



Unveiling the role of long non-coding RNAs in chicken immune response to highly pathogenic avian influenza H5N1 infection

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ABSTRACT

Avian influenza viruses (AIVs) pose a significant threat to global poultry production, necessitating effective control strategies to mitigate economic losses and ensure animal welfare. Long non-coding RNAs (lncRNAs) have emerged as crucial regulators of immune responses, yet their roles in AIV-infected chickens remain poorly understood. This study aimed to investigate the expression profiles of lncRNAs and their targets in Vietnamese Ri chickens infected with the highly pathogenic AIV (HPAIV) H5N1. Through RNA sequencing, we identified novel lncRNAs and analyzed differentially expressed (DE) transcripts at 1 and 3 days post-infection (dpi) in chicken lung tissue. Our results revealed a higher number of DE lncRNAs and mRNAs at 1 dpi and 3 dpi, respectively, compared to control, with resistant chickens exhibiting a notably stronger immune response than susceptible chickens at 3 dpi. Functional analysis implicated these lncRNAs in immune-related pathways crucial for host responses to H5N1 viral infection. Furthermore, we identified lncRNA-mRNA interactions associated with antiviral responses and immune function. Notably, several genes involved in antiviral resistance and immune responses showed higher expression in resistant chickens, confirming their stronger antiviral response. Overall, our study provides insights into the role of lncRNAs in the host's response to HPAIV H5N1 infection in chickens and highlights potential candidates for further investigation into host-pathogen interactions. These findings could drive the development of novel control strategies for AIVs, significantly enhancing poultry health and biosecurity.

Introduction

The family *Orthomyxoviridae* comprises four genera of influenza viruses: influenza A, B, and C, as well as *Thogotovirus* (Lamb, 2001). Influenza viruses typically possess a single-stranded, segmented, and negative-sense RNA structure (Swayne and Suarez, 2000). Among these, influenza A viruses are the only type that naturally infect birds (Capua and Marangon, 2006). Avian influenza viruses (AIVs) have crossed species barriers, moving from avian hosts to mammals, including humans (Kopp and Mendell, 2018; Smith, et al., 2009). Recent avian influenza outbreaks have caused substantial losses in various animal populations, including poultry, wild birds, and certain mammals (Wille and Holmes, 2020). According to the World Organisation for Animal Health (WOAH), surveillance data since 2022 across ten countries on

three continents highlight a notable increase in infection rates among mammals (World Health Organization, 2023). Therefore, developing effective methods to control AI viruses, which can cause significant economic losses and adversely affect animal welfare, is imperative.

Non-coding RNAs (ncRNAs), including miRNAs and long ncRNAs (lncRNAs), typically do not encode proteins and play crucial roles in regulating various biological processes (Zhang, et al., 2019). lncRNAs, defined as RNAs longer than 200 nucleotides, have attracted increasing attention due to their potential immunomodulatory roles (Kopp and Mendell, 2018). In our previous studies, we analyzed the potential functions of DE mRNAs, miRNAs, and exosomal miRNAs, focusing on their roles in antiviral response and immune functions (Hong, et al., 2021b; Vu, et al., 2023a; Vu, et al., 2023b; Vu, et al., 2022a). While several studies have investigated lncRNA expressions in response to

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H5N1 AIV infection in mice, ducks, and chicken cells (Hu, et al., 2018; Lu, et al., 2019; Wang, et al., 2022), none have examined lncRNA expressions in chicken samples. Therefore, further studies are required to better understand the potential roles of lncRNAs following H5N1 AIV infection, particularly in chickens.

lncRNAs can regulate gene expression through various mechanisms, including transcriptional and post-transcriptional regulation, protein activity modulation, and nuclear organization and scaffold function (Statello, et al., 2021). Notably, they regulate biological processes in recipient cells by either promoting or inhibiting target gene expression (Mattick, et al., 2023; Statello, et al., 2021).

In this study, we aimed to identify novel lncRNAs associated with host responses to AIV H5N1 infection and examine DE transcripts, including lncRNAs, in chicken lung tissue. Specifically, we analyzed DE lncRNAs and their targets in both highly pathogenic AIV (HPAIV)-resistant and -susceptible indigenous Vietnamese Ri chickens. Ri chickens of resistant (Mx/A; BF2/B21) and susceptible (Mx/G; BF2/B13) lines were selected by genotyping the Mx dynamin-like GTPase (Mx) and major histocompatibility complex class I antigen BF2 genes. Subsequently, we identified potential lncRNA candidates and their targets related to antiviral responses and immune function.

Materials and methods

Experimental design and sample preparation

The HPAIV-H5N1-resistant and -susceptible Ri chickens utilized in this study were obtained from the National Institute of Animal Science (NIAS), Hanoi, Vietnam (Vu, et al., 2022a). For the H5N1 infection experiment, five chickens per group were intranasally administered allantoic fluid, containing 1×10^4 of a 50% egg infectious dose (EID₅₀) of the A/duck/Vietnam/QB1207/2012 (H5N1) strain, harvested from infected eggs (Table S1). All chicken infection experiments were conducted in a biosafety level 2 plus (BSL2+) facility at our collaborative laboratory in the Department of Biochemistry and Immunology, National Institute of Veterinary Research, Vietnam (Hong, et al., 2022). In accordance with institutional ethical guidelines, chickens exhibiting severe clinical signs of infection were humanely euthanized using CO₂ asphyxiation followed by cervical dislocation, ensuring rapid loss of consciousness and minimizing animal distress. Lung tissue samples were collected 1 and 3 days post-infection (dpi) (Table S1), immediately flash-frozen in liquid nitrogen, and stored at -80°C until further processing. All experimental procedures involving animals were approved by the National Institute of Veterinary Research (NIVR), Vietnam (TCVN 8402:2010/TCVN 8400-26:2014).

