DOI: 10.5965/223811712112022066

Revista de Ciências Agroveterinárias 21 (1): 2022
Universidade do Estado de Santa Catarina

Transferability of microssatellite markers to *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae)

Transferibilidade de marcadores microssatélites para Trichogramma pretiosum Riley, 1879 (Hymenoptera: Trichogrammatidae)

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Submission: 16/08/2021 | Acceptance: 03/11/2021

ABSTRACT

The biological pest control has expanded in Brazil with the *Trichogramma pretiosum* as the main natural enemy. The microsatellite molecular markers Simple Sequence Repeat (SSR) have been the most used, as they are multiallelic, robust and reproducible, in several species. In order to optimize future processes of identification and analysis of the parasitoid's genetic diversity, twenty markers, isolated and characterized for the parasitoid wasp *Trichogramma dendrolimi*, were tested in 15 generations of *T. pretiosum*. Those markers, ten have been transferred and can be used to evaluate the genetic variation of *T. pretiosum*.

KEYWORDS: biological control; genetic variation; molecular markers; parasitoid wasp.

RESUMO

O controle biológico de pragas tem expandido no Brasil com o *Trichogramma pretiosum* como o principal inimigo natural. Os marcadores moleculares microssatélites de repetição de sequência simples (SSR) tem sido os mais utilizados, por serem multialélicos, robustos e reprodutíveis, em várias espécies. No intuito de otimizar futuros processos de identificação e análise de diversidade genética do parasitoide, 20 marcadores, isolados e caracterizados para a vespa parasitoide *Trichogramma dendrolimi*, foram testados em 15 gerações de *T. pretiosum*. Destes marcadores, dez foram transferidos e podem ser utilizados para avaliar a variação genética de *T. pretiosum.*

PALAVRAS-CHAVE: controle biológico; variação genética; marcadores moleculares; vespa parasitoide.

Biological control is a fundamental component of the balance of nature, whose essence is based on the reciprocal density mechanism, where natural enemies are mortality agents dependent on the population density of the host/prey. Among the natural enemies, there is the egg parasitoid of the genus *Trichogramma* Westwood, 1833 (Hymenoptera: Trichogrammatidae) as an alternative to be used in biological control of agricultural pests, especially the lepidopterans. The choice of strains of *Trichogramma* spp. in biological control programs is conducted by the selection of individuals who have desirable characteristics, such as aggressiveness to the host (insect pest) and adequate persistence in the field. The *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) has been the most polyphagous and abundant species in Brazil, present in regions of different altitudes (FRAGOSO et al. 2020, BARBOSA et al. 2021).

The selection process of the strains of this species can be optimized with the help of specific segments of deoxyribonucleic acid (DNA), defined as molecular markers. The amplification of microsatellite molecular markers Simple Sequence Repeat (SSR), is performed by Polymerase Chain Reaction (PCR) in specific regions of the genome, using a specific primers pair (LITT & LUTY 1989, WEBER & MAY 1989). These markers are preferred for applications in genetical enhancement studies, mainly because they are abundant in the genome, easy to automate, codominant, multiallelic, robust and reproducible, so that the polymorphism patterns displayed by SSR are larger than any other contemporary marker system (SANTOS et al. 2020, ZORTÉA et al. 2021).

The limitation of SSR markers as a tool in a genetic breeding program for *T. pretiosum*, consists of the

need for isolation and development of specific primers for this species, or have primers of related species available to assess transferability. The first polymorphic microsatellite markers for the parasitoid *Trichogramma dendrolimi* Matsumura, 1926 (Hymenoptera: Trichogrammatidae) were isolated and characterized (LÜ & HAN 2016). Therefore, the objective of this work was to analyze the transferability of SSRs developed for *T. dendrolimi* in accesses of *T. pretiosum* for 15 generations in the laboratory.

The *T. pretiosum* was in the 15th generation maintained in laboratory on the Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças (NUDEMAFI)[1] from the Universidade Federal do Espírito Santo (UFES), which provided all these generations for the study of transferability of microsatellite markers.

