

Improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems through CRISPR-Cas9 technology - EditGrass4Food

**“EditGrass4Food”, ID No EEA-RESEARCH-64, Contract No EEZ/BPP/VIAA/2021/4
is financially supported by European Economic Area (EEA) grants**

Agenda

Administrative topics

- Next project report to be prepared by May 31st (partner information to be submitted by May 15)
- 1st report and all the expenses have been approved by the Latvian Council of Science

Steering committee meeting in April:

- Data to be decided (Doodle)
- Preparation for the 2nd project report

Next face-to-face meeting on 13- 14th October, 2022 in Tallinn, Estonia

2nd call of EEA-NOR scholarship activity

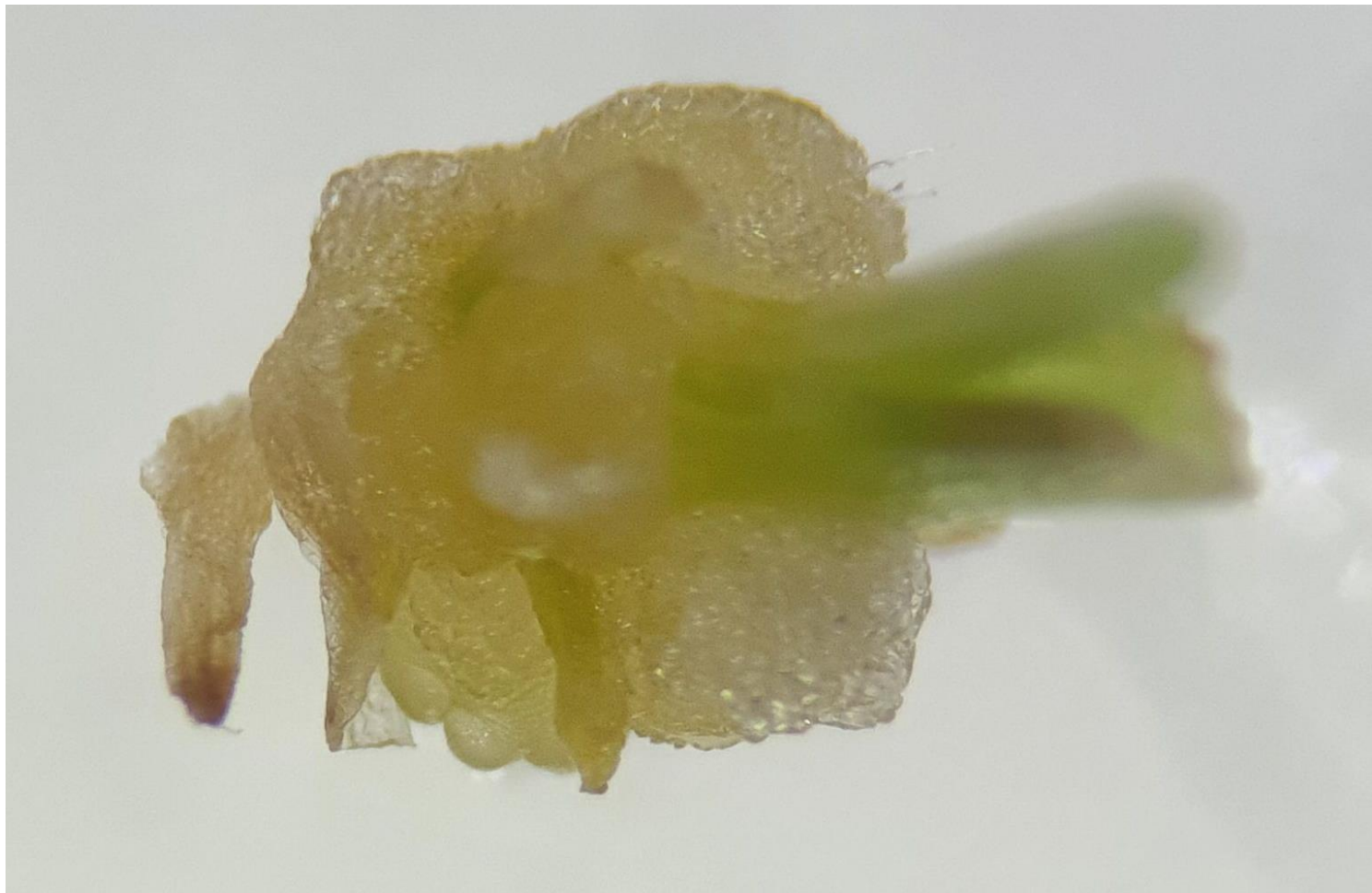
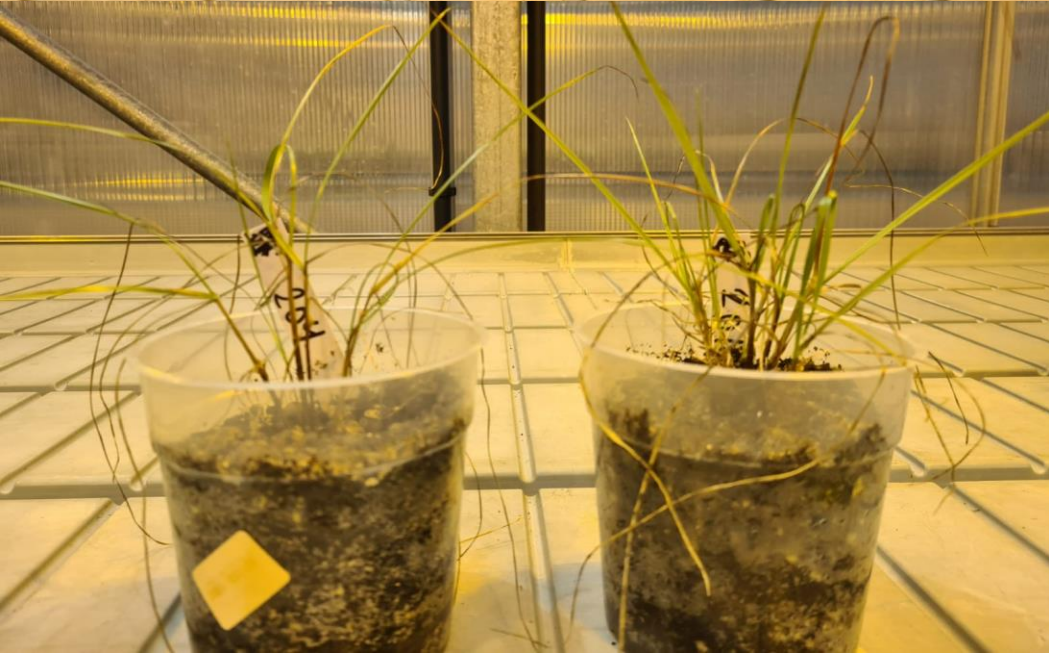
- <https://eeagrants.lv/en/2022/02/15/2nd-call-of-scholarships-activity/>

