

Life at high latitudes: evolution of cold acclimation and its role in the successful adaptation of the grass subfamily Pooideae to temperate regions

Grass adaptation and diversification in the temperate zone: Grasses comprise a monophyletic lineage of around 12000 species distributed across every terrestrial habitat on Earth. Although the majority of grasses are tropical, a few lineages have successfully diversified within the temperate zone, including Bambusoideae, Danthonioideae, and Pooideae. However, the evolutionary innovations underlying these major niche transitions are poorly understood.

Plant adaptation to cold: Freeze tolerance is the ability of plant tissues to survive below zero temperatures through biochemical and morphological adjustments. The latter is a highly complex trait that encompasses tolerance to direct effects of low temperatures on biomolecule stability and function and effects of ice formation in and surrounding the plant (e.g. freeze dehydration, intracellular ice-crystal growth). Although plants have some innate tolerance to a sudden exposure to frost, additional freeze tolerance can, in many species, be induced through cold acclimation, i.e. an extended exposure to cold non-freezing temperatures.

Molecular mechanisms in cold climate adaptation: Cold acclimation involves major changes in the biochemical and physiological state of the plant. Proteins and compounds with various protective functions accumulate (e.g. sugars, dehydrins, chaperons, ROS-scavengers and ice binding proteins), while photosynthesis and several other metabolism-related biochemical pathways undergo adjustments to account for the novel climatic conditions. Together, these molecular responses form the basis for plants to survive extremes of low-temperature encountered in temperate climates.

Rationale: Pooideae evolved and diversified in a period of gradually cooling and drying climate. We hypothesize that cold acclimation was an important contributing factor to the adaptation of Pooideae to temperate regions. In this thesis we will investigate how cold acclimation evolved in the subfamily. One alternative is that cold acclimation evolved in the Pooideae ancestor. Alternatively, cold acclimation response has evolved multiple times in Pooideae. To study how evolution of cold adaptation has happened in Pooideae, we will determine the phylogenetic distribution and ancestral state of cold acclimation across Pooideae using a combination of growth experiments and characterization of physiological responses.

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Evolution of regulatory mechanisms of gene expression in the grass subfamily Pooideae.

Differences in gene regulation are now believed to be the primary mode of evolution since they allow for differences in how much or when a gene product is expressed without the potential negative effects of altering the protein product itself. *Cis*-regulatory elements consist of binding sites, generally upstream of a transcription start site (but they can be intronic), where promoters, repressors and enhancers bind to influence transcription. Changes in regulatory sequence, in the form of SNPs or INDELS, have been shown to affect vernalization requirements and timing of flowering in wheat. Bioinformatic approaches such as searching for motifs in upstream regions of transcription start sites can be a cost-effective first step compared to molecular approaches, such as ChiP-Seq, for identifying regulatory differences across taxa of interest. Recently, transcription factor binding sites for genes involved in photoperiod and vernalization were predicted from genome sequences in *Arabidopsis*, *Brachypodium*, wheat, and barley. This approach will be applied to the *CONSTANS* genes across representative Pooidea species for which we have both genome and transcriptome data. The *CONSTANS* genes are a gene family where the members are involved in regulation of flowering time.

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