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Polymorphism of prolactin receptor gene in indigenous ducks of Assam

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Abstract
Prolactin receptor (PRLR) genes are found to be associated with the molecular basis of useful production traits in poultry. The present study was conducted to study the polymorphism of PRLR gene in 101 indigenous ducks of Assam. PCR-RFLP analysis of PRLR gene using *PciI* restriction enzyme revealed two genotypes, arbitrarily designated as AA, AB. Following digestion of 367 bp amplified product of PRLR gene yielded two fragments (108 bp and 259 bp) of AA genotype and three fragments (108 bp, 259bp and 367 bp) of AB genotype. The frequencies of A and B alleles were found to be 0.956 and 0.045, respectively and genotypic frequencies 0.911 and 0.089 for AA and AB genotype, respectively. A variant of PRLR gene was found to be predominant in the indigenous ducks of Assam with the highest frequency of AA genotype followed by AB genotype (AA>AB). Chi-square (χ^2) test revealed that the population under study was in Hardy-Weinberg Equilibrium for PRLR gene.

Keywords: Duck, PCR-RFLP, *PciI*, Polymorphism, PRLR

1. Introduction

Genetic improvement of reproductive traits of farm animals and poultry is generally aimed to enhance production efficiency, product quality and maximizing economic performance. Advanced research approaches and methods spanning from fundamental studies of molecular genetic mechanisms through the applied methodologies has enabled scientists to obtain vast amounts of new genomic information that allows for improved estimation of an animal's genetic and breeding values with greater accuracy. The identification and utilization of potential candidate genes associated with reproduction and significant effects on economically important traits have become increasingly important in poultry breeding programmes. Ducks are mostly reared among the rural masses for egg and meat production to improve the socio-economic status. Meat and eggs of waterfowl belong to the food with high nutritional quality. Ducks are well known for their economical importance varies between the continents and the countries [1]. PRLR is found to be associated with oocyte maturation [2, 3]. Polymorphism of the PRLR gene is reported to be significantly associated with egg production traits in the Erlang Mountainous chicken and found as a strong candidate gene that affects egg production traits in this breed [4]. Consistent research on this gene, which is involved in many physiological pathways, contributes to understanding the molecular basis of useful production traits. The use of this molecular genetic markers potentially will increase the intensity of selection and will most effectively uncover the productive potential of birds. The pituitary hormone of PRL is a ligand of PRLR [5]. The PRLR is an important regulator gene in the process of cell growth and differentiation. It is regarded as a candidate genetic marker for reproductive traits. It is also speculated that PRLR gene may be the major gene responsible for the attainment of sexual maturity [6]. In hens, PRLR gene is located on the Z chromosome [7-9]. This gene consists of 15 exons and 14 introns [5], encoding 831 amino acids [10] and is closely associated with production traits [11]. This gene was reported to have significant effects on egg production [12, 13]. The objective of the present study was to identify polymorphism in genomic sequences of PRLR gene and to estimate their gene and genotypic frequencies in indigenous ducks of Assam, India.

2. Materials and Methods

The present study was conducted on indigenous ducks of Assam from 9 different districts, *viz.*, Dhubri, Bongaigaon, Barpeta, Nalbari, Kamrup, Morigaon, Nagaon, Karbi Anglong and Sivasagar.

