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Original Article

Phylo-geographic analysis of whitefly on the basis of mitochondrial cytochrome oxidase 1 gene

Análise filogeográfica da mosca-branca com base no gene citocromo oxidase 1 mitocondrial

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Abstract

Bemisia tabaci is a species complex that causes damage to its broad range of plant hosts through serious feeding. It transmits plant viruses of different groups to important agricultural crops. Some important cash crops of Pakistan are sugar cane, rice, tobacco and seed oil. It shows high genetic variability and is differentiated as races or biotypes. Biotypes are, biotype Q, biotype B, biotype B2, biotype M, biotype L, biotype A, biotype H, biotype C, biotype K, biotype N, biotype R, biotype E, biotype P, biotype J, biotype S, biotype AN. Although the current report based on the Bayesian study of mitochondrial cytohrome oxidase gene1 (CO1) DNA sequences has classified the different populations of whiteflies into twelve genetic groups which are Mediterranean, Sub-Saharan Africa silverleafing, Indian Ocean, Asia II, Asia I, Australia, New World, Italy, China, Sub-Saharan Africa non-silverleafing, Mediterranean/ Asia Minor/Africa and Uganda sweet potato. Begomoviruses is largest group of viruses transmitted by *B. tabaci* and cause major diseases of crops such as tomato and chili leaf curl disease, cassava mosaic disease; yellow mosaic disease of legumes and cotton leaf curl disease. The main objective of current study is to inculpate knowledge regarding genetic diversity of whitefly in cotton fields across Pakistan *via* analysis of partial DNA sequence of mitochondrial gene Cytochrom Oxidase I (mtCO1).

Keywords: *Bemesia tabaci*, biotyping, mitochondrial gene, biodiversity, cloning vector, plasmids, cytochrom oxidase I.

Resumo

Bemisia tabaci é um complexo de espécies que causa danos a uma ampla gama de hospedeiros vegetais por meio de alimentação séria. Ele transmite vírus de plantas de diferentes grupos para importantes safras agrícolas. Algumas safras comerciais importantes do Paquistão são cana-de-açúcar, arroz, tabaco e óleo de semente. Apresenta alta variabilidade genética e é diferenciado em raças ou biótipos. Os biótipos são: biótipo Q, biótipo B, biótipo B2, biótipo M, biótipo L, biótipo A, biótipo H, biótipo C, biótipo K, biótipo N, biótipo R, biótipo E, biótipo P, biótipo J, biótipo S, biótipo AN . Embora o relatório atual baseado no estudo bayesiano das sequências de DNA do gene 1 da oxidase do citocromo mitocondrial (CO1) tenha classificado as diferentes populações de moscas-brancas em doze grupos genéticos, que são Mediterrâneo, África Subsaariana com folha de prata, Oceano Índico, Ásia II, Ásia I, Austrália, Novo Mundo, Itália, China, África Subsaariana sem folha prateada, Batata-doce Mediterrâneo / Ásia Menor / África e Uganda. Os begomovírus são o maior grupo de vírus transmitidos por *B. tabaci* e causam as principais doenças de culturas, como a doença do cacho do tomate e da pimenta-malagueta, doença do mosaico da mandioca, doença do mosaico amarelo de leguminosas e doença do enrolamento da folha do algodão. O principal objetivo do presente estudo é inculpar conhecimento sobre a diversidade genética da mosca-branca em campos de algodão em todo o Paquistão por meio da análise da sequência parcial de DNA do gene mitocondrial Citocromo Oxidase I (mtCO1).

Palavras-chave: *Bemesia tabaci*, biotipagem, gene mitocondrial, biodiversidade, vetor de clonagem, plasmídeos, citocromo oxidase I.

1. Introduction

The haplo-diploid species of a sap-feeding insect, *Bemisia tabaci* (*Hemiptera: Aleyrodidae*) belonged to a cluster of insects generally identified like whiteflies. This can complete a generation within two to three weeks in meadow crops, where it is a pest of hot climate among 30° south and north of the equator (De Barro, 1995).

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Eggs were laid on the surface of leaves by winged mobile adults, as in white flies different changes (metamorphosis) occurs for the development of an adult organism in which crawler stage is followed by three different stages such as, after hatching immature instar, pupa and then an adult (Gill, 1990). It prefers transitory herbaceous hosts together with several horticultural and dicotyledonous agricultural species. Within these climates, crops commonly grown-up in below than six months and a characteristic harvesting establishment engages recurring superimpose planting. Subsequently harvesting the crop is ruined and it necessitates the whiteflies to go away to younger crops or wild plants. Throughout the survival of a crop, invasion includes an early immigration stage, chased through regional, inside the crop dispersion and populace enhance, moreover after that a last migration stage, when the crop come to an end. About 1300 whitefly species in over 120 genera have been described (CABI, 2001).

Bemisia tabaci also known as tobacco or cotton whitefly is broadly dispersed within temperate, sub-tropical and tropical agriculture zones (Byrne and Bellows Junior, 1991; Brown, 2001). Now it is globally dispersed and established at all continents apart from Antarctica (Martin, 1999).

Bemisia tabaci was described for the first time in 1889 in Greece (Gennadius, 1889). Due to the number and variety of natural enemies found in the region, the Indian sub-continent was considered to be the epicenter of *B. tabaci* (Banks et al., 2001). It has had a universal pest within the previous 20 years (Qiu et al., 2008; Brown et al., 2012).The most significant feature of the *Bemisia tabaci* is the presence of highly genetically variable characters amongst its population. This inconsistency is manifested through the presences of populaces which differ in their capacity of virus transmission and to feed or reproduce on particular host's characteristics. *B. tabaci* has over 900 host plants. A great amount of plant species fostered and non-cultivated yearly and eternally are documented as adequate nourishing and/or reproductive hosts. Mound and Halsey (1978), listed plant species, among them 50% assimilate to five families: Asteraceae, Malvaceae, Solanaceae, Euphorbiaceae and Fabaceae.

