Development and Testing of Novel Chloroplast Markers for Perennial Ryegrass from *De Novo* Sequencing and *In Silico* Sequences

S. McGrath
Teagasc, Ireland

T. R. Hodkinson
University of Dublin, Ireland

S. Barth
Teagasc, Ireland

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

Development and testing of novel chloroplast markers for perennial ryegrass from *de novo* sequencing and *in silico* sequences

S. McGrath[1,2], T.R. Hodkinson[2] and S. Barth[1]
[1]Teagasc Crops Research Centre, Oak Park, Carlow, Ireland; [2] Department of Botany, University of Dublin, Trinity College, Ireland, E-mail: smcgrath@oakpark.teagasc.ie

Keywords perennial ryegrass, chloroplast SSR markers, cross species amplification

Introduction Chloroplast DNA is uniparentally inherited and non-recombinant in *Lolium perenne*. These properties make the chloroplast genome a useful tool for studying inter- and intra- specific relationships. Previous genetic studies on *L. perenne* have used chloroplast sequence data. However, the relative lack of variation in the chloroplast genome limits its usefulness for analysis at the single individual level within a species. However, chloroplast SSR markers have recently been shown to have high levels of polymorphism (Provan *et al*., 2004). This is the first study to design and employ such markers for *L. perenne.* The objectives of this study are (1) to design and (2) optimise novel chloroplast SSR markers and (3) use them to analyse variation and diversity in *L. perenne* and related grass species.

Materials and methods DNA from *L. perenne, L. multiflorum, Festuca pratensis* and *Festuca arundinacea* was extracted using a modified CTAB method. These DNA samples were amplified and sequenced using the primers c and f for the *trn-L* intron and *trn-F* intergenic spacer region (Taberlet *et al*., 1991), and the primers 1R and 2R for the *atpB-rbcL* intergenic spacer region (Samuel *et al*., 1997), on an ABI 310 automated DNA sequencer. Further plastid DNA sequences from *L. perenne* and related species were also obtained from GenBank. All *de novo* and *in silico* sequences were analysed for microsatellite motifs using a modified version of the MISA perl script (Thiel, 2003). 27 pairs of primers were designed using Primer 3 software (Rozen & Skaletsky, 1998). PCR conditions were optimised to amplify loci for each primer pair. Of these, 19 were chosen for further analysis. 12 primer pairs were optimised as 5'=fluorescently labelled primers. These primers were used to amplify DNA from various populations of *L. perenne* and other grass species. PCR products were analysed using an ABI 3100 automated DNA sequencer and sized using GeneMapper™ v3.0 software.

Results The optimised primers showed little variation within *Lolium* species (Table1). The markers amplified across a broad range of grass species, *e.g.* from nine species for marker TeaCpSSR1 to 23 for marker TeaCpSSR11. Some of the alleles were shared between *L. perenne* and other species. Certain alleles were species specific, *e.g.* an allele of marker TeaCpSSR1 was specific to *Arundo donax.*

Table 1 Example for the allelic range of a set of four chloroplast SSR markers

Primer name	Gene region	*L. perenne*		Other grass species	
		# alleles	Size range in b.p.	# alleles	Size range in b.p.
TeaCpatpssr2	*atpB-rbcL*	4	217 - 229	3	227 - 230
TeaCpssr1	*23S-5S*	3	193 - 196	5	193 - 200
TeaCptrnssr2	*trnL-trnF*	5	178 - 195	4	176 - 200
TeaCpssr11	*trnV*	3	193 - 196	5	193 - 201

Conclusions As a next step, these optimised primers will be used to investigate the phylogeography of perennial ryegrass including mode and time-point of introduction of *L. perenne* into Ireland. They can also be used to test for maternal inheritance in festulolium hybrids. These primers are also a valuable tool to control cross pollination and selfings in breeding programs. Furthermore, their greatest potential lies in species differentiation, such as in seed testing or at the taxonomic level.

Acknowledgements We are grateful to the grass breeding group in Oak Park for their help and to Dr. Nicolas Salamin for his bioinformatics assistance. SMcG is supported by a Walsh Fellowship.

References

Provan, J., Biss,P.M., McMeel, D., and Mathews S. (2004) Universal primers for the amplification of chloroplast microsatellites in grasses (Poaceae). Molecular Ecology Notes 4: (2), 262-264

Rozen, S., Skaletsky, H.J. (1998) Primer3. http://www.genome.wi.mit.edu/genome_software/other/primer3.html.

Samuel, R., Pinsker, W., Kiehn, M. (1997) Phylogeny of some species of Cyrtandra (Cesneriaceae) inferred from the atpB/rbcL cpDNA intergene region. Botanica Acta 110(6) 503-510

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105-1109

Thiel, T. (2003) MISA - Microsatellite identification tool. http://pgrc.ipk-gatersleben.de/misa/