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# SCREENING OF GENE EXPRESSION ASSOCIATED WITH SALINITY AND UVB TOLERANCE IN LOCAL TOMATO LANDRACES

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***Abstract***

*Rapid climate change in recent years may significantly impact future generations through various climate factors affecting crop production. As a result, a primary global concern is developing new crop plant varieties that are more resistant to abiotic stress factors such as high temperatures, salinity, drought, and UVB radiation. These new plant varieties may be selected from local landraces that thrive in the affected soils. Therefore, the present study focuses on several local tomato landraces from northwestern Romania, cultivated in soils impacted by salinity, drought, and high temperatures. This research evaluated the gene expression of selected genes related to abiotic stress, aiming to identify valuable molecular traits among the local landraces that confer resilience to fluctuating environmental conditions. This approach could allow for the breeding of new tomato varieties to meet the food industry and consumers' quantitative and qualitative demands.*

***Key words****: climatic changes, DREB, HSP70, gene expression, PSIID2, SOD.*

## INTRODUCTION

A synthesis report from the Intergovernmental Panel on Climate Change (IPCC) states that human activities, primarily greenhouse gas emissions, undoubtedly cause the warming trend. Current environmental data shows that the global surface temperature has increased by 1.1˚C compared to the levels recorded between 1850 and 1900 from 2011 to 2020 (https://www.ipcc.ch/).

These adverse environmental conditions seriously affect the distribution and growth of crop plants (Guo & Wang, 2011). Therefore, considering the global population is estimated to double by the year 2050, it is imperative to identify viable solutions to ensure food safety and security (www.fao.org).

The discovery and development of new crop plant varieties that have improved resistance to abiotic stresses, such as heat, drought, salinity, and UVB radiation, are major global priorities (Machado & Seralheiro, 2017; Ors & Suarez, 2017; Martinez et al., 2018; Nowicka et al., 2018).

Plants face various abiotic stresses, which force them to develop a complex array of tolerance mechanisms for survival (Koyro, 2012; Zhang et al., 2022). To resist these environmental conditions, such as drought, extreme heat, salinity, and increased UVB radiations, plants generate several physiological and metabolic responses (Knight & Knight, 2001; Hirayama & Shinozaki, 2010; Mantri et al., 2012; Chen et al., 2022).

Plant protective mechanisms against UVB radiation include increasing the expression levels of genes in the oxidative defense system, synthesizing secondary metabolites, repairing DNA, and producing protective compounds that can absorb UVB radiation, such as flavonoids and phenolic compounds. They also accumulate osmoprotectants like proline and glycine betaine (Jordan, 2002; Ballaré, 2003; Ashraf and Foolad, 2007; Zlatev et al., 2012; Sharma et al., 2017; Fraikin, 2018; Chen et al., 2022).

Individual or combined abiotic stress factors differentially induce the expression of the HSP70 (heat shock protein 70) and *PSIID2* (photosystem II D2) genes (Polenta et al., 2020).

These characteristics enhance crop plants' potential to significantly broaden the range of viable growing environments (Ramonell & Somerville, 2002; Broccanello et al., 2023).

Plants can demonstrate phenotypic plasticity under various environmental conditions (Tester & Langridge, 2010). Quantitative analyses of plant traits are essential for quickly selecting crop varieties that thrive in resource-limited environments and challenging soil conditions (Fiorani & Schurr, 2013).

Romania has a significant amount of soil affected by salinization. In Bihor County, located in the northwestern part of the country, approximately 9.11% of the land is impacted by varying degrees of salinity: weak salinity affects 38122 hectares, moderate salinity affects 900 hectares, and strong salinity impacts 400 hectares (http://apmbh.anpm.ro/). Local tomato landraces have been identified growing in these saline-affected soils. These local landraces enable cultivation on soils already impacted by elevated salinity or drought conditions.

By incorporating traits that improve resilience to these stressors, farmers or breeders can continue to develop crops in areas that were previously unsuitable for cultivation.

From a literature review we selected four genes that we consider relevant in investigating resistance to both saline and UVB stress (He et al., 1993; Guo et al., 2011; Gadi, 2018; Ors et al., 2021). After confirming the presence of the target genes: *SOD* (superoxide dismutase), *DREB* (dehydration responsive element-binding protein), *HSP70* (heat shock protein 70), and *PSIID2* (photosystem II D2), a comparative analysis of their expression levels was conducted. This analysis compared the studied landraces with a control variety under varying salt concentrations and UVB stress conditions, aiming at identifying valuable molecular traits in studied landraces that confer resilience to varying environmental conditions.

