Final Report

Project no.: NN-110962

Title: Role of heat shock transcription factors and MAP kinases in regulation of plant stress responses.

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Introduction

Plant responses to extreme environmental conditions are controlled by a complex regulatory system. Perception of the environmental stimuli, transduction and propagation of the signal, change of the expression of specific gene sets leads to particular alterations in metabolism and development. Transcription factors (TFs) are key components in this regulatory mechanism as they control the transcription of specific target genes. Activity of transcription factors is controlled at several levels including transcription, post-translational modifications, intracellular transport and degradation.

In our earlier research several transcription factors were isolated by a novel genetic screen, using random transformation of a cDNA library into Arabidopsis and regulated overexpression of the integrated cDNA clones (Conditional cDNA Overexpression System, COS) combined with specific genetic screens (Papdi et al., 2008, Plant Physiol 147: 528-542). The screens resulted in the identification of various classes of transcription factors which modulate responses to different environmental stresses. The heat shock factor A4A (HSFA4A) was identified due to its capacity to enhance salt tolerance in overexpressing plants (Pérez-Salamó et al., 2014 Plant Physiol 165: 319-334). The ERF/AP2-type RAP 2.12 factor upregulated the ADH1-LUC target gene and was shown to be implicated in responses to anoxia and osmotic stress (Papdi et al., 2008, Plant Physiol 147: 528-542). The ZnF-type factor ZFP3 modulate ABA sensitivity and responses to light (Joseph et al., 2014, Plant Physiol 165: 1203-1220). Further characterization of these factors could help to understand the stress-related signaling pathways and help to decipher the complex regulation of abiotic stress responses.

In this project we focused on the characterization of the HSFA4A factor, deciphering its role in controlling the responses to stress combinations. To better understand the regulation of complex stress responses, our studies included other stress-related TFs such as RAP2.12 or MYB-type PHR1 and upstream signaling factors including MAP kinases and CRK-type kinases, which can modulate TF activities.

Aims of the research program

The aim of the proposed project was the better understanding how multiple abiotic stresses are integrated through ROS signals, how such signals are sensed and transduced through specific MAPK signalling pathways and HSF transcription factors to confer stress tolerance to plants.

Results

Regulation of HSFA4A

HSFA4A was shown to be induced by a number of stresses, including salinity, oxidative, osmotic, wound, heat and cold stresses, UV-B light, various elicitors and pathogens (Pérez-Salamó et al., 2014 Plant Physiol **165:** 319-334). Effect of stress combination however was not investigated. Our results indicate that salt or heat stress and their combination can induce HSFA4A. Transcription activation is however different by salt or heat and their combination (Figure 1, Andrási et al., 2019).

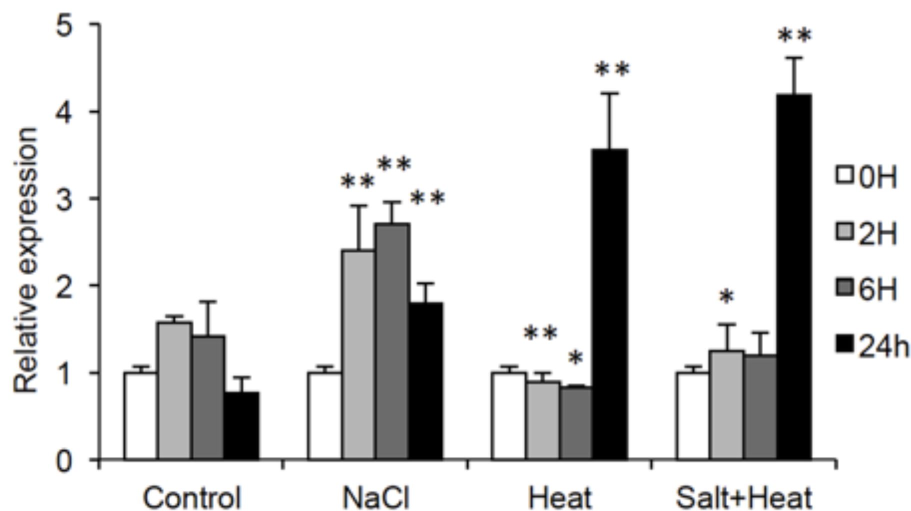


Figure 1. Regulation of HSFA4A transcription in wild type Arabidopsis subjected to salt, heat and combined stresses. Bars on diagrams indicate standard error, * and ** show significant differences to control at $p<0.05$ and $p<0.01$, respectively (Student t-test).

To study intracellular localization of HSFA4A during stress, the pHSFA4A::HSFA4A-YFP gene construct was introduced into wild type Arabidopsis. The HSFA4a-YFP expressing roots were treated with salt and change of YFP-derived fluorescence was monitored. Our results suggest rapid transfer of HSFA4A to nuclei which starts within minutes upon onset of salt stress, and probably happens before de novo biosynthesis. Prolongated salt stress lead to particularly strong fluorescence in nuclei, suggesting nuclear accumulation of HSFA4A during stress (Figure 2, Andrási et al., 2019).

