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Full Length Research Paper

Genetic diversity of *Iris lactea* var. *chinensis* germplasm detected by inter-simple sequence repeat (ISSR)

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The genetic diversity of 24 *Iris lactea* var. *chinensis* were analysed by using 100 ISSR primers of which only 11 primers generated distinct amplified products. Among the 24 materials, a total of 214 bands were detected, of which 170 bands were polymorphic. The percentage of polymorphic bands (PPB) was 79%. The ISSR-based genetic similarity (GS) values among 24 accessions ranged from 0.400 to 0.929. Abundant genetic diversity among *I. lactea* var. *chinensis* accessions was revealed by ISSR markers. Based on the UPGMA cluster analysis and principal components analysis (PCA), the 24 materials investigated were divided into four groups, most of genetic variation was within a group. Genetic distance was found to be related to geographic distance among *I. lactea* var. *chinensis*.

Key words: *Iris lactea* var. *chinensis*, inter-simple sequence repeat (ISSR) marker, genetic diversity.

INTRODUCTION

Iris lactea var. *chinensis* is a wild perennial grass native to China. Its Chinese botanical name is "Ma Lin" and it is prevalent in desert steppe and saline meadow and widely distributed in northern and western China, Siberian regions, east of Russia, Far East, and Mongolia (Xu et al., 2006). It has proved that this plant has high tolerance to drought, salt, plant diseases, and insect pest, and the propagation and management of the plant is easy during later growth stage (Bai et al., 2008; Wang, 2002). Many previous studies of this plant have focused on the botanical characteristics, planting and reproduction, resistance, cytology and breeding, etc (Rui et al., 2007; Shen et al., 2008; Wang et al., 2007; Mu et al., 2005). However, relatively few studies have examined on the molecular biology of it (Veerle et al., 2002), and extensive ISSR (Inter-simple sequence repeat) marker studies have not yet been used to assess genetic diversity among the species.

Inter-simple sequence repeat (ISSR) amplification is a PCR based method that can rapidly differentiate closely related individuals, and it has been proved to be useful as novel DNA markers in studies on purposing plant improvement, such as genomic fingerprinting, phylogenetic analysis, gene tagging, etc. This technique uses a single primer composed of a microsatellite sequence anchored at the 3' or 5' end by 2 to 4 arbitrary and often degenerated nucleotides, and it does not require prior knowledge of DNA sequence for primer design (Zietkiewicz, 1994). Although ISSR markers have similar benefits to random amplified polymorphism of DNA (RAPD) markers which are one of the representative low cost DNA markers, ISS R markers are more reliable than RAPD ones because of comparatively longer length of the primers and higher annealing temperature (Leila et al., 2007). And it could be used widely, especially on evaluating plant germplasm and genetic diversity (Liu and Cordes, 2004).

The objective of this present investigation was to study the effect of geographic distance on genetic diversity of *I. lactea* var. *chinensis* using ISSR markers, and to provide important evidence for genetic improvement and evaluate the applicability of this analysis for assessing the

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Table 1. The number and source of materials used in this study.

No.	Code	Locality	No.	Code	Locality
1	BJ001	South-west of Beijing	13	IM007	South-central of Inner Mongolia
2	XJ001	Turpan, Xinjiang Province	14	IM008	South-central of Inner Mongolia
3	XJ002	Turpan, Xinjiang Province	15	XJ003	Eastern of Xinjiang Province
4	JL001	North-west of Jilin Province	16	IM009	South-central of Inner Mongolia
5	IM001	Central of Inner Mongolia	17	XJ004	North-central of Xinjiang Province
6	SX001	Northern of Shanxi Province	18	IM010	Southern of Inner Mongolia
7	IM002	Eastern of Inner Mongolia	19	XJ005	Northern of Xinjiang Province
8	IM003	Western of Inner Mongolia	20	KS001	Northern of Korea South
9	GS001	North-west of Gansu Province	21	KS002	Central of Korea South
10	IM004	South-central of Inner Mongolia	22	RF001	South-east of Russian federation
11	IM005	South-central of Inner Mongolia	23	K001	Eastern of Kazakhstan
12	IM006	South-central of Inner Mongolia	24	RF002	West-central of Russian federation

Table 2. Name of primers and sequences of 11 effective primers.

