Genomic Dissection of Local Adaptation in Elymus sibiricus: Integrating SLAF Sequencing, Genetic Diversity, Genotype-Environment Association, and Common Garden Experiments

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Research Article

Keywords: adaptative evolution, GEA, GWAS, common garden experiment, Elymus sibiricus, local environment

Posted Date: January 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8447544/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on May 27th, 2026. See the published version at https://doi.org/10.1186/s12870-026-09100-w.

Abstract

Background

Unraveling the genetic basis underlying local adaptation represents a pivotal area of research within ecological and evolutionary biology. In the present study, we explored the genomic mechanisms that underpin the local adaptation of *Elymus sibiricus*, an indigenous grass species predominantly distributed in the temperate alpine regions of the Eurasian continent.

Results

Population structure analyses categorize 318 accessions of *E. sibiricus* into three distinct groups: XJ (Xinjiang), NC (North of China), and the QTP (Qinghai-Xizang Plateau). Furthermore, through selection sweep analysis, we have pinpointed specific genes that show evidence of selection across these three groups, with these genes implicated in pathways related to local climate adaptation. A total of six gene exchange events among the 13 subgroups were detected, and the dynamics of population history revealed marked fluctuations in the effective population size of each subgroup in the recent past. Notably, we observed significant genetic differentiation (as measured by Fst) among the subgroups, suggesting that local climate acts as a primary driver of population structure in different groups of *E. sibiricus*. Consequently, we performed genetic–environment association (GEA) analysis and successfully identified a substantial number of candidate genes that correlate with temperature and rainfall, predominantly involved in pathways such as Phenylpropanoid biosynthesis. In addition, we conducted a three-year common garden experiment in Hongyuan county, Sichuan, assessing six morphological indicators, including adaptive traits such as biomass, followed by genome-wide association (GWAS) analysis.

Conclusion

The genes selected across populations, as well as those associated with local climate and adaptive phenotypes through association analysis, provide insights into the mechanism underlying the broad climate adaptability of *E. sibiricus*.

Introduction

In recent years, there has been an increasing focus within the field of evolutionary biology on understanding the genomic variations that occur during populations differentiation and the various evolutionary forces that drive the differentiation of plant genomes (Seehausen et al., 2014; Strasburg et al., 2012). Adaptive evolution assumes a pivotal role in propelling biological evolution, allowing organisms to adapt and survive in diverse environments, ultimately shaping the process of species evolution and enhancing biodiversity. Local adaptation refers to the occurrence wherein certain essential adaptive traits exhibit genetic differentiation among populations, leading to the individual populations evolving differently in response to the shared environmental stressors, due to their unique genetic

makeup (Kawecki and Ebert, 2004). The current global biodiversity showcases the extraordinary adaptability of numerous species, enabling them to successfully confront and adapt to historical fluctuations in climate (Hampe et al., 2013). This remarkable ability to adapt to evolving environmental conditions has not only ensured the survival but also facilitated the flourishing of these species in diverse habitats around the globe. Nevertheless, concerns are mounting that the current pace of climate change exceeds the capacity of many species to adequately track and adapt to suitable climate habitats. This concern arises from the recognition that the current rate and scale of climate change are unparalleled in recent history. It encompasses habitat fragmentation, climate change itself, and elevated levels of UV-B radiation, all of which pose substantial obstacles for numerous organisms (Hampe et al., 2013). Moreover, limited gene flow between populations experiencing distinct environmental pressures will further enhance their genetic divergence, leading to local adaptation (Slatkin, 1987).

The potential for populations to effectively respond and adapt to shifting selection pressures depends on their ability to generate well-suited phenotypes, such as biomass, that are suitable for their new environments (Tigano and Friesen, 2016). This capacity, in turn, hinges on the presence of genetic variation within a population, which serves as the basis for natural selection to operate upon (Aitken et al., 2008). The existence of significant genetic diversity expands the range of potential traits available for natural selection to act upon, thereby increasing the probability of successful adaptation to new environmental conditions. Conversely, populations with limited genetic variation may encounter challenges in generating the requisite phenotypic modifications that are vital for their survival and reproductive success when confronted with novel selection pressures (Escoda et al., 2022). Thus, the level of existing genetic variation within a population assumes a crucial role in determining its adaptive potential and its capacity to endure and thrive in dynamic environments.

Species with broader ecological niches and distribution are generally regarded as less vulnerable to the impacts of climate change when compared to endemic species with limited distribution (Casazza et al., 2014). *Elymus*, the largest genus in cool-season Poaceae grasses, is renowned for its extensive distribution spanning across Eurasia and northern North America (Sun, 2014). Given its vast geographic range and diverse range of ecological habitats, *Elymus* serves as an ideal model for investigating the adaptability of plants to various environmental conditions. Furthermore, the allopolyploid nature of all *Elymus* species makes them valuable subjects for investigating evolutionary patterns, thereby providing insights into the origin, historical dynamics, and selection processes of other polyploid species. As a model plant of the *Elymus* genus, *Elymus sibiricus* exhibits a wide distribution across Eurasia and is considered as the dominant species in the alpine areas, notably the Qinghai-Tibet Plateau (Xiong et al., 2021). *E. sibiricus* demonstrates robust ecological adaptability, flourishing in diverse habitats such as meadows, bushes, riverbanks, mountain slopes, and valleys. Its natural occurrence extends across a broad range of altitudes, spanning from 1,000 to 4,000 meters (Ma et al., 2012). The remarkable ecological success of *E. sibiricus* in such diverse environments renders it a prime candidate for investigating plant adaptability and exploring the mechanisms that underpin its capacity to thrive in distinct ecological niches. Furthermore, *E. sibiricus* is increasingly regarded as a promising energy crop for cultivation on marginal land. This recognition stems from its attributes, including high biomass

output, effective fermentation properties, and a fiber content that exceeds that of common energy grasses like *Phalaris arundinacea* and *Avena fatua* (Li et al., 2012; Cui et al., 2011).

Through an analysis of the genetic diversity and population structure of various *E. sibiricus* populations, we can gain valuable insights into their gene flow dynamics and potential factors impeding gene exchange. This information is of utmost importance for assessing the potential for genetic exchange and population connectivity, which in turn has implications for the long-term survival and adaptability of the *E. sibiricus*. The development of high-throughput sequencing technology has provided a powerful means to obtain SNP loci at the whole genome level, and based on this, we can identify adaptive loci, elucidate underlying mechanisms, and unveil the selection and evolution patterns of *E. sibiricus*. Here, a total of 318 wild accessions encompassing the main distribution areas of the *E. sibiricus* were conducted with the whole-genome specific-locus amplified fragment sequencing (SLAF-seq). We systematically analyzed the genetic diversity, population structure, population historical dynamics, and gene flow among populations in various geographic populations while exploring SNP variations at the genome-wide level. The selective sweep analysis (combining Fst and Pi) was also used to explore the selected genes of various geographical groups of *E. sibiricus*. In addition, we also conducted gene-environmental association analysis (GEA) to identify the main candidate genes responsible for adapting to the climate factors in the original habitat of the *E. sibiricus*. Ultimately, a subset of 181 accessions was selected for common garden experiments in Hongyuan, located in Aba Prefecture, Sichuan, China. Over a period of three years, six morphological indicators were continuously measured and genome-wide association analysis (GWAS) was performed.