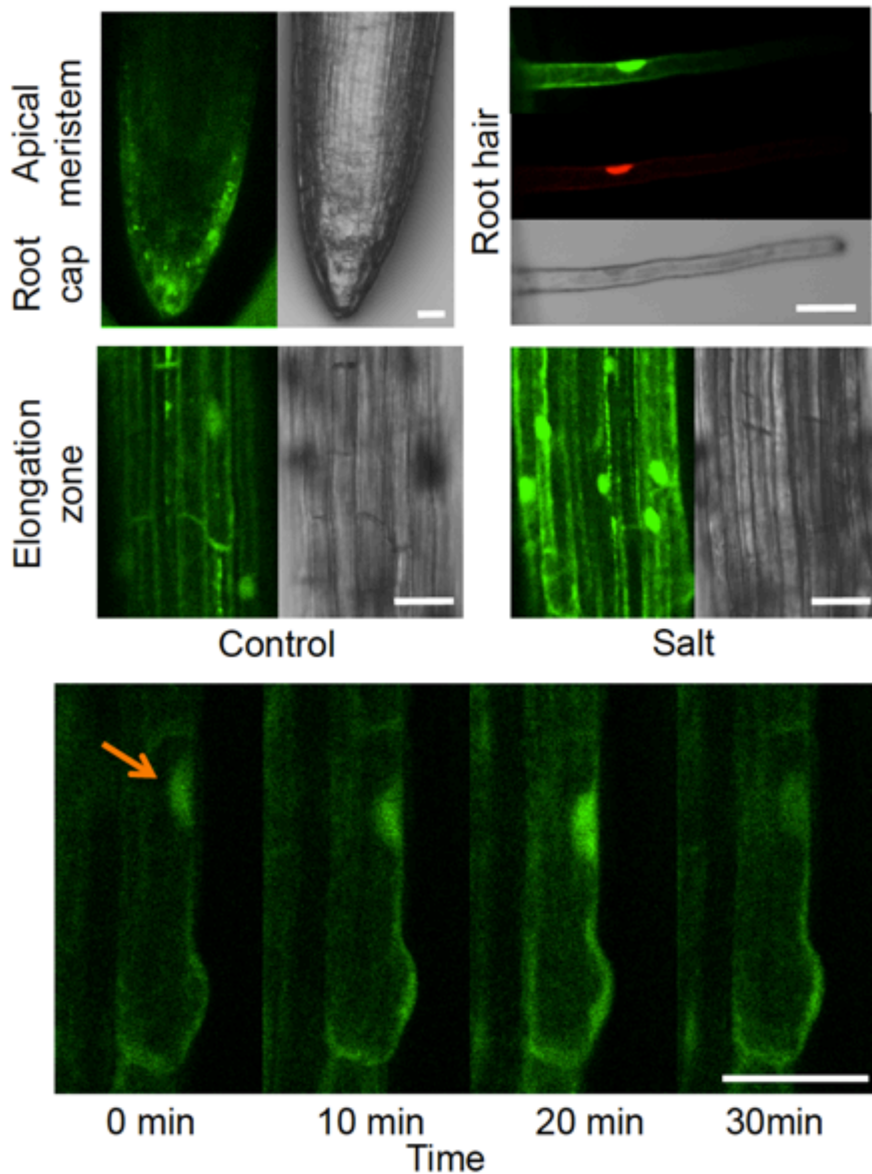
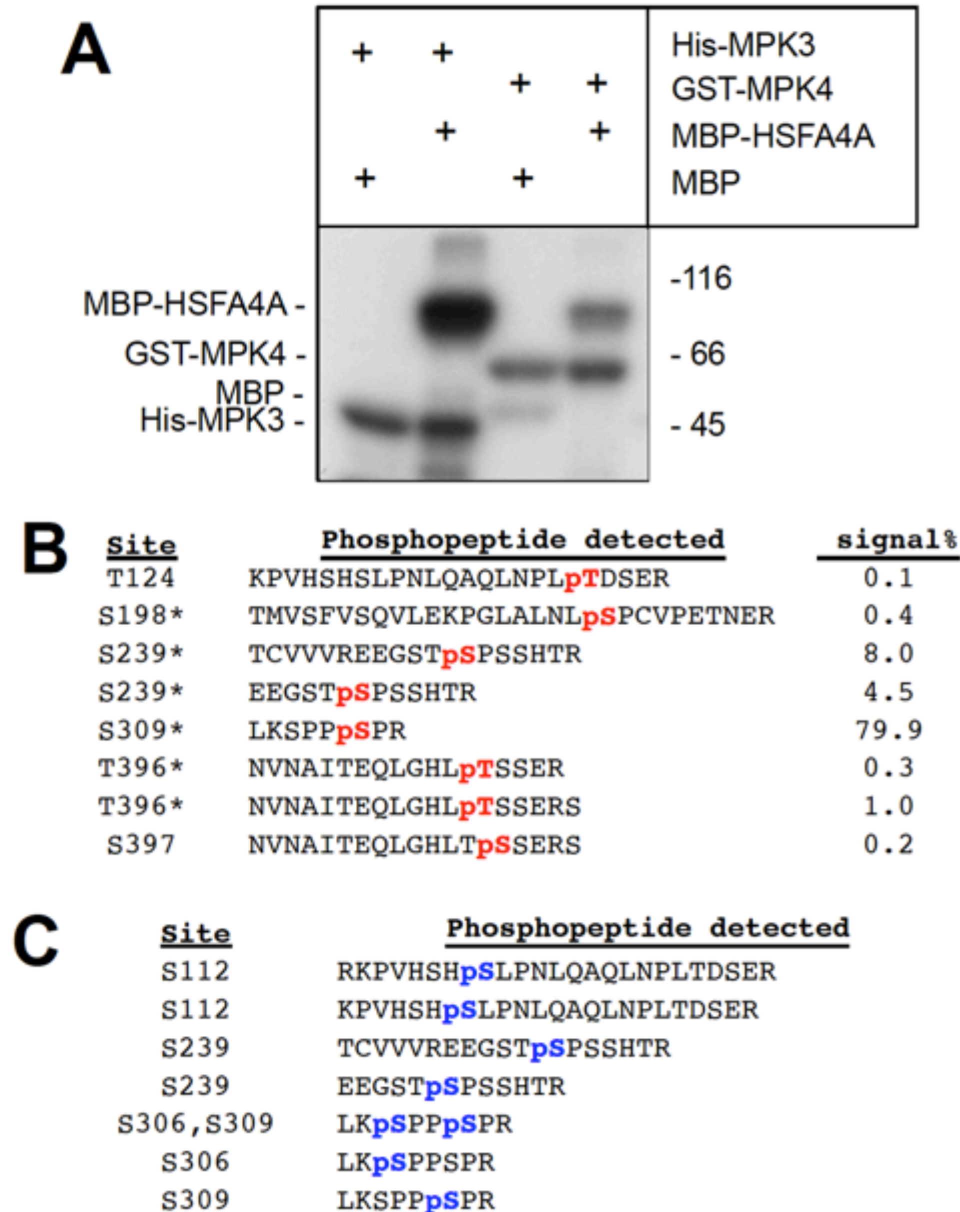


Figure 2. Intracellular localization and transfer of HSFA4A. Confocal microscopic detection of the HSFA4A-YFP fusion protein in roots. HSFA4A is transported into nuclei (arrow) during salt stress. Roots were treated with 100 mM NaCl and HSFA4A-YFP-derived fluorescence was monitored in individual cells at regular intervals.

Phosphorylation of HSFA4A

Phosphorylation of transcription factors and other regulatory proteins is an important post-translational modification which can profoundly influence the activities of these factors. In our previous paper we have reported that HSFA4A can be phosphorylated by MAP kinases MPK3 and MPK6 (Pérez-Salamó et al., 2014, Plant Physiol **165:** 319-334). An alternative stress-related MAP kinase signaling pathway is controlled by MPK4 which is implicated in pathogen responses, controls ROS homeostasis and regulates a

number of TFs. We have therefore tested the capacity of MPK4 to phosphorylate HSFA4A.



B

Site	Phosphopeptide detected	signal%
T124	KPVHSHSLPNLQAQLNPLpTDSER	0.1
S198*	TMVSFVSQVLEKPGLALNLpSPCVPETNER	0.4
S239*	TCVVVREEGSTpSPSSHTR	8.0
S239*	EEGSTpSPSSHTR	4.5
S309*	LKSPPpSPR	79.9
T396*	NVNAITEQLGHLpTSSER	0.3
T396*	NVNAITEQLGHLpTSSERS	1.0
S397	NVNAITEQLGHLTpSSERS	0.2

C

Site	Phosphopeptide detected
S112	RKPVHSHpSLPNLQAQLNPLTDSER
S112	KPVHSHpSLPNLQAQLNPLTDSER
S239	TCVVVREEGSTpSPSSHTR
S239	EEGSTpSPSSHTR
S306,S309	LKpSPPpSPR
S306	LKpSPPSPR
S309	LKSPPpSPR

Figure 3. Phosphorylation of HSFA4A. A) *In vitro* phosphorylation of HSFA4A by MAP kinases MPK3 and MPK4. MBP-tagged HSFA4A was phosphorylated *in vitro* by His-MPK3 or GST-MPK4. B) List of phosphopeptides identified by mass spectrometry. Phosphorylated amino acids are indicated with red letters. Phosphopeptide signal % was calculated from MS signal areas of the unmodified and phosphorylated peptides detected in the tryptic digest without phosphopeptide enrichment. C) Detection of phosphopeptides *in vivo*. HSFA4A-YFP fusion protein was immunoprecipitated from transgenic plants and phosphopeptides were detected by mass spectrometry. Blue letters indicate phosphorylated amino acids.

