Indian J. Plant Genet. Resour. 29(3): 388-391 (2016)
DOI 10.5958/0976-1926.2016.00080.2

Genomic Resource Development for Aquaculture Species in India

Vindhya Mohindra, Basdeo Kushwaha, Rajeev K Singh and JK Jena
ICAR-National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow–226002 India

India is the second highest cultured global fish producer country and freshwater aquaculture contributes to over 95 percent of the total aquaculture production. It comprises of the culture of carp fishes, catfishes (air breathing and non-air breathing), freshwater prawns, pangasius and tilapia. In addition, in brackishwater sector, the aquaculture includes culture of shrimp varieties, mainly the native giant tiger prawn (*Penaeus monodon*) and exotic whiteleg shrimp (*Penaeus vannamei*). Thus, the production of carp in freshwater and shrimps in brackishwater form the bulk of major areas of aquaculture activity (FAO, 2016, http://www.fao.org/fishery/countrysector/naso_india/en). Among the carps, major contribution is by three species of Indian major carps, *viz.,* catla *(Catla catla),* rohu (*Labeo rohita)* and mrigal (*Cirrhinus mrigala),* (contributing about 70-75%), supplemented by three exotic species viz., silver carp (*Hypophthalmichthys molitrix),* grass carp (*Ctenopharyngodon idella)* and common carp (*Cyprinus carpio).* It is followed by exotic Pangas catfish, *Pangasianodon hypophthalmus (Pangasius sutchi),* and freshwater prawn *(Macrobrachium rosenbergii)* from freshwater aquaculture and shrimps from coastal farming. Among the brackishwater/marine finfish species, the Asian seabass (*Lates calcarifer*) and cobia (*Rachycentron canadum*) are considered as among the most important candidate species suitable for farming in ponds and cages in brackish and marine water ecosystem. (http://www.ciba.res.in/index.php/candidate-species). In addition, important food fishes of cold waters are mahseer and schizothoracids belonging to the indigenous species and trouts among the exotic varieties, contributing around 1% to the food basket.

Over the past 10-15 years, rapid development in the area of agricultural genomics has taken place in India, focussed on innovative genomic applications towards improving the important production and performance traits. Genome research requires the development of a number of resources that facilitate both structural and functional analysis of the genome. For this purpose, wide-ranging genomic tools are now been generated in fish species. These include large number of molecular markers including genetic sequence polymorphisms, Cell lines, Expressed sequence tags (ESTs), transcriptomes and Gene expression profiles under different conditions, Genetic/ linkage and physical maps and Whole genome sequences resources. This wealth of new genomic resources has caused a paradigm shift in research, by reducing the dependence on the model systems and working directly on the aquaculture species.

Whole Genome Sequencing and Development of Allied Genomic Resources

Whole genome sequencing of two commercially important fish i.e. *Labeo rohita* (rohu) and *Clarias batrachus* (magur) has been in progress in a network project mode (DBT, New Delhi sponsorship) involving ICAR-NBFGR, ICAR-CIFA, AAU Anand and ICAR-IASRI (www.nbfgr.res.in). Under this initiative, multi platform whole genome sequencing up to more than 100x coverage in both rohu (genome 1.4 GB) and magur (1 GB) has been accomplished using more than five sequencing platforms and the *de novo* assembly is in progress (http://mail.nbfgr.res.in/RohuMagur_Genome/index.html). Complete mitogenomes of both the species have been deciphered. A 5x coverage BAC library in magur has been developed. In addition, heterozygous SNPs and SSRs, Repeat elements, genes involved in anti-microbial peptide family and miRNAs mining have been done. A total of 483830 SNVs were identified from the assembled contigs in all the three populations of *C. batrachus*.

Programmes on whole genome sequencing of an the anadromous hilsa shad, *Tenualosa ilisha* and Indian white shrimp, *Penaeus indicus*, have been initiated under the ICAR Consortium Research Platform (CRP) on Genomics in 2015, for structural and functional genomics in these two species with commercial potential to discover variants of genes, from hitherto unexplored germplasm (ICAR, http://www. icar. org.in/ en/ node/10866).