Materials and methods

Sampling and genomic DNA isolation

Our sample set included 318 *E. sibiricus* accessions across Eurasia, thoroughly covering the geographical range and bioclimatic niche of *E. sibiricus* (Table S1). The specimens are deposited in the Sichuan Experimental Base of the *Elymus* Research Institute, Sichuan Agricultural University with the deposition numbers beginning with "ES" (Table S1). All specimens were identified by Prof. Xiao Ma and Prof. Xing Fan of Sichuan Agricultural University. The leaf material of each accession was used to DNA isolation using the CTAB method, and the quality and quantity of DNA were detected using 1% agarose gel electrophoresis and a NanoDrop 2000 UV-Vis spectrophotometers (Thermo Fisher Scientific, Waltham, MA, USA).

SLAF sequencing and variation calling

The strategy of SLAF-seq was performed based on the Sun et al. (2013), which included chromosomal DNA digestion, SLAF fragment tailing with A at the 3' end, ligation of Dual-index adapter, gel electrophoresis, and universal primer PCR amplification. Two enzymes (*Rsa*□ and *Hae*□, New England

Biolabs, Ipswish, MA, USA) were selected to digest the genomic DNA. Illumina Hiseq 2500 platform (Illumina, Inc., San Diego, CA, USA) was used to sequence the libraries using 125-bp paired-end reads. The high-quality reads were mapped onto the reference genome of *E. sibiricus* (Li et al., 2025) using BWA software, the shared SNPs obtained by GATK and SAMtools software were considered as the final SNP dataset, in a vcf format. The vcf file was analyzed using vcfR packages (Knaus and Grünwald, 2017). The SNPs with missing data ≥ 10% were filtered, and SNPs with the minor allele frequency > 5%, and depth > 4 were collected for subsequent analysis.

Population genetic analysis

To infer the genetic relationships of all the 319 samples, phylogenetic inferences, principal components analysis (PCA), discriminant analysis of principal components (DAPC), and STRUCTURE were used. Phylogenetic relationships of individuals of *E. sibiricus* were constructed using the neighbor-joining method in the program MEGA version 10.0 (Kumar et al., 2018). The PCA and DAPC were analyzed using Plink2 and R package 'adegenet' (Jombart et al., 2010), respectively. The Bayesian-based STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to infer the genetic memberships of the test accessions, with the number of genetic clusters (K) was setting from 1 to 10 with 10 replicates for each value of K. The number of Markov Chain Monte Carlo replications and the length of the burn-in period were set as 50,000 and 10,000. The optimal K value was identified using average likelihood and ΔK statistics (Evanno et al., 2005). Finally, CLUMPAK (Kopelman et al., 2015) was used to estimate the membership coefficients at the individual level. The Pi values of each subgroup were calculated using vcftools (Danecek et al., 2011).

Demographic history and gene flow

The effective population size of each group was estimated using smc^{++} (Terhorst et al., 2017), which possessed a higher accuracy in recent past. To gain an understanding of the overall population structure, the population genetic distance was calculated and the phylogenetic tree was constructed using vcfR package. The population divergence degree was reflected by pairwise Fst and gene flow events among those populations. The Fst values between two subgroups were calculated based on vcfR (Knaus and Grünwald, 2017), adegenet (Jombart, 2008), and hierfstat (Goudet, 2005) R packages. The migration patterns among the tested populations were inferred using TreeMix v 1.13 based on the vcf file. The possible migration events (m) were set as 0–10.

Bioclimatic data

The 19 bioclimatic variables of the 318 *E. sibiricus* accessions were extracted in DIVA-GIS software (Hijmans et al., 2001) based on their longitude and latitude. The correlation plot was conducted using PerformanceAnalytics R package (Peterson et al., 2018). The density distributed based on the bio1

(annual mean temperature) and bio12 (annual precipitation) of the tested accessions was plotted using ggpointdensity R package (https://github.com/LKremer/ggpointdensity).

Association analysis

The optimal model was performed with Q + K using GEMMA pipeline (Vogt et al., 2022). The genetic relationships of the tested individuals were taken into consideration based on the kinship and Q matrix generated from Admixture (Alexander and Lange, 2011). GEC software (http://pmglab.top/gec/#/) was used to estimate the suggested *P* value which indicated that the suggested *P* is 1e-5. The gens within 100 Kb of a significant SNP's flanking region were considered as candidate genes.

Selective sweep

The population divergence index (Fst) and nucleotide divergency (Pi) were combined to identify the selected regions between two groups. The pairwise Fst values and Pi of each group were both calculated using vcftools (Danecek et al., 2011) with window-size of 1,0000 and window-steps of 1,000. Then the Fst and Pi were combined to identify the selected regions and genes using fst_pi_select_sweep.r R package.

Common garden experiment and phenotypic indicator determination

The 181 *E. sibiricus* accessions were randomly selected from the 318 samples and cultivated in a common garden at Hongyuan, Sichuan (with the altitude of 3620 m) in 2019 year. The biomass (dry yield, BM), plant height (PH), stem thick (ST), Flag leaf length (FLL), flag leaf width (FLW), internode number (IN) of individual were measured in boot stage during 2020–2022 years, with six replications.

Results

SLAL-seq data set

After the filtering process, each accession produced about 1.0 Gb of high-quality clean reads, totaling 6,481,696 reads, with a Q30 ratio of 95.09% and a GC content of 46.91% (submission number, Table S1). Sequence alignment with the reference genome of *E. sibiricus* identified approximately 7,535,251 SLAF tags distributed across all 14 chromosomes (Fig S1A). Of these SLAF tags, 3,359,774 (44.59%) were found to be polymorphic. The average depth of SLAF tags varied from 5.05 to 36.84-fold for each accession of *E. sibiricus*. The distribution of the obtained SNPs across all the chromosomes of *E. sibiricus* resulted in a total of 260,281 high-quality variants (Fig S1B), confirmed by the high mapping quality of the vcf file, considering parameters such as read depth, mapping quality, and Phred-scaled quality (Fig. 1).

Information and population structure of 318 E. sibiricus accessions

We collected 318 wild accessions from the main distribution areas of *E. sibiricus*, including Xinjiang (XJ, China), the Qinghai-Tibet plateau (QTP, including Qinghai (QH), Sichuan (SC), Gansu (GS), and Tibet (Xizang, XZ)), North China (NC), North Asia (NA), Europe (EU), and central Asia (CA). Based on their geographical distribution, those accessions were further divided into 13 sub-populations (Fig. 2A, Table S1), among which EU and CA groups each contained only one accession, respectively. The main climate factors with significant variations in the collection sites of these wild germplasms include annual mean temperatures of 1.516 to 2.421℃, annual precipitation of 385.757 to 438.867 mm, and 5.777 to 6.645 ℃, and 366.709 to 409.014 mm (Fig. 2B).

