ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia
SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Animal Sciences 10 (1): 59-67, 2016
ISSN 1819-1878 / DOI: 10.3923/ajas.2016.59.67
© 2016 Knowledgia Review, Malaysia

CrossMark
click for updates

Studies on the Genetic Variability of Three Fish Species (*Cyprinus carpio specularis*, *Cyprinus carpio communis* and *Oncorhynchus mykiss*) Collected from Kashmir (India) Using Random Amplified Polymorphic DNA (RAPD) Technique

Hilal Ahmad Ganaie and Md. Niamat Ali
Cytogenetics and Molecular Biology Research Laboratory, Centre of Research for Development (CORD), University of Kashmir, Srinagar, 190 006, J and K, India

Corresponding Author: Md. Niamat Ali, Cytogenetics and Molecular Biology Research Laboratory, Centre of Research for Development (CORD), University of Kashmir, Srinagar, 190 006, J and K, India

ABSTRACT

The present study evaluated the patterns of morphometric and genetic variation using RAPD-PCR techniques for the first time on two species of *Cyprinus* and single species of *Oncorhynchus mykiss* from Srinagar, Kashmir, India. The Random Amplified Polymorphic DNA (RAPD) analysis was used to estimate the genetic relationships and diversity of three species of local fishes (Family Cyprinidae). A large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the present study, genetic variability of three local fish species (*Cyprinus carpio specularis*, *Cyprinus carpio communis* and *Oncorhynchus mykiss*) collected from Dal Lake and local Trout Fish Farm (Harwan, Srinagar, India) was analyzed using the RAPD-PCR technique. Amplified DNA fragments with monomorphic profile were not found in the studied species, except for *Cyprinus carpio specularis* and *Cyprinus carpio communis* which presented monomorphic bands as both belong to the same genus. All species showed high levels of genetic variability among individuals. *Cyprinus carpio communis* and *Cyprinus carpio specularis* are more similar in comparison to the other *Oncorhynchus mykiss* based on morphological approach.

Key words: Genetic polymorphism, *Cyprinus carpio specularis*, *Cyprinus carpio communis*, *Oncorhynchus mykiss*, RAPD, Kashmir, India

INTRODUCTION

The aquatic ecosystems are among those where life diversity expresses itself in an intense manner. The wide ecological diversity, representing a hot spot of fish biodiversity. The natural environment is damaged and populations of several species are either overexploited or depleted, the necessity of useful genetic information for conservation management increases in order to provide an accurate measure of biodiversity (Affonso and Galetti Jr., 2007; Bay *et al*., 2006). The diversity in the genetic structure among different species should be taken into consideration for the conservation of eventual evolutionary units along the Kashmir Province (India). The famous Dal Lake of Kashmir in India, has a total area of approximately 25 km^2, being situated in heart of Srinagar city. Recent studies on the Dal Lake described several fish species based on morphologic criteria. The aquatic ecosystems have suffered aggressions like predatory fishing, introduction of exotic species, deforestation, pollution and hydroelectric dam implementations. They have caused

deep modifications in environmental dynamics, jeopardizing the rich fish variety (Sole-Cava, 2001). The advances in DNA techniques have had a great impact in addressing problems in many aspects of biology. Assessment of genetic variations is based upon information at the molecular level by various sensitive molecular techniques such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR), Randomly Amplified Polymorphic DNA (RAPD) etc. Ultimately RAPD, markers generated by Polymerase Chain Reaction (PCR) is widely used since 1990's to assess intra specific genetic variation at molecular level (Welsh and McClelland, 1990). Kumari *et al.* (2013) used RAPD for the population based study. This molecular approach by RAPD has been providing useful information for species management and genetic conservation of several freshwater as well as marine fishes (Wasko and Galetti Jr., 2002; Hatanaka and Galetti Jr., 2003; Wasko *et al*., 2004). The main objective of genetic resources conservation is to maintain genetic integrity and natural levels of genetic diversity and to enhance genetic diversity in population and species where it has been eroded (Rajora and Mosseler, 2001). The genetic diversity is essential for the long term survival of the species by populations because it provides the raw material for adoption and evolution, especially when environmental conditions have changed (Nandani and Thakur, 2014). The ability of the RAPD technique to reveal intra-specific variation can be used in screening for the degree of inbreeding in commercial plant and animal species to prevent an increase in the frequency of deleterious recessive alleles in populations (Bay *et al*., 2006; Craig *et al*., 2007; Galetti Junior *et al*., 2006; Govindaraju and Jayasankar, 2004; Rohfritsch and Borsa, 2005).

