VISCEA
Vienna International Science
Conferences and Events Association

International Conference

Plant Abiotic Stress Tolerance VI

Programme and Abstracts

Vienna, Austria
February 21-22 , 2020

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Organizing Committee

Local Organizing Committee	International Organizing Committee
Alisher Touraev (Local Organizer, Austria) Teun Munnik (Conference Co-Chair, The Netherlands)	Eduardo Blumwald (USA) Heribert Hirt (Saudi Arabia) Martijn van Zanten (The Netherlands) Sergey Shabala (Australia) Mark Aarts (The Netherlands) Kaisa Kajala (The Netherlands) Michael Wrzaczek (Finland) Claudia Jonak (Austria)

Welcome to the 6th International Conference on "Plant Abiotic Stress Tolerance"!

Abiotic stresses, defined as the negative impact of non-living factors on the living organisms in a specific environment are the primary causes of crop loss worldwide. Abiotic stresses include high and low temperatures, salinity, drought, flooding, heavy metal stress and many other environmental factors. Plant tolerance to these stresses is dependent on the molecular networks involved in stress perception, signaling, and the expression of specific stress-related genes and metabolites.

The **6th International Conference "Plant Abiotic Stress Tolerance"** to be held on **February 21-22, 2020**, in Vienna, Austria will discuss the most recent advances in understanding and combating plant abiotic stress and tolerance mechanisms and to define new frontiers in this field.

This two-day event will provide leading academy and industry scientists a platform to communicate recent advances in "**Plant Abiotic Stress Tolerance**", and an opportunity to establish multilateral collaboration.

The **6th International Conference on "Plant Abiotic Stress Tolerance"** will cover the following research topics:

- ***Plant Response to Heat Stress***
- ***Plant Response to Temperature & Drought***
- ***Plant Response to Salt & Osmotic Stress***
- ***Plant Response to Nutrient Stress & Heavy Metals***
- ***Plant Response to Oxidative- and Flooding Stress***
- ***Plant Abiotic Stress Signaling***
- ***Breeding & Engineering of Abiotic Stress Tolerance***

Approximately 150 participants are expected to attend this exciting scientific forum including almost 30 lectures delivered by worldwide known invited speakers and young, talented speakers selected from submitted abstracts. The program combines plenary lectures, poster sessions, a unique Conference Dinner Party and sightseeing tours of Vienna.

Prof. Alisher Touraev (VISCEA, Austria, Local Organizer)

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6th International Conference on “Plant Abiotic Stress Tolerance”
(February 21 - 22)

February 21 (Friday)

08.00 - 17.00	***Registration***
09.30 - 09.40	**Opening** Welcome address by **Alisher Touraev (Local Organizer, Austria)** Welcome address by **Teun Munnik (Conference Co-Chair, The Netherlands)**
09.40 - 10.30	**Keynote Lecture:** **Eduardo Blumwald (USA):** CHLOROPLAST VESICULATION Mediates Chloroplast and Peroxisome Turnover, Source-sink Relationships and Stress Tolerance in Crop Plants
10.30 - 11.00	***Coffee break***
11.00 - 12.30:	**Session I: Plant Response to Heat Stress**
Chairs	*Eduardo Blumwald (USA) & Heribert Hirt (Saudi Arabia)*
11.00 - 11.25 (+5)	**Heribert Hirt (Saudi Arabia):** Microbiome-induced Epigenetic Mechanism of Heat Stress Tolerance in Plants
11.30 - 11.45 (+5)	**Sylva Prerostova (Czech Republic):** Heat Acclimation and Inhibited Cytokinin Degradation positively Affect Heat Stress Tolerance of Arabidopsis
11.50 - 12.05 (+5)	**Guido Domingo (Italy):** Heat Stress Response in Tobacco BY-2 Cells impaired in cAMP Content
12.10 - 12.25 (+5)	**Sona Pandey (USA):** Modulation of Heat Stress Response by Heterotrimeric G Proteins in Arabidopsis
12.30 - 14.00	***Lunch + Poster Session (all numbers), Conference Photo***
14.00 - 15.35	**Session II: Plant Response to Temperature & Drought**
Chairs	*Martijn van Zanten (The Netherlands) & Sergey Shabala (Australia)*
14.00 - 14.25 (+5)	**Martijn van Zanten (The Netherlands):** Thermomorphogenesis Control by Histone Deacetylase 9; Optimal Plant Performance under Suboptimal Temperature Conditions
14.30 - 14.50 (+5)	**László Szabados (Hungary):** Small paraquat Resistance Proteins (SPQ) modulate paraquat, ABA and Drought Responses in Arabidopsis
14.55 - 15.10 (+5)	**Katarzyna Lechowicz (Poland):** Insight into Mechanisms of Drought Resistance in Lolium multiflorum/Festuca arundinacea Introgression Forms
15.15 - 15.30 (+5)	**Liudmyla Kozeko (Ukraine):** Kinetics of HSP70 and HSP90 Expression and Drought Tolerance of Arabidopsis thaliana
15.35 - 16.00	***Coffee break***
16.00 - 17.35	**Session III: Plant Response to Salt & Osmotic Stress**
Chairs	*Sergey Shabala (Australia), Martijn van Zanten (The Netherlands)*
16.00 - 16.25 (+5)	**Sergey Shabala (Australia):** Strategies and Cost of Plant Osmotic Adjustment

16.30 - 16.45 (+5)	**Femke de Jong (The Netherlands):** Identification of PIP2-interacting Proteins in Response to Salt- and Heat Stress
16.50 - 17.00 (+5)	**Simone Cantamessa (Italy):** An Endophytic Bacterium improves Salt Stress Tolerance in Tomato Plants
17.05 - 17.15 (+5)	**Zoltan Takacs (Austria):** Hot water Treatment modifies Redox Status and Phytohormone Synthesis in Apple Fruit during short-term Storage
17.20 - 17.30 (+5)	**Chwan-Yang Hong (Taiwan):** Molecular Characterization and Subcellular Localization of Salt-inducible Lipid Transfer Proteins in Rice
17.35 - 19.00	***Welcome Reception + Poster Session (all numbers)***
19.00 - 22.00	**Conference Dinner Party** Traditional Austrian food and wine, located in one of Vienna's famous 'Heurigen' **Cost: 50,- EUR**

February 22 (Saturday)

08.00 - 17.00	***Registration***
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Chairs	*Mark Aarts (The Netherlands) & Michael Wrzaczek (Finland)*
09.00 - 09.25 (+5)	**Mark Aarts (The Netherlands):** Exploring the Genetics Underlying Arabidopsis Zn Deficiency Response
09.30 - 09.50 (+5)	**Gabor Feigl (Hungary):** Alyssum lesbiacum Populations from Distinct Habitat show Different Nitro-Oxidative Response to Nickel Stress
09.55 - 10.10 (+5)	**Manon Sarthou (France):** Identification of Uranium Root Absorption Pathways in Arabidopsis thaliana
10.15 - 10.30 (+5)	**Julian Preiner (Austria):** Increased levels of phenolic compounds, polyamines and amino acids indicate potential symbiont induced reduction of tungsten (W) induced stress in glycine max
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Chairs	*Kaisa Kajala (The Netherlands) & Michael Wrzaczek (Finland)*
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11.25 - 11.45 (+5)	**Michael Wrzaczek (Finland):** CRK2 is involved in the Control of Salt Stress-Induced Callose Deposition
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Chairs	*Claudia Jonak (Austria) & Vibha Srivastava (USA)*
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16.30 - 16.45 (+5)	**Inês Leitão (Portugal):** Effects on antioxidant Defense Mechanisms of Lettuce Plants Exposed to Contamination by Acetaminophen and Carbamazepine
16.50 - 17.05 (+5)	**Michal Lieberman-Lazarovich (Israel):** Pollen flavonols in a Tomato high-pigment Mutant grown under Heat Stress Conditions
17.10 - 17.25 (+5)	**Tom Rankenberg (The Netherlands):** Dying to Survive: Molecular Regulation of Senescence during Submergence and Recovery in Arabidopsis
17.30 - 17.45 (+5)	**Galina Shevchenko (Ukraine):** Changes of Protein Expression in A. thaliana from Chernobyl Zone
17.50 - 18.00	***Closing Ceremony***

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Abstracts of Oral Presentations

CHLOROPLAST VESICULATION mediates chloroplast and peroxisome turnover, source-sink relationships and stress tolerance in crop plants

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The earliest detectable event during stress-induced senescence is the loss of photosynthetic activity and degradation of the chloroplasts. The protein CV (CHLOROPLAST VESICULATION) targets the chloroplast, promoting the formation of vesicles (containing stroma and thylakoid proteins) that are released from the chloroplast and transported into the vacuole through an autophagy-independent pathway. CV encodes a non-classical adaptor protein that recruits clathrin heavy chain-2, inducing the budding of CVVs (CV-contained vesicles). While OsCV overexpression caused leaf yellowing and a decrease in photosynthetic activity, OsCV silencing contributed to the maintenance of chloroplast integrity. CV-silenced plants displayed enhanced source fitness (i.e. carbon and nitrogen assimilation) and photorespiration, leading to water-deficit stress tolerance. Since high atmospheric CO2 conditions diminished photorespiration, we tested whether CV-silencing might be a viable strategy to improve the effects of high CO2 on grain yield and N-assimilation in rice. Under high CO2, OsCV expression was induced and OsCV was targeted to peroxisomes, facilitating peroxisome biogenesis factors (OsPEX11) degradation, reducing peroxisome number. At elevated CO2, CV-silenced rice plants maintained peroxisome proliferation, photorespiration and higher N assimilation. Co-immunoprecipitation of OsCV-interacting proteins indicated that, similar to its role in chloroplast protein turnover, OsCV acted as a scaffold, binding peroxisomal proteins. From a biotechnological perspective, CV silencing offers a suitable strategy for the generation of stress-tolerant transgenic crops.

