Acta Phytopathologica et Entomologica Hungarica 36 (3–4), pp. 365–368 (2001)

Molecular Polymorphism between Population of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) Propagating on Tobacco and Onion

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The molecular polymorphism between the specimens of *Thrips tabaci* Lindeman collected from tobacco *(Nicotiana tabacum)* and from onion *(Allium cepa)* was observed by RAPD-PCR-analysis. The amplified DNA-banding patterns of *T. tabaci* populations propagate on tobacco were significantly different from those propagate on onion. In total, 54 amplified DNA-bands were scored. Of these, 39 (72.0%) were different, and 7 (13.0%) bands were mutual between the two populations. A slight molecular dissimilarity between females and larvae of *T. tabaci* populations propagating on tobacco and onion were observed on the basis of one (1.9%) discriminating PCR-fragment and six ones (11.1%), respectively. The results presented indicate an intraspecific molecular variability between populations of *T. tabaci* propagating on tobacco as well as on onion.

Keywords: *Thrips tabaci communis, Thrips tabaci tabaci,* PCR., tobacco, onion.

According to the statement of Zawirska (1976) *Thrips tabaci* Lindeman comprises two "types", from among which the populations of *T. tabaci tabaci* living on *Nicotiana tabacum,* and out of the vegetation period on different weed species, propagate by arrhenotoky and are able to transmit tomato spotted wilt tospovirus. The populations of *T. tabaci communis* living on different plant species, mainly on onion, they propagate with teletoky and their specimens are not virus vectors. Schliephake and Klimt (1979) use the subspecies expression. Since Klein and Gafni, (1996) as well as Kraus and coworkers (1998) had found intraspecific molecular differences between Thysanoptera species as well as Thysanoptera populations occurring in different agrobiotopes, we have begun to investigate whether there are possible intraspecific molecular differences between the two *T. tabaci* types (subspecies), in spite of morphological similarity of adults.

0238–1249/2001/$ 5.00

Materials and Methods

Sampling of thrips

Thrips individuals were collected from two populations from tobacco and onion. Specimens living on tobacco (70 females and larvae), were collected at Kállósemjén, (Hungary), July 13 2000, comprising about a fifty sampling sites across the 10 ha tobacco plantation. Specimens living on onion (70 females and larvae) were collected at Tordas (Hungary), July 19 2000 across a 1/2 ha plantation comprising about twenty sampling sites. Sexes, and developmental stages were selected under dissecting microscope. Samples were stored in DNA extraction buffer at –20 °C until DNA extraction.

DNA extraction

DNAs of pooled samples of females and larvae of both populations were extracted according to the CTAB-protocol of Doyle and Doyle (1990) with the modification of Mendel et al. (1994). Thrips were ground to fine powder under liquid nitrogen prior to adding 400 µl of DNA extraction buffer followed by an incubation in water bath for 30 min at 60 °C. An equal volume of 400 µl chloroform: isoamyl alcohol (24:1) were added to each sample at room temperature followed by a separation in microcentrifuge at 12000 r.p.m., 4 °C, for 3 min. DNA samples were precipitated from the aqueous phase by an addition of a 2/3 volume of isopropanol, washed by 70% ethanol, let them dry for 30 min. at room temperature, and resuspended in 100 µl $T_{10}E_1$ buffer (10 mM Tris, 1 mM EDTA). The quality of isolated DNA was determined on a 0.8% agarose (SIGMA) gel stained with ethidium bromide (0.5 ng/µl).

RAPD-PCR analysis

For PCR analysis thirteen decamer primers (Operon Tech) were used: OPA/01 (CAGGCCCTTC), OPA/08 (GTGACGTAGG), OPA/09 (GGGTAACGCC), OPA/11 (CAATCGCCGT), OPA/16 (AGCCAGCGAA), OPA/19 (CAAACGTCGG), OPH/11 (CTTCCGCAGT), No/11 (ACGGCATATG), OPAB/09 (GGGCGACTAC), OPAI/21 (CACGCGAACC), OPAL/20 (AGGAGTCGGA), PAL/1 (GCGATGTAGCG), PAL/2 (CCAGGTGGACC). All of these primers were taken from a pre-tested primer collection which produced extremely reproducible PCR band patterns in a previous study. PCR amplification was carried out in a 25 µl reaction mixture containing 10–20 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 0.75 µM d NTPs, 60 nM primer, 1.5 U Taq DNA polymerase (Gibco). Cycling was carried out in a Perkin-Elmer 9600 thermocycler with the following steps: 1 cycle of 94 °C for 2 min.; 40 cycles of the steps of 94 °C for 10 sec, 36 °C for 30 sec, 72 °C for 1 min.; and closed by a cycle of 72 °C for 2 min. Electrophoresis was performed in 1.6% agarose (Gibco) gels. PCR fragments at low intensities were only scored as present when they were reproducible in repeated experiments. A negative control which contained all the necessary PCR components except template DNA was also included in PCR runs. Each successful reaction with scorable bands was repeated at least twice. Fragment sizes were estimated by comparison to DNA molecular weight markers (Boehringer Mannheim, and Fermentas).

