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Research Article

POLYMORPHISMS OF GROWTH HORMONE GENE IN HARINGHATA BLACK CHICKEN

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ABSTRACT: The present study was carried out with an aim to investigate the genetic variability of growth hormone gene in Haringhata Black chicken. Blood samples were collected from 82 experimental birds and genomic DNA was extracted using the modified high salt method. Amplification of specific DNA fragment of intron 4 of growth hormone gene yielded a product size of 713 bp and was analyzed for polymorphism using PCR-SSCP technique. The banding pattern of present investigation revealed two SSCP variants AA and BB genotype in all experimental birds. In the analysed flock of Haringhata Black Chicken, the genotype frequencies were found to be 0.915 for AA and 0.085 for BB genotype. The frequencies of A and B alleles were 0.915 and 0.085 respectively which indicated A allele was predominant in the studied Haringhata Black Chicken population of the farm. The Chi Square Test revealed that studied population was not in accordance with Hardy Weinberg equilibrium with respect to intron 4 of Growth hormone gene.

Key words: Haringhata Black Chicken, Growth Hormone gene, Polymorphism, PCR-SSCP.

INTRODUCTION

The chicken growth hormone (cGH) gene is one of the important genes that influence the performance traits because of its crucial role in growth and metabolism (Nie *et al*. 2005). In addition, it has an important role in innate and acquired immune systems. Specially, it regulates thymulin excretion, growth of thymus, proliferation of lymphoid cells, activity of phagocytic cells and haemopoiesis (Gala 1991). The cGH gene located on chromosome number 19 in avian (Stephen *et al*. 2001) consists of 5 exons and 4 introns with an overall length of 4.1 kb (Kansaku *et al*. 2008). The products of cGH gene consist of 191 amino acid mature growth hormone protein and 25 amino acid signal peptides (Tanaka *et al.* 1992). Scientists have found clear examples of 'functional nuclear introns' that can accommodate sequences important for the expression of the gene on which the intron resides. Nuclear introns can also be important in a process called alternative splicing, which can produce multiple types of messenger RNA from a single gene. While introns do not encode protein products, they are integral to gene expression regulation and are involved in virtually every step of mRNA processing. Kuhnlein *et al*. (1997) reported that DNA polymorphism in the chicken growth hormone gene has a positive correlation with resistance to disease and egg production. Despite of the importance of Haringhata Black Chicken in rural areas of Nadia and 24 Parganas District of West Bengal, India, scanty information is available with respect to their performance, adaptability, resistance to diseases, genetic variability and genetic relationships. Therefore, the present study was performed to evaluate the genetic variability of growth hormone gene in Haringhata Black chicken using PCR-SSCP technique.

MATERIALS AND METHODS

The present research work was carried out at Haringhata Poultry Farm, Mohanpur, located in Nadia district of West Bengal and at the Department of Animal Genetics and Breeding, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata-700037.

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Experimental bird

The present research work was carried out on a total of eighty-two (82) randomly selected Haringhata Black chicken at 22nd weeks of age reared at intensive management system of the Haringhata Poultry Farm, Mohanpur, located in Nadia district of West Bengal and their genomic DNA were used as principal material for investigation of growth hormone gene polymorphism in this present study.

Collection of blood samples

About 2 ml of blood sample was collected aseptically from wing vein of each bird in a vacutainer tube containing 200-300 µl of 10 percent EDTA as anticoagulant. The tubes were shaken gently to facilitate thorough mixing of blood with the anticoagulant. The samples were then transported to the laboratory in an icebox containing ice packs and stored at 4^0 C till further processing for DNA isolation.

DNA samples

In this study, genomic DNA were isolated from blood samples of native Haringhata Black Chicken using salting out procedure as described by Miller *et al.* (1988) with few modifications. The genomic DNA samples were stored at -20^0 C. DNA analysis was conducted at the Department of Animal Genetics and Breeding of West Bengal University of Animal and Fishery Sciences, Kolkata 700 037, West Bengal, India.

DNA amplification

Amplification of specific DNA fragments was carried out in intron 4, size 713 bp, spanning from the 2479 base to 3192 of the chicken cGH gene (GenBank: D10484.1). The amplification process was performed using a pair of primers (forward: 5'- CGCTCTGCTATTTCTCTTAC-3' and reverse: 5'- ATGGAACCGTGGAATGATGG-3'). The primers for Growth Hormone gene were selected from the published reports of Muin and Lumatauw (2013). DNA was quantified spectophotometrically and electrophoresed. Genomic DNA (2 µl) was amplified with 1 µl *Taq* DNA polymerase, 2 µl $MgCl_2$, 2 µl dNTPs, 2.5 µl PCR buffer and 1 µl of each primer in a total volume of 25 µl PCR mixture. The PCR mixture was properly mixed until homogeneous then inserted into the PCR machine. PCR conditions was programmed as follows: initial denaturation at 94^0 C for 5 min, followed by 30 cycles with each cycle, denaturation 94^0 C for 60 secs, annealing 60° C for 60 secs, extension 72^0 C for 60 sec and final extension at 72^0 C for 10 min. The amplified products or amplicons were run at 2% agarose gel electrophoresis at 70 volts for 90 min. To visualize the PCR product, gel was stained using ethidium bromide.

