Poultry Science®

Whole genome resequencing reveals the adaptability of native chickens to drought, tropical and frigid environments in Xinjiang

Lihua Zhang ,*,† Haiying Li,*,[1] Xiaoyu Zhao,* Yingping Wu,* Jiahui Li,* Yingying Yao,* Yang Yao,* and Lin Wang*

*College of Animal Science, Xinjiang Agricultural University, Urumqi, China; and †State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang 310021, China

ABSTRACT Chickens exhibit extensive genetic diversity and are distributed worldwide. Different chicken breeds have evolved to thrive in diverse environmental conditions. However, research on the genetic mechanisms underlying chicken adaptation to extreme environments, such as tropical, frigid and drought-prone regions, remains limited. In this study, we conducted whole-genome sequencing of 240 individuals from six native chicken breeds in Xinjiang, China, as well as 4 publicly available chicken breeds inhabiting regions with varying annual precipitations, temperatures, and altitudes. Our analysis revealed several genetic variants among the examined breeds. Furthermore, we investigated the genetic diversity and population structure of breeds residing in extreme drought and temperature environments by comparing them. Notably, native chicken breeds exhibited different genetic diversity and population structures. Moreover, we identified candidate genes associated with chicken adaptability to the environment, such as *CORO2A, CTNNA3, AGMO, GRID2, BBOX1, COL3A1, INSR, SOX5, MAP2* and *PLPPR1*. Additionally, pathways such as lysosome, cysteine and methionine metabolism, glycosaminoglycan degradation, and Wnt signaling may be play crucial roles in regulating chicken adaptation to drought environments. Overall, these findings contribute to our understanding of the genetic mechanisms governing chicken adaptation to extreme environments, and also offer insights for enhancing the resilience of chicken breeds to different climatic conditions.

Key words: chicken, genetic variant, candidate gene, whole genome sequencing

2024 Poultry Science 103:103947
https://doi.org/10.1016/j.psj.2024.103947

INTRODUCTION

Chicken rank among the foremost poultry species globally, providing humans with plentiful meat and eggs daily. With origins dating back 10,000 years, domestic chickens stand as among the earliest domesticated poultry species (Xiang et al., 2014). In addition to the domestication by human, geographical isolation, climate shifts, disease, and human interventions have contributed to the diversification of chickens across various regions and epochs, resulting in the emergence of distinct breeds and subspecies. According to statistics from the United Nations Food and Agriculture Organization in 2007, China has 116 chicken breeds, accounting for 10% of all breeds globally (Marsjan, 2008).

The genome sequence of the red jungle fowl, the wild progenitor of domestic chickens, was unveiled in 2004. The chicken genome contains approximately one billion base pairs of sequence and an estimated 20,000 to 23,000 genes (ICGSC,2004). The chicken genome has undergone numerous changes during the evolutionary process, including mutations, recombination events, and selection pressures. These changes have bestowed upon chickens a spectrum of diverse biological characteristics and behaviors (Boschiero et al., 2018; Yang et al., 2019). Selective pressures during domestication have led to the retention of specific genes, influencing traits such as egg production, growth rate, meat quality, and disease resistance in chickens (Liu et al., 2019; Podisi et al., 2013). Furthermore, some genes have undergone adaptive evolution, endowing various chicken breeds adapted to diverse environmental and feeding conditions (Zhao et al., 2022).

Chickens serve as a pivotal species for investigating genetic adaptation. Across the globe, native chicken breeds distributed worldwide have adapted to various environmental conditions and production systems

Received February 27, 2024.
Accepted June 3, 2024.

*Corresponding author: lhy-3@163.com

through natural and artificial selection. Understanding the genetic mechanisms underlying chickens adaption to extreme environments not only sheds light on the cold adaptation of poultry but also furnishes a theoretical basis for improving chicken breeds.

Whole-genome resequencing has been widely used with the advent of next-generation sequencing technology. An in-depth comparison and analysis of the genomes across various chicken breeds of chickens revealed some genes related to chicken evolution and adaptive mechanisms have been unveiled. Genes related to body size, such as *IGF1, SOX5, SATB2* and *IGF2BP1*, have been discovered (Chu et al., 2023). A study involving 119 individuals from 4 native Chinese chicken breeds inhabiting diverse climates, ranging from tropical to frigid environments, elucidated genetic differences in the *SLC33A1, NDUFS4* and *TSHR* genes, which may contribute to their tolerance and survival in extreme environments (Shi et al., 2023). Additionally, the *ME3* and *ZNF536* genes, implicated in fat metabolism and the nervous system in cold adaptation, may affect the cold adaptation of chickens (Xu et al., 2021). While numerous studies have explored genetic factors controlling growth, development, reproduction, and production traits (Rubin et al., 2010). However, the genetic mechanism of the unique adaptation of chickens to drought and cold environments remain incompletely understood.

The native chicken breeds living in Xinjiang encompass the Yemili Chicken (**YC**), Baicheng Fatty Chicken (**BY**), Hetian Black Chicken (**HB**), Fuyun Black Chicken (**FB**), Yili Chicken (**YL**) and Xiaowan Black Chicken (**XB**). The Yemili Chicken is native to the Wuer Kazha Mountain in Emin County, exhibiting strong adaptability and flexible body. Found mainly in the vicinity of Baicheng County in the Aksu area, the Baicheng Fatty Chicken is a versatile breed renowned for its fat and beautiful body, petite bones, and substantial meat. Hotan Black Chickens possess the characteristics of strong resistance to disease, rough feeding, and high temperature. Fuyun Black Chicken has the excellent characteristics of strong resistance to dryness, heat and cold, boasting a fully black plumage. Yili Chicken showcases strong physique, uniform shape, compact structure, and good flying. Xiaowan Black Chicken boasts petite feathers on its toes and exhibits heightened adaptability to diverse environments. These breeds collectively exhibit exceptional attributes in withstanding dry, heat, and severe cold climates.

This study involved whole-genome sequencing of 240 chickens from six native Xinjiang breeds in China that inhabit areas with extreme rainfall and temperature conditions. The resequencing data from 27 chickens present in the public database were downloaded (Supplementary Tables S1−S2). We then analyzed their population structure and genome diversity based on the genomic data of these chicken breeds. We identified genes associated with the adaptability of these local chicken breeds to drought, tropical, and frigid environments through whole-genome selective scanning analysis. These results provide valuable insights into the genetic basis of the adaptability of chickens to extreme environments and will be beneficial to the improvement of chicken breeds.

MATERIALS AND METHODS

Experimental Animals

Genomic DNA was extracted from 40 individuals of each population from YC, BY, HB, FB, YL, and XB chickens following the protocol of the DNeasy Blood & Tissue Kit protocol (Qiagen, Valencia, CA). A 1 μg DNA per individual served as input material for sample preparations, and sequencing libraries were generated using the Truseq Nano DNA HT Sample Preparation Kit (Illumina, San Diego, CA) according to the manufacturer's guidelines. Index codes were assigned to each sample to attribute the sequences. Initially, the DNA samples were sonicated to fragment to 350 bp. The DNA fragments underwent end polishing, A-tailing, and ligation with full-length adapters for Illumina sequencing accompanied by PCR amplification. Subsequently, PCR products underwent purification using the AMPure XP system, and library size distribution was measured via an Agilent 2100 Bioanalyzer (Agilent Technologies, CA). Additionally, libraries underwent quantification using real-time PCR and subsequently sequenced on DNBSEQ-T7 (Novogene Co. Ltd, Tianjin, China).

