

USE OF SSR MARKERS FOR CLUSTER ANALYSIS OF ALFALFA CULTIVARS TO DEFINE POSSIBLE HETEROTIC GROUPS.

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In alfalfa breeding, it is critical to combine genetically diverse genotypes for capitalizing heterosis and preventing inbreeding depression. In the development of new synthetic varieties, breeders usually chose their parental material based on the phenotype. However, the choice of parents according to their genetic diversity may provide a powerful tool for combining genotypes capable of providing higher biomass production through a more distant genetic base. In this context, molecular marker technology, like SSR, could be used to estimate genetic distance among cultivars or genotypes, and then defining heterotic groups. The objectives of this work were to characterize genetic diversity in a group of non-dormant alfalfa cultivars using SSR markers, and to identify possible heterotic groups among them.

Thirteen FD 9 commercial cultivars (ACA 903, CW 194, DK 191, GAPP 969, Magna 901, Milonga II, ProINTA Mora, Salado, SPS 9000, Super Sequel, WL 903, Zaino and 59N59), each one represented by 20 plants, were analyzed using six SSR markers: FMT13, MTIC451, B14B03, MTIC432, AFca1, and AFct11. These markers were chosen by their satisfactory PCR amplification and resolution in 6% polyacrylamide denaturing gels. Banding patterns were codified and analyzed using Gene 4X (1) and Atetra (2) software in order to estimate F_{ST} matrix and Nei's genetic distance matrix, respectively. Data were then utilized to perform two cluster analyses for all cultivars using InfoGen/P-2006 statistical package (3). Four and three clusters were defined by the F_{ST} matrix and Nei's genetic distance, respectively; however, cultivar grouping were similar between both methods. Overall, three main groups were identified: a) Magna 901 alone; b) WL 903, ProINTA Mora and SPS 9000; and c) GAPP 969, DK 191, Super Sequel and Salado. The remaining cultivars exhibited varied grouping patterns depending on cluster methodology, tending to be related as a group by itself under Nei's genetic distance, but individually related to other cultivars under F_{ST} matrix criteria.

It was concluded the SSR markers can be used in the definition of crossing blocks for developing alfalfa cultivars with higher genetic base. The use of 20 genotypes to represent the variability of each cultivar appears to be adequate and in agreement with some other works. However, the use of only six SSR markers seems insufficient for genetic diversity studies and for a more consistent definition of heterotic groups.

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(2) Van Puyvelde, K. *et al.* 2010. *Molecular Ecology Res.* 10: 331-334.

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