Table 1. Frequency of SSCP patterns of Intron 4 region of GH gene in Haringhata Black Chicken.

Pattern	Genotype	Frequency
Two band	AA	75
Four band	BB	7

Table 2. Genotype and Gene frequencies of Intron 4 region of GH gene in Haringhata Black Chicken.

Genotype	No. of birds	Genotype frequency	Allele	Allelic frequency
AA	75	0.915	A	0.915
AB	-	-	B	0.085
BB	7	0.085		

Single Strand Conformational Polymorphism

Following PCR amplification of genomic DNA from each of the samples under study, the products *i.e.* the double stranded DNA fragments were subjected to SSCP analysis as per Orita *et al*. (1989) with minor modifications to study the sequence variation in intron 4 of Growth Hormone gene of Haringhata Black Chicken. An amount of 4 µl PCR product and 12 µl SSCP loading buffer dye were taken into 200 µl PCR tubes. Samples were denatured at 95ºC for 10 min. After denaturation samples were kept immediately in ice for 10 min to make the single strand DNA for its conformation. The denaturated PCR products were run in a non-denaturing 10% polyacrylamide gel for 12 hours at a constant volt (70 V) in room temperature. After electrophoresis, SSCP gels were fixed and stained in a solution containing 10% ethanol, 0.5% acetic acid and 0.2% silver nitrate for 20 minutes to identify the DNA sequence variations. The silver stained gel was kept on transilluminator and SSCP variants were recorded for genotyping of Haringhata Black Chicken.

Genotypes identification

The procedure for identification of genotypes used in this study was adapted from the banding pattern of single strand conformational polymorphism (SSCP). The genotypes were detected accordingly by observing at every possible combination of the SSCP patterns that could provide identification of alleles. Each pattern was designated as a particular genotype.

Statistical Analysis

Genotype frequencies of different SSCP patterns were estimated from the combination of various alleles generated based on the movement of DNA molecules.

Table 3. Observed and Expected number of genotypes of Intron 4 region of GH gene in Haringhata Black Chicken.

Genotype	Genotypes		$\frac{(O -- E)^2}{E}$
	Observed no.	Expected no.	
AA	75	68.65	0.587
AB	0	12.76	12.760
BB	7	0.59	69.640
	d.f. = 1		χ^2 value=82.987

Different genotypes were identified on the basis of different patterns. Gene frequencies were calculated from genotypic frequencies. The Genotype and gene frequencies were estimated following standard procedure (Falconer and Mackay 1996). The Chi-Square (χ^2) test for goodness of fit was used to find out difference among various genotypes and tested for Hardy-Weinberg equilibrium. The significance of calculated value was adjudged from the Table values of Snedecor and Cochran 1994.

RESULTS AND DISCUSSION

Genomic DNA isolation

DNA samples of Eighty two (82) Haringhata Black Chicken were isolated from their blood samples using salting out procedure as described by Miller *et al.* (1988). From 0.5 ml of blood sample approximately 50-100 mg of genomic DNA was isolated. DNA extraction and quality determination with electrophoresis and spectrophotometer was done for all Chickens and obtained result was acceptable (Fig. 1).

PCR of Growth Hormone (GH) gene

Amplification of specific DNA fragment of Intron 4 of GH gene was successfully performed for total samples using the primer pair (forward and reverse) under PCR condition that was explained in previous section. The results of electrophoresis of PCR products obtained in this study (Fig. 2) appeared as a clear single band, and occupied the appropriate position with approximately 713 bp of amplicon size. The amplified products were consistent with the target fragments and had a good specificity, which could be directly analyzed through PCR-SSCP technique.

The present research finding of intron 4 of growth hormone gene was in agreement with the result of Muin and Lumatauw (2013), Bingxue *et al*. (2003) in Indonesia native chickens population and F_2 chicken derived from Broilers crossing to Silky in China, respectively. However, Shahnaz *et al*. (2008), Thakur *et al.* (2009), Khoa *et al*. (2013), Makhsous *et al*. (2013) and Rahmadani *et al.* (2014) reported the product size of 1200 bp, 1216 bp, 563 bp, 1164 bp and 367 bp respectively. This difference of base pairs in the PCR fragment suggests the possibility of insertion/duplication of the sequence, which can only be confirmed by sequencing.