The comprehension of genetic differences of native fish populations is fundamental for long term fisheries management. Information derived from molecular genetic techniques shall contribute significantly for the preservation of aquatic genetic resources and sustainable development (Martins *et al*., 2003). Randomly Amplified Polymorphic DNA (RAPD) is a type of Polymerase Chain Reaction (PCR) reaction, but the segments of DNA that are amplified are random. The molecular biologists performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction (Erlich, 1989; Williams *et al*., 1900; Welsh and McClelland, 1990; Wada *et al*., 1995; Griffiths *et al*., 1996; Johnson *et al*., 1996; Lyons *et al*., 1997; Postlethwait *et al*., 1998, 1994; Ohtsuka *et al*., 1999).

The results indicated that RAPD could be effectively used for genetic diversity analysis in wild species of prospective value as it is reliable, rapid and superior to those based on pedigree information (Raghunathachari *et al*., 2000). The RAPD procedure has been instrumental to understand the genetic variability of fish populations (Hatanaka and Galetti Jr., 2003; Kapila and Mishra, 2006; Barat *et al*., 2008). The information revealed from the analysis of genetic polymorphism for fish species and subspecies is widely used in the management of aquatic genetic resources (Rashed *et al*., 2011; Saad *et al*., 2009; Eknath, 1994). These activities are documentation of genetic resources and the variety of ecosystems; characterization to determine the genetic structure, evaluation to estimate economic potential and utilization in sustainable breeding schemes (Wasko *et al*., 2004; Bay *et al*., 2006; Affonso and Galetti Jr., 2007; Nandani and Thakur, 2014).

The RAPD technology provides a quick and efficient screen for DNA sequence based polymorphism at a very large number of loci. The major advantage of RAPD includes that, it does not require pre-sequencing of DNA. It involves no blotting or hybridization steps, hence, it is quick,

simple and efficient, it requires only small amounts of DNA (about 10 ng per reaction), the procedure can be automated, high number of fragments, arbitrary primers are easily purchased, unit costs per assay are low compared to other marker technologies (Dinesh *et al*., 1993; Wasko *et al*., 2004). The RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding.

The RAPD can detect high levels of polymorphism and produce genetic markers (Welsh and McClelland, 1990; Williams *et al*., 1990). To evaluate the genetic variability of *Cyprinus carpio specularis, Cyprinus carpio communis* and *Oncorhynchus mykiss* from Kashmir, RAPD analysis were performed. The results described here could be used for stocks maintenance of the studied species in hatchery programs. However, many factors can affect the changes in RAPD profiles, a proper optimization is required in order to generate reliable results (Atienzar and Jha, 2006).

MATERIALS AND METHODS

Fish samples: Fish samples from different locations from the Dal Lake, Srinagar and Trout farm Harwan, Srinagar, India were collected in two years duration. Fishes after collection were transported in specially designed containers with water to the laboratory and kept in aquariums. The Rainbow trout was collected from the Trout farm at Harwan, Srinagar and was brought to the lab. After collection, the fishes were acclimatized to the laboratory conditions for 15 days (APHA/AWWA/WPCF., 1998). Specimens were kept in big aquariums each with 2-3 individuals/50 L of water. Water was kept oxygen saturated by aeration. The aquariums were cleaned and the water was renewed regularly. Qualities of water (pH = 7.3±0.6, Dissolved Oxygen = 7.3±0.4 ppm, Free CO_2 = 5.8±0.4 ppm, Alkalinity = 106±6.8 ppm) were maintained according to the standard methods of APHA/AWWA/WPCF (1998). Fishes were fed with commercial feed daily at least one hour prior to the replacement of the water.

