

HETEROZYGOSITY TREND ESTIMATED BY SSR MARKERS IN THE FIRST STEP OF ALFALFA FREE-HYBRIDS CONSTRUCTION

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In the construction process of 4-constituents alfalfa free-hybrids (Rotili et al., 1999), the first step is the crossing of partially inbred (S_2) parental plants, selected for dry matter production and other traits of interest during two cycles of selfing, to obtain Simple Hybrids (SH) $S_2 \times S_2$. These hybrids, once multiplied with selection till Syn3 generation, will constitute the parents ($2S_2$ Syn3) of the 4-constituents free-hybrids.

Aims of the present work are to study the genetic diversity of the S_2 parents and to estimate the trend of heterozygosity recover following the crossing $S_2 \times S_2$ by means of SSR molecular markers; besides, to investigate the relationship between these parameters and the yielding performance of SH progenies.

The parental population was represented by S_1 progenies of plants from somatic hybridization *M. sativa* x *M. falcata* (Téoulé, 1983) crossed to non-inbred *M. sativa* of various origins. Fourteen S_2 mother plants (MP) belonging to 4 families were chosen by means of positive selection for dry matter (DM) yield and total stem height and divergent selection for average internode length during two selfing cycles. SH progenies were obtained by manual crossing without emasculation of the S_2 MPs and the corresponding synthetics (Syn2 generation) by manual polycross of the same plants. Four SHs (640 plants in total) and the corresponding synthetic (1600 plants) were studied for two years in miniplots 25 cm diameter 80 cm height at the density of 400 plants m^{-2} with not limiting irrigation. Within each SH progeny, the best performing individuals (mean + 1.5s) with the desired stem morphology were selected (5, 6, 3 and 3 respectively). The 17 SH individuals and the 14 S_2 MPs were analyzed by means of 67 SSR loci derived from both *M. sativa* and *M. truncatula* to estimate genetic similarity (DICE coefficient) and heterozygosity level.

The four Simple Hybrids differed significantly for total dry matter yield (11 cuts in two years): with reference to the production of the corresponding synthetic (Syn2), the best SH showed a gain of +30% and the weakest one a decrease of -28%. Final mortality was consistent with the productive data resulting 5% and 47.5% respectively for the highest and the weakest SH. The analysis of SSR data is currently being performed.