PCR-SSCP Analysis

Good quality PCR amplified products (sufficiently intense and without any spurious bands) as assessed by horizontal submarine agarose gel electrophoresis were used for SSCP analysis. The obtained electrophoretic picture of different banding patterns of 713 bp of intron 4 of GH gene in the present study were compiled in a photograph and have been depicted in Fig. 3. The banding pattern of present investigation revealed two SSCP variants which were arbitrarily assigned as AA and BB genotype. The AA and BB genotypes were detected by the presence of two bands and four bands respectively.

The frequencies of different SSCP patterns for intron 4 of GH gene are presented in Table 1. It was found that out of 82 studied Haringhata Black Chicken 75 birds had AA genotype and 7 birds had BB genotype. The research finding in this present investigation revealed that 91.5 percent of the studied birds had AA genotype while only 8.5 percent had BB genotype for intron 4 of GH gene in Haringhata Black Chicken population. The AB genotype could not be detected among the birds examined. This finding was well in agreement with the findings of Muin and Lumatauw (2013) in native Indonesian chicken. However, in contrast with the research carried out by Bingxue *et al*. (2003) who reported three genotypes: AA (253 chickens), AB (71 chickens) and BB (9 chicken) in a population of hybrid chickens (Broilers Star X Silky). The finding of present study was also in contrast to those of Thakur *et al.* (2009) who reported genotypes AA and AB in Kadaknath and Kulibaba (2015) who reported genotypes AB and BB in local chicken breeds of Vietnam. The present study revealed that the AA genotype was predominantly found in the studied Haringhata Black Chicken population. The absence of AB genotype in the studied population might be due to limited sample size used in the present investigation.

Genetic Structure of the Population

Genotypic Frequency

In the analysed flock of Haringhata Black Chicken, the genotype and gene frequencies with respect to intron 4 of GH gene were calculated and have been depicted in Table 2. The genotype frequencies were found to be 0.915 for AA and 0.085 for BB genotype in Haringhata Black Chicken with respect to intron 4 of GH gene. These findings were in close conformity with those of Muin

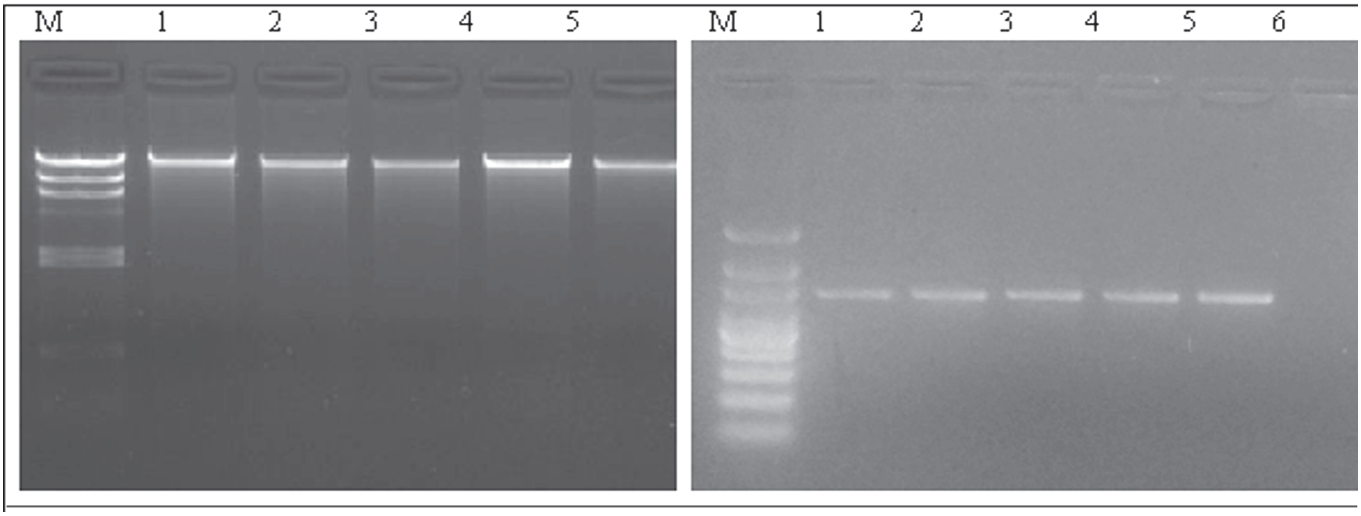

Fig. 1. Genomic DNA of Haringhata Black Chicken on 0.8% agarose gel.
M: DNA Ladder, Lane 1-5: Working DNA samples.

Fig. 2. PCR amplified product of Intron 4 region of Growth Hormone gene on 2% agarose gel.
Lane 1-5: PCR product (713 bp), Lane 6: Negative control, M: 100 bp DNA Ladder

and Lumatauw (2013) who reported the frequencies of AA and BB genotypes as 0.9028 and 0.0972 respectively in Indonesian native chicken.

