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Progress in Molecular Breeding of *Portunus trituberculatus*

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Abstract

The swimming crab (*Portunus trituberculatus*) is a commercially important marine species in China. Overfishing, environmental pollution, and intensive aquaculture have led to germplasm degradation, slow growth, and disease susceptibility. Recent advances in high throughput sequencing have enabled molecular breeding approaches to accelerate genetic improvement. This opinion synthesizes key progress in germplasm evaluation, genomics, marker development, and marker assisted selection (MAS) for P. trituberculatus. We also discuss the potential of genome wide association studies (GWAS), genomic selection (GS), and gene editing, and propose strategies to overcome current bottlenecks. An integrated environmental-genetic perspective—combining sustainable aquaculture practices with genetic management—is essential for the long-term viability of this resource.

Keywords: *Portunus trituberculatus*; Molecular breeding; Genomic resources; Marker assisted selection

Abbreviations: MAS: Marker Assisted Selection; GWAS: Genome Wide Association Studies; GS: Genomic Selection; SSR: Simple Sequence Repeat; RAPD: Randomly Amplified Polymorphic DNA; RFLP: Restriction Fragment Length Polymorphisms; AFLP: Amplified Fragment Length Polymorphism; SNP: Single Nucleotide Polymorphism; QTL: Quantitative Trait Locus; AHPND: Acute Hepatopancreatic Necrosis Disease; MSTN: myostatin; GBS: genotyping-by-sequencing

Introduction

The swimming crab *(Portunus trituberculatus)* is widely distributed along the coasts of China, Korea, Japan, and Southeast Asia. In 2024, China produced 97,696 tons through aquaculture and 452,555 tons through marine capture [1]. However, overfishing, habitat pollution, and high density farming have reduced wild resources and caused slow growth, increased size variation, and poor disease resistance [2,3]. Although several improved varieties (e.g., "Huangxuan No. 1", "Huangxuan No. 2") have been released, conventional breeding is slow and environment sensitive. Molecular breeding offers a faster, more precise path. This opinion highlights major advances and future directions, while also acknowledging the broader environmental context—ocean pollution and antimicrobial resistance—as critical challenges for sustainable crab farming [4].

Germplasm Resources and Genetic Diversity

Early studies used morphometrics and low resolution markers (e.g., RAPD, RFLP, AFLP) to assess population structure [5-10]. However, these markers have inherent limitations: RAPD and AFLP are dominant, making it impossible to distinguish

heterozygotes from homozygotes, while RFLP is technically laborious and time-consuming [11]. By contrast, simple sequence repeat (SSR) markers are co-dominant, highly polymorphic, abundant, and amenable to high-throughput automation, making them more powerful for population genetic analysis [12]. Using these SSR markers, wild populations were shown to maintain moderate to high genetic diversity. For example, Xu et al. [13] reported expected heterozygosity (He) as high as 0.9348 in Zhoushan and Xiamen populations. In contrast, cultured stocks exhibit significantly lower diversity [14,15], underscoring the value of wild germplasm for breeding. Interestingly, SNP based studies found lower diversity (He = 0.261) and less differentiation (Fst = 0.016) [16], highlighting marker dependent differences. To reliably guide conservation and breeding, we recommend integrating multi marker data and considering population history and environmental pressures.

Genomic Resources: Linkage Maps, QTLs, and Genome Assembly

High density genetic linkage maps are essential for quantitative trait locus (QTL) mapping and molecular marker-assisted selection (MAS). Early AFLP based maps had low coverage (~50%) [17]. Subsequent AFLP+SSR maps improved coverage to 74 75% [18]. Using SLAF seq, Lv et al. [19] constructed an SNP-based map with 10,963 markers and 98.85% genome coverage, enabling QTL mapping for growth, salinity tolerance, and sex determination. For example, two QTLs for low salinity tolerance were identified on linkage group 17, with 79 candidate genes [20]. Growth related QTLs explained 12 36% of phenotypic variation [19]. Sex linked SNP markers confirmed an XX/XY sex determination system [21,22]. The current reference genome is fragmented, but a Nanopore Hi C assembly has greatly improved contiguity [23,24]. Future efforts should aim for a telomere to telomere, haplotype resolved assembly and a pan genome to capture structural variation.

Molecular Markers and Marker Assisted Selection

High throughput sequencing has revolutionized marker discovery. Transcriptome sequencing identified >22,000 SSRs and >66,000 SNPs [25], and GBS yielded 155,971 high quality SNPs [16]. For growth traits, markers such as PTR8a (SSR) and comp58070 R31 (SNP) are significantly associated with body weight and carapace width [26-28]. For disease resistance (e.g., against *Vibrio alginolyticus*), SNPs in PtCrustin, PtcSP, and PtSPH have been linked to susceptibility/tolerance [29-31]. A GWAS on fatness and acute hepatopancreatic necrosis disease (AHPND) has been reported [32,33], but studies on growth remain scarce due to high costs and difficulty in phenotyping. We advocate for standardized family populations, automated phenotyping (computer vision), and low cost genotyping (RAD seq, 2b RAD) to accelerate GWAS in this species.

Despite progress, molecular breeding of *P. trituberculatus* is still in its infancy. We offer the following opinionated recommendations:

a) Integrate GWAS and genomic selection (GS). Build a reference population of at least 1,000 individuals phenotyped for growth, disease resistance, and stress tolerance. Use machine learning (e.g., deep learning) to improve prediction accuracy. Develop a medium density SNP chip to reduce genotyping costs.

b) Explore gene editing. CRISPR/Cas9 has not been systematically applied in crabs due to technical barriers (e.g., embryo microinjection). Pilot studies on sperm mediated transfection or electroporation are needed. Candidate target genes could include MSTN (myostatin) for growth enhancement and *Crustin* for improved immunity.

c) Address environmental drivers. Ocean pollution—including antibiotics, heavy metals, and microplastics—drives the spread of antimicrobial resistance in marine environments. Resistant bacteria have been isolated from crabs and their habitats [34]. Breeding for disease resistance should go hand in hand with improved water quality management and reduced antibiotic use in aquaculture.

d) Strengthen germplasm conservation. Wild populations are the ultimate source of genetic variation. Periodic monitoring using SNP panels, together with spatially structured harvest quotas, will prevent further erosion of diversity.

e) Promote open data and collaboration. Many research groups work in isolation. A public database for QTLs, candidate genes, and GWAS summary statistics would greatly accelerate progress.

Conclusion

Molecular breeding holds great promise for improving growth, disease resistance, and stress tolerance in *P. trituberculatus*. High quality genomic resources and marker technologies are already available. The remaining challenges are organizational (phenotyping, cost, data sharing) and environmental (pollution, antimicrobial resistance). By combining advanced genetics with ecosystem management, we can achieve sustainable and profitable crab farming.

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