




















PROJECT REPORT

Responses to abiotic and biotic stresses - from the cellular level to fruit development - contributions of the Czech Centre for Experimental Plant Biology

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Abstract

The “Centre for Experimental Plant Biology”, a joint project of the Institute of Experimental Botany of the Czech Academy of Sciences and CEITEC (represented by Mendel and Masaryk Universities), focused on elucidating the mechanisms of plant responses to abiotic and biotic stresses and their combinations at the cellular level, in intact plants during vegetative and reproductive stages, and fruit development. The consortium demonstrated the importance of shared research facilities, complementary approaches, and knowledge exchange, addressing demanding questions in plant biology. The consortium made breakthrough in plant-pathogen interactions, including identification of exocyst-syntaxin cooperation in non-host resistance. The results confirmed the fundamental role of phytohormones in stress responses, including negative correlation of leaf bioactive gibberellins with drought stress, and the role of cytokinins in ROS homeostasis, sulphur metabolism, and heat stress responses, including volatile emission. Molecular analyses revealed expansin-mediated cell wall remodelling, brassinosteroid-mediated regulation of root growth through PIN2, the role of ALBA and LARP6C proteins in pollen development under abiotic stress, and heat stress impact on fertilization rate, embryo and seed development. Gene Set Enrichment and RNA-Seq analyses allowed to identify crucial genes involved in the apple scab resistance network. The main results obtained during the five-year project are summarised here.

Keywords: apple breeding, exocyst, gametophyte, pathogen, phytohormone, stress tolerance.

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Introduction

As sessile organisms, plants are frequently exposed to many abiotic and biotic stresses. Only rarely do plants cope with a single stress. More often, they must defend themselves against combinations of stresses. Understanding plant stress responses and the mechanisms of effective defence are prerequisite for improving plant stress tolerance, which is especially critical for elite cultivars, where high yields are often associated with stress sensitivity. Considering that plant interactions with the environment, especially during exposure to combined abiotic and biotic stresses, are very comprehensive, this research topic required the synergistic efforts of several groups with complementary expertise encompassing phytopathology (including viruses), deep knowledge of regulatory mechanisms controlling exocyst activity, gametophyte development, embryogenesis, cell wall properties and experience in the analysis of hormones, proteins, transcripts, volatile compounds, and lipids. The project reflects the current situation: due to global warming, crop production faces several limiting factors. In addition to temperature and drought extremes, plants are exposed to a wide range of pathogenic microorganisms that represent an additional burden for stressed plant lines that have long been bred for maximum yield.

Partners

The Institute of Experimental Botany of the Czech Academy of Sciences (IEB, <http://www.ueb.cas.cz/en>) focuses on basic research in plant genetics, physiology, pathology, and biotechnology. The project team consisted of nine Prague laboratories, a group from Olomouc, and the Střížovice apple breeding station. The groups were involved in the characterisation and regulation of intracellular vesicle transport by the secretory complex exocyst, elucidation of the mechanisms of function of phytohormones and other regulatory substances, phospholipid signalling pathways, and pollen developmental biology. The responses of intact plants (at the organ level) were monitored to elucidate plant defence mechanisms against abiotic and biotic stress conditions (covering pathogens including plant viruses). Research at the apple breeding station in Střížovice focused on uncovering monogenic and polygenic resistance to the most severe apple diseases - scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*).

The Central European Institute of Technology (CEITEC, <https://www.ceitec.eu/>) is a consortium of four leading Brno universities and two research institutes combining life sciences, advanced materials, and nanotechnologies. The Department of Molecular Biology and Radiobiology (DMBR) is part of the Faculty of Agricultural Sciences, MENDELU. The CEITEC research group Developmental and Production Biology - Omics approaches (<https://www.ceitec.cz/vyvojova-a-produkcni-biologie-omicke-pristupy/rg52>, <https://umbr.af.mendelu.cz/>) aims to determine the molecular mechanisms of hormonal regulations and their functions during plant development and stress response. This research group

has elucidated the role of the plant hormone cytokinin in modulating plant responses to temperature shocks at proteomic, transcriptomic and growth response levels.

