MOLECULAR CHARACTERIZATION OF B-Lβ II FAMILY ALLELES IN CARIBRO-VISHAL CHICKEN BY PCR-SSP

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ABSTRACT

The present study was planned to investigate the polymorphism of MHC B-Lβ II family (class II) alleles and genotyping using one of the variants of polymerase chain reaction (PCR), the PCR-SSP (Polymerase Chain Reaction with sequence specific primers) in Caribro-Vishal (Caribro-91) chickens. Genomic DNA was isolated from blood of 30 birds maintained at experimental poultry unit at College of Veterinary Science and Animal Husbandry, Jabalpur. A 235 bp, exon-2 region of chicken MHC B-Lβ II family was amplified using a set of degenerating primers. The amplicons of the first round of amplification were subjected to PCR-SSP at the specified annealing temperatures and replication cycles. The primers designed for B_2, B_{13}, B_{15}, B_{19} and B_{21} haplotypes amplified the target sequences producing a 222 bp, 141 bp, 222 bp, 213 bp and 213 bp fragments, respectively. The findings of the research revealed 10 genotypes. It was observed that the alleles B_{19} and B_{15} exhibited predominance in this genetic group. Moreover, genotypic distribution also revealed higher occurrence of $B_{15}B_{19}$, $B_{19}B_{19}$ and $B_{15}B_{15}$ heterozygotes in the studied population. The highly significant Chi-square values ($P<0.01$) showed that the selected population of birds was not in Hardy Weinberg equilibrium.

KEY WORDS: Caribro-Vishal, MHC B-Lβ II family gene, PCR-SSP

INTRODUCTION

The major histocompatibility complex (MHC) in the chicken was first discovered as a blood group locus and is also termed as the B complex. The MHC encodes for three classes of proteins named B-F, B-L and B-G. The MHC gene seems to be closely associated with both disease resistance and immune responsiveness. The genes in the chicken MHC exert major genetic control over host resistance to viral, bacterial and parasitic diseases (Shirley and Lillehoj, 2012). Caribro-Vishal (Caribro-91) is a commercial broiler chicken developed at Central Avian Research Institute (CARI), Izzatnagar, Bareilly (U.P.), which is most suitable bird for tropical climate.

Genotyping for single nucleotide polymorphisms (SNPs) is relevant to various research investigations. Nowadays, allele-specific PCR amplification techniques using sequence-specific primers (PCR-SSP) are widely employed for the genotyping of single nucleotide polymorphisms (SNPs) in both routine diagnosis and medical research (Welsh and Bunce, 1999; Hurd *et al*., 2002). Literature on MHC gene polymorphism in indigenous breeds of poultry is very scanty. Therefore, the present investigation was planned to explore the genetic polymorphism at MHC B-Lβ II family gene using PCR-SSP in Caribro-Vishal chicken.

MATERIALS AND METHODS

Blood sampling and DNA extraction

About one ml blood was collected from 60 Caribro-Vishal birds, reared under Madhya Pradesh Biotechnology Council Project, Bhopal, in the Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University

(NDVSU), Jabalpur. Genomic DNA was extracted from the venous blood following the method of John *et al.* (1991) with slight modification (0.5 ml of blood was taken instead of 5 ml) . The concentration and purity of DNA were checked by Nano drop Spectrophotometer (ND-1000). DNA samples with an OD 260/280 ratio of 1.7 to 1.9 were considered and were further subjected to 0.8 % agarose gel electrophoresis for quality check.

MHC Gene primer sequence

A pair of primers specific to MHC B-Lβ (class II) gene as reported by Zheng *et al*. (1999) was custom synthesized at Integrated DNA Technology (IDT) for first round of amplification. The primer sequences used were FP 5'-CG TTC TTC TTC TRC GGT RBG AT-3' and RP 5'-TA GTT GTG CCG GCA GAM CSYG-3', where, R=A or G, M=A or C, S=G or C, Y=C or T, B=G,C or T.

Five pairs of chicken MHC B-LβII family haplotype specific primers (B2, B13, B15, B19 and B21 alleles specific) as reported by Zheng *et al.* (1999) were used for PCR-SSP. These primers were custom synthesized at Integrated DNA Technology (IDT) for haplotyping of chicken.

Genotyping by PCR-SSP

The B-Lβ family specific PCR products were diluted 1:100 times and 2 µl of diluted samples were subjected to PCR-SSP with annealing temperature of 55^{0}C for B2, B19 and B21; 60^{0}C for B13 and 50^{0}C for B15 alleles. To confirm the targeted PCR amplification, 5 µl of PCR product from each tube was mixed with 1 µl of 6X loading dye buffer and was visualized as a single compact band by UV transilluminator and photographed (Gel documentation system, Bio-Rad, USA). Sample(s), which yielded a single compact band for any one of the five pairs of primers, were considered homozygous for that B allele(s) targeted by the designed sequence specific primer. However, samples that yielded amplification products with two sets of primers were considered heterozygous for the targeted alleles.