## MATERIAL AND METHODS

1. ***Experimental design and stress treatments***

Four different varieties of tomatoes were used in the experiments, with seeds sourced from local farmers in Bihor County. The landraces were named after their collection sites: 'Ateaș 136', 'Ateaș 37', and 'Cefa 7', while 'Marmande', a conventional cultivar, served as the control group for comparison with local landraces. The seeds were sown in pots (Ø 10 cm) filled with Terra peat soil. After germination, the plants were grown under a 16/8 hours light/dark cycle until they developed their first layer of true leaves.

Four groups of plants, each group with eight plants, were established for the treatments and control group, respectively. The treatments consist of two different salt concentrations and a combination of salt (NaCl) and UV-B radiation as follows: 300 mM NaCl – variant 1 (V1), 300 mM NaCl and UV-B radiation – variant 2 (V2), 450 mM NaCl – variant 3 (V3). The control groups (untreated plants, Ctrl) were regularly watered to maintain soil moisture at 60% of its total water-holding capacity and treated plants were watered in the same manner but with a saline solution. UVB radiation was applied for three consecutive days, two hours per group of plants, with three treatment cycles. The UVB radiation level used (0.45-0.55 $mW/cm^2$) represented an approximate 30% increase over the UVB radiation levels measured outdoors on a sunny day at noon when solar intensity peaks.

2. ***Determining the expression of target genes in tomato landraces subjected to saline and UVB stress***

To determine the expression of the target genes (the antioxidant gene *SOD*, stress-related genes *DREB* and *HSP70*, and the photosynthesis-associated gene *PSIID2*) in the studied tomato landraces, plant leaves were collected for total RNA isolation, cDNA synthesis, and quantitative Real-Time PCR using a modified protocol from Sicora et al. (2006) and McGinn (2003). Briefly, the leaf material (apx. 80 mg) were homogenized in Trizol (Invitrogen) using a SpeedMill homogenizer (Analitik Jena) and then the RNA extraction protocol was followed as described previously (Sicora et al., 2006, McGinn et al., 2003). For cDNA synthesis was used SensiFAST cDNA Synthesis kit (Meridian Bioscience).

The SsoAdvanced Universal SYBR Green Supermix kit (BioRad) was utilized for RT-PCR, along with the BioRad CFX 96 real-time amplification and detection system with specific fluorophores. All genes were tested with the appropriate primers, using tubulin (*TB*) as the reference gene. The primer sequences employed are similar to those used by Raja et al. (2020) (Table 1).

Table 1. Primer sequences used in genetic characterization experiments

| Gene name | Primer sequence (5'– 3) | Product size |
|---|---|---|
| SOD | | |
| F | ACT ATC TTC TTC ACC CAG GA | 281 |
| R | GAG TTT GGT CCA GTA AGA GG | |
| DREB | | |
| F | TGG CGT TAG GGT TTT CCG AT | 193 |
| R | GCG GGT GCT TTT CGA GTT TT | |
| HSP 70 | | |
| F | GCA CCA TCC ACT TCA CCC AA | 220 |
| R | CCC TGA AGT CCA ATG ATC CCA | |
| PSII D2 | | |
| F | TCC TAG GGC GGT TTT GAT GG | 205 |
| R | AGT GGC AAA CCT GGA ATC CT | |
| TB | | |
| F | GAT AAC TGT ACT GGA CTG GAA GG | 250 |
| R | GGA TGG CTT CGT TAT CCA AGA G | |

Note: F – forward primer, R – reverse primer

After confirming the presence of target genes, the relative abundance of transcripts was mathematically calculated from the gene-specific amplification curves.

A comparative analysis quantified the expression levels of each target gene in the studied landraces and the control variety, which were exposed to different concentrations of salt stress and intensities of UVB radiation.

3. ***Data analysis***

In all experiments three technical replicates were used and an average of the Ct value was plotted.

The gene expression data were presented as (i) expression of the studied genes in different landraces and in the conventional cultivar in relative transcription units and the normalization was done to control sample hence the gene expression of the control samples has the value of 1 (Figures 1-4).