The results of the phylogenetic tree revealed the categorical division of test accessions into three distinct groups: Group I, Group II, and Group III. Notably, Group I primarily comprised accessions from NC, Group II consisted mainly of accessions from XJ, and Group III mainly included accessions from the QTP region (Fig. 2C). Group Ⅱ could be further divided into Group Ⅱ-1 and Group Ⅱ-2. Additionally, a principal component analysis (PCA) was conducted, demonstrating that PC1 accounted for 45.75% of the total variance, while PC2 explained 8.79% of the total variance (Fig. 2D). The PCA plot illustrated that PC1 could separate the test accessions into three groups. The population structure analysis also exhibited three distinct genetic memberships, with accessions from the QTP region displaying a notably different genetic background compared to those from NC and XJ regions (Fig. 2E and 2F).

Genetic differentiation between different subgroups

The observed Pi values in each subgroup demonstrated higher genetic diversity in *E. sibiricus* accessions from the QTP region, as evidenced by the relatively higher Pi values in the GS, QH, and SC subgroups (Fig. 3A). The Fst values calculated between each subgroup indicated that the largest genetic divergence was observed between the EU subgroup and the other eight subgroups, with the Fst values higher than 0.25. High genetic divergence was also observed between the QH and CA subgroups, as well as between the QH and NA1 subgroups. A TreeMix analysis was conducted to infer the historical relationships and migration patterns between these populations, identifying a total of six gene flow events among subgroups (Fig S2, Fig. 3B). At the early stage, relatively strong gene flow events from SC to XZ and NA2 were observed, while relatively weak gene flow was detected from QH to NC1 during this time.

Demographic analysis

According to SLAF-sequencing data, past demographic patterns of 13 subgroups revealed relatively stable population effective size (*Ne*) in the past. However, complex demographic fluctuations were observed in more recent times (Fig. 4). A unique demographic pattern emerged in the GS subgroup, characterized by three significant declines in *Ne* occurring approximately between 50 million years ago (Mya) and 2.85 years ago. In recent years, however, the *Ne* of the GS subgroup has experienced a continuous increase. The NA2 subgroup displayed a similar *Ne* pattern to NA3, with both experiencing two decreases followed by a slowly gradual increase in recent times. The *Ne* patterns of the remaining

subgroups remained consistent until approximately 3.52 years ago. Among them, only the XJ (Xinjiang) and XZ (Tibet) subgroups showed a trend of increase in *Ne*.

Bioclimatic data and gene-environment association (GEA) study

The bioclimatic and geographical datasets used in this study included 19 bioclimatic factors, longitude, latitude, and altitude for 318 wild accessions of *E. sibiricus*. A pairwise correlation analysis was performed on these data and its results are presented in Fig S3. According to the criterion of a correlation coefficient greater than 0.8 or lower than − 0.8, only nine bioclimatic factors with relatively important biological significance were retained from the initial datasets (Fig. 5A). These retained factors were considered to have a strong influence on the biological characteristics of *E. sibiricus*, with bio12 (annual precipitation) recognized as a critical factor influencing plant growth and development. To further explore this, a gene-environment association (GEA) study was conducted to identify SNP markers associated with the bio12 (Fig. 5B). The QQ plot showed a significant departure from the expected distribution, indicating the presence of genetic variants associated with the bio12 (Fig. 5C). A total of 47 significant SNP markers associated with 159 genes were identified, mostly contributing in H03, H07, and S04 chromosome. Seventy of these genes showed differential expression in the drought transcriptome (Xiong et al., 2024, Table S2). Additionally, KEGG enrichment analysis showed that these genes mainly enriched in metabolism, especially carbohydrate and carbon metabolism (Fig. 5D). Especially, signal transduction pathways were also enriched in environmental information processing.

Following the initial analysis, the variables bio1, bio2, bio5, bio6, bio12, bio14, and bio15 were further categorized into two groups: the first consisting of bio1, bio2, bio5, and bio6, associated with temperature, and the second consisting of bio12, bio14, and bio15, related to precipitation. Further, a total of 36, 550, 24, and 173 genes were identified as candidate genes associated with temperature factors including the bio1, bio2, bio5, and bio6, among which 70 genes were successfully annotated in the KEGG database (Table S3). The most enriched pathways consisted of Ribosome (10 genes), Phenylpropanoid biosynthesis (9 genes), Ubiquitin mediated proteolysis (8 genes), and Protein processing in endoplasmic reticulum (8 genes). Especially, we found that two genes *EsiH02g0025590* and *EsiH02g0025600* were shared in the association result of bio2 and bio6 (Fig. 6A, B), annotated with Zinc finger and Myb/SANT-like DNA-binding domain in the Pfam database. Moreover, a total of 77, 95, and 74 genes were associated with precipitation factors including bio12, bio14, and bio15, respectively, among which 67 genes were annotated in the KEGG database (Table S3). The most enriched pathways consisted of Phenylpropanoid biosynthesis (6 genes) and Biosynthesis of amino acids (5 genes). We also found two genes *EsiH02g0043840* and *EsiH02g0043850* shared in the association result of bio 12 and bio15 (Fig. 6C, D). Overall, the candidate genes associated with these important climatic factors, particularly the shared genes, were crucial in the environmental adaptation process of *E. sibiricus*.

Selective sweep analysis

Based on the results of the PCA and phylogenetic tree, the tested accessions of *E. sibiricus* could be divided into three distinct groups, namely, XJ, QTP, and NC. To investigate the evolutionary and adaptational mechanisms of these groups, the selected regions and genes of each group were identified by combined the Fst and Pi values (Fig S4). A total of 215, 86, and 67 genes were identified as the selected genes between NC vs XJ, QTP vs NC, and XJ vs QTP, respectively. KEGG annotation results indicated that the genes selected between NC vs XJ were mostly annotated in Carbon metabolism (5 genes), Phenylpropanoid biosynthesis (4 genes), and Fatty acid biosynthesis (4 genes) (Table S4). The genes selected between QTP vs NC, and XJ vs QTP were significantly enriched in Glycerophospholipid metabolism and Plant-pathogen interaction (both containing 2 genes), and beta-Alanine metabolism and Plant-pathogen interaction (both containing 2 genes).

Common garden experiment and genome-wide association study (GWAS)

In the common garden experiment and genome-wide association study (GWAS), the Pearson correlation analysis of biomass (BM), plant height (PH), stem thickness (ST), flag leaf length (FLL), flag leaf width (FLW), and internode number (IN) of 181 accessions is shown in Fig. 7A, indicating that all pairwise correlation coefficients are less than 0.8. The PCA analysis revealed distinct patterns and relationships among these phenotypic indicators (Fig. 7B). The first and second components accounted for 44.1% and 25.8% of the total variance, respectively. In particular, the phenotypic indicators can be categorized based on the first component. Our focus was on the biomass (BM) associated genes, and the Manhattan map and QQ plot were displayed in Fig. 7C and D. A total of 180 genes were considered as the candidate genes associated with the BM, and were mostly annotated in Oxidative phosphorylation, Protein export, Pyrimidine metabolism, and Starch and sucrose metabolism (Table S5).

