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Research Article

ASSESSMENT OF GENETIC VARIABILITY OF TWO FRESHWATER PRAWNS, *MACROBRACHIUM DAYANUM* AND *MACROBRACHIUM LAMARREI* FROM JAMMU REGION BY USING ISSR MARKERS

***Raman Jasrotia., Seema Langer., Palaq., Nipoon Sharma and Rakesh K Panjaliya**

Department of Zoology, University of Jammu (J&K) India

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ABSTRACT

The present study evaluated the genetic variations using ISSR-PCR technique for the first time on two species of *Macrobrachium* from Jammu region of J&K, India. The Inter Simple Sequence Repeat Polymorphic DNA (ISSR) analysis was employed to estimate the genetic relationships and diversity among two species of local prawns (Family Palaemonidae). The specimens for present investigation were collected from water bodies of Kathua district, Samba district and Jammu district of Jammu and Kashmir. Genomic DNA was extracted from pleopods using salt extraction method. The quality and quantity of extracted DNA were analysed by Agarose Gel Electrophoresis as well as Spectrophotometry. DNA was amplified using ISSR primers in the Polymerase Chain Reaction (PCR). Values obtained from the bands on Agarose gel (1.8%) in Tris Borate EDTA buffer (TBE) were scored and analyzed. Results showed that the bands were in the range between 350 and 1000 bp. Maximum number of bands were recorded for *M. dayanum* of Kathua district. When a comparison was drawn within the populations of *M. dayanum* and with population of *M. lamarrei*, it has been found that the population of *M. dayanum* of Kathua district showed higher degree of genetic polymorphism. Amplified DNA fragments with monomorphic profile were also evident. *Macrobrachium dayanum* showed high levels of genetic variability at inter and intra-specific level.

INTRODUCTION

Freshwater ecosystems are among those where biodiversity express itself in an intense and vivid manner. Jammu and Kashmir is the Northernmost state of India surrounded largely by Himalayan mountains, endowed with rich aquatic resources such as rivers, streams, lakes, ponds and springs that are enriched with prosperous faunal diversity. Prawns (shrimp like shellfishes) constitute a major component of the crustacean biodiversity of stream ecosystems of Jammu. They are rich source of high quality proteins, vitamins (A and D), important minerals and Omega-3 fatty acids which are good for our health. The local prawn *Macrobrachium dayanum* is a commercially important shellfish and is a potential candidate for raising its culture (Jhingran, 1994) as nutritionally also it stands at par with other culturable fish species (Langer *et al*. 2004). Due to changes in their natural environment by pollution as well as by anthropogenic pressures, several faunal species are either exploited or depleted. Even *M. dayanum* once readily available in a number of water bodies in Jammu (Kailoo, 1984) has observed a significant population decline in the recent years (Chalotra, 2002; Kumari, 2008). The genetic diversity is crucial for the long term survival of the species because it provides the raw material for adoption and evolution, especially when environmental conditions have changed (Kumari and Thakur, 2014; Ganaie and Ali, 2016). Thus assessment of genetic diversity present within a species is a prerequisite for developing a sustainable conservation program and efficient management plan.

Variations at the molecular (genetic) level form a key aspect for the characterization of species. Molecular markers play an important role in the evaluation of genetic diversity and genetic variability within and between species and populations (Powell *et al*. 1996; Guasmi *et al*. 2012). Several advance techniques based on DNA polymorphism to study genetic diversity are provided by molecular biology. The choices include Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Microsatellite Polymorphism (RAMP) and Inter Simple Sequence Repeat Polymorphic DNA (ISSR).

Inter-Simple Sequence Repeats (ISSRs) are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. ISSR markers amplify inter-microsatellite sequences at multiple loci throuhout the genome

Corresponding author:* **Raman Jasrotia
Department of Zoology, University of Jammu (J&K) India

(Wang *et al*. 2002; Chistiakov *et al*. 2006; Yang *et al*. 2010). The genotyping by ISSR analysis involves the detection of polymorphism in microsatellite and intermicrosatellite loci without prior knowledge of DNA sequences (Yang *et al*. 2010). ISSR analysis has been used extensively to assess genetic diversity and population genetic structure of both plant (Tanyolac, 2003; Guasmi *et al*. 2012) and animal species (Luque *et al*. 2002; Wang *et al*. 2002; Shouhani *et al*. 2014) as it yields better results over RAPD and allozyme analyses (Esselman *et al*. 1999).

The present investigation is an attempt to determine the genetic variations among three populations of *M. dayanum* and its comparison with a population of *M. lamarrei*. The data so generated will serve as a baseline for developing potential selective breeding program and culturing of these species.

