Molecular polymorphism among populations of *Frankliniella intonsa*

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Abstract: Molecular polymorphism of five distinct populations of *Frankliniella intonsa* (Trybom) was investigated by RAPD-PCR analysis. The individuals of both males and females were sampled from two different host plants of lucerne (*Medicago sativa*), and red clover (*Trifolium pratense*) at five sampling sites around Valkó and Nagykovácsi (Hungary). Fifteen primers were applied. On the basis of the PCR-fragments generated by the primer of OPQ14 populations of *F. intonsa* were discriminated molecularly as a result of either the locations, or sexes. Our result seemed to prove a high level of intraspecific molecular diversity in *Fr. intonsa*.

Keywords: molecular polymorphism; RAPD; Thripidae; Thysanoptera.

Introduction

Molecular analyses have been applied to characterise subspecies or forms of the same Thysanoptera species. Cenis and Beitia (1994) compared *Frankliniella occidentalis* with species of *Limothrips* and *Aeolothrips*, also several Diptera and Homoptera, detecting typical species-specific DNA-fingerprints. Klein and Gafni (1996) used RAPD markers to discriminate three different morphological types of onion thrips, *Thrips tabaci*. In Thripidae, species-specific distinctive PCR-fragments were detected in a comparison of *F. occidentalis* with *T. tabaci* (Kraus et al. 1999). Perring et al. (1993) successfully separated the morphologically indistinguishable silver-leaf type of *Bemisia tabaci* from the sweet-potato type by using molecular markers. Kraus et al. (1999) also used RAPD-PCR to detect intraspecific variability of *F. occidentalis*, detecting a DNA fragment suitable to distinguish *F. occidentalis* populations originated from Switzerland and Australia. The aim of the study presented is to analyse the molecular diversity of different populations of *Frankliniella intonsa* in Hungary.

Materials and Methods

Sampling sites. Thrips were collected from two host plants, lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*), at five localities around Valkó and Nagykovácsi (Hungary). Individuals were collected and separated to sexes under a stereomicroscope according to Jenser (1982), and Mound and Kibby (1998).

DNA extraction. For DNA extraction 100 individuals of each population were collected into five populations (Table 1). Until DNA isolation, thrips pools were stored in Eppendorf tubes containing 1 ml of DNA extraction buffer of 1% hexadecyltrimethylammonium bromide (CTAB, Sigma) at –20 °C. The extraction was performed according to the CTAB procedure (Doyle and Doyle 1990) as modified by Mendel et al. (1994). Thrips were ground to fine powder under liquid nitrogen. 400 µl of DNA extraction buffer at 60 °C was added to each tube. The homogenate was incubated at 60 °C for 30 min, followed by an addition of equal volume of chloroform:isoamyl alcohol (24:1) and separated by a microcentrifuge

Nº.	Species	Sampling site	Host plant	Sex
1	*F. intonsa*	Nagykovácsi	*Medicago sativa*	female
2	*F. intonsa*	Nagykovácsi	*M. sativa*	male
3	*F. intonsa*	Valkó	*Trifolium pratense*	female
4	*F. intonsa*	Valkó	*M. sativa*	male
5	*F. intonsa*	Valkó	*M. sativa*	female

Table 1. The sampling sites, host plants, and sexes of the populations of *Frankliniella intonsa* studied

at 13000 r.p.m. for 1 min. Total DNA was precipitated with isopropanol to the aqueous phase, washed in 70% ethanol, dried and resuspended in 100 µml $T_{10}E_1$ buffer (10 mM Tris, 1 mM EDTA). The quality of isolated DNA was determined on a 0.8% agarose gel stained with ethidium bromide.