Genomic DNA extraction: Total genomic DNA was obtained from fish blood and scales using GenElute Genomic DNA Extraction kit (Sigma Aldrich; Cat. No. NA2000) and lysis buffer (Wasko *et al*., 2003). Purified DNA was preserved at 4ºC for immediate use or stored at -20°C. The quantification of DNA was done by UV spectrophotometer analysis. The quantity of DNA was measured by obtaining the absorbance reading at 260 nm and the purity of DNA was checked by calculating the ratio of absorbance readings at 260 and 280 nm.

RAPD-PCR of the extracted pure DNA: The DNA amplifications were performed in a final volume of 25 μL containing approximately 2 μL of genomic DNA, 1 μL of primer (Invitrogen), 1.2 μL of each dNTPs, 1.5 μL of PCR buffer, 1 μL $MgCl_2$, 0.4 μL of Taq DNA polymerase (Invitrogen) and 17.9 μL of double distilled water. Primer P-12 was used to generate RAPD fragments (Table 1). The PCR reactions were carried out in a thermal cycler (Mini Cycler Eppendroff) programmed for 94°C for 2 min, 40 cycles of 94°C for 1 min, 36°C for 30 sec, 72°C for 2 min and finally 72°C for 7 min. This programme was run for 40 cycles. DNA amplified fragments were separated by electrophoresis at 100 V on 1.2% agarose gel with Tris-borate-EDTA buffer

Table 1: Primer sequence and number of DNA fragments obtained for the analyzed species

		No. of DNA fragments		
Primers	Sequence	*Cyprinus carpio specularis*	*Cyprinus carpio communis*	*Oncorhynchus mykiss*
P-12	5'CCGAGCACCG3'	06	05	04

(Sambrook *et al*., 1989; Asensio *et al*., 2009). Most parameters (concentration of reaction components, additives, different polymerases and thermal profiles) affecting RAPD-PCR were examined, in order to increase pattern complexity (Diakou and Dovas, 2001). After PCR, DNA fragments were stained with ethidium bromide and photographed under ultraviolet light with a digital photosystem of Gel Doc. The DNA fragments length was estimated by comparison with 1 kb ladder (Step Up^{TM} 50 bp DNA Ladder (Merck).

RESULTS

The profile of amplified DNA primarily depends on nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. Therefore, amplification products from the same alleles may differ in length and will be detected as presence and absence of bands in the RAPD profile.

***Cyprinus carpio specularis*:** Six RAPD fragments were obtained after the amplification with P-12 primer and all of them were polymorphic for *Cyprinus carpio specularis*. Size of DNA fragments were 840, 860, 930, 920, 980 and 1000 bp (Table 2).

***Cyprinus carpio communis*:** Five RAPD fragments were obtained after the amplification with P-12 primer and all of them were polymorphic for *Cyprinus carpio communis*. Size of DNA fragments were 850, 910, 930, 940 and 1000 bp.

***Oncorhynchus mykiss*:** Four RAPD fragments were obtained after the amplification with P-12 primer and all of them were polymorphic for *Oncorhynchus mykiss*. Size of DNA fragments were 840, 930, 940 and 980 bp.