Phosphorylation experiments showed that HSFA4A can be phosphorylated not only by MPK3 but also by MPK4 (Figure 3A). Subsequent analysis by mass spectrometry identified six amino acid residues of HSFA4A which were phosphorylated by MPK4: Thr124, Ser198, Ser239, Ser309, Thr396 and Ser397. Four of the identified sites coincided with amino acid residues phosphorylated also by MPK3 (Ser198, Ser239, Ser309, Thr396) (Pérez-Salamó et al., 2014, Plant Physiol 165: 319-334). Calculation of phosphopeptide signal frequencies suggested that Ser309 is the primary phosphorylation site for MPK4 (Figure 3B, Andrási et al., 2019).

To test phosphorylation of HSFA4A *in vivo*, YFP-tagged HSFA4A was purified from control and salt-treated transgenic plants expressing the pHSFA4A::HSFA4A-YFP gene construct, and a number of phosphopeptides were identified by mass spectrometry. Three phosphorylated amino acid residues coincided with results of *in vitro* phosphorylation experiments (Thr238, Ser239 and Ser309), confirming that these amino acids are i*n vivo* targets of MAP kinases (Figure 3C). These residues are present only in HSFA4A-type proteins of closely related plant species which belong to the Brassicaceae family. Phosphorylation of other amino acid residues suggested, that they can be phosphorylated by several protein kinases, such as PKA, PKC, CDK, CRK, CK1 or GSK3-type kinases (Andrási et al., 2019). Among these kinases phosphorylation of HSFA4A was tested by CRK-type kinases (Baba et al., 2018).

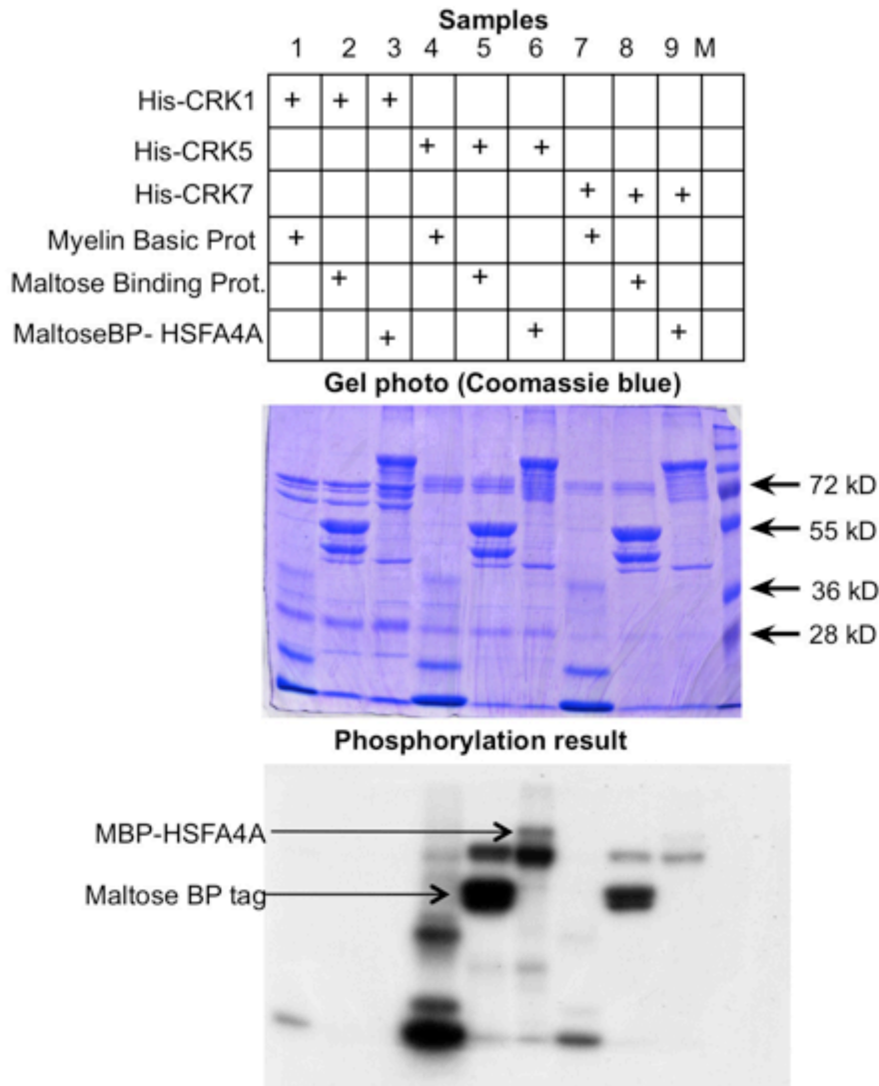


Figure 4. Phosphorylation of HSFA4A with CRK1, CRK5 and CRK7 kinases. HSFA4A was tagged with maltose binding protein and purified MBP-HSFA4A was used for in vitro phosphorylation. Weak phosphorylation of HSFA4A with CRK5 protein kinase could be detected.

CDPK-related kinases (CRKs) are closely related to calcium-dependent kinases (CDPKs) with no conserved Ca binding domains. To characterize their function CRK genes were cloned, characterized and their function was studied with T-DNA insertion mutants (Baba et al., 2018).

HSFA4A phosphorylation was tested with three representative members of the CRK kinase family using in vitro phosphorylation assays. While CRK5 could weakly phosphorylate HSFA4A, CRK1 and CRK7 were inefficient in these assays Figure 4). These results confirm, that HSFA4A is target of different classes of protein kinases and are under complex post-translational control.

Interactions of HSFA4A

Recognition of heat shock elements and transcriptional activation of target genes requires trimerisation of heat shock factors. Intramolecular dimerization of HSFA4A has previously been demonstrated, and conserved Cys residues were shown to be essential to stabilize such interactions (Pérez-Salamó et al., 2014, Plant Physiol **165:** 319-334). In this project we could demonstrate, that in plant cells most of the HSFA4A protein exist in high molecular weight complexes. Such complexes are however sensitive to redox changes and can be monomerized in reducing environment (Andrási et al., 2019).

To test the effect of MAPK-mediated phosphorylation on HSFA4A multimerisation in vivo, intramolecular interactions of wild type and phosphorylation mutants of HSFA4A proteins were compared. Bimolecular fluorescence complementation (BiFC) assays demonstrated that MAPK-mediated phosphorylation has positive influence on BiFC signals, determined by the intramolecular interactions of HSFA4A (Figure 5, Andrási et al., 2019).