The genomic DNA was extracted using the protocol developed for *Trichogramma* spp. in bulks by BORBA et al. (2005) adapted from OLERUP & ZETTERQUIST (1994) in two independent repetitions (samples) per generation for analysis of ISSR (Inter simple sequence repeat). In each sample, the bulks of 100 females with larger size, without deformation and with up to six hours old, were macerated with the aid of a steel rod, directly into 250 µl of extraction buffer (Tris HCl 10 mM pH 8.0; NaCl 100 mM; EDTA 10 mM pH 8.0; SDS 0.5%) added of proteinase K (20 $\mu g.ml^{-1}$).

Posteriorly, the samples were incubated for 2 h at 37 ºC and centrifuged for six minutes at 14000 g, at room temperature. Then 130 µl of TE and 120 µl of NaCl 5M were added. New centrifugation by 15 minutes at 14000 g was proceeded and the supernatant was transferred to a new tube after 10 minutes of decantation, adding 500 µl of ethanol 95%, followed by further centrifugation for 15 minutes at 14000 g, wash with 200 µl of ethanol 70% chilled and centrifuged for five minutes at 14000 g. The DNA was resuspended in 50 µl of TE pH 8.0 with RNAse (40 $\mu g.ml^{-1}$) and quantified in the NanoDrop™ 2000/2000c Spectrophotometers Thermo Scientific.

The Laboratório de Genética e Melhoramento Vegetal (LGMV) from the UFES had twenty markers SSR (TD 01, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 16, 17, 19, 20, 21, 23, 24 and 26) developed for *Trichogramma dendrolimi* by LÜ & HAN (2016) (Table 1). All of them were provided for the transferability test of this work.

The reactions of PCR were performed according to the protocol described hereafter: 0.4 mM of markers; 1 unity of Taq polymerase; 10 $ng.\mu l^{-1}$ of DNA and 1X of buffer, 1.5 mM of $MgCl_2$ and 1mM of dNTP.

The amplification program was performed with the denaturation phase at 94ºC for 2 minutes; 10 cycles at 94ºC for 30 seconds, 58°C subtracted 1°C each cycle for 30 seconds, and 72ºC for 30 seconds, 58 °C subtracted 1 °C each cycle for 30 seconds and 72 ºC for 30 seconds. Twenty eight cycles at 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds. The last step consisted of a final extension phase at 72 ºC for 8 minutes (LÜ & HAN 2016). The amplified fragments were separated by electrophoresis in polyacrylamide 10%, for 4h, and at constant electric power of 90W. The gel was stained with ethidium bromide (25 $\mu l.L^{-1}$) for 30 minutes.

The OD 260/OD 280 ratio is a parameter to assess the quality of nucleic acid preparations (REGITANO 2007). Values less than 1.8 result from contamination with protein, residual phenol, guanidine or other reagent used in the extraction protocol. This can cause drag in the amplification of the bands, making it impossible to analyze the data. In the present work, the A260/A280 ratio was approximately 2 ± 0.07 for all samples (Table 2), which was suitable for analysis of SSR. The concentration of DNA was 63.67 ± 18.64 $ng.\mu l^{-1}$. The amplification of consistent bands occurred in 10 out of 20 primers evaluated (TD 04, 05, 07, 10, 11, 14, 19, 20, 21 and 26), in a total of 40 alleles, 19 being polymorphic (Table 3), characterized by the permanent appearance of bands (Figure 1A), permanent disappearance of bands (Figure 1B) and disappearance and appearance of bands (Figure 1C) from certain generations. This be associated with the occurrence of mutations in the flanking regions, due to the uneven pairing of homologous chromosomes in meiosis, or occurrence of null alleles that were not amplified due to DNA polymerase slippage during replication, thus preventing annealing of the primer (SCHLÖTTERER & TAUTZ 1992, CHAPUIS & ESTOUP 2007). The number of alleles per *locus* ranged from two to eight. The consistency degree of the technique was measured by analyzing two independent samples from each generation. The average of the analyzed loci in the two independent samples was 94.93%, with a range of 73.33-100% for each prime (Table 3), characterizing the high consistency degree of the SSR technique in the study of DNA polymorphism in *T. pretiosum*. Therefore, the microsatellite molecular markers transferred to *T. pretiosum* can be used for future evaluation of the genetic variation of this parasitoid.

