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Genetic diversity analysis of *Bemisia tabaci* populations in Pakistan using RAPD markers

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Abstract Background: The *Bemisia tabaci* is one of the most devastating pests of agricultural crops and ornamental plants worldwide. The genetic diversity and biotype status of the *Bemisia tabaci* in Pakistan was assessed by using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). A total 80 samples of *B. tabaci* collected from 14 districts of the Punjab province and 7 districts of the Sindh province were included.

Results: All 10 primers screened in this study generated 151 scorable amplification products, of which 117 or 77% were polymorphic. Pairwise Nei and Li's similarity had ranged from 0.25 to 0.88 among all individuals analyzed. Based on Nei and Li's similarity coefficients *Bemisia* populations were grouped into 3 main clusters and clearly distinguished the non B biotype from the B biotype.

Conclusion: The level of similarity among populations of same biotypes was high whereas between populations of non B and B biotypes appeared to be less closely related. This analysis showed that non B biotype is prevalent in both provinces however B biotype is restricted to few locations in Sindh. This monitoring of the spread of *B. tabaci* in Pakistan will assist in the establishment of appropriate management strategies.

Keywords: Asia II 1, genetic diversity, UPGMA, whitefly.

INTRODUCTION

The whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), has a long history as a serious pest and virus vector of agricultural crops worldwide. *B. tabaci* is expected to be a complex of 11 well defined groups with 24 distinct species (Dinsdale et al. 2010; De Barro et al. 2011). In Pakistan, *B. tabaci* has increased in importance since 1987/1988, when major outbreaks of cotton leaf curl disease had occurred. Cotton leaf curl virus (CLCuV) was recognized as a cause of severe losses in cotton, vectored by *B. tabaci* (Hameed et al. 1994) and the presence of the B biotype was first reported in Punjab in 1996 (Hameed et al. 1996). Further studies revealed Pakistani Bemisia population into Indian, Southeast Asian, and Mediterranean African groups. The Indian clade type appeared more dominant in the Punjab and the Mediterranean African clade type in Sindh (Simon et al. 2003).

Different genetic markers have been originated with distinct properties that have exposed important biological information in *B. tabaci.* Among the molecular markers, Random Amplified Polymorphic DNA (RAPD) is extensively being employed in studies of genetic diversity as it is a simple and inexpensive

technique. The RAPD markers were used in revealing the genetic structure of the *B. tabaci* populations (Abdullahi et al. 2003; Khasdan et al. 2005), for the identification and spread of invasive biotypes (Horowitz et al. 2003; Delatte et al. 2005; Tahiri et al. 2006) and for distinguishing indigenous populations from those of introduced B biotype (Rekha et al. 2005). It was considered that the B and Q biotypes are genetically isolated as less gene flow was observed between the two biotypes under laboratory conditions when RAPD data was quantified by molecular variance (AMOVA). This was later confirmed by many studies based on mitochondrial cytochrome oxidase 1 gene analysis and now B and Q biotypes are thought to be separate species (Dinsdale et al. 2010; De Barro et al. 2011). In the present study, estimation of genetic diversity and monitoring the presence of species/biotype among *B. tabaci* biotypes was conducted using RAPD markers.

MATERIALS AND METHODS

Adult whitefly populations were collected from 40 cotton growing areas of Punjab and Sindh provinces. A total of 80 samples of *B. tabaci* were used in this study. For comparison populations (B-1) of B biotype (Middle East Asia Minor 1) was provided by South China agricultural university and indigenous population (A-11) of *B. tabaci* sampled from Multan in 2006 and maintained in the NARC, Islamabad were also included. The indigenous population was identified as non B (Asia II 1) by mtCO1 sequence (HM488014) and the collection sites are summarized in Table 1.

DNA extraction

Total genomic DNA was extracted from individual white flies by crushing them in a glass homogenizer containing 60 µL of ice cold lysis buffer. Lysis buffer was prepared according to a standard protocol defined by Frohlich et al. (1999). The homogenate was transferred to 0.5 ml microtube and incubated at 65ºC for 15 min and 95ºC for 10 min, in thermocycler. The processed samples were stored at -20ºC.

