Title: Engineering of rapeseed redox pathways to improve environmental stress tolerance.

Aims of the proposal

Unpredictable weather changes and global warming can have negative impacts on agricultural productivity. Shorter or longer periods of water deficit and heat waves can develop, to which many crops cannot adapt properly. Rapeseed, one of the most important oil crops, reacts particularly sensitively to these adverse changes. Responses to drought and heat are controlled by numerous genes that can adjust metabolic processes during adverse conditions. Information on these genes can be explored to understand the stress tolerance of plants better. Using our preliminary results, we began to research several Arabidopsis and Lepidium genes that control ROS levels and/or signals and influence tolerance to drought, salinity, heat, or combined stresses. Due to the high level of homology between genes of the Brassicaceae family, we can predict, that rapeseed genes have the same or similar function to their Arabidopsis or Lepidium counterparts. Therefore, engineering the rapeseed genes might lead to similar changes in stress tolerance. As genetic transformation and genome editing have already been known for this species, we can design molecular tools for rapeseed, which can be suitable to enhance tolerance to different types of stress conditions.
The principal aim of our research program is the establishment of scientific ground for a translational research program to improve abiotic and biotic stress tolerance of rapeseed, one of the most important sources of vegetable oil in Hungary and the world. Our objective is to use the knowledge obtained on model plants and particular genes identified in model and extremophile organisms to develop genetic and molecular strategies to enhance tolerance to salinity, drought, and if possible, a combination of these stresses with high temperatures.
The specific objectives of the project were:
- Characterize candidate genes from Arabidopsis and the halophyte plant *Lepidium crassifolium* to improve stress tolerance in other plants.
- Evaluate the capacity of these genes to enhance tolerance to abiotic and combined stresses (salt, drought, and heat) in the model plant.
- Validate identified genes and gene forms to improve tolerance of rapeseed to drought, salinity, heat and/or a combination of them.
- Establish genome editing protocols for targeted mutagenesis of the rapeseed genome.
- Use of a new automatic phenotyping platform to characterize tolerance traits.

Results

1. Small paraquat resistance proteins confer drought tolerance to overexpressing Arabidopsis plants.

During our previous research, we identified a gene encoding a small peptide from *Lepidium crassifolium* (**SPQ - S**mall **P**ara**q**uat resistant protein), which caused paraquat resistance when overexpressed in Arabidopsis plants (Rigó et al., 2016, doi: 10.1111/pce.12768). Sequence homology search with LcSPQ revealed that a highly similar gene is present in Arabidopsis thaliana, the *AT3G52105*, which encodes a previously unknown protein of 70 amino acids (AtSPQ). To compare the functions of Lepidium and Arabidopsis SPQ proteins, full-length cDNA of *AT3G52105* have been cloned and overexpressed in *Arabidopsis thaliana*. Paraquat resistance of AtSPQ and LcSPQ overexpressing plants was similar in growth assays using different paraquat-containing mediums (Figure 1.1 A). Moreover, the paraquat-induced oxidative damage was reduced by LcSPQ and AtSPQ overexpression as lipid peroxidation rates

in paraquat-treated LcSPQ and AtSPQ plants were significantly lower than in wild type plants. (Figure 1.1 B).

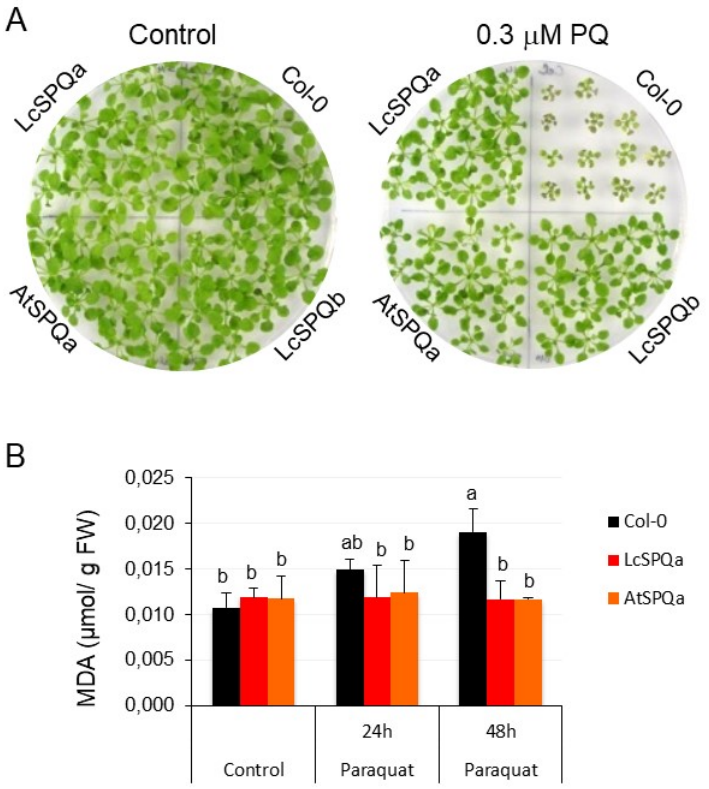


Figure 1.1 Paraquat resistance of SPQ overexpressing plants. AtSPQa, LcSPQa, LcSPQb: independent overexpressing SPQ lines. A) Homozygous SPQ overexpressing and wild type (Col-0) plants grown on paraquat-containing culture media. B) Lipid peroxidation rates of Col-0 and SPQ overexpressing plants, treated with 7 µM paraquat for 24 or 48 hours.

To better understand the function of SPQ, we performed root and hypocotyl growth assays using LcSPQ, AtSPQ and *spq1* mutant plants. The root elongation of LcSPQ, AtSPQ, and *spq1* were tested on paraquat containing media. Inhibition of root growth was alleviated by overexpression of AtSPQ and LcSPQ but was not significantly different in *spq1* when compared to wild type (Figure 1.2. A). To test paraquat sensitivity in the absence of light, hypocotyl elongation of dark-germinated seedlings was tested. Hypocotyl elongation of etiolated Col-0 and *spq1* seedlings was similarly reduced by paraquat but was less affected in AtSPQa and LcSPQa plants (Figure 1.2. B). These results suggest that overexpressing SPQ proteins of Lepidium and Arabidopsis species can cause similar paraquat resistance in Arabidopsis.

In addition to testing paraquat tolerance, we performed in vitro growth tests using PlantSize software (developed earlier in our group) under different stress conditions (salt, osmotic, and ABA treatments). We observed that LcSPQ and AtSPQ lines showed ABA sensitivity compared to the wild type (data not shown). Based on these results, we completed the characterizations of AtSPQ and LcSPQ overexpressing and *spq1* mutant lines with ABA induced stomata opening experiments and gene expression studies. Stomata apertures were similar in all plants in control conditions and were significantly smaller in ABA-treated guard cells of AtSPQ and LcSPQ overexpressing plants (Figure 1.3. A). Stomata aperture of the *spq1* mutant remained larger than the wild type only when a high ABA concentration was used. In case of the gene expression experiments, 2-week-old in vitro-grown plants were treated with 50 µM ABA and transcript levels were determined by qRT-PCR. RD29A and RAB18 levels

were significantly higher in AtSPQ and LcSPQ plants under ABA treatments but were not altered in the *spq1* mutant (Figure 1.3. B). These results revealed that LcSPQ and AtSPQ overexpression enhances ABA sensitivity.

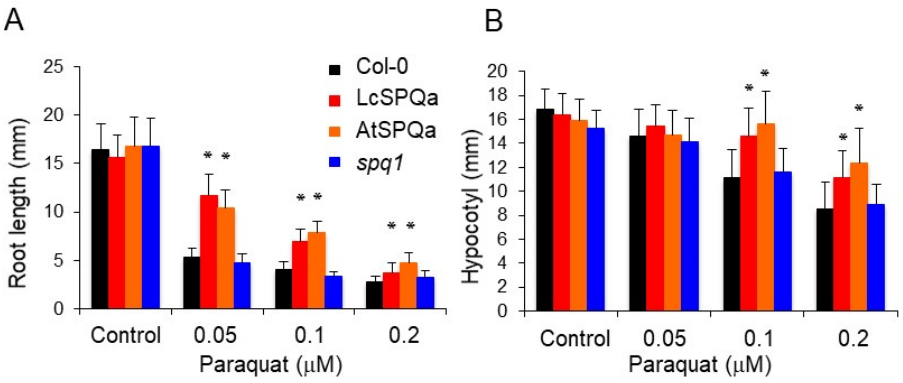


Figure 1.2 Paraquat sensitivity of non-photosynthetic organs. A) Root growth of Col-0, LcSPQ (LcSPQa), and AtSPQ (AtSPQa) lines and the *spq1* mutant in the presence of paraquat. Root lengths were measured 8 days after germination. B) Hypocotyl elongation of etiolated LcSPQa and AtSPQa and *spq1* seedlings (germination in dark). Hypocotyl lengths were measured 5 days after germination.

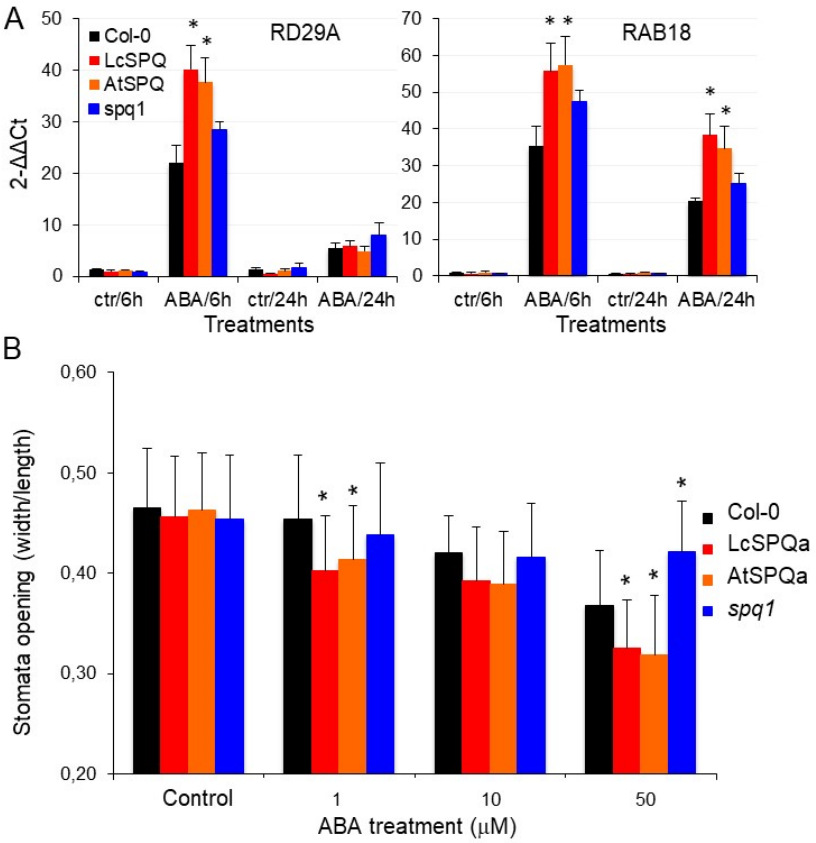
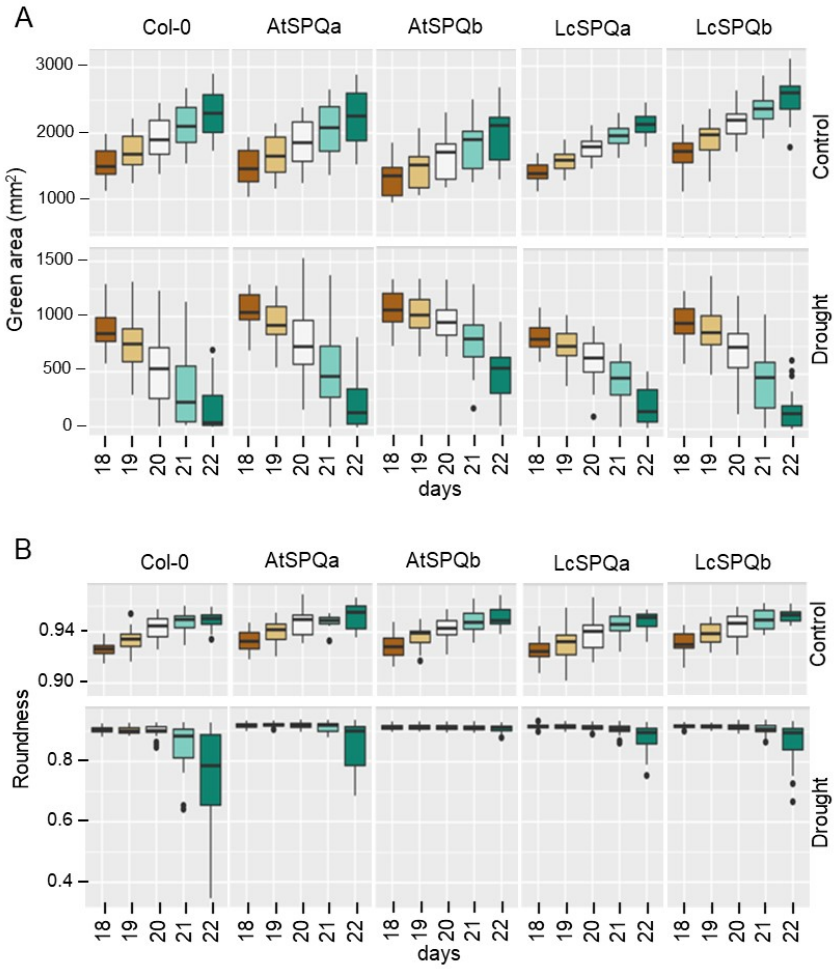


Figure 1.3. ABA sensitivity of wild type, LcSPQ or AtSPQ overexpressing plants and the *spq1* mutant. A) Expression of ABA-induced genes *RAB18* and *RD29A* in ABA-treated and control plants. Relative expression is shown where 1 corresponds to transcript levels of Col-0 in 6 hours control samples. B) The ratio of stomata diameter and length indicates the degree of stomata opening. Larger numbers indicate more open stomata.