Results

All of the thirteen RAPD primers applied resulted in a highly scorable PCR fragment patterns. The number of amplified DNA bands varied from 1 (primer OPA-11, *Fig. 1*) to 9 (primer No/11, *Fig. 1*) per primer with molecular weights from about five hundred to 2000 base pairs. In total, 54 (100%) amplified DNA bands were scored. Of these, 39 (72.0%) were found to be significantly different, and 7 (13.0%) bands were mutual between the specimens of two populations. The molecular differences between females and larvae of *T. tabaci* populations propagating on tobacco and on onion resulted in 1 PCR-fragment (1.9%), and 6 ones (11.1%), respectively *(Fig. 1).*

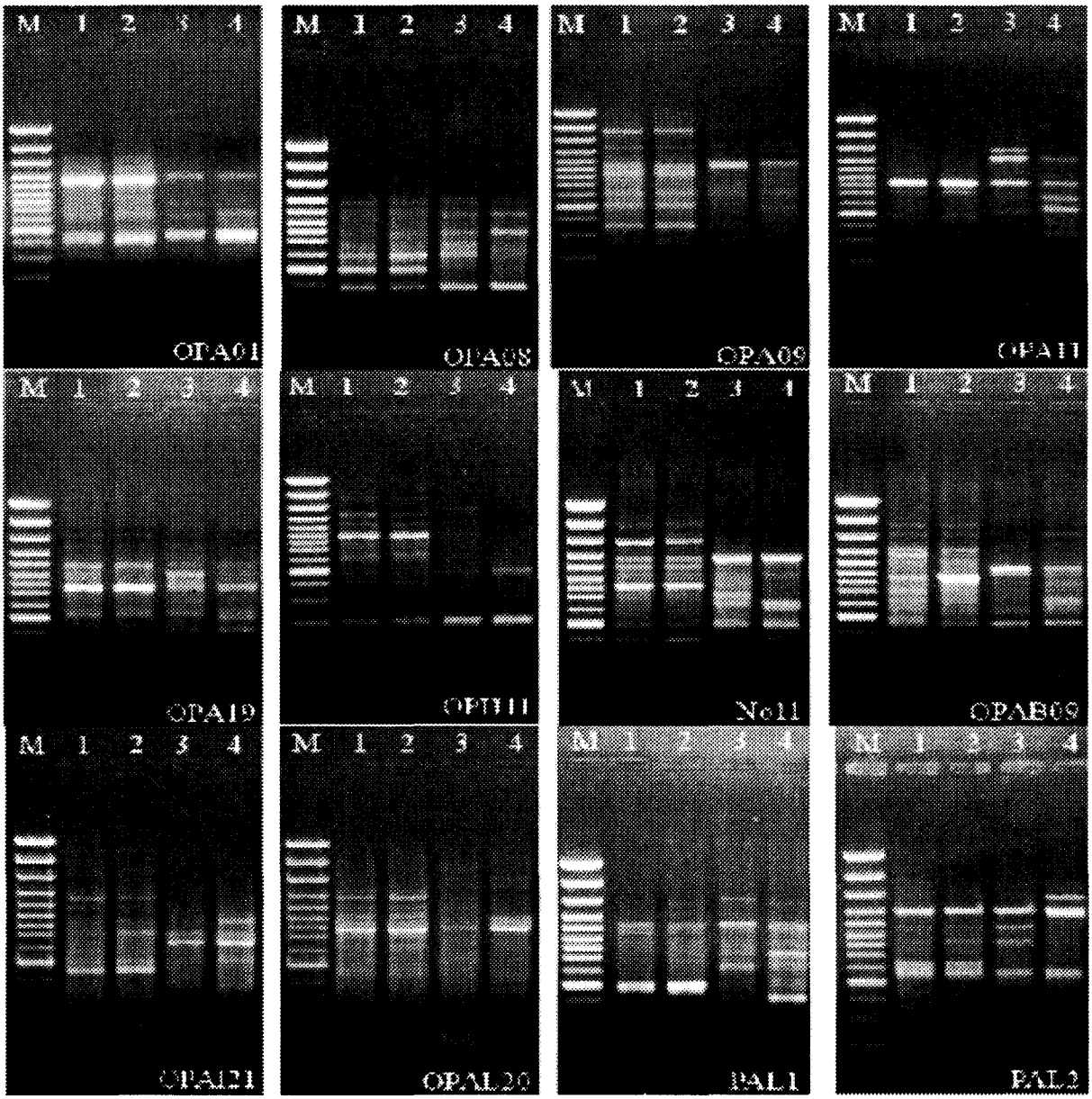

Fig. 1. Twelve samples of agarose gel electrophoresis of PCR amplifications (generated by primers from OPA01 to PAL2) of the populations of *Thrips tabaci* propagating on tobacco or on onion. Lanes, M: Mw, molecular weight marker (arrows show significant Mw fragments in bp); 1 (female adults ♀) and 2 (larvae): *T. tabaci* propagating on tobacco; 3 (female adults ♀) and 4 (larvae): *T. tabaci* propagating on onion

Discussion

According to the available data there are new possibilities in the field of taxonomy both in botany (Gyulai et al., 2000) as well as in entomology in detail in the thysanopterology (Klein and Gafni, 1996; Kraus et al., 1998). Molecular markers such as izoenzyme differences, restriction fragment length polymorphism (RFLP), and especially the PCR-based molecular polymorphisms were found to be helpful to distinguish ambiguous taxa (Klein and Gafni, 1996; Kraus et al., 1998), and also to determine species-specific RAPD-fragments as done in the comparative study of *T. tabaci* and *Frankinellia occidentalis* (Kraus et al., 1998).

