Acta Bot. Croat. 80 (1), 35–42, 2021
DOI: 10.37427/botcro-2021-010

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Expression of genes for selected plant aminoacyl-tRNA synthetases in the abiotic stress

Jurica Baranašić[1#], Anita Mihalak[1], Ita Gruić-Sovulj[1], Nataša Bauer[2], Jasmina Rokov-Plavec[1*]

[1] University of Zagreb, Faculty of Science, Department of Chemistry, Division of Biochemistry, Horvatovac 102a, HR-10000 Zagreb, Croatia

[2] University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Horvatovac 102a, HR-10000 Zagreb, Croatia

[#]Current address: Laboratory for advanced genomics, Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia

Abstract – Plants, as sessile organisms, have evolved intricate mechanisms to adapt to various environmental changes and challenges. Because various types of stress trigger significant decrease in global translation rates we examined the stress-related expression of aminoacyl-tRNA synthetases (aaRSs), enzymes that participate in the first step of protein biosynthesis. We have analyzed promoters of genes encoding cytosolic seryl-tRNA synthetase (SerRS), cytosolic aspartyl-tRNA synthetase (AspRS) and cytosolic cysteinyl-tRNA synthetase (CysRS) in *Arabidopsis thaliana* L., and examined SerRS, AspRS and CysRS gene expression in seedlings exposed to different abiotic stressors. Although global translation levels are repressed by stress, our results show that plant aaRSs expression is not decreased by osmotic, salt and heavy metal/cadmium stress. Moreover, during exposure to stress conditions we detected increased AspRS and CysRS transcript levels. SerRS gene expression did not change in stress conditions although participation of SerRS in stress response could be regulated at the protein level. Expression of the examined aaRS genes under stress correlated well with the length of their predicted promoters and the number of available binding sites for the stress related transcription factors. It thus appears that during stress it is important to keep steady state levels of aaRSs for translation of specific stress-related mRNAs and furthermore to rapidly continue with translation when stress conditions cease. Importantly, increased levels of plant aaRSs during stress may serve as a pool of aaRS proteins that can participate directly in stress responses through their noncanonical activities.

Keywords: aminoacyl-tRNA synthetase, abiotic stress, gene expression, heavy metal stress, osmotic stress, plant, salt stress, translation

Introduction

As sessile organisms, plants have evolved elaborate mechanisms to adapt to adverse environmental changes and challenges. The molecular responses in plants to abiotic stresses are probably more advanced and prominent than in animals (Qin et al. 2011). Research on how plants adjust to various environmental stresses is crucial not only to plant biologists but also to agronomists, because abiotic stress has a particularly negative impact on crop production (Cramer et al. 2011). The need to understand the mechanisms involved in plant abiotic stress responses and the generation of plants tolerant to stress has accordingly gained much attention in recent years (Qin et al. 2011). In plants, as in all eukaryotes, gene expression reprogramming plays a pivotal role in the response to abiotic stress. This reprogramming is a highly complex process, including transcription, mRNA processing, mRNA transport and stability, translation or protein turnover; each participating but having a different weight in the final protein levels (Yángüez et al. 2013). One of the earliest plant metabolic responses to abiotic stresses is the inhibition of protein biosynthesis and an increase in

* Corresponding author e-mail: rokov@chem.pmf.hr

chaperone levels controlling protein folding and processing. Repression of global protein biosynthesis is often accompanied with selective translation of mRNAs encoding proteins that are vital for cell survival and recovery from stress (Liu and Qian 2014, Merchante et al. 2017).

