Locating, and Utilising *Festuca Pratensis* Genes for Winter Hardiness for the Future Development of More Persistent High Quality *Lolium* Cultivars

M. W. Humphreys
Institute of Grassland and Environmental Research, UK

D. Gasior
Agricultural University of Craców, Poland

A. Kosmala
Institute of Plant Genetics Polish Academy of Sciences, Poland

O. A. Rognli
The University of Norway, Norway

Z. Zwierzykowski
Institute of Plant Genetics Polish Academy of Sciences, Poland

See next page for additional authors

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

Presenter Information

M. W. Humphreys, D. Gasior, A. Kosmala, O. A. Rognli, Z. Zwierzykowski, and M. Rapacz

Locating, and utilising *Festuca pratensis* genes for winter hardiness for the future development of more persistent high quality *Lolium* cultivars

M.W. Humphreys[1], D. Gasior[2], A. Kosmala[3], O.A. Rognli[4], Z. Zwierzykowski[3] and M. Rapacz[2]
[1]Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, UK; [2]Agricultural University of Craców, Department of Plant Physiology, Faculty of Agriculture, Podłużna 3, Kraków, Poland; [3]Institute of Plant Genetics Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland.Agricultural [4]The University of Norway, Department of Chemistry and Biotechnology, P.O. Box 5040, 1432 Ås, Norway; Email: mike.humphreys@bbsrc.ac.uk

Keywords: *Festulolium*, introgression-mapping, QTL-mapping, cold-hardening, photosystem II (PSII)

Summary Genes for freezing-tolerance and winter hardiness were located in *Festuca pratensis* by QTL analysis and introgression-mapping. QTL for freezing-tolerance on *F. pratensis* chromosome 4 were orthologous to rice chromosome 3, and Triticeae chromosome 5. Increased energy dissipation during the autumn through a lower maximum quantum yield of photosystem II (PSII) was correlated with improved winter survival. Freezing-tolerance in *Lolium* was achieved by the transfer and subsequent expression of *F. pratensis* genes from chromosome 4 that govern the expression of a non-photochemical (NPQ) mechanism for the dissipation of excess light energy under low temperature.

Introduction We describe outcomes from field tests for winter hardiness in Norway and Poland and simulated freeze testing and cold acclimation studies for adaptations to low temperatures. Quantitative trait loci (QTL) for winter hardiness were assigned to chromosomes and the first QTL map of *Festuca pratensis* constructed. Introgression-mapping in *Lolium* x *Festuca* hybrids is a particularly efficient mechanism for trait "dissection" as it harnesses the high frequencies of chromosome recombination inherent in these hybrids with the capability to locate sites of chromosome introgression by genomic in situ hybridisation (GISH). This has led to the location of *F. pratensis* genes associated with winter survival and freezing-tolerance.

Materials and methods Plant populations used were: a *F. pratensis* mapping family (Alm *et al.,* 2003); backcross populations involving *Lolium perenne x F. pratensis* and *L. multiflorum* x *F. pratensis* hybrids; a series of monosomic chromosome addition lines where a *F. pratensis* chromosome replaces its *L. perenne* homoeologue (King *et al.,* 2002). GISH techniques were used to locate sites of *Festuca* introgressions on *Lolium* chromosomes (e.g. King *et al.,* 2002). Simulated freeze-testing involved use of a climatic cooler (Cambridge Scientific UK, Ltd.) and followed cold acclimation for 2w at 2˚C, 8h light, 400µmol $m^{-2}s^{-1}$. Chlorophyll fluorescence measurements were as in Rapacz *et al.* (2004).

Results and discussion Two major QTL for freezing tolerance and four QTL for winter survival in the field were located. QTL for freezing tolerance, *Frf4* on chromosome 4 and *Frf5* on chromosome 5 explained a total of 34.3% of the total phenotypic variation. *Frf4* was orthologous to the frost tolerance *Fr1* and *Fr2* loci in wheat which are also associated closely with the *Vrn-1* (vernalization) gene and 2 regulatory genes *Rcg1* and *Rcg2* known to have a major role in expression of cold-responsive (*cor*) genes. A monosomic chromosome addition line for *F. pratensis* chromosome 4, or introgression lines of *Lolium spp* with *F. pratensis* genes from chromosome 4, displayed cold acclimatory adaptations of PSII typical of *F. pratensis.* The presence of *Festuca*-derived NPQ mechanisms for protection against high-light induced inactivation of PSII at low temperature, in *Lolium* led to improved freezing-tolerance. Norwegian and Polish field trials and simulated freezing-trials led to the selection of *Lolium*-like diploid (2n = 2x = 14) backcross derivatives with good winter hardiness and freezing-tolerance. Plants from the Norwegian field study were found repeatedly with introgressed *F. pratensis* genes at the same location on *Lolium* chromosomes 3 and 4. A *F. pratensis* introgression on chromosome 2 of *Lolium* led to the breakdown of mechanisms required for effective cold acclimation.

References

Alm V., C. Fang, C.S. Busso, K.M. Devos, K. Vollan, Z. Grieg, & O.A. Rognli (2003). A linkage map of meadow fescue (*Festuca pratensis* Huds.) and comparative mapping with other Poaceae species. *Theoretical and Applied Genetics*, 108:25-40

King J., I.P. Armstead , I.S. Donnison, H.M. Thomas, R.N. Jones, M.J. Kearsey, L.A. Roberts, A. Thomas, W.G. Morgan, & I.P. King (2002). Physical and genetic mapping in the grasses *Lolium perenne* and *Festuca pratensis*. *Genetics*, 161:315-324

Rapacz M., D. Gasior, Z. Zwierzykowski, A. Lesniewska-Bocianowska, M.W. Humphreys, & A.P. Gay (2004). Changes in cold tolerance and the mechanisms of cold acclimation of photosystem II to cold hardening generated by anther culture of *Festuca pratensis* x *Lolium multiflorum* cultivars. *New Phyt.*, 162:105-114