The Indian Journal of Veterinary Sciences & Biotechnology ***(2017) Volume 12, Issue 3, 132-137***

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

http://dx.doi.org/10.21887/ijvsbt.v12i3.7112

Submitted : 5-01-2017 ***Accepted : 13-01-2017*** ***Published : 15-02-2017***

Molecular Characterisation of MHC B-Lβ (Class II) Family Alleles in Jabalpur Dual Coloured Type Chicken using PCR- SSP

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Abstract

The present investigation was taken up to study the polymorphism at MHC B-Lβ (CLASS II) family alleles using PCR- SSP in sixty Dual Coloured type chicken in the Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur. An amplified PCR product of 235 bp size observed on amplification of exon 2 of MHC B-Lβ II family gene further underwent PCR-SSP using five pairs of chicken MHC B-Lβ (class II) family haplotype specific primers. The allelic frequency of B_2,B_{13},B_{15}, B_{19} and B_{21} were 0.375, 0.217, 0.192, 0.217 and 0.000, respectively. The frequency of allele B_2 was found to be the highest while of B_{15} was observed to be the lowest. The allele B_{21} was absent in the present studied population of Dual Coloured type birds. Chi-square values for testing correspondence between observed and expected genotypic frequencies at this locus were found to be non-significant (P>0.05) revealing Hardy -Weinberg equilibrium at this MHC locus for this populations.

Key Words: Haplotype, Dual Coloured type, PCR, PCR-SSP

Introduction

Improvement of animal health is one of the major objectives in current animal breeding strategies. Selection for genetic resistance provides a potential avenue for improving health status of farm animals, increasing productivity, reducing the need for pharmaceutical intervention and thus reducing cost of production. A major goal in poultry industry is resistance to diseases and enhancement of innate immune responsiveness. For the improvement of disease resistance using genomic approaches, the criterion of selection is shifted from phenotypically expressed disease status to allele status at DNA level known as Marker Assisted Selection (MAS). MAS can enable selection for disease resistance without exposure to disease challenges and allows high level of selection accuracy (Soller and Andersson, 1998). Major Histocompatibility Complex (MHC) has been used as a set of candidate genes for association between DNA markers and antibody response (Yonash *et al.,* 2000). The MHC haplotype nomenclature was standardized initially using serological reagents (Briles *et al*., 1982). But traditional serology for MHC identification has several limitations, including subjectivity in interpretation of serological reactions and expertise required for production

of new reagents (Wang *et al*., 2014). In addition, the cost and time involved in serological reagent development are huge. However, allelic typing can be simplified using polymerase chain reaction using sequence specific primers (PCR-SSP). With this method large number of birds can be easily analyzed for allelic variation without the use of labeled probes for MHC Class II molecules. Therefore, information of MHC can be used as a means of altering allelic frequencies selectively to improve correlated traits, primarily disease resistance and immune response (Kaufman and Lamont, 1996).

The MHC affects resistance and susceptibility to certain poultry diseases (Heller *et al*., 1991) as well as immune responses (Yonash *et al*., 1999). For genetic improvement of economic traits and disease resistance, a breeder can identify or use identified haplotypes of MHC conferring resistance to particular diseases for optimum gains. Owing to the paucity of literature available on the MHC typing in Jabalpur Dual Coloured Type birds and in view of the above facts, the present investigation was taken up to study the polymorphism at MHC B-Lβ (class II) family alleles using PCR- SSP in Jabalpur Dual Coloured Type Chicken.

Materials and Methods

The present work was conducted on sixty Dual Coloured Type chicken 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. About 1 ml blood was collected from each of the experimental birds from wing vein in ethylene diamine tetra-acetate (EDTA) vacutainers. Genomic DNA was extracted from venous blood according to the method described by John *et al.* (1991) with minor modifications. The concentration, purity and quality of DNA were checked by UV spectrophotometer and horizontal agarose gel electrophoresis.

Quality of DNA was assessed through 0.8 per cent horizontal submarine agarose gel electrophoresis A pair of primers specific to MHC B-Lβ (class II) (Zheng *et al*., 1999) was custom synthesized by Integrated DNA Technology (IDT), USA for first round of amplification. The primer sequences are given below.

