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## Analysis of stress response genes in moss *Dicranum scoparium*

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## INTRODUCTION

Mosses are the ancient terrestrial organisms representing a branch of the evolution of higher plants, where the haploid gametophyte dominates in the life cycle. They can survive in extremely adverse conditions. The mechanisms of plant stress resistance include the expression of genes encoding defence proteins, such as antioxidant enzymes, chaperones, transcription factors.

## AIM

In present work, we analyzed differential gene expression in the moss *Dicranum scoparium* in response to various abiotic stresses, such as heavy metals, temperature stress, prooxidants, an inhibitor of photosynthesis, dehydration, for 1 and 12 h.

## MATERIALS AND METHODS

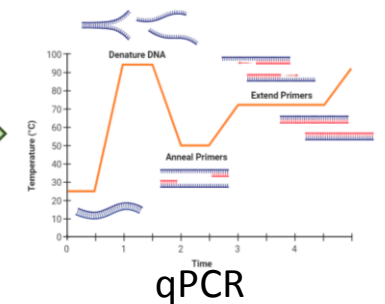
- Heavy metals ( $\text{CdCl}_2$ )
- Temperature stress
- Paraquat
- Dehydration
- 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)



Qiagen Rnase plant mini kit



cDNA synthesis (MMLV Kit)



qPCR

**Bioinformatic analysis:** the transcriptomic data (RNA-Seq) of the moss *D. scoparium* were taken from the NCBI SRA database. The library quality score was checked using FASTQC, then trimming of the reads with Trimmomatic and *de novo* assembly of the transcriptome using Trinity. From the file with the collected transcripts, a database was created to search for target genes using BLAST+. Protein sequences of target stress genes of the mosses with annotated genomes were used as a request. In *D. scoparium*, the target stress genes were discovered and found to be present in single homologous sequences.

The **F-box** is a component of the SCF E3 ubiquitin ligase complex that mediates ubiquitination and subsequent proteasomal degradation of target proteins.

**PHR1** (Protein Phosphate Starvation Response 1) is a transcription factor involved in phosphate starvation.

**FTSH** (ATP-dependent zinc metalloprotease) - plays a role in quality control of integral membrane proteins.

**ATG8** (Autophagy-related protein 8) - Ubiquitin-like modifier involved in autophagy and autophagosomes formation.

**DBF1** (DRE binding factor 1) involved in dehydration.

**Lea3** (late embryogenesis abundant 3) - proteins are produced during seed embryogenesis and in vegetative tissue in response to various abiotic stressors

Table 1. Characteristics of putative proteins, data obtained through the Protparam service

	DsATG8-1	DsDBF-1	DsF-box	DsFTSH-9	DsLea-3	DsPHR1
Number of amino acids	122	238	494	814	150	565
Molecular weight	14004.22	25573.03	54738.26	88777.70	14833.00	64037.98
Theoretical pI	8.59	5.98	8.38	7.21	5.53	9.02
Total number of negatively charged residues (Asp + Glu)	18	25	58	105	18	73
Total number of positively charged residues (Arg + Lys)	20	23	65	105	17	85
The instability index (II) is computed	30.62	64.99	45.76	38.07	26.09	38.56
This classifies the protein as	stable	unstable	Unstable	stable	stable	stable
Aliphatic index	80.74	70.34	81.15	88.42	45.33	72.51
Grand average of hydropathicity (GRAVY)	-0.388	-0.224	-0.376	-0.265	-0.724	-0.612

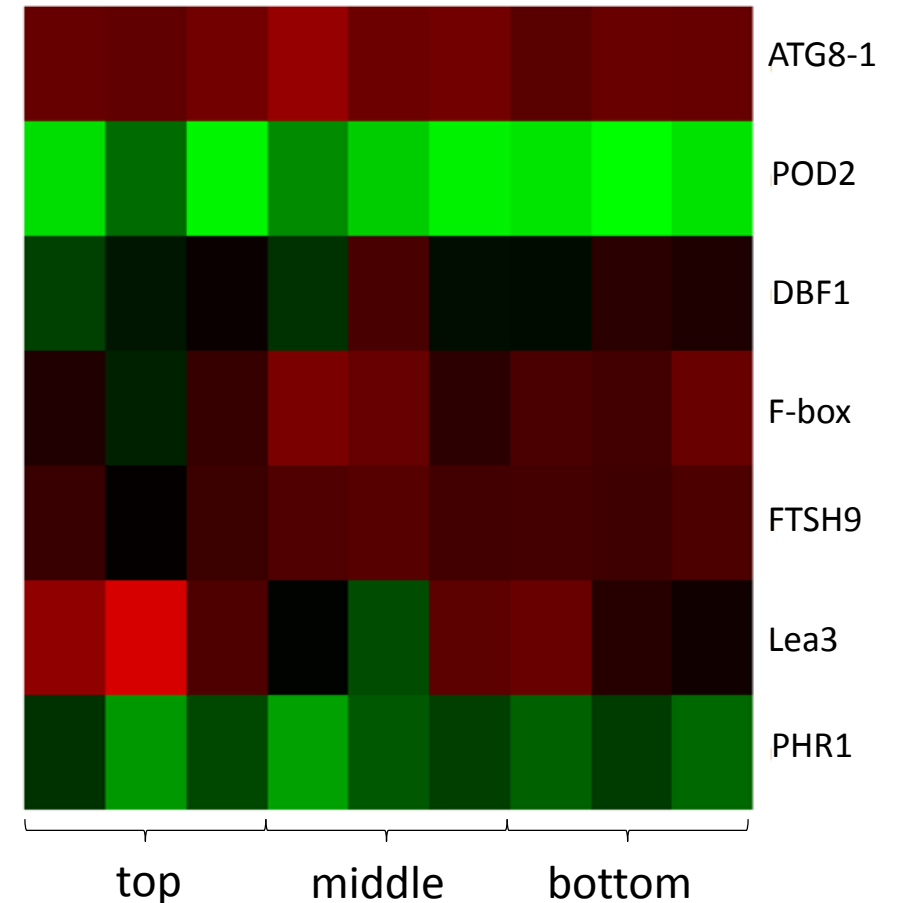
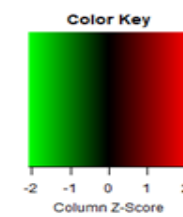