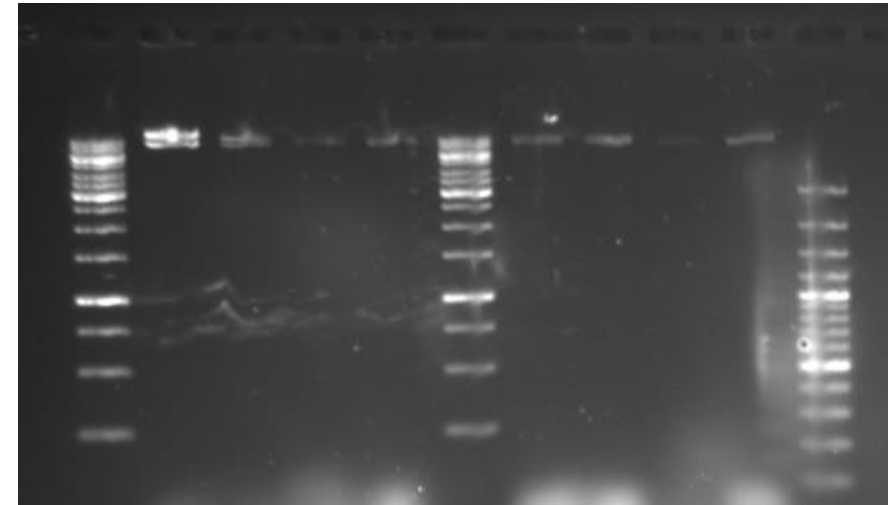
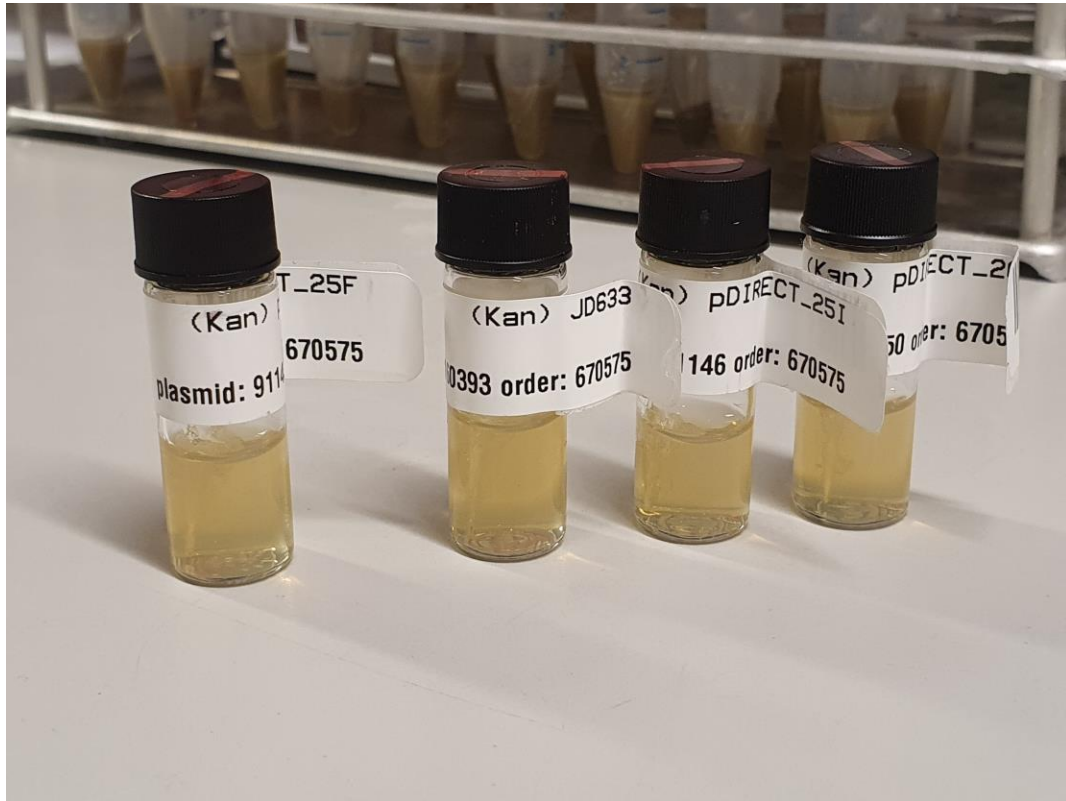
Agenda