[1]According to NUDEMAFI, the *T. pretiosum* was originally obtained from an area cultivated with maize (S 20°52'28.75" W 41°29'1.65") at Alegre county, Espírito Santo State, Brazil in September 2016.

Table 1. Characterization of microsatellites in *Trichogramma dendrolimi*.

Names	Primer sequence (5′–3′)	Size (bp)
TD01	F:AAGGCTGGCAACAAAAAAG R:CCAGATCGCAAATAACATC	150-158
TD03	F:CCGAAGTTCGTTTTTAGCAATC R:AACTCGGCGGGGATTTTCTTAC	76-90
TD04	F:TAAGGCAGAGCTTGTAAGTGGC R:GATACGCTGGTGTATGTTGGC	110-128
TD05	F:ACTTCATTAATCGCATCGG R:CCTGTAATCTTGTTTGTCTCTC	128-132
TD06	F:GACCTCGTGCTTTTTCTCTCTT R:CAATGTCGTTGTGTGTCTGTGTT	116-120
TD07	F:CAGGAATATCACAGGCTTACCATC R:AGAGTGTCAGCGAGAGCGAAC	120-140
TD09	F:GCATATACAGACACACATACATTGG R:GCGGCTCGTTTATCTTGACTT	244-256
TD10	F:TATAGCTCTTGCTCGTCAGTGG R:CGAATTTTCTTTCTCGCTCTCTC	219-227
TD11	F:ATGCATTGGCTGCCGGAAGAG R:CACATACCGCGGGAATAGAAAAC	170-184
TD12	F:TGGGATAGTAAAGTGCCTCGTG R:CAAGATCGTCGTGCCTACATAAT	138-147
TD13	F:TTGGCGGAGGCGGATGAAAA R:ACGAGAGCAGGAAAAAGGGAACG	217-225
TD14	F:AATACTTGAGCGGCGACAT R:TTCCTACCTCTACCTCCCACC	210-229
TD16	F:AAAGACCGACACACAGAGGAAAG R:TGTGCAGTAAGAGGACTTGAATGAC	160-176
TD17	F:TTGGGATAGTAAAGTGCCTCGT R:CAAGATCGTCGTGCCTACATAAT	142-151
TD19	F:CCGCACATACACCCACAT R:GAACGCTTCAAACCACTCG	130-140
TD20	F:ACTGGAATATCCCGCTGGTGT R:GGAAACTCGGACTTTTATCGTGG	100-116
TD21	F:CTGGCAAGCGCACTTATATCTT R:AGCTCAGCCTGGGAAAATCT	132-147
TD23	F:CATCATCAACACCATCAGCAGCAGT R:AAGCGACAGTGGCAGGATCTCAGG	188-200
TD24	F:AGCCGGTGTCCTGTTCTTTG R:CCTTCGACTTTTTGAATCGTGAG	182-190
TD26	F:AGTTTACAACTATCCCGAATG R:TACTACTAATACTACCACACCACAG	190-197

Research source: LÜ & HAN (2016).

Table 2. Analysis of purity and quantity of genomic DNA from 100 females of *Trichogramma pretiosum*.

Samples	Generations	OD260/OD280	DNA concentration (ng.µl^{-1})
1	1	1.94	41.3
2	1	1.99	58.1
1	2	2.00	33.7
2	2	1.68	83.9
1	3	2.03	26.1
2	3	2.01	66.3
1	4	2.03	20.4
2	4	1.97	59.9
1	5	2.03	28.9
2	5	1.98	72.8
1	6	2.03	30.1
2	6	1.99	68.6
1	7	1.99	40.8
2	7	1.94	63.1
1	8	1.97	49.0

To be continued...