Primer selection and RAPD assays

Initially, 5 randomly selected samples were used to choose the suitable primers. In total 25 10mer primers were used which were previously selected for measuring genetic diversity and biotypes identification by Moya et al. 2001; Lima et al. 2002; Simon et al. 2003. After preliminary experiments, 10 primers were selected on the basis of their ability to detect the polymorphism and production of the scorable banding patterns in *B. tabaci*: OPA-04, OPA-09, OPA-11, OPB-13, OPC-01, OPC-04, OPC-10, OPC-15, OPO-06 and OPO-18.

RAPD-PCR reactions were performed in 25 µl of reaction mixture containing 10 mM Tris-HCl, (pH 8.8), 50 mM KCl, 3.0 mM $MgCl_2$, 200 µM of each deoxynucleotide triphosphate (dNTP), 0.8 µM of primer, 1.25 units Taq polymerase (Fermentas UAB Lithuania) and 2 µl of template DNA. Amplifications were carried out in a thermocycler using the following parameters: Initial denaturation for 1 min at 94ºC followed by 45 cycles of denaturing for 1 min at 92ºC, Primer annealing for 1 min at 35ºC and extension for 2 min at 72ºC and final extension of 7 min at 72ºC, and then held at 4ºC. After amplification, PCR products were separated by electrophoresis using 1.4% agarose gel containing 0.5 ug ml-1 of ethidium bromide in 1 X Tris-borate EDTA (TBE) buffer.

Data analysis

DNA fragment amplified by a given primer was scored as 0 for the absence and 1 for the presence of a band. Pair-wise comparisons of the *B. tabaci* populations based on the presence or absence of distinctive and shared amplified products were used to generate similarity coefficients. From the resulting similarity coefficients, a dendrogram was constructed using unweighted pair group method with arithmetic means (UPGMA). This analysis was carried out using the NTSYS-pc, version 2.2 package (Rohlf, 2005).

RESULTS AND DISCUSSION

DNA amplification and biotype identification

Among the 25 decamer oligonucleotide primers, 10 were selected on the basis of clear and variable bands (Table 2). A total of 151 scorable amplification products were generated by 82 samples. The number of amplification products generated by each primer varied from 9 (OPA-11) to 23 (OPO-06) with an average of 15 bands per primer. The size of amplified fragments had ranged from 150 bp to 3000 bp. A total of 117 (77%) polymorphic bands were observed ranging from 6 to 20 fragments per primer. The average number of polymorphic fragments per primer among them was 11.7. The primers OPO-06 provided the highest number of polymorphic fragments (20) while the minimum number of polymorphic bands (6) was observed in OPA-11 primer. The study showed that the primer OPA-04 gave the unique and unambiguous DNA profiles that clearly distinguished biotype B from indigenous non B individuals (Figure 1). The banding pattern of reference population B-1 (B biotype) and samples SdMk07-1 (lane 73) and SdMk07-2 (lane 74) were identical and produced two polymorphic fragments of 150 bp and 450 bp. The RAPD products of all the other samples were similar to the A-11 (non B biotype) and produced 490 bp and 1250 bp size unique products (Figure 1).

Similarity matrix

A similarity matrix based on the proportion of shared RAPD fragments was used to establish the level of relatedness between populations of *B. tabaci*. Pair-wise estimates of similarity ranged from 0.25 to 0.88. The A-11 (non B biotype) and PjMu07-6 were the closest genotype with the highest similarity index of 88%. This was followed by 83% similarity between B-1 (B biotype) and SdMk07-2. The lowest level of similarity at, 25% was obtained between SdMk07-01 and PjBp07-04. Among the Punjab *B. tabaci* populations, similarity coefficients had ranged from 0.32 to 0.75 whereas Sindh *B. tabaci* populations from 0.30 to 0.72 excluding the controls A-11 and B-1. This shows that the range of similarity coefficients across both provinces has little variation. The similarity coefficients among B biotype had ranged from 0.42 to 0.83 with an average of 0.57 whereas non B biotype showed similarity coefficients from 0.26 to 0.88 with an average of 0.62. The level of similarity among the B biotype populations is high whereas the populations of non B and B biotypes appeared to be less closely related. Similar outcomes were previously reported in Brazilian Populations using RAPD (Lima et al. 2002).

