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Effects of *STAT5B* and *BMPR-1B* genes on growth and production traits in Red Jungle Fowl, Fayoumi, Hilly chickens, and their crossbreeds

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Abstract

This study explored the genome sequence, genetic diversity, and single nucleotide polymorphisms (SNPs) within the *STAT5B* and *BMPR-1B* genes, and their association across Red Jungle Fowl (RJF), Fayoumi (Fay), and Hilly chickens, as well as in Hilly Reddish Brown (RB) × Fay crossbreed chickens. A cohort comprising 40 cocks and 320 hens of each chicken genotype (except for RJF, where 4 cocks and 12 hens were utilized), was raised from day-old to 1-y of age. Genotyping of 30 chickens of each population except RJF for SNPs was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, with genotype frequencies compared using the chi-square test. Marker-trait association analyses were carried out using estimated breeding values. Genetic flow among breeds and diversity was assessed using molecular co-ancestry and polymorphic information criteria. The AG genotype predominated over GG and AA genotypes in the *STAT5B* gene, whereas heterozygote genotype frequency exceeded homozygote genotype frequency in the *BMPR-IB* gene. Recessive gene frequencies surpassed dominant gene frequencies. Chi-square tests confirmed Hardy–Weinberg equilibrium for all genotypes. Mutations were detected in Hilly genotypes, with polymorphic information criteria values of 0.27–0.37 for *STAT5B* and 0.34–0.39 for *BMPR-1B*. The SNP marker of *BMPR-1B* was associated with mature live weight but not egg production. Phylogenetic trees of both genes indicated that the genetic distance among the genotypes was close. These findings suggest a shared ancestry among the studied chickens, with the *STAT5B* SNP emerging as a potential genetic marker for enhancing productive traits.

Keywords: native chicken, diversity, marker-assisted selection, *BMPR-1B, STAT5B*, traits
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Introduction

Bangladesh has several indigenous chicken breeds, such as non-descriptive Deshi (ND), Aseel, Naked Neck, and Hilly varieties, as well as some exotic pure breeds, such as Rhode Island Red (RIR), Fayoumi (Fay), White Leghorn, and the crossbreed Sonali (RIR × Fay) (Bhuiyan, 2008; Khan *et al.,* 2017). Hilly chickens, known for their productivity, are predominantly found in the Chattogram Hill Tract (CHT) region of Bangladesh (Faruque *et al.,* 2015; Khan *et al.,* 2017). According to Khan *et al*. (2017; 2018), Hilly chickens exhibit two main types: Spotted White and Black (SWB) and Reddish Brown (RB), with RB chickens demonstrating superior performance to SWB. Despite several phenotypic studies,

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genotypic research on chicken breeds in Bangladesh remains limited (Faruque *et al.,* 2011; Khan *et al.,* 2017; Das *et al.,* 2018).

The bone morphogenetic proteins (BMPRs) belonging to the transforming growth factor-β (TGF-β) family play a key role in ovarian physiology of domestic animals (Dube *et al*., 1998; Shimasaki *et al*., 1999). A nonconservative substitution (Q249R) in the BMPR-IB sequence is associated with the proliferation character of some ewe breeds (Mulsant *et al*., 2001; Souza *et al*., 2001; Wilson *et al*., 2001). In the chicken ovary, granulosa cells are a major target for BMPs, and it was suggested that mRNA levels for BMPR-IB in granulosa cells were higher than in theca cells (Onagbesan *et al*., 2003). BMPRs are also involved in the formation of primordial follicles in the hamster ovary (Wang *et al*., 2009).

Growth, reproduction, and egg production are polygenic traits controlled by many genes. Among them, the signal transducer and activator of transcription (*STAT*) gene contains five conserved domains and exists in two isoforms (*STAT5A* and *STAT5B*), which are activated by a wide variety of cytokines, growth hormone, and prolactin (Hennighausen *et al*., 2008; Hennighausen & Robinson, 2008; Zhao *et al.,* 2012), *STAT* is notably associated with growth and cytokine expression. Another important group of genes, bone morphogenetic proteins (BMPs), belonging to the transforming growth factor-β (TGF- β) subclass, play a vital role in ovarian physiology (Shimasaki *et al*., 1999). BMPs are involved in numerous biological pathways, ranging from pulmonary traits to bone remodeling (Hinck *et al*., 2016; Li & Quigley, 2024). The major target for BMPs, mRNA levels for BMPR-IB in granulosa cells are higher than in theca cells (Onagbesan *et al*., 2003) therefore BMPR-IB mainly controls egg production by linking to ovulation rate (Zhang *et al.,* 2008). Thus, both *BMPR-IB and STAT5B* genes have significant impacts on growth, reproduction, and egg production traits.

Previous studies (Nedup *et al.,* 2012; Okumu *et al.,* 2017) used genetic characterization with microsatellite markers and/or modern techniques (such as SNPs, marker-assisted selection (MAS), genome sequencing) to analyse different native chicken populations. However, in Bangladesh, such information is scarce or entirely absent. Utilizing molecular tools could enhance the conservation and management of available poultry resources (Boettcher *et al.*, 2010). Genetic diversity in chicken breeds offers an opportunity for improvement, requiring an initial assessment of candidate genes using MAS (Pagala *et al.*, 2017). Understanding the genetic diversity of indigenous chicken breeds of Bangladesh is crucial for conservation and improvement through breeding. Therefore, the study was undertaken to investigate gene sequencing, genetic diversity, and SNPs of the *STAT5B* and *BMPR-1B* genes, and their association with candidate genes and breeding values of RJF, Fay, and Hilly chickens, as well as the Hilly RB × Fay crossbreed chicken in order to conserve and genetically improve these indigenous chickens.

Materials and Methods

The research was conducted at the Department of Genetics and Animal Breeding laboratory, and Poultry Research and Training Centre (PRTC) of Chattogram Veterinary and Animal Sciences University (CVASU), at the Chittagong Hill Tract areas of Bangladesh from March 2019 to June 2020. Animal ethics approval was granted for the study (CVASU Ethical Committee approval no: CVASU/Dir (R&E) EC/2021/273 (3), dated: 22/09/2021).