Primer no.	Sequence (5'-3')	Optimum T_m	Total no. of bands	No. of polymorphic bands	The percentage of polymorphic bands (%)
811	GAGAGAGAGAGAGAGAC	56	20	15	75
834	AGAGAGAGAGAGAGAGYT	52	18	13	72.2
835	AGAGAGAGAGAGAGAGYC	54	24	19	79.2
842	GAGAGAGAGAGAGAGAYG	52	25	21	84
845	CTCTCTCTCTCTCTCTRG	53	30	24	80
847	CACACACACACACACARC	52	15	13	86.7
855	ACACACACACACACACYT	57	17	13	76.5
857	ACACACACACACACACYG	52	19	15	78.9
859	TGTGTGTGTGTGTGTGRC	51	14	12	85.7
864	ATGATGATGATGATGATG	51	17	13	76.5
880	GGAGAGGAGAGGAGA	55	15	12	80
Sum			214	170	79.4
Mean			19.5	15.5	

phylogenetic relationships.

MATERIALS AND METHODS

Plant material

A total of 24 accessions of *I. lactea* var. *chinensis* were used, of which nineteen (19) were collected from six provinces in China, two (2) from South Korea, two from Russian, and one (1) from Kazakhstan (Table 1). They were provided by Beijing Research Development Center for Grass and Environment.

DNA extraction and ISSR-PCR

DNA extractions were performed from young leaves of each accessions by the cetyl trimethyl ammonium bromide (CTAB) method with a minor modification. DNA quality and quantity were checked using 1.0% agarose gel electrophoresis and spectrophotometer respectively.

The PCR reaction was carried out in 25 µl of mixture containing 50 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.3 mM dNTP, 0.4 µM primer, 1.5 U Taq DNA polymerase and 1 × buffer. The PCR cycling conditions were as follows: 94℃ for 3 min (initial denaturation), then followed by 35 cycles at 94℃ for 30 s (denaturation), 51~56℃ (different primer, different temperature) for 30 s (annealing), 72℃ for 1 min (extension), with a final 10 min extension at 72℃ and then a cool down to 4℃ (Wang et al., 2008).

One hundred ISSR primers from the Biotechnology Laboratory, University of British Columbia, were screened in this study. Eleven ISSR primers that gave clear and polymorphic bands were used in subsequent experiments (Table 2). The amplifications were repeated twice for each primer analyzed. The PCR products were separated by electrophoresis on 1.5% agarose gels containing ethidium bromide in a 0.5 × TBE buffer, and then photographed under UV light. And they were also separated on 8% non-denaturalization polyacrylamide gels for 4 h at 200 V, followed by staining with 0.1% $AgNO_3$, and then photographed under white light.