RNA sequencing and transcriptomic analysis

Total RNA was extracted from the lung tissue samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol. The total RNA samples with a 28S:18S ratio greater than 1 and an RNA Integrity Number (RIN) over 6 (Vu, et al., 2022a) were used for cDNA preparation (Table S2). cDNA libraries were independently prepared using the MGIEasy RNA Directional Library Prep Set (Illumina, Inc., San Diego, CA, USA). The resulting libraries were subjected to paired-end sequencing (with a read length of 150 base pairs (bp)) on the Illumina MGISEQ platform.

The quality of the raw reads was assessed using FastQC software version v0.11.9 (<http://www.bioinformatics.babraham.ac.uk/project/s/fastqc/>). Subsequently, adaptors, low-quality reads, and poly-N sequences were removed using Trimmomatic version v0.39 (<http://www.usadellab.org/cms/?page=trimmomatic>). The clean reads were then mapped to the chicken reference genome (Gallus gallus. GRCg7b) using Hisat version v2.2.1 (<https://daehwankimlab.github.io/hisat2/manu>), and the mapped reads were sorted using Samtools version v1.19 (<https://www.htslib.org/download/>). Transcripts were assembled from

the mapped reads using StringTie version v2.2.1 (<https://github.com/gpertea/stringtie>), and the resulting GTF files containing transcripts for each sample were merged and compared using GffCompare version v0.12.7 (<https://github.com/gpertea/gffcompare>). Fig. 1 presents a schematic of the pipeline for identifying lncRNAs, including mapping and assembly processes.

Identification of lncRNAs

The merged transcript data was screened to identify lncRNAs. Initially, transcripts shorter than 200 nucleotides (nt) in length and those with an open reading frame (ORF) length less than 300 amino acids (aa) were filtered out. Subsequently, assembled transcripts were identified as potential novel lncRNAs based on the following criteria: falling entirely within a reference intron (i), having multiple exons with at least one junction match (j), overlapping with reference exons on the same strand (o), representing unknown intergenic transcripts (u), and showing exonic overlap with reference exons on the opposite strand (x). The coding potential of these putative lncRNAs was then predicted using four tools: predictor of lncRNAs and mRNAs based on k-mer scheme (PLEK) (cutoff value < 0), coding potential calculator (CPC) 2 (cutoff value < 0), coding-potential assessment tool (CPAT) (cutoff value < 0.532), and coding non-coding index (CNCI) (cutoff value < 0). Pfam-scan was employed for further validation. Subsequently, predicted lncRNAs overlapping with only one sample were removed. Fig. 1 depicts a schematic of the pipeline for identifying lncRNAs, including lncRNA filtering and counting processes.

DE lncRNAs and mRNAs

edgeR version 4.0.16 was utilized to compute the trimmed mean of M-value (TMM) for the lncRNAs and mRNAs in each sample. DE lncRNAs and mRNAs in comparison groups were defined based on a log₂ fold-change (FC) of ≥ 1 and a false discovery rate (FDR) of < 0.05. The secondary structures of lncRNAs were generated using RNAfold with minimum free energy (MFE) (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>) (Fig. 5).

lncRNA-mRNA interactions

Co-expression networks of lncRNA-mRNA interactions were constructed using the WGCNA package (version v1.72) in R. The network was established based on TMM values, and each module was visualized. Interactions among transcripts within each module of the background networks were calculated using mutual information based on TMM values through ARACNE, implemented via the 'bnlearn' package (version 4.9.3) in R. The connections between lncRNAs and mRNAs were visualized using Cytoscape (version 3.10.1). Gene ontology (GO) category and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses were performed using the database for annotation, visualization, and integrated discovery (DAVID).

Cis and trans-acting lncRNAs and target genes analysis

Protein-coding genes located within 100 kb upstream and downstream of the identified lncRNAs on the same chromosome were considered cis-acting target genes. Genes outside this range or on different chromosomes were reclassified as trans-acting target genes (Engreitz, et al., 2016; Quinn and Chang, 2016). Fig. 1 presents a schematic of the pipeline for analyzing predicted lncRNAs, including cis and trans-acting lncRNAs and their target genes.

Reverse transcription-quantitative PCR (RT-qPCR)-based validation of DE lncRNAs and mRNAs

