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Genetic diversity assessment of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) whitefly in vegetable and crop fields of Hormozgan province using ISSR markers

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Abstract

Bemisia tabaci (Gennadius) is one of the most significant agricultural pests worldwide due to its extensive distribution and highly polyphagous nature, with over 500 host plant species reported. During the 2017–2018 cropping season, we assessed the genetic diversity of *B. tabaci* populations across 12 major vegetable-growing regions in Hormozgan Province, Iran. Genetic variation was analyzed using six ISSR primers. Binary data analysis revealed genotype similarity values ranging from 0.09 to 0.94, with an average similarity of 0.52. The overall high genetic similarity suggests limited genetic diversity among the sampled populations based on the selected primers. Nevertheless, the broad range of similarity values indicates that the ISSR markers were effective in detecting genetic variation. The highest similarity (94%) was observed between tomato field samples from Minab and Fin, followed by 88% similarity between eggplant samples from Minab and tomato samples from Fin, and 83% similarity between tomato and eggplant samples within Minab. Cluster analysis showed that groups with larger sample sizes exhibited greater genetic similarity. The occurrence of samples from the same geographic region in separate clusters, and samples from different regions within the same cluster, suggests that geographic isolation is not a primary factor influencing genetic differentiation in these populations.

Keywords: Biotype, Genetic diversity, Whitefly, ISSR marker

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بررسی تنوع ژنتیکی سفیدبالک *Bemisia tabaci* (Gennadius) (Hem.: Aleyrodidae) در مزارع سبزی و صیفی استان هرمزگان با استفاده از نشانگر ISSR

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چکیده

سفید بالک پنبه *Bemisia tabaci* (Gennadius)، یکی از مهم‌ترین آفات محصولات کشاورزی با گسترش جهانی است. این حشره بسیار چندخوار بوده و تاکنون بیش از ۵۰۰ گونه گیاهی به عنوان میزبان آن به ثبت رسیده است. در سال زراعی ۹۷-۱۳۹۶، با هدف بررسی تنوع ژنتیکی این حشره در مزارع سبزی و صیفی، از ۱۲ منطقه مختلف صیفی‌کاری استان هرمزگان نمونه‌برداری به عمل آمد و برای بررسی تنوع ژنتیکی در بین مناطق مورد مطالعه از شش آغازگر ISSR استفاده شد. بر اساس نتایج به دست آمده از تجزیه داده‌های دوتایی، ماتریس تشابه بین ژنوتیپ‌های مورد مطالعه از ۰/۰۹ (کم‌ترین میزان تشابه) تا ۰/۹٤ (بیش‌ترین میزان تشابه) (با میانگین تشابه ۰/۵۲) متغیر بود. میزان بالای تشابه ژنتیکی یاد شده، نشان‌دهنده پایین بودن تنوع ژنتیکی در بین نمونه‌ها بر اساس آغازگرهای مورد استفاده است، اما با توجه به دامنه بالای تشابه در این بررسی، می‌توان بیان کرد که نشانگرها به‌خوبی توانسته‌اند تنوع ژنتیکی میان نمونه‌های *B. tabaci* را نشان دهند. بیش‌ترین تشابه (۹٤ درصد) بین نمونه جمع‌آوری شده از محصول گوجه‌فرنگی در مناطق میناب و فین و در مرتبه بعدی بین نمونه‌های برگرفته از محصول بادمجان در منطقه میناب و نمونه برگرفته از محصول گوجه‌فرنگی در منطقه فین (با تشابه ۸۸ درصد) و بین نمونه برگرفته از محصول گوجه‌فرنگی و بادمجان در منطقه میناب (با تشابه ۸۳ درصد) مشاهده شد. بر اساس گروه‌بندی انجام شده، گروه‌هایی دارای تعداد نمونه بیش‌تر، دارای تشابه ژنتیکی بالایی هستند. قرار گرفتن نمونه‌های یک منطقه جغرافیایی در دو گروه مستقل و نمونه‌های مناطق مختلف در یک گروه نشان‌گر این مطلب است که عامل جدایی جغرافیایی نمی‌تواند یک عامل تعیین کننده در جدایی ژنتیکی جمعیت‌ها باشد.

