



## Genetic Diversity in the STAT1 Gene of River Buffalo Populations in North Sumatra, Indonesia

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### ABSTRACT

Signal Transducer and Activator of Transcription 1 (STAT1) is a crucial transcription factor in interferon signaling pathways, playing a vital role in immune responses against viral, bacterial, and parasitic infections. This study aimed to identify polymorphisms in the STAT1 gene among river buffalo populations in Indonesia to assess genetic diversity and its potential for improving disease resistance and productivity. A total of 100 river buffaloes from four regions (Lubuk Pakam, Pancur Batu, Sunggal, and Tapanuli Utara) were analyzed. Genomic DNA was extracted from hair samples, and three SNPs (g.15856G>T, g.16211C>T, and g.16252C>G) were genotyped using PCR and Sanger sequencing. Genetic parameters, including allele frequency, heterozygosity, Hardy-Weinberg equilibrium (HWE), and Polymorphism Information Content (PIC), were calculated. Results revealed that SNP g.16211C>T had the highest PIC value (0.302), indicating its usefulness as a genetic marker, though it deviated from HWE, suggesting influences from selection or genetic drift. The UPGMA dendrogram clustered Lubuk Pakam and Pancur Batu together, while Sunggal and Tapanuli Utara formed a separate group, reflecting genetic relationships among populations. Further research is needed to explore the functional implications of these polymorphisms and their role in immune regulation.

**Keywords:** Genetics diversity, STAT1 gene, River buffalo, North Sumatra

### Article History

Article # 25-422  
Received: 23-Jul-25  
Revised: 27-Aug-25  
Accepted: 04-Nov-25  
Online First: 18-Nov-25

### INTRODUCTION

Signal Transducer and Activator of Transcription 1 (STAT1) is a crucial transcription factor in the interferon (IFN) signaling pathways, particularly mediating type I and type III IFN responses (Stanifer et al., 2020; Tolomeo et al., 2022). In livestock species, including the river buffalo (*Bubalus bubalis*), STAT1 has garnered significant attention due to its role in modulating immune defenses against viral, bacterial, and parasitic infections (Zhang et al., 2025). River buffalo, a key species for milk, meat, and draught power in tropical and subtropical regions, are often exposed to a range of infectious diseases that can severely impact their productivity and economic value (Di Stasio & Brugiapaglia, 2022; Saputra & Anggraeni, 2023).

River buffalo in Indonesia, based on microsatellite

analyses, exhibit low genetic diversity, appearing monomorphic (Saputra et al., 2020). Recent genomic studies employing single nucleotide polymorphism (SNP) markers have consistently revealed low genetic diversity within buffalo populations in Indonesia, Vietnam, and China (Pauciullo et al., 2025). This limited genetic variability poses challenges for breeding programs aimed at improving traits such as disease resistance, productivity, and adaptability. Identifying and characterizing key genes, such as STAT1, which play a critical role in immune responses, could provide valuable insights into the genetic potential of Indonesian river buffalo populations. By exploring polymorphisms in the STAT1 gene, this study aims to uncover genetic markers that could serve as a foundation for enhancing genetic diversity and improving the overall quality of this economically important species.

**Cite this Article as:** Hasan F, Khaerunnisa I and Saputra F, 2026. Genetic diversity in the STAT1 gene of river buffalo populations in North Sumatra, Indonesia. International Journal of Agriculture and Biosciences 15(1): 328-332. <https://doi.org/10.47278/journal.ijab/2025.190>



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Recent studies have highlighted the importance of STAT1 in the innate immune response, particularly its role in interferon signaling, which is vital for antiviral defense (Jung et al., 2020; Li et al., 2023; Metwally et al., 2024). In cattle, a closely related species, polymorphisms in STAT1 have been associated with resistance to diseases such as bovine tuberculosis and mastitis (Khan et al., 2020). These findings suggest that STAT1 could serve as a valuable genetic marker for selective breeding programs aimed at improving disease resilience in livestock. However, despite its potential, research on STAT1 in river buffalo remains limited, with few studies exploring its genetic variability, expression patterns, and functional implications in this species (Sharma et al., 2024). The primary objective of this study was to identify polymorphisms in the STAT1 gene among river buffaloes in Indonesia.

## MATERIALS & METHODS

### Samples

A total of 100 river buffaloes were included in this study, comprising 40 individuals from Lubuk Pakam, 22 from Pancur Batu, 16 from Sunggal, and 22 from Tapanuli Utara. Hair samples were collected from each animal, and genomic DNA was extracted using the Tissue/Blood DNA Mini Kit (Geneaid, Taiwan) following the manufacturer's protocol.