Two ml of blood was aseptically collected from 101 apparently healthy indigenous ducks using EDTA (2.7%) as an anticoagulant. The samples were properly labelled and transferred to the laboratory. Genomic DNA was isolated from 20 µl of whole blood using DNeasy blood & tissue Kit (Qiagen, Netherlands). Purity of DNA was judged on the basis of optical density (OD) ratio, at 260:280 nm. The samples having OD ratio (260 nm/ 280 nm) 1.7 to 1.9 were used for the experiment. The primer sequences used for the amplification of PRLR gene (F: TTCCATTGTTCAGTAGCGTT and R: GATTTGCAATGGTATCCCTG) were designed using sequence template downloaded from NCBI, accession no. DQ345782.1. The primers were diluted for a working concentration of 10 pmol/ µl. The PCR reaction was carried out in 0.2 ml PCR tubes in a thermal cycler (BIO-RAD Model S100, USA). PCR was carried out in a final reaction volume of 25µl. Each reaction volume contained 1.0 µl of DNA template, 0.5 µl each of forward and reverse primers of concentration 10 pmol/µl, 12.5 µl of master mix (Dream taq, 2X) (Thermo Scientific) and 10.5 µl of nuclease free water. PCR reaction was carried out in a thermal cycler under the following condition: initial denaturation at 95°C for 4 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 30 sec, extension at 72°C for 45 sec with a final extension at 72°C for 7 min. PCR amplification was confirmed by 2% agarose gel electrophoresis and amplified product was visualized as single compact fluorescent band of expected size under UV light and documented by gel documentation system (Gel Doc XR+, BIO-RAD, USA). The 4 µl PCR products was digested with 1 µl of *Pci*I enzyme in 1X REs buffer followed by incubation at 37°C for 12 hours. The digested products were analyzed by 12% neutral polyacrylamide gel electrophoresis (PAGE) and the gel was visualized in a Gel Doc system. Identification of genotypes was done according to the restriction fragment patterns The amplified PCR products were sequenced by outsourcing (First Base, Malaysia). The identity of the sequences were confirmed by using NCBI-BLAST tool and the results of sequencing were analysed by using BioEdit. Gene and genotypic frequencies were calculated as described by Falconer and Mackay (1996). Chi-square (χ^2) test was performed to test if the population was in Hardy-Weinberg equilibrium.

3. Results and Discussion

The yield and purity of extracted DNA samples were estimated using UV Spectrophotometer (Nanodrop Spectrophotometer, Model-UV/VIS 916). The yield of DNA extracted from 20 µl of whole blood ranged from 361.2 ng/µl to 502.0 ng/µl with a mean of 431.6 ng/µl. The OD ratio (260nm/280nm) was in the range of 1.7 to 1.9 indicating the purity of the extracted DNA. After quantification of each DNA sample, a uniform final concentration of 100 ng/µl was prepared by further dilution of the entire sample in 2.7% Tris EDTA (TE) buffer. 1% agarose gel electrophoresis of the isolated DNA yielded distinct bands with no smearing (Fig.1). By PCR amplification, a 367 bp amplified product was obtained in all samples from indigenous ducks of Assam (Fig.2). The PCR-RFLP studies on PRLR gene in indigenous ducks of Assam using *Pci*I revealed two types of fragment patterns, arbitrarily designated as AA and AB genotype. Following digestion of the PCR products, AA genotype yielded two fragments (108 and 259 bp) and AB genotype yielded three fragments (108, 259 and 367 bp) (Fig.3). The frequencies of both the alleles are presented in (Table1). A nucleotide transition (A→C) was observed at position 233 bp. In the population studied, two groups of ducks exhibiting polymorphism of PRLR gene could be distinguished: AA and AB. Notably, there were greater numbers of AA homozygotes in comparison to AB heterozygotes. The frequencies of AA and AB genotypes were 0.911 and 0.089, respectively and the frequencies of ‘A’ and ‘B’ alleles were 0.956and 0.045 respectively. Gene frequencies showed that the PRLR ‘A’ variant was predominant in indigenous ducks of Assam. The Chi-square (χ^2) test revealed that the calculated value for PRLR gene (0.019) was found to be lesser than that of the tabulated values at 5% level of significance with 1 degree of freedom and are presented in (Table2). Hence the population under study was found to be in Hardy-Weinberg Equilibrium for this gene. Digestion of 367 bp PCR products of PRLR gene with *Pci*I revealed two types of fragment patterns, arbitrarily designated as AA and AB genotype. The AA genotype yielded two fragments 108 bp and 259 bp, AB genotype yielded three fragments 108 bp, 259 bp and 367 bp in indigenous ducks. Polymorphism of PRLR gene was also observed among White Leghorns, Hy-Line brown egg layers, Avian broilers and Chinese local breeds of chicken including Silkie [14], Wanjiang white goose [11], indigenous chickens of Mazandaran province [5]. However, Poltavskaya Glinistaya breed of chicken was reported to be monomorphic [15] for this gene. Bidirectional sequencing was done for the representative samples of the two available genotypes, i.e. AA and AB. The allele ‘A’ sequence was found to have one *Pci*I restriction site at nucleotide position 108 yielding two fragments of 108 bp and 259 bp after digestion. On the other hand, the ‘B’ allele of the gene was not having any restriction site for the used enzyme yielding a single fragment of 367 bp even after digestion because of the A→C mutation involving the restriction site. Gene and genotypic frequencies were calculated as per the method described [16]. The frequencies of A and B alleles of PRLR gene were 0.956 and 0.045, and those of AA and AB genotypes were found to be 0.911 and 0.089, respectively. The present findings revealed a higher frequency of AA genotype followed by AB for PRLR gene. These data were in conformity with the findings of three genotypes GG, GT and TT, coded by two different alleles G and T in PRLR gene in Khaki Campbell ducks, where the frequencies of genotype GG and allele G were found to be the highest 0.56 and 0.74, respectively [17]. Chi-square (χ^2) test revealed that the calculated value for PRLR gene was lesser than that of the tabulated values at 5% level of significance with 1 degree of freedom indicating that the population under study was in Hardy-Weinberg Equilibrium for PRLR gene. Thus the analysis of actual and theoretical distributions of individuals with different genotypes for PRLR gene did not reveal any disturbance of genetic equilibrium in the experimental duck population, which indicated the absence of selection pressure. However, these findings were in contrast with the findings in Mazandaran native fowls [4].