Drought stress reduced the growth of green leaf areas, and the most dramatic changes happened between the 18th and 22nd days of water stress. Therefore, we performed a comparative analysis of the different genotypes in these days. The changes in green areas of the wild type and overexpressing lines were similar in well-watered and water-limited conditions (Figure 1.4 A). In contrast, we observed morphological differences in advanced drought conditions when the rosette roundness of wild type plants declined faster than LcSPQ and AtSPQ (Figure 1.4. B). These differences in maintenance of the regular rosette shape indicate that the overexpressing lines are more tolerant to water-deficient conditions. To compare the photosynthetic efficiency of well-watered and drought-stressed plants, we measured the Fv/Fm parameter of Photosystem II. Fv/Fm values were similar in all genotypes in well-watered conditions, and until 18-19 days after water withdrawal. These values decreased in the later stage of the experiments, but the changes were smaller and happened 2 days later in LcSPQ and AtSPQ plants than in the wild type (Figure 1.4 C). Phenotyping data showed that morphological changes due to wilting and reduction of photosynthetic parameters were delayed in SPQ-overexpressing plants compared to the wild type, indicating that SPQ can contribute to maintaining the viability of transformed Arabidopsis under drought conditions.



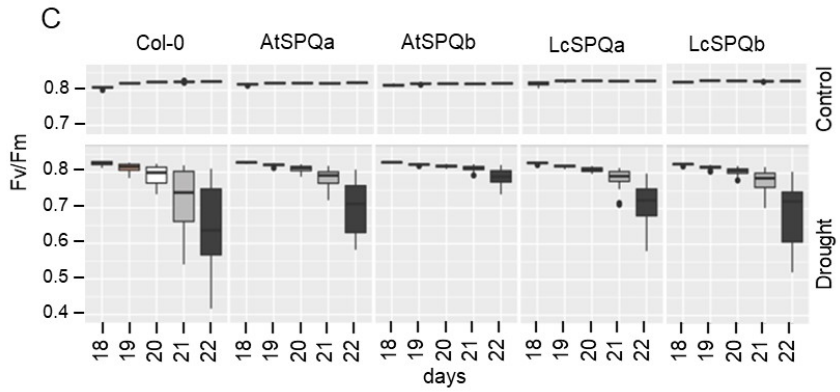


Figure 1.4. Drought tolerance of SPQ overexpressing Arabidopsis plants measured by plant phenotyping system (Control: uninterrupted watering, Drought: water withdrawal). RGB images of 30 plants were used to show average rosette sizes as green areas (A) and shapes as roundness (B). The effect of drought stress on photosynthetic parameters of Col-0 wild type and SPQ overexpressing plants was determined by chlorophyll fluorescence imaging (C, Fv/Fm parameter).

To supplement our phenotyping data, we examined 4-week-old plants grown in a greenhouse or growth chamber after exposure to drought stress. We measured the relative water content of the plants after 9 and 11 days of water deprivation. We found that while the water content of well-watered (control) and plants dried for 9 days was similar, on day 11th the relative water content of LcSPQ and AtSPQ decreased less compared to the wild types (Figure 1.5. A).

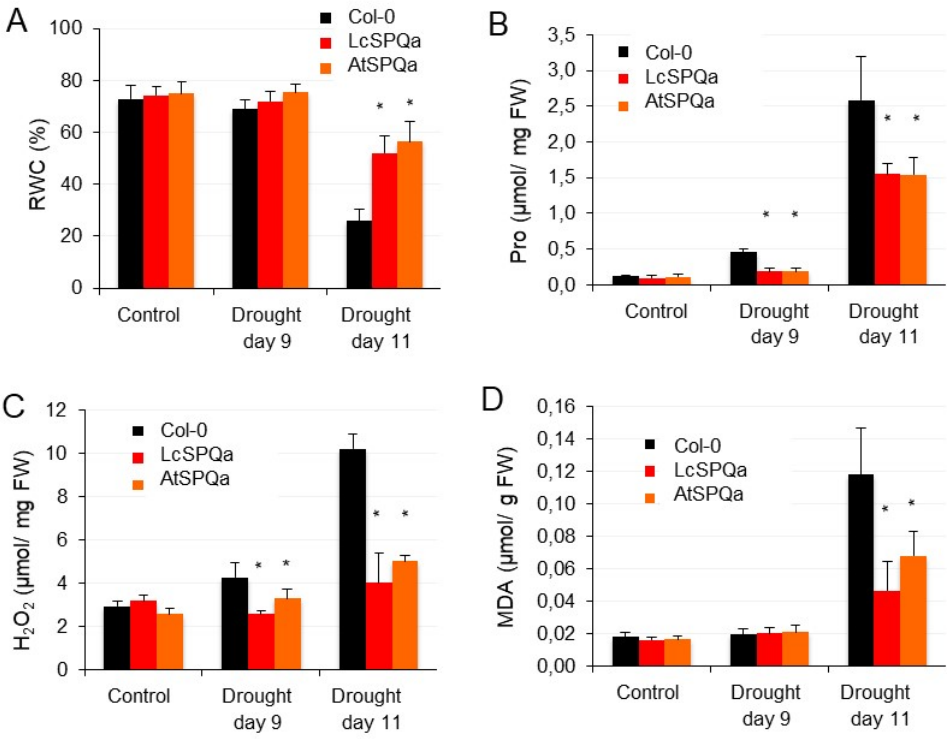


Figure 1.5. Physiological parameters of Col-0 wild type and LcSPQ or AtSPQ overexpressing plants subjected to drought stress. Relative water content (A), proline content (B), hydrogen peroxide levels (C), and lipid peroxidation were measured as MDA content (D) in leave samples of control and drought-stressed plants.

Several stress-related parameters were studied in drought-treated plants to understand better the physiological and molecular background of the drought tolerance of LcSPQ and AtSPQ lines. To test redox balance in AtSPQ and LcSPQ overexpressing lines we measured H_2O_2 content and lipid peroxidation level (MDA content) under drought stress. We observed less hydrogen peroxide formed in AtSPQ and LcSPQ (Figure 1.5. C), and lipid peroxidation level was also lower (Figure 1.5. D) compared to the wild type plants. The proline accumulation was also inferior in the SPQ overexpressing lines under drought stress (Figure 1.5. B). These data suggested that the LcSPQ and AtSPQ overexpression can alleviate oxidative damage of drought-stressed plants by containing ROS accumulation in water-limited conditions.

As a summary of these results, we published our data supplemented with more detailed characterizations in Plant Cell and Environment in April 2022 (doi: 10.1111/pce.14338).
The above results were a significant (50%) part of Dóra Faragó's dissertation, who obtained her PhD on 12.12.2019 (https://doktori.hu/index.php?menuid=193&lang=HU&vid=20818).

2. Increased expression of AtSPQ protein can improve drought tolerance in *Brassica napus*

Previously we identified that overexpression of the Small Paraquat resistant protein (SPQ) from *Lepidium crassifolium* or *Arabidopsis thaliana* could enhance tolerance to paraquat and drought by hypersensitivity to abscisic acid (ABA) in overexpressing Arabidopsis plants. As Arabidopsis and rapeseed (*Brassica napus*) are closely related members of the Brassicaceae family, we tested the effect of AtSPQ on rapeseed stress tolerance. *Brassica napus* transformation was done using tissue culture techniques. The methods found in the publications available did not prove too effective, so we requested a protocol from the MATE Institute of Genetics and Biotechnology, which we modified slightly during the hypocotyl transformation. In addition, we developed a new type of selection system for the primary testing of the presumed transformants, which significantly helped to confirm the transformation in the T2 and T3 generations. These experiments resulted in several T1 seedlings which were achieved for further growth and molecular testing. Expression studies were carried out to determine transgene expression (AtSPQ and LcSPQ). We found T1 overexpression lines in both cases (Figure 2.1. A and B) but only for the AtSPQ was the level of expression sufficiently high in two lines (AtSPQ 1, AtSPQ 3) to work with further later. T2 generations were produced from the selected independent lines and further experiments were performed on 2-2 sublines. The copy number of the inserted transgene was determined, and putative homozygous lines were selected.

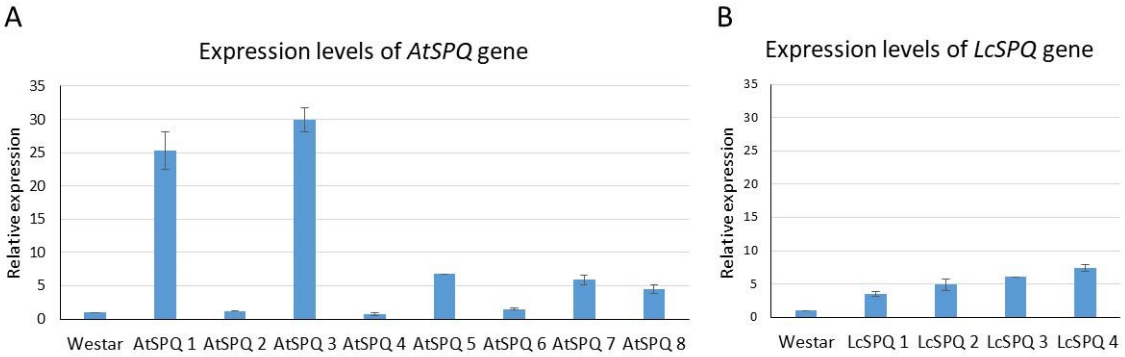


Figure 2.1. AtSPQ and LcSPQ overexpressing T1 lines
AtSPQ 1-8: pTCO272 35S::AtSPQ transformed T1 lines (A), LcSPQ 1-4: pTCO272 35S::LcSPQ transformed T1 lines (B), Westar: *Brassica napus* wild type, Westar cultivar

Based on our result that overexpression of AtSPQ in Arabidopsis causes paraquat resistance, we first examined root growth and hypocotyl elongation of T2 sublines in the presence of different concentrations of paraquat. To determine paraquat sensitivity, we used a special closed, but not sterile system that we developed. Based on examining the 2-2 sublines of two independent transformants, we measured that the AtSPQ overexpression lines have less paraquat-inhibited root growth and hypocotyl elongation than the wild type (Figure 2.2.). During these experiments, we observed that high concentration paraquat can cause hypocotyl damage (Figure 2.3. A). We measured the survival rate of the plants and the results were similar to that in the growth assays (Figure 2.3. B), the overexpression lines were more resistant to the treatments. These data are consistent with the results obtained for Arabidopsis SPQ overexpression lines.

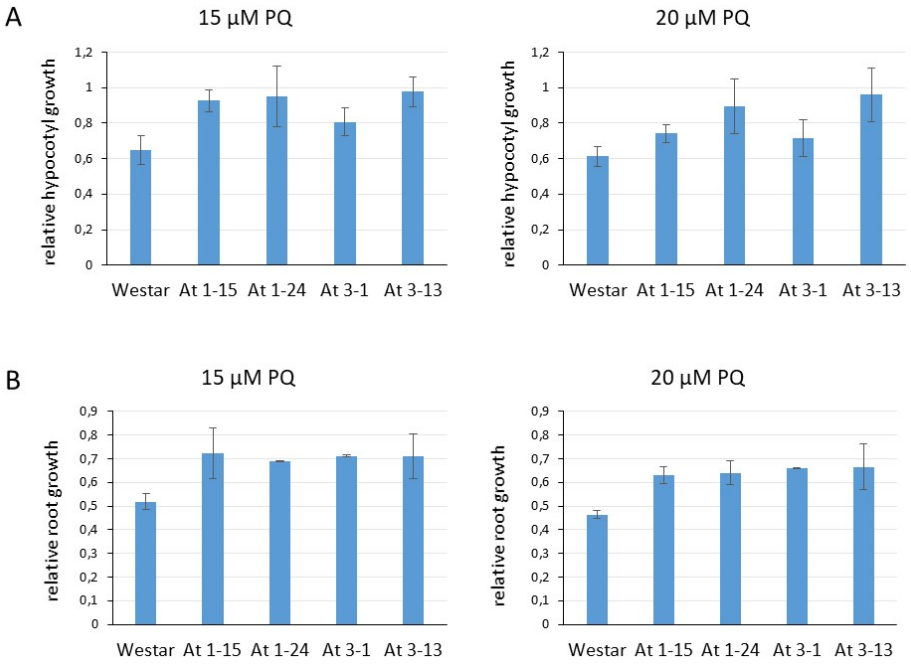


Figure 2.2. Effects of paraquat treatments on hypocotyl elongation and root growth. Relative hypocotyl (A) and root growth (B): Relative hypocotyl and root growth indicate growth change relative to non-treated. Westar: Brassica napus Westar cult., At 1-15: AtSPQ 1-15 T2 subline, At 1-24: AtSPQ 1-24 T2 subline, At 3-1: AtSPQ 3-1 T2 subline, At 3-13: AtSPQ 3-13 T2 subline.