In the first step of protein biosynthesis, cells need to aminoacylate tRNAs with their respective amino acids in a process catalyzed by aminoacyl-tRNA synthetases (aaRSs) (Perona and Gruic-Sovulj 2014). During protein biosynthesis on the ribosome, amino acid is transferred from the tRNA onto a growing peptide according to the genetic code. Aminoacyl-tRNA synthetases are therefore housekeeping proteins that play an important role in the expression of genes to create proteins. Besides that, aaRSs may be involved in a myriad of other cellular processes exerting their noncanonical function beyond translation (Guo and Schimmel 2013, Mocibob et al. 2016). It is well documented that aaRSs participate in cellular stress responses in bacteria and metazoa. For example, vertebrate tyrosyl-tRNA synthetase has been shown to translocate to the nucleus during oxidative stress and protects against DNA damage by activating transcription factor E2F1, which promotes expression of DNA repair genes (Wei et al. 2014, Cao et al. 2017). During oxidative stress, phosphorylated methionyl-tRNA synthetase (MetRS) enhances the mischarging of methionine on non-methionyl tRNAs. Methionine carried by noncognate tRNAs is incorporated into growing polypeptides during translation and is used as scavanger of reactive oxygen species, protecting cells from oxidative damage and apoptosis (Lee et al. 2014). During oxidative stress, oxidized phenylalanyl-tRNA synthetase (PheRS) exhibits increased proofreading activity against cytotoxic tyrosine isomers, noncognate amino acids that are increased during oxidative stress. Increased proofreading of PheRS maintains high fidelity of translation even under conditions of oxidative stress (Steiner et al. 2019).

Research on plant aaRSs has revealed their cellular localization (Rokov-Plavec et al. 2008, Duchêne et al. 2009, Kekez et al. 2016), substrate specificity (Rokov et al. 1998, Rokov and Weygand-Durasevic 1999, Rokov-Plavec et al. 2002, 2004, Aldinger et al. 2012), fidelity (Rokov-Plavec et al. 2013, Lee et al. 2016, Hoffman et al. 2019) and protein interactors (Yang et al. 2018, Kekez et al. 2019). Thus far, only one crystal structure of plant aaRS has been reported (Kekez et al. 2019). As enzymes essential for protein biosynthesis in the cytosol, chloroplast and mitochondria, plant aaRSs appeared important for plant growth and development, and disruption of their function was shown to be either lethal or cause severe defects early in plant development (Berg et al. 2005, Kim et al. 2005, Kitagawa et al. 2019, Zheng et al. 2019). Participation of aaRSs in plant stress responses is poorly characterized. A few reports have indicated aaRS involvement in plant responses to stress, but the exact molecular mechanisms were not revealed. Wheat MetRS was shown to be highly expressed during biotic stress imposed by the fungal pathogen *Fusarium graminearum* resulting in significant resistance of the plant to this pathogen (Zuo et al. 2016). A recent genetic screen identified aspartyl-tRNA synthetase (AspRS) as a new master regulator of resistance to pathogens induced by beta-aminobutyric acid (BABA). This non-proteinaceous amino acid is a well-known priming agent that protects many plants from various biotic and abiotic stresses. BABA interferes with AspRS canonical activity resulting in the activation of cellular defense mechanisms (Luna et al. 2014). High throughput proteomic screen for proteins involved in the early responses of *Arabidopsis thaliana* L. to oxidation stress due to cadmium exposure identified, among other proteins, several aminoacyl-tRNA synthetases (Sarry et al. 2006).

Prompted by findings that various types of stress significantly decrease global translation rates (Merchante et al. 2017, Zhao et al. 2019) we sought to determine stress related expression levels of aaRSs, the crucial enzymes in protein biosynthesis. We have analyzed promoters of genes encoding cytosolic seryl-tRNA synthetase (SerRS), cytosolic AspRS and cytosolic cysteinyl-tRNA synthetase (CysRS), and examined SerRS, AspRS and CysRS gene expression in *A. thaliana* seedlings exposed to different abiotic stressors for 3, 6, 8 and 24 hours. Although stress induces global translation repression, our results show that expression of plant aaRS genes is not decreased. Moreover, during exposure to stress conditions we detected increased AspRS and CysRS transcript levels, while SerRS gene expression did not change. This indicates that aaRSs are required for translation of specific stress-related mRNAs, as well as for resumption of translation after stress. Furthermore, plant aaRSs may participate directly in plant stress responses through their noncanonical activities.

Materials and methods

Analysis of transcription factor binding sites in promoter region

The occurrence of transcription factor (TF) binding site motifs in the promoter of a gene may be used to predict whether the gene is regulated by a specific TF. Here we analyzed promoter sequences of genes for cytosolic SerRS (At5g27470), cytosolic AspRS (At4g31180) and cytosolic CysRS (At5g38830). EPD, AGRIS AtcisDB and AthaMap databases were used for promoter analysis and detection of TF binding sites (Yilmaz et al. 2011, Hehl et al. 2016, Dreos et al. 2017). The databases are suitable for a comprehensive analysis of genomic sequences for potential TF binding sites and provide information about experimentally validated cis-regulatory elements, as well as predicted motifs present in a specific promoter.