MATERIAL AND METHODS

Prawn samples

The specimens of *Macrobrachium dayanum* (Figure 1a) were collected from Nagri stream (32° 30' N, 75° 44' E) and Chadwal stream (32° 28' N, 75° 19' E) of Kathua district and R.S. Pura streams (32° 30' N, 74° 43' E) of Jammu district and that of *Macrobrachium lamarrei* (Figure 1b) from stream fed ditches of Kheri (32° 37' N, 74° 52' E) (Samba district). The streams at Nagri and Chadwal are rivulets of river Ravi whereas Kheri and R.S. Pura streams are rivulets of river Tawi. For collection, cast net of mesh size 5mm x 5mm were used. The live specimens were brought to the Department of Zoology, University of Jammu in the plastic containers and were maintained in the departmental ponds equipped with aerators and dead specimens were preserved in 70% alcohol until DNA was isolated for genetic analysis.

Figure 1a *Macrobrachium dayanum*

Figure 1b *Macrobrachium lamarrei*

Table 1 ISSR primers tested in this study

Primers	Sequence of primers (5'-3')
ISSR 1	CACACACACACACACAAT
ISSR 2	CACACACACACACACAAC
ISSR 3	CACACACACACACACAGT
ISSR 4	CACACACACACACACAGA
ISSR 5	GGTCACACACACACACAC

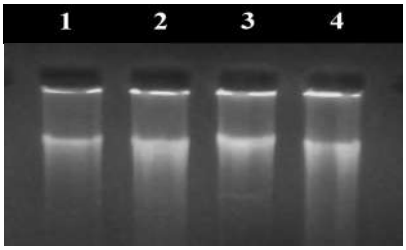

Figure 2 Genomic DNA (Lane 1 to 4) of *Macrobrachium dayanum* after agarose gel electrophoresis

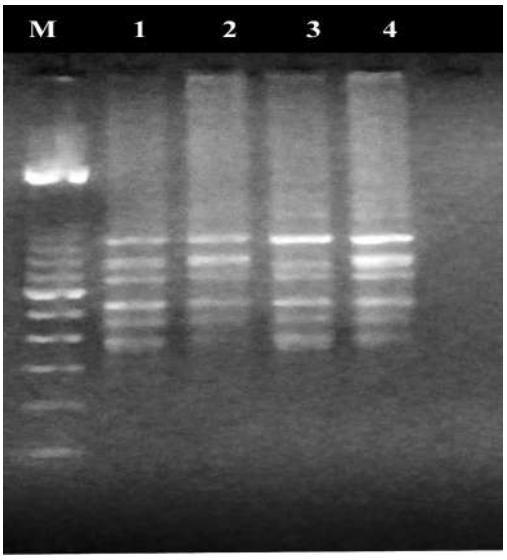

Figure 3 ISSR banding pattern of Nagri population of *M. dayanum* (Lane 1 to 4) after PCR with primer 1
Lane M: 100bp DNA ladder
Lane 1: 390, 480, 520, 700, 800, 1000 bp
Lane 2: 390, 480, 520, 700, 800, 1000 bp
Lane 3: 390, 480, 520, 700, 750, 1000 bp
Lane 4: 390, 480, 520, 700, 800, 1000

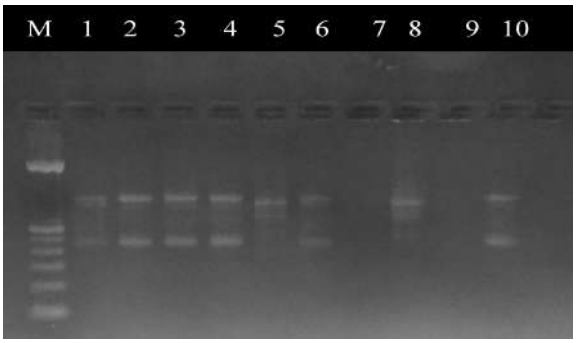

Figure 4 ISSR banding pattern of Kheri population of *M. lamarrei* (Lane 1 to 4), Chadwal population of *M. dayanum* (Lane 5 to 8) and R.S. Pura population of *M. dayanum* (Lane 9 & 10) after PCR with primer 1