Cluster analysis

A dendrogram was constructed based on Nei and Li's similarity coefficients using UPGMA. A total 82 *B. tabaci* populations including the reference populations were grouped into 3 main clusters I, II and III corresponding to non B Punjab, non B Punjab and Sindh and B Sindh respectively (Figure 2). The clusters formed by the non B biotype populations of *B. tabaci* are clearly distinct from the B biotype cluster. The first cluster included only Punjab non B *B. tabaci* populations with the A-11 reference population. This group clearly showed that populations from the same district are grouped together for example most of the populations of Khanewal, RahimYaar Khan and Vihari districts were set together. The second cluster contained all non B biotype populations of both Punjab and Sindh provinces. Geographically this is a more diverse group with populations from different locations being clustered as one. In this case, populations from the same district formed different groups unlike the first group. The B biotype populations formed an independent third main cluster which contained B biotype reference population with two populations (SdMk07-1 and SdMk07-2) and both from Mirpurkhas district (Sindh). This suggests that B biotype is currently in very few locations of Sindh province while the non B biotype was observed in Punjab as well as Sindh Province and interestingly, most of the *B. tabaci* populations originating from different areas of the same district fall into the same sub clusters. This analysis showed that genetic distances between populations of non B and B are considerably higher than distances between B biotype populations (Lima et al. 2002).

Cluster analysis of RAPD data separated the *B. tabaci* samples on the basis of B and non B biotypes (Rabello et al. 2008) with the same populations as identified with RAPD banding patterns generated by OPA-04. This primer can be considered further for the development of biotype specific SCAR marker. If this analysis is evaluated according to Dinsdale nomenclature (Dinsdale et al. 2010), the B biotype is Middle East Asia Minor 1 and non B is Asia II 1, and it shows that Asia II 1 is more diverse and significantly present in both the provinces whereas MEAM 1 is observed in a small number of locations

in Sindh. Additional study is required to determine why B biotype has not been established to any significant degree in Sindh even though its strong presence had been documented before (Hameed et al. 1996, Simon et al. 2003). The information gained from this study will help in developing and promoting future control strategies.

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REFERENCES

ABDULLAHI, I.; WINTER, S.; ATIRI, G.I. and THOTTAPPILLY, G. (2003). Molecular characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava. *Bulletin of Entomological Research*, vol. 93, no. 2, p. 97-106. **[CrossRef]**

DE BARRO, P.J.; LIU, S.S.; BOYKIN, L.M. and DINSDALE, A.B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, vol. 56, p. 1-19. **[CrossRef]**

DELATTE, H.; REYNAUD, B.; GRANIER, M.; THORNARY, L.; LETT, J.M.; GOLDBACH, R. and PETERSCHMITT, M. (2005). A new silverleaf-inducing biotype Ms of *Bemisia tabaci* (Hemiptera: Aleyrodidae) indigenous to the islands of the south-west Indian Ocean. *Bulletin of Entomological Research,* vol. 95, no. 1, p. 29-35. **[CrossRef]**

DINSDALE, A.; COOK, L.; RIGINOS, C.; BUCKLEY, Y.M. and DE BARRO, P.J. (2010). Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. *Annals of the Entomological Society of America*, vol. 103, no. 2, p. 196-208. **[CrossRef]**

FROHLICH, D.R.; TORREZ-JEREZ, I.; BEDFORD, I.D.; MARKHAM, P.G. and BROWN, J.K. (1999). A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology*, vol. 8, no. 10, p. 1683-1691. **[CrossRef]**

HAMEED, S.; KHALID, S.; UL-HAQ, E. and HASHMI, A. (1994). Cotton leaf curl disease in Pakistan caused by a whitefly transmitted Geminivirus. *Plant Diseases*, vol. 78, no. 5, p. 529. **[CrossRef]**

HAMEED, S.; KHALID, S. and NAQVI, S.M. (1996). Occurrence of B-biotype of *Bemisia tabaci* in Pakistan. In: *Brighton Crop Protection Conference: Pests and Diseases Proceedings of an International Conference*, (18th-21th November, Brighton, UK, 1996), vol. 1, no. 2, p. 81-85.