Microbiome-induced epigenetic mechanism of heat stress tolerance in plants

Kirti Bhatt, Arsheed Sheik, Kiruthiga Mariappan and **Heribert Hirt**
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In response to stress, plants induce a set of stress genes for survival and recurrent stress induces epigenetic changes in stress memory genes to overcome otherwise lethal conditions. We study the contribution of the microbiome of desert plants to their extraordinary capacity to survive under extreme conditions of heat, drought or salt stress. We show that Enterobacter sp. SA187, a microbial strain of endophytic microbiome the indigenous desert legume Indigofera argentea, can confer multi-stress tolerance to non-host plants and crops. Using Arabidopsis thaliana as a genetic model system, we uncovered that microbial-induced plant heat stress tolerance is an epigenetic mechanism that enables plants to grow under extreme conditions. These findings have important implications for crop production on arid lands and under changing conditions of global warming.

Heat acclimation and inhibited cytokinin degradation positively affect heat stress tolerance of Arabidopsis

Sylva Prerostova[1], Petre Dobrev[1], Barbara Kramna[1], Alena Gaudinova[1], Vojtech Knirsch[1], Lukas Spichal[2], Marek Zatloukal[2], Radomira Vankova[1]
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The effect of acclimation (1 h 37°C followed by 2 h 20°C) on heat stress (HS; 3 h 45°C) was compared with the impact of direct HS. Phytohormones levels and antioxidant enzymes activities were evaluated in Arabidopsis thaliana shoot apices, leaves and roots after HS and after 24-h recovery. Acclimation diminished negative effects of HS in apices and roots, not in leaves. Stimulated antioxidant enzymes indicated suppression of reactive oxygen species. Acclimation stabilized growth of apices, showed by cytokinins (CK), auxin, abscisic acid (ABA) and jasmonic acid levels. Presence of ABA catabolites suggested an early response. INCYDE (CK oxidase/dehydrogenase inhibitor) in combination with acclimation slightly elevated HS tolerance. It increased CK and auxin content after HS and suppressed ABA and ethylene content during recovery. Non-acclimated plants treated by INCYDE (before or after HS) showed worse stress response due to delay of the repair process.
The work was supported by MEYS CR project no. LTAUSA17081.

Heat stress response in Tobacco BY-2 cells impaired in cAMP content

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Cyclic AMP (cAMP) is involved in thermotolerance, causing a calcium influx which promotes the expression of HSPs (1). To study more in detail the role of cAMP in heat stress (HS) response, we used tobacco BY-2 cells overexpressing the cAMP-sponge protein (cAS cells) which is able to reduce cell cAMP content (2). cAS cells were less tolerant to a moderate HS (35°C) than wild type (WT) ones. Despite the higher total antioxidant activity in cAS than in WT cells already at 27°C, an imbalance in redox homeostasis occurred after HS. The low cAMP content also prevented the activation of proteases and proteasome in response to HS. Proteomic and phosphoproteomic profiling showed that genetic buffering of cAMP at 27°C leads an alteration in signaling and protein degradation. In response to HS we observed an additional impairement of proteasome while the chaperone mediated protein folding increased. Moreover, metabolism and translation were strongly regulated and the involvement of these proteins in the altered heat stress response will be discussed.

(1) DOI:10.1111/j.1365-313X.2012.04969.x
(2) DOI:10.1007/s11103-016-0431-5

Modulation of heat stress response by heterotrimeric G proteins in Arabidopsis

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Plants being sessile have developed adaptive mechanisms to sense and respond to a variety of environmental cues. One such adaptive mechanism is operated through heterotrimeric G-proteins, which are key signaling intermediates in eukaryotes. In Arabidopsis, heterotrimeric G-proteins consists of one canonical and three extra-large G alpha, one G beta and three G gamma proteins. Analysis of knockout mutants missing one or more G-protein subunits demonstrate that the G beta protein, in association with the extra-large G alpha proteins regulate heat stress response, with no role of the canonical G alpha protein. The three G gamma proteins also contribute to this regulatory pathway. The heat stress tolerant phenotype of G protein mutants is associated with the changes in stomatal development and movement, which is also linked to their higher photosynthetic efficiency, lower leaf temperatures, and less ROS accumulation. The mutants also exhibit higher chlorophyll and electrolyte leaching, suggesting alterations in their membrane integrity. Additional molecular mechanisms suggest involvement of specific transcription factors and hormone transporters. Given the general role of G-proteins in mediating multiple stresses including heat, these could be a potential target for developing heat tolerant crops in the future.

Thermomorphogenesis control by Histone Deacetylase 9; Optimal plant performance under suboptimal temperature conditions

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[2] Institute of Molecular, Cell & Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom
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Many plant species can respond to high ambient temperature conditions by adjusting their architecture. This is known as thermomorphogenesis and enables optimal plant performance under suboptimal conditions. Thermomorphogenesis includes leaf movement and elongation growth of stems and hypocotyls and allows enhanced evaporative cooling and heat flux avoidance. We found that the chromatin modifying enzyme HISTONE DEACETYLASE 9 is required for thermomorphogenesis but not the shade avoidance response. At warm temperatures, HDA9 induces the expression of YUCCA8, a rate-limiting enzyme in auxin biosynthesis. Specifically, HISTONE DEACETYLASE 9 mediates histone deacetylation at the TSS of YUCCA8, which stimulates the eviction of the repressive histone H2A.Z variant from nucleosomes of the YUCCA8 promoter. Our work assigns a novel role to histone deacetylation in activating gene expression.

Small paraquat resistance proteins (SPQ) modulate paraquat, ABA and drought responses in Arabidopsis

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The Small Paraquat resistance Protein of Lepidium crassifolium (LcSPQ) and Arabidopsis thaliana (AtSPQ) confers paraquat resistance to overexpressing transgenic Arabidopsis plants. The knockout Arabidopsis mutant was found to be hypersensitive to this herbicide. The AtSPQ and LcSPQ proteins are composed of 70 and 69 amino acids respectively, and has a signal peptide but no other recognizable sequence domain. Overexpression of the SPQ proteins has not altered polyamine contents, neither sensitivity to polyamines. Besides being implicated in paraquat response, overexpression of SPQs enhanced sensitivity to abscisic acid (ABA), while the knockout Arabidopsis mutant was found to be insensitive to ABA. SPQs can considerably improve drought tolerance by reducing water loss, stabilizing photosynthetic electron transport and enhancing plant survival in water-limited environment. The conserved SPQ proteins seem to connect multiple regulatory pathways in stress responses.
Research was supported by grants: NKFI NN-118089, NKFI FK-128920, KH-129510.

Insight into mechanisms of drought resistance in Lolium multiflorum/Festuca arundinacea introgression forms

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Lolium multiflorum is a forage grass species characterized by a relatively high yielding capacity but significantly low abiotic and biotic stress resistance, while F. arundinacea expresses high ability to avoid and/or to tolerate water deficit in soil. The hybridization of both species enables the assembly of their complementary characters within a single genotype. Here, two L. multiflorum/F. arundinacea introgression forms with different capacity in response to drought, were examined during drought treatment in pots. The main objective of the presented research was to identify the key components of leaf metabolism associated with resistance to water deficit and/or with the capacity to regenerate after stress cessation. The physiological parameters, lipid composition as well as accumulation of antioxidants and the Calvin cycle enzymes, were analyzed. The analysis at transcriptome level to identify genes differentially expressed under drought, was also performed.
The research was performed within the project funded by Poland National Science Centre (no. 2016/23/B/NZ9/00820).

