# 1 Investigating the role of mRNA alternative splicing for the thermoresponse in seeds

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## 1.1 SUMMARY

At CEITEC, our team explores the effects of warm temperatures of seed development in Arabidopsis thaliana and Brassica napus. We grow the plants under conditions mimicking a daily rhythm, cooler nights, and warmer days with a midday temperature above 30oC, which is 10oC above the normal growth temperature. We identified various changes in traits, including major defects in embryo morphogenesis. Our recent data suggest that regulators of alternative splicing (AS) are transcriptionally activated in the temperature-treated seeds and that such seeds carry defective embryos. We aim at investigating if key genes in the seed thermoresponse are targeted by an altered AS output. What is the consequence of this temperature-dependent increase in AS activity, e.g., does it produce alternative protein isoforms, or does it contribute to quantitative control of these genes' expression, and how do warm temperatures regulate AS?

## 1.2 SCIENTIFIC BACKGROUND

Pre-mRNA splicing is a house-keeping activity occurring for most genes in higher eukaryotes. The spliceosome is a multimeric complex that catalyzes two sequential transesterification reactions to excise the interspaced intron (Will and Lührmann, 2011). The spliceosome is a multi-subunit complex comprised of the U2-dependent small nuclear RNPs (snRNPs), namely U1, U2, U5, U4/U6, and numerous non-snRNP proteins. Few proteins remain as core components of the tri-snRNP complex, including Snu114, Brr2, and PRP8. PRP8a interacts with a preformed U4/U6.U5 tri-snRNP complex to form a larger catalytically active spliceosomal B complex (Bartels et al., 2002; Maeder et al., 2009). Besides constitutive splice site choice, the generation of AS variants can increase transcriptome complexity. In A. thaliana, about 60% of intron-containing genes produce AS variants (Reddy et al., 2013). To select an alternative splice site, constitutive splicing factors (SF) or splicing regulators bind to exon/intron enhancer or suppressor sequence elements to influence recruitment of the spliceosome complex resulting in AS (Staiger and Brown, 2013). Some components of the spliceosome, such as PRP8a (Kulichová et al., 2020), or of the NineTeen Complex (NTC), such as CWC15 (Slane et al., 2020), are also involved in embryo development, as demonstrated by the embryonic defects in the respective mutants.

Essential genes involved in embryo morphogenesis via the regulation of production and signaling of the phytohormone auxin were shown to produce AS variants (Kriechbaumer et al., 2012; Cucinotta et al., 2020). How this is affecting embryo development in normal and stress conditions are yet to be investigated. Moreover, AS has been proposed as one of the regulatory mechanisms for abiotic stress response in plants (Staiger and Brown, 2013; Laloum et al., 2018). To support this observation, we have shown that in Brassica napus, young seeds developed at 34oC express more mRNAs of a few AS regulators (SCL30A and SLU7) (Mácová et al., 2021). And it was shown that one of the heat shock factors, HSFA2, produces splicing variants upon heat activation (Liu et al., 2013) as a self-regulatory mechanism to the heat shock

response. Also, the embryonic defects we observed in young A. thaliana and rapeseed seeds developed at high temperatures (Mácová et al., 2021; Sánchez López et al., unpublished) are similar to the ones in the prp8a and cwc15 mutant embryos (Kulichová et al., 2020; Slane et al., 2020), further supporting a link between splicing activity and embryo development.

## 1.3 AIMS

Given the importance of a fine-tuned regulation of the AS for plant development, we aim to explore further its upregulation in the thermoresponse in A. thaliana seeds. We would like to address three main questions:

1> Are the transcripts of HSFs and other critical morphogenic embryonic genes regulated by AS in seeds, and are the HSF variants involved in response to high temperatures?

2> What are the molecular regulators of the AS machinery responding to the increased growth temperatures?

3> What is the function of the altered AS output in the thermoresponse?

This small collaborative project will initiate our research into the putative impact of AS on the seed thermoresponse. We will try to answer the first research question. Based on the results of the proposed analysis performed during this INTEG RNA collaboration, a project covering the other research questions may be prepared.