Plant material and stress conditions

Wild-type seeds of *Arabidopsis thaliana* (ecotype Columbia) were surface-sterilized for one minute in 70% (v/v) ethanol and then 10 minutes in 1% (w/v) Izosan (Pliva, Croatia), rinsed 5 times with distilled water. Seeds were planted on half-strength Murashige and Skoog (MS5519, Sigma, USA), 0.5% (w/v) sucrose and 0.8% (w/v) agar medium. Af-

ter three days of stratification, seeds were grown for eight days in a growth chamber under 16 h light / 8 h dark conditions (120 to 130 μmol m^{-2} s^{-1}) at 24 °C. Half of the eight-day-old seedlings were planted on the same medium as described above for control and on the medium with added stressor (200 μM $CdCl_2$, 200 mM mannitol or 200 mM NaCl). After 8 h and 24 h the seedlings were collected and snap-frozen. The other half of the eight-day-old seedlings were placed in liquid half-strength Murashige and Skoog medium with 0.5% (w/v) sucrose as control and in liquid half-strength Murashige and Skoog medium with 0.5% (w/v) sucrose and added stressor (200 μM $CdCl_2$, 200 mM mannitol or 200 mM NaCl). After 3 h and 6 h the seedlings were collected and snap-frozen. To evaluate stressor effect on seedlings we applied short term exposure in liquid medium, and long term exposure on solidified medium. Treatment in liquid medium is more reliable and reproducible since seedlings are completely immersed in the stressor. However, long term incubation of *A. thaliana* seedlings in a liquid medium per se triggers stress response. To avoid this stress-related effect of liquid medium, for any longer exposure of seedlings to stressors we used a solidified medium. For each stressor, the entire experiments were performed three times using different batches of seedlings (three biological replicates).

RNA isolation, quality control and cDNA synthesis

To isolate the total RNA, 50 mg of plant material was frozen with liquid nitrogen and homogenized with glass beads. Isolation of total RNA using RNAzol (Sigma-Aldrich, USA) was performed according to the manufacturer's protocol. Total RNA was resuspended in 50 μL of DEPC-treated water. The integrity of RNA was checked by measuring the absorbance using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and using agarose gel-electrophoresis. DNA was removed with TURBO DNA-free™ Kit (Ambion) according to the manufacturer's protocol. Up to 12.5 μL of total RNA was incubated with 2 U of TURBO DNase for 30 minutes at 37 °C in 1x TURBO DNase buffer, followed by inactivation of TURBO DNase. The integrity of DNase-treated RNA was checked by measuring the absorbance using a NanoDrop ND-1000 spectrophotometer and using agarose gel-electrophoresis. cDNA was synthesised using High Capacity cDNA Reverse Transcription Kit (Ambion) according to the manufacturer's protocol. Reverse transcription was performed with 1 μg of DNase-treated total RNA in a thermal cycler (Eppendorf) under the following conditions: 10 minutes at 25 °C, 120 minutes at 37 °C and 5 minutes at 85 °C.

Real-time PCR and data analysis

Gene-specific primers were designed using NCBI Primer-BLAST (Ye et al. 2012) and are listed in Tab. 1. Quantitative PCR was performed in an optical 96-well plate with 7500 Fast Real-Time PCR system (Applied Biosystems) and universal cycling conditions (10 min 95 °C, 40 cycles of 15 s at 95 °C and 60 s at 60 °C) followed by the generation of a dissociation curve to check for specificity of amplification. Reactions contained SYBR Green Master Mix (Applied Biosystems), 300 nM of a gene-specific forward and reverse primer and 2 μL of 5x diluted cDNA in each 20 μL reaction. No template controls (NTC) contained 2 μL RNase-free water instead. In qPCR, samples from various biological replicates were run in triplicate (technical replicates). PCR amplification efficiencies were calculated using LinRegPCR software (Ruijter et al. 2009). PCR amplification efficiencies for reference gene and genes of interest were comparable and are shown in Tab. 1. The relative quantification of gene expression was calculated using the comparative $\Delta\Delta C_T$ method with actin 2 gene (At3g18780) as reference gene. The relative expression of specific genes was calculated by the following formulas: $\Delta C_T = C_T$ specific gene - C_T reference gene and $\Delta\Delta C_T = \Delta C_T$ treated sample - ΔC_T untreated sample. The relative expression level was calculated as $2^{-\Delta\Delta CT}$.

