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Xiaoli Yang
Lanzhou University, China

Ting Ban
Lanzhou University, China

Peng Han
Lanzhou University, China

Xiang Liu
Lanzhou University, China

Xiaojuan Wang
Lanzhou University, China

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To identify alfalfa cultivars based on rapd markers using bulked and individual DNA

Xiaoli Yang, Ting Ban, Peng Han, Xiang Liu and Xiaojuan Wang*

College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China,

* Correspondence author: xiaojuanwang@lzu.edu.cn

Key words: alfalfa, cultivar, bulked DNA, individual DNA, RAPD

Introduction The genetic relationship and distance among alfalfa cultivars is of great interest for breeding programmers as well as for cultivar identification. However, cultivated alfalfa (*Medicago sativa* L.) is tetraploidy out-breeding species with a self-incompatibility system, which causes a high degree of genetic variation among individuals within populations. The aim of this study was to identify alfalfa cultivars based on RAPD markers using bulked and individual DNA, respectively.

Materials and methods Sixteen alfalfa cultivars were used in this study. Genomic DNA was isolated using CTAB method. 20, 40 and 60 individual plants per population and bulked DNA were analyzed, respectively. A total of 10 RAPD primers were used for routine screening. The bands visualized on the gels were scored manually and each band in the RAPD profile was considered as 0 (absent) or 1 (presence).

Results and discussion Using bulked genomic DNA samples, four out of 10 primers detected the specific bands belong to 5 cultivars respectively, which could be used to identify among alfalfa varieties. Figure 1 (a) showed the patterns of a bulked genomic DNA samples from 20 individuals per population using RAPD primer OPEL-6, two specific bands of cultivar "Longdong" (780 bp) and "Jindera" (950 bp) were appeared, and there were 60% individuals of cultivar "Longdong" to have this specific band. The polymorphic bands did not increase with the numbers of the individuals bulked, which indicated that 20 was enough to identify "Longdong" and "Jindera" from other cultivars. To identify alfalfa cultivars using individual genomic DNA with 10, 20, 40 and 60 plants per population, we found that it was difficult for the high variation within each population (Figure 1, b). According to the results of this study, data based on RAPD markers were useful in identifying alfalfa cultivar varieties using bulked genomic DNA samples from 20 plants per population.

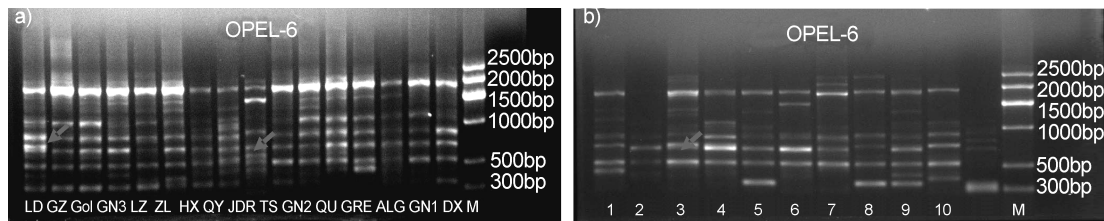


Figure 1 RAPD patterns obtained from bulked genomic DNA samples of 16 cultivars (a) and from 10 individual genomic DNA samples of cultivar "Longdong" (b) with primer OPEL-6.

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