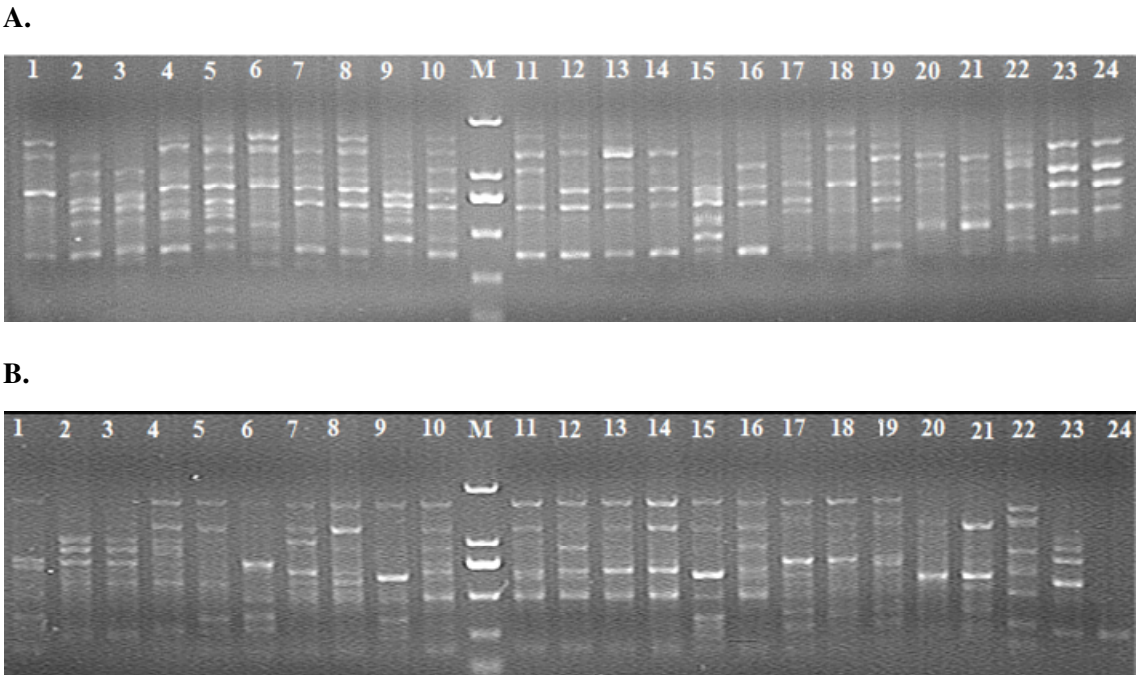

Figure 1. ISSR band profile of primer 834 (A) and primer 842 (B) on 1.5% agarose gels.

Data analysis

The ISSR bands were scored using the binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. Typical bands scored were bright and well-separated from other bands, and faint bands were not scored to avoid the scoring of artificial bands. Data analyses were performed using the NTSYSpc version 2.0 computer program package (Mark et al., 2007; Houda et al., 2007; Xia et al., 2007). Pairwise comparisons were calculated using the Jaccard similarity coefficient. The similarity values were used to generate a dendrogram via the unweighted pair group method with arithmetic average (UPGMA). The data were also computed to perform a principal components analysis (PCA).

RESULTS

Polymorphism of ISSR markers

Eleven of the one hundred ISSR primers used yielded banding patterns that were clear and could be scored with confidence (Table 2), eight of which (811, 834, 835, 842, 847, 855, 857 and 880) were those consisting of “A+G” and “A+C” repeat motifs. Satisfactory results in terms of band resolution were obtained by using primers with different 2-base. It showed that the genome of *I. lactea* var. *chinensis* has many simple sequence repeat such as “AC”, “AG” and “GA”.

In order to get the highest polymorphism, the PCR products were also separated on 8% non-denaturalization polyacrylamide gels, and the study of genetic diversity was on the base of them. Figures 1 and 2 showed the amplified bands using primer 834 and 842 on different gels.

Clearly detectable and reproducible bands from 100 to 2,000 bp in size were amplified. Eleven ISSR primers yielded a total of 214 bands, among which 170 bands (79%) were polymorphic. The number of polymorphic bands varied from 12 to 24 with an average of 15.5 bands per primer (Table 2).

Genetic diversity of *Iris lactea* var. *chinensis*

The genetic diversity analysis revealed that the genetic similarity (GS) coefficients between *I. lactea* var. *chinensis* ranged from 0.400 to 0.929. The highest similarity of 0.929 was observed in no.12 and no.13, whereas the lowest similarity of 0.400 was observed between no.7 and nos. 2, 3 (Table 3).

Based on the GS coefficients between individuals, a cluster analysis was carried out and a dendrogram was generated that represented the genetic relationships among 24 accessions (Figure 3).