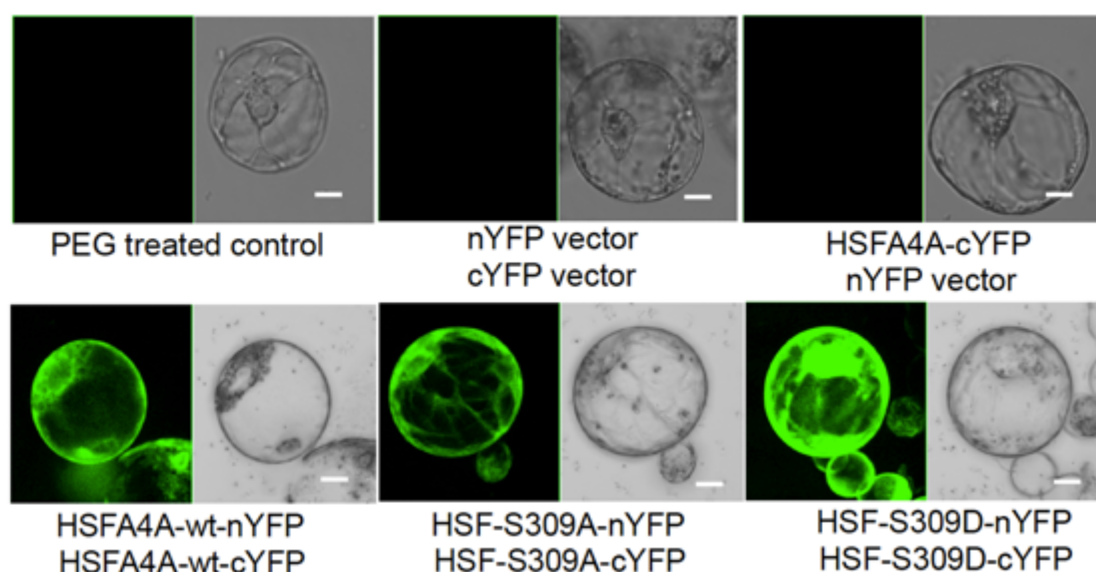


Figure 5. Multimerisation of HSFA4A in vivo. BiFC assay of wild type HSFA4A (HSFA4A-wt), and mutants in which Ser309 was changed to Ala (HSF-S309A) or Asp (HSF-S309D). nYFP and cYFP indicates N and C terminal half of YFP protein. Controls include PEG-treated protoplasts without plasmids, protoplasts transformed with plasmids having nYFP and cYFP fragments or protoplasts expressing HSFA4A-cYFP in combination with the empty nYFP.

To identify HSFA4A interacting proteins, YFP-tagged HSFA4A (encoded by the pHSFA4A::HSFA4A-YFP construct, see above) was used in affinity purification. Identity of HSFA4A-associated proteins was determined by mass spectrometry. Proteomic analysis revealed interaction of HSFA4A with several other transcription factors such as HSFA4C, HSFA5, WRKY1 and chromatin-associated proteins such as DEL3 and GYRB2 (Table 1). These results suggest, that HSFA4A is part of a complex regulatory system, characterized by multiple interactions with different factors.

Table 1. HSFA4A-interacting proteins identified by affinity purification coupled with mass spectrometry.

Gene	AGI code	Protein	Localization
HSFA4A	***AT4G18880***	***Heat stress transcription factor A-4a***	***nucleus***
HSFA5	AT4G13980	Heat stress transcription factor A-5	nucleus
HSFA4C/RHA1	AT5G45710	Heat stress transcription factor A-4c, ROOT HANDEDNESS 1	nucleus
AHL4	AT5G51590	AT-hook motif nuclear-localized protein 4	nucleus
WRKY1	AT2G04880	WRKY transcription factor 1	nucleus
DEK3	AT4G26630	DEK-DOMAIN CONTAINING PROTEIN 3, chromatin-associated	nucleus
GYRB2	AT5G04130	DNA GYRASE B2	mitochondrion/ nucleus

Identification of several HSFA4A-interacting transcription regulators in affinity purification experiments (Table 1) prompted us to compare possible interactions with other, stress-related factors, which were characterized in our laboratory and shown to modulate responses to different types of stresses. The ERF-VII class transcription factors RAP2.2, RAP2.3 and RAP2.12 regulate responses to osmotic stress, low oxygen conditions and modulate ABA sensitivity (Papdi et al., 2015). The MYB-type transcription factors PHR1 and PHL1 are implicated in phosphate starvation, and control proline metabolism in stress conditions (Alexa et al., 2017). Interaction of these transcription factors with HSFA4A could however be not demonstrated in biochemical assays (not shown).

Genetic interactions

Heat shock factors are known for their capacity to form homo or heterotrimers. Our results suggested that HSFA4A can interact with HSFA5 and HSFA4C, less-known members of the plant heat shock factor family. Previously interaction of HSFA4 and HSFA5 factors was described suggesting that HSFA5 might act as repressor in such interaction (Baniwal et al., 2007, J Biol Chem **282:** 3605-3613).

In order to study genetic interactions of these heat shock factors, a double *hsfa4a,hsfa5* mutant line was generated by crossing *hsfa4a* and *hsfa5* mutants. In this experiment a *hsfa4a* mutant in Col-0 background was used. Growth of all these mutants in standard *in vitro* conditions was similar to wild type plants. To evaluate stress responses, survival and damage of plants exposed to different doses of salt, high temperature and combined salt and heat stresses was recorded. While growth of wild type plants, individual and double mutants was similar under control conditions, differences were detected in respect of stress tolerance. Heat stress affected the mutants in similar degree to wild type plants. Based on survival rates, the *hsfa4a* mutant and the double *hsfa4a,hsfa5* mutant were hypersensitive to salt and combined, salt and heat stresses. The *hsfa5* mutant however did not display enhanced stress sensitivity (Figure 6).

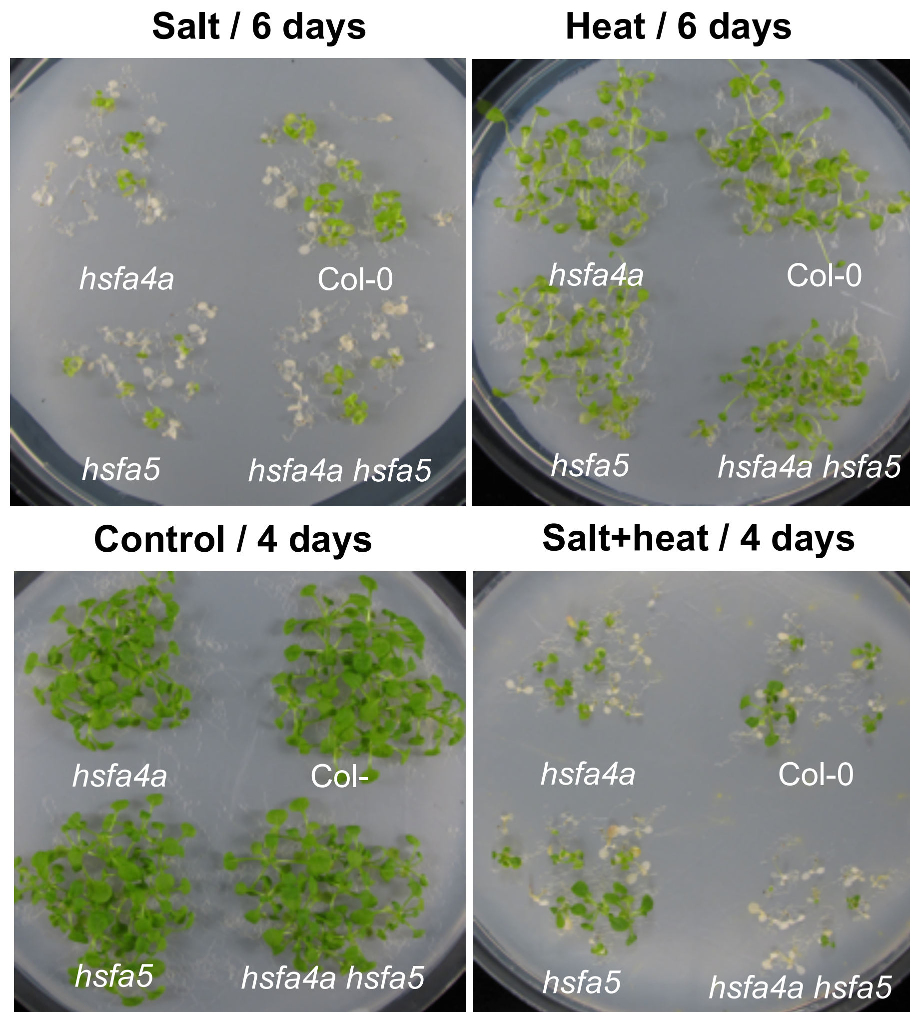


Figure 6. Comparison of stress responses of single and double hsfa4a and hsfa5 mutants. Plants were grown on standard culture medium for 2 weeks, and were stressed by transferring them to thin layer of culture medium with or without 150mM NaCl. Part of the plants were subjected to heat stress (37^{c}C). For recovery, plants were transferred to standard culture medium and growth condition. Survival rates were scored 10 days after.