This study used two types of Hilly chickens (SWB and RB) and RJF, Fay, and crossbred Hilly RB male × Fay female chickens. All chickens were reared using the same management, hygiene, and climatic conditions, following a semi-scavenging rearing system from 1-d old to 1 y of age. Random mating was allowed at a sex ratio of 1:8 (40 cocks and 320 hens) for all chickens except RJF, where the sex ratio was 1:3 (4 cocks and 12 hens).

All equipment was cleaned, disinfected, and fumigated before use. Foot baths and other biosafety measures were properly followed. Chickens had a diet of *ad libitum* fresh water and a formulated ration with the ingredients of broken corn, polished rice, wheat, soybean meal, di-calcium phosphate, protein concentrate, vitamin–mineral premix, soybean oil, and common salt. In the ration, which was given twice a day, chicks received 2,950 kcal/kg; growers 2,800 kcal/kg, and layers 2,660 kcal/kg. The protein amounts were 20%, 17%, 16%; calcium was 1.0%, 0.75%, and 3.5%; and phosphorus was 0.5%, 0.5%, and 0.7% for chicks, growers, and layers, respectively. Supplements such as vitamin C, glucose, and salt were supplied with water as needed. A feeder was placed for every six birds (1:6) and a waterer for every eight birds (1:8) in the laying house. The chicks were brooded using an electric brooder, and the proper brooding temperature was maintained. The brooding period was 3 w during summer and 6 w during winter. The lighting management was 24 h for chicks, 20 w for growers, and 16 h during the laying period. Standard vaccination schedules (Baby Chick Ranikhet Disease, BCRDV; Ranikhet Disease, RDV; Newcastle Disease, ND; Fowl Cholera) were followed, and other farm operations like debeaking and deworming were also carried out as per the appropriate procedure.

The traits evaluated included day-old chick (DOC) weight; live weight at weeks 8, 12, and 16; weight at sexual maturity (WSM); age at sexual maturity (ASM); mature live weight (MLwt); yearly egg production (YEP); egg weight (EWt); age at first egg; and the reproductive parameters of fertility and hatchability. An electric balance was used to measure live weight up to ASM and MLwt (g) at 35 and 36 w for males and females, respectively. The eggs were collected and recorded twice daily from the onset of lay until 52 w of age.

The calculation of gene and genotype frequency was based on the direct gene count method following Nei (1987). Hardy–Weinberg (H-W) balance was counted according to Hartl & Clark (1997). To test H-W equilibrium, a chi-square test was used.

Regardless of sex, 60 chickens of each genotype were blood sampled from wing veins, except RJF (14 chickens for the *STAT5B* gene and 12 chickens for *BMPR1B*) using a vacutainer tube containing 0.5M EDTA (pH = 8). A FavorPrep™ blood genomic DNA extraction kit (Favorgen Biotech Corporation, Taiwan) was used to extract DNA from whole blood samples. After 1 min of centrifugation, the DNA was stored at -20 °C. Of all the samples, 50% samples were positive for both genes and the remaining 50% were damaged, due to improper storage of the blood samples and degeneration. From the positive samples, a 10% sample was sequenced and the best sequence was presented.

The primers for the PCR of the *STAT5B* gene and polymorphisms were identified from exon 6, a method adopted by Ou *et al.* (2009), Niknafs *et al.* (2014), and Charoensook *et al.* (2016a). *BMPR-IB* genes were designed based on a chicken mRNA sequence (GenBank accession no. NM_205132) (Zhang *et al.,* 2008) to amplify the exon 6 to 7 fragments. Both genes were then amplified using the following primers: 5′-CCATCCCTTCCTGGTGCAGT-3′ as forward and 5′-ACTGCTGCCATTTCCCTTTG-3′ as reverse for *STAT5B*, and 5′-GCTATGGGGAAGTCTGGATG-3′ as forward and 5′-TGCCTTTAATGTCTGCCGC-3′ as reverse for *BMPR-IB*. The resulting product size was 554 bp and 581 bp, respectively, with a final PCR reaction volume of 25 µl, composed of 12.5 µl master mix, 2.5 µl of each primer (forward and reverse), 5 µl buffer, and 2.5 µl prepared DNA template (FavorPrep™). The PCR amplification was conducted in an MJ PTC-200. During the thermal–temporal protocol, 95 °C was used for initial denaturation for 10 min, followed by 50 cycles. In each cycle, 30 s was spent at 95 °C for denaturation, 30 s at 65 °C for annealing, and 2 min at 72 °C for extension (Kerje *et al*., 2004; Noor *et al.,* 2021). Finally, this was followed by 10 min at 72° C for final extension.

A microcentrifuge tube containing 15 ml of digestion mixture, which consisted of 10 ml of PCR product, 1.5 ml of NE buffer (1× diluted from 10× buffer), 2.5 ml of deionized water, and 1 ml of the restriction enzyme, *HindIII*, was used to digest the *STAT5B* gene. The final digestion mixture, volume 15 µl, was mixed thoroughly and was digested using *HindIII* restriction enzymes for 1 h, incubated at a temperature of 37 °C, and inactivated at 80 °C for 20 min.

For the *BMPR-IB* gene, a 15 µl digestion mixture [10 µl of the PCR product, 1.5 µl of recommended buffer (Tango buffer 10× buffer), 2.5 µl of deionized water, and 1 µl (10 units) of the restriction enzyme, *MspI*] was placed in a 0.5 ml microcentrifuge tube and was digested by restriction enzymes for 12 h, incubated at a temperature of 37 °C, and inactivated at 80 °C for 20 min.