An important genome resource with chromosome level assembly of whole genome sequence of an important

---

**Author for Correspondence: Email- vindhyamohindra@gmail.com* candidate species, the *Asian seabass* (*Lates calcarifer*) was generated, which would serve for development of genomic assays for establishing molecular aquaculture in this species as well as related species (Vij *et al*., 2016).

Trait Associated Transcriptome Profiling

Transcriptome/EST sequencing can boost genetic and genomic research of target species and genetic mechanism underlying the production and performance traits can be studied. It is also a fast and efficient means for novel genes identification and genetic marker development.

The Indian major carp, *Labeo rohita*, genomic resources for reproduction-research were generated through transcriptome profiles from twelve-tissues from pre-spawning rohu by mRNAseq and identified identified 940 reproduction-related genes (Sahu *et al*., 2015). In an another study by a collaborative project of ICAR-CIFA and Nofima Ås Norway, transcriptome profiles were used for identification of expressed genes and polymorphisms in lines of rohu selected for resistance or susceptibility to an important bacterial pathogen, *Aeromonas hydrophila* (Robinson *et al*., 2012).

The mechanism of abiotic stress tolerance has also been studied through transcriptome studies. In Indian catfish, *Clarias magur*, a hypoxia tolerant species, the genomic resources pertaining to molecular processes facilitating its adaptation to hypoxia stress were generated. The analysis of differential expression of transcripts from six tissues suggest that *C. magur* combat low O_2 level by, in part, through transcription factors, altering the expression of a series of genes involved in metabolism, signaling and immune responses, interlinked with other several pathways (Mohindra *et al*., 2016). In the hypoxia susceptible fish, *Catla catla*, oxidative stress conditions were defined (Singh *et al*., 2016) and the important role of TLRs and NOD receptors signaling pathway in sterile inflammation and pathobiology of fish in hypoxic stress was observed and thus their role in abiotic stress management in aquaculture (Basu *et al*., 2016). Differentially expressed genes from shrimp (*Penaeus monodon*) in response to low and high salinity stress were also identified through EST analysis (Shekher *et al*., 2013, 2014). Transcriptional response to heat shock in liver of snow trout (*Schizothorax richardsonii*) through deep RNA sequencing characterised the genes and molecular pathways involved in heat shock response (Barat *et al*., 2016).

The black tiger shrimp, *Penaeus monodon,* is the second-most widely cultured prawn species in the world.. However, disease and poor reproductive maturation in captivity is one of the serious threats to sustainability of the shrimp farming industry. The information on host immune gene response to WSSV pathogenesis was studied by Shekhar *et al*. (2015) through microarray analysis with Shrimp cDNAs of 40,059 unique sequences. It revealed the altered expression of numerous genes represented diverse functions such as immune response, osmoregulation, apoptosis, nucleic acid binding, energy and metabolism, signal transduction, stress response and molting (Shekhar *et al*., 2015). Another study by Soonthornchai W (2016) to understand the immune responses in the stomach of black tiger shrimp (*Penaeus monodon*) during acute Hepatopancreatic Necrosis Disease (AHPND), caused by a pathogenic strain of *Vibrio parahaemolyticus,* differentially expressed transcripts (DETs) in the stomach during strain 3HP infection was examined using Ion Torrent sequencing.

Genetic/Linkage Map and Mapping QTL for the Traits

Linkage map with wide marker coverage is an essential resource for genetic improvement study for any species. Genetic linkage map of *Labeo rohita* was developed by placing 68 microsatellite markers (Sahoo *et al*., 2015) spaning a sex-averaged total length of 1462.2 cM, in 25 linkage groups. A linkage map of 3193 transcribed single nucleotide polymorphisms in rohu was constructed (Robinson *et al*., 2014) and QTL associated with resistance to *Aeromonas hydrophila,* with 21 SNPs mapping to ten linkage groups showed significant associations with the traits hours of survival and dead or alive.

To scan the *P. monodon* genome for loci associated with resistance to white-spot syndrome virus and markers associated with sex determination, a high density linkage map for black tiger shrimp (*Penaeus monodon*) was developed under a collaborative project of ICAR-CIBA and Nofima Ås Norway, based on genotyping array containing 6,000 cSNPs (Baranski *et al*., 2014) and a total of 3959 SNPs were mapped to 44 linkage groups. Linkage groups were identified to contain QTLs significantly associated with hours of survival after white spot syndrome virus infection and SNPs significantly associated with sex (Baranski *et al*., 2014).

