AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/aeb.html

Genetic diversity of *Bemisiatabaci* in Pakistan

[1]Sadiqullahkhan, [1]Mushtaq Ahmad, [2]Nawshad Muhammad, [1]Rahmat Ali Khan, [3]Saad Ullahkhan, [4]Ayesha Yousuf

[1]Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan
[2]Interdisciplinary Research Center in Biomedical Materials, COMSATS Institute of Information Technology, Lahore, Pakistan
[3]Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Khyber Pakhtunkhwa, Pakistan
[4]Institute of Biotechnology Bahauddin Zakariya University Multan, Punjab, Pakistan

ARTICLE INFO

Article history:
Received 23 January 2014
Received in revised form 19 April 2014
Accepted 6 April 2014
Available online 15 May 2014

Key words:
Bemisiatabaci, viruses transmission, biotype, B and Q

ABSTRACT

Bemisiatabaci (*hemipteraaleyrodidae*) belongs to a broad group of whiteflies insects that consists of 1500 species. *Bemisiatabaci*is a species complex and consists of 24 well known biotypes. These biotypes have differences in many aspects like biochemical and genetic aspects. The capacity to transmit the plant viruses has made the *Bemisia tabaci* an important economic threat insect. The most important virus transmitted by *Bemisia tabaci* is cotton leaf curl virus that causes the cotton leaf curl disease of cotton. Similarly many viruses causing diseases of vegetables are also carried by *Bemisiatabaci*.In this study, *Bemisiatabaci* population collected in various areas of Pakistan has been characterized on molecular basis using mitochondrial DNA cytochrome oxidase I marker. The results obtained from the locus mtCOI amplification in different samples shows that the elecropherogram of insects belonging to biotype B displays a band of 661 and those of insects belonging to the biotype Q shows a band of 400bp. The sample from different locations shows that the population of *Bemisia tabaci* is mixture of B and Q.

To Cite This Article: Sadiqullahkhan, Mushtaq Ahmad, Nawshad Muhammad, Rahmat Ali Khan, Saad Ullahkhan, Ayesha Yousuf., Genetic diversity of Bemisiatabaciin Pakistan. ***Adv. Environ. Biol., 8(5),*** *1339-1344, 2014*

INTRODUCTION

Bemisia tabaci (whitefly) is one of the most serious attacking insect of several field and greenhouse crops in the world [1, 2]. Both the larvae and adult whitefly feeds on the phloem sap of many plant hosts. During feeding they cause the excretion of honeydew, falling on leaves and fruits. This honeydew serves as suitable growth medium for fungi. The fungi then covering the plant area, hinders the photosynthesis of plants and also affects the yield and quality of fruits. It also contaminates the cotton lint and decreases the value of cotton. Many important plant viruses (111 plant viruses) are transmitted by *Bemisia tabaci* [3].One of the most important viruses is cotton leaf curl virus (CLCuV) that causes cotton leaf curl disease [4,5]. CLCuD was reported in Pakistan during 1960s that remained a minor but sporadic problem in following twenty years [6].

During 1998,CLCuD destroyed 60-ha area near Multan region, that was a signal for beginning of CLCuD in Pakistan. The economic loss was estimated to US$ 5 billion in 1992-1997[7]. Cotton (Gossypiumhirsutum) is one of the most important economic crops in Pakistan. Besides textile industries cotton is an important raw material in many food processing industries in Pakistan. Due to huge export demand for cotton is increasing in Pakistan. Similarly many regions of this country are suitable for vegetable production.

The whitefly has broad host range as 600 hundreds species,so called as broadly polyphagous in nature [1, 8-10]. This host includes ornamentals, vegetables and fibrous species. Among these plants, cotton is a plant of economic priority worldwide and especially in Pakistan as it is a third largest cotton exporting country internationally (US Foreign agriculture Service, 2008). In production it is fourth largest producer worldwide. Farmers that grows ranges to about 26%, representing 15% cultivated lands in country. About 55% foreign exchange is earned by cotton.