Three research groups from the Mendel Center for Genomics and Proteomics of Plants, a plant research department of CEITEC Masaryk University (CEITEC MU), were involved in the project. The molecular mechanisms underlying hormonal regulation, in particular hormonal signal transduction and its functions in plant development, were the main target of the Functional Genomics and Proteomics of Plants research group. The Hormonal Crosstalk in Plant Development group investigated hormonal crosstalk in plant organogenesis, lateral root formation, and the role of local auxin biosynthesis in seed and embryo development. The Proteomics Core Facility was involved in all steps of proteomic analysis - protein isolation, separation of protein mixtures, protein characterisation by mass spectrometry, and bioinformatics data processing (Fig. 1).

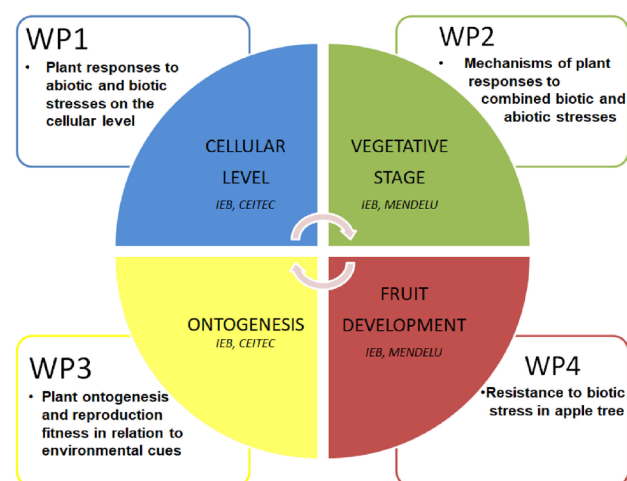


Fig. 1. Structure of the project organisation.

WP1 “Plant responses to abiotic and biotic stresses at the cellular level”

WP1 focused on elucidating the mechanisms of plant responses to simultaneous abiotic and biotic environmental stresses at the cellular level (Fig. 2). This goal required a complex approach combining the study of phytohormone regulation (metabolism, mobility/transport, and signalling), mathematical modelling, and intrinsic cellular events such as secretion, vesicle trafficking, and cell wall biogenesis. WP1 aimed to study the crosstalk between secretory pathways (especially exocytosis regulated by the exocyst complex) and phytohormone action in relation to cell wall modulation and defence responses (callose, defensive papillae, xylem biogenesis). The development of infection symptoms, amplification and virulence of the pathogen in a particular mutant host, and the expression of defence marker genes or pathogenesis-related proteins, mainly PR1, at the RNA and protein level were followed.

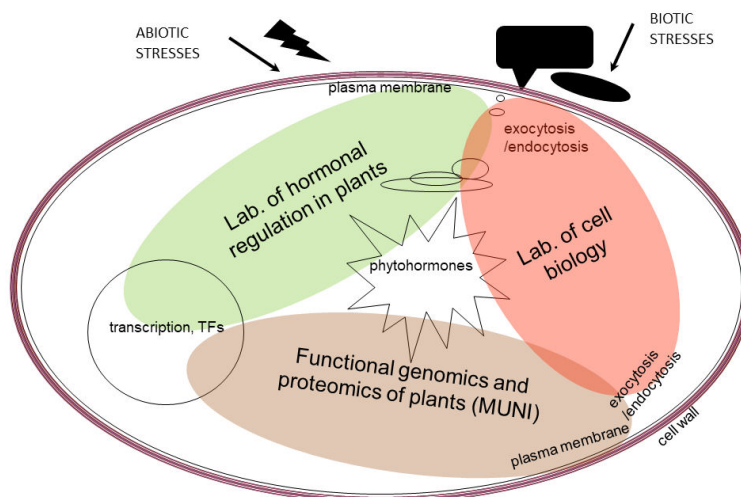


Fig. 2. Scheme of Working Package 1.

Plant responses to combined abiotic (temperature, light and drought) and biotic stresses were assessed at the molecular level. In WP1, the role of plant hormones in the control of cell wall properties was investigated. The aim was to clarify the hormonal control of secondary cell wall formation, its importance for water conductivity, and the role of hormonal control in regulating cell wall properties with implications for the (a)biotic stress response.

