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RESEARCH ARTICLE

MOLECULAR BIOLOGY

GENETIC CHARACTERIZATION OF *BACTROCERA (DACUS)* FLIES (DIPTERA: TEPHRITIDAE) BASED ON RAPD-PCR

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ABSTRACT

Genetic characterization of two species of fruit flies belonging to the genus *Bactrocera* (=*Dacus*), namely, *B. cucurbitae* and *B. dorsalis* was carried out using ten RAPD markers. In *B. cucurbitae* and *B. dorsalis* twenty four and twenty one fragments were amplified ranging from 150bp-2000bp, respectively. Four out of ten primers were monomorphic. Mean heterozygosity in *B. cucurbitae* and *B. dorsalis* was 0.119 and 0.093, respectively. Nei's genetic identity value (0.831), calculated using Tools For Population Genetic Analysis (TFPGA) software, reveals very close genetic similarity between the two species.

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KEYWORDS

RAPD-PCR, *Bactrocera,* molecular markers, heterozygosity, genetic identity.

INTRODUCTION

Fruit flies belonging to the family Dacinae, are biologically interesting and economically very important group of Diptera, as larvae belonging to this family are known to infest a wide range of plant species causing severe loss to fruits and vegetable crops.[1,2]

Several molecular markers e.g. allozymes, Random Amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR), Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP), sequencing of mitochondrial DNA sequences (COI, COII, ND-1 and ND-5), Single Stranded Conformation Polymorphism (SSCP), Single Nucleotide Polymorphism (SNP) and microsatellites have been extensively used to unravel intra and interspecific genetic variations, population structure, migration pattern and phylogenetic relationships among fruit flies *Rhagoletis, Anastrepha, Ceratitis* and *Bactrocera (=Dacus)* of the family Tephritidae.[3-19]

In the present study, genetic variation has been analyzed in two species of fruit flies of the genus *Bactrocera (=Dacus)* using RAPD-PCR markers.

MATERIALS AND METHODS

Larvae of *B. cucurbitae* (Coquillett) and *B. dorsalis* (Hendel) were collected from sponge gourd (*Luffa aegyptiaca*) and guava (*Psidium guajava*), respectively, and laboratory colonies were established and maintained at 26±1ºC.

Genomic DNA was amplified using ten random decamer primers (Table 1). For Random Amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) studies DNA extraction, PCR amplifications, visualization of gels and interpretation of the bands were performed following the method adopted by Bajpai and Tewari[20].

A negative control amplification reaction was also performed for each primer. For each individual the amplification reactions were repeated twice to determine the consistency and reproducibility of bands. Individuals exhibiting a particular band were assumed to be homozygous or heterozygous for a dominant allele at that locus while the individuals which failed to exhibit a band at that locus were interpreted as homozygous recessive[21]. Average heterozygosity, Nei's[22] genetic identity and distance were calculated by using Tools For Population Genetic Analysis (TFPGA) software[23].

RESULTS

Out of the ten primers used primer numbers 5 and 7 did not produce any amplicons, while rest of the primers produced discernible bands. The oligonucleotide primers used, number of fragments amplified, heterozygosity, range of size of amplicons and polymorphic loci (%) are presented in Table 1. In *B. cucurbitae* twenty four fragments were amplified ranging from 150 bp - 2000 bp with a mean heterozygosity of 0.119, while in *B. dorsalis* twenty one fragments were amplified which ranges from 150 bp -1864 bp with a mean heterozygosity of 0.093 (Table 1). The genetic identity value was found to be 0.831. The primers used in the present study were able to generate specific banding pattern considered to be ideal for identification of species. RAPD-PCR amplification pattern of two species with primer number 2 and 4 are shown in Fig. 1a and b.

Table : 1

Number of amplified fragments, range of size of amplicons, heterozygosities and polymorphic loci (%) for all the primers.

Primer number	Sequence (5' - 3')	*B. cucurbitae* (n = 20)		*B. dorsalis* (n = 20)		Range of size of amplified fragments in base pair (bp)
		No. of amplified fragments	Polymorphic loci (%)	No. of amplified fragments	Polymorphic loci (%)	
1.	CCAGCCGAAC	5 [1] (0.300)	85.71	2[1] (0.143)	42.86	250 - 2000
2.	GACTAGGTGG	4 [3] (0.078)	16.67	5[3] (0.148)	33.33	300 - 1864
3.	GGGACGTTGG	3 [3] (0)	0	2 [2] (0)	0	233 - 500
4.	AGGGTCGTTC'	3 [1] (0.252)	66.67	3 [1] (0.224)	66.67	560 - 1000
5.	CTCTGTTCGG	0 (0)	--	0 (0)	--	--
6.	TGCGTGCTTG	4 [4] (0)	0	4 [4] (0)	0	250 - 1055
7.	GTCCCGACGA	0 (0)	--	0 (0)	--	--
8.	TGATCCCTGG	1[1] (0)	0	1[1] (0)	0	250
9.	CAGGCCCTTC	1[1] (0)	0	1[1] (0)	0	550
10.	TGCCGAGCTG	3 (0.329)	100	3 (0.336)	100	150 - 1000
	Mean	**0.119**	**33.85**	**0.093**	**30.85**	

n = sample size; Values under square bracket represent monomorphic band;

