Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; SP-8(2): 103-106

Received: 22-01-2020
Accepted: 25-02-2020

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Molecular characterization of native chicken: prospects and challenges

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Abstract
Molecular characterization of native chicken is the most important tools for the conservation and proper utilization of genetic resources. Indigenous chicken became hardy and resistance to various diseases due to natural selection under free range system. Their survivability is more than the crossbred under village condition. Native chicken plays very important role in rural areas but there is lacking of information on their genetic makeup, performance, adaptability and resistance to diseases. Marker identification will help to enhance selection of quality genotypes for breeding to improve important traits as tolerance to diseases and resistance to environmental stresses. The performance of birds can be improved by selection, upgrading or crossbreeding programme by utilizing desired traits with the help of molecular markers. Some of the limitations of this application are lack of funding, poor infrastructure, scarcity of technical manpower and poor laboratory services.

Keywords: molecular characterization, native chicken, conservation, disease resistance

Introduction
Poultry farming using native breeds in rural area is being practiced in many developing and underdeveloped countries throughout the world. Importance of native birds for rural economy is immense in our country under backyard farming. The number of backyard poultry birds has risen by a staggering 46 per cent during 2012 to 2019 (20th livestock census, 2019). In addition to livelihood it fulfills the requirement of protein in the form of egg and meat at low cost. They have long shank and multicolor plumage which provides protection against predators. They can thrive well on kitchen waste, leftover food, insects and worms etc. Despite of their significance in backyard poultry production, their genetic potential has not been properly utilized. Therefore it is important to assess the native genetic resources for their conservation. In our country there are 19 breeds of chicken registered by NBAGR, Karnal till today including Ankaleshwar, Aseel, Busra, Chittagong, Danki, Daothigir, Ghagus, Harringhata Black, Kadaknath, Kalasthi, Kashmir Favorolla, Miri, Nicobari, Punjab Brown, Tellichery, Mewari, Kaunayen, Hansli and Uttara.

Importance of conservation and characterization of genetic resource
The chicken genetic resources have been originated from their wild ancestor i.e. Red Jungle Fowl and evolved in diverse environment. In poultry more than fifty percent breeds of turkey, domestic duck, chicken, muscovy duck and goose are thought to be endangered [9]. In India and other developing countries without proper characterization or study many of the breeds would be lost [8]. If a breed extinct it means loss of its unique germplasm that is responsible for special adaptive measures which arises due to interactions between the genotype and the environment. The major threat to genetic diversity is due to mixing of high yielding germplasm in poultry sector by indiscriminate cross breeding of local chicken with less adapted exotic germplasm to evolve highly productive breed which adversely affect the native chicken population. So, there is an urgent need to document the diversity of native genetic resources and utilize the local fowl as genetic pool for further selection and breeding strategies. So, it is important to characterize different breeds of native populations to preserve the maximum amount of genetic diversity and further set conservation priorities, to know how unique or different a breed is from other [5].

Molecular characterization
Molecular or genetic characterization can be defined as the procedures used to investigate the

genetic pattern of phenotypes, their mode of inheritance from generation to the next generation, relationships between breeds, levels of variability and within-breed genetic structure.

Importance of Molecular Characterization

- To assess the origin of birds and the geographic distribution of their diversity.
- Providing new information to guide and prioritize conservation decisions for poultry as locally adapted chicken genetic resources could become future assets in breeding programmes.
- Created new possibilities for the selection and rapid genetic improvement of chicken.

Techniques for molecular characterization

Characterization at the molecular level based on molecular markers may be used to estimate genetic variability at genome level and determining the biodiversity with high levels of accuracy and reproducibility. It has important role in estimating the genetic diversity among individuals by comparing the genotypes at a number of polymorphic loci.

Molecular markers: It is variation in DNA base sequence in any locus in the genome of an organism. In a population this marker varies among the different individuals. There are mainly two types of molecular markers on the basis of their chemical nature including biochemical and genetic. Biochemical marker was the first marker used in the livestock for characterization of species, in which a particular protein is visualized as bands of different mobility on a gel. However, its applicability is limited due to its low level of polymorphism. Now DNA based polymorphisms are the markers of choice for molecular-based surveys of genetic diversity. The genetic marker studies the gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. There are two techniques to identify genetic markers:

1. **Non PCR based technique:** This method includes the technique in which no amplification of DNA is required. e.g RFLP.

Restriction Fragment Length Polymorphism (RFLP): In this method cleavage pattern of DNA is analysed to differentiate organism. The DNA sample is digested by restriction endonuclease enzyme results in restriction fragments which are separated by gel electrophoresis according to their length and then southern blot by using specific probe. The advantage of RFLPs is that they are co-dominant markers and are very useful in breeding and linkage analysis. Its limitation is that it requires large amount of DNA. It is hazardous and expensive due to the use of a radioactive isotope. PCR-RFLP assay is a two-step reaction to identify multiple species after restriction enzyme digestion of PCR amplified DNA sequence [12, 2].

