Closing report of NKFIH PD 131884
(Role of GPXL3 in ER-related stress responses)

Content

I. Scientific background

The endoplasmic reticulum (ER) is a highly specialized and dynamic organelle housing enzymes and chaperones responsible for guiding the folding and assembly of newly synthesized proteins. Within the ER, disulfide bonds are typically introduced through a two-category enzymatic system. Thiol oxidases (e.g., ER oxidoreductase 1 [ERO1]) are responsible for creating the disulfide bonds, while carrier proteins (e.g., protein disulfide isomerase [PDI]) facilitate their transfer to specific substrate proteins [1,2]. The process of creating and rearranging disulfide bonds in proteins found in oxidative subcellular compartments is termed oxidative folding. In plants, correct disulfide formation is vital for the proper functioning of essential proteins, including phytohormone receptors (such as ETR1 and BRI1), disulfide-rich storage proteins, antimicrobial proteins (like puroindolines, lipid-transfer proteins, and thionins), and small peptide hormones (such as defensins, EPF1, and EPF2) [2-4]. During the oxidative protein folding process, the ER accumulates H_2O_2 and oxidized glutathione (GSSG) [5]. H_2O_2 serves a dual role: in higher concentrations, it can lead to lipid peroxidation and protein oxidation [6]. However, in a different context, it acts as a secondary transducer, triggering the antioxidant defence system to maintain redox balance [7].

In mammals, it is well-confirmed that disorders in the protein folding process can have severe consequences. ER stress has been involved in several human neurological disorders, including Parkinson's disease, Alzheimer's disease, and prion diseases, as well as various cardiovascular syndromes [8,9]. In plants, the importance of the ER is equally noticeable. Environmental stressors like high temperatures, salinity, osmotic stress, drought, heavy metals, intense light, and biotic factors such as viruses can induce ER stress by interfering with the protein folding machinery. This often results in the accumulation of misfolded proteins and generates the unfolded protein response (UPR) [10]. The UPR involves the upregulation of ER chaperones and components of the degradation system, along with an expansion of the ER itself [11,12]. Luminal binding protein (BiP) is a crucial ER chaperone and is often referred to as a UPR sensor. It plays a vital role in binding to nascent proteins, preventing their aggregation [13]. Many genes that are upregulated in response to ER stress possess consensus promoter sequences that correspond to ER stress-responsive transcription factors, such as IRE1/bZIP60 and bZIP28 [14].

The enzyme family known as glutathione peroxidase (GPX) comprises proteins with evolutionary ties to non-heme thiol peroxidases. These enzymes catalyse the reduction of H_2O_2 or organic hydroperoxides into water or the corresponding alcohols, using reduced glutathione.

In plants, there exist proteins similar to GPXs, referred to as plant glutathione peroxidase-like (GPXL) enzymes. These GPXLs share close phylogenetic relationships with animal phospholipid hydroperoxide glutathione peroxidases [15]. However, unlike most animal GPXs, the GPXL isoenzymes feature cysteine in their active sites rather than selenocysteine [16]. Some of these GPXLs exhibit dual functions, serving both as glutathione peroxidases and thioredoxin peroxidases. Interestingly, they tend to favour the thioredoxin (TRX) regenerating system *in vitro* over the glutathione system. Consequently, they are often classified as thioredoxin peroxidases [17-20]. GPXLs play a significant role as antioxidant enzymes, but their activity levels are comparatively lower than those of animal GPXs. This has led to the suggestion that they might have distinct functions, possibly impacting redox balance, growth, and development [21,22]. Notably, GPXL3 is found in the endoplasmic reticulum (ER) membrane of *Arabidopsis thaliana* [23]. Since the ER lacks TRXs, GPXL3 might rely on alternative electron donors, such as protein disulfide isomerases (PDIs). Another possibility is that GPXL3 could directly oxidize newly synthesized proteins, thus acting as a thiol oxidase within the oxidative protein folding process [2].

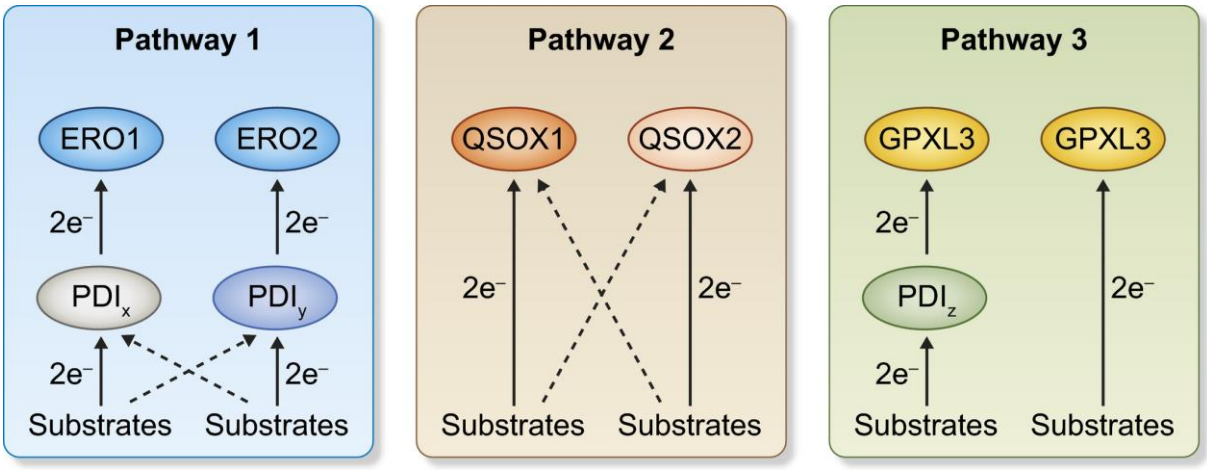

Figure 1: Pathways for oxidative protein folding in the endoplasmic reticulum (ER) of plants [2].

It's essential to note that such functions of GPXs have only been conclusively demonstrated in yeast and mammals [24,25]. Because of that, we wanted to investigate the relationship between the AtGPXL3 and ER stress. Moreover, an AtGPXL3 orthologue protein can be found in *Brassica napus*, and in the light of the earlier localisation results conducted in *Arabidopsis thaliana*, BnGPXL3 is a promising candidate also having a role in ER stress under environmental stress. As far as we know, BnGPXL3 has not been investigated before.

Our main aims were:

1. The investigation of the relationship between the AtGPXL3 and the ER stress using *Arabidopsis thaliana* Col-0 wild type and *Atgpxl3* knockout mutants.

2. Determination of the subcellular localisation of the AtGPXL3 homolog protein in *Brassica napus*.
3. Examination of the role of BnGPXL3 in ER stress using different treatments.

