Characterisation of Perennial Ryegrass Parental Inbred Lines for Generating Recombinant Inbred Lines for Fine Mapping and Gene Cloning

U. C. M. Anhalt
Teagasc, Ireland

S. Barth
Teagasc, Ireland

T. Schwarzacher
University of Leicester, UK

J. S. Heslop-Harrison
University of Leicester, UK

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Characterisation of perennial ryegrass parental inbred lines for generating recombinant inbred lines for fine mapping and gene cloning

U.C.M. Anhalt[1,2], S. Barth[1], T. Schwarzacher[2] and J.S. Heslop-Harrison[2]
[1]Teagasc, Crops Research Centre, Oak Park, Carlow, Ireland; [2]University of Leicester, Department of Biology, Leicester LE1 7RH, UK, Email: uanhalt@oakpark.teagasc.ie

Keywords: inbred lines, SSRs, AFLP, phenotype, inter-specific markers

Introduction Intermated recombinant inbred lines (IRIs) are a powerful tool for fine mapping and cloning of genes. Such population structures have been particularly helpful for cloning of genes in the model genetic plant *Arabidopsis thaliana*. IRIs or recombinant inbred lines (RILs) would be valuable for perennial ryegrass (*Lolium perenne*), but its allogamous character means that the construction of RILs is a difficult task. The international *Lolium* community would benefit from the development of such lines. The aims of the projects are to characterise the parental lines and initial generations at the (1) phenotypic, (2) molecular and (3) molecular cytogenetic level.

Materials and methods Two contrasting highly inbred perennial ryegrass lines were used as parental material for the further construction of recombinant inbred lines. These lines originated from an inter-specific cross between meadow fescue and perennial ryegrass. This original material was backcrossed for several generations to the ryegrass parent and subsequently selfed for ten generations. Plants from two genetic backgrounds were crossed to make an F1.

DNA was isolated from the parental lines, meadow fescue, tall fescue and pure perennial ryegrass with a modified CTAB protocol.

In total, 236 SSRs markers were tested and PCR conditions optimised. The SSR marker set comprises 54 ryegrass specific SSRs markers (CRC Australia) and 25 inter-specific grass SSR markers (Warnke *et al*., 2004). In addition 157 tall fescue SSRs were used (The Samuel Roberts Noble Foundation and Warnke *et al*., 2004). 60 EcoRI/MseI enzyme primer combinations were tested and a subset chosen for further analyses. Furthermore some grass RFLP probes are being converted into CAPS markers to enable connection with existing ryegrass maps based on RFLPs. Ten markers are already converted. In addition phenotypic scoring for the description of the parental plant material was carried out.

Results and discussion The perennial lines displayed different phenotypic characteristics, e.g. in plant architecture, levels of disease resistance and nutrient use efficiency. The SSR markers were polymorphic and applicable to perennial ryegrass. Of the 236 used, 127 had not been applied to perennial ryegrass before. The Noble Foundation fescue SSR markers differentiated the parental lines, fescue and *Lolium* material from each other. Cornell and CRC markers were also very useful for molecular characterisation. AFLP markers were appropriate to identify polymorphism between the different parental lines and the different grass species. To improve connections with more genetic linkage maps additional RFLP-CAPS markers will be designed. Further studies at the phenotypic, molecular and molecular cytogenetic level are in progress. The work will yield a set of characterised recombinant inbred lines that will be helpful for the construction of detailed linkage maps, and for the subsequent cloning of genes. Furthermore this work will allow insights into the genome organisation of perennial ryegrass.

Acknowledgments
We are grateful for a research license for 54 SSRs markers to the CRC for Molecular Plant Breeding, Australia and for 157 fescue markers to The Samuel Roberts Noble Foundation, USA.
Especially we acknowledge the contribution of Dr. Vincent Connolly for developing the parental lines. We thank Teagasc for award to a Research Fellowship to UCMA.

References

Warnke, S.E; Baker, R.E; Jung, G; *et al*. (2004) Genetic linkage mapping of an annual x perennial ryegrass population. Theor. Appl. Genet. 109:2, 294-304