Statistical analysis

Data analysis was conducted with Student's *t*-test to detect the differences between control and treated groups (Yuan et al. 2006). Standard deviation (SD) was calculated with the use of the results of the $2^{-\Delta\Delta CT}$ method. The results are presented as mean ± SD from three independent experiments. Differences were considered significant at $P < 0.05$. Asterisk symbols (*) indicate significant differences: $^{**}P < 0.01$ and $^{*}P < 0.05$.

Tab. 1. Primer pairs used for quantitative real-time PCR. ACT2 primer sequences were taken from Czechowski et al. 2005 and Remans et al. 2008.

Gene	Strand	Sequence	PCR efficiency
ACT2 (At3g18780)	Forward	5' - CTTGCACCAAGCAGCATGAA - 3'	1.886 ± 0.007
	Reverse	5' - CCGATCCAGACACTGTACTTCCTT - 3'	
SerRS (At5g27470)	Forward	5' - AGCCCGTAGTTGCTGATACC - 3'	1.898 ± 0.008
	Reverse	5' - AATTTCAAGAAAACAGAAGAGTCGT - 3'	
AspRS (At4g31180)	Forward	5' - TCCCAGAAGTCTTGGAGCAAC - 3'	1.875 ± 0.014
	Reverse	5' - CAAATCCACCGTGTAGAGGC - 3'	
CysRS (At5g38830)	Forward	5' - CGCAGCTAGAGAGTTCGTCA - 3'	1.888 ± 0.004
	Reverse	5' - TCCACCATCTTCAGACAATGC - 3'	

Results

We examined genomic regions upstream of the genes encoding cytoslic SerRS, AspRS and CysRS using the *A. thaliana* cis-regulatory element database AtcisDB, which considers a promoter to be an intergenic region upstream of the gene of interest, excluding any coding region of upstream genes (Yilmaz et al. 2011). According to the AtcisDB database, upstream intergenic regions of all three aaRS genes were identified as predicted promoters, since no experimentally validated binding of TFs to these regions is documented so far. Analysis using AtcisDB revealed that the predicted promoter of SerRS gene is only 194 base pairs long and that transcription factors of WRKY family are predicted to bind to SerRS promoter and regulate transcription of SerRS gene. The promoter sequence of AspRS gene is predicted to be 1039 bp long, with putative binding sites for transcription factor families BHLH, Homeobox, bZIPs and ABI3VP1, while CysRS promoter is the longest (2391 bp) and contains binding motifs for transcription factor families BHLH, Homeobox, WRKY, MADS, MYB, bZIP, ABI3VP1 and LFY. Furthermore, using AthaMap database (Hehl et al. 2016) we examined the region from -1,000 bp to +200 bp relative to the transcription start of a gene considering that, in general, majority of gene regulatory sequences (86%) are found inside this area (Yu et al. 2016). Analysis showed that this region in SerRS, AspRS and CysRS genes contains many potential places for binding of TFs belonging to major stress-related TF protein families AP2/ERF, NAC, MYB, bZIP and WRKY (Baillo et al. 2019) (Tab. 2). Altogether, bioinformatics analysis showed that promotors of three aaRS genes contain many potential binding sites for stress-related TFs, implying that abiotic stress may influence expression of the aaRS genes examined.

In order to determine the impact of abiotic stress conditions on the expression of selected aaRS genes, seedlings of *A. thaliana* were exposed to osmotic stress imposed with 200 mM mannitol, salt stress imposed with 200 mM NaCl or heavy metal stress imposed with 200 μM cadmium. Expression of SerRS, AspRS and CysRS genes was measured at four different time points. The transcript levels in control and treated samples were measured using RT-qPCR and normalized by using the expression of actin2 gene as endogenous control. The effect of 200 mM mannitol is shown in Fig. 1. The SerRS gene expression did not differ significantly from SerRS gene expression in control, untreated seedlings (Fig. 1A). On the other hand, in certain conditions osmotic stress significantly enhanced AspRS and CysRS gene expression. Expression of AspRS gene was significantly elevated 8 hours after osmotic stress application (Fig. 1B), while CysRS gene expression rose immediately after mannitol application and remained significantly increased for 3, 6 and 8 hours (Fig. 1C). The effect of 200 mM NaCl is shown in Fig. 2. The SerRS gene expression did not significantly differ from that in controls (Fig. 2A), while the expression level of the AspRS gene significantly increased 8 hours after addition of NaCl and stayed induced even 24 hours following salt application (Fig. 2B). Salt stress imposed with 200 mM NaCl significantly enhanced CysRS expression 6 and 8 hours after application, while after prolonged exposure (24 hours) CysRS gene expression decreased (Fig. 2C).

