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Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.)

Wen-Gang Xie [a], Xin-Quan Zhang [a,*], Hong-Wei Cai [b], Wei Liu [a], Yan Peng [a]

[a] Department of Grassland Science, Animal Science and Technology College, Sichuan Agricultural University, Ya'an 625014, PR China
[b] Department of Plant Genetic & Breeding, College of Agronomy and Biotechnology, China Agricultural University, 2 Yuanmingyuan West Road, Beijing 100094, PR China

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ABSTRACT

The diversity and genetic relationships among 74 orchardgrass accessions were analyzed using cereal EST-SSRs and orchardgrass SSR markers in order to estimate genetic variability and compare the level of diversity. In total, 190 polymorphic bands were detected with an average of 6.3 alleles per SSR loci. The average polymorphic rate (P) for the species was 84.63%, suggesting a high degree of genetic diversity. The molecular variance analysis (AMOVA) showed that the proportion of variance explained by within- and among-geographical groups diversity was74.87% and 25.13%, respectively. The distinct geographical divergence of orchardgrass was revealed between Americas and Oceania. The ecogeographical conditions such as climate and soil, genetic drift and mating system could be the crucial factors for genetic divergence. Furthermore, the study also indicated that northern Africa, Europe and temperate Asia might be the diversity differentiation center of orchardgrass. The result will facilitate the breeding program and germplasm collection and conservation.

1. Introduction

Orchardgrass (*Dactylis glomerata* L.) is one of the most important cool-season forage grasses, which is native to northern Africa, western and central Europe, temperate and tropic Asia and has exhibited remarkable local adaptation and ecotype differentiation (Hultén, 1968; Tolmachev et al., 1995; Gauthier et al., 1998). Orchardgrass has been widely used as forage for more than 100 years, especially in North America, Europe (Casler et al., 2000) and Japan (Mitui, 1981), owing to its good nutrition, high yield and good tolerance to shadow. Orchardgrass germplasm resources are rich in China, where it is primarily distributed in the southwest and northwest areas, mainly growing on forest edges, shrubs and sub alpine meadows at elevations ranging from 1000 to 3600 m (Peng et al., 2008). And it have been widely utilized in cultivated pastures with favorable forage yield, improved quality and good adaptability to the local environment and plays an important role in animal husbandry and environmental sustenance in China.

Genetic diversity is an important precursor in a study of any species because its amount and distribution are likely to affect the evolutionary potential of species (Futuyma, 1986). It is particularly useful in the characterization of individuals, accessions, and cultivars in determining duplications in germplasm collection and for the choice of parental genotypes in breeding programs. In the past, indirect estimates of similarity based on morphological information have been widely used in many species including orchardgrass (Gauthier and Lumaret, 1999). However, phenotypic variation does not reliably reflect genetic

* Corresponding author. Tel.: +86 835 2882270; fax: +86 835 2886080.
E-mail address: zhangxq8@hotmail.com (X.-Q. Zhang).

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variation because of the role of environmental interaction in determining the phenotype. In recent years, the number of molecular assays available for application in this area has increased dramatically. Genetic variation of wild orchardgrass accessions and nature populations had been evaluated by randomly amplified polymorphic DNA (RAPD) (Kolliker et al., 1999; Tuna et al., 2004; Zeng et al., 2006a,b), amplified fragment length polymorphism (AFLP) (Reeves et al., 1998; Peng et al., 2008), inter-simple sequence repeats (ISSR) (Zeng et al., 2006a,b) and sequence-related amplified polymorphism (SRAP) markers (Zeng et al., 2008). Compared with other molecular markers, microsatellites (simple sequence repeats, SSRs) are highly polymorphic, abundant and are accessible to other research laboratories via published primers sequences, they have become the marker of choice for genetic mapping, gene tagging, genetic diversity study, genomic and cDNA fingerprinting. Currently, SSR markers have been used to determine genetic diversity and relationships in many plant species (Kubik et al., 2001; Gethi et al., 2002; Burnham et al., 2002; Zoghlami et al., 2009; Cheng and Huang, 2009; Tahan et al., 2009). During the last two decades, a large number of expressed sequence tags (EST) have been generated in several cereal crop species and transfer of SSR markers across species or genera has been reported in several cereal crops (Zhao and Kochert, 1993; Roder et al., 1995; Brown et al., 1996; Cordeiro et al., 2001; Thiel et al., 2003) as well as several minor grass species (Wang et al., 2005; Narasimhamoorthy et al., 2008; Sim et al., 2009). However, little research has been conducted on the transferability of SSR markers from major cereal crops to orchardgrass. Meanwhile, although previous diversity studies provided some valuable information for researchers and breeders working with *D. glomerata*, they were limited in restricted regions and a small number of accessions. Therefore, further analyses at a larger region and a large number of germplasm collections may produce new insights and give a better understanding of the distribution of genetic diversity at the regional level.

