1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: The Role of Nuclear Transcriptome in the Formation of Stress Granules in Plants

1.1. Project goals

The research project focuses on the molecular mechanisms of plant responses to abiotic stress. The main objective of the project is to understand the composition of stress granules (RNA) and the role played by the nuclear transcriptome in their formation.

Specific Objectives:

- Understanding the transcriptome of stress granules (SG) to reveal the mechanisms of their formation and functioning.
- Deciphering the interactions between the nucleus and SG during different stages of stress to understand the role of nuclear transcripts in stress granule biogenesis.
- Identification of RNA binding proteins (RBPs) involved in the selective transport of RNA from the cell nucleus to SG.

1.2. Outline

Extreme environmental conditions demand exceptional genomic responses that enhance adaptation to stress. Plants are organisms whose habitats are intimately linked to their germination sites. Therefore, the plasticity of gene expression in response to stress is crucial. These processes are regulated simultaneously at both transcriptional and post-transcriptional levels (PTGR) (Litholdo and Bousquet-Antonelli 2019). Recently, it has been demonstrated that one of the important PTGR mechanisms in adverse environmental conditions in plants is the spatial organization of the transcriptome within cells. It is believed that the formation of SG directly impacts gene expression during stress (Guzikowski et al. 2019), as the stored RNA indirectly regulate the rate of protein synthesis. Additionally, it is suggested that the cell may utilize the RNA stored in these granules after stress conditions have ceased.

Our preliminary studies revealed a surprising finding that the inhibition or even reduction in the efficiency of nuclear-cytoplasmic transport disturb the formation of SGs. This was accompanied by a decrease in the amount of poly(A) RNA in the cytoplasm. This suggests the involvement of certain nuclear transcripts in the formation of stress granules. Our results also confirm existing literature data: (i) actinomycin D (ActD) treatment in cells infected with cricket paralysis virus 1A (CrPV-1A) inhibits host transcription, thereby preventing SG formation (Khong et al. 2017); (ii) ActD inhibits SG assembly under arsenate stress; ActD induces the nuclear protein HuR translocation to the cytoplasm; HuR protein translocation to the cytoplasm leads to stress granule disassembly (Bounedjah et al. 2014).

The above data indicate the involvement of nuclear RNA in SG formation. To verify this hypothesis, the doctoral candidate will conduct: (i) identification of nuclear-derived RNA present in SGs; for this purpose, cells subjected to stress will be incubated with BrU (uridine analog); when newly

transcribed RNA exits the nucleus, SGs will be isolated and BrU-containing RNA will be sequenced; (ii) comparison of the nuclear transcriptome of stressed plants treated and untreated with leptomycin B (an inhibitor of nuclear-cytoplasmic transport) to identify potential RNAs whose retention in the nucleus inhibits SG formation; (iii) investigation of whether nuclear RNA "seeds" are present in SGs; examination of A. thaliana mutants with a disrupted expression of potential RNA "seeds."

I propose a doctoral project focusing on plant response mechanisms to abiotic stress, which integrates several experimental pathways and significantly enhances our understanding of the role of SGs in this phenomenon. In the presented project, we aim to explore a completely new issue that has not been addressed previously: the involvement of nuclear transcriptome in the formation of SGs, structures that are the main sites of RNA accumulation in the cytoplasm during stress.

1.3. Work plan

- Cultivation of A. thaliana seedlings in in vitro cultures under physiological conditions and hypoxic stress.
- Isolation and analysis of the SG transcriptome (RIP techniques, RNA-seq).
- Tracking and identification of nuclear transcripts in SGs (techniques involving BrU, RIP, BrU-RNA-seq).
- Intracellular localization of transcripts present in SGs and the nucleus (in situ hybridization).
- Investigation of the nuclear transcriptome under stress conditions in plants treated and untreated with nuclear transport inhibitors (isolation of cell nuclei using the INTACT technique and RNA-seq).
- Analysis of stress granule presence in A. thaliana mutants with knockout genes encoding RNA "seeds" (FISH technique).
- Identification of RNA-associated proteins remaining in the nucleus and transported from the nucleus to SGs during stress (isolation of selected RNPs and protein identification using bottom-up LC-MS/MS technique).

1.4. Literature *(max. 10 listed, as a suggestion for a PhD candidate)*

2. Maruri-López et al. (2021) Front Plant Sci. 12:722643;
3. Niedojadło et al. (2016) RNA Biol. 13:531-543;
4. Parker et al. (2020) eLife 9:e49658;
5. Guzikowski et al. (2019) Wiley Interdiscip.
6. Sorenson and Bailey-Serres (2014) Proc. Natl. Acad. Sci. USA 111:2373-2378;
7. Sorenson and Bailey-Serres (2015) Methods Mol. Biol. 1284:209-219;

7.1. Required initial knowledge and skills of the PhD candidate

The candidate must hold a Master's degree in molecular biology, biotechnology, biology, or related fields. The candidate should be prepared for new challenges and approach them with enthusiasm. The doctoral candidate should be able to handle minor setbacks in scientific work and consistently strive to achieve set goals. The individual applying for this position should demonstrate commitment to scientific work and be available for travel related to research. Additionally, skills in cell and molecular biology are desirable, including techniques such as in situ hybridization, RNA work, genotyping and floral dip transformation method . The candidate must hold a Master's degree in molecular biology, biotechnology, biology, or related fields.

7.2. Expected development of the PhD candidate's knowledge and skills

The doctoral candidate is expected to continuously develop their knowledge and skills during the doctoral studies. The work in this position primarily requires acquiring new competencies, interpreting results, and developing new concepts within the scope of the conducted research.

In the first year of work, the doctoral candidate should master the substantive basics of their research and learn techniques for isolating cell nuclei and stress granules, extracting RNA from them, and generating expression libraries. In the following year, they should achieve initial results from the transcriptome analysis of isolated structures and the localization of specific RNA. By the end of the third year of work, the doctoral candidate should have sufficient results to write the first publication. During this time, they should also identify proteins associated with specific RNA and analyse mutants.

Throughout the doctoral work, the doctoral candidate will be under the scientific supervision of the supervisor and will have the opportunity to develop their skills through scientific trips and participation in scientific conferences.