

2 Discussion

In this study, seven polymorphic microsatellite primers with clear gel electrophoresis patterns and stable amplification across all populations were successfully selected, and corresponding DNA fingerprint profiles were established. These markers provide a valuable reference for future studies on the origin and evolution of *Platycladus orientalis* varieties, molecular-level identification and classification, hybrid breeding, and parental selection for genetic mapping. As an ideal type of molecular marker, microsatellites exhibit advantages such as good reproducibility, simplicity, efficiency, and high primer transferability in the identification of *P. orientalis* varieties. However, because the detection and application of SSR polymorphism largely depend on PCR amplification efficiency, different primers may require different reaction conditions. Therefore, it is essential to conduct preliminary optimization experiments for each primer and adopt appropriate strategies to maintain PCR reaction conditions at an optimal level.

In plant variety identification and classification studies, a more scientific approach is to integrate multiple methods and use them to complement and validate one another (Yuan et al., 2014). Identification results based on morphological traits and molecular markers are not always completely consistent. For example, some morphologically similar *P. orientalis* individuals were identified as hybrids in STRUCTURE clustering analyses. This phenomenon may be attributed to genetic variation caused by backcrossing and introgression, or to morphological variation resulting from convergent evolution and environmental selection (Rieseberg et al., 1999; Schwarzbach et al., 2001; Lexer et al., 2003). Similar patterns have also been observed in other populations exhibiting natural hybridization (Rieseberg, 1995). Any single method has inherent limitations, and relying on a single approach for species identification and classification makes it difficult to ensure the scientific rigor and reliability of the results.

Numerous studies have shown that factors such as genetic drift and gene flow have a substantial impact on population genetic structure. In recent years, parameters such as genetic differentiation coefficients have been widely used as important indicators for evaluating population genetic structure and kinship relationships among varieties (Song et al., 2011; Xu et al., 2014). Genetic variation analysis of *P. orientalis* populations using seven SSR markers showed that the average number of alleles (N_a) among the 100 samples from four sampling regions was 3.714, the average effective number of alleles (N_e) was 1.900, the average Shannon index (I) was 0.818, and the average expected heterozygosity (H_e) was 0.440. These results indicate that the 100 sampled accessions possessed relatively rich genetic diversity. The fixation index (F_{st}), which reflects the level of allelic heterozygosity among populations and is used to measure the degree of population differentiation, was 0.0371. This value falls within the range of 0–0.05, indicating high similarity among populations, small genetic distances, and very low genetic differentiation.

Results from principal coordinate analysis (PCoA), UPGMA clustering, and STRUCTURE clustering based on microsatellite data consistently showed that the 100 *P. orientalis* accessions were not strictly clustered according to their geographic origins. This suggests that the genetic backgrounds of the germplasm resources are relatively similar and that varying degrees of natural hybridization may occur among *P. orientalis* germplasm from different sampling regions. Hybridization promotes gene flow among populations and contributes to genetic evolution, thereby influencing population genetic structure and altering its overall pattern.

The formation of this spatial genetic variation pattern may be the result of the combined effects of long-distance gene flow, natural climatic conditions, and geographic isolation. In theory, geographically proximate regions tend to have similar soil conditions and environmental climates, resulting in less pronounced differences in natural selection pressures and increased opportunities for interpopulation gene exchange. Consequently, populations located closer to each other tend to have smaller genetic distances and higher genetic similarity.

At present, only a portion of *P. orientalis* resources has been collected, and the limited sample size may introduce bias into the analyses. Therefore, a more comprehensive evaluation and utilization of *P. orientalis* germplasm resources will require further investigation and research. To adapt to diverse ecological conditions and geographic environments, wild plant resources have undergone prolonged evolutionary processes involving intense survival