Closing report of NKFIH PD 131909

Relationship between glutathione transferases and the abiotic stress tolerance of tomato

I. Scientific background

Glutathione transferases (GSTs) are one of the most versatile enzymes, which in plants were originally described as the members of detoxification pathways (Frear, Swanson, 1974). GSTs catalyse a wide range of reactions from which one of the well-known is the conjugation of glutathione (γ-Glu-Cys-Gly, GSH) to electrophilic compounds. Moreover, several GSTs also have glutathione peroxidase (GPOX) activity, thus reduce organic hydroperoxides. However, GSTs are participating in a variety of other reaction with numerous alternative substrates (Edwards and Dixon 2005, Dixon et al. 2010, Cummins et al. 2011, Labrou et al. 2015, Estévez and Hernández 2020). Several studies have shown that they are involved in the response to various stresses (Mittova et al. 2003, Kilili et al. 2004, Gallé et al. 2009, Sappl et al. 2009, Chen et al. 2012, Xu et al. 2015, Horváth et al. 2015a, Benyó et al. 2016, Islam et al. 2017, Horváth et al. 2019, Gallé et al. 2021, Ding et al. 2022, Otulak-Kozieł et al. 2022,).
Abiotic stresses are the key factors which negatively influence plant development and productivity (Chikkaputtaiah et al. 2017, He et al. 2018). Among those stresses, drought causes the most serious problem, but high salinity also extensively affects plants life cycle (He et al. 2018). When salt concentration reaches a critical level it causes at first osmotic stress which is followed by ionic toxicity if plants cannot maintain ion homeostasis (Munns and Tester 2008). Consequently these stresses are the main cause of extensive agricultural production losses worldwide (Chikkaputtaiah et al. 2017). Moreover, primary stresses can induce other secondary stresses, especially oxidative stress (Zhu 2001, Munns and Tester 2008, Liang et al. 2018).

It is well demonstrated that the level of reactive oxygen species (ROS), like superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) are generally increases under different abiotic and biotic stress conditions (Hasanuzzaman et al. 2020). These highly reactive molecules may be harmful for the cells, but plants utilize ROS as a signalling molecule under stress conditions. To mitigate stress induced oxidative damages and support the maintenance of a proper ROS level and reduction/oxidation (redox) state within the cells a strict control between ROS production and scavenging is crucial (Hasanuzzaman et al. 2020).

Several essential antioxidant mechanisms were evolved to regulate ROS-, especially H_2O_2 levels, such as ascorbate-glutathione (AsA-GSH) pathway and ROS-processing enzymes (Štolfa et al. 2016). The total amounts of non-enzymatic antioxidants (e. g., GSH, AsA and flavonoids) and their reduced-oxidized status (ascorbate/dehydroascorbate: ASC/DHA and glutathione (γ-Glu-Cys-Gly; reduced/oxidized forms of glutathione: GSH/GSSG) are fundamental elements of the redox homeostasis of cells but glutathione is the master regulator (Potters et al. 2010, Foyer and Noctor 2011, Noctor et al., 2011, Gill et al. 2013).

Thiol redox biochemistry is considered to have crucial role in cellular processes, thus preservation of a high GSH level and reduced/oxidized glutathione (GSH/GSSG) ratio are

important for proper function of the cells and organs. Maintenance of a highly negative redox potential for glutathione (around -310 mV) is achieved through continuous reduction of glutathione disulfide by glutathione reductase (GR) enzyme and/or due to increased de novo synthesis of GSH (Aller et al. 2013, Csiszár et al. 2018).

A multiplicity of reactions may contribute to GSH oxidation during oxidative stress, leading to modifications in the status of this potentially important cellular redox signal. Among the molecules able to oxidize GSH to produce GSSG are ROS, dehydroascorbate, and dehydroascorbate reductase (DHAR) in the AsA-GSH pathway, which is an outstanding GSSG-generating enzyme (Rahantaniaina et al. 2013). However, several studies suggest that other enzymes, like glutaredoxins (GRXs) and glutathione transferases (GSTs) may also contribute to GSH oxidation to GSSG (Rahantaniaina et al. 2013). Using Arabidopsis T-DNA insertional *gst* mutants, previously we have found that several specific AtGSTs are involved in determination of the redox potential of GSH and modify the GSH content of the cells (Horváth et al. 2015b, Horváth et al. 2019).

GSTs can be grouped into ten classes based on their conserved amino acid sequences namely tau (GSTU), phi (GSTF), zeta (GSTZ), theta (GSTT), lambda (GSTL), iota, dehydroascorbate reductase (DHAR), hemerythrin, γ-subunit of the eukaryotic translation elongation factor 1 B (EF1 Bγ) and tetrachloro-hydroquinone dehalogenase (TCHQD) (Lallement et al. 2014, Liu et al. 2013). GSTFs, GSTUs, GSTLs and DHARs are plant specific classes, and the majority of GSTs belong to the tau and phi classes (Estévez and Hernández 2020).