II. Results

1. Recognition of the specific changes in the physiological parameters and transcription of selected genes during ER stress, depending on the presence or the lack of GPXL3 in *Arabidopsis thaliana*

(Part of the results presented in the BSc dissertation and OTDK competition assays of Dávid Milodanovic, 2020 and 2021)

The experiments were started with *Arabidopsis thaliana* Col-0 wild type and *Atgpxl3* knockout mutants and performed preliminary experiments to optimize the concentration of the treatments. After analysing the preliminary results, we selected 200 mOsm polyethylene glycol (PEG), 5 µg/ml tunicamycin (TUN), 1 mM 4-phenylbutyrate (PBA), 2 mM dithiothreitol (DTT) and 5 mM L-azetidine-2-carboxylic acid (AZC) as final treatment concentrations and treated the hydroponically grown 6-week-old Arabidopsis plants for 24 hours. PEG was used to trigger unspecific osmotic stress, and we could with it investigate the common physiological responses. TUN triggers ER stress and commonly used for this purpose, AZC is a proline analogue thus indirectly causes ER stress, DTT is a strong reducing agent and blocks disulphide formation, while PBA is a molecular chaperone and inhibits ER stress [26-29].

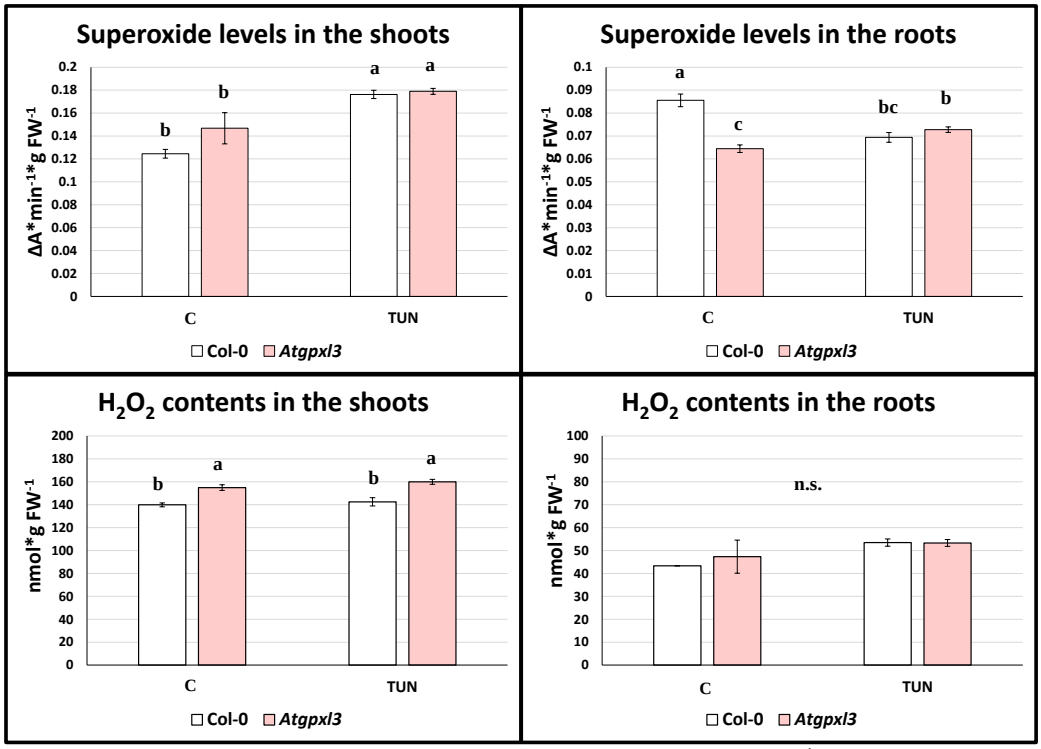

Figure 2: Changes in the levels of superoxide and H_2O_2 content after 24h 5 mg·L^{-1} tunicamycin (TUN) treatment in the shoots and roots of wild type (Col-0) and *Atgpxl3* mutant plants. Data denoted by different letters indicate a significant difference between them at a $p<0.05$ level according to Duncan's test. (means±SE, n=9) (Bela et al., unpublished data)

Investigation of the physiological parameters such as pigment contents, electrolyte leakage (EL%), lipid peroxidation, superoxide radical, hydrogen peroxide (H_2O_2) level and relative water content (RWC%) was carried out under control conditions and following stress treatments. Our results showed no difference between the Col-0 and mutant plants in the content of different pigments and RWC%. Reactive oxygen species (ROS) and malondialdehyde (MDA) levels were increased after the different treatments, and in the mutant plants higher level of H_2O_2 was detected than in the Col-0, even under control conditions (Figure 2).

We measured the activities of different antioxidant enzymes after different treatments in Col-0 and *Atgpxl3* mutants. Most of our treatments (except for PEG) caused decreased superoxide dismutase, catalase, guaiacol peroxidase and glutathione reductase activities. Interestingly, ER stress caused stronger inhibition of antioxidant enzyme activities in the mutant plants. Determination of ascorbate and glutathione contents revealed, that ER stress resulted in elevated non-enzymatic antioxidant levels, but no significant differences were detected between the Col-0 and the *Atgpxl3* mutants. Because of the elevation of the reduced form of glutathione, the redox potential (E_{hc}) became more negative in the shoots and roots of treated plants (Table 1).

Table 1: The calculated redox potential values (E_{hc}) after 24 h 5 mg·L^{-1} tunicamycin (TUN) treatment in the shoot and the root of *Arabidopsis thaliana* Col-0 and *Atgpxl3* mutant plants. (from BSc dissertation of Dávid Milodanovic, 2021)

E_{hc} (mV)		SHOOT	ROOT
Control	Col-0	-231±18	-239±14
	Atgpxl3	-234±10	-241±23
TUN	Col-0	-248±8	-249±3
	Atgpxl3	-255±23	-253±9

We checked the expression level of the 8 *GPXLs*, as well as some selected important ER stress-related genes. Most of the ER-related genes were activated after the treatments. We experienced large differences in the transcript levels of the selected genes (for example *BIP1, -2, -3*, between Col-0 and *Atgpxl3* mutant plants, almost in every case the expression rates were higher in the mutants (Figure 3).

As we expected, ER stress treatment caused a higher level of protein oxidation and proteasome activity, in the mutant plants the proteasome activity was similarly higher. The level of BiP protein was determined by Western-blot analysis, using actin as a reference protein. ER stress treatment resulted in a higher amount of BiP protein, but in some cases (using tunicamycin) in the mutant shoots the BiP protein level was lower than in Col-0. Interestingly, the lack of

AtGPXL3 protein led to severe ER stress, where the BIP protein degradation was intensified, or the protein was used up (Figure 4).

