Poster Abstract - 5.12

BIPARENTAL PLASTID INHERITANCE IN *MEDICAGO SATIVA* **REASSESSED USING PLASTOME POLYMORPHISMS**

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alfalfa, chloroplast, microsatellites, Plastome inheritance

Medicago sativa is known to have bi-parental plastid inheritance with paternal bias, as demonstrated using chloroplast (CP) mutations. With the final objective to investigate if this peculiar type of plastid inheritance is also present in other *Medicago* species, we are developing plastid DNA markers suitable for this purpose. Two unrelated *M. sativa* genotypes, named CLA and SAL, were reciprocally crossed and evaluated for 10 published CP microsatellites that, surprisingly, were all monomorphic. Two previously described CP DNA hypervariable regions (Skinner 2000) were then amplified and one of them (named *Hind*III region) was polymorphic giving an amplicon of about 500bp in CLA and 700 in SAL.

Progeny plants from the reciprocal crosses were so far screened with the *Hind*III polymorphism. Of 94 SAL x CLA progenies, 87 (92.6%) had the paternal marker, 5 (5.3%) had the maternal marker, and two was heteroplasmic, displaying both markers. The genomic DNA was re-extracted from these two individuals and heteroplasmy was confirmed. One plant had the paternal band plus a shorter amplicon of about 400 bp, probably the result of a replication-related deletion that has previously been documented for this region containing direct repeats. Of 76 CLAxSAL progenies, 61 (80.3%) had the paternal marker, 14 (18.4%) had the maternal marker, and one, displaying both markers. The alfalfa CP inheritance pattern is therefore confirmed in this cross.

The second hypervariable region (named *Hae*II) appeared to be monomorphic after agarose gel electrophoresis, giving an amplicon of about 1300 bp. Because the sequence of this region is not published, we sequenced it. The high polymorphism previously found in this region among alfalfa germplasms is probably related to the presence of an AT-rich direct repeat of about 64 bp present in 5 copies. We found a 5 bp and two single base deletions in SAL, that can be used to design primers for an allele-specific PCR test.

The PCR-based markers used in this preliminary experiment will be tested in *Medicago truncatula*, a model species in which, to our knowledge, plastid inheritance has not yet been investigated. The sequence of most of the primers developed for alfalfa CP markers was found in the *M. truncatula* published plastome sequence.

Reference

Skinner (2000) Non random chloroplast DNA hypervariability in *Medicago sativa*. Theor. Appl. Genet. 101:1242-1249