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## Genetic diversity of orchardgrass germplasm detected by molecular markers

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**Key words:** AFLP, genetic diversity, ISSR, orchardgrass, RAPD

**Introduction** Orchardgrass (*Dactylis glomerata* L.) is one of the most important grasses in temperate zones with high forage yield and good quality. In this study we investigated the genetic diversity in 60 orchardgrass accessions with a combination of three molecular markers: AFLP (Amplified fragment length polymorphism), ISSR (Inter-simple sequence repeat) and RAPD (Random amplified polymorphic DNA).

**Materials and methods** Sixty DNA samples were extracted from accessions by the CTAB method. Nine AFLP primer pairs were used in genetic diversity analysis based on Vos's AFLP protocol and PCR reaction system (Vos et al., 1995). Twelve ISSR primers and twenty RAPD primers were used for further analysis, the protocol and PCR reaction system were from Wang (2003) and Kölliker (1999), respectively. All the fragments from three markers were scored manually for band presence (1) or absence (0). The resulting data matrix was analyzed by NTSYS-pc 2.1.

**Results** According to the analysis of three molecular markers, the following results were obtained: (1) A total of 400 AFLP DNA fragments were amplified, among which 336 (84.0%) were polymorphic. Genetic similarity coefficient (GS) ranged from 0.5786 to 0.9308 among accessions, suggesting rich genetic variation, especially in Chinese accessions. According to the AFLP marker, the genetic variation of orchardgrass accessions was closely associated with ploidy levels and geographical distributions. (2) 116 bands were produced in ISSR analysis, of which 101 (87.07%) were polymorphic. The GS ranged from 0.6116 to 0.9231, which showed rich genetic diversity among accessions. ISSR dendrogram could reveal the geography distribution of orchardgrass. (3) 120 bands were produced in RAPD marker and 97 (80.83%) were polymorphic. The GS ranged from 0.6154 to 0.9254, also showing a high level of diversity in orchardgrass.

**Conclusions** The PCR products showed abundant Genetic polymorphism by three molecular markers, and the genetic comparability analysis suggested rich genetic diversity of orchardgrass germplasm, which was consistent with Kölliker research result (Kölliker 1999). Three molecular markers were considered useful tools for evaluating the genetic diversity for orchardgrass. However, AFLP detected more variance and revealed ploidy levels of accessions, which suggested AFLP marker could be better for genetic diversity research.

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