ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 10, October 2021

Determination of Genetic Diversity in Cichlid Ornamental Fishes Using RAPD Molecular Markers

[1] Sai Bindu Gadde
[1] B. Tech Biotech, Vignan University, Guntur, Andhra Pradesh
[1] *Saibindu.811@gmail.Com*

***Abstract:* - CICHLID fishes have diversified and innovative characteristics. Identification of chromosomal variation is more upgraded than the identification of morphological character of this fish species. Recognition of special biogeographic patterns is important to understand the genotype of these fishes. This research article will focus on genetic diversity and topologies relationship among the Cichlid fishes. Analysis of different kinds of cichlid variation is essential for enhancement of economic prosperity from the fish cultured ornamental fishes are one of the innovative and wonderful creatures with different characteristics. Demand for ornamental fishes is increasing day by day, though it is providing a huge opportunity for enhancement of economic stability. Nowadays, government agencies have introduced different promotional schemes for boosting entrepreneurship activities. Discussion on ornamental fish, cichlid variety and RAPD will be useful to justify this researched article.**

The use of RAPD PCR is one of the innovative techniques to determine the subspecies of the selected fish. DNA (RAPD-PCR) is one of the innovative molecular tools to identify the characteristics of genetic diversity. In this case, RAPD fingerprinting is the most effective way to demonstrate the differences of the family, kingdom, species of fish. Genetic variation is enabled to focus on the genetic taxonomic to enhance knowledge on fishes' biodiversity. The proportion of genetic diversity, polymorphic activities helped to acquire knowledge on breeding activities. Genomic DNA of some particular Cichlid Species such as Blue peacock, green jewel has been mentioned in this paper. DNA isolation of the selected fishes has been discussed. DNA fragments of these fishes have been discussed in this project. Recognition of the ecological opportunity helps to increase the adaptability of these fishes. Genetic diversity helps to acquire knowledge on the biodiversity of the fishes. This project will determine the importance of DNA finger printing to enhance knowledge on this topic.

Keywords: DNA, RAPID, UPGMA, Ornamental fish, Cichlid

I. INTRODUCTION

Ornamental fishes are occupying a second position to give pleasure to people. Increasing demand for this fish is increasing the entrepreneur's profitability. DNA fingerprints help to eradicate the practices of taxonomic methods, this method is used to identify fishes according to their morphological characteristics.

Ornamental fish

Ornamental fishes are known as living jewels in the aquatic system. There are about 30,000 fish spices, among that, near about 800 are from ornamental fish. Due to biodiversity, climate changes and colour availability, India has a great potential for the development of ornamental fish culture in India. There are two different characteristics of ornamental species, such as exotic and indigenous. Nearly 90% of native species are collected to continue the export business. According to Santos et al. (2016) [7], effective indigenous species are Zebrafish, honey gourami, black shark, black knife fishes are important fishes. According to Usman et al. (2013) [8], DNA fingerprints are one of the most effective tools for the identification of genetic variation.

Moreover, it helps to identify the genetic background of ornamental fishes. Enhancement of technology helps to emerge innovative technological tools such as real-time PCR, microarray chips for getting effective results on DNA fingerprinting (Henning et al. 2017) [2]. Nowadays, the use of DNA (RAPD-PCR) is increasing for the detection of ornamental fish's genetic polymorphism. DNA fingerprints, RAPD techniques and DNA analysis helped to identify the diversity of fishes.

Cichlid variety

Genomes and adaptive radiation are two important parts of biological organisation. According to Koh et al. (1999) [4], cichlids belong from the vertebrate diversity, there are near about 2500 cichlid fishes. Due to small radiation, India has 3 species, Madagascar has 35 species and South Africa has 400 species. Strawberry Cichlid is from "captive-bred variety", it is from the Animalia kingdom. Different types of cichlid will be discussed in the following section.

