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Advances in Animal and Veterinary Sciences
Research Article

# Association of *PRL/Xba*I Polymorphisms with Reproductive Performances in Vietnamese BT and TB Duck Lines

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**Abstract** | The *prolactin* (*PRL*) gene polymorphisms in the intron 1 region and their association with reproductive traits in the reciprocal TB and BT duck lines were assessed. The 441 bp segment of the *PRL* gene was amplified from the genomic DNA samples derived from total of 225 ducks (111 TB and 114 BT), and the PCR-RFLP technique to examine the genetic variations in the *PRL* gene among individuals. The restriction enzyme, *Xba*I, was digested to cleave the 441-bp PCR products and revealed the TT, TG, and GG genotypes. The TT genotype was most prevalent, with frequencies of 0.820 in TB and 0.754 in BT lines. Allele frequencies of T and G were 0.906 and 0.094 in TB, and 0.868 and 0.132 in BT, respectively. The effective population size (Ne) was 1.205 in TB and 1.297 in BT, while expected heterozygosity (He) values were 0.170 in TB and 0.229 in BT, with polymorphic information content (PIC) values at 0.203 and 0.229, respectively. Ducks with the TT/*Xba*I genotype exhibited significantly higher (P<0.05) egg weight compared to those with the TG/*Xba*I genotype in both lines. In the BT line, eggs from TT genotype ducks weighed 76.4 grams versus 74.3 grams from TG genotype ducks (P<0.05). In the TB line, TT genotype ducks had significantly higher egg weight compared to for TG genotype ducks (72.4 grams *v.s* 69.9 grams; P < 0.05). Furthermore, the TT/*Xba*I genotype ducks in the BT line (76.4 grams) showed significantly higher (P<0.01) egg weight than those in the TB line (72.4 grams). Polymorphisms were identified at the *PRL/Xba*I locus, with the TT genotype dominant in the population. Female ducks with the TT genotype showed higher egg weights, indicating that the polymorphism at *PRL/Xba*I locus may serve as a potential selection genetic marker to improve egg production. Further study is required to explore the impact of this polymorphism on efficacy of breeding selection and application. In addition, maintaining the T allele and TT genotype can be considered in practical conditions for breeding program selections to enhance the egg yield.

**Keywords** | Climate change, Duck, Egg production, PCR-RFLP, Prolactin gene, Reciprocal crossbred

**Received** | October 10, 2024; **Accepted** | November 21, 2024; **Published** | February 17, 2025
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**Citation** | Le TL, Trang TTV, Hoang TT, Tang PC, Nguyen NT (2025). Association of *PRL/Xba*I polymorphisms with reproductive performances in Vietnamese BT and TB duck lines. Adv. Anim. Vet. Sci. 13(3): 608-617.
**DOI** | https://dx.doi.org/10.17582/journal.aavs/2025/13.3.608.617
**ISSN (Online)** | 2307-8316; **ISSN (Print)** | 2309-3331

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## INTRODUCTION

The production of duck meat is expanding globally, while in Vietnam, traditional practices like wet rice cultivation and duck farming hold significant importance in the Agri-economy. The country is ranked second behind China, with over 83.7 million ducks as 7% of the global total (FAOSTAT, 2022). Poultry provide vital human nutrition, with duck meat and egg products offering superior nutritional values compared to chicken (Biswas *et al*., 2019; Weng *et al*., 2022). Vietnam has successfully created numerous high egg-yield duck lines, notably the TC duck strain, a hybrid between the Chinese super-egg-laying duck (Zhejiang/Triet Giang) and the Vietnamese native duck (Co) breed. The TC duck line has a high egg yield, averaging 280.65 to 282.68 eggs/duck over a 52-week laying period, with egg weight of 68 to 70 grams and a feed conversion ratio of 2.04 to 2.10 kg per 10 eggs (Nguyen *et al*., 2011). The Bien duck, domesticated by Vietnamese scientists at the National Institute of Animal Science, is the first duck line in Vietnam that can live and lay eggs in the marine environment. These ducks drink seawater and eating many kinds of forages in coastal areas, producing around 240 eggs per year (Henning *et al*., 2013; Vuong *et al*., 2019). Crossbreeding the Bien salt-tolerant and high egg-yielding TC duck lines by reciprocal crosses shows promise for improving adaptability to face the growing challenges of climate change and salt-water intrusion. These environmental shifts present significant challenges for duck farming in Vietnam's coastal areas, where developing salt-tolerant, high-yield TB and BT crossbred is essential for maintaining stable egg production and ensuring resilience against these evolving conditions.