Kinetics of HSP70 and HSP90 expression and drought tolerance of Arabidopsis thaliana

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The accumulation of heat shock proteins (HSPs) that stabilize cellular proteostasis is a key mechanism of plant adaptation to water deficiency. Plants are unusual in having multi genes HSP families. To assess the contribution of different HSP70s and HSP90s into drought tolerance, we subjected Arabidopsis thaliana seedlings to increasing water deficit in agar medium. RT-PCR analysis of kinetics of gene expression of 12 HSP70s and 7 HSP90s with the different subcellular localization revealed specific patterns in the expression profiles concerned to the basic level, range and rate of changes. At that, the genes of mitochondrial isoforms were the most reactive. Among the stress-inducible cytosolic genes, only AtHSP70-4 showed high induction under the certain level of water deficiency. A response of other inducible genes AtHSP70-5 and AtHSP90-1 was very weak. Nevertheless, knockout mutants Athsp70-5 and Athsp90-1 confirmed the contribution of the corresponding members to seedling drought tolerance. The obtained data pointed out that AtHSP70-4 is the main inducible member of the HSP families providing drought tolerance of A. thaliana, whereas AtHSP70-5 and AtHSP90-1 are additional but also essential inducible components.

Strategies and cost of plant osmotic adjustment

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Exposure to hyperosmotic environment lowers the external water potential and compromises a plant's ability to take up water. To deal with this issue, plants must adjust osmotically, e.g. to accumulate solutes to balance extra osmotic pressure in the soil solution to maintain turgor. Plants may achieve osmotic adjustment via two major avenues, i.e., by de novo synthesis of organic osmolytes and by increased uptake of inorganic ions. In this talk, I compare the energy cost associated with each strategy. I argue that the carbon cost of osmotic adjustment via inorganic ion uptake is an order of magnitude lower than that via organic osmolyte synthesis and is generally preferred, even by glycophyte species. I argue that relying on Na+ will be the most optimal option assuming plants possess efficient tissue tolerance mechanisms. I then discuss the underlying mechanisms conferring such tissue tolerance in plant cells, with a major focus on vacuolar Na+ sequestration. I argue that an active removal of Na+ from the cytosol into vacuole is only one component of the vacuolar sequestration mechanism. Another (often-neglected) component is Na+ retention in vacuoles. I show that the failure to control Na+ -permeable slow (SV) and fast (FV) vacuolar channels may result in a futile cycle. I then discuss different scenarios of operation of SV and FV channels in plant cells and their energy cost for plants.

Identification of PIP2-interacting proteins in response to salt- and heat stress

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Phosphoinositides play multiple roles in various cellular processes, including vesicular trafficking, cytoskeleton organisation, and signal transduction. In plants, the level of phosphatidylinositol-4,5-bisphosphate (PIP2) is extremely low, yet the model plant Arabidopsis thaliana contains 11 genes encoding the enzyme that makes PIP2, i.e. PIP 5-kinase (PIP5K). KO mutants of various PIP5K genes have implicated roles for PIP2 in cell division and development, but so far, very few PIP2 targets have been identified. We know that PIP2 synthesis is dramatically enhanced upon salt- and heat stress, and that this predominantly occurs at the plasma membrane. To find out the signaling functions of PIP2, we have developed a protein pull-down assay using PIP2-affinity beads, coupled to mass spectrometry analysis using protein extracts enriched in peripheral-membrane proteins isolated from salt-, heat- or control-treated Arabidopsis cell suspensions. So far, we have identified various PIP2-binding proteins, including PATELIN1 and 2 that are known to bind PIP2 and to be involved in salt stress. A list of new potential PIP2 targets will be presented.

An endophytic bacterium improves salt stress tolerance in tomato Plants

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Global climate changes strongly affect crop growth conditions: they are, therefore, a significant constraint to the world food request. Soil salinization and aridity are among the most significant problems. It is well known that Plant Growth-Promoting Bacteria (PGPB) improve plant health status under both biotic and abiotic stresses. In this research work, we investigated the possible plant protection effects induced in tomato by a PGPB, the endophyte Pseudomonas migulae 8R6, in salinity conditions. We choose this bacterium for its capability of synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves ACC to α-ketobutyrate and ammonia and thereby decreases ethylene levels in the host plants. A low level of ethylene can alleviate plant stress symptoms. In a first, short time experiment, the degree of colonization by the mutant 8R6 expressing GFP and its localization in the plant tissues of tomato by fluorescence, confocal, and image analysis techniques were evaluated. Our results confirmed that 8r6 is a facultative endophyte. In the first 30 days, plants elicited with 8r6 (both mutant expressing GFP, both wild-type) showed a decreased growth. Histological analysis on stems revealed a reduction of the diameter of epidermal and parenchymatic cells. In a second experiment, we assessed the effects of 8R6-GFP and 8r6-wild type on the growth and the health of tomato in the presence/absence of salt stress by microbiological and physiological techniques. Under salt stress, 8r6 wild type stimulated plant growth with a significant reduction of symptoms. Moreover, the OJIP test of tomato leaves showed a positive effect of 8r6 wild type under salt stress, compared to control plants. Finally, we explored the production of seeds containing 8R6-GFP to allow a simple and direct use of this endophyte: tomato seeds contained 8R6-GFP and raised new plants, already colonized by 8R6-GFP.

Hot water treatment modifies redox status and phytohormone synthesis in apple fruit during short-term storage

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Hot water treatment (HWT) of fruits is an approved method to reduce post-harvest fruit damage by pathogen infection. While the beneficial effects of HWT on storability of various fruits have been well documented, HWT induced cellular and molecular responses are not fully understood. Here, we examined the effect of HWT on the redox status and the gene expression of defence hormones and pathogenesis-related (PR) proteins of apples. While apple fruits showed a gradual, but treatment independent, reduction in H2O2 and ascorbate levels during storage, HWT led to an increase in the amounts of glutathione compared to untreated controls. This correlated with enhanced glutathione reductase activity, resulting in a more reduced glutathione state after HWT. To examine stress signalling, changes in MAPK activity were analyzed in an early time course experiment after HWT. HWT also led to a persistent increase in MAPK activity, suggesting the induction of downstream signalling pathways. Interestingly, HWT resulted in a decrease in ripening-associated ethylene biosynthesis transcripts and activated salicylic acid biosynthesis and pathogenesis-related gene expression. Taken together, these results suggest that post-harvest HWT of apple fruits activates stress signalling and induces changes in the oxidative metabolism and phytohormones biosynthesis, which might contribute to protect fruits against pathogen infection and thus extend its shelf-life.

Molecular characterization and subcellular localization of salt-inducible lipid transfer proteins in rice

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Plant lipid transfer proteins (LTPs) are abundant lipid binding proteins that are important in membrane vesicle biogenesis and trafficking, however, the biological importance of LTPs on salt-stress response in rice remains unclear. Therefore, salt responsive rice LTPs were identified and characterized in this study. Microarray analysis showed seven genes positively regulated by salinity, including five Ltp genes (LtpII.3, LtpII.5, LtpII.6, LtpV.1, and LtpV.2) and two Ltp-like (LtpL; LtpL1, and LtpL2) genes. Amino acid alignment revealed that all these Ltp and LtpL genes contained the N-terminal signal peptide. Apart from LtpL1, all salt-inducible Ltp genes had the conserved eight cysteine residue motifs backbone. Verification of gene expression to different stimuli in rice seedlings revealed that salt-regulated Ltp genes differentially responded to drought, cold, H2O2, ABA and CaCl2. Furthermore, the expression of Ltp and LtpL genes was tissue-specifically regulated by ABA-dependent and independent pathway. Three LtpII subfamily genes, including LtpII.3, LtpII.5, and LtpII.6, were strictly expressed in flowers and seeds, and LtpIII.1 mRNA strongly accumulated in stem tissue. Subcellular localization analysis of LTP-DsRed fusion proteins revealed that the five LTPs and two LTPLs localized at the endoplasmic reticulum. The results provide new clues to further understanding the biological functions of Ltp genes.

Exploring the genetics underlying Arabidopsis Zn deficiency response

Valeria Ochoa Tufiño, Maria Almira Casellas, Ana Carolina Campos, Khadija Aaliya, Joost van den Heuvel, Ana G.L. Assunção, Robert Akkers, Henk Schat, **Mark G.M. Aarts**
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Zn is an essential element and the most abundant transition metal in plants. To cope with Zn deficiency, plants adjust their Zn homeostasis by inducing expression of several genes. In order to learn more about their role and function in relieving Zn deficiency, we have analysed some of them in more detail. We visualized the distribution of Zn depletion in Zn deficient roots, determined the expression of Zn transporter genes in response to Zn deficiency in time, mapped their cellular specificity of expression in roots based on promoter-nYFP fusions, and examined the effects of their loss of function using single and double mutants. Zn transporter genes ZIP1, ZIP3, ZIP4, ZIP5, ZIP9, ZIP11, ZIP12, IRT3, MTP2, HMA2 and YSL3 were studied. In addition, we studied the role of N-ALPHA-TERMINAL ACETYLTRANSFERASE 25 (NAA25) in Zn response and performed a Genome Wide Association analysis of the Zn deficiency ionome, which revealed the involvement of a cluster of HIPP genes in Zn deficiency response. Our findings generally confirm the complexity of the Zn deficiency response, and the high redundancy with respect to different Zn transporters, but also shows that there are several other genes that play a role, which will help in further unravelling the different components of the plant Zn deficiency response.