HOROWITZ, A.R.; DENHOLM, I.; GORMAN, K.; CENÍS, J.L.; KONTSEDALOV, S. and ISHAAYA, I. (2003). Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica,* vol. 31, no. 1, p. 94-98. **[CrossRef]**

KHASDAN, V.; LEVIN, I.; ROSNER, A.; MORIN, S.; KONTSEDALOV, S.; MASLENIN, L. and HOROWITZ, A.R. (2005). DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population dynamics. *Bulletin Entomological Research*, vol. 95, no. 6, p. 605-613. **[CrossRef]**

LIMA, L.H.C.; CAMPOS, L.; MORETZSOHN, M.C.; NÁVIA, D. and DE OLIVEIRA, M.R.V. (2002). Genetic diversity of *Bemisia tabaci* (Genn.) populations in Brazil revealed by RAPD markers. *Genetics and Molecular Biology*, vol. 25, no. 2, p. 217-223. **[CrossRef]**

MOYA, A.; GUIRAO, P.; CIFUENTES, D.; BEITIA, F. and CENIS, J.L. (2001). Genetic diversity of Iberian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA-polymerase chain reaction. *Molecular Ecology*, vol. 10, no. 4, p. 891-897. **[CrossRef]**

RABELLO, A.R.; QUEIROZ, P.R.; SIMÕES, K.C.C.; HIRAGI, C.O.; LIMA, L.H.C.; OLIVEIRA, M.R.V. and MEHTA, A. (2008). Diversity analysis of *Bemisia tabaci* biotypes: RAPD, PCR-RFLP and sequencing of the ITS1 rDNA region. *Genetics and Molecular Biology,* vol. 31, no. 2, p. 585-590. **[CrossRef]**

REKHA, A.R.; MARUTHI, M.N.; MUNIYAPPA, V. and COLVIN, J. (2005). Occurrence of three genotypic clusters of *Bemisia tabaci* and the rapid spread of the B biotype in south India. *Entomologia Experimentalis et Applicata*, vol. 117, no. 3, p. 221-233. **[CrossRef]**

ROHLF, F.J. (2005). NTSYSpc (Numerical Taxonomy and Multivariate Analysis System). Version 2.2, Exeter Software, *Applied Biostatistics Inc.* New York, USA.

SIMON, B.; CENIS, J.L.; BEITIA, F.; KHALID, S.; MORENO, I.M.; FRAILE, A. and GARCIA-ARENAL, F. (2003). Genetic structure of field populations of begomoviruses and of their vector *Bemisia tabaci* in Pakistan. *Phytopathology*, vol. 93, no. 11, p. 1422-1429. **[CrossRef]**

TAHIRI, A.; SEKKAT, A.; BENNANI, A.; GRANIER, M.; DELVARE, G. and PETERSCHMITT, M. (2006). Distribution of tomato infecting begomoviruses and *Bemisia tabaci* biotypes in Morocco. *Annals of Applied Biology*, vol. 149, no. 2, p. 175-186. **[CrossRef]**

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Tables

Table 1. Sampling locations of *Bemisia tabaci* populations from Punjab and Sindh Provinces, Pakistan.