Sequence Quality Control and Filtering

The raw data reads in fastq format underwent a series of quality control (**QC**) procedures using in-house C scripts to ensure the reliability and absence of artificial bias, such as low-quality paired reads resulting from base-calling duplicates and adapter contamination. The QC procedure comprised the following steps: Firstly, reads containing $\geq$ 10% unidentified nucleotides (N) were removed. Subsequently, reads with > 20% of bases having a phred quality <5 were discarded. Reads with > 10 nt aligned to the adapter, allowing for $\leq$10% mismatches were eliminated. Finally, putative PCR duplicates, which corresponded to completely identical pairs of read 1 and read 2 in 2 paired-end reads generated during PCR amplification in library construction, were removed.

Genome-Wide Variant Calling and Annotation

The remaining high-quality paired-end reads were mapped to the chicken reference genome GRCg7b (https://www.ncbi.nlm.nih.gov/datasets/geno me/GCF_016699485.2/) using the Burrows-Wheeler Aligner (Version: 0.7.8) with the command line "BWA mem -t 8 -k 32 −M." To minimize mismatches generated by PCR amplification before sequencing, duplicated reads were removed using SAMtools (Li and Durbin

2009; Li et al., 2009) to reduce mismatches generated by PCR amplification before sequencing. Additionally, genomes of four Red Junglefowl (**RJF**), 10 Sri Lankan (**SL**), 5 Saudi Arabian (**SA**), and 8 Tibetan (**ST**) chickens were retrieved from the public database, resulting in an average genome mapping rate of 99.67% with an approximate average depth of 15.29-fold average depth (Supplementary Tables S1−S2).

After aligning the reads, we conducted SNP calling on a population scale using a Bayesian approach implemented in the GATK software (McKenna et al., 2010). This involved calculating the genotype likelihoods for each individual at every genomic location and determining allele frequencies with the sample using Bayesian methods. We employed the HaplotypeCaller module, the GVCF model, and conducted a joint genotyping step to integrate variations across all samples from a combined gVCF file. Filtering parameters were set as follows: GQ > 5 || QD < 2.0 || FS > 60.0 || SOR > 3.0 || MQRankSum < 12.5 || ReadPosRankSum < 8.0. Subsequently, only high-quality SNPs meeting the criteria (coverage depth ≥ 4, RMS mapping quality ≥ 20, miss < 0.1) were retained for further analysis to exclude SNP calling errors stemming from incorrect mapping or InDels.

Annotation of the variations was performed using the ANNOVAR (Version: 2013-05-20) package by referencing the chicken GRCg7b genome (Wang et al., 2010). SNPs were categorized based on their location within the genome, including exonic regions (overlapping with coding exons), intronic regions (overlapping with introns), splicing sites (within 2 bp of splicing junction), upstream and downstream regions (within a 1 kb region upstream or downstream from the transcription start site), and intergenic regions. SNPs located in coding exons were further classified as synonymous SNPs (those that did not cause amino acid changes) or nonsynonymous SNPs (those that caused amino acid changes). Additionally, mutations leading to stop gains or stop losses were also included in this category. The identified population-based InDels were similarly functionally annotated using this method.

Population Structure and Genetic Diversity

We used the ADMIXTURE program to estimate individual ancestry and admixture proportions based on whole-genome high-quality SNPs (Tang et al., 2005). Before this analysis, LD-pruning was performed using plink software with the parameter of "−indep-pairwise 50 10 0.1" (Purcell et al., 2007). We predefined the number of genetic clusters from K = 2 to 10 with 200 bootstrap replicates for each run and default method and setting in the ADMIXTURE analysis. Subsequently, an individual-based neighbor-joining (**NJ**) tree was constructed using the *p*-distance in TreeBest software (Vilella et al., 2009) to elucidate the phylogenetic relationships from a genome-wide perspective. Phylogenetic trees were visualized using MEGAX software (Tamura et al., 2011). Additionally, principal component analysis (**PCA**) was conducted to assess genetic structure using the GCTA software (Yang et al., 2011). The significance level of the eigenvectors was determined using the Tracey−Widom test.

Identification of Regions of Homozygosity (ROHs)

ROHs are defined as a continuous or uninterrupted stretches of a DNA sequences without heterozygosity in the diploid state. To quantify the ROHs for each individual, we employed the PLINK v1.9 program (Purcell et al., 2007), with the following command "−homozyg-window-snp 20 −homozyg-window-het 1 −homozyg-snp 10 −homozyg-kb 100 −homozyg-density 10 −homozyg-gap 100".

Linkage Disequilibrium Analysis

The dataset provided us with the opportunity to examine the linkage disequilibrium (**LD**) pattern among 240 chickens using a set of 16 million high-quality SNPs. To estimate LD decay, we employed Haploview v4.2 and calculated the squared correlation coefficient (***r*2**) between pairwise SNPs (Barrett 2009). The program parameters were set as follows: '-n -dprime -minMAF 0.05'. We calculated the average *r*2 value for pairwise markers within a 500 kb window and then averaged it across the entire genome.

Detection of Selective Signatures

We used allele frequencies at variable sites to identify signals of selection in 40 kb windows, with a step size of 20 kb, using the VCFtools (v 0.1.14) software with the following approaches: for each window, we calculated the average fixation index (***F*ST**) and nucleotide diversity ($\theta\pi$) between the populations of chicken from tropical and frigid environments (SA and SL populations versus YC, BY, FB, and YL populations), drought, and abundant rainfall environments (RJF and SL populations versus HB and XB populations). We calculated the genome-wide distribution of *F*ST, where *F*ST was calculated from the allele frequencies (not from the allele counts) using the standard equation:

$$\theta\pi\_\text{within} = (\theta\pi\_\text{population1} + \theta\pi\_\text{population2})/2$$

$$F_{ST} = (\theta\pi\_\text{total} - \theta\pi\_\text{within})/\theta\pi\_\text{total}$$

$\theta\pi$_population1: the group of HB and XB or the group of YC, BY, FB and YL; $\theta\pi$_population2: the group of RJF and SL or the group of SL and SA; $\theta\pi$: 1 - fA^2 - fT^2 -fC^2 - fG^2, fN: frequency of nucleotide N; $\theta\pi$_total: for the total $\theta\pi$, the allele frequencies of the 2 populations are averaged (Han et al., 2022). Any windows with significantly high *F*ST and $\log_2$ ($\theta\pi$ ratio) values were considered outliers under strong selective sweeps. All outlier regions were assigned to corresponding SNPs and

annotated genes. Putatively selected regions were located by extracting windows from the extreme tails of the *F*ST and $\log_2(\theta\pi$ ratio) distributions by applying a threshold of the 5% highest genes. Additionally, we estimated the cross-population extended haplotype homozygosity (XP-EHH; https://github.com/joepickrell/xpehh) statistic for adaptation to tropical, frigid and drought environments, using the control group as a reference. For tropical environment, the control groups were YC, BY, FB and YL populations. For frigid environment, the control groups were SL and SA populations. While for drought environments, HB and XB populations were the control groups. The average XP-EHH values were computed with a 40-kb window size and a 20-kb step size. Windows with the 5% highest XP-EHH values were selected.

