

Characterization of *Lolium perenne* antifreeze proteins and their potential for conferring freezing stress tolerance.

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Freezing is a major stress encountered by plants. *Lolium perenne* (Lp), a freezing-tolerant plant, expresses four antifreeze proteins (AFPs) which control extracellular ice formation through their ice recrystallization inhibition activity (IRI). We have initiated the characterization of LpIRI3 (the native version of the mature protein, LpAFP) and LpIRI2 (a naturally-occurring truncated protein which lacks the signal peptides found in the other LpIRI proteins). To this end we have cloned the coding regions of these proteins into an expression vector in order to assay the recombinant protein for thermal hysteresis and IRI activity, and also into binary vectors either as fusions with orange fluorescent protein (OFP), or as single or multiple expression cassettes for overexpression in *Arabidopsis thaliana*. The OFP fusion proteins will facilitate localization studies and the overexpression analyses will allow us to assess possible low temperature tolerance conferred to the non-freezing tolerant *Arabidopsis*. Preliminary experiments appear promising and we hypothesize that by expressing LpAFP, LpIRI2 and LpIRI3 together – as these proteins may have different intra- or extracellular localizations – we can maximize their effects on freezing tolerance.

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