For cDNA synthesis, 2 µg of total RNA was treated with 1.0 U of

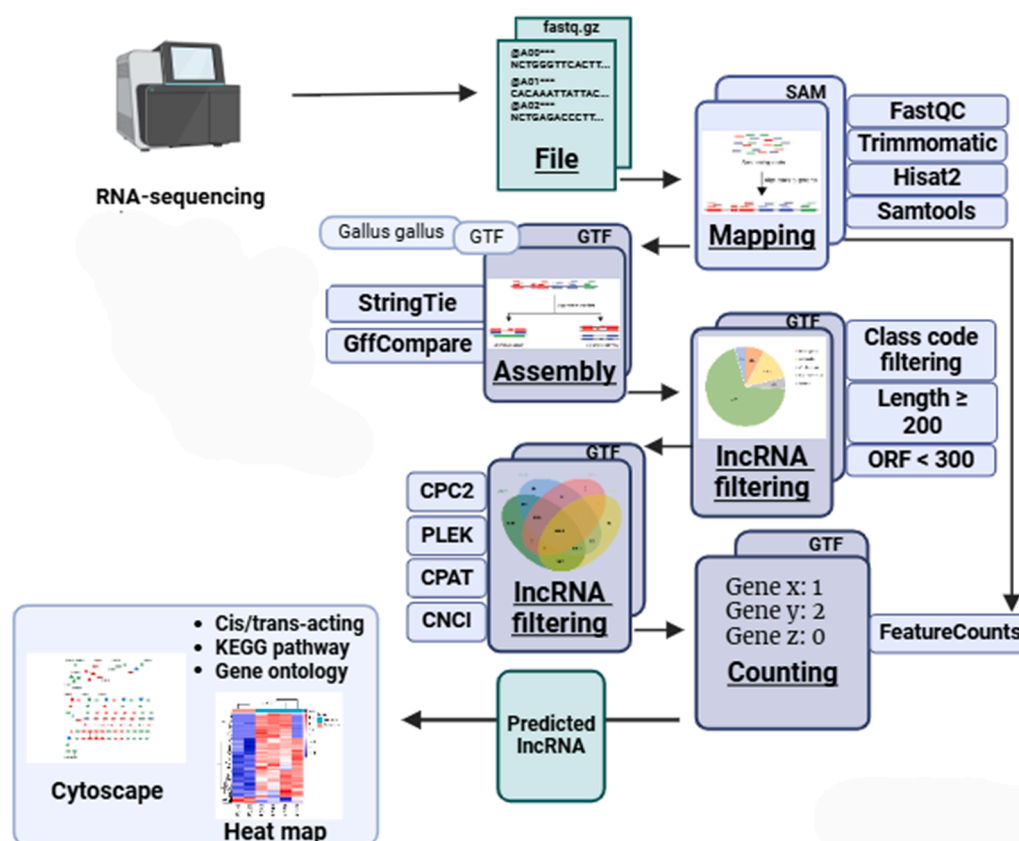


Fig. 1. Schematic of the pipeline for identifying lncRNAs in response to H5N1 infection in chickens.

DNase I (RNase-free). Subsequently, cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Invitrogen). RT-qPCR analysis was conducted using Dyne qPCR 2x PreMIX (SYBR Green with low ROX; Dyne Bio INC, Korea) in a CFX Connect Real-time PCR system (Bio-rad, Hercules, CA, USA), following the manufacturer's instructions. Primers were designed using NCBI Primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (Table S3). Briefly, a 20 μ L reaction mixture, containing 10 μ L of 2 $\times$ SYBR Green Master Mix, 1 μ L each of 10 pmol forward and reverse primers, 1 μ L of template cDNA, and 7.0 μ L of nuclease-free water, was prepared. The amplification protocol consisted of an initial denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50–65°C for 30 s, and extension at 72°C for 30 s. The lncRNA and mRNA expression levels were normalized to GAPDH. All experiments were independently performed in triplicate, and the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) was used to calculate relative expression levels.

Statistical analysis

The RT-qPCR was conducted in triplicate, and the results are presented as mean $\pm$ standard error of the mean (SEM; $N = 3$). Statistical analyses were performed using IBM SPSS statistical software (SPSS 26.0 for Windows; IBM, Chicago, IL, USA). Differences between groups were assessed using the Student's *t*-test, with $p < 0.05$ indicating statistical significance.

Results

RNA sequencing analysis

Twenty-nine of the forty lung tissue samples collected from resistant and susceptible Ri chickens post-H5N1 infection passed quality control

checks and underwent high-throughput RNA sequencing. Each of the twenty-nine libraries generated approximately 7 GB of cDNA sequences per chicken, with 90% of bases having a quality score above Q30. Additionally, over 88% of the filtered reads from each library were successfully mapped to the reference genome (Table S2).

Characteristics of putative lncRNAs

Several criteria and filtering steps were applied to identify novel lncRNA candidates. Initially, 517,633 assembled transcripts were generated using StringTie. These transcripts were then filtered based on transcript length, resulting in 516,773 candidate lncRNAs with class codes i, j, o, u, and x. Further filtering based on ORF length yielded 332,728 novel candidate lncRNAs. Subsequently, we refined the list using CPC, PLEK, CPAT, CNCI, and Pfam to obtain 62,335 identified lncRNA transcripts (Fig. 2A). Finally, focusing on transcripts present in at least two samples, we identified 12,398 novel lncRNA transcripts. In the overall distribution of 24,344 lncRNAs (comprising 12,399 novel and 11,945 known lncRNAs), the majority of lncRNA loci were bidirectional (70%), while 14% were intronic, 8% intergenic, 5% antisense, and 4% sense (Fig. 2B). Analysis of the length and exon number distributions of both lncRNAs and mRNAs revealed distinct patterns for known and novel lncRNAs compared to mRNAs. Among known lncRNAs, 39% exhibited 4 exons, while novel lncRNAs predominantly had 2 exons, accounting for 60%. In contrast, 48% of the mRNAs had over 10 exons (Fig. 2C). Additionally, the length distribution showed that the majority of known lncRNAs, novel lncRNAs, and mRNAs exceeded 1,000 bp in length, accounting for 99.4%, 69.3%, and 98.9%, respectively (Fig. 2D).