In tomato (*Solanum lycopersicum*) 90 *SlGST* genes were identified, and based on the protein sequences 57 GSTU, 7 GSTL, 6-6 GSTF and DHAR, 4 GSTT, 3 EF1Bγ, 2-2 zeta (GSTZ) and glutathionyl-hydroquinone reductase (GHR) and 1 tetrachloro-hydroquinone dehalogenase (TCHQD) isoenzymes can be classified (Islam et al., 2017). Earlier studies identified tau class GSTs in tomato that were able to mitigate the adverse effect of salt-, osmotic-, cold- and oxidative stresses (Kampranis et al. 2000, Kilili et al. 2004, Xu et al. 2015, Ding et al. 2022). A specific set of *GST* genes is induced during response to salt or osmotic stresses, and elevated GST and/or GPOX activities are part of successful osmotic, dehydration or salt stress responses (Csiszár et al. 2014, Islam et al. 2017, Gallé et al. 2021). Furthermore, increased GST and GPOX activities, accumulation of GSH and elevated GSH:GSSG ratio characterized resilient tomato plants (Mittova et al. 2003, Gallé et al. 2021).

The aim of this study is to compare the abiotic stress tolerance of 3 tomato (*Solanum lycopersicum* L.) genotypes which are cultivated in Hungary (such as ‘Mobil’ and ‘Elán F1’) or commonly used in research (‘Moneymaker’). In this project we studied the genotype-specific pattern of stress-inducible *SlGSTs* and glutathione- or redox-related genes that are involved in pathways regulating stress tolerance. The main results can be summarized as follows:

II. Results

Differences were detected in tomato cultivars already under control conditions

The fresh weight and length of 4-week-old hydroponically grown plants were similar in all three cultivars. **Contents of H_2O_2 and the lipid peroxidation marker malondialdehyde (MDA) also were similar in leaves and roots of the investigated cultivars,** except for the higher H_2O_2 levels in 'Moneymaker' leaves compared to other cultivars.

Investigation of non-enzymatic antioxidant content showed that **the reduced AsA and GSH level was the lowest in leaves of 'Mobil'** under control condition. Furthermore, **the half-cell reduction potential (E_{hc})** calculated from measured glutathione concentrations, using the formula of Schafer and Buettner (2001) **were more positive in 'Mobil' than in the other two cultivars** (Table 1).

Investigation of GR and glutathione transferase-related enzyme activities revealed that 'Mobil' had significantly higher GR activity in the roots and slightly higher GST activities in leaves and roots under control condition compared to 'Moneymaker' and 'Elán F1', but its leaves possessed the lowest DHAR activity.

Expression of the two *GR* and 27 *GST* genes was detected in leaves and roots of 5-week-old plants by high-throughput quantitative real-time PCR (HT-qPCR, Avidin Ltd.). Compared to the 'Mobil' control samples (in which the transcript level was considered to be 0 for each gene on a log2 scale), **22 and 3 genes were expressed on a higher level in leaves of 'Moneymaker' and 'Elán F1', respectively**, and 2 sequences showed lower expression in 'Elán F1', under control conditions. In roots, less difference was found among the tomato cultivars than in leaves. Three genes showed higher and 2 lower expression in 'Moneymaker', and 6 had lower abundance in 'Elán F1' than in 'Mobil'. Despite of the differences found in the expressions, the GST activity was even slightly higher in 'Mobil' than in 'Moneymaker'.

Differences in osmotic stress response of tomato cultivars were observed

The effect of different concentrations of polyethylene glycol (50, 100, 200 and 300 mOsm of PEG 6000) treatment for one week were tested on hydroponically grown, 4-week-old tomato plants. Seven days of 100, 200 and 300 mOsm PEG treatment was lethal and plants wilted already after a few days. For further investigations gradually increased 50 mOsm (7%) PEG treatment was chosen because plants could survive this degree of osmotic stresses (Fig. 1).

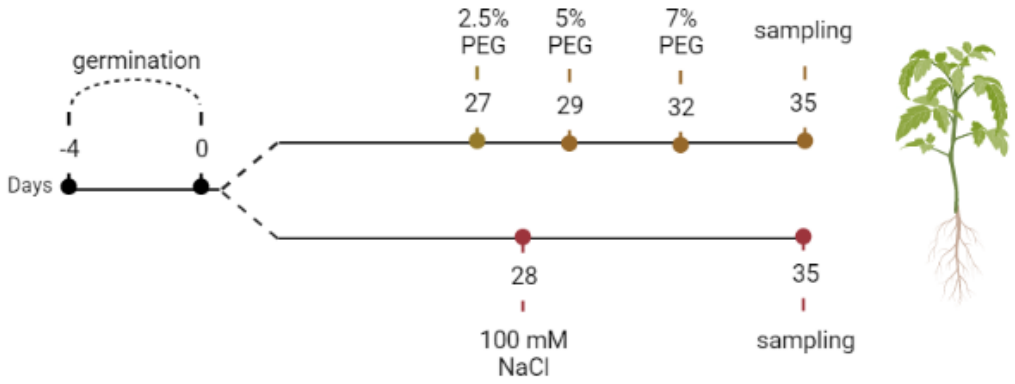

Fig. 1 The scheme of the treatments of tomato plants with NaCl and polyethylene glycol 6000 (PEG). Created by BioRender.com

One week long 50 mOsm PEG treatment decreased the length of shoots and the fresh weight (FW) of shoots and roots in all genotypes. **The relative water content decreased** in leaves and roots of all cultivars, but **the most in 'Mobil'**. One week of osmotic stress induced H_2O_2 and MDA accumulation both in leaves and roots of tomato plants, in 'Moneymaker' somewhat lower MDA levels was observed compared to 'Mobil' or 'Elán F1'.