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 10, October 2021

Figure 1: Blue peacock
CLASS: ACTINOPTERYGII
GENUS: AULONOCARA
SPECIES: A. NYASSAE

Figure:2 Yellow morph
CLASS: ACTINOPTERGII
GENUS:AULONOCARA
SPECIES:A.BAENSCHI

Figure:3 Green terror
CLASS: ACTINOPTERGLL
GENUS:ANDINOACARA
SPECIES:A.RLVULATUS

Figure:4 Green jewel
CLASS:ACTINOPTERGII
GENUS: HEMICHROMINI
SPECIES:HEMICHROMINI

Figure:5 BI COLOR Peacock
CLASS: ACTINOPTERGII
GENUS:MAULANA
SPECIES:AULONOCARA

Figure:6 red peacock
Class: ACTINOPTERGII
Genus: AULONOCARA
Species: A.JACOBFRELBERGI

Figure: 7 TILAPIINE CICHLID
Class: ACTINOPTERYGII
Genus: PSEUDOCRNILABRINAE
species: TILAPIINI

Figure :8 tiger Oscar
Class: ACTINOPTERYGII
GENUS:ASTERONOTUS
Species: A.OCELLATUS

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 10, October 2021

Figure:9 white cichlid
class: ACTINOPTERGII
genus: ALBINO
species: PINDANI

Figure:10 straw berry cichlid
Class: ACTINOPTERYGII
Genus: ALBLNO
Species: ALBLNO

DNA ISOALTION:

S.NO	Primers	Length	Sequence
1	OPA-05	10	5'AGGGGTCTTG3'
2	OPA-07	10	5'GAAACGGGTG3'
3	OPA-10	10	5'GTGATCGCAG3'
4	OPA-4	10	5'AGTCAGCCAC3'
5	OPA-3	10	5'AATCGGGCTC3'

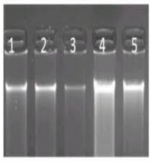
Table.5. Shows PCR reaction mixture composition :

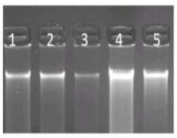
PCR Components	Working Concentration
10* PCR buffer	1*
10mM DNTPS	0.2mM
MgCl2	1.5mM
Primer	1uM
3U/UL TAQ DNA polymerase	1U/UL
Template DNA	25-300ng

Experiments	Chemicals	Stock Concentration
DNA Isolation	Tris HCL	1M
	EDTA	0.5M
	Nacl	5M
	SDS	20%
	Ammonium acetate	7.5M
	Chloroform : Isoamyl alcohol	24:1
	Isopropanol	99.9%
	Chilled ethanol	70%
	Autoclave distilled water	----
Agarose Gel Electrophoresis	Agarose powder	0.8% & 1.2%
	TAE Buffer	50X
	Ethidium bromide	10mg/ml
	Loading dye (Bromophenol blue)	6X
RAPD-PCR	Taq Buffer	10x
	dNTPS	10mM
	Primers (OPA5,OPA7 & OPA1)	20pM
	Template DNA	500ng
	DNA polymerase enzyme	3U/ml

Fig :1 shows DNA isolation of five Cichlid species (1-blue peacock). species(1redpeacock) (2- yellow morph ,3-green terror,) (4-bi color peacock 5-green jewel)

Fig: 2shows DNA isolation of Five cichlid (2-tilapiine cichlid 3-tigeroscar) (4-white cichlid 5-strawberry)





RAPD and its application

Random Amplified Polymorphic DNA (RAPD) is an effective technique that can be used for molecular ecology for the identification of taxonomy characteristics. According to Jacobs et al. (2019) [3], this technique is enabled to analyse of mixed genome samples and helps to create specific probes. It can be used for different purposes, such as studying closely related species and genetic identity and gene mapping studies. This technique includes DNA Amplification Fingerprinting (DAF) and Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) for extensive primers. The primary advantage of this technique is, it requires a low quantity of DNA and is easy to access. RAPD test images

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 10, October 2021

and lane test images will be provided in the following section.

