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Research Article

PROLACTIN GENE POLYMORPHISM IN HARINGHATA BLACK CHICKEN BREED

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ABSTRACT: PCR-SSCP analysis was carried out to investigate the polymorphism of prolactin gene in Haringhata Black chicken. The amplified fragment (497 bp) of promoter region of prolactin gene revealed three types of SSCP banding pattern arbitrarily assigned as CC, CD and DD genotypes on 10 percent polyacrylamide gel. The heterozygous CD genotypes showed presence of maximum five bands while rest two homozygous genotypes CC and DD showed three and two bands, respectively. In the present study, genotype frequencies were found to be 0.622 for CC, 0.341 for CD and 0.037 for DD genotype with respect to promoter region of prolactin gene. The frequencies of C and D alleles were estimated as 0.793 and 0.207, respectively. The calculated Chi square value revealed that the population was in Hardy Weinberg Equilibrium with respect to promoter region of prolactin gene in Haringhata Black Chicken.

Key words: Haringhata Black Chicken, Prolactin Gene, Polymorphism, PCR-SSCP.

INTRODUCTION

Poultry is an integral part of human being for meat and egg as source of animal protein since the dawn of civilization. Because of the minimum requirement for feed, water and other inputs, poultry plays a significant role on rural economy as well as the national economy of the country. Haringhata Black chicken, an important registered breed reared in traditional backyard system in the northern part of North 24 Parganas and southern part of Nadia districts of West Bengal, India. Although ample information is available on their performance traits but scanty information is available on their genetic polymorphism especially for prolactin gene. Keeping this aspect in view the present investigation was carried out with an aim to investigate the polymorphism of prolactin gene in Harianghata Black Chicken breed.

MATERIALS AND METHODS

Experimental Bird

The present research work was conducted on randomly selected eighty two (82) Haringhata Black chicken at 22nd weeks of age reared at intensive management system of the Haringhata Poultry Farm located at Mohanpur in Nadia district of West Bengal, India. In this study the genomic DNA of these birds were used for investigation of prolactin gene polymorphism.

Collection of blood samples

About 2 ml of blood sample was collected aseptically from wing vein of each bird in a vaccutainer 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 brought to the laboratory in an icebox containing ice packs and stored at 4°C till further use.

DNA samples

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 available at the Department of Animal Genetics and Breeding of West Bengal University of Animal and Fishery Sciences, Kolkata 700 037, West Bengal, India.

DNA amplifications

PCR conditions including the cyclic condition and the concentration of different ingredients of PCR reaction mixture was standardized for target gene fragment after carrying out PCR using several combinations. Then standardized PCR reaction mixture and standardized PCR thermocyclic conditions used for amplification of target fragment. Genomic DNA (2 µl) was amplified with 1 µl Taq DNA polymerase, 2 µl $MgCl_2$, 2 µl dNTPs, 2.5 µl

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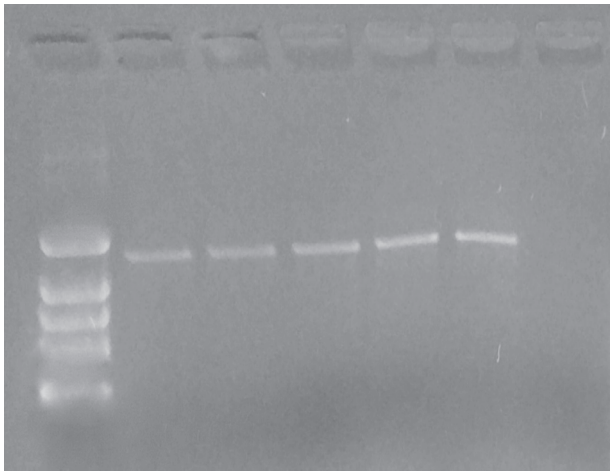

Fig.1 PCR amplified product on 2% agarose gel
Lane 1-5: PCR product (439 bp)
Lane 6: Negative control, M: 100 bp DNA Ladder.