Alyssum lesbiacum populations from distinct habitat show different nitro-oxidative response to nickel stress

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Nickel (Ni) hyperaccumulator endemic species of Lesbos Island (Greece) Alyssum lesbiacum (Candargy) possesses an exceptional Ni tolerance. In our study, Alyssum seeds from two geographically separated study sites (Ampeliko and Loutra) were germinated and grown on control and Ni-containing soil in a rhizotron system. Root length and meristem viability of plants from both habitats were decreased by Ni; however lateral root number decreased only of plants from Ampeliko site. Behind the different morphological response, the level of nitric oxide (NO) and nitration changed in different ways. In our system NO acts as a signaling molecule, not as a stress indicator, and the decreasing protein tyrosine nitration in roots of plants from Ampeliko site could be associated with higher Ni tolerance.
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Identification of uranium root absorption pathways in Arabidopsis Thaliana

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Uranium (U) is a naturally occurring trace metal element and radionuclide that may locally accumulate at concentrations that pose potential risks to ecosystems, agrosystems and, ultimately, human health. Uranium is not essential for plants, but it is taken up from the soil, incorporated into root tissues, and then translocated at low levels to shoot organs. Uranium is chemotoxic and potentially radiotoxic to all living organisms. It triggers oxidative stress and interferes with root growth and architecture, mineral nutrition, homeostasis of essential elements such as phosphate and iron, and photosynthesis. Despite significant efforts to decipher the mechanisms underlying U toxicity in plants, little is known about the mechanisms that contribute to the absorption of the radionuclide from the environment. In this study, we used a combination of strategies to elucidate the routes used by U to be taken up by the roots of Arabidopsis thaliana. Taken together, our data combining starvation experiments, competition assays, mutants and inhibitors analyses, showed that U uptake by roots probably operates through different transport systems, among which calcium has a central role.

Increased levels of phenolic compounds, polyamines and amino acids indicate potential symbiont induced reduction of tungsten (W) induced stress in glycine max

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The transition metal tungsten (W) shares certain chemical properties with the essential plant micro nutrient molybdenum (Mo), and is proposed to inhibit enzymatic activity of molybdoenzymes such as nitrate reductase, by replacing the Mo-ion bound to the molybdopterin co-factor (Mo-MPT). It has been shown that roots and nodules of symbiotically grown soy bean plants exhibit higher levels of proteins involved in hormone and flavonoid biosynthesis in the presence of high concentrations of W (0.5 mM Na2WO4). Flavonoids are thought to be involved in radical scavenging as well as chelation and sequestration of various HM. The aim of this study was to clarify if a symbiotically induced increase of secondary metabolites affects the plant's tolerance to tungsten. Our metabolomic study showed that symbiotically grown (Nfix symbiont B. japonicum) exhibit a stronger metabolic response compared to their non-symbiotic (10 mM KNO3) counterparts in presence of tungsten. We found an increase in phenolic compounds, flavonoids and soluble sugars in Nfix roots and leaves exposed to tungsten which resulted in a higher antioxidant capacity in comparison to Nfed plants. Furthermore, we could show an increase in organic acids, polyamines (i.e. putrescine, spermidine) and amino acids (i.e. Proline, Alanine) in Nfix plants in response to 0.5 mM W. Our results strongly indicate a symbiont induced alleviation of tungsten stress via enhanced radical-scavenging, metal-chelating and osmo-protective capacity.

Evolutionary flexibility in flooding response circuitry in angiosperms

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Flooding due to extreme weather threatens crops and ecosystems. To understand variation in gene regulatory networks activated by submergence, we conducted a high-resolution analysis of chromatin accessibility and gene expression at three scales of transcript control in four angiosperms, ranging from a dryland-adapted wild species to a wetland crop. The data define a cohort of conserved submergence activated genes with signatures of overlapping cis regulation by four transcription factor families. Syntenic genes are more highly expressed than nonsyntenic genes, yet both can have the cis motifs and chromatin accessibility associated with submergence up-regulation. Whereas the flexible circuitry spans the eudicot-monocot divide, the frequency of specific cis motifs, extent of chromatin accessibility, and degree of submergence activation are more prevalent in the wetland crop and may have adaptive importance.

CRK2 is involved in the control of salt stress-induced callose Deposition

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The cysteine-rich receptor-like kinases (CRKs) form one of the largest groups of RLKs in plants. The distinguishing feature of CRKs is their extracellular domain which carries two copies of the domain of the unknown function 26 (DUF26, also known stress-antifung PF01657) containing three strictly conserved cysteine residues (C-8X-C-2X-C). We have identified CRKs, as essential signal transduction components in response to biotic and abiotic stress. In particular, CRK2 is involved in the organization of early signaling events at the plasma membrane following signal perception. CRK2 interacts with several known components of abiotic stress responses including phospholipases and callose synthases. In response to salt stress, plasma membrane-localized CRK2 relocalizes to plasmodesmata where it interacts with callose synthases and regulates callose deposition. This relocalization is dependent on phosphatidic acid production and calcium signaling. Salt-induced callose deposition is reduced in the crk2 mutant, which is impaired in salt tolerance. A role for callose deposition in salt stress is also highlighted by the cals1 mutant, which is also displays reduced resistance to salt stress. We propose that CRK2 serves as a organizing element at the plasma membrane during the responses to abiotic but also biotic stress and integrates calcium signaling, callose deposition and also reactive oxygen species production.

Regulation of stress tolerance under different light conditions

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Light and temperature are two main factors that enable plants to sense seasonal changes and adjust growth, defense, and transition to flowering according to the prevailing conditions. In recent years, phytochrome signaling has emerged as key regulatory pathway(s) coordinating developmental processes and stress responses. Beyond its role in light-signaling and pathogen defense, phyB has recently been demonstrated to act as temperature sensor, thus forming a signaling hub in acclimatory responses to changes in the environment. We are investigating regulatory components within this signaling network. Experimentally, we are using ozone to induce apoplastic ROS production to treat plants grown under different light conditions. Under long day conditions, we observed a faster and stronger activation of molecular defenses (including protein phosphorylation cascades, and hormone signaling) as well as programmed cell death (PCD), as compared to plants grown under short day conditions. We see that salicylic acid plays an important role in PCD in a day-length-sensitive manner. Furthermore, we are proposing protein phosphatase PP2A as novel regulator within the phytochrome signaling network.

Engineering ethylene biosynthetic pathway leads to flood tolerance in Plants

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The rising atmospheric temperature, along with elevated carbon dioxide, can cause catastrophic natural disasters, including floods. The flood can cause about $19 billion crop loss worldwide. Plants grown in flooded areas suffer from anoxia related symptoms, including a decrease in ATP content and ethanol formation. Conversion of ethanol to acetaldehyde can be toxic and ultimately lead to cell death. Plants can tolerate anoxia by swollen aerenchyma, the formation of adventitious roots, programmed cell death, etc. It is well documented by researchers that the production of ethylene in roots leads to flood tolerance. Ethylene is produced in plant roots from the oxidation of aminocyclopropane carboxylic acid (ACC) by ACC oxidase. However, ethylene production is interrupted in plant roots due to a lack of oxygen. We tested the hypothesis that overexpression of the ACC oxidase gene in Arabidopsis thaliana will lead to the production of ethylene and impart flood tolerance. We reported that ACC oxidase gene expression in Arabidopsis thaliana provided flood tolerance to transgenic plants for 35 days. In contrast, control plants showed symptoms of flood-related injuries from the eight-day. Currently, we are evaluating the potentials of developing flood-tolerant economically important plant species sunflower and lentil by genetic transformation of sunflower and lentil with the same ACC oxidase gene. We have developed an in vitro regeneration system of sunflower and lentil, and we are in the process of transforming these plants with the ACC oxidase gene.