Discussion

High-throughput sequencing technology has emerged as an invaluable tool for unraveling population structure and demographic history. By harnessing this technology, we can gain a comprehensive understanding of the intricate genetic relationships among different populations and the evolutionary forces that have shaped their genetic diversity over time. Here, the whole-genome SLAF sequencing was used to explore population architecture, demographic history, and genetic differentiation of *E. sibiricus* in different populations and/or wild accessions. Furthermore, GEA and GWAS analysis using morphological indicators identified abundant candidate genes in shaping the evolutionary patterns and controlling the phenotypic diversity of *E. sibiricus*.

Selective sweeps and polygenic adaptation drive genomic architecture of E. sibiricus

This study indicates that local adaptation to climate in *E. sibiricus* influenced by ongoing diversifying and stabilizing natural selection acting on multiple genes associated with local environmental variables, highlighting a complex adaptive architecture. Climate adaptation in *E. sibiricus* is primarily governed by

numerous genes with small individual effects that are dispersed throughout the genome, implying the presence of pervasive genomic alterations in line with polygenic adaptation.

We found that 318 *E. sibiricus* accessions could be significantly divided into three groups (XJ group, NC group, and QTP group), based on their chloroplast sequences (Xiong et al., 2020). The pairwise Fst values across 13 subgroups showed clear genetic differentiation among these subgroups, suggesting that the primary climatic factors exert selective pressures on various geographical populations. The QTP group has a unique genetic background, as confirmed in other population structure studies of *E. sibiricus*, including chromosome karyotype analysis (De et al., 2014), RAPD marker (Ma et al., 2012), and chloroplast DNA study (Xiong et al., 2020). The population in the QTP underwent significant natural pressures to adapt to the harsh environmental conditions found in high-altitude regions (Zhang et al., 2022). These pressures include low atmospheric pressure, intense radiation exposure, rapid temperature fluctuations, and the challenges of surviving winters. A total of 86 and 67 genes were under selection between NC vs QTP and XJ vs QTP, mainly responding to Phenylpropanoid biosynthesis, Porphyrin and chlorophyll metabolism, Propanoate metabolism, and oxidative phosphorylation. These pathways have been proven to be important in resisting low temperature (Pappi et al., 2021; Zhang et al., 2022), UV-B (Liu et al., 2015), and hypoxia stress (Fan et al., 2013).

The unique terrain conditions of Xinjiang, known as the "three mountains sandwiched between two basins," have allowed the native plant in this region to have their own unique evolution and differentiation models, leading to geographical isolation from other regions. In addition, mountain landforms play a role in species distribution and population isolation. As such, the XJ population can be clearly distinguished in phylogenetic tree and PCA analysis, with no gene flow detected between XJ and other subpopulations. The largest number of selected genes (215 genes) were found in XJ vs NC group, with several pathways including Phenylpropanoid biosynthesis and Fatty acid biosynthesis playing an important role in plant resistance to drought stress (Fini et al., 2012; Zhao et al., 2021; Gu et al., 2020). This may be due to the low rainfall in Xinjiang, which has led to the evolution of genes related to drought resistance in the local *E. sibiricus* accessions.

Selection signals are scanned between XJ, NC and QTP, indicate that directional or diversified natural selection exists among different geographical groups of *E. sibiricus* (XJ, NC, and QTP), as reflected in the diverse genes related to many key metabolic processes. This suggests that the genome of *E. sibiricus* has the potential to actively respond to rapid climate change, which benefits its long-term survival. This adaptive response is most likely aided by standing genetic variation, commonly expected in long-generation species (Chhatre et al., 2019), with QTP groups possessing stronger adaptability, as seen in their generally higher Pi values. However, according to the population historical dynamics, certain regions of the QTP, particularly SC and QH, have undergone a significant decrease in their effective population size over time. Additionally, recent climate data indicates abnormal high temperatures during the summer and frequent occurrences of extreme weather events poses a formidable challenge to the survival of the indigenous *E. sibiricus* in these areas, diminishing its ability to adapt. In this case, gaining an understanding of the specific regions and genes selected in response to the local climate

environment among different geo-populations can provide a foundation for expanding the adaptable regions of *E. sibiricus*.

Functional genes shaped by the environmental factors and biomass

Selective environmental factors play a crucial role in the local adaptation, genetic diversity, population structure of wild accessions (Cortés and Blair, 2018). Thus, employing climatic variables as indicators of selective environmental pressure can be a valuable approach to capture fundamental aspects of the mechanisms underlying resistance and tolerance to abiotic stress (Gómez-Espejo et al., 2022). Evidence of local adaptation patterns has been detected across various genomic regions spanning all 14 *E. sibiricus* chromosomes. It is worth noting that each climate variable has different biological significance in forming adaptive responses, exhibiting different genomic associations.

Temperature and rainfall are the main environmental factors affecting the growth and development of plants, and reflect their adaptation to high/low temperature and drought/waterlogging. Furthermore, according to the Maxent model of *E. sibiricus*, temperature and rainfall are the main limiting factor for the distributions of *E. sibiricus* (Xiong et al., 2020). This study detected abundant SNPs and candidate genes significantly associated with temperature and rainfall in the original habitat of *E. sibiricus* through GEA analysis. The genes associated with temperature-related variables mainly involved in Phenylpropanoid biosynthesis, Ubiquitin mediated proteolysis, and Protein processing in endoplasmic reticulum, and their role in resisting high/low temperature stress in multiple species has been confirmed (Meng et al., 2022; Ding et al., 2020). Furthermore, genes associated with rainfall have also enriched many important biological pathways. It is worth noting that the Phenylpropanoid biosynthesis has been enriched in GEA analysis of temperature and rainfall, suggesting its importance in the climate adaptation of *E. sibiricus*.

Phenotypic plasticity, which refers to the genotype's ability to exhibit various phenotype (morphological, physiological, and behavioral traits) in response to different environmental conditions, is crucial to a plant's adaptability (Sommer, 2020). Biomass serves as a comprehensive indicator of plant growth status and adaptability in specific environments (Mehlferber et al., 2022), making it an important breeding objective pursued by numerous grass breeders. This study performed a common garden experiment and GWAS analysis of *E. sibiricus* cultivated in high-altitude areas for three years, detecting 180 genes associated with biomass, with these genes mainly participating in Oxidative phosphorylation, Protein export, Pyrimidine metabolism, and Starch and sucrose metabolism.