Marker-assisted selection (MAS) can significantly decrease the time and cost involved in the selection process by eliminating the need for elaborate large-scale experiments, thereby reducing the risks and enhancing overall efficiency (Wakchaure *et al*., 2015). MAS has numerous advantages compared to the conventional Best Linear Unbiased Prediction (BLUP) method by the targeted selection of specific genetic traits, providing flexibility in integrating diverse genetic data. MAS reduces the time and financial resources required for selection (Fritsche-Neto *et al*., 2021; Zhang *et al*., 2022) as a crucial and efficient tool for enhancing the quality and productivity of crops and livestock (Gutierrez-Reinoso *et al*., 2021).

Prolactin (PRL), a hormone produced by the anterior pituitary gland, plays various biological roles across all vertebrates (Yurnalis et al., 2019). In ducks, PRL is composed of 229 amino acids, encoded by the *prolactin* gene (Kansaku *et al*., 2008). Studies have shown that polymorphisms in intron 1 of the duck *prolactin* gene are associated with egg weight (Li *et al*., 2009; Bagheri *et al*., 2013; Bai *et al*., 2019), while polymorphisms in exon 5 are linked to annual egg production (Wang *et al*., 2011; Osman *et al*., 2017; Nguyen *et al*., 2023). Haplotype analysis revealed that each mutation correlated with egg production and reproductive traits. Studies on various Chinese local duck breeds have identified a C→A mutation at position 381 in intron 1, detected using the *Xba*I enzyme (Wang *et al*., 2011). In Mulard ducks, the *PRL/Xba*I polymorphism significantly influences body weight at 10 and 12 weeks of age (Mazurowski *et al*., 2016). Variation at the *PRL/Xba*I locus within intron 1 significantly influences egg production in Khaki Campbell ducks, particularly benefiting individuals with the GT genotype, which exhibit the highest egg yield (Chuekwon and Boonlum, 2017).

This study firstly assessed prolactin gene polymorphism in intron 1 region using the PCR-RFLP technique in two reciprocal crossbred duck lines, known for their high egg yield and salt tolerance (referred to as TC×B or the TB line, and B×TC or the BT line). The impact of gene polymorphism on several egg productivity traits of these crossbred duck lines was analyzed to serve as a molecular database to support future selection for enhancing egg production and shortening the time for breeding selection compared to traditional methods, which are crucial for enhancing egg productivity in hybrid duck lines that are created for adaptability in salt tolerance condition.

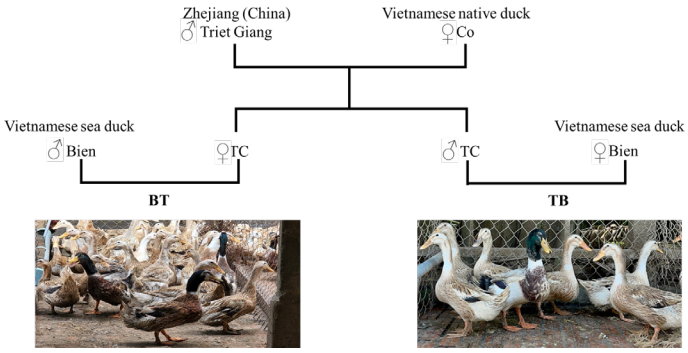


**Figure 1:** Breeding scheme illustrating the origins of reciprocal crossing between the TC and B duck lines used in the study. Images of BT (left) and TB (right) ducks at 18 weeks of age.

## MATERIALS AND METHODS

### Birds

A total of 225 ducks comprised 111 from the TB line and 114 from the BT line (Figure 1) was used. The ducks were raised at the VIGOVA Poultry Research and Development Center. They were kept based on created family units (one male with eight females) with 25 families for one duck line was designed, identified by a wing tag number, and fed according to the standard diet for egg-laying ducks. Commercial feed was provided based on individual family accordingly stages of development: 20-22% crude protein

(CP) and 2,850-2,900 kcal metabolizable energy (ME) ad libitum for the starter phase (0 to 8 weeks), 15.5-16.5% CP and 2,700-2,750 kcal ME with restricted feeding for the growing phase (9 to 15 weeks), and 18% CP and 2,700 kcal ME ad libitum for the laying phase (18 to 70 weeks). Clean water was also freely available.

Reproductive parameters recorded included the age at first egg (AFE; in days), the total number of eggs (TNE; eggs) calculated as the number of eggs laid up to 38 weeks of age by the female ducks using individual trap nests, the mean weight of eggs (MEW; in grams) measured by weighing each egg on a digital scale accurate to ±0.01 g, and the total egg weight collected during weeks 37 and 38 (Ibrahim *et al.*, 2018; Nguyen *et al.*, 2023).