WP2 “Mechanisms of plant responses to combined biotic and abiotic stresses”

The research in WP2 focused on a complex investigation of plant responses to combined abiotic and biotic stress factors (Fig. 3). The aim was to explore the mechanisms of plant defence against the combination of two strong stress factors with different characteristics. Due to the complexity of plant responses to stress factors, close collaboration of several teams with complementary expertise was required. Expertise included phytopathology and virology, monitoring of phospholipid signalling pathways, analyses of phytohormones (auxins, cytokinins, gibberellins, brassinosteroids, jasmonic, salicylic and abscisic acids and their metabolites), proteome, transcriptome, volatile signalling compounds and lipids. The effect of subsequent stresses was compared with that of simultaneous stress factors. The knowledge gained from the model plant *Arabidopsis thaliana* was applied to studying the defence mechanisms of economically important crops (barley, rape, tomato) against compatible pathogens. In addition, the possibility of increasing the stress tolerance of plants by applying selected biologically active substances (developed by team members) was tested.

WP3 “Plant ontogenesis and reproduction fitness in relation to environmental cues”

Plant growth, development and reproduction heavily depend on the correct response to various and changing

environmental cues at the level of organs, tissues, cells and subcellular compartments. Having no fixed body plan, plants display adaptive growth patterns that are tuned to the ever-changing environmental conditions. The joint effort of five research groups aimed to investigate the coordination and integration of plant growth and development with genetically pre-programmed steps and environmental conditions by 1) characterisation of the role of auxin and its dynamics in relation to organ development and environmental cues, 2) characterisation of the anterograde and retrograde cytonuclear interactions in response to stress treatment, 3) elucidation of the regulation of the development and function of reproductive organs (especially gametophytes) development and function under stress conditions and the function of selected regulatory pathways and candidate proteins in this process, and 4) characterising the role of L-AFL transcription factors in abiotic stress responses during post-fertilisation development (Fig. 4).

WP4 “Resistance to biotic stress in apple trees”

Apple scab is the most devastating disease in apple trees worldwide. The main objective of WP4 was to utilise molecular approaches, including next-generation sequencing, genomics and transcriptomics, to identify genes that confer resistance to abiotic and biotic stresses in apple trees and to identify genetic markers for disease and stress resistance that apple breeders can utilise in marker-assisted breeding programmes (Fig. 5). Using RNA-Seq, a gene co-expression network was created, and genes involved in the process of apple scab infection were characterised. Several apple varieties with promising breeding potential, including apple scab-susceptible and mono- and polygenic-resistant trees, were evaluated at the molecular level. Single nucleotide polymorphism (SNP) was utilised to identify markers for polygenic scab-resistant apple genes. SNPs proved to be universal and the most common forms of genetic variation among individuals of the same species. The ultimate goal of our