The adaptability of plants is their ability to cope with changing environments, which is a complex process achieved through genetic variation and evolution (Anderson et al., 2011). Therefore, understanding the genetic diversity of as many genotypes as possible for a specific species can provide a theoretical basis for its adaptability research. Additionally, the common garden experiment provides insights into whether the adaptability of plants to their natural environment can keep up with climate

change (Anderson and Wadgymar, 2020). The combination of these two factors can provide a comprehensive understanding of the existing and potential adaptability of a species.

Abbreviations

QTP
Qinghai-Xizang Plateau
GEA
genetic–environment association
GWAS
genome-wide association analysis
SLAF-seq
specific-locus amplified fragment sequencing.

Declarations

Ethics approval and consent to participate

All plant materials used in this study were collected and utilized in compliance with relevant laws, regulations, and ethical guidelines. Permission for the field collection of wild germplasm was obtained through an official permit issued by the Sichuan Forestry and Grassland Bureau. The remaining wild materials were sourced from the publicly available collections of the National Plant Germplasm System (NPGS) of the United States Department of Agriculture Agricultural Research Service (USDA-ARS). No collections were made on private land.

Consent for publication

Not applicable.

Availability of data and materials

The raw data of SLAF sequencing has been uploaded to the China National GeneBank DataBase (CNGBdb, https://db.cngb.org/cnsa/submit/) with the CNGBdb Project of CNP0004533.

Competing Interests

The authors declare that they have no competing interests.

Funding

This study was funded by National Natural Science Foundation of China (32471764), National Key Research and Development Program (2024YFD1301202), and Sichuan Forestry and Grassland Science and Technology Innovation Team-Grass Breeding and Innovation (CXTD2025005).

Authors' contributions

YX, QQY, and YLX conceived and supervised the study. YX and QQY performed the experiments and analyzed the data. YX, YLX, JMZ and XM drafted the manuscript. XFJ, XL, JBZ and XM revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank Genepioneer Biotechnologies for their technical support. We also are grateful to the editors for processing the manuscript and to the reviewers for their constructive comments.

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Figures

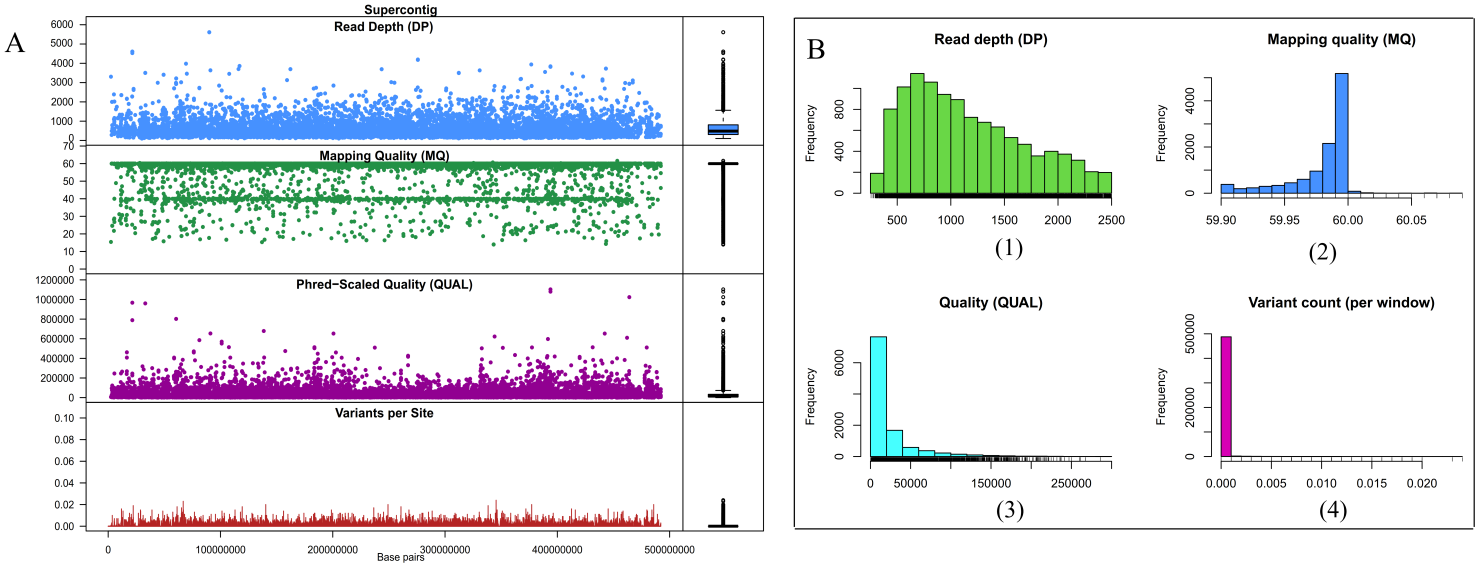

Figure 1

The quality statistics of the vcf file (A). x-axis indicated the position of the *E. sibiricus* genome. B, the distribution histogram of the read depth (1), mapping quality (2), quality (3), and variant count (4).