Binding of HSFA4A to target promoters

Three HSFA4A-induced genes were selected to test promoter binding: the *HSP17.6A, ZAT12* and *WRKY30* genes encoding a small heat shock protein, a zinc finger domain factor and a WRKY-type TFs, respectively (Pérez-Salamó et al., 2014, Plant Physiol 165: 319-334). Promoter regions of these genes contain several HSE motifs, binding sites of heat shock factors, suggesting that they can be direct targets of HSFA4A.

Promoter binding was demonstrated *in vivo* by chromatin immunoprecipitation (ChIP) assays. Heat treatment enhanced enrichment on all three promoters, while salt promoted HSFA4A binding to ZAT12 and WRKY30 promoters but not to HSP17.6A. Combination of both stresses was additive on promoter binding on ZAT12 and WRKY30 genes, while binding on HSP17.6A promoter was similar to heat treatment (Figure 7). These results

confirmed that these genes are direct targets of HSFA4A, and binding is influenced by the environmental conditions (Andrási et al., 2019).

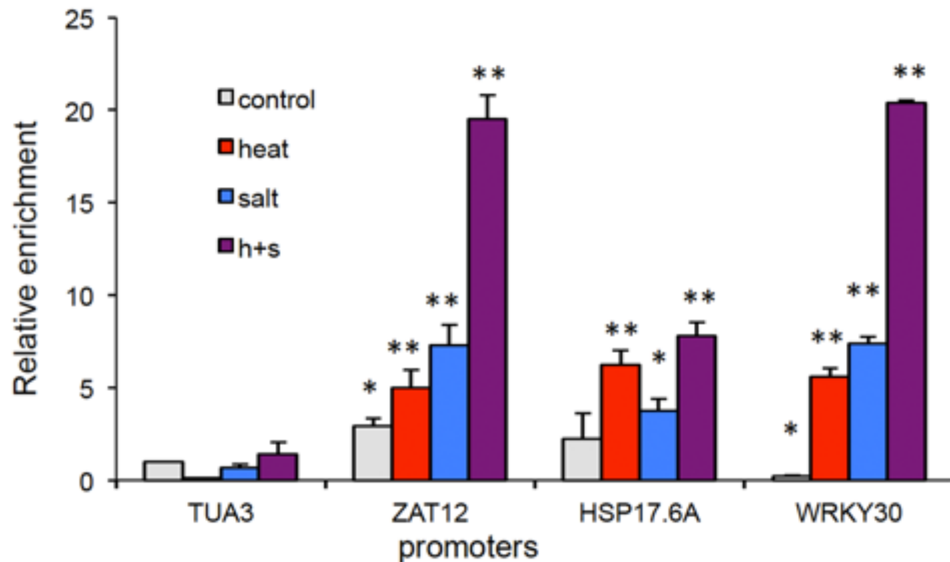


Figure 7. Binding of HSFA4A on target gene promoters. ChIP assay with YFP-tagged HSFA4A. Plants were treated by salt (150 mM NaCl, 6 h), heat stress (37°C, 6 h), and their combination before ChIP assay. ChIP results are shown as relative enrichment by q-PCR, where reference (value 1) is the q-PCR value of the TUA3 promoter, which lacks any HSE motif. * and ** show significant differences to ChIP values of TUA3 promoter at $p<0.05$ and $p<0.01$, respectively (Student t-test).

HSFA4A can enhance tolerance to combined salt and heat stresses

In natural environments stress conditions usually develop in combinations which can be more harmful to plants than individual stresses. To evaluate responses to combined salt and heat stresses, tolerance of Col-0 wild type and HSFA4A overexpressing lines were tested. Survival and cellular damage was tested in plants exposed to different doses of such stresses. Heat treatments had only minor effect on plant viability in our experimental conditions, while salt stress affected plants in a concentration-dependent manner. In mild stress conditions overexpression of the phosphorylation-mimicking HSFA4A mutant was slightly more efficient to contain lipid peroxidation, while both forms of HSFA4A reduced oxidative damage when higher doses of stress were imposed (Figure 8). Plant survival was clearly increased by overexpression of both forms of HSFA4A, when plants were exposed either to higher doses of salinity or when salt was combined with elevated temperature. Our results indicate that HSFA4A can reduce oxidative damage and improve plant tolerance not only to individual salt or heat stresses, but also by stress combinations (Figure 10, Andrási et al., 2019).

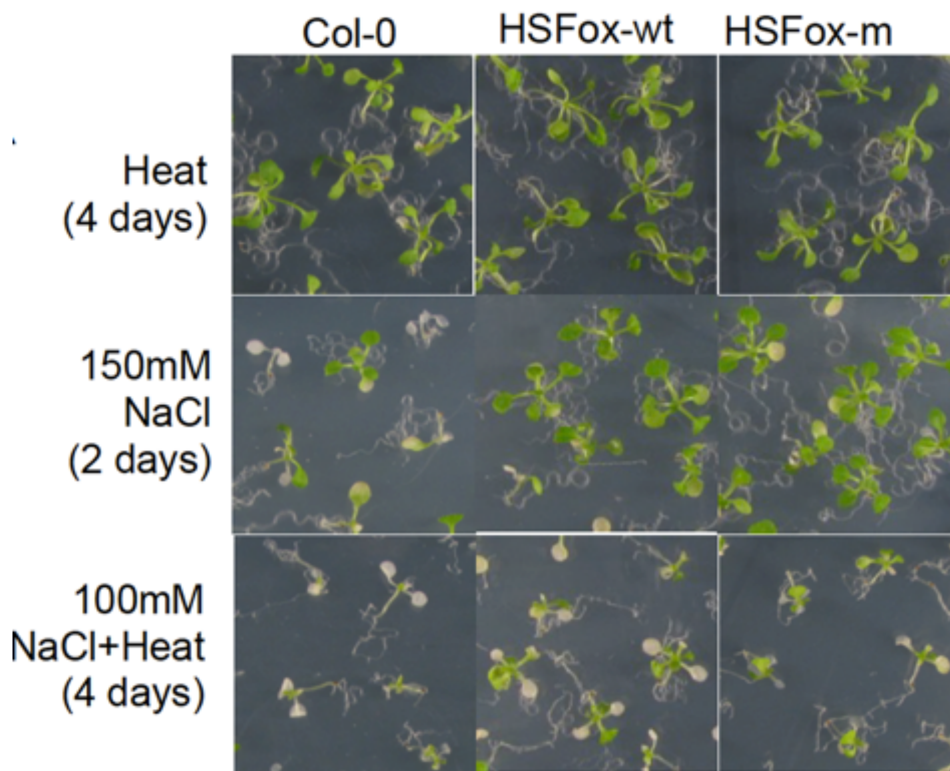


Figure 8. HSFA4A overexpression enhances tolerance to heat and salt stresses. 10 days-old *in vitro*-grown plantlets were treated by salt (100 mM, 150 mM NaCl), heat (37°C in light, 30°C in dark) or their combinations for two or four days. Rates of surviving healthy (vigorous growth with several new green leaves), damaged (small plants with retarded growth and/or chlorotic leaves) and dead plants (completely chlorotic with no green leaves) were scored 10 days after recovery.