Two well-known haplotypes are Q and B that collectively proved greatly insidious. (Frohlich et al., 1999). B. biotype has only currently been recorded in the sub- continent of India (Banks et al., 2001). It has been stated that the origin of the *B. tabaci*, biotype may be the North Eastern Africa-Middle East-Arabian Peninsula. Q biotype appearance and establishment is anticipated, or was possible expected to already have occurred in at least certain locations, and is expected to have important and across-the-board economic relevance. In Pakistan, cotton leaf curl disease (CLCuD) for the first time was noticed near Multan in 1967. No attention was paid to this disease because of its informal incidence and less cost-effective significance. It has become a solemn danger to cotton crop of Pakistan since 1987. The area affected by this disease was recorded as 97,580 hectares with a collapse of 543,294 bales of cotton throughout 1992-93 period in the Punjab (Aslam et al., 2000). When harvest fatalities in many fields increased 80% (Ali and Aheer, 2007) and the country's economy was critically affected, the disease-hit area of cotton has exceeded from about 60 hectares in 1988-1989 to almost 900,000 hectares in 1993-1994.

It has been observed that two indigenous species of *B. tabaci*, Asia II 1, (biotypes PCG-1, SY, PK1, ZHJ2, K and P) and Asia 1(also mentioned in the literature as biotypes NA, M, PCG-2 and H) are accountable for the elevated frequency of CLCuD in Punjab province. Whereas in Sindh province the Middle East-Asia Minor 1 (usually identified as biotype B2 and B) was detected which show low frequency of CLCuD (Simón et al., 2007) based on this it is alluring to propose that *Bemisia tabaci* all over the Sindh seem not as similar as those establish in the province of Punjab and that those in Sindh are not much proficient CLCuD. Though, on the whole the information of species geographic distribution is missing in provinces. For effective management of *Bemisia tabaci* it is essential to identify the vector(s) accurately (Ahmed et al., 2011). So the use of phylogenetic markers such as mitochondrial cytochrome oxidase I gene (mtCO1) (Kirk et al., 2000) and ITS1 region of rDNA (Abdullahi et al., 2003) has been much improved by knowledge of the variation of *B. tabaci*. To understand the epidemics of viruses transmitted by this insect, accurate assessment of the difference of *B. tabaci* populations is required. Application of gene marker Mitochondrial cytochrome oxidase gene1(mtCO1) has permitted mapping the allocation of the haplotypes of *B. tabaci* in Pakistan with related results for recognizing the epidemiology of Cotton leaf curl virus CLCuV (Simón et al., 2003).

2. Materials and Methods

2.1. Sampling of whiteflies

Samples of adult whiteflies were collected from different areas of Punjab like Bahawalpur, Multan, Arifwala, Muzafargarh, Sahiwal, Kabirwala, Tibasultan, Narowal, Rajanpure, Khairpur from cotton crop. (Table 1) Whiteflies samples were preserved in 95% ethanol in 1.5 mL eppendorf tubes which were properly labeled and kept at 4 °C unless utilized.

2.2. Genomic DNA extraction from whiteflies

Using De Barro et al. (2006)genomic DNA were extracted from the whiteflies with minor modification. Single adult whitefly from the sample was rinsed by sterile double distilled (SDDW) water to eliminate the ethanol completely. Peculiar whitefly was crushed in 5uL of freshly prepared Lysis Buffer (1uL of 0.5 M EDTA [pH 8.0], 5 uL of 1 M Tris-HCl [pH 8.0], 5uL of Nonident P-40, 50uL of 1.0 mg/mL proteinase K and 989uL SDDW to make the final volume 1mL) on a Petri dish covered with an aluminium foil. 35uL Lysis Buffer was added again to the crushed sample and then whole mixture was shifted to a 0.5 mL microcentrifuge tube. The tube was kept on ice for 5 minutes. After ice incubation the mixture in tube was placed at 65 °C for 15 minutes and then at 95 °C for 10 minutes to deactivate the proteinase Kinase. The sample was centrifuged at 14000 rpm for one minute

Table 1. Whitefly sampling location of Punjab province, along with their genetic group, number of nucleotides and accession numbers of mtCOI gene.

Sample code	Location	Number of nucleotides	Biotypes/Genetic group	Accession number
RI-1	Arifwala	866	Asia II unresolved	HG792860
RI-3	Multan	866	Asia II unresolved	HG792861
RI-4	Muzafargarh	865	Asia II unresolved	HG792862
RI-6	Toba Tek Singh	866	Asia II unresolved	HG792866
RI-7	Okara	866	Asia II unresolved	HG792867
RI-8	Faisalabad	866	Asia II unresolved	HE862289
RI-9	Sahiwal	866	Asia II unresolved	HG792863
RI-10	Kabirwala	866	Asia II unresolved	HG792864
RI-11	Tiba Sultan Pur	866	Asia II unresolved	HG792865

and supernatant was used directly for Polymerase Chain Reaction (PCR) amplification.

2.3. Polymerase chain reaction (PCR)

The mitochondrial marker gene CO1 (*cytochrome oxidase 1*) was amplified using primers C1-j-2195-5'TTGATTTTTTGGTCATCCAGAAGT3'and L2-N301045'TCCAATGCACTAATCTGC CATATTA 3'(Frohlich et al., 1999). All reactions (25ul) contained 5uL DNA extracted from whitefly, 2.5uL of 10X Taq polymerase buffer (Fermentas), 2.5uL of 2mM dNTPs, 0.25uL of Taq DNA polymerase (Fermentas), 1.5uL of 25mM $MgCl_2$, 0.5uL of each primers and 12.25uL of SDDW was prepared. PCR reactions were carried out on Eppendrof Mastercycler at 5 min pre-denaturation at 94 °C, followed by 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 50 °C and 1 min amplification at 72 °C. Finally 72 °C extension temperature was provided for 10 min. PCR product were tested on 1% agarose gel.