No.	Populations	Place	Identification (Biotype)
		Punjab	Non B
1	PjFd07-l	Faisalabad/Faisalabad	Non B
2	PjFd07-2	Faisalabad/Faisalabad	Non B
3	PjJh07-1	Jhang/Jhang	Non B
4	PjJh07-2	Jhang/Jhang	Non B
5	PjJh07-3	Shorkot/Jhang	Non B
6	PjJh07-4	Shorkot/Jhang	Non B
7	PjKw07-1	Saraisidhu/Khanewal	Non B
8	PjKw07-2	Saraisidhu/Khanewal	Non B
9	PjKw07-3	Kabirwala/Khanewal	Non B
10	PjKw07-4	Kabirwala/Khanewal	Non B
11	PjKw07-5	Talamba/Khanewal	Non B
12	PjKw07-6	Talamba/Khanewal	Non B
13	PjKw07-7	Khanewal/Khanewal	Non B
14	PjKw07-8	Khanewal/Khanewal	Non B
15	PjKw07-9	Mianchnnu/Khanewal	Non B
16	PjKw07-10	Mianchnnu/Khanewal	Non B
17	PjMu07-1	BastiMaluk/Multan	Non B
18	PjMu07-2	BastiMaluk/Multan	Non B
19	PjMu07-3	Multan/Multan	Non B
20	PjMu07-4	Multan/Multan	Non B
21	PjMu07-5	Shujabad/Mulatn	Non B
22	PjMu07-6	Shujabad/Mulatn	Non B
23	PjLd07-1	Lodhran/Lodhran	Non B
24	PjLd07-2	Lodhran/Lodhran	Non B
25	PjBp07-1	Bahawalpur/Bahawalpur	Non B
26	PjBp07-2	Bahawalpur/Bahawalpur	Non B
27	PjBp07-3	Nurpur/Bahawalpur	Non B
28	PjBp07-4	Nurpur/Bahawalpur	Non B
29	PjBp07-5	Ahmadpur East/Bahawalpur	Non B
30	PjBp07-6	Ahmadpur East/Bahawalpur	Non B
31	PjBp07-7	HasilPur/Bahawalpur	Non B
32	PjBp07-8	HasilPur/Bahawalpur	Non B
33	PjRy07-1	Liaqatpur/Raheem Yaar Khan	Non B
34	PjRy07-2	Liaqatpur/Raheem Yaar Khan	Non B
35	PjRy07-3	Khanpur/Raheem Yaar Khan	Non B
36	PjRy07-4	Khanpur/Raheem Yaar Khan	Non B
37	PjRy07-5	R Yaar Khan/Raheem Yaar Khan	Non B
38	PjRy07-6	R Yaar Khan/Raheem Yaar Khan	Non B
39	PjRy07-7	Sadiqabad/Raheem Yaar Khan	Non B
40	PjRy07-8	Sadiqabad/Raheem Yaar Khan	Non B
41	PjVi07-1	Tibba/Vehari	Non B
42	PjVi07-2	Tibba/Vehari	Non B
43	PjVi07-3	Vehari/Vehari	Non B
44	PjVi07-4	Vehari/Vehari	Non B
45	PjVi07-5	Burewala/Vehari	Non B
46	PjVi07-6	Burewala/Vehari	Non B
47	PjVi07-7	Gaggo/Vehari	Non B
48	PjVi07-8	Gaggo/Vehari	Non B
49	PjVi07-9	Luddan/Vehari	Non B
50	PjVi07-10	Luddan/Vehari	Non B
51	PjBn07-1	ChistianMandi/Vehari	Non B
52	PjBn07-2	ChistianMandi/Vehari	Non B
53	PjBn07-3	Bahawalnagar/Bahawalnagar	Non B
54	PjBn07-4	Bahawalnagar/Bahawalnagar	Non B

55	PjPa07-1	Pakpattan/Pakpattan	Non B
56	PjPa07-2	Pakpattan/Pakpattan	Non B
57	PjPa07-3	Nurpur/Pakpattan	Non B
58	PjPa07-4	Nurpur/Pakpattan	Non B
59	PjPa07-5	Jehan Khan/Pakpattan	Non B
60	PjPa07-6	Jehan Khan/Pakpattan	Non B
61	PjSh07-1	Arifwala Sahiwal	Non B
62	PjSh07-2	Arifwala Sahiwal	Non B
63	PjSh07-3	Sahiwal/Sahiwal	Non B
64	PjSh07-4	Sahiwal/Sahiwal	Non B
65	PjSh07-5	Chichawatni/Sahiwal	Non B
66	PjSh07-6	Chichawatni/Sahiwal	Non B
67	SdNs07-1	Nawabshah/Nawabshah	Non B
68	SdNs07-2	Nawabshah/Nawabshah	Non B
69	SdSg07-1	Sanghar/Sanghar	Non B
70	SdSg07-2	Sanghar/Sanghar	Non B
71	SdTa07-1	TandoAllahYaar/TandoAllahYaar	Non B
72	SdTa07-2	TandoAllahYaar/TandoAllahYaar	Non B
73	SdMk07-1	MirpurKhas/MirpurKhas	B
74	SdMk07-2	MirpurKhas/MirpurKhas	B
75	SdHd07-1	Hyderbad/Hyderbad	Non B
76	SdHd07-2	Hyderbad/Hyderbad	Non B
77	SdKh07-1	Khairpur/Khairpur	Non B
78	SdKh07-2	Khairpur/Khairpur	Non B
79	SdNf07-1	Nowsheroferoz/Nowsheroferoz	Non B
80	SdNf07-2	Nowsheroferoz/Nowsheroferoz	Non B

Host Plant: Cotton.

Table 2. Primers used for generating RAPD profiles in *B. tabaci*.

