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Orijinal araştırma (Original article)

Detection of genetic polymorphism by RAPD-PCR in two *Trichogramma* (Hymenoptera: Trichogrammatidae) species in Turkey

Türkiye'deki iki *Trichogramma* (Hymenoptera: Trichogrammatidae) türünde RAPD-PCR ile genetik polimorfizmin belirlenmesi

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Summary

In biological control, identification of species is the first step. It is especially difficult for the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) because of their minute size and lack of reliable morphological characters. In the study, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) banding patterns were used for characterization of three laboratory cultures that represented two *Trichogramma* species [*Trichogramma brassicae* (Bezdenko) ve *Trichogramma euproctidis* (Girault)]. Thirteen of the fifteen primers were used individually to discriminate the species. Genetic variation and distance within and between species with respect to proportion of shared bands were clarified. Genetic distances within species were much lower (0.8%) than between species (68%). Our results suggest that the RAPD technique could be used to improve identification and to better understand the genetic polymorphism of *Trichogramma* spp. Potential uses of RAPD-PCR in genetic analyses on parasitic Hymenoptera are discussed in the study.

Key words: Parasitoid, *Trichogramma brassicae*, *Trichogramma euproctidis*, genetic polymorphism, molecular marker

Özet

Biyolojik mücadelede, tür teşhisi ilk basamağı oluşturur. Bu durum, mikroskobik boyutları ve güvenilir morfolojik karakterlerinin olmayışından dolayı özellikle *Trichogramma* cinsi için zordur. Bu küçük arıların tanımlanması için farklı moleküler belirteçler kullanılmıştır. Bu çalışmada iki *Trichogramma* türünü temsil eden [*Trichogramma brassicae* (Bezdenko) ve *Trichogramma euproctidis* (Girault)] üç laboratuvar kültüründen elde edilen rastgele çoğaltılmış polimorfik DNA-polimeraz zincir reaksiyonu (RAPD-PCR) bantlaşma şekilleri karşılaştırılmıştır. Onbeş primerden onüçü bireysel olarak tür ayırt edilmesinde kullanılmıştır. Tür içi ve türler arası varyasyonu ölçmek için, genetik uzaklıklar, ortak skorlanabilen bantların oranına dayanarak hesaplanmıştır. Tür içi genetik uzaklığın (%0.8), türler arası genetik uzaklıktan (68%) çok daha düşük olduğu bulunmuştur. Sonuçlar, RAPD yönteminin, tür teşhisinin ilerlemesi ve *Trichogramma* türlerinin genetik çeşitliliğinin daha iyi anlaşılması için etkili olarak kullanılabileceğini ortaya koymaktadır. Bu çalışmada, parazitik Hymenoptera üzerindeki genetik incelemelerde RAPD-PCR'ın potansiyel kullanımı tartışılmıştır.

Anahtar sözcükler: Parazitoit, *Trichogramma brassicae*, *Trichogramma euproctidis*, genetik çeşitlilik, moleküler belirteç

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Introduction

Trichogramma is a large genus of Hymenopteran parasitoids. Species belong to the genus *Trichogramma* (Trichogrammatidae: Hymenoptera) have been used in biological control of many harmful pests in a variety of crops. There are about 180 recognised *Trichogramma* species used in biological control programs (Pinto, 1998). Correct identification of species is crucial to success in biological control. However, there are systematic and genetic problems in identifying species of *Trichogramma.* Many closely related species are also problematic to differentiate morphologically (Li & Shen, 2002). Selection of the appropriate *Trichogramma* species is based on inter- and intraspecific variation. Use of the male genitalia to identify species of *Trichogramma* is still a problem because of parthenogenesis and having similar genital structures (Pinto et al., 1989; De Almeida, 2004). It should be emphasized that taxonomic studies on *Trichogramma* can not be carried out in the absence of good type specimens (Nagarkatti & Nagaraja, 1977) so clarifying the taxonomy of *Trichogramma* and correct identification is essential. Incorrect identification of natural enemies causes ineffective biological control. Hence, the molecular method based on DNA techniques including polymerase chain reaction (PCR) and random amplified polymorphic (RAPD) techniques has been used for the rapid and correct identification of these parasitoids (Laurent et al., 1998; Silva et al., 1999).

In Turkey, taxonomic studies of *Trichogramma* are relatively rare. In the study, RAPD analysis was evaluated for its ability to detect polymorphism among *Trichogramma* populations of a corn field in the south of Turkey. Specifically, the technique was used to detect the intraspecific and interspecific variability of two *Trichogramma* species, *Trichogramma brassicae* (Bezdenko) and *T. euproctidis* (Girault).