2.4. Agarose gel electrophoresis

For 1% [w/v] gel in a small apparatus (6x10x0.5cm), 0.7 g agarose was added into 70 mL of 1x TAE buffer ((0.5 mM EDTA [pH 8.0] and 20 mM Tris-acetate) was heated until an obvious, unambiguous solution was obtained. Cool it down about 60C, add 0.5ug ethidium bromide. Then melted gel was poured into a flatbed gel tray and wells were made by placing appropriate comb. After removing the comb carefully gel was placed into the electrophoresis tank having enough 1X TAE buffer just to cover the gel. DNA was mixed with 1/6 volume (v/v) of 6x loading dye (0.4% xylene cyanol FF, 25% ficoll and 0.4% bromophenol blue] 400). DNA samples along with DNA size marker (vivantis, 1kb) were loaded in the congealed gel flooded in 1x TAE buffer. Electrophoresis was taken out at 60 volt until the necessary DNA partition was attained. Gel within the DNA bands was visualized using short waves ultraviolet rays providing by trans-illuminator (UVP, upland CA91786 USA, Model LMS-26E).

2.5. Ligation

PCR amplified product was cloned by using an InsTAclone PCR cloning kit (Fermentas) according to the information provided by the manufacturer.

2.6. Preparation of competent Escherichia coli (DH5 α) competent cells

The *Escherichia coli* cells (DH5α strains) were made competent by Cohen et al. (1974)process with minor modification. Single colony was picked from freshly streaked LB agar plate and inoculated into 5 mL of LB broth. The broth was placed at shaking incubator at 37 °C at 140 rpm (14-16 hrs). Next day 1 mL of the incubated broth was inoculated into 200 mL flask having 50 mL of LB medium and placed in shaking incubator at 37 °C for 2 hours. After 2 hours the cells were then pelleted by centrifugation at 6000 rpm for 5 mints at 4 °C. The supernatant was discarded and the pellet was dissolved in 10-15 mL of 0.1M $CaCl_2$ solution and kept on ice for 40 minutes. The cells were centrifuged again and pellet was

resuspended in 1 mL of 0.1M $CaCl_2$ solution and stored at 4 °C until needed (not more than 36 hours).

2.7. Transformation of competent E.Coli cells (DH 5α)

Following standard procedure (Sambrook and Russel, 2001) competent *E.Coli* cells were transformed. Total volume of 130ul was prepared using 100 ul of competent cells and 30ul of ligation mixture in a 1.5 mL eppendorf tube. After mixing gently left it on ice for 40 min. The cells were heat shocked at 42 °C in a heat block for 90sec. and kept on ice for further 2 min. 800ul of LB media was added into the mixture, mixed and incubated at 37 °C for one hour. After one hour the mixture was taken out from the incubator, centrifuged at 14000 rpm for 2 min. The supernatant was discarded in a way that 200ul of medium left behind the tube. To resuspende the pelleted cells the tube was tapered gently and spread on to a solid LB medium (1.2% agar, 1% tryptone, 0.5% yeast extract and 1% NaCl) plate containing 40 µL IPTG isopropyl -α-D-thiogalactopyranoside (24 mg/mL), 40 µL X-Gal 5-bromo-4-choloro-3-indolyl-β- D-galactopyranoside (50 mg/mL) and 100µg/mL ampicillin for PTZ57R/T. The plate was incubated at 37°C for 16 hours. Positive colonies were identified by blue- white-selection next day.

2.8. Culturing and plasmid isolation

A single positive colony was picked by using sterile loop and inoculated into a test tube containing 5 mL LB broth with appropriate antibiotic. The test tube was placed in shaking incubator at 37°C for overnight. Next day the culture was decanted into 1.5 mL microfuge tube and centrifuged at 14000 rpm for 10 min. The supernatant was discarded and pellet made as dry as possible by using blotting paper. The pellet was again dissolved into 150ul Resuspension solution (50 mM glucose, 0.45g/50 mL, 10mM EDTA 186g/50 mL pH 8.0 25mM tris.Hcl, 0.151 g/50 mL pH 8.0). 200 uL lysis solution (0.2 N NAOH, 8 g/20 mL and 1% SDS, 20 uL) (1% [w/v] SDS, 0.2 M NaOH) and mixed gently. After adding and mixing thoroughly 200 µL Neutralization solution (3.0 M potassium acetate [pH 5.5]), centrifuged at 1400 rpm for 10 minutes in a microfuge. The supernatant was shifted into new microfuge tube and two volumes of absolute chilled ethanol was added into the tubes and again centrifuged at 14000 rpm for 15 min to pellet out the DNA. The pellet was washed with 150 uL 70% ethanol and centrifuged at 14000 rpm for 3 minutes after discarding the supernatant. After removing the supernatant carefully by micropipette the tubes having plasmid pellet was dry by keeping in an oven and pellet was dissolved in 30-50 uL water according to the size of pellet and kept at -20 °C.

2.9. Restriction digestion

The clones in plasmid vectors was confirmed by double digestion with specific restriction endonucleases *EcoR1* and *Pst1* with compatible buffer having optimal activities for both enzymes. For digestion 10 µl reaction mixture contained 10 units of *EcoR1* (0.5 µl) and 10 units of *Pst1* (0.5 µL) 3 ul plasmid in 10X buffer V3 (1 uL) and 5 µL of sterile distilled water. The reaction mixture was kept at 37 °C for 1 hr. the samples were electrophoresed on 1% agarose gel and were examined under UV light after staining with ethidium bromide.

2.10. Plasmid isolations purification

Confirmed positive clones after restriction digestion experiment were taken to plasmid isolation by using Quiagen Kit (QIAprep Spin). Overnight grown culture (1.5 mL) was got and centrifuged at 14000 rpm for 3 min and discarded the supernatant. Repeated this step again for the healthy pellet. 250uL of buffer P1 was used to resuspended the pellet by vortex. 250uL of Solution P2 was added and mixed by inverting the tubes. Then centrifuged for 10 min at 14000 rpm after adding 350ul of N3 solution. The supernatant was shifted in labeled column and centrifuged for 1 min at 14000 rpm. Discarded the supernatant 700 uL wash buffer was added and centrifuged at 14000 rpm for 1 min to remove the traces of impurities. Supernatant discarded and transfered the column in new labeled eppendrof. For DNA elution 50uL elution buffer was added at the centre point in such a way that tip did not touch the column. Tubes were centrifuged at 14000 rpm for 1 min after staying for 2 min at room temperature.