Mitogenomes

Mitochondrial genome sequences have been extensively used for population genetics/genomics, evolutionary and phylogenetic studies and mitogenomes have been sequenced for *Labeo rohita*, *Catla caltla*, *Cirrhinus mrigala*, *Labeo calbasu, Labeo gonius, L. frimbriatus, Clarias magur (earlier name C. batrachus), Channa marulius, Chitala chitala, Pangasius pangasius, Puntius denisonii, P. chalakkudiensis, Schizothorax richardsonii, Tor putitora, Tor tor* and many other species.

Development of Genomic Resources for Utilisation in Genetic Differentiation of Wild Fish Populations

Development of genetic/genomic resources for utilisation in estimation of genetic variation, genetic differentiation and population structure for wild populations of aquaculture as well as potential aquaculture fish species have been studied at ICAR-NBFGR and other ICAR fisheries institutes using molecular markers including mitochondrial sequences and SSR (gene associated as well as anonymous) markers across the natural range of distribution in Indian rivers. These species include *Tor putitora, Tor tor, Catla catla, Cirrhinus mrigala, Labeo rohita, L. calbasu, L. dero, L. dyocheilus, L. dussumieri, Clarias batrachus, Chitala chitala, Tenualosa ilisha, Puntius denisonii, Horabagrus brachysoma, Gonoproktopterus curmuca, Channa marulius, Macrobrachium rosenbergii and Etroplus suratenesis*. Using the genomics tools as well as the morphological characters, attempts are made to study the phylogeography and delineate evolutionary significant units or genetic stocks existing in natural population.

Genomic Resource Databases

The generation of vast amounts of biological data has fuelled the need for computational systems and techniques to manage and analyse such data, which has led to the development of bioinformatics tools. The concept of public accessibility of data (‘open data’) is generally adopted for all genomics projects and most genomics data are posted online in publically accessible databases. Genomic resource Databases under the Scheme 'Centre for Agricultural Bioinformatics: Fisheries Domain' at ICAR-NBFGR, Lucknow have been prepared and major ones are Fish Karyome, The Fish Barcode Information System (FBIS) for Indian fishes; Fish and Shellfish Microsatellite Database for Indian Fishes and Fish Mitogenome Resource (FMiR).

National Repository of Fish Cell Lines (NRFC)

NRFC is located at ICAR-NBFGR, Lucknow, which was established with the financial support from Department of Biotechnology, Govt. of India, New Delhi. NRFC aims to receive, authenticate, store and supply the cell lines to the scientific community for R & D work. NRFC also aims to provide support for training and education to stake holders and to serve as a National Referral Centre of Indian and exotic fish cell lines in the country. At present, 50 fish cell lines are being held at NRFC (www.nbfgr.res.in).

Future Perspective

The vast information being generated through the aquaculture fish genome projects, in the form of a vast array of structural and functional genomics data, would form a baseline data for further research on genetic improvement for production and performance traits, including fish health management. Identification of genetic markers surrounding quantitative trait loci (QTL) forms the basis for MAS. These molecular markers can help in genetic management of the species to optimize the production. Genomics can help to overcome problems related to infectious diseases by better understanding host defence systems and identifying QTL or candidate genes. Further, the use of SNPs associated with disease/immunity related traits in aquaculture species can facilitate the selection of fish with superior genetic material as well as a better understanding of host-pathogens interaction and disease resistance. This genome information may uncover a mine of genetic information in the form of gene and regulatory sequences, SNPs, STRs etc., which can further be used for documenting genetic variability and identifying DNA regions for early and efficient selection.

In aquaculture, with the passage of time, significance of wild fish genetic resources is enhanced, as sources of genetic diversity for use in fish breeding programmes and related research of fish genomics technologies. The genetic diversity is needed for meeting the future challenges of climate change, new diseases or further improving the production traits. Thus for future sustainability and profitability of aquaculture, it is important to characterise and conserve the wild genetic resources.