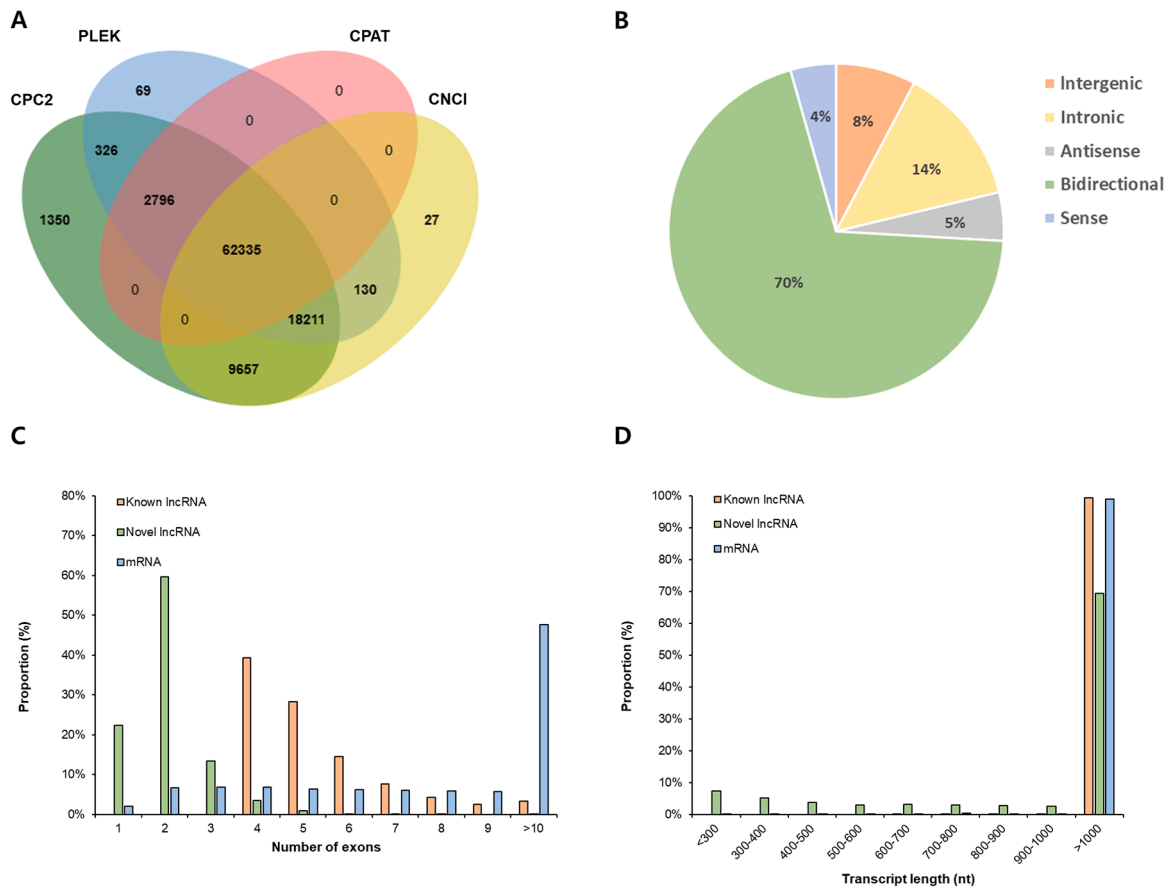


Fig. 2. Characterization of lncRNAs. A) Venn diagram illustrating overlapping lncRNAs predicted using the following tools: predictor of lncRNAs and mRNAs based on k-mer scheme (PLEK), coding potential calculator (CPC) 2, coding-potential assessment tool (CPAT), and coding non-coding index (CNCI). B) Pie chart depicting the categorical distribution of lncRNAs. C) Distribution of exon numbers per transcript. D) Distribution of transcript lengths.

Identification of DE lncRNAs and functional analysis of target genes

DE analysis of lncRNAs and mRNAs was conducted at 1 dpi and 3 dpi compared to control, as well as between the two chicken lines. The number of DE transcripts (both lncRNAs and mRNAs) was higher at 3 dpi following H5N1 infection in both resistant and susceptible chickens, whereas the number of DE transcripts in these chickens was lower at 1 dpi post-H5N1 infection (refer to Fig. S1, Table S4, and Table S5).

Additionally, seven modules (yellow, red, blue, green, brown, turquoise, and grey) were identified based on the lncRNAs and mRNAs in control and infected chickens at 3 dpi (Fig. S2). Interactions between lncRNAs and mRNAs in resistant and susceptible chickens at 3 dpi with H5N1 in each module are shown in Fig. S3. The analysis primarily focused on the different responses of resistant and susceptible chickens at 3 dpi, revealing a total of 99 DE lncRNAs, comprising 92 up-regulated and 7 down-regulated lncRNAs (refer to Table S4). The volcano plot and heatmap illustrating the DE lncRNAs are presented in Fig. 3A–B. GO and KEGG pathway analyses of the lncRNA target genes are shown in Fig. 3C–D. The predicted target genes are involved in various immune-related and signal transduction pathways, such as transforming growth factor beta (TGF- β), mitogen-activated protein kinase (MAPK), retinoic acid-inducible gene I (RIG-I), influenza A, herpes simplex virus 1 infection, and cytokine-cytokine receptor signaling pathways (Fig. 3D).

Cis and trans-acting lncRNAs and immune-related target genes

At 3 dpi, 70 lncRNA–mRNA interactions related to H5N1 infection were confirmed between the two chicken lines, as shown in Fig. 4 and

Table S6. These interactions indicated both cis and trans regulation types and were categorized into network types (yellow, red, blue, brown, turquoise, or green). The results showed 18 cis-acting lncRNAs and their targets, 14 trans-acting lncRNAs and their targets, and 6 lncRNAs exhibiting both cis and trans regulation (Fig. 4). Two of these novel lncRNAs and their target genes were considered potential candidates for further H5N1 infection research. The lncRNAs TCONS_00228212 (1,031 bp) and TCONS_00308667 (1,538 bp) target DEXH-box helicase 58 (*DHX58*, also known as *LGP2*) and eukaryotic translation initiation factor 2 alpha kinase 2 (*EIF2AK2*, also known as *PRK*), respectively, both of which are related to antiviral immune responses. The secondary structures of these lncRNAs, generated based on base-pair probabilities, are shown in Fig. 5. The results showed that TCONS_00228212 and TCONS_00308667 have MFE values of -498.70 kcal/mol and -342.90 kcal/mol, respectively. Notably, these novel lncRNA target genes are associated with antiviral responses, suggesting their potential role in H5N1 infection.