Technical developments

Improvement of in vitro selection tools

Genetic screens lead to the identification of a number of regulatory genes through mutant isolation. In order to enhance efficiency and apply microbial selection criteria, screening conditions were optimized for Arabidopsis cell cultures randomly transformed with the COS library. The system allowed rapid screening of hundreds of thousands transformed cell colonies in defined and well controlled conditions. Inserted cDNA clones could subsequently isolated by small amounts of genomic DNA by PCR amplification of the cDNA insert. The system is suitable for the isolation of such traits which can be identified at cell level (Pérez-Salamó et al., 2016).

Development of the PlantSize image analysis system

At the initiation of the project some preliminary data were available about the biological function of HSFA4A. Non-destructive measurement of

growth rates and color can however give more precise data about tolerance/sensitivity of overexpressing lines or knockout mutants. We have therefore developed the PlantSize image analysis system, which was used to compare growth rates, changes in chlorophyll and anthocyanine content of HSFA4A overexpressing lines and the *hsfa4a* mutant (Figure 9, Faragó et al., 2018).

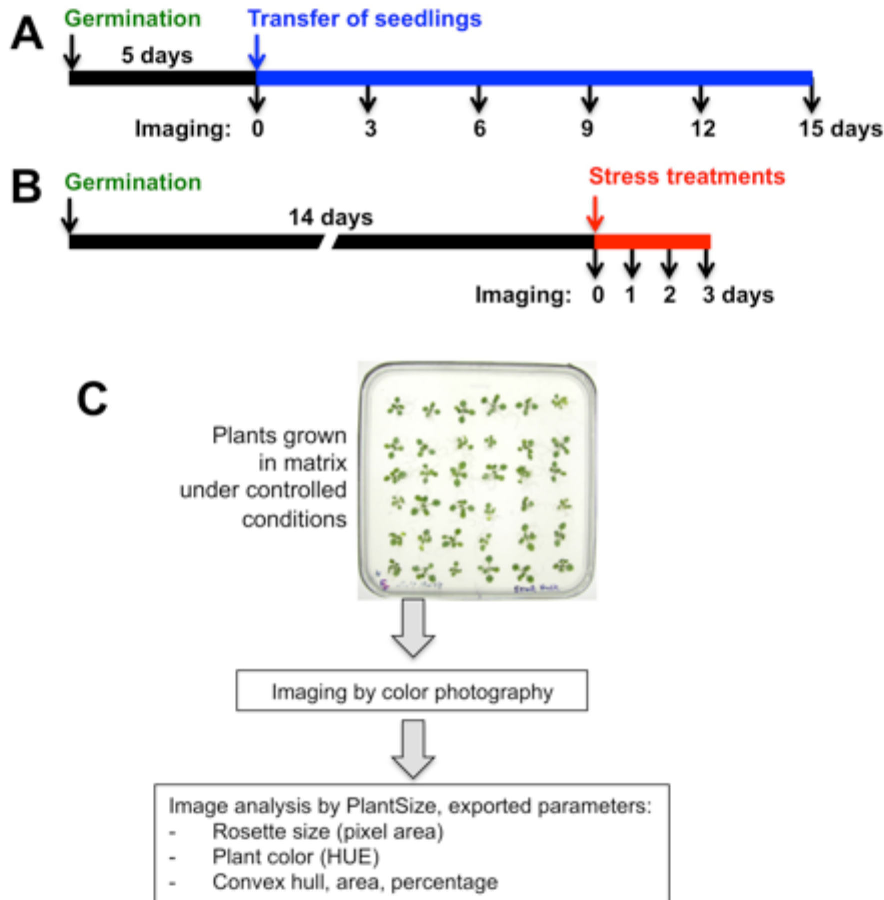


Figure 9. Image analysis by PlantSize. A) Experimental setup to monitor plant growth by repeated imaging in sublethal stress conditions. B) Experimental setup to monitor physiological changes in stress conditions. C) Work flow of image analysis by PlantSize and subsequent data processing.

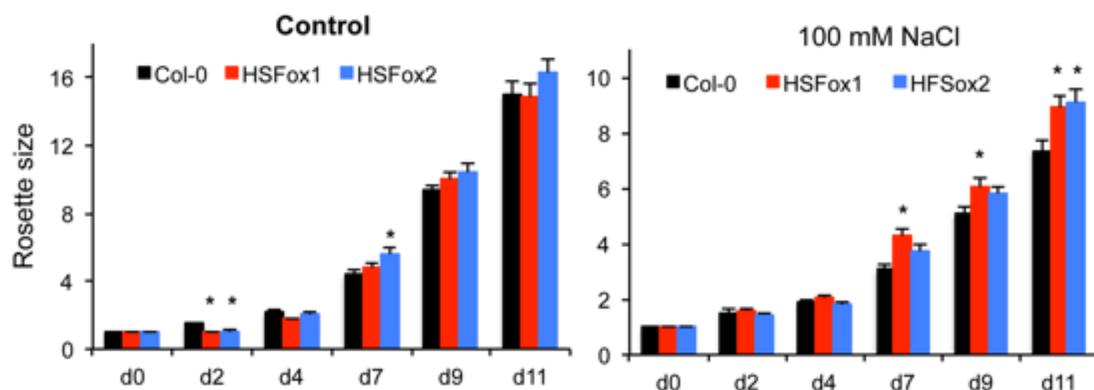


Figure 10. Growth analysis of HSFA4A overexpressing lines on salt-containing medium (100 mM NaCl). Growth was assessed by measuring rosette sizes with PlantSize.

Effect of HSFA4A overexpression on plant growth in stress conditions was investigated by evaluating changes of rosette sizes, chlorophyll and anthocyanin accumulation of two independent *HSFA4A* overexpressing plants. Growth of transgenic lines was significantly superior on media containing sublethal concentration (100 mM) of NaCl, while it was similar in control conditions (Figure 10, Faragó et al., 2018).

On the other hand, the *hsfa4a* insertion mutant was found to be hypersensitive to salt (NaCl), oxidative stress or heavy metals (eg. $CdCl_2$). The non-destructive image analysis revealed that relative growth rate and chlorophyll accumulation was inferior, while anthocyanine accumulation was higher in the salt and heavy metal -treated mutant than in wild type plants, confirming its hypersensitivity to these stress conditions (Figure 11, Faragó et al., 2018).