The digested products for both genes were run on a 2.5% agarose gel (Lonza, USA) at 90 V for 1.5–2 h and stained with ethidium bromide in 1×TAE buffer, and their sizes were estimated using a 100-bp-plus DNA ladder. The bands were visualized under an ultraviolet light trans-illuminator and photographed using a computer. The genotype patterns of RB, SWB, and the crossbreed were examined by digesting the *STAT5B* gene with the *MspI* restriction enzyme at the position of the G4533815A SNP. Additionally, PCR-RFLP was performed using the *HindIII* restriction enzyme at the position of the A287G SNP in the *BMPR-IB* gene. The desired PCR product was cleaned, sterilized, and excised quickly to minimize exposure of the DNA to UV light. The minimum agarose slice was transferred to a 1.5 ml microcentrifuge for purification using a gel extraction kit (Fermentas, Thermo Fisher Scientific, USA). Then, 5 µl of a post-PCR reaction product was mixed with 2 µl of ExoSAP-IT (enzyme: ExoASP-IT) for a combined 7 µl reaction volume. This was incubated at 37 °C for 15 min to degrade the remaining primers and nucleotides. Finally, to inactivate the ExoSAP-IT enzymatic reaction, the mixed sample was incubated at 80 °C for 15 min.

Bidirectional sequencing was done using Sanger sequencing with a BigDye Terminator v3.1 sequencing kit and a 3730Xl automated sequencer (Applied Biosystems, Foster City, CA), using both the forward and reverse primers of PCR amplification. After that, nucleotide sequences were determined on both strands of the PCR amplification products at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea) using an ABI PRISM 3730XL Analyzer (96 capillary types). Finally, the obtained sequences were edited using MEGA6 software (Tamura *et al.,* 2013), and sequence alignment using muscle was conducted to identify nucleotide polymorphisms.

The sequence of *BMPR-IB* and *STAT5B* genes were taken from the NCBI gene bank. Similar sequences and their nucleotides were investigated using the BLAST tool (http://ncbi.nlm.nih.gov). The

polymorphisms of these genes were detected based on sequence alignment using muscle, and the phylogenetic tree was created using the neighbour-joining method (MEGA6 software; Tamura *et al.,* 2013).

The observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated within the population between breeds to determine the total number of alleles and average alleles per locus across breeds. The observed heterozygosity was calculated using Equation 1 (Botstein *et al.,* 1980):

$$H_O = \text{f(Aa) + frequency of heterozygotes} \quad (1)$$

The molecular co-ancestry between two individuals, *i* and *j,* at a given locus was computed using the scoring rules described by Eding and Meuwissen (2001) and Calballero & Tora (2002):

$$f_{ij}l = \frac{1}{4}[I_{11} + I_{12} + I_{21} + I_{22}] \quad (2)$$

where I_{xy} is 1 when allele x on locus 1 in individual *i* and allele y in the same locus in individual *j* are identical, and zero otherwise. Note that this figure can only have four values: 0, $^1/_4$, $^1/_2$, and 1.

The breeding values of different studied traits were estimated using average information restricted maximum likelihood (AIREML), based on restricted maximum likelihood (REML), following Johnson & Thompson (1995). Breeding values of the studied traits were estimated following the individual animal model:

$$Y = Xb + Zu + e \quad (3)$$

where Y is the vector of all observations,
b is the vector of fixed effects,
u is the vector of the breeding value of the individual's (random), and
e is the vector of residual effects.
X and Z are design matrices connecting to the fixed and random effects, respectively.

Allele and genotype frequencies were calculated based on SNP information, and Hardy–Weinberg equilibrium was tested using a chi-square test. We used the following model to analyse the association between markers and traits, taking breeding values as the dependent variable and genotypes as the independent variable. We estimated the least square means for the different parameters of chickens using PROC GLM and PROC MIXED in SAS (SAS, 2010), following a randomised block design (RBD).

The studied model for computing the least square means was:

$$Y_{ijk} = \mu + G_i + B_j + e_{ijkl} \quad (4)$$

where Y_{ijk} is the estimated breeding values of the traits,

μ is the population mean,

G_i is the fixed effect of G4533815A SNP genotypes,

B_j is the fixed effect of A287G SNP genotypes, and

e_{ijkl} is the random error associated with each record, distributed as N (0, σ^2).

There was no significant interaction between the genes' additive effects, so interactions were not considered in the model.

Results and Discussion

The size of the amplicon was 554 bp and 581 bp for the *STAT5B* and *BMPR1B* genes, respectively (Figures 1 and 2). Using the PCR-RFLP technique, the genotypes of *STAT5B* were analysed at the SNP position G4533815 G>A by digesting with the FastDigest *MspI* restriction enzyme, and three genotype patterns were observed: AA, AG, and GG (Figure 3). The nucleotide substitution, A287G, was highly polymorphic. For the *STAT5B* SNP's non-digested (554 bp) and digested (477/77 bp) fragments, alleles A and G were detected, where the genotypes were AA (554), AG (554, 477, and 77), and GG (477 and 77). For the *BMPR1B* SNP's non-digested (581 bp) and digested (287 bp) fragments, alleles C and B were detected, where the genotypes were BB (287), BC (581, 287), and CC (581) (Figure 4).

