Analysis of Genetic Changes in Single-Variety Ryegrass Swards

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Introduction Ryegrass varieties are synthetics, with a wide within-variety genetic variance for most traits. Ryegrass swards are likely to experience genetic changes with seasons and years because of plant death, asymmetric vegetative reproduction or plant recruitment through reproduction or seed immigration. These changes may be related to or induce changes in agronomic traits, such as biomass production or dry matter composition. The present research was undertaken to measure genetic changes in swards obtained from sowings of a single variety of ryegrass. These changes were evaluated using both neutral molecular markers and morphological traits. The present paper deals with the molecular markers.

Material and methods A diploid ryegrass variety, cv Herbie, was cultivated under 12 different treatments combining different environmental conditions (5 locations in France), duration of cultivation (from 2 to 7 years) and exploitation regimes (grazing or cutting). In spring 2003, samples of 100 independent tillers were taken from each treatment to provide different populations. Two 100 seed lots of Herbie were used as controls. Every individual plant from the field samplings and the seed lots may be considered as a different genotype. DNA was extracted from each individual initial tiller. Each genotype was characterised with 13 molecular markers homogeneously distributed over the 7 chromosomes of the ryegrass haploid genome. The primers used for revealing marker polymorphism were obtained from INRA Lusignan or the Noble Foundation (Ardmore, OK, USA). PCR reactions were performed and markers were revealed using an ABI 3100 capillary sequencer on the Genotyping Laboratory in INRA Clermont-Ferrand. The software used for population genetic analysis included: Genetix, Genepop (Raymond & Rousset, 1995) and Geneclass (Piny *et al.,* 2004). Allelic frequency for each marker was analysed as well as the panmictic status of each population. Analysis of allelic polymorphism makes it possible to detect the recruitment of new individuals. Wright F-statistics (Wright, 1965) are being calculated for the whole set of populations. Distance among populations and the initial seed batches are also being calculated.

Results Data analysis is still in progress. As expected from the structure of the initial polycross of the variety Herbie, wide polymorphism was observed within the initial seed lots of the variety with the number of alleles varying from 2 to 15 depending on the markers, with a prevalence of a few alleles for each marker (Fig 1). Small but not significant differences among initial seed batches were observed. Panmictic equilibrium was reached for all markers. This is relevant to the four generations of multiplication in the commercial seed production of this variety. However, a small heterozygous deficiency was detected for most markers. New alleles were detected for 6 markers at a low frequency, in some cultivated swards. It is relevant to the low immigration rate observed in grassland swards. This extra genetic variation may originate from either the pollination of plants from the swards by foreign pollen (Nurminiemi *et al.*, 1998) or seed immigration (Mouquet *et al.*, 2004)
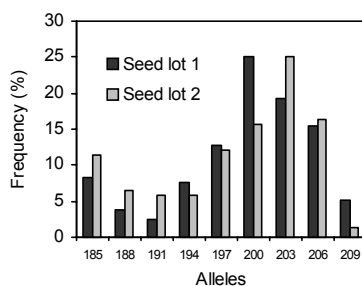

Figure 1 Distribution of allele frequency in the two seed batches for the microsatellite marker NFFA027

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