There are 41 biotypes of *Bemisia tabaci* so called species complex based on allozymeand molecular data[3, 11-13].Biotype is a morphologically unidentifiable but molecularly distinct group of Bemisia tabaci. The biotypes differs in various aspects of biotic and abiotic factors including ability to induce phototoxic disorders, plant virus transmission capability, host specialization, resistance to insecticides, geographic distribution range,

Corresponding Author: Dr. Nawshad Muhammad, Assistant Professor Interdisciplinary Research Center in Biomedical Materials, COMSATS Institute of Information Technology, Lahore, Pakistan
E-mail: nawshadchemist@yahoo.com

degree of fecundity, host range etc.Many phylogenetic (Bayesian) techniques have been used to elucidate genetic basis for *Bemisia tabaci* species complex. Out of 41, 24have been molecularly identified and 17 in remaining unlabelled up to now. Molecular markers like mt cytochrome oxidase I, internally transcribed spacer I sequences, microsatellites markers and 18s ribosomal (nuclear) and 16s ribosomal (mitochondrial) supports the biotype identification.

The lack of ecological and behavioral data seriously hampers the development of non-chemical methods for whitefly management.The effective and sustainable control strategy can be designed only when whitefly biotypes are clarified and the interaction between whiteflies, host plants and natural enemies are thoroughly studied. This study aims to differentiate *Bemisia tabaci* populations in Pakistan especially in cotton growing regions and to determine genetic diversity based on mtCOI markers.

MATERIALS AND METHODS

Whitefly collection:

The study was conducted in agriculturally cultivated area, containing vegetables and cotton of Pakistan. The survey covered a wide area distributed in three provinces (Punjab, Khyber Pakhtunkhwa, and Sindh) and North Waziristan Agency of Pakistan. The*Bemisia tabaci* were collected through power aspirator and some time by using handmade aspirator through sucking. In the field collection were made from random sites and plants. These samples were carefully placed in the test tubes consisting 100% ethanol. The test tubes consisting of samples were carefully labeled and were stored at $5C^{o.}$

DNA extraction:

The DNA extraction was carried out according to that of described by De Barro anddriver[14]. The*Bemisia tabaci* weretaken out from ethanol, washed with distilled waterand allowed to dry on filter paper for few seconds. They were crushed (homogenized) using micropistle in 5µl of extraction buffer in 0.2ml Eppendroftube. The DNA extraction buffer consists of 5µl of TrisHCl (800µl),1µl of EDTA(0.5 M, pH8), 1% SDS (10%), 789µl of SDDW and 5µl of proteinase K (1mg/ml). After crushing is completed, 15µl of extraction is again added so as total summing up to 20µl. The homogenate wasagitated for few seconds, incubated at $54C^0$ for 15 min, at $95C^o$ for 10 min. Finally the homogenate containing crude mixture was centrifuged at 14000 rpm for one minute at room temperature and finally the pellets were removed and supernatant contained DNA of *Bemisia tabaci*was then collected and stored at $4C^0$ for further use in PCR analysis.

PCR Reaction:

A portion of mtCOI was amplified with a polymerase chain reactionusing primers, BaQ F (5′GAAGCAACGACTACTTACAA-3′**) ,** BaQ R (5′TTCTCGGCGTTTTTACCAA-3′) and BaB F (5′-CCACTATAATTATTGCTGTTCCCACA-3′),L2N-3014R(5′TCCAATGCACTAATCTGCCCATCTGCCCATATTA-3′).The PCR was performed using 5µl of DNA extract in 25µl of reaction mixture containing 4.5 µl of distilled water, 12.5 µl of Taqmix (buffer, $Mgcl_2$, dNTPs, TaqDNA polymerase)(MBI Fermentas, Life Sciences,York, UK), 1.5 µl each of forward and reverse primer and 5µl of DNA.The reaction mixture was vortexed and centrifuged for few seconds for thorough mixing. The reaction mixture was taken through thermo cycling conditions consisting: 5 minutes of 95°C for template DNA denaturation followed by 30 cycles of amplification each consisting of 3 steps: one minute at 95°C for DNA denaturation into single strands;1minute at 56-60°C for markers to hybridize or "anneal" to their complementary sequences on either side of the target sequence: and one minute at 72°C for extension of complementary DNA strands from each primer, final 10 minutes at 72°C for Taq DNA polymerase to synthesize any unextended strands left. PCR was carried out in T3 thermocyclers (Biometra, Gottingen, Germany).