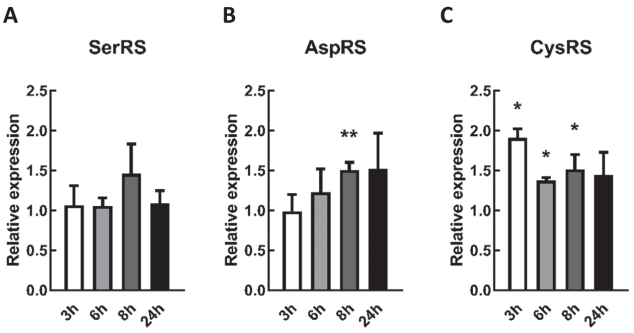

Fig. 1. Expression of cytosolic seryl-tRNA synthetase (SerRS), cytosolic aspartyl-tRNA synthetase (AspRS) and cytosolic cysteinyl-tRNA synthetase (CysRS) under osmotic stress induced by 200 mM mannitol. The expression level for each gene in stress condition was calculated relative to its expression in the control, untreated sample. The gene expression was normalized with the housekeeping actin 2 transcript. Bars represent mean values ± SD from independent experiments (n = 3). Asterix indicate significant difference between treated and control samples, *P < 0.05, **P < 0.01.

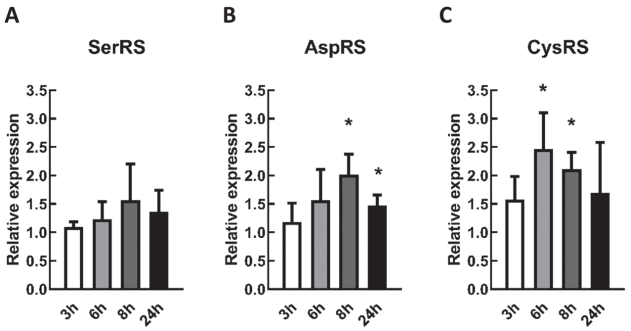

Fig. 2. Expression of cytosolic seryl-tRNA synthetase (SerRS), cytosolic aspartyl-tRNA synthetase (AspRS) and cytosolic cysteinyl-tRNA synthetase (CysRS) under salt stress induced by 200 mM NaCl. The expression level for each gene in stress condition was calculated relative to its expression in the control, untreated sample. The gene expression was normalized with the housekeeping actin 2 transcript. Bars represent mean values ± SD from independent experiments (n = 3). Asterix indicate significant difference between treated and control samples, *P < 0.05.

Protein levels of SerRS, AspRS and CysRS were shown to be increased during early responses of *A. thaliana* due to cadmium exposure (Sarry et al. 2006). In order to examine whether accumulation of these proteins is a result of enhanced transcript accumulation we tested the gene expression of selected aaRSs after exposure to cadmium for 3, 6, 8 and 24 hours. The effect of 200 μM cadmium on aaRSs gene expression in the *A. thaliana* seedlings is shown in Fig. 3. Compared with control seedlings, the treated seedlings did not show a statistically significant change in SerRS transcript levels during different exposure times (Fig. 3A). The same results were obtained during analysis of AspRS and CysRS transcript levels (Fig. 3B, C).

