单环刺螈耐高温性状全基因组关联分析及全基因组选择研究

Genome wide association study and genomic selection of heat tolerance in *Urechis unicinctus*

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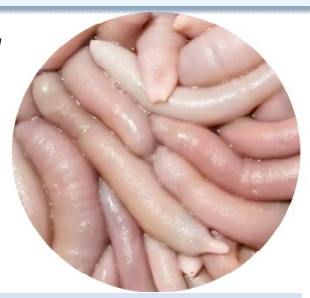
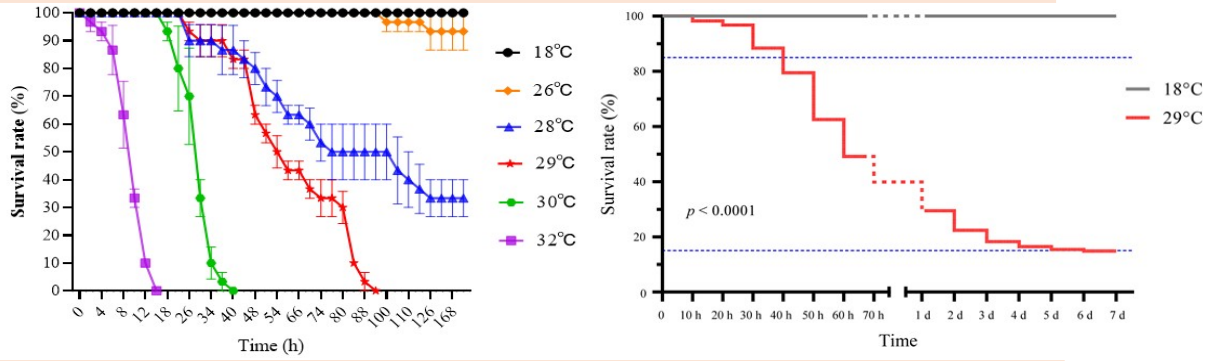


Abstract

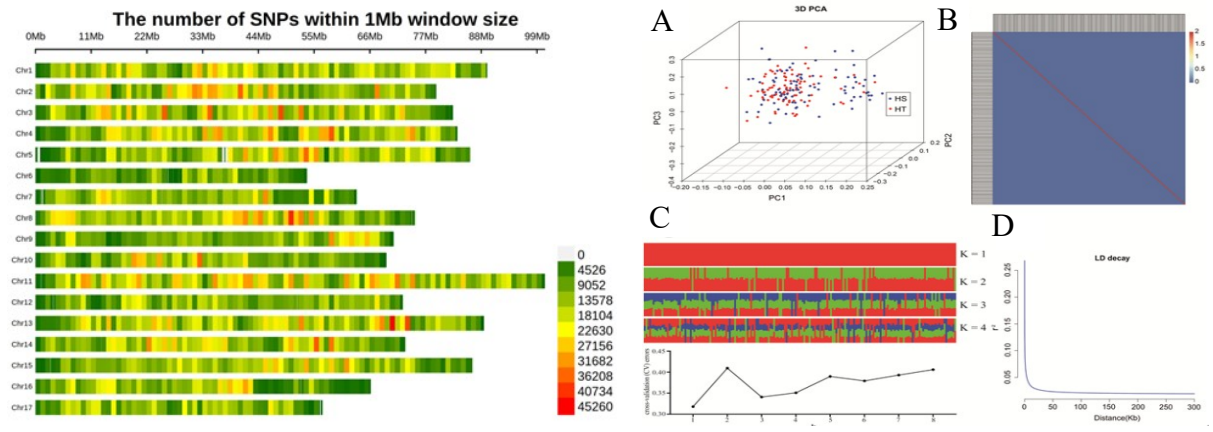
Urechis unicinctus is an emerging aquaculture species with high economic value. It is rich in protein and essential amino acids, and its nutritional value is similar to that of sea cucumbers, showing very high nutritional quality. Due to the influence of temperature, *U. unicinctus* is mainly distributed along the coastal areas of the Bohai Sea in China. In addition, high temperatures in summer can cause severe mortality in aquatic animals, which poses a challenge to global aquaculture and also restricts the development of *U. unicinctus* farming. To identify SNPs and candidate genes related to heat tolerance will help in breeding heat tolerant *U. unicinctus*, enabling better adaptation to high summer temperatures and expansion of its cultivation range. This study first evaluated the heat tolerance of *U. unicinctus* and selected 29℃ for screening heat tolerant individuals. Genotyping was performed using whole-genome resequencing. After filtering, a total of 17,693,070 high quality SNPs were obtained for genome wide association analysis (GWAS). A total of 209 heat tolerance related SNPs and 318 candidate genes were identified. Fine-mapping was used to analyze the significant heat tolerant SNPs and identify causal variants. Sanger sequencing was performed on a validation population to confirm the genotyping results. The results showed that this SNP is significantly associated with heat tolerance, providing a reference for marker-assisted selection (MAS). By integrating GWAS and transcriptome data, six genes that contained significant heat tolerant SNPs were determined to be related to heat tolerance. Additionally, RNA interference was performed on the screened heat tolerant *CAD* gene. The results showed that the heat tolerance of *U. unicinctus* was significantly reduced after *CAD* gene interference, confirming its important function in heat resistance. Furthermore, a comparative evaluation of seven genomic selection (GS) models (RRBLUP, GBLUP, BayesA, BayesB, BayesC, Bayes LASSO, and Bayesian Ridge Regression) revealed that RRBLUP was more suitable for genomic prediction of heat tolerance in *U. unicinctus*. These results provide a foundation for understanding the molecular mechanisms of heat tolerance in *U. unicinctus*, marker assisted selection, and the breeding of heat tolerant varieties, which will accelerate the development of its aquaculture industry.