Electrophoresis Products:

Amplified PCR products of DNA samples were analyzed on 1% agarose gel prepared by melting 0.5g of agarose in 50 ml 1 X TBE (0.89 M Tris-Borate, 0.032 M EDTA, pH 8.3) in a microwave oven for two minutes. 5 µl of ethidium bromide (0.5 µg/ml final concentrations) was added to stain DNA.

Five µl of PCR samples were mixed with loading dye (0.25% bromophenol blue with 40% sucrose) and loaded into the wells. Electrophoresis was performed at 110 volts (80 mA) for one hour in 1 X TBE buffer. Amplified products were visualized by placing the gel on UV transilluminator (Biometra, Gottingen, Germany).

Results:

The results obtained from the locus mt COI amplification in different samples shows that the elecropherogram of insects belonging to biotype B displays a band of 661 and those of insects belonging to the biotype Q shows a band of 400bp (Fig. 1). The sample from different locationsshows that the population of *Bemisia tabaci* is mixture of B and Q.

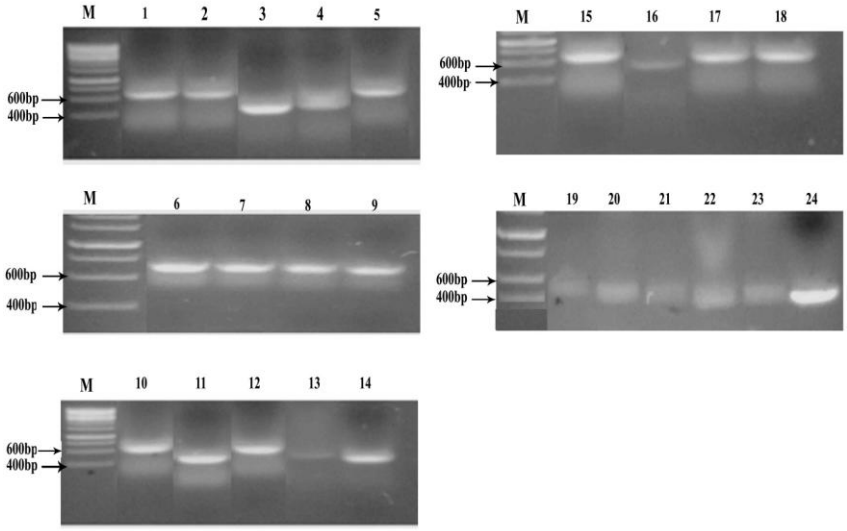

Fig. 1: Agarose Gel Electrophoresis of PCR product of Bemisia tabaci biotypes. Q type marker has 400bp product while B type has 661bp amplified product. M represent a molecular weight marker (I Kb DNA ladder). Lane 1-18 represent different regions of Punjab province showing mixture of Q & B biotypes including, Lane 1: Kabirwala, B biotype; Lane 2: Faisalabad, B biotype; Lane 3, Tobatake Singh, Q biotype; Lane 4: Gojra, Q biotype; Lane 5: Jhang, B biotype;Lane 6: Khanewall, B biotype; Lane 7: Shujaabad, B biotype: Lane 8: Multan, B biotype; Lane 9: Jalalpur, B biotype; Lane 10, Melsi, B biotype; Lane 11Ahmadpur, Q biotype; Lane 12Rahimyar khan, B biotype; Lane 13:Khanpur, Q biotype; Lane 14: Bahawalpur, Q biotype; Lane 15:D.Gkhan, B biotype; Lane 16:Kottaddo, Q biotype; Lane 17:Rajanpure, B biotype; Lane 18:Liaeh, B biotype. Lane 19-24 represent different regions of Khyber Pakhtunkhwa province and North Waziristan Agency showing only Q biotypes including Lane 19: D.I. Khan, Q biotype; Lane 20: Bannu, Q biotype; Lane 21:Naurang, Q biotype; Lane 22:Mirali (NWA), Q biotype; Lane 23: Peshawar (Tarnab form), Q biotype; Lane 24:Miranshah (NWA), Q biotype.