In the dendrogram, only one major cluster was formed comprising four groups at a GS coefficient 0.62 and all the accessions in each region clustered together; but in some instances, accessions in one region clustered into different groups.

Group I consists of 15 accessions formed into five sub groups: The first sub group consisted of three strains namely, no.1, no.6 and no.18 at a GS coefficient 0.74; the second sub group was formed with no.17 and no.19 at a

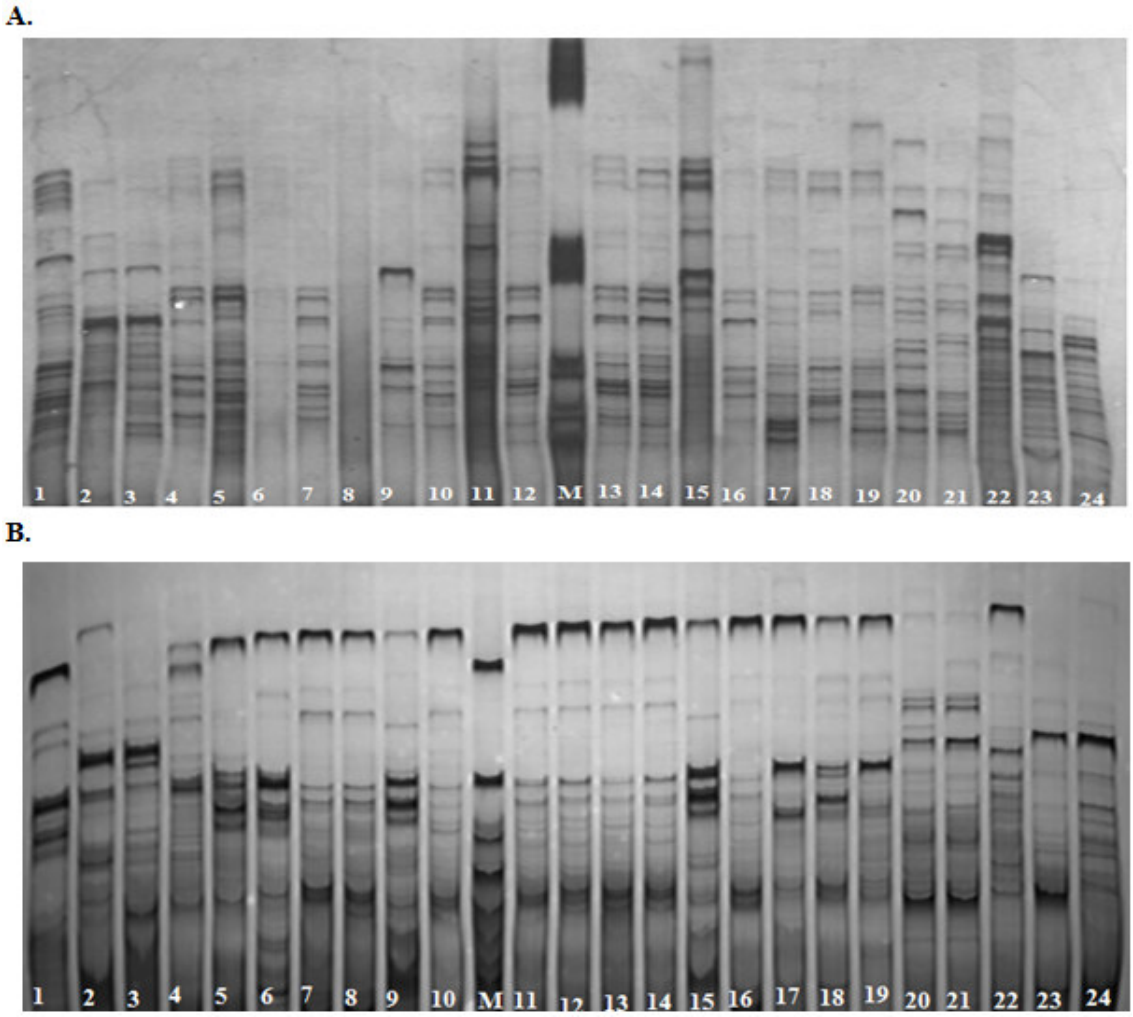

Figure 2. ISSR band profile of primer 834 (A) and primer 842 (B) on 8%. non-denaturalization polyacrylamide gels.