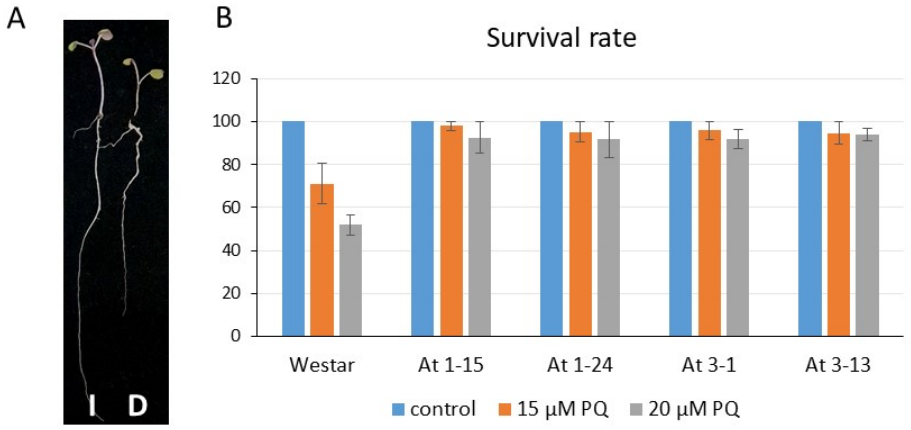


Figure 2.3. The survival rate of the AtSPQ overexpressing lines. I: intact, D: damaged plant (A); Survival rate: survived/all (B). Westar: Brassica napus Westar cult., At 1-15: AtSPQ 1-15 T2 subline, At 1-24: AtSPQ 1-24 T2 subline, At 3-1: AtSPQ 3-1 T2 subline, At 3-13: AtSPQ 3-13 T2 subline.

Plant phenotyping revealed that AtSPQ overexpressing Arabidopsis plants tolerated better water deprivation than wild type plants. We investigated the behavior of overexpressing rapeseeds with reduced water availability using our plant phenotyping platform. Drought assays were performed under a 16/8 hours light/dark cycle, and ~250 µmol m^{-2} s^{-1} photon flux density using 4-week-old plants. We applied two types of phenotyping systems, in one we tested the plants exposed to slow, continuous drying (24 days water deprivation), and in the other, we examined the consequences of rapid and intense drying (13 days water deprivation). No difference was found in the case of longer drying when the green area was measured (data not shown), neither for well-watered plants nor for water-deficient plants. In the case of rapid and intense drying, we found that the overexpressing lines were better than the wild types, their leaves kept their turgor longer (Figure 2.4.). Unfortunately, this system could not measure the leaf area of the plants due to the size of the rapeseed, but the leaf temperature was measured with an infrared camera during the drought period. We observed that from day 7 to day 9 after the last irrigation, the leaf surface temperature of the AtSPQ overexpressing lines was significantly lower compared to Westar plants (Figure 2.5. A and B), while no difference was found at the other stages of drying and well-watered plants.

Figure 2.4. The phenotype of AtSPQ overexpressing plants under water deficit conditions. Images of representative Westar wild type, AtSPQ 1-15 and AtSPQ 3-13 overexpressing plants, day 10 after last watering.

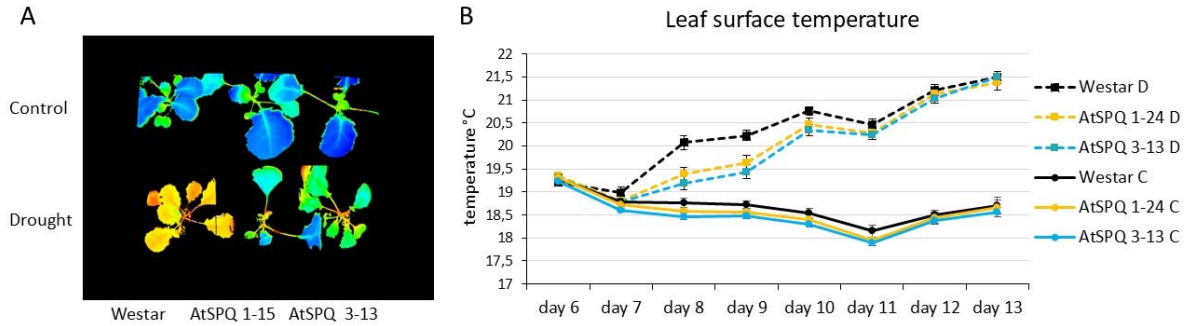


Figure 2.5. The temperature of the leaves measured by an infrared camera of the plant phenotyping system. Images of representative Westar wild type, AtSPQ 1-15 and AtSPQ 3-13 overexpressing plants, day 9 after last watering (A). The changing of the leaf surface temperature during the drought period from day 6 to day 13 after the last watering (B).

Drought influences many physiological parameters, and photosynthesis is one of the most sensitive metabolic responses it affects. The change can be monitored by measuring the Fv/Fm value using chlorophyll fluorescence detection. We measured the light-adapted Fv/Fm values through chlorophyll fluorescence change during the drought period. In well-watered conditions, Fv/Fm was not different between wild type and AtSPQ overexpressing plants.

Fv/Fm was reduced in all drought-treated plants but remained significantly higher in AtSPQ overexpressing plants than in wild types (Figure 2.6.).

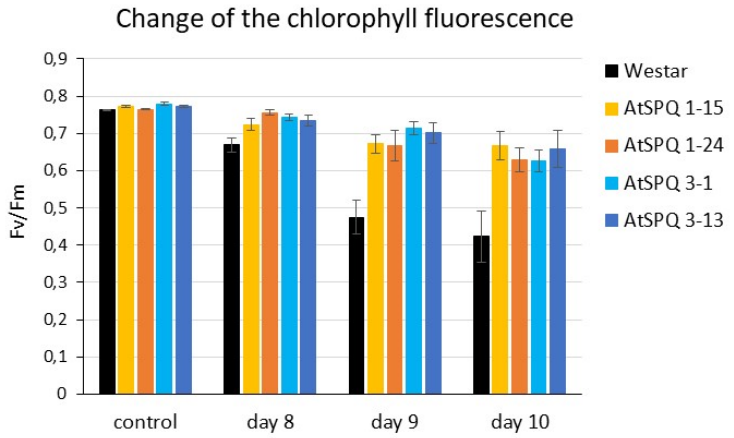


Figure 2.6. Change of the chlorophyll fluorescence (Fv/Fm parameter) in well-watered and drought-stressed plants; control: well-watered; day 8, day 9, and day 10 after the last watering. Fv/Fm values were measured on 250 µmol m^{-2} s^{-1} photon flux density.

Our plant phenotyping experiments revealed that AtSPQ overexpressing rapeseed tolerated better water deprivation than wild types. Water retention improved, plants wilted less, and photosynthetic activity was more stable under water deprivation.

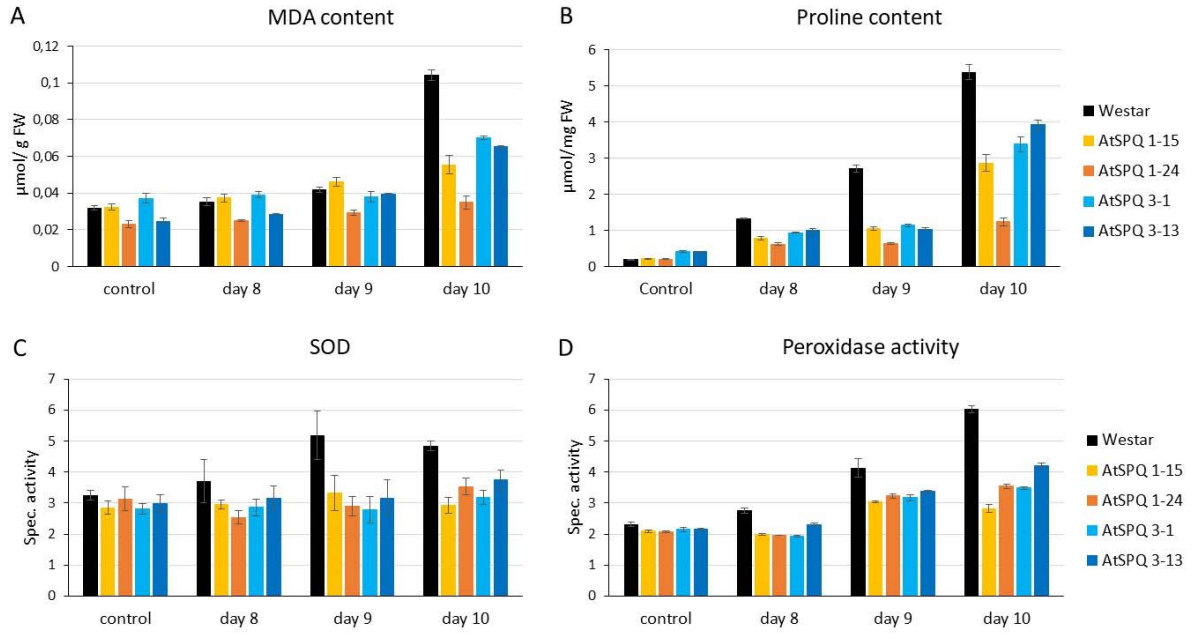


Figure 2.7. Oxidative responses and proline accumulation under drought. Control: well-watered, day 8, day 9, and day 10 after the last watering. Lipid peroxidation level (MDA content) of Westar and AtSPQ overexpressing plants (A). Proline content of Westar and AtSPQ overexpressing plants (B). Superoxide dismutase (SOD) and peroxidase activity of Westar and AtSPQ overexpressing plants (C, D).

Drought stress increases ROS accumulation, alters the activity of antioxidant enzymes, and can lead to proline accumulation. We measured the lipid peroxidation level (MDA content) under drought stress and we found that the MDA accumulation was lower in the AtSPQ lines compared to the wild type rapeseeds (Figure 2.7. A). The proline accumulation was inferior in the AtSPQ overexpressing lines during the water shortage period (Figure 2.7. B). The superoxide dismutase (SOD) and peroxidase activity were also measured, and similar to our

previous results, these were either not or less elevated in the overexpression lines when significant increase was observed in the wild type (Figure 2.7. C and D).
In addition, we performed transcript analysis to test the expression of several stress-induced genes (NCED3, P5CS1, RD29A). For the three genes tested, we found that while their expression was similar under control conditions, drought stress significantly increased their expression levels in the wild type compared to AtSPQ lines.

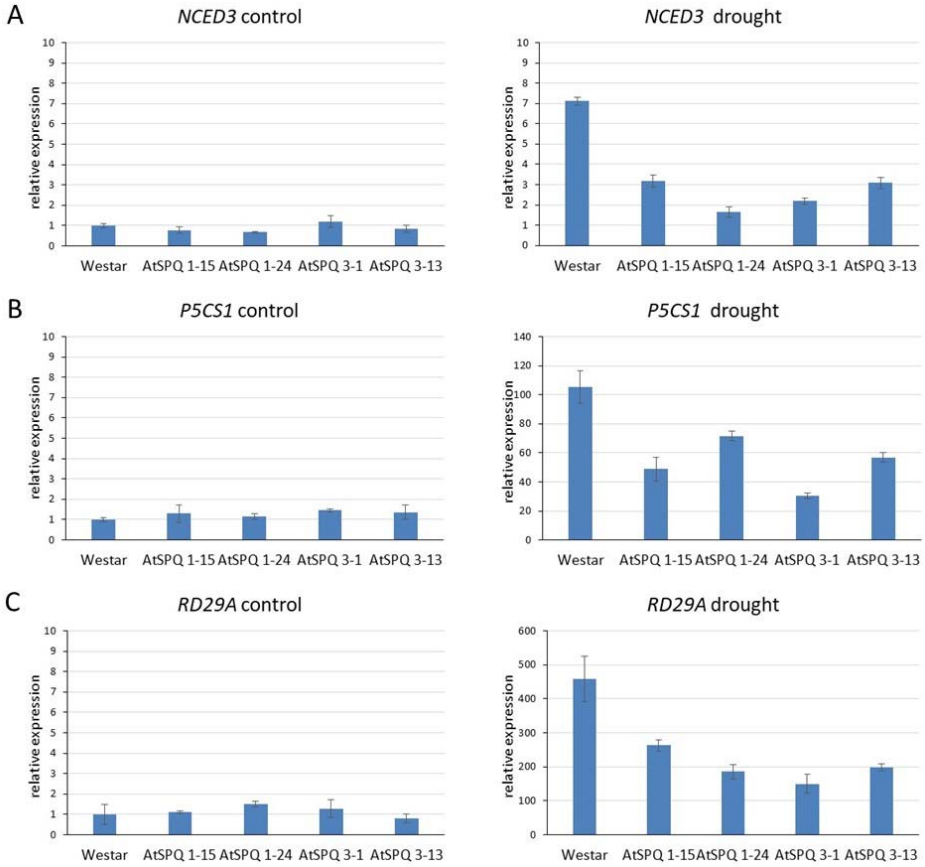


Figure 2.8. Expression of stress-induced genes in drought-treated and control plants. Control: well-watered, drought: 9 days after the last watering.

Summarizing our results, we found that overexpression of AtSPQ in rapeseed enhanced paraquat tolerance as measured in root elongation and hypocotyl length assays. Plant phenotyping revealed that AtSPQ overexpressing rapeseed tolerated better water deprivation than wild type plants. The photosynthetic activity improved, and oxidative damage and proline accumulation were reduced. The expression data of stress-induced genes also supported these observations. We conclude that the overexpressing of AtSPQ also improves the drought tolerance of Brassica napus similar to Arabidopsis plants.

We present our results at the 16th International Conference on Reactive Oxygen and Nitrogen Species in Plants (POG 2024) on 29-31 May 2024, and the manuscript is being prepared.

