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Single Nucleotide Polymorphism C-2161G in Promoter Region of *Prolactin* Gene in 'N' Strain of White Leghorn

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A B S T R A C T

Single nucleotide polymorphisms are the most commonly observed genetic variation among particular population, generally known as SNPs (pronounced "snips"). The main objective of the current research was recognition of SNP C-2161G in promoter region of prolactin gene in 'N' strain of White Leghorn and its interrelation with production traits. A total of 200 birds of 'N' strain of White Leghorn were randomly selected from All India Co-ordinated Research Project (AICRP) on Poultry Improvement, Mannuthy, Thrissur, Kerala. Blood samples were collected from the randomly selected birds and isolation of genomic DNA was done using DNA isolation kit. PCR and PCR-RFLP analysis was accomplished to detect a SNP at C-2161G site in promoter region of *prolactin* gene. The genotypes are represented as CC, CG and GG for C-2161G. All the 200 birds were noticed with same genotype (CC) and production performance.

Introduction

Prolactin (PRL) is a polypeptide hormone secreted by the adenohypophysis gland and it has been shown to have a broad spectrum of biological functions in all vertebrates. The chicken prolactin hormone plays a major role in egg production. At present marker assisted selection (MAS) is a blooming technology in poultry breeding programme to choose the superior individual. In order to achieve the better production level in poultry MAS can be performed rather than conventional method of selection. Commonly used markers include, Single Nucleotide Polymorphism (SNP) and Microsatellite marker etc. Obtained results

based on these polymorphisms are compared with phenotypic variation among the individual birds. Various methods viz., Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP), Single Strand Conformation Polymorphism (SSCP) and Microsatellite marker analysis are used to identify the polymorphic patterns of genes and to identify the markers.

It has been reported that prolactin gene has a crucial role on production traits like age at sexual maturity, egg weight and egg number. The main objective of the present study was recognition of single nucleotide polymorphism C-2161G in promoter region of *prolactin* gene in 'N' White Leghorn and its interrelation with production traits.

Materials and Methods

Experimental birds

A total of 200 birds each belonging to 'N' strain of White Leghorn (IWN) which had undergone 28 generations of continuous selection were randomly selected from All India Research Co-ordinated Project (AICRP) farm on poultry improvement, Mannuthy.

Collection of blood samples

From each bird, 0.5-1 ml of blood was collected from the wing vein using 2.5 ml disposable syringe in a EDTA vial under aseptic condition. The samples were brought to the laboratory at 4ºC in ice pack.

Isolation of genomic DNA

Isolation of Genomic DNA was done from the whole blood according to the standard procedure using ODP304 Origin Genomic DNA isolation kit. The yield and quality of the DNA obtained was checked by 0.8% agarose gel electrophoresis as well as by Nano-drop spectrophotometer. The DNA samples showing the OD260/OD280 value between 1.7 and 1.9 was used for further investigation.

PCR assay

Polymerase chain reaction was carried out using specific set of forward (F-5'AGAGGCAGCCCAGGCATTTTAC3') and reverse primer(R-5'CCTGGGTCTG GTTTGGAAATTG3') to amplify the 439bp fragment of *prolactin* gene containing single nucleotide polymorphism C-2161G in promoter region. Each diluted primer (10 pM/µl) was added to the template DNA [working solutions prepared from stock solution by diluting with sterile distilled water (Millipore) to get a final concentration of 100 ng/µL] and 2X PCR Smart Mix (origin) in a PCR tube and made upto the final volume of 20 µL using ultra-filtered Millipore water.

PCR was done in Bio-Rad thermal cycler and standardization was done for each reaction by mild adjustment of concentration of ingredients and annealing temperature with the following profile: initial denaturation of 5 min at 94°C; 35 cycles of 94°C for 30 s, annealing at 67.7°C for 30 s, and 72°C for 30 s with a final elongation of 5 min at 72°C. PCR amplicon was subjected to 2% agarose gel.