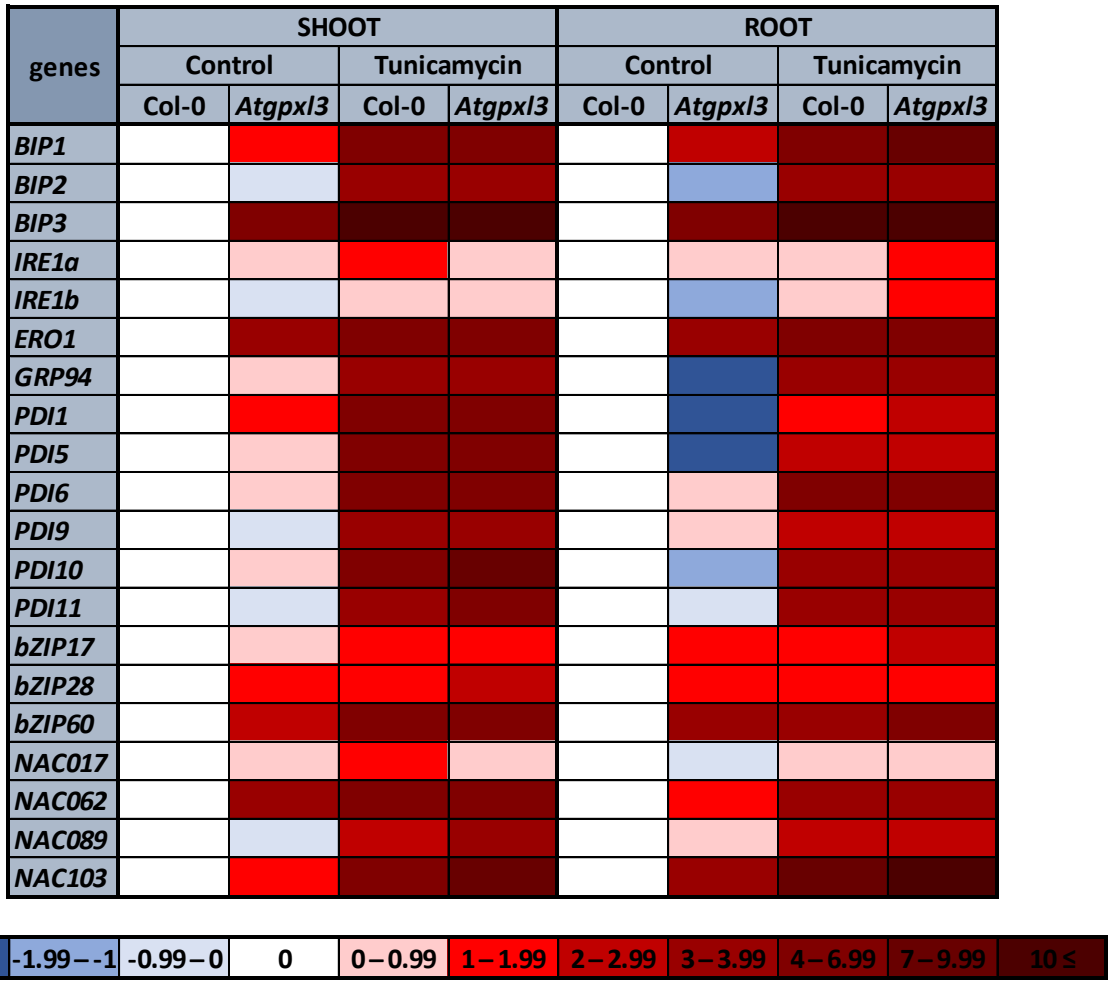

Figure 3: The relative transript levels of selected ER stress-related genes after 24h 5 mg·L^{-1} tunicamycin treatment in the shoots and roots of wild-type (Col-0) and *Atgpxl3* mutant plants. *ACTIN2* (*At3g18780*) gene was used as internal control, data were normalized to the control wild type values (0) and presented on a log2-scale as a heatmap. Blue colours show repression, while red colours show activation, according to the colour scalebar. (Bela et al., unpublished data)

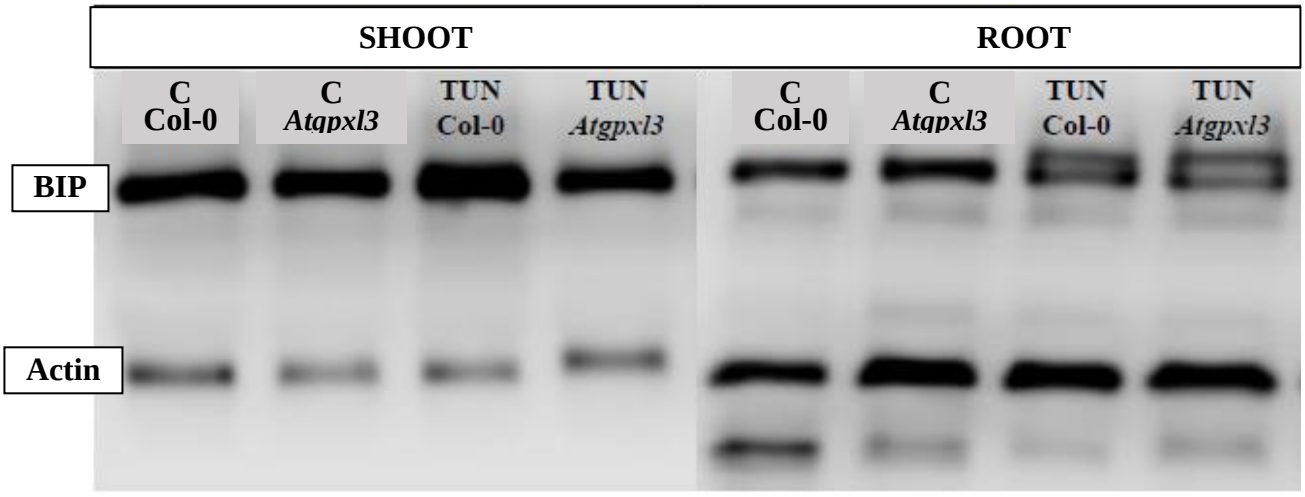

Figure 4: Changes in the levels of BIP protein after 24h 5 mg·L^{-1} tunicamycin (TUN) treatment in the shoots and roots of wild type (Col-0) and *Atgpxl3* mutant plants. (from BSc dissertation of Dávid Milodanovic, unpublished data)

Based on the observed differences, we can conclude that the GPXL3 protein likely plays a significant role in the ER stress response and may have an impact on signalling pathways. However, further investigations are required to determine its precise role. We started to check the interactions of AtGPXL3 with different PDIs using the yeast two-hybrid method. It turned out only recently that, unfortunately, we used an inadequate vector, thus now the cloning processes are repeated.

