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Genetic Diversity of Himalayan Region Chicken through SSR Molecular Markers

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Abstract: ***Samples of chicken blood are collected from the lower Himalayan region (Red fowl and Black fowl) to study the genetic diversity on the basis of microsatellite ADL0306 whose phenotypic properties are very helpful to chicken breeders. Simple Sequence Repeats PCR technique is a reliable technique to study the difference and similarity among different species of gallusgallus which is based on repeating motifs. SSR-PCR gel electrophoresis image is analyzed using Total lab Quant version 12.4 software. Software analyses gel image with respect to ladder run along with samples and generates reports in term of dendrogram and molecular weight of each electrophoresis band. Dendrogram shows close relatedness and diversity distance among all samples.***

Keywords: Gallus gallus, repeating motif, dendrogram, microsatellite

1. Introduction

Chicken meat is one of the most famous and delicious kitchen ingredients in Northern India. Even though they are given same environmental, dietary and other climatic conditions, they develop with different weight. Less weight chicken is a cause of concern. Therefore, it is a highly important issue to identify the reason behind it. There must be a genetic reason as to why they grow with different size and weight. Several new DNA markers have been developed to detect genetic variety within and between chicken populations. DNA based markers have dominant impact on gene mapping. DNA molecular markers are useful in measuring local gene flow and migration [1].

The chicken, domesticated at present time, are the descendants of red jungle fowl hybrid along with the grey jungle fowl, first raised long past in the northern parts of the Indian subcontinent. Chicken is a major source of protein in our daily diet, containing 54% protein. Chicken belongs to the subspecies *Gallus gallus domesticus* and is raised all over the world for its meats and eggs.

Native breeds are considered a national asset and a key factor in creating sustainable agriculture in developing countries. Therefore, precise assessment of such native genetic resources is of great importance and could be utilized for the purpose of their conservation, management, reproduction and exploitation. Native chickens are known to be good foragers and efficient mothers with minimal care required for their growth. They are, therefore, most suited for raising under village conditions. These birds do, however, need special attention with respect to their conservation and improvement [2, 3].Information about the genetic characterization of these strains and the amount of genetic diversity among them are minimal.

Knowledge of molecular genetics provides modern tools for chicken breeding and enhanced selection progress with fast and precise identification and selection at gene level with better performance [4]. Genetic variation found in chicken strains permits breeders to improve new characteristics in response to environmental changes [5]. Molecular analysis of genetic diversity and relatedness among chicken strains are an important prerequisite for the recognition of genetic resources that are economically important [6].

In this experiment, twenty chicken blood samples were collected from the lower Himlalayan region. Genomic DNA of 15 samples was isolated successfully. Ten different SSR primers were used for PCR of which one primer produced successful result for further analysis. Ten microsatellites were selected, out of which microsatellite ADL0306 was used.

Ten microsatellite sequences representing four chicken chromosomes were studied in the experiment. The choice of microsatellite sequences was guided by the results of other studies, in which the high expected heterozygosity (HE) and Polymorphism Information Content (PIC) of the selected microsatellite sequences in chickens were reported.

2. Literature Survey

2.1 Microsatellite ADL0306

Microsatellite ADL0306 has repeat motif $(tg)_{11}$(accession no. G01721.1), responsible for phentotypic aspects like Body Weight, skin fat weight, Egg weight, and Height [7].

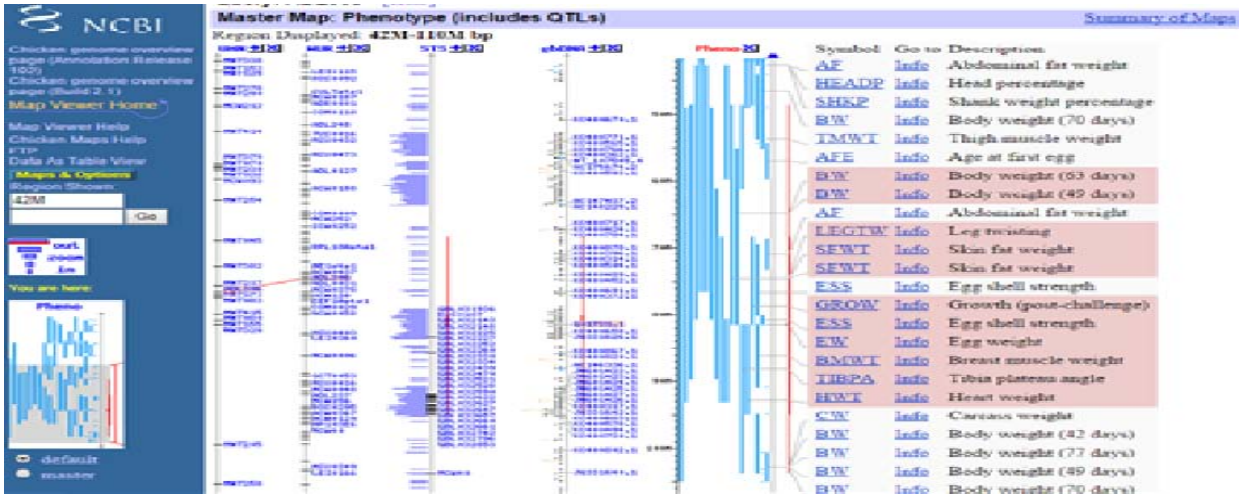

Figure 1: Selected phenotypes are a result of corresponding gene product for Microsatellite ADL0306, taken from NCBI site.