Measuring the non-enzymatic antioxidant levels revealed bigger differences of osmotic stress response among the investigated cultivars. The ascorbate levels of 'Moneymaker' was on control level after one week of 50 mOsm PEG, however total ascorbate content increased in leaves and decreased in roots of 'Mobil' and 'Elán F1' compared to the control. Interestingly, the **ratio of reduced and oxidized ascorbic acid (AsA/DHA) increased in roots of 'Moneymaker' and decreased in 'Mobil' and 'Elán F1'** (Table 1). Glutathione level increased in leaves of 'Moneymaker' and 'Mobil', and decreased in roots of 'Moneymaker' and 'Elán F1' after one week of PEG treatment, whereas it did not change in leaves of 'Elán F1' and in roots of 'Mobil'. The calculated redox potential became more positive compared to the control in leaves and roots of 'Elán F1' by 6 mV and 21 mV, respectively. **The E_{hc} values** increased **in roots of 'Moneymaker'** by 16 mV, it means **became more oxidized, but decreased in leaves of 'Moneymaker' and in leaves and roots of 'Mobil' after PEG treatment compared to the control** (Table 1).

Table 1 Ratio of ascorbic acid and dehydroascorbate (AsA/DHA), reduced and oxidized glutathione (GSH/GSSG) and glutathione redox potential (E_{hc}) of hydroponically grown 5-week-old *Solanum lycopersicum* 'Moneymaker', 'Mobil' and 'Elán F1'.

	Leaves		Roots	
	Control	**PEG**	**Control**	**PEG**
AsA/DHA				
Mm	6.98 ± 0.88	5.31 ± 0.72	1.35 ± 0.65	2.04 ± 0.9
Mo	12.57 ± 0.74	17.24 ± 1.93	2.62 ± 0.26	2.42 ± 0.72
El	7.44 ± 0.56	7.62 ± 1.73	2.35 ± 0.74	1.18 ± 0.47
GSH/GSSG				
Mm	89.64 ± 7.32	87.80 ± 1.25	41.50 ± 8.04	22.10 ± 11.04
Mo	59.20 ± 10.98	109.14 ± 7.94	28.54 ± 4.31	34.61 ± 12.02
El	54.70 ± 22.36	28.71 ± 2.01	39.94 ± 17.85	16.98 ± 4.36
E_{hc}				
Mm	-322.77 ± 0.46	-326.08 ± 5.32	-299.19 ± 5.16	-283.97 ± 13.97
Mo	-310.84 ± 2.09	-327.15 ± 6.94	-290.23 ± 3.79	-292.18 ± 5.27
El	-311.82 ± 11.3	-305.74 ± 4.81	-298.36 ± 8.77	-277.69 ± 7.25

After 1 week of 50 mOsm PEG treatment GR and glutathione peroxidase (GPOX) activities decreased in leaves and increased in roots of all cultivars. **The highest GPOX and GR activities were measured in 'Moneymaker'.** Glutathione transferase (GST) activity increased in leaves and roots of 'Mobil' and 'Elán F1', whereas did not change in 'Moneymaker' after osmotic stress treatment.

Although tomato plants survived 50 mOsm (7%) PEG treatment for several days, wilting of plants was observed and the elevated H_2O_2 and lipid peroxide levels may trigger further damages to the cells in all cultivars. The elevated AsA and GSH levels contributed to a successful osmotic stress tolerance, but were insufficient for the alleviation of growth inhibition. Based on the results of growth parameters, the 'Moneymaker' tolerates best the applied 50 mOsm PEG treatment, in which the higher GR and GPOX activities also may participate (manuscript under preparation).

GSTs have important roles in successful stress response of tomato plants under salt stress

We tested the effect of different concentrations of NaCl (100, 150 and 200 mM) treatments and found that 200 mM NaCl applied for one week was lethal for 4-week-old tomatoes. However plants could tolerate 100 mM NaCl, thus this concentration was chosen for further investigations, and used for salt stress treatments as shown in Fig. 1.

Comparison of the salt stress responses of three tomato cultivars revealed that '**Moneymaker' efficiently preserved the growth in the presence of 100 mM NaCl** for one week. Only

slight biomass decrease was observed in ‘Elán F1’, but significant growth inhibition was detected in ‘Mobil’ after one week of salt stress. According to our results ‘Moneymaker’ and ‘Elán F1’ were capable to restore their growth during the 7 days of 100 mM NaCl treatment, indicating the higher stress tolerance of these cultivars. Interestingly, increased H_2O_2 levels were measured in all cultivars after salt stress, which was not associated with MDA contents, showing that the elevated ROS may serve other, signalling purposes.

One week long 100 mM NaCl treatment uncovered differences in the relevance of ASA-GSH pool of tomato cultivars. **Lower AsA and GSH levels measured in leaves of ‘Mobil’ compared to the other cultivars resulted in the smallest AsA/DHA and GSH/GSSG ratios and more positive E_{hc} values in leaves and roots under control conditions and after salt stress** (Fig. 2). The elevation of DHAR and the most pronounced decrease in GR activities in ‘Mobil’ could confer to the higher redox potential of GSH after salt stress compared to the other cultivars (Fig. 2, 3). It is important to highlight that glutathione level increased only in the roots of ‘Moneymaker’ after salt treatment. Although the GR activity of roots decreased, **elevated GSH/GSSG ratios and a more reduced GSH pool characterized the roots of all cultivars. The higher DHAR activities and increased AsA levels could promote the protection of GSH pool.**