Comparison of results: The banding patterns of the three fishes were different. The banding patterns of *Cyprinus carpio specularis* and *Cyprinus carpio communis* were somewhat more similar than the banding patterns of the *Oncorhynchus mykiss* (Fig. 1). The number of bands per individuals ranged from 04-06 (Table 1) and bands amplified ranged in size from 840-1000 bp (Table 2). The molecular size of the bands was estimated with a molecular DNA ladder (Step Up^{TM} 50 bp DNA Ladder), which is of 50-1000 bp in length. The genetic variability of the fishes as analyzed from the Fig. 1 can be interpreted in the following points:

- There was a wide difference between the band patterns of the *Cyprinus* fishes to that of the Rainbow trout. This may be due to the fact that the *Cyprinus* fishes are the warm water fishes and the trout being the cold water fish
- There was also a close pattern of the bands in the *Cyprinus carpio specularis* and *Cyprinus carpio communis* as they have much similarity as both of them occur in the same family Cyprinidae
- Some of the bands in all the fishes were thick: in *Cyprinus carpio specularis,* a prominent band of 980 bp, in *Cyprinus carpio communis,* a prominent band of 930 bp and in *Oncorhynchus mykiss*, three successive bands (980, 940 and 930 bp), which indicates that these bands are highly amplified in comparison to the others

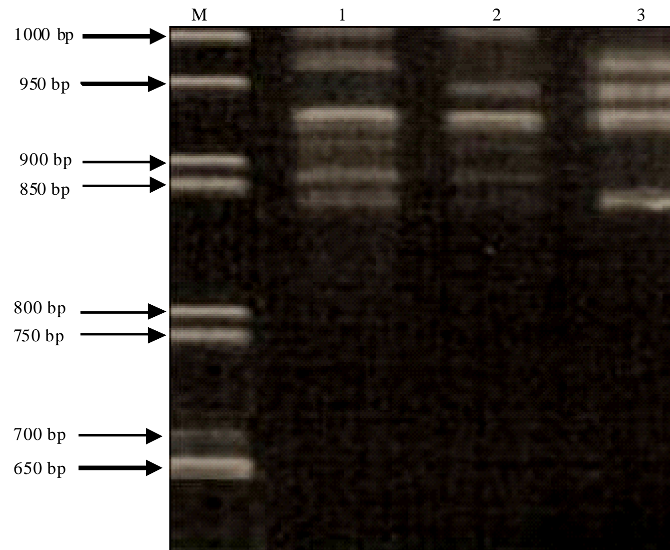

Fig. 1: A typical RAPD banding pattern amplified with primer P-12 that resolved in 1.2% agarose gel and stained with ethidium bromide. Lane M: Template DNA was from Step up™ 50bp DNA ladder, Lane 1: *Cyprinus carpio specularis*, Lane 2: *Cyprinus carpio communis* and Lane 3: *Oncorhynchus mykiss*

Table 2: Size of the bands determined by comparing with the step up™ 50 bp DNA ladder

Marker size in bp	*Cyprinus carpio specularis*	*Cyprinus carpio communis*	*Oncorhynchus mykiss*
1000	1000	1000	-
950	980	940	980
900	930	930	940
850	920	910	930
800	860	850	840
750	840	-	-
700	-	-	-
650	-	-	-
600	-	-	-

DISCUSSION

Genetic approaches offer powerful tools for examining the current status of populations, for understanding the population changes for its conservation (Belfiore and Anderson, 2001). The RAPD technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Garg *et al*., 2009a, b).

In the present study, two *Cyprinus* species (*C. carpio specularis* and *C. carpio communis*) and single *Oncorhynchus mykiss* were identified using a molecular technique (RAPD) to detect genetic variability of these economic fish. The RAPD was chosen because this is cheaper, simple and fast techniques for detecting genetic polymorphism at a molecular level. In addition, just only one primer could obtain the different profiles for genomic analysis (Raina *et al*., 2001; Antunes *et al*., 2010). The RAPD PCR-based method is used for fish species identification because it is a simple, specific and sensate method for genetic characterization (Asensio *et al*., 2009). The DNA markers were polymorphic reflecting a rich allelic diversity in the applied fish species. So, the primers of these loci are recommended to detect the genetic polymorphism and inferring the genetic variations for the applied fish species. It is necessary to estimate intra and inter population variations and

phylogenetic relationships among fish genomes (Saad *et al*., 2011) to help the breeder in designing suitable breeding programs for fish improving and/or conservation. In addition, this will be useful in detecting any genetic contamination in these fish genomes.