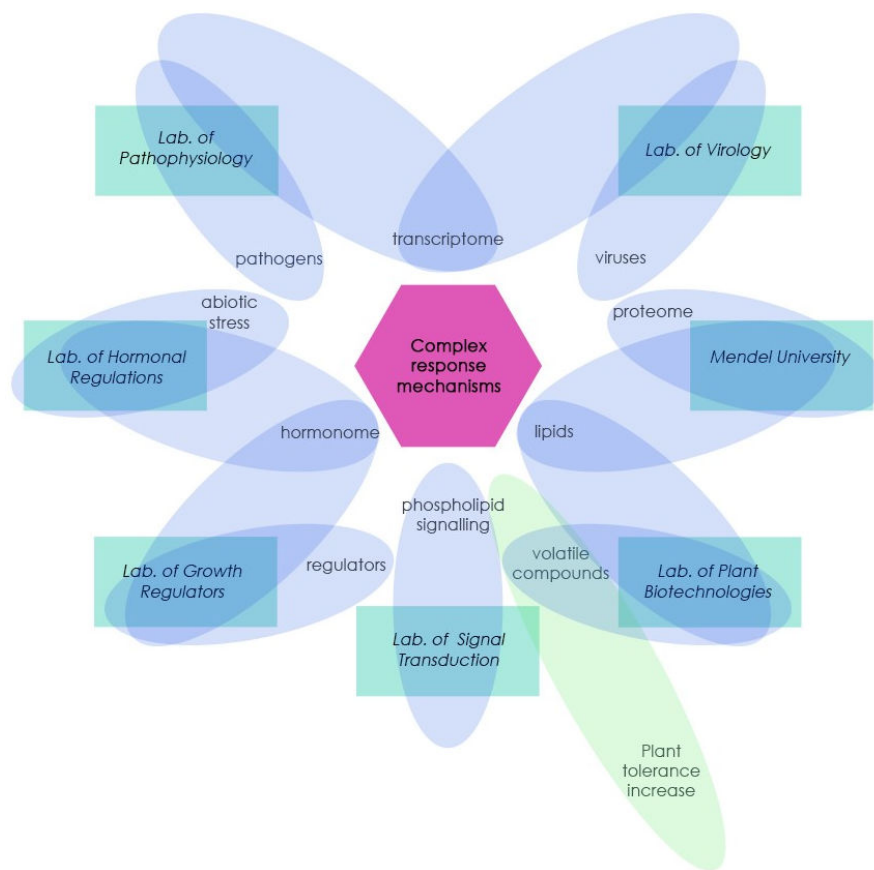


Fig. 3. Scheme of Working Package 2.

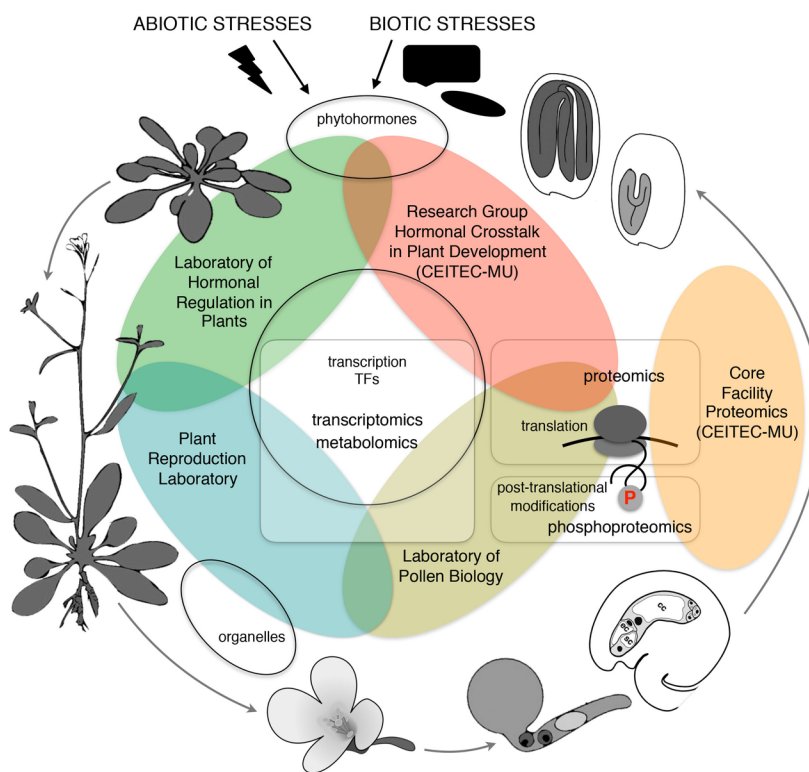


Fig. 4. Scheme of Working Package 3.

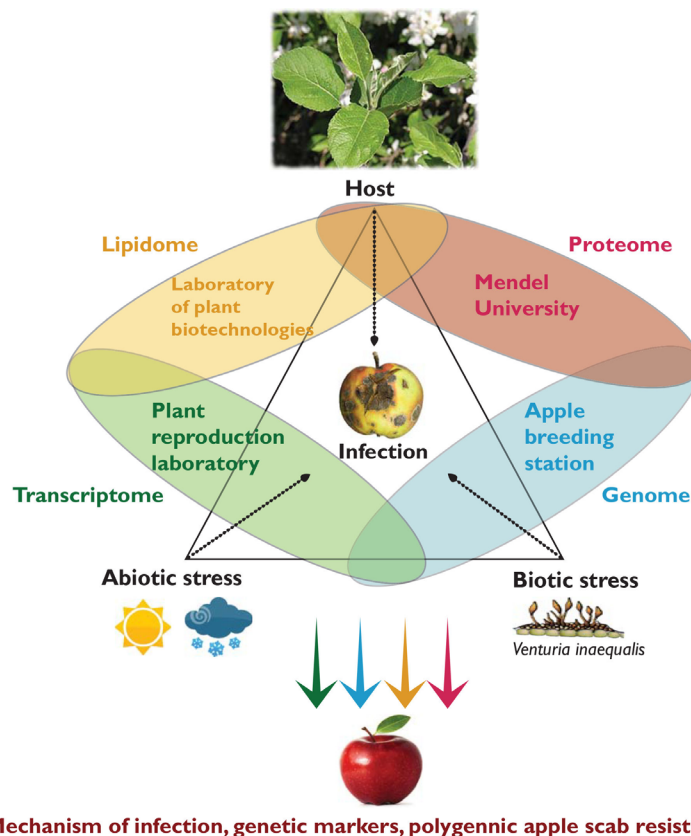


Fig. 5. Scheme of Working Package 4.

apple breeding in terms of resistance to fungal diseases has been achieved by polygenic (pyramidal) resistance (*i.e.*, a variety of resistant genes cooperating against fungal intruders).