## 1.4 WORK PROGRAM

### *1.4.1 The experimental approach including key techniques or technologies*

Dataset of known splicing variants induced by stresses and by mutations in genes involved in AS will be screened for a list of candidate transcripts. These transcripts are encoded by genes such as splicing factors/regulators, HSFs, and few other temperature-responsive genes involved in the auxin production, transport, response, and embryonic morphogenesis.

The most interesting transcripts displaying AS will be experimentally tested in our study models in the partner lab during a one-month internship of J.F. Sánchez López, CEITEC Ph.D. student. As warm temperatures induce morphogenic defects, samples will be taken for developmental stages before and after embryonic morphogenic changes, e.g., seeds containing embryos at early globular and heart stages. Seeds from wild-type plants grown at control conditions, 28/18, 34/18, and 28/24 (day and night temperatures in °C), will be collected for the experiments. Following RNA extraction, reverse transcription with oligo dT and random hexamer primers will be performed to cover transcripts with and without poly-A tails. Furthermore, samples from RNA decay mutants (various types available in Andreas Wachter's lab) can be included to cover variants with relatively low steady-state levels. Using primer sets for the most promising AS regions, PCR amplification, cloning, and sequencing will be performed to identify AS variants of interest. For a final selection of AS events, a quantitative analysis of AS ratio outputs in the various samples will be conducted, using either PCR co-amplification and ratio quantitation via a Bioanalyzer or quantitative PCR of individual AS variants.

Selected plant lines affected in AS regulation will be phenotypically analyzed during seed development at warm temperatures. The experiments will be performed by CEITEC during the secondment of an Andreas Wachter group's lab member. Plants will be grown in the CEITEC Plant Sciences core facility. Seeds will be dissected at globular and heart stages and cleared in

a chloral hydrate-based clearing solution. Embryo morphology will be assessed using light microscopy from the CEITEC Cellular Imaging core facility. The effects of warm temperatures will be scored as the changes in any phenotype penetrance in the various tested genetic backgrounds compared to what is observed in wild-type plants.

### *1.4.2 What will be done by which partner and the expected synergies*

In combination with this project, we will apply for an internship of one month for J.F. Sánchez López, CEITEC Ph.D. student involved in the project. Based on the phenotyping observations performed at CEITEC, the list of candidate transcripts will be prepared at CEITEC. Juan Francisco will also prepare the samples to be tested and performed any necessary preliminary testing to optimize his stay at the partner lab.

Andreas Wachter's lab at JGU Mainz will screen their datasets for splicing variants with the provided candidate transcripts. During the internship in the partner lab, AS on the selected transcripts will be experimentally tested.

At CEITEC, the lab offers the possibility to phenotypically analyze various genetic materials, plants with altered expression in genes involved in the AS regulation. This material can be tested for seed development defects in plants grown at warm temperatures.

### *1.4.3 The benefit of this research project to RNA research at CEITEC*

Our group at CEITEC established the growth conditions to monitor the thermoresponse in seeds in rapeseed (Mácová et al., 2021) and A. thaliana (Sánchez López et al., unpublished). Our preliminary analysis hinted at us to look further into the regulation of splicing. However, we have not any genetic material nor the expertise to do so. The INTEG RNA project provides the opportunity to team up with Andreas Wachter's lab at JGU Mainz in Germany to expand our toolbox and expertise in this direction.

### *1.4.4 Future collaborations or joint funding expected to result from work proposed*

Hopefully, this collaborative project will provide a ground for further collaboration to further explore this proposal topic. One of the possibilities is the application for funding for a collaborative project with the Weave initiative, a LA project between GACR in the Czech Republic and DFG in Germany.

## 1.5 BUDGET

We ask for bench fees covering the experiments that J.F. Sánchez López will perform during his internship in Andreas Wachter's lab at JGU Mainz. We will, therefore, apply for a secondment for J.F. Sánchez López to cover the fees related to his travel to Mainz and his one-month stay. We also plan to apply for a 2-week secondment of a lab member of the partner lab to CEITEC for the phenotyping screen.

## 1.6 REFERENCES


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