## Sample Collection and Genomic DNA Extraction Procedures

Blood samples were collected from the ducks at the age of 8 weeks. The management procedures followed best practices to ensure minimal discomfort for the animals, following the guidelines for the best practice of using animals in research based on EU directive 2010/63. A professional technician drew 1 mL of fresh blood from the wing vein of each duck, then placed in EDTA-treated tubes and stored at 4°C on ice. The samples were transported to the laboratory within 12 hours and stored in a refrigerator at 4°C until extraction, which occurred within one week (Huang *et al.*, 2017). Total DNA was extracted using a TopPURE® blood DNA extraction kit (ABT-Vietnam), following the supplier's guidelines. Total extracted DNA was quantified using two methods: 1% agarose gel electrophoresis and optical density (OD) measurement at wavelengths 260 nm and 280 nm with a NanoBioDrop (BioDrop, UK) spectrophotometer and then preserved at -80°C for further use.

## PCR Amplification, PCR-RFLP Assay and Electrophoresis

The PCR amplification of the intron 1 *PRL* gene region was performed using a Thermo Scientific™ DreamTaq Green PCR Master Mix (2X) (Thermo Scientific, USA), nuclease-free water (Thermo Scientific, USA), agarose (Bioline, UK), a 100-bp ladder (Thermo Scientific, USA), and 0.5X TAE buffer (Vietnam). The PCR reaction was carried out in a total volume of 12.5 µL of chemical components as 0.4 µL of each primer (10 pmol/µL), 3.45 µL of nuclease-free water (Thermo Scientific), 2 µL of DNA template (50 ng/µL) and 6.25 µL of Master Mix (a ready-to-use mixture of Taq DNA polymerase, PCR buffer, MgCl2, and dNTPs). The primer design tool on the NCBI website (https://www.ncbi.nlm.nih.gov/) was used to design a primer based on the sequence identified by accession number AB158611.1. The designed primers had sequences of 5'- ATCGAGGTAAACTCCACGAC -3' (forward primer) and 5'- TTCAGTGACACTGCTCAGTG-3' (reverse primer) with a 441 bp fragment length. The thermal cycling program of the PCR reaction included pre-denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, extension at 72°C for 30 seconds and a post-extension at 72°C for 7 minutes (Nguyen *et al.*, 2023). The negative control for PCR involved running a reaction without adding any template DNA. After electrophoresis, the PCR products were visualized using 1% agarose gel stained with GelRed. A 100 bp DNA ladder was run alongside the samples, and the gel was visualized using a GelDoc It2 system (UVP, USA).

Each digestion reaction had a total volume of 11 µL comprising 5 µL of nuclease-free water, 2 µL of PCR products, 3 µL of 10X CutSmart® Buffer, and 1 µL of *Xba*I restriction enzyme (25 units/µL). A negative control with all components except the *Xba*I enzyme was not included. The reaction mixture was incubated at 37°C overnight. The restriction enzyme was then deactivated by adding 4 µL of 2X Gel Loading Dye, Purple (New England Biolabs) to each reaction and incubating at room temperature for 15 minutes following the manufacturer's instructions. The results were verified by electrophoresis using a 2% agarose gel for 30 minutes. The expected outcomes after the digestion of the three genotypes were TT yield two fragments as 274/167 bp (both alleles are digested), TG yield three fragments of 441/274/167 bp (one allele is digested and other one is undigested), and a GG yield one fragment of 441 bp (none digested by enzyme).

## Data Analysis

The restriction enzymes were identified using the NebCutter program version 3.0 (https://nc3.neb.com/NEBcutter/). Allele and genotypic frequencies were calculated following the method described by Abdolhay *et al.* (2012). Observed heterozygosity (Ho) and expected heterozygosity (He) were determined using the formulas from Cui *et al.* (2023). A Chi-square ($\chi 2$) test was conducted to assess whether the population was in Hardy-Weinberg equilibrium, as described by Rosalinda et al. (2024). The polymorphic information content (PIC) was assessed according to the method described by Serrote *et al.* (2020). The effective number of alleles (Ne) is a crucial metric for genetic diversity. This value represents the number of effective alleles in a population, equivalent to the number of alleles that, if they had equal frequencies, would produce the same level of genetic diversity observed in the population (Ahmadi *et al.*, 2007). The Ne was calculated using the formula:

$$Ne = \frac{1}{\sum_i^k p_i^2}$$

Where; $p_i$ is the frequency of the $i^{th}$ allele and k is the total number of alleles in the population (Nei, 1978).