The expression of the studied genes was also presented (ii) relative to the control cultivar, hence the expression of Marmande cultivar always has the value of 1 (Figures 5-8).

For data analysis the Microsoft Excel for MAC 2011 data processing program was used.

## RESULTS AND DISCUSSION

Salinity and UVB stress, both alone and in combination, are critical environmental factors in current climate change. When these conditions exceed a plant's protective capacity, they trigger intrinsic molecular protection mechanisms that help mitigate the negative physiological effects of the stressor (Mladin et al., 2018). At the cellular level, numerous genes respond by altering their expression in reaction to these stressful conditions (Agarwal et al., 2007; Rampino et al., 2012; Johnson et al., 2014; Harb et al., 2015; Zandalinas et al., 2017). The level of response and its amplitude is directly correlated with the perceived intensity of the stressor. These protective mechanisms can be broadly categorized into two groups based on their mode of action: some attenuate the impact of the stressor, while others induce metabolic changes within cells to counteract the harmful effects (Reddy et al., 2016). In the present study, in 'Marmande's control variety, the *SOD* gene's expression is minimal under control conditions. Post-treatment gene expression was measured, normalized against the control value. Thus, the highest expression of the *SOD* gene is observed after treatment with 300 mM salt, while the lowest is seen following treatment with 450 mM salt. In the case of the combined salt and UVB treatment, the gene expression level is higher than that observed with the high salt concentration but lower than that observed with 300 mM NaCl treatment (Figure 1a).

The expression of the *DREB* gene follows the same pattern as that of the *SOD* gene, with the lowest expression observed in the control and the highest after treatment with 300 mM NaCl. The combined treatment leads to an overexpression of the *DREB* gene, with a level slightly lower than that observed after treatment with 300 mM NaCl, but much higher than after the 450 mM NaCl treatment (Figure 1b).

The expression of the *HSP70* gene is lowest under control conditions, and with the administration of 300 mM NaCl, a significant increase in its expression is observed. In the case of the combined salt and UVB treatment as well as after 450 mM NaCl, *HSP70* expression is very low, yet still higher than in the control (Figure 1c).

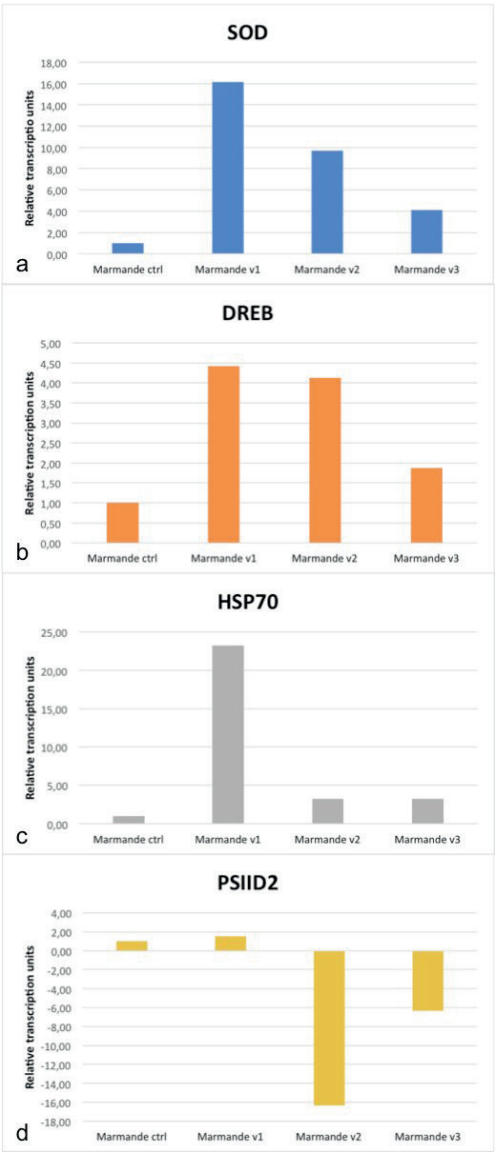


Figure 1. Gene expression of studied genes *SOD*, *DREB*, *HSP70* and *PSIID2*, in the control 'Marmande'; compared to the control plants and the three treatments variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

In the case of the PSIID2 gene, following treatment with 300 mM NaCl, its expression is slightly higher than in the control. Under the combined treatment and the higher salt concentration treatment, expression drops dramatically, with the greatest decrease observed in the combined salt and UVB treatment (Figure 1d).