RESULTS

Genome Resequencing and Variation Detection Among Different Breeds of Chickens

In this study, we sequenced 240 individuals from six native chicken breeds from Xinjiang. Additionally, we downloaded RJF (*Gallus gallus spadiceus*), SL and SA chickens from a public database. The average precipitation in the habitats of the sequenced RJF, ST, and YL breeds exceeded 400 mm, whereas the average precipitation for SA, HB, and XB breeds was below 100 mm. The average temperatures for SL and SA habitats ranged between 26 and 27°C, while the temperature in the HB habitat was recorded at 18.5°C. In contrast, YC, BY, FB, and YL habitats experienced temperatures below 8°C. It is worth noting that the altitude of RJF and ST habitats exceeded 3,000 m, which is higher compared to other chicken breeds (Supplementary Table S1).

The sequencing depth for the 6 native chicken breeds ranged from 10X to 21X, with genome coverage exceeded 96% (Supplementary Table S2). A total of 16,759,127 SNPs were detected, with 8,486,449 SNPs located in the gene spacer region and 7,654,748 SNPs found in the intronic region. Among these, there were 67,267 nonsynonymous sites, 45 stop-loss sites, and 381 stop-gain sites. The ts/tv was 2.492 (Supplementary Table S3). A total of 4,758,841 InDel sites were obtained, comprising 1,625,503 insertions, 3,133,338 deletions, 4,722,096 intergenic sites, 33 stop-gain sites, and one stop-loss site. Furthermore, 896 frameshift deletions and 326 frameshift insertion sites were observed (Supplementary Table S4). These mutations were distributed across chromosomes 1−28, 31, 33, 34, W, and Z (Figure 1).

Population Structure and Admixture Analysis

We constructed a phylogenetic tree using genome-wide SNP data from all populations to examine the genetic relationship among these native chicken breeds (Figure S1). Based on this analysis, all individuals were categorized into 10 distinct monophyletic groups (Figure 2A). The XB, FB, HB, BY, YL, ST, RJF, YC, SL, and SA populations clustered into independent evolutionary clades. Specifically, the YL, ST, and RJF populations exhibited close genetic relatedness, while the YC, SL, and SA populations have close genetic

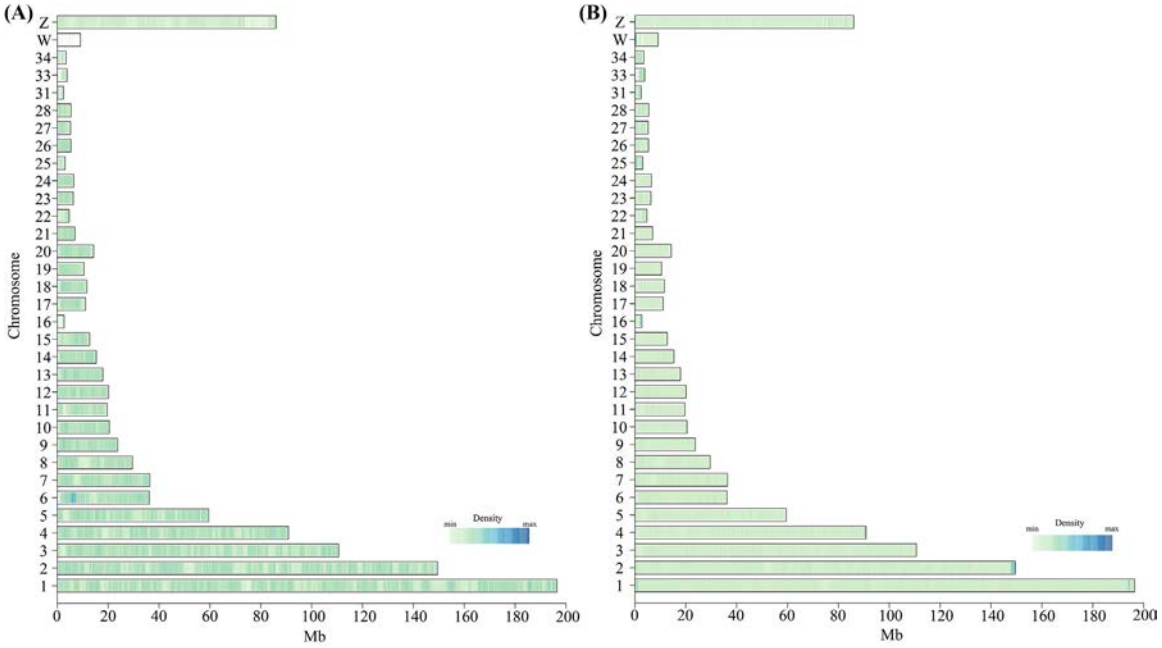

Figure 1. The distribution of SNP and InDel variants in the 267 chicken population. (A) The density distribution of SNPs from different chromosomes. (B) The density distribution of InDels on each chromosome.