References

Barat A, PK Sahoo, R Kumar, C Goel and AK Singh 2016) Transcriptional response to heat shock in liver of snow trout (*Schizothorax richardsonii*) – a vulnerable Himalayan Cyprinid fish. *Funct. Integr. Genomics* **16(2):** 203-13.

Barat A, PK Sahoo, R Kumar and V Pande (2016) Solute carriers (SLCs) identified and characterized from kidney transcriptome of golden mahseer (*Tor putitora*) (Fam: Cyprinidae). *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **200:** 54-61.

Basu M, M Paichha, SS Lenka, R Chakrabarty and M Samanta (2016) Hypoxic stress: impact on the modulation of TLR2, TLR4, NOD1 and NOD2 receptor and their down-stream signalling genes expression in catla (*Catla catla*). *Mol. Biol. Rep*. **43(1):** 1-9.

Mohindra V, RK Tripathi, A Singh, R Patangia, RK Singh, KK Lal and JK Jena (2016) Hypoxic stress-responsive genes in air breathing catfish, *Clarias magur* (Hamilton 1822) and their possible physiological adaptive function. *Fish Shellfish Immunol.*

Robinson N, M Baranski, KD Mahapatra, JN Saha, S Das, J Mishra, P Das, M Kent, M Arnyasi and PK Sahoo (2014) A linkage map of transcribed single nucleotide polymorphisms in rohu (*Labeo rohita*) and QTL associated with resistance to *Aeromonas hydrophila*. *BMC Genomics* **15:** 541.

Robinson N, PK Sahoo, M Baranski, Mahapatra K Das, JN Saha, S Das, Y Mishra, P Das, HK Barman and AE Eknath (2012) Expressed sequences and polymorphisms in rohu carp (*Labeo rohita*, Hamilton) revealed by mRNA-seq. *Mar Biotechnol (NY)* **14(5):** 620-33.

Sahoo L, A Patel, BP Sahu, S Mitra, PK Meher, KD Mahapatra, SK Dash, P Jayasankar and P Das (2015) Preliminary genetic linkage map of Indian major carp, *Labeo rohita* (Hamilton 1822) based on microsatellite markers. *J. Genet.* **94(2):** 271-7.

Sahu DK, SP Panda, PK Meher, P Das, P Routray, JK Sundaray, P Jayasankar and S Nandi (2015) Construction, De-Novo Assembly and Analysis of Transcriptome for Identification of Reproduction-Related Genes and Pathways from Rohu, Labeo rohita (Hamilton). *PLoS One* **10(7):** e0132450.

Shekhar MS, A Gomathi, G Gopikrishnaand AG Ponniah (2015) Gene expression profiling in gill tissues of White spot syndrome virus infected black tiger shrimp *Penaeus monodon* by DNA microarray. *Virusdisease* **26(1-2):** 9-18.

Shekhar MS, J Kiruthika and AG Ponniah (2013) Identification and expression analysis of differentially expressed genes from shrimp (*Penaeus monodon*) in response to low salinity stress. *Fish Shellfish Immunol.* **35(6):** 1957-68.

Shekhar MS, J Kiruthika, S Rajesh and AG Ponniah (2014) High salinity induced expression profiling of differentially expressed genes in shrimp (*Penaeus monodon*). *Mol. Biol. Rep.* **41(9):** 6275-89.

Singh SP, J Sharma, T Ahmad and R Chakrabarti (2016) Oxygen stress: impact on innate immune system, antioxidant defence system and expression of HIF-1α and ATPase 6 genes in *Catla catla*. *Fish Physiol. Biochem.* **42(2):** 673-88.

Soonthornchai W, S Chaiyapechara, S Klinbunga, W Thongda S Tangphatsornruang, T Yoocha, P Jarayabhand and P Jiravanichpaisal (2016) Differentially expressed transcripts in stomach of Penaeus monodon in response to AHPND infection. *Dev. Comp. Immunol.* **65:** 53-63.

Vij S, H Kuhl, Kuznetsova *et al.* (2016) Chromosomal-Level Assembly of the Asian Seabass Genome Using Long Sequence Reads and Multi-layered Scaffolding. *PLoS Genet*. **12(4):** e1005954.