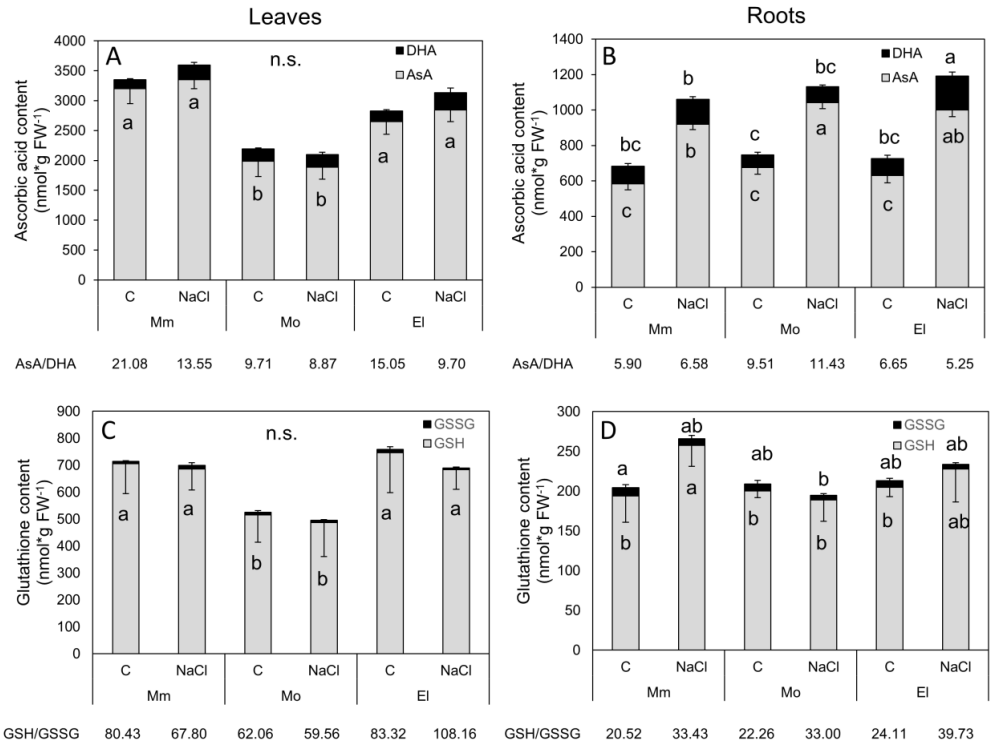

Fig. 2 Ascorbic acid (reduced – AsA, oxidized – DHA) and glutathione (reduced – GSH, oxidized – GSSG) contents and their ratio in 5-week-old tomato cultivars under control conditions and after one week of 100 mM NaCl treatment (NaCl). Mm – ‘Moneymaker’, Mo – ‘Mobil’, El – ‘Elán F1’. Means ± SD. Columns with different letters (regular font for GSH and AsA, italic font for GSSG and DHA) are significantly different at $p \leq 0.05$, determined by Duncan’s test. n.s.–not significant.

The GST activity was higher compared to the control conditions in 'Moneymaker' and 'Elán F1' leaves (by 160.1 % and 91.7 %, respectively) and roots (by 44.7 % and 81.6 %, respectively) **after one week of 100 mM NaCl treatment**. On the other hand, in leaves of 'Mobil' the GST activity decreased by 27.7 % and increased in roots only by 25.5 % due to applied salt stress compared to control conditions (Fig. 3E, F). The smallest difference between the cultivars after salt stress was measured in the GPOX activity. While there were no significant changes in the GPOX activity of leaves, it was increased in roots. **The lowest GPOX activity after salt stress was detected in 'Mobil' roots** (Fig. 3G, H).