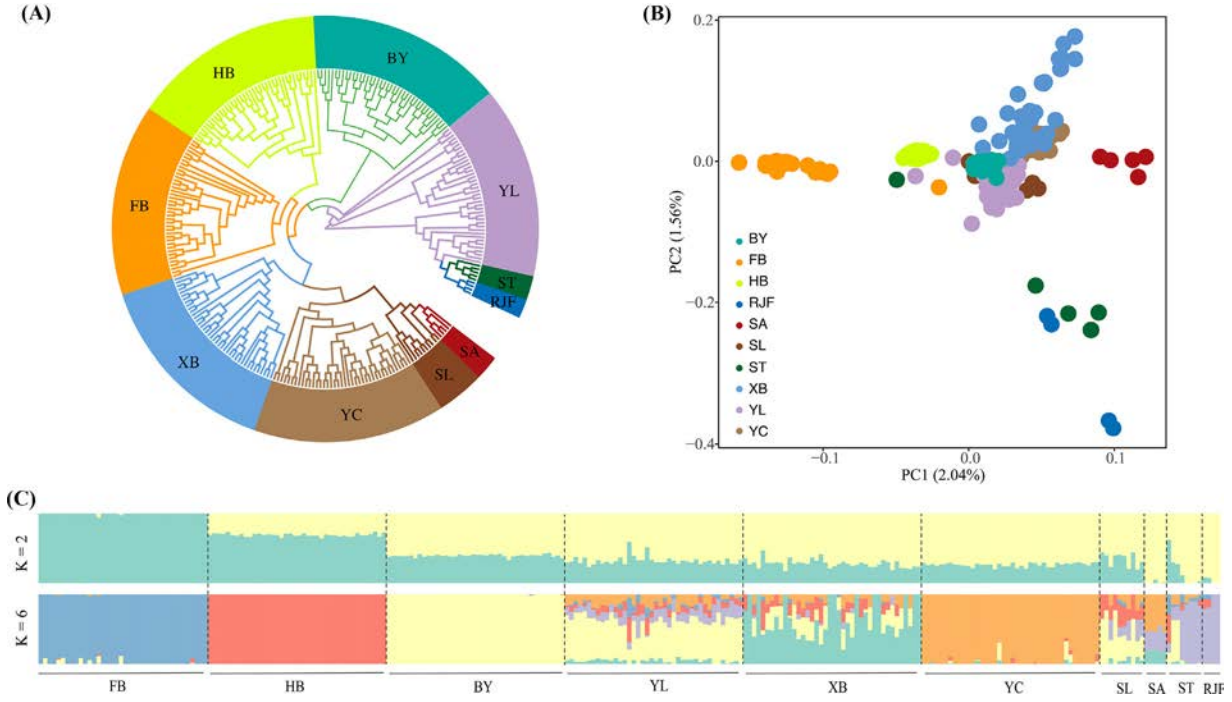

Figure 2. Population genetic analyses of ten chicken breeds. (A) Neighbor-joining tree (B) Principal component analysis. (C) Genetic structure of chicken breeds. The length of each colored segment represents the proportion of the individual's genome inherited from ancestral populations (k = 2 and 6).

relationships. In order to further understand the genetic structure, a principal component analysis (**PCA**) was performed on the genomes of all 267 chickens. The total interpretative variance for PC1 and PC2 was 2.04% and 1.56%, respectively. According to the PCA analysis results (Figures S2−S4), there was an overlap between YL, ST, and RJF populations, as well as between SL, SA, YC, RJF, and YL overlapped. However, the FB population separated from the others (Figure 2B).

To further investigate the admixture events and phylogenetic relationships, we conducted an unsupervised admixture analysis with K sets of 2 and 6. At K = 2, the HB, BY, YL, XB, YC, SL, and RJF populations showed similar ancestral components. When K was set to 6, extensive genetic introgression was observed among the YL, XB, YC, SL, SA, ST, FB, and RJF populations. In contrast, the HB and BY breeds had a single ancestral component that differed from other chicken breeds (Figure 2C).

Analysis of Population Genetic Structure

LD estimates based on R^2 values varied across the six native populations in Xinjiang, China. Short-distance LD was observed in all populations, but the rate of LD decay varied between them. YL group declined more rapidly than the others, while FB exhibited the slowest decay (Figure 3A). This result indicates that the YL population had high genetic diversity, whereas the FB population had low genetic diversity, a high degree of domestication, and considerable selection pressure. With regard to the distribution of inbreeding coefficient, YL and XB presented wider inbreeding coefficient distributions than FB, HB, and BY. In contrast, FB and HB showed higher inbreeding coefficients than BY, suggesting that inbreeding may contribute to decreased genetic diversity in the FB population (Figure 3B).

The genetic diversity of FB and HB was lower than that of BY, while YL and XB displayed slightly higher genetic diversity than other breeds within the six native Xinjiang chicken breeds (Figure 3C). These findings were consistent with LD and inbreeding coefficient results. Specifically, YL and XB breeds had longer ROH fragments and numbers, indicating high levels of generation inbreeding inherited from a common ancestor (Figure 3D).

Selective Signatures in Tropical, Frigid, and Drought Adaptability Environments

We compared 2 relatively extreme groups (SA and SL populations versus YC, BY, FB, and YL populations) to understand the adaptive genetic mechanisms of native chicken varieties in tropical and frigid environments. First, the adaptation to tropical environment was performed based on the overlap of the F_{ST} (5% high-ranking outlier, $F_{ST} > 0.063$) and $\theta\pi$ ratio analysis (5% high-ranking outlier, $\theta\pi$ ratio > 0.151), a total of 260 candidate regions were screened, and 174 positive selection genes were identified after gene location and functional annotation (Figure 4A, Supplementary Table S5). Meanwhile, 1,187 candidate intervals were screened using the 5% high-ranking XP-EHH values (Figure 4B, Supplementary Table S6). There were 48 overlapping

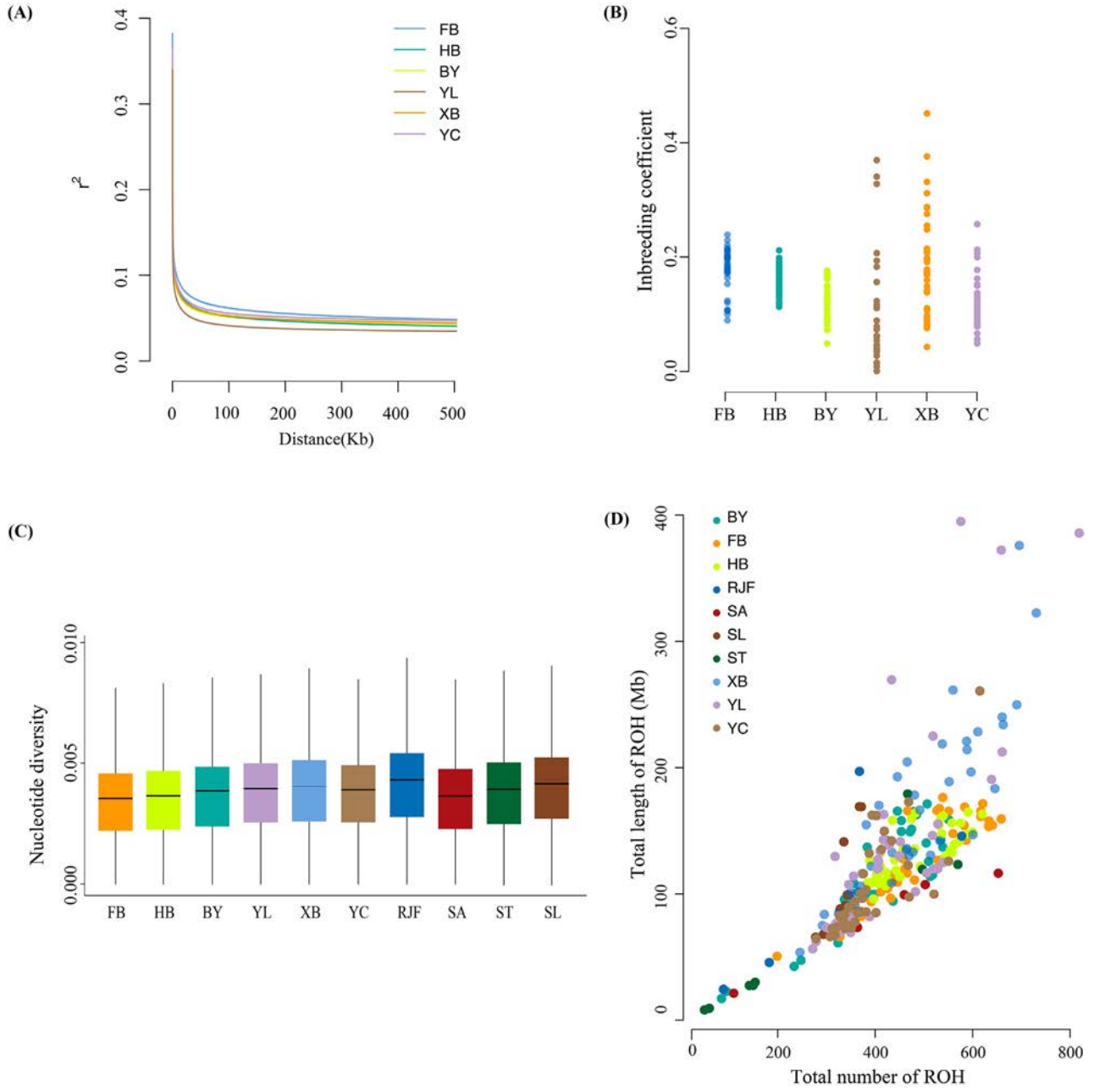

Figure 3. Genetic diversity among different chicken populations. (A) Decay of linkage disequilibrium estimated from each breed. (B) Inbreeding coefficient for each individual from different chicken populations. (C) Box plots of the nucleotide diversity for each group. Points on the outside of the whiskers are outliers. (D) Estimation of the total number of runs of homozygosity (**ROH**) for each group.

