Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Multilevel response to heat and cold stress in cauliflower (Brassica oleracea var. botrytis)

Magdalena Taube

magdalena.taube@ump.edu.pl

Poznan University of Medical Sciences

Anna Kasprowicz-Maluśki

Adam Mickiewicz University in Poznań

Dawid Bielewicz

Adam Mickiewicz University in Poznań

Ewelina Stolarska

Adam Mickiewicz University in Poznań

Michał Taube

Adam Mickiewicz University in Poznań

Władysław Polcyn

Adam Mickiewicz University in Poznań

Witold Nowak

Adam Mickiewicz University in Poznań

Michał Rurek

Adam Mickiewicz University in Poznań

Przemysław Wojtaszek

Adam Mickiewicz University in Poznań

Research Article

Keywords: heat stress, cold stress, stress response, stress tolerance, cauliflower

Posted Date: October 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11231577/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Abiotic stress, including heat and cold stress, are among the most detrimental to crop production. Despite previous studies, our understanding of how physiological and molecular responses enable crop plants to adapt to temperature stress remains unsatisfactory and requires further exploration. Under cold and heat stress, as well as during recovery after direct stress treatments, we observed increased electrolyte leakage in cauliflower leaves. Furthermore, after exposure to heat, a decrease in chlorophyll levels and photosynthetic conversion capacity was observed, indicating chloroplast senescence induction progressed throughout. This decrease was also observed after the recovery phase, suggesting irreversible changes in chloroplast function. Interestingly, chloroplasts under cold stress do not exhibit functional and most likely, structural changes. At the molecular level, heat stress, particularly heat recovery, had a negative impact on the cauliflower transcriptome by decreasing the abundance of numerous transcripts in cauliflower leaves. Cold and heat stress also affected a variety of transcription factors (e.g., MYB and WRKY family members). In the future, our results may contribute to the development of cauliflower varieties with improved tolerance to both high and low temperatures. That may be crucial for the global crop industry and food safety.

INTRODUCTION

Plants must constantly cope with environmental stresses throughout their life cycle. They cannot escape from detrimental conditions, so they need complex molecular adaptations to survive in a harsh environment [1]. Through evolution, plants have developed the ability to acclimate and adapt to fluctuating environmental conditions [2]. These responses to stress stimuli are present at the developmental, biochemical, physiological, and molecular levels [3].

Among abiotic stressors, plants are highly responsive to temperature alterations, even to differences of 1°C. In general, temperatures above the optimum are perceived as heat stress, which can disrupt cellular homeostasis and lead to cell death. During exposure to high temperatures, plants exhibit changes in photosynthetic activity, cell structure, and the proteome [4].

Besides elevated temperatures, plants also have to cope with the detrimental impact of cold, and temperature-related stressors are among the most critical factors limiting yields and global plant distribution [5], [6]. Stress associated with low temperatures above zero is referred to as cold stress or cold acclimation, whereas temperatures below zero cause freezing stress [7], [8]. Cold responses involve changes in plasma membrane and cell wall properties, synthesis of cryoprotectants, and enhanced scavenging of reactive oxygen species [6]. Moreover, cold acclimation induces differential expression of numerous cold-responsive genes, which is essential for establishing cold resistance and improving low-temperature adaptation [8], [9].

Many genes and proteins play a crucial role in plant tolerance to cold and heat stress [10]. Different gene families and stress-inducible genes are involved in highly complex signal transduction networks [6]. TFs regulate the expression of a dozen stress-induced genes [11]. Heat stress response genes (HSR) play a

crucial role in heat tolerance. Heat stress response genes, including heat stress TFs (Hsfs) and heat shock proteins (Hsps), are central to heat tolerance (Dong et al. 2015). Notably, Hsps also contribute to cold stress responses [12]. Proteins involved in temperature stress responses act not only as structural components but also as regulators of the metabolome, transcriptome, and epigenome [13]. In addition to molecular changes, temperature stress induces morphological and physiological alterations in many plant species [14], [15]. Plant response to thermal stress, which increases at optimum temperatures and decreases below them, triggers a complex cascade of signals and their transduction. Thermosensing alters the expression level of plant RNAs and the accumulation of specific proteins, which may help plants cope with unfavorable conditions [16]. Plants also produce a wide range of regulatory molecules to adjust growth and development under stress [17]. Abiotic stresses cause the loss of approximately half of the annual global crop production [18]. Crops exhibit discrete temperature thresholds, and elevated temperatures can severely affect growth, development, and yield, posing a threat to global food security [19], [20]. Cauliflower (*Brassica oleracea* var. *botrytis*) is a major horticultural crop with annual production exceeding 2.5 million tons in Europe and is widely cultivated worldwide (“FAOSTAT” n.d.). Its high content of health-promoting compounds, including glucosinolates, makes cauliflower an important component of a balanced diet [21].

Cauliflower genotypes originate from the Mediterranean horticultural region, and some of them are sensitive to temperature stress, which precluded the selection of plant material for our study (cold- and heat-sensitive cultivars). Reports describing transcriptomic, proteomic, metabolomic, and physiological responses to heat and cold stress in cauliflower remain limited. Therefore, the objective of this study was to identify differentially expressed genes (DEGs), proteins (DEPs), and metabolites (DEMs) involved in temperature stress responses in cauliflower. RNA-seq, qRT-PCR, LC-MS/MS, and phenomic analyses were performed to evaluate the effects of cold and heat stress on cauliflower curds and leaves. Compared with major model and crop species, such as wheat, rice, and Arabidopsis, temperature stress tolerance in cauliflower remains poorly characterized.