2. Determination the subcellular localisation of BnGPXL3

(The results presented in the BSc dissertation of Máté Görbe, 2021)

In our ongoing experiments, we set out to investigate the *Brassica napus* (rapeseed) GPXL enzymes, since there is currently limited information available about their characteristics. The precise description of their location and function remains unclear. The closest relatives in which the localization and functions of these GPXL enzymes have been better examined are the model plant *Arabidopsis thaliana*. Among the isoenzymes found in Arabidopsis, AtGPXL1 and -7 are located in the chloroplasts, AtGPXL2 and -8 in the cytoplasm and the nucleus, AtGPXL3 in the ER membrane, AtGPXL4 and -5 are anchored to the plasma membrane, and AtGPXL6 localizes in the cytoplasm and mitochondria [23]. AtGPXL3 is presumed to play a role in ER stress processes and the proper formation of protein tertiary structures [2].

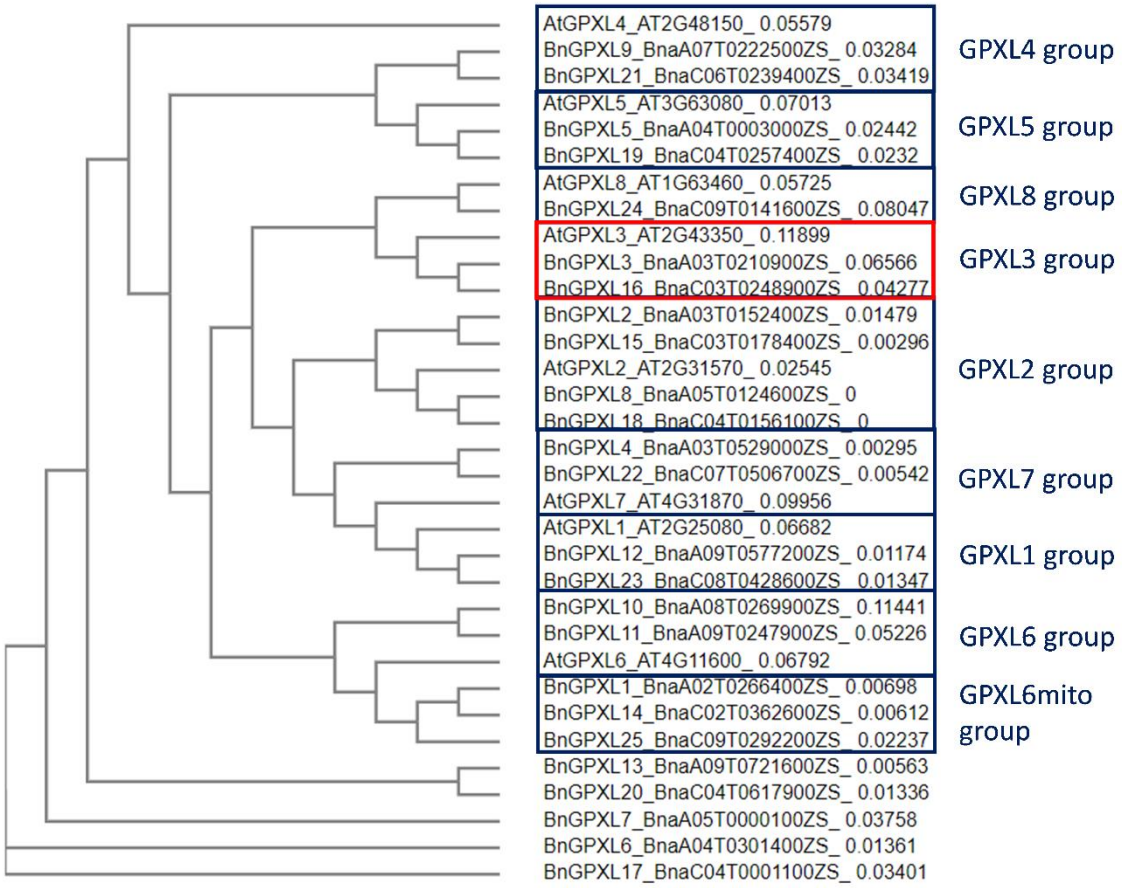

Figure 5: Phylogenetic tree of *Arabidopsis thaliana* and *Brassica napus* GPXLs. In the phylogenetic tree the BnGPXL nomenclature is according to Li et al. 2021 [30]. As the sequence of the homologue proteins on ChromosomeA and -C are very similar, we were unable to design gene specific oligonucleotides for quantitative real-time PCR or cloning, in our experiments we used groups, naming from the homologue Arabidopsis GPXLs (blue rectangles).(Unpublished data)

Since we planned to investigate the relationship between rapeseed BnGPXL3 and ER stress in future experiments, our first step was to demonstrate that, like AtGPXL3 in Arabidopsis, whether BnGPXL3 in rapeseed is also located in the ER membrane (Figure 5). To achieve this, we have fused a redox-sensitive green fluorescent protein (roGFP2) to the isoenzyme, which will enable us to confirm the protein's localization by taking advantage of the differing redox states of cell compartments.

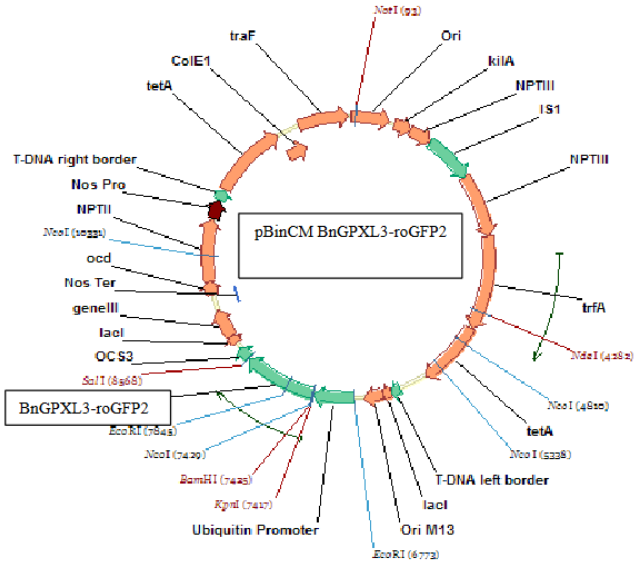

Figure 6: The prepared BnGPXL3-roGFP2:pBinCM construct. The original construct (GRX1-roGFP2:pBinCM) was provided by Prof. Andreas Meyer [31] (from BSc dissertation of Máté Görbe, 2021)

We used the GRX1-roGFP2:pBinCM plasmid, and with the use of BamHI and BcuI restriction enzymes, we changed the *GRX1* sequence to the sequence of *BnGPXL3* (Figure 6). After the cloning process, Lines 29, 37, 54, and 58 were the four lines from our checked constructions in GV3101 Agrobacteria that we chose to use for the transient expression experiment. For microscopic investigations, transformed tobacco leaf segments were cut off and placed between two coverslips. Using a confocal laser scanning fluorescence microscope, we looked at the localization of the produced BnGPXL3 using the roGFP2 signal, as it was used in the case of the Arabidopsis GPXLs [23]. The advantage of the fusion with roGFP2 is that it not only allows us to determine the position of the produced protein using the green fluorescent signal but also provides more precise information based on the redox potential it can determine. The redox potential of intracellular organelles is distinctive and varies from one another. For example, the ER is in a strongly oxidized state, it is characterised by a high redox potential (-208 mV), which aids in the process of protein structure formation within it [32].