Discussion

Land plants are anchored in one place for most of their life cycle and therefore must constantly be able to adapt their growth and metabolism to abiotic stresses such as changes in light intensity and temperature, high salinity conditions, heavy metal exposure and the lack of water and essential minerals (Floris et al. 2009). Transcription factors are key regulators of gene expression that control cellular and developmental processes and the response to environmental stimuli. The number of transcription factor genes in *A. thaliana* genome is >1500 which is 3 times more than in animals with similar genome size (*Drosophila melanogaster* (Diptera) and *Caenorhabditis elegans* (nematode); Riechmann et al. 2000), which suggests that transcriptional regulation plays a more important role in plants then in animals. Hence, plants' survival depends on their ability to rapidly regulate gene expression in order to adapt their physiology to their environment. To gain more insight into aaRSs in stress response we analyzed promoters of genes encoding cytosolic SerRS, AspRS and CysRS. The rationale was to reveal whether promoters of these genes contain binding site motifs for the transcription factors important in plant stress response. In addition, we determined stress related expression levels of selected aaRS genes.

We exposed *A. thaliana* seedlings to osmotic, salt and heavy metal stress and analyzed transcript expression patterns of selected aaRS genes (SerRS, AspRS and CysRS) 3, 6, 8 and 24 hours after the application of various stressors. Our results show that expression of none of the investigated aaRS genes changes during stress induced by exposure to cadmium (Fig. 3). This observation is surprising considering that previous proteomic investigation showed that protein levels of all three investigated aaRSs are increased during early responses of *A. thaliana* cells to oxidation stress due to cadmium exposure (Sarry et al. 2006). The discrepancy between previous proteomic data and our gene expression study may be related to the increased stability of aaRS proteins or mRNAs, as well as increased translation of aaRS mRNAs on ribosomes during stress. It is known that increased protein levels are not always correlated to transcript levels and selective mRNA translation is documented especially during exposure to stress (Sablok et al. 2017). It is interesting to note that results of the mentioned proteomic investigation indicated that SerRS undergoes posttranslational modification during cadmium stress. We have recently solved crystal structure of *A. thaliana* SerRS and identified the disulfide link in this cytosolic protein (Kekez et al. 2019). Considering that cadmium induces oxidative stress in cells, it is plausible that cysteines in SerRS are oxidized and form a disulfide link, thus creating a new, posttranslationally modified form of SerRS. This oxidized form of SerRS may possess higher stability due to introduction of a covalent disulfide link and may participate in plant stress response mechanisms. In this work we did not observe a statistically significant change in SerRS transcript levels during exposure to osmotic, salt and heavy metal stress, although promoter analysis revealed many potential binding sites for stress related transcription factors in the region from -1,000 bp to +200 bp relative to the transcription start site (Tab. 2). Although this region is regulatory for many *A. thaliana* genes (Yu et al. 2016), it appears that regulation of SerRS gene is controlled by its short promoter (196 bp) with only few putative TF binding sites (as predicted with AtcisDB database) and that expression of SerRS gene is not responsive to stress

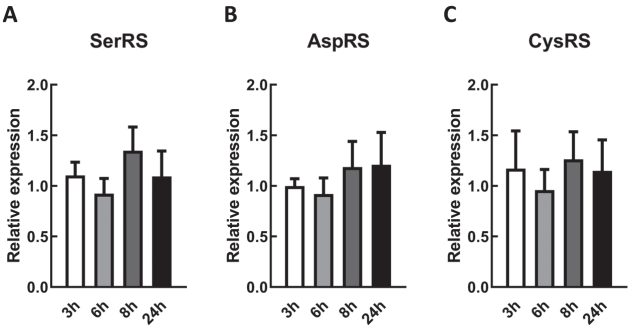

Fig. 3. Expression of cytosolic seryl-tRNA synthetase (SerRS), cytosolic aspartyl-tRNA synthetase (AspRS) and cytosolic cysteinyl-tRNA synthetase (CysRS) under heavy metal stress induced by 200 μM cadmium. The expression level for each gene in stress condition was calculated relative to its expression in the control, untreated sample. The gene expression was normalized with the housekeeping actin 2 transcript. Bars represent mean values ± SD from independent experiments (n = 3).

Tab. 2. Number of potential transcription factor (TF) binding sites in region -1000 bp to +200 bp from transcription start site of SerRS, AspRS and CysRS genes. Analysis was performed using AthaMap and plant transcription factor database (PlnTFDB, Pérez-Rodríguez et al. 2010).