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A one-way analysis of variance (ANOVA) was conducted to evaluate the impact of prolactin gene polymorphism on reproductive traits, followed by Tukey's test for post-hoc comparisons. A significance level of $P<0.05$ was used for statistical tests conducted with Minitab software version 22.0.

## RESULTS AND DISCUSSION

### DNA Amplification and Genotyping of the Prolactin Gene

A 441-base pair (bp) fragment from intron 1 of the prolactin gene was successfully amplified across all the duck samples, with the representative electrophoresis image shown in Figure 2. This successful amplification across all the samples demonstrated the consistency and reliability of the procedure. At the negative control position, the absence of a band indicated no contamination during the PCR process.

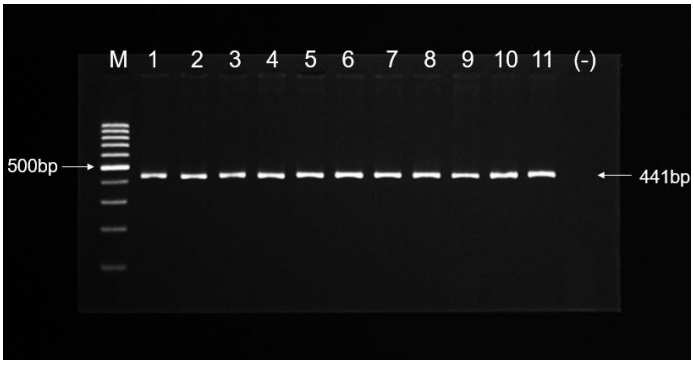


**Figure 2:** Representative electrophoresis results of PCR products using primer pair L441. M: 100 bp marker. Lanes 1-11: representative samples. (-) negative control.

The next step in the PCR-RFLP technique involved digesting all the PCR products with the restriction enzyme *Xba*I. The resulting electrophoresis image is presented in Figure 3.

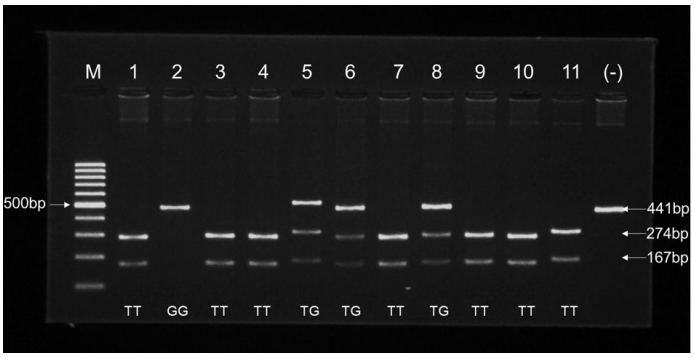


**Figure 3:** Representative electrophoresis PCR-RFLP pattern of the PRL gene digested with *Xba*I. M: 100 bp marker. Lanes 1,3,4,7,9,10,11: TT/*Xba*I genotype. Lanes 5,6,8: TG/*Xba*I genotype. Lane 2: GG/*Xba*I. (-) negative control.

As showed in the Figure 3, the G and T alleles and three genotypic (TT, GT, GG) were detected across all the TB and BT crossbred duck groups. The *Xba*I restriction enzyme was used to identify three genotypes within the studied duck populations: 441 bp for the GG genotype, 441/274/167 bp for the TG genotype, and 274/167 bp for the TT genotype. This research focused on the SNP 376 C>A (accession number AB158611) to identify genetic polymorphisms in the two studied duck populations following that suggested by several studies (Wang *et al*., 2011; Mazurowski *et al*., 2016; Chuekwon and Boonlum, 2017; Yurnalis et al., 2019).

