A High-Density SSR Linkage Map of Red Clover and Its Transferability to Other Legumes

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Introduction A high-density linkage map of red clover was constructed based on SSR and RFLP markers. In order to construct a linkage map with user (breeder) friendly markers; i.e. informative and easy detection, two policies were adopted for marker development. One was that the markers should be derived from cDNA or gene-rich regions, and the other was that the SSR markers should be detected polymorphisms on agarose gels.
We also discuss the transferability of the markers on the map to other red clover germplasm and legumes. Such highly transferable markers could be used to screen anchor markers for both on a consensus map of red clover and other legume species.

Materials and methods One hundred and eighty eight mapping individuals were derived from cross progenies between "HR" (a clone from Swiss variety "Renova" x Japanese variety "Hokuseki") and "R130" (A clone from Russian variety x wild accession collected in the Archangelsk region). SSR-enriched cDNA libraries, normalized cDNA libraries, SSR-enriched genomic libraries and methyl filtrated genomic libraries of red clover were constructed to develop SSR markers. SSR regions were detected by sequencing the libraries, and then primer pairs were designed. Polymorphic bands of SSR markers were detected on 3% agarose gels. A combined genetic linkage map was calculated using JoinMap 3.0. Eighty red clover germplasms and thirteen kinds of legumes including white clover, alfalfa, *Medicago truncatula*, *Lotus japonicus* and soybean were tested by PCR amplification using SSR markers on the map.

Results and discussion A total of 7,244 SSR marker primer pairs were successfully designed from a total of 83,172 sequences derived from the red clover genomic and cDNA libraries. Twenty percent of the primer pairs exhibited polymorphisms within the mapping population on 3% agarose gels. A combined genetic linkage map was constructed with a total of 457 markers, including 338 SSR and 119 RFLP markers.

Approximately 70% of the SSR markers on the map amplified strong bands at corresponding positions in white clover DNA. Also, 33% and 27% SSR markers amplified strong bands at corresponding positions in *Medicago truncatula* and Alfalfa DNA, respectively. This result indicated that the SSRs of red clover were highly conserved between *Trifolium* and *Medicago* species, and that the SSR markers of the present map were transferable to the other forage legumes. The present map should allow us to obtain useful genetic information on red clover, other *Trifolium* species and other members of the legume family.

Conclusions A high-density linkage map of red clover based on SSR and RFLP markers was constructed. The markers were developed taking informative and easy detection into consideration for user-friendly use. The SSR markers were transferable to *Trifolium* and *Medicago* species and could be used as anchor markers in legume species.