



# Transcriptome profiling reveals insight into the cold response of perennial ryegrass

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Outline



> Importance of perennial ryegrass & current challenges

Winter survival (freezing stress & its impacts)

Freezing stress responses

Transcriptome analysis

Candidate genes to improve freezing tolerance in ryegrass

➤ Conclusion

#### Importance of perennial ryegrass & current challenges





- Grasslands occupy ~40% agricultural land is europe
- Backbone for livestock production
- Perennial ryegrass most important

high tillering, quick regrowth, high nutritive value

tolerance to grazing & frequent cuts

- Well adapted to costal climate of western europe
- Low persistance in harsh environments (cold,drought) restricting its wide spread commercial cultivation in north and eastern europe
- Opportunity: Prolonged growing season (1-3 months) in northern europe due to climate change
- Scope: develop cultivars with improved drought & cold tolerance



#### Freezing stress & its impacts





Forage grass covered in ice crystals during early winter at Ås, Norway



Differences in winter survival between two forage grasses

#### > Winter hardiness is complex (freezing temperatures, ice, snow mould etc)

Freezing tolerance – primary deperminant of winter survival





#### Freezing stress responses





> Changes in temperature & light intensity – cold acclimation

cease growth

accumulate carbohydrates

 Frost– synthesis of sugars, proteins and metabolites to stabilize cellular functions

Changes at physiological & sub-cellular level – gene expression

Goal: Identify key genes & regulators to improve winter hardiness





#### Case study:

Transcriptome wide responses during cold acclimation and freezing stress in resistant and susceptible perennial ryegrass genotypes

#### **Pre-experiment**



	No.of genotypes	EL (%) min	EL (%) max	Mean	SD
Exp -14	142	16.7	57.8	37.3	8.84
Exp -12	157	9.76	61.0	25.3	10.2



Genotypes selected for transcriptome analysis



#### **Experimental setup**





- sampling point

4 genotypes : 2 resistant & 2 susceptible in 3 reps



#### **RNA extraction & sequencing**







#### Validation of experimental setup





 Clustering along x and y axis according to experimental factors

#### Differentially expressed genes





Sus	Up	Down	Total
T4 vs T1	1462	1961	3423
T5 vs T4	2805	1015	3820
T6 vs T4	3779	2447	6226



### Differences in cold acclimation ?

Res	Up	Down	Total
T4 vs T1	3474	2632	6106
T5 vs T4	2538	2175	4713
T6 vs T4	2502	2213	4715



#### Functional enrichment analysis



KEGG analysis of genes upregulated during CA and FT



#### Differences at cold acclimation



#### Functional enrichment analysis



KEGG analysis of genes downregulated during CA and FT



Differences at cold acclimation

#### Comparative transcriptomic analysis





Direct comparision of treatments between cultivars

Res vs Sus	Up	Down	Total
T2	2144	2166	4310
Т3	1890	3291	5181
T4	3087	1758	4845
Т5	2082	2259	4341
Т6	2325	2321	4646

Huge differences across different treatments - candidate genes ?



#### Search for candidate genes



- Goal find candidate genes to improve freezing tolerance by comparing resistant and susciptible cultivars
- > Problem: many DE genes at CA & FT and huge differences between cultivar
- > Filtering strategy: Differentially expressed under freezing stress & differential expression between cultivars









#### **Co-expression** analysis



#### Module-trait correlation



V3.Lp\_chr5\_0G16846



### V3.Lp\_chr4\_0G5610 V3.Lp\_chr4\_0G622 V3.Lp\_chr1\_0G4476 V3.Lp\_chr3\_0G3734 15 0

2

3

Susceptible Resistant



#### V3.Lp\_chr7\_0.1G12236

- DREB 1B-like
- LEA 2 subgroup
- HSF transcription factor
- ERD 7
- Cold-shock protein CS120-like (dehydrin)
- Dehydrin DHN3-like
- Cold-shock protein CS120-like (dehydrin)
- Acyl-CoA dehydrogenase
- protein ETHYLENE-INSENSITIVE 2
- Cold-shock protein CS120-like (dehydrin)
- Sucrose:sucrose 1-fructosyltransferase-like
- Cold-responsive protein kinase 1-like
- Cold-responsive protein kinase 1-like
- Cold-regulated 413 protein





#### Conclusion



- Comparative transcriptomic analysis instrumental for identifying candidate genes
- Differences in cold acclimation between resistant and susceptible cultivars
- > Huge variation in expression of core FT genes between resistant and susceptible cultivars
- Potential candidates for gene editing :

Cold-responsive protein kinase 1-like - knockout (sus)

Cold-shock protein CS120-like (dehydrin) - Overexpression (sus)

Furture work: identify more candidate gene and literature review

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