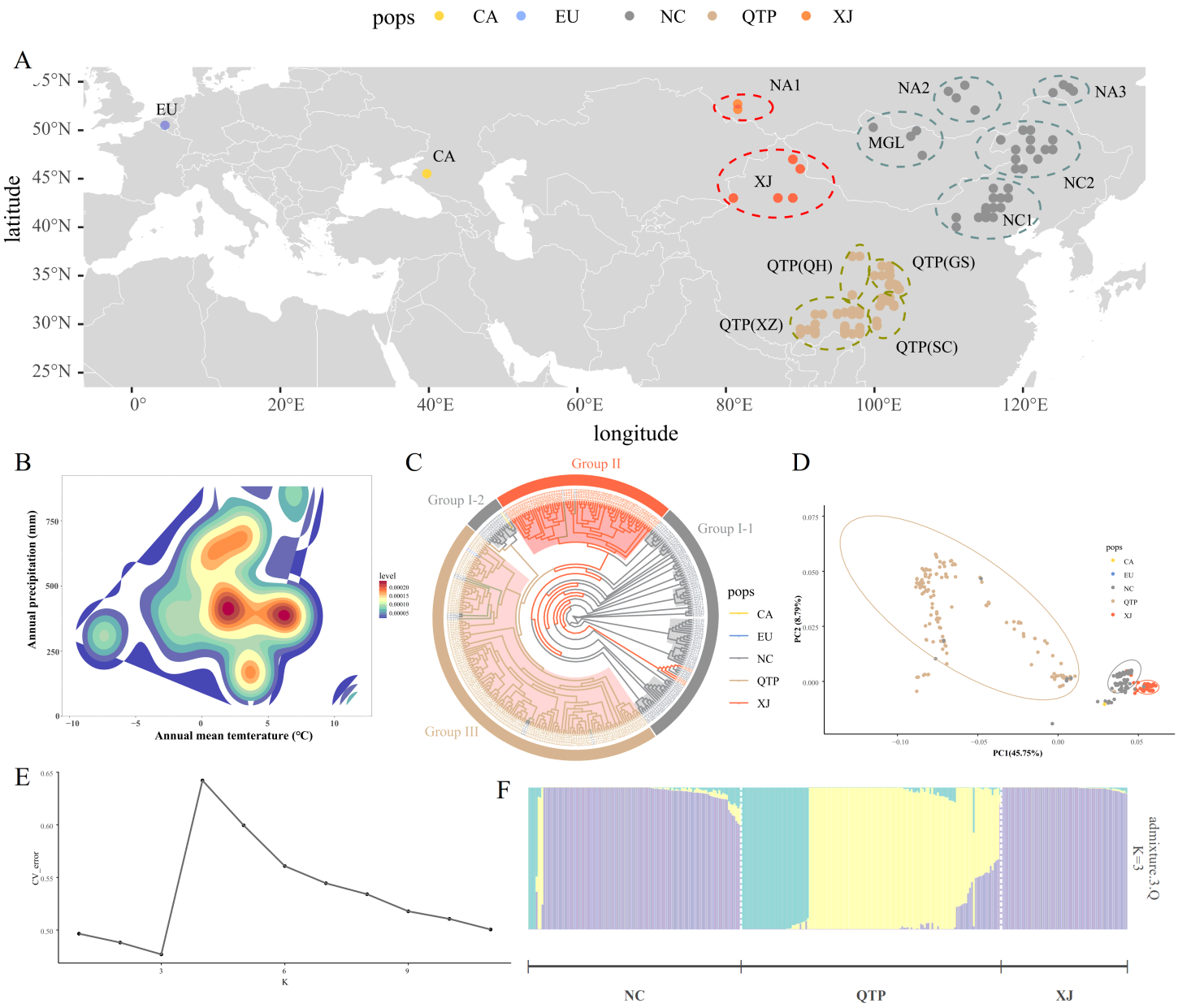

Figure 2

The information of the tested accessions and their population structure. A, the geographical location of the 318 *E. sibiricus* accessions; CA, central Asia; EU, Europe; NC, north China; QTP, Qinghai-Xizang plateau; XJ, Xinjiang, China. B, density distribution of the tested accessions based on their annual mean temperature and annual precipitation. C, the phylogenetic tree of the *E. sibiricus* accessions. D and F, the PCA plot and genetic memberships of the tested accessions. E, the optimal of the K value was 3.

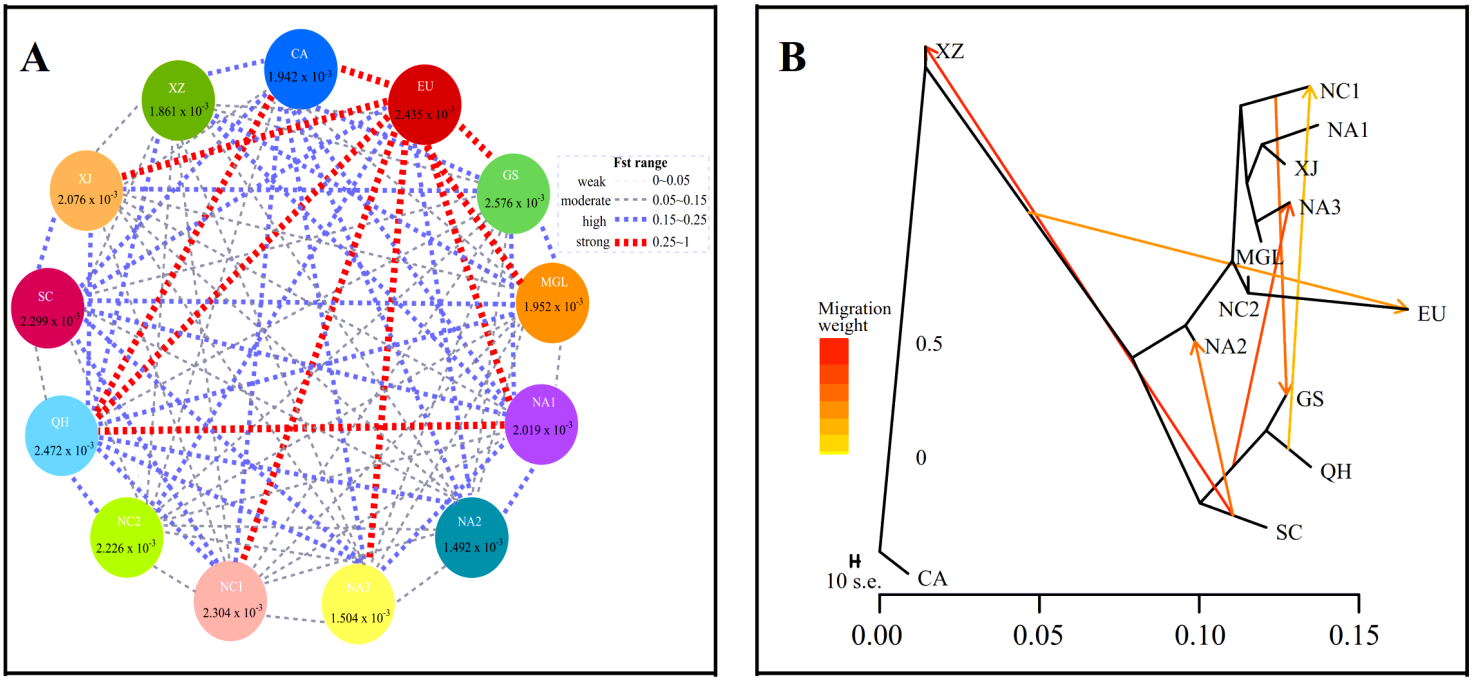

Figure 3

A, the pairwise population divergence index among 13 subgroups. The redder and thicker the line, the greater the differentiation coefficient between populations. The numbers inside each circle represent the Pi values of each subgroup. B, the Treemix result of the subgroups with the outgroup of the CA. Arrows indicate the direction of gene flow.