3. Mutation in Arabidopsis PPR40 gene affects tolerance to water deficit

The *Arabidopsis thaliana* PPR40 identified in our previous research can regulate mitochondrial electron transport and contribute to stress responses (Zsigmond et al., 2008, doi: 10.1104/pp.107.111260). The knockout *ppr40-1* and the knockdown *ppr40-2* mutant exhibited different degrees of hypersensitivity to ABA in germination assays. In addition, the enhanced ABA sensitivity in *ppr40-1* led to faster stomata closure. Using our previous results, we compared the survival rates of the *ppr40* mutant to wild type plants after 13 days of water deprivation, where the *ppr40-1* mutant showed significantly higher recovery rate (Figure 3.1.) Additionally, the *ppr40-2* mutant displayed an intermediate survival rate, while plants overexpressing PPR40 showed recovery similar to wild type. These results highlight the role of PPR40 in ABA responses and plant survival under drought conditions.

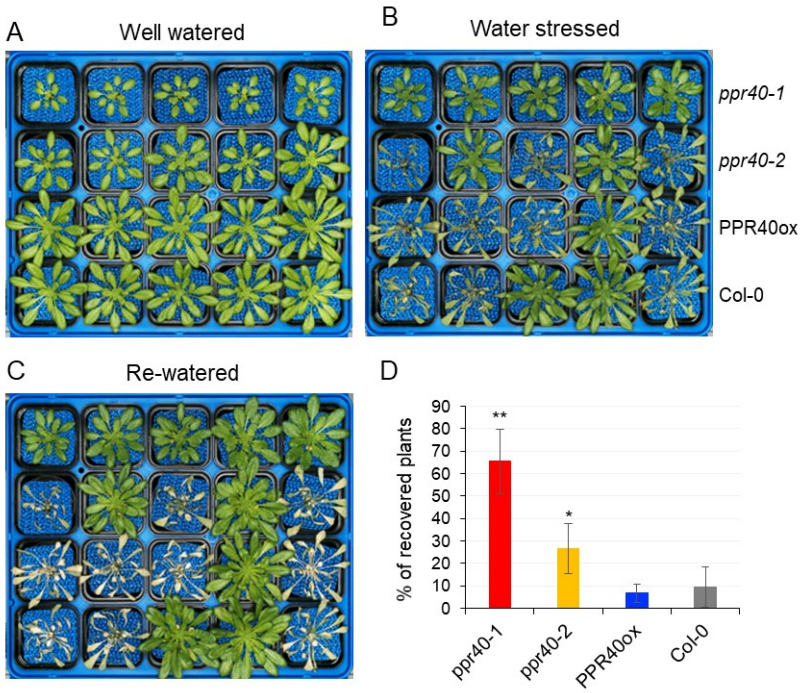


Figure 3.1. Survival of wild type, PPR40 overexpressing plants, and *ppr40* mutants after rewatering. Plants grew in the plant phenotyping system. 4-week-old plants were deprived of water for 13 days and subsequently re-watered. Representative images of each genotype grown in a phenotyping tray (A, B, C). Plants before watering were stopped (day 0, A). Plants after 12 days of water stress (B). Plants 6 days after re-watering (C). Survival rates of drought-treated plants in a typical experiment (D). The percentage (%) of recovered plants is shown.

The growth and morphology of the well-watered and water-stressed plants were compared using the automatic plant phenotyping platform. Rosette size and morphological parameters were estimated from RGB images. Under control conditions, *ppr40-1* and *ppr40-2* plants were substantially smaller than the wild type, confirming their semidwarf growth phenotype. Water stress severely inhibited rosette growth and the green area of wild-type plants showed a rapid decline after day 9 of water withdrawal (Figure 3.2. A). Conversely, the *ppr40* mutants showed delayed declines in the green area, indicating sustained viability under water-limited conditions. Changes in the slenderness of leaf (SOL) were slower in *ppr40* mutants compared to wild type (Figure 3.2. B), indicating better preservation of plant morphology during drought stress.

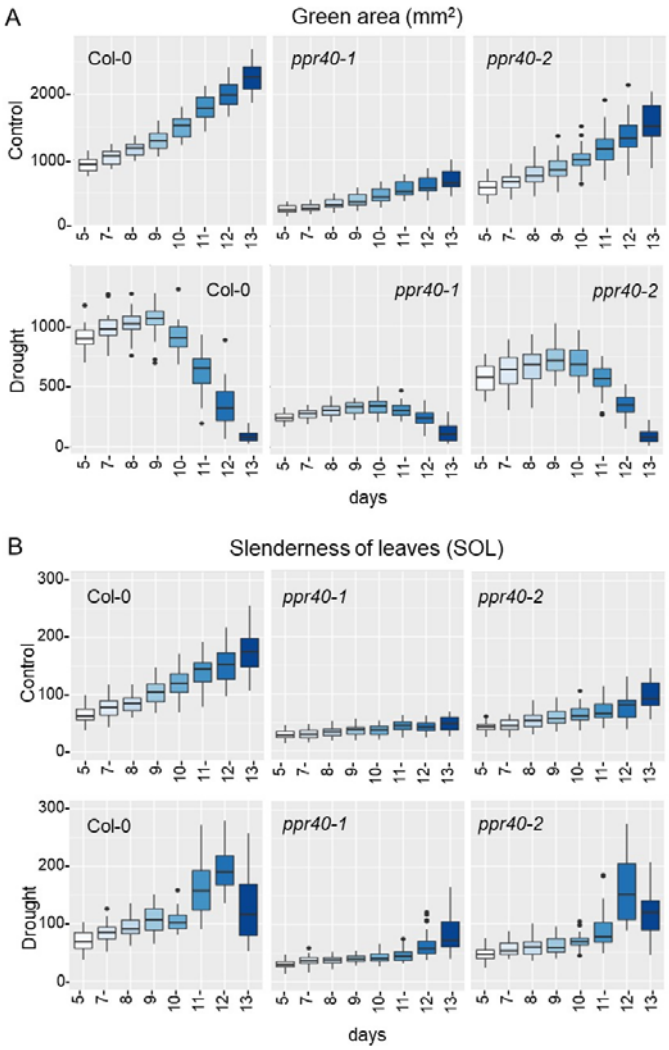


Figure 3.2. Phenotyping alteration in plant growth and development under drought. Change of rosette size as displayed by green area (A). Change of plant morphology as displayed by slenderness of leaves (SOL) parameter (B). Control: uninterrupted watering; Drought: water withdrawal. Days: number of days after the last watering.

Enhanced ABA sensitivity in plants can result in faster stomatal closure to support water preservation during drought. Stomatal conductance, indicative of gas exchange and transpiration, was compared between water-stressed wild type and mutant plants. We observed lower stomatal conductance in the *ppr40-1* mutant even in well-watered conditions and up to three days after finishing the watering (Figure 3.3. A). Although initially higher in wild type plants, stomatal conductance dropped faster under water-restricted conditions than the *ppr40-1* mutant. Severe water deprivation led to a significant reduction in stomatal conductance for both genotypes. Relative water content (RWC) measurements revealed similar levels in well-watered plants but a substantial reduction under water stress, particularly in wild type plants (Figure 3.3. B). The *ppr40-1* mutant retained more water under stress than the wild type, correlating with slower wilting and higher viability.

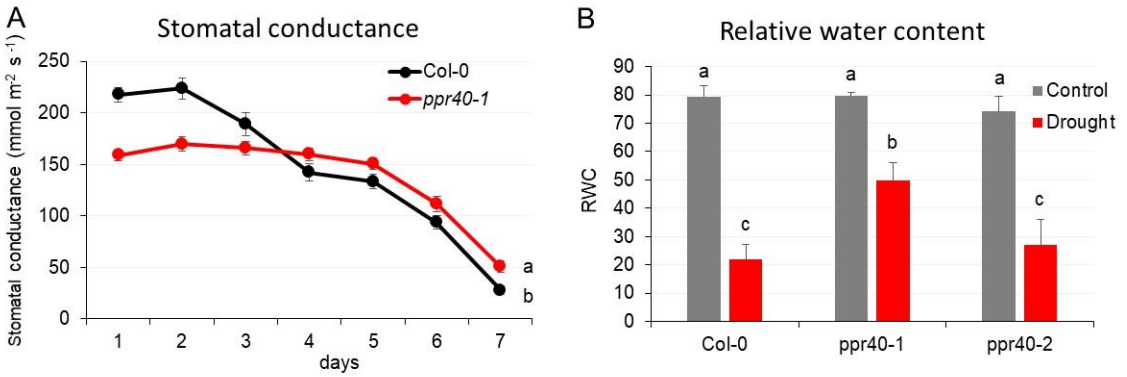


Figure 3.3. Effects of drought stress on stomatal conductance and relative water content (RWC) in Arabidopsis mutants. Change of stomatal conductance of Col-0 wild type and *ppr40-1* mutant plants (A). 4-week-old plants were used. During water withdrawal, stomatal conductance was measured at daily intervals from the first day.RWC in Arabidopsis *ppr40-1* and *ppr40-2* mutants and Col-0 wild-type plants stressed by water deprivation for 10 days (B).

Reactive oxygen species (ROS) generation is a common response to environmental stresses in plants. Elevated ROS levels lead to oxidative stress, resulting in the peroxidation of macromolecules and lipids. To supplement the growth and survival data under drought stress, we measured the lipid peroxidation level (MDA content). Under well-watered conditions and early in the drying period (day 7), no difference in MDA levels was observed in the plants (Figure 3.4. A). On days 8 and 9, the *ppr40-1* mutant had lower MDA levels than the wild type and the *ppr40-2* mutant.

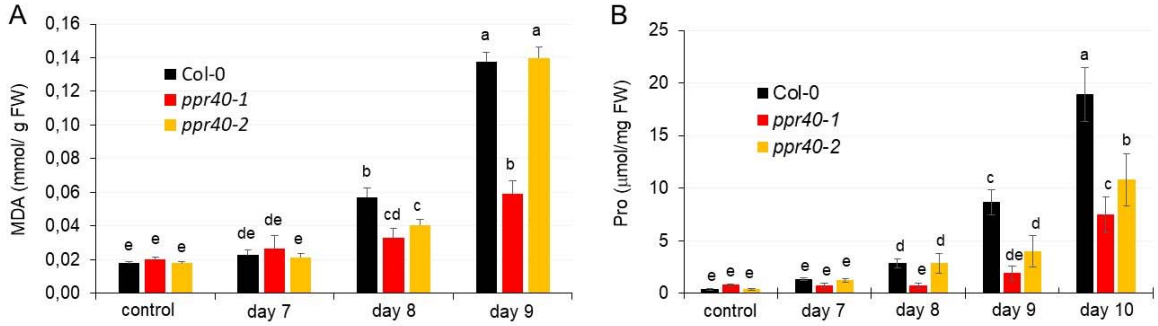


Figure 3.4. Oxidative response during drought. Time scale: number of days after the last watering. Lipid peroxidation (MDA content) rates of Col-0 wild type and *ppr40* mutant plants in well-watered and water-stressed conditions (A). Change in proline content of wild type and mutant plants stressed by water withdrawal for up to 10 days (B).

Proline accumulation is a well-known physiological response to water deprivation in plants and is related to defenses during oxidative stress. The proline content of *ppr40-1* was slightly higher in well-watered conditions, while *ppr40-2* was similar to the wild type (Figure 3.4. B). Proline levels in wild type and *ppr40-2* increased after 7 days without water but remained stable in *ppr40-1* until day 8. By day 10, proline content in *ppr40-1* and *ppr40-2* mutants was lower than wild type. These results indicate significantly lower drought-induced oxidative damage in *ppr40-1* than in *ppr40-2* and wild-type plants.

Plant mitochondria are crucial for energy supply and metabolic pathways through respiration. We performed a metabolomic survey focusing on the TCA cycle, sugars, and amino acids to understand the effect of the *ppr40-1* mutation on mitochondrial metabolism.

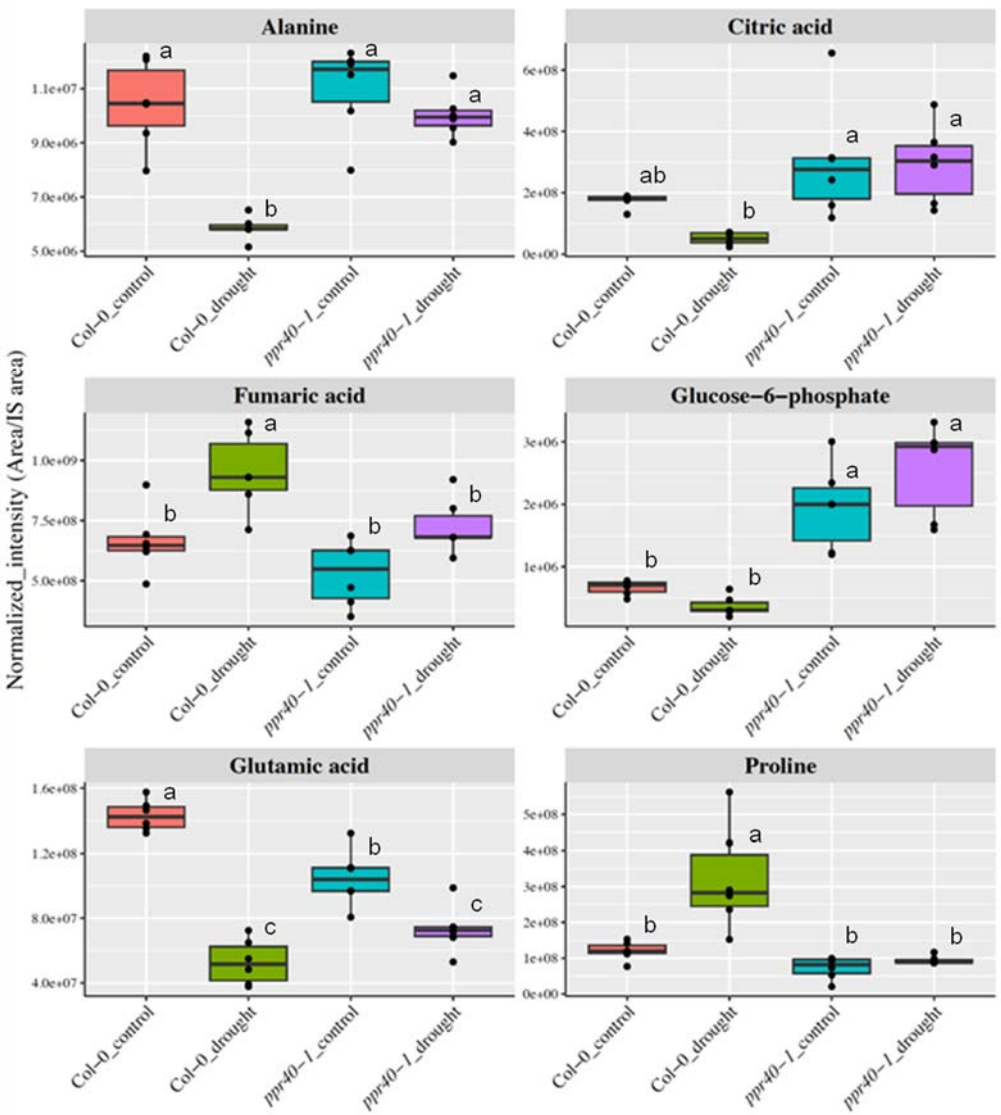


Figure 3.5. Metabolic profiles of six selected metabolites in Col-0 wild type and *ppr40-1* mutant plants in well-watered (control) and water-restricted (drought) environments.