3. Material and Methods

DNA isolation: DNA isolation method includes lysis buffer (50mM Tris-HCl, 20mM EDTA pH 8.0, 1% SDS, 10uL proteinase K 20mg/mL), saturated phenol, PCI (Phenol: chloroform: Iso-amyl alcohol, 25:24:1), Chloroform, Isopropanol, Sodium Chloride 5M.

200ul of blood is taken into 1.5ul eppendorftube. After that, 700ul of lysis buffer is added, vortexed briefly and left for 2 days for incubation at 55°C. After incubation, centrifuge samples at 12000 rpm for 10 minutes, collect supernatant and discard the pellet. Add saturated phenol in equal volume to supernatant, centrifuge at 12000 rpm for 10 minutes, collect top layer in fresh eppendorf tube, add chloroform in equal volume, centrifuge at 12000 rpm for 10 minutes. Collect top layer in a fresh eppendorf tube and add equal volume of isopropanol and 170ul NaCl(0.5M). Centrifuge at 12000 rpm for 10 minutes, collect pellet, wash it with 70% ethanol and finally suspend the pellet in 100ul of $T_{10}E_{0.5}$ buffer for long storage in -20°C.

3.1 Quantity Analysis of DNA

Table 1: Spectrophotometer Reading

Sample	*Absorbance at 260 nm*	*Absorbance at 280 nm*	*O.D.*	*Yield (ug/ul)*
S1	2.272	1.620	1.402	11.35
S2	0.211	0.113	1.860	1.055
S3	0.430	0.389	1.105	2.15
S4	2.280	1.624	1.403	11.4
S5	0.172	0.107	1.607	0.86
S6	0.010	0.008	1.200	0.05
S7	0.224	0.210	1.060	1.12

SSR-PCR Conditions: Following is the cocktail for ssr reaction : 10xPCR buffer (including Mg^{++}) 5.0ul, dNTPs 4.0 ul, Forward primer 2.5 ul, Reverse primer 2.5 ul, Template DNA 2.0ul, Taq polymerase 0.2 ul (5U/ul), ddH2O 33.8 ul.

PCR program : Initial denaturing temperature is set at 95°C for 5 minutes followed by 35 times loop of 98°C temperature for 20 seconds. Annealing temperature of 49°C for 20 seconds with final extension temperature of 72°C for 4 minutes including holding temperature of 4°C for 1 hour is provided.

Agarose Electrophoresis: 2.5% Agarose gel is prepared in 1X TBE buffer to run PCR samples at 95V for 45 minutes along with 100bp ladder.

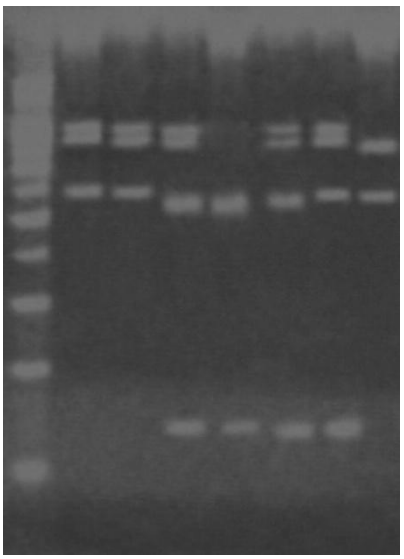

Figure 2: Primers used : Forward : TCAGTTTGACTTTCCTTCAT, Reverse : GTTACTGTATCTTGGCTCAT,
Tm: 49°C

4. Result

Table 2

Sample	**Ladder**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Lane No.	1	2	3	4	5	6	7	8
No. of bands	12	3	3	4	2	4	4	2

The above electrophoresis image is analyzed by totallab software which calculates molecular weight of each band with respect to ladder. Data is generated by Totallab software for seven samples.

Table 3: Totallab software result

	Bands			
Samples	**1**	**2**	**3**	**4**
S1	941.839	878.485	582.534	
S2	952.454	870.696	600	
S3	934.915	857.242	571.096	127.443
S4	557.006	125.047		
S5	970.582	870.696	575.71	127.443
S6	924.809	870.696	582.534	125.999
S7	848.551	582.534		

Comparative analysis of electrophoresis bands on the data generated by Totallabquant version 12.4

Totallab quant version 12.4: It is a gel image analyzing software, which takes electrophoresis image as a input. Ladder specification along with number of bands and their respective sizes are also provided as input. On the basis of these inputs, the software calculates the relative molecular weight of bands.

