RESEARCH ARTICLE

Genetic Polymorphism of *PRL* and *PRDM16* Genes in Synthetic Backyard Poultry Birds Popular in Andhra Pradesh

Oguru Sai Sruthi*, Kopparthi Sakunthala Devi[1], Kroviddi Sudhakar[2], Kamisetty Aswani Kumar[3]

Abstract

A total 317 synthetic backyard poultry birds namely Rajasri, Vanaraja, Native chicken, Commercial layer (BV-300) and broiler (COBB-400) were randomly selected for determining the genetic polymorphism of *PRL* and *PRDM16* genes using PCR-RFLP method. The restriction fragment sizes of 160, 144, 81, 54 bp (4 bands), 304, 160, 144, 81, 54 bp (5 bands) and 304, 81, 54 bp (3 bands) were observed for *PRL/Alu1* and 157, 313 bp (2 bands), 470, 313, 144 bp (3 bands) and an uncut fragment of 470 bp for *PRDM16/Bgl*1 locus. For *PRL* gene the genotypic and allelic frequencies of *CC, CT, TT* and *C, T* were found to be 0.07, 0.37, 0.56 and 0.26, 0.74 (Rajasri); 0.41, 0.59, 0.00 and 0.70, 0.30 (Commercial layer); 0.16, 0.56, 0.28 and 0.44, 0.56 (Native chicken). For *PRDM16* gene they were found to be 0.46, 0.46, 0.08 and 0.69, 0.31 (Vanaraja); 0.51, 0.43, 0.06 and 0.73, 0.27 (Commercial broiler); 0.50, 0.46, 0.04 and 0.73, 0.27 (Native chicken). The observed (H_o) and expected heterozygosity (H_e) values ranged from 0.37 to 0.59 ; 0.38 to 0.49 for *PRL* and 0.43 to 0.46;0.39 to 0.42 for *PRDM16* genes. Chi-square differences in genotypic and allelic frequencies at *PRL/AluI* locus showed significant differences ($p \leq 0.01$) between Rajasri, Commercial layer (BV-300) and Native chicken.

Keywords: Backyard poultry birds, Genetic polymorphism, *PRDM16, PRL*.

Ind J Vet Sci and Biotech (2025)**:** 10.48165/ijvsbt.21.3.15

Introduction

Egg production and broodiness are two important traits in modern layers which determine the productivity of the bird. Egg production in chicken is the result of expression of many genes and gene interactions. Broodiness is observed in most common breeds of domestic fowl and has a fundamental role in avian reproduction and production (Bhattacharya *et al*. 2011). Increased plasma prolactin concentration is associated with occurrence of broodiness. Most of the genotypic studies for reported polymorphism have largely been carried out with Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) type markers. In chicken prolactin (*PRL*) gene is located on chromosome number 2 and is composed of 5 exons & 4 introns. Polymorphic studies conducted over the past two years indicated that the *PRL* gene partakes directly and indirectly in shaping many production traits in poultry. Both single nucleotide polymorphism and mutation caused by the insertion or deletion (Indel-mutation) are found in the flanking region of this gene and coding region of the single peptide (Zhou *et al*. 2001; Cui *et al*. 2006).

In commercial broiler farms, body weight and meat yield become one of the major concern to improve the profitability because they affect both effectiveness and efficiency of production cost directly. The chicken *PRDM16* gene resides on chromosome 21 and is composed of 11 exons (locus NC_006108). The PRD1-BF1- RIZ1 homologous domain containing *16 (PRDM16)* genes has been known to be correlated with growth, fatness and meat quality of Chinese Native chicken (Han *et al*., 2012). *PRDM16* suppressed the expression of genes selective for white fat cells.DNA pool sequencing and PCR-RFLP techniques have been used to identify molecular markers for the purpose of determining genetic variation in the *PRDM16* gene. Therefore, the aim of present study was to investigate distribution of allelic and genotypic frequencies, to find polymorphism of *PRL* (prolactin) gene in Rajasri bird and to examine the role of *PRDM16* gene on growth and development in Vanaraja

[1] Department of Animal Genetics and Breeding, College of Veterinary Science, Proddatur-516360, Sri Venkateswara Veterinary University, Andhra Pradesh, India

[2] Department of Animal Genetics and Breeding, NTR College of Veterinary Science, Gannavaram-521101 Sri Venkateswara Veterinary University, Andhra Pradesh, India