Water deprivation caused significant alterations in the TCA cycle and amino acid concentrations, with *ppr40-1* showing less impact (Figure 3.5.). Proline accumulation was reduced in *ppr40-1* under drought stress, while citric acid and glucose-6-phosphate levels were elevated in *ppr40-1* compared to wild type plants. These results highlight alterations in various metabolic pathways, including the mitochondrial TCA cycle, in the *ppr40-1* mutant.
Photosynthesis can respond sensitively to drought stress. Therefore, we performed chlorophyll fluorescence analysis to monitor photosynthesis in wild type and mutant plants under standard and water-deprived conditions. The Fv/Fm values remained stable in wild type plants until day 10 of water deprivation, after that they declined sharply (Figure 3.6.). In contrast, the *ppr40-1* mutant showed more stable Fv/Fm values, declining later and remaining around 0.77 on day 13. The *ppr40-2* mutant exhibited intermediate Fv/Fm kinetics, dropping faster than *ppr40-1* but remaining more stable than the wild type. Differences in Fv/Fm changes indicated that the photosynthesis remained functional for more extended periods in the drought-stressed *ppr40* mutants than in wild type plants.

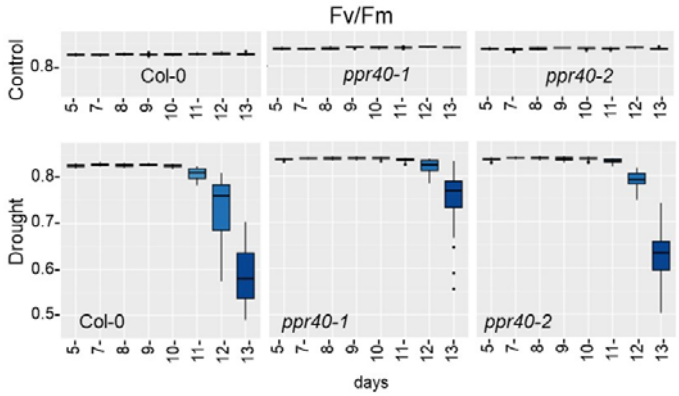


Figure 3.6. Change of the Fv/Fm value of photosystem II in well-watered and drought-treated plants. Control: uninterrupted watering; Drought: water withdrawal. Days: number of days after the last watering.

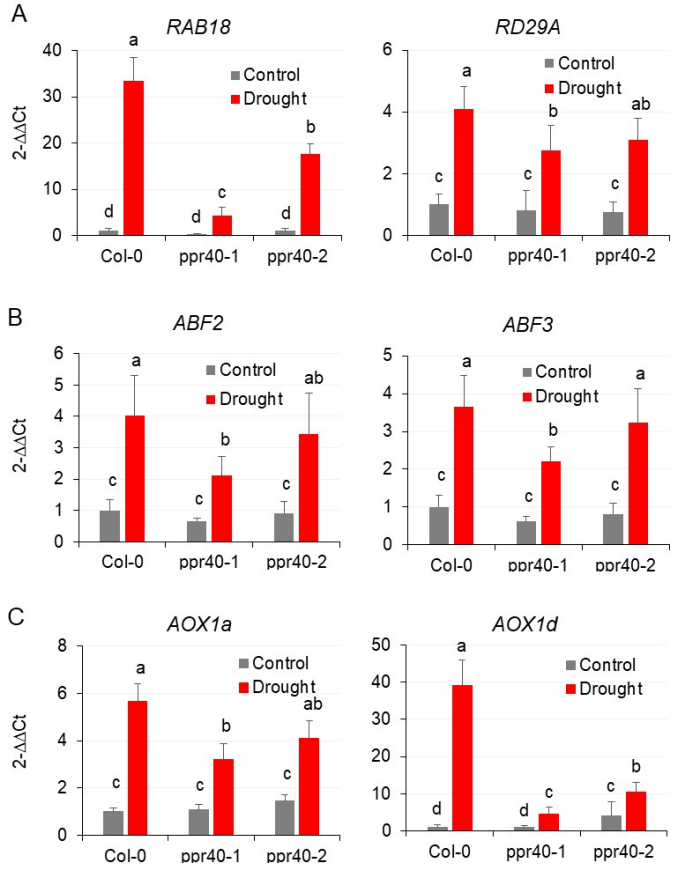


Figure 3.7. Expression of selected stress-induced genes. Transcript levels of *RAB18, RD29A* (A), *ABF2, ABF3* (B), and *AOX1a, AOX1d* (C) genes were measured by qRT-PCR. Relative expression levels are shown, with a value of 1 corresponding to transcript levels of Col-0 wild type in control plants.

Water deprivation induces significant changes in gene expression profiles, with numerous genes being either up or downregulated in dehydrated plants. ABA, a crucial stress hormone, promotes rapid physiological responses like stomata closure and induces the expression of dehydration-responsive genes. Transcript levels of target genes, including *RAB18* and *RD29A*, were monitored in drought-stressed plants, revealing significant differences between wild type and *ppr40* mutants. Expression of ABA-responsive transcription factors *ABF2* and *ABF3* increased in response to water stress, with *ppr40* mutants showing intermediate induction compared to wild type plants. Additionally, transcript levels of stress-responsive *AOX* genes,

particularly *AOX1d*, were less activated in *ppr40* mutants than in wild type plants, indicating alterations in mitochondrial functions in response to drought stress.

As a summary of these results, we published our data supplemented with more detailed characterizations in PLANTA in March 2024 (doi: 10.1007/s00425-024-04354-w).
The above results are a defining part of Kamal Kant's dissertation, who is defending his PhD thesis on 10.05.2024.

4. Targeted mutagenesis with genome editing in *Brassica napus*

Using our research results of *Arabidopsis thaliana ppr40* mutants (see above, Kamal et al. 2024, doi: 10.1007/s00425-024-04354-w) to study the effect on drought tolerance we applied the CRISPR/Cas9 genome editing technology in *Brassica napus*. We first identified the possible homologous region to design CRISPR primers for the Arabidopsis PPR40 (*At3g16890*) gene homolog in rapeseed. The *Brassica napus* is not a traditional diploid it is an amphidiploid (or allotetraploid) plant. In *Brassica napus,* the Arabidopsis *PPR40* homologs encode in two chromosomes: Bna_A01 (*BnaA01g27520D*), and Bna_C01 (*BnaC01g44090D*). We designed primer pairs, which could edit both genes. For CRISPR primer design we used the first 500 bp sequences upward from the ATG start codon. To minimize the off-target effect, we used a well-referenced online CRISPR primer designing software: https://crispr.dbcls.jp/ As we got the results, we chose one primer pair: located 288-310 bp region (Figure 4.2. A). The planned modification targeted amino acids 96-103 upstream of the PPR domains (Figure 4.1.) which could be similar to the Arabidopsis *ppr40-1* mutation. After the synthesis, we cloned the annealed primer pairs into the pGGH7_AtCas9 vector. To initiate genome editing of rapeseed, we transformed wild type rapeseed plants with the constructed pGGH7-AtCas9-PPR40 vector.

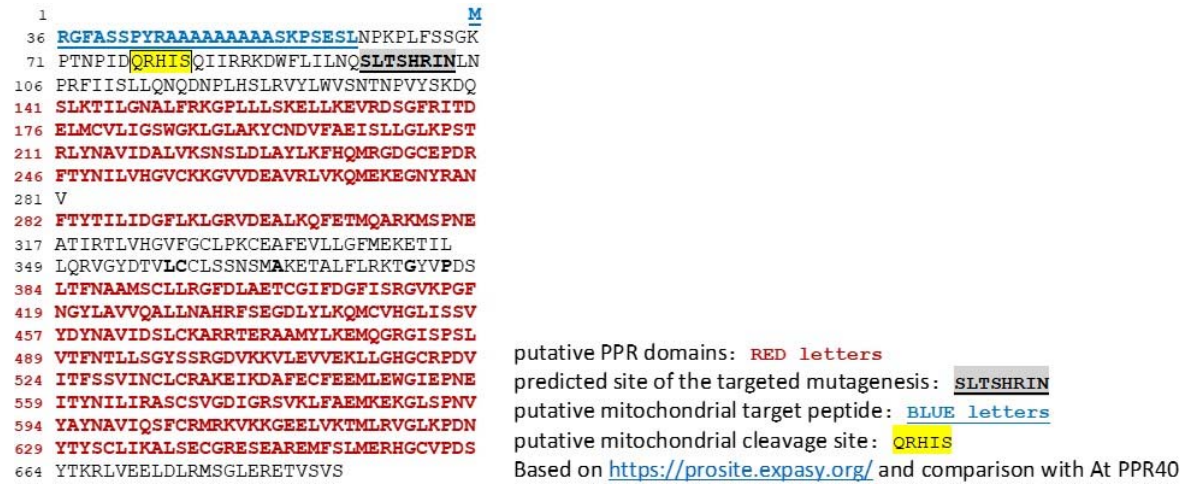


Figure 4.1. Editing site of the *Brassica napus* putative pentatricopeptide repeat-containing protein. Possible protein structure of BnaC01g44090D.

We had problems with the *in-vitro* tissue culture-based transformation, as only a few kanamycin and hygromycin-resistant shoots could be regenerated in the selection medium. To obtain a higher probability of putative transformant rapeseeds, we introduced a different type of transformation technique. We optimized an in-planta Agrobacterium-mediated method for *Brassica napus* transformation without the need for *in-vitro* plant regeneration. We initiated experiments that are based on our experience with Arabidopsis flower-infiltration technology

with combining bud-injection. As a new technology, we adapted methods based on flower bud infiltration, which was originally developed for *Solanum lycopersicum* and *Brassica campestris* (Sharada et al. 2017, doi: 10.1007/s11240-017-1178-7; Yan et al. 2004, Agricultural Sciences in China, CBA: 441784). For the bud injection, we used approx. 3 mm buds which were still completely closed. The concentration of the Agrobacterium solution was OD_{600}: 0.3-0.4. The injection medium contained: 5% sucrose, 0.004% Silvet L-77, and 100 µM acetosyringone. Agrobacterium was injected directly into the buds with a syringe, and then the plants were covered overnight. The siliques that developed from the injected buds were collected, and the seeds were selected on antibiotic-containing medium (Figure 4.2. B). The experiments resulted in several hygromycin-resistant seedlings which were planted into the greenhouse for further growth and molecular testing. Progenies of these plants were tested for T-DNA insertion and transgene expression with PCR and qPCR, respectively. We found that only a moderate number of the originally selected antibiotic-resistant plants can carry the T-DNA insert. We identified 10 independent T1 transformed lines whose T2 progeny also carried the T-DNA insert. We had started the detailed characterization of the T2 lines to identify the presence of genome editing in both *PPR40* genes of *Brassica napus*. The involvement of chromosomes A and C was tested separately by PCR (Figure 4.2 A, C) and sequencing reactions. Based on preliminary results we found a deletion in the case of chromosome C, but there had not been a line where both chromosomes (A and C) were affected. We also sequenced the affected region of 42 lines (both A and C chromosomes) but the result was not clear in all cases. In order to clarify these results, we propagated a T3 generation from the T2 lines in question, which are examined by sequencing.