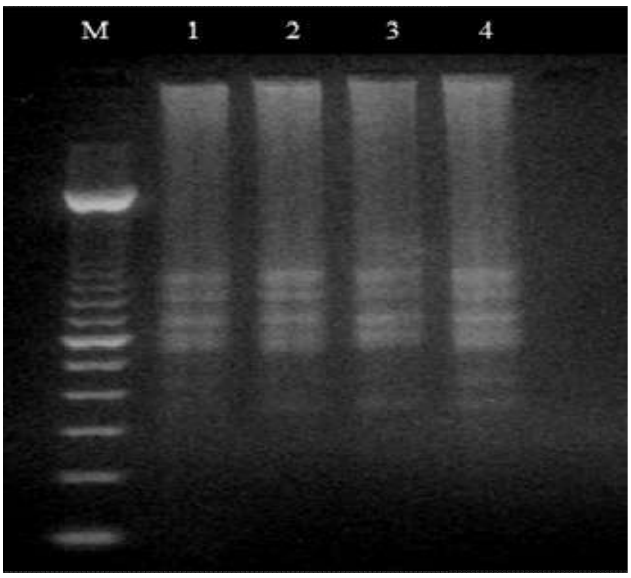

Figure 5 ISSR banding pattern of Nagri population of *M. dayanum* (Lane 1 to 4) after PCR with primer 2

DNA extraction

The genomic DNA (Figure 2) was isolated from the pleopodal tissue using Salt Extraction method (Aljanabi and Martinez, 1997) with slight modifications. The extracted DNA was stored at -20^{o} C till further analysis. The qualitative and quantitative analysis of the genomic DNA was done by using 1.2% agarose gel electrophoresis in TBE and Spectrophotometry at 260nm.

ISSR-PCR Analysis

A set of five ISSR Primers (Table 1) were tested for the present investigation. Out of five primers, we got good and scorable results for first two primers only and for rest three primers we got unclear bands. Further detailed analysis was therefore done by using ISSR 1 and ISSR 2. PCR reaction was performed in thermal cycler of Applied Biosystems thermal cycler (Make Veriti by Life Technology, Singapore), using 25µl of reaction mixture containing 2 µl DNA, 2.5 µl reaction buffer, 1.0 µl dNTPs (10 mM), 1.0 µl Taq Polymerase (1U/µl), 2.5 µl $MgCl_2$ (25 mM), 2.0 µl primer and 14 µl PCR water to make up the final volume of 25 µl. The conditions used for the amplification were as follows: an initial denaturation step at 95°C for 5 minutes; followed by 45 cycles of 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute; and finally elongation (extension) at 72°C for 10 minutes.

The PCR products were separtaed according to size by electrophoresis at 80 V on 1.8% agarose gel in 0.5x TBE and visualized under ultraviolet light after ethidium bromide staining. One hundred base pair DNA ladders were used as markers to estimate the size of amplified products. Clear and intense bands were scored while faint bands against background smear were not considered for the further analysis. The ISSR-PCR data was then statistically analysed using Jaccard's similarity coefficient and dendrogram was constructed.

Table 2 Showing PCR products after ISSR genotyping

Primer	*Macrobrachium dayanum* (PCR Products (bp))			*Macrobrachium lamarrei* (PCR Products (bp))
	Nagri stream	Chadwal stream	R.S.Pura Stream	Kheri Stream
ISSR1	1000	1000	1000	1000
	-	-	-	900
	800	800	-	-
	700, 750	700	-	-
	520	500	-	-
	480	480	480	480
	390	-	-	-
ISSR 2	950	-	-	-
	810	800	-	-
	700	700	700	700
	600	600	600, 650	600, 650
	580	-	-	-
	410	-	-	-
	350	-	-	-

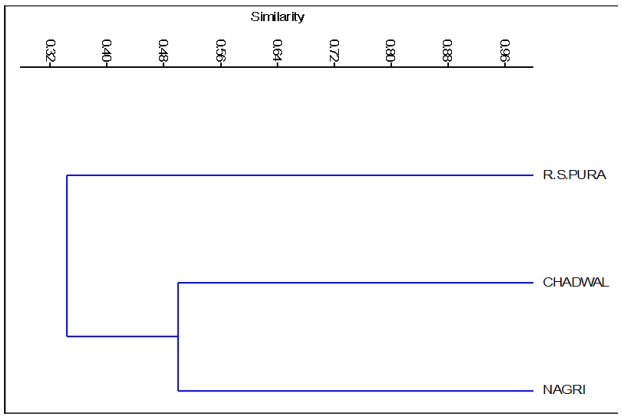

Figure 6 Dendrogram based on UPGMA cluster analysis (PAST Software 2.09) for three populations of *M. dayanum*: Nagri population, Chadwal population and R.S. Pura population using ISSR-PCR data for ISSR Primer 1.

RESULTS

For each specimen, each fragment/band that was amplified using ISSR primers was treated as unit character. The presence of bands on each and all the loci of the population indicates monomorphism while the presence of bands in some and not all the loci indicates polymorphism.