[3] Department of Veterinary Biochemistry, NTR College of Veterinary Science, Gannavaram-521101, Sri Venkateswara Veterinary University, Andhra Pradesh, India

***Corresponding author:** Dr. Sai Sruthi O., M.V.Sc student, Department of Animal Genetics and Breeding, College of Veterinary Science, Proddatur-516360, SVVU, Andhra Pradesh, India. E-mail: saisruthi73852@gmail.com

How to cite this article: Sai Sruthi, O. Sakunthala Devi, K., Sudhakar, K., & Aswani Kumar, K. (2025). Genetic Polymorphism of *PRL* and *PRDM16* Genes in Synthetic Backyard Poultry Birds Popular in Andhra Pradesh. Ind J Vet Sci and Biotech, 21(3), 73-77.

Source of support: Nil

Conflict of interest: The author(s) declare that there is no conflict of interest.

Submitted 29/01/2025 **Accepted** 20/03/2025 **Published** 10/05/2025

broiler bird and to perform comparative screening for genetic polymorphism of both these genes in Native chicken.

MATERIALS AND METHODS

In order to identify the polymorphism related to *PRL* and *PRDM16* genes, around 1 mL of whole blood sample was aseptically collected from medial metatarsal and brachial vein of each bird into a sterile polypropylene centrifuge tube and the genomic DNA was isolated from whole blood by Proteinase-K: Phenol: Chloroform: Isoamyl alcohol extraction method (Sambrook and Russell, 2001) with slight modifications to suit under lab conditions. The purity of genomic DNA was verified using 1% agarose gel electrophoresis at 80V for about 45 min and gel was visualized under Gel Documentation system. DNA was quantified by using NanoDrop$^{\text{TM}}$ 2000/2000c (Thermo Fisher) and purity of DNA was judged on the basis of OD ratio at 260 and 280 nm. Primer sequences were designed for *PRL* and *PRDM16* using NCBI primer designing tool as shown below (Table 1).

Table 1: Primer sequences designed for *PRL* and *PRDM16* genes

Gene	Primer Sequence (5' - 3')	T_m	GC%	Length(bp)	Product (bp)	Reference
PRL	F: AGAGGCAGCCCAGGCATTTTAC	57.00	54.54	22	439bp	Cui *et al.* (2006)
	R: CCTGGGTCTGGTTTGGAAATTG	55.00	50.00	22		
PRDM16	F: GATTTTCCCACCATCTCTATACC	53.00	43.47	23	470bp	Han *et al.* (2012)
	R: GAGGAAAGAAGGAATGCTGAGAAT	54.00	41.66	24		

Table 2: Details of restriction enzyme and their recognition sites

Gene	Enzyme	Genetic group	Recognition sequence
PRL	*Alu*I	Rajasri, Native chicken, Commercial layer	5′ --- AC′GA--- 3′ 3′ --- TC′GA--- 5′
PRDM16	*Bgl*I	Vanaraja, Native chicken, Commercial broiler	5′ ---GCCNNNN′NGGC--- 3′ 3′ ---CGGN′NNNNCCG --- 5′

PCR was performed for amplification of *PRL* and *PRDM16* genes using Thermocycler at initial denaturation temperature of 94°C/5 min followed by 35 cycles of denaturation (94°C/30 sec), annealing (62°C/30 sec) and extension (72°C/30 sec), with final extension of 72°C/10 min. The restriction enzymes used in present study (Table 2) to digest PCR product were obtained from New England Biolabs (NEB).

The PCR product of 3 µL from each tube was digested with 3 units/µL and 4 units /µL of *Alu*I and *Bgl*I restriction enzymes, respectively, and 10X Assay buffer was added to make up the final reaction volume of 10 µL. PCR products were digested with respective combination of restriction enzymes and corresponding reaction buffer in sufficient quantity of nuclease and protease free water (Himedia, Mumbai). The reaction mixture was spinned for few seconds for uniform mixing and incubated overnight at 37°C in water bath. T he fragment size of PCR-RFLP products was estimated by comparison with 50 bp and 100 bp DNA ladder (O 'Gene Ruler DNA Ladder, Thermoscientific). RFLP pattern revealed the restricted fragments of different sizes corresponding to different genotypes. Genetic group wise the number of restriction sites and fragment sizes were noted down.