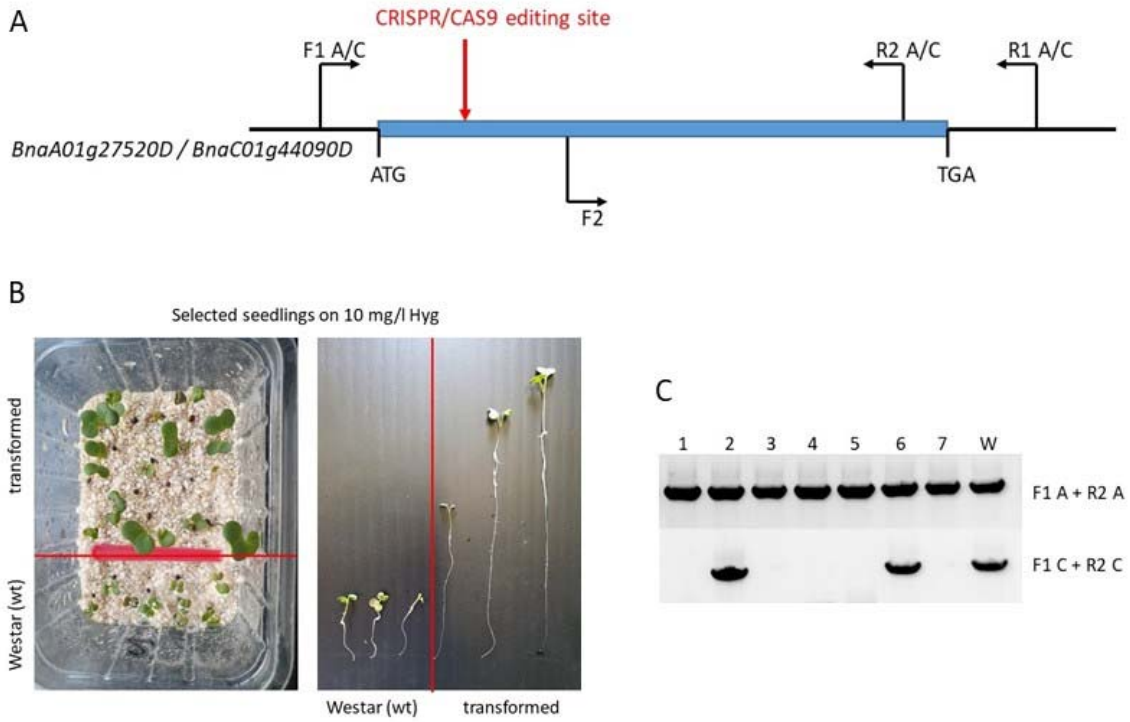


Figure 4.2. Genom editing in *Brassica napus*. A) Schematic map of the genome editing site and the location of primers used for identification. B) Antibiotic selection of transformed seedlings. C) PRC reactions using chromosome-specific primers. 1-7: T2 sublines of transformed; W: *Brassica napus* Westar cultivar (wild type); F1A, R2A: A01 chromosome-specific primers; F1C, R2C: C01 chromosome-specific primers.

5. Effects of heat and combined stress on *hsfa* mutants

The heat shock factor A4A (HSFA4A) is a positive regulator of ROS signaling and promotes stress tolerance (Perez-Salamo et al. 2014, doi: 10.1104/pp.114.237891). HSFA5 (heat shock factor A5) was reported to interact with HSFA4A and functions as a repressor of it (Baniwal et al., 2007, doi: 10.1074/jbc.M609545200). We assumed that the knockout *hsfa5* mutant would be expected to have enhanced HSFA4A-dependent regulation. Therefore, we crossed *hsfa5* with the *hsfa4a* mutant to study their genetic interaction. Homozygous *hsfa4axhsfa5* lines were generated to compare their tolerance to single mutants. To analyze the responses to salt, osmotic, and oxidative stresses of these mutants, we first tested the germination rate and root growth ability using in vitro assays. Unfortunately, we found no difference in these parameters, both single and double mutants showed a phenotype similar to the wild type (data not shown). We also performed *in vitro* analyses using PlantSize software (developed earlier in our group, Faragó et al., 2018, doi: 10.3389/fpls.2018.00219) under different stress conditions (100 and 125 mM NaCl, 200 and 300 mM sorbitol, 0.3 µM paraquat, 32 °C, and combined stresses). We analyzed various physiological parameters of the mutant lines (*hsfa4a, hsfa5, hsfa4axhsfa5)*: rosette size, roundness, convex size and ratio, growth rate, chlorophyll and anthocyanin content, and fresh weight. In the case of 100 mM NaCl + heat stress, we have observed a slightly increased stress tolerance in the *hsfa4a*hsfa5* double mutant compared to the wild type and single mutants (Figure 4.1.). No differences were observed for all other treatments (data not shown).

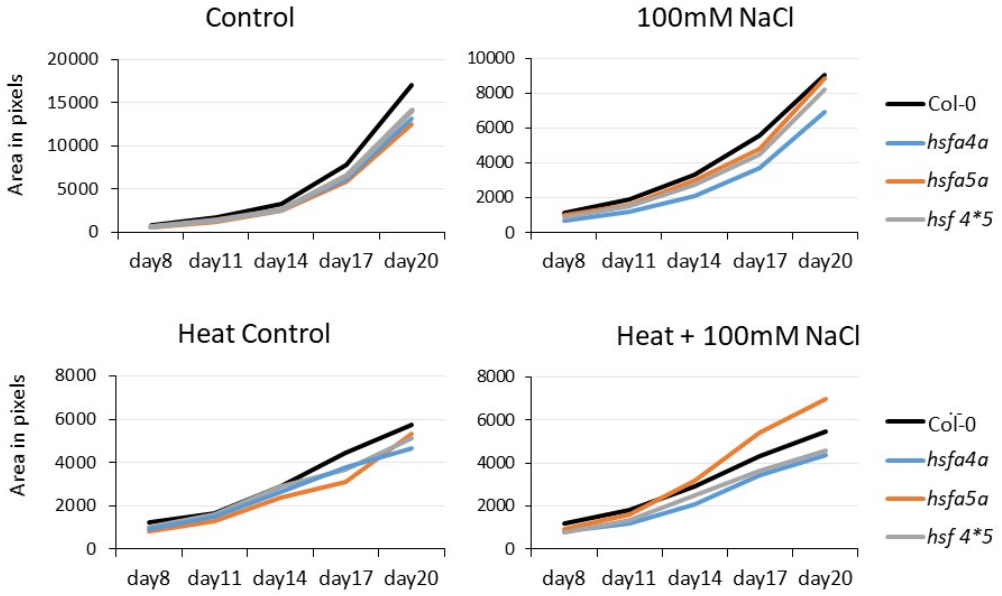


Figure 4.1. Growth of *hsfa4a*, *hsa5*, and *hsfa4a*hsfa5* (*hsf 4* 5*)mutant plants. Seeds were germinated on standard culture medium with 22 °C in light, and 18 °C in dark period (8/16 hours light/dark cycle) and 7-day-old seedlings were used for various stress treatments. We monitored the growth for 12 days (day 8 - day 20). Control: standard culture medium in 22/18 °C; 100 mM NaCl: standard culture medium supplemented with 100 mM NaCl in 22/18 °C; Heat control: standard culture medium in 32/28 °C; Heat + 100 mM NaCl: standard culture medium supplemented with 100 mM NaCl in 32/28 °C.

Moreover, we tested the *hsfa* single and double mutants' survival capability using *in vitro* assays. For the survival test, the *hsfa4a, hsfa5, hsfa4a*hsfa5,* and wild type plants were grown on standard culture medium for 10 days after germination at 22 °C in light and 18 °C in dark. 10-day-old seedlings were transferred onto the liquid standard culture medium. 150 mM NaCl was added for salt stress. For the heat stress, the plates were kept in a chamber at 37 °C in light and 30 °C in dark. After 2 days, 4 days, and 6 days of stress treatments, the seedlings were

transferred onto the solid standard culture medium. Survival rate and fresh weights were calculated after a 10-day recovery period (Figure 4.2.). We observed a slightly increased survival rate and lower fresh weight loss in *hsfa4a*hsfa5* plants compared to the wild type under combined stress after 4 and 6 days of treatment.

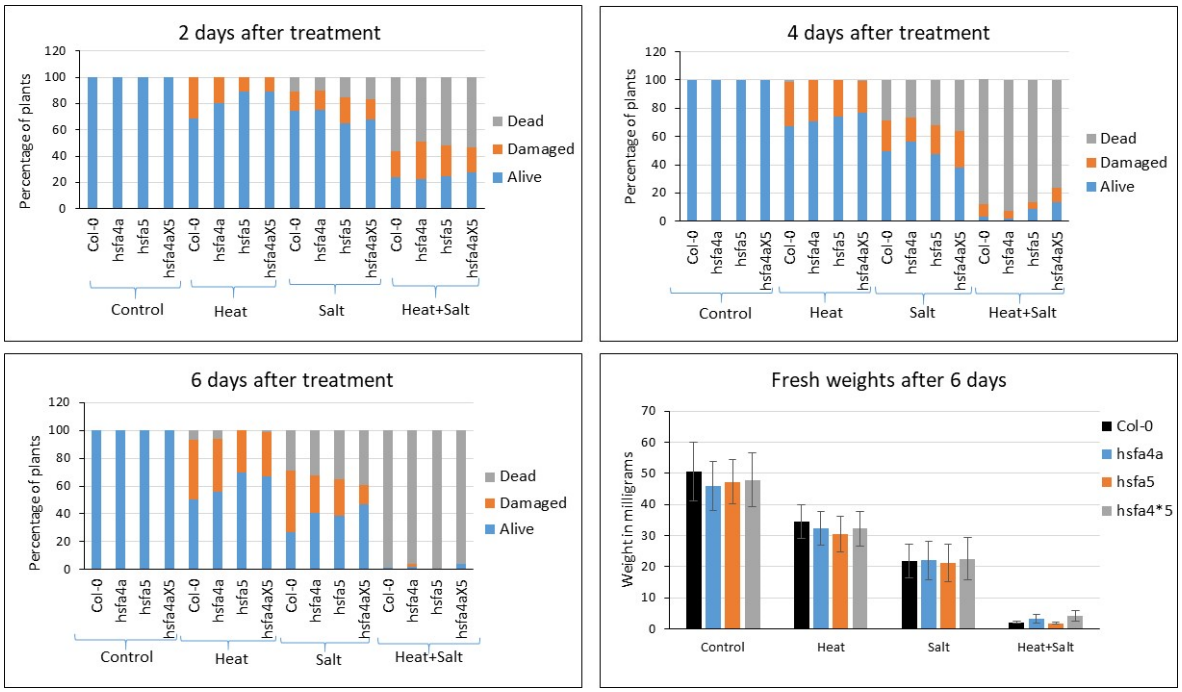


Figure 4.2. Survival rates and fresh weights of *hsfa4a*, *hsfa5*, *hsfa4a*hfsa5* (*hsfa4*5*) and wild type (Col-0) plants. Control: liquid medium without treatment at 22°C in light, 18°C in dark. Salt: 150 mM NaCl. Heat: 37 °C in light, 30 °C in dark. Rates of surviving healthy (vigorous growth with several new green leaves), damaged (small plants with retarded growth and/or chlorotic leaves) and dead plants (completely chlorotic with no green leaves) were scored 10 days after recovery. Fresh weights were measured after 6 days of single and combined stress treatments and a 10-day recovery period.

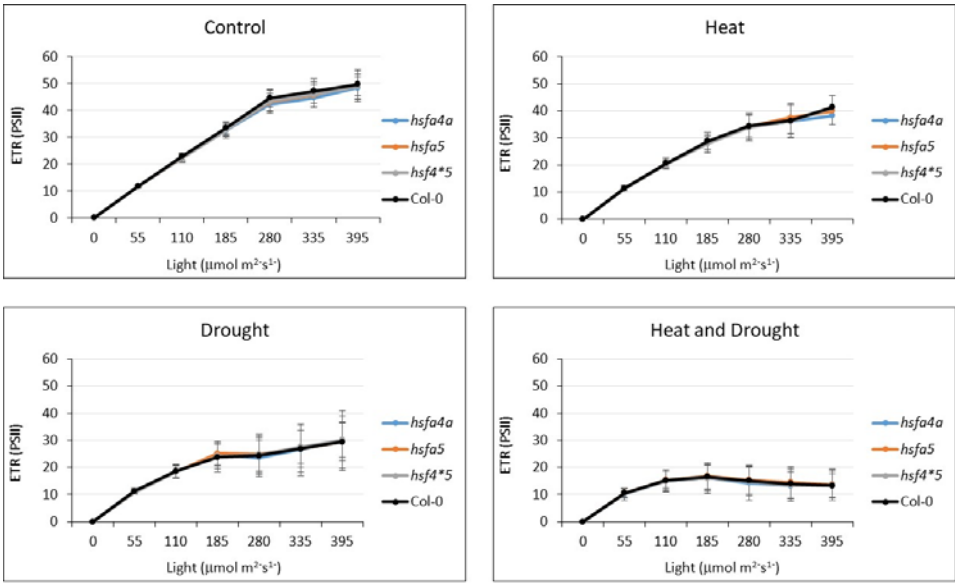


Figure 4.3. Change of the chlorophyll fluorescence (ETR) in well-watered, heat, drought, and combined stressed *hsfa4a*, *hsfa5*, *hsfa4a*hfsa5* (*hsfa4*5*) and wild type (Col-0) plants. Control: well-watered; heat: 37 °C/ 32 °C; drought: 12 days of water deprivation. ETR values were measured on different light intensities.

To test the drought, heat, and drought+heat combined stress tolerance of double and single mutant lines we performed a rapid stress assay (drought: 12 days water deprivation, heat: 12 days 37 °C in light, 32 °C in dark) measuring the growth parameters and change of the chlorophyll fluorescence values (ETR – electron transport rate of PSII). Moreover, we also measured the survival rates of *hsfa4a, hsfa5, and hsfa4a*hsfa5* mutants after 14 days of stress treatments. Regarding growth parameters (data not shown) and chlorophyll fluorescence changes, we observed no difference in mutant lines compared to the wild type under drought, heat, and drought+heat combined stresses (Figure 4.3.). When examining the survival rate, we found that the *hsfa4a* and *hsfa4a*hsfa5* showed increased survival rates than the wild type under the drought and drought+heat combined stresses (Figure 4.4.).