3. Results

Samples of whitefly were collected from different areas of Punjab (Figure 1 and Table 1). Total DNA was extracted from each sample of single whitefly. For whitefly biotypes analysis through PCR, primers C1-j-21955' TTGATTTTTTGGTCATCCAGAAGT3' and L2-N-30104 5' TCCAATGCACTAATCTGC CATATTA 3' (Frohlich et al., 1999) were used which are designed on cytochrome oxidase subunit1.These primers produced approximately 850bp product (Figure 2 and 3). Amplified products were cloned in a cloning vector PTZ57R/T and confirmed by restriction analysis. Selected clones (RI-1,RI-3,RI-4,RI-6,RI-7,RI-8,RI-9,RI-10and RI-11) were sent to Macrogen, Korea for sequencing. Sequencing results were subjected to BLAST at NCBI web page and analysis showed that all sequences are partial DNA of cytochrome oxidase subunit 1gene of whitefly *Bemisia tabaci*. Comparison conducted with worldwide sequences taken from the Bemisia tabaci database showed that clone RI-1(HG792861) had maximum percentage identity to AJ867557-China (100%). Likewise RI-3 (HG792861), RI-4 (HG792862), RI-8(HE862289), RI-9 (HG792863) RI-10 (HG792864) and RI-11 (HG792865) had maximum percentage identity to HM590140-India and HM590142-India (99.8%). RI-6(HG792866) had maximum percentage identity to HM590140-India (99.9%). RI-7 had maximum percentage identity to HM590140-India and HM590142-India (99.6%).Although sequences of clones obtained in this study had between 98.8% to 99.9% identity among themselves (Figures 4, 5).

The phylogenetic relations between the whitefly populations gathered from the Punjab province of Pakistan, as well as amongst few descriptive sequences for whitefly downloaded from GenBank. On the basis of their partial cytochrome oxidase subunit 1 sequences, the whitefly populations were categorized into various groups and each group is representing distinct genetic group such

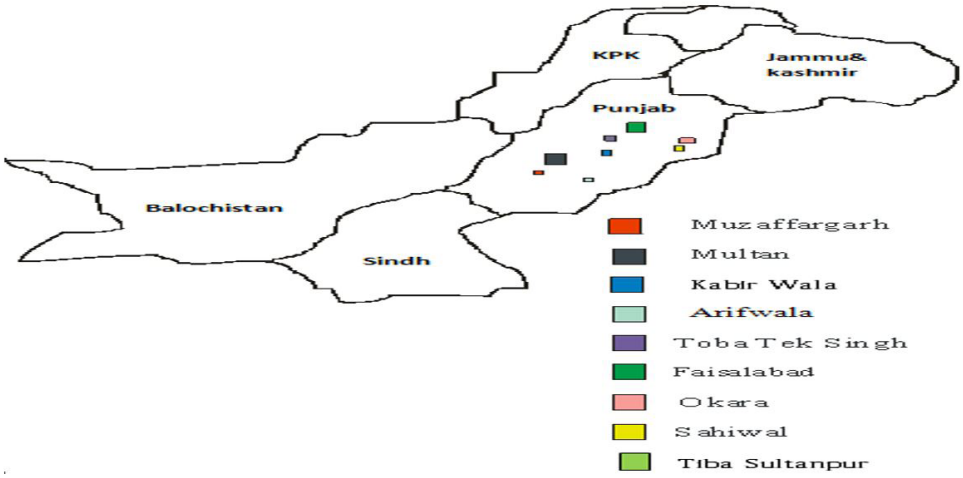

Figure 1. Pakistan's map representing regions (colored boxes) from where sampling of whiteflies was done.

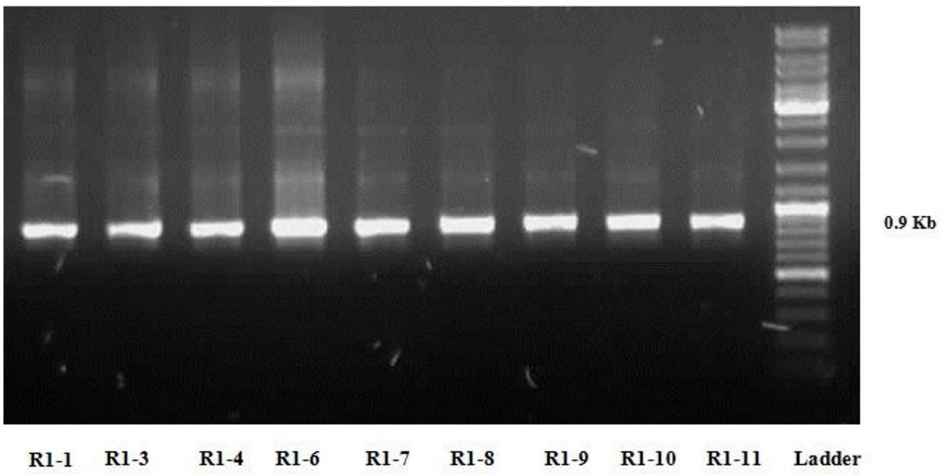

Figure 2. PCR amplified product (RI-1,RI-3,RI-4,RI-6, RI-7,RI-8,RI-9, RI-10 and RI-11) of whitefly's mitochondrial DNA's partial cytochrome oxidase gene 1 . The product size is approx. 0.9 kb which was compared with 1 kb DNA ladder.