Statistical Analysis

Gene and genotypic frequencies were estimated from the combination of various RFLP alleles. Chi-square test using POPGENE 1.32 software (Yeh *et al.,* 1999), was used to test whether the population is under Hardy Weinberg equilibrium or not.

RESULTS AND DISCUSSION

The electrophoretic profile of RFLP analysis revealed three different genotypes namely *CC*, *CT*, and *TT* and two alleles *C* and *T*. The PCR product of *PRL* and *PRDM16* genes were digested with suitable restriction enzymes namely *Alu*I and *Bgl*I, which recognizes the SNP C-2402T in the *PRL* and 1161C>T in *PRDM16* gene. Total three RFLP patterns were observed, that is in individuals with 4 bands (160, 144, 81, 54 bp), 5 bands (304, 160, 144, 81, 54 bp), 3 bands (304, 81, 54 bp), and in *PRDM16/Bgl*I as a result of SNP 1161 *C>T* individuals with 2 bands (157, 313 bp), 3 bands (470, 313, 157 bp) and an uncut fragment at 470 bp, and were designated as *CC*, *CT*, and *TT,* respectively. Gel image showing different genotypes of *PRL* and *PRDM16* genes obtained using PCR-RFLP are shown in Fig 1&2. Results revealed that the exon 2 of *PRL* gene which harbours the polymorphic site was amplified. The substitution of C→T (i.e.. C with T) occurs in position 2402 of the gene (AB011438.2) which is a site of *Alu*I restriction enzyme.

Genotypic and Allelic Frequencies of *PRL* and *PRDM16* Genes

Distribution of *PRL/Alu*1 and *PRDM16/Bgl*1 allelic, genotypic frequencies and Chi-square test values between different breeds are presented in Tables 3 and 4. Within the *PRL* locus the *CT* and *CC* genotypes have highest genotypic frequency in Commercial layer (BV-300) (0.59 and 0.41) followed by Native chicken (0.56 and 0.16) and Rajasri (0.37 and 0.07). The *TT* genotype was absent in Commercial layer (BV-300), but was present in Rajasri (0.56) and Native chicken (0.28). The abundance of *TT* genotypes (0.56) in Rajasri birds corroborated with findings of Kulibaba and Podstreshnyi (2012) in Ukranian synthetic line G-2 (0.71) and Rashidi *et al.* (2012) in Native birds. However, more *TT* genotypes were observed in indigenous breeds by Vu and Ngu (2016) in Vietnam chicken (0.69); contrarily absence of TT genotypes was reported in Azarbaijan native chicken by Abdi *et al.* (2014). Roy *et al.* (2019) observed the non-existence of *TT* genotypes in commercial White Leghorn chicken and lower frequencies by Cui *et al.* (2006) and Al-Sheik and Ismail (2017).

Fig 1: Gel image showing PCR-RFLP of PRL/ALU locus: Lane 6: 50 bp DNA Ladder, Lane 1, 5, 8, 9, 10: TT genotypes, Lane 2, 3, 4, 7, 11: CT genotypes, Lane 12: Negative control

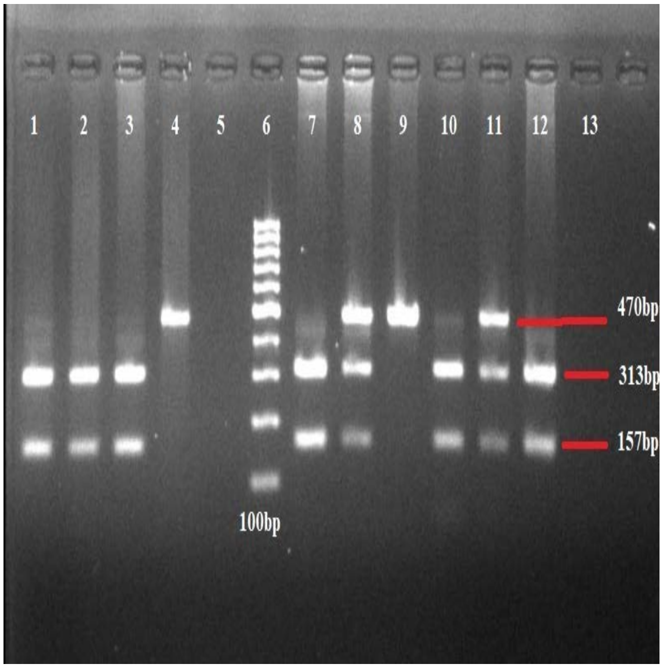

Fig 2: Gel image showing PCR-RFLP of PRDM16/BglI locus: Lane 6: 100 bp DNA Ladder, Lane 5, 13: Negative controls, Lane 1, 2, 3, 7, 10, 12: *CC* genotypes, Lane 8, 11: *CT* genotypes, Lane 4, 9: *TT* genotypes