Values under common bracket represent heterozygosity

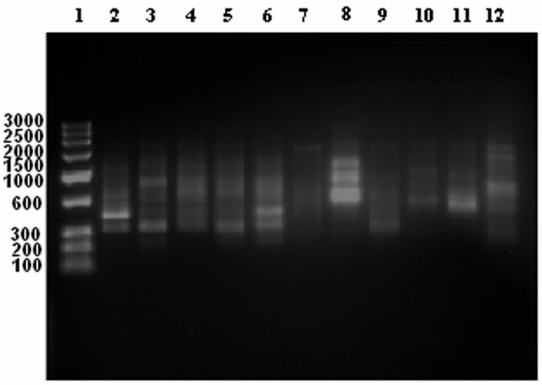

Fig. 1-(a) RAPD pattern with primer 2, Lane 1: Molecular weight marker (Low range DNA ruler), Lane 2-6: *B. cucurbitae* , Lane 7-12: *B. dorsalis*

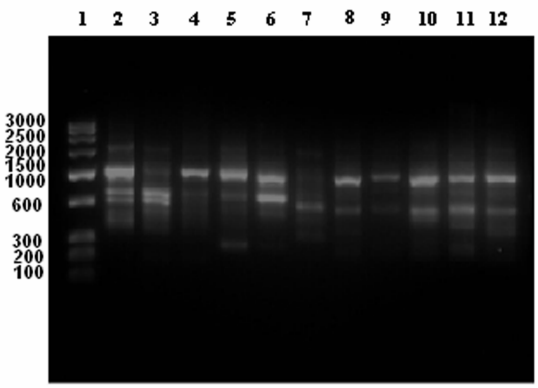

Fig. 1-(b) RAPD pattern with primer 4, Lane 1: Molecular weight marker (Low range DNA ruler), Lane 2-6: *B. cucurbitae* , Lane 7-12: *B. dorsalis*

DISCUSSION

Amplification with ten primers produced a total of twenty four fragments ranging in size from 150 bp - 2000 bp, out of ten primers used four primers have been found to be monomorphic. However bands reported as monomorphic may hide genetic heterogeneity because of the dominance of RAPD markers[24].

The mean heterozygosity found at RAPD loci in *B. cucurbitae* is 0.119 and in *B. dorsalis* is 0.093, suggesting that the genetic variation in these species is low. The genetic identity between the two species was found to be 0.831 indicating that they are very closely related. Such a close level of similarity is suggestive of the fact that the differentiation between the two species has been accomplished with relatively little genetic change[25,26]. Such a low level of heterozygosity and high genetic identity has also been observed in other families of Diptera also (Table 2).

Table: 2

Average heterozygosity, genetic identity and dissimilarity index for members belonging to families Tephritidae, Oestridae, Calliphoridae and Muscidae.

Name of the family/ Species	Average heterozygosity (range)	Genetic identity (range)	Genetic divergence (range)	Reference
Tephritidae				
Ceratitis capitata population	0.08-0.29	-	0.158-0.357	Baruffi et al[27]
Oestridae				
Dermatobia hominis	0.151-0.168	0.708-0.734	-	Azeredo-Espin and Lessinger[28]

population				
Calliphoridae				
Cochliomya hominivorax population	0.006-0.140	0.582-0.653	-	Infante-Malachias et al[21]
Muscidae				
Haematobia irritans populations	-	-	0.041-0.204	Vasconcelos et al[29]
Sarcophagidae				
Sarcophaga sp.	0.110-0.130	0.680-0.785	0.242-0.381	Bajpai and Tewari[20]

However, an extensive survey among dacine fruit flies, with the help of RAPD-PCR and other DNA based molecular markers needs to be carried out to unravel genetic variations and genetic relatedness among these economically important flies.

CONCLUSION

RAPD-PCR is found to be most easy and robust technique to distinguish two *Bactrocera* species. It will provide support to taxonomist, as it reveals information based on genetic constitution. The amplification pattern obtained by RAPD primers were considered to be ideal for identification of species. This technique has been widely used for systematic and population genetics studies in dipterans of medical, veterinary and economic importance.

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