TF family	SerRS	AspRS	CysRS	TF family function (according to PlnTFDB)
AP2/ERF	88	30	46	Plant-specific transcription factors, key regulators of floral organ identity determination and of leaf epidermal cell identity, key regulators of several abiotic stresses and respond to multiple hormones.
NAC	68	51	47	Plant-specific transcription factors, key regulators of stress perception and developmental programmes.
MYB	56	24	43	Involved in the control of the cell cycle, involved in control of plant secondary metabolism, as well as the identity and fate of plant cells.
bZIP	21	1	8	Regulate processes including pathogen defence, light and stress signalling, seed maturation and flower development.
WRKY	3	1	0	Play significant roles in responses to biotic and abiotic stresses, and in development.

conditions used in this work. However, as explained above, SerRS expression in stress can be regulated posttranscriptionally, at the mRNA or protein level.

The expression level of the AspRS gene significantly increased during osmotic and salt stress, induced by the application of 200 mM mannitol or 200 mM NaCl, respectively. The increase in AspRS gene expression was observed 8 hours after stress application, and increased levels of AspRS mRNA were present even after 24 hours in the case of salt stress. The observed increased AspRS gene expression is in agreement with our detailed analysis of AspRS promoter which indicated 51, 30 and 24 binding site motifs for TFs belonging to NAC, AP2/ERF and MYB families, respectively (Tab. 2). These TF families are important regulators of stress response (Nuruzzaman at al. 2013, Baillo et al. 2019). It was previously shown that transcription of *A. thaliana* AspRS gene was enhanced during biotic stress imposed by pathogen *Hyaloperonospora arabidopsidis* (Peronosporaceae) infection and that the enzyme can switch from canonical AspRS activity to noncanonical defense activity upon pathogen infection (Luna et al. 2014). Here we show that the level of AspRS transcripts increases during osmotic and salt stress indicating that AspRS also participates in abiotic stress responses. Therefore, it appears that AspRS can serve as a general stress factor in plants.

The expression level of the CysRS gene significantly increased for 3, 6 and 8 hours after the application of 200 mM mannitol and with the application of 200 mM NaCl in duration for 6 and 8 hours. Our analysis showed that the promoter of CysRS gene contains 47, 46 and 43 possible binding sites for NAC, AP2/ERF and MYB transcription factors, respectively (Tab. 2). This high number of stress-related TF binding sites and large promoter size (2391 bp) is in accordance with prompt and enhanced CysRS gene expression after exposure to stress. It is interesting to note that an increase in free cysteine levels has been reported as a response to various abiotic stress factors, including cadmium, salt and drought stress (Zagorchev et al. 2013). Cysteine is mainly needed for the biosynthesis of sulfur-rich compounds with anti-stress activity, such as GSH, phytochelatins (oligomers of GSH) and stress-related proteins (Zagorchev et al. 2013). If cysteine is excessively used for synthesis of anti-stress compounds, cysteine levels are presumably not sufficient to support translation and therefore CysRS is overproduced to mitigate that effect. On the other hand, recent studies have shown that bacterial and human CysRS use cysteine to produce persulfides, such as CysSSH or longer chain sulfur compounds (polysulfides, including CysS/GS–(S)n–H) (Akaike et al. 2017). These persulfides have antioxidative effects. It is plausible to assume that plant CysRS participates in similar processes through its noncanonical function.

Although stress triggers a rapid downregulation of general protein biosynthesis (Merchante et al. 2017), our results show that expression of plant aaRSs that participate in translation is not decreased. Moreover, during exposure to stress conditions we detected increased AspRS and CysRS transcript levels, while others reported increased SerRS, AspRS and CysRS protein levels (Sarry et al. 2006). Considering that during stress, translation of stress related proteins is enhanced we may assume that the levels of aaRSs are kept at steady-state to pursue translation of specific mRNAs encoding proteins that are essential for stress resistance and survival. Also, when stress conditions cease and global translation starts again, it may be advantageous to have sufficient levels of aaRSs to sustain translation. Finally, increased protein levels of plant aaRSs during stress may serve as a pool of aaRS proteins that can participate directly in stress responses through their noncanonical activities. Further research on plant aaRS role in stress is required to reveal the underlying molecular mechanisms that link aaRSs to plant stress response.

Acknowledgments

The authors thank Vlatka Zoldoš for use of qPCR instrument. This work was supported by the grants from the Croatian Science Foundation (IP-2016-06-6272), the Foundation of Croatian Academy of Sciences and Arts and University of Zagreb.

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