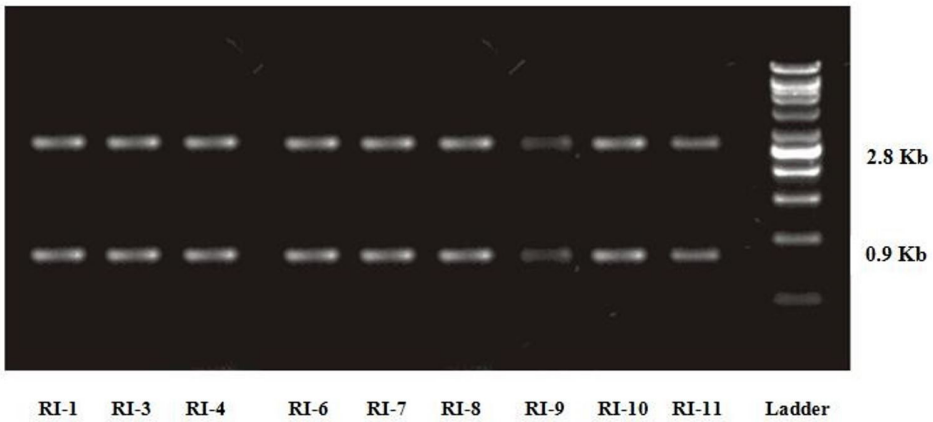

Figure 3. Digestion of mtCO1 inserts pTZ57R/T plasmid. On the right panel indicated the size of PCR products. Size 0.9kb that was compared with 1 kb DNA ladder (Ladder).

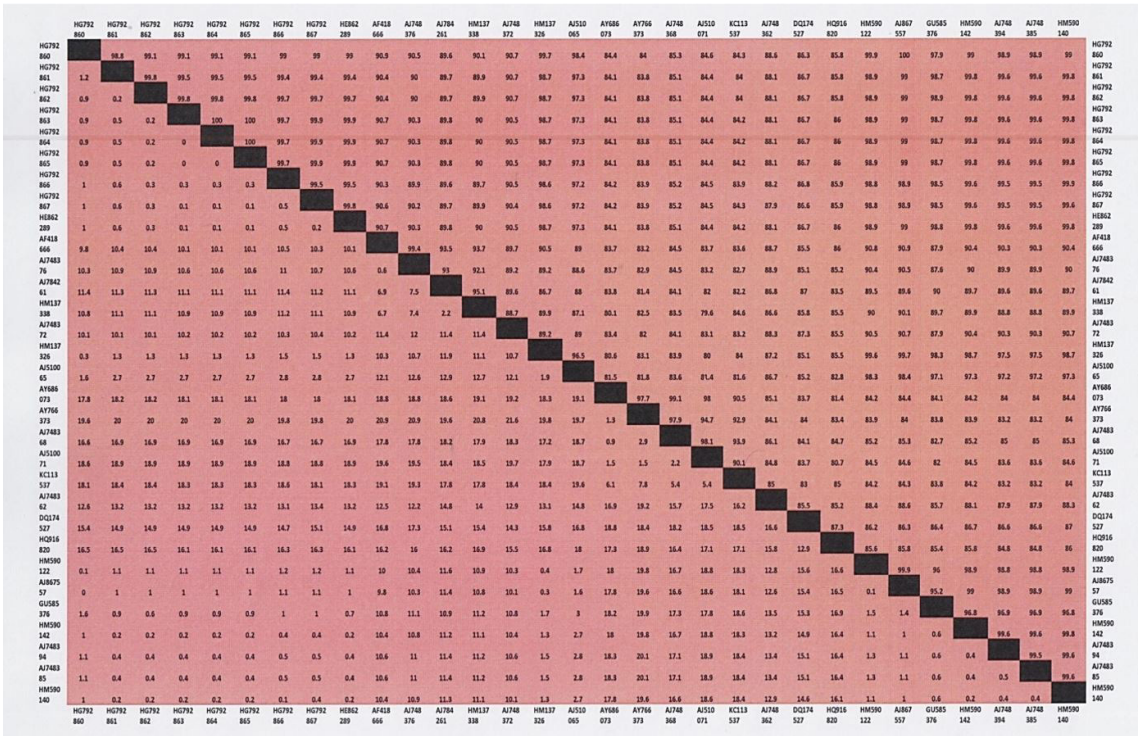

Figure 4. Percentage homology of sequences of *b. tabaci* with NCBI sequences.

as MEAM1,MED, Asia III, China 4* and Asia II. Sequences collected in this study were clustered into Asia II unresolved group. Clade representing Asia II is further divided into two sub-clades. One sub-clade contains sequences from Taiwan, India, and Nauru and second sub-clade contains sequences from India, China, Nepal and Bangladesh. Sequences of this study are associated with second sub-clade.

4. Discussion

For greater than 20 years, *B. tabaci* recorded as a main insect of horticultural and agricultural crops in numerous states together with Pakistan (Qiu et al., 2008). It was considered that Pakistan perhaps is one of the hubs of the beginning of this pest, but worldwide genetically variable records proposed that it is more possible that Africa is the center from where *Bemisia* originated. That is why this study unifies our information of the variety and distribution of *B. tabaci* across Pakistan and gives the data concerning the haplotype organization of the *B. tabaci* complex in Pakistan's various areas. Two biotypes, Q and B, are worldwide dispersed (Martinez-Carrillo and Brown, 2007) amongst the 12 genetic groups of *B. tabaci* described by Boykin et al. (2007). The results achieved from the present study, although, show that all the *B. tabaci* populaces collected from the Pakistan's Punjab Province related to Asia II genetic group (an unresolved biotype) explained in Boykin et al. (2007). None of these biotypes, B and Q discovered in the samples of *B. tabaci* which were gathered from Pakistan. This suggests that B and Q biotype does not exist in Pakistan. The patterns of distribution of indigenous *B. tabaci* across Pakistan in the present study suggest that most of the diversity is associated with the Nepal, India and Bangladesh. In Pakistan's economy agriculture plays an important role. It constitutes almost 25% of GDP and recriuts approximately 1/2 of human resources of the country. Cotton is an essential crop and an important contributor to the nationwide economy. CLCuD is only spreaded by *B. tabaci* that is a big danger to cotton yield. To the great extend cotton leaf curl disease (CLCuD) infections related to a new biotype of *B. tabaci* had been discovered in the previous researches. While the current study revealed the presences of this new haplotype in Punjab. It is also found that it is an alleged species within *B. tabaci* species complex renowned as Asia II. It is present in somewhere else within various distinct haplotype names. It also has been indicated that the occurrence of CLCuD is effectively linked with the abundance of the vector and which species of vector exits. In Punjab, higher profusion of Asia II represents the higher frequency of CLCuD.