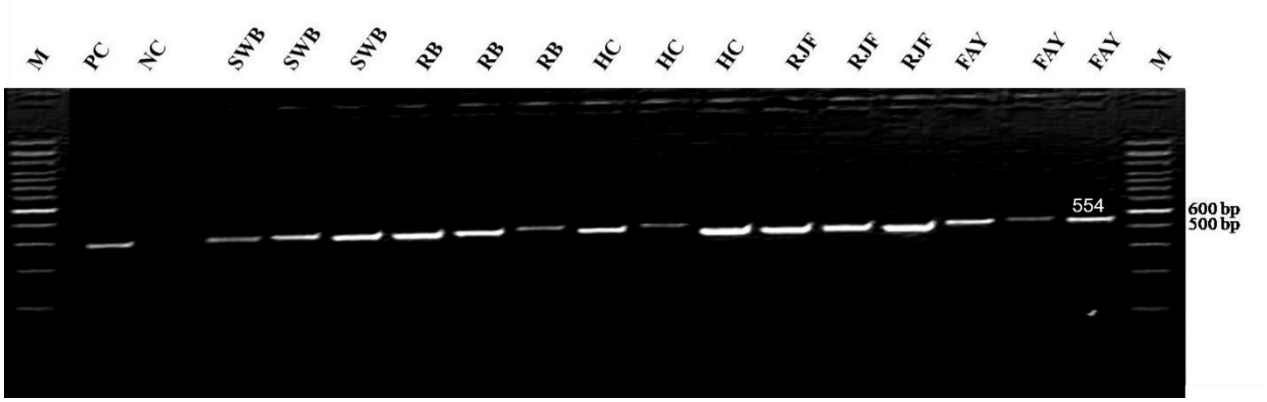

Figure 1 Gel electrophoresis image for amplicon (554 bp) of *STAT5B*
Lane (M) is a DNA marker/ladder; Lane PC is a positive sample and Lane NC is a negative sample; Lanes L1–L15 represent the PCR product of the *STAT5B* (554 bp) gene of five types of chickens. L1–L3 represent the Spotted White and Black (SWB) chicken, L4–L6 represent RB chickens, L7–L9 represent crossbred chickens, L10–L12 represent RJF, L13–L15 represent Fayoumi chickens
RB = Reddish brown; SWB = Spotted White and Black; Crossbred = RB × Fay; Fay = Fayoumi; RJF = Red Jungle Fowl

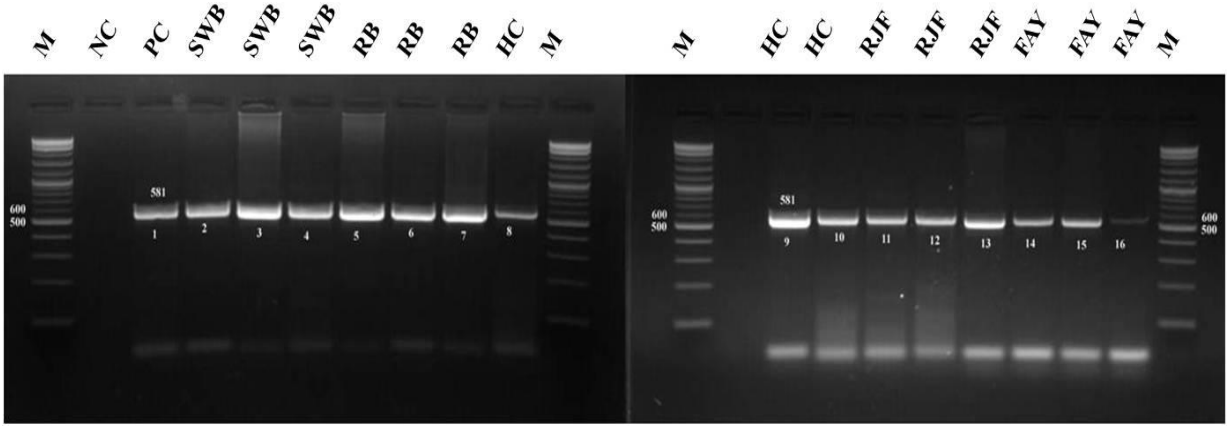

Figure 2 Gel electrophoresis image for amplicon (581 bp) of *BMPR-1B*
Lane (M) is a DNA marker/ladder; Lane 1 is a positive control and Lane NC is a negative control; Lane L2–L16 represent the PCR product of *BMPR1B* (581 bp) gene of five types of chickens. L2–L4 represent the SWB chicken, L5–L7 represents RB chickens, L8–L10 represents crossbred chickens, L11–L13 represents RJF, L14–L16 represents the Fayoumi chickens
RB = Reddish brown; SWB = Spotted White and Black; Crossbred = RB × Fay; Fay = Fayoumi; RJF = Red Jungle Fowl

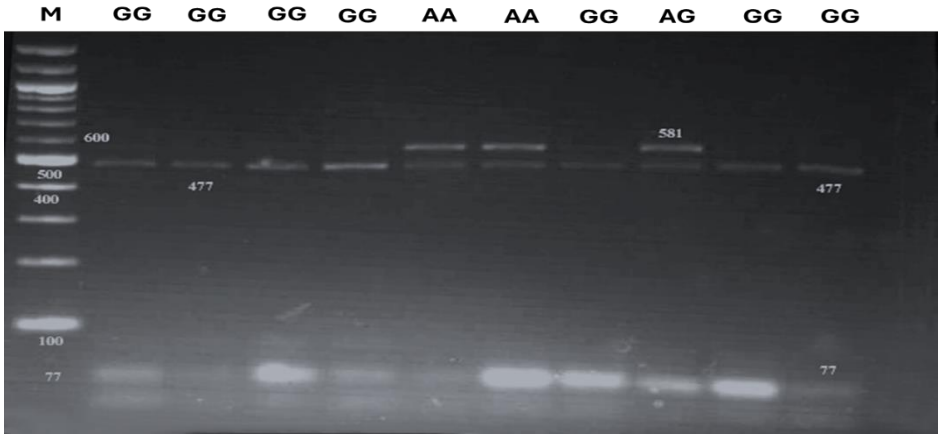

Figure 3 Genotyping of A4533815G single nucleotide polymorphism (SNP) in chicken *STAT5B* gene
Polymerase chain reaction fragment length polymorphism (PCR-RFLP) patterns with agar gel electrophoresis of *STAT5B* gene in five types of chickens. Non-digested (554 bp) and digested (477/77 bp) fragments were considered respectively as alleles A and G, where the genotypes are AA (554), AG (554, 477, and 77), and GG (477 and 77). (M = 100 bp DNA ladder