In TB hybrid ducks, three genotypes TT, TG, and GG were observed with the T allele frequency reaching 0.914 and the G allele frequency 0.086 in females (Table 1). In males, only the TT and TG genotypes were detected, with T and G allele frequencies of 0.875 and 0.125, respectively. For the BT crossbred duck line, the T allele frequency in females was 0.876 and the G allele frequency was 0.124, while the T allele frequency was 0.84 and the G allele frequency was 0.16 in males. Females from the TB line exhibited higher T allele frequency (0.914) than females from the BT line (0.876). Similarly, males from the TB line had a higher T allele frequency (0.875) than those from the BT line (0.84). These data indicated that the T allele was dominant in TB and BT hybrid ducks, aligning with trends reported in various duck breeds in China (Wang *et al*., 2011). By contrast, Chuekwon and Boonlum (2017) reported that the frequency of TT, TG, and GG genotype was 0.07, 0.37, and 0.56, respectively, with a predominance of the G allele at a frequency of 0.74 on Khaki Campbell ducks that showed a markedly different pattern with current study. Whole, monomorphism at the SNP position 376 C>A (accession number AB158611) was observed in certain duck breeds including Muscovy (Mazurowski *et al*., 2016) and Perkin (Sabry et al., 2020). The controversial data among studies could be due to the different of breed sources examined. In current study, the dominant of T allele frequency in TB and BT hybrid ducks could be due to high selection pressure that applied to select the birds in advance for increasing of egg production leading to inbreeding aimed at increasing homozygosity with imbalance allele frequency, it is a necessary step in developing these hybrid lines (Qin *et al*., 2024).

The newly created duck population exhibited an effective number of alleles (Ne) of approximately 1.2 across both lines. Such a low Ne (Ne<5) signifies very limited genetic diversity, primarily resulting from extensive inbreeding (Debnath *et al*., 2023) carried out over multiple generations to develop specific parental lines for hybridization, leading to reduced allelic diversity (Trakovická *et al*., 2014; Zhang *et al*., 2016). The polymorphic information content (PIC) values in the BT and TB duck lines were less than 0.25,


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**Table 1:** Analysis of prolactin gene polymorphism at the *PRL/Xba*I locus.

| Line | Parameter | | Genotype frequency | | | Allelic frequencies | | He | Ne | PIC | χ2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TT | TG | GG | T | G | | | | |
| BT | M | N | 18 | 6 | 1 | 0.84 | 0.16 | 0.269 | 1.368 | 0.233 | 0.14 |
| | | Observed frequency | 0.72 | 0.24 | 0.04 | | | | | | |
| | | Expected frequency | 0.706 | 0.269 | 0.025 | | | | | | |
| | F | N | 68 | 20 | 1 | 0.876 | 0.124 | 0.217 | 1.278 | 0.194 | 0.05 |
| | | Observed frequency | 0.764 | 0.225 | 0.011 | | | | | | |
| | | Expected frequency | 0.767 | 0.217 | 0.016 | | | | | | |
| | Total | N | 86 | 26 | 2 | 0.868 | 0.132 | 0.229 | 1.297 | 0.203 | 0.0005 |
| | | Observed frequency | 0.754 | 0.228 | 0.018 | | | | | | |
| | | Expected frequency | 0.753 | 0.229 | 0.018 | | | | | | |
| TB | M | N | 18 | 6 | 0 | 0.875 | 0.125 | 0.218 | 1.28 | 0.195 | - |
| | | Observed frequency | 0.75 | 0.25 | 0 | | | | | | |
| | | Expected frequency | 0.766 | 0.218 | 0.016 | | | | | | |
| | F | N | 73 | 13 | 1 | 0.914 | 0.086 | 0.157 | 1.187 | 0.145 | 0.07 |
| | | Observed frequency | 0.839 | 0.149 | 0.012 | | | | | | |
| | | Expected frequency | 0.835 | 0.157 | 0.008 | | | | | | |
| | Total | N | 91 | 19 | 1 | 0.906 | 0.094 | 0.17 | 1.205 | 0.156 | 0.0001 |
| | | Observed frequency | 0.82 | 0.171 | 0.009 | | | | | | |
| | | Expected frequency | 0.821 | 0.17 | 0.009 | | | | | | |

**Note: M:** male; **F:** female; χ 2 tabulated (0.05, 1) = 3.841; **He:** Expected heterozygosity; **Ho:** Observed frequency.

indicating low polymorphism levels. A low PIC value reflects the selective breeding process, where individuals were predominantly chosen for high egg production, leading to diminished molecular diversity (Abdalhag *et al*., 2015; Chesnokov and Artemyeva, 2015; Nguyen *et al*., 2023). These factors underscore the risks associated with reduced genetic diversity and inbreeding within these newly established lines.

To test whether a population follows Hardy-Weinberg equilibrium (HWE), the chi-square test ($\chi^2$) is based on allele frequencies in the population. Since this population has 2 alleles, the degrees of freedom (df) will be 1 (Debnath *et al*., 2023). The computed $\chi^2$ value in Table 1 was less than the critical $\chi^2$ value at the 0.05 significance level for df = 1, suggesting that the genotype distribution adhered to Hardy-Weinberg equilibrium (Basumatary *et al*., 2019; Sari *et al*., 2022; Sanni *et al*., 2024). The adherence to HWE may be attributed to factors such as a sufficiently large population size and the process of creating the TB and BT hybrid lines being random mating. This result suggested that the BT and TB populations were in genetic equilibrium and less influenced by selection, mutation, migration, or genetic drift affecting allele frequencies, it could be due to randomly and rotational male used for creating the new line applied, although the imbalance of allele was found as above.