کلید واژه‌ها: بیوتیپ، تنوع ژنتیکی، سفیدبالک، نشانگر ISSR

دبیر تخصصی: دکتر احسان رخشانی

Introduction

Human survival is closely tied to the health and productivity of plants, with approximately 90% of global food sources derived from plant-based products. However, an estimated 30% of annual crop production is lost due to pest infestations (Oerke, 2006). Among these destructive pests, whiteflies are particularly significant. Belonging to the family Aleyrodidae, whiteflies comprise over 1,550 identified species worldwide (Liu et al., 2015). One of the most economically important species is the cotton whitefly, *Bemisia tabaci* (Gennadius), a globally distributed and highly polyphagous pest (Fekrat & Shishehbor, 2004; Parrella et al., 2012). First reported in 1889 from Greece on cotton plants, *B. tabaci* earned its common name "cotton whitefly" (Gangwar & Gangwar, 2018). It feeds on a broad spectrum of host plants—including ornamental, vegetable, cucurbit, agricultural, and rangeland species—affecting more than 500 plant species across 60 botanical families. Major host plants include tomato, potato, peanut, soybean, cotton, pepper, squash, cucumber, bean, eggplant, watermelon, cabbage, and various ornamentals (De Barro et al., 2011; Liu et al., 2015). The individuals of *B. tabaci* can transmit a destructive viral disease, namely Tomato yellow leaf curl virus, to different host plant speices, including tomato and pepper. TYLCV is a species of the genus Begomovirus in the family Geminiviridae, with a single-stranded DNA genome with double-stranded particles, transmitted by this pest in a persistent, circulating, and non-propagative manner. The virus has a wide distribution in countries of the Mediterranean region, the Middle East, and Africa, including Cyprus, Israel, the Ivory Coast, Jordan, Lebanon, Senegal, Egypt, and Tunisia (H El-Sappah et al., 2022). The disease has been reported from Israel and the Jordan Valley since the early 1960s and is currently of significant economic importance in many countries (Prasad et al., 2020).

Understanding the genetic diversity of *B. tabaci* is crucial for developing effective pest management strategies (Basij et al., 2019). Numerous studies have explored this aspect across different regions. For instance, Yu et al. (2012) examined indigenous *B. tabaci* populations in Fujian Province, China, using RAPD and ISSR markers. Their findings revealed polymorphic bands at 2,000 and 1,728 base pairs, indicating high genetic diversity and a correlation between geographic and genetic distances. Similarly, Park et al. (2019) analyzed *B. tabaci* MED populations in Korea using microsatellite markers, reporting a significant correlation ($P < 0.05$) between genetic differentiation (FST) and geographic distance, alongside relatively high genetic diversity. These variations are attributed to factors such as local adaptation, insecticide resistance, and human-mediated dispersal.

In China, genetic analyses using mtCOI and cDNA-AFLP markers identified two invasive species (MEAM1 and MED) and four cryptic native species (Asia II 1, Asia II 3, China 3, and Asia II 7), with polymorphic group percentages ranging from 91.9% to 98.7%. MED exhibited the lowest similarity to other species (Xiao-Jun et al., 2012). In South Sudan, Misaka et al. (2020) identified six distinct genetic groups of *B. tabaci* on sweet potato and cassava. Comparative studies in China using ISSR markers revealed higher genetic diversity in biotype Q than in biotype B, suggesting greater ecological adaptability (Dong et al., 2008). These structural differences were further confirmed by ISSR analysis.

In Pakistan, a RAPD marker study (Hameed et al., 2012) showed Nei and Li's similarity coefficients ranging from 0.25 to 0.88, clustering populations into three major groups. The non-B biotype exhibited a wider geographic distribution than biotype B, which was confined to fewer locations. Queiroz et al. (2016) used SCAR

markers to differentiate biotypes B, Q, and native Brazilian biotypes, successfully distinguishing the latter from the invasive types.

Given that Hormozgan Province ranks third nationally in vegetable crop production—particularly for tomato and eggplant (Anonymous, 2018)—and considering these crops are primary hosts of *B. tabaci*, it is essential to investigate the genetic structure and diversity of this pest in the region to enhance local pest management strategies.