Sl.	Primer'S	Sequence	Amplified	Polymorphic	Percent	Fragment
1	OPA-04	AATCGGGCTG	11	8	73	150-2000
2	OPA-09	GGGTAACGCC	13	10	77	350-2300
3	OPA-11	CAATCGCCGT	9	6	67	500-3000
4	OPB-13	TTCCCCCGCT	18	15	83	250-2500
5	OPC-01	TTCGAGCCAG	15	12	80	200-1500
6	OPC-04	CCGCATCTAC	12	9	75	250-2000
7	OPC-10	TGTCTGGGTG	12	10	83	200-1500
8	OPC-15	GACGGATCAG	18	13	72	300-1500
9	OPO-06	CCACGGGAAG	23	20	87	250-1800
10	OPO-18	CTCGCTATCC	20	14	70	250-2300
Total			151	117		

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Figures

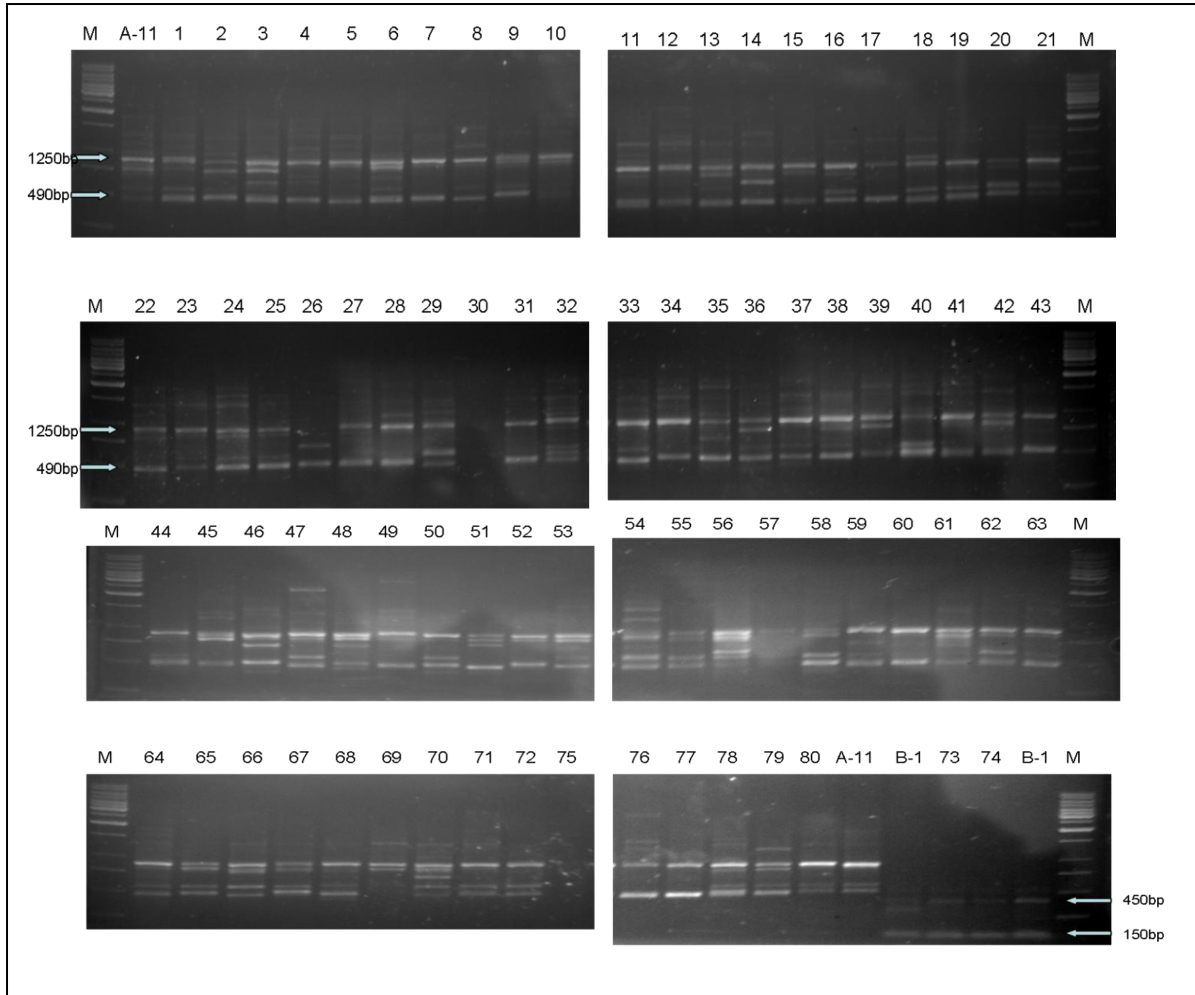

Fig. 1 RAPD banding profiles of 80 *B. tabaci* of Pakistan with reference populations, A-11 (indigenous non B) and B-1 (B biotype) generated with primer OPA-04. The lanes represents M is 1 Kb marker. The lanes abbreviation are described as in **Table 1**.

