

Isolation and Characterisation of Genes Encoding Ice Recrystallisation Inhibition Proteins (IRIPs) in the Cryophilic Antarctic Hair-Grass (*Deschampsia Antarctica*) and the Temperate Perennial Ryegrass (*Lolium Perenne*)

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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

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Presenter Information

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Isolation and characterisation of genes encoding ice recrystallisation inhibition proteins (IRIPs) in the cryophilic antarctic hair-grass (*Deschampsia antarctica*) and the temperate perennial ryegrass (*Lolium perenne*)

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Keywords: antarctic hair-grass, perennial ryegrass, ice recrystallisation inhibition proteins (IRIPs), freezing tolerance

Introduction Antarctic hairgrass (*D. antarctica* Desv.), the only grass species indigenous to Antarctica, has a well developed tolerance of freezing, strongly induced by cold-acclimation. In response to low temperatures *D. antarctica* exhibits recrystallisation inhibition (RI) activity, localised to the apoplast, that prevents further growth of ice crystals following freezing.

Materials and methods A gene family from *D. antarctica* encoding ice recrystallisation inhibition proteins (IRIPs) and orthologues from perennial ryegrass were isolated and comparatively characterised. IRIP genes are found in a range of Pooidae species but the products they encode vary widely in length due to apparent plasticity in the number of LRRs.

Results and conclusions Cold acclimation induces RI activity more than 64-fold and 8-fold in leaves and roots of *D. antarctica* respectively. Similarly, RI activity is induced by cold-acclimation in leaves and roots of perennial ryegrass (*L. perenne* L.) in excess of 16- and 4-fold respectively. Leaf apoplastic extracts from plants of both species grown at 22°C possess no RI activity while activity is induced in response to cold-acclimation by factors of at least 73-fold in *D. antarctica* and 1.7-fold in *L. perenne*. The genomes of *D. antarctica* and *L. perenne* both appear to harbour multiple IRIP-related sequences. IRIPs are apoplastically-targeted proteins with two potential ice-binding motifs: between 1 and 9 leucine rich repeats (LRRs), and 16 IRIP repeats. Three-dimensional structures of IRIPs from *D. antarctica* (*Da*IRIP) and *L. perenne* (*Lp*IRIP) were constructed by comparative homology modelling. *Da*IRIPs are predicted to adopt conformations with two ice-binding surfaces that are better matched to the prism face of ice than those of *Lp*IRIPs (Figure 1). An *Lp*IRIP was genetically mapped on perennial ryegrass LG1 using gene-associated single nucleotide polymorphisms. Levels of IRIP transcripts are greatly enhanced (c. 47-fold) in leaves of *D. antarctica* following cold-acclimation, but only moderately so (c. 4-fold) in roots of *L. perenne*. Moreover in extracts from *E. coli* expressing heterologous proteins RI activity is specifically conferred by *Da*IRIP. It is proposed that enhancement of IRIP-mediated RI activity relative to *L. perenne* and other temperate grasses and cereals has contributed to the cryo-tolerance of *D. antarctica* and thus its ability to colonise Antarctica.

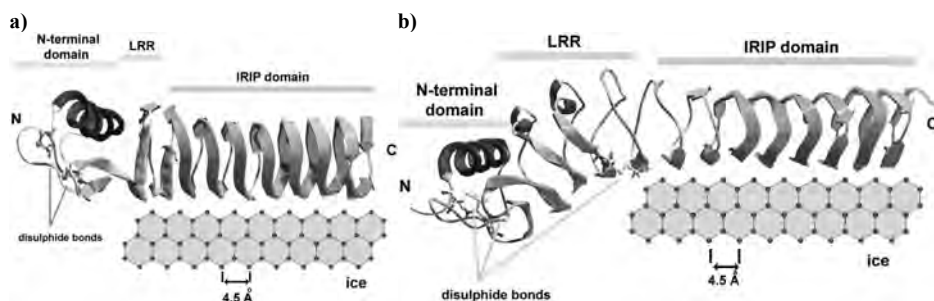


Figure 1 Structural models of IRIPs. a) Ribbon backbone diagram of theoretical structure of *Da*IRIP and b) *Lp*IRIP, aligned along the prism face of ice (parallel to the *a*-axis)