broodstock numbers and inadequate pedigree management can lead to excessive contributions from certain families, accelerating the loss of genetic diversity (Sonesson et al., 2023). Meanwhile, although hybrid breeding can enhance growth performance, the absence of molecular marker-assisted management may result in genetic background confusion and biased evaluation.

On the other hand, wild germplasm resources are also under continuous pressure. Overfishing, habitat degradation, and environmental disturbances have led to population declines and reduced genetic diversity in some species (Fadli et al., 2023; Ybanez and Gonzales, 2023; Nurdin et al., 2025). Some populations have shown signals of historical contraction and even low levels of genetic variation (Vaini et al., 2021; Chen et al., 2025). Additionally, management challenges remain, including insufficient genetic assessment, incomplete broodstock renewal mechanisms, lack of germplasm databases, and inadequate coordination of conservation policies (Li, 2022; Sonesson et al., 2023). Therefore, it is necessary to establish a full-chain management system covering “wild resources—conservation populations—breeding populations—commercial seed,” integrating molecular monitoring, broodstock management, and ecological conservation to ensure the long-term stability and sustainable utilization of grouper germplasm resources (Li, 2022).

3 Theoretical Basis for Genetic Diversity Assessment in Groupers

3.1 Concept and evaluation indices of genetic diversity

Genetic diversity refers to the quantity and distribution patterns of genetic variation within a species and among different populations. It is an important component of biodiversity and forms the basis for aquatic organisms to adapt to environmental changes, maintain population stability, and support genetic improvement (Hassanien and Al-Rashada, 2020; Yang et al., 2022). For marine aquaculture species such as groupers, genetic diversity is primarily reflected at the DNA level, including the number and frequency distribution of alleles, haplotype composition, and variation at polymorphic loci across the genome. These genetic differences may further manifest as phenotypic variations in traits such as growth rate, body shape, disease resistance, and environmental adaptability. Therefore, a high level of genetic diversity generally indicates stronger environmental adaptability, greater evolutionary potential, and lower risk of population decline, making it an important criterion for evaluating the quality and breeding potential of grouper germplasm resources (Chen et al., 2025).

Compared with traditional morphological indicators, molecular marker technologies can more directly reflect genetic variation and are less affected by environmental factors; thus, they have become core tools in studies of genetic diversity in groupers. Currently, commonly used methods include microsatellite markers (SSR), mitochondrial DNA (mtDNA) haplotype analysis, single nucleotide polymorphisms (SNPs), ISSR, as well as rapidly developing high-throughput genotyping and whole-genome resequencing technologies (Hassanien and Al-Rashada, 2020; Hsu et al., 2023; Wu et al., 2024). These techniques have enabled genetic diversity assessment to evolve from early low-resolution morphological or limited-locus analyses to comprehensive multi-locus, genome-wide, and functionally associated analyses, improving both the accuracy of population comparisons and the interpretation of the genetic basis of germplasm resources (Houston et al., 2020).

In practice, genetic diversity assessment typically relies on a set of quantitative indices to form a comprehensive analytical framework. Common within-population diversity indices include the number of alleles (Na), effective number of alleles (Ne), allelic richness, observed heterozygosity (Ho), and expected heterozygosity (He), which reflect variation at the allele and genotype levels (Hassanien and Al-Rashada, 2020). Among these, Na represents the total number of alleles detected at a locus, whereas Ne emphasizes the contribution of allele frequency distribution to effective variation. Ho and He represent the actual proportion of heterozygous individuals and the theoretically expected heterozygosity, respectively. In addition, polymorphism information content (PIC), nucleotide diversity (π), and haplotype diversity (Hd) are commonly used to evaluate marker polymorphism and sequence-level variation (Chen et al., 2025). For comparisons among populations, indices and methods such as FST, ΦST, GST, DEST, genetic distance, principal component analysis (PCA), and Bayesian clustering are used to