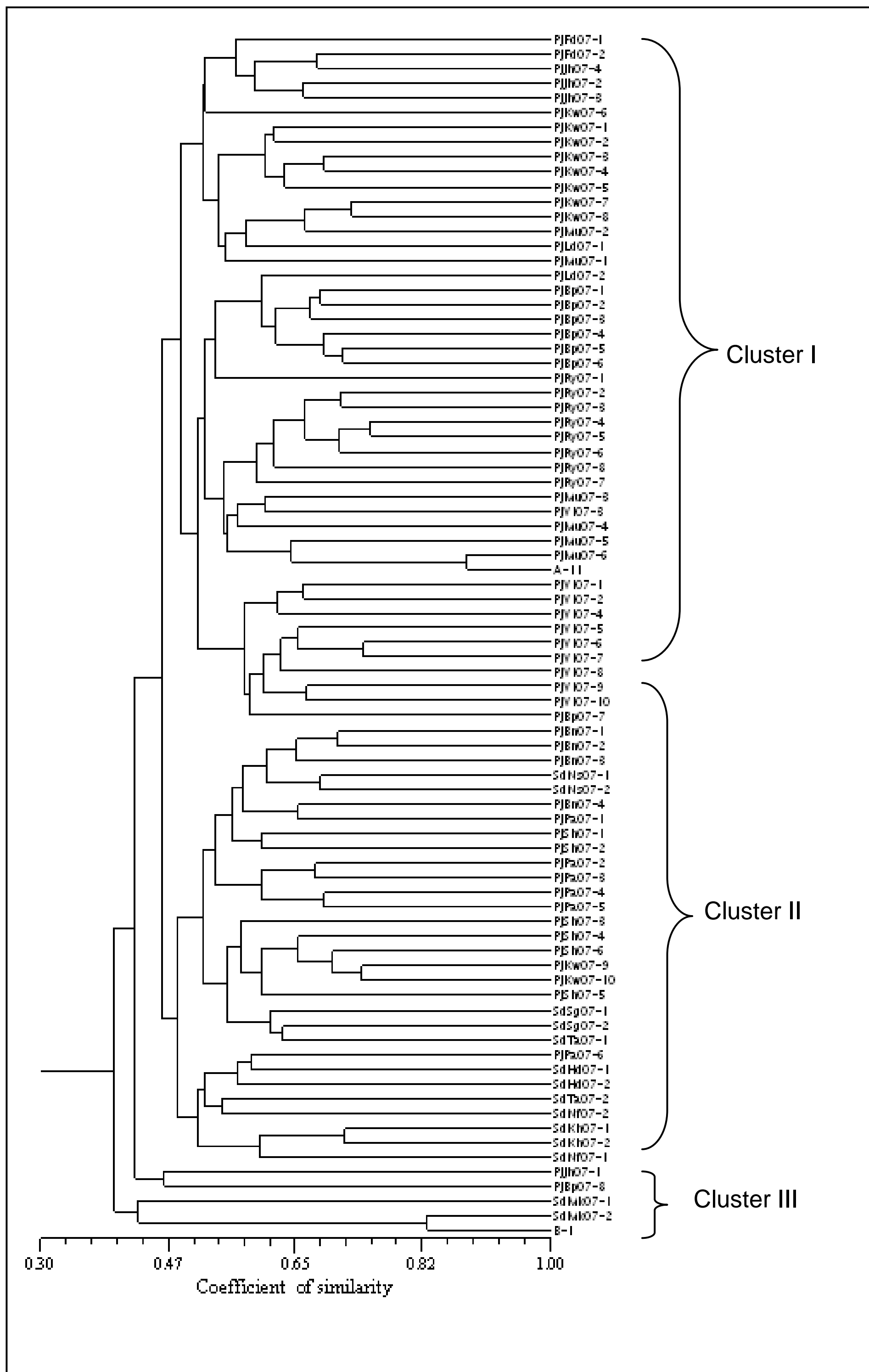

Fig. 2 UPGMA based dendrogram showing the grouping and relationship of 82 Bemisia populations based on 151 RAPD fragments.