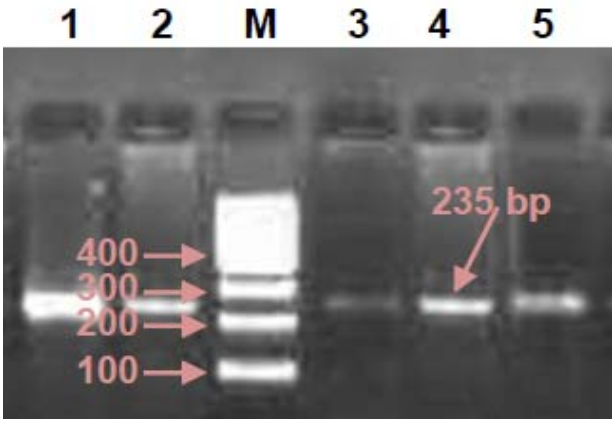
Statistical Analysis

Gene and genotype frequencies were estimated using Popgene 32(version1.32), Microsoft Windows-based freeware for population genetic analysis retrieved from http://www.ualberta.ca/~fyeh/fyeh (Yeh *et al.,* 1999) and the population was tested for genetic equilibrium at this locus.

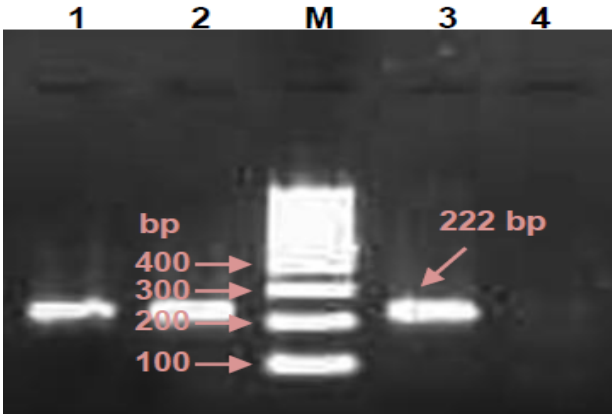
RESULTS AND DISCUSSION

An amplified PCR product of 235 bp sizes was obtained in Caribro-Vishal birds on amplification of exon-2 of MHC B-Lβ II family gene of chicken. The samples that yielded a single compact band size of 235 bp of exon 2 of B-L β II family genes in birds were further processed for PCR-SSP. Diluted (1:100) product of primary PCR was separately amplified for all the standard haplotypes, following standard protocols (Zheng *et al*., 1999). Amplification of targeted haplotypes i.e. B2, B13, B15, B19 and B21 resulted in amplicons of 222 bp, 141 bp, 222 bp, 213 bp and 213 bp sizes, respectively. Thus, ten different genotypes were observed in Caribro-Vishal birds. There is no report available on MHC B-Lβ II gene polymorphism in Caribro-Vishal chicken. However, similar degenerating primers and amplicons of 235 bp size were reported by Zheng *et al*. (1999) in White Leghorn chicken (WLH), Shanaz *et al.* (2005) in Bantam, Bantamised White leghorn (BWLH) and WLH chicken and Xu *et al*. (2007) in Chinese indigenous chickens.

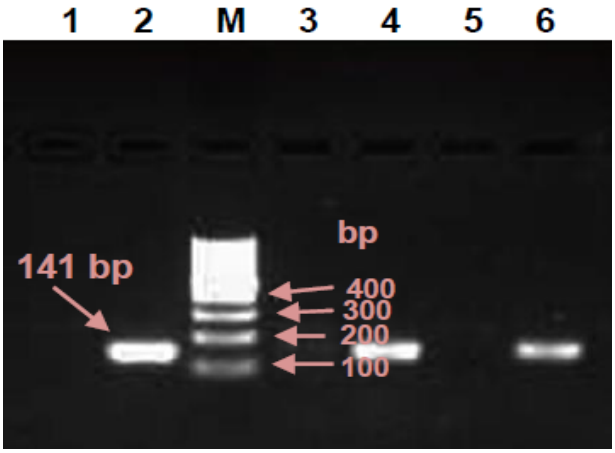
The allelic frequency at MHC B-Lβ II family gene locus in Caribro-Vishal reveals that highest frequency (0.358) was observed in B19 allele which was followed by (0.317) in B15, (0.150) in B13, (0.142) in B21 and (0.033) in B2 in the present studied population. Out of total 15 possible genotypes with the combination of 5 targeted alleles, a total of 10 genotypes were observed in Caribro-Vishal population under study. The five genotypes viz., B_2B_2, B_2B_{15}, B_2B_{19}, B_2B_{21} and $B_{13}B_{21}$ were found absent in the present studied population. The genotypic frequency of $B_{15}B_{19}$ was found