The frequency of *C* allele in commercial layer (0.70) was found to be highest compared to *T* allele (0.30) which was similar to earlier reports on commercial chicken (Al-Sheik and Ismail, 2017; Kulibaba and Podstreshnyi, 2012). The frequency of *T* alleles was more in Rajasri (0.74). Similar observation was noticed in indigenous Nongdahe (Cui *et al.*, 2006) and Mazandaran chicken (Rashdi *et al.*, 2012). The substitution of the nucleotide *C* to *T* at 2402 was responsible for higher egg production as reported by Cui *et al.* (2006), Kuilbaba and Podstreshnyi (2012), and Al-sheik and Ismail (2017) in White leghorn, Ukranian chicken line-A, Hyline brown and IWH strain of White leghorn, whereas Shulika and Kulibala (2018) reported that *CC* and *TT* genotypes were associated with higher egg number and egg weight in Rhode-Island Red.

The avian *PRL* gene is highly conserved and polymorphism is observed to occur predominantly at 5′ flanking region, 3′ flanking region and coding region of the signal peptide (Kansaku *et al.*, 2008). The earlier studies established that the *PRL* gene is closely associated to the onset and maintenance of broody behaviour and could be a genetic marker for egg production (Luan *et al.*, 2014) in breeding chicken.

It has been reported that *PRDM16* gene could be a genetic marker for myoblast development (Seale *et al.*, 2008). The genotypic frequency (Table 4) of *CC*, *CT* and *TT* in Vanaraja birds observed was 0.46, 0.46, and 0.08; in Native chicken 0.50, 0.46, and 0.04; in Commercial broiler (COBB-400) 0.51, 0.43 and 0.06. The allele frequency of *C* and *T* in Vanaraja, Native chicken, Commercial broiler (COBB-400) was 0.69, 0.31; 0.73, 0.27; 0.73, 0.27. The *C* allele frequency in the studied populations was higher than *T* though not significant (p>0.05), Contrarily, Han *et al.* (2012) reported low frequencies in Chinese native chicken and reported that 1161 C>T, a synonymous SNP influencing growth and meat quality traits indicating that the SNP could be a potential marker for selection.

Population Genetic Indices

A Chi-square test was performed to test whether the distribution of the *PRL* genotypes in the flocks followed Hardy-Weinberg equilibrium or not and the analysis revealed that the distribution of genotypes differed significantly ($p \leq 0.01$) in Commercial layer birds, but not in Rajasri and Native chicken, indicating selection favouring *C* allele was practiced in Commercial layers for increased egg production. Though Rajasri bird were also selected for increased egg production (Rao Viroji *et al.*, 2012) the allele frequency observed in it is not indicative, which could be due to difference in sample size. However, the differences for genotypic and allelic frequencies showed significance between the populations of Rajasri ($p \leq 0.01$) with Commercial layer and Native chicken.

For *PRDM16* gene no significant difference was observed for Chi-square test in genotypic and allelic frequencies among different breeds. The differences among genotypes for Vanaraja, Native chicken and Commercial broiler were found to be non-significant, which indicated that these populations were under Hardy-Weinberg Equilibrium. It shows that the alleles at this locus have remained free from changing gene and genotypic frequencies.

Heterozygosity

The population indices of *PRL* and *PRDM16* genes for different breeds are shown in Table 5. Observed heterozygosity was calculated based upon number of heterozygotes at respective loci over total number of individuals. The expected heterozygosity was based on assumption that the population was under Hardy Weinberg Equlibrum (Nei, 1978).

The distribution of *PRL* genotypic frequencies in the studied population revealed a observed heterozygosity (H_o) value of 0.36, which was less than the expected heterozygosity (H_e) (0.38) in Rajasri, suggesting a high degree of homozygosity. Whereas, observed heterozygosity was more than expected in Native chicken (0.56) and Commercial layer (0.58).

