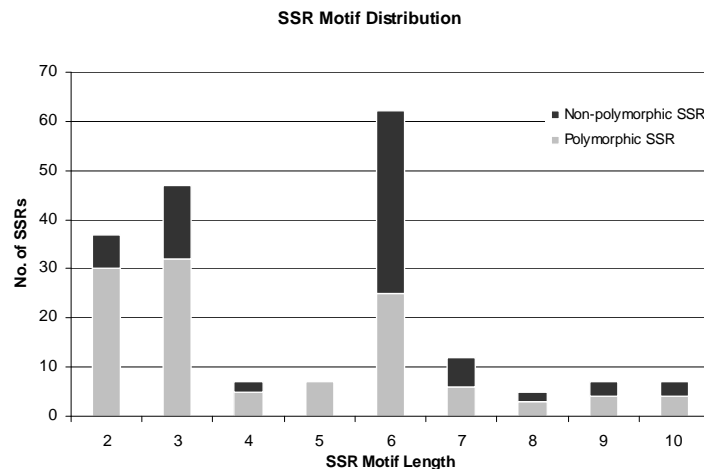


Genome-wide Identification of Microsatellites in White Clover (*Trifolium repens* L.) using FIASCO and phpSSRMiner

Yan Zhang, Ji He, Patrick X. Zhao, Joseph H. Bouton, Maria J. Monteros
The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401

Allotetraploid white clover (*Trifolium repens* L.) is an important forage legume widely cultivated in most temperate regions. The objectives were to develop an integrated approach for microsatellite development and evaluate the approach for the development of new SSR markers for white clover. Genomic libraries were constructed using a modified Fast Isolation by AFLP of Sequences COntaining repeats (FIASCO) procedure (Zane et al., 2002) and phpSSRMiner was used to develop the microsatellite markers. Sequences containing simple sequence repeat (SSR) motifs were isolated using two biotin-labeled probes, (CA)₁₇ and (ATG)₁₂. The sequences of 6,816 clones were assembled into 1,698 contigs, 32% of which represented novel sequences based on BLASTN searches. Approximately 32%, 28%, and 16% of these SSRs contained hexa-, tri-, and di-nucleotide repeats, respectively. The most frequent motifs were the CA and ATG complementary repeats and the associated compound sequences. Primer pairs were designed for 859 SSR loci based on sequences from these genomic libraries and from GenBank white clover nucleotide sequences. A total of 191 SSR primers developed from the two libraries were tested for polymorphism in selected clones of the parental genotypes GA43 ('Durana') developed by Bouton et al. (2005), SRVR developed by Gibson et al. (1989), and six F1 progeny from a mapping population. Ninety two percent produced amplicons and 66% of these were polymorphic. The approach described here could be readily adapted and utilized in other non-related species with none or limited genomic resources.



References

- Bouton, J.H., Woodfield, D.R., Caradus, J.S., Wood, D.T., 2005. Registration of 'Durana' white clover. *Crop Sci.* 45, 797.
- Gibson, P.B., Barnett, O.W., Pederson, G.A., McLaughlin, M.R., Knight, W.E., Miller, J.D., Cope, W.A., Tolin, S.A., 1989. Registration of Southern Regional Virus Resistant white clover germplasm. *Crop Sci.* 29, 241-242.
- Zane, L., Bargelloni, L., Patarnello, T., 2002. Strategies for microsatellite isolation: A review. *Mol. Ecol.* 11, 1-16.