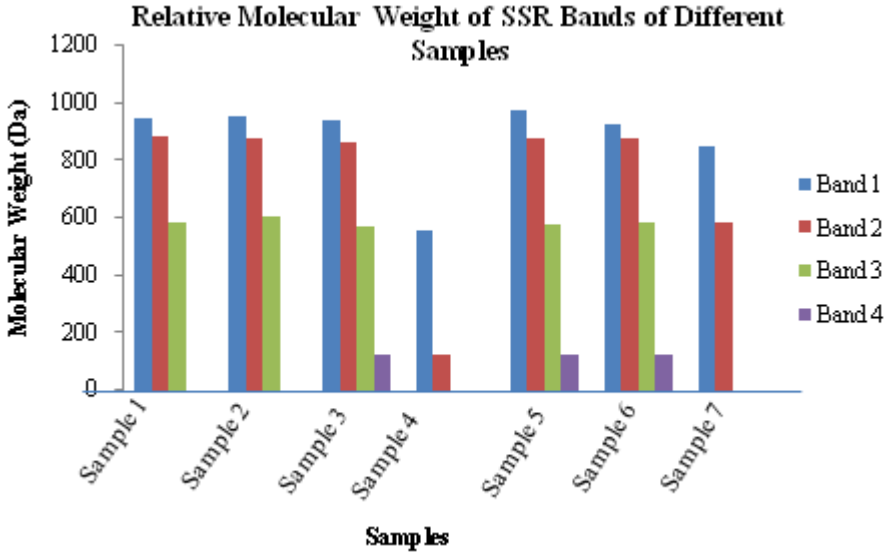

Figure 3: Histogram (Molecular Weight Vs Samples)

Totallab software also generates dendrogram which helps in segregation among different species of chicken and can be used to identify similarity as well. The values of similarity indices reflect the genetic distance among studied genotypes. Phylogenetic relationships and genetic distance can be used to determine the relatedness among different chicken strains.

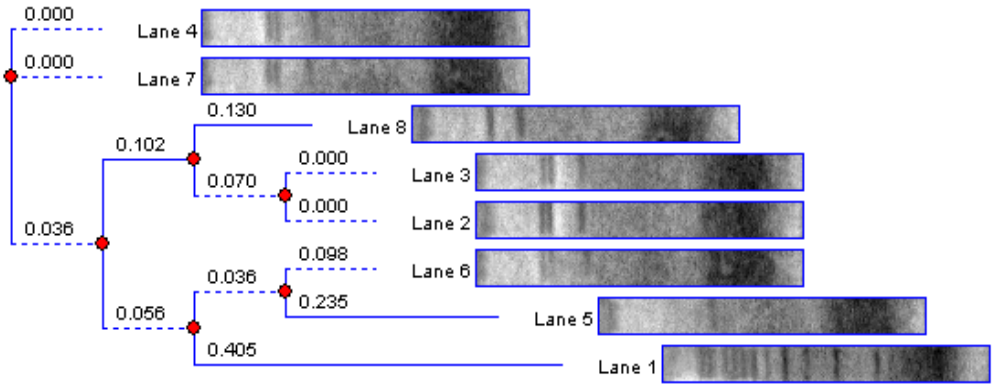

Figure 4: Dendrogram showing relatedness between different samples

5. Result and Conclusion

In fig.4, Lane1 is ladder which shows much difference from rest of the samples. But sample 4 and sample 7 are having very close relatedness but are different from the other samples. Apart from this, sample 3 and sample 2 are genetically similar. Knowing phenotypic characteristics, it will be possible to segregate them. The values of similarity were estimated across all possible pair wise comparisons of individuals among strains.

6. Future Scope

This Data compiled to show genetic variation among different varieties of chicken is quite useful and can be used by Breeders to sort out the problem of variation in body weight of chicken and hence select desired species of chicken for breeding. The selection and optimization of molecular markers is a fundamental step towards full success in genetic studies.

References

[1] Rahimi, G., A. Khanahmadi, A. Nejati-Javaremi and S. Smailkhanian, 2005. Evaluations of genetic variability in a breeder flock of native chicken based on randomly amplified polymorphic DNA markers. Iran. J. Biotechnol., 3: 231-234.
[2] Kaya, M. and M.A. Yildiz, 2008. Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. Biochem. Genet, 46: 480-491.
[3] Zhou and Lamount 1999, Wimmers et al. 2000, Wardęcka et al. 2002, Kerje et al. 2003, Jacobsson et al. 2004, Lujiang et al. 2006, Tadano et al. 2007a, b.
[4] Fulton J.E. (2008): Molecular genetics in a modern poultry breeding organization. World's Poult. Sci., 64: 171-176.
[5] Ola A. Galal, Medhat R. and Ragaa E. Abd-El- Karim (2013): Analysis of genetic diversity within and among four rabbit genotypes using biochemical and molecular genetic markers. Afri. J. Biotechnol., 12 (20): 2830-2839.
[6] Nikkhoo M., Hadi S., Ghodrat R., Mozhdeh N., Farnaz F. and Minoo K. (2011): Measurement of genetic parameters within and between breeder flocks of Arian broiler lines using randomly amplified polymorphic DNA (RAPD) markers. Afri. J. Biotechnol., 10 (36): 6830-6837.
[7] http://www.ncbi.nlm.nih.gov

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