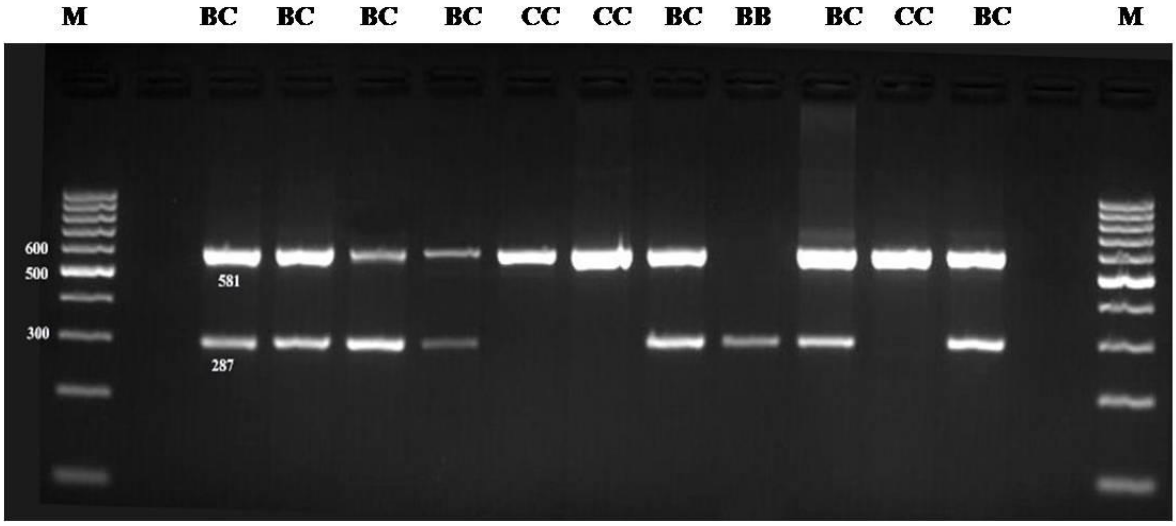

Figure 4 Genotyping of A287G single nucleotide polymorphism (SNP) in chicken *BMPR-1B* gene
Polymerase chain reaction fragment length polymorphism (PCR-RFLP) patterns with agar gel electrophoresis of *BMPR1B* gene in five types of chickens. Non-digested (581 bp) and digested (287 bp)

The *BMPR1B* fragments of the Hilly and Hilly-crossbreed obtained more than 99% (581) homology with the reference chicken's genomic sequence (accession no. EF530593.1) of Zang chicken, whereas the *STAT5B* fragments displayed more than 99% (554) homology with the *Numida meleagris* genomic sequence (accession no. XM 010724872.3) for the same genotype. However, the other two genotypes, RJF and Fay, showed comparatively lower values (Table 1). These results aligned with the results of Kerje *et al*. (2004). Of the sequences, no polymorphism was found for RJF, but mutation was detected in Hilly and Hilly-crossbred chickens at 287 bp (G to A) for *BMPR1B* and at 4533815 bp (A to G) for *STAT5B* (Figure 5 & 6). Hidayati and Saragih (2020) did not report polymorphism of *BMPR1B* in populations of Arabian chickens, Indonesian native chickens, or laying hens.

Table 1 The scoring of similarity and matching rate of different sequences of different chicken genotypes in the case of *STAT5B* and *BMPR1B* genes

Genes	Genotypes	Hilly Cross (HC)		Reddish Brown (Rb)		Spotted White (SW)		RJF		Fayomi	
		Max Score	Identify	Max Score	Identify	Max Score	Identify	Max Score	Identify	Max Score	Identify
STAT5B	*Numida meleagris* transcript variant X4 mRNA XM 010724872.3	888	99.6	894	99.5	790	99.2	901	98.4	875	97.8
BMPR1B	*Gallus gallus* (Zhang *et al.,* 2008), exon 6,7 EF530593.1	893	99.6	889	99.4	760	99.3	952	98.8	883	97.3

**E value was 0 for all the breeds for both genes

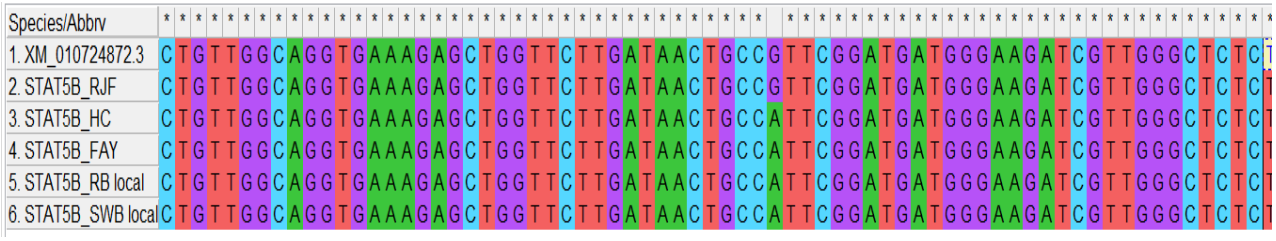

Figure 5 Sequence alignment with reference sequence at position 4533815A/G compared with Gene Bank accession number XM 010724872.3 for *STAT5B* gene from BLAST using MEGA 6 program

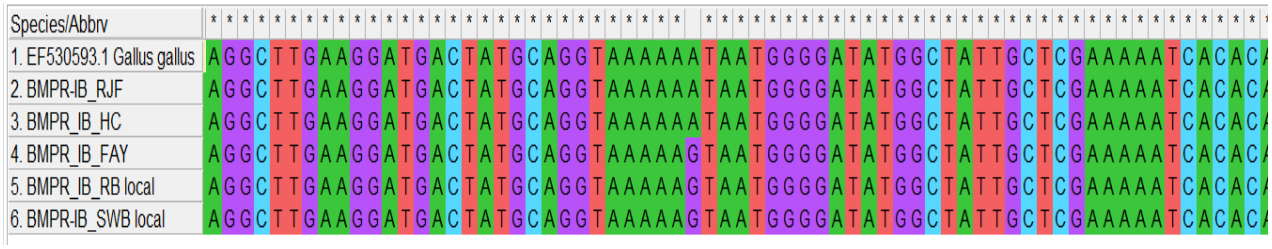

Figure 6 Sequence alignment with reference sequence at position 287 A/G compared with Gene Bank accession number EF530593.1 for *BMPR1B* gene from BLAST using MEGA 6 program