RT-qPCR analysis of lncRNA and their target genes

RT-qPCR analysis of the lncRNAs and their target genes confirmed the differential expression patterns observed in the RNA sequencing results. The results showed that the up-regulated lncRNAs (TCONS_00228212, TCONS_00308667, TCONS_00309948, TCONS_00383093, TCONS_00487609, TCONS_00487694, and TCONS_00059268) and their immune-related target genes (*DHX58*, *EIF2AK2*, tumor necrosis factor ligand superfamily member 13B (*TNFSF13B*), mitogen-activated protein kinase kinase kinase 6 (*MAP3K6*), mothers against decapentaplegic homolog 2-like

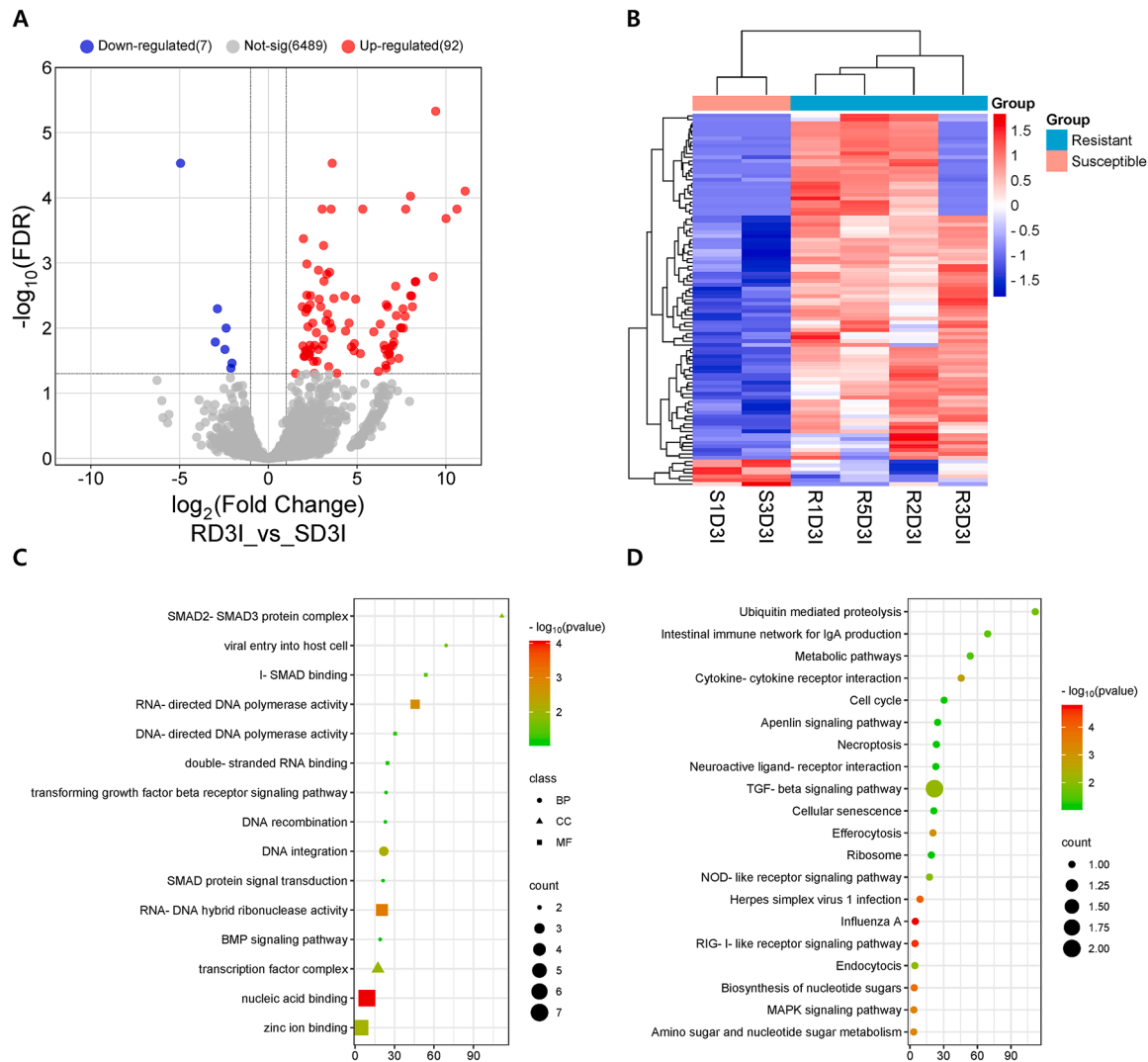


Fig. 3. Analysis of lncRNAs and target genes in resistant and susceptible chickens at 3 days post-infection (dpi) with H5N1. A) Volcano plot of lncRNAs. B) Heat map of differentially expressed (DE) lncRNAs. Up-regulated and down-regulated DE lncRNAs are marked in red and blue, respectively. C) Gene ontology (GO) term analysis of lncRNA target genes. D) Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of lncRNA target genes.

(*SMAD2W*), chromodomain helicase DNA binding protein 1W (*CHD1W*), and TGF-beta signal pathway antagonist Smad7 (*SMAD7B*) exhibited significantly higher expression in resistant chickens compared to susceptible chickens (Fig. 6A–B). Conversely, the down-regulated lncRNAs (TCONS_00059268 and TCONS_00423704) and their target genes (sushi domain containing (*SUSD1*), transmembrane protein 81 (*TMEM81*), and solute carrier family 25 member 42 (*SLC25A42*)) exhibited significantly lower expression in resistant chickens compared to susceptible chickens (Fig. 6A–B and Table S6). These RT-qPCR data were consistent with RNA sequencing results, validating the differential expression observed and supporting the role of these lncRNAs and their target genes in the immune response to H5N1 infection.

Discussion

lncRNAs play pivotal roles as gene expression regulators across various biological processes, including immune responses to viral infections (Mattick, et al., 2023; Statello, et al., 2021). While several studies have investigated lncRNAs in the context of H5N1 AIV infection, a notable gap remains in understanding their role in chicken samples (Hu, et al., 2018; Lu, et al., 2019; Wang, et al., 2022). Therefore, our study aimed to address this gap by comprehensively analyzing lncRNAs

and their regulatory targets in Ri chickens infected with HPAIV H5N1.