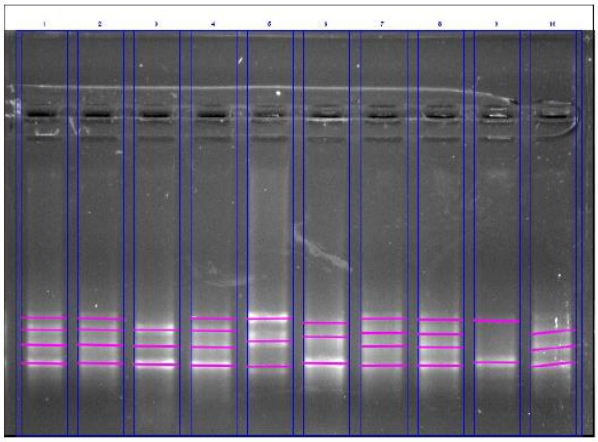


Figure 12: OPA-03

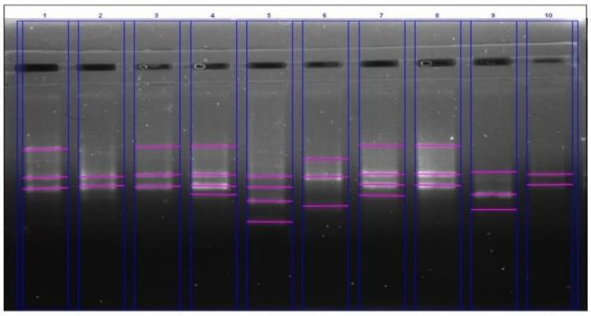


Figure 13: 0PA-05

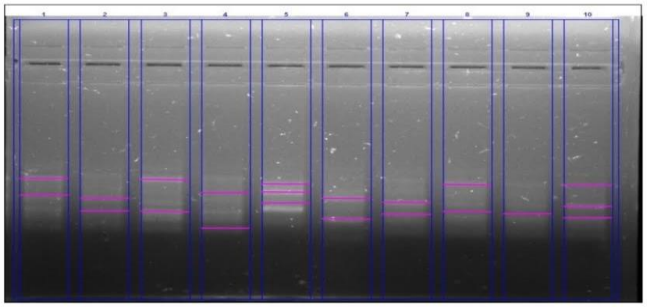


Figure: 0PA-04

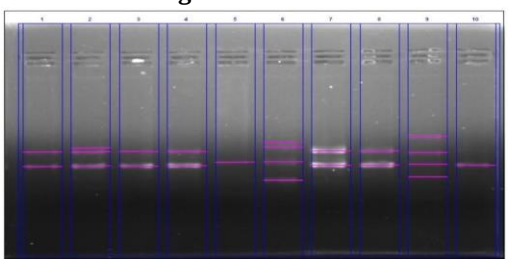


Figure: 0PA-10

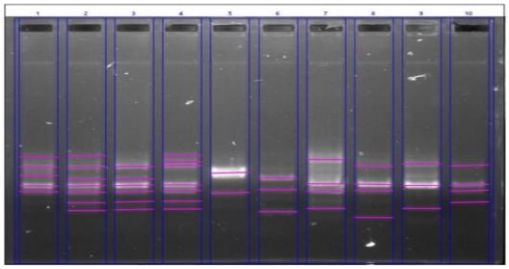


Figure:0PA-07

Previous work on cichlids and related work

Nowadays, there are numerous molecular marker techniques, which are used to identify the characteristics of cichlid fishes. According to REGMI et al. (2021) [6], DNA fingerprints are one of the most effective techniques for the identification of genetic variation.

II. MATERIALS AND METHOD

During the data collection process, the primary research method has been used for the isolation of Cichlid species.

Ten different types of samples of cichlid have been used to collect information. Different equipment, such as PH meter, autoclave, vortex Mixer, PCR, gel tank and many others. After the collection of samples, genomic data has been extracted. After that, agarose gel has been produced. The next stage is electrophoresis of DNA and DNA visualisation to get appropriate results from the collected samples.

III. RESULTS

	1	2	3	4	5	6	7	8	9	10
1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2		1	1.000	1.000	0.000	0.000	1.000	1.000	0.000	1.000
3			1	0.000	1.000	1.000	0.000	0.000	0.000	1.000
4				1	0.000	0.000	1.000	1.000	1.000	1.000
5					1	1.000	1.000	1.000	0.000	0.000
6						1	0.000	0.000	0.000	0.000
7							1	1.000	1.000	1.000
8								1	0.000	0.000
9									1	0.000
10										1

Figure : similarity matrix and DENDOGRAM analysis (Source: Provided)

Uses of genomic data helped to identify the presence and absence of amplified fields for different 10 cichlid samples throughout this RAPD test. RAPID binary data matrix has been used for the identification of similarities among cichlid samples. Throughout this test, a dendrogram has been generated to complete this test. Identification of the coefficient values of 10 cichlids has provided separate

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 10, October 2021

clusters with variation

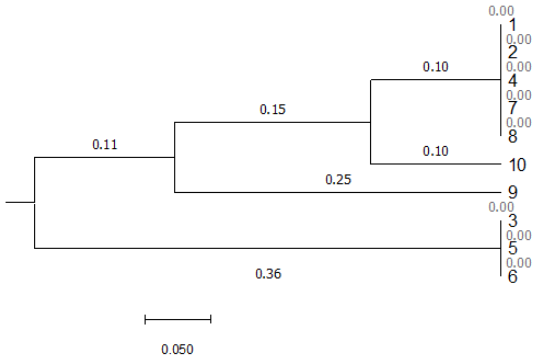


Figure 69: PHYLO GENETIC TREE

(Source: provided)