In local landrace 'Ateaș 37', in the control, the expression of the *SOD* gene is the lowest compared to its expression following the treatments. Thus, the highest expression of the *SOD* gene is observed after the 450 mM salt treatment, while the lowest expression is observed after the 300 mM NaCl and UVB treatment (Figure 2a).

The expression of the *DREB* gene follows the same pattern as that of the *SOD* gene, with the lowest expression observed in the control and the highest following treatment with 450 mM NaCl. The treatments with 300 mM NaCl and the combination of 300 mM NaCl and UVB result in similar levels of *DREB* gene (Figure 2b).

The expression of the *HSP70* gene is the lowest in the control, while a significant increase in its expression is observed after treatment with 450 mM NaCl. Under the combined NaCl and UVB treatment, as well as after 300 mM NaCl salt stress, *HSP70* expression is low, but still higher than in the control (Figure 2c).

In the case of the *PSIID2* gene, during the combined salt and UVB treatment, expression is repressed, with higher expression values observed in the 300 mM NaCl and 450 mM NaCl treatments compared to the control (Figure 2d).

In the local landrace 'Ateaș 136', the expression of the *SOD* gene is repressed under the 300 mM saline solution and UVB treatment, as well as the 450 mM saline treatment. However, gene expression following the 300 mM saline treatment is slightly higher than in the control plants (Figure 3a).

The expression of the *DREB* gene is also repressed under the 300 mM NaCl treatment and the 300 mM NaCl combined with UVB. The highest expression, significantly greater than in the control plants, is observed under the 450 mM NaCl treatment (Figure 3b).

For the *HSP70* gene, expression levels are relatively similar across all treatments, yet significantly higher than in the control plants (Figure 3c).

The expression of the *PSIID2* gene is visibly repressed under all treatments compared to the control plants, with consistently low expression levels (Figure 3d).

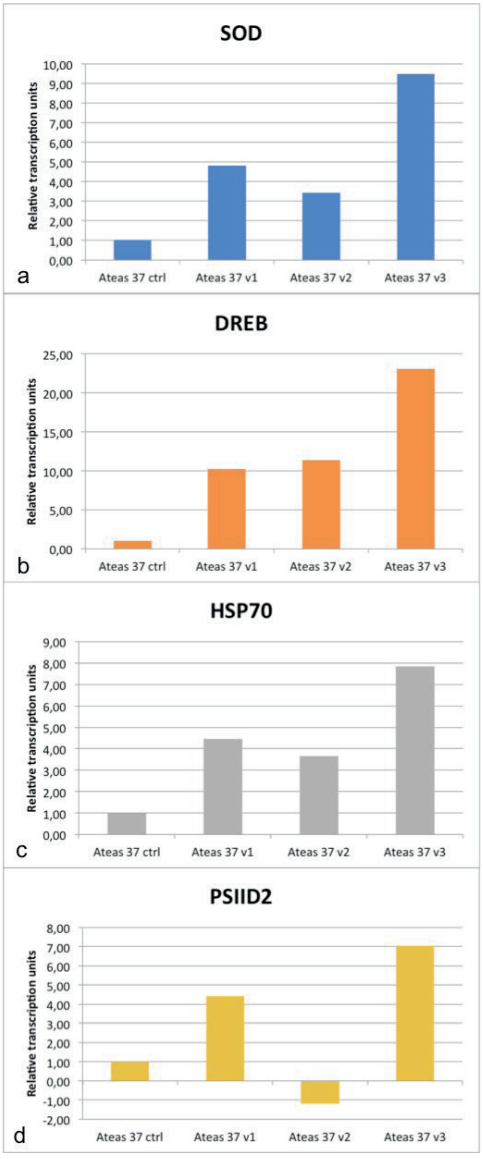


Figure 2. Expression of the studied genes – *SOD*, *DREB*, *HSP70*, and *PSIID2* in the local landrace 'Ateaș 37', compared with the control plants and the three treatment variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