## Comparative Analysis of Reproductive Parameters and Association of PRL/XbaI Genotypes with Egg Production in Reciprocal Hybrid Duck Lines (BT and TB)

**Comparison of the reproductive parameters of duck lines:** The reproductive parameters of the two reciprocal hybrid duck lines (BT and TB) demonstrated statistically


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significant differences in key egg production (Table 2). The age at first egg (AFE) was earlier in the TB line (148.08 days) compared to the BT line (150.91 days) but no statistical significance was found (P > 0.05). Egg yield (EY) up to 38 weeks of age was significantly higher in the BT line than in the TB line (102.16 vs. 96.63 eggs; P < 0.01). Furthermore, the average egg weight (EW) at 38 weeks was substantially greater in the BT line, averaging 75.95 grams, with the TB line exhibiting a significantly lower average of 72 grams (P < 0.01). These findings highlighted the superior performance of the BT line in terms of egg yield and egg weight, with differences between the lines showing strong statistical significance.

**Table 2:** Reproductive parameters within the examined duck populations.

| Reproductive traits | BT N (Mean ± SE) | TB N (Mean ± SE) | P-value |
|---|---|---|---|
| AFE (days) | 88 (150.91$^{b}$±1.15) | 86 (148.08$^{a}$±1.33) | 0.1081$^{*}$ |
| EY (eggs) | 88 (102.16$^{a}$±1.00) | 86 (96.63$^{b}$±1.25) | 0.0007$^{***}$ |
| EW (grams) | 88 (75.95$^{a}$±0.38) | 86 (72,00$^{b}$±0.39) | 0.0001$^{***}$ |

Note: **AFE:** Age at first egg; **EY:** egg yields up to 38 weeks of age; **EW:** egg weight at week 38; **N:** number of individuals; **a, b:** Values in the same row without common superscripts differ significantly; ***:** P < 0.05; *****:** P < 0.01.

**Table 3:** Association of PRL/*Xba*I genotypes on reproductive parameters.

| Parameter | Duck line | Genotypes of PRL/XbaI locus | | P-value |
|---|---|---|---|---|
| | | TT N (Mean ± SE) | TG N (Mean ± SE) | |
| AFE (days) | BT | 68 (150.7$^{B}$±1.38) | 20 (151.6$^{B}$±2.03) | 0.7497 |
| | TB | 73 (147.9$^{A}$±1.51) | 13 (149.3$^{A}$±2.37) | 0.6997 |
| | P-value | 0.1758 | 0.4846 | |
| EY (eggs) | BT | 68 (101.9$^{A}$±1.21) | 20 (103.3$^{A}$±1,76) | 0.5877 |
| | TB | 73 (97.1$^{B}$±1.37) | 13 (94.75$^{B}$±2.94) | 0.3765 |
| | P-value | 0.0086$^{***}$ | 0.0069$^{***}$ | |
| EW (gam) | BT | 68 (76.4$^{a, A}$±0.43) | 20 (74.3$^{b, A}$±0.74) | 0.0183$^{*}$ |
| | TB | 73 (72.4$^{a, B}$±0.42) | 13 (69.9$^{b, B}$±0.88) | 0.0245$^{*}$ |
| | P-value | 0.0001$^{***}$ | 0.0007$^{***}$ | |

**Note: AFE:** Age at first egg; **EY:** egg yields up to 38 weeks of age; **EW:** egg weight at week 38; **N:** number of individuals; **a, b:** Values in the same row without common superscripts differ significantly; **A, B:** Values in the same column of each parameter without common superscripts differ significantly; ***:** P < 0.05; *****:** P < 0.01.