Materials and Methods

In this study, systematic sampling of *B. tabaci* was conducted during the 2017–2018 cropping season across twelve regions of Hormozgan Province, including Rodan, Minab, Hasht bandi, Ghaleh ghazi, Eisin, Tazian, Dasht emam, Sarkhon, Finn (Sarzeh), Shamil hajiabad, Kehoristan, and Parsian (Gusmao et al., 2005). Sampling sites were selected based on the cultivated area of vegetables and cucurbits, in consultation with experts from the Agricultural Jihad Organization and local farmers. Sampling was carried out from October to April, coinciding with the main cultivation season and the peak period of pest activity in the fields. Environmental parameters, including temperature and relative humidity, were continuously monitored, with average temperatures ranging from 25 to 35 ℃ and mean relative humidity recorded between 45% and 65%. Specimens were collected using a handheld aspirator and were preliminarily identified morphologically at the collection sites. Each sample was immediately transferred into 1.5 mL microtubes containing acetone (Razi Company) to prevent DNA degradation. Samples were then stored at low temperatures under controlled conditions and transported to the laboratory for molecular analyses, including DNA extraction, sequencing, and genetic diversity assessments. This protocol contributed to the standardization of sample collection across all regions, allowing for accurate population comparisons and reliable molecular investigations.

Genomic DNA was extracted using a modified CTAB protocol (Reineke et al., 1998). Insect tissues were frozen in liquid nitrogen, dried, and ground in 2 mL microtubes. Each sample was mixed with 500 µL of extraction buffer (25 mM EDTA, 25 mM NaCl, 10 mM Tris-HCl, pH 8.0, and 1% SDS), followed by the addition of 5 µL proteinase K (Pishgam Biotechnology Co.) to facilitate tissue digestion. Salt concentration was adjusted with 140 µL of 5 M NaCl. An equal volume (710 µL) of cold chloroform: isoamyl alcohol (24:1) was added, gently mixed, and incubated on ice for 30 minutes. Samples were centrifuged at 13,000 rpm for 20 minutes, and the supernatant was transferred to a new tube. Subsequently, 225 µL of 5 M ammonium acetate was added, mixed, and incubated on ice for another 30 minutes. A second centrifugation at 13,000 rpm for 20 minutes was performed to remove proteins and debris. The supernatant was transferred to a fresh tube, and 0.7 volumes of cold isopropanol were added to precipitate DNA. Samples were incubated on ice for 30 minutes or at 4°C overnight. DNA pellets were obtained by centrifugation at 13,000 rpm for 30 minutes, washed twice with 500 µL of 70% ethanol, vortexed, and centrifuged at 13,000 rpm for 10 minutes. Pellets were dried at 37°C and resuspended in 50 µL of TE buffer or double-distilled water. DNA quality was assessed by electrophoresis on a 0.8% agarose gel.

To evaluate genetic diversity, six ISSR primers were selected (Table 1). PCR amplification was performed in 25 µL reaction volumes containing 5.5 µL Mastermix, 1 µL DNA template, 1 µL primer, and 17.5 µL distilled water. Reactions were carried out in a Biorad thermal cycler following a modified protocol from Perring

et al. (1993): initial denaturation at 94°C for 5 minutes (1 cycle), followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 48–52°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes.

Amplified products were separated by electrophoresis on 1% agarose gels at 65 V for 90 minutes. Fragment sizes were estimated using a 100 bp DNA ladder (DNA Ladder Plus 100 bp, Pishgam Biotechnology Co.), and bands were visualized using a Gel Documentation System (Intas model).

Due to the qualitative nature of the data, a binary matrix was constructed in Excel 2016, scoring band presence as "1" and absence as "0." Genetic similarity was calculated using the Jaccard coefficient in NTsys version 2.02. Cluster analysis of *B. tabaci* genotypes was performed using the UPGMA method in the same software.

Table 1. Sequence of primers used to study *Bemisia tabaci* samples (Gong et al., 2001)

Sequence of primers	Temperature (°C)	Primers
(GACAC)3	49	ISSR1
(GACA)4	48	ISSR2
(TCC)5	52	ISSR3
(AGG)5	52	ISSR4
(ACTG)4	48	ISSR5
(5´-AGAGGTGGGCAGGTG-3´)	52	ISSR6

Results

All 24 whitefly specimens collected from various regions of Hormozgan Province—including Rudan, Minab, Fin (Sarzah), Dasht Emam, Hasht Bandi, Isin, Qaleh Qazi, Kahurestan, Parsian, Sarkhoon, Shamil Hajji Abad, and Tazian—were morphologically identified as *Bemisia tabaci* using established taxonomic keys and diagnostic features (Weber, 1931; Martin, 1987; Anonymous, 2004).