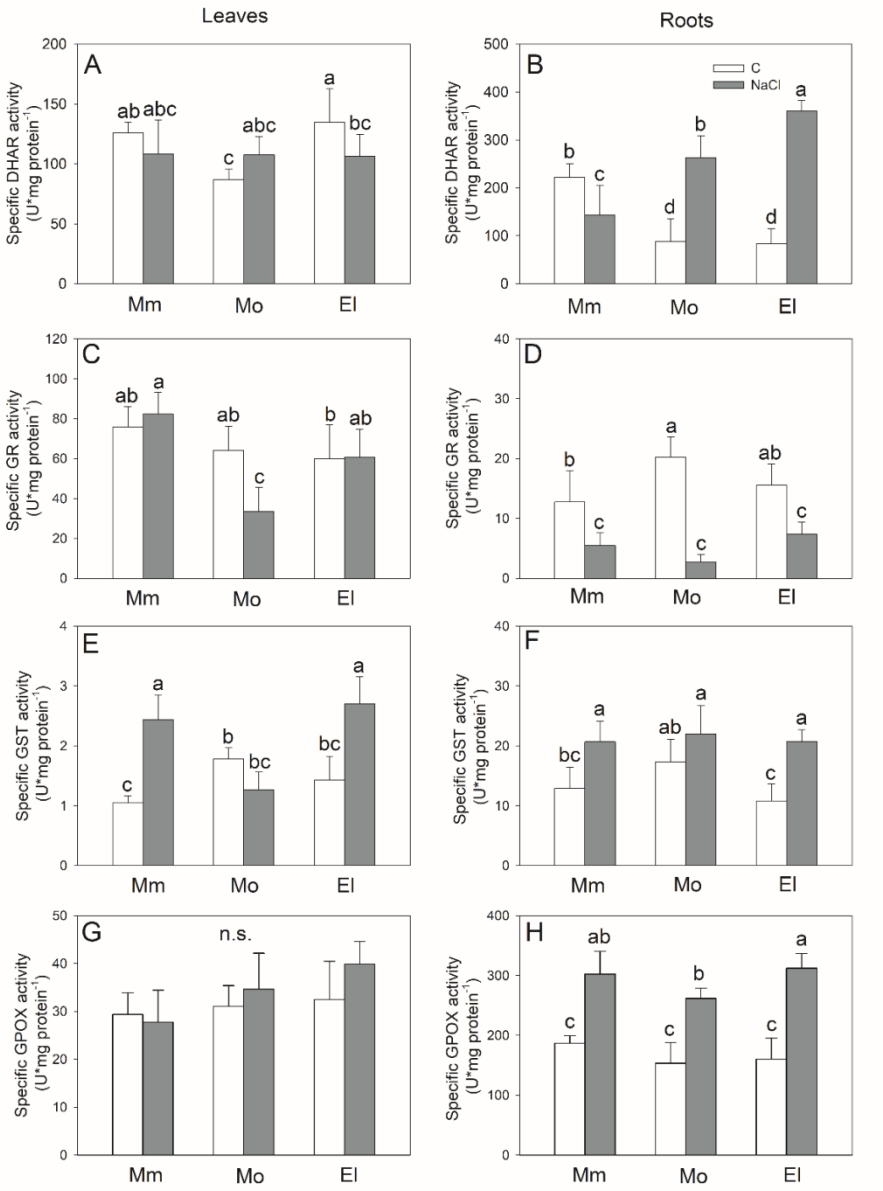

Fig. 3 Specific glutathione reductase (GR), transferase (GST) and peroxidase (GPOX) activities of tomato in 5-week-old tomato leaves (A, C, E, G) and roots (B, D, F, H, respectively) under control conditions and after one week of 100 mM NaCl treatment. Mm – 'Moneymaker', Mo – 'Mobil', El – 'Elán F1'. Means ± SD. Columns with different letters are significantly different at $p \leq 0.05$, determined by Duncan's test. n.s.–not significant.

HT-qPCR and quantitative real-time PCR (qPCR, qTOWER Real-Time qPCR System, Analytik Jena, Jena, Germany) were used to determine the expression of 29 selected genes in the leaves and roots of three investigated cultivars. After one week of salt stress treatment **induction of *GR1* and *GR2* genes was detected in 'Elán F1'**. Furthermore, **upregulation of 2, 1 and 1 *GSTs* and repression of 10, 6 and 9 genes was measured in leaves of 'Moneymaker', 'Mobil', and 'Elán F1', respectively**. In roots, induction of *GSTU15*, *-32* and *-47* was measured in all three cultivars after the treatment, in addition expression of 6 genes (*GSTU5*, *-U14*, *-U26*, *-U27*, *-U51*, *-U54*) was higher in 'Elán F1' than in control plants and that of two genes (*DHAR5*, *GSTU25*) in 'Mobil'(Fig. 4A, B). Among the investigated genes, repression due to salt stress was detected only in two cases in roots: at *GSTF3* in 'Moneymaker' and *DHAR3* in 'Mobil'. **Although only 3 *GSTUs* expression increased in roots of 'Moneymaker' after salt stress, the GST and GPOX activities were elevated on a similar manner than in 'Elán F1', where 9 genes were induced** (Fig. 4B). It can be supposed that the GSH/GSSG ratio which increased the most in 'Elán F1' roots may contribute to the more explicit upregulation of genes. However, 5 *GST* genes also were induced in 'Mobile' roots, which showed smallest increase in GST and GPOX activities (Fig 3, 4).