genes identified using these 3 methods, including *CORO2A, CTNNA3, AGMO, GRID2* and *LRP*2, genes that may be related to adaptation to tropical conditions (Supplementary Table S11). We then identified 406 selection regions associated with the adaptation to frigid environment for native chickens by comparing F_{ST} and $\theta\pi$ ratios (Supplementary Table S7). The 457 candidate regions were identified using the top 5% XP-EHH values (Figure 4B, Supplementary Table S8), and 99 overlapping genes, including *BBOX1, COL3A1, INSR, SOX5, MAP2,* and *PLPPR1*, were obtained using these 3 methods, which may affect the adaptation to frigid environment for native chickens (Supplementary Table S11).

We conducted a selective sweep analysis to identify the functional genes responsible for the drought adaptability differences between 2 extreme groups (RJF and SL populations versus HB and XB populations) using F_{ST} (5% high-ranking outlier, $F_{ST} > 0.063$) and $\theta\pi$ ratio analysis. There were 643 regions harboring 400 genes identified as selected regions (Supplementary Table S9). Additionally, 846 candidate regions were identified using XP-EHH (Figure 5, Supplementary Table S10), and a total of 740 genes were found to overlap using all 3 methods (Supplementary Table S11). Notably, 27 of these candidate genes, such as *BBOX1, COL3A1*, and *INSR,* were also identified as selective genes associated with the drought adaptation of native chickens.

Functional enrichment analysis of these candidate genes was performed. The genes associated with the frigid environments of native chicken breeds exhibited

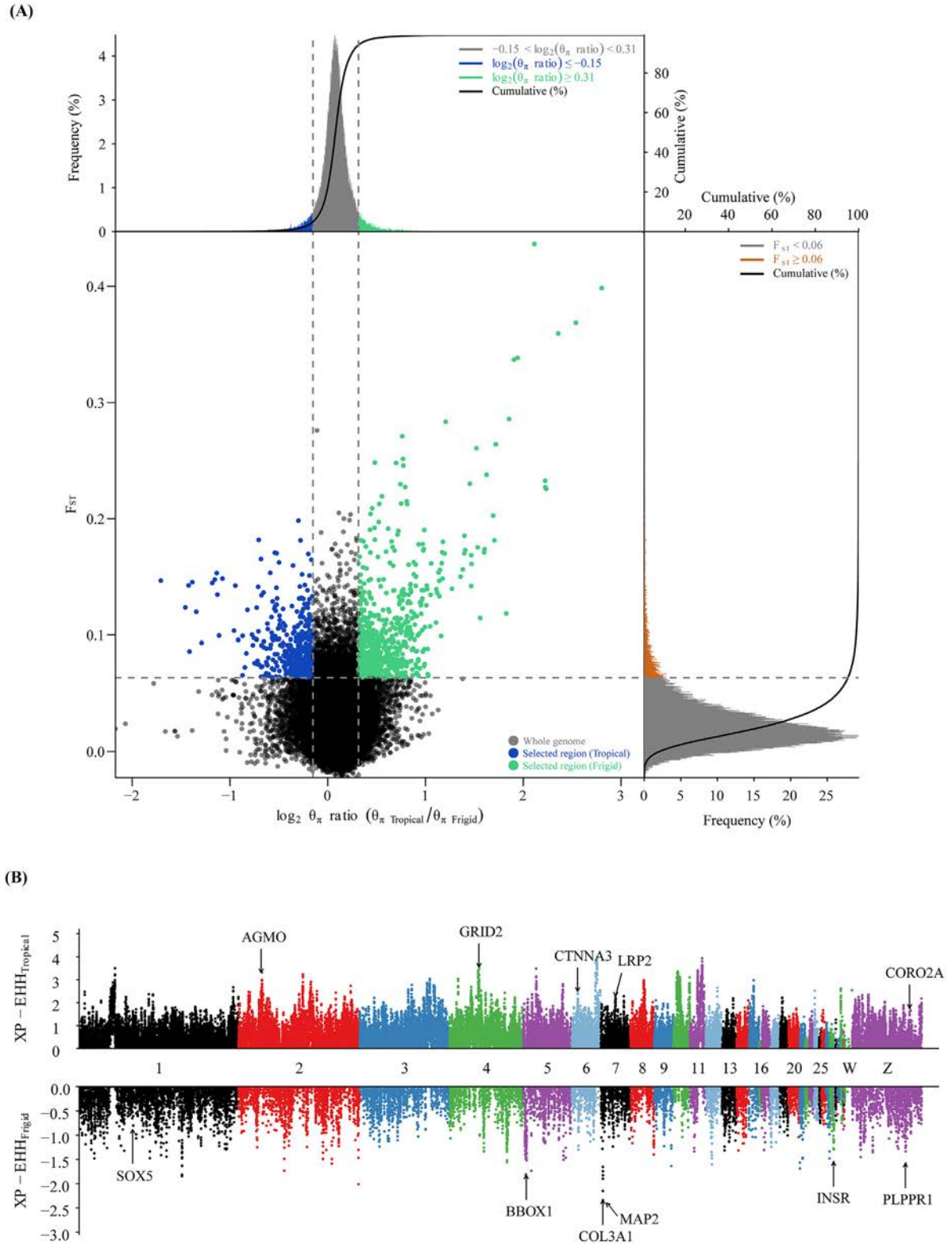

Figure 4. Genomic regions with strong selective sweep signals of adaptation to tropical and/or frigid environments of chicken populations using a combination of the top 5% F_{ST} values, log2$\theta\pi$ ratios and XP-EHH values. (A) Distribution of dot plot of $\theta\pi$ and F_{ST} values (top 5% outliers). The selected regions for adaptation to tropical and/or frigid environments are indicated with blue and green dots. (B) Distribution of Manhattan plot of XP-EHH values (top 5% outliers). The candidate gene using a combination of the top 5% F_{ST} values, log2$\theta\pi$ ratios and XP-EHH values for adaptation to tropical and/or frigid environments are indicated.

racemase and epimerase activities, which regulated the VEGF and MAPK signaling pathways, as well as the apoptosis pathway. Metabolic pathways associated with drought environments in native chicken breeds were found to be involved in lysosome, cysteine and methionine metabolism, glycosaminoglycan degradation, and the Wnt signaling pathways (Figure S5, Supplementary Table S12).