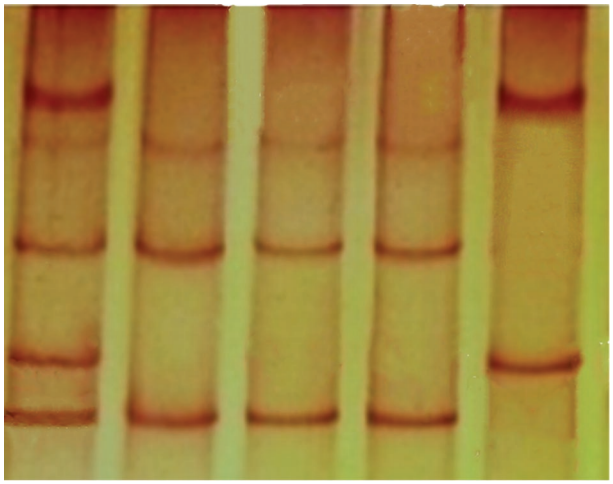

Fig. 2. Different SSCP patterns at 10% polyacrylamide gel
Lanes 2, 3, 4 – CC genotype
Lane 5 – DD genotype, Lane 1- CD genotype.

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 for amplification. PCR conditions was programmed as follows: initial denaturation 94 ^{0}C for 5 min followed by 30 cycles with each cycle denaturation 94 ^{0}C for 30 sec, annealing 62 °C for 30 sec, extension 72 ^{0}C for 30 sec and final extension at 72 ^{0}C for 5 min. The amplified amplicons were run at 2% horizontal agarose gel electrophoresis at 70 volt for 90 minutes. Agarose gel was stained using ethidium bromide to visualize the PCR product.

Single strand conformational polymorphism

The procedure of SSCP used in this investigation was as per Orita *et al*. (1989) with minor modifications to study the sequence variation. PCR products (4 µl) from each individual sample were thoroughly mixed with 12 µl volume of SSCP loading buffer dye into 200 µl PCR tubes. The tubes were heated in water bath at 94 °C for 10 minutes for denaturation. The tubes were immediately snap cooled on ice for 10 minutes to make the single strand DNA for its confirmation. The samples were then loaded immediately onto 10% polyacrylamide gel. The electrodes were connected properly with the power pack and the gel was run at the rate of 70 volt for 12 hours at room temperature. Silver staining of polyacrylamide gel was carried out following the method as described by Byun *et al.* (2009) with few modifications. The gel was stained with a solution containing 10% ethanol, 0.5% acetic acid and 0.2 % silver nitrate for 20 minutes to identify the DNA sequence variations. The stained gel was then placed on the U-V transilluminator within the gel documentation system (Syngene) and photographs were taken for identification of different SSCP banding patterns.

Genotypes identification

The procedure for identification of genotypes used in this study was adapted from the different 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. 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

PCR amplification

In this present investigation approximately 50-100 mg of genomic DNA were isolated from each blood samples of Haringhata Black Chicken under study. All these samples under this present investigation showed a single genomic DNA band on 0.8 percent agarose gel electrophoresis gel. The best quality of DNA samples were subjected to Polymerage Chain Reaction (PCR) for further study. Amplification of specific DNA fragment of prolactin gene was successfully performed for total samples using the primer pair (forward and reverse) under optimum PCR condition as explained in previous section. The result of electrophoresis of PCR products obtained

Table 1. Frequency of SSCP patterns of Promoter Region of Prolactin Gene in Haringhata Black Chicken.

Pattern	Genotype	No. of Birds
Three band	CC	51
Five band	CD	28
Two band	DD	3

Table 2. Genotype and Gene Frequencies of Promoter Region of Prolactin Gene in Haringhata Black Chicken.

Genotype	No. of Birds	Genotype Frequency	Allele	Allelic Frequency
CC	51	0.622	C	0.793
CD	28	0.341	D	0.207
DD	3	0.037	—	—

in this study appeared as a clear single band in each amplicon (Fig. 1). The amplification promoter region of prolactin gene yielded in a product size of approximate 439 bp length on 2 percent agarose gel electrophoresis when visualized under the UV trans-illuminator in all the samples of Haringhata Black Chicken.

The PCR amplified product obtained for promoter region of prolactin gene in the present study was in accordance with those obtained by Cui *et al.* (2006), Alipanah *et al.* (2011) and Abdi *et al.* (2014). However, Begli *et al.* (2010) and Bhattacharya *et al.* (2010) reported 130 or 154 bp and 277 bp product size, respectively.