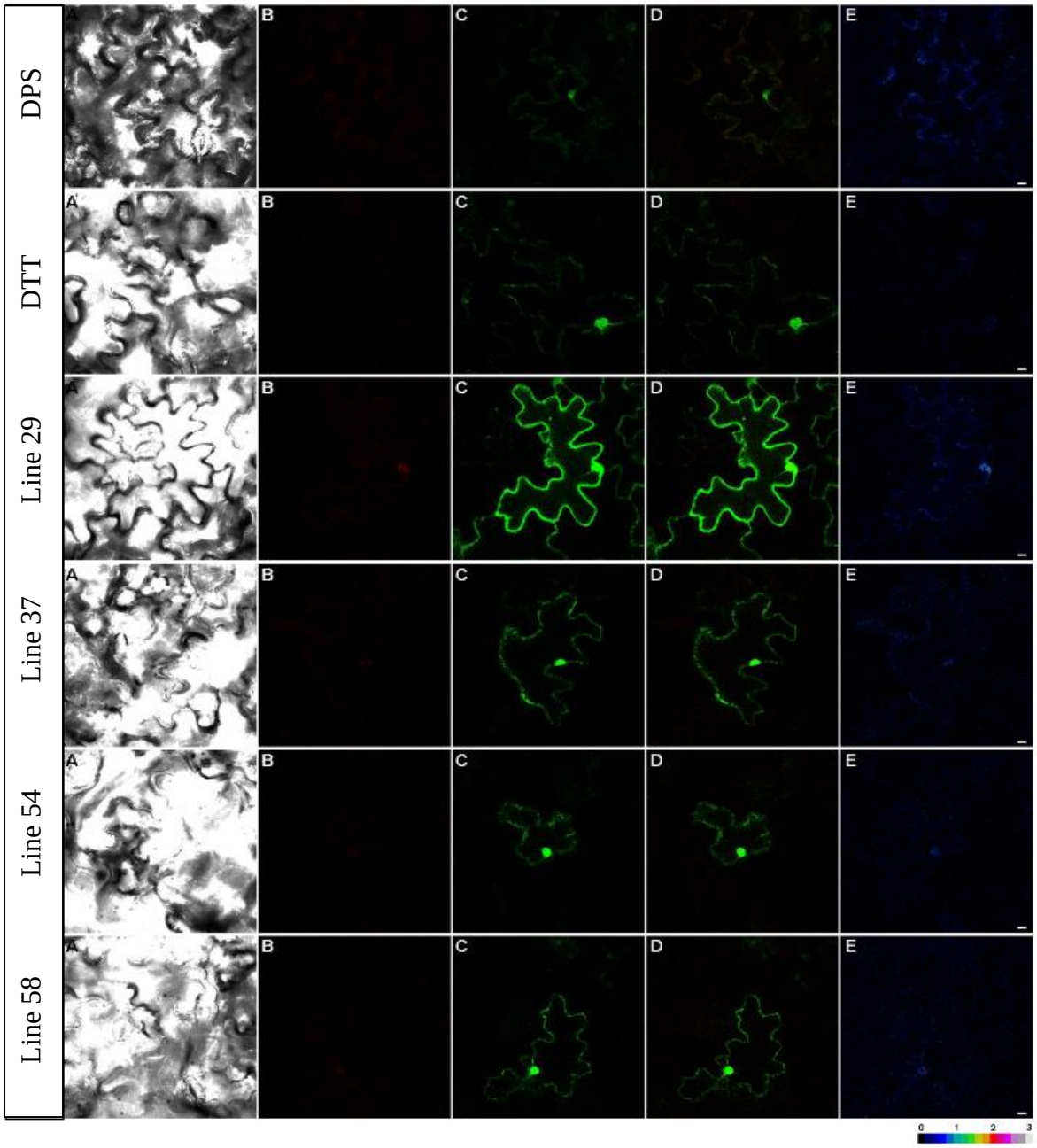

Figure 7: Representative confocal microscopic images of tobacco plants transiently expressing BnGPXL3-roGFP2 in its leaf segments (DPS: Aldritiol^{TM}, DTT: dithiothreitol). **A**: light microscopic images; **B**: fluorescence intensity, λ_{exc}=405 nm and λ_{em}=505-530 nm; **C**: fluorescence intensity, λ_{exc}=488 nm and λ_{em}=505-530 nm; **D**: merge of "**A**" and "**B**" images; **E**: Ratiometric image from the fluorescence intensities (I_{405}/I_{488}), values represented by colours according to the colour scale bar at the right bottom of the figure. The scalebar in pictures "**E**" are 10 μm. (from BSc dissertation of Máté Görbe, unpublished data)

We obtained surprising results. Arabidopsis GPXL3 has been proven to be located in the ER and Golgi membranes, with its N-terminal active region facing the lumen [23]. However, the homologous BnGPXL3 that we were currently examining showed no pattern typical of ER localization and, in fact, the calculated redox potential values did not fall within the range characteristic of the ER lumen. Based on the microscopic images, we observed clear

cytoplasmic and nuclear organelle localization in all four lines (Figure 7). The redox potential results, calculated from fluorescence intensity values ranged from -293.29 to -274.39 mV in different lines (Table 2), indicating a distinctly reduced state of the cellular organelles. The judged cytoplasmic and nuclear localization based on visual assessment may also be accurate in this case, as both cell components are evidently characterized by a reduced redox status [27].

Table 2: The calculated redox potential values, determined in the four examined lines (29, 37, 54, 58).

Line number	Redox potential (mV)
29	-282.49±3.5
37	-274.39±3.8
54	-285.12±4.1
58	-293.29±5.9

As a next step, we planned to investigate the protein localization in stable rapeseed transformant plants to confirm or possibly, to prove it is false. This is because the outcomes obtained through transient expression in tobacco plants do not always align with the results obtained from stable transformant original plants. To achieve this, we attempted to introduce the BnGPXL3-roGFP2:pBinCM constructs through rapeseed floral transformation. After multiple transformation attempts, none of the 1502 seeds obtained were found to be transformants yet.