Results and Discussion

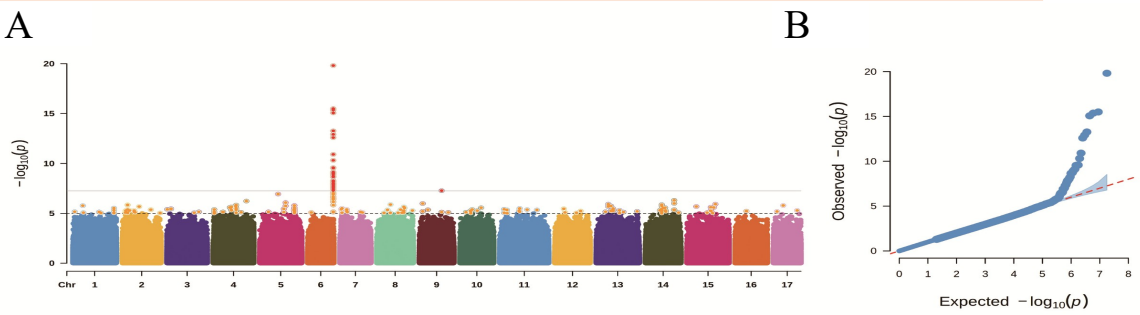
1 Identification of heat sensitive and heat tolerant individuals under 29°C heat stress



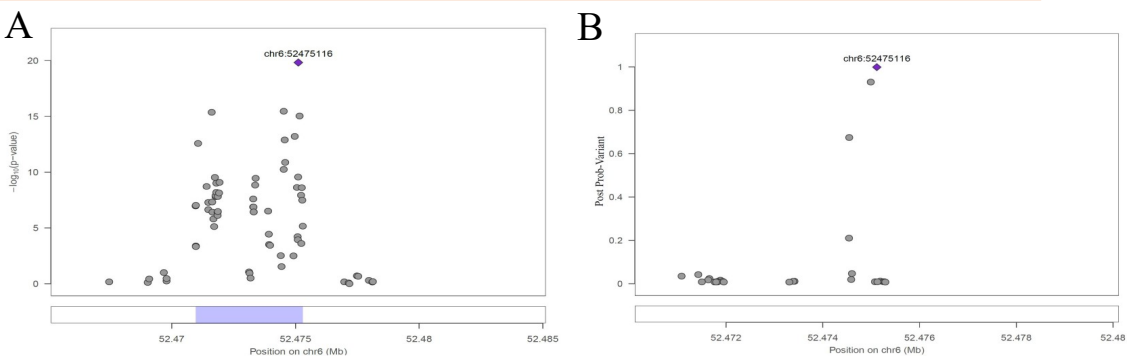
2 Population genetic structure and linkage disequilibrium



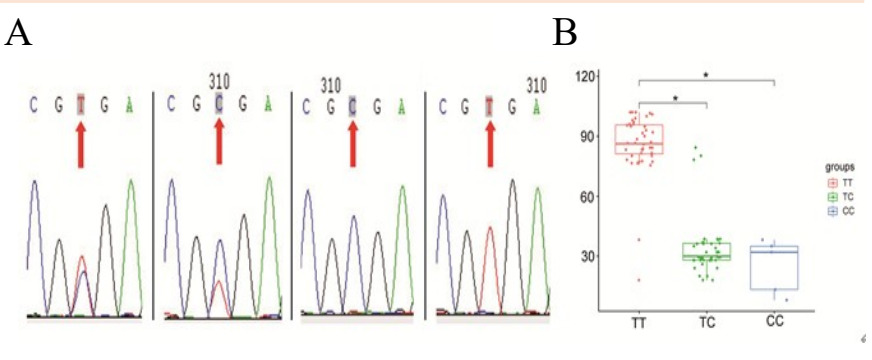
3 Genome wide association study of heat tolerance in *U. unicinctus*