2. **PCR based technique:** In this technique amplification of DNA is required to identify the product. e.g. AFLP, RAPD, Microsatellite, SNP etc.

Amplified Fragment Length Polymorphism (AFLP): The AFLPs are dominant biallelic markers. In this method the genomic DNA is digested with the help of restriction endonucleases. The restriction fragment that is obtained is selectively amplifying by PCR. It is highly reproducible and very useful in analyzing between breed variations but requires more DNA (300-1000ng per reaction). AFLP markers enhance framework linkage maps of chicken and other avian genomes economically [14].

Random amplified polymorphic DNA (RAPD): This technique utilizes PCR amplification with a random and short (usually 10 nucleotides) primer. Under low annealing temperature, typically ≤35°C primer anneals randomly at multiple sites on the genomic DNA. Almost all RAPD markers are dominant. It is rapid, simple, user friendly and does not require any prior knowledge of the target sequence. RAPD markers identify low genetic distance among native chicken [1, 17, 13]. High genetic similarity was observed in Aseel than Kadaknath breed [22]. Genetic diversity was higher between Aseel and Brahma than within breed [7].

Microsatellite: Microsatellite is multiple copies of short tandem repeats, generally 1-6 base pair (bp) long, located in both non-coding and coding regions and evenly distributed throughout the genomes. In chickens many microsatellite loci are available so it is regarded as most convenient tool for determination of genetic distances and heterozygosity. Microsatellites exhibit a high degree of polymorphism among breeds and individuals [18, 4, 15]. High genetic diversity was observed in native breeds [26], Hazra and Kaunayen chicken [23, 24]. It was found that chickens of Chamrajnagara and Ramanagra were more distant whereas chickens of Mysore and Bangalore rural were least distant [20].

Single-nucleotide polymorphism (SNP): It is variations in sequence of DNA that results from a change in the nucleotide at a single location in the genome. In a simpler form it is a DNA sequence occurring when single nucleotide (A, T, G or C) differs among members of a species. It is recently emerged as new generation molecular markers for various applications because of abundance in both plant and animal genomes. It is a co-dominant marker and can work with extremely degraded DNA sample. It is efficient rapid and cost effective genetic tool for study of chicken population. It efficiently provides information regarding genetic diversity both within and between breeds. The frequency of SNP in chicken genome is 1 SNP per 225 bp, which is 5 times more than the humans [27]. Disease resistance genes [6, 16] and fat deposition gene in chickens [3] is identified by using SNP. Among the six Italian chicken breeds SNP markers showed the genetic and genomic variability [25].

New technique for molecular characterization

Copy Number Variants (CNV): These are genomic structural variations found over the whole genome in all species and refer to genomic segments of at least 50 bp in size. CNVs contribute significantly to both disease susceptibility/resistance and normal phenotypic variability in humans and animals. Four major mechanisms have been found to be related to CNV formation including non-allelic homologous recombination, non-homologous end joining, Template Switching and Fork Stalling and LINE1 Retro transposition. In comparison to SNPs it is less frequent in terms of absolute numbers but CNVs cover a larger proportion of the genome therefore, a high potential effect on phenotypic variability. Besides these some CNVs are also

found to be the genetic basis of phenotypic variation in chickens. Partial duplication of the *PRLR* is related to the late feathering. Chicken pea-comb phenotype is associated with a duplicated sequence close to the first intron of *SOX5* and dermal hyper pigmentation with an inverted duplication containing *EDN3*. A number of methods may be used to detect Copy number variations, including single nucleotide polymorphism (SNP) arrays, sequencing and array comparative genome hybridization (aCGH). CNV detection becomes more reliable and accurate at whole-genome level by using Recent advances in next-generation sequencing (NGS) technology [21]. On the other hand study on Mexican Creole chicken population suggest that there is not a clear division in classifiable subpopulations based on the CNVR characterization and the chicken population can be considered a unique mix of genetics [11].

Challenges

In developing countries like India one of the biggest challenges is lack of successful breeding programmes, small flock sizes, incorrect assessment of phenotypic variability between farms, low reproductive efficiencies and poor animal health management programmes. The most important resource limitation is lack of basic research facilities and availability of funds to set up research. Molecular genetics research is highly sophisticated and also required skilled manpower as well as technologist. Other limitations include inadequacy of scientific equipments to support research and technical manpower [10], poor essential utilities like power and water, no support services such as gene bank, in vitro storage facilities, storage facilities, animal holding facilities, radiation huddling, disposal facilities and computing facilities and ICT services [19].

Conclusions

The native breed of chicken possesses better feed conversion ability for non conventional feed material, disease resistance, tropical adaptability and local preference. They are being used for rural backyard poultry production but their genetic potential has not been fully exploited. During past few decades, the development of tools for the analysis of DNA enabled our capacity enormously to characterize variation within and between breeds, and form the basis for conservation and genetic improvement of the native poultry breeds. Conserving the poultry breeds will be enabled by the existing and emerging molecular technology.

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