Materials and Methods

Trichogramma samples: Populations of *Trichogramma brassicae* and *Trichogramma euproctidis* were collected in the eggs of the European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera: Crambidae) from corn fields in the south of Turkey (Table 1). Females emerged from parasitized corn borer eggs were used to initiate isofemale lines. The cultures were reared at the Biology Department of Erciyes University, Turkey in eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Oztemiz, 2007).

Table 1. Origins of two *Trichogramma* species used in the study

Population	Locality/Origin	Original Host	Date collected
1: *Trichogramma euproctidis* (Girault)	N 37° 2' 28.0'' E 35° 24' 46.0'' Elevation: 220m	*Ostrinia nubilalis* Hbn.	September 2006
2: *Trichogramma brassicae* (Bezdenko)	N 37° 13' 34.0'' E 35° 5' 37.0'' Elevation: 173m	*Ostrinia nubilalis* Hbn.	September 2006
3: *Trichogramma brassicae* (Bezdenko)	N 37° 6' 17.0'' E 35° 6' 39.8'' Elevation: 86m	*Ostrinia nubilalis* Hbn.	September 2006

DNA Extraction and RAPD-PCR Analysis: DNA was extracted from one wasp of each species. They were ground in 60µl 5% Chelex-100 and 2µl Proteinase K (20mg/ml). Then they were incubated for 1h at 55 ^{o}C, followed by 10min at 96 ^{o}C. The RAPD-PCR was performed in a total volume of 15µl and contained 3µl DNA template, 1.5µl PCR buffer (10X buffer with $(NH_4)_2$ SO_4, Fermentas), 0.5µl dNTPs (10mM stock solution), 2µl random primer (10µM, Bio Basic Inc.), 0.25µl Taq Polymerase (5 u/µl, Fermentas), 1.5µl $MgCl_2$ (25mM stock solution, Fermentas), 1.2µl BSA (10mg/ml) and 5.05µl of

sterile distilled water. The temperature profile for the RAPD-PCR was a pre-denaturing step of 2.5 min at 94 °C, followed by 38 cycles of 45 s at 94°C, 45 s at 35°C and 45 s at 72°C, with a final extension step of 5 min at 72°C. The PCR products were electrophoresed in a Tris-Asedic Acid-EDTA buffer by 1% agarose gel for 1.5h at 80V. The DNA was stained with ethidium bromide and the bands were photographed under UV light. Fifteen oligomers (Bio Basic Inc.) containing 10 nucleotides were used for amplification reactions. The sequences of oligomers used in the study are shown in Table 2.

The amplicons produced were scored as present (1) or absent (0), data were then converted to a distance matrix and a dendogram was constructed with the UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) method (Sneath & Sokal, 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf, 1988).

Table 2. RAPD primers used in DNA genomic amplifications of *Trichogramma brassicae* and *Trichogramma euproctidis*

No	Sequence (5'→3')	%GC
1 (S17)	AGGGAACGAG	60
2 (S18)	CCACAGCAGT	60
3 (S19)	ACCCCCGAAG	70
4 (S20)	GGACCCTTAC	60
5 (S21)	CAGGCCCTTC	70
6 (S22)	TGCCGAGCTG	70
7 (S23)	AGTCAGCCAC	60
8 (S24)	AATCGGGCTG	60
9 (S25)	AGGGGTCTTG	60
10 (S26)	GGTCCCTGAC	70
11 (S27)	GAAACGGGTG	60
12 (S28)	GTGACGGGTG	60
13 (S29)	GGGTAACGCC	70
14 (S30)	GTGATCGCAG	60
15 (S31)	CAATCGCCGT	60

Results and Discussion

Polymerase chain reaction (PCR) was carried out using fifteen decamer random primers to define the genetic variability between three laboratory cultures of *Trichogramma brassicae* and *Trichogramma euproctidis*. Primers, with the exception of S19 and S26, effectively differentiated the cultures (Figure 1).