BLβII-F 5' – CG TTC TTC TTC TRC GGT RBG AT – 3'

BLβII-R 5' – TA GTT GTG CCG GCA GAM CSY G – 3'

Where, R = A or G, M = A or C, S = G or C, Y = C or T, B = G, C or T.

PCR amplification of MHC gene was carried out in a final reaction volume of 25 µl. A master mix for desired number of samples was prepared and 22 µl aliquot was transferred in each PCR tube. Three µl genomic DNA (30 ng/µl) was added in each tube to make the final volume 25 µl. A negative control, containing all the reaction components except the template DNA was also made to check any contamination of the foreign DNA in the reaction components. The PCR tubes were kept in a pre programmed thermo cycler (Master cycler gradient, Eppendorf) and standardized at an annealing temperature of 54 °C for 40 seconds.

To confirm the targeted PCR amplification, 5 µl of PCR product from each tube was mixed with 1 µl of 6X loading dye buffer from each tube on 2 per cent agarose gel containing ethidium bromide (1% solution @ 5 µl / 100 ml) along with 100 bp DNA ladder (O'GeneRuler™, Fermentas) at a constant voltage of 70 V for 45 min in 0.5 X TBE buffer. The amplified product was visualized as a single compact band by UV trans illuminator and photographed (Gel documentation system, Bio-Rad, USA). The B-L family specific PCR products were diluted 10:100 times and 2 µl of diluted samples were subjected to PCR-SSP with cycling condition mentioned in Table 1.

To confirm the targeted PCR amplification, 5 µl of PCR product from each tube was mixed with 1 µl of 6 X loading dye buffer and was visualized as a single compact band by UV trans-illuminator

Table 1: PCR -SSP cycling Profile

Target haplotype	up-primer	dn-primer	Annealing T emp (^oC)	Cycles	Product size (bp)
B_2	4up8	1DN69	55	18	222
B_{13}	1up32	1DN65	60	15	141
B_{15}	3up8	2DN69	50	18	222
B_{19}	2up8	1DN66	55	15	213
B_{21}	1up8	2DN66	55	18	213

and photographed (Gel documentation system, Bio-Rad, USA).

Gene and genotype frequencies were estimated using Popgene 32 (version 1.32), microsoft Windows-based free ware 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. Homogeneity of distribution of various polymorphic variants at exon- 2 of MHC B-Lβ (class II) gene across the three breeds was studied using chi-square test (Steel and Torrie, 1980).

Results and Discussion

PCR amplification of exon 2 of B-Lβ II family gene

An amplified PCR product of 235 bp size (plate 1) was observed on amplification of exon 2 of MHC B-Lβ II family gene. No traceable report on MHC B-Lβ II gene polymorphism was available in Dual Coloured type lines of poultry. However, similar degenerating primers and amplicons of same base pair size were also used by Pharr *et al.* (1993), Zoorob *et al.* (1993), Zheng *et al.* (1999) in White Leghorn chicken; Shanaz *et al.* (2005) in Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) and Xu *et al.* (2007) in Chinese indigenous chickens.

PCR-SSP of exon 2 of B-Lβ II family alleles

The secondary PCR amplification i.e. sequence specific polymerase chain reaction for targeted haplotypes i.e. B_2, B_{13}, B_{15}, B_{19} and B_{21} yielded amplicons of 222 bp (plate 2 a), 141 bp (plate 2 b), 222 bp (plate 2 c), 213 bp (plate 2 d) and 213 bp (plate 2 e) size, respectively in the present study. Similar findings pertaining to base pair size of the above haplotypes were also reported by Zheng *et al.* (1999) who also used the PCR-SSP method to type B-Lβ II family alleles in broiler chicken. They observed that haplotypes with identical B-Lβ II family sequences produced identical reaction patterns in PCR-SSP: B_{21}, B_{A4} and B_{A7} haplotypes reacted identically, despite differing in the B-G regions. In their study most of the B homozygotes yielded strong amplification products with only one primer pair with the exception of B_{A8}, B_{A9} and B_{A12} haplotypes. Findings similar to the present one have also been reported by Shanaz *et al.* (2005) in Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) with regards to amplification pattern of standard haplotypes used in the present study.

Allelic and genotypic frequency at exon-2 MHC B-LβII family gene locus

The allelic frequency of B_2, B_{13}, B_{15}, B_{19} and B_{21} were 0.375, 0.217, 0.192, 0.217 and 0.000, respectively. The frequency of allele B_2 was found to be the highest while of B_{15} was observed to be the lowest. The allele B_{21} was absent in the present studied population of Dual Coloured type birds.