GS coefficient 0.77; the third sub group was formed with no.4, no.7 and no.8 at a GS coefficient 0.77; the fourth sub group consisted of six cultivars at a GS coefficient 0.80; and no.5 was isolated in the sub group. Group II consists of two accessions namely, no.9 and no.15 forming a cluster at a GS coefficient 0.78. Group III has two sub groups, one sub group consisted of no.2 and no.3 at a GS coefficient 0.78, the other sub group consisted of no.23 and no.24 at a GS coefficient 0.86. Group IV also consists of two sub groups, of which one was formed of no.20 and no.21 at a GS coefficient 0.84, and no.22 as a separate isolate in the sub group.

Figure 4 presents the first two axes of the principal components analysis, which revealed similar grouping of individuals. It was obvious that four groups were divided by PCA, and they were similar with the dendrogram. The first and second sub groups of Group I clearly fell into one group, the others fell into another group. Group III and Group IV fell into third group, and Group II was also one group. It showed that the more the difference in genetic distance, the bigger the geographic distance.

DISCUSSION

The knowledge of the genetic diversity within a species may be useful for management programs, since the presence of sub-groups in the range of a species makes necessary to use standards for resource conservation that consider each genetic unit independently from another. Identification and analysis of the genetic diversity were traditionally based on the morphological characteristics, which required careful observation of the plants in field gene banks. In addition, this kind of analysis also required a long period especially for ligneous plant, and easily be affected by developmental stage, environmental conditions, cultivation conditions, and even sampling error. Several markers have been designed for establishing

Table 3. Genetic similarity coefficients of 24 *Iris lactea* var. *chinensis* based on ISSR analysis.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.000																							
2	0.524	1.000																						
3	0.500	0.788	1.000																					
4	0.588	0.465	0.476	1.000																				
5	0.641	0.435	0.435	0.735	1.000																			
6	0.800	0.453	0.500	0.577	0.629	1.000																		
7	0.571	0.400	0.400	0.771	0.706	0.618	1.000																	
8	0.582	0.435	0.435	0.771	0.718	0.641	0.882	1.000																
9	0.594	0.494	0.518	0.582	0.647	0.629	0.553	0.553	1.000															
10	0.571	0.424	0.471	0.724	0.729	0.629	0.741	0.777	0.647	1.000														
11	0.618	0.459	0.459	0.724	0.706	0.618	0.777	0.765	0.635	0.824	1.000													
12	0.606	0.459	0.482	0.759	0.694	0.618	0.777	0.777	0.647	0.812	0.847	1.000												
13	0.606	0.459	0.482	0.747	0.706	0.629	0.777	0.812	0.612	0.835	0.871	0.929	1.000											
14	0.600	0.441	0.465	0.729	0.724	0.624	0.747	0.771	0.594	0.806	0.865	0.877	0.912	1.000										
15	0.606	0.565	0.506	0.559	0.553	0.606	0.553	0.529	0.788	0.600	0.612	0.624	0.577	0.594	1.000									
16	0.588	0.429	0.453	0.706	0.688	0.624	0.759	0.771	0.559	0.794	0.818	0.818	0.865	0.882	0.571	1.000								
17	0.700	0.482	0.459	0.641	0.659	0.653	0.647	0.694	0.624	0.612	0.656	0.682	0.671	0.653	0.635	0.677	1.000							
18	0.782	0.482	0.447	0.629	0.647	0.747	0.647	0.659	0.577	0.588	0.588	0.624	0.612	0.594	0.553	0.629	0.753	1.000						
19	0.694	0.500	0.465	0.659	0.677	0.647	0.629	0.677	0.606	0.594	0.629	0.629	0.665	0.647	0.571	0.635	0.771	0.712	1.000					

Table 3. contd.