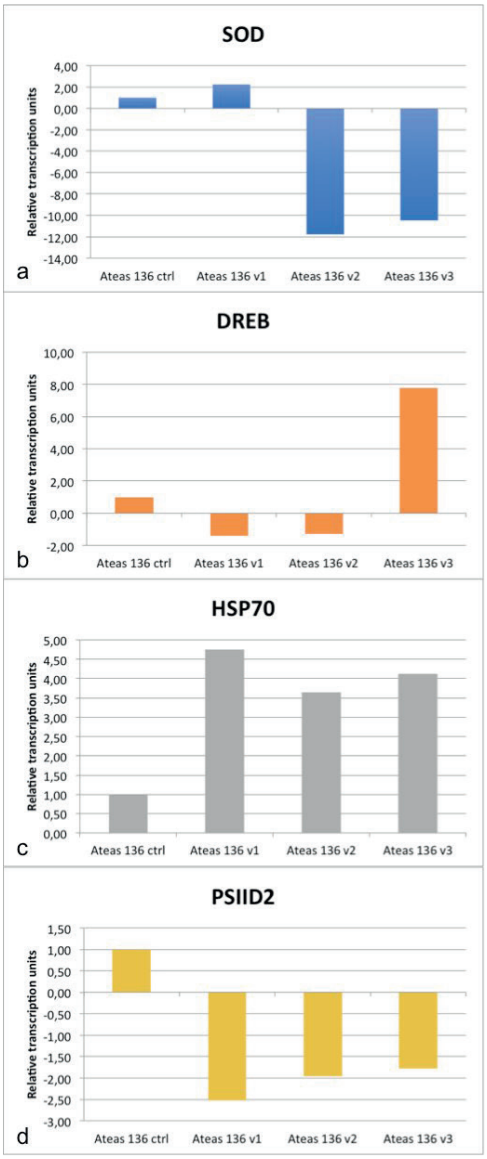


Figure 3. The expression of the studied genes - *SOD*, *DREB*, *HSP70* and *PSIID2* in the local landrace 'Ateaș 136', compared to the control plants and the three treatments variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

In the local landrace 'Cefa 7', the highest expression of the *SOD* gene is observed under the 300 mM saline solution combined with UVB treatment.

Meanwhile, in the 300 mM and 450 mM saline treatments, expression is slightly higher than in the control plants (Figure 4a).

The expression of the *DREB* gene follows the same pattern as the *SOD* gene but with higher values. The highest expression is observed under the 300 mM saline solution combined with UVB treatment, while the values resulting from the other two treatments are very close to those of the control plants (Figure 4b).

The expression of the *HSP70* gene is highest under the 300 mM saline solution combined with UVB treatment, while the lowest values, which are very similar, are observed in the 300 mM saline treatment and the control plants (Figure 4c).