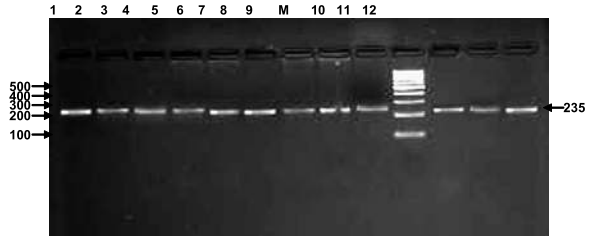

Plate 01: Amplified PCR product of Dual coloured type electrophoresed on 2% agarose gel
M: 100 bp DNA ladder, Lanes: 1-12 are amplified PCR products

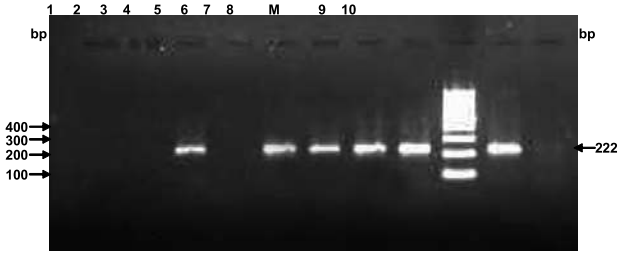

Plate 02(a): Amplified PCR – SSP product of B_2 haplo type electrophoresed on 2% agarose gel
M: 100 bp DNA ladder, Lanes: 3,5-9 are amplified PCR – SSP products

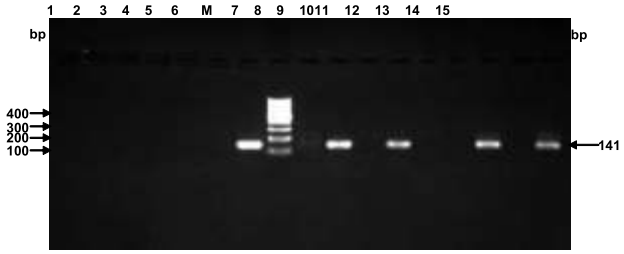

Plate 02(b): Amplified PCR – SSP product of B_{13} haplo type electrophoresed on 2% agarose gel
M: 100 bp DNA ladder, Lanes: 6,8,10,13,15 are amplified PCR–SSP products

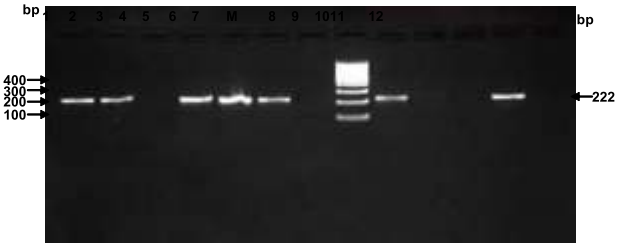

Plate 02(c): Amplified PCR – SSP product of B_{15} haplo type electrophoresed on 2% agarose gel
M: 100 bp DNA ladder, Lanes: 1-2,4-6,8,11 are amplified PCR–SSP products

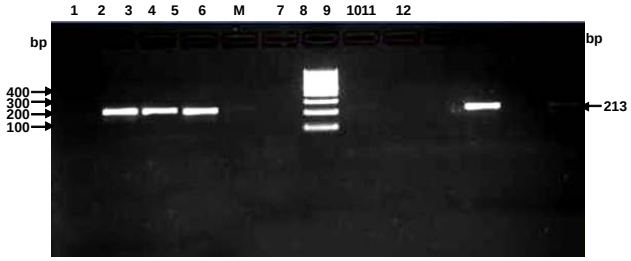

Plate 02(d): Amplified PCR – SSP product of B_{19} haplo type electrophoresed on 2% agarose gel
M: 100 bp DNA ladder, Lanes: 2-4, 10,12 are amplified PCR – SSP products