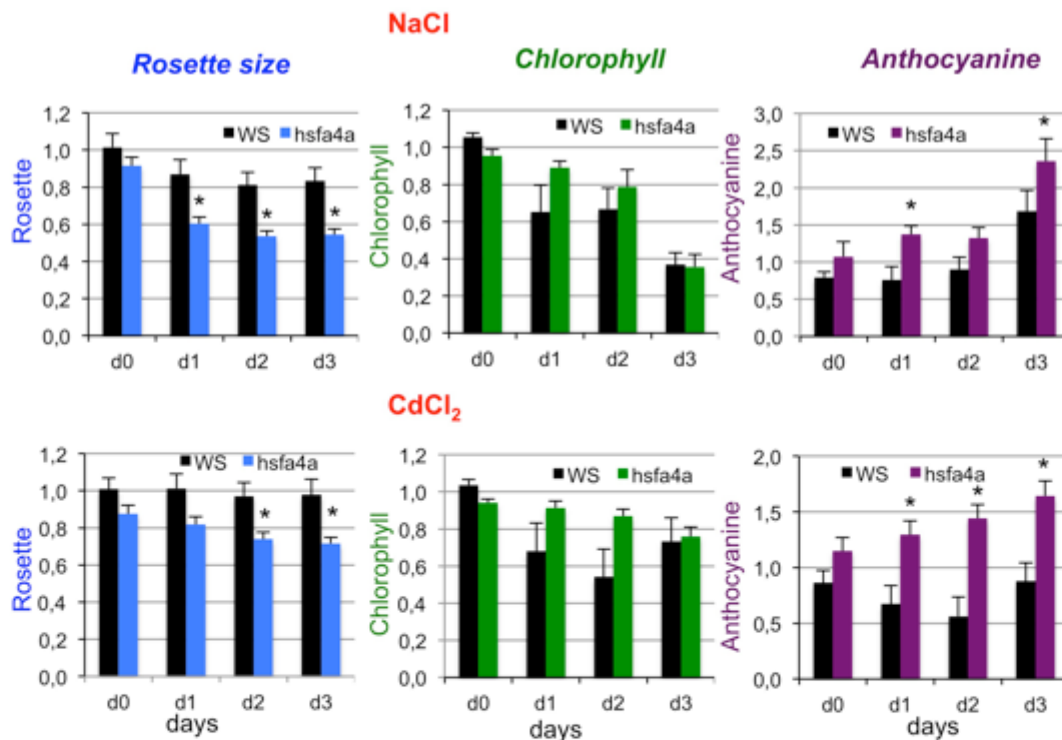


Figure 11. The *hsfa4a* mutant is hypersensitive to salt and heavy metal. Analysis of rosette size, chlorophyll content and anthocyanine accumulation with PlantSize.

Conclusions.

The research program could enhance our knowledge on the signaling system which includes the HSFA4A transcription factor. We showed that the stress-induced HSFA4A is mobilized into the nuclei in stress conditions, transmits ROS signals and activate target genes. We demonstrated that MAP kinases MPK3, MPK4, MPK6 and probably a CRK-type kinase can

phosphorylate HSFA4A in vivo. REDOX state and MAPK-mediated phosphorylation influences intramolecular interactions, which is important for transcriptional activation of target genes. HSFA4A binds to promoters of a group of target genes, and binding is enhanced by different stress conditions. We showed, that HSFA4A is not only implicated in salt stress responses, but controls tolerance to combined stresses also. Evaluation of stress tolerance and sensitivity of mutants and overexpressing transgenic lines was greatly facilitated by technical developments including the simple to use image analysis system, PlantSize, which is able to monitor small changes in plant growth and color, important indicators of tolerance and sensitivity.

Among other TFs, HSFA5, HSFA4C and WRKY1 were found to interact with HSFA4A *in vivo* and might influence its action. Other transcription factors can either be targets of HSFA4A (eg. ZAT12, WRKY30), or can influence stress responses in parallell and interconnecting regulatory pathways (eg. ERFVII and MYB-type regulators, Papdi et al., 2015, Aleksza et al., 2017). Model of HSFA4A action in stress signaling is depicted in Figure 12.

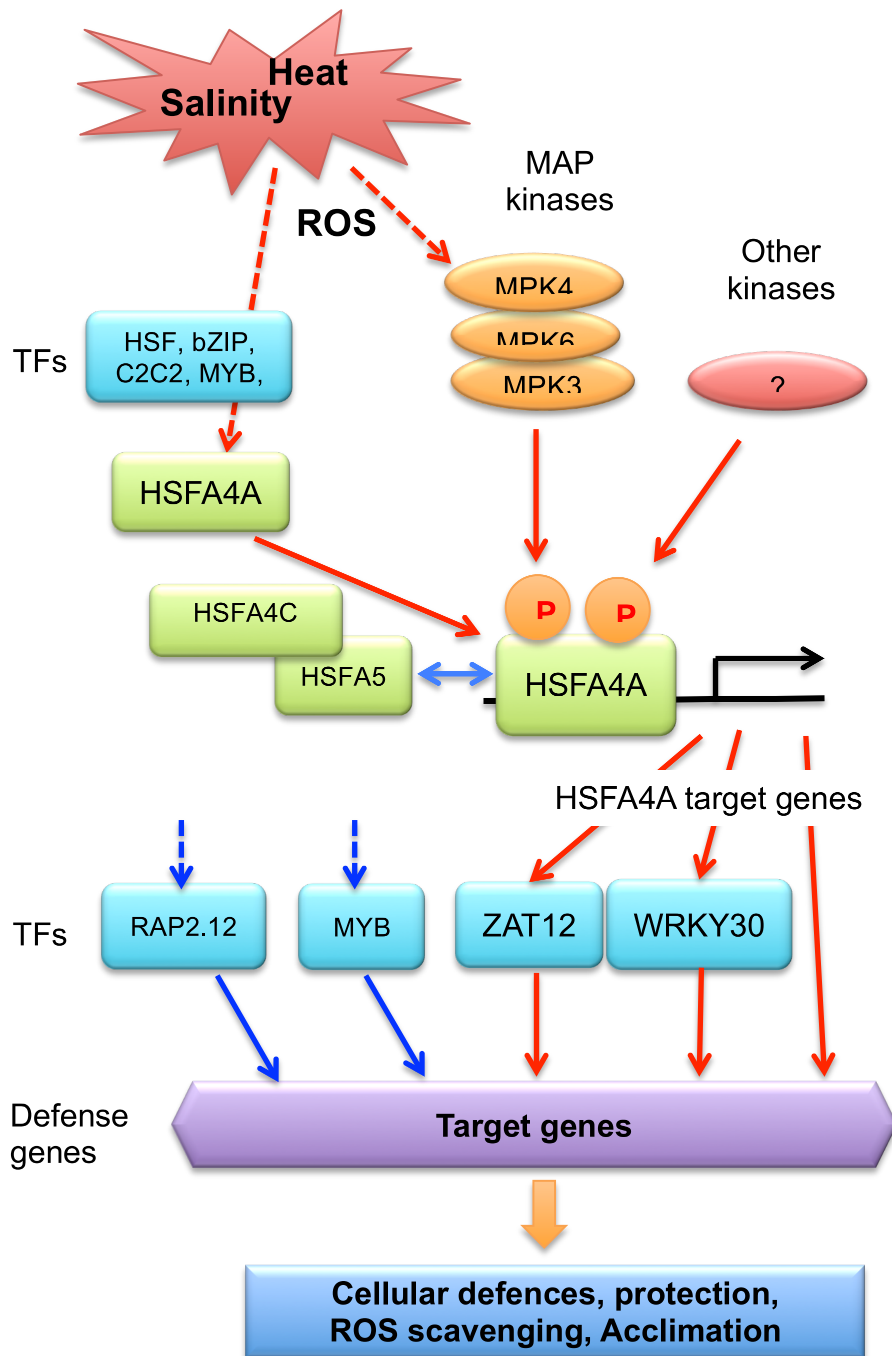


Figure 12. Model of complex stress signal transduction and transcription regulation mediated by HSFA4A. Possible involvement of other transcription factors and upstream regulators such as protein kinases are indicated.