Table: Samples of Bemisiatabaci collected from different areas of Pakistan

S. No.	Location	Host Plant	Biotype	S. No.	Location	Host Plant	Biotype
Punjab Province				26	Silanwali	GossypiumHirsutum	B
1	Khanewal	GossypiumHirsutum	B	27	Abdul Khakeem	GossypiumHirsutum	B
2	Multan (CRC)	GossypiumHirsutum	B	28	Muzzafargarh	GossypiumHirsutum	Q
3	Jhang	GossypiumHirsutum	B	29	Jalalpur	GossypiumHirsutum	B
4	Biopark (BZU)	GossypiumHirsutum	Q	30	Bahawalpur	GossypiumHirsutum	B
5	Mailsi	GossypiumHirsutum	B	31	Multan (BZU fields)	GossypiumHirsutum	B
6	Rahimyar Khan	GossypiumHirsutum	B	32	Samudari	GossypiumHirsutum	B
7	MukhdoomPur	GossypiumHirsutum	B	33	Multan (Nawabpur road)	GossypiumHirsutum	Q
8	Khanpur	GossypiumHirsutum	Q	34	Multan (Band Boson road)	GossypiumHirsutum	Q
9	Kotaddo	GossypiumHirsutum	Q	35	Tobbatak Singh	GossypiumHirsutum	Q
10	Ahmadpur	GossypiumHirsutum	Q	**Khyber Pakhtunkhwa Province**			
11	Bahawalpur	GossypiumHirsutum	Q	36	Bannu (BaistKhel)	Hybiscusesculantus	Q
12	Lieah	GossypiumHirsutum	B	37	Bannu (Township)	Cucurbitopepo	Q
13	Vahshrif	GossypiumHirsutum	Q	38	Naurang (Lakki)	Solonummelogena	Q
14	Alipur	GossypiumHirsutum	Q	39	D.I.Khan (ARI.CC)	GossypiumHirsutum	
15	Sahiwal	GossypiumHirsutum	Q	40	Peshawar (Tarnab farm)	Physiolus vulgaris	Q
16	Rajanpur	GossypiumHirsutum	B	41	Peshawar (Cantt.)	Brassica oleracae	Q
17	Burewala	GossypiumHirsutum	B	**North Waziristan Agency (FATA)**			
18	Kabirwala	GossypiumHirsutum	B	42	Mirali (Haddi)	Hybiscusesculantus	Q
19	Vehari	GossypiumHirsutum	B	43	Mirali (Tappi)	Hybiscusesculantus	Q

20	D.G Khan	GossypiumHirsutum	B	44	Mirali (Edak)	Hybiscusesculantus	Q
21	Khangarh	GossypiumHirsutum	B	45	Miranshah	Hybiscusesculantus	Q
22	Shujabad	GossypiumHirsutum	B	**Sindh Province**			
23	Faisalabad	GossypiumHirsutum	B	46	Nawabshah (Cotton Research Institute Sindh)	GossypiumHirsutum	B
24	Gojra	GossypiumHirsutum	Q	47	Mirpurkhas (Sindh Horticulture Research Institute)	GossypiumHirsutum	B
25	Peermehal		Q				

The results of our study indicate that in Punjab province of Pakistan 60-70% population was B biotype while 40-30% population was that of Q biotype. So a higher level of diversity of Q and B biotype has been observed in cotton growing regions of Punjab. The whitefly samples collected from Khyber Pakhtunkhwa (KPK) province and North Waziristan Agency depicts the unique results in this study. The collection was made from vegetables crops. The analysis of all the samples clearly gives the amplified band of 400bp that is specific to Q biotype. *Bemisia tabaci* collected from Sindh province has B biotype.Table 1 shows the detail results of the study presented here.