In this study, we employed RNA sequencing to analyze lncRNAs and predict their targets in HPAIV-infected Ri chickens. HPAIV-resistant and -susceptible Ri chicken lines were selected based on their *Mx* and *BF2* genotypes and subsequently infected with HPAIV H5N1 (Vu, et al., 2022a; Vu, et al., 2022b). Preliminary experiments and previous findings have indicated significant immune responses in both resistant and susceptible chickens at 3 dpi with H5N1 (Hong, et al., 2021b; Lee, et al., 2022; Vu, et al., 2023a; Vu, et al., 2022a; Vu, et al., 2022b). The results of the present study also showed similar patterns, with DE transcripts (both lncRNAs and mRNAs) being higher at 3 dpi compared to 1 dpi following H5N1 infection in both chicken lines (Fig. S1, Table S4, and Table S5). A total of 12,398 novel lncRNA transcripts were identified in lung tissues following H5N1 infection in the two chicken lines. The majority (70%) of these lncRNA loci are bidirectional and exceed 1,000 bp in length (Fig. 2B and D). These characteristics align with observations from previously reported lncRNAs within the genome (Goyal, et al., 2017; Kern, et al., 2018; Srinivas, et al., 2023). Additionally, the distribution of transcript lengths and exon numbers differed substantially between lncRNAs and mRNAs, highlighting distinct features of lncRNA biology. However, the 150 bp read length limitation of our sequencing platform likely posed challenges in identifying longer

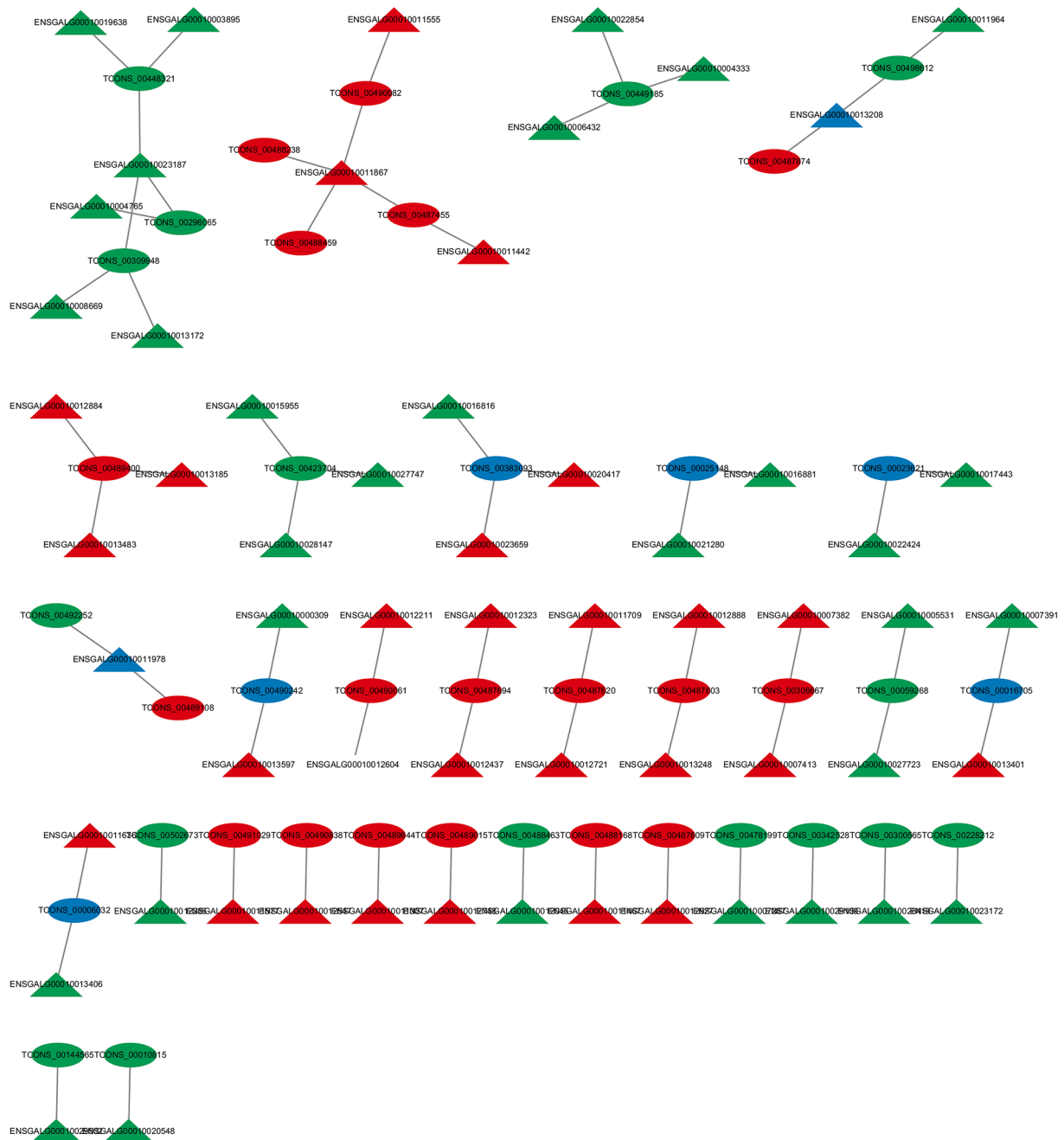


Fig. 4. LncRNA–mRNA interactions in resistant and susceptible Ri chickens at 3 dpi with H5N1. Triangles and ellipses indicate mRNAs and lncRNAs, respectively. Red indicates cis-acting, green indicates trans-acting, and blue indicates both cis and trans-acting lncRNAs or target genes.

lncRNAs. Future studies will aim to utilize longer-read sequencing platforms.

Additionally, we acknowledge the ongoing discussion regarding the retention of lncRNAs with ORFs shorter than 300 amino acids (aa). In our study, we applied stringent criteria to identify potential novel lncRNAs, filtering out transcripts shorter than 200 nucleotides and those with ORFs of less than 300 aa to focus on non-coding transcripts, as shorter ORFs are less likely to code for functional proteins. Nonetheless, further validation of these identified lncRNAs is crucial to confirm their non-coding potential and to elucidate their functions in AIV infection. To enhance accuracy, we employed multiple coding potential prediction tools, including PLEK, CPC2, CPAT, and CNCI, alongside Pfam-scan, and removed predicted lncRNAs that overlapped with only one sample to ensure robustness. Future studies involving knockdown or

overexpression experiments, as well as functional assays, will be necessary to clarify the biological roles of these lncRNAs.