3. Identification of the correlations between physiological changes and transcription of selected genes during ER stress measured in *Arabidopsis thaliana* or *Brassica napus*

(Part of the results presented in the MSc dissertation of Anna Farkas, 2022)

We also performed ER stress related experiments with *Brassica napus* cv. GK GABRIELLA, with a very good adaptability under different environmental conditions. Investigation of the physiological parameters such as pigment content, EL%, lipid peroxidation, superoxide radical, H_2O_2 level and RWC% was carried out under control conditions and after the different treatments (TUN, PBA, DTT, AZC and PEG). Our results showed that all treatments increased the ROS and MDA levels, but pigment content, RWC% and EL% changed only in case of PEG treatment.

Measurement of different antioxidant enzyme activities revealed that in the shoots of the plants superoxide dismutase activity increased and catalase activity decreased in case of TUN and DTT treatments, peroxidases and glutathione reductase activities increased in case of AZC and PEG treatments. In the roots of the plants TUN, AZC and PEG treatments caused significant activation of superoxide dismutase of peroxidases enzymes. Determination of ascorbate and glutathione contents revealed, that stress caused elevated non-enzymatic antioxidant levels, but the reaction was different for each treatment. While PEG was able to induce both ascorbate and glutathione production, TUN and DTT treatments increased glutathione content, mainly reduced glutathione, therefore decreased the redox potential of the plants. AZC treatment only caused changes in ascorbate levels (Bela et al., unpublished results).

As we expected, stress treatment caused higher level of protein oxidation and proteasome activity, but there were no significant differences between the treatments, only PBA caused lower protein oxidation. The level of BiP protein was determined by Western-blot analysis, using actin as reference protein. Only the ER stress treatment (using TUN) resulted in higher amount of BiP protein, suggesting that the other stresses did not cause severe effect on protein folding in rapeseed.

Plant	Gene	SHOOT		ROOT	
		Control	TUN	Control	TUN
Arabidopsis thaliana	*AtGPXL1*	0,00	0,10	0,00	-3,01
	AtGPXL2	0,00	0,75	0,00	0,17
	AtGPXL3	0,00	-1,96	0,00	0,05
	AtGPXL4	0,00	0,05	0,00	-0,35
	AtGPXL5	0,00	1,63	0,00	-0,30
	AtGPXL6	0,00	1,22	0,00	0,68
	AtGPXL7	0,00	-0,74	0,00	-0,43
	AtGPXL8	0,00	1,32	0,00	0,57
	AtBIP1	0,00	7,17	0,00	4,91
	AtERO1	0,00	6,42	0,00	3,94
	AtPDI1	0,00	5,46	0,00	0,62
Brassica napus	*BnGPXL1*	0,00	0,78	0,00	0,71
	BnGPXL2	0,00	0,08	0,00	0,66
	BnGPXL3	0,00	-1,18	0,00	1,48
	BnGPXL4	0,00	0,00	0,00	1,63
	BnGPXL5	0,00	-1,90	0,00	1,85
	BnGPXL6	0,00	-0,02	0,00	0,48
	BnGPXL6mito	0,00	-0,40	0,00	0,81
	BnGPXL7	0,00	4,22	0,00	-1,65
	BnGPXL8	0,00	0,38	0,00	2,05
	BnBIP1-2	0,00	5,78	0,00	7,56
	BnBIP3	0,00	2,77	0,00	3,20
	BnERO1	0,00	2,30	0,00	2,90
	BnPDI1-1	0,00	1,49	0,00	2,02
	BnPDI1-2	0,00	3,29	0,00	3,93
	BnPDI1-3	0,00	0,75	0,00	0,62
	BnPDI1-4	0,00	-0,37	0,00	-1,21
	BnPDI1-5	0,00	-1,08	0,00	0,23
	BnPDI2-1	0,00	2,10	0,00	2,02
	BnPDI2-2	0,00	2,65	0,00	3,04
	BnPDI2-3	0,00	2,54	0,00	3,29
	BnPDI5-1	0,00	-1,55	0,00	-0,67
	BnPDI5-2	0,00	0,12	0,00	-0,76
	BnPDI5-3	0,00	-0,70	0,00	-0,96
	BnPDI5-4	0,00	0,14	0,00	0,08
	BnPDILQY1	0,00	-0,52	0,00	-0,38
	BnPDISCO2	0,00	-0,01	0,00	-0,92

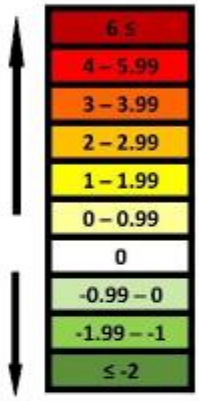

Figure 8: The relative transript levels of *GPXL* genegroups and selected ER stress-related genes after 24h 5 mg·L^{-1} tunicamycin treatment in the shoots and roots of *Arabidopsis thaliana* and *Brassica napus* plants. *AtACTIN2* (*At3g18780*) and *BnACTIN7 (LOC106382989)* genes were used as internal control, data were normalized to the control values (0) and presented on a log2-scale as a heatmap. Green colours show repression, while yellow/red colours show activation, according to the colour scalebar. (from MSc dissertation of Anna Farkas, unpublished data)

Most of the ER-related genes (*BIPs, PDIs, ERO*) were activated after TUN and DTT treatments, while AZC and PEG did not cause similar activation. The changes in the expression of *BnGPXL* genes were more interesting. *BnGPXL1* expression did not change significantly. AZC increased

the transcript amount of *BnGPXL2*. Only TUN treatment increased *BnGPXL3* expression. *BnGPXL4* expressed only in the roots of the plants, but all treatments were able to induce its expression. *BnGPXL5* transcript level was detectable in shoots and roots also, but only increased in the roots after treatments. DTT and PEG treatments were able to upregulate *BnGPXL6* expression. All the treatments increased the transcript level of *BnGPXL7* in the shoots of the plants. Only TUN treatment increased the expression of *BnGPXL8* both in shoot and root (Figure 8). So, the treatment with the proved ER-stress inducer TUN caused specific changes in the expression of *BnGPXL3* and *BnGPXL8*, therefore these genes and their translated proteins can be interesting candidates in the investigation of ER stress responses.