PCR amplification. For RAPD analysis 15 decamer primers were applied (Table 2). PCR amplification was carried out in 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 dNTPs, 60 nM primer, 1.5 U Taq DNA polymerase (Promega). Cycling was carried out in a Perkin-Elmer 9600 thermocycler: 1 cycle of 94 °C for 2 min; 40 cycles of these steps of 94 °C for 10 sec, 36 °C for 30 sec, 72 °C for 1 min, and closed by 1 cycle of 72 °C for 2 min. Electrophoresis was performed in 1.2% agarose (FMC) gels. Fragment sizes were estimated by comparison to DNA molecular weight marker (GeneRuler™ 100bp Ladder Plus, Fermentas). *Analysis of PCR amplification profiles*. PCR amplification bands were scored as present (1) or absent (0). The similarity was analysed on the basis of the number of shared amplification products according to simple matching method. A dendrogram based on similarity coefficient indices was generated with the program Syn-Tax-pc 5.0 by means of the unweighted pair group method of arithmetic means (UPGMA) (Podani 1993).

Results and Discussion

All the fifteen RAPD primers tested produced scorable PCR-band pattern in the five *F. intonsa* pools. The number of bands varied from 2 to 15 per primer and the sizes of the bands varied from several hundreds of base pairs (bp) up to 1500 bp. On the basis of the PCR-fragments generated by the primer of OPQ14 populations of *F. intonsa* were discriminated molecularly as a result of either the locations, or sexes (Figs 1-2). Our results are similar to those of Kraus et al. (1999) using the primer OPA07, who found a distinct DNA fragment in *F. occidentalis* populations derived from Switzerland and Australia.

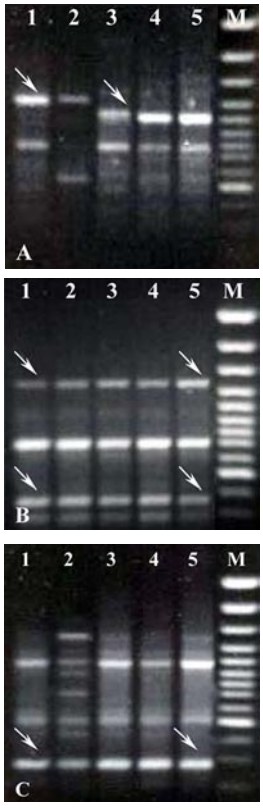

Figure 1. Samples of PCR-amplification patterns of populations of *Frankliniella intonsa*. Lanes (location/host plant/sex): 1 – Nagykovácsi/*M. sativa*/female, 2 - Nagykovácsi/*M. sativa*/male, 3 - Valkó/*T. pratense*/female, 4 – Valkó/*M. sativa*/male, 5 – Valkó/*M. sativa*/female. Primers: OP-Q-14 (A), OP-B-06 (B), OP-D-05 (C), M (Mw): 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp in length). Arrows indicate polymorphic- (A), and characteristic (B, C) PCR-fragments.

Primer	Sequence (5′-3′)	Primer	Sequence (5′-3′)	Primer	Sequence (5′-3′)
OPA01	CAGGCCCTTC	OPD05	TGAGCGGACA	OPAB09	GGGCGACTAC
OPA07	GAAACGGGTG	OPJ09	TGAGCCTCAC	NO08	ATCCGCGTTC
OPA08	GTGACGTAGG	OPQ14	GGACGCTTCA	NO11	ACGGCATATG
OPB06	TGCTCTGCCC	OPX11	GGAGCCTCAG	UBC354	CTAGAGGCCG
OPC15	GACGGATCAG	OPAI21	CACGCGAACC	PAL2	CCAGGTGGACC

Table 2. RAPD primers and their sequences applied

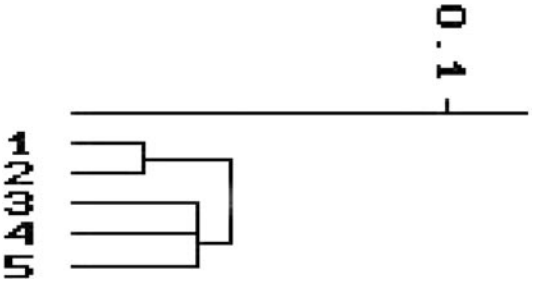

Figure 2. Genetic distances among populations of *F. intonsa* based on simple matching coefficient of similarity, Syn-Tax-pc Programme. Populations 1-2: Nagykovácsi, Populations 3-5: Valkó.

Acknowledgement

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