Main results obtained during the project

Exocyst interaction with syntaxin: The study of the function of the plant exocyst in biotic stress/interactions revealed an important function of the exocyst in reinforcing the cell wall of papillae or fungal haustoria isolation in encasements. Using non-adapted *Blumeria graminis* f. sp. *hordei* (*Bgh*), the plant syntaxin SYP121/PEN1 and the exocyst subunit EXO70B2 of the vesicle tethering complex were shown to be involved in the formation of papillae and affect their morphology. SYP121 was found to interact directly with EXO70B2. The *exo70B2/syp121* double mutant was impaired in defence cell wall modifications formation (papillae, haustoria encasement) in response to *Bgh*, suggesting an additive role of the exocyst and SYP121 in non-host resistance, *i.e.*, cooperation of exocyst and a SNARE protein in penetration resistance (Ortmannová *et al.* 2022).

Pathogenesis-related protein1 processing in *Arabidopsis*: Pathogenesis-related protein 1 (PR1) family is an evolutionarily conserved eukaryotic type of cysteine-rich secretory protein used mostly as a marker for salicylic acid-dependent processes (due to transcriptional

up-regulation). We found that PR1 secretion starts from the endoplasmic reticulum. PR1 protein moves through the multivesicular body and undergoes partial proteolytic processing dependent on an intact C-terminal motif. Interestingly, only full-length PR1 variants (*i.e.*, nonmutated or processing-mimicking) are secreted into the cell wall/apoplast. The C-terminal protein fragment was released after proteolytic cleavage functions as a modulator of plant defence responses, including local control of programmed cell death (Pečenková *et al.* 2022).

Shootward auxin transport counteracts root growth deviation from the vertical, which is triggered by direct root illumination: Direct illumination is perceived as stress by roots and leads to pleiotropic growth adaptation processes (Lacek *et al.* 2021). We found that root illumination triggers an increased deviation of growth from the vertical, which is even more pronounced in mutants with impaired modulation of shootward auxin distribution along the root tip (García-González *et al.* 2021a). Furthermore, we have shown that this light-triggered mechanism of root escape is more enhanced when sugar levels are elevated (García-González *et al.* 2022). We observed that shootward auxin transport modulated by the auxin influx carrier AUXIN RESISTANT 1 is required to reduce growth rate during directional growth adaptation of gravi-stimulated roots, which is even more pronounced when the root is supplied with energy in the form of sucrose (García-González *et al.* 2021b).

Crosstalk between brassinosteroid and auxin controls directional root growth by fine-tuning PIN2 sorting upon gravi-stimulation:

Exogenous stimuli often trigger root growth deviation from the vertical. Directional growth along the gravity vector is controlled by auxin distribution along the root tip mediated by the auxin efflux transporter PIN-FORMED2 (Retzer *et al.* 2023). We deciphered the tight interplay between brassinosteroid signalling and regulation of PIN2 abundance at the plasma membrane, which modulated auxin gradient formation in gravi-stimulated roots (Retzer *et al.* 2019). By tracking the intracellular trafficking of a constitutively degraded version of PIN2, we demonstrated that brassinosteroid signalling acts antagonistically to PIN2 endocytosis. Brassinosteroid-mediated regulation of PIN2 sorting determined the rate of gravity-induced root curvature *via* attenuation of differential cell elongation (Retzer *et al.* 2019).

Expansins induce cell wall remodelling: The specificity of expression and localisation of several alpha-expansins was studied in *Arabidopsis thaliana* (Samalova *et al.* 2023).

Using a new non-invasive technique, Brillouin imaging, as well as well-established atomic force microscopy (AFM), we discovered the surprising effect of *EXPA1* overexpression leading to cell wall stiffening. Based on the results of Fourier transform infrared spectroscopy (FTIR) and RNAseq analyses, we propose a novel role for *EXPA1* in regulating cell wall biomechanics by inducing cell wall remodelling.