**Association of PRL/XbaI genotypes with egg production in reciprocal hybrid duck lines:** Statistical data from analyzing the association of *PRL/Xba*I genotypes with reproductive traits are presented in Table 3. There were no statistically significant differences (P>0.05) between TT/*Xba*I and TG/*Xba*I genotypes for age at first egg and egg yield in BT and TB lines (Table 3). In the BT line, the age at first egg was 150.7 days for TT/*Xba*I and 151.6 days for TG/*Xba*I, with an egg yield of 101.9 and 103.3 eggs, respectively. In the TB line, the age at first egg was 147.9 days for TT/*Xba*I and 149.3 days for TG/*Xba*I, with an egg yield of 97.1 and 94.75 eggs, respectively. Several published studies reported that the *PRL/Xba*I polymorphism in intron 1 of Khaki Campbell ducks showed a significant impact on egg production. For egg yield, ducks with the GT/*Xba*I genotype (53.32 eggs) exhibited significantly higher egg production compared to those with GG/*Xba*I (37.50 eggs) and TT/*Xba*I (36.67 eggs) genotypes (Chuekwon and Boonlum, 2017). The controversial results could be come from the different source of duck breeds, previously selection pressure of duck population examined, further study is required.

In the BT line, the average egg weight was 76.4 ± 0.43 grams for the TT genotype and 74.3 ± 0.74 grams for the TG genotype. In the TB line, the average egg weight was 72.4 ± 0.42 grams for the TT genotype and 69.9 ± 0.88 grams for the TG genotype. These differences were statistically significant (P<0.05), suggesting that the *PRL/Xba*I locus may influence egg weight in both duck lines. These polymorphisms in the *PRL* gene within intron 1 that affect duck egg weight were consistent with findings from various previous studies (Li *et al*., 2009; Bai *et al*., 2019). Investigation on the Gaoyou duck breed in China revealed that polymorphisms in intron 1 influenced egg weight at the *PRL/Dra*I locus, with the AB/*Dra*I genotype (74.51 g) exhibiting the lowest egg weight compared to the AA/*Dra*I (76.8 g) and BB/*Dra*I (76.72 g) genotypes (Li *et al*., 2009). Similarly, Bai *et al*. (2019) reported that polymorphisms in intron 1 of the *PRL* gene affected egg weight in two Chinese domestic duck lines, Jinding and Youxian, using single-strand conformation polymorphism (PCR-SSCP) analysis. In general, intron 1 does not directly code for proteins but several hypotheses have been proposed to explain its influence, ex: Li *et al*., (2009) indicated that some introns contain nucleotide sequences that can be translated into novel peptides, which may interact with peptides from the N-terminal exons. Furthermore, the A/C mutation in the non-coding region can also impact gene expression by affecting regulatory elements. Certain intronic SNPs may also activate hidden splice sites, leading to alternative splicing (Chang *et al*., 2012; Chuekwon and Boonlum, 2017). Therefore, while introns are not involved in protein synthesis, variations within intron 1 could potentially affect translation through mechanisms that warrant further investigation. Many studies have shown that gene sequences in intron regions influence the protein expression of these genes (Gallegos and Rose, 2019; Li *et al*., 2022; Lin *et al*., 2024). Intron sequences, once excised during the splicing process, can play a significant role in the formation of microRNAs (miRNAs) (MacFarlane and Murphy, 2010).

These miRNAs are small RNA molecules, derived from intronic regions of pre-mRNA. The miRNAs are involved in post-transcriptional regulation by binding to complementary sequences in target mRNAs, leading to their degradation or inhibition of translation (Dong *et al*., 2023). This regulatory mechanism allows miRNAs to modulate gene expression and influence protein function (Huang *et al*., 2024). We hypothesized that the influence of the intron 1 region on egg weight differences could be explained by the formation of miRNAs during transcription, where introns are excised. These intron-derived miRNAs are associated with the prolactin gene expression regulation in ducks, thereby affecting egg weight, however, further research is needed to elucidate this hypothesis.

In the same genotype, the BT duck line exhibited higher egg yield and egg weight values compared to the TB duck line (Table 3). Specifically, for the TT genotype, the egg yield in the BT line was 101.9 ± 1.21, whereas in the TB line, it was 97.1 ± 1.37 ($P < 0.01$). Similarly, for the TG genotype, the BT line demonstrated an egg yield of 103.3 ± 1.76, compared to 94.75 ± 2.94 in the TB line ($P < 0.01$). When comparing the egg weight of the two duck lines with the same TT/*Xba*I genotype (Figure 4), the BT duck line showed a significantly higher egg weight compared to the TB duck line (76.4 ± 0.43 grams *v.s* 72.4 ± 0.42 grams; $P<0.01$) and this difference was statistically significant at $P < 0.01$, indicating that the BT duck line produced notably heavier eggs than the TB duck line, resulting in an increase in the total egg yield in terms of quantity. Furthermore, TB line originated from TC male with high egg number in reproductive cycle, meanwhile, BT line originated from Bien breed with less egg number but high egg weight.