To know about the genetic polymorphism of *B. tabaci*, molecular markers have been used and mitochondrial cytochrome oxidase 1 gene (mtCO1) is the most broadly used part of DNA for examining the genetic makeup of *B. tabaci*. Furthermore, especially *B. tabaci*, recognition is centered entirely upon the application of genetic markers, while all other suggested methods be lacking in some type of solid quantification or stability of severance. Previously, phylogenetic investigations had been utilized to attempt to allocate individuals to specific clades. Although, this method has been challenging, because no definite set of

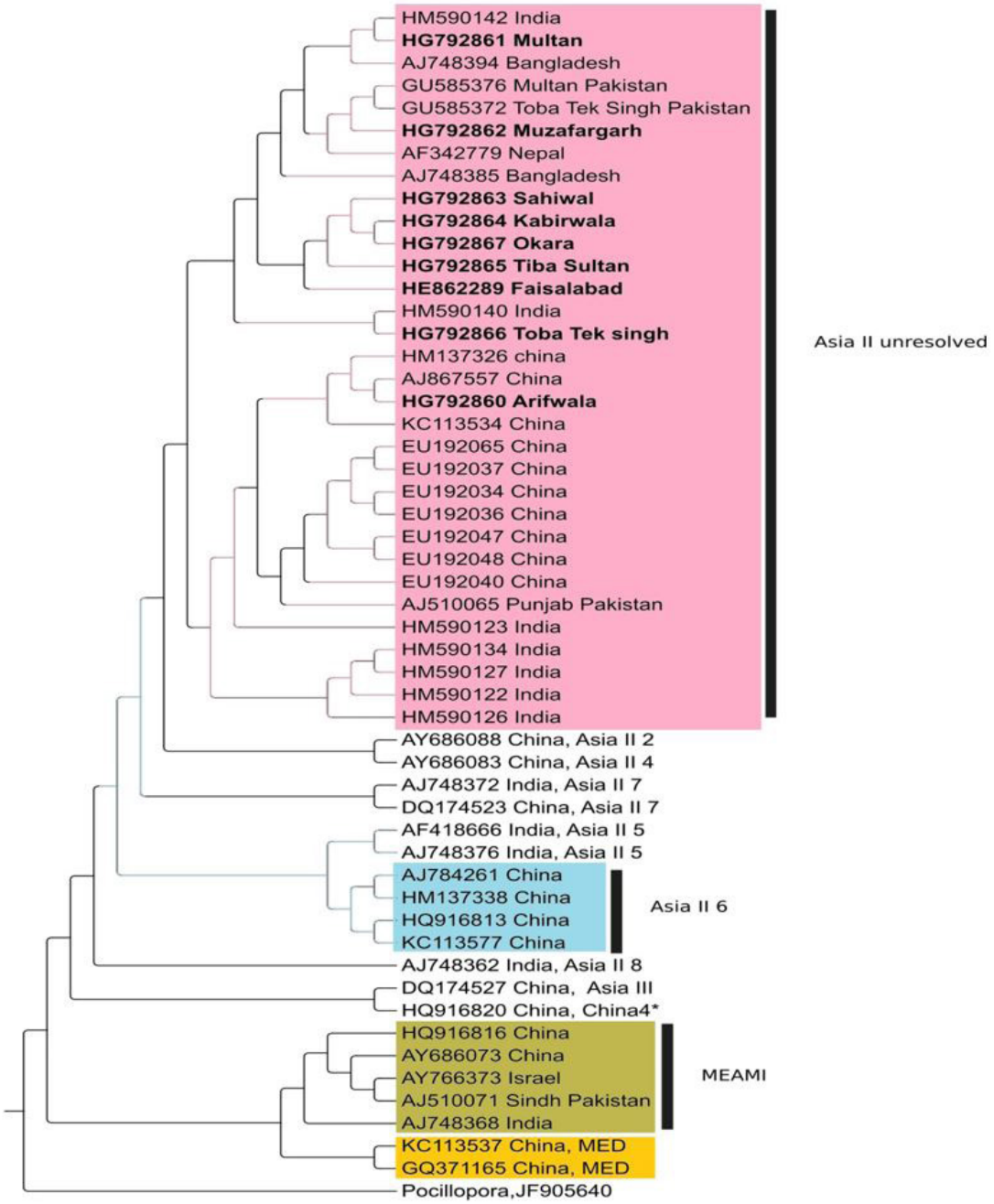

Figure 5. Phylogenetic analysis on the basis of partial sequences of cytochrome oxidase gene obtained in this study (in bold font) and extracted from NCBI databank. Perpendicular branches are arbitrary, parallel branches are proportional to calculated mutation distances. Bootstrap value is 1000 replicates.

rule has been built-up to give direction as to what the limits are for any group. As a result, task has often been random and not consistent, and frequently influenced by the supplement of biotypes selected for the analysis. However, a method proposed by Dinsdale et al. (2010) puts few means to conquer this by giving a definite set of rule to identify genetic limits. Furthermore, utilization of concord sequences organized on the basis of the utilization of the definite set of rule ensuing that through a broad sample of the renowned variety makes task of recognition faster, arguably and simply extra precise (Dinsdale et al., 2010). An outstanding example of this is the resolution of the molecular identification of the vector of CLCuV in Punjab's cotton.

Bedford et al. (1994), for the first time reported *B. tabaci* from Pakistan, who applied esterase to differentiate various haplotypes of *B. tabaci*. The analysis of these haplotypes shows that haplotypes K and P are from Pakistan and Nepal respectively. While De Barro and Bourne (2010) demonstrated by utilizing the ITS1 region of ribosomes, that the whiteflies recognized as K and P by Bedford et al. (1994) were extremely densely linked. In a subsequent study, *B. tabaci* of Pakistan divided into three groups: Mediterranean-African, Indian and South-east Asian, utilizing RAPD-PCR and mtCO1 by Simón et al. (2003). Although, De Barro et al. (2006) once more applying ITS1 region of ribosomes, allocated the two K and P to an unresolved group of *B. tabaci* that, formerly mentioned as Indian Subcontinent on the basis of mtCO1 (Brown, 2001) China, Pakistan and Thailand cluster'. Ahmed et al. (2011)described that the great occurrence of infestation of cotton in Punjab was because of new biotype of *B. tabaci*, by again using Mitochondrial CO1 and named it PK1.While the current study suggests that it is H, P and K biotype which exists in Punjab province. Generally moderate to broad host ranges of the whitefly vector have recorded, while the viruses transmitted by them are characterized by moderate to narrow host abilities. This deficient in lucidity and reliability about recognition of *B. tabaci* has debatably mystified current knowledge of aspects behind the outburst.