Publications in which the project support is acknowledged:

Scientific papers

1. Papdi Cs, Pérez-Salamó I, Joseph MP, Giuntoli B, Bögre L, Koncz Cs, Szabados L (2015) The low oxygen, oxidative and osmotic stress responses synergistically act through the Ethylene Response Factor-VII genes RAP2.12, RAP2.2 and RAP2.3. Plant J. 82: 772-784 (IF: 5.468).

2. Perez-Salamó I, Boros B, Szabados L (2016) Screening Stress Tolerance Traits in Arabidopsis Cell Cultures. In: *Methods in Molecular Biology,* New York: Humana Press. 1398:235-246 (IF: 0.85).

3. Aleksza D, Horváth GV, Sándor Gy, Szabados L (2017) Proline accumulation Is regulated by transcription factors associated with phosphate starvation. Plant Physiology 175:555-567 (IF: 5.949)

4. Faragó D, Sass L, Valkai I, Andrási N, Szabados L (2018) PlantSize offers an affordable, non-destructive method to measure plant size and color in vitro. Front Plant Sci. Vol. 9: 219 (IF: 3.678).

5. Baba AI, Rigó G, Ayaydin F, Rehman AU, Andrási N, Zsigmond L, Valkai I, Urbancsok J, Vass I, Pasternak T, Palme K, Szabados L, Cséplő Á (2018) Functional analysis of the Arabidopsis thaliana CDPK-Related kinase family: AtCRK1 regulates responses to continuous light. Int. J. Mol. Sci. 19: 1282 (IF: 3.687).

6. Andrási N, Rigó G, Zsigmond L, Pérez-Salamó I, Papdi C, Klement É, Pettkó-Szandtner A, Baba AI, Ayaydin F, Dasari R, Cséplő Á, Szabados L (2019) The MPK4-phosphorylated Heat Shock Factor A4A regulates responses to combined salt and heat stresses. J. Exp. Bot. (in press) doi.org/10.1093/jxb/erz217 (IF: 5.354)

Conference presentations (lecture, poster):

1. Szabados László, Mary Prathiba Joseph, Boros Bogáta, Hornung Ákos, Papdi Csaba, Kozma-Bognár László (2015) *Az ABA, stressz és fény jelátvitel kapcsolata növényekben.* 16. Kolozsvári Biológus Napok, Kolozsvár, Románia.

2. Csaba Papdi, Imma Pérez-Salamó, Mary Prathiba Joseph, Beatrice Giuntoli, László Bögre, Csaba Koncz and László Szabados (2015) *The Ethylene Response Factor-VII genes RAP2.12, RAP2.2 and RAP2.3 regulate low oxygen, oxidative and osmotic stress responses.* 26th International Conference on Arabidopsis Research, ICAR, Paris, France. P528.

3. Zsigmond L, Boros B, Koerber N, Fiorani F, Szabados L (2015) *Stress phenotyping of Arabidopsis mutants disrupting mitochondrial electron transport genes.* European Plant Phenotyping Network's Plant Phenotyping Symposium, Barcelona, Spain, 11-12th of November, 2015.

4. Zsigmond L, Kiss T, Boros B, Koerber N, Fiorani F, Szabados L (2015) Mitochondrial proteins influence stress responses in Arabidopsis thaliana. COST FA1306: *The quest for tolerant varieties - Phenotyping at plant and cellular level.* 1st General Meeting, June 22-24, 2015, IPK Gatersleben, Germany.

5. László Szabados, Csaba Papdi, Imma Pérez Salamó, Gábor Rigó, Mary Prathiba Joseph, Ildikó Valkai, Norbert Andrási, Dóra Faragó, Matthew Hannah, Csaba Koncz (2016) Functional identification of stress regulatory genes in model and extremophile plants using the Conditional Overexpressing System (COS). International Conference on "EMERGING BIOTECHNOLOGIES", Kakatiya University, Warangal, India.

6. Andrási Norbert, Rigó Gábor, Cséplő Ágnes, Imma Pérez-Salamó, Szabados László (2016) *A MAP kinázok szerepe az Arabidopsis HSFA4A hősokk factor intramolekuláris kölcsönhatásaiban*. 17. Kolozsvári Biológus Napok. Kolozsvár, Románia.

7. Norbert Andrási, Gábor Rigó, Ágnes Cséplő, Immar Pérez-Salamó, László Szabados (2016) MAP kinase-mediated phosphorylation regulates intramolecular interactions of the Arabidopsis Heat Shock Factor A4A. Plant Biology Europe, EPSO/FESPB 2016 Congress, Prague, Czech Republic,

8. László Szabados, Gábor Rigó, Ildikó Valkai, Dóra Faragó, Edina Kiss, Sara Van Houdt, Nancy Van de Steene, Matthew A. Hannah (2017) Gene mining in extremophile plants: stress tolerance genes from *Lepidium crassifolium.* Hungarian Molecular Life Sciences 2017 Conference, Eger, Hungary.

9. Abu Imran Baba, Gábor Rigó, Ferhan Ayaydin, Norbert Andrási, Ateeq Ur Rehman, János Urbancsok, Laura Zsigmond, Ildikó Valkai, Imre Vass, Taras Pasternak, Klaus Palme, László Szabados and Ágnes Cséplő (2018) Functional analysis of the *Arabidopsis thaliana* CDPK Related Kinase (CRK) family. Straub Days, Szeged, 2018.05.11-12.

10. László Szabados, Norbert Andrási, Laura Zsigmond, Gábor Rigó, Ágnes Cséplő, Imma Pérez-Salamó, Csaba Papdi, László Bögre (2018) Tolerance to combined salt and heat stress is alleviated by heat shock factor a4a (HSFA4A) from *Arabidopsis thaliana*. 12th Congress Of The International Plant Molecular Biology, Montpellier, France

11. Baba AI, Rigó G, Ayaydin F, Andrási N, Ur-Rehman A, Urbancsok J, Zsigmond L, Valkai I, Vass I, Pasternak T, Palme K, Szabados L, Cséplő Á (2018) Functional analysis of CDPK Related Kinase (CRK) family in *Arabidopsis thaliana* plant. Plant Biology Europe FESPB/EPSO Congress 2018, Copenhagen, Denmark.

12. Faragó D, Sass L, Valkai I, Andrási N, Szabados L. (2018) PlantSize: an affordable, non-destructive method to measure plant size and color in vitro. Plant phenotyping for future climate challenges, COSTFA1306 Meeting, 2018.03.20-21. Leuven, Belgium.

13. Norbert Andrási, Gábor Rigó, Ágnes Cséplő, Laura Zsigmond, Imma Pérez-Salamó, Abu Imran Baba, Eva Klement, Aladár Pettkó-Szandtner, Sahiba Siddiqui, László Szabados (2019) The Heat Shock Factor A4A regulates responses to combined salt and heat stresses. Hungarian Molecular Life Sciences 2019 Conference, Eger.

14. Annabella Erdélyi, Ildikó Valkai, Gábor Rigó, Ágnes Szepesi, Dávid Alexa, Niklas Koerber, Fabio Fiorani, László Szabados, Laura Zsigmond (2019) The role of *Arabidopsis thaliana* mitochondrial proteins in stress responses. Hungarian Molecular Life Sciences 2019 Conference, Eger.