Genetic similarities between alfalfa cultivars based on an analysis using sequence related amplified polymorphism (SRAP) DNA markers

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Sequence related amplified polymorphisms (SRAPs) are relatively simple and highly reproducible DNA markers that are useful for both mapping and gene tagging in plants. The reaction is based on amplification of template DNA with primers that consist of three sequence domains: 1. The first 10-11 nucleotides at the 5' end of the primer are random, followed by 2. The sequence CCGG in the forward primer and AATT in the reverse primer, and finally 3. Three selective nucleotides at the 3' end of each primer. The CCGG sequence in the forward primer results in preferential annealing to exon sequences, while the AATT domain causes the reverse primer to preferentially anneal to non-coding regions This amplification strategy tends to amplify open reading frames (Li and Quiros, 2001).

We isolated DNA from commercial alfalfa cultivars and nine sources of alfalfa germplasm: Ladak, *M. varia*, Chilean, Peruvian, Indian, Turkestan, Flemish, African, and *M. falcata*. The DNA samples were amplified using 38 different SRAP primer pairs. Amplifications were performed in 25µl reactions containing 50 ng DNA, 200µM each dNTP, 1.5 mM $MgCl_2$, 2.5 U Amplitaq Gold DNA polymerase, and 37 ng each forward and reverse primer. Thermocycling profile was as described by Li and Quiros (2001). Polymorphisms were resolved on 15% polyacrylamide-TBE gels.

Primer pairs varied considerably in the number of amplicons produced and in detectable polymorphisms. The 38 primer pairs produced over 400 amplicons. The nine sources of germplasm could all be clearly discriminated using as few as four different primer pairs. Currently we are using SRAPs to detect variation both within and between alfalfa cultivars representing a wide range of parental materials and zones of adaptation. The markers should be quite useful for linkage mapping and marker-assisted-selection in alfalfa.

Li, G. and C. Quiros. 2001. Sequence-related amplified polymorphisms (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor. Appl. Genet. 103:455-461.