DE analysis revealed a higher number of DE lncRNAs and mRNAs at 3 dpi compared to 1 dpi in both resistant and susceptible chickens. Additionally, the number of DE transcripts was higher in resistant chickens compared to susceptible chickens at both time points post-infection (Fig. S1). Moreover, the expression levels of cytokines, chemokines, interferons, and interferon-stimulated genes were higher in H5N1-resistant chickens compared to H5N1-susceptible chickens (Table S5). Specifically, several key immune-related genes, including *TLR*, *JAK-STAT*, *IFN-kappa*, *IL-6*, *IL-18*, *CCL4*, *CCL19*, *Mx1*, *PKR*, *TRIM25*, *viperin*, and *MDA5*, were upregulated in resistant chickens at 3 dpi. These DE genes are associated with antiviral defense mechanisms and inflammatory responses (Table S5). They play crucial roles in

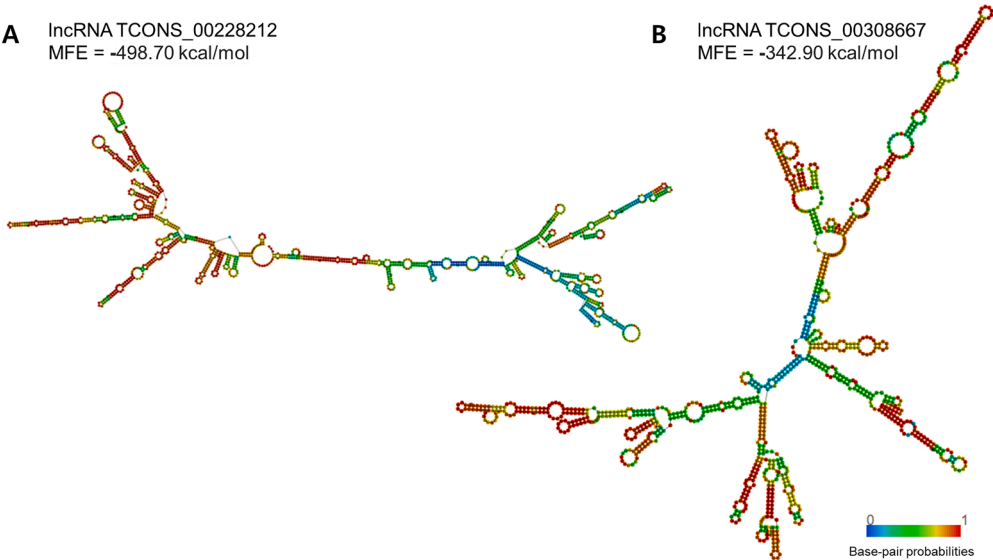


Fig. 5. Minimum free energy (MFE) secondary structure and positional entropy of the lncRNAs. A) lncRNA TCONS_00228212, which targets the *DHX58* gene. B) lncRNA TCONS_00308667, which targets the *EIF2AK2* gene.

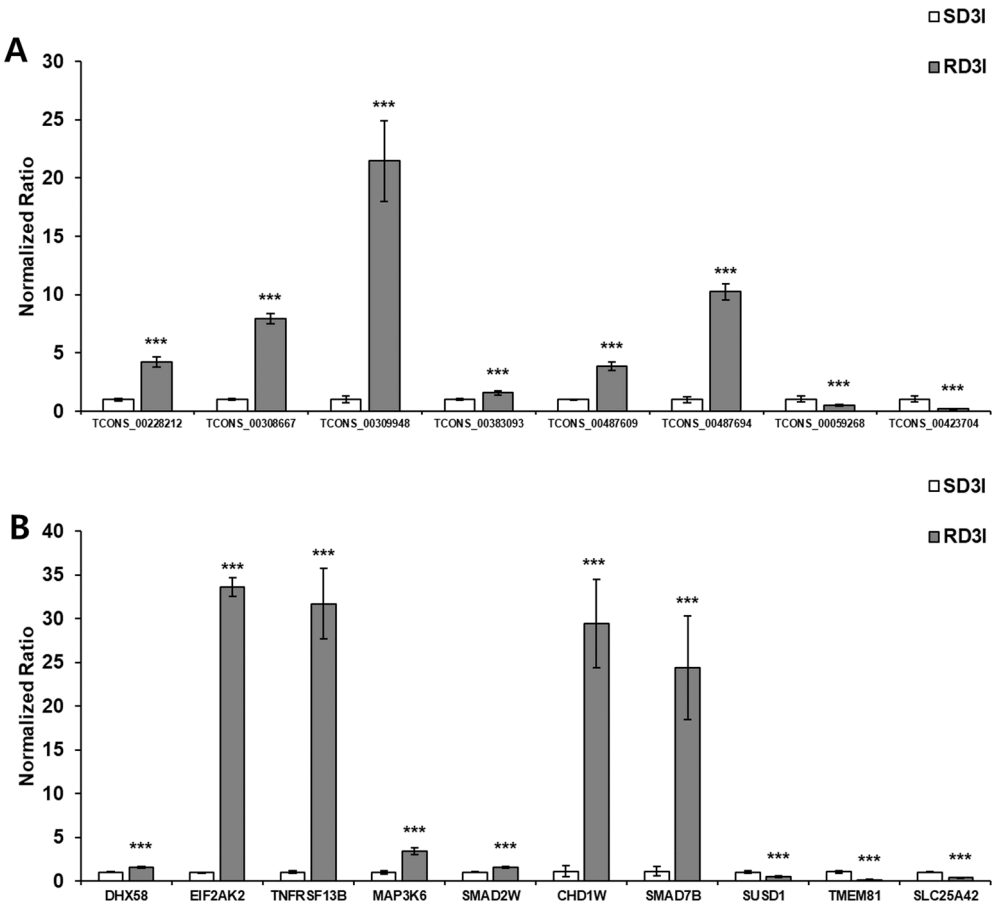


Fig. 6. RT-qPCR-based validation of DE (A) lncRNAs and (B) target genes. Data represent the mean $\pm$ standard error of the mean (SEM) of three independent experiments: *** $p < 0.05$.

