

## Structuration of genetic diversity among and within alfalfa varieties using microsatellite markers

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The analysis of genetic diversity among alfalfa cultivars is complex because of the high within-cultivar variation and the autotetraploidy of the species. Eight codominant SSR markers (1) were used to analyse the genetic structuration among and within a set of populations including seven cultivars registered by a single breeder and his breeding pool. F-statistics, extended to autotetraploids, were applied (2). Overall populations, the number of alleles per locus varied from 3 to 24, and the mean number of alleles per plant (A) varied from 1.92 to 3.25 depending on the locus. Except for rare alleles, all the alleles were present in all cultivars. The mean gene diversity (H) was high, ranging from 0.630 to 0.684 in the cultivars and in the breeding pool. This parameter of within-cultivar diversity was not related to the year of registration of the cultivars, indicating that there was no loss of genetic diversity and no genetic shift during selection. Similarly the within-cultivar diversity was not related to the number of parents (from 3 to 15 half-sib families). All loci were at a panmictic equilibrium in the cultivars, except one, probably because of presence of null alleles. With seven SSR loci, each cultivar was at panmictic equilibrium. The parameters  $F_{ST}$  and  $\rho$  were small, but significant for six of the seven markers. The  $F_{ST}$  for each cultivar compared to the breeding pool ranged from 0.003 to 0.008.  $F_{ST}$  ranged from 0.001 to 0.012 between the 21 pairs of cultivars, 15 of them being significant. It indicates that selection was made with little change in the genetic background, even if, as indicated by the agronomic evaluation, significant progress was obtained on disease or lodging resistance and protein content. The use of non neutral markers would have probably given a more significant structuration among cultivars. A complementary analysis of diversity with varieties of broader origins is needed to assess the possibility of using microsatellite markers for variety distinction.

Table 1.  $F_{ST}$  and  $\rho$  estimators of genetic differentiation for 7 SSR locus and over all loci, evaluated on 7 alfalfa cultivars (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.0001$ ; NS: not significant)

Locus	$F_{ST}$	$\rho$
FMT13	0.0040 NS	0.0145
MTIC451	0.0068 **	0.0259
MTIC189	0.0095 ***	0.0349
MAA660456	0.0050 ***	0.0202
B14B03	-0.0009 *	-0.0032
MTIC93	0.0282 **	0.1060
MTIC432	0.0060 ***	0.0238
Over all loci	0.0064 ***	0.0242

### Literature cited

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2. Ronfort J, Jenczewski E, Bataillon T, Rousset F (1998). Analysis of population structure in autotetraploid species. Genetics 150:921-930.