In this study, we comprehensively evaluated the response of cauliflower to cold stress (CS) and heat stress (HS), as well as the recovery phase after temperature stress (CR and HR, respectively), at the transcriptomic, proteomic, metabolomic, and physiological levels. This approach enables us to perform a more accurate characterization of multilevel changes in cauliflower under detrimental thermal conditions. Through our study, we also defined the transcriptional network governing the temperature-stress response in cauliflower, protein accumulation patterns, and metabolic pathways potentially contributing to enhanced thermotolerance.

RESULTS

The permeability of cauliflower membranes in stress and recovery

Following both CS and HS, an approximately 3- to 5-fold increase in electrolyte leakage was observed (from approximately 10% in the control variant to over 30% under CS and approximately 50% under HS). During HR, a significant decrease in electrolyte leakage was observed, compared to the HS, from approximately 50% to 20%. A much smaller decrease in electrolyte leakage was observed in the CR compared to CS, from over 30% to less than 30% (Fig. 1A).

Chlorophyll content in cauliflower leaves in response to CS and HS

During the CS treatment in cauliflower leaves, the relative chlorophyll content did not change significantly (Fig. 1B). The results obtained suggest that the chloroplasts of these plants functioned stably under CS. However, HS resulted in much more severe physiological changes, as chlorophyll levels in cauliflower leaves were significantly reduced (Fig. 1B). This effect manifested with a delay after heat exposure, but the green pigment continued to degrade throughout the recovery period, indicating irreversible damage to the photosynthetic apparatus.

Evaluation of the effectiveness of photochemical and non-photochemical processes occurring in cauliflower leaves in response to CS and HS

Analysis of photosynthetic conversion capacity (FV/FM, ΦPSII, QPD3, Rfd) indicates that there is no damage to photosystem II centers at reduced temperatures. Furthermore, the adaptation of the photosynthetic apparatus to the 10-day cold period was observed, as evidenced by a 25% increase in Rfd – the index corresponding to the overall efficiency of the entire photosynthetic process, not just the initial efficiency of PSII [22]. Rfd measurement exposes the stressed leaves to saturating light, which stimulates stomatal opening; therefore, it is a straightforward indicator of the net CO2 assimilation rate [23] (Fig. 1B, panel III). The NPQ parameter, which relates to non-photochemical quenching of fluorescence, remained unchanged across all experimental variants. The results suggest that during CS, photosynthesis in cauliflower leaves is not significantly disturbed, and chloroplasts show no loss at either the structural or functional level. Moreover, any significant changes in the relative chlorophyll content were observed. Accordingly, under the conditions described, the cauliflower plants do not activate their essential photoprotective mechanisms.

On the other hand, during HS, photosynthetic efficiency changes significantly, as the Rfd index decreases by a factor of 3 (Fig. 1B, panel IV). FV/FM drops dramatically in leaf 1, indicating extensive damage to the PSII active site. Recovery after HS partially restores these losses, indicating that the damage was reversible in at least some chloroplasts in leaf 1. Leaf 2 proved less sensitive to HS. Rfd drops in both leaves and does not recover, indicating an inability to restore the efficiency of all photosynthetic processes. Thus, HS disrupts photosynthetic efficiency in cauliflower, leading to electrolyte leakage, and the active center of photosystem II appears highly sensitive to heat.

Analysis of the photochemical parameters during heat treatment revealed a significant decrease in the effectiveness of photochemical reactions (FV/FM, ΦPSII, Rfd). During the recovery phase, an irreversible

decline in photosynthetic conversion efficiency was observed. Negligible changes in the value of the NPQ parameter (Fig. 1B, panel IV) indicate that, in the HS conditions, the mechanisms of thermal fluorescence quenching are not activated. This means that the conditions under which the experiment was conducted did not cause (as in the case of cold stress) the appearance of light stress for photosynthesis.

1. The level of electrolyte leakage from cauliflower leaves in all experimental variants

(BI-BIV) the relative content of chlorophyll (Chl, panels BIA, BIIA), flavonoids (Flv, panels BIC, BIIC), and the value of the NBI index (panels BIIB, BIIB) in cauliflower leaves. Changes in photosynthetic parameter values (photochemical and non-photochemical) in cauliflower leaves in response to CS and CR are presented on panel BIII and for HS and HR on panel BIV

Statistically significant data with $p < 0.05$ after one-way ANOVA analysis and post hoc Tukey's HSD test. The different letters next to the standard deviation bars indicate statistically significant differences between the variants. Ctr- control, CS- cold stress, CR- cold recovery after 48 h, CR120h- cold recovery after 120 h, HS- heat stress, HR- heat recovery after 48 h, HR 96h- heat recovery after 96 h

Measurement of the maximum photochemical yield of photosystem II (PSII)

The maximum photochemical efficiency of PSII was determined by measuring the Fv/Fm ratio; according to literature data [24], it can be used as a reliable indicator of the photochemical activity of the photosynthetic apparatus. Interestingly, cauliflower under cold stress did not exhibit significant changes in Fv/Fm ratio compared with plants grown under control conditions (Fig. 1). On the other hand, in the leaves of cauliflower plants subjected to elevated temperature, a significant decrease in the variable-to-maximum fluorescence ratio was observed (after photochemical adaptation of the leaves to darkness). Interestingly, in the case of the older leaf (No. 1), the changes in the maximum photochemical yield of PSII were much more noticeable than in the case of the younger leaf (No. 2) (Fig. 1).

