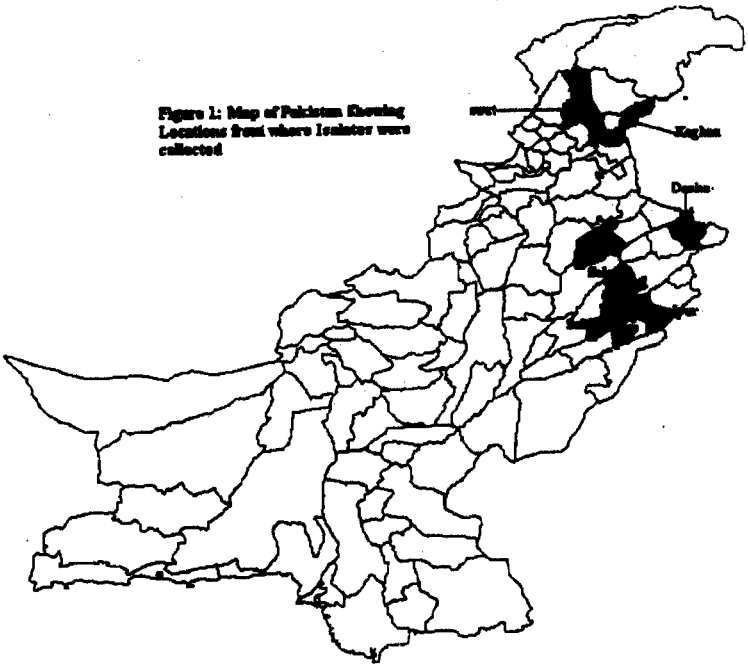

Figure 1: Map of Pakistan Showing Locations from where Isolates were collected

Figure 2. Polyacrylamide gel pattern obtained by elecrtophoretic separation of soluble proteins from the isolates of *Phytophthora infestans*.

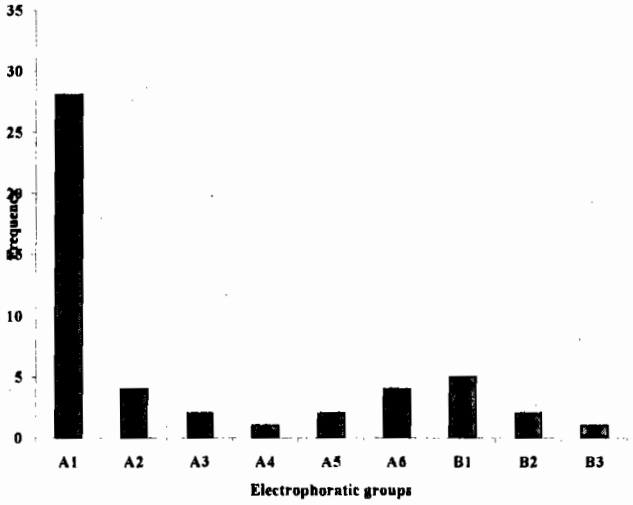

Figure 3. Frequency distribution of electrophoratic groups of *Phytophthora infestans* isolates.

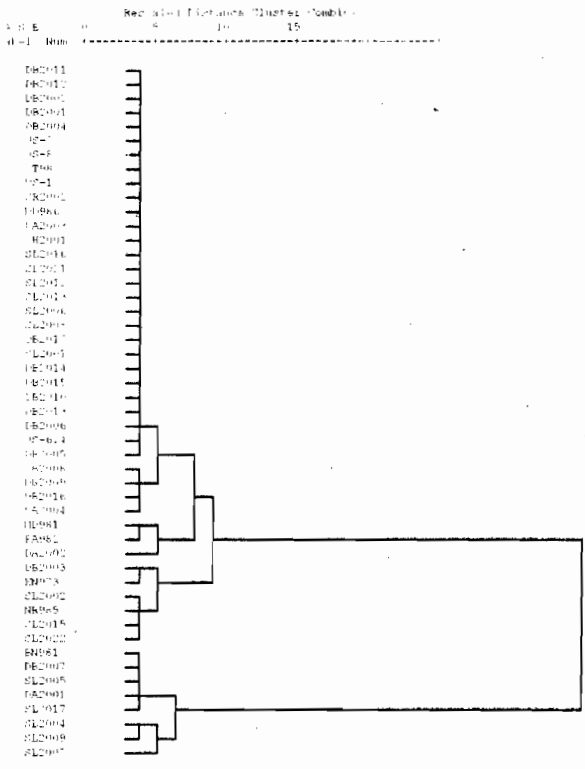

Fig 4. A dendrogram depicting relationship among forty-nine isolates of *Phytopthora infestans.*

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