Abiotic Stress-Induced Lipid Signaling

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Phospholipids are crucial membrane components. They are responsible for the bilayered structure and fluidity of membranes, which prevents charged molecules from entering and escaping, and allow cells and cellular compartments to build up a membrane potential as electrical source of energy as well as to maintain water. Besides structural functions, phospholipids are also crucial for cell signalling. The latter typically include polyphosphoinositides (PPIs), derivatives of phosphatidylinositol (PI) that are phosphorylated at the D-3, -4 and/or -5 position of the inositol ring, and phosphatidic acid (PA). These signalling lipids only represent a minor fraction of the total cellular phospholipid pool, yet control many aspects of eukaryotic cell functioning. Over the years, we have generated various genetically-encoded lipid-biosensor lines that can monitor lipid signalling in living cells and tissues. They were generated by fusing DNA of specific lipid-binding domains with those of fluorescent proteins (FPs), and stably expressing them Arabidopsis. In combination with KO- and OE mutants, we are exploring how lipid second messengers, the enzymes involved in their metabolism, and protein targets, integrate stress- and developmental signals in plants. In this lecture, our latest results on the signalling role of phosphatidylinositol-4,5 bisphosphate (PIP2) and PA will be addressed.

Linking Salt Stress Signalling to the Chromatin

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Dynamic adjustment of chromatin organization constitutes one of the key responses of plants to stress. DEK3 is an evolutionarily conserved chromatin-architectural protein involved in modulation of chromatin function and plant stress tolerance. DEK3 levels regulate in vivo nucleosome occupancy and DNA accessibility and contribute to transcriptional regulation of its target genes. Furthermore, fine-tuned DEK3 levels are important for salt stress tolerance. Following up on the role of DEK3 in stress tolerance, we identified a stress-responsive, chromatin-associated protein kinase that phosphorylates DEK3. Evidence for a novel mechanism to regulate chromatin function under high salinity stress will be presented.

Glucose-TOR signaling controls thermotolerance/thermomemory

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Global warming exhibits profound effects on plant fitness and productivity. To withstand stress, plants sacrifice their growth and trigger activation of protective stress responses in ensuring survival. However, the switch between growth and stress responses is largely elusive. In the past decade, emerging role of Target of Rapamycin (TOR) has been studied linking energy and stress signaling. In the present study, we have identified the crucial role of glucose (Glc) mediated TOR signaling in plant adaptation to heat stress (HS). Glc epigenetically governs the transcription of core HS signaling genes in a TOR-dependent manner. TOR acts in concert with Histone acetyltransferase 1 (HAC1) and dictates the epigenetic landscape of HS loci. Arabidopsis plants defective in TOR and HAC1 exhibited reduced thermotolerance with a decrease in expression of core HS signaling genes. Further, TOR overexpressing plants grown under high light conditions showed increased thermotolerance than tor RNAi lines further substantiating the requirement of energy signaling in stress mitigation. In addition, Glc-TOR signaling governs the transcriptome reprogramming of myriad set of genes involved in HS protection and recovery. Moreover, TOR promotes the accumulation of histone H3K4me3 marks at the promoters of thermomemory-related genes and therefore, governs thermomemory. Collectively, our findings thus reveal the crucial function of Glc-TOR signaling dictating integration of stress and energy signaling in mediating thermotolerance/thermomemory.

Unraveling the role of the enigmatic Zea mays PIP1;1 aquaporin

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Plasma membrane aquaporins (PIPs) cluster into two groups (PIP1 and PIP2). When expressed alone, PIP1s are localized in the endoplasmic reticulum (ER), while PIP2s are found in the plasma membrane. However, when co-expressed, PIP1s and PIP2s physically interact within a heterotetramer, leading to the relocalization of the PIP1s from the ER to the plasma membrane and to an increase in the membrane permeability (Pf). The most widely expressed PIP in maize is PIP1;1. However, this isoform does not behave like the other PIP1s. The aim of this project is to elucidate the specificity, the regulation and the role of PIP1;1 using complementary approaches. While the co-expression of PIP1;1 and PIP2;5 in oocytes did not increase the cell Pf, both isoforms physically interacted to regulate their plasma membrane localization. However, when expressed in maize suspension cells, YFP-PIP1;1 enhanced the Pf compared to the control cells. These results point to a synergistic interaction between PIP1;1 and PIP2;5, but the mechanism underlying the enhanced activity is still under study. Finally, deregulation of PIP1;1 expression in maize plants modified the stomatal behavior and the hydraulic parameters under water stress.

Modulation of ERECTA for improving stress tolerance in rice

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Breeding stress tolerance is a daunting task due to low heritability of the trait. ERECTA (ER) has been implicated in environmental stress responses in the recent studies on tomato and rice. In this study, a truncated Arabidopsis ER (ΔER), defective in ER signaling, was expressed in rice, and the resulting transgenic lines were evaluated by qPCR at V3 (seedlings), R2 (panicle inside the stem), R3 (early flowering), R4 (flowering), R5 (5 days after flowering), and R6 (milk grain) stages. Expression of rice ER-like genes Os06g0203800 (OsERL1), Os02g0777400 (OsERL2), and Os06g0130100 (OsERL3) was also evaluated. The development phases that showed the highest expression of transgene ΔER and OsERL1, OsERL2 and OsERL3 were the reproductive phases R2, R3 and R4, an important criterion for the rice plant tolerance to water stress, since reduced moisture or high heat before anthesis or during fertilization greatly compromises grain yield. The expression of ΔER gene in rice resulted in 4 – 50x upregulation of OsERL1, OsERL2 and OsERL3 genes in the reproductive phases. Most importantly, this upregulation was significantly higher in transgenic lines expressing high levels of ΔER compared to the lines expressing lower levels. Therefore, rice compensates for the dominant-negative mutation in ER function by overexpressing its native ER (OsERL) genes. As ER genes play important role in stress tolerance, this study illuminates a strategy for enhancing stress tolerance in rice.

Effects on antioxidant defence mechanisms of lettuce plants exposed to contamination by acetaminophen and carbamazepine

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Pharmaceuticals are currently increasing their occurrence in rivers and streams. This indicates that these compounds may reach irrigation waters and consequently edible vegetables. The main objective of this work is to understand how lettuce plants cope with the exposure to different concentrations of acetaminophen (ACT) and carbamazepine (CBZ). The presence of both pharmaceuticals in roots and leaves was confirmed by HPLC-MS techniques. The minerals' contents were determined by ICP-OES. Cu, Zn, K and P contents decreased for longer periods with CBZ, and Fe content declined in roots under CBZ. Antioxidant defence system was evaluated by CAT, GPOD, SOD, APX, GR and GPX activities. H2O2 content increased for longer periods of exposure to ACT and CBZ and a significant increase was found in CAT and GPOD activities. In case of SOD, an increase was only found for ACT exposure. APX activity revealed differences in case of CBZ contamination. In both contaminations, roots and leaves exhibited different responses to oxidative stress. Anthocyanins content increased in contaminated plants, which indicate a response induced by oxidative stress due to its antioxidant properties.

Pollen flavonols in a tomato high-pigment mutant grown under heat stress conditions

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The significance of pollen quality for plant reproduction under heat stress conditions is well established. When flowers develop under heat stress conditions, pollen viability, pollen germination and pollen tube elongation are hampered, leading to reduced fertility and fruit set. Identifying factors that contribute to improved pollen performance under heat stress could potentially lead to the development of heat tolerant crop varieties. Flavonols, a class of secondary metabolites with antioxidative property, were formerly suggested to have a protective role under heat stress conditions in tomato. Since the tomato high-pigment mutants are well known for their increased levels of secondary metabolites, we sought to investigate pollen flavonols in these mutants in the context of thermotolerance. Using DPBA-staining and flow cytometry, we show that under both normal and heat stress conditions, flavonols are found in higher levels in pollen of the high-pigment mutant. Importantly, this is correlated with a higher proportion of viable pollen under heat stress conditions in the mutant. We hypothesize that pollen flavonols may support pollen functionality and therefore reproductive success under heat stress conditions in tomato.

Dying to survive: molecular regulation of senescence during submergence and recovery in Arabidopsis

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Most terrestrial plants are extremely flood sensitive. Flooding events thus pose a large threat to crop yields worldwide. An aqueous environment severely reduces gas exchange and light exposure, limiting plant photosynthesis and energy metabolism during a flood, and eventually prove fatal. Fast recovery following a flood is essential for plant survival. However the post-submergence phase can present additional stressors for a plant. Following desubmergence, there is excessive ROS production, rapid dehydration and accelerated senescence. We aim to find the signals and networks controlling submergence and recovery. Regulation of recovery processes depends on submergence acclimation and signals (ethylene, ABA, ROS) generated upon emersion. Signals occurring during submergence and recovery are systemic, but post-submergence stress symptoms are manifested in an age-dependent manner: oldest leaves die first, whereas the youngest leaves and the meristem survive the longest. In this context I will discuss (1) age-dependent shoot transcriptomic responses during submergence and recovery (2) the role of the transcription factor ORE1 and how its tissue-specific regulation contributes to the coordinated breakdown of Arabidopsis leaves (3) the relevance of senescence for flooding survival.