Discussion:

Since more than two decades *Bemisia tabaci* has been a serious pest of many agricultural zones of the world including Pakistan [2,13,15,16]. As an agricultural pest *Bemisia tabaci* is threatening the economy of agriculturally dependent countries. The biodiversity of *Bemisia tabaci* variesfor each country. According to global genetic diversity data Africa is the most suitable region for *Bemisia tabaci* populations [17]. There are 24 biotypes of *Bemisia tabaci* but two biotypes(B, Q) are found worldwide[16-18]. The origin of this pest also lies in Pakistan [1]. Previously data are available on the biodiversity of *Bemisia tabaci* in Pakistan but our data completely elaborates the *Bemisia tabaci* population structure.So this study provides the first data to describe the biotypes (species complex) mainly in cotton and vegetable growing areas of Pakistan.

In this study we tried several different types of primer pair but only B biotype and Q biotype specific primers produced amplified bands. The polymerase chain reaction, the molecular technique being as a convenient tool is used to perform the preliminary examinations of whitefly populations. The B and Q biotype specific primers used in this study were designed based on sequences found in populations all over the world [2, 19].

The B and Q biotypes have been introduced recently in Pakistan as none of them were present before 2009[20].But from the results of our study it is clear that the B biotype is found more than 50% in Punjab province of Pakistan. Similarly Q biotype is found upto 50%. This shows many underlying facts. For example Q biotype has high survival ability at high temperature [21]. The insecticide résistance is also very high as compared to B biotype. The insecticides neonicotinoids and pyriproxyfen have been tested against Q biotypes that have showed negative effects on it [22]. So that's why Q biotype is gradually displacing the B[23].Previous data also shows that Q biotype has been more successfully replaced the B biotype and invaded e.g. more than 50% the other *Bemisia tabaci* biotypes like in Southeastern provinces of china. Biotype Q was not found in many of the cotton growing regions of Punjab province like Multan but was found in the collections from Kotaddo etc. The regions where the insecticides are regularly used like BZU were consist of Q biotype as Q biotype is resistant to insecticides.

The samples analyzed in this study may not cover whole regions but it may be concluded from our data that a number of factors is involved for increasing proportion of biotype Q in Pakistan. Additionally human activities like transportation in the country might be the cause of introduction, spread and increasing population of Q biotype in Punjab province of Pakistan. Besides it may also be suggested that the interaction between whitefly species, begomovirus and host plants have role in specific population increase of Bemisia tabaci.Costa *et al.* reported that B biotype (Middle EastAsiaMinor 1) has high survival on Pumpkin that is infected with squash leaf curl virus as compared to uninfected plant[3]. It has been also observed by Legg and Thresh that *Bemisia tabaci* has high fecundity and abundance on cassava that was infectedwith cassava mosaic virus. Also the distribution of the vector and in turn the virus was enhanced by the mutual interaction of the virus and vector.

The molecular analysis based on COI markers, both Q biotype and B biotype have been detected in the populations of *Bemisia tabaci* from Pakistan but with increasing trend of Q biotype as compared to B biotype. For the identification of *Bemisia tabaci* two types of oligonucleotide primers were used (B biotype[661bp] and Q biotype[400bp] specific primers). The *Bemisia tabaci* samples collected from the vegetable hosts from the different localities of KPK and North Waziristan agencies were identified as biotype Q as the give the amplified product of 400bp.

Previous data also shows the coexistence of both biotypes (B& Q) in many regions of the world like Spainand Japan[24, 25]. As natural competition is increasing due to temperature and insecticide use that in turn determines the natural selection of *Bemisia* population in agricultural zones of the world. The biotype A that was previously adapted in USA has been displaced biotype B[2]. In china the fluctuation of B biotype and Q biotype has also been found with increasing trend of Q biotype [26]. In our study the displacement of B biotype by Q biotype has been notified in many districts. Similarly it is clear from the results of this study; it was assumed that partial displacement of biotype B by Q has occurred in many localities of Pakistan. The resistance of Q biotype to insecticides is perhaps the principal factor explaining the distribution of biotype Q in many regions of the world. The same situation has been found in Khyber Pakhtunkhwa where the biotype Q is fully dominated. The possible explanations for the presence biotype Q in KPK is that Biotype Q may be ancestral or might be introduced through transportation activities to this region. It is also possible that KPK is a vegetable growing zone and biotype Q is most usually found on the vegetable hosts.