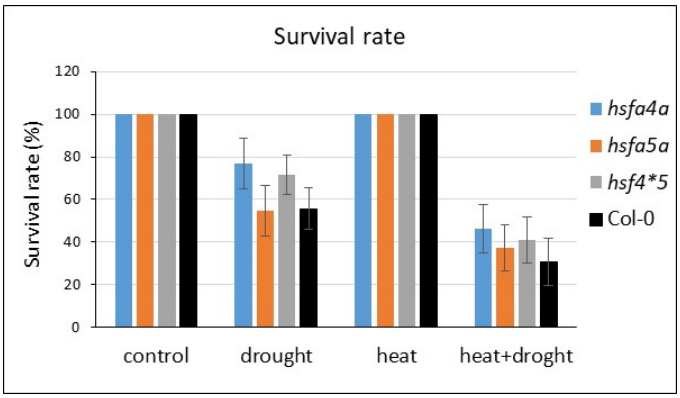


Figure 4.4. Survival rate of *hsfa4a*, *hsfa5*, *hsfa4a*hfsa5* (*hsfa4*5*) and wild type (Col-0) plants. . Control: well-watered; heat: 37 °C/ 32 °C; drought: 14 days water deprivation.

Rank	Uniq Pep	Acc #	Gene	AGI	Protein MW	Protein Name
[5]		O49403	HSFA4A	AT4G18880	46245	Heat stress transcription factor A-4a
[5-1]	18	Q94BZ5	HSFA5	AT4G13980	52355,5	Heat stress transcription factor A-5
[673]		Q9SSD2	PRP8/EMB14/SUS2	AT1G80070	275429,3	Pre-mRNA-processing-splicing factor 8
[427]		Q9FJZ7	TIM-barrel	AT5G66420	80719,9	TIM-barrel signal transduction protein
[1510]		A0A178UYN6	HSFA5	AT4G13980	37416,9	Heat stress transcription factor A-5
[1085-1	8	Q9FNL5	GBPL3	AT5G46070	120326,4	Guanylate binding protein-like 3
[825-1]	13	Q9FNX5	DL1E/DRP1E/EDR3	AT3G60190	69804,4	Dynamin-related protein 1E, ENHANCED DISEASE RESISTANCE 3
[5-3]	4	Q9FK72	HSFA4C/RHA1	AT5G45710	39648,1	Heat stress transcription factor A-4c, ROOT HANDEDNESS 1
[1125]		Q9CAF6	GYRA	AT3G10690	104538,7	DNA gyrase subunit A, DNA GYRASE A,
[193-1]	1	Q9LNJ1	REC1	AT1G01320	163814,6	REDUCED CHLOROPLAST COVERAGE 1
[477]		Q9LVA0	BAG7	AT5G62390	51567,8	BAG family molecular chaperone regulator 7, BCL-2-ASSOCIATED ATHANOGENE 7
[314-6]	7	O65570	VLN4	AT4G30160	109328,3	Villin-4
[491-1]	15	Q5XEP2	HOP2	AT1G62740	64520,2	Hsp70-Hsp90 organizing protein 2
[493]		A0A178UIJ0	MYOSIN XI K	AT5G20490	172554,9	MYOSIN XI K, MYOSIN XI-17, XIK
[1385]		Q9C865	SH3P1	AT1G31440	49258,5	SH3 domain-containing protein 1
[243]		*Q9LSK7*	*HON4*	*AT3G18035*	*51524,9*	*A linker histone like protein/winged-helix DNA-binding transcription factor family protein*
[1419]		*Q8VZS7*	*AT4G18070*	*AT4G18070*	*27778,2*	*suppressor;*
[619]		*Q8LE50*	*CC1-like*	*AT2G16940*	*63012,3*	*Putative splicing factor, Splicing factor, CC1-like protein*
[9-1]	*11*	*Q9C7V7*	*myosin*	*AT1G64330*	*64495*	*myosin heavy chain-like protein*
[563]		O23629	HTB9	AT3G45980	16436,5	Histone H2B.6, H2B
[306]		P59259	H4	AT1G07660	11409,5	Histone H4, Histone superfamily protein
[563-3]	1	Q0WS50	H2B	AT2G37470	15081,9	Histone H2B.4
[303]		Q93YR3	HIP1/TPR11	AT4G22670	46621,8	HSP70-INTERACTING PROTEIN 1, TETRATRICOPEPTIDE REPEAT 11
[21-5]	14	P29515	TBB7/TUB7	AT2G29550	50747,5	Tubulin beta-7 chain
[21-8]	8	A0A178UKM5	TUB8	AT5G23860	50607,4	Tubulin beta-8 chain
[129-8,	4	F4JVB9	GRP8, CCR1,	AT4G39260	10863,8	Cold, circadian rhythm, and RNA binding 1
[247-2]	15	A0A178UWB3	PAB2	AT4G34110	68673	Polyadenylate-binding protein

Table 4.1. Possible interacting partners of HSFA4A protein

To find possible new interacting partners for the HSFa4a, immunoprecipitation experiments were performed using the previously established HSFA4A-YFP fusion protein. Several possible interacting partners were detected by MALDI-TOF mass spectrometry analysis available at our research institute (Table 4.1.) Bimolecular fluorescence complementation

(BiFC) assays were used to verify possible interactions by applying protoplast-based and *Nicotiana bentamiana* leaf infiltration transient expression systems. We have tested the interaction of HSFA4A with HSFA4C and BAG7 proteins, among others, but have not yet been able to prove it using BIFC assays.

6. Mitochondrial NDUFS8.2 modulates responses to stresses associated with reduced water availability

Various mitochondrial processes play a crucial role in plants' defense against abiotic stress. Although chloroplasts are the main source of ROS, mitochondria are also important in the maintenance of cellular redox homeostasis, where Complex I and III of the mitochondrial electron transport chain (mETC) are major sites for ROS production. To reveal the importance of genes encoding the mitochondrial proteins in stress responses, we analyzed insertion mutants of 12 *Arabidopsis thaliana* genes, encoding the subunits of Complex I and III of mETC. Phenotypes of the mutants were characterized in osmotic, salt, and oxidative stress conditions. Morphological alterations and differences in tolerance to drought and salinity were revealed through germination and growth tests by complex phenotyping in collaboration with the European Plant Phenotyping Network (data not shown). During our research, two mutant alleles of the *NDUFS8.2* gene were characterized in detail. NDUFS8.2 is a core part of the Complex I. The knockout *ndufs8.2-1* and the knockdown *ndufs8.2-2* mutant exhibited different degrees of tolerance to sorbitol and paraquat in growth assays (Figure 6.1.). While *ndufs8.2-1* showed better growth habits under osmotic and oxidative stress, the *ndufs8.2-2* mutant had a phenotype either similar to wild type plants, or intermediate between wild type and *ndufs8.2-1.* Therefore, we used the *ndufs8.2-1* allele in further characterization of *NDUFS8.2* function.

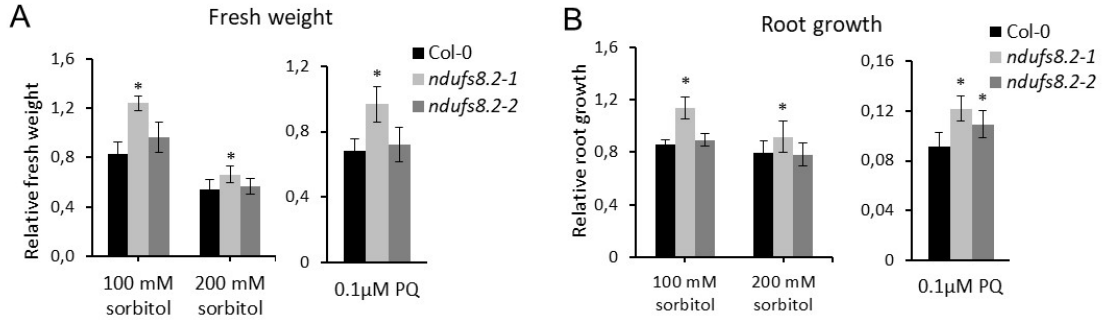


Figure 6.1. Growth of the *ndufs8.2-1* and *ndufs8.2-2* mutants and wild type plants in osmotic and oxidative stress conditions. 2-week-old in vitro-grown seedlings were transferred to and cultured on media supplemented with sorbitol or paraquat for 10 days. A) Relative fresh weight of wild type and mutant plants. Relative fresh weight: treated/non-treated. B) Root elongation of wild type and mutant plants. Relative root growth: treated/non-treated.

The genetic complementation of the *ndufs8.2-1* mutant was performed with the p35S::NDUFS8.2:GFP construct that was introduced into the *ndufs8.2-1* mutant by Agrobacterium-mediated gene transfer. To study mutant complementation, the ndufs8/C1 homozygous line was subsequently used, in which the *NDUFS8.2* transcript level was similar to the wild type. We tested ROS accumulation and the effect of oxidative damage in the *ndufs8.2-1* mutant and ndufs8/C1 complemented mutant (Figure 6.2.). The H_2O_2 content and lipid peroxidation level of *ndufs8.2-1* plant was similar to wild type and ndufs8/C1 plants in control conditions. Osmotic and oxidative stresses led to lower H_2O_2 and MDA accumulation in the mutants than in wild type or ndufs8/C1 plants (Figure 6.2. A, B). To test the effect of the *ndufs8.2-1* mutation on photosynthetic efficiency, Fv/Fm value was compared in sorbitol and

paraquat-treated wild type, complemented, and mutant plants. The Fv/Fm were similar in standard growth conditions. The sorbitol or paraquat treatment reduced Fv/Fm values in wild type plants and the complemented mutant, but had only negligible effects on the *ndufs8.2-1* mutants (Figure 6.2. C).

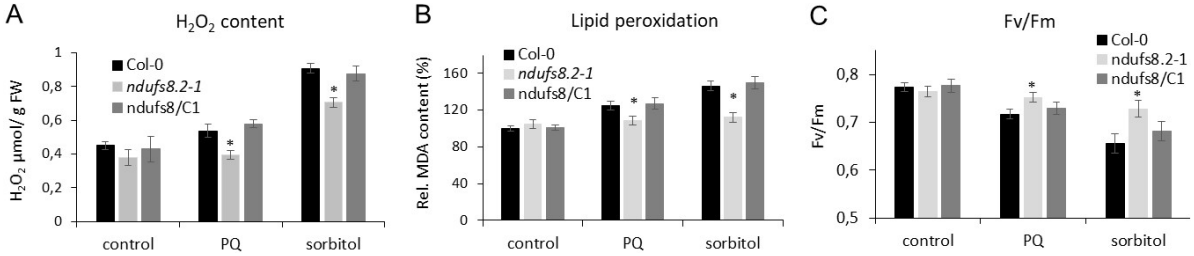


Figure 6.2. ROS accumulation, oxidative damage, and photosynthetic performance of wild type plants, *ndufs8.2-1* mutant and the complemented mutant under oxidative or osmotic stresses. 2-week-old plants were subjected to osmotic and oxidative stress (200 mM sorbitol or 2 µM paraquat) for 24 h. A) H_2O_2 accumulation. B) Lipid peroxidation rates determined by MDA accumulation. Relative values are shown where 100% corresponds to MDA content of non-treated wild type plants. C) Fv/Fm values of PSII.

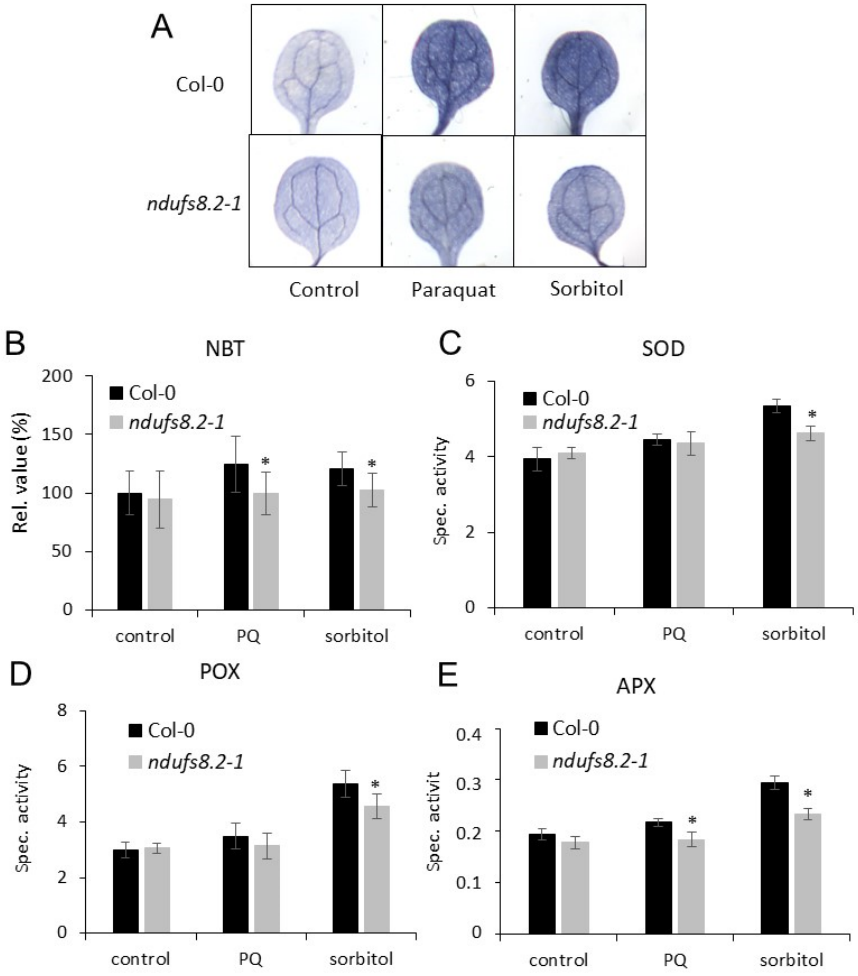


Figure 6.3. ROS generation and detoxification in Col-0 wild type and *ndufs8.2-1* mutant.
A) Nitroblue tetrazonium (NBT) staining of leaves of paraquat or sorbitol-treated plants. 2-week-old in vitro grown plants were treated with 2 µM paraquat (PQ) or 300 mM sorbitol for 24 h. B) Quantitative evaluation of NBT staining. C-E) Activities of superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) in paraquat or sorbitol-treated leaves.