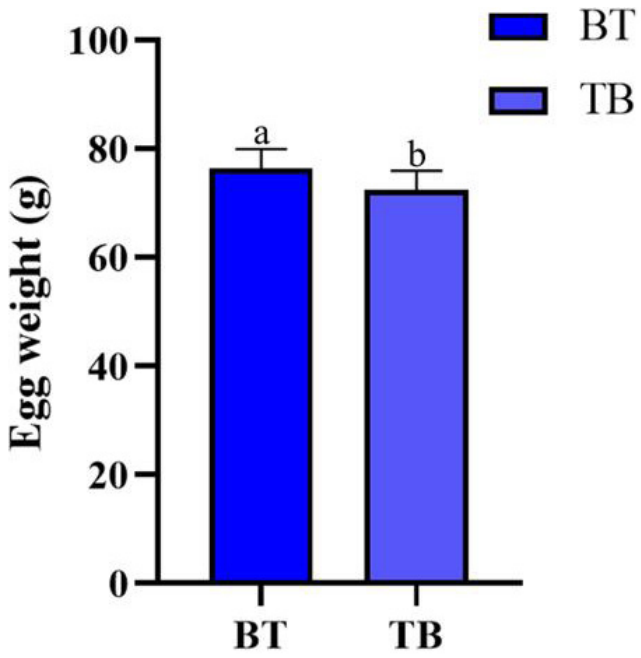


**Figure 4:** Chart comparing egg weight of individuals with TT/*Xba*I genotype in BT and TB lines. [a,b]: $P < 0.001$.

Egg weight in ducks is known to influence various aspects of egg quality, including the yolk-to-albumen ratio, shell weight, shell thickness, and hatchability of ducklings (Kokoszyński *et al*., 2007; Abd El-Hack *et al*., 2019; Jalaludeen and Churchil, 2022). Heavier duck eggs tend to have a higher yolk proportion compared to lighter eggs (Applegate *et al*., 1998; Onbasilar *et al*., 2011; Kuru *et al*., 2023). Consequently, eggs with larger yolks offer greater nutritional value, as yolks are primarily composed of proteins and lipids (Ahn *et al*., 1997; Liu *et al*., 2022). Pekin ducks in China showed an inverse relationship between egg weight and shell thickness (Ipek and Sozcu, 2017; Nasri *et al*., 2020), implying that as egg weight increased, the eggshell became thinner, making it harder for the ducklings to hatch (Galić *et al*., 2019). The mortality rate of early stage of embryonic development and hatchability rate are higher in heavier eggs compared to lighter ones (Onbaşılar *et al*., 2011; Kuru *et al*., 2023). Therefore, depending on the production goals, ducks with the TT/*Xba*I genotype can be selected from either the BT or TB line. For breeding purposes, the association of *PRL/Xba*I polymorphism with the yolk egg rate and hatchability rate require further study in both lines.

## CONCLUSIONS AND RECOMMENDATIONS

This study identified prolactin gene polymorphisms in intron 1 for the first time across two reciprocal crossbred duck lines, BT and TB, with three genotypes and two alleles (SNP 376 C>A). Results revealed the impact of the polymorphism at *PRL/Xba*I locus on egg weight, with the TT/*Xba*I genotype producing a heavier egg weight. Our findings suggested that this marker can be utilized in selective breeding to improve egg yield through egg weight component. Additionally, maintaining the T allele and TT genotype in the practical condition should be considered and further studies are required to explore these associations as clear comprehensively.

## ACKNOWLEDGMENTS

We are deeply grateful to all the VIGOVA staffs for collecting of duck blood samples for testing and diligent recording of duck phenotype data during this study.

## NOVELTY STATEMENT

This study is the first to investigate the polymorphism of the prolactin gene in intron 1 within reciprocal crosses (BT, TB), demonstrating the positive association of *PRL/Xba*I in intron 1 on egg production in the new created crossbred ducks with high adaptability to climate change conditions in Vietnam.

## AUTHOR'S CONTRIBUTIONS

Tan Loi Le, designed the primers, conducted the genotyping, analyzed the genotype data, and drafted the manuscript. Thi Tuong Vi Trang, analyzed the association of genotype with phenotype data. Tuan Thanh Hoang, handled the collection of phenotypic data. Ngoc Tan Nguyen and Pin-Chi Tang supervised the study, reviewed the manuscript, and ensured that the text was free from plagiarism. All authors have reviewed and approved the final version of the manuscript.

### Conflict of Interest

The authors confirm that they have no conflicts of interest.

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