PCR-RFLP assay

Five micro litre of the amplified product of promoter region of *prolactin* gene C-2161Gwas added with 5 units of *CsP6I*restriction enzyme and incubated at 37^0C for 1hr. The composition of reaction mixture with the final volume of 12µl contains 5µl PCR product, 1.2µl of 10X buffer, 0.5µl of *CsP6I* (10U/µl) and 5.3µl of distilled water. Restriction digestion was

carried out for all the PCR amplicons. After restriction digestion, the digested PCR products were separated by electrophoresis in 3% agarose gel in 1X buffer with 50 bp DNA size marker. The restriction pattern was visualized under UV trans-illuminator and documented in gel documentation system. PCR amplicons were sequenced using respective forward and reverse primers in an automated sequencer using Sanger's dideoxy chain termination method at AgriGenome Labs Pvt. Ltd., Cochin.

According to the polymorphic pattern birds were categorized in to three different genotypes. Genotypes and allelic frequency was calculated. The production traits viz., age at sexual maturity, egg weight at $28^{th,}32^{nd}$ and 40^{th} weeks of age and egg number up to 40 weeks of age were measured in the randomly selected birds of 'N' strain of White Leghorn and their association with SNP of *prolactin* gene was analyzed by one way ANOVA using the software SPSS (Version 21.0).

Results and Discussion

Lane 1 to 8: 439bp uncut fragment of CC genotype

Lane 9: 50bp Ladder

SNP at C-2161G position of the promoter region of *prolactin* gene was produced an uncut 439bp fragment represents CC genotype (Figure 1). All the 200 birds of White Leghorn were observed with the same genotype CC. The genotypic frequency of CC, CG and GG of C-2161G site were1.000, 0.000 and 0.000, respectively and the allelic frequency of C and G were 1.000 and 0.000, respectively in White Leghorn. The phenotypic performance viz., age at sexual maturity, egg weight at 28^{th}, 32^{nd} and 40^{th} weeks and egg number up to 40 weeks of age of all the birds were found to be at the same level. All the birds were represented the same genotype and phenotypic performance. Hence, it has not been taken up for further association study.

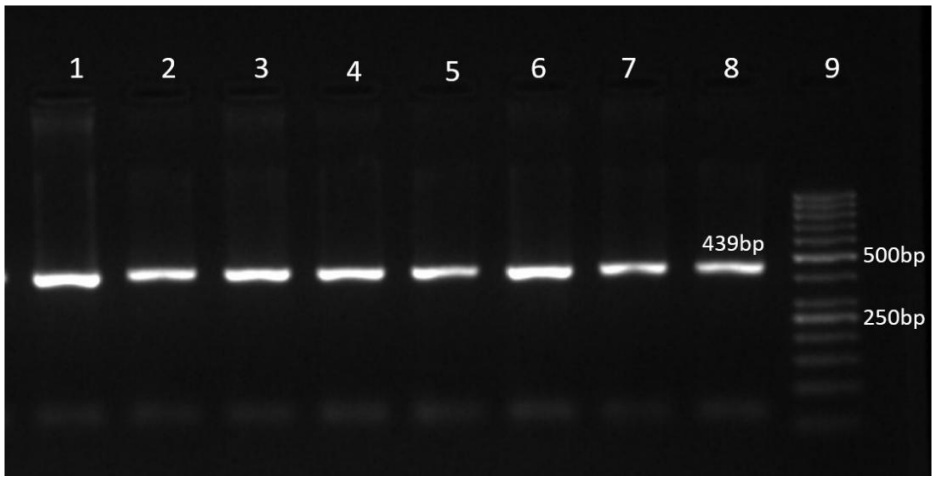

Figure.1 PCR-RFLP analysis of promoter region (C-2161G) of *prolactin* gene on 3% agarose gel (*CsP6I*)

This result suggests that all the birds with same genotype and phenotypic performance is mainly because of the intense selection of breeding for past 30 years in AICRP on Poultry improvement, Mannuthy. It indicates the level of selection efficiency of the AICRP form. This SNP C-2161G of promoter region of *prolactin* gene can be used as a molecular

marker to determine the efficiency of selection in a particular population. Further research study could be performed in other breeds of chicken to find out the interrelation of this SNP C-2161G with production traits.

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