Efficacy of Microsatellite Markers for Delegating Genetic Variations between two Poultry Breeds of India for the Conservation of Germplasm

Abhishek Sharma[1&3], Shiv Om Pratap[1], Ruchi Gaur[2], Praval Pratap[1], Mritunjai Singh[3] and Archana Tiwari[3]

Dr MPS Group of Institutions, Agra (UP)
ITM University, Gwalior
Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal (MP)
Corresponding author- drshivompratap@gmail.com

ABSTRACT

Molecular markers are the identical DNA sequences, found at particular sites in the genome, and transferred from one generation to another generation with the laws of inheritance. With the advancements of molecular tools for the assessment of genetic variations to explore genetic information which turn out to be possible through molecular markers. These molecular tools are reliable as they are easily identifiable, ease of genotyping and their passage to consecutive generations. Previously the molecular markers are based on the phenotypic traits, after words the biochemical markers came into existence with their protein expression as the result of gene activity. The advancements in molecular markers like RFLP, VNTR, RAPD, AFLP, SNP, STR etc. being used for the many researches across the plants and animals impartiality. These markers play an important role in establishment of more efficient breeding regimens and conservation strategies in the poultry breeds. Microsatellites markers having a repeats of 1-6 nucleotides found dispersed among the genome are now widely preferred in the study of genetic variations in contrast to other genetic markers. The use of DNA markers in poultry has supported the many research as means of biodiversity and QTLs assessment for the determination of traits of economic benefits in different avian species such as: chicken, quails, ducks, goose, turkey and other birds.

***Keywords*:** Molecular markers, microsatellites, genetic variations, sequences, poultry etc.

Introduction:

Effectiveness of Molecular advances has explored a new horizon for the identification and confirmation specific nucleotides sequences for the determination of genetic variations in respect of livestock improvement programs. These molecular tools have greatly facilitated the analysis of various population parameters among the inter-breed and intra-breed genetic variations of commercial and native chickens. As far as concerned the need of determining genetic diversity *via* unique alleles across the genome. These molecular markers plays an important role by exploring the existence of such alleles in a particular chickens for establishing table birds to fulfillment the current needs of nutrition, researches and breed establishment. In the genome, these molecular markers are considered as constant landmarks, identical nucleotides sequences, heritable as per Mendelian laws, co-dominance in nature, highly reproducibility, ease of genotyping and cost effective without manual inputs (**Pratap *et al*., 2013**).
The first report on the DNA marker based technology was made by **Botstein *et al.* (1980)**, who realized that a DNA based marker known as restriction fragment length polymorphism (RFLP) had provided much greater efficacy for mapping various human genes than anything previously available. Molecular markers have the ability to find out the variabilities in both coding and non-coding sequences **(Fulton *et al*., 2006),** placed in the Table No.1 briefly.Inheritance of plumage color variations in a large flock of Japanese quail (**Mishra *et al.* 2008**) along with the development of Microsatellite DNA markers for duck (**Christ *el et al.* 2006**).

Microsatellite Markers: *A novel tool for accessing diversity*
Microsatellite markers known as a short tandem repeat (STR) is a genomic element that consists of repeats of nucleotides sequences multiple times in a tandem array. STRs can be classified according to the number of nucleotides in the repeat unit. These are multi-allelic, highly polymorphic, co-dominant and assayable by PCR. Perfect microsatellites are those that contain a single uninterrupted repeat element flanked on both sides by non-interrupted sequences **(Weber 1990** and **Ruchi *et al*., 2017).**The STRs qualify well as a polymorphic and robust marker system which has proven to be more versatile, particularly for population analysis (**Ahlawat*et al*., 2008: Pratap *et al*., 2013, Ruchi *et al*., 2017).** Microsatellites have been successfully used in many chicken studies because of their co-dominant nature and availability in different allelic forms across the genome. **(Fulton *et al*., 2006; Chatterjee *et al*., 2010**and **Sridevi *et al*., 2018).**

Various Models of STR Mechanism:

Microsatellites tend to mutate with mutation rates of up to 10^{-2} per generationamong the genome.The simplest popular model of microsatellite evolution is the classical stepwise mutation model (SMM) in which, upon a mutation, 1 repeat unit is either gained, resulting in an expansion, or lost, resulting in a contraction. However, mutations have been observed to change the repeat length by >1 unit. The two-phase model (TPM) allowing mutations of 1 repeat unit (one-phase) with probability $p$and mutations of ≥1 unit(s) (two-phase) with probability $1 - p$, while the distribution of the lengths of multiunit mutations is geometric**(Sainudiin*et.al***

2004). There are various other markers which are widely preferring in the developed countries as depicted in the **Table No. 1** but in India still breed characterization is attaining on the basis of morphological and biochemical basis mostly.