Genetic similarity between two different genotypes has been observed for the development of meaningful taxonomies to complete this project. As per the "UPGMA Jaccard's similarity matrix RAPID test," gDNA rate has been determined. The highest and least amount of gDNA rate helps in species study to complete this project (DOENZ et al. 2019)[1]. Identification of complex genus is essential to get accuracy in the RAPD test. This polygenetic tree helped in generation of the UPGMA method

DISCUSSION

Screening of genotypes is enabled got to represent ten different cichlids. This analysis helped to acquire knowledge on genetic diversity for the fulfilment of demand in the global market. The UPGMA method is dependent on two different clusters, such as cluster I and cluster II. subdivision of (clusters I) helped to get results in the form of clusters and clusters (Levin et al. 2019) [5]. This analysis process helped to enhance knowledge on DNA fingerprints. Recognition of the genetic relationship helped to enhance knowledge on the researched topic.

CONCLUSION

DNA fingerprinting is an innovative laboratory test for the identification of biological evidence. This evidence is required for molecular ecology. This research paper has dissected ornamental fish, cichlid and RAPID test. Gathering knowledge on these sections was necessary for the enhancement of knowledge on biodiversity. Primary research methods have been used to collect information on ten different cichlids. The methodology section has briefly discussed this topic. Results and discussion helped to acquire knowledge on the UPGMA method. It discussed the decision and subdivision of clusters in this project. Moreover, it helped to identify the gDNA rate to conclude.

REFERENCE

[1] DOENZ, C.J., KRAHENBUHI, A.K., Walker, J., Seehausen, O. and BRODERSEN, J., 2019. Ecological opportunity shapes a large Arctic CHARR species radiation. Proceedings of the Royal Society B, 286(1913), p.20191992.

[2] Henning, F., Machado-SCHIAFFINO, G., Baumgarten, L. and Meyer, A., 2017. Genetic dissection of adaptive form and function in rapidly speciating cichlid fishes. Evolution, 71(5), pp.1297-1312.

[3] Jacobs, A., Carruthers, M., ECKMANN, R., YOHANNES, E., Adams, C.E., BEHRMANN-GODEL, J. and Elmer, K.R., 2019. Rapid niche expansion by selection on functional genomic variation after ecosystem recovery. Nature ecology & evolution, 3(1), pp.77-86.

[4] Koh, T.L., Khoo, G., Fan, L.Q. and PHANG, V.P.E., 1999. Genetic diversity among wild forms and cultivated varieties of discus (SYMPHYSODON spp.) as revealed by random amplified polymorphic DNA (RAPD) fingerprinting. Aquaculture, 173(1-4), pp.485-497.

[5] Levin, B.A., CASAI-López, M., Simonov, E., DGEBUADZE, Y.Y., MUGUE, N.S., TIUNOV, A.V., DOADRIO, I. and GOLUBTSOV, A.S., 2019. Adaptive radiation of barbs of the genus LABEROBARBUS (CYPRINIDAE) in an East African river. Freshwater Biology, 64(10), pp.1721-1736.

[6] REGMI, B., Douglas, M.R., EDDS, D.R. and Douglas, M.E., 2021. Geometric morphometric analyses define riverine and lacustrine species flocks of Himalayan SNOWTROUT (CYPRINIDAE: SCHIZOTHORAX) in Nepal. Aquatic Biology, 30, pp.19-31.

[7] Santos, L.N., SALGUEIRO, F., Sampaio Franco, A.C., Marques, A.C.P. and NOBREGA, F., 2016. First record of the invasive blue peacock cichlid CICHLA PIQUITI KULLANDER and Ferreira 2006 (CICHLIFORMES: CICHLIDAE) in the PARAIBA do Sul river basin, south eastern Brazil. BIOINVASIONS Record, 5(4).

[8] Usman, B.A., AGBEBI, O.T., Bankole, M.O., OGUNTADE, O.R. and Popoola, M.O., 2013. Molecular characterisation of two cichlids populations (Tilapia GUINEENSIS and SAROTHERODON MELANOTHRERON) from different water bodies in Lagos State, Nigeria. International Journal of Biotechnology and Molecular Biology Research, 4(5), pp.71-77.