Continuation

2	8	1.94	57.2
1	9	1.92	26.7
2	9	2.01	77.0
1	10	2.01	34.2
2	10	2.02	64.1
1	11	1.97	28.5
2	11	1.98	65.0
1	12	1.92	36.1
2	12	2.03	54.3
1	13	2.02	32.9
2	13	1.97	79.4
1	14	1.98	46.9
2	14	2.03	75.1
1	15	2.04	34.6
2	15	1.94	57.0
Average		2.00 ± 0.07	63.67 ± 18.64

Table 3. Primers amplified in *Trichogramma pretiosum*.

Markers	NA[1]	NPA[2]	CD (%)[3]
TD04	2	0	100.00
TD05	2	2	86.67
TD07	2	2	73.33
TD10	2	0	100.00
TD11	8	2	100.00
TD14	3	1	91.11
TD19	7	5	99.05
TD20	8	6	99.17
TD21	3	0	100.00
TD26	3	1	100.00
	Total = 40	Total = 19	Average (Amplitude) = 94.93 (73.33-100)

[1]NA = number of alleles. [2]NPA = Number of Polymorphic Alleles. [3]CD = Consistency Degree.

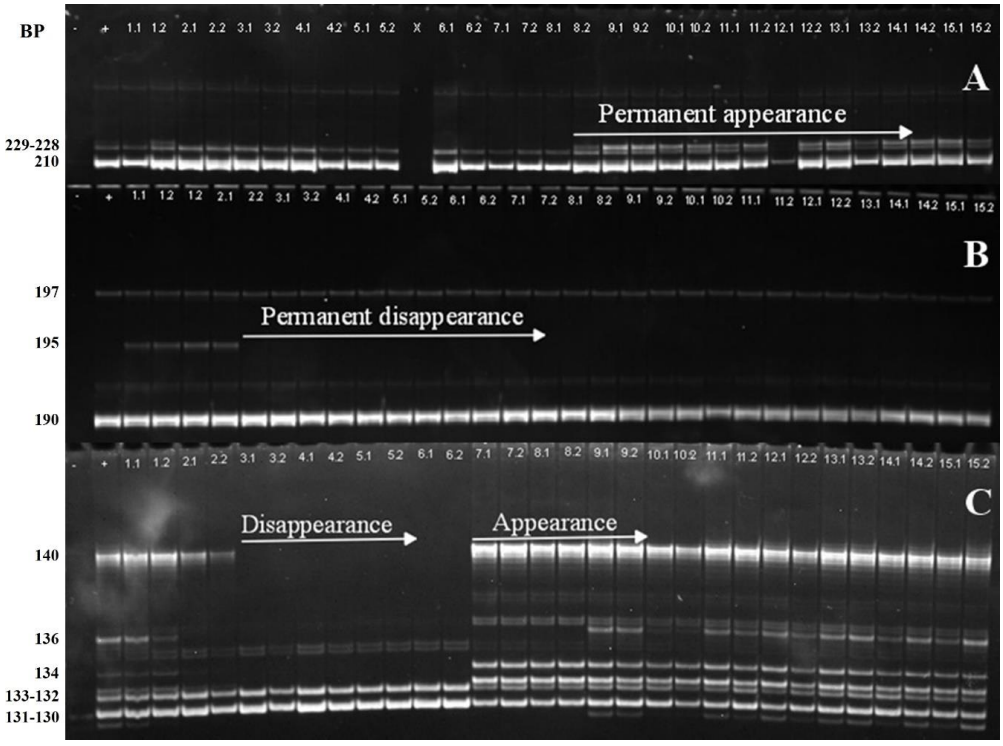

Figure 1. Visualization of band effects in DNA of *Trichogramma pretiosum* by successive generations. (A) Bands permanent appearance on primer TD 14. (B) Bands permanent disappearance permanent on primer TD 26. (C) Band disappearance/appearance on primer TD 19 (C). BP = Size (nitrogen base pairs). Negative control (-) = water instead DNA. Positive control (+) = Standard DNA. (n.y) = (Generation. Sample). x = No sample, as it was accidentally punctured.

ACKNOWLEDGEMENTS

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES).

The identification of *Trichogramma pretiosum* Riley, 1879 was accomplished by the DSc. Débora Ferreira Melo Fragoso.

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