Changes of protein expression in A. thaliana from Chernobyl zone

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Chernobyl today is characterized by the presence of plants adapted to chronic irradiation and soil pollution with heavy metals. An understanding of the molecular mechanisms of plant resistance is essential for agriculture and biotechnology of stress tolerance. We studied three A. thaliana accessions from Chernobyl area with different tolerance to radiomimetic and response of their proteomes to cadmium. The accessions were grown under controlled conditions on a MS medium with or without 100uM CdCl2. Subsequent proteome changes were determined by 2D electrophoresis and mass spectrometry, and analyzed by bioinformatics. Specific reaction was found in the proteome of the tolerant Chernobyl genotype (Ch_07). In particular, the mechanism preserving photosynthesis (RUBISCO, glutamate-1-semialdehyde 2,1-aminomutase 1) and energy production was activated, enzymes for the biosynthesis of sulfur-containing amino acids probably associated with the biosynthesis of phytochelatins were accumulated, alterations in proteins active in protein synthesis/folding, ascorbate-glutathione cycle (glutathione reductase) and stress responses (annexinD1) were observed.

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Abstracts of Poster Presentations

Poster №1:

Transcription factor OsERF106 negatively modulates salt stress response in rice

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Abiotic stress affects rice production and quality. The regulatory networks of gene expression that mediated by transcription factors is especially important for rice in response and tolerance to environmental stresses. The supergene family of rice transcription factors, Ethylene Response Factors (OsERFs), have been known to involve in growth development; hormone response; pathogen interaction and abiotic stresses. We found that OsERF106 gene expression was induced under salinity stress. However, the function of OsERF106 under salt stress is still unknown. In this study, we demonstrated that over-expression OsERF106 in rice leads to the retardation of growth and hypersensitive to osmotic and salinity stresses. The in vitro ROS staining (NBT and DBA) and the measurement of MDA content in transgenic rice showed higher accumulation of $H2O2$ and $O2.-$. Moreover, the OsERF106-overexpressing rice increased the expression level of ion transporter OsSOS1 and decreased OsHKT1; 4 gene expression, resulted in higher Na+ and K+ accumulation in rice shoots. The transcriptome analysis showed that OsERF106 down-regulated the gene expression levels of OsHKT1;4 and stress-related transcription factors OsbZIP16, OsWRKY42, OsWRKY45, while the gene expression of potassium transporters OsTPKb, OsHAK12 and chloride transporter OsCCC1 were up-regulated. Taken together, OsERF maybe a negative regulator of rice in response to salt stress. OsERF106 may change the oxidative stress state and Na+/K+ homeostasis to affect rice growth and salt stress tolerance.

Poster №2:

Physiological adaptation of eight Red sea halophytes to their natural saline habitats

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Different halophytes employ diverse but integrated physiological mechanisms to withstand high soil salinity. The relative contribution and the ecological significance of each of these mechanisms vary among plant species. Here, ecological parameters, physiological and metabolic responses to salt stress were compared among eight halophytes in a Red sea eastern coast salt marsh in Saudi Arabia. Plant taxa included Suaeda monoica, S.vermiculata, S. schempri, Tamarix aphylla, Heliotropium crispum, Zygophyllum coccineum, Halopeplis perfoliata, and Avicennia marina. The rhizospheric soils surround the tested species differed significantly in their salinity levels. The tested species differed in a number of C and N metabolism-related parameters such as photosynthetic pigments, carbohydrates, leaf total N, protein and amino acids. They also differed in MDA and non-enzymic antioxidant metabolites. More details and correlations among the tested parameters will be presented.

Poster №3:

Assessing the role of bZIP10 transcription factor in the response of Arabidopsis plants to prolonged dark periods

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bZIP transcription factors play an important role in plant responses to various environmental factors. Several bZIPs from groups C and S1, which preferentially form heterodimers, have been previously shown to be both regulated by divergent adverse factors and involved in plant reactions to these factors (Mair et al., eLIFE, 2015; Hartmann et al., Plant Cell, 2015; Droege-Laser and Weiste, TiPS, 2018). Here we show that Arabidopsis lines with ectopic expression of C-group member bZIP10 demonstrate hypersensitivity to dark treatment: they showed a distinctly higher leaf damage and cell death compared to wild-type plants, whereas the knock out and dominant negative lines seemed to be less affected by prolonged darkness. Such a damage was especially observed in the younger leaves. The induction of cell death in bZIP10-overexpressing plants during prolonged darkness overlapped with the change in redox potential of the glutathione cytoplasmic pool, as assessed by the redox sensitive roGFP2 fused to the human glutaredoxin GRX1 (Meyer et al., Plant J, 2007). These results agree with the suggested role of bZIP10 as a positive regulator of cell death in plant hypersensitive response (Kaminaka et al., Embo J, 2006). Our preliminary data point toward redox-dependent regulation of bZIP10, which contains three cysteine residues in its' sequence.

Poster №4:

HOS15 controls flowering time through COP1-mediated GI degradation in Arabidopsis thaliana under cold ambient temperature

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Flowering time, one of many important physiological traits is regulated by environmental inputs including light and temperature. A key photoperiodic flowering promoter, GIGANTEA (GI), controls its downstream floral activators such CONSTANS (CO) and FLOWERING T LOCUS (FT) positively. The protein abundance of GI is regulated by CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1), a RING finger type ubiquitin E3 ligase in a 26S proteasome-dependent manner. Here, we demonstrate that a WD repeat protein, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE15 (HOS15) also regulates flowering at cold ambient temperature by controlling the degradation of GI in cooperation with COP1. Loss of HOS15 function mutant, hos15-2 was insensitive to low ambient temperature in terms of flowering. When we analyzed the epistatic relationship with HOS15 and flowering genes, GI, CO, and FT, we found that gi-2, co-1, and ft-1 are epistatic to hos15-2, which indicates that GI, CO and FT act downstream of HOS15 in a thermosensory pathway. In fact, cold-induced degradation of GI is impaired in hos15-2 mutant and HOS15 forms a complex with COP1. Moreover, the protein accumulation of GI is highly increased in hos15-2 cop1-4 double mutant similarly to hos15-2 and cop1-4 mutants. Thus, HOS15-COP1-triggered GI turnover or degradation results in delayed flowering at cold ambient temperature. Taken together, our findings suggest that HOS15 functions as an integrator of photoperiod and ambient temperature signaling in Arabidopsis.

Poster №5:

Transcriptome-wide screening and functional characterization of Drought-Induced Long Noncoding RNAs in Rice

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Long noncoding RNAs (lncRNAs) have appeared as critical regulatory factors of various biological processes. In rice (Oryza sativa L.), it has been known that several lncRNAs regulate key biological processes. However, systematic examination of rice lncRNAs involved in abiotic stress responses has not been reported. Here, we re-analyzed the expression profile of lncRNAs in publicly available rice transcriptome datasets derived from abiotic stress treatments. Overall, we identified 10,831 rice lncRNAs that were significantly altered under four different abiotic stresses. Strong cross-talks between different stress signaling pathways were observed for lncRNA expression changes. Real-time RT-PCR analysis confirmed the differential expression patterns of these lncRNAs under various stress conditions. To determine the regulatory role of lncRNAs in abiotic stress signaling, lncRNAs were transiently overexpressed in rice protoplasts. As a result, DRIL4 overexpression increased the expression levels of stress marker genes such as WRKY71, ATPase, OsTPS, and Wsi18. Our results show the comprehensive identification and functional characterization of a group of abiotic stress-responsive lncRNAs in rice.

Poster №6:

Exploring Camelina sativa stress tolerance mechanisms for future breeding approaches

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Plants frequently encounter adverse growth conditions. Climatic factors, such as extreme temperatures, drought and contamination of soils by high concentrations of salts, are major abiotic environmental stressors that delay growth and development and thus reduce crop yield. It is now accepted that in future European agriculture will face severe losses in quality and quantity from abiotic stresses. Camelina sativa (false flax, gold-of-pleasure) is an ancient, low-input European oil seed crop which has a high adaptability to changing environmental conditions and an inherent resilience compared to other crops. Thus, Camelina is a promising resource to identify previously unknown tolerance mechanisms and harness them. To study the abiotic stress tolerance of Camelina sativa, a set of different camelina genotypes are analyzed under high salinity conditions. This approach will be complemented by assessing the response to different water/drought regimes. Phenotypic changes, physiological parameters as well as molecular markers will be analyzed and correlated with the stress response/tolerance of the different camelina lines. As plant growth and development, and thus the agronomic value of crops, are tightly linked to their metabolism, a focus will be laid on key carbohydrate metabolic enzymes. To do so, we recently established a platform for multiple metabolic and enzymatic measurements in a microplate format in our lab. The overall goal of the project is to decipher the stress tolerance mechanisms of Camelina sativa to support innovative breeding.

