Genetic Diversity Among Alfalfa Cultivars Using SSR Markers

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Introduction Alfalfa (*Medicago sativa*) is an autotetraploid, allogamous and heterozygous species. Cultivated varieties are synthetic cultivars, usually obtained through 3 or 4 generations of panmictic reproduction of a set of various numbers of parents. The parents can be clones, half-sib or full-sib families. The breeders apply selection pressure for some agronomic traits, to induce changes in the genetic background. The objective of this study was to investigate the differentiation level among seven cultivars originating from one breeding program, and between these cultivars and the breeding pool, with eight SSR markers.

Materials and methods We focused on seven varieties and the breeding pool of a French private breeder (GIE GRASS, formerly GIE Verneuil), registered between 1990 and nowadays. Each cultivar was represented by 20 plants, except one that was represented by 40 plants. The Breeding pool was represented by 49 plants. Eight SSR primer pairs originating from *M. truncatula* and mapped in alfalfa (Julier *et al*., 2003) were selected to assay genetic variation in the cultivars. Genotypes were scored according to band intensity, since an allele can be present in more than one dose. The within population genetic diversity was estimated as the number of alleles (*A*) and the expected heterozygosities according to Hardy-Weinberg expectations (H_e) for each SSR locus. To test for a departure from Hardy-Weinberg expectations in the different studied populations, we used the software AUTOTET (Thrall and Young, 2000) to calculate the parameter F_{IS}. The level of differentiation between the cultivars was measured using the differentiation parameter F_{ST} computed in an ANOVA framework extended to autotetraploids, with the software Gene4X (Ronfort *et al*., 1998).

Results The number of alleles per locus ranged from 3 to 24 depending on the locus, with an average of 14.9 alleles per locus. The number of alleles tended to be higher in the Breeding pool, but this tendency was not true after correction of the allelic richness for sample size. The mean number of alleles per plant (*A*) per locus ranged from 1.92 to 3.25 depending on the locus. For all SSR loci, the most frequent and infrequent alleles were the same among all the cultivars and the Breeding pool whatever the number of alleles per locus. One SSR locus, that showed a significant heterozygote deficiency related to the presence of null alleles, was removed from the data for the following analyses. Globally, all the studied cultivars and the Breeding pool showed no departure from Hardy-Weinberg equilibrium. The mean genetic diversity (H_e) for each cultivar and for the Breeding pool ranged from 0.665 to 0.717, indicating high within-population variability. H_e was not related to the year of registration nor to the number of parents of the cultivars. Over all loci and all populations, F_{ST} was highly significant ($P<0.001$), but the estimate were low (F_{ST} = 0.0048). The differentiation between each pair of cultivars was significant for 15 of the 21 pairs of cultivars, with F_{ST} ranging from 0 to 0.005.

Conclusions The SSR loci, with a scoring of the allelic doses, gave us the possibility to exploit the whole genotypic information. A large within-cultivar variation was observed, but the level of differentiation among cultivars was low, even if significant. Our results also show that the sampling effect due to the selection within the breeding pool of the female parental plants has a reduced impact on the differentiation between each cultivar and the breeding pool. Finally, this breeding procedure produces cultivars with small differences in allelic frequencies for neutral markers. Non neutral markers, related to breeding traits, would have probably given a stronger differentiation among cultivars. This type of description of genetic diversity in alfalfa populations is useful for the analysis of breeding programs or genetic resources management. The use of SSR markers to detect differences among cultivars (that usually is a critical step in variety registration due to low among-cultivar distinction for quantitative traits) requires further studies based on a wider range of variation.

References

Julier B, Flajoulot S, Barre P, Cardinet G, Santoni S, Huguet T, Huyghe C (2003) Construction of two genetic linkage maps in cultivated tetraploid alfalfa (*Medicago sativa*) using microsatellite and AFLP markers. BMC Plant Biol 3:9

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

Thrall PH, Young A (2000) AUTOTET: A program for analysis of autotetraploid genotypic data. J Hered 91:348-349