Table No.1: Relevancy of other Molecular Markers for the Chicken Genetic Diversity

S.No	Author	Marker Used	Citations
1.	S.J Lamont	RFLP	Poultry Science, January 1987
2.	S.P.S Ahlawat	RAPD – PCR	British Poultry Science April 2004
3.	M. De Marchi	AFLP	Animal Genetics, October 2005
4.	T. Twito	SNPs	Animal Genetics, December 2010

Microsatellite Markers for Study of Genetic Diversity in Poultry Breeds:

Poultry production is an important livestock sector playing a major role as food source for the world. Chicken (*Gallus gallus*) belong to order Galliformes, class of Aves of the animal kingdom. Chicken being domestic for us from years from different continents and sub-continents the breeds from India , USA, China, Malaysia, Kenya, Thailand and other parts of the world. The genome of chicken has a diploid number of 39 chromosomes, eight pairs of macro chromosomes, one pair of sex chromosomes (Z & W), and 30 pairs of micro chromosomes. The Chickens like other avian species differ from mammals in respect of sex chromosomes, the female is heterogametic (ZW) and male is Homogametic (ZZ) and Z&W chromosomes display hetero-polymorphism. The size of the chicken genome is estimated to be 1.2 x 10^9 bps and approximately 4000 cm in length. The Chicken Genome consist total 586 functional genes and 2386 loci along with the numerous Microsatellite Markers for the study for unraveling the genome (**Groenen *et al*., 2000** and **Chattergee *et al*., 2010).**

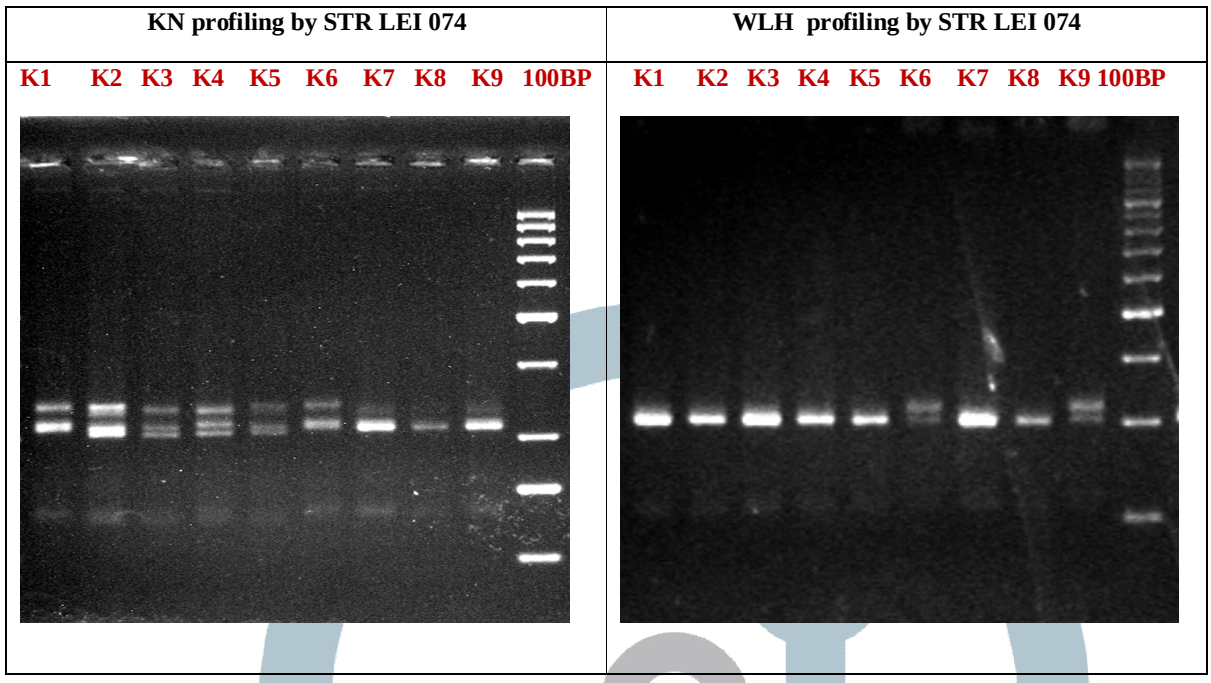
The smaller size, which is less than half the size of mouse and human genome which makes the chicken genomics more interesting, as the evolution appears to have novel genome to minimal size and chicken genome, like other animals consist of repetitive sequences with variable degree of repetition across the genome.The first chicken genome sequence assembly was released in March 2004 (International Chicken Genome Sequencing Consortium, 2004), revealed that sequence of 907 Mb covers approximately 86% of the Chicken genome on the 38 autosomes, 10 micro-chromosomes do not have an assigned sequence (**Pirani *et al*., 2007; Rajkumar *et al*., 2008; Pratap *et al*., 2013** and **Ruchi *et al*., 2017**).