The present study examines both the level of genetic diversity in orchardgrass and its variation distribution. 74 orchardgrass accessions from 27 countries and 9 geographical regions, comprising cultivars, wild materials, advanced breeding lines and subspecies were investigated by cereal EST-SSRs and orchardgrass SSRs. The specific objectives were: (1) to investigate genetic relationships among 74 orchardgrass accessions originating from various geographic locations, (2) to study the genetic diversity within and among geographical regions, (3) to formulate appropriate strategies for the conservation and utilization of the orchardgrass genetic resources available.

2. Materials and methods

2.1. Plant materials

A total of 74 orchardgrass accessions from 27 countries and 9 geographical regions, comprising cultivars, wild materials, advanced breeding lines and subspecies were chosen for analysis by means of SSR markers (Table 1, Fig. 1). They were obtained from the USDA-ARS, Unite States; Sichuan Agricultural University, China. Among these accessions, thirteen Chinese accessions were from Sichuan, Yunnan, Guizhou, Jiangxi, Chongqing and Xinjiang, representing the main distribution regions in China. Other plant introduction (PI) accessions were from Asia, Europe, Africa, Oceania and Americas. In addition, all the accessions were also grouped in nine geographical regions: EA, WA, SA, NE, SE, EE, AM, OC, NF according to their origin. The vouchers of the materials were deposited at the Department of grassland science, Sichuan Agricultural University, China. Seeds with a pretreatment at a low temperature were germinated on absorbent filter paper in Petri dishes with a temperature 23 °C. Germinated seeds were transplanted into a sand-peat mixture and the plants maintained in a greenhouse.

2.2. DNA extraction

Each accession was represented by 25 plants, and 100 mg of fresh young leaves from each plant was used to create a pool. Fresh young leaves were powdered in liquid nitrogen and genomic DNA was extracted using a modified CTAB method (Doyle, 1991). The quality and concentration of the DNA were determined by comparing the sample with known standards of lambda DNA on 1% (w/v) agarose gels. The isolated genomic DNA was diluted to 10 ng/μL and stored at −20 °C for use.

2.3. Primer selection and PCR amplification

A total of 50 cereal EST-SSRs were randomly selected, including 10 maize EST-SSRs (M), 15 wheat EST-SSRs (W), 10 sorghum EST-SSRs (S) and 15 pairs of sorghum genomic SSR primers (SG). These primer sequences were published in Wang et al. (2005) (Table 2). And 15 orchardgrass SSR primer pairs were kindly provided by Dr. Cai, China Agricultural University. The PCR amplification and SSR genotyping were carried out as described by Wang et al. (2005) and Xie et al. (2010). Then amplification fragments were separated on 6% denatured polyacrylamide gels. After electrophoresis, the gel was stained by $AgNO_3$ solution. Then gel was photographed by Gel Doc (TM) XR System.

2.4. Data analysis

For the statistical analysis, the patterns at all SSR loci were scored for each polymorphic band as 1 for band presence and 0 for band absence. The resulting present/absent data matrix was analyzed using POPGENE v.1.31 (Yeh et al., 1999). The genetic identity (GI) and the genetic distance (GD) among 74 accessions were also computed using the same program. Genetic diversity was evaluated with the polymorphic information content (PIC) $PIC = 1 - \sum P_{ij}^2$, where P_{ij} is the frequency of the jth