In our results presented *T. tabaci* propagating on tobacco showed a characteristic level of molecular differences (72%) compared to those propagating on onion. The significant molecular variations established by these investigations confirmed the opinion of Zawirska (1976) regarding the two *T. tabaci* types (subspecies). Concerning the molecular variations among the *T. tabaci* populations occurring on onion in different agrobiotopes (Klein and Gafni, 1996) as well as the results of the investigations of Kraus and coworkers (1998) and our further investigations are require on *T. tabaci* populations.

Acknowledgements

The project was supported in part by Hungarian Research Grants OTKA T-026559, T-030267 and by the SZPÖ-Gyulai-73/99. Authors thank to Prof. Alan York (Purdue University) for his comments on the manuscript.

Literature

Doyle, J. J. and Doyle, J. L. (1990): Isolation of plant DNA from fresh tissue. Focus, 12, 13–15.

Gyulai, G., Gémesné, J. A., Sági, Zs., Heszky, L., Venczel, G. and Zatykó, L. (2000): Doubled haploid development and PCR-analysis of F1 hybrid derived DH-R2 paprika (*Capsicum annuum* L.) lines. J. Plant Physiol. 156, 168–174.

Klein, M. and Gafni, R. (1996): Morphological and molecular variations in thrips population collected on onion plants in Israel. Folia Entomol. Hung. 57 (Suppl.), 57–59.

Kraus, M., Schreiter, G. and Moritz, G. (1998): Moleculargenetic studies of thrips species. In: Vierbergen, G. and I. Tunç (eds): Proceedings of the Sixth International Symposium on Thysanoptera. Antalya, Turkey. 77–80.

Mendel, Z., Nestel, D. and Gafni, R. (1994): Examination of the origin of the Israeli population of the *Matsucoccus josephi* (Homoptera: Matsucoccidae) using random amplified polymorphic DNA-polymerase chain reaction. Ann. Entomol. Soc. Amer. 87, 165–169.

Schliephake, G. and Klimt, K. (1979): Thysanoptera, Fransenflügler, in Tierwelt Deutschlands. No. 66. VEB Gustav Fischer Verlag, Jena. pp. 477.

Zawirska, I. (1976): Untersuchungen über zwei biologische Typen von *Thrips tabaci* Lind. (Thysanoptera, Thripidae) in der VR Polen. Arch. Phytopathol. Pflanzenschutz 12, 411–422.