Molecular Characterization of selected Poultry Breeds have been carried out in terms of molecular parameters like; Na (Average Number of Alleles), Ne (Effective Numbers of Alleles), PIC (Polymorphism Information Content) Ho(Observed Heterozygosity) and He (Expected Heterozygosity) by using a panel of 06 Microsatellite Markers as compare to morphological parameters to delineate genetic difference for the establishment of breeding conservation strategies in the current study as placed in the **Table No.2**. Allelic Pattern showing clear cut molecular differences through Gel photographs *via* PCR amplification by using Microsatellite Markers to differentiate these chicken breeds on molecular level as depicted in the **Figure No. 1** in the current study.

Table No.2: Comparative Molecular Parameters among the Selected Poultry Breeds based on Microsatellite Marker profiling

Microsatellite Marker	Population	Na	Ne	PIC	Ho	He
ADL 0145	KN	2	2.4	0.375	0.111	0.529
	WLH	2	2.0	0.372	0.444	0.523
ADL 0210	KN	2	2.5	0.286	0.099	0.366
	WLH	2	1.6	0.375	0.333	0.529
GCT 0053	KN	4	2.4	0.624	0.889	0.725
	WLH	2	1.9	0.321	0.556	0.425
LEI 074	KN	4	2.9	0.631	0.667	0.732
	WLH	2	1.2	0.178	0.222	0.209
MCW 0005	KN	3	3.8	0.448	0.889	0.582
	WLH	2	2.5	0.346	0.667	0.471
MCW 0104	KN	4	2.6	0.558	0.889	0.647
	WLH	2	2.0	0.362	0.333	0.503

Figure No.1: Allelic Pattern of Selected Poultry Breeds: KN and WLH by LEI 074 Microsatellite Markers



In this way the efficacy of Molecular Markers have proved not only in the diversified breeds of chickens even intra breed genetic differences can also established *via* Microsatellite Markers as compared to Morphological or Biochemical basis which needs more manual inputs.

Need of Molecular Markers for the Study of Genetic Diversity:

The microsatellites are reputed DNA-marker systems of choice, as they provide a polymorphic and robust marker system, being abundant, co-dominant, randomly available across genome, having high information content due to variable repeat length, high mutation rate, ability to decipher moderate to high level of variability, readily amenable to PCR, ease of genotyping and easy allele assess ability on electrophoresis **(Kaya and Yildiz, 2008).**Microsatellites act as genetic markers by detecting the length of polymorphism in the core repeat units. Microsatellite has been registered by many authors as reliable markers in chickens **(Romanov and Weigend, 2001)**. The possible linkage of STRs polymorphism with growth, egg production, and immune-competence traits is an advent tool of Quantitative genetics. **(Chattergee *et al.* 2010).**

The conservation of native germplasm is need of the our due to their uniqueness in the hardiness, ability to thrive under adverse climatic conditions and desirable taste and flavor of eggs and meat in contrast to commercial egger breed like as WLH. Hence there is a significant demand for the products of native chickens like as; Aseel and Kadaknath breeds. However, in order to increase the productivity of backyard/rural farming, improved/exotic birds are being introduced in the rural areas or in their breeding tracts leading to dilution of their genetic purity or complete replacement of native germplasm and hence these breeds are under threat of extinction **(Haunshi *et al.*, 2011)**. Advancements of Biotechnological tools and techniques have simplified the understanding of inherent-variability between various breeds of economic importance in Poultry for the goodquality of protein in term of egg and meat to overcome the malnutrition of our society**(Parmar *et al.*, 2006, Mishra *et al.*, 2008 and Pathak *et al.*, 2015).**

Conclusion:

The review illustrates that analysis of genetic diversity of chicken breeds *via* Microsatellite Markers are best suitedin the current molecular characterization of a breed. The improvements in Poultry products may be more useful through molecular breeding for enhancing production traits, meat and more numbers of eggs for the better human welfare. Chicken can also serve as a very good model of researches due to numerous of advantages due to its variant breeds, shorter generation time, valuable nutritional products etc. and also supporting global economy. The current review has given an idea for the establishment of unique breeds through molecular estimates and future conservation policies further. Therefore, it was concluded that microsatellite-based genomic analysis through polymorphic STR markers can efficiently delineate population-differences as per given citations in chicken population, arisen due to unique breeding histories and evolutionary forces.

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