Gene-Associated Single Nucleotide Polymorphism (SNP) Discovery in Perennial Ryegrass (*Lolium Perenne* L.)

J. W. Forster
La Trobe University, Australia

N. O. I. Cogan
La Trobe University, Australia

A. C. Vecchies
La Trobe University, Australia

R. C. Ponting
Molecular Plant Breeding Cooperative Research Centre, Australia

M. C. Drayton
La Trobe University, Australia

See next page for additional authors

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J. W. Forster, N. O. I. Cogan, A. C. Vecchies, R. C. Ponting, M. C. Drayton, J. George, J. L. Dumsday, G. C. Spangenberg, and K. F. Smith

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J.W. Forster[1,3], N.O.I. Cogan[1,3], A.C. Vecchies[1,3], R.C. Ponting[1,3], M.C. Drayton[1,3], J. George[1,3], J.L. Dumsday[1,3], G.C. Spangenberg[1,3] and K.F. Smith[2,3]

[1]Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia [2]Primary Industries Research Victoria, Hamilton Centre, Hamilton, Victoria 3300, Australia [3]Molecular Plant Breeding Cooperative Research Centre, Australia Email: john.forster@dpi.vic.gov.au

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Introduction Perennial ryegrass (*Lolium perenne* L.) is the most important grass species for temperate pasture systems world-wide. Varietal improvement programs for this obligate outbreeding species are based on polycrossing of multiple parents to produce heterogeneous synthetic populations. The complexity of breeding systems creates challenges and opportunities for molecular marker technology development and implementation. Previous research has led to: the generation of a comprehensive suite of simple sequence repeat (SSR) markers, reference genetic map construction, comparative genetic studies, QTL identification, and population structure analysis. Emphasis has now shifted from the use of anonymous genetic markers linked to trait-specific genes to the development of functionally-associated genetic markers based on candidate genes. The successful implementation of this approach will allow effective selection of parental plants in germplasm collections based on superior allele content.

Materials and methods A hierarchical system for candidate gene identification has been devised, and targets in a range of functional categories have been defined, on the basis of association with key output traits. At present, over 150 perennial ryegrass genes have been introduced into an *in vitro* single nucleotide polymorphism (SNP) discovery process based on amplicon cloning from the heterozygous parents of the second generation F_1(NA_6 x AU_6) two-way pseudo-testcross genetic mapping family. This method has the benefits of permitting discrimination of paralogous sequences and direct determination of haplotype structure. SNP variation within and between the parental genotypes is based on amplicon sequencing and alignment, followed by validation using the single nucleotide primer extension (SNuPe) assay.

Results and conclusions Proof-of-concept for SNP detection, validation and subsequent mapping has been obtained using the *Lp*ASRa2 abiotic stress tolerance associated gene. Putative SNPs have been identified for 76 genes, over a total of c. 78 kb of resequenced DNA, and a high proportion of these SNPs have been validated. SNP frequency in perennial ryegrass is high compared to other Poaceae species, at c. 1/57 bp across all components of the transcriptional unit. SNP haplotype and haplogroup structures have been defined for *Lp*ASRa2 and other genes, and variability has been surveyed across diverse genotypic sources. A strategy has been developed for validation of associations between candidate gene haplotype variation and phenotypic variation for correlated agronomic characters. Herbage quality and abiotic stress tolerance genes will provide 'proof-of-concept' for this procedure, involving measurements of transcriptional activity, metabolite levels and field character expression. This information, in concert with detailed understanding of within- and between-population genetic variation, will inform future strategies for marker implementation in pasture grass breeding.

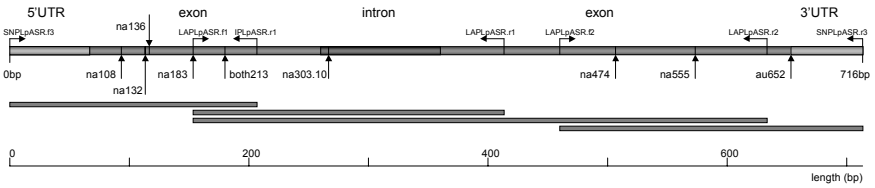

Figure 1 Location of SNP loci within the transcriptional unit of the *Lp*ASRa2 gene, showing the locations of amplicon-specific primers and heterozygous SNPs relative to intron, exon and untranslated region (UTR) components. SNP loci detected as heterozygous within the NA_6 parental genotype of the F_1(NA_6 x AU_6) mapping family are indicated with the prefix na; the converse for the AU_6 parental genotype is indicated with the prefix au. A single SNP locus (at coordinate 213) was heterozygous between the two parents.