PCR-SSCP Analysis

PCR-SSCP technique was used to identify genetic variants of promoter region of prolactin gene in Haringhta Black Chicken. The result revealed different SSCP variants for PCR products in 10% poly acrylamide gel. The different banding patterns of SSCP variant on gel were compiled in a photograph and have been depicted in Fig. 2. The banding pattern of present investigation revealed three SSCP variants which were arbitrarily assigned as CC, CD and DD genotypes. The heterozygous CD genotypes showed presence of maximum five bands while rest two homozygous genotypes CC and DD showed three and two bands respectively. From different SSCP patterns for promoter region of prolactin gene, it was found that out of 82 studied Haringhata Black Chicken 51 birds had CC genotype, 28 had CD and 3 birds had DD genotype (Table 1). The research finding in this present investigation revealed that 62.2 percent of the studied birds had CC genotype, 34.1 percent had CD while only 3.7 percent had DD genotype. The present finding revealed higher frequency of CC genotype followed by CD and DD genotype for promoter region

Table 3. Observed and Expected Number of Genotypes with respect to the Promoter Region of Prolactin Gene in Haringhata Black Chicken.

Genotype	Observed	Expected	$\frac{(O - E)^2}{E}$
CC	51	51.56	0.006
CD	28	26.92	0.043
DD	3	3.52	0.076
	d.f. = 1	χ^2 value=0.125	

of prolactin gene in Haringhata Black Chicken population. This finding was in agreement with those of Jiang *et al.* (2005), Liang *et al.* (2006) and Begli *et al.* (2010) who also reported all the three genotypes and demonstrated that the CC genotype was commonly found in poultry populations with respect to promoter region of promoter gene. However, the present finding was in contrast to that of Bhattacharya *et al.* (2010) who reported five genotypes *viz*. CC, CD, CE, DD and DE in four strains of White Leghorn Chicken. The absence of CE and DE genotypes in the present study might be due to less number of Haringhata Black Chicken considered in the present investigation.

Genetic structure of the population

In the present study, genotype frequencies were found to be 0.622 for CC, 0.341 for CD and 0.037 for DD genotype in Haringhata Black Chicken with respect to promoter region of prolactin gene (Table 2). These findings were in close conformity with those of Jiang *et al.* (2005), Liang *et al.* (2006) and Begli *et al.* (2010). Jiang *et al.* (2005) reported the genotypic frequencies of CC, CD and DD genotypes as 0.60, 0.34 and 0.06 in Chinese Blue-shell chicken breed, respectively. Liang *et al.* (2006) observed the genotypic frequencies of CC, CD and DD genotypes as 0.67, 0.25 and 0.08 for promoter region of PRL gene in Yuehuang Chineese chicken breed. Begli *et al.* (2010) also reported almost similar genotype frequencies of CC (0.566), CD (0.390) and DD (0.044) genotypes in native fowl of Yazd province, Iran.

In case of promoter region of prolactin gene, the frequencies of C and D alleles were estimated as 0.793 and 0.207, respectively (Table 2). The present finding revealed a higher frequency of C allele which was predominant in the studied Haringhata Black Chicken population of the farm. Higher frequency of C allele (0.761) than D allele (0.239) was also reported by Begli *et al.* (2010) in native fowl of Yazd province, Iran. Jiang *et al.* (2005) also found higher allele frequency of C allele (0.77) than D allele (0.23) in Chinese Blue-shell chicken. Liang *et al.* (2006) observed higher allele frequency of C allele (0.80) than D allele (0.20) for promoter region of

PRL gene in Yuehuang Chineese chicken.

Three genotypes CC, CD and DD were observed in the present investigation for promoter region of prolactin gene. The observed number and expected number of birds with CC, CD and DD genotypes have been depicted in Table 3. From the Table, it is evident that the observed and expected number of CC genotype was 51 and 51.56 respectively. The observed and expected number of CD genotype was 28 and 26.92 respectively and that of DD genotype was 3 and 3.52, respectively. The calculated Chi square value of 0.125 at 5 percent level of significance at 1 degree of freedom revealed that the population was in Hardy Weinberg equilibrium with respect to promoter region of PRL gene. It may be due to the inherit advantage of the genes on the adaptability and because of the long term evolution and breeding. The result obtained in the present study was in agreement with those obtained by Begli *et al*. (2010), Bhattacharya *et al*. (2010) and Tempfli *et al*. (2015) but in contrary to the results obtained by Abdi *et al*. (2014).

Research finding of this present investigation revealed that prolactin gene of Haringhata Black Chicken is polymorphic. Further attempt needs to require finding out the association of this polymorphic candidate gene with economic traits and this will help the breeders to search some genetic marker for economic traits. This may be used as an aid to the selection of this poultry breed at an early stage and can save huge economic loss for rearing the birds till maturity. Hence, polymorphism of prolactin gene may be used as a candidate gene for marker assisted selection for improvement of Haringhata Black Chicken.

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