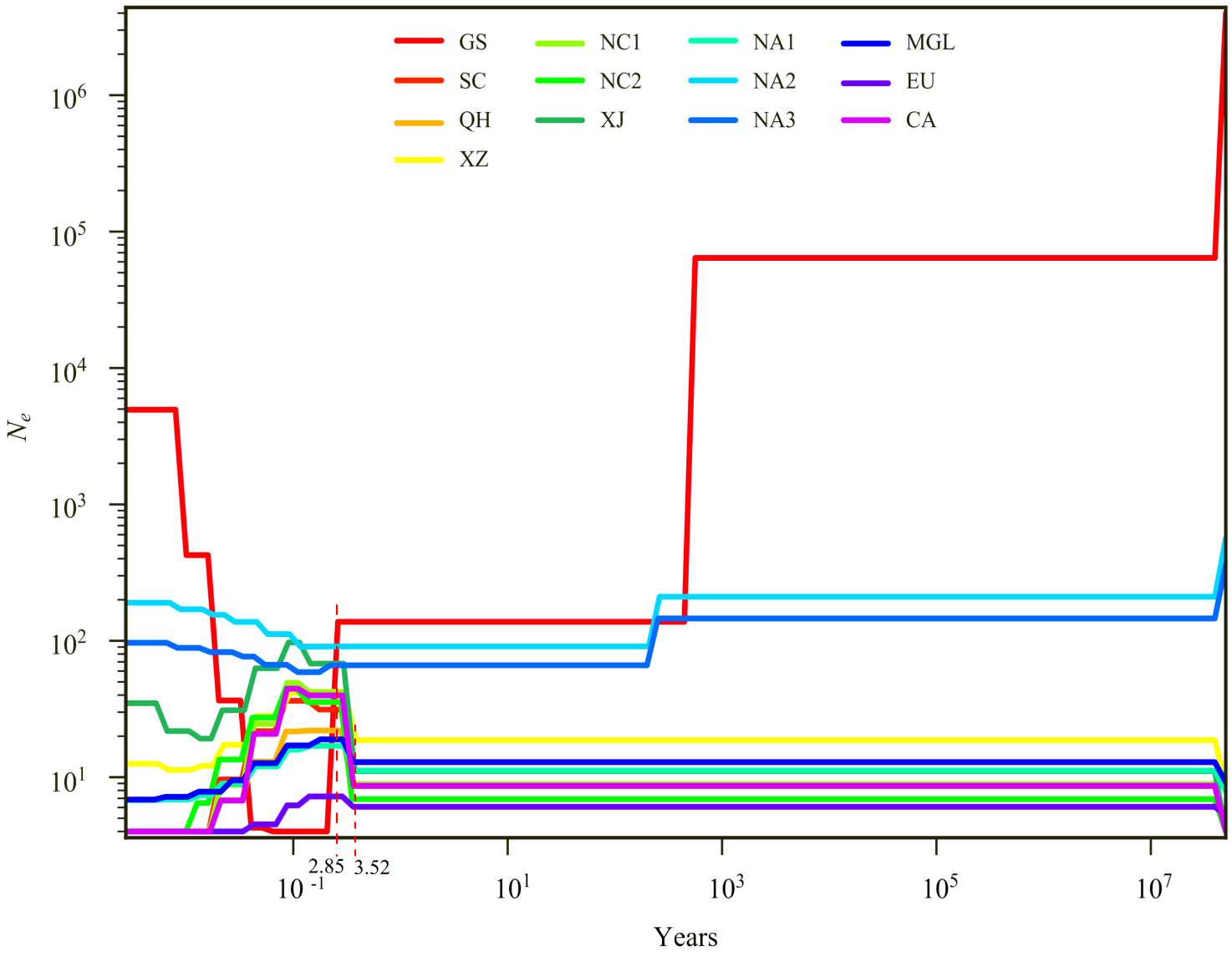

Figure 4

The effective population size of the 13 subgroups of the *E. sibiricus*.

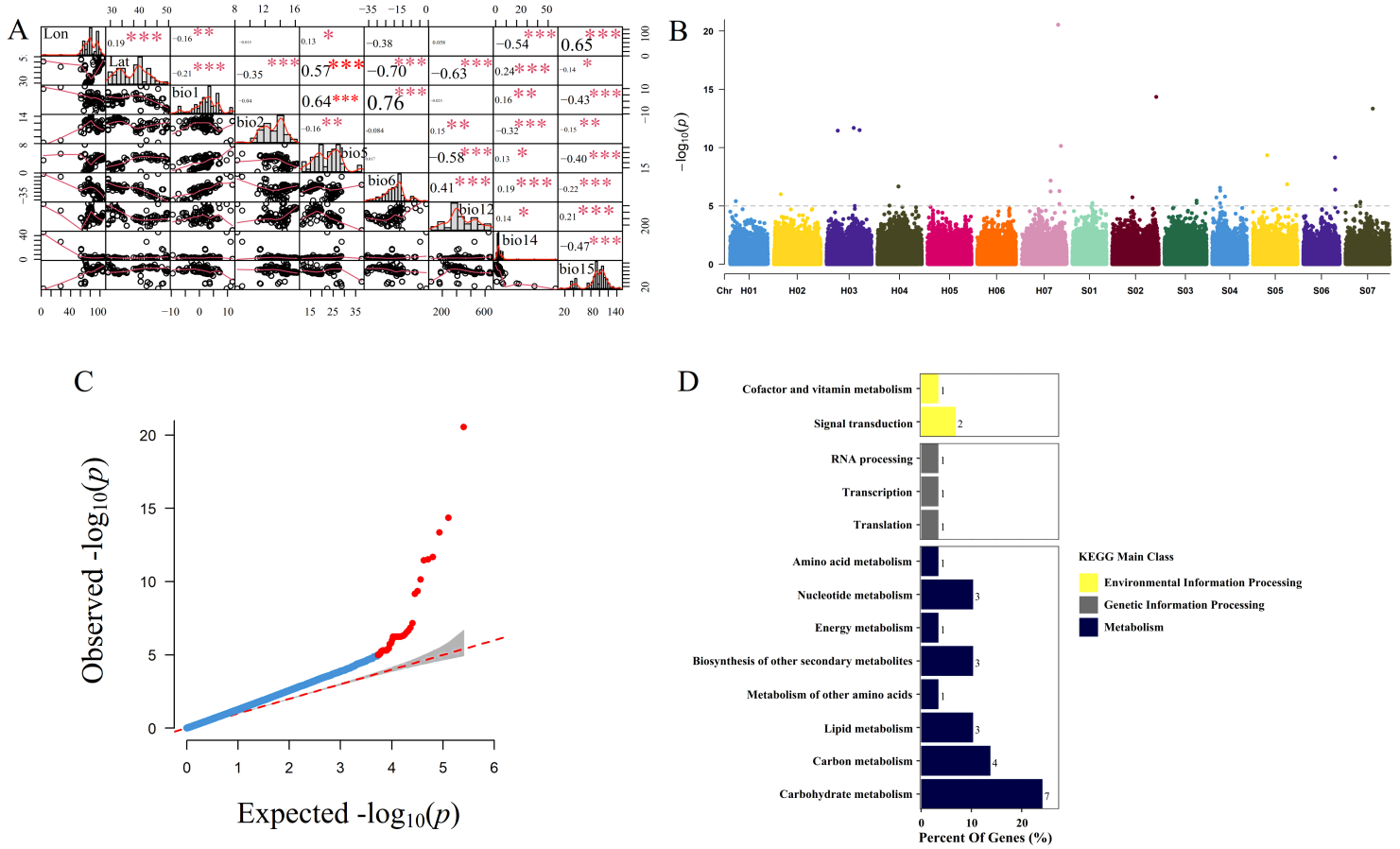

Figure 5

The correlation among the latitude, longitude, and seven climatic factors of the tested accessions (A). The Manhattan map and QQ plot based on the bio 12 (B, C). The KEGG annotation of the shared genes between GWAS and transcriptome under drought stress (D).