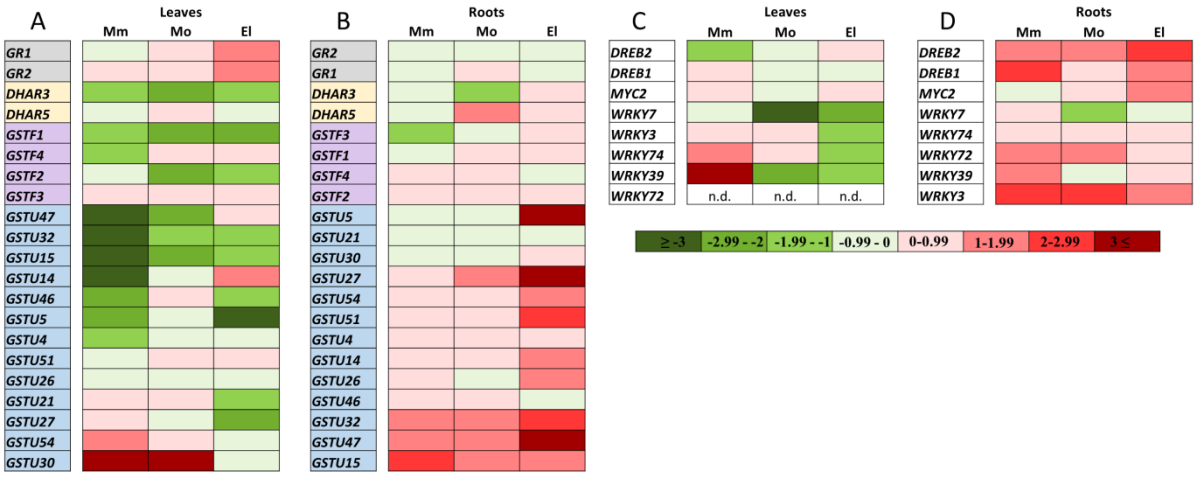

Fig. 4 Heat map of the expression levels of two *Solanum lycopersicum* glutathione reductases (*GR1*, *GR2*) and 19 selected genes belonging to 3 GST classes (*DHAR*, *GSTF*, *GSTU*; A, B), and 8 transcription factors (C, D) determined in salt treated five weeks old 'Moneymaker' (Mm), 'Mobil' (Mo) and 'Elán F1' (El) tomato cultivars. Relative transcript amounts of the genes in leaves (A, C) and roots (B, D) were determined by HT-qPCR after 1 week of 100 mM NaCl treatment. The expression of genes was normalized fist by the average of *actin2* and *elongation factor 1α* genes, and second to the average transcript amount of each gene in untreated cultivars. Genes were arranged according to the increasing expression level measured in 'Moneymaker'. Green colours show repression, while red colours show activation, as it is indicated in the colour scale bar. Log_2 of $2^{-\Delta\Delta Ct}$ data are presented in the heat map. The presented data are from two biological replicates.

To reveal the relationship between redox potential and gene expression changes, Pearson's correlation was determined. We used E_{hc} and ΔCt values for calculation, thus a strong positive

correlation means that as the root tissues become more reduced, the expression level of a given gene increases, while in case of strong negative correlation the transcript level of a specific gene increases as the root tissues become more oxidized. **The correlation analysis revealed that there are a lot of differences in the correlation coefficients (R values) in the investigated organs and cultivars.** For example, in 'Moneymaker' leaves strong positive correlation can be found between the redox potential and the expression of *GSTF1* gene, while in roots the transcript level of *GSTF1* has strong negative correlation with the redox potential. Similarly **opposite changes occurred in R values in** other **cultivars** (Fig. 5A, B), **which elucidate the importance of other signalling pathways along with redox regulation**.

Even more alterations can be found if we compare the R values of the cultivars. For example, strong positive correlations were found between the changes in expression of investigated sequences and E_{hc} in almost all cases in 'Moneymaker' leaves, while in the case of 'Elán F1' strong negative correlations were detected. While in 'Moneymaker' leaves *DHAR3*, *GSTU4*, *-5*, *-27*, *-46* showed positive correlations, in 'Elán F1' a very strong negative correlation was determined. The transcription of several *GSTs* related differently to the redox state in roots also (Fig. 5A, B).

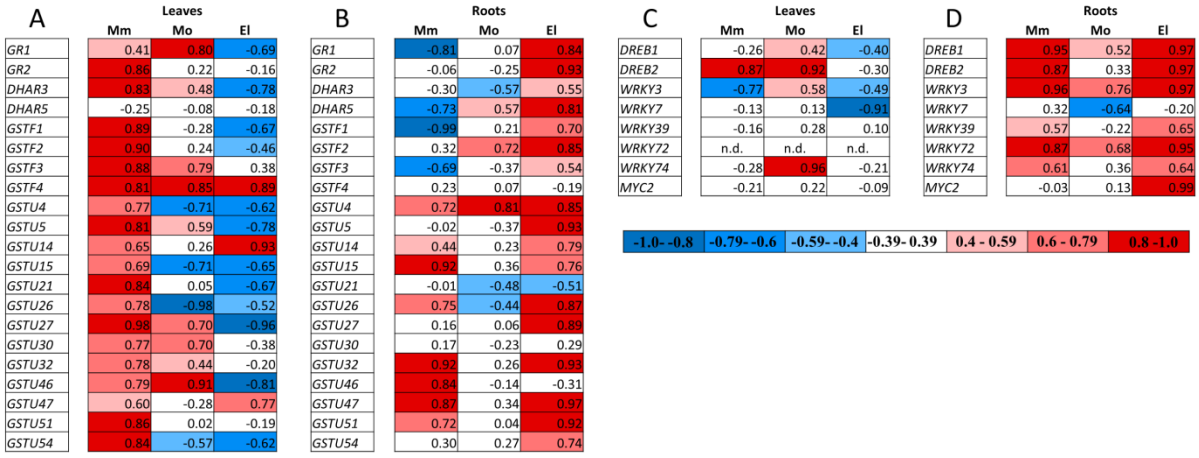

Fig. 5 Correlation analysis on the basis of the redox potential (E_{hc}) and expression of selected genes (ΔCt values) measured under different conditions in the leaves (A, C) and roots (B, D) of 'Moneymaker' (Mm), 'Mobil' (Mo) and 'Elán F1' (El) tomato plants. Red colour highlights show positive, blue colour highlights show negative correlations. n.d. – not detected

Transcriptional responses are regulated in plants either by stress- or ROS-derived changes in the signal transduction mechanisms that alter transcription factor function, or by direct or indirect ROS-induced redox regulation (Mittler et al. 2022). Screening of *cis*-regulator sequences in the 5' regulatory regions of the investigated genes (1500 bp up–stream from the start codon) found in the Sol Genomics Network (SGN) database (http://solgenomics.net) was performed using New PLACE database (https://www.dna.affrc.go.jp/PLACE/?action=newplace). The *in silico* analysis revealed different occurrence of redox-responsible *cis*-elements, such as