20	0.512	0.588	0.565	0.559	0.529	0.535	0.494	0.518	0.494	0.506	0.471	0.482	0.471	0.512	0.529	0.524	0.482	0.565	0.535	1.000			
21	0.506	0.547	0.559	0.588	0.571	0.518	0.500	0.500	0.535	0.465	0.512	0.512	0.488	0.529	0.524	0.482	0.488	0.524	0.565	0.841	1.000		
22	0.447	0.571	0.559	0.482	0.465	0.482	0.465	0.441	0.488	0.406	0.429	0.465	0.429	0.471	0.524	0.471	0.441	0.465	0.459	0.618	0.635	1.000	
23	0.535	0.682	0.624	0.500	0.529	0.535	0.459	0.471	0.565	0.447	0.471	0.471	0.459	0.441	0.541	0.477	0.424	0.494	0.477	0.565	0.582	0.571	1.000
24	0.577	0.688	0.618	0.471	0.512	0.541	0.429	0.453	0.547	0.406	0.465	0.465	0.453	0.459	0.524	0.482	0.465	0.535	0.518	0.571	0.600	0.553	0.865	1.000

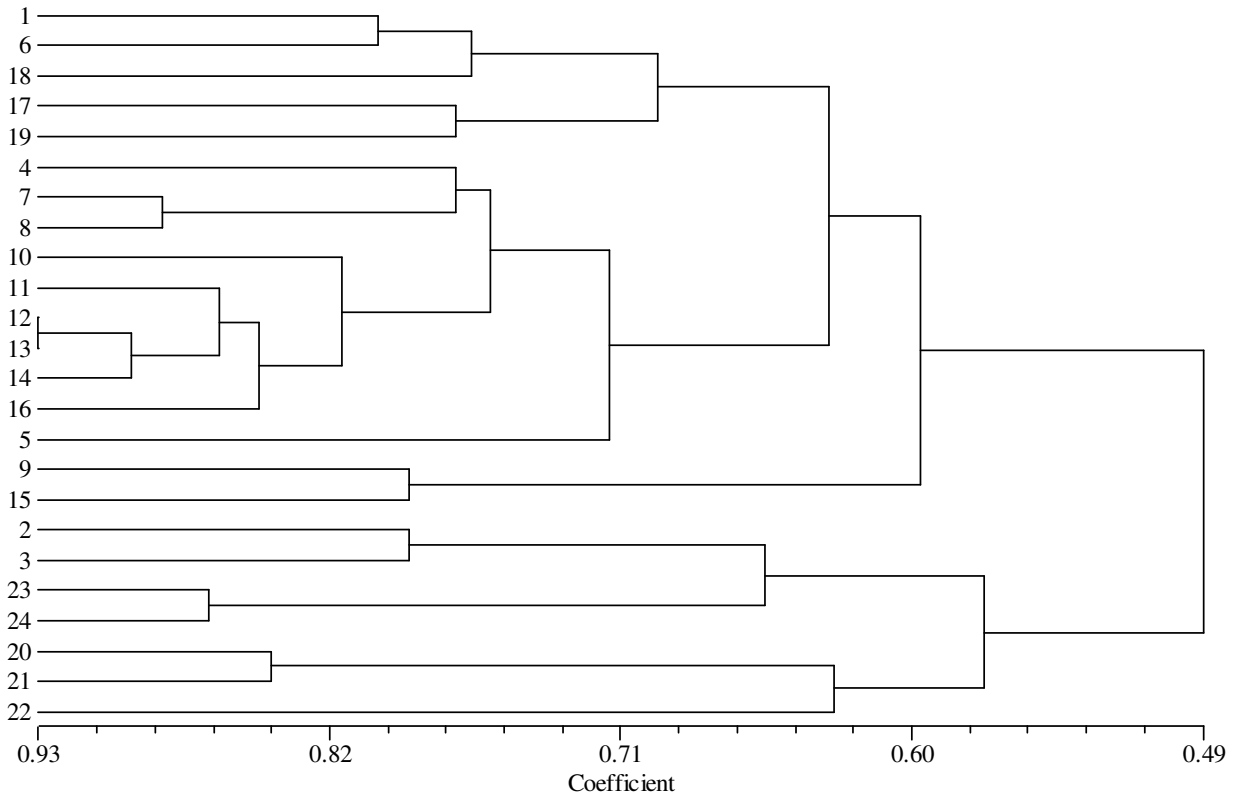

Figure 3. UPGMA-derived dendrogram of 24 individuals based on genetic similarity coefficients.