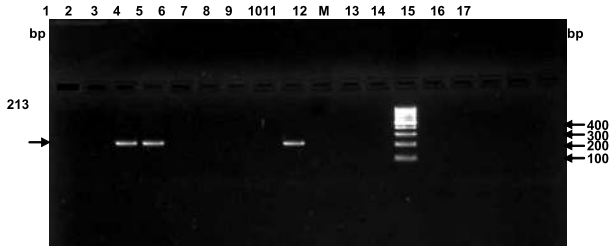

Plate 02(e):Amplified PCR – SSP product of B_{21} haplo type electrophoresed on 2% agarose gel
M: 100 bp DNA ladder, Lanes: 3,4, 9 are amplified PCR – SSP products

Similar study for MHC haplotype conducted on Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) was reported by Shanaz *et al.* (2005). Under this study different frequency for different haplotypes were reported. Allele B_{19} (0.680) was predominant followed by B_{15} (0.263) and B2 (0.055) in Bantam. Allele B_{21} was absent in this genetic group. In Bantamised White leghorn (BWLH), B_{19} (0.486) and B_{15} (0.444) were observed predominantly followed by B_2 (0.065,) whereas in WLH, B_{15} (0.402) had the highest frequency followed by B_{19} (0.333), B_2 (0.138) and B_{21} (0.125). Allele B_{21} was observed only in WLH at lower frequency.

Out of 60 Dual Coloured birds 7 belonged to genotype B_2B_2, 13 to genotype B_2B_{13}, 7 to genotype B_2B_{15}, 11 to B_2B_{19}, 3 each to $B_{13}B_{13}$, $B_{13}B_{19}$, 4 to $B_{13}B_{15}$, 5 to $B_{15}B_{15}$ and $B_{19}B_{19}$ and 2 to $B_{15}B_{19}$. The

genotypic frequencies of 0.117, 0.217, 0.117, 0.183, 0.050, 0.050, 0.067, 0.083, 0.083 and 0.033 were for B_2B_2, B_2B_{13}, B_2B_{15}, B_2B_{19}, $B_{13}B_{13}$, $B_{13}B_{19}$, $B_{13}B_{15}$, $B_{15}B_{15}$, $B_{19}B_{19}$ and $B_{15}B_{19}$, respectively. A test for genetic equilibrium was carried out by comparing observed genotypic frequencies with expected genotypic frequencies calculated from gene frequencies. The non-significant chi-square value observed in the present study revealed that Dual Coloured type population was in Hardy-Weinberg equilibrium. In a similar study for MHC haplotype conducted on Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) was reported by Shanaz *et al.* (2005). Under this study different frequency for different genotypes were reported. 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. Other two genotypes, B_2B_2 and $B_{15}B_{15}$ were observed at the equal frequencies (0.055). In Bantamised WLH, five genotypes were observed, among them heterozygote $B_{15}B_{19}$ (0.832) was predominant while B_2B_2, $B_{19}B_{19}$ and B_2B_{19}, $B_{15}B_{15}$ were observed at low frequencies. In WLH, nine genotypes were observed, in that homozygous $B_{15}B_{15}$ (0.249) and $B_{19}B_{19}$ (0.194) were predominant followed by $B_{15}B_{19}$ (0.138), B_2B_{15}, B_2B_{19} and $B_{15}B_{21}$, equally at 0.082 and B_2B_2, $B_{19}B_{21}$ and $B_{21}B_{21}$ equally at 0.55.

The differences in allelic frequencies observed under the present study when compared with that of Shanaz *et al.* (2005) might be due to the fact that the different populations of different breeds maintained under the different sets of environmental conditions are subject to different evolutionary forces to varying degree. In addition, sampling fluctuations might also have contributed to the differences in allelic frequencies in different breeds and populations. Further, intermixing of populations from different geographical locations and hybridization accompanied by genetic difference might have also contributed to this high degree of genetic diversity among breeds/lines. Similar study for test of genetic equilibrium was conducted on Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) by Shanaz *et al.* (2005). Under this study, alleles in three strains of poultry were not in Hardy Weinberg equilibrium.

The genetic equilibrium condition in Dual Coloured type may be due to random mating for MHC genotypes over the generations in this breed. The other probable reasons for this may be non selective advantages for the different MHC alleles over each other, different reproductive and survival rates of different genotypes and state of balance between different forces which change the gene frequencies. However, to point out which of these causes might have been working to maintain equilibrium condition in addition to random mating, could not be ascertained.

Conflict of Interest: All authors declare no conflict of interest.

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