Our results presented some species-specific RAPD markers. These markers could be used in two different ways. In the first way these markers will be used, as a species genetic signature. The second way, these markers are useful as marker assisted selection (Rashid *et al*., 2009) in breeding (to develop local fish breeds) and restocking programs.

Restocking and stock enhancement programs are now recognized as an important tool for the management of fishery resources. It is important, however, to have an adequate knowledge on the genetic population structure of both the released stock and the wild population before carrying out such programs (Pereira *et al*., 2010). So, the genetic markers should be conducted to provide the information needed for a sound management of economic aquatic resources in wild fish stocks and/or farms (Saad *et al*., 2011). In addition, development of local *Cyprinus* and trout species breeds is important because many of them will be resilient to climatic stress and represent a unique source of genes for improving these species production and conservation in the future.

These results are useful in studying the genetic structure of *Cyprinus* and trout species because the genetic structure of *Cyprinus* and trout species and populations are not fully maximized and clear. Generally, genetic diversity which is required for populations to be more adaptive with the environmental changes can be measured using an array of molecular methods (Rashed *et al*., 2008). The present study showed that RAPD primers were informative in detecting species specific DNA markers.

CONCLUSION

In conclusion, the present investigation revealed the morphometric and genetic variation of two *Cyprinus* and single trout species. The results of morphological approach revealed that *Cyprinus carpio communis* and *Cyprinus carpio specularis* are more similar in comparison to the other *Oncorhynchus mykiss*. The latter exhibits distinct variation both in the morphological character and genetic fragments. The present investigation contributes to the knowledge on morphological and genetic variation to the *Cyprinus* and Trout fish species. However, much specific molecular biomarkers are required for understanding the taxonomical relations of many other species of this group, which are widely distributed in various fresh water streams, lakes and fish farms of Kashmir.

ACKNOWLEDGMENTS

The author is very thankful to Director, Centre of Research for Development, University of Kashmir and Dr. Mohd. Ashraf Bhat, Incharge Centre of Plant Biotechnology, Division of Biotechnology, SKUAST-K for providing all the necessary facilities including the laboratory facilities for conducting the work in a smooth way.

REFERENCES

APHA, AWWA and WEF., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC., USA., ISBN-13: 9780875532356, pp: 6-139.

Affonso, P.R.A.M. and J.P.M. Galetti Jr., 2007. Genetic diversity of three ornamental reef fishes (Families Pomacanthidae and Chaetodontidae) from the Brazilian coast. Braz. J. Biol., 67: 925-933.

Antunes, R.S.P., V.N. Gomes, S.M.A.P. Prioli, R.A. Prioli and H.F. Julio Jr. *et al*., 2010. Molecular characterization and phylogenetic relationships among species of the genus *Brycon* (Characiformes: Characidae) from four hydrographic basins in Brazil. Genet. Mol. Res., 9: 674-684.

Asensio, L., I. Gonzalez, M. Rojas, T. Garcia and R. Martin, 2009. PCR-based methodology for the authentication of grouper (*Epinephelus marginatus*) in commercial fish fillets. Food Control, 20: 618-622.

Atienzar, F.A. and A.N. Jha, 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. Mutation Res./Rev. Mutation Res., 613: 76-102.

Barat, A., G.K. Sivaraman, R. Maikhuri and R. Kapila, 2008. Studies on genetic diversity of mahseer (*Tor putitora*) population using RAPD-PCR assay. In 8th Indian Fisheries Forum, Kolkata, November 2008, pp: 22-26.

Bay, L.K., R.H. Crozier and M.J. Caley, 2006. The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Mar. Biol., 149: 1247-1256.

Belfiore, N.M. and S.L. Anderson, 2001. Effects of contaminants on genetic patterns in aquatic organisms: A review. Mutation Res./Rev. Mutation Res., 489: 97-122.

Craig, M.T., J.A. Eble, B.W. Bowen and D.R. Robertson, 2007. High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). Mar. Ecol. Prog. Ser., 334: 245-254.

