The Use of Molecular Markers in Genetic Variability Analysis of a Collection of *Dactylis Glomerata* L.

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Introduction *Dactylis glomerata* L. is one of the three most used perennial grasses in Europe (jointly with *Lolium perenne* and *Festuca arundinacea)*. The main qualities of *Dactylis glomerata* are high productivity in pure cultures, high level of proteins and tolerance to drought, cold and shade (Mousset & Chosson, 1986). In the last ten years, techniques that allow direct discrimination at the DNA level have encouraged the study of the genetic variability within cultivated populations as well as identification of the diversity available in germplasm banks. DNA polymorphisms have been shown to be efficient in the identification of genetic variability in several groups of plants. They can be used as an auxiliary tool in breeding programs through obtaining genetic maps and in the identification of molecular markers useful for assisting selection. The aims of this work were: (1) to study the genetic variability of 100 genotypes in a collection of *Dactylis glomerata* from several areas of Portugal, grown in the experimental fields of ENMP-INIAP, using PCR based molecular markers obtained with RAPD and ISSR primers, together with morphological and agronomic characterization, (2) to select genotypes that may function as parents of new synthetic varieties.

Materials and methods DNA was extracted from fresh leaves of 100 genotypes of *Dactylis glomerata* using a modified CTAB method. DNA polymorphism was revealed using random amplified DNA (RAPD) based on oligonucleotides of 10 bases and a technique that requires the use of a primer composed by a repetitive sequence and an arbitrary sequence of 1 to 3 nucleotides in one of the extremities (ISSR). Markers were analysed with NTSYS-PC software (version 2.01b). A matrix of genetic variability was constructed with the data using the DICE coefficient (D). The grouping method UPGMA (Unweighted Pair Group Method with Arithmetic Average) was used to construct dendrograms.

Results and discussion A total of 89 RAPDs and 47 ISSRs were obtained and analysed using the grouping methods of UPGMA. The analysis of the RAPD dendogram showed that the genotypes could all be separated. However the similarity level was very high (94%) due to the fact that the majority of genotypes were from the same sub-species *Dactylis glomerata* ssp.*hispanica.* This high level of similarity is in accordance with data obtained from the morphological and agronomic characterisation of the genotypes. With regard to the ISSR dendrogram, the number of markers appeared not to be sufficient to separate all the genotypes. However those genotypes that were considered the best according to morphological and agronomic characterisations were all located in the same area of the dendogram and were relatively separated from other genotypes. In a joint analysis of the results, as expected the RAPD markers had more influence than the ISSRs. Genotypes were not aggregated according to geographic origin and it appears that genotypes that are not close geographically may be close genetically and vice-versa. The genotypes of most interest with regard to high yield, long growing season and the greatest spring and winter vigour are positioned in the lower section of the dendrogram (Figures 1 and 2). From this group of about 25 genotypes, parents were selected to form a new synthetic variety. The chosen genotypes were 2, 33, 40 and 41 and these are already established in the experimental field following the third year of crosses.

Reference

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