The phylogenetic study of members of Middle East-Asia of Pakistani represents that it relats to a distinctive subclade made up of biotypes from India, Bangladesh, China and Nepal. Howeve the phylogenetic rebuilding shows that they occure earlier from those which compose this subclade. This proposes that major part of the habitat of this species is constructed by Pakistan, that is constant with the deduction of Simón et al. (2007). Furthermore the formation of distinctive subgroup from the haplotypes of Pakistan along with Middle East-Asia and that these biotypes have yet to disperse out of this area is interesting. This is alluring to consider that they might be short of some of the biologic character connected with enveloping biotypes of this species, but it is ill-timed and needs further meticulous analysis.

DNA sequencing of amplified part of the mtCO1, are the most definitive and informative approach, producing constructive phylogeographical information concerning the existing origin of particular biotypes (and haplotypes). Resultant mtCO1 sequence estimations through a variety of reference of published and unpublished records, allows distinctions not only among haplotypes of *B. tabaci*, however also creates promising finding of other whitefly species that inhabit the similar horde and might unintentionally be accumulated within *B. tabaci*. Yet, the capability to differentiate general biological kinds does not have expectant the formation of quarantines versus the obstinate *B. tabaci* variations, as the identification on the basis of morphology persist the most realistic source for common recognition of insects at harbors of entrance. In fact, a main insufficiency for progress the biotype notion is the lack of biological records obtainable for increasing amount of distinct genetic variations. Here is also an essential thing that is to collect a long term data about plant and whitefly samples on a regular basis for a number of years and analyze them for virus population in whitefly, biotypes of whitefly, and evolution of new species/strains (Colvin et al., 2006). Though, for correct recognition of virus vectors and insect's pests is a requirement for their helpful control activities. Mitochondrial cytochrome oxidase I (mtCO1) gene based analysis of the widespread compilations of the whitefly have significantly enhanced the knowledge of the genetic variation of the *B. tabaci* species complex (Dinsdale et al., 2010). The knowledge obtained from mtCO1 is an important means that can help to prohibit the introduction of exotic *B. tabaci* biotypes. It may be necessary to study genetic of *B. tabaci* in Pakistan using various techniques which are available, such as mitochondrial CO1 sequences, esterase pattern and SSL character in order to obtain more and the most necessary information that can be used to designate groups to *B. tabaci* populations and separate them from other populations. It was suggested that information derived from using multiple techniques is the best for grouping or categorizing biotype for any given population.

5. Conclusion

As this study aimed to identify *B.tabaci* biotypes in the Punjab province of Pakistan. It was found that only population belongs to Asia II unresolved genetic group, which include H, K and P biotypes is present here. There is no existence of B and Q biotype. This study provides the basis for molecular identification of *B. tabaci* based on partial DNA sequence of mitochondrial cytochrome Oxidase gene I (mtCO1) in Pakistan.

6. Future Work

As a vector, whitefly is an important organism for many of viral disease. Studies have shown that changes in whitefly populations have significantly influenced the prevalence of viral diseases. Pakistan, more specifically Punjab, is the hub of many of economically important viruses vectored by whiteflies. Therefore additional geographical and temporal studies of whiteflies are important for good agricultural management.

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, pp. 97-106. http://dx.doi.org/10.1079/BER2003223. PMid:12699530.

AHMED, M.Z., DE BARRO, P.J., GREEFF, J.M., REN, S.-X., NAVEED, M. and QIU, B.-L., 2011. Genet identity of the *Bemisia tabaci* species complex and association with high cotton leaf curl disease.*Pest Management Science*, vol. 67, no. 3, pp. 307-317. http://dx.doi.org/10.1002/ps.2067. PMid:21308956.

ALI, A. and AHEER, G.M., 2007. Varietal resistance against sucking insect pests of cotton under Bahawalpur ecological conditions. *Journal of Agricultural Research*, vol. 45, no. 3, pp. 1-5.

ASLAM, M., GILANI, A., SATTAR, M., SARWAR, M., RAHOO, G.M. and RIZVI, S.N.H., 2000. Resistance of different cotton varieties to cotton leaf curl virus under field conditions.*Journal of Research (Science)*, vol. 11, pp. 42-45.

BANKS, G.K., COLVIN, J., REDDY, R.V.C., MARUTHI, M.N., MUNIYAPPA, V., VENKATESH, H.M., KUMAR, M.K., PADMAJA, A.S., BEITIA, F.J. and SEAL, S.E., 2001. First report of the Bemisia tabaci B biotype in India and an associated Tomato leaf curl virus disease epidemic.*Plant Disease*, vol. 85, no. 2, pp. 231. http://dx.doi.org/10.1094/PDIS.2001.85.2.231C. PMid:30831961.

BEDFORD, I., BRIDDON, R.W., BROWN, J.K., ROSELL, R.C. and MARKHAM, P.G., 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from di erent geographic regions.*Annals of Applied Biology*, vol. 125, no. 2, pp. 311-325. http://dx.doi.org/10.1111/j.1744-7348.1994.tb04972.x.

BOYKIN, L.M., SHATTERS JUNIOR, R.G., ROSELL, R.C., MCKENZIE, C.L., BAGNALL, R.A., DE BARRO, P. and FROHLICH, D.R., 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences.*Molecular Phylogenetics and Evolution*, vol. 44, no. 3, pp. 1306-1319. http://dx.doi.org/10.1016/j.ympev.2007.04.020. PMid:17627853.