Comparative analysis of cauliflower leaf transcriptome in HS and HR responses

High-throughput analysis of the cauliflower transcriptome was performed only in response to elevated temperature treatments. During the analysis of the total transcriptome of cauliflower, RNA samples isolated from the leaves of 30-day-old seedlings subjected to HS and from the seedlings grown under control conditions (Ctrl) were used. The NovaSeq 6000 experimental platform from Macrogen Inc. (South Korea) was used and *de novo* sequencing strategy was employed.

The percentage of unigenes annotated using specific databases, the analysis of functional terms (GO: terms) assigned to the obtained unigenes and the *in-silico* prediction of the localization of proteins

encoded by the cauliflower unigenes is provided in the Supplementary File S1 (details in the Materials & Methods section and in the Supplementary Material & Methods File). Although the most of assembled unigenes were annotated particularly from NT database, other databases appeared also relevant in this process and that the overall annotating efficiency was very high. Moreover, over 6.5% unigenes appeared stress-responsive and almost 16% of them seem to encode organellar proteins, which underline the relevance of plastids and mitochondria in temperature stress response in cauliflower leaves (Supplementary File S1).

The contig pools obtained for the regulated transcripts under the analyzed experimental conditions were presented in Supplementary Table 1. They were compared pairwise: HS vs. Ctr and HR vs. Ctr (Fig. 2, Supplementary File S1). The results obtained clearly indicate the diversity of responses to HS and HR conditions in cauliflower leaves.

The analysis of DEGs showed that in the investigated samples, there were more than 12700 contigs, which together met the following conditions: -2 = > FC > = 2 (the cut-off level of the fold change in accumulation in relation to the control conditions was equal to 2) and the value of the *p* coefficient in the nbinomWaldTest test < 0.05. Interestingly, comparing the number of unique contigs between the HS conditions (6657) and the HR phase (4083) revealed a limited pool of common transcripts, the levels of which are regulated in both experimental variants. This demonstrates the significant specificity of the cauliflower transcriptomic response during HS and after HR. Generally, more genes show altered expression levels after direct HS, with more downregulated than upregulated unigenes. In contrast, after HR phase, the increase in expression levels affects a larger gene pool than during after HS (Fig. 2, Supplementary File S1).

Results of the analysis of GO: terms enrichment using g: *Profiler*, *AgriGO* and *ShinyGO* tools are presented in Supplementary Table 2 and were summarized on Fig. 2. Analysis of differential gene expression has shown that genes encoding numerous TFs, proteins necessary for signal transduction, proteins related to intracellular transport, including vesicular transport and secretory processes, and chaperones are involved in HS response of cauliflower leaves. During HS, mainly genes encoding proteins involved in nitrogen and phosphorus metabolism, regulatory, signaling, and developmental proteins, transport proteins, lipid and certain amino acid metabolism proteins, nucleotide-binding proteins, DNA-binding proteins, and calcium chaperones were particularly regulated. However, after HR, the genes encoding proteins involved in sulfur metabolism, glucosinolate synthesis, enzymes necessary for hormone biosynthesis, cell wall proteins, enzymes of the flavonoid, chlorophyll, and other pigment metabolic pathways, oxidoreductases, and the response to reactive oxygen species (ROS) were affected. Changes in the metabolism of starch and other carbohydrates, as well as

1. (A) Heat map of the one-way clustering using Z-scores for normalized expression values (log_2-based) of 3000 unigenes that were randomly selected from the total pool of 12757 raw contigs, satisfying with FC 2 and raw.*p*. value (details in the text)
2. (B) Changes in the level of gene expression in cauliflower leaves in response to HS and HR compared to the expression level under control conditions (Ctr). Ovals indicate gene pools with particularly higher (*the pink oval*) or lower (*the blue oval*) expression levels than the average expression level under HS (*left plot*) or after HR (*right plot*). Gray dots aligned with the diagonal line indicate genes whose expression level does not change under investigated conditions. FC- fold change in accumulation: stress conditions (HS or HR)/control. Filtered (red and orange dots) as well as unfiltered (gray dots) data were displayed (conditions shown at the right)
3. (C) Statistics on all, annotated, and annotated and unique (non-redundant) unigene quantities (upregulated and downregulated pools are indicated by red and blue on histograms)
4. (D) The number of unique (non-redundant) unigenes regulated during heat stress (HS; yellow circles) and heat recovery (HR; red circles); on the left - the total pool of unique (non-redundant) unigenes, in the center - unigenes upregulated or (right) downregulated during HS and HR
5. (E) Summary of main GO: terms related to KEGG pathways that appeared enriched among HS- and HR-responsive cauliflower contigs comparing to *Brassica* data. For the analysis *g: Profiler* was used. Only significant GO: terms (adjusted $p < 0.05$) are presented. In this graph, *p*-values for each term were also indicated. ALL- all contigs, *Bole- Brassica oleracea*, *Bna- Brassica napus*, *Bra- Brassica rapa*, DOWN- downregulated contigs, UP- upregulated contigs. Full data is available in Supplementary Table 2g.
6. (F) Enriched reactome pathways among HS- and HR-responsive cauliflower contigs comparing to *Brassica* data from ShinyGO tool. NR protein sequence accession.version numbers were used as a query (*column D, Supplementary Table 1a*). Only significant pathways (adjusted $p < 0.05$) were presented. Results for all HS-/ HR-regulated contigs (ALL) as well as for the upregulated (UP) and downregulated (DOWN) contigs were shown. For the clarity, as reference only *Brassica oleracea* STRING database resources were used (full results are presented in *Supplementary Table 2g*). Due to the lack of enriched pathways for upregulated HS-responsive contigs with *B. oleracea* as reference, the data were compared to *B. rapa* resources the biosynthesis of glutathione, as well as relevant amino acids (Trp, Val, Leu, and Ileu), and the metabolism of ubiquinone, were also observed. According to KEGG and reactome pathway analysis, under HS unigenes for proteins necessary for fatty acid degradation and carbohydrate metabolism are specifically enriched. In contrast, HR engages particularly broadened list of functional categories, including transport of small molecules as well as aminoacid and secondary metabolism that are particularly enriched. Among upregulated unigenes in HS, the ones coding for proteins ER-processed and responsive for protein import contrast with the downregulated genes for secondary metabolite synthesis and carbohydrate metabolism. On the other hand, after HR, among upregulated unigenes the ones for proteins of secondary metabolism and transmembrane transport are distinctive and downregulated ones code proteins for biological oxidations and aminoacid degradation (Fig. 2, Supplementary Table 2).