The distribution of *PRDM16* genotypic frequencies in the studied population revealed that observed heterozygosity (H_o) in general was greater than expected heterozygosity (H_e) in Vanaraja (0.46), Commercial broiler (0.43) and Native chicken (0.46), due to the more variation in the breeds. Shulika and Kulibala (2018) reported similar findings for observed heterozygosity (H_o) and expected heterozygosity (H_e) in Rhode Island chicken.

Table 3: Distribution of *PRL/Alu*1allelic, genotypic frequencies and Chi-Square test values between different breeds

Breed	Genotypic frequency			Allele frequencya		Chi-square value (χ^2)
	CC	CT	TT	C	T	
Rajasri	0.07 (07)	0.37 (33)	0.56 (50)	0.26	0.74	0.277
Commercial layer (BV-300)	0.41 (21)	0.59 (30)	0.00 (00)	0.70	0.30	8.500**
Native chicken	0.16 (08)	0.56 (28)	0.28 (14)	0.44	0.56	0.797

Values in parentheses indicate number of genotypes. ** Significant at 1% L.S (P≤0.01)

Table 4: Distribution of *PRDM16/Bgl*1 allelic, genotypic frequencies and Chi-Square test values between different breeds

Breed	Genotypic frequency			Allele frequency		Chi-square value (χ^2)
	CC	CT	TT	C	T	
Vanaraja	0.46 (35)	0.46 (35)	0.08 (06)	0.690	0.310	0.389
Commercial broiler (COBB-400)	0.51 (26)	0.43 (22)	0.06 (03)	0.730	0.270	0.275
Native chicken	0.50 (25)	0.46 (23)	0.04 (02)	0.730	0.270	1.243

Values in parentheses indicate number of genotypes.

Table 5: Population Indices of *PRL* and *PRDM16* genes for different breeds

Gene	Breed	He	Ho	PIC	F_{IS}
PRL	Rajasri	0.38	0.36	0.31	0.05
	Commercial layer (BV-300)	0.41	0.58	0.32	-0.41
	Native chicken	0.49	0.56	0.37	-0.13
PRDM16	Vanaraja	0.42	0.46	0.33	-0.07
	Commercial broiler(COBB-400)	0.39	0.43	0.31	-0.08
	Native chicken	0.39	0.46	0.31	-0.16

PIC = Polymorphic information content; F_{IS} = Fixation index;
He = Expected heterozygosity; Ho = Observed heterozygosity

Polymorphic Information Content (PIC) and Fixation Index (F_{IS})

The PIC is an indication of the degree of the informativeness of the marker (low polymorphism: < 0.25, medium polymorphism: 0.25 to 0.50 and high polymorphism: > 0.50). The PIC values of the present study revealed medium polymorphism and varied from 0.311 to 0.371 for *PRL*, 0.316 to 0.336 for *PRDM16* gene among the different genetic groups (Table 5).

Fixation index (F_{IS}) is a measure of deviation of genotypic frequencies from panmictic frequencies in terms of heterozygous deficiency/excess. The value of F_{IS} was found to be negative (Table 5) for all genetic groups -0.41 to -0.07, except Rajasri (0.05). The negative F_{IS} values indicate that the population is outbred in nature. The negative F_{IS} values in commercial layers and native chicken are indicative of heterozygote excess (outbreeding) compared with Hardy-Weinberg equilibrium and inbreeding was evident in Rajasri population.

CONCLUSION

The present study successfully identified genetic polymorphisms in *PRL* and *PRDM16* genes among different genetic groups of backyard poultry using the PCR-RFLP technique. The variation in genotypic and allelic frequencies observed across Rajasri, Vanaraja, Native chicken, Commercial layer, and broiler birds indicates the existence of genetic diversity at these loci. Significant genotypic differences at the *PRL* gene locus suggest its potential association with traits like broodiness and egg production. Similarly, polymorphism in the *PRDM16* gene points to its probable role in growth and meat quality. The observed heterozygosity values further support the presence of moderate genetic variation within the studied populations. These findings contribute valuable molecular insights for future selection and breeding strategies in backyard poultry improvement programs.