BROWN, J.K., 2001. Molecular markers for the identification and global tracking of whitefly vector/begomovirus complexes.*Virus Research*, vol. 71, no. 1-2, pp. 233-260. http://dx.doi.org/10.1016/S0168-1702(00)00221-5. PMid:11137175.

BROWN, J.K., FAUQUET, C.M., BRIDDON, R.W., ZERBINI, M., MORIONES, E. and NAVAS-CASTILLO, J., 2012. Geminiviridae. In: A.M.Q.KING, M.J.ADAMS, E.B.CARSTENS and E.J.LEFKOWITZ, eds. *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses.Amsterdam*: Elsevier, pp. 351-373.

BYRNE, D.N. and BELLOWS JUNIOR, T.J.R., 1991. Whitefly biology. *Annual Review of Entomology*, vol. 36, no. 1, pp. 431-457. http://dx.doi.org/10.1146/annurev.en.36.010191.002243.

CAB INTERNATIONAL – CABI. (2001) *Crop Protection Compendium: global module*.3rd ed. Wallingford: CAB International. CD-Rom Database.

COHEN,S.N., 1974.Transformation of *Escherichia coli* by Recombinant Plasmid Replicons Constructed *in Vitro*. In: R.F.Grell, ed.*Mechanisms in Recombination.Boston, MA*: Springer. https://doi.org/10.1007/978-1-4684-2133-0_15.

COLVIN, J., OMONGO, C.A., GOVINDAPPA, M.R., STEVENSON, P.C., MARUTHI, M.N., GIBSON, G., SEAL, S.E. and MUNIYAPPA, V., 2006. Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications.*Advances in Virus Research*, vol. 67, no. 6, pp. 419-452. http://dx.doi.org/10.1016/S0065-3527(06)67011-5. PMid:17027686.

DE BARRO, P.J., 1995. *Bemisia tabaci* biotype B, a review of its biology, distribution and control.*CSIRO Division Entomology Technical Paper*, vol. 36, pp. 1-58.

DE BARRO, P.J. and BOURNE, A., 2010. Oviposition host choice by an invader accelerates displacement of its indigenous competitor. *Biological Invasions*, vol. 12, no. 9, pp. 3013-3023. http://dx.doi.org/10.1007/s10530-010-9691-1.

DE BARRO, P.J., BOURNE, A., KHAN, S.A. and BRANCATINI, V.A.L., 2006. Host plant and biotype density interactions their role in the establishment of the invasive B biotype of Bemisia tabaci. *Biological Invasions*, vol. 8, no. 2, pp. 287-294. http://dx.doi.org/10.1007/s10530-005-1261-6.

DINSDALE, A., COOK, L., RIGINOS, C., BUCKLEY, Y.M. and BARRO, P., 2010. Refined global analysis of Bemisia tabaci (Hemiptera: Sternorrhyncha: Aleyrodoiea: Aleyrodidae) mitochondrial cytochrome oxidase I to identify species level genetic boundaries. *Annals of the Entomological Society of America*, vol. 103, no. 2, pp. 196-208. http://dx.doi.org/10.1603/AN09061.

FROHLICH, D.R., TORRES-JEREZ, I.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, pp. 1683-1691. http://dx.doi.org/10.1046/j.1365-294x.1999.00754.x.PMid:10583831.

GENNADIUS, P., 1889. Disease of tobacco plantations in the Trikonia. The aleyrodide of tobacco.*Ellenike Georgia*, vol. 5, pp. 1-13.

GILL, R.J., 1990. The morphology of whiteflies. In: D.GERLING, ed. *Whiteflies, their bionomics, pest status and management*.Andover: Intercept, pp. 13-46.

KIRK, A.A., LACEY, L.A., BROWN, J.K., CIOMPERLIK, M.A., GOOLSBY, J.A., VACEK, D.C., WENDEL, L.E. and NAPOMPETH, B., 2000. Variation in the Bemisia tabacis.l. species complex (Hemiptera: Aleyrodidae) and its natural enemies leading to successful biolo- gical control of Bemisia biotype B in the USA.*Bulletin of Entomological Research*, vol. 90, no. 4, pp. 317-327. http://dx.doi.org/10.1017/S0007485300000444. PMid:11020790.

MARTIN, J.H., 1999. *The Whitefly Fauna of Australia (Sternorrhyncha: Aleyrodidae): a taxonomic account and Identification guide. Canberra*: CSIRO. CSIRO Entomology Technical Paper, no. 38.

MARTINEZ-CARRILLO, J.L. and BROWN, J.K., 2007. First report of the Q biotype of *Bemisia tabaci*in southern Sonora, Mexico. *Phytoparasitica*, vol. 35, no. 3, pp. 282-284. http://dx.doi.org/10.1007/BF02981162.

MOUND, L.A. and HALSEY, S.H., 1978. *Whitefly of the world, a systemic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data*.Chichester: British Museum (Natural History).

QIU, B.L., MANDOUR, N.S., XU, C.X. and REN, S.X., 2008. Evaluation of entomopathogenic nematode, Steinernemafeltiae as a biological control agent against the whitefly *Bemisia tabaci. Journal of Insect Pest Management*, vol. 54, pp. 247-253.

SAMBROOK, J., andRUSSELL, D.W., 2001. *Molecular cloning: A laboratory manual.NY*: Cold Spring Harbor Laboratory Press.

SIMÓN, B., CENIS, J.L. and DE LA RUA, P., 2007. Distribution patterns of the Q and B biotypes of *Bemisia tabaci* in the Mediterranean Basin based on microsatellite variation. *Entomologia Experimentalis et Applicata*, vol. 124, no. 3, pp. 327-336. http://dx.doi.org/10.1111/j.1570-7458.2007.00586.x.

SIMÓN, B., CENIS, J.L., BEITIA, F., KHALID, S., MORENO, I.M., FRAILE, A. and GARCÍA-ARENAL, F., 2003. Genetic structure of field populations of begomoviruses and of their vector *Bemisia tabaci* in Pakistan.*Phytopathology*, vol. 93, no. 11, pp. 1422-1429. http://dx.doi.org/10.1094/PHYTO.2003.93.11.1422. PMid:18944071.