In addition, we investigated GO term enrichment among clustered (by FC value) cauliflower unigenes that were significantly responsive (-2 < = FC > = 2, p < 0.05) under HS and after HR, compared to Ctr conditions (Supplementary Table 3). We performed clustering twice. The first one was for HS/Ctr-responsive unigenes versus HR/Ctr-responsive ones, and the second one was done for HR/Ctr-responsive unigenes versus HS/Ctr-responsive ones). Such a procedure enabled deeper analysis of unigenes clustered similarly by FC and specifically for the given treatment (HS or HR). For clarity, the NT records listed in Supplementary Table 3be, along with their respective FC values, were used for GO term enrichment analysis. Such analysis of enrichment GO terms within unigenes in each cluster confirmed results from general enrichment analyses and additionally highlighted the diversity of stress responsive genes in cauliflower leaves. A short summary of the most relevant GO terms within each cluster was presented in Supplementary Table 3a. It appeared that HS-responsive genes coded mainly transferases and proteins targeted to organelles, as well as proteins for valine, leucine and isoleucine degradation, protein folding, oxidative phosphorylation and response to stress. In contrast, HR-responsive genes coded proteins for lyase activity, hormonal regulation, Ca homeostasis, biosynthesis of secondary metabolites and small molecule metabolism.

Changes in the level of TFs and transcripts accumulation in cauliflower during HS and after HR

Using the *Plant TFDB* v5.0 database, the accumulation of cauliflower TF transcripts (identified from RNA-seq data) was assessed in response to HS and during the HR phase. This analysis revealed that, under the analyzed stress conditions, the accumulation of up to 212 diverse TFs differed significantly from that under control conditions (Supplementary Fig. 1, Supplementary File S2).

Among transcripts with the highest level of accumulation after HS, the ones encoding Hsfs belonging to the family of heat shock TFs, proteins WRKY28 and WRKY48 belonging to the WRKY and MYB families were all dinstictive. In addition, an increase in the level of transcripts encoding TFs from C3H, ERF, and bHLH families was observed. Among the transcripts for TFs with the reduced level in response to HS in cauliflower leaves, MYB, NAC, and ERF family- encoding transcripts were notable (Supplementary File S2).

Under HR, the increase in transcript abundance for TFs belonging to the bHLH, C3H, C2H2 and bZIP families (distinct from the previously mentioned ERF, MYB, and NAC families) was evident. During the HR phase, we can also distinguish transcripts for TFs, whose levels were significantly reduced compared to the control). Interestingly, transcripts encoding TFs from the ARR-B, C3H, MYB, NF-YB, and Trihelix families exhibited an opposite accumulation trend in response to direct HS and during the HR phase (see Supplementary File S2).

Comparative analysis of the total proteome of cauliflower leaves and curds in response to CS and HS

We decided to extend the transcriptomic analysis by comparing the cauliflower proteome. For this purpose, experimental shotgun proteomics approaches were employed without protein separation on polyacrylamide gels (mainly the *non-label* LC-MS/MS technique). The quantitative and qualitative

changes in the total proteome of cauliflower plant leaves and curds under control conditions, temperature stress (CS and HS), and the recovery phase following the end of the thermal stressor action (CR and HR).

In addition, the reproducibility between biological and technical replicates was assessed using scatterplots and correlation analysis. For total proteomic data from curds, the Pearson correlation coefficient across the three technical replicas ranged from 0.92 to 0.98, while across the three biological replicas, it ranged from 0.81 to 0.99.