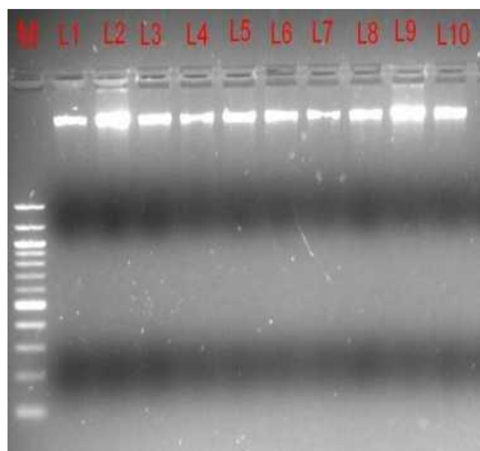

Fig 1: 1% Agarose gel electrophoresis M: 100 bp ladder, L1- L10: Genomic DNA

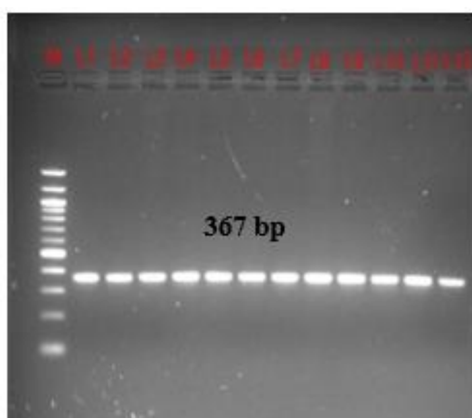

Fig 2: 2% Agarose gel electrophoresis M: 100 bp ladder,

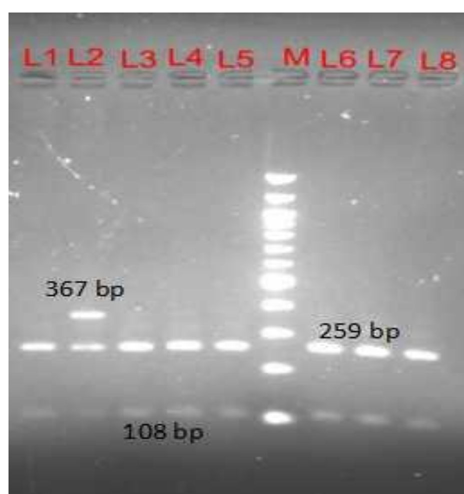

Fig 3: 2% Agarose gel electrophoresis M: 100 bp ladder, L_1, L_3, L_4, L_5, L_6, L_7, L_8: AA Genotype

Table 1: Genotype and gene frequencies for PRLR *g*ene

Loci	Genotype frequencies		Gene frequency	
PRLR	AA	AB	A	B
	0.911 (92)	0.089 (9)	0.956	0.045

Table 2: Chi-Square test for Hardy-Weinberg equilibrium in *PRLR* genotypes

Loci	Genotype	Observed	Expected	$(O-E)^2/E$	χ^2
PRLR	AA	92	92.213	0.001	0.019^{NS}
	AB	9	8.605	0.018	

4. Conclusion

The population of Indigenous ducks of Assam under study was polymorphic in respect of the *PRLR* gene, having 2 alleles with frequencies 0.956 and 0.045, respectively.Chi-Square (χ^2) test revealed that the population under study was in Hardy-Weinberg equilibrium for PRLR gene.

5. Acknowledgements

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