activating immune pathways such as the MAPK, JAK-STAT, and NF- κ B pathways, which are essential for the host's defense against viral infections. These results indicate a stronger immune response in resistant Ri chickens, consistent with findings from previous studies (Hong, et al., 2021b; Lee, et al., 2022; Vu, et al., 2023a; Vu, et al., 2022a; Vu, et al.,

2022b).

At 3 dpi, we identified 99 DE lncRNAs, the majority of which were upregulated, in resistant chickens compared to susceptible chickens (Fig. 3A–B and Table S4). Functional analysis revealed their involvement in various immunity-related signal transduction pathways crucial

for the host's response to H5N1 infection, including TGF- β , RIG-I, MAPK, influenza A infection, herpes simplex virus 1 infection, and cytokine-cytokine receptor signaling pathways (Fig. 3D). Notably, all upregulated lncRNAs and their immune-related targets exhibited significantly higher expression levels in resistant chickens compared to susceptible chickens (Fig. 4 and Table S6). Moreover, we identified 70 lncRNA-mRNA interactions related to H5N1 infection between resistant and susceptible chicken lines. These interactions included both cis- and trans-acting lncRNAs and their target genes involved in antiviral response and immune function. RT-qPCR analysis confirmed the differential expression patterns observed in RNA sequencing results, further supporting the role of these lncRNAs in host immune responses to H5N1 infection.

The identified lncRNA-mRNA interactions include several genes associated with antiviral resistance and immune responses. For instance, protein kinase R (EIF2AK2) inhibits the translation of viral mRNAs, including those from influenza A viruses (Garcia, et al., 2006), while DHX58 (or LGP2) positively regulates antiviral responses mediated by RIG-I and melanoma differentiation-associated protein 5 (MDA5) (Satoh, et al., 2010). Furthermore, TNFSF13 is known for its involvement in B cell and immunoglobulin A immune system function (Jo, et al., 2019), and MAP3K6 is part of a protein kinase signal transduction cascade involved in antiviral defense mechanisms (Guan, et al., 2023). Additionally, SMAD7B and SMAD2W are involved in the transforming growth factor beta (TGF- β) pathway, a crucial signaling system in immune regulation and cell development (Hu, et al., 2021; Takimoto, et al., 2010). On the other hand, SUSD1 negatively regulates MHC-I in genome-wide screens of diffuse large B cell lymphoma 3 (Dersh, et al., 2021). Our results indicate higher expression of these genes in resistant chickens compared to susceptible chickens, suggesting a stronger antiviral response in the resistant group.

In previous studies, we observed significant differences in the expressions of genes related to the cytokine-cytokine receptor, MAPK, and influenza A infection signaling pathways upon H5N1 infection using the same Ri-resistant and -susceptible chicken animal models. These pathways are essential for innate immune defense and foreign DNA sensing (Hong, et al., 2021a; Lee, et al., 2022; Vu, et al., 2022a; Vu, et al., 2022b).

In conclusion, our study provides valuable insights into the role of lncRNAs in the host response to HPAIV H5N1 infection in chickens. The identified lncRNAs and their targets represent potential candidates for further investigation into the molecular mechanisms underlying host-pathogen interactions and the development of novel strategies for controlling AIVs. Specifically, lncRNAs TCONS_00228212 and TCONS_00308667 were identified as potential positive regulators of DHX58 and EIF2AK2, respectively. Further research is warranted to validate and elucidate the functions of these lncRNAs in AIV infection. Future studies will focus on experimentally validating these lncRNAs through knockdown and overexpression experiments, as well as functional assays, to further investigate their roles in antiviral immune responses.

Abbreviations

lncRNA: Long-noncoding RNA
 HPAIV: Highly pathogenic avian influenza virus
 KEGG: Kyoto encyclopedia of genes and genomes
 GO: Gene ontology
 RNA-seq: RNA sequencing
 DE: Differentially expressed
 DAVID: Database for annotation, visualization, and integrated discovery
 NF- κ B: Nuclear factor kappa B
 PLEK: Predictor of lncRNAs and mRNAs based on k-mer scheme
 CPC: Coding potential calculator
 CPAT: Coding-potential assessment tool

CNCI: Coding non-coding index
 RIG-I: Retinoic acid-inducible gene I
 TGF- β : Transforming growth factor beta
 MAPK: Mitogen-activated protein kinase
 DHX58: DEXH-box helicase 58
 EIF2AK2: Eukaryotic translation initiation factor 2 alpha kinase 2
 TNFSF13B: Tumor necrosis factor ligand superfamily member 13B
 MAP3K6: Mitogen-activated protein kinase kinase kinase 6
 SMAD2W: Mothers against decapentaplegic homolog 2-like
 CHD1W: Chromodomain helicase DNA binding protein 1W
 SMAD7B: TGF-beta signal pathway antagonist Smad7
 SUSD1: Sushi domain containing
 TMEM81: Transmembrane protein 81
 SLC25A42: Solute carrier family 25 member 42
 MDA5: Melanoma differentiation-associated protein 5

Consent for publication

Not applicable.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary files.

Ethics approval

All experimental protocols involving animals were approved by the National Institute of Veterinary Research (NIVR), Vietnam (TCVN 8402:2010/TCVN 8400-26:2014).

Declaration of competing interests

The authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104524.

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