We also tested the ROS generation and the operation of detoxification systems under osmotic and oxidative stress. Under stress conditions, the mutant showed less intense NBT staining compared to the wild type, indicating lower superoxide accumulation (Figure 6.3. A, B). Although the mutant displayed similar superoxide dismutase (SOD) and peroxidase (POX) activity to the wild type under control and paraquat-treated conditions, it exhibited slightly lower SOD and POX activity when subjected to sorbitol treatment (Figure 6.3. C, D). Ascorbate peroxidase (APX) activity was lower in the mutant after oxidative or osmotic stresses (Figure 6.3. E). These results suggest that attenuated ROS generation but not enhanced antioxidant activities can be responsible for lower peroxide accumulation and oxidative damage in the stress-exposed *ndufs8.2-1* mutant.

Drought-induced osmotic and oxidative stresses pose significant challenges to plants leading to damage. A comparison between the *ndufs8.2-1* mutant and wild type plants revealed that the mutant exhibited less damage under mild drought stress conditions (Figure 6.4.). The photosynthetic activity analysis showed that the mutant maintained higher effective PSII yield and electron transport rates under water stress compared to wild type plants (Figure 6.4. A, B). Moreover, after a prolonged period of water deprivation followed by re-watering, a higher percentage of *ndufs8.2-1* mutant plants survived compared to wild type plants, indicating greater drought tolerance in the mutant (Figure 6.4. C).

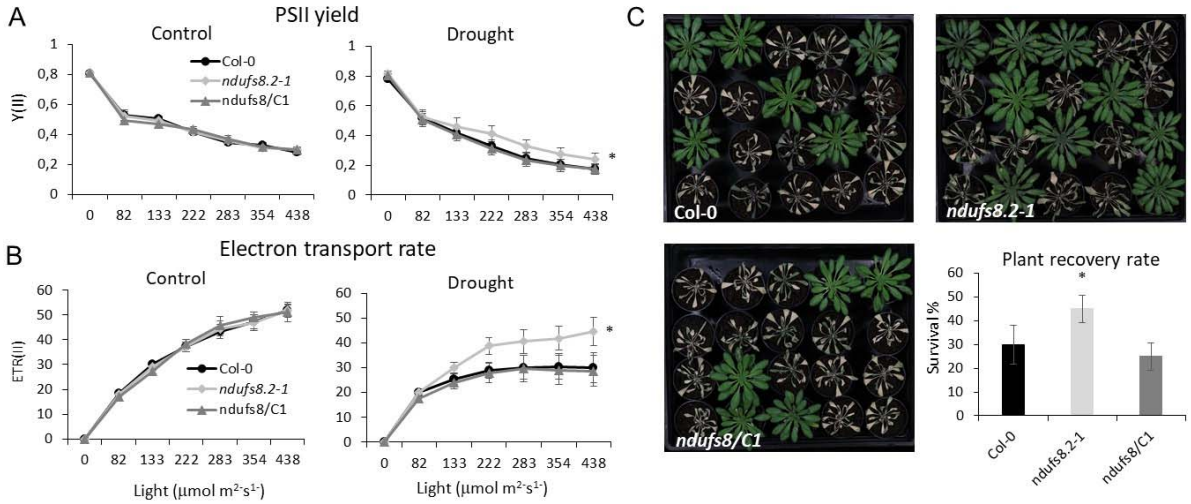


Figure 6.4. Tolerance of the *ndufs8.2-1* mutant to water deprivation. 5-week-old plants were grown in standard conditions, and watering was suspended for 11 days. Photosynthetic parameters were determined through chlorophyll fluorescence detection. A) PSII yield (Y(II)) and B) electron transport rates of PSII (ETR(II)) were determined in wild type (Col-0), *ndufs8.2-1* mutant, and complemented mutant (ndufs8/C1) plants. C) Survival of Col-0 wild type, *ndufs8.2-1* mutant, and complemented mutant plants (ndufs8/C1) after 13 days of water withdrawal and subsequent rewatering. The plant recovery rate was calculated 7 days after rewatering.

We characterized the alterations of the mitochondrial functions in the *ndufs8.2-1* mutant. Mitochondrial respiration was measured with O_2 consumption, and we found that the measured value was lower in the mutant except when succinate was used as a substrate (Figure 6.5. A). While no significant differences were observed in whole plant H_2O_2 content between the mutant and wild type under standard conditions, isolated mitochondria from the mutant exhibited higher superoxide dismutase (SOD) activity and H_2O_2 accumulation (Figure 6.5. B, C), indicating the potential elevation of mitochondrial ROS due to alterations in Complex I. Additionally, ATP generation was compromised in the *ndufs8.2-1* mutant, with lower ATP

content and ATP/ADP ratio compared to wild type plants (Figure 6.5. D), suggesting reduced mitochondrial function in the mutant.

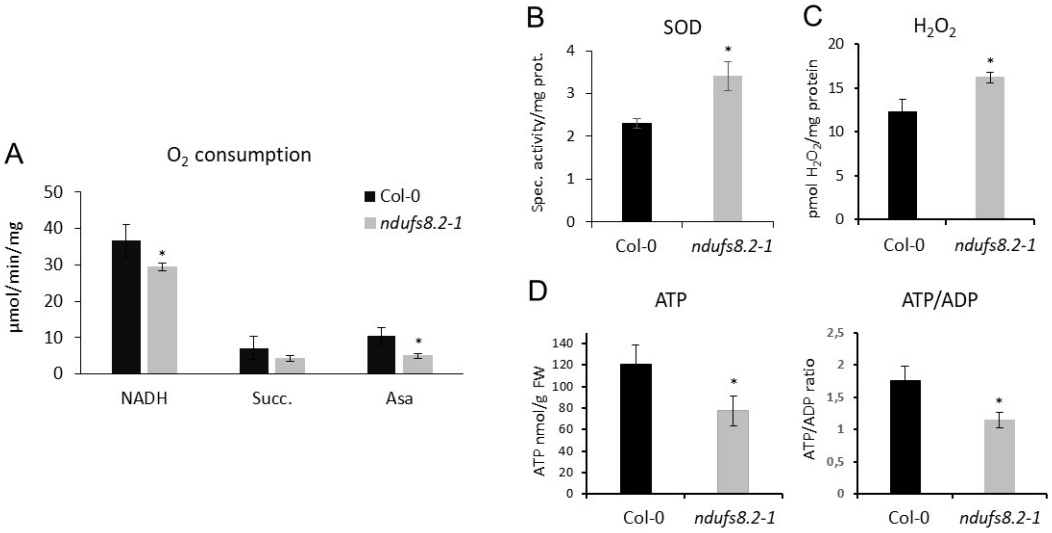


Figure 6.5. Mitochondrial functions of Col-0 wild type and *ndufs8.2-1* mutant plants. O_2 consumption, SOD activity, and H_2O_2 content were measured on isolated mitochondria. A) O_2 consumption of mitochondria using various substrates: NADH, succinate (Succ), or ascorbate (Asa). B) SOD activities in Col-0 wild type and *ndufs8.2-1* mitochondria. C) H_2O_2 content in wild type and *ndufs8.2-1* mitochondria. D) ATP content and ATP/ADP ratio of Col-0 and *ndufs8.2-1* plants.

Under stress conditions, the alternative oxidase (AOX) and alternative NAD(P)H dehydrogenases (altNDs) play a crucial role in diverting excess electrons to O_2 through non-energy-conserving pathways, helping to alleviate over-reduction of the mETC and prevent harmful ROS accumulation. Transcript levels of *AOX1a*, *NDA2*, *NDB1*, *NDB2*, and *NDB4* were 2 to 3-fold higher in the *ndufs8.2-1* when compared to wild type plants (Figure 6.6. A, B). These data suggest that the non-phosphorylating electron transport pathway mediated by altNDs can work with higher efficiency in the mutant which may be associated with the observed stress tolerance.

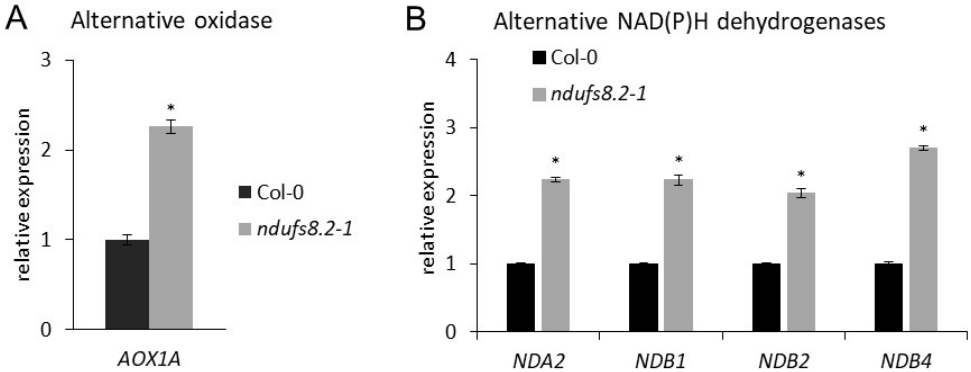


Figure 6.6. Relative expression levels of the genes encoding enzymes in non-phosphorylating pathways. Relative transcript levels are shown, where 1 corresponds to Col-0 plants in control conditions. A) Relative expression of *AOX1A* genes in Col-0 wild type and *ndufs8.2-1* mutant plants. B) Relative expression of alternative NAD(P)H Dehydrogenase (ND) genes: *NDB1, NDB2, NDB4,* and *NDA2* as determined by qRT-PCR.

As a summary of these results, we published our data supplemented with more detailed characterizations *in* Plant Physiology and Biochemistry in March 2024 (doi: 10.1016/j.plaphy.2024.108466).

Additional publications:

Posters:
Hungarian Molecular Life Sciences 2019, Eger March 29-31
The role of *Arabidopsis thaliana* mitochondrial proteins in stress responses
A Erdélyi, I Valkai, G Rigó, Á Szepesi, D Aleksza, N Koerber, F Fiorani, L Szabados, L Zsigmond

14th International Conference on Reactive Oxygen and Nitrogen Species in Plants (14th POG Conference) July 10 - 12, 2019 in Munich, Germany
Regulation of mitochondrial respiratory pathways during stress conditions
A Juhász-Erdélyi, I Valkai, G Rigó, Á Szepesi, D Aleksza, N Koerber, F Fiorani, L Szabados, L Zsigmond

Magyar Szabadgyök-Kutató Társaság X. Kongresszusa. Szeged, 2019. augusztus 29-30
Arabidopsis mitokondriális légzési lánc szerepe a stresszválaszokban
A Juhász-Erdélyi, I Valkai, G Rigó, Á Szepesi, D Aleksza, N Koerber, F Fiorani, L Szabados, L Zsigmond

Magyar Növénybiológiai Társaság XIII. Kongresszusa. Szeged, 2021. augusztus 24-27.

1. The AtCRK5 protein kinase is required to maintain the ROS NO balance affecting the PIN2-mediated root gravitropic response in Arabidopsis

Cséplő Á, Zsigmond L, Andrási N, Baba AI, Labhane NM, Pető A4, Kolbert Z, Kovács HE, Steinbach G, Szabados L, Fehér A, Rigó G

2. Small Paraquat resistance proteins modulate paraquat and ABA responses and confer drought tolerance to overexpressing Arabidopsis plants

Faragó D, Zsigmond L, Benyó D, Ayaydin F, Alcazar R, Rigó G, Szabados L

3. Analysis of abiotic stress tolerance in selected genes of *Arabidopsis thaliana*

Kant K, Rigó G, Norbert A, Szabados L, Zsigmond L

Redox Biology Congress 2022, SFRR-E and POG meeting 24-26 August 2022 Ghent, Belgium
The Arabidopsis mitochondrial protein PPR40 modulates drought tolerance.
K Kant, G Rigó, D Benyó, D Faragó, L Szabados, L Zsigmond

Straub-Days 2022, Biological Research Centre, Szeged May 25-26-27, 2022:

1. Analysis of abiotic stress tolerance in selected genes of *Arabidopsis thaliana*

K Kant, G Rigó, N Andrási, L Szabados, L Zsigmond

2. The role of *Arabidopsis thaliana* mitochondrial proteins in stress responses

A Juhász-Erdélyi, I Valkai, G Rigó, Á Szepesi, D Aleksza, N Koerber, F Fiorani, L Szabados, L Zsigmond

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Small paraquat resistance proteins modulate ABA sensitivity and can confer drought tolerance to overexpressing plants
D Faragó, L Zsigmond, SA Rabilu, D Benyó, K Kant, G Rigó, L Szabados

Lectures:
49. Membrán-Transzport Konferencia, Sümeg 2019. május 14-17.
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Free Radical Biology and Medicine Vol. 189, Supplement 1, 20 August 2022, Page 52
Characterization of mitochondrial electron transport mutants under stress conditions
A Juhász-Erdélyi, I Valkai, G Rigó, Á Szepesi, D Aleksza, K Kant, N Koerber, F Fiorani, L Szabados, L Zsigmond

Straub-Days 2022, Biological Research Centre, Szeged May 25-26-27, 2022:
Small paraquat resistance proteins confer drought tolerance to overexpressing Arabidopsis plants by interfering with paraquat and ABA responses
D Faragó, L Zsigmond, D Benyó, R Alcazar, G Rigó, F Ayaydin, SA Rabilu, A Pettkó-Szandtner, L Szabados