In terms of experimental design, a combined analysis using microsatellite markers (SSR) and selected SNP loci was adopted to achieve both high polymorphism detection and high-resolution population structure analysis. Previous studies indicate that grouper genetic analyses typically use 8-20 SSR loci supplemented by mitochondrial genes or SNP data derived from reduced-representation sequencing, enhancing the detection of within-population diversity, kinship, and differentiation patterns (Weng et al., 2021; Yang et al., 2022; Wu et al., 2024). In this study, DNA was extracted using the standard phenol-chloroform method, followed by PCR amplification of target fragments. SSR genotyping was performed using capillary electrophoresis platforms, while SNP loci were detected using high-throughput sequencing. Data analysis was conducted using software such as PopGen, Arlequin, and STRUCTURE to calculate indices including allele number, heterozygosity, polymorphism information content, inbreeding coefficient, and Hardy-Weinberg equilibrium. Further analyses such as FST, AMOVA, PCA, and clustering were used to characterize population differentiation. If known parent-offspring relationships were included, kinship and parentage analyses were also performed to estimate effective breeding population size and identify unequal reproductive contributions (Hassanien and Al-Rashada, 2020; Weng et al., 2021; Yang et al., 2022).

4.2 Molecular marker results and population structure analysis

The molecular marker results indicated clear differences in genetic diversity among grouper populations from different sources. Overall, wild populations exhibited higher allele numbers, allelic richness, and heterozygosity than cultured populations, suggesting greater genetic variation. In contrast, cultured populations showed allele loss, reduction of rare alleles, and imbalanced allele frequencies at certain loci, indicating genetic variation loss during artificial breeding and seed propagation. This pattern is consistent with previous studies showing that cultured populations are more susceptible to founder effects and genetic drift under domestication and limited broodstock conditions, resulting in a narrower genetic base.

From the perspective of heterozygosity and inbreeding parameters, wild populations generally showed higher expected heterozygosity (He) than cultured populations. In cultured populations, observed heterozygosity (Ho) at some loci was lower than expected, leading to elevated FIS values and suggesting heterozygote deficiency (Yang et al., 2022). This pattern may result from inbreeding, family structure, Wahlund effects, or the presence of mixed subpopulations. Notably, some hatchery studies have shown that even when offspring heterozygosity is similar to that of parents, rare alleles are significantly reduced and genetic bottleneck signals are detectable. This suggests that genetic drift may first manifest as a reduction in allelic richness rather than an immediate decline in heterozygosity (Wenne, 2023). Therefore, interpreting genetic status in cultured populations requires a multi-indicator approach rather than reliance on a single heterozygosity metric.

Population structure analyses further revealed that FST values among cultured populations were generally low, indicating frequent germplasm exchange or shared broodstock sources among farms. In contrast, FST values between cultured and wild populations were relatively high, indicating a certain degree of genetic differentiation. AMOVA results typically supported this pattern, showing that a significant proportion of genetic variation was attributable to differences among populations, particularly between “wild” and “cultured” groups. STRUCTURE, PCA, and PCoA analyses commonly separated samples into two main genetic clusters corresponding to wild and cultured origins, although some individuals exhibited admixture (Chen et al., 2025). Such admixture may reflect gene flow caused by stock enhancement, cross-regional seed transfer, introduction of wild broodstock, or historical germplasm exchange. Additionally, selection signals detected at certain SNP loci suggest that artificial selection has left functional genetic footprints in genomic regions associated with growth, stress resistance, or disease resistance (Wu et al., 2024).

4.3 Genetic diversity evaluation and germplasm conservation recommendations

Based on the comprehensive analysis of genetic diversity indices in this case, wild grouper populations in the study area still maintain relatively high genetic diversity, whereas cultured populations have shown varying degrees of genetic variation decline, reduced allelic richness, and increased differentiation from wild populations.