In total, 951 non-redundant (unique) proteins were identified in cauliflower curds (Supplementary File S3). Analysis of data overrepresentation using Fisher's test revealed that, among the proteins of cauliflower curds, we can distinguish various plastid, cell membrane, ribosomal, nuclear, and cytoplasmic proteins across all experimental variants. It should be added that proteins with catalytic activity, metal ion-binding (including Cu^{2+}), and DNA-binding proteins (including TFs) were notably overrepresented among all stress-responsive cauliflower proteins. These proteins performed various functions, including involvement in responses to stressors such as light intensity (Supplementary File S3). In CS, ribosomal and mRNA-binding proteins as well as proteins for ribosomal subunit assembly and cold response proteins, some dehydrins, redox homeostasis proteins together with selected glycine-rich proteins (GRPs) were responsive in abundance. Among CS-responsive proteins, cytosolic and plasma membrane ones predominated. In contrast to that, in CR, aminoacid synthesis enzymes and thioredoxins, inter alia, were distinctive (Supplementary File S3). A total of 27 proteins were identified in the curds, and their levels were regulated during HS and HR. Under these conditions, the accumulation of 16 proteins decreased, including 4 to undetectable levels. On the other hand, the accumulation of 11 proteins was increased, of which 7 were detected only after heat stress. In HS response (similarly to CS response, but with another protein set) plasma membrane and cytosolic proteins were in number, including structural ribosome constituents (various ribosomal proteins) and mRNA-binding proteins, HSPs for the proper protein folding, dehydrins and GRPs responded in abundance and after HS various translation factors, transporters and heat-responsive proteins were in addition notable (Supplementary File S3).

Interestingly, among the differentially expressed proteins (DEPs) in cauliflower curds, 6 heat shock proteins were induced under both HS and, especially, under HR. They were undetectable under control conditions. In addition, no changes in the accumulation of the proteins were observed during CS and CR (Supplementary Fig. 3).

In total, 862 unique proteins have been identified in the total proteome of cauliflower leaves.

Under CS, various proteins, including cytoplasmic and plastid proteins. The latter ones were involved in photosynthesis (e.g. thylakoid lumen proteins, various PS subunits and enzymes). Transporter proteins also appeared distinctive. During HS, organellar (mitochondrial and plastid) proteins were enriched. mRNA-binding and Cu and ADP-binding proteins as well as cold-, Cd- and light-responsive proteins were also notable (Supplementary File S3). In these conditions, the accumulation of numerous proteins that

neutralize reactive oxygen species (ROS) was observed in cauliflower leaves. At the same time, under HS in leaves, a decrease in the accumulation of various ribosomal proteins was observed. HS also increased the accumulation of one of the glycine-rich proteins (GRP2A). Interestingly, in another member of the GRP protein family (RZ1C), the level of accumulation was higher after HR in cauliflower leaves than under elevated-temperature conditions (Supplementary Fig. 2, Supplementary File S3).

Comparison of the results of proteomic and transcriptomic analyses (association of protein and mRNA accumulation)

The next step in characterizing the cauliflower response to elevated temperature stress was to investigate the relationship between the proteomic and transcriptomic responses. The aim of this research step was therefore to focus on the accumulation of transcripts coding for those proteins that showed a significantly altered level in response to HS and after HR. lists of abundantly affected proteins for each stress variant were compared with the corresponding selected transcripts (based on browsing lists of all contigs regulated under HS and after HR). In this way, it became possible to check whether all the significantly regulated cauliflower proteins under stress conditions are accompanied by a statistically significant accumulation of their corresponding transcripts.

In response to high-temperature stress, 8,110 genes with varying expression levels were identified in cauliflower leaves (a statistically unfiltered list of contigs). From the comparative proteome analysis, 68 proteins were selected that showed significant differences in their levels during the HS response. Of these 68 proteins, transcripts for 16 proteins were identified. The results of this comparison are presented in Fig. 3.

Under HR, a total of 3,346 genes with varying expression levels were observed in cauliflower leaves (a statistically unfiltered list of contigs). In the HR phase, 76 proteins showed significant differences in accumulation levels. Among these 76 proteins, transcripts were identified for 8 of those proteins (Fig. 3).

We also decided to assess the correlation between transcript and protein levels by comparing log2FC values from our transcriptomic and proteomic data for HS-treated cauliflower leaves. Among the selected proteins, only 9 correlated with the transcriptomic data. For 8 of 9 proteins, the level of their transcripts decreased statistically as their protein levels increased (transcript and protein FCs were inversely correlated). In the case of remaining one of the analyzed proteins (protein containing a region with a TPR domain; Bo9g028630), the trend was opposite. As the accumulation of transcripts of this protein increased, the level of the protein itself decreased during heat stress (Fig. 4).

Comparative analysis of the metabolome of cauliflower leaves and buds in response to stress of reduced and elevated temperature

The response of the cauliflower curd metabolome after CS included accumulation of several metabolites, such as disaccharides and fatty acids, which were significantly enriched among responsive

metabolites. In particular, levels of oleic acid, L-serine, glycerol, β-lactose, β-alanine, and dehydroascorbic acid were significantly increased. In contrast, the broadened set of metabolites displayed elevated levels during the recovery phase following CR, including pyrroline carboxylic acids as well as mono- and disaccharides as the significantly enriched metabolites (Supplementary File S4). In this case, it was found, inter alia, that the levels of hydroxybutyric acid, N-butylalanine, lactose, lactulose, hydroquinone, maltotriose, D-pannose, fumaric acid, caffeic acid, cadaverine, maltitol, galactonic acid, L-arginine, 6-phosphate-D-fructose, and nicotinic acid were significantly increased (for more details regarding metabolomic changes under temperature stress see Supplementary File S4).

In cauliflower curds under HS influence, a large group of metabolites was observed. Among responsive ones, pyrroline carboxylic acids (similarly to CR) as well as TCA acids and various carbohydrates were notable. After HR, pyranones appeared top first group of enriched responsive metabolites in addition to groups enriched in HS (Supplementary File S4). Some of metabolites decreased in abundance in HS, however, after HR, their levels increased again to values similar to those present in control conditions or significantly higher. This group included, inter alia, L-methionine, L-5-oxoproline, D-glucuronic acid, D - (-) - sorbitol, sinapic acid, sinapic acid, D-maltose, ribose, D-ribose, lauric acid, inosine, and L - (-) - phenylalanine.

