Estimating genetic relationships among semi-dormant and nondormant alfalfa cultivars with Sequence Related Amplified Polymorphisms (SRAPs).

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The objective of the present study was to assess the utility of molecular marker data generated by Sequence Related Amplified Polymorphisms (SRAPs) to assess genetic relationships among semi-dormant and non-dormant modern alfalfa cultivars using bulked DNAs. Marker data was also used to examine relative levels of genetic diversity within cultivars. Five bulk DNA samples representing 20 plants per bulk were isolated from 29 semi-dormant and nondormant alfalfa cultivars and the nine historically recognized alfalfa germplasms (PIs). Bulked DNA samples were amplified with seven different primer pairs. Dice similarity coefficients were derived from amplicon presence/absence data. Unweighted pair-group arithmetic average (UPGMA) cluster analysis was conducted to generate a dendrogram representing genetic similarity between bulked DNA samples.

The seven primer pairs generated 188 markers ranging in frequency from 100.0 to 0.5%. The average number of markers per primer pair combination was 27. Only six markers (3.9%) were monomorphic (i.e. present in all DNA bulks) and 32 markers were present at a <10% frequency in the 189 DNA bulks included in the study. The highest numbers of markers were associated in ≤10% and ≥90% of the DNA bulks. In general, most bulks of a given cultivar or population clustered in close proximity with other bulks of the same cultivar or population. Cluster analysis revealed two main clusters: 1) all five bulk DNA samples of *M. falcata* and a single bulk sample of *M. varia*, and 2) all other cultivars and PIs. Of the cluster that included the majority of the samples included in the study, fall dormancy classes 6, 7, and 8 separated clearly from FDCs 9, 10, and 11 with one node including all cultivars with FDC of 6, 7 and 8, and another containing the remaining FDCs (9, 10 and 11) and the historically recognized germplasm sources, with the exclusion *M. falcata*. In order to assess relative contribution of the markers generated in the study, cluster analyses were conducted using subsets of the marker data. Analysis of markers present in ≤90%, ≤60%, and 10%-90% of the bulked DNAs did not provide better resolution of cultivars/populations nor were higher bootstrap support values obtained than the results based on all 188 markers. The highest mean genetic similarity estimate among bulks within cultivars/populations was observed in 13R Supreme while the lowest mean genetic similarity was seen in Ameristand 801S. The highest mean genetic similarity between populations was seen between Lobo and 5681 and the lowest mean genetic similarity estimate was observed between Turkistan and DK 194. SRAP markers are relatively easy to generate, have extremely high reproducibility, and can be easily resolved using simple electrophoresis. These results suggest that SRAPs may be useful in identifying marker-phenotype associations in alfalfa with bulked plant samples.