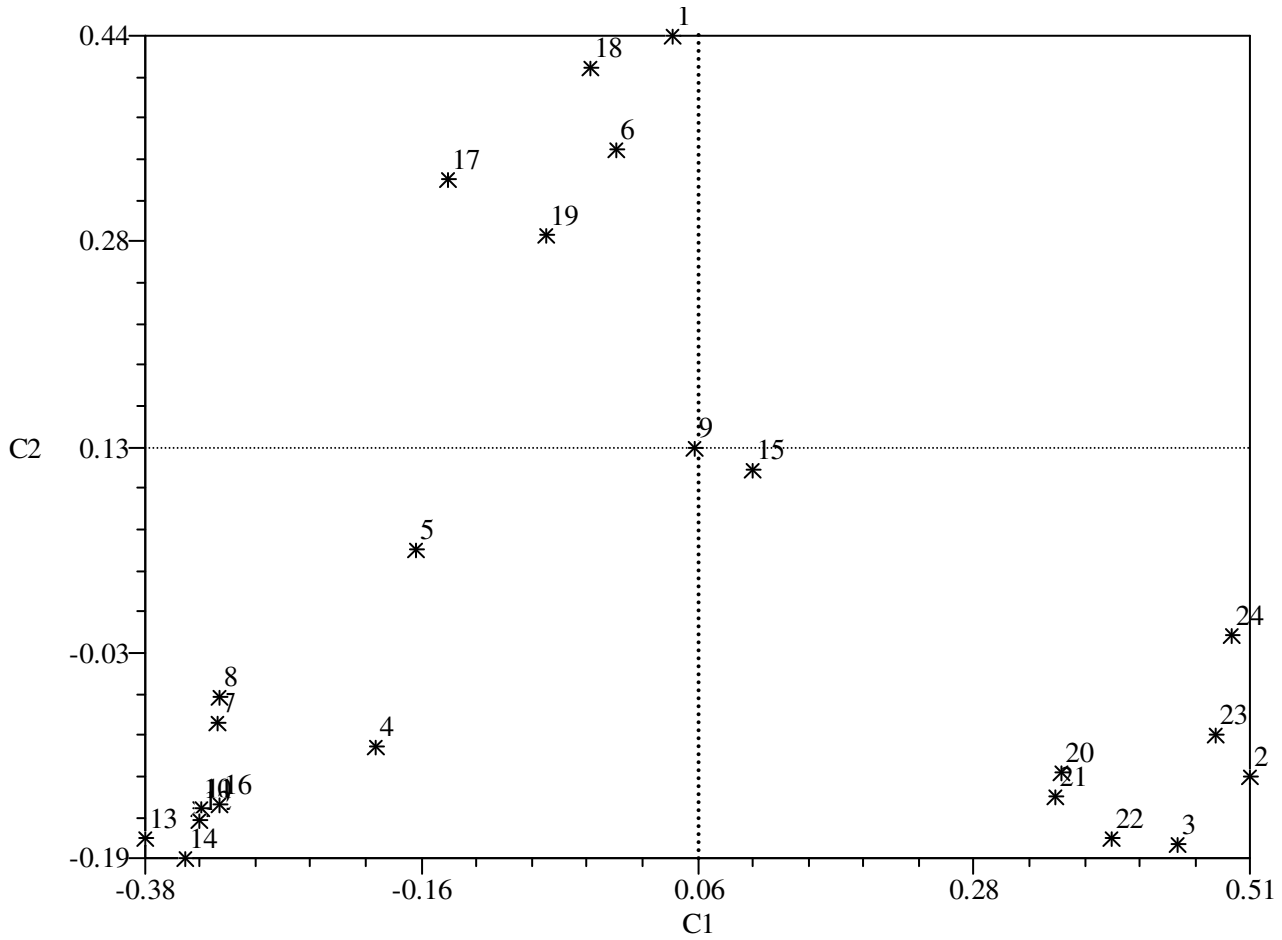

Figure 4. Principal component analysis (PCA) based on ISSR polymorphism of 24 individ

the genetic diversity. However, the disadvantage of biochemical markers, such as isozymes, was their limitation in numbers and thereby only a part of genome can be detected. Molecular markers, which detected variation at the DNA level, overcame most of the limitations of morphological and biochemical markers.

In this study, performing PCR analysis by using ISSR primers has shown to be a useful tool in studies concerning genetic diversity, either at intra- or inter-specific level, phylogenies and evolutionary biology. They were advantageous because no genomic information was required for their use. And it could give reliable results with an elevated reproducibility, since the long lengths and high annealing temperatures of primers decreased the amount of primer mismatched artifacts and had high performances even with very small amounts of tissues. It provided a convenient and rapid assessment of the differences in genetic composition of closely related individuals at the DNA level, and it has been employed in a large number of plant species for characterization and assessment of genetic diversity because of their speed and ease in handling. Recently, similar results have been reported in gerbera plants (Bhatia et al., 2009), cashew (Thimmappaiah et al., 2008), Mediterranean faba bean (Terzopoulos and Bebeli, 2008), *Gynostemma pentaphyllum* (Thunb.) Makino (Wang et al., 2008), sweet potato (Li et al., 2008), *Oryza meyeriana* (Wan et al., 2008), *Nelumbo* (Hong et al., 2008) and eggplant (Shiro et al., 2008).