Poster №7:

Morphological and molecular characterization of mutants with abnormal hairs in tomato

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Trichomes are hair-like structures on the aerial surface of many plant species. Despite the important roles of trichomes in diverse abiotic and biotic plant defense, most of the genes for multicellular trichome formation remain unknown. To identify genes related to trichome development, we screened Micro-Tom mutant population generated by EMS (Ethylmethane sulfonate) mutagenesis and obtained four mutants with distinct trichome phenotypes compared with wild-type Micro-Tom plants. All four mutants have a similar trichome morphology with distorted and curled trichomes. Previously, we showed that hairless and inquieta mutants have distorted trichomes and the corresponding genes are involved in the polymerization of actin cytoskeleton. So we hypothesized that the new four mutants are also related to actin polymerization. To compare the expression levels of genes involved in the polymerization of actin cytoskeleton between the four mutants and wild-type plants, RT-PCR analysis was performed. Among 16 genes tested, one gene showed different size between one mutant and wild-type plants. To find mutation region from the mutant, we are currently doing cDNA and genomic DNA sequencing of this gene from the mutant and wild-type plants.

Poster №8:

Uncovering the role of Glucose-TOR signaling in modulating cold stress tolerance

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Low temperature stress not only limits the geographical distribution of plants but also affects the productivity of crops. Role of sugars as an osmoprotectant or a membrane stabilizer during cold stress is well known. However, the role of sugars in capacity of being a signaling molecule to provide tolerance to cold stress still remains elusive. This study reveals that glucose-stimulated TARGET OF RAPAMYCIN (TOR) acts as a key player in regulating cold tolerance. Our microarray analysis demonstrated that glucose leads to changes in the global transcriptomic landscape in the presence of cold and activates innumerable genes responsive to cold stress. Also, glucose promotes the recovery of Arabidopsis seedlings from cold stress and enhanced the expression of cold stress signaling genes. Physiological and transcriptional studies showed that tor RNAi was sensitive to cold stress and displayed reduced expression of core cold responsive genes while TOR overexpression lines exhibited enhanced tolerance. Previous reports suggest that HISTONE ACETYLTRANSFERASE1 (HAC1) is a plant homologue of mammalian Acetyltransferase p300 and mammalian mTORC1 interacts with p300. Arabidopsis seedlings defective in HAC1 showed reduced cold stress recovery together with decreased expression of cold stress signaling genes. Thus, the investigation highlights an interplay of Glucose-TOR mediated signaling and epigenetic modulation via HAC1 in governing cold stress response in Arabidopsis.

Poster №9:

Analysing the role of the protein kinase ASKα in the low-energy response in plants

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Environmental stress is among the most significant factors responsible for substantial and unpredictable losses in crop production worldwide. Improving plant stress resistance and crop yield under adverse environmental conditions is thus a major goal in sustainable agriculture. Both abiotic and biotic stresses induce a metabolic shift from growth to stress response and can lead to energy depletion. Previously, we have demonstrated that plant GSK3 protein kinases contribute to the regulation of carbohydrate metabolism under stress conditions (Jonak C et al. 2000, Dal Santo S. et al. 2012). Furthermore, we have shown that the protein kinase ASKα (Arabidopsis GSK3/Shaggy-like kinase α) positively regulates salt-stress tolerance and pathogen resistance (Dal Santo S et al. 2012, Stampfl H. et al. 2016). To gain new insights into the cross-talk between stress tolerance and energy metabolism in plants, we are currently investigating the potential role of ASKα in the energy starvation response in Arabidopsis thaliana.

Poster №10:

Heat stress memory at the shoot apical meristem of Arabidopsis thaliana

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Plants, being sessile, are more prone to the harmful effects of the rising global temperatures. During evolution, plants have evolved several mechanisms to combat different environmental stresses. One such mechanism is the 'thermomemory', in which plants are able to 'memorize' the exposure to a first, moderate and non-lethal stress (priming) and the information about the stress event is stored during the memory phase in order to ensure a faster/stronger response to the next, more severe stress (triggering). In our group we demonstrated that the shoot apical meristem (SAM) is primable and displays a transcriptional memory of heat stress (HS) (Olas et al., unpublished). RNA-seq analysis revealed that eight out of the 21 reported HEAT SHOCK TRANSCRIPTION FACTORS (HSFs) are involved in the regulation of thermomemory at the SAM; one of them being HSFmeristem2b (HSFm2b). We found that the expression of the HS memory genes at the SAM is dependent on HSFm2b, suggesting that HSFm2b is one of the key components involved in regulating the SAM's thermomemory response.

Poster №11:

Effect of omeprazole on seed germination and grow parameters of Pisum sativum subsp. arvense in stress conditions

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Many agricultural plants must deal with stress conditions for a germination or grow in the environment that is affected by natural or artificial factors in the soil and water. However, some of the pharmaceutical compounds could take positive effect in the seed priming. The grow characteristics of the field pea, genotype Arvika, seeds were determined after a treatment by omeprazole, proton-pump inhibitor, or H2O2 as another control for 24 h before sowing. For salt and drought stress simulation, the seeds were also cultivated in several solutions of NaCl or PEG 6000, which reduced root and shoot length. Regarding preliminary results, the 1 mM solution of omeprazole was chosen for further priming of seeds. The cultivation in 150 mM NaCl or 30 mM PEG medium showed that the seeds grew in distilled water without negative impact by applied drug, but there were changes in a case of stress treatments. The roots of sprouts were more sensitive to the salt, when both omeprazole and H2O2 were able to prolong them. However, only H2O2 significantly increased the root size in medium with PEG 6000. The effect of omeprazole will be also further determined in juvenile plants.

Poster №12:

Improving drought stress-relevant root development through biostimulant priming in Arabidopsis thaliana

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An improvement of root architecture is crucial for the adaptation of plants to abiotic stress, in particular drought. Seaweed extracts act as biostimulants, and are widely used as plant strengtheners in agriculture and horticulture. In this research, the priming effect of the biostimulant Super Fifty (an Ascophyllum nodosum extract) on root architecture, especially primary root development under conditions of drought stress, was studied in Arabidopsis thaliana. By analyzing growth phenotype and transcriptome data, together with fluorescence microscopy methods, we gained first insight into the molecular pathways affected by Super Fifty in the Arabidopsis root. Our findings will provide a better understanding of the molecular basis of the priming effect triggered by Super Fifty, and how this knowledge may be employed in future breeding approaches to develop crops with a superior tolerance to water deficit.

Poster №13:

The nitrosative signalling is associated with nano-ZnO tolerance in Brassica species

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Zinc oxide nanoparticles (ZnO NP) may exert toxic effects on plants. Our study revealed ZnO NP (~8 nm)-induced increase in nitric oxide and peroxynitrite levels and in protein tyrosine nitration in tolerant Brassica juncea seedlings, but not in sensitive Brassica napus. This was accompanied by a reduction of S-nitrosoglutatione (GSNO) level and decreased GSNO reductase activity. Our result show that the ZnO NP exposure triggers nitrosative processes especially in the tolerant Brassica species indicating the involvement of nitrosative signalling in plant ZnO NP tolerance.
This work was supported by the National Research, Development and Innovation Fund (NKFI-6, K120383, NKFI-8 KH 129511. A. Molnar was supported by UNKP-19-3-SZTE-201 New National Excellence Program of the Ministry for Innovation and Technology.

Poster №14:

Effects of salt stress on gas exchange and chlorophyll fluorescence of beet plants

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Salinity is a significant environmental factor affecting physiological processes in plants. This study monitors the effect of salt stress induced by the NaCl solution (0 – deionized water; 50, 150, 250, 350 mmol/L) in sugar beet ´Dobrovická A´, chard ´Lucullus´ and fodder beet ´Hako over the course of 40 days. The container experiment was established under partially greenhouse conditions. The diverse reactions of these monitored species to salt stress are well apparent from the results. The results show that salinity affects the rate of photosynthesis and transpiration, stomatal conductivity and fluorescence. Plants of selected beet species are more effective in water management at concentrations of 250 mM NaCl and 350 mM NaCl. Compared to the control group, net photosynthetic rate, transpiration and stomatal conductance and decreased at 350 mmol/L NaCl. A decrease of Fv/Fm took place from the concentration of 350 mmol/L NaCl, too. Sugar beet proved as the most sensitive to salt stress, showing a significant reduction of photosynthesis, stomatal conductance and chlorophyll fluorescence. The obtained results therefore prove the tolerance of the chard to salt stress.