ACKNOWLEDGEMENT

Authors express sincere gratitude to the livestock farm complex of Gannavaram, Tirupathi, Proddatur, SVVU, and Srinivasa hatcheries for granting permission and their cooperation for collecting blood samples from the chicken breeds used in this study.

REFERENCES

Abdi, M., Seyedabadi, H., & Gorbani, A. (2014). Gene polymorphism and its associations with production and reproductive traits in West-Azarbaijan Native chicken. *Bulletin of Environment, Pharmacology and Life sciences, 3,* 39-45.

Al-Sheik, A.A., & Ismail, I. H. (2017). Effect of prolactin gene polymorphisms on egg weight of White Leghorn and Hy-line brown laying hen strains. *Iraqi Journal of Biotechnology, 16,* 2.

Bhattacharya, T.K., Chatterjee, R.N., Sharma, R.P., Niranjan, M., & Rajkumar, U. (2011). Associations between novel polymorphisms at the 5′-UTR region of the prolactin gene and egg production and quality in chickens. *Theriogenology, 75,* 655-661.

Cui, J.X., Du, H. L., Liang, Y., Deng, X.M., Li, N., & Zhang, X.Q. (2006). Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poultry Science, 85,* 26-31.

Han, R., Wei, Y., Kang, X., Chen, H., Sun, G., Li, G., & Huang, Y. (2012). Novel SNPs in the *PRDM16* gene and their associations with performance traits in chickens. *Molecular Biology Reports, 39,* 3153-3160.

Kansaku, N., Hiyama, G., Sasanami, T., & Zadworny, D. (2008). Prolactin and growth hormone in birds: protein structure, gene structure and genetic variation. The *Journal of Poultry Science, 45,* 1-6.

Kulibaba, R.A., & Podstreshnyi, A.P. (2012). Prolactin and growth hormone gene polymorphisms in chicken lines of Ukrainian selection. *Cytology and Genetics, 46*, 390-395.

Luan, X., Liu, D., Cao, Z., Luo, L., Liu, M., & Gao, M. (2014). Transcriptome profiling identifies differentially expressed genes in Huoyan goose ovaries between the laying period and ceased period. *Public Library of Science One, 9,* 1-25.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*(3), 583-590.

Rao Viroji, S.T.,Chinni Preetham, V., Sakunthala Devi, K., Narasimha, J. (2012). Rajasri - a designer fowl introgressed with genes from native birds evolved for backyard production system. *Indian Journal of Poultry Science, 47,* 388-390.

Rashidi, H., Rahimi-Mianji, G., Farhadi, A., & Gholizadeh, M. (2012). Association of prolactin and prolactin receptor gene polymorphisms with economic traits in breeder hens of indigenous chickens of Mazandaran province. *Iranian Journal of Biotechnology, 10,* 129-135.

Roy, B.G., Vishesh, K.S., Usha, R., & Mahendra, C.K. (2019). PCR-RFLP study of candidate genes for egg production in layer chicken. *Archives of Animal and Poultry Sciences*, ***1***, 555-563.

Sambrook, J., & Russell, D.W. (2001). *Molecular Cloning: A Laboratory Manual*. 3rd edn., Cold Spring Harbor Lab (CSHL), New York, International Standard Book Number, 0879695773.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conre, H.M., Erdjument-Bro-mage, H., Tempst, P., Rudnicki, M.A., Beier, D.R., & Spiegelman, B.M. (2008). *PRDM16* controls a brown fat/skeleton muscle switch. *Nature, 454,* 961-967.

Shulika, L.V., & Kulibaba, R.O. (2018). Genetic structure of Rhode-Island Red chicken breed population on *PRL* and *INS* loci. Associations between genotype and chicken productivity. *Archiva Zootechnica, 21,* 51-59.

Vu, C., & Ngu, N.T. (2016). Single nucleotide polymorphisms in candidate genes associated with egg production traits in native Noi Chicken of Vietnam. The *International Journal of Plant, Animal and Environmental Sciences,6,* 162-169.

Yeh, F.C., Yang, R., & Boyle, T. (1999). *PopGene. Version 1.32.* Microsoft window–based freeware for population genetic analysis. University of Alberta. Edmonton, AB, Canada.

Zhou, M., Zhang, X.Q., Shi. Z.D., & Cao,Y.C. (2001). Cloning and sequencing of prolactin gene cDNA in three chicken breeds. *Yi Chuan Xue Bao, 28,* 614-620.