A novel method for Reverse Transcription PCR: Reverse transcription PCR (RT-PCR) is the preferred method for detecting plant RNA viruses. It offers high sensitivity, specificity and multiplexing capability. However, the traditional RT-PCR method is time-consuming, prone to cross-contamination and expensive. To overcome these limitations, we used the synthetic DNA polymerase RTX. RTX is a unique enzyme with proofreading ability, insensitivity to inhibitors and the ability to recognise both RNA and DNA templates. We have developed a simplified protocol using RTX to quantify RNA viruses in plant tissues, reducing the time from two days to two hours and increasing throughput (Hoffmeisterová *et al.* 2022). This breakthrough has the potential to revolutionise plant virology research.

Evaluation of environmental risks caused by pharmaceuticals released into the environment:

The environmental risk from the anthelmintic praziquantel was found to be much higher than that from the anti-inflammatory drug naproxen (Landa *et al.* 2018). Praziquantel caused massive up-regulation of genes encoding heat shock proteins and down-regulation of genes involved in cell proliferation and transport of water, lipids, and amino acids. We also identified several candidates (cytochromes P450, methyltransferase, glycosyltransferases, ABC and MATE transporters) involved in naproxen and praziquantel metabolism. The glutathione transferases GSTU24, GSTU25 and

GSTU22 were upregulated. It seems possible that these three genes are involved in the metabolism of a broader spectrum of organic pollutants and their overexpression could lead to improved plant tolerance.

Environmental impacts on seed composition: Seed composition has a direct influence on seedling establishment and early growth. We harvested barley seeds at locations with suitable temperature and precipitation gradients to determine environmental changes in their protein and metabolite contents. Our results showed that at least a quarter of the total grain protein content was modulated by the maternal environment. Positive correlations were found between temperature and ribosomal proteins, proteins involved in translation and lipid droplet formation, CAZymes and heat shock proteins (HSPs). Interestingly, the abundance of HSP70s correlated negatively with seed yield, but the seeds with the highest HSP70 abundance showed the best performance in the accelerated ageing test. Molecular analyses showed that these seeds also differed from the others in terms of gibberellin contents and ROS metabolism (Dufková *et al.* 2023).

Cytokinins impact biomass accumulation, ROS homeostasis, temperature perception and sulphur metabolism:

Cytokinins (CK) are multifaceted plant hormones that play a role in diverse plant growth and development processes. Our integrative omics approach, combining transcriptomics, proteomics and phenomics data, has shown that CK excess in the cell expansion phase promotes shoot biomass by stimulating primary metabolism, thereby increasing the cell expansion rate (Skalák *et al.* 2019). We also found that CK affect sulphur metabolism by depleting sulphate and free glutathione contents. These results position CK as a modulator of sulphur uptake, assimilation and remobilisation in plant defence and as a putative regulator of ROS homeostasis (Pavlů *et al.* 2022). The role in the regulation of ROS metabolism was confirmed in experiments using mutants with negative components of the CK signalling pathway (type-A ARR), which exhibited enhanced heat stress tolerance associated with the accumulation of enzymes of ROS metabolism, including glutathione peroxidase, glutathione S-transferases, and superoxide dismutase (Jindal *et al.* 2022).

Cytokinins act synergistically with heat acclimation to improve rice thermotolerance:

Cytokinin role in heat stress responses of rice was evaluated using meta-topolin-9-(tetrahydropyran-2-yl)purine (mT9THP). Treatment with cytokinin partially substituted heat acclimation. The combined application of cytokinin and acclimation significantly improved plant thermotolerance, including increases in jasmonic acid and indole-3-acetic acid, shifts in lipid composition, and production of protective compounds and volatiles (*e.g.*, butanediol and the LOX-derived ones). Stimulation of volatile production appears to contribute significantly to the cytokinin beneficial effects. Our data suggest an efficient strategy for promoting plant heat stress tolerance (Prerostova *et al.* 2023).

