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J. R. Yu

*Southwest University, China*

D. Q. Liu

*Kunming University of Science and Technology, China*

L. M. Chen

*Kunming University of Science and Technology, China*

K. Z. Li

*Kunming University of Science and Technology, China*

Yongxiong Yu

*Southwest University, China*

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## Genetic diversity among alfalfa (*Medicago sativa* L.) cultivars using ISSR markers

J.R. Yu<sup>1,2</sup>, D.Q. Liu<sup>2</sup>, L.M. Chen<sup>2</sup>, K.Z. Li<sup>2</sup>, Y.X. Yu<sup>1</sup>

<sup>1</sup>College of Zoological Science and Technology, Southwest University, Chongqing, 400716, China, E-mail: yuyongxiong8@126.com, <sup>2</sup>Biotechnology Research Center, Kunming University of Science and Technology, Kunming, 650224, China

**Key words** : genetic diversity, *Medicago sativa* L., ISSR, cultivar, fall dormancy

**Introduction** The cultivated alfalfa is an autotetraploid and allogamous species ( $2n=4x=32$ ). Alfalfa germplasms are invaluable genetic resources for improving and breeding the alfalfa cultivars. Due to the allogamous behaviour, much genetic variation exists in the alfalfa germplasm. Obviously, it is very necessary to evaluate the genetic diversity and relationship among the alfalfa cultivars through molecular markers analysis. The inter-simple sequence repeat (ISSR) is a kind of molecular marker which exhibits the length polymorphism between two microsatellite loci. In order to provide new insight into the genetic diversity and relationship of alfalfa germplasms, 30 accessions of *Medicago sativa* from China and other four countries were investigated by the technique of ISSR.

**Materials & methods** Thirty cultivars (16 from China, 10 from USA, 2 from Australia, 1 from Canada, and 1 from Germany) of alfalfa germplasms were employed in this study. Alfalfa seeds were surface-sterilized and germinated in the MS medium and grown in a growth chamber for 4 weeks. For each cultivar, about 0.1g of young seedlings were taken and used for genomic DNA isolation with a modified CTAB (Cetyltrimethylammonium bromide) method. Totally 68 ISSR primers were used in the present study. ISSR primers were commercially synthesized according to the sequences released by University of British Columbia, and the procedure for ISSR assay was conducted as described by Narayanan *et al.* (2007). The data analysis was performed with the method of Liu *et al.* (2006).

**Results & discussion** A total of 10 primers which generated clear and distinguishable DNA fragments were screened out among the 68 ISSR primers and applied to the 30 alfalfa accessions. As a result, 10 primers displayed totally 169 alleles, of which 76 alleles were polymorphic, and the ratio of polymorphic alleles was 62.28%. The Jaccard's genetic similarity coefficients among 30 alfalfa accessions ranged from 0.26 to 0.96 with an average of 0.61. On the UPGMA dendrogram constructed from genetic similarity coefficients, the 30 alfalfa accessions were divided into three clusters. Cluster I contained only one accession, Gradullar, which was from America. The cluster II was composed of all 13 accessions, of which fall dormancy classes  $\geq 3$  from southern regions of China, Australia, Germany and America. While all the 16 accessions, of which fall dormancy classes  $< 3$  from northern regions of China, Canada and America fell into the cluster III.

**Conclusions** The results of the present study indicated that there were high genetic diversities existing among alfalfa cultivars throughout the world, especially for the alfalfa cultivars developed in America, which had relatively broader genetic background. A less fall dormant group and a strong fall dormant group were divided under the UPGMA dendrogram constructed from genetic similarity coefficients.

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