Another distinctive group of metabolites included chemical compounds whose levels were significantly elevated only after HR. It included, among others, stearic acid, oleic acid, arbutin, hydroxyproline, homoserine, uracil, cyanoalanine, arabitol, salicylic acid, palmitic acid, uridine, benzoic acid, g-aminobutyric acid (GABA), adenine, caffeic acid, malonic acid, and nicotinic acid. Compounds such as lactic acid, putrescine, 2-oxoglutaric acid, glutaric acid, phenylalanine, itaconic acid, butyrolactam, glycine, fumaric acid, glycolic acid, N-acetyl-D-glucosamine, threonine, raffinose, maltose, lactulose, lactose, and aconitic acid are examples of metabolites detected at elevated levels both after HS and HR.

Interestingly, among the compounds identified in cauliflower curds subjected to elevated temperatures, some levels decreased significantly only after HR. These compounds were present in curds under control conditions; their level decreased during the HS treatment, and then this decrease was most pronounced after HR. This group of compounds includes, among others, glucose, lactobionic acid, turanose, galactinol, D-(-)-xylose, D-fructose, L-serine, glutamic acid, allose, and citric acid.

In cauliflower leaves, after CS, TCA acids, disaccharides and cyclic alcohols were significantly enriched as responsive metabolites including the increased levels of pentadecanoic acid, lactitol, fructose-6-phosphate, N-acetylmannosamine, galactinol, melibiosis, D-fructose-6-phosphate, and glucose β-D - (+). Interestingly, after CR, δ-valerolactones in addition to disaccharides were enriched (Supplementary File S4). In the case of metabolites such as itaconic acid, fumaric acid, 4-hydroxypyridine, oleic acid, α-lactose, maltotriose, lactose, and L-glucose, both during CS and after CR, a clear decrease in their accumulation was observed. The group of metabolites can also be clearly separated; their levels decreased significantly during CS in cauliflower leaves. However, after the CR ended, they increased

again to the control level. This group includes such metabolites as 3-hydroxyisovaleric acid, 2-deoxytetronic acid, maltose, shikimic acid, glyceric acid, and succinic acid.

Under HS, numerous metabolites accumulated in cauliflower leaves, from which TCA acids as well as monosaccharides and δ-valerolactones (the latter ones similarly to CS) were among the top significantly enriched ones (Supplementary File S4). The responsive metabolites included amino acids such as L-serine, L - (+) - lysine, L-tyrosine, leucine, isoleucine, phenylalanine, or tryptophan, but also succinic acid, trans-cinnamic acid, and cadaverine. Interestingly, many metabolites showed lower accumulation than in the control conditions. This happened for, inter alia, 2-oxoglutaric acid, glycolic acid, fumaric acid, xylonic acid, itaconic acid, chlorogenic acid, glutaric acid, as well as D - (+) - xylose, D - (+) - fucose, and L - (+) – arginine. Cinnamic acids (which dominated in the metabolomic profile), together with TCA acids and monosaccharides were also significantly enriched among responsive metabolites after HR phase (Supplementary File S4).

Discussion

Acclimation to temperature extremes requires activation of regulatory mechanisms that reshape cellular and molecular processes [25]. Because translation, folding, and turnover are time-dependent, changes in mRNA abundance often precede—and do not necessarily predict—protein accumulation. Multi-omics interpretation must therefore consider temporal offsets and regulation beyond transcription, including RNA processing, translation, and post-translational modification, which together contribute to weak transcript–protein correlations [25], [26]. Metabolites further complicate inference because they act both as protective compounds and as signals. Accordingly, integrated transcriptomic, proteomic, and metabolomic analyses are essential for resolving abiotic-stress networks and their regulation [27], [28], [29].

Heat stress (HS) and cold stress (CS) are major constraints on plant growth and productivity and can cause developmental disorders and yield losses in crops such as cauliflower [30], [31], [32]. Although temperature-responsive mechanisms are well studied in model species, integrative datasets remain comparatively scarce for cauliflower. Here, we combined transcriptomics (including HS and recovery), proteomics, metabolomics, and physiological/morphological phenotyping to characterize responses to HS and CS. For cauliflower transcriptome sequencing we have deliberately applied *de novo* strategy (see Materials and Methods), although cauliflower full genomic sequence was recently released [33] and extended earlier published sequence draft [34]. However, Chen *et al.* [33] study based in Chinese C8_BobV2 isolate from Asiatic-origin inbred line, whereas our cultivar belongs to the Mediterranean-origin heterozygous hybrid variety F1 generated by crossing two inbred lines. Nevertheless, Chen *et al.* [33] have also compared genomes of distinct cauliflower lines, even early ripening cultivars, whereas our cultivar ('*Oviedo*') belongs even to super-early cultivars and in fact it was not involved in Chen *et al.* [33] and Sun et al. [34] analyses. Owing to the genetic variability within cauliflower cultivars and lines of distinct geographical origins, we decided to rely on the broad genomic *Brassica* data (with emphasis on *B. oleracea*, *B. napus* and *B. rapa* species) rather than on single genomic sequence coming from the

isolate far from the European center of cauliflower breeding and cultivation, the more so the genomic data resources of the cauliflower cultivar from this region are still limited. Henceforth we have applied *de novo* mapping strategy based on the genomic resources of the entire *Brassica* genus, which allows for the reduction of reference bias, correct consideration of organellar reads, and is consistent with generally accepted practices in functional and transcriptomic studies conducted on heterogeneous material.