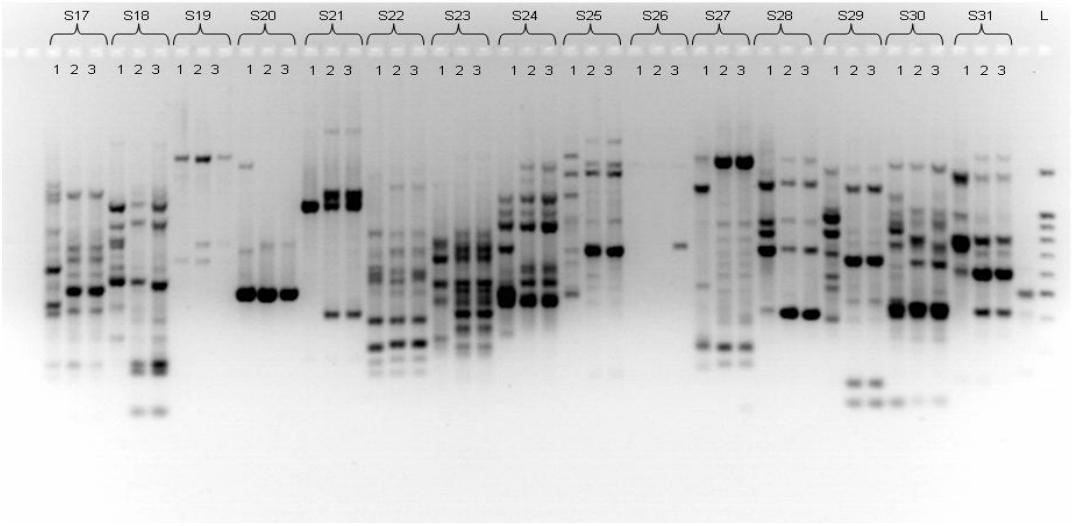

Figure1. Electrophoretic banding patterns produced by the primers S17-S31: 1 and 2 represent *Trichogramma euproctidis* and *Trichogramma brassicae*, respectively; 3 represents *Trichogramma brassicae*, (Two *Trichogramma brassicae* strains were taken from different cultures), L: low ladder size standard.

Table 3. Genetic distances between *Trichogramma* species

	Distance matrix		
Species	Te	Tb1	Tb2
Te	0		
Tb1	0,679	0	
Tb2	0,681	0,008	0

(Te: *Trichogramma euproctidis*, Tb1: *Trichogramma brassicae*-first culture, Tb2: *Trichogramma brassicae*-second culture).

Table 3 shows genetic distances calculated among three *Trichogramma* cultures from the RAPD data. Clearly, the genetic distances show that *T. euproctidis* is different from *T. brassicae,* but two *T. brassicae* strains were closely related to each other. In spite of the same banding patterns within *T. brassicae* cultures, banding profiles were completely different between *T. euproctidis* and *T. brassicae* for the S20, S21, S27, S28 and S29 primers. At the same time, S19 and S26 primers did not distinguish the two species. Nine of eleven bands from S17, six of eight bands of S18, ten of twelve bands of S23, three of six bands of S24 and three of five scorable bands of S31 primer showed variation between the two species. These polymorphic and monomorphic band numbers are given in Table 4. The within-species genetic distances were much lower (0.8%) than the between-species genetic distances (68%).

The RAPD-PCR technique has been reported as an effective method to discriminate geographically and genetically isolated populations (Jain et al., 2010) and can be used for identifying parasitic Hymenoptera (Roehrdanz, 1996). While identification of *Trichogramma* species is done by the investigation of male genital characters (Nagarkatti & Nagaraja, 1977), the use of molecular markers in RAPD can be effectively applied. Li (2007) found 3 reproducible major bands within *Trichogramma dendrolimi* (Matsumura) for only one RAPD primer. We found 76 distinct major bands between *T. euproctidis* and *T. brassicae* for the fifteen RAPD primers. One polymorphic band did not differentiate the two species and eighteen bands were monomorphic (Table 4).

Table 4. Observed polymorphism with 15 primers used according to RAPD analysis in *Trichogramma euproctidis* and *Trichogramma brassicae*

Primer	Number of total bands	Number of monomorphic bands	Number of polymorphic bands
S17	11	2	9
S18	8	2	6
S19	1	1	0
S20	2	0	2
S21	2	0	2
S22	10	2	8
S23	12	2	10
S24	6	3	3
S25	6	2	4
S26	1	0	1
S27	12	0	12
S28	5	0	5
S29	9	0	9
S30	5	2	3
S31	5	2	3
Total	95	18	77

Edwards & Hoy (1993) showed that RAPD-PCR could be effectively used for analyzing genetic variations in the hymenopteran parasitoids *Trioxys pallidus* (Haliday) and *Diglyphus begini* (Ashmead). Of the 120 primers tested, 92 produced a total of 342 scorable bands for *T. pallidus*. Twenty five primers were tested for *D. begini* and 18 produced a total of 53 scorable bands. Barnay et al. (2001) used only two oligomers containing 10 nucleotides for amplification and they identified one strain of *Trichogramma cacoeciae* (Marchal) and three different strains of *Trichogramma evanescens* (Westwood) using RAPD profiles. In our study, 13 of 15 primers identified one strain of *T. euproctidis* and two different strains of *T. brassicae*.

Before using parasitoids in a biological control program, it is important to identify the species so they can be correctly matched to the target species. The RAPD technique could provide quick, simple, effective discrimination and better understanding of the genetic variability of the corn borer egg parasitoids, *T. euproctidis* and *T. brassicae*. RAPD analysis could also be used in quality control of mass-rearing of these insects for biological control.

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