Administrative topics – spending of direct costs

- Costs of inventory, tools and materials
- Publication costs
- Conferences

Overheads (25% of already spent direct costs)





- 2. and 3. - pJD633 HindIII (8975, 12692 bp)
- 4. and 5. - pDIRECT_25F HindIII (8311 and 8975 bp)
- 7. and 8. - pDIRECT_25I HindIII (8929 and 11023 bp)
- 9. and 10. - pDIRECT_26H HindIII (8929 and 10532 bp)

To be done: sequencing of AarI cloning site region

Structural type	Expression pattern	Characteristic features	Corresponding dehydrins in barley and wheat
K _N	Low temperatures (cold, frost), drought, salt, ABA, SA	Cryoprotective	Barley: DHN5 (K ₉) Common wheat: WCS120 family – WCS120 (K ₆); low-molecular K _N types – WDHN13 (K ₂)
SK _N	Cold (chilling, frost)	Acidic; membrane lipid interactions	Barley: DHN8 (SK ₃) Common wheat: WCOR410a,b,c (SK ₃)
K _N S	Chilling-induced	Reproductive tissues (anthers)	Barley: DHN13 (KS) Common wheat: at least one ortholog to barley DHN13 (KS type)
Y _N SK _m	Strong dehydrative stresses (drought, frost, salt), ABA, GA, MeJA, SA	Basic; accumulation in cytoplasm and nucleus; phosphorylation of S-segment (NLS); allelic variation (barley <i>Dhn4</i>); some are embryo-specific	Barley: DHN1,2,3,4,6,7,9,10,11,12 Common wheat: several drought- and ABA-inducible homologs, e.g., RAB15 (YSK ₂)
Y _N K _m	Cold	Antifreeze, cryoprotective	Durum wheat: DHN5 (YSK ₂) Not present in barley and wheat genomes

ABA, abscisic acid; GA, gibberellic acid; MeJA, methyl jasmonate; NLS, nuclear localization signal; SA, salicylic acid.

Wheat and barley dehydrins under cold, drought, and salinity – what can LEA-II proteins tell us about plant stress response?

Klára Kosová*, Pavel Vitámvás and Iľja T. Prášil

Plant Stress Biology and Biotechnology, Department of Plant Genetics, Breeding and Product Quality, Crop Research Institute, Prague, Czech Republic

Edited by:

Juan Francisco Jiménez-Bremont,
Instituto Potosino de Investigación
Científica y Tecnológica, Mexico

Reviewed by:

Urs Feller, University of Bam,
Switzerland
Margarita Rodríguez-Kessler,
Universidad Autónoma de San Luis
Potosí, Mexico

*Correspondence:

Klára Kosová, Plant Stress Biology and
Biotechnology, Department of Plant
Genetics, Breeding and Product
Quality, Crop Research Institute,
Dmova 507, 161 06 Prague
6 – Ruzyně, Czech Republic
e-mail: kosova@vur.v.cz