### PCR Amplification

A PCR premix was prepared with a total reaction volume of 15 µL, consisting of 0.5 µL DNA template, 0.5 µL of each primer (10nM), 0.5 reaction volume of GoTaq® Green Master Mix (Promega, USA), and nuclease-free water to adjust the final volume. Amplification was performed using a SimpliAmp Thermal Cycler (ThermoFisher, USA) under the following conditions: initial denaturation at 95°C for 5min, followed by 35 cycles of denaturation at 95°C for 10 seconds, annealing at 61°C for 30s, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The PCR products were then visualized on a 1% agarose gel stained with ethidium bromide and examined under UV light to confirm successful amplification. Genotyping was conducted using the Sanger sequencing method, performed by Macrogen (South Korea). The sequencing results were analyzed to identify specific polymorphisms within the STAT1 gene.

### Statistical Analysis

Observed and expected heterozygosity, Hardy-Weinberg equilibrium and Polymorphism Information Content (PIC), were calculated using the Cervus software (version 3.0.7) (Kalinowski et al., 2007). Genotype frequency, Allele frequency, and UPGMA dendrogram were analyzed using the POPGENE software (version 1.32) (Yeh & Boyle, 1997).

## RESULTS AND DISCUSSION

The STAT1 gene in buffalo is structurally conserved, located on chromosome 2, spanning 40,612 base pairs and comprising 25 exons. This organization mirrors that found

in other mammalian species such as cattle (*Bos taurus*) and humans (*Homo sapiens*), reflecting the gene's evolutionary conservation and critical role in immune function. STAT1 is a key component of the JAK-STAT signaling pathway, which is essential for interferon-mediated immune responses and host defense against pathogens (Das et al., 2024). The gene encodes a 748-amino acid protein in Murrah buffalo, a length comparable to that observed in other livestock species, reinforcing the functional conservation of STAT1 across mammals (Deng et al., 2015).

The identification of mutations in intronic regions of the STAT1 gene, specifically at positions 15,856 (Intron 9), 16,211 (Intron 10), and 16,252 (Intron 10). Although introns are non-coding sequences, they play critical roles in gene expression, including the regulation of mRNA splicing, stability, and transcriptional efficiency (Shi et al., 2020; Haddad-Mashadrizheh et al., 2024). Mutations in intronic regions can affect these processes, potentially altering the function or expression levels of the gene (Li et al., 2025).

The absence of the GG genotype at g.16252C>G in the Pancur Batu population and the CG genotype in the Lubuk Pakam, Sunggal, and Tapanuli Utara populations suggests limited genetic variability at this locus in these buffalo populations (Table 1). This could be attributed to genetic drift, selection pressures, or the founder effect, which are common in isolated or small populations (Judson et al., 2024; Marfurt et al., 2024). The dominance of the G allele at g.15856G>T and the C allele at g.16211C>T and g.16252C>G across all populations indicates a potential selective advantage or neutral fixation of these alleles. Such patterns of allele distribution are often observed in populations under similar environmental or disease pressures, where certain alleles may confer a survival or reproductive advantage (Lockwood et al., 2024).

The observed genetic patterns highlight the importance of understanding population-specific genetic diversity for effective breeding and conservation strategies. The absence of certain genotypes in specific populations underscores the need to maintain genetic diversity to prevent inbreeding and preserve adaptive potential (White et al., 2025). Additionally, these findings provide a foundation for future research on the association between STAT1 polymorphisms and disease resistance in buffalo populations, which could inform selective breeding programs aimed at improving livestock health and productivity (Zhu et al., 2025).

The identification of multiple alleles at SNPs g.15856G>T, g.16211C>T, and g.16252C>G highlights the genetic diversity present in the STAT1 gene among the studied buffalo populations (Table 2). SNPs are the most common form of genetic variation and are widely used as molecular markers in genetic studies due to their abundance and stability across generations (Tian et al., 2024). The presence of alleles A and T at g.15856G>T, C and T at g.16211C>T, and C and G at g.16252C>G suggests that these loci are polymorphic, which is a key characteristic of SNPs. The presence of these SNPs in the buffalo population provides valuable insights into the genetic architecture of STAT1 and its potential role in disease resistance and immune regulation.

**Table 1:** Genotype frequency of three SNPs in STAT1 gene

Location	g.15856G>T		g.16211C>T			g.16252C>G		
	GG	TT	CC	CT	TT	CC	CG	GG
Lubuk Pakam (n=40)	0.92	0.08	0.70	0.12	0.18	0.97	0	0.03
Pancur Batu (n=22)	0.95	0.05	0.68	0.23	0.09	0.85	0.15	0
Sunggal (n=16)	0.87	0.13	0.56	0.25	0.19	0.87	0	0.13
Tapanuli Utara (n=22)	0.86	0.14	0.45	0.41	0.14	0.95	0	0.05
Overall (n=100)	0.91	0.09	0.62	0.23	0.15	0.93	0.03	0.04