Evaluation of the possibility to replace vernalisation with heat stress: The effect of vernalisation in *Cichorium endivia*, which is necessary for flowering during the second year of their life cycle, was characterised. Vernalisation decreased the production of ethylene, abscisic acid (ABA) and total jasmonates (JA), while the levels of salicylic acid (SA), benzoic acid and spermidine polyamine increased (Mathieu *et al.* 2020). The effect of heat stress on phytohormone content was different in non-vernalised and vernalised plants, with higher levels of ABA and JA and lower levels of 1-aminocyclopropane, a precursor of ethylene biosynthesis. These data suggest that heat stress response requires pathways other than vernalisation to accelerate the flowering process.

Elucidation of gibberellin role in wheat drought responses: The effect of progressive drought on the gibberellin content was investigated in *Triticum aestivum* (Ptošková *et al.* 2022). Five days of progressive drought reduced the leaf length, together with a significant reduction in the levels of the bioactive gibberellins GA₁ and GA₄, their catabolites and biosynthetic precursors. Wheat root length was not affected by drought, and gibberellin concentrations even increased slightly in drought-stressed root tips. The other hormones analysed responded to drought similarly in leaves and roots.

The effect of rhizobacteria on the maize response to salinity and phosphorus deficiency: Inoculation of maize roots with either of three strains of *Rhizobacteria*, *Arthrobacter* and *Bacillus* mitigated adverse effects of salinity, phosphorus deficiency and their combination, associated with the reduced uptake of nutrients and diminished growth. *Rhizobacteria* reduced the uptake of sodium cations, increased the uptake of potassium cations, and similarly enhanced plant growth. When the bacterial strains were cultured *in vitro*, each was shown to be capable of producing the phytohormones auxin, abscisic acid, gibberellins and cytokinins (Tchuisseu Tchakounté *et al.* 2020).

Temporary heat stress suppresses PAMP-triggered immunity and resistance to bacteria in *Arabidopsis thaliana*: Simultaneous biotic and abiotic stress was studied on *A. thaliana*. When plants were subjected to temporary heat stress prior to treatment with the flg22 mimicking pathogen-associated molecular patterns (PAMPs), this resulted in a strong reduction in the production of ROS and reduced *FLS2* transcription, suggesting that short exposure to high temperature suppresses PAMP-triggered immunity, which subsequently leads to the higher susceptibility of plants to pathogens (Janda *et al.* 2019).

The independent mode of action of phosphatidylinositol 4-kinases $\beta 1\beta 2$ and salicylic acid in biotic stress responses: *A. thaliana* mutant, deficient in phosphatidylinositol 4-kinases $\beta 1\beta 2$ (an important player in phospholipid signalling and trafficking) constitutively accumulates high levels of salicylic acid, which itself drives further hormonal and transcriptomic alterations.

These mutations were associated with increased resistance to the adapted pathogens *Hyaloperonospora arabidopsidis*, *Pseudomonas syringae*, and *Botrytis cinerea*, but had the opposite effect on penetration resistance to non-adapted *Blumeria graminis*, likely relying on targeted callose accumulation (Kalachova *et al.* 2020).

Stress-responsive genes are involved in cytoplasmic male sterility: Cytoplasmic male sterility (CMS) is caused by a mitochondrial-nuclear interaction that leads to pollen non-viability but does not affect vegetative growth. The mechanisms are very diverse, and only a few have been studied. We analysed the transcriptomes of male-fertile and male-sterile individuals of *Silene vulgaris*, a model plant for the study of reproductive system gynodioecy. We found many stress response genes differentially expressed between male-fertile and male-sterile flower buds (*e.g.*, F-box or jasmonate-induced proteins), likely involved in pollen abortion. Down-regulation of alternative oxidase in female flower buds causes oxidative stress and may have triggered deleterious changes interfering with pollen development (Krüger *et al.* 2020).

Long days induced stress-responsive phytohormones but did not accelerate the flowering of *Chenopodium ficifolium*, the close relative of the cultivated plant *Chenopodium quinoa*: High levels of abscisic, jasmonic, and salicylic acids were found in *Chenopodium ficifolium* cultivated under long-day conditions (18-h photoperiod) (Gutierrez-Larruscain *et al.* 2022a,b). The increased concentrations of these stress-responsive phytohormones were associated with the upregulation of numerous genes involved in the response to oxidative stress. In contrast to some examples of stress-induced flowering (*e.g.*, in *Lemna*), stress did not accelerate flowering in *Chenopodium ficifolium*.

