Analysis of Genetic Diversity in Alfalfa with Molecular Markers

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Introduction There is increasing interest in the application of molecular technologies in alfalfa (*Medicago sativa* L .) improvement programs in China . Molecular markers and linkage maps have great utility in crop breeding programs to aid in increasing the efficiency of selection . Examining relationships of genetic distance among germplasm is useful to select parents for mapping populations of alfalfa . This study was conducted to assess the levels of genetic diversity of alfalfa including the domestic Chinese varieties and some exotic alfalfa lines .

Materials and methods The field trial was conducted to assess the levels of genetic diversity of 84 lines of alfalfa including the domestic Chinese varieties and some lines from South Australia , North America and Europe . The trial plots were established in Lanzhou , China . The plot design was a randomized block design with three replications . Yields were measured by hand harvesting plots three times a year for five years . The growth rate and regeneration capacity were tested . The natural height and stem height were measured when cutting . The procedure of molecular markers based on PCR have been described . PCR primers derived from soybean , *Medicago truncatula* etc . Analysis system of Randomly Amplified Polymorphic DNA (RAPD) , Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) on genomic DNA of alfalfa(*Medicago sativa*) were established . Data analysis was processed by NTsys 2 .01 .

Results The data show significant differences in height , cutting height and fresh yield among different alfalfa lines . Fifty five alfalfa entrees were divided into seven types by RAPD , SSR and ISSR analysis . The result of cluster analysis showed there is small distance and close relationship existing in Chinese domestic cultivars . Tianshui , a Chinese domestic cultivar is not in the same cluster with the other domestic alfalfa . Also , Tianshui exhibited distinctive varietal characteristics during the field experiments . We obtained 120 polymorphic bands by PCR amplification with screened 32 RAPD primers , 8 SSR primers and 12 ISSR primers from 55 domestic and exotic alfalfa varieties . The results of SSR-PCR on alfalfa showed that there is large difference in SSR polymorphism among the alfalfa varieties . Polymorphism of alfalfa can be detected by a fingerprinting assay . The ISSR which shows abundant polymorphism in the fingerprint of alfalfa was steadier than RAPD .

Conclusions The genetic diversity of the 55 alfalfa cultivars was evaluated by RAPD , SSR and ISSR fingerprinting . The results of fingerprinting assay indicated that polymorphism of alfalfa can be detected and the alfalfa cultivars can be distinguished by the 3 or 6 primers screened . The molecular markers can be effectively applied in studying genetic diversity of alfalfa .

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