Figure 1. Heat map of transcript expression in different parts of the thallus of *D. scoparium* (top - middle - bottom), according to SRA data

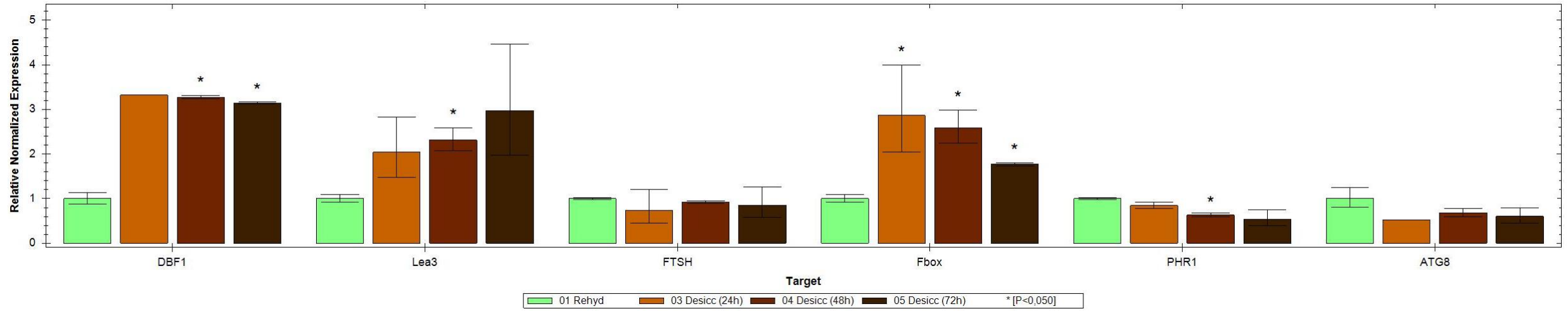


Figure 2. Gene expression in the moss *D. scoparium* in response to dehydration

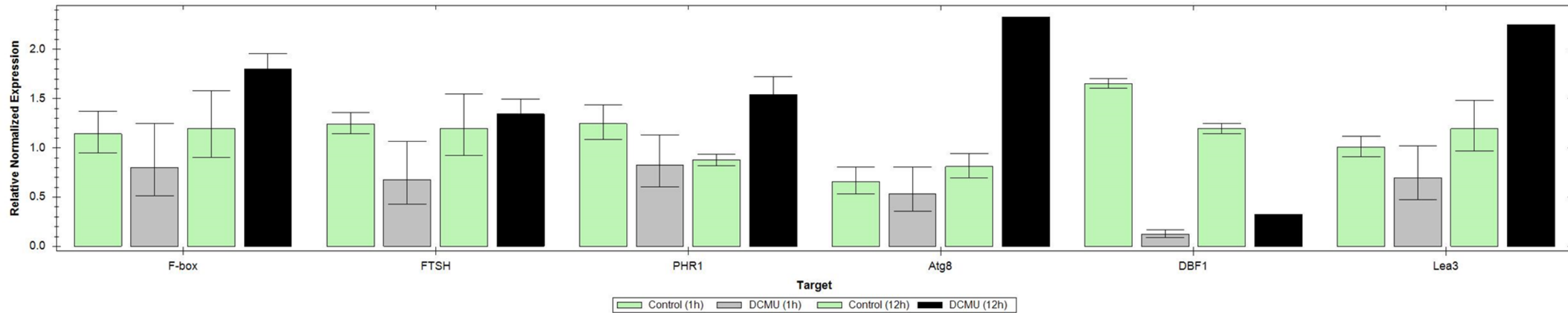


Figure 3. Gene expression in the moss *D. scoparium* following DCMU treatment inhibitor

Table 2. Overview of the expression of stress genes in the moss *D. scoparium* to various abiotic stresses

Genes	DCMU		PQ		CdCl <sub>2</sub>		Dehydration	Heat		Low temperature	
	1 h	12 h	1 h	12 h	1 h	12 h	24/ 48/ 72 h	1 h	12 h	1 h	12 h
<i>Atg8</i>	-	+	-	-	-	+	-	-	-	-	-
<i>DBF1</i>	-	-	+	-	+	-	+	+	-	-	-
<i>F-BOX</i>	-	-	-	-	-	-	+	-	-	+	-
<i>Lea3</i>	-	+	-	-	-	-	+	-	+	-	-
<i>PHR1</i>	-	-	-	-	-	-	-	-	-	+	-
<i>FTSH</i>	-	-	-	-	-	-	-	-	-	-	-

# Conclusions

1. Time dependence of the upregulation of stress genes occurs in the moss *Dicranum scoparium*. Genes encoding transcription factor *DBF1*, phosphate starvation *PHR1* and *F-Box* are involved in the early stress response, while genes encoding autophagy marker *Atg8* and *Lea* protein are involved in the late stress response.
2. Stress genes display both specific and universal responses to different abiotic stresses.
3. The involvement of these genes in the stress response of *Dicranum* suggests their evolutionary importance for the survival of higher plants under harsh environment.

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