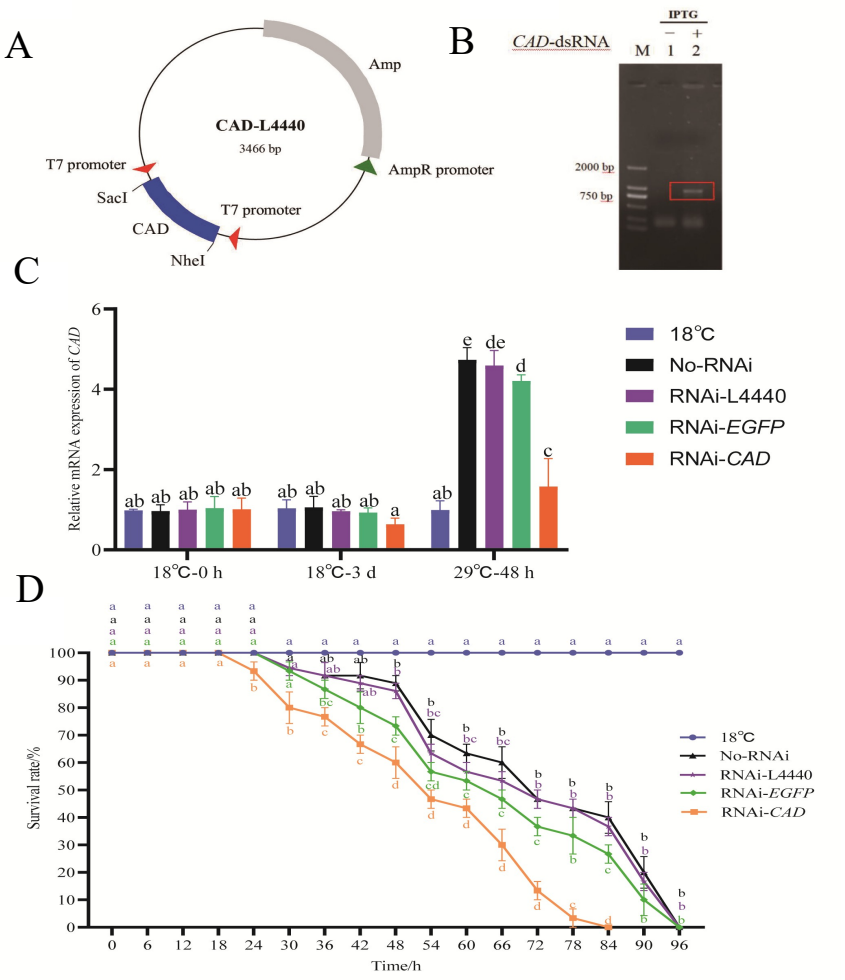
4 Fine-mapping of significant SNP



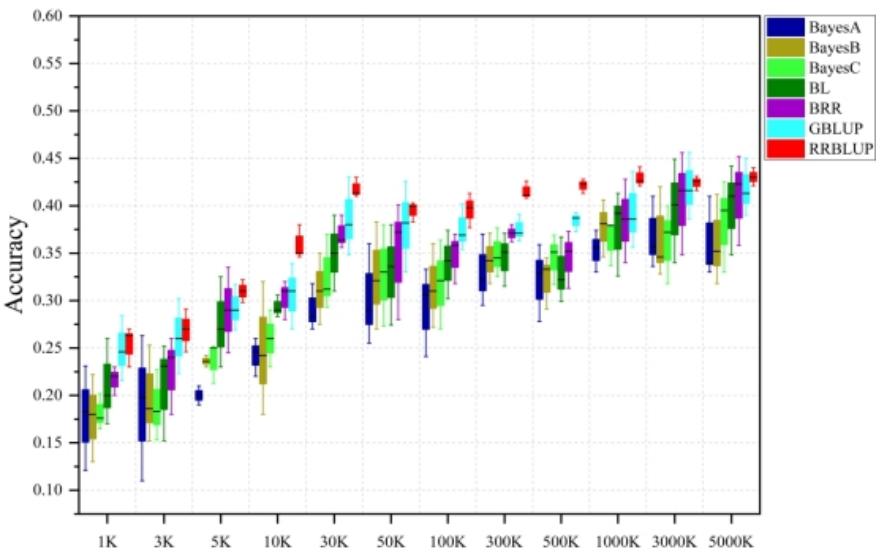
5 Genotypic validation of significant SNPs by Sanger sequencing



6 Functional validation of the CAD gene in heat tolerance



7 Genomic selection for heat tolerance in *U. unicinctus*



Conclusion

The results of this study showed that 29 °C was selected as the best temperature to find heat sensitive and heat tolerant *U. unicinctus* individuals. A total of 209 SNPs and 318 candidate genes related to heat tolerance were identified by GWAS. The Fine-mapping result showed that the SNP chr6:52475116 had a posterior probability of over 95%. Sanger sequencing in a separate population confirmed that this SNP was significantly linked to heat tolerance. The RNA interference result showed that the CAD gene is functionally important for heat tolerance in *U. unicinctus*. The genomic selection result showed that the RRBLUP model was the most suitable for genomic prediction of heat tolerance in *U. unicinctus*.

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