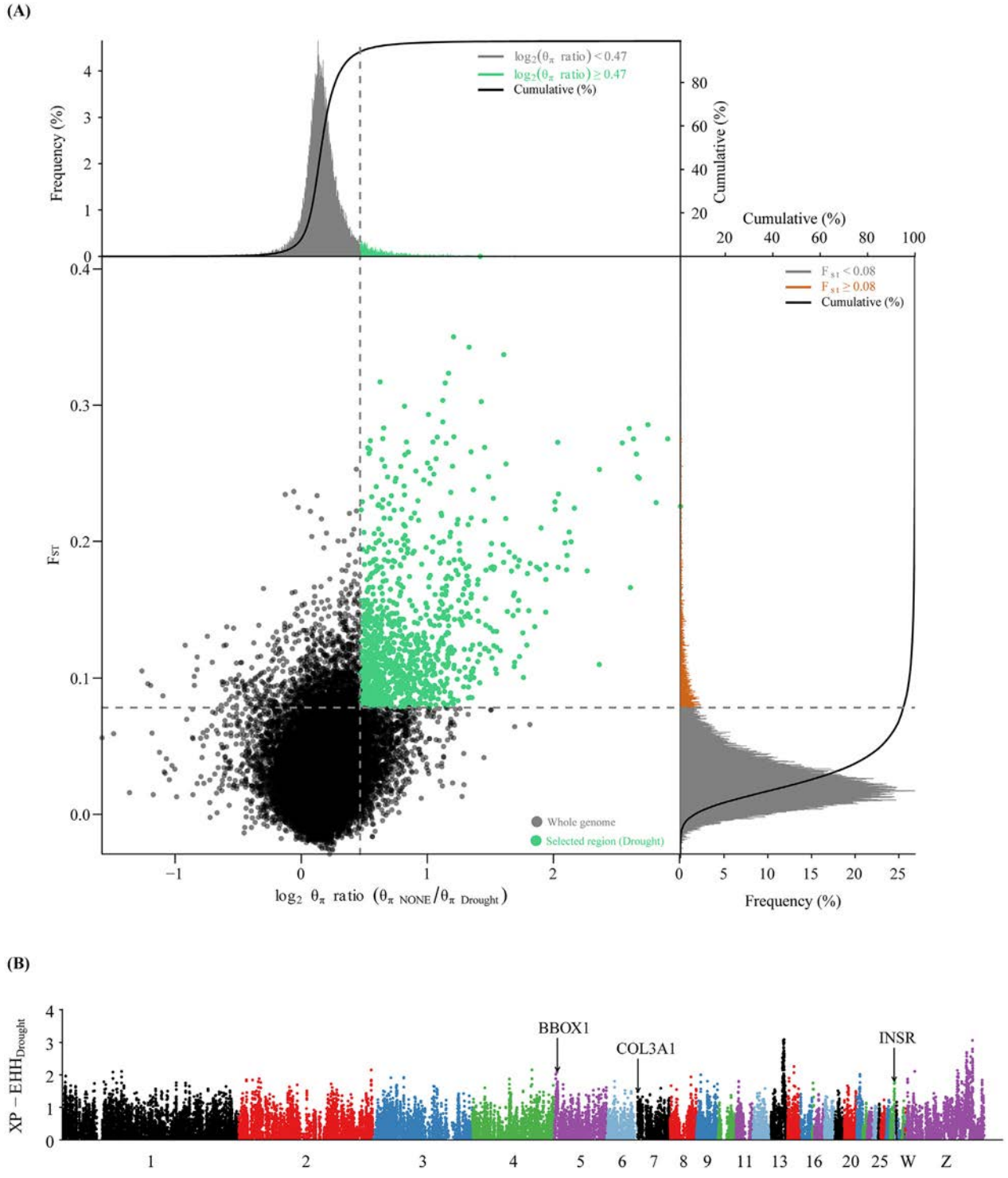

Figure 5. Genomic regions with strong selective sweep signals of adaptation to drought environments of chicken populations using a combination of the top 5% F_{ST} values, log2$\theta\pi$ ratios and XP-EHH values. (A) Distribution of Manhattan plot of $\theta\pi$ and F_{ST} values (top 5% outliers). The selected regions for adaptation to drought environments are indicated with red dots. (B) Distribution of Manhattan plot of XP-EHH values (top 5% outliers). The candidate gene using a combination of the top 5% F_{ST} values, log2$\theta\pi$ ratios and XP-EHH values for adaptation to drought environments are indicated.

DISCUSSION

In this study, we conducted whole-genome sequencing of 240 chickens and identified a total of 16,759,127 SNPs and 4,758,841 InDel variants. Among these, there were 16,141,197 SNPs and 4,752,775 InDels were located in the introns and intergenic regions, accounting for 96.31% and 99.87% of the total variation, respectively. The proportion of variation occurring in the functional regions of genes was relatively small, suggesting that gene regulation may have a greater impact on the differences in environmental adaptability among these chicken breeds.

BY chickens and YL chickens are geographically close to each other, with no significant difference in the

average annual temperature of their habitats. However, the average annual rainfall in the habitat of YL chickens was significantly higher than that of BY chickens. An unsupervised mixed analysis revealed that BY chickens exhibited single ancestral components. In contrast, YL chickens displayed mixed genetic infiltration, including the ancestral lineages of BY chickens, hinting at potential gene flow between the 2 populations.

The six chicken breeds in this study inhabited different environments characterized by variations in annual precipitation and temperature. For example, the habitats of BY had high precipitation than that of HB. In contrast, the environments for HB had a higher temperature. BY and HB had different single ancestral lineages, and YL and HB had extreme living environments similar to BY versus HB. However, the ancestral components of YL and XB were much more complex and had high genetic diversity. These findings suggest that different chicken breeds may possess additional genetic mechanisms for adapting to different environments.

Selective scanning analysis revealed genes associated with adaptability to tropical environments in native chickens. These genes are involved in various pathways such as VEGF, MAPK, and apoptosis. *CTNNA3* encodes a protein belonging to the vascular protein/alpha-catenin family, which plays a role in cell adhesion of muscle cells. The *CTNNA3* gene was downregulated in the testis of heat-stressed chickens during the investigation of the effect of acute heat stress on the testis gene expression of a broiler-type Taiwan village chicken (Wang et al., 2015). Adenylyl cyclase 2 (***ADCY2***) is a B member of adenylyl cyclase, is crucial in accelerating phosphorylation, glycogen synthesis, and breakdown (Li et al., 2014). Mutations in the *AGMO* gene, an enzyme dependent on tetrahydrobiopterin and iron, have been linked to reduced glucose-stimulated insulin response, type 2 diabetes, and susceptibility to intracranial aneurysms. This gene may have enabled Indonesian chickens to challenge hot and humid climates (Xu et al., 2022). Additionally, the glutamate receptor ionophil delta 2 gene (***GRID2***) is a multichannel membrane protein, that plays a significant role in regulating mitochondrial morphogenesis, dendritic development, and synaptic formation in Purkinje cells (Huang et al., 2022). The low-density lipoprotein-associated protein 2 (***LRP2***), function as a polygonate receptor and is essential for the reuptake of various ligands, including lipoproteins, sterols, vitamin-binding proteins, and hormones. The expression of the *LRP2* gene has been found to impact the incubation of chicken embryos (Dayan et al., 2020). These genes may play similar role in chickens and contribute to their adaptation to tropical environments.

