C. R. Biologies xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Genetics/Génétique

AFLP assessment of genetic variability and relationships in an Asian wild germplasm collection of *Dactylis glomerata* L.

Ming Sun [a], Chenglin Zhang [a], XinQuan Zhang [a], Yan Fan [b], Kaixin Fu [a], Wendan Wu [a], Shiqie Bai [c], Jianbo Zhang [c], Yan Peng [a], Linkai Huang [a], Yanhong Yan [a], Xiao Ma [a,*]

[a] *Department of Grassland Science, Animal Science and Technology College, Sichuan Agricultural University, Chengdu, Sichuan 611130, China*
[b] *Chongqing Municipal Institute of Animal Husbandry, Chongqing 400039, China*
[c] *Sichuan Academy of Grassland Science, Chengdu, Sichuan 611731, China*

ARTICLE INFO

Article history:
Received 10 October 2016
Accepted after revision 29 December 2016
Available online xxx

Keywords:
Dactylis glomerata
AFLP
Geographic groups
Genetic structure
Environmental adaptation
Climate

ABSTRACT

Orchardgrass (*Dactylis glomerata* L.), an excellent perennial and cool season forage species distributed in most temperate regions, has been cultivated widely in Western China. Amplified fragment length polymorphism markers were employed to determine the genetic variability and population structure among 41 indigenous orchardgrass accessions from Central Asia and Western China. On the basis of 531 polymorphic fragments resulted from eight primer combinations, polymorphic information content (PIC), marker index (MI) and resolving power (RP) averaged 0.252, 16.34 and 25.27 per primer combination, respectively, demonstrating the high efficiency and reliability of the markers used. We found relatively low differentiation ($Fst = 0.135$) for three geographical groups, where Central Asia (CA) and Southwest China (SWC) group exhibited higher intra-population diversity ($He = 0.20$ and 0.21) than that of the Xinjiang (XJ) group ($He = 0.14$). We also did not detect a clear pattern of isolation by distance with a low value of $r = 0.301$ in the Mantel test. STRUCTURE, FLOCK, UPGMA clustering and PCoA analyses showed that CA group is more related to the SWC Group rather than to the XJ Group. In addition, this study strongly suggests that geographical and ecological environmental factors together could better explain the genetic differentiation between different geographical regions than geographic isolation alone, especially for Xinjiang accessions. The present study also could support that Southwest China might be the internal diversity center of *D. glomerata* in China. The knowledge about the genetic variability of the Asian accessions examined contributes to rapid characterization, defining gene pools of wild accessions, and selecting appropriate germplasms for plant improvement.

1. Introduction

The genus *Dactylis* contains only a single species and at least 18 subspecies [1]. *Dactylis glomerata* L. (orchardgrass or cocksfoot), a long-lived and perennial grass species, has been considered as the fourth most economically significant forage grass in the world based on its high

---

* Corresponding author.
E-mail address: maroar@126.com (X. Ma).

http://dx.doi.org/10.1016/j.crvi.2016.12.003
1631-0691/© 2017 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

Please cite this article in press as: M. Sun, et al., AFLP assessment of genetic variability and relationships in an Asian wild germplasm collection of *Dactylis glomerata* L., C. R. Biologies (2017), http://dx.doi.org/10.1016/j.crvi.2016.12.003

productivity and its disease resistance under varying climatic conditions [2,3]. *D. glomerata* can be diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$), or hexaploid ($2n = 6x = 42$), and are separated into subspecies by morphological characteristics, geographical distribution, and/or chromosome ploidy [2]. Most populations are diploid [2,4]. Continuous outcrossing by wind pollination, natural selection and adaptation processes has resulted in a wide geographic range and large morphological variability [5]. It has been widely disseminated and become naturalized in many parts of the temperate and subtropical world, including Europe, Asia, North Africa, and the Canary Islands, with remarkable local adaptation and ecotype differentiation [6]. It is suggested that a successful Central Asian diploid progenitor of *Dactylis glomerata*, similar to today's ssp. *altaica*, expanded its range to cover a broad region [4] and Chinese ssp. *sinensis* might evolve from European ssp. *aschersoniana* which originated from Central Asia, although many aspects of the *Dactylis* species' original colonization remain to be elucidated [7]. In particular, orchardgrass occurs indigenously in mountain slopes or light forest shade of Xinjiang and Southwest China. Moreover, hence most of the Chinese orchardgrass varieties were domestic (such as cv. GuLin, BaoXing, and ChuanDong) and developed from wild local ecotypes [8]; these varieties have played an important role in pasture husbandry due to high yield and good adaptability to the local environment.