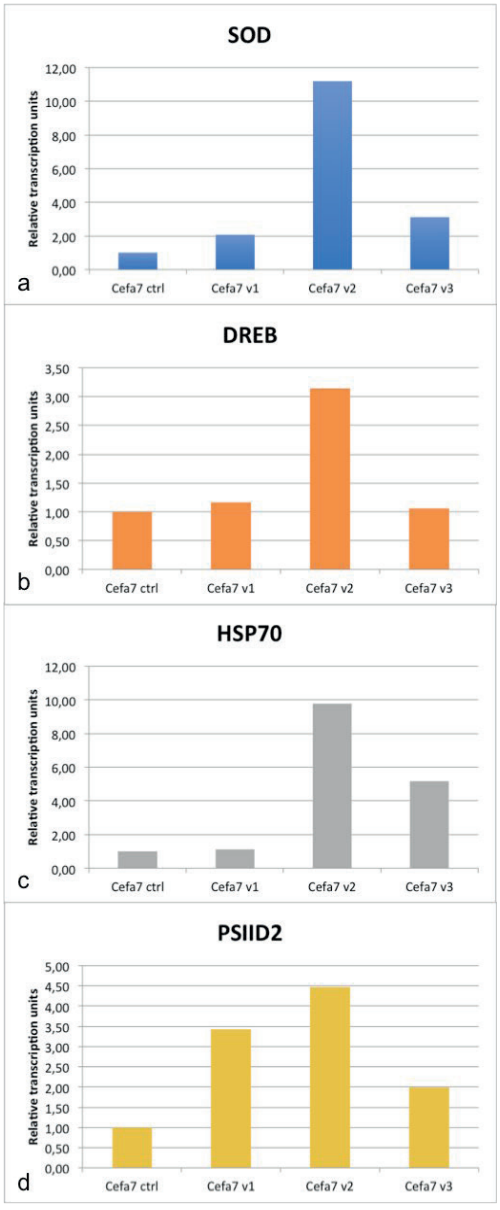


Figure 4. The expression of the studied genes, *SOD*, *DREB*, *HSP70* and *PSIID2* in the local landrace 'Cefa 7' compared to the control plants and the three treatments variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

The highest expression of the *PSIID2* gene is observed in plants treated with 300 mM saline solution combined with UVB, followed by those treated with 300 mM saline solution. Plants treated with 450 mM saline solution have gene expression levels similar to those of the control plants (Figure 4d).

The expression of the *SOD* gene (Figure 5) is generally repressed across all treatment variants. The highest expression level is observed in the control plants of the local landrace 'Ateaș 136'. The same landrace, when treated with 300 mM saline solution**,** shows positive expression values similar to those of the local landrace 'Ateaș 37' treated with 450 mM saline solution.

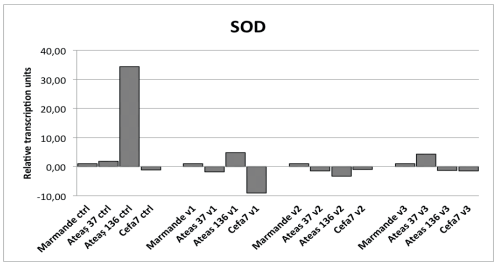


Figure 5. *SOD* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

For the *DREB* gene (Figure 6), expression is repressed in the control plants of the 'Ateaș 37' and 'Ateaș 136' landraces, in all local landraces treated with 300 mM saline solution, as well as in the 300 mM saline + UVB and 450 mM saline treatments for the same landraces.

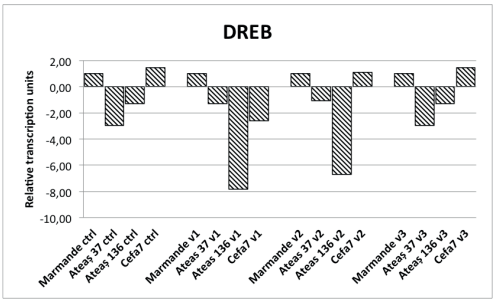


Figure 6. *DREB* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl și UVB, V3 – 450 mM NaCl)

Low but positive values are observed in the control plants of the 'Marmande' reference variety and the local landrace 'Cefa 7'. Following treatment with 300 mM saline solution, positive gene expression values are recorded in the 'Marmande' variety. The 300 mM saline + UVB treatment induced *DREB* gene expression in both the 'Marmande' variety and the 'Cefa 7' landrace**,** with values similar to those obtained in plants treated with 450 mM saline solution**.**

The *HSP70* gene exhibits low positive values (ranging from 0 to 8 relative transcription units) in nearly all the plants studied. The exception is observed in the repression of the gene in all the plants of the local landraces treated with 300 mM saline solution (Figure 7).

The expression of the *PSIID2* gene shows significant positive values in the local landraces 'Ateaș 136' and 'Cefa 7' treated with 300 mM saline solution and UVB. Minimum positive values are observed in the control plants of the 'Marmande' variety and the local landrace 'Ateaș 136' (Figure 8).

Similar values were obtained in the 'Marmande' variety plants treated with 300 mM saline solution, in the **'**Marmande' variety and 'Ateaș 37' landrace plants treated with 300 mM saline solution and UVB**,** and in the 'Marmande' variety and 'Ateaș 136' landrace plants treated with 450 mM saline solution. The gene is repressed in the control plants of the 'Ateaș 37' and 'Cefa 7' landraces, as well as in the 300 mM saline-treated plants of the 'Ateaș 37', 'Ateaș 136', and 'Cefa 7' landraces**,** and in the 450 mM saline-treated plants of the 'Ateaș 37' and 'Cefa 7' landraces.