Evaluation and identification of germplasms using ISSR markers play an important role in studies of genetics and breeding. In this paper, seventeen ISSR primers were used for estimating genetic diversity of 24 accessions. Using these primers, 214 discernible DNA fragments were generated and 170 of them were polymorphic. The present study revealed relatively higher polymorphism (79.4%) in 24 accessions of *I. lactea* var. *chinensis* based on the statistical data. The mean genetic similarity coefficient was 0.5924, indicating that the accessions had high genetic diversity, and particularly, there were high genetic variations among those accessions that came from different countries.

The dendrogram obtained by the UPGMA method consisted of four major groups and ten sub groups (Figure 3). Group I, including five sub groups, had common pedigree from Inner Mongolia province, especially the

fourth sub group, which was probably due to the frequent gene exchanges of germplasm. Group II distributed at the boundary between Gansu and Xinjiang province. Group III had the biggest geographical distance, which came from three countries. And group IV maybe had a same origin from Korea South. Their geographical isola-tion may cause the distinctness in their clustering behavior. That is to say, gene exchange with external varieties did not occur and hence the remote relation-ships were maintained between them. Reif et al. (2005) proved that the genetic diversity of cultivars intended to decline in wheat breeding process may be related to the narrow use of a few parents and the emphasis of several target characteristics.

In conclusion, ISSR markers could be successfully employed to assess the level of polymorphism and diversity in *I. lactea* var. *chinensis*. The above results obtained by ISSR analysis provided useful information for molecular identification, pedigree analysis, genetic improvement, germplasm conservation, and construction of core collections in *I. lactea* var. *chinensis* (Zhao et al., 2006; Shahsavar et al., 2007).

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