Screening Genes for Association with Loci for Nitrogen-Use Efficiency in Perennial Ryegrass by Pyrosequencing™

O. Dolstra
Plant Research International B.V., The Netherlands

D. Dees
Plant Research International B.V., The Netherlands

J. -D. Driesprong
Plant Research International B.V., The Netherlands

E. N. van Loo
Plant Research International B.V., The Netherlands

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

Screening genes for association with loci for nitrogen-use efficiency in perennial ryegrass by pyrosequencing™

O. Dolstra, D. Dees, J.-D. Driesprong and E.N. van Loo
Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, Netherlands. Email: oene.dolstra@wur.nl

Keywords: *Lolium perenne*, breeding, nitrogen-use efficiency, SNPs, QTL, DNA pooling, pyrosequencing™

Introduction The application of marker-assisted selection to improve quantitative traits in perennial ryegrass (*Lolium perenne*) is cumbersome. It requires a priori knowledge on the association of markers and genes. The knowledge on the chromosomal location of major genes for quantitative traits as well as on gene sequences is rapidly growing. However, determination of the genetic constitution of parents prior to their use in breeding still is impractical. More realistic is to collect association data along with the testing activities needed for breeding new varieties. This study uses changes in allele frequency due to selection as a criterion for gene-trait association. Selection-dependent changes are detected with single nucleotide polymorphisms (SNPs) of candidate genes using DNA-pools of F2 plants differing in nitrogen-use efficiency (NUE). The procedure and its feasibility are outlined for one locus.

Materials and methods DNA pools were established by mixing equal quantities of DNA from two selections of about 45 F2 plants obtained by selfing #1331. The F2 selections, i.e. N+ and N-, differ in NUE (Dolstra *et al*., 2003). A pyrosequencer, PSQ96 is used to determine allele frequencies (cf Neve *et al*., 2002). Muylle (2003) reported the gene polymorphism, SNP3, which was used here to test the procedure. To this end PCR amplification with a biotinylated forward primer (5'-TGG GAA GAC AAC GCT TAA-3') and the reverse primer 5'-TTC CGA CAT CAA ACT CCT GC-3' was done to get a pyrosequencing template of 185bp. The sequence of the primer for the actual pyrosequencing of SNP3 was 5'-GGT CAC CCA TGC AA-3'. The incorporation order of nucleotides was set to be AC/GATGAAG.

Results and conclusions Genomic DNA of the parents of #1331, both homozygous for a SNP3 allele, was mixed in different ratios to get variation in allele frequencies. Figure 1 shows the presence of a highly significant relationship between the expected and observed frequency of the C allele. The repeatability of the measurements is good as can be deduced from the data presented in Table 1. Small differences between expected and observed values are probably mainly due to incomplete background correction. The pools showed no significant change in allele frequencies, indicating the absence of any effect of selection for NUE (Table 1). There is probably no major NUE-QTL present in a chromosome area of 25 cM at each side of the SNP3 locus. This conclusion is supported by the finding that mapping showed SNP3 to be located on linkage group 2 where no NUE-QTLs were detected. A set of 25 markers like SNP3, evenly spread over the *Lolium* genome, would be sufficient to do a quick genome scan for the presence of major QTLs.

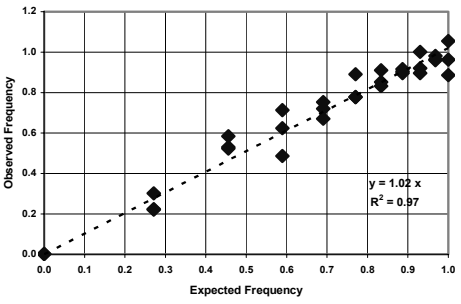

Figure 1 Relation between observed and expected

Table 1 Estimates of allele frequencies for the two contrasting F2 selections and their ancestors.

Materials	Allele Frequency (G/G+C)	
	Mean	SD
Parents		
P413	0.00	0.042
P510	1.01	0.059
F1		
#1331	0.56	0.009
F2 Selections		
N+	0.57	0.008
N-	0.41	0.045

References

Dolstra O., C. Denneboom, A.L.F. de Vos & E.N. van Loo (2003) Marker-assisted selection in improvement of quantitative traits of forage crops. In: International Workshop 'Marker Assisted Selection: a fast track to increase genetic gain in plant and animal breeding?', October 17-18, 2003 Villa Gualino, Torino, Italy, p1-5 (http://www.fao.org/biotech/docs/dolstra.pdf)

Muylle, H. (2003) Genetic analysis of crown rust resistance in ryegrasses (Lolium spp.) using molecular markers. Thesis Universiteit Gent, Gent, Belgium, p89

Neve B., P. Froguel, L. Corset, E. Vaillant, V. Vatin, P. Boutin (2002) Rapid SNP allele frequency determination in genomic DNA pools by Pyrosequencing ™. BioTechniques 32: 1138-1142