**Table 2:** Allele frequency of three SNPs in STAT1 gene

Location	g.15856G>T		g.16211C>T		g.16252C>G	
	G	T	C	T	C	G
Lubuk Pakam (n=40)	0.93	0.07	0.76	0.24	0.93	0.02
Pancur Batu (n=22)	0.95	0.05	0.80	0.20	0.80	0.20
Sunggal (n=16)	0.88	0.12	0.69	0.31	0.88	0.12
Tapanuli Utara (n=22)	0.86	0.14	0.66	0.34	0.95	0.05
Overall	0.91	0.09	0.74	0.26	0.95	0.05

**Table 3:** Heterozygosity, hardy-weinberg, and PIC

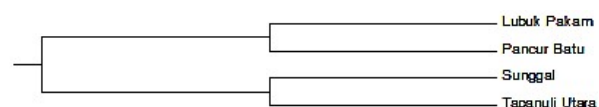
SNP	Observed Heterozygosity	Expected Heterozygosity	Hardy-Weinberg	Polymorphism Information Content
g.15856G>T	0.0000	0.1646	nd	0.150
g.16211C>T	0.2300	0.3915	***	0.302
g.16252C>G	0.0300	0.3915	nd	0.099

Understanding the distribution and frequency of these alleles can aid in the development of marker-assisted selection (MAS) strategies to improve traits such as disease resistance and productivity in buffalo populations (Mou et al., 2024). Furthermore, these findings contribute to the broader understanding of genetic diversity in livestock species, which is essential for conservation and breeding programs aimed at maintaining healthy and resilient populations (Kichamu et al., 2025).

The higher PIC value of SNP g.16211C>T (0.302) compared to SNPs g.15856G>T and g.16252C>G suggests that this locus has a greater degree of informativeness for genetic diversity studies (Table 3). PIC values are a measure of the usefulness of a marker for detecting polymorphism, with values above 0.25 generally considered moderately informative (Botstein et al., 1980). This makes g.16211C>T a valuable marker for evaluating genetic variation and population structure in river buffalo populations. However, the deviation from Hardy-Weinberg equilibrium (HWE) at g.16211C>T raises important considerations for its use in association studies. HWE is a fundamental principle in population genetics, and deviations from equilibrium can arise due to factors such as selection, genetic drift, migration, or non-random mating (Neamatzadeh et al., 2024). Markers not in HWE may introduce bias in association analyses, as they do not reflect the expected distribution of genotypes under random mating conditions (Graffelman et al., 2013). Therefore, while g.16211C>T is informative for genetic diversity, its deviation from HWE limits its suitability for phenotype association studies unless the underlying causes of the deviation are understood and accounted for. For future studies, it is recommended to identify additional markers in HWE that can complement g.16211C>T in association analyses.

The UPGMA dendrogram revealed two distinct clusters: the first cluster comprised individuals from Lubuk Pakam and Pancur Batu, while the second cluster included individuals from Sunggal and Tapanuli Utara (Fig. 1). This clustering pattern indicates that the genetic distance between Lubuk Pakam and Pancur Batu is relatively small,

suggesting a closer genetic relationship. Similarly, Sunggal and Tapanuli Utara exhibit genetic closeness, forming a separate cluster.

**Fig. 1:** UPGMA based on STAT1 with Three SNPs.

## Conclusion

This study identified and characterized polymorphisms in the STAT1 gene among river buffalo populations in Indonesia, revealing significant insights into their genetic diversity and population structure. The analysis of three SNPs (g.15856G>T, g.16211C>T, and g.16252C>G) demonstrated varying levels of polymorphism, with SNP g.16211C>T exhibiting the highest Polymorphism Information Content (PIC) value (0.302), making it a valuable marker for assessing genetic diversity. However, its deviation from Hardy-Weinberg equilibrium (HWE) suggests potential influences from selection, genetic drift, or non-random mating, which may limit its utility in association studies without further investigation.

## DECLARATIONS

**Funding:** This research was funded by the Universitas Sumatera Utara with contract number: 433/UN5.2.3.1/PPM/SPP-TALENTA USU/2021.

**Acknowledgement:** The authors would like to thank Universitas Sumatera Utara for their support.

**Conflict of Interest:** None

**Data Availability:** The raw sequencing data and processed files are available from the corresponding author upon reasonable request.

**Ethics Statement:** This study did not require ethical approval, as no invasive procedures or experiments involving live animals were conducted. Hair samples from river buffalo were collected non-invasively, without causing harm, distress, or discomfort to the animals. Therefore, ethical clearance from an animal care and use committee was not necessary.

**Author's Contribution:** FH: Acquired funds for the study, collected samples, designed research methodology, reviewed manuscript. IK: performed the experiments, analyzed the data, wrote the first draft, project administration. FS: performed the experiments, analyzed the data, wrote the first draft, and reviewed the manuscript. All authors have read and approved the final manuscript

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