Mutation in Arabidopsis mitochondrial pentatricopeptide repeat 40 gene affects tolerance to water deficit

Ph.D. Dissertation

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Introduction:

Climate change poses a direct threat to global food security, with severe droughts becoming increasingly common in regions such as Europe, the United States and China. Underscoring the urgency of developing strategies to mitigate its effects, these events are characterized by dwindling river flows, rampant wildfires and crop failures. Central to these efforts is the need for drought resistant crops and sustainable agricultural practices. In this context, *Arabidopsis thaliana* is proving to be a valuable model organism for studying plant genetics and elucidating stress response mechanisms. With its rapid life cycle, self-compatibility and manageable genome size, Arabidopsis offers unique advantages for genetic research. Arabidopsis has served as a cornerstone for understanding gene function in dicotyledons since its genome was sequenced in 2000. The advent of T-DNA insertion mutants has further facilitated studying the role of specific genes, opening up opportunities for agricultural and industrial applications.

Drought, a consequence of reduced rainfall, is a major stress for plants, leading to reduced water availability, soil degradation and increased susceptibility to various environmental hazards. Plant responses to drought include a spectrum of physiological, biochemical and molecular adaptations orchestrated to mitigate the deleterious effects of water scarcity. These responses, aimed at maintaining cellular homeostasis and ensuring survival under adverse conditions, include changes in photosynthesis, gene expression and antioxidant metabolism.

The role of reactive oxygen species (ROS) is critical for plant adaptation to drought stress. ROS are signaling molecules involved in both defense mechanisms and stress response pathways. Abscisic acid (ABA), a phytohormone, is a key regulator of drought responses. It orchestrates stomatal closure and activates stress-responsive genes to conserve water and enhance stress tolerance. On the other hand, mitochondria, vital organelles in plant cells, play multiple roles in cellular metabolism, producing energy and sensing stress. Mitochondrial alternative oxidases (AOX) act as key modulators of energy balance. They remove excess electrons to reduce ROS production and maintain cellular redox homeostasis under stress conditions.

The pentatricopeptide repeat (PPR) family of proteins emerges as a critical regulator of mitochondrial gene expression and stress adaptation within this intricate network of stress responses. PPR proteins, characterized by repeated pentatripeptide domains, modulate RNA metabolism, splicing and translation, thereby influencing several physiological processes, including ABA- and ethylene-mediated stress signaling pathways.

The PPR40 protein is associated with complex III of the mitochondrial electron transport chain. It has been suggested that PPR40 contributes to the maintenance of mitochondrial electron flow in salt-stressed plants. Two insertional mutations of the *PPR40* gene were previously identified: *ppr40-1* and *ppr40-2* alleles, proposed as knockout and knockdown mutations, respectively. In germination assays, *ppr40-1* and *ppr40-2* showed stronger and weaker ABA hypersensitivity, respectively, suggesting that the two alleles affect ABA signaling to different extents. The electron transport of the *ppr40-1* mutant was particularly sensitive to perturbations that resulted in increased ROS production. This modulates ABA signaling and sensitivity to this hormone. Overexpressing *PPR40* improved salt stress germination in vitro and reduced ABA sensitivity While the *ppr40-1* mutant has been extensively characterized in vitro, the phenotype of the *ppr40* mutant alleles and their responses to water deficit in soilgrown plants have not been investigated. Here we show that two mutant alleles of the *PPR40* gene, *ppr40-1* and *ppr40-2*, are more tolerant to gradual water stress, and have a better ability to conserve water, reduce oxidative damage and survive water deficit.

Objectives:

The general objective of the study was to investigate how *ppr40* mutants which affect mitochondrial electron transport influence responses to water deficit, and what is their role in modulating drought tolerance in model plant *Arabidopsis thaliana*.

- Assess the survival rates of *ppr40-1*, *ppr40-2*, and wild type (Col-0) plants during drought stress.
- Measure the physiological parameters including Electron Transport Rate, proline content, lipid peroxidation, and relative water content in *ppr40-1, ppr40-2*, and Col-0 plants under drought stress.
- Examine the expression levels of drought-related genes in *ppr40-1, ppr40-2,* and Col-0 plants under drought stress.

- Utilize advanced phenotyping system to evaluate leaf characteristics such as green area, compactness, area ratio, slenderness, and various PSII parameters (e.g. QY max) in *ppr40-1*, *ppr40-2,* and Col-0 plants under drought stress.

Materials and Methods:

Plant material and growth conditions:

ppr 40 mutants, *PPR40* overexpression, wildtype (Col-0).

22 °C, an 8/16 h light/dark cycle, and an approximate photon flux density of 150 µmol m^{-2} s^{-1}

Physiological Assays:

Survival rate, Relative water content, Proline estimation, MDA estimation, stomatal conductance, Chlorophyll fluorescence measurements

Phenotypic Measurements:

Green area, slenderness of leaves, compactness

Gene Expression Studies:

Expression of stress-related genes like *RD29A, RAB18, ZAT12, ABF2, ABF3, AOX1a, AOX1d.*

Summary of the results:

Climate change poses adverse effects on plants, particularly crucial agricultural crops. One of the most pressing concerns is drought which can cause severe food scarcity in the world; therefore, it is necessary to understand the intricate plant mechanisms to develop resilient crop varieties. The findings presented in this thesis provide significant insights into the role of PPR40 in enhancing drought tolerance in Arabidopsis plants. Through a comprehensive analysis of various physiological, biochemical, and molecular parameters, we have demonstrated the role of PPR40 in conferring resilience to water stress.