Table 2 presents the genotype frequencies, allele frequencies, and chi-square (χ^2) tests for goodness of fit for the *STAT5B* and *BMPR1B* genes. In both genes, the crossbred RB × Fay chickens exhibited the dominant genotype frequencies; Fayoumi did not possess this genotype. The genotype frequencies of the *STAT5B* gene across the five genotypes demonstrated that the Hilly crossbreed showed a higher frequency for the GG genotype (0.6) compared to the AA and AG genotypes (0.0 and 0.4, respectively). Additionally, the genotype frequency of AG was higher than GG and AA in the Fay, RB, and RJF genotypes. In crossbred and SWB chickens, the genotype frequency of AG was lower than in the other chickens. The heterozygous genotype (AG) was predominant across the studied population. The allele frequency of G was higher than A in all the chickens, whereas the highest allelic frequency (0.80) was obtained in the crossbreed. The dominant allele frequency was higher than the recessive allele frequency in chicken populations (Ou *et al.,* 2009; Charoensook *et al.,* 2016b).

For the BMPR-*IB* gene, the BC genotype frequency was higher than BB and CC in all the chicken populations. Furthermore, this SNP displayed a higher allele frequency of C than the B allele in all five populations, with the highest allele frequency (0.67) found in RJF chickens (Table 2).

The A and G allele frequencies in this study were within the range reported by Zhang *et al.* (2008) for Chinese native chickens and Niknafs *et al.* (2014) for Mazandaran native chickens. The chi-square value for all the genotypes was lower than the tabulated value (5.99; P <0.05) at two degrees of freedom for both the *STAT5B* and *BMPR1B* genes, which confirms that the population in this study were in Hardy–Weinberg equilibrium. Similar results were obtained by Bhuiyan *et al.* (2013) and Charoensook *et al.* (2016b). The value of the recessive allele (G) was higher than the dominant allele (A) in the present study. Niknafs *et al*. (2014) and Awad & El-Tarabany (2015) reported similar results (dominant allele, A = 0.62; recessive allele, G = 0.38).

Table 2 Genotype and allele frequency of *STAT5B* and *BMPR-1B* genes in five chicken populations and the chi-squared (χ^2) test value

Gene	Genotype[1]	N	Genotype observed			Genotype frequency %			Allele frequency		χ^2 (2)
			AA	**AG**	**GG**	**AA**	**AG**	**GG**	**A**	**G**	
STAT5B	RB×Fay	60	-	24	36	-	0.40	0.60	0.20	0.80	0.153
	RB	60	6	42	12	0.10	0.70	0.20	0.45	0.55	0.005
	SWB	60	6	24	30	0.10	0.40	0.50	0.30	0.70	0.934
	Fay	60	-	45	15	-	0.75	0.25	0.38	0.62	0.000
	RJF	14	-	10	4	-	0.71	0.29	0.35	0.65	0.111
			BB	**BC**	**CC**	**BB**	**BC**	**CC**	**B**	**C**	
BMPRP1B	RB×Fay	60	3	42	15	0.05	0.70	0.25	0.40	0.60	0.000
	RB	60	6	39	15	0.10	0.65	0.25	0.43	0.57	0.039
	SWB	60	3	36	21	0.05	0.60	0.35	0.35	0.65	0.047
	Fay	60	-	51	9	-	0.85	0.15	0.43	0.57	0.000
	RJF	12	-	8	4	-	0.67	0.33	0.33	0.67	0.237

[1] RB = Reddish brown; SWB = Spotted White and Black; Crossbred = RB × Fay; Fay = Fayoumi; RJF = Red Jungle Fowl; N = number of chickens

[2] Chi-square (χ^2) with 2 degrees of freedom, tabulated value of χ^2 is 5.99 at a 5% level of significance

Table 3 shows the genetic variability (observed (H_O) and expected (H_E) heterozygosity), molecular co-ancestry (f_{ij}), and polymorphism informative content (PIC) of different genotypes of chickens for the *STAT5B* and *BMPR-1B* genes. The H_O values varied from 0.40 to 0.75 for the *STAT5B* gene and from 0.60 to 0.85 for the *BMPR-1B* gene in all populations of chickens. The highest H_O value was observed in Fay for both *STAT5B* and *BMPR-1B* SNPs. The lowest H_O value was found in crossbred and SWB chickens (0.40) for the *STAT5B* gene and in SWB (0.60) for the *BMPR-1B* gene. For both the *STAT5B* and *BMPR-1B* genes, no differences (P >0.05) were found between H_O and H_E among the populations.

The current findings were similar to Bangladeshi, Chinese, Ethiopian, and Tanzanian chicken populations (Lyimo *et al.,* 2014; Khan *et al.,* 2018; Rashid *et al.,* 2020), however, their results were at different gene loci. Charoensook *et al.* (2016b) reported values that ranged from 0.108 to 0.400, which is lower than the values found in the present study. A comparison of data from different studies is partially problematic due to the different genetic backgrounds of the chickens and the different markers used (Halima *et al.,* 2009). In addition, the variation in expected heterozygosity (H_E) may be affected by differences in location, sample size, population structure, and the use of microsatellite markers (Wei *et al.,* 2009).

The co-ancestry (f_{ij}) values ranged from 0.125 to 0.437 for both the *STAT5B* and *BMPR-1B* genes in all studied chickens. The within-breed molecular co-ancestry (f_{ij}) values for these two genes indicated that these chickens have minimal genetic differentiation. Similar findings have been reported for native Japanese poultry (Tadano *et al.,* 2008), local Italian chickens (Zanetti *et al.,* 2010), and Hilly chickens of Bangladesh (Khan *et al.,* 2018).