Populations of M. dayanum

Nagri stream

For primer 1, seven bands/fragments were obtained after PCR amplification and size of DNA fragments were 390, 480, 520, 700, 750, 800 and 1000bp (Figure 3). For primer 2, seven bands of the size of 350, 410, 580, 600, 700, 810 and 950 bp were obtained (Figure 5).

Chadwal stream

For primer 1, four bands were obtained after PCR amplification and size of DNA fragments were 480, 500, 700, 800 and 1000bp (Figure 4). For primer 2, three bands of the size of 600, 700 and 800 bp were obtained.

Stream of R.S. Pura

For primer 1, only two bands of the size of 480 and 1000bp were obtained (Figure 4). For primer 2, three bands of the size of 600, 650 and 700 bp were obtained.

Population of M. lamarrei

For primer 1, three bands of the size of 480, 900 and 1000 bp were obtained (Figure 4). For primer 2, three bands of the size of 600, 650 and 700 bp were obtained.

The banding patterns of the three populations of *M. dayanum* and single population of *M. lamarrei* were different. The number of bands per individual ranged from 2-7 (Table 2) and bands amplified ranged in size from 350-1000 bp. The molecular size of the bands was estimated with a molecular DNA ladder of 100 bp. The genetic results of the studied species can be interpreted in the following points:

- Among three populations of *M. dayanum* the two monomorphic bands (bands shared by all the populations) (480 and 1000 bp) were obtained using primer 1 and two monomorphic bands (600 and 700 bp) were obtained using primer 2.
- For Nagri stream, we got three polymorphic bands /alleles (390, 520 and 750 bp) with primer 1 and five polymorphic bands (350, 410, 580, 810 and 950 bp) with primer 2. For Chadwal stream, we got one polymorphic band each with primer 1(500 bp) and with primer 2 (800 bp) whereas for R.S. Pura stream no polymorphism was observed. Thus the comparison reveals that the Nagri population exhibits highest degree of genetic polymorphism among the three populations.
- The banding profile also unveil that the Nagri and Chadwal stream populations have close similarity as compared to the third stream. *M. lamarrei* when compared with the populations of *M. dayanum*, two bands were similar and one band of 900 bp was different which may serve as diagnostic allele to distinguish between the two species. The polymorphism was more in *M. dayanum* than *M. lamarrei*.
- The closeness of bands of *M. lamarrei* of Kheri stream and *M. dayanum* of R.S. pura stream was also found.
- Dendrogram based on Jaccard's similarity coefficient was also constructed in PAST Software 2.09 (Figure 6) for three populations of *M. dayanum* for ISSR marker 1. It showed that the two populations (Nagri and Chadwal) are genetically similar compared to population of R.S. Pura stream.

DISCUSSION

The present study represents first attempt to investigate the genetic variations of the two local prawn species of Jammu region of J&K State by ISSR markers. When compared with other genetic markers, ISSR has many advantages such as quick, easy to handle, highly reproducible and polymorphic, and requiring no prior genome sequence information. In the current study, it has been found that at the inter population level, the population of *M. dayanum* of Nagri stream exhibits greater genetic polymorphism and genetic diversity than that of other two streams. There were several alleles found in the nagri population which may be used to distinguish it from other populations. At interspecific level i.e. between *M. dayanum and M. lamarrei* also, the former is more genetically variable than the later. The maintenance of genetic polymorphism reflects the process of adaptation to environmental heterogeneity.

Among the three populations, the nearness of banding profile indicated that nagri population and Chadwal population of *M. dayanum* are genetically most similar. The reason for this similarity can be attributed to the fact that these two streams are the rivulets of the same River Ravi and the surprising similarity between *M. dayanum* of R.S. Pura and *M. lamarrei* of Kheri can also be possibly explained by the fact that these two streams have common source i.e. River Tawi.

Our results are comparable to the study of genetic diversity of *M. nipponense* of China using ISSR primers by Yang *et al*. (2010) in which Poyang lake population exhibits great level of variability whereas Yangtze River population exhibits the lowest level of variability. Rebello *et al.* (2015) investigated the genetic variability in East and West coast penaeid prawn, *Penaeus monodon* from two wild natural stocks using RAPD technique. Results indicated that these two populations may be distinct genetic stock which is quite similar to our results as three studied populations of *M. dayanum* also exhibited different bands.

The genetic polymorphism in the population can be due to variations in environmental factors such as temperature, alkalinity and pollution (Ponniah, 1989). The role of temperature in maintaining alleles at different frequencies has been proved in natural populations (Nyman and Shaw, 1971) as well as experimentally (Mitton and Koeh, 1975).

The pattern of genetic variability and genetic diversity observed in the present study in two local prawns can be used as baseline information for protection, management of population and selective breeding programmes.

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