Utilization of SSR to Distinguish Alfalfa Cultivars

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Introduction Simple sequence repeat (SSR) or microsatellite markers are co-dominant, abundant and hyper-variable molecular markers from eukaryotic genomes that are being widely used in genetic mapping and phylogenetic studies. Currently, the number of available SSR markers is still very limited for use in alfalfa (*Medicago sativa*). Thus, this study was conducted to develop SSR from alfalfa genomic libraries and EST and BAC sequence data from *M. truncatula* for use in distinguishing the nine historically recognized U.S. germplasm sources and eleven fall dormancy check cultivars of alfalfa.

Materials and methods The nine historical cultivars of alfalfa included: African, Chilean, Falcata, Flemish, Indian, Ladak, Peruvian, Turkistan and Varia types as well as wild tetraploid M. falcata, two very non dormant *M. sativa* accessions and diploid *M. coerulea*, *M. sativa* ssp. *falcata* and *M. truncatula*. The SSR markers were also used for phylogenetic analysis for the 11 standard cultivars (Maverick, Vernal, 5246, Legend, Archer, ABI 700, Dona Ana, Pierce, CUF 101, UC-1887 and UC-1465) for autumn dormancy. The alfalfa genomic derived SSRs was developed from genomic DNA which was extracted from the alfalfa population W10, digested with Sma I, Alu I, Rsa I, Nac I, Hinc II and Xmn I, and fragments were cloned into pUC19. Colonies were probed with repeats of AC, AT, CT, CTT, GAT and GGT motifs. SSR derived from *M. trucatula* EST and BAC sequenced data were primer analyzed based on primers from several sources (Eujayl *et al*., 2004; Julier *et al*., 2003; Diwan *et al*., 2000). Primers were identified based on the nomenclature used in Diwan *et al*. (2000) or by the corresponding Genbank accession or tentative contig name (www.medicago.org). The PCR amplifications of SSR primers were based on the method of Diwan *et al*. (1997). All reactions utilized 30 ng of DNA in a final reaction volume of 10µl. Fluorescent labelling was used whereby a sequence-specific M13 forward primer is labelled with one of three fluorescent dye labels: FAM, HEX or NED. These fragments were then run on a 3100 DNA Analyzer (ABI) and scored using the Gene Scan and Genotyper software (ABI).

Results A dendrogram was constructed from these data, representing three main clusters: 1) diploid ssp. *falcata*; 2) *M. truncatula*; and 3) all remaining entries. *M. truncatula* (Jemalong), Ladak (Ladak), Arabian (UC-1465), Indian (Sirsa Type 9), Flemish (DuPuit), Peruvian (Hairy Peruvian), African 2 (Moapa) and Turkistan (Kayseri) were separated through multiple correspondence analysis. The remaining germplasms within the ssp. *sativa* species could not be separated due to a limited number of SSR markers used in this study. The results of phylogenetic analysis of the fall dormancy standards showed that the cultivars which are non-dormant tended to be clustered together separate from the cultivars which are dormant.

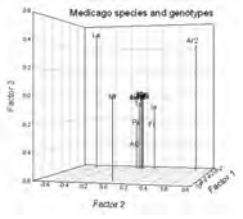

Figure 1. 3D scatter plot after multiple corres-pondence analysis of genetic distance among historically recognized alfalfa germplasm sources. Mt=*M. trucatula*, La=Ladak, Ar2=Arabian, In=Indian, Af2=African, Pe=Peruvian, and Tu=Turkistan

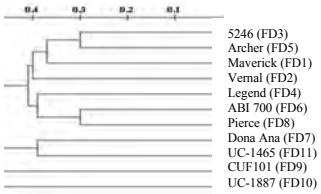

Figure 2 Dendogram of the 11 fall dormancy check cultivars of alfalfa.

Conclusions SSR can be utilized to discriminate a number of alfalfa cultivars, although additional SSR will need to be developed to clearly identify individual cultivars of alfalfa.

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