Consistent with expectations, transcript abundance was not uniformly predictive of protein levels [35], reinforcing the need to interpret stress responses across molecular levels. Our patterns also agree with previous evidence that chilling severity and duration shape cauliflower performance more strongly than in some related Brassica crops, pointing to species-specific acclimation strategies [36].

At the gene and pathway levels, HS activated canonical proteostasis programs, including strong induction of heat shock proteins (HSPs) and associated chaperone systems, consistent with their conserved role in maintaining protein homeostasis [37], [38]. HS also influenced secondary metabolic routes, including phenylpropanoid- and pigment-related pathways, which contribute to protection and signaling [38]. CS responses are typically phased, progressing from early transcriptional shifts to broader remodeling of lipid metabolism, oxidative stress defenses, carbohydrate metabolism, and photosynthetic adjustment [39]. Our integrated profiles support this framework and indicate that stress phase and organ identity (leaf vs. curd) strongly condition response magnitude and direction.

Physiological measurements revealed membrane sensitivity under both stress types. Electrolyte leakage increased across treatments, indicating compromised membrane integrity, plausibly driven by ROS accumulation and ROS-mediated lipid perturbation that elevates membrane permeability [40]. Pigment responses were stress-type dependent: chlorophyll decline was more evident under cold-related conditions, and cold recovery (CR) further reduced chlorophyll compared with CS, suggesting incomplete restoration and/or a shift toward senescence-like processes. This cold-associated chlorophyll loss was particularly apparent in younger leaves, consistent with differential developmental sensitivity. By contrast, PSII efficiency was comparatively stable under CS in our conditions, whereas HS is frequently associated with PSII impairment across species [31], [41], consistent with a more direct heat-driven destabilization of photosynthetic components.

Secondary metabolite profiles pointed to divergent carbon-allocation strategies. Flavonoids accumulated during HS, albeit gradually, consistent with photoprotection and broader stress mitigation and with their relatively high basal levels under control conditions [42]. Flavonoids can also represent a flexible carbon reservoir [43]. In contrast, CS reduced flavonoid content and the decrease persisted or intensified during CR, a pattern compatible with senescence-associated carbon remobilization and resource reallocation [44]. Together with chlorophyll decline during CR, these trends suggest that recovery from cold may entail continued metabolic costs rather than rapid re-establishment of the pre-stress state.

Transcription factor (TF) remodeling provides a mechanistic context for these coordinated shifts. TFs are central regulators of acclimation and de-acclimation, acting with additional transcriptional and

chromatin-level factors to control stress-responsive gene expression [45], [46]. During HS, we observed pronounced accumulation of HsfA2 and HsfA3 transcripts, consistent with their roles as core regulators of HS signaling and HSP gene induction, including links to oxidative stress mitigation and DREB2A-associated regulation [47], [48], [49]. HS also increased WRKY transcripts, including WRKY39, implicating hormone-integrative modules (salicylic acid/jasmonate) that contribute to thermotolerance [50]. Upregulation of MYB factors (including MYB111) supports engagement of TF networks connecting abiotic stress with flavonoid metabolism [51], and our data suggest MYB111 may also participate in HS acclimation in cauliflower. Finally, strong induction of NAC TFs aligns with evidence that NAC factors can enhance heat tolerance through activation of HS-responsive genes, highlighting potential targets for thermotolerance improvement [52].

Metabolomic reprogramming emphasized redox control and osmoprotection. Temperature stress elevates ROS and challenges metabolic homeostasis; tolerance depends on antioxidant pools (e.g., ascorbate, glutathione) and ROS-scavenging systems [53]. In curds, CS increased dehydroascorbic acid during direct stress and elevated p-coumaric/galactonic acids during CR, indicating enhanced antioxidant and phenolic-linked protection. In leaves, CS increased soluble sugars and proline (e.g., glucose/mellibiose and proline), consistent with osmotic adjustment and protection of macromolecules. Proline accumulation is a widely reported hallmark of low-temperature acclimation across species, including cereals and Brassica crops [32], [54]. Prior work additionally links CS acclimation to induction of ROS-detoxifying enzymes and enzymes supporting ascorbate biosynthesis [55], consistent with our redox-related shifts. Under HS, leaves accumulated multiple amino acids, and curds accumulated amino acids and soluble sugars (including raffinose-family and related carbohydrates) during HS and heat recovery (HR), consistent with roles in osmoprotection, carbon buffering, and signaling [27]. Detection of additional stress-associated metabolites (e.g., γ-aminobutyric acid, hydroxybutyric acid, and nicotinic acid derivatives) further supports activation of conserved abiotic-stress metabolic routes [56]. Notably, many metabolite levels returned to—or exceeded—controls during de-acclimation, indicating that metabolic adjustment continues beyond the acute stress window and may reflect compensatory recovery metabolism.

Proteomics corroborated with major remodeling of stress-responsive and catalytically active proteins (Supplementary Fig. 9). During HS, HSP70/HSP101/HSP90.1 and related chaperones were among the most informative proteins, consistent with their established roles in folding, trafficking, and degradation [57], [58]. Several HSPs were detected specifically in curds after HS and persisted through HR, supporting HS-specific induction and sustained protective capacity. Proteins involved in ROS detoxification also increased; ascorbate peroxidase, a central component of the ascorbate–glutathione cycle, has been proposed as a marker of HS responsiveness in *Brassicaceae* [59] and likely contributes to maintaining redox balance during heat exposure.