The genetic diversity of the studied chickens was assessed by calculating the PIC values, which ranged from 0.27 to 0.37 for the *STAT5B* gene and from 0.34 to 0.39 for the *BMPR-1B* gene (Table 3). In interpreting these figures, PIC >0.50 indicates a highly informative locus, 0.25< PIC <0.50 indicates a reasonably informative locus, and PIC <0.25 indicates a slightly informative locus (Lyimo *et al.,* 2014). The PIC values found in the present study indicate the low diversity of these chickens. This observation was lower than the findings of Seo *et al.* (2001) and Rashid *et al*. (2020), who obtained PIC values of 0.598–0.865 and also lower than Khan *et al.* (2018), who found PIC values of 0.46–0.86. Various factors

may account for the differences in H_O, H_E, PIC, and co-ancestry (f_{ij}) values between previous findings and the present study, such as the size of the sample, the structure of the population, the number of markers used, the type and number of alleles identified by the marker, the method of allele scoring (null allele or allele drop-out), and the sampling strategy.

Table 3 Expected (H_E) and observed (H_O) heterozygosity, within breed molecular co-ancestry (f_{ij}) for each type analysed and the PIC for the different types of chicken (Hilly, crossbred, Fayoumi, and RJF)

Gene	Genotype	H_O	H_E	PIC	f_{ij}
STAT5B	RB × Fay	0.40	0.32	0.27	0.125
	RB	0.70	0.49	0.37	0.375
	SWB	0.40	0.42	0.33	0.437
	Fay	0.75	0.53	0.36	0.125
	RJF	0.71	0.55	0.35	0.125
BMPR-1B	RB × Fay	0.70	0.48	0.36	0.375
	RB	0.65	0.49	0.37	0.437
	SWB	0.60	0.45	0.35	0.375
	Fay	0.85	0.49	0.39	0.125
	RJF	0.67	0.44	0.34	0.125

[1] RB = Reddish brown; SWB = Spotted White and Black; Crossbred = RB × Fay; Fay = Fayoumi; RJF = Red Jungle Fowl; N = number of chickens

The mean breeding values (BVs) of different traits for the *STAT5B* and *BMPR-1B* genes in the studied genotypes are displayed in Table 4. These results showed significant differences in the BVs of the G4533815A SNP genotype of *STAT5B* and the A287G SNP genotype of *BMPR-IB*. Of the three genotypes, the heterozygous genotype, BC, showed higher values in females than the two homozygous classes, BB and CC. The highest BVs were found in the BC genotype for ASM (2.6 ± 0.6) for males, which differed from other genotypes (P <0.05). Homozygous BB and CC genotypes showed higher BVs for live weight in BB genotypes and for WSM and MLwt in CC genotypes. The sex differences in the BVs differed substantially within and between populations. However, the BVs of different genotypes were similar for egg weight and DOC (P >0.05).

For the *STAT5B* gene (SNP G4533815A), association analysis revealed that among the three genotypes, the heterozygous genotype AG was higher for females for growth, reproduction traits, and egg production than the GG and AA classes (P <0.05). In male chickens, by contrast, the highest breeding value was found in the GG genotype for live weight, which differed from the other genotype classes (P <0.05). The dominance effect was also marked in different traits across genotypes for the studied traits. Similar results were found by Niknafs *et al.* (2014), who observed that the G4533815A SNP of the chicken *STAT5B* gene was associated with the additive genetic effect of body weight at 8 and 12 w of age, and that the homozygous genotype (CC) was higher than the BB genotype. The phenotypic performance, including additive and non-additive and environmental factors, might affect the results of marker-trait association analysis (Zhao *et al.,* 2012; Niknafs *et al.,* 2014), The *STAT5B* gene may be involved physiologically in growth hormone actions and body weight (Rosenfeld *et al.,* 2007). The results found here indicate that the genetic marker of the *STAT5B* gene might be useful for selection programmes for the consecutive development of traits concerning chicken growth and egg production.

Table 4 Breeding value (means ± SEM) for genotypes of SNP G4533815A (*STAT5B*) and SNP 287 G>A (*BMPR-1B*) genes