The impact of long-term high-temperature stress on embryo and seed development in *Brassica napus*: Phenotyping analysis of unfertilised ovules and seeds bearing embryos at the 8-cell and globular stages from three cultivars exposed to high temperatures revealed reduced fertilisation rate, increased abortion rate, defective embryonic development and preharvest sprouting (Máková *et al.* 2022). Transcriptomic analysis revealed up-regulation of genes related to heat stress, protein folding and binding to heat shock proteins, and down-regulation of cell metabolism. Heat-tolerant cultivar stimulated genes encoding peroxidases, lipocalin or SAG21/LEA5, while heat-sensitive cultivars were characterised by heat-induced cellular damages with up-regulation of jasmonate repressors (Jedličková *et al.* 2023).

Specific functions of the ALBA proteins: Pollen development and pollen tube growth are the most sensitive part of the plant reproductive cycle to stress conditions (Chaturvedi *et al.* 2021). Among the adaptation to heat stress emerges a post-transcriptional redistribution of mRNA to protect sensitive transcripts and to enable the specific translation of stress-related proteins (Hafidh and

Honys 2021). The engagement of ALBA proteins was demonstrated in male reproductive development and the heat stress response, highlighting the involvement of ALBA4 and ALBA6 in RNA metabolism, storage and/or translational control in pollen during heat stress (Náprstková *et al.* 2021). The dynamic re-localisation of ALBA proteins reflects their redundancy but also their possible functional diversification in plants.

The functions of the LARP6C protein: We demonstrated that the evolutionarily conserved RNA-binding protein LARP6C is required for the transition from dry pollen to pollen tubes and the guided growth of pollen tubes towards the ovule (Billey *et al.* 2021). *In planta* transient analysis of reporter mRNAs designed from the LARP6C target MGD2 provided evidence that LARP6C can shift from a repressor to an activator of translation as the pollen grain enters the rehydration phase. We propose that LARP6C orchestrates the storage and timely posttranscriptional regulation of a subset of mRNAs to overcome the dehydration stress response and shift from the quiescent to the active state and along the progamic phase to promote male fertilisation in plants.

Identification of genes conferring resistance to apple scab: Using next-generation sequencing, genomics and transcriptomics, we have identified genes that confer resistance to abiotic and biotic stresses (particularly apple scab) in apple trees, as well as genetic markers and molecular signatures for disease and stress resistance that can be utilised by apple breeders in marker-assisted breeding programmes. An important role in the defence response against scab is played by pathogenesis-related proteins) and reactive oxygen group, including antioxidant enzymes as well as metallothionein and lipid transfer genes. The key role in defence was played by salicylic acid and, to a lesser extent, jasmonic acid and MAP kinase signalling pathways.

Identification of SNPs associated with monogenic and polygenic resistance: Single nucleotide polymorphism (SNP) was used to identify markers for polygenic apple scab resistance genes. Polymorphic SNPs were identified in the variety Allegro, which carries a polygenic apple scab resistance (origin from cv. Discovery), and then converted into CAPS markers for easy detection. Since the saturation of the population with these SNP markers was relatively low, we complemented this method by detection by molecular signature. Weighted co-expression analysis was used with Gene Set Enrichment Analysis and RNA-Seq differential expression analysis allowed to identify crucial genes involved in the apple scab resistance network. Now we are able to detect and distinguish polygenic and Rvi6-based monogenic resistance in subsequent apple crosses.

The positive influence of cold pre-treatment on apple scab resistance: The influence of cold stress on apple scab infection was investigated on the *Venturia*-susceptible apple var. Gala. The fungal spread slowed

at four days post inoculation on plant leaves kept at 4°C for 10 h prior to infection. RNA-Seq expression profiling showed increased expression of several enzymes involved in the SA signalling pathway (such as NPR1, Enhanced Disease Susceptibility 1, PR1 or PAL), as well as some transcriptional enhancers (WRKY). The data suggest that both cold stress and *Venturia* infection involve the SA response pathway. Hence, its elevation by cold stress is probably responsible for the slightly reduced infection in cold-treated Gala samples.

Conclusions

The project stimulated a fruitful collaboration among 15 teams from three partner organisations. The results include *ca.* 200 publications in impacted journals, in various areas (Fig. 1S). It provided a unique opportunity for both undergraduate and postgraduate students to join teams of experienced scientists and enhance their research skills. The project also enabled the acquisition of sophisticated equipment, a prerequisite for competitive research.

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