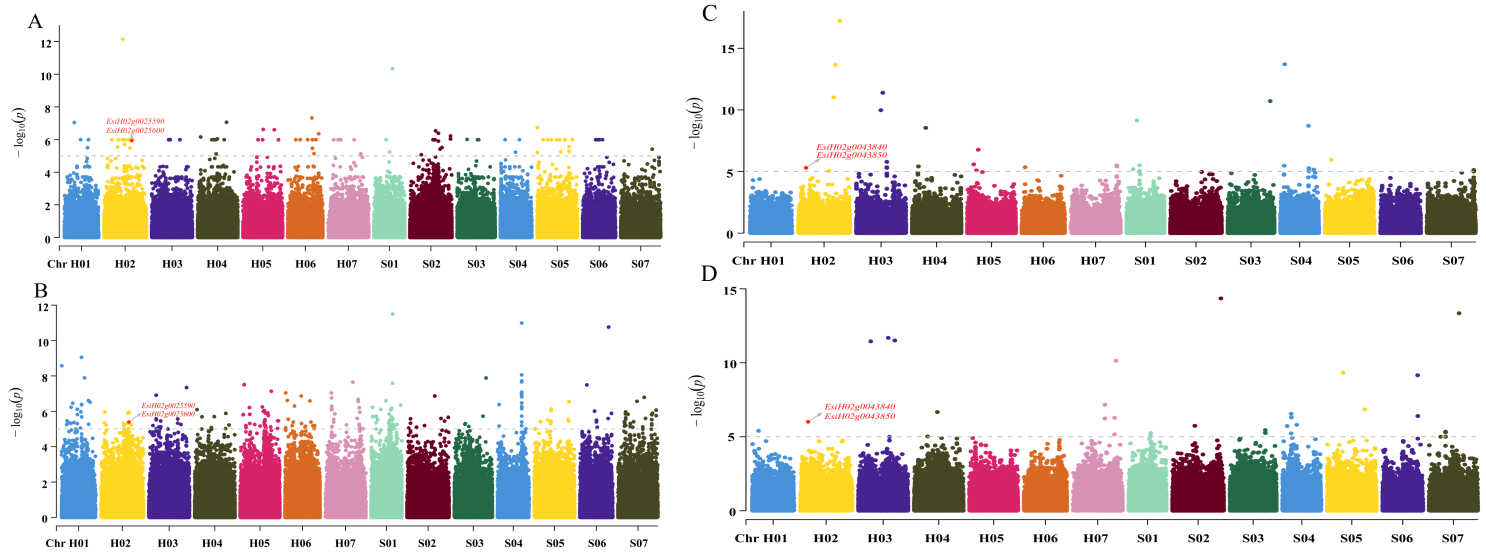

Figure 6

The Manhattan plots based on the bio2 (A) and bio6 (B), and bio12 (C) and bio15 (D). The shared associated genes between bio2 and bio6, and bio12 and bio15 were showed.

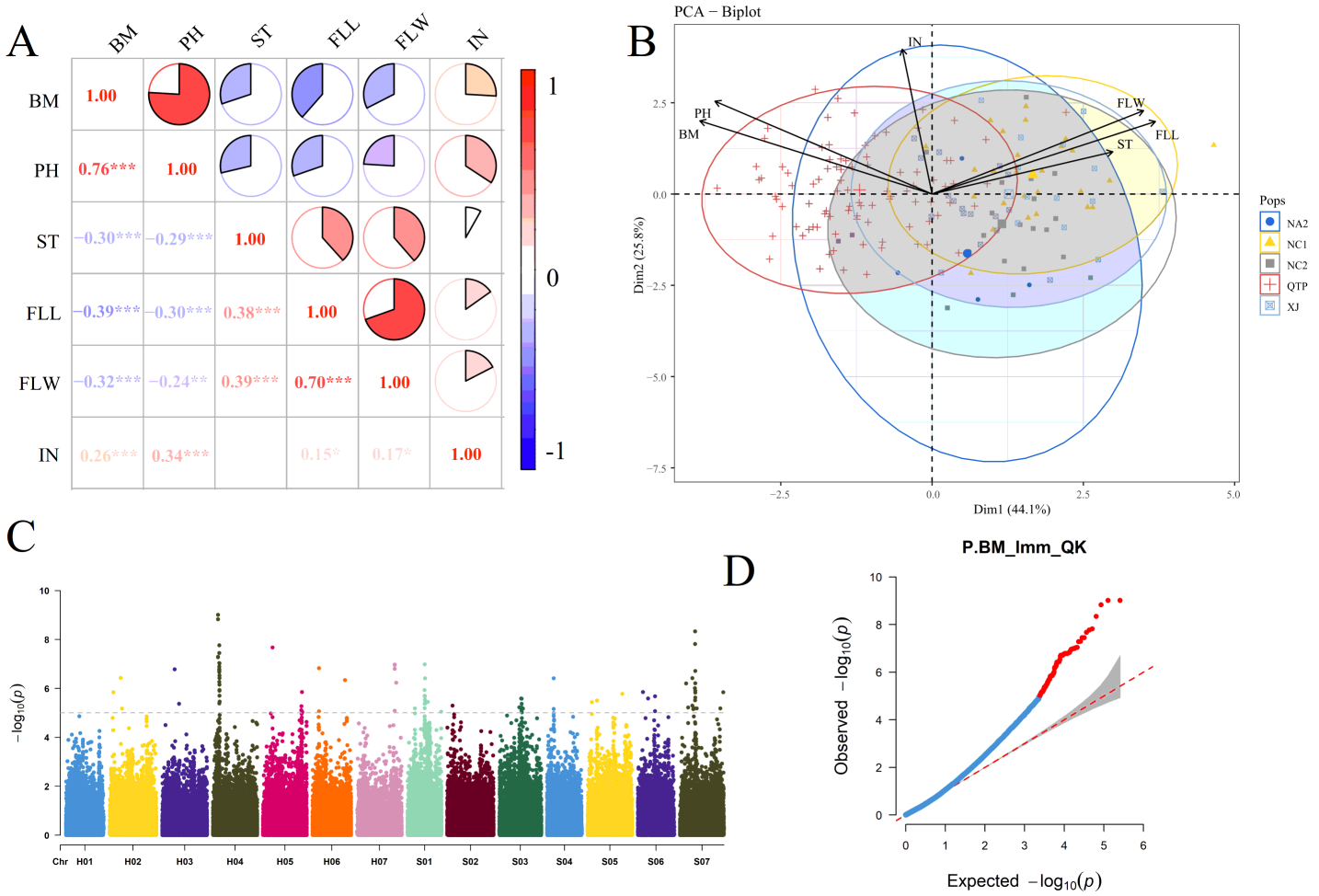

Figure 7

The correlation and principal components analysis plot of the six phenotype indicators (A). BM, biomass; PH, plant height; ST, stem thick; FLL, Flag leaf length; FLW, flag leaf width; IN, internode number. The Manhattan map and QQ plot based on the BM.

Supplementary Files

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- TableS2.xlsx
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