Dehydrins as a group of late embryogenesis abundant II proteins represent important dehydration-inducible proteins whose accumulation is induced by developmental processes (embryo maturation) as well as by several abiotic stress factors (low temperatures, drought, salinity). In the review, an overview of studies aimed at investigation of dehydrin accumulation patterns at transcript and protein levels as well as their possible functions in common wheat (*Triticum aestivum*), durum wheat (*T. durum*), and barley (*Hordeum vulgare*) plants exposed to various abiotic stress factors (cold, frost, drought, salinity) is provided. Possible roles of dehydrin proteins in an acquisition and maintenance of an enhanced frost tolerance are analyzed in the context of plant developmental processes (vernalization). Quantitative and qualitative differences as well as post-translational modifications in accumulated dehydrin proteins between barley cultivars revealing differential tolerance to drought and salinity are also discussed. Current knowledge on dehydrin role in wheat and barley response to major dehydrative stresses is summarized and the major challenges in dehydrin research are outlined.

Keywords: dehydrin dynamics, proteins, transcripts, abiotic stress, barley, wheat

INTRODUCTION

Abiotic stress factors – cold, frost, drought, salinity – severely limit plant growth and development as well as the final yield in crops including cereals from the tribe Triticeae. Low temperatures, drought, and salinity represent stress factors associated with plant cell dehydration. Dehydration stress factors induce profound cellular response aimed at an elimination of water loss. Plant cell response to dehydration includes an accumulation of osmotically active compounds including hydrophilic proteins such as dehydrins. Several physiological studies focused on plant stress response have reported a positive relationship between the level of accumulation of dehydrin transcripts or proteins and plant stress tolerance (Kosová et al., 2010). More tolerant cultivars or genotypes usually reveal a higher level of dehydrin transcripts or proteins than the less tolerant ones although the relationship between plant stress tolerance and dehydrin transcript or protein accumulation is not always obvious (linear) due to a complex nature of plant stress tolerance mechanisms.

The aim of the study lies in a summary of the research focused on dehydrin transcript and protein accumulation in wheat and barley plants exposed to various abiotic stresses with a dehydrative component – cold, drought, salinity. We have tried to summarize recent results gained on cultivated common wheat (*Triticum aestivum*), durum wheat (*T. durum*) and barley (*Hordeum vulgare*) exposed to cold, frost, drought, and salt stress factors.

DEHYDRINS IN WHEAT AND BARLEY

There has already been published a complete annotation of barley genome sequence (The International Barley Genome Sequencing

Consortium, 2012) while common wheat (*T. aestivum*) complete genome sequencing is still in progress (www.wheatgenome.org). Barley (*H. vulgare*) represents the relatively most tolerant cultivated Triticeae species with respect to dehydrative stresses, especially drought and salinity (Colmer et al., 2006). There have been 13 dehydrin genes *Dhn1* to *Dhn13* identified in the barley genome (Tommasini et al., 2008). Common wheat genome is much larger than in barley due to its allohexaploid nature resulting in the presence of orthologs and also paralogs (Sarhan et al., 1997; Danyuk et al., 1998). Recently, Wang et al. (2014) have identified 54 dehydrin unigenes in common wheat genome by a search of wheat EST database.

Dehydrin structural types and expression patterns: both barley and wheat contain four (K_N, SK_N, Y_NSK_m, K_NS) out of five dehydrin structural types (Close, 1997) while they lack dehydrins of Y_NK_m type (Table 1). The largest group of dehydrins in barley (10 out of 13 *Dhn* genes) as well as in common wheat belong to Y_NSK_m type which encompass basic dehydrins induced by strong dehydration stresses (drought, salt, frost) as well as by abscisic acid (ABA) due to an occurrence of several ABRE elements in their promoters (Choi et al., 1999). Some of Y_NSK_m type dehydrins are embryo-specific such as barley *Dhn12* (Choi and Close, 2000). K_N type dehydrins DHN5 in barley and several K_N type dehydrins (WCS120 family as well as low-molecular K_N type dehydrins such as WDHN13) in common wheat are induced by both cold and drought as well as by ABA (Choi et al., 1999; Tommasini et al., 2008; Wang et al., 2014). There has also been described an induction of barley *Dhn5* by moderate levels of salicylic acid (SA) up to 0.25 mM while an inhibition

Bioinformatics approach to identify abiotic stress candidate genes in *Lolium*

1. Identify known genes in grasses, e.g., barley or wheat
2. Identify homologous sequences in *Lolium* genome through BLAST search at Ryegrass genome browser
<https://ryegrassgenome.ghpc.au.dk/>
3. Identify candidates, do phylogenetic analysis, identify exon-intron structure, target 5' exon(s) for sgRNA design