In conclusion this study helps in crop management by controlling invasive agricultural pest. Further study is needed on many aspects like onmigration, distribution and interaction of *Bemisia tabaci* with host and virus. Molecular techniques and DNA markers that can easily differentiate the *Bemisia tabaci* biotypes are needed to achieve these goals.

Aragones-Beltran, P., J.P. Pastor-Ferrando, F. Garcia-Garcia, and A. Pascual-Agullo, 2010. An Analytic Network Process approach for sitting a municipal solid waste plant in the Metropolitan Area of Valencia (Spain). Environmental Management, 91: 1071-1086.

REFERENCES

[1] Mound, L.A., S.H. Halsey, 1978. Bemisiatabaci (Gennadius). In Whitefly of the World, A Systematic Catalog of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data. British Museum (Natural History) and John Wiley & Sons, Chichester, New York, Brisbane, Toronto, pp: 118-124.

[2] Brown, J., D. Frolich, R. Rosell, 1995. The sweet potato or silver leaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annual Review of Entomology, 40: 511-534.

[3] Costa H.S., J.K. Brown, D.N. Byrne, 1991. Life history traits of the whitefly, *Bemisiatabaci* (Homoptera; Aleyrodidae) on six virus infected or healthy plant species. Environmental. Entomololgy, 20(4): 1102-1107.

[4] De barro, P.J., 1995. Bemisiatabaci biotypes: a review of its biology, distribution and control. Commonwealth Scientific and Industrial Research Organization Technical Paper, 33: 58.

[5] Martinez, S.S., A.O.R. de Carvalho, L.G. Vieira, L.M. Nunes, A. Bianchini, 2000. Identification, geographical Distribution and host plants of *Bemisiatabaci* (Genn.) Biotypes (Homoptera: Aleyrodidae) in the state of Paranov Brazil. Anais-da-Sociedade-Entomologica-do-Brasil, 29(3): 597-603.

[6] Hussain, T., T. Mahmood, 1992. Pak. Cottons, 1988, 32, 248. Govt. of the Punjab, "Assessment of damage due to Cotton Leaf Curl Virus in the Punjab", Report, Govt. of the Punjab.

[7] Briddon, R.W., M.S. Pinner, J. Stanley, P.G. Markham, 1990. *Gemini virus* coat protein replacement alters insect specificity. Virology, 177: 85-94.

[8] Butler, G.D., T.J.T. Henneberry Jr, E. Clayton, 1983. *Bemisiatabaci* (Homoptera: Aleyrodidae): Development, Oviposition, and Longevity in Relation to Temperature. Ann. Entomol. Soc. America, 76(2): 310-313.

[9] Coudriet, D.L., N. Prabhaker, A.N. Kishaba, D.E. Meyerdirk, 1985. Variation in Developmental Rate on Different Hosts and Overwintering of the Sweetpotato Whitefly, *Bemisiatabaci* (Homoptera: Aleyrodidae). Environmental entomology, 14: 516-519.

[10] Greathead, A.H., 1986. Host Plants. Chapter 3, pp. 17-25. In: *Bemisia tabaci* - a literature survey on the cotton whitefly with an annotated bibliography (Ed. M.J.W. Cock). CAB International Institute of Biological Control, Ascot, UK, 121 pages.

[11] Frolich, D.R., I. Torres-Jerez, I.D. Bedford, P.G. Markham, J.K. Brown, 1999. A phylogeographical analysis of the Bemisia tabaci species complex based on mitochondrial DNA markers. Molecular Ecology, 8: 1683-1691.