Poster №15:

AIRP6 is a Microtubule-Associated RING-type E3 Ubiquitin Ligase That is involved in ABA-Mediated Guard Cell Movement

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Abscisic acid (ABA) is a phytohormone which is a key regulator of various cellular mechanisms including defensive response to environmental stresses such as drought. A series of RING-type E3 ubiquitin (Ub) ligase genes were reported to be involved in ABA-mediated drought response, namely AIRPs (ABA Insensitive RING Proteins). Here, we report the sixth of the genes, named AIRP6. Firstly, the E3-ligase activity of AIRP6 was confirmed by in vitro self-ubiquitination assay. However, unlike other AIRPs, the transcripts of AIRP6 were not elevated neither by drought nor ABA. Nevertheless, the knock-out plants of AIRP6 showed compromised response to ABA during germination, reminiscent of other AIRPs. Plus, ABA-mediated stomatal closure was hampered in the knock-out mutants of AIRP6. Interestingly, subcellular localization of AIRP6 seemed to be microtubule (MT) structure, which was disrupted by MT-depolymerizing chemical oryzalin. Moreover, ABA-mediated MT depolymerization in guard cells was greatly hindered in the knock-out mutant of AIRP6. Collectively, these results suggest AIRP6 to be a viable E3 Ub ligase involved in ABA-mediated MT depolymerization during stomata closure.

Poster №16:

Arabidopsis protein quality factors MPSR1 and molecular chaperone HSP90.1, are inversely correlated E3 ubiquitin ligase

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MPSR1 (Misfolded Protein Sensing RING protein 1) is a RING-type E3 ubiquitin (Ub) ligase that was reported to eliminate misfolded proteins arising from proteotoxicity. In this study, we show that two protein quality factors MPSR1 and HSP90.1, a well-characterized molecular chaperone, are under reciprocal suppressive regulation according to the progress of proteotoxicity. At the early-stage of proteotoxicity, the levels of MPSR1 increase while cellular levels of HSP90.1 stay unchanged. As the proteotoxic stress continues, however, the cellular levels of HSP90.1 increase while the levels of MPSR1 decrease. Addition of bacterially-expressed HSP90.1 interrupts in vitro binding of MPSR1 and its target misfolded protein, Δ2GFP in pull-down assay, and restore the self-ubiquitination activity of MPSR1 which is decreased by addition of Δ2GFP in ubiquitination assay. Overexpression or knock-down of MPSR1 represses or promotes the accumulation of HSP90.1 respectively. Collectively, we propose that MPSR1 suppresses translation of HSP90.1 at the early-step of proteotoxicity while HSP90.1 promotes self-ubiquitination/degradation of MPSR1 at the late-step.

Poster №17:

ABA-insensitive RING Protein 5 is a Positive Regulator of the ABA-mediated Drought Stress Response in Arabidopsis

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The ubiquitin-26S proteasome system is a crucial protein degradation mechanism that involved in regulating diverse cellular processes. In this system, proteins that are designated for degradation are mostly determined by E3 ubiquitin (Ub) ligase. The Arabidopsis ABA insensitive RING protein 5 (AtAIRP5) is a RING-type E3 Ub ligase that was induced by ABA, NaCl, and drought treatments. The transiently expressed AIRP5-GFP protein was localized to the cytosolic fraction with a punctate expression pattern in Arabidopsis protoplasts. Germination and stomatal closure of airp5 knock-out mutant plants were less sensitive relative to the wild-type plants in response to ABA. In contrast, germination and stomatal movement of AtAIRP5-overexpressing transgenic plants showed sensitive phenotypes in response to ABA. The 35S:AtAIRP5-2xFlag plants also exhibited increased tolerance to severe drought stress, as opposed to airp5. Together, these results suggest that AtAIRP5 plays a positive role in ABA-mediated drought stress response in Arabidopsis.

Poster №18:

Effects of aminophenol on photosynthesis and chlorophyll fluorescence of lettuce

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The effect of different doses of aminophenol (0, 5 µM, 50 µM, 500 µM, 5 mM) in the acute (1x application) and chronic (regular application) variants on the photosynthesis rate (Pn) and chlorophyll fluorescence of lettuce was studied in a greenhouse experiment over the course of 40 days. The obtained results show that with increasing concentration of aminophenol in both application variants the Pn and chlorophyll fluorescence decreases in comparison with control plants. In the event of an acute paracetamol effect, the Pn is reduced by the control (6.03 µmol CO2/m2/s) and 5 mM (4.89 µmol CO2/m2/s). In chronic effects of medicines on the Pn, depending on the variant. The Pn in the control plants was 6.02 µmol CO2/m2/s and at the highest concentration of aminophenol was 4.91 µmol CO2/m2/s. Also, the chlorophyll fluorescence, as determined by Fv/Fm, decreased after treatment with aminophenol. In lettuce plants growing in acute medicines treatment, fluorescence was in control plants 0.827 and at the highest medicines concentration 0.758 In chronic aminophenol treatment, the Fv/Fm interval was from 0.827 (control) to 0.736 (5 mM).

Poster №19:

In vivo and Ex vivo Magnetic Resonance Spectroscopy (MRS) for the measurment of GABA and Glutamate functions in the root system

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For their survival, plants depend on a growth pattern that is adaptive to their surroundings and is regulated by various growth hormones. Under the ground, this growth pattern is manifested in a plastic response of the root system. Together with the well-known growth hormones (Auxin and Cytokinin), other secondary metabolites (e.g., Ethylene and Abscisic acid) act Eo ipso as growth hormones. Together, they form an intricate regulation network that controls the plant growth pattern. Interestingly, most of the known classical neurotransmitters, i.e., molecules that operate for the advancement of plastic response mechanisms in animals' central nervous system, also operate as part of this intricate growth regulation network. Recently, we initiate a project to utilize magnetic resonance spectroscopy (MRS), a measurement technique that is key to biomedical research but is rarely used in plant research, to study the function and interaction pattern of native plant neurotransmitter molecules. We concentrate on the root system characterization of GABA and Glutamate, the most abundant neurotransmitters of animals. For the study, we are developing an MRS receiver coils for in vivo and ex vivo measurements using the most advanced MR scanners that are used in chemistry and biomedicine. Our research will pave the way to understanding the functions of these molecules in the root system, and on a broader level, for the understanding of general plasticity principles across biological kingdoms.

Cyclic nucleotide-gated ion channel 14 and 16 promote basal tolerance to heat and chilling in rice seedlings

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Calcium signaling has been postulated to be critical for both heat and chilling tolerance in plants, but the molecular events in calcium signaling in response to neither of the temperature stresses are fully understood. Here, we investigated the function of two cyclic nucleotide gated calcium channel (CNGC) proteins OsCNGC14 and OsCNGC16 in temperature stress tolerance in rice by examining the loss of function mutants of these two genes generated by genome editing. These mutants have reduced survival rate, higher accumulation of hydrogen peroxide and heightened cell death under heat and chilling. Furthermore, the loss of either OsCNGC14 or OsCNGC16 reduces or abolishes cytosol calcium signals induced by either heat or chilling. This study also reveals for the first time a critical role of CNGC in chilling tolerance in plants and suggests an overlapping molecular mechanism in calcium signaling in responses to heat stress and chilling stress.

The impact of the xenoestrogen bisphenol-A exposure on the seagrass Cymodocea nodosa various part elongation: A preliminary note

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The effects of environmentally relevant bisphenol A (BPA) concentrations (0.03-3 μg L-1) on the seagrass Cymodocea nodosa various part elongation were tested for 10 days. In all plant parts the first effect was detected at the 0.3 μg L-1 concentration on the 10th day of BPA exposure. The elongation gradually decreased, whereas the respective "Toxicity Indexes" increased by increasing BPA concentrations. Based on the elongation as toxicity assessor, all C. nodosa parts and specifically, the plagiotropic rhizomes and the adult blades, followed by the intermediate blades, adult sheaths and juvenile blades appear to be sensitive to BPA toxicity. These findings suggest that the elongation of various seagrass parts, in this particular order, could be used as an early warning signal, and it constitutes a valuable tool to derive the seawater quality criteria and to be used in BPA monitoring programs of coastal environments.

Physiological responses of the seagrass Halophila stipulacea to AgNP stress: A preliminary note

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The present study provides new information with regard to AgNP phytotoxicity on seagrasses. Changes in leaf Halophila stipulacea oxidative stress parameters and cell viability exposed to environmentally relevant AgNP concentrations (0.0002-0.02 mg L-1) for 8 days were examined to identify sensitive biomarkers. AgNP characterization was conducted following TEM and DLS analyses, suggesting aggregation. The overproduction of H2O2 at 0.0002 mg L-1 AgNP-treated plants, causing oxidative damage, resulted in lipid peroxidation (MDA) and stimulated antioxidant defense mechanisms, as indicated by SOD and peroxidase activity increase. Dead epidermal cells firstly occurred at 0.02 mg L-1, while no dead vein cells were detected. Hence, the above changes appear to be sensitive markers of AgNP toxicity. These findings suggest the significance of further research on seagrass-AgNP interactions and could be applied in biomonitoring programs for the protection and management of coastal environment.

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