The Arabidopsis thaliana *ppr40-1* mutant showed hypersensitivity to ABA, salt and sugar in germination assays, was identified in a T-DNA insertion mutagenesis program. The knock-out mutant plants exhibit a semi-dwarf growth phenotype. In addition, another allele was identified, *ppr40-2*, whose mutation did not completely abolish the *PPR40* gene function but resulted in a phenotype intermediate between *ppr40-1* and the wild type. These mutations in the PPR40 gene affect mitochondrial electron transport chain reducing electron transport rates. Our investigation began by characterizing the *ppr40* mutant's response to drought conditions. The ABA hypersensitivity is correlated with improved survival rates in water-limited conditions, as the *ppr40-1* mutant showed significantly higher survival rates compared to Col-0 wild-type plants, while the *ppr40-2* mutant exhibited intermediate survival rates.

Complex phenotyping of the *ppr40* mutants in an automatic plant phenotyping platform provided valuable information on their growth and morphology in well-watered and water-limited conditions. Under standard growth conditions, the *ppr40-1* mutant showed semi-dwarf phenotype and reduced growth rates when compared to Col-0 (wild type) and *ppr40-2* mutant. However, under water stress, the *ppr40* mutants exhibited delayed declines in rosette size and sustained their viability for longer periods compared to wild-type plants. The analysis of hue ratios and slenderness of leaves further supported the superior tolerance of the *ppr40* mutants to water deprivation, as their rosette morphology was better preserved during water stress.

We further investigated the stability of photosynthesis in the *ppr40* mutants under drought conditions. Chlorophyll fluorescence analysis indicated that photosynthetic electron transport remained functional for more extended periods in the drought-stressed *ppr40* mutants compared to wild-type plants. The maximum quantum yield analyzed under automatic phenotypic system, dropped faster in Col-0 when compared to *ppr-40* mutants under water deficit conditions, again supporting our results.

The higher water content during drought conditions affects a range of physiological parameters, eventually influencing the stress tolerance of plants. Physiological parameters, such as Relative water content, proline accumulation, and MDA revealed important insights into the drought tolerance mechanisms of the *ppr40* mutants. *ppr40* mutants showed better water retention capabilities, with significantly reduced RWC reductions under water stress compared to wild-type plants, due to faster stomata closure in *ppr40* mutants. Proline accumulation, a well-known response to water deprivation in plants, was also higher in the Col-0 when compared to *ppr40* mutants, suggesting that these mutants experience less stress. The lower level of *P5CS1* gene expression in *ppr40* mutants correlates well with compromised proline accumulation. Additionally, we observed reduced oxidative damage in the *ppr40* mutants, as evidenced by lower lipid peroxidation rates (MDA levels) and attenuated ROS-induced gene activation.

The modulation of drought-induced gene expression, ABA-responsive genes, and stress-responsive Alternative Oxidases (AOX) further highlights the role of *PPR40* in regulating plant responses to water stress. The *ppr40-1* mutant displayed significantly reduced transcript levels of the ABA-induced dehydrin gene *RAB18* under drought stress, with *ppr40-2* exhibiting intermediate expression compared to wild-type plants. Transcript levels of the ROS-induced gene regulator *ZAT12* were also significantly lower in *ppr40-1*, indicating attenuated activation of ROS-dependent gene expression under drought treatment. Expression analysis revealed lower *AOX1a* and *AOX1d* transcript levels in drought-induced *ppr40* mutants compared to wild type plants, suggesting that these mutants experienced limited stress and their non-phosphorylating respiratory pathways were less active. The expression levels of bZIP-type *ABF* transcription factors, which are key regulators of ABA-induced transcription and showed similar patterns to *AOX* genes. These lower levels in *ppr40* mutants were basically because the decreased water reduction prevented dehydration hence there was lower accumulation of reactive oxygen species and lower oxidative stress. We examined the transcript levels of specific genes involved in auxin, salicylic acid (SA), and other hormone-related genes, but this effect was less pronounced or absent in the *ppr40* mutants.

Overall, the comprehensive analysis of the *ppr40* mutants revealed that PPR40 can play a critical role in modulating drought tolerance through multiple interconnected mechanisms. The mutants exhibited improved water retention, reduced oxidative damage, and stabilized photosynthetic electron transport, resulting in better survival rates under water stress. These findings shed light on the complex interplay between ABA signaling, photosynthesis, ROS homeostasis, and stress-responsive gene regulation, all of which can be influenced by the absence of PPR40 protein.

A deeper understanding of the function of PPR40 and related proteins in enhancing drought tolerance could have an impact on crop improvement. Such an understanding holds great promise for addressing escalating water scarcity and mitigating the adverse effects of climate change. Harnessing the potential of the PPR40 protein in ongoing plant breeding initiatives offers the opportunity to develop more resilient and productive crop varieties. Such efforts are key to strengthening future food security and promoting sustainable agricultural practices.

List of Publications: (MTMT: 10074888)

1. Mutation in Arabidopsis mitochondrial Pentatricopeptide repeat 40 gene affects tolerance to water deficit. Kamal Kant, Gábor Rigó, Dóra Faragó, Dániel Benyó, Roland Tengölics, László Szabados*, Laura Zsigmond*; Planta. (2024). IF 4.3

2. Mitochondrial complex I subunit NDUFS8.2 modulates responses to stresses associated with reduced water availability. Annabella Juhász-Erdélyi, Ildikó Valkai, Gábor Rigó, Agnes Szepesi, Dávid Alexa, Kamal Kant, Niklas Körber, Fabio Fiorani, László Szabados, Laura Zsigmond*; Plant Physiology and Biochemistry. (2024). IF 5.6

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As the corresponding and/or contributing author of the mentioned publications, I declare that the authors have no conflict of interest related to this study. I also declare that the Ph.D. candidate Kamal Kant worked under my supervision and his contribution was prominent in obtaining the results, and his first author publication was not used for Ph.D. defense by any of the co-authors.

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