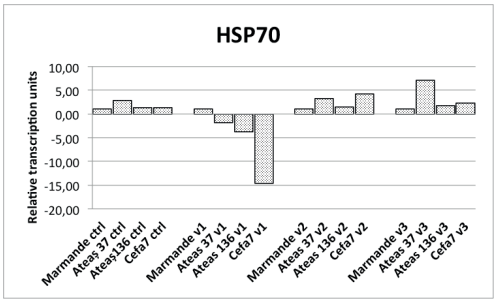


Figure 7. *HSP70* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

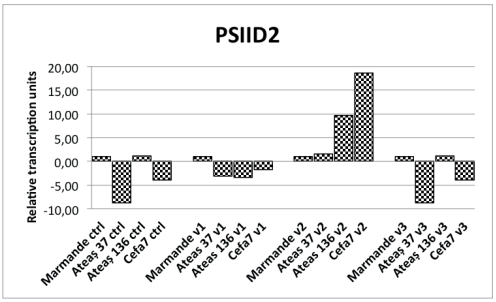


Figure 8. *PSIID2* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl și UVB, V3 – 450 mM NaCl)

*SOD* gene (superoxide dismutase) encodes a protein that protects the cell from the effect of abiotic stress (water stress) by protecting the cell structures from the reactive oxygen species (Teixeira et al., 2006; Lata & Prasad, 2011; Zandalinas et al., 2017).

ABA is a phytohormone that regulates a series of metabolic processes and is a signal transductor for stress signaling in plants. *DREB* (dehydration responsive element binding) is a transcription factor that regulates stress signal transduction in an ABA - independent manner and is involved in modulating gene response in plants that are exposed to abiotic stress (Nakashima et al., 2000; Agarwal et al., 2007).

It was shown previously that in plants treated with different abiotic stresses or a combination of stress factors the expression of heat shock proteins (HSPs) along with other enzymes involved in reactive oxygen species detoxification, glycolysis and photosynthesis are induced (Rampino et al., 2012; Johnson et al., 2014; Raja et al., 2020). Elevated levels of *HSP70* gene expression during our study for two of studied tomato landraces is in line with other studies that also reported increased transcript expression of *HSP70* (Rizhsky et al., 2004). In our study, the gene responsive to abiotic stress are induced when the cell enters in stress - mode. At the local landraces, the amount of transcript of stress induced genes is not so high as in the case of control variety 'Marmande'. This can be explained by the higher intrinsic resilience to stress showed by the local varieties. However, when the stress level exceeds the response threshold, the local

varieties respond better than ‘Marmande’ by inducing the stress response specific genes.

Plants exposed to different abiotic stresses showed suppression of many photosynthetic genes. Some studies showed induced expression of *PSIID2* gene under single or combined stress (Raja et al., 2020) while other showed down-regulated expression of genes involved in photosynthetic process (Rampino et al., 2012; Johnson et al., 2014). It was suggested that this variation in the expression profiles of photosynthetic genes is due to the fact that photosynthesis is more affected by stress conditions than other cellular processes. In our study, the expression level of *PSIID2* transcript is downregulated in the case of combined stress and high salt level in ‘Marmande’ variety and ‘Ateaş 37’ only for combined stress, and in ‘Ateaş 136’ landrace the expression is downregulated for all treatments. In ‘Cefa 7’ in all treatment variants and ‘Ateaş 37’ in the case of combined stress, the expression is up regulated. These findings suggest a more sensitivity to applied stresses for ‘Ateaş 136’, ‘Marmande’ and ‘Ateaş 37’, whilst ‘Cefa 7’ is more resilient to stress treatments.

Overall, the local landraces that were cultivated for many years on salt-enriched soil display a higher response threshold to stress as an acclimation effect. When the level of stress triggers mechanisms of response, they also present an increased capacity to genetically induce the genes involved in cellular stress protection.

## CONCLUSIONS

Following the comparative analysis of the evolution of the expression of target genes, an increase in expression values is observed for the genes involved in the plant's response to stress.

The ‘Marmande’ variety generally shows a higher level of induction of stress-response genes compared to samples from the local landraces. This effect is likely related to the greater tolerance of these local landraces to saline stress**,** UVB stress, and the combined application of both stress types. Thus, plants from the local landraces perceive the applied stress as less intense and activate the molecular protection mechanisms at a lower level compared to the ‘Marmande’ variety plants.

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