The selection analysis also revealed genes associated with adaptation to frigid environments in native chickens. For example, the *INSR* gene encodes a member of the receptor tyrosine kinase family proteins that regulates the uptake and release of glucose, as well as the synthesis and storage of carbohydrates, lipids, and proteins. Studies have shown that the insulin receptor (*Insr*) gene was upregulated in the brown adipose tissue (**BAT**) of rats under low-temperature exposure (Wang and Wahl 2014). Furthermore, the *BBOX1* gene encodes gammabutylbetaine hydroxylase, and environmental stress has been found to significantly downregulate this gene in Penaeus vannamei (Rashidi-Nezhad et al., 2014). Additionally, *COL3A1*, a type III collagen, is present in the extracellular matrix and is the most abundant protein in animals. It plays a crucial role in hypoxia adaptation and cold tolerance in Tibetan sheep (An et al., 2023). Lastly, the *SOX5* gene influences the shape of the comb, which is a skin appendage and a sexual dimorphism characteristic of chickens. This gene has been identified as a candidate for cold adaptation (Romanov et al., 2023).

The genes adapted to the frigid environment, as screened by the 5% high-ranking XP-EHH value analysis, were found to be enriched. These genes are involved in racemase and epimerase activity pathways, specifically *AMACR, GALM, PEL1*, and *TSTA3*, which play a role in carbohydrates and energy metabolism. The KEGG pathway enrichment analysis showed their association with the VEGF and mitogen-activated protein kinase (**MAPK**) signaling pathways, as well as apoptosis. The VEGF signaling pathway is a crucial signaling sensor for both physiological and pathological angiogenesis. Exposure to cold temperatures leads to a transient increase in VEGF gene expression in brown adipose tissue due to the release of norepinephrine (ASANO et al., 1997). The MAPK cascade is a highly conserved module involved in various cellular functions, including cell proliferation, differentiation, and migration. Transcriptome and proteome analysis have demonstrated that the MAPK cascade regulates the cold tolerance of chrysanthemum (Zhang et al., 2023). Temperature also affects the expression of genes related to porcine embryo apoptosis (Jin et al., 2007). Autophagy, a double-membrane autophagosome is formed around the cytoplasmic component and is passed to the vacuole or lysosome for degradation. Autophagy-deficient plants are more sensitive to salt and drought conditions than wild-type plants, indicating the role of autophagy in coping with these stresses (Liu et al., 2009). The metabolism of cysteine and methionine (map00270) was the most affected pathway by a comprehensive and large-scale single omics analysis of young oil palm tree leaves experiencing water shortage (Bittencourt et al., 2022). Glycosaminoglycans (**GAGs**) are long-chain unbranched-chain anionic polysaccharides composed of repeating disaccharide units. They are involved in biologically relevant processes of the extracellular matrix, such as cell proliferation and communication (Bojarski and Samsonov 2021). The Wnt signaling pathway is critical for adult tissue maintenance, remodeling and regeneration, embryonic development, and many cellular processes including cell motility and cytoskeletal recombination. Upregulation of the Wnt signaling pathway may be necessary as an adaptive response to increased salinity (Jeffries et al., 2019).

CONCLUSIONS

This study conducted whole-genome sequencing of 240 native chicken breeds in Xinjiang, and identified several genetic variation sites. Chicken breeds from regions with different annual precipitation and average temperatures were compared according to their living environment, and their genetic diversity and population structure were analyzed. Candidate genes, such as *CORO2A, CTNNA3, AGMO, GRID2, BBOX1, COL3A1, INSR, SOX5, MAP2* and *PLPPR1*, associated with adaptation to tropical, frigid, and drought adaptability environments in native chickens were identified. The results of this study will help us recognize the genetic mechanism of adaptation to extreme environments in chickens. The insights gained from the identification of candidate genes have potential applications in the improvement of chicken breeds in the future.

ACKNOWLEDGMENTS

This research was funded by the 2024 Xinjiang poultry breeding technology integration demonstration and promotion project (2024 xjjq-z-01).

The original data reported in this article have been deposited with the NCBI database under project no. PRJNA1077999.

Ethics Statement: The animal study was reviewed and approved by the Animal Welfare and Ethics Committee of Xinjiang Agricultural University, Urumqi, Xinjiang, China (Approval number 2023007).

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103947.

REFERENCES

An, L., Y. Li, L. Yaq, Y. Wang, Q. Dai, S. Du, Y. Ru, Q. Zhoucuo, and J. Wang. 2023. Transcriptome analysis reveals molecular regulation mechanism of Tibet sheep tolerance to high altitude oxygen environment. Anim. Biotechnol. 34:5097–5112.

ASANO, A., M. MORIMATSU, H. NIKAMI, T. YOSHIDA, SAITO, and Masayuki. Adrenergic activation of vascular endothelial growth factor mRNA expression in rat brown adipose tissue: implication in cold-induced angiogenesis. Biochem. J. 328:179–183.

Barrett, J. C. 2009. Haploview: visualization and analysis of SNP genotype data. Cold Spring Harb Protocip71 2009:pdb.

Bittencourt, C. B., T. L. Carvalho da Silva, J. C. Rodrigues Neto, L. R. Vieira, A. P. Leao, J. A. de Aquino Ribeiro, P. V. Abdelnur, C. A. F. de Sousa, and M. T. Souza Jr. 2022. Insights from a Multi-Omics Integration (MOI) Study in oil palm (Elaeis guineensis Jacq.) response to abiotic stresses: part one-salinity. Plants (Basel) 11:1755.

Bojarski, K. K., and S. A. Samsonov. 2021. Role of oligosaccharide chain polarity in protein-glycosaminoglycan interactions. J. Chem. Inf. Model 61:455–466.

Boschiero, C., G. C. M. Moreira, A. A. Gheyas, T. F. Godoy, G. Gasparin, P. Mariani, M. Paduan, A. S. M. Cesar, M. C. Ledur, and L. L. Coutinho. 2018. Genome-wide characterization of genetic variants and putative regions under selection in meat and egg-type chicken lines. BMC Genomics 19:83.

Chu, J., Y. Ma, H. Song, Q. Zhao, X. Wei, Y. Yan, S. Fan, B. Zhou, S. Li, and C. Mou. 2023. The genomic characteristics affect phenotypic diversity from the perspective of genetic improvement of economic traits. iScience 26:106426.

Dayan, J., N. Reicher, T. Melkman-Zehavi, and Z. Uni. 2020. Incubation temperature affects yolk utilization through changes in expression of yolk sac tissue functional genes. Poult Sci 99:6128–6138.

