

Comparison of genetic diversity among perennial ryegrass varieties (*Lolium perenne*) revealed by AFLP and RAPD markers

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Introduction

Knowledge of germplasm diversity and of relationships among elite breeding materials has a significant impact on the improvement of crop plants (Hallauer *et al.* 1988). In grass, this information is useful in planning crosses for hybrid and line development, in assigning lines to heterotic groups, and in plant variety protection. It can be obtained from pedigree and heterosis data, from morphological traits or using molecular markers, which detect variation at the DNA sequence level (Smith and Smith 1992). In particular, DNA-based polymorphisms are a powerful tool in the assessment of the genetic similarity between breeding stocks.

The objectives of the present study were: (1) to determine the genetic similarity obtained with RAPD-PCR (Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction) and AFLP (Amplified Fragment Length Polymorphism) techniques in eighteen ryegrass varieties on the Irish Recommended Breeder's list and (2) to compare their effectiveness in estimating genetic diversity.

Materials and Methods

RAPD-PCR Technique

10ng of DNA from each sample to be analysed was added to a 25µl PCR reaction containing 1xPCR buffer (MgCl₂ free), 1.5mM MgCl₂, 50mM each of dATP, dCTP, dGTP and dTTP, 0.36µM random primer (Operon Technology) and 2.5 units of Taq DNA polymerase (Promega). RAPD-PCR amplification was carried out at 95°C for 1 minute, 36°C for 1 minute and 72°C for 1 minute for 45 cycles followed by a 10-minute extension step at 72°C using a Hybaid PCR Express™ thermal cycler. Products created during amplification were visualised by electrophoresis on a 1.2% TBE agarose gel.

AFLP Technique

AFLP templates were prepared by simultaneous digestion of 500 ng of genomic DNA with *EcoRI* and *MseI*. Restricted genomic DNA fragments were ligated to *EcoRI* and *MseI* adapters. This constituted the template DNA for further amplification. Template DNAs were amplified using pre-amplification primers having one selective nucleotide. Pre-amplification products were diluted (1:10) using TE and used as templates for selective amplification. Combinations of *EcoRI* and *MseI* AFLP primers containing three selective nucleotides that were supplied by the manufacturer were used for selective amplification. Following amplification, the PCR products were analysed by ABI PRISM GeneScan Analysis software.

Data analysis

Only clear and unambiguous DNA bands were included in the analyses. Markers were scored for presence (1) and absence (0) of the band in both AFLP and RAPD analyses. Bands of different electrophoretic mobilities were assumed to be non-allelic, while bands of the same mobility were assumed allelic. Pairwise similarity matrices were calculated using MVSP v3.0 (Multi Variate Statistical Program). UPGMA cluster analysis was used to identify genetic variation patterns among the perennial ryegrass varieties.

Results

AFLP and RAPD Analysis

Presence (1) or absence (0) binary data matrices containing polymorphic AFLP fragments for each primer combination were used to generate the genetic similarity estimates. The similarity co-efficient ranged from 0.6 to 0.85, indicating considerable genetic distance among the varieties.

Matrices generated for polymorphic RAPD fragments produced genetic similarity estimates ranging from 0.77 to 1.0 (1.0 indicating that no differences were detected).

Comparison of AFLP and RAPD Analysis

Both sets of dendrograms agree quite well in terms of separation of the most diverse varieties in terms of genetic variation. While the amount of polymorphic data generated by the AFLP process is far greater than that of RAPDs both processes consistently identify 'Yatsyn' as being an outsider in terms of genetic similarity. This is not at all surprising, as 'Yatsyn' is derived from New Zealand and is the only southern hemisphere-derived variety in this study. Characterisation of more closely related varieties is more difficult using RAPDs due to the fact that there may be only 8 to 15 amplified products generated per variety. The AFLP process, on the other hand generates an average of 50 to 60 products per sample thereby facilitating much more accurate analysis of genetic similarity.

Conclusions

Of these two methods, RAPD is the least accepted because of the relatively low degree of complementarity between primer and target DNA sequence. This can make the test difficult to standardise but satisfactory repeatability can be attained for samples amplified within at least the same laboratory provided immense care is taken. It is also a useful tool as an indicator of genetic diversity among more distantly related varieties. AFLP technology appears to circumvent most of these difficulties by using high stringency PCR primer annealing conditions to known DNA sequences that are ligated onto restriction fragments. Generated data is highly reproducible with less than 3% error as opposed to 16.1% with RAPDs.

References

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