Diakou, A. and C.I. Dovas, 2001. Optimization of random-amplified polymorphic DNA producing amplicons up to 8500 bp and revealing intraspecies polymorphism in *Leishmania infantum* isolates. Anal. Biochem., 288: 195-200.

Dinesh, K.R., T.M. Lim, K.L. Chua, W.K. Chan and V.P. Phang, 1993. RAPD analysis: An efficient method of DNA fingerprinting in fishes. Zoolog. Sci., 10: 849-854.

Eknath, A., 1994. Managing aquatic genetic resources. Management Example 4: The Nile Tilapia, International Centre for Living Aquatic Resources Management. Makati, Metro Manila, Philippines.

Erlich, H.A., 1989. PCR Technology: Principles and Applications for DNA Amplification. Macmillan Publishers, New York, USA., ISBN-13: 9780935859560, Pages: 246.

Galetti Junior, P.M., W.F. Molina, P.R.A.M. Affonso and C.T. Aguilar, 2006. Assessing genetic diversity of Brazilian reef fishes by chromosomal and DNA markers. Genetica, 126: 161-177.

Garg, R.K., P. Sairkar, N. Silawat and N.N. Mehrotra, 2009a. Genetic polymorphism of two populations of catfish *Aorichthys seenghala* (Sykes) using RAPD fingerprinting. Int. J. Zool., 3: 130-134.

Garg, R.K., P. Sairkar, N. Silawat, N. Vijay, N. Batav and N.N. Mehrotra, 2009b. Genetic diversity between two populations of *Heteropneustes fossilis* (Bloch) using RAPD profile. Int. J. Zool. Res., 5: 171-177.

Govindaraju, G.S. and P. Jayasankar, 2004. Taxonomic relationship among seven species of groupers (genus *Epinephelus*; family Serranidae) as revealed by RAPD fingerprinting. Mar. Biotechnol., 6: 229-237.

Griffiths, A.J.F., J.H. Miller, D.T. Suzuki, R.C. Lewontin and W.M. Gelbart, 1996. An Introduction to Genetic Analysis. 6th Edn., W.H. Freeman, New York, USA., Pages: 916.

Hatanaka, T. and P.M. Galetti Jr., 2003. RAPD markers indicate the occurrence of structured populations in a migratory freshwater fish species. Genet. Mol. Biol., 26: 19-25.

Johnson, S.L., M.A. Gates, M. Johnson, W.S. Talbot and S. Horne *et al*., 1996. Centromere-linkage analysis and consolidation of the zebrafish genetic map. Genetics, 142: 1277-1288.

Kapila, R. and D.P. Mishra, 2006. Randomly Amplified DNA (RAPD) Fingerprinting of coldwater fish, *Schizothorax richardsonii* (Gray). Indian J. Fish., 53: 219-224.

Kumari, N., L.B. Singh and S. Kumar, 2013. Molecular characterization of goats using random amplified polymorphic DNA. Am. J. Anim. Vet. Sci., 8: 45-49.

Lyons, L.A., T.F. Laughlin, N.G. Copeland, N.A. Jenkins, J.E. Womack and S.J. O'Brien, 1997. Comparative Anchor Tagged Sequences (CATS) for integrative mapping of mammalian genomes. Nat. Genet., 15: 47-56.

Martins, C., A.P. Wasko, C. Oliveira and F. Foresti, 2003. Mitochondrial DNA variation in wild populations of *Leporinus elongatus* from the Parana River basin. Genet. Mol. Biol., 26: 33-38.

Nandani, K. and S.K. Thakur, 2014. Randomly amplified polymorphic DNA-a brief review. Am. J. Anim. Vet. Sci., 9: 6-13.

Ohtsuka, M., S. Makino, K. Yoda, H. Wada and K. Naruse *et al*., 1999. Construction of a linkage map of the medaka (*Oryzias latipes*) and mapping of the DA mutant locus defective in dorsoventral patterning. Genome Res., 9: 1277-1287.