[12] De barro, P.J., F. Driver, J.H. Truaman, J. Curran, 2000. Phylogenetic relationships of world populations of Bemisiatabaci (Gennadius) using ribosomal ITS1. Molecular Phylogenetics and Evolution, 16(1): 29-36.

[13] Perring, T.M., 2001. The *Bemisiatabaci*species complex. Crop Protaction, 20: 725-737.

[14] De Barro, P.J., F. Driver, 1997. Use of random amplified polymorphic DNA polymerase chain reaction (RAPDPCR) to distinguish the B biotype from other biotypes of *Bemisiatabaci* (Gennadius) (Hemiptera: Aleyrodidae). Australian. Journal of Entomology, 36: 149-152.

[15] Qiu, B.L., S.A. Coats, S.X. Ren, A.M. Idris, C.X. Xu, J.K. Brown, 2007. Phylogenetic relationships of native and introduced Bemisiatabaci(Homoptera: Aleyrodidae) from China and India based on

mtCOIDNA sequencing and host plant comparisons. Progress in Natural Science: Materials International, 17(6): 645-654.
[16] De Barro, P.J., J.W.H. Trueman, D.R. Frohlich, 2005. *Bemisiaargentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. Bulletin Entomology Research, 95: 193-203.
[17] Boykine, L.M., R.G. Shatters, R.C. Rosell, C.L. Mckenzie, R.A. Bagnall, P.J. De barro, D.R. Frolich, 2007. Global relationships of *Bemisiatabaci* (Hemiptera: Aleyrodidae. revealed usingBayesian analysis of mitochondrial COI DNA sequences. Molecular Phylogenetics and Evolution, l44: 1306-1319.
[18] Chu, D., T. Jiang, G.X. LIiu, D.F. Jiang, Y.L. Tao, Z.X. Fan, H.X. Zhou, 2007. BI YP: Biotype status and distribution of *Bemisiatabaci* (Hemiptera: Aleyrodidae. in Shandong Province of Chinabased on mitochondrial DNA markers. Environmental. Entomology, 36: 1290-1295.
[19] Belllows, T.S., T.M. Perring, R.J. Gill, D.H. HeadRick, 1994. Description of a species of Bemisia (Homoptera: Aleyro-didae) infesting North American agriculture. Annals of the Entomological Society of America, 87: 195-206.
[20] Ahmed, M.Z., M.N. Maruthi, S.X. Ren, N.S. Mandour, M. Naveed, B.L. Qiu, 2009. Genetic variations of Bemisia tabaci populations in cotton growing area from Pakistan, Egypt and China. Journal Pest Science, Doi: 10.1007/s10340-009-0279-4.
[21] Bonato, O., A. Lurette, C. Vidal, J. Fargues, 2007. Modelling temperature-dependent bionomics of *Bemisia tabaci* (Q biotype). Physiology. Entomology, 32: 50-55.
[22] Horowitz, A.K., G.R. Gormon, I. Denholm, 2003. Inheritance of pyriproxyfen in the whitefly, *Bemisiatabaci* (Q biotype). Arch Insect Biochemistry and Physiology, 54(4): 177-186.
[23] Pascual, S., 2006. Mechanisms in competition, under laboratory conditions, between Spanish biotypes B and Q of *Bemisiatabaci* (Gennadius). Spanish Journal Agriculture Research, 4: 351-354.
[24] Guiro, P., F. Beita, J.L. Cenis, 1997. Biotype determination in Spanish populations of *Bemisatabaci* (Hemiptera: Aleyrodidae). Bulletin Entomology Research, 87: 559-587.
[25] Ueda, S., J.K. Brown, 2006. First report of the Q biotype of *Bemisia tabaci* in Japan by mitochondrial oxidase I sequence analysis. Phytoparasitica, 34: 405-411.
[26] Chu, D., F.H. Wan, Y.J. Zhang, J.K. Brown, 2010. Change in the biotype composition of *Bemisiatabaci* in Shandong Province of China from 2005 to2008. Environmental Entomology, 39(3): 1028-36.