Cross-omics comparisons revealed both coordination and decoupling between transcript and protein dynamics. Under HS, a subset of proteins changed in parallel with their transcripts, including thioredoxin (Bo9g058820), linked to oxidative stress protection, and tryptophan synthase (Bo7g111250), consistent

with elevated amino-acid pools. Conversely, coordinated decreases included Psb28, a PSII-associated factor implicated in PSII function under temperature stress [60]. During post-HS de-acclimation, fewer transcript–protein pairs remained concordant, with a marked increase in GRP2A suggesting that recovery engages regulatory priorities distinct from those of acute HS, and that post-stress states should be considered biologically active phases rather than passive reversals.

In this study, we aimed towards identification of key molecular responses in the most responsive and biologically relevant tissue-stress combinations. Our data limitations concern (1) the lack CS and CR responses and (2) focusing on single organ (leaves) in case of RNA-seq. However, our transcriptomic analysis was intentionally limited to leaf under HS and HR to provide a focused, statistically robust transcriptomic assessment of the conditions central to our hypothesis and showing the most pronounced responses at physiological level (Fig. 1). Leaves were also prioritized due to their primarily involvement in multiple aspects of plant life, including temperature sensing, photosynthetic impairment, transpiration, and early visible stress responses. The impact of some stressors, including high temperature on the whole ‘omes’ in Brassica genus is recognized at various extents. For the chosen cauliflower cultivar, especially during the earliest stages of cultivation, heat stress appeared to be more detrimental than other stressors. Furthermore, leaves tended to show the clearest and comparable stress responses with proteins, metabolites, and physiological patterns under HS and especially under HR.

Our omics data, particularly transcriptomic and proteomic responses of cauliflower leaves are only modestly correlated, which can be results of multiple factors, including, among others (1) post-translational protein modifications affecting dynamic protein turnover and stability, (2) transcript degradation rate dynamically fluctuating across investigated stress conditions. Consequently, all those factors will influence the activation and/or inhibition of specific metabolic pathways in cauliflower. However, building the full integrative map of metabolic pathways from transcriptomic, proteomic, and metabolomic responses would be challenging task in our case, mostly because of data heterogeneity and temporal and spatial dynamicity of the data. Instead, we propose concise model of concerned omical replies under temperature stress acclimation (Fig. 5).

In summary, cauliflower responses to HS and CS are multilevel, phase-dependent, and organ-specific, integrating membrane stability, photosynthetic and pigment status, TF-centered transcriptional reprogramming, proteostasis maintenance, antioxidant defenses, and metabolic reallocation. Temperature stress activated networks of abiotic stress-related genes and metabolites required for redox balance and homeostasis, while frequent transcript–protein decoupling underscores the necessity of integrated multi-omics to resolve acclimation and recovery mechanisms in non-model crops.

MATERIALS & METHODS

Growth conditions of the plant material

Seeds of analyzed cauliflower (*Brassica oleracea* var. *botrytis*) cultivar 'Oviedo' were obtained from Bejo Zaden (Poland). Leaves were taken from plants cultivated for 30 days in a growth chamber at 22°C with 70% humidity under a 16-hour light/8-hour dark photoperiod with a photon flux density of 150 μmol m^{-2} s^{-1}. Curds (inflorescence 'heads') were taken from cauliflower plants cultivated for 3 months in growth chambers as described elsewhere [61].

Stress treatment

For cold stress (CS) treatment, plants were incubated for 10 days at 10°C. After CS, half of the plants were immediately frozen in liquid nitrogen and stored at -80°C for further analysis. The second half of the plants, after CS, were incubated for 48 h at 22°C (control conditions, Ctr) for cold recovery (CR) and were then collected as described above. For heat stress (HS) treatment, plants were incubated for 2 hours at 42°C. After HS, half of the plants were collected, and the other half were placed under heat recovery (HR) conditions for 48 hours. The sampling was performed according to the same procedure as for cold stress. Three replicates were performed for each experimental variant.

Electrolyte leakage test

Membrane damage was assessed by measuring electrolyte leakage from HS-treated, CS-treated, heat and cold-recovered (HR and CR, respectively), and Ctr-grown plants immediately after the given treatment. Ten leaf discs were randomly selected from four plants after excision from fully expanded leaves (10 discs mixed from 4 plants) and placed into 50ml Falcon tubes with 10mL ultrapure water. All samples were incubated on an orbital shaker at 100rpm for 24 hours at room temperature. After incubation, the initial conductivity (C1) was measured using a waterproof conductivity meter (CC-411, Elmetron, Poland). Then the Falcon tubes with leaf discs were placed in a boiling water bath for 10 minutes. They were then cooled on ice and subsequently warmed to room temperature. The final conductivity (C2) was then measured. The relative electrolyte leakage was calculated using the formula (C1/C2) × 100. Three replicates were performed for each experimental variant.

Measurement of chlorophyll a fluorescence induction kinetics

Chl fluorescence induction kinetics (the Kautsky effect) of cauliflower plants (Ctr-grown, after HS, CS, HR, and CR) were performed on leaf fragments (0.5-cm area cut from 2–3 mm below the leaf tip) by using a pulse amplitude