Pereira, J.C., P.G. Lino, A. Leitao, S. Joaquim and R. Chaves *et al*., 2010. Genetic differences between wild and hatchery populations of *Diplodus sargus* and *D. vulgaris* inferred from RAPD markers: Implications for production and restocking programs design. J. Applied Genet., 51: 67-72.

Postlethwait, J.H., S.L. Johnson, C.N. Midson, W.S. Talbot and M. Gates *et al*., 1994. A genetic linkage map for the zebrafish. Science, 264: 699-703.

Postlethwait, J.H., Y.L. Yan, M.A. Gates, S. Horne and A. Amores *et al*., 1998. Vertebrate genome evolution and the zebrafish gene map. Nature Genet., 18: 345-349.

Raghunathachari, P., V.K. Khanna, U.S. Singh and N.K. Singh, 2000. RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). Curr. Sci., 79: 994-998.

Raina, S.N., V. Rani, T. Kojima, Y. Ogihara, K.P. Singh and R.M. Devarumath, 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome, 44: 763-772.

Rajora, O.P. and A. Mosseler, 2001. Challenges and opportunities for conservation of forest genetic resources. Euphytica, 118: 197-212.

Rashed, M.A., Y.M. Saad, M.M. Ibrahim and A.A. El-Seoudy, 2008. Genetic structure of natural Egyptian *Oreochromis niloticus* evaluated using dominant DNA markers. Global Veterinaria, 2: 87-91.

Rashed, M.A., Y.M. Saad, A.H. Atta and N.E. Ahmed, 2011. Reconstruction of phylogenetic relations among four Tilapia species. World Applied Sci. J., 14: 456-462.

Rashid, M.A., Y.M. Saad, A.A. Al-Suudi and M.M. Ibrahim, 2009. Gene flow in some *Oreochromis niloticus* populations based on SSR linked markers to MHC loci class I. J. Biol. Chem. Environ. Sci., 4: 319-331.

Rohfritsch, A. and P. Borsa, 2005. Genetic structure of Indian scad mackerel *Decapterus russelli*: Pleistocene vicariance and secondary contact in the Central Indo-West Pacific Seas. Heredity, 95: 315-326.

Saad, Y.M., M.S. Hanafi, M.A. Essa, A.A. Guerges and S.F. Ali, 2009. Genetic signatures of some Egyptian *Clarias gariepinus* populations. Global Veterinaria, 3: 503-508.

Saad, Y.M., N.M.A. Shabana, N.A. El-Ghazaly, M.H. Fawzy and A.M. Mohamed, 2011. Conservation of some sea bream (*Sparus aurata*) fish populations. World J. Fish Mar. Sci., 3: 489-495.

Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., USA., ISBN-13: 9780879695774, Pages: 397.

Sole-Cava, A.M., 2001. Biodiversidade Molecular e Genetica da Conservacao. In: Biologia Molecular e Evolucao, Matioli, S.R. (Ed.)., Holos Editora, Ribeirao Preto, Brazil, pp: 172-192.

Wada, H., K. Naruse, A. Shimada and A. Shima, 1995. Genetic linkage map of a fish, the Japanese medaka *Oryzias latipes*. Mol. Mar. Biol. Biotechnol., 4: 269-274.

Wasko, A.P. and P.M. Galetti Jr., 2002. RAPD analysis in the Neotropical fish *Brycon lundii*: Genetic diversity and its implications for the conservation of the species. Hydrobiologia, 474: 131-137.

Wasko, A.P., C. Martins, C. Oliveira and F. Foresti, 2003. Non-destructive genetic sampling in fish. An improved method for DNA extraction from fish fins and scales. Hereditas, 138: 161-165.

Wasko, A.P., C. Martins, C. Oliveira, J.A. Senhorini and F. Foresti, 2004. Genetic monitoring of the Amazonian fish matrincha (*Brycon cephalus*) using RAPD markers: Insights into supportive breeding and conservation programmes. J. Applied Ichthyol., 20: 48-52.

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.