W–box, TGACG–motif and ABRE element in the promoter regions of the tomato *GST* genes. Based on promoter analysis and the literature expression of 8 stress responsive (*DREB1*, *DREB2*, *MYC2*, *WRKY3*, *-7*, *-39*, *-72* and *-74*) transcription factors (TFs) was investigated using qPCR in control plants and after one week of 100 mM NaCl treatment to reveal potential regulatory differences between investigated cultivars. **In leaves, induction of the selected TF genes was detected only in 'Moneymaker'. The expression level was higher after salt stress in case of 5, 4 and 3 TFs in roots of 'Moneymaker', 'Elán F1' and 'Mobil', respectively.** Induction of *WRKY74* was detected in leaves and elevated expression of *WRKY39* was measured in leaves and roots of 'Moneymaker' (Fig. 4C, D). Previous study showed that there are 81 *WRKY* genes in tomato, wherein a number of *SlWRKY* genes were significantly up-regulated under stress treatments (Huang et al. 2012). Among several others *SlWRKY39*, *SlWRKY72* and *SlWRKY74* showed induction during salt, drought and biotic stresses (Huang et al. 2012). *SlWRKY39* is a positive regulator in tomato against biotic and abiotic stresses, moreover overexpression of this TF activates the expression of pathogenesis- and environmental-stress related genes (Sun et al., 2015).

Relationship of redox potential and gene expression changes was calculated in case of TFs also, and similar results were found in all cultivars. However a strong correlation was found between *DREB2* and E_{hc} values in 'Moneymaker' and 'Mobil', but only very week in 'Elán F1'. *DREB2* is a dehydration-responsive element-binding TF, which affects the development of vegetative and reproductive organs, participates in abscisic acid signalling and mediates salt stress tolerance in tomato (Hichri et al. 2015). In roots strong correlations were detected in 'Moneymaker' and Elán F1' (Fig. 5C, D).

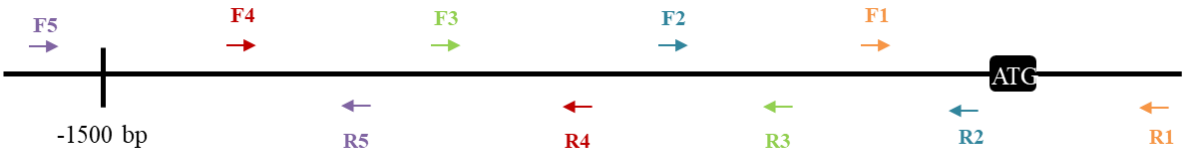

Fig. 6 Schematic representation of the **s**ites of primer pairs used in the sequencing study. Primer pairs are labelled with the same number and colour. F – forward, R – reverse, ATG – start codon.

Since our results indicated different regulation of the homologous genes in the investigated cultivars with different stress tolerance, further analysis of promoter region of selected tomato genes has been performed. Five overlapping primer pairs were used to amplify approximately 1500 bp long region upstream from the start codon as shown in Fig. 6. The circa 500 bp long PCR fragments were sequenced using the forward primers. The 5' regulatory regions of *GSTF4*, *GSTU26* and *GSTU47* genes, expressed differently in leaves or roots of tomato cultivars were determined and compared to the sequence found in the SGN database (http://solgenomics.net). The investigated region of *GSTF4* and *GSTU26* was almost identical in all cultivars and in reference sequence found in SGN, thus the same *cis*-regulator elements were identified. However, **in *GSTU47*** between -665 and -390 bp positions up–stream from

the start codon **a variable region was identified, where several nucleotide mismatches were detected in all cultivars.** Although the differences in the sequences did not change the number and position of W–box elements (WRKY binding sites), **alterations in gibberellic acid-responsive elements or in DOF (DNA binding with one finger) protein binding sites may affect the regulation of the gene expression** under control conditions and after salt stress.

As a summary, according to our results 'Moneymaker' and 'Elán F1' were capable to restore their growth during one week long salt stress but decreased growth was measured in 'Mobil'. The most sensitive 'Mobil' cultivar was characterized by lower GSH and AsA levels, and decreased GR and GST activities after salt treatment compared to 'Moneymaker' and 'Elán F1'. The gene expression pattern of *GR*, *GST* and *TF* genes was unique in all tree cultivars. Several strong and cultivar specific correlations were found among the changes in redox potential and gene expressions, which highlighted the complexity of the stress response of the tomato. Our results indicate that lower GSH and AsA levels, and more positive redox potential of glutathione influenced negatively the salt stress response of 'Mobil'. Since 'Moneymaker' and 'Elán F1' had higher GR and inducible GST activities, it is suggested that these, along with elevated non-enzymatic antioxidant levels supported the salt tolerance of plants.

III. References

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IV. Dissemination of the results

Publications:

1. Horváth E, Bela K, Gallé Á, Riyazuddin R, Csomor G, Csenki D, Csiszár J (2020) Compensation of mutation in *Arabidopsis glutathione transferase* (*AtGSTU*) genes under control or salt stress conditions. International Journal of Molecular Sciences, 21(7): 2349. doi: 10.3390/ijms21072349 (impact factor: 4.556)
2. 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. (impact factor: 5.437)
3. Horváth E, Bela K, Kulman K, Fargó N, Riyazuddin R, Gallé Á, Puskás LG, Csiszár J (2023) Glutathione Transferases are Involved in Salicylic Acid-Induced Transcriptional Reprogramming. Journal of Plant Growth Regulation. https://doi.org/10.1007/s00344-023-10915-2 (impact factor: 4.640)

Manuscript under publication:

Horváth E, Kulman K, Hajnal Á, Tompa B, Pelsőczi A, Bela K, Gallé Á, Csiszár J: Identification of *glutathione transferase* genes participating in a successful salt stress response of tomato plants

