

## Genetic relationships of alfalfa cultivars based on simple sequence repeat (SSR) molecular markers

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Alfalfa cultivars are difficult to distinguish based upon morphological traits. Only a few traits have been used such as multifoliolate leaves and flower color. However, most of the cultivars are characterized by screening for multiple pests including diseases, insects and nematode resistance; salt tolerance, forage quality and a few agronomic traits such as fall dormancy, winter survival, and tolerance of intensive grazing. Molecular markers especially simple sequence repeats (SSR) have not been used extensively in alfalfa to characterize alfalfa cultivars. This study was conducted to determine if simple sequence repeat (SSR) DNA markers could be used to distinguish modern alfalfa cultivars from several commercial companies. Forty-two cultivars were studied with fall-dormancy rating of FD 2-11. Three differently derived SSR were utilized. Genomic SSR (gSSR) derived from a genomic library of *Medicago sativa*; SSR derived from express sequence tag (EST) from *M. truncatula* and bacterial artificial chromosome (BAC) derived SSR also from *M. truncatula* were all used. SSR primer pairs were fluorescently labeled and sequenced on a 3730 DNA Analyzer. Nineteen SSR were utilized to distinguish the cultivars. The sequence data were analyzed and a dendrogram was developed to determine relationships among the alfalfa cultivars. Based upon the results from this study the cultivars could be distinguished from each other. Analysis of the dendrogram and the split tree neighbor-net diagrams demonstrate that the alfalfa cultivars clustered together although the non-fall dormant cultivars were more closely related to each other. We have shown that a small number of SSR can be utilized to distinguish alfalfa cultivars.