A total of 46 alleles were amplified using six ISSR primers. The allele size ranges for each primer are presented in Table 2. The number of loci detected per primer was as follows: ISSR1 (10), ISSR2 (6), ISSR3 (8), ISSR4 (5), ISSR5 (6), and ISSR6 (11). Primer ISSR6 yielded the highest number of alleles, while ISSR4 produced the fewest. The average number of polymorphic bands across all primers was 8.3. Polymorphism Information Content (PIC) values ranged from 0.3533 (ISSR1) to 0.4339 (ISSR5), indicating moderate levels of polymorphism. Marker index values were also calculated, with ISSR6 showing the highest index (4.26), suggesting its superior discriminatory power among the tested samples. Representative ISSR banding patterns are shown in Figure 1.

Genetic similarity among the populations, based on binary data analysis, ranged from 0.09 (lowest) to 0.94 (highest), with an average similarity of 0.52. The relatively high similarity values suggest limited genetic diversity among the sampled *B. tabaci* populations using the selected ISSR markers. The highest similarity (94%) was observed between tomato field samples from Minab (Mi-t) and Fin (F-t), followed by 88% similarity between eggplant samples from Minab (Mi-e) and tomato samples from Fin (F-t), and 83% similarity between tomato and eggplant samples within Minab (Mi-t and Mi-e).

Table 2. Statistical characteristics of ISSR primers in the studied *Bemisia tabaci* populations

Marker index	Level of primer polymorphism	Polymorphism%	Number of polymorphic bands	Total number of bands per primer	Allele size	Primer sequences (5′-3′)	Primer
3.53	0.3533	100	10	10	300-2000	(GACAC)3	ISSR 1
2.38	0.3971	100	6	6	300-1250	(GACA)4	ISSR 2
3.20	0.3997	100	8	8	300-3000	(TCC)5	ISSR 3
1.86	0.3724	100	5	5	300-1500	(AGG)5	ISSR 4
2.60	0.4339	100	6	6	300-2000	(ACTG)4	ISSR 5
4.26	0.3872	100	11	11	200-2000	(5´AGAGGTGG GCAGGTG-3´)	ISSR 6
2.97	0.3780	100	8.3	8.3	-	Mean	

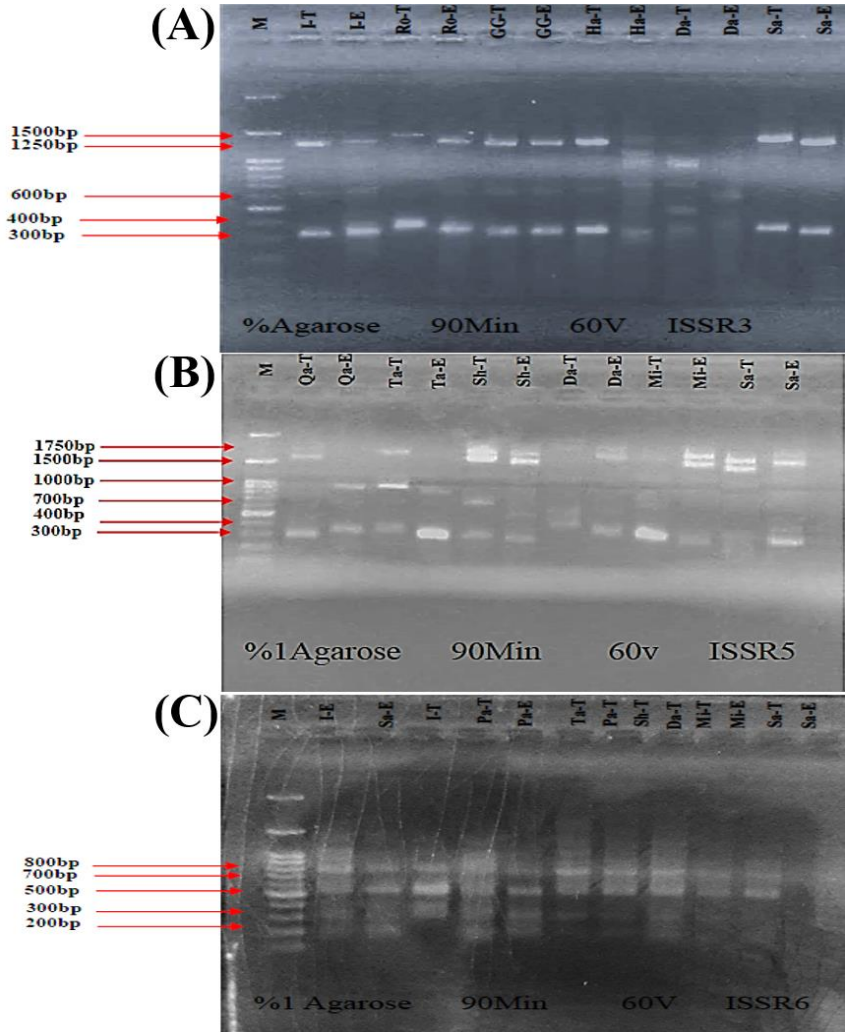


Figure 1. DNA electrophoresis profile from ISSR3 (A), ISSR5 (B); and ISSR6 (C) primers amplification in *Bemisia tabaci* samples of the study areas (In the upper part of each well, the sample code is used and also the letter M in the first well indicates the size of the marker. The size of the marker used is 100 pairs, and the size of each band is 100 pairs. In the lower part, the concentration of 1% agarose gel and duration indicate the electrophoresis time of 90 minutes with a voltage of 60).

Cophenetic correlation analysis revealed that the Jaccard similarity coefficient combined with the UPGMA clustering method yielded the highest correlation values, and was therefore used for dendrogram construction (Table 3).

The resulting UPGMA dendrogram (Figure 2) illustrated genetic relationships among the 24 *B. tabaci* samples. At a similarity threshold of 0.59, eighteen distinct groups were identified: sixteen single-member groups, one two-member group, and one six-member group. Larger clusters reflected greater genetic similarity among their constituent samples.

Table 3. Cofenetic coefficient calculated for *Bemisia tabaci* populations

Dice	Simple matching (SM)	Jaccard (J)	Similarity coefficient
0.78781	0.7937	0.83724	Cophenetic coefficient

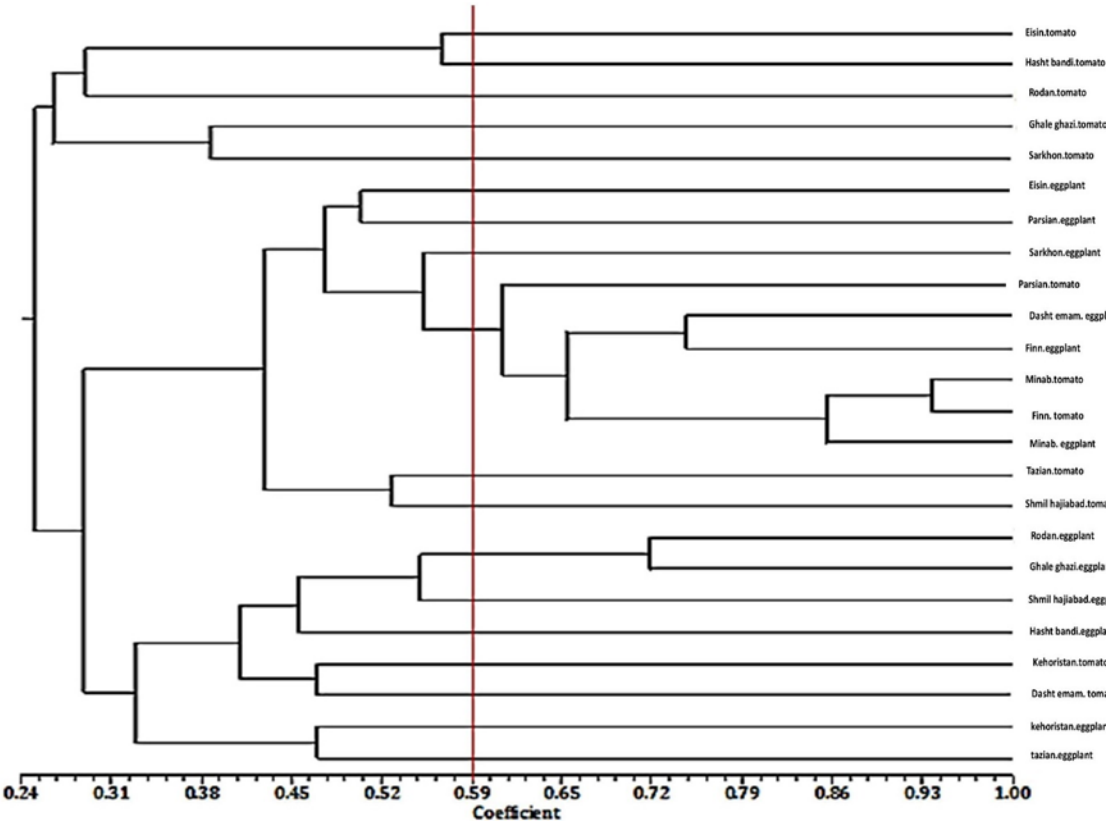


Figure 2. Dendrogram Genetic association between 24 *Bemisia tabaci* genotypes using ISSR marker by UPGMA method and Jaccard similarity coefficient

Discussion

In recent years, the damage rate of whitefly pest species has increased dramatically in Hormozgan province, making it difficult for many available insecticides to control the pest properly. On the other hand, the ability of the pest to transmit the associated viral disease has been significantly affected, and many greenhouses producing tomatoes and peppers have been infected with the viral disease (Personal communication with Mahmoudzadeh). One of the existing hypotheses regarding this challenge was the introduction of genetically different

populations, and another was the effect of spraying pressure on the genetic drift of the existing populations feeding on different host plant species. In Hormozgan Province, different tomato cultivars are cultivated with different degrees of susceptibility to TYLCV (a range of varieties from highly susceptible to highly resistant cultivars). Accordingly, a high number of insecticides from different groups is used on TYLCV-susceptible cultivars to control the vector, which over time can lead to selection and genetic drift in different populations of the pest (Personal communication with Mahmoudzadeh). This study was conducted to investigate the genetic pattern of *B. tabaci* in Hormozgan province. Given the high rate of TYLCV infection in Bushehr province, it is likely that this province has also experienced conditions similar to those in Hormozgan province, which need to be investigated.

Polymorphism Information Content (PIC) is a measure of marker informativeness, ranging from 0 to 1, and reflects the degree of genetic variation detected (Roy et al., 2023). In this study, the observed range of genetic similarity among *Bemisia tabaci* samples suggests that the selected ISSR markers were effective in revealing genetic diversity, despite the overall high similarity values.

The clustering of samples from the same geographic region into separate groups, and the grouping of samples from different regions together, indicates that geographic location alone is not a definitive factor in population differentiation. This pattern may be influenced more strongly by host plant selection pressure or by anthropogenic dispersal mechanisms (Restoux et al., 2010). The movement of infested agricultural products between regions likely facilitates gene flow, contributing to genetic homogeneity across geographically distinct populations (Cordeiro et al., 2019).

Gene flow plays a critical role in shaping genetic diversity within and among populations (Oyarieme et al., 2024). In its absence, genetic drift can lead to divergence at neutral loci. The low genetic differentiation observed in this study may be attributed to the frequent movement of produce within Hormozgan Province, which enables the spread of *B. tabaci* and homogenizes genetic variation. Additionally, widespread use of similar insecticides across regions may exert uniform selection pressure, further reducing genetic divergence.

Interestingly, genetic variation was detected between *B. tabaci* populations collected from tomato and eggplant within the same locality. This may support the hypothesis that the introduction of host plant-adapted populations or long-term host association in these regions may drive genetic differentiation, even in the absence of geographic isolation (Restoux et al., 2010).

Comparable findings have been reported in other regions. Fontes et al. (2012) identified 2 to 13 alleles in *B. tabaci* populations using microsatellite markers, with an average FST of 0.13. Populations on beans exhibited distinct genetic profiles, likely influenced by host plant and insecticide exposure. American populations showed lower diversity compared to Brazilian populations, supporting the hypothesis of biotype B introduction via trade routes.

Sami et al. (2007) used RAPD markers to assess genetic diversity in Iranian *B. tabaci* populations, revealing substantial variation not solely attributable to geographic separation. High genetic variability was observed among populations with differing levels of invasiveness.

Here, we found geographically close populations in genetically distinct clades, showing these populations may have experienced high selection pressures. Park et al. (2019) reported significant genetic differentiation ($P < 0.05$) among *B. tabaci* MED populations in Korea, with clustering patterns influenced by geographic distance,

adaptation, and insecticide resistance. Tamilnayagan et al. (2019) found multiple genetic groups coexisting within single fields in Tamil Nadu, India, based on mitochondrial COI gene sequencing.

In Georgia, USA, Gautam et al. (2020) observed low genetic diversity among *B. tabaci* populations, attributing this to extensive gene flow and the dominance of a pandemic population associated with widely cultivated host plants. Similarly, Thangavel Boopathi et al. (2019) demonstrated that high genetic diversity in *Aleurodicus dispersus* may result from multiple introductions from different source regions.

Given the potential for human-mediated dispersal of *B. tabaci* (Gippet et al., 2019), implementing quarantine measures to restrict the movement of infested produce is essential to prevent the spread of resistant biotypes. Furthermore, reducing reliance on chemical control and promoting integrated pest management strategies could mitigate the emergence of resistance and preserve genetic diversity within local populations.

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