To better understand the genetic diversity, interrelatedness and differentiation of wild accessions or populations of the species is a critical step towards appropriate breeding programs developing superior varieties. Previous studies have shown that considerable variation for various agromorphological characters exist among orchardgrass accessions of ecotypes, cytotypes, and subspecies, with a clear relationship between geographical areas and observed diversity [9–13]. However, the morphological characters are not only sensitive to environmental factors, but also require labor intensive evaluation over long periods of time [14,15]. Molecular markers are widely used as efficient tools to investigate genetic variability in orchardgrass germplasm collections including random amplified polymorphic DNA (RAPD) [16,17], inter-simple sequence repeat (ISSR) markers [18], sequence-related amplified polymorphism (SRAP) [19], simple sequence repeat (SSR) [20] and amplified fragment length polymorphism (AFLP) [21]. Among the various marker systems, the AFLP technique is considered as a robust and highly informative DNA fingerprinting method used to assess a large number of markers without prior sequence knowledge [22]. Until now, however, only few efforts using AFLP markers were undertaken to study genetic diversity in wild Chinese orchardgrass accessions [21]. Marker analysis of individual genotypes was considered to be more informative in detecting population genetic structure, but this method is not practical if a large number of populations are to be characterized [23]. In view of its cheapness and effectiveness, bulking strategy has been successfully employed to investigate the genetic variation between accessions or cultivars of allogamous species [20,24,25]. It been suggested that bulks of 20 plants per accession seem sufficient to represent the genetic variation within and between accessions for most forage grass species [26]. Moreover, the non-detection of rare alleles or bands using the pooling approach is compensated by the fact that a larger number of populations can be analyzed with the same amount of effort [27,28].

Many previous studies on the genetic structure and diversity in orchardgrass have been carried out, involving a variety of populations and molecular marker technologies [3,18–21,29], while the common features of the majority of these studies are exclusion of Central Asian germplasm as well as available ecogeographical data of collection sites, and that they could simply reveal genetic relationships and diversity within the tested materials, the internal diversity center of *D. glomerata* in China remaining unclear. It is widely acknowledged that plant genetic structure is usually resulting from geographical isolation or local adaptation (i.e. environmental factors, including temperature, precipitation and elevation, etc.) [30–34]. In addition, ecogeographically distinct populations can differ in their level of genetic diversity or in the distribution of diversity within and among regions, and this diversity could be used for the *in situ* conservation of natural populations from genetic erosion and stratification or utilization of larger plant germplasm collections [35].

Since Central Asia has been suggested to be the origin center of orchardgrass and since Southwest China and Xinjiang are the typically native distribution areas of orchardgrass in China, we focused on 41 wild *D. glomerata* accessions from the above three regions in the present study. The aims of this work are:

- to assess polymorphic AFLP markers with highly informative value and to characterize the genetic diversity in selected accessions;
- to investigate genetic relationships among wild accessions and specifically to estimate the extent of variation between and within ecogeographical groups and subgroups;
- to elucidate the effects of geographical isolation and environmental factors on the genetic diversity and pattern of the population structure.

2. Material and methods

2.1. Plant materials

The plant material was collected from different localities of four Asian countries including China, Kazakhstan, Kyrghyzstan, Tajikistan (Fig. 1). A total of 41 indigenous orchardgrass accessions were considered, which represented three geographical groups, CA (Central Asia, 13), XJC (Xinjiang of China, 10), SWC (Southwest China, 18). The sampling locations and their geographic coordinates are shown in supplementary data, Table S1. The seeds of Chinese accessions were collected from different sites, and other accessions were obtained from the National Plant Germplasm System, USA. Seeds from each locality were germinated directly and maintained at Sichuan Agricultural University, Ya'an, Sichuan. Each accession was represented by a bulk sample. For each accession,

Please cite this article in press as: M. Sun, et al., AFLP assessment of genetic variability and relationships in an Asian wild germplasm collection of *Dactylis glomerata* L., C. R. Biologies (2017), http://dx.doi.org/10.1016/j.crvi.2016.12.003