III. Evaluation and summary of the results

While animal GPXs are well-characterized enzymes, there is limited information available on plant GPXLs. Although several enzymes have been purified and their biochemical properties have been analysed, their *in vivo* roles and significance remain largely unexplored. It was initially believed that their primary function was the conversion of lipid hydroperoxides into less toxic compounds, thus contributing to membrane integrity maintenance. In recent years, their involvement in redox homeostasis, H_2O_2 homeostasis, and thiol/disulfide balance modulation has gained prominence [33]. Meyer et al. [2] proposed that thiol peroxidases connect ROS to functional redox signalling. GPXLs can oxidize Cys-containing proteins involved in signalling, such as phosphatases, kinases, and transcription factors, thereby regulating various pathways. Furthermore, the significance of ER-localized GPXL3 in oxidative protein folding, disulfide bridge formation, and/or regeneration of participant enzymes, all while managing H_2O_2, has been suggested. GPXL enzymes may be involved in signalling crosstalk during abiotic stress responses through redox signal transduction, epigenetic regulation, transcription factors, and direct protein-protein interactions [2]. Based on the literature, GPXLs can interact with other proteins, suggesting their potential signalling functions [33]. With GPXL3 as the first described peroxidase in the plant secretory pathway, further analysis of its precise biochemical function and the exact definition of oxidizing substrates and reducing metabolites or proteins was necessary. **In the frame of the present project, we demonstrated that the lack of GPXL3 interferes with ER stress responses, and to pinpoint the exact place of GPXL3 in this pathway, we initiated yeast two-hybrid experiments to identify its interaction partners.**

In addition to their role in stress tolerance, GPXLs regulate plant growth and development under normal and adverse conditions. The relevance of GPXLs in growth and development became apparent after reports of high transcript levels of *GPXL* genes in *Arabidopsis thaliana*, with their expression depending on tissue and developmental stage [34]. According to our results, the lack of AtGPXL5 enzyme activity negatively impacted plant growth and development under normal environmental conditions [35]. It led to a reduction in primary root length, biomass, chlorophyll and anthocyanin pigment contents, rosette size, and leaf convex area compared to wild types and overexpressing lines. Furthermore, the importance of GPXL5 in the development and skotomorphogenesis of dark-grown Arabidopsis seedlings was evident as knock-down *Atgpxl5* mutants exhibited defective phenotypes, including reduced hypocotyl and radical growth compared to four-day-old dark-grown wild type and AtGPXL5 overexpressing

plants. Although the elevated ROS levels and more oxidized redox status in *Atgpxl5* mutants could trigger increased ET production, changes in ET-related gene expression patterns in both the insertional mutant and AtGPXL5-overexpressing plants suggested crosstalk between AtGPXL5 and ethylene signalling [35]. The significance of GPXLs in proper model plant growth and development has paved the way for applying this knowledge to crop plants. Recently, tissue-specific GPXL gene expression levels have been reported in several crop plants. Among the 25 *BnGPXLs* genes, group II genes, such as *BnGPXL1, -14, -8, -18, -11, -25, -12,* and *-23*, were upregulated in shoots, roots, leaves, flowers, siliques, and seeds, with the exception of *BnGPXL8, -12-18*, which were downregulated in seeds. On the other hand, genes belonging to other groups were downregulated except for *BnGPXL2, -4, -15, -22*, which were significantly higher in leaves, flowers, seeds, and siliques. The high *GPXL* expression levels indicate the importance of these genes in the developmental processes of rapeseed [30]. **According to our results, *AtGPXL3* and *-8* homologues (*BnGPXL3, -16, -24*), although usually exhibiting low expression levels in plant organs, they and their translated proteins are promising candidates in stress-, and especially in ER stress research.**

GPXL overexpression holds promise in molecular or traditional breeding to develop stress-resistant crop plants. However, several unresolved questions remained. Firstly, it is essential to explore the species-specific roles and regulatory networks of GPXLs in various crop plants under normal and stress conditions. Secondly, further intensive research is required to investigate the crosstalk between GPXLs and other components of the antioxidant system and hormone signalling, given their influence on stress responses, growth, and developmental processes. For example, the significance of GPXL activation by de-crotonylation or their involvement in ferroptosis remains unknown. Finally, it is conceivable that plant GPXLs, with their ability to catalyse redox reactions of different lipid hydroperoxides, may be utilised in the development of analytical and diagnostic kits. Their multifaceted involvement in regulating physiological processes throughout a plant's life ensures that plant GPXLs will gain more significant recognition and application in the future [33].

IV. References

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V. Dissemination of the results

4. Publications

a) Gallé Á, **Bela K**, Hajnal Á, Faragó N, Horváth E, Horváth M, Puskás L, Csiszár J (2021) Crosstalk between the redox signalling and the detoxification: GSTs under redox control?, PLANT PHYSIOLOGY AND BIOCHEMISTRY, 169: 149-159. **IF: 5.437**
b) **Bela K**, Riyazuddin R, Csiszár J (2022) Plant Glutathione Peroxidases: Non-Heme Peroxidases with Large Functional Flexibility as a Core Component of ROS-Processing Mechanisms and Signalling, ANTIOXIDANTS 11: (8) p. 1624. **IF:7.000**
c) Riyazuddin R, **Bela K**, Poór P, Szepesi Á, Horváth E, Rigó G, Szabados L, Fehér A, Csiszár J (2022) Crosstalk between the Arabidopsis Glutathione Peroxidase-Like 5 Isoenzyme (AtGPXL5) and Ethylene, INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 23: (10) 5749. **IF:5.600**
d) Horváth E, **Bela K**, Kulman K, Faragó N, Riyazuddin R, Gallé Á, Puskás L, Csiszár J (2023) Glutathione Transferases are Involved in Salicylic Acid-Induced Transcriptional Reprogramming, JOURNAL OF PLANT GROWTH REGULATION 42: (7) pp. 4497-4510. **IF:4.640**

5. Book Chapter

a) **Bela K**, Riyazuddin R, Horváth E, Hajnal Á, Gallé Á, Bangash SAK, Csiszár J (2020) A növényi glutation-peroxidáz-szerű enzimek szerepe az oxidatív stresszválaszban. In: Poór, Péter; Mézes, Miklós; Blázovics, Anna (szerk.). Oxidatív stressz és antioxidáns védekezés a növényvilágtól a klinikumig. Budapest, Magyarország: Magyar Szabgyök-Kutató Társaság, pp. 12-19. , 8 p. SZTE Publicatio