Han, B. X., S. S. Yan, Q. Xu, J. J. Ni, X. T. Wei, G. J. Feng, H. Zhang, B. Li, L. Zhang, and Y. F. Pei. 2022. Mendelian randomization analysis reveals causal effects of plasma proteome on body composition traits. J. Clin. Endocrinol. Metab. 107:e2133–e2140.

Huang, S., C. Zhang, X. Xie, Y. Zhu, Q. Song, L. Ye, and Y. Hu. 2022. GRID2 aberration leads to disturbance in neuroactive ligand-receptor interactions via changes to the species richness and composition of gut microbes. Biochem. Biophys. Res. Commun. 631:9–17.

International Chicken Genome Sequencing Consortium. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432:695–716.

Jeffries, K. M., R. E. Connon, C. E. Verhille, T. F. Dabruzzi, M. T. Britton, B. P. Durbin-Johnson, and N. A. Fangue. 2019. Divergent transcriptomic signatures in response to salinity exposure in two populations of an estuarine fish. Evol Appl 12:1212–1226.

Jin, Y. X., J. Y. Lee, S. H. Choi, T. Kim, X. S. Cui, and N. H. Kim. 2007. Heat shock induces apoptosis related gene expression and apoptosis in porcine parthenotes developing in vitro. Anim Reprod Sci 100:118–127.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. S. Genome Project Data Processing. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Li, Y. X., H. G. Jin, C. G. Yan, C. Y. Ren, C. J. Jiang, C. D. Jin, K. S. Seo, and X. Jin. 2014. Molecular cloning, sequence identification, and gene expression analysis of bovine ADCY2 gene. Mol Biol Rep 41:3561–3568.

Liu, Y., Y. Xiong, and D. C. Bassham. 2009. Autophagy is required for tolerance of drought and salt stress in plants. Autophagy 5:954–963.

Liu, Z., N. Yang, Y. Yan, G. Li, A. Liu, G. Wu, and C. Sun. 2019. Genome-wide association analysis of egg production performance in chickens across the whole laying period. BMC Genet 20:67.

Marsjan, P. A., and J. K. Oldenbroek. 2008. The state of the world's animal genetic resources for food and agriculture. Section C: molecular markers, a tool for exploring genetic diversity. 319-333

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, and M. A. DePristo. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303.

Podisi, B. K., S. A. Knott, D. W. Burt, and P. M. Hocking. 2013. Comparative analysis of quantitative trait loci for body weight, growth rate and growth curve parameters from 3 to 72. BMC Genet 14:22.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81:559–575.

Rashidi-Nezhad, A., S. Talebi, H. Saebnouri, S. M. Akrami, and A. Reymond. 2014. The effect of homozygous deletion of the BBOX1 and Fibin genes on carnitine level and acyl carnitine profile. BioMed Central 75, doi:10.1186/1471-2350-15-75.

Romanov, M. N., A. S. Abdelmanova, V. I. Fisinin, E. A. Gladyr, N. A. Volkova, O. A. Koshkina, A. N. Rodionov, A. N. Vetokh, I. V. Gusev, D. V. Anshakov, O. I. Stanishevskaya, A. V. Dotsev, D. K. Griffin, and N. A. Zinovieva. 2023. Selective footprints and genes relevant to cold adaptation and other phenotypic traits are unscrambled in the genomes of divergently selected chicken breeds. J. Anim. Sci. Biotechnol. 14:35.

Rubin, C. J., M. C. Zody, J. Eriksson, J. R. Meadows, E. Sherwood, M. T. Webster, L. Jiang, M. Ingman, T. Sharpe, S. Ka, F. Hallbook, F. Besnier, O. Carlborg, B. Bed'hom, M. Tixier-Boichard, P. Jensen, P. Siegel, K. Lindblad-Toh, and L. Andersson. 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464:587–591.

Shi, S., D. Shao, L. Yang, Q. Liang, W. Han, Q. Xue, L. Qu, L. Leng, Y. Li, X. Zhao, P. Dong, M. Walugembe, B. B. Kayang, A. P. Muhairwa, H. Zhou, and H. Tong. 2023. Whole genome analyses reveal novel genes associated with chicken adaptation to tropical and frigid environments. J Adv Res 47:13–25.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739.

Tang, H., J. Peng, P. Wang, and N. J. Risch. 2005. Estimation of individual admixture: Analytical and study design considerations. Genet Epidemiol. 28:289–301, doi:10.1002/gepi.20064.

Vilella, A. J., J. Severin, A. Ureta-Vidal, L. Heng, R. Durbin, and E. Birney. 2009. EnsemblCompara GeneTrees: Complete, duplication-aware phylogenetic trees in vertebrates. Genome Res 19:327–335.

Wang, K., M. Li, and H. Hakonarson. 2010. ANNOVAR: Functional Annotation of Genetic Variants From High-Throughput Sequencing Data. Nucleic Acids Res. 38:e164, doi:10.1093/nar/gkq603.

Wang, S. H., C. Y. Cheng, P. C. Tang, C. F. Chen, H. H. Chen, Y. P. Lee, and S. Y. Huang. 2015. Acute heat stress induces differential gene expressions in the testes of a broiler-type strain of Taiwan country chickens. PLoS One 10:e0125816.

Wang, X., and R. Wahl. 2014. Responses of the insulin signaling pathways in the brown adipose tissue of rats following cold exposure. PLoS One 9:e99772.

Xiang, H., J. Gao, B. Yu, H. Zhou, D. Cai, Y. Zhang, X. Chen, X. Wang, M. Hofreiter, and X. Zhao. 2014. Early Holocene chicken domestication in northern China. Proc Natl Acad Sci U S A 111:17564–17569.

Xu, N. Y., Z. Y. Liu, Q. M. Yang, P. P. Bian, M. Li, and X. Zhao. 2022. Genomic analyses for selective signatures and genes involved in hot adaptation among indigenous chickens from different tropical climate regions. Front Genet 13:906447.

Xu, N. Y., W. Si, M. Li, M. Gong, J. M. Lariviere, H. A. Nanaei, P. P. Bian, Y. Jiang, and X. Zhao. 2021. Genome-wide scan for selective footprints and genes related to cold tolerance in Chantecler chickens. Zool Res 42:710–720.

Yang, J., S. H. Lee, M. E. Goddard, and P. M. Visscher. 2011. GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88:76–82.

Yang, S., Z. Shi, X. Ou, and G. Liu. 2019. Whole-genome resequencing reveals genetic indels of feathered-leg traits in domestic chickens. J. Genet. 98:47.

Zhang, Q., Y. Li, K. Cao, H. Xu, and X. Zhou. 2023. Transcriptome and proteome depth analysis indicate ABA, MAPK cascade and Ca(2+) signaling co-regulate cold tolerance in Rhododendron chrysanthum Pall. Front Plant Sci 14:1146663.

Zhao, X., J. Zhang, H. Wang, H. Li, C. Qu, J. Wen, X. Zhang, T. Zhu, C. Nie, X. Li, G. Muhatai, L. Wang, X. Lv, W. Yang, C. Zhao, H. Bao, J. Li, B. Zhu, G. Cao, W. Xiong, Z. Ning, and L. Qu. 2022. Genomic and transcriptomic analyses reveal genetic adaptation to cold conditions in the chickens. Genomics 114:110485.