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Genetic diversity analysis of sedges (*Carex* spp.) in Shandong, China based on inter-simple sequence repeat

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ABSTRACT

As the plants of turfgrass, forage and environment protecting plants, *Carex* L. has important economic value. The aims of the study were to construct ISSR-PCR amplification reaction system on *Carex* and to investigate the genetic diversity of 16 *Carex* populations belonging to 10 species using inter-simple sequence repeat (ISSR) makers. A total of 120 polymorphic amplified bands were obtained from 6 primers, and the percentage of polymorphisms was 100%. Genetic similarity between accessions ranged from 0.4250 to 0.8667 with an average of 0.6459, suggesting that the collected accessions are genetically diverse. All accessions were grouped into 3 clusters according to the UPGMA dendrogram. Most of the populations from the same regions can be basically clustered together and molecular grouping of *Carex* spp. correlates with geographical distribution and ecological environment. However, a few appeared to be divergent with the geographical distribution. The results showed that ISSR maker is an effective tool for the study of genetic diversity in *Carex*. As for the genus *Carex*, such information is needed for successful management and preservation of species to ensure the maintenance of genetic variation.

1. Introduction

Carex L., with about 2000 species recognized worldwide, is one of the largest genera in the Cyperaceae and the most ecologically important genera of vascular plants. The genus is widely distributed in a wide range of habitats from tropical areas to the high arctic, and the majority of the species in North America and eastern Asia occur in the north temperature zones (Bernard, 1990; Reznicek, 1990). In China, the genus *Carex* involves 500 species which are abundant in all parts of the country, and 27 species and 3 varieties of them are in Shandong Province, China (Li, 2004).

Carex populations play an important role in restoring and maintaining the ecological environment (Li and Zhou, 1998; Ji et al., 2006; Liu et al., 2009). Some species of *Carex* plants are extensively used to feed livestock; and others are used in turf management they can adapt to different environments and can spread easily (Ratliff and Westfall, 1988; Bernard, 1990; Schütz, 2000). In view of their economic importance, it is necessary to study the genetic relationship and diversity among species of *Carex* so that they can be used as genetic resources in breeding programmes.

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Previous studies on *Carex* mainly focused on geographical distribution, taxonomy, reproduction, biological and ecological characteristics (Reznicek, 1990; Schütz, 2000; Marlena and Pawel, 2011). The methods of achene epidermis (Zhang et al., 2000), micromorphology of allozyme variation (Huh, 2001), peroxidase isoenzyme and molecular makers (Roalson and Friar, 2004; Hipp et al., 2007) have been used to analyze the diversity and relationship of *Carex* plants. Although, the studies mentioned above have provided useful information about the plants, more related knowledge of genetic variation and diversity is prerequisite for exploiting *Carex* resources.

Molecular makers have been used as a preferred method for evaluating the genetic diversity of plant germplasms in the past years because it could distinguish between highly related genotype. Inter-simple sequence repeat (ISSR) makers (Zietkiewicz et al., 1994) which target microsatellite motif and do not require gene sequence information, can produce more reliable and reproducible bands and it is simple cost effectiveness and low cost per polymorphism than AFLP or RAPD (Godwin et al., 1997; Jones et al., 1997; Li et al., 2011).

At present, ISSR makers have been widely used as high efficient molecular marker methods for plants species identification, genetic mapping, gene localization, and genetic diversity analysis of plants, such as *Pelargonium reniforme* (De Wet et al., 2008), *Citrullus lanatus* (Djè et al., 2010), *Cynodon radiates* (Huang et al., 2010) and the genus *Cerasus* (Shahi-Gharahlar et al., 2011). The clonal and genetic diversity of different *Carex moorcroftii* populations on the Qinghai-Tibet Plateau was studied previously (Liu et al., 2009); however, further information is needed to understand species-level genetic relationship of the genus *Carex* in China. Therefore, the objective of this study was to analyze genetic diversity among 16 accessions of *Carex* distributed in Shandong province using ISSR makers.

2. Materials and methods

2.1. Plant materials

Sixteen natural *Carex* accessions were collected from different locations of Shandong Province. Reference specimens were deposited as vouchers at gardening experimental station of Shandong Agricultural University, China. The information of all the accessions was shown in Table 1.

2.2. DNA extraction

For each accession, young and healthy leaves were collected, stored in ice box and immediately were taken to the lab. In each accession, 3 samples were randomly selected for the ISSR analysis. Genomic DNA was extracted using the CTAB method described by Huang et al. (2010) with slight modification.

The quality and integrity of genomic DNA were examined on 1% agarose gel electrophoresis by comparing to size maker (DM2000 plus DNA Maker). DNA concentration was determined through Eppendorf Bio-Photometer plus nucleic acid analyzer. A portion of the DNA was diluted to 60 ng/μL with ddH_2O as templates for PCR amplification and the stock. Portions were stored at −20 °C.

2.3. ISSR-PCR amplification reaction system and amplification conditions

The ISSR-PCR components were optimized with varying concentrations of genomic DNA (30, 60, 90, 120 ng) and primers (0.125, 0.25, 0.375, 0.5 μM) were performed to optimize PCR conditions (Table S1, Supplementary data). The polymerase chain

Table 1
Experimental materials.

No.	Population[a]	Latin name	Source	Voucher number[b]
1	TS	*Carex neurocarpa*	Taishan	TS-1
2	TS	*C. japonica*	Taishan	TS-2
3	TS	*C. forficula*	Taishan	TS-3
4	TS	*C. leiorhyncha*	Taishan	TS-4
5	TS	*C. duriuscula*	Taishan	TS-5
6	TS	*C. breviculmis*	Taishan	TS-6
7	TS	*C. lanceolata*	Taishan	TS-7
8	LS	*C. lanceolata*	Laoshan	LS-1
9	LS	*C. lanceolata* var. *subpediformis*	Laoshan	LS-2
10	LS	*C. pisiformis*	Laoshan	LS-3
11	LS	*C. humilis*	Laoshan	LS-4
12	CLS	*C. breviculmis*	Culaishan	CLS-1
13	CLS	*C. lanceolata*	Culaishan	CLS-2
14	CLS	*C. humilis*	Culaishan	CLS-3
15	JRS	*C. heterolepis*	Jiurushan	JRS-1
16	BS	*C. breviculmis*	Bashan	BS-1

[a] TS, LS, CLS, JRS, BS stand for Tai, Lao, Culai, Jiuru and Ba mountains, respectively.
[b] Reference specimens were deposited as vouchers at gardening experimental station of Shandong Agricultural University.