6. Lectures

a) Csiszár J, Gallé Á, Horváth E, **Bela K**, Hajnal ÁB, Erdei L, Tari I, Fehér A (2021) A stresszválaszok és redox folyamatok fiziológiai és molekuláris vizsgálatai az SZTE Növénybiológiai Tanszéken, In: Györgyey, János (szerk.) XIII. Magyar Növénybiológiai Kongresszus, Szegedi Biológiai Kutatóközpont p.40.
b) Riyazuddin R, **Bela K**, Horváth E, Poór P, Szepesi Á, Rigó G, Szabados L, Fehér A, Csiszár J (2021) Crosstalk between the membrane localised Arabidopsis glutathione peroxidase-like isoenzymes-5 (AtGPXL5) and ethylene signaling, In: Györgyey, János (szerk.) XIII. Magyar Növénybiológiai Kongresszus, Szegedi Biológiai Kutatóközpont p.36.
c) Riyazuddin R, **Bela K**, Horváth E, Poór P, Szepesi Á, Rigó G, Szabados L, Fehér A, Csiszár J (2021) Involvement of the membrane localised Arabidopsis glutathione peroxidase-like isoenzymes-5 (AtGPXL5) in ethylene signaling, In: Hagymási, Krisztina; Poór, Péter (szerk.) A Magyar Szabadgyök-Kutató Társaság XI. Kongresszusa, PROGRAM ÉS ÖSSZEFOGLALÓK, p.18.
d) Csiszár J, **Bela K**, Horváth E, Gallé Á, Hajnal Á, Tompa B, Dobai D, Riyazuddin R, Ayaydin F, Rigó G, Szabados L, Fehér A (2023) From stress physiology to molecular biology: Glutathione and glutathione related enzymes as key antioxidants and redox signalling compounds, In: Kolozsvári Biológus Napok - 2023, p.14.
e) Csiszár J, Riyazuddin R, Szepesi Á, Farkas A, Milodanovic D, Horváth E, Poór P, Gémes K, Szabados L, Rigó G, Fehér A, **Bela K** (2023) Polyamines in Arabidopsis glutathione peroxidase mutants: Function as antioxidants or growth regulators?, In: 3rd Plant Polyamine Research Workshop. Program and Abstracts, p. 22.

7. Posters

a) **Bela K**, Riyazuddin R, Milodanovic D, Horváth E, Hajnal Á, Gallé Á, Csiszár J (2020) Detection of the in vivo redox state of Atgpxl2 and -3 mutant seedlings, using roGFP2 redox probe. In: Baltic redox workshop Greifswald, p. 60.
b) Horváth E, Kulman K, **Bela K**, Riyazuddin R, Gallé Á, Csiszár J (2020) Mutation of glutathione transferase tau 19 (AtGSTU19) altered salicylic acid response in Arabidopsis seedlings. In: Baltic redox workshop Greifswald, p. 64.
c) **Bela K**, Milodanovic D, Riyazuddin R, Farkas A, Horváth E, Gallé Á, Bangash SAK, Poór P, Csiszár J (2021) ER stress response of Atgpxl3 plants, In: Plant Biology Europe 2021 Abstract Book, p.241.
d) **Bela K**, Milodanovic D, Riyazuddin R, Farkas A, Lkhagvadorj O, Horváth E, Gallé Á, Bangash SAK, Poór P, Csiszár J (2021) Van-e az AtGPXL3-nak szerepe az ER stresszválaszban?, In: Györgyey, János (szerk.) XIII. Magyar Növénybiológiai Kongresszus, Szegedi Biológiai Kutatóközpont p.60.
e) Csiszár J, **Bela K**, Hajnal Á, Horváth E, Faragó N, Puskás L, Gallé Á (2021) Glutathione transferases in roots as antioxidants and redox modulators, In: Plant Biology Europe 2021 Abstract Book, p.278.
f) Hajnal Á, Csiszár J, **Bela K**, Horváth E, Faragó N, Puskás L, Gallé Á (2021) The connection between glutathione transferases and redox state – a comparative analysis of two tomato cultivars, In: Plant Biology Europe 2021 Abstract Book, p.288.
g) Horváth E, Kulman K, Gaál M, **Bela K**, Hajnal Á, Csiszár J (2021) Salt stress response of tomato cultivars: focussing on glutathione and related processes, In: Plant Biology Europe 2021 Abstract Book, p.156.
h) Horváth E, **Bela K**, Hajnal Á, Feigl G, Kulman K, Gaál M, Csiszár J (2021) Paradicsom fajták sóstressz válaszának összehasonlító vizsgálata, In: Györgyey, János (szerk.) XIII. Magyar Növénybiológiai Kongresszus, Szegedi Biológiai Kutatóközpont p.65.
i) Csiszár J, **Bela K**, Riyazuddin R, Farkas A, Milodanovic D, Szepesi Á, Horváth E, Szabados L, Rigó G, Fehér A (2023) Arabidopsis glutathione peroxidase-like enzymes: Non-heme ROS scavengers in the signal transduction network connect redox regulation to plant development, In: Christophe, ROBAGLIA; Cécile, LECAMPION; Thomas, DELCOURT; Alexandra, MARAVAL (szerk.) Plant Biology Europe 2023, p. 150.

8. MSc dissertation

a) Farkas Anna (2022) Arabidopsis és repce glutation peroxidáz-szerű fehérjék szerepének összehasonlítása a tunikamicin által kiváltott ER stresszben. Témavezető: Dr. Bela Krisztina

9. BSc dissertations

a) Farkas Anna (2020) Az Arabidopsis GPXL-4 és -5 enzimek szerepe a hajtás fejlődésében. Témavezető: Dr. Bela Krisztina
b) Görbe Máté (2021) Brassica napus GPXL3 enzim sejten belüli lokalizációjának meghatározása redox érzékeny GFP2 segítségével. Témavezető: Dr. Bela Krisztina
c) Milodanovic Dávid (2021) Tunikamicin által kiváltott ER stress vizsgálata *Atgpxl3* mutáns növényeken. Témavezetők: Dr. Bela Krisztina és Dr. Poór Péter

10. OTDK competitions

a) Milodanovic Dávid (2020) AtGPXL3 szerepének vizsgálata tunikamicin indukált ER stresszválasz folyamán. Témavezetők: Dr Bela Krisztina és Dr. Poór Péter. Szegedi

helyi Tudományos Diákköri Konferencia, Biológia VI. (Növénybiológia) szekció, 2020. november 26. ONLINE

b) Milodanovic Dávid (2021) AtGPXL3 szerepének vizsgálata tunikamicin indukált ER stresszválasz folyamán. Témavezetők: Dr Bela Krisztina és Dr. Poór Péter. Országos Tudományos Diákköri Konferencia, Növényélettan 1. szekció, 2021. május 4. ONLINE (received a special award)

VI. Justification of the modifications compared to the original research and work plan

The original research plan was scheduled for December 2019 to August 2022. While we successfully completed all the planned experiments, including several additional ones like the yeast two-hybrid method, the timing of these experiments and the presentation of the results were slightly modified.

Due to COVID-related regulations, the experiments for the first year were delayed as orders arrived late. Restrictions also led to the cancellation or online format of all planned conferences. This naturally affected the budget, as there was no expenditure on travel and daily allowances. We reallocated these saved funds within the main budget, allocating a significant portion to cover the increased cost of chemicals and using the amount designated for daily allowances to pay for additional assistance.

The schedule for the grant was also adjusted because I was on maternity leave from April 2022 to May 2023. Although my research students continued to conduct experiments, and I was supervising them, I was unable to actively work in the laboratory. Therefore, I requested a temporary suspension of the grant. As a result, the active period of the grant eventually completed in September 2023.