The genotype frequencies observed in present investigation differed from those of Bingxue *et al*. (2003) who found three distinct genotypes AA, AB and BB with genotypic frequencies of 0.759, 0.213 and 0.027, respectively in a population of hybrid chickens (Broilers Star X Silky). The finding of present study also differed from those of Thakur *et al.* (2009) and Kulibaba (2015) who reported two genotypes AA and AB in Kadaknath and AB and BB in local chicken breeds of Vietnam respectively. The frequencies of AA and AB in Kadaknath were 0.4151 and 0.5849 respectively while the frequencies of AA and AB were 0.070 and 0.930 in local chicken breeds of Vietnam respectively.

Allelic Frequency

The frequencies of A and B alleles in the present study were 0.915 and 0.085 respectively. The present finding revealed a higher frequency of A allele which was predominant in the studied Haringhata Black Chicken population of the farm. Higher frequency of A allele (0.9028) than B allele (0.0972) was also reported by Muin and Lumatauw (2013) in Indonesian native chicken. Bingxue *et al*. (2003) also observed higher frequency of A allele (0.8655) than B allele (0.1335) in a population of hybrid chickens (Broilers Star X Silky). Thakur *et al.* (2009) also observed higher allele frequency of A allele (0.7075) than B allele (0.2925) in Kadaknath. However, Kulibaba (2015) observed higher frequency of B allele (0.964) than A allele (0.036) in local chicken breeds of Vietnam.

H-W Equilibrium

Two genotypes AA and BB of Haringhata Black Chicken for intron 4 of GH gene were observed in the present investigation. The observed and expected number of birds with AA and BB genotypes has been depicted in Table 3. It is evident from the table that the observed and expected number of AA genotype was 75 and 68.65, respectively. The observed and expected number of BB genotype was 7 and 0.59 respectively and that of AB genotype was 0 and 12.76 respectively. The calculated Chi Square value obtained was 82.987 which is higher than the tabulated value of 3.84 at 5 percent level of significance at 1 degree of freedom. Therefore, the studied

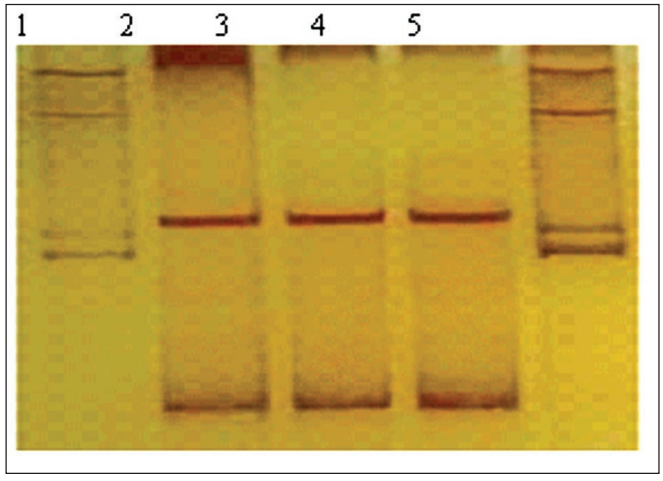

Fig. 3. Different SSCP banding patterns of 713 bp fragment of Intron 4 region of Growth Hormone gene of Haringhata Black Chicken.
Lane 1, 5: BB Genotype, Lane 2, 3, 4: AA Genotype.

population was not in accordance with Hardy Weinberg equilibrium with respect to intron 4 of Growth hormone gene. This result indicated that evolutionary forces like migration, mutation and selection might have acted on the studied Haringhata Black population for this locus. This present finding was in contrary to the findings of Thakur *et al.* (2009), Khoa *et al.* (2013), Makhsous *et al.* (2013), Rahmadani *et al.* (2014) and Kulibaba (2015).

A preliminary analysis of SSCP polymorphic patterns in exon 4 of Growth Hormone gene with the genomic profiles derived from the PCR-SSCP analysis was performed and revealed that a particular allele is more prevalent in Haringhata Black Chicken. This finding may be used in order to adaptability as well as to improve their production, preserving genetic diversity of the population. Furthermore, it may be used for association study which may help in marker assisted selection in Haringhata Black Chicken, also taking into account short and long-term effects on population structure and rates of inbreeding, in order to improve poultry production preserving genetic diversity of the population.

In conclusion, the presence of genetic variants in growth hormone gene can be exploited using PCR-SSCP technique. Association of such alleles with the traits of economic importance will help the poultry breeders to search genetic markers for desired traits. The knowledge of these polymorphisms will also be useful in phylogenetic analysis as well as in design of breeding programs. Hence, growth hormone gene may be used as a candidate gene in marker assisted selection to improve the performance of Haringhata Black chicken.

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