Manuscript under preparation:

Horváth E, Kulman K, Hajnal Á, Tompa B, Pelsőczi A, Bela K, Gallé Á, Csiszár J: G*lutathione transferase* genes participate in the successful osmotic stress response of tomato plants

Book chapter

1. 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

Lectures:

1. Csiszár J, Gallé Á, Horváth E, Bela K, Hajnal ÁB, Erdei, Tari I, Fehér A. A stresszválaszok és redox folyamatok fiziológiai és molekuláris vizsgálatai az SZTE Növénybiológiai Tanszéken. In: Györgyey János (szerk.), XIII. Magyar Növénybiológiai Kongresszus: Összefoglaló kötet. Konferencia helye, ideje: Szeged, Magyarország 2021.08.24. - 2021.08.27. p. 40. (2021). ISBN: 9786150123509
2. Csiszár J, Bela K, Horváth E, Gallé Á, Hajnal Á, Tompa B, Riyazuddin R, Rigó G, Szabados L, Fehér A (2022) New hub in the signal transduction network connecting redox regulation to plant development: the glutathione-linked antioxidant enzymes. BRC, Szeged "Straub napok 2022"
3. Horváth E (2022) A glutation transzferázok szerepe a stresszválaszban és a redox állapot fenntartásában. MATE GBI, Gödöllő, "MTA MBTB Köztestületi tagok fóruma"

Posters:

1. 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.
2. 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.
3. Horváth E, Bela K, Hajnal Á, Feigl G, Kulman K, Gaál M, Csiszár J. Paradicsom fajták sóstressz válaszának összehasonlító vizsgálata. In: Györgyey János (szerk.), XIII. Magyar Növénybiológiai Kongresszus: Összefoglaló kötet. Konferencia helye, ideje: Szeged, Magyarország 2021.08.24. - 2021.08.27. p. 65. (2021). ISBN: 9786150123509
4. Bela K, Milodanovic D, Riyazuddin R, Farkas A, Lkhagvadorj O, Horváth E, Gallé Á, Bangash SAK , Poór P, Csiszár J. Van-e az AtGPXL3-nak szerepe az ER stresszválaszban? In: Györgyey János (szerk.), XIII. Magyar Növénybiológiai Kongresszus: Összefoglaló kötet. Konferencia helye, ideje: Szeged, Magyarország 2021.08.24. - 2021.08.27. p. 60. (2021). ISBN: 9786150123509
5. Horváth E, Feigl G. Different nitro-oxidative response of tomato cultivars to salt- and osmotic stress. In: Zsuzsanna Kolbert, Gábor Feigl, Árpád Molnár, Ágnes Szepesi, Attila Bodor, Attila Fehér (szerk.). 8th Plant Nitric Oxide International Meeting: Program & Book of Abstracts. Konferencia helye, ideje: Szeged, Magyarország 2021.07.07. - 2021.07.09. p. 59. (2021)
6. Horváth E, Bela K, Kulman K, Gallé Á, Riyazuddin R, Csiszár J. Early response in salicylic acid-treated roots of Arabidopsis *glutathione transferase* (*Atgstf8* and *Atgstu19*)

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8. Bela K, Milodanovic D, Riyazuddin R, Farkas A, Horváth E, Gallé Á, Bangash SAK, Poór P, Csiszár J. ER stress response of *Atgpxl3* plants. In: Plant Biology Europe 2021 Abstract Book. Konferencia helye, ideje: Torino, Olaszország 2021.06.28. - 2021.07.01. p. 241. (2021)
9. Csiszár J, Bela K, Hajnal Á, Horváth E, Faragó N, Puskás L, Gallé Á. Glutathione transferases in roots as antioxidants and redox modulators. In: Plant Biology Europe 2021 Abstract Book. Konferencia helye, ideje: Torino, Olaszország 2021.06.28. - 2021.07.01. p. 278. (2021)
10. Hajnal Á, Csiszár J, Bela K, Horváth E, Faragó N, Puskás L, Gallé Á. The connection between glutathione transferases and redox state – a comparative analysis of two tomato cultivars. In: Plant Biology Europe 2021 Abstract Book. Konferencia helye, ideje: Torino, Olaszország 2021.06.28. - 2021.07.01. p. 288. (2021)
11. Horváth E, Kulman K, Gaál M, Bela K, Feigl G, Hajnal Á, Tompa B, Gallé Á, Csiszár J (2022) Focusing on the role of glutathione and related processes in salt stress response of tomato cultivars. In: 8th International Symposium on Structure and Function of Roots, Book of Abstracts. Horný Smokovec, Szlovákia 2022.06.12. - 2022.06.16. p. 26.

MSc dissertations:

Kulman Kitti: Glutation transzferázok szerepe a paradicsom fajták sóstressz válaszában

BSc dissertations:

Gaál Marcell: Az antioxidánsok szerepe különböző paradicsom fajták ozmotikus stresszválaszában

V. Justification of the modifications compared the original research plan and work plan

Research activities:

Some delay occurred at the experiments, due to the unexpected circumstances caused by the COVID-19 pandemic as well as the increase in utilities, which resulted in the temporary closures of the University of Szeged.

Changes in the Budget plan:

An administrative modification of the project was approved on July 14, 2022 and it was extended to January 31, 2023. In the meantime, the legal contribution rate of salaries was decreased, and the remaining sum was spanned on material costs.