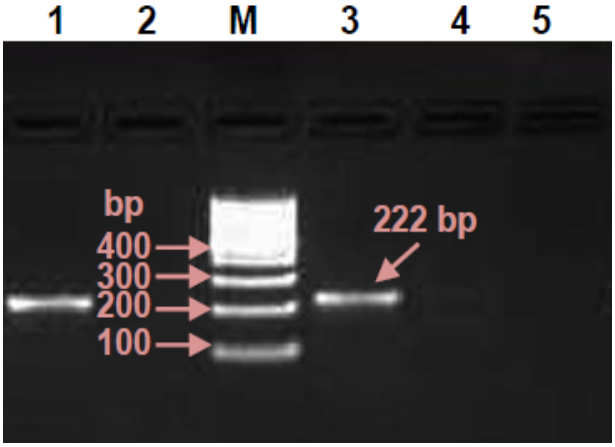
(a). PCR product



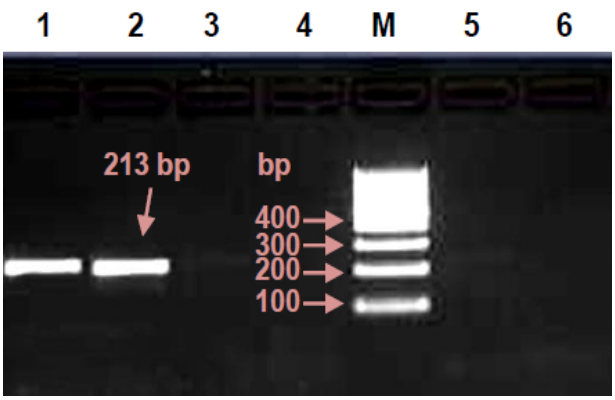
(b). B2 haplotype



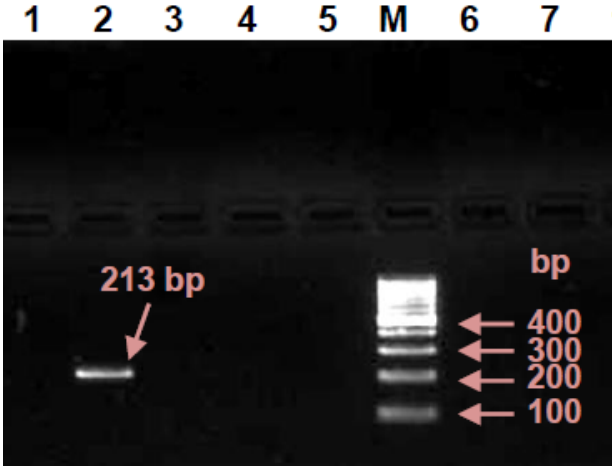
(c). B13 haplotype



(d). B15 haplotype



(e). B19 haplotype



(f). B21 haplotype

Plate 1: Amplified (a) PCR product of exon-2 of MHC B-L?II family gene and (b-f) PCR-SSP product of targeted haplotypes electrophoresed on 2% agarose gel (M-100bp DNA ladder)) in Caribro-Vishal chickens.

to be highest (0.200) while those of $B_{13}B_{19}$ and $B_{15}B_{21}$ were lowest (0.033) among the ten observed genotypes. The rest genotypic frequency was in the following decreasing order B19B19, 0183 >; B15B15, 0.150 >; B19B21, 0.117>; B13B15, 0.100 >; B21B21 and B2B13, 0.067; and >B13B13, 0.050. The highly significant Chi-square values (43.167**, P<0.01) showed that the studied population was not in Hardy Weinberg equilibrium at this locus.

Similar findings were reported by Zheng *et al*. (1999) who reported different haplotypes with identical B-LβII family sequences using PCR-SSP in broiler chicken. Similar study for MHC haplotypes conducted on Bantam, BWLH and WLH chicken was reported by Shanaz *et al.* (2005). In their study frequencies for different haplotypes were reported. Allele B_{19} was predominant followed by B_{15} and B_2 in Bantam. Allele B_{21} was absent in Bantam and was observed only in WLH at lower frequency. In Bantam genetic group predominantly two genotypes, $B_{15}B_{19}$ and $B_{19}B_{19}$ were observed with respective frequencies of 0.416 and 0.472. In BWLH, five genotypes were observed, among them heterozygote $B_{15}B_{19}$ was predominant. Whereas, in WLH, nine genotypes were observed and the two homozygotes $B_{15}B_{15}$ and $B_{19}B_{19}$ were predominantly observed. Their studied population of three strains of poultry was not in Hardy Weinberg equilibrium.

These differences in allelic frequencies observed in the present study and with those reported by Shanaz *et al.* (2005) in Bantam, BWLH and WLH chickens might be due to the fact that the different breeds and size of populations maintained under different sets of environmental conditions are subject to different evolutionary forces to varying degree as detected by utility and market demands. Further, intermixing of populations from different geographical locations and hybridization accompanied by genetic drift might have also contributed to this high degree of genetic diversity among breeds. The genetic disequilibrium condition in Caribro-Vishal population may be due to non-random mating for MHC genotypes over the generations. The other probable reasons for this state of disequilibrium in this breed may be due to selective advantages for the different MHC alleles over each other, resulting into unequal reproductive and survival rates of different genotypes, and state of different genetic forces which change the gene frequencies.

Therefore, it can be concluded that the allele B_{19} and B_2 exhibited predominance in this genetic group while genotypic distribution revealed abundance of $B_{15}B_{19}$ followed by $B_{19}B_{19}$ in the present population of birds which was not in Hardy-Weinberg equilibrium at this MHC locus. The MHC locus is highly polymorphic in this breed of poultry.

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