Traits[1]	Sex[2]	*STAT5B* gene					*BMPR-1B* gene				
		AA	AG	GG	Additive	Dominant	BB	BC	CC	Additive	Dominant
DoC	M	0.02±0.2	0.3±.0.1	-0.0±0.2	-0.1±0.1	0.3±0.2	-0.0±0.1	-0.2±0.1	-0.1±0.1	0.1±0.1	0.1±0.2
	F	0.1±0.2	0.2±0.1	0.1±0.15	-0.1±0.1	0.1±0.2	-0.2±0.1	0.2±0.1	-0.003±0.1	-0.003±0.1	0.2±0.14
BW 8 w	M	-0.9±5.6	6.8±11.7	0.9±5.5	1.9±5.5	-0.9±9.4	9.8^{bx}±3.0	9.1^{bx}±7.3	-10.5^{ay}±6.2	-4.3±4.5	3.9±7.9
	F	-1.5±7.8	-1.4±8.6	1.2±5.5	-5.9±5.2	18.8±8.6	-1.5^{ay}±5.3	-5.4^{ay}±3.3	6.7^{bx}±8.5	0.7±4.3	-12.3±7.2
BW 12 w	M	8.7^{ay}±16.6	-8.9^{ay}±5.6	27.8^{by}±11.9	-9.0±8.8	13.2±15.3	21.2^{bx}±17.4	2.6^{ay}±9.4	2.5^{ax}±19.3	-10.2±10.8	-11±18.9
	F	-8.2^{ax}±16.9	15.4^{bx}±9.6	-7.9^{ax}±8.9	-7.3±9.4	-15.2±16.5	-3.7^{ay}±13.2	9.7^{bx}±6.1	-13.3^{ay}±23.3	17.6±10.7	-2.8±19.4
BW 16 w	M	-1.6^{by}±15.2	-41^{ay}±13.0	26.0^{cy}±16.0	-7.9±11.1	22.4±18.8	35.5^{bx}±19.7	-80.0^{ay}±10.9	15.6^{b}±23.2	-3.2±14.0	-23±23.9
	F	5.7^{bx}±11.8	37.4^{cx}±12.9	-25.2^{ax}±6.4	-3.4±9.9	34.9±16.2	-9.9^{ay}±19.3	47.4^{bx}±26.7	14.0^{ab}±20.4	0.5±11.2	-23±18.9
ASM	M	0.2^{b}±0.4	0.7^{b}±0.7	-0.8^{a}±0.5	0.3±0.4	-1.0±0.6	-0.7^{b}±0.4	2.5^{c}±0.7	-1.5^{a}±0.6	0.2±0.5	1.04±0.9
	F	-0.7^{a}±0.3	-1.2^{a}±0.3	0.4^{b}±0.8	-0.1±0.4	0.4±0.7	0.2^{b}±0.6	-1.79^{a}±0.8	1.1^{b}±1.03	0.03±0.6	-0.4±1.08
WSM	M	12.2^{b}±23.1	-112^{ay}±28.5	35.5^{b}±34.1	-25.±24.4	17.4±42.5	62.4^{bx}±28.2	-143.1^{ay}±20.6	93.3^{bx}±30.1	-27.1±30.3	-51±51.9
	F	-2.0^{b}±19.9	52.8^{cx}±18.3	-55.6^{a}±30.7	-12±19.7	14.9±34.9	-40.6^{ay}±15.5	108.1^{bx}±15.6	-80.6^{ay}±23.8	3.5±22.8	0.03±39.5
MLWT	M	-4.4^{b}±20.1	-122^{ay}±16.4	137^{cy}±32.7	-6.9±27.3	32.9±46.6	5.3^{bx}±45.2	-184.1^{ay}±25.5	148.3^{cx}±39.6	-5.3±41.1	-60±70.3
	F	2.6^{b}±14.9	77.5^{cx}±9.2	-73^{ax}±16.6	-2.0±18.1	21.1±31.9	-32.2^{by}±18.6	133.4^{cx}±11.8	-110.2^{ay}±11.6	-16.5±25.3	33.9±43.7
Egg wt		0.1^{b}±0.2	-0.3^{a}±0.3	0.1^{b}±0.3	0.0±0.2	-0.7±0.3	-0.1±0.2	-0.110.25	0.1±0.2	0.1±0.2	-0.17±0.2
Egg Production		-3.9^{a}±0.4	4.2^{b}±0.5	-0.4^{a}±0.5	0.0±0.9	0.6±1.5	-2.7^{a}±0.4	3.5^{c}±0.28	0.0^{b}±0.3	-0.2±0.6	0.03±1.1

[1] DOC = day-old chick, BW = body weight, ASM = age at sexual maturity, WSM = weight at sexual maturity, MLWT = mature live weight, Egg wt = egg weight

[2] M = male and F =female

Different letters between (a, b and c) for genotype differences; x and y for male and female differences, respectively, indicate significant differences at $P < 0.05$

For SNP A287G, a strong association between the *BMPR-1B* gene and the BVs of investigated traits was found in different genotypes. Regarding this SNP, of the three genotypes, the heterozygous genotype, BC, had a higher value (P <0.05) at weeks 12 and 16 and for WSM, MLwt, and egg production in females than the other two homozygous genotypes, BB and CC (Table 4). Awad & El-Tarabany (2015) found that the A287G SNP of the *BMPR-1B* gene was strongly associated with body weight from weeks 2–8. The A287G SNP of *BMPR1B* was associated with egg production from 47–56 w, and the dominant effect was also significant. There was no association between BMPR1B and reproductive traits (P >0.05). Similar findings were reported elsewhere (Zhang *et al*., 2008). Onagbesan *et al.* (2003) stated that the *BMPR-1B* gene was involved in follicular differentiation and maintenance of the follicular hierarchy. The phylogenetic tree for the study of evolutionary relationships of taxa of *STAT5B* and *BMPR-1B* genes is shown in Figure 5 a, and b, respectively.

Figure 5 (a) Phylogenetic tree drawn based on nucleotide sequence of *STAT5B* gene and 5(b) *BMPR-IB* gene, where XM 010724872.3 is the *Meleagris gallopava* (turkey) and EF530593.1 is the *Galas galas* (Wild Red Jungle fowl)
FAY = Fayoumi, RJF = Red Jungle Fowl, RB local = Reddish Brown local Hilly chicken, SWB local = Spotted White and Black local Hilly chicken, HC = is Hilly × Fayoumi crossbred chicken

In the *STAT5B* gene, the sample sequence, XM_010724872.3, was from the *Meleagris gallopava* (turkey), which was close to both RB and SWB local Hilly chickens but differed from crossbred, Fay, and RJF chickens. The *BMPR-IB* gene, EF530593.1, was from the *Galas galas* (Wild Red Jungle fowl) and was close to SWB and RB local Hilly chickens but differed from SWB local and HC chickens.

The SNPs located in *STAT5B* and *BMPR-1B* genes are important genetic markers that link growth and egg production characteristics in chickens, with these effects appearing to arise from a dominant allele. The association between the *BMPR-1B* (A287G) and the *STAT5B* genes demonstrates that the growth and production traits in the studied chickens are polymorphic. The phylogenetic tree showed that the genetic distance among the genotypes is close for both genes, suggesting that these markers have the potential to enhance marker-assisted breeding programmes. However, the study's relatively small sample size, particularly of Wild Red Jungle Fowl (RJF), may restrict the generalizability of the findings to the broader population. The smaller sample size may also affect the study's ability to detect statistically significant differences in gene and genotype frequencies. Despite these limitations, the implication of this study provides basic molecular data that will be useful for future research on growth, reproduction, and egg production in chickens. Additionally, it will assist in undertaking a genetic improvement programme for indigenous chickens. However, for a definitive conclusion, an in-depth study with a larger sample size is needed to confirm these results and to support efforts for the conservation and genetic improvement of the studied chickens.

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