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Genetic variability of *Bemisia tabaci* in the Mediterranean and Sahel Regions

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Abstract

The whitefly *Bemisia tabaci* is regarded as a complex species because of its high genetic variability, and at least 20 haplotypes/biotypes with varying degrees of biological characterization are currently recognized within the species. In the Mediterranean Basin and the Sahel Region the whitefly is widespread on a high number of wild and cultivated plant species. We analysed the genetic variability of *B. tabaci* by means of sequencing and restriction analyses of the mtCOI sequence. The results show the presence of five different biotypes in the Mediterranean Basin, B, Q, M, S, and T and of two haplotypes/biotypes in the Sahel Region, J and Sub-Saharan VI. According to their sequence analyses B, Q, and J belong to the Mediterranean/North African clade, S and Sub-Saharan VI to the African clade, while T falls within the Southeast/Fareast Asian clade. A diagnostic PCR-RFLP of mtCOI can be applied to identify most of these genetic variants.

Key words: *Bemisia tabaci*, mtCOI, haplotype/biotype, Mediterranean Basin, Sahel Region.

Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera Aleyrodidae) is one of the most important pests in the world, being the known vector of more than 100 plant viruses (Jones *et al*., 2003). This insect shows a high intraspecific biological and genetic variability, and it is regarded as a species complex (Brown *et al*., 1995). This variability is revealed by the existence of populations that differ in their ability to feed or reproduce on particular hosts and in their virus transmission characteristics. Different biotypes are usually recognized by the presence of specific phytotoxic reactions, esterase markers and several DNA fingerprinting techniques. To date, about 20 biotypes have been identified and characterized to differing degrees (reviewed by Perring, 2001; Simòn *et al*., 2003). We carried out surveys of *B. tabaci* on wild and cultivated plants and we analysed the samples for the mtCOI gene sequence. Five genetically distinct biotypes have been identified in the Mediterranean, B, Q, S, M and T. Three biotypes/haplotypes, Q, J and Sub-Saharan VI have been found in the Sahel Region. B and Q are polyphagous and coexist in the same areas in the Mediterranean Basin, sharing their habitat, while T can only be found in limited spots in the underbrush of pine forests in mountain areas of Sicily and Apulia regions.

Materials and methods

Surveys were carried out in the open field and in greenhouses on cultivated and spontaneous herbaceous plants in Italy, Spain and Mali (Western Africa). Adult whiteflies were collected with an aspirator and stored in 70% ethanol until the analyses. Nymphs were collected with the leaves where they fed on and transferred to the laboratory for morphological observations and to establish laboratory rearings in climate-controlled chambers on different host-plants. Samples of whiteflies from other Mediterranean countries were kindly provided by several colleagues. DNA was purified from single insects either by boiling the samples in Tris-EDTA or by using Chelex (BioRad). mtCOI was amplified in PCR with primers C1-J-2195 MTD-10 and L2-N-3014 MTD-12 (Simon *et al*., 1994). Amplicons were purified using a kit (Qiagen) and sequenced or were singly restricted with *Tru*9I or *Taq*I endonucleases at 65 °C for 2 h. Restricted DNA was electrophoresed on 7% acrylamide/bisacrylamide (29:1) gel and stained with ethidium bromide. The sequences were input in the MEGA3 software (Kumar *et al*., 2004), and a phylogenetic tree was obtained using Kimura two-parameters with the Neighbour-Joining method.

Results and discussion

Amplicons of about 880 bp were obtained in PCR from all the samples of *B. tabaci* analysed.

Sequencing analyses

In the Mediterranean Basin five different haplotypes were present and, according to their different biological properties, they have been defined as biotypes B, Q, M, S, and T. According to the phylogenetic analyses they belong to three distinct genetic clades, Mediterranean/Asian/African with the biotypes B and Q, African Sub Saharian clade with the S biotype and Asian clade with M and T biotypes. The T biotype, monophagous on *Euphorbia characias* L. in Sicily and Apulia (Southern Italy), resulted correlated with the Asian clade, even if the average genic distance from the other individuals belonging to this clade is similar to the distance among different clades.

In the Sahel Region we identified samples belonging to the Mediterranean/Asian/African (biotypes Q and J)

and to the African clade (Sub-Saharan VI haplotype). Genic distance between Q and J haplotypes found in the Sahel are the lowest we found among our different haplotypes.

Biotypes B and Q were found on a huge variety of host plants, cultivated and spontaneous, in the open field or in greenhouse conditions. When reared in climate-controlled chambers they developed on cucumber, zucchini, tomato, bean, cotton and poinsettia. The T biotype was only found on *E. characias*, in two isolated spots in Sicily, Nebrodi-Peloritani Mountains, and in the Foresta Umbra in Apulia. In both sites whiteflies of this biotype fed on *E. characias* in the undergrowth of pine forests, at 600–1000 m a.s.l. in Sicily and at 400-500 m a.s.l. in Apulia. In these areas winter is relatively cold and plants are sometimes covered by snow. When reared in climate-controlled chambers the T biotype whiteflies developed only on its natural host-plant, *E. characias* and, for few generations, on *Datura stramonium* L. The J biotype was found in the Sahel Region on several Solanaceae, Fabaceae, and Cucurbitaceae, as for the biotypes B and Q, but it was also found on some additional host plants of the genera: *Indigofera* (Fabaceae), *Cassia* (Caesalpiniaceae), *Ricinus*, *Jatropha*, *Manihot*, *Securinega*, and *Euphorbia* (Euphorbiaceae), *Gossypium*, *Hibiscus*, and *Sida* (Malvaceae), *Leptadenia* (Asclepiadaceae), *Leonotis* and *Leucas* (Lamiaceae), and *Ipomoea* (Convolvulaceae). When reared in climate-controlled chambers the J biotype proved to be polyphagous and fed on the same host-plants described for B and Q biotypes. The Sub-Saharan VI haplotype was found on cassava. We did not rear this haplotype in controlled conditions. B and Q biotype whiteflies died within 2 days when caged on cassava cuttings and no viable eggs were laid on this plant.

RFLP analyses

Restriction with *Tru*9I provided a diagnostic assay for the identification of the five Mediterranean biotypes, since the profiles obtained from the different biotypes, regardless of their geographic origin, were unique. The same enzyme was able to differentiate the monophagous cassava haplotype from Mali from J and Q, but the latter haplotypes shared the same profile. When digested with *Taq*I the 880 bp mtCOI amplicons provided diagnostic profiles for B and Q biotypes, which showed clearly different patterns. The same amplicons from T and S biotypes were not cut by *Taq*I.

Conclusion

This research reveals a complex pattern of high genetic variability within the *B. tabaci* species complex in the Mediterranean and Sahel Regions. B, Q, and J can be considered as polyphagous biotypes belonging to the Mediterranean-African clade, sharing the same geographical areas (B and Q) or divided by the relatively recent barrier of the Sahara desert (J) (De La Rua *et al.*, 2006). Understanding phylogenetic relationships for the other biotypes/haplotypes is more difficult. Biotypes S and T are monophagous biotypes whose geographical range is larger than initially suspected, but they have different phylogenetic relationships, the former being close to the African Sub-Saharan clade (Sub-Saharan VI subgroup) and the latter to the Southeast Far East Asian clade. The presence in Western Africa of a monophagous haplotype on cassava, grouped within the African clade, Sub-Saharan subgroups (Berry *et al*., 2004), points out that in this area the risk of cassava mosaic virus (ACMV) epidemics is important, due to the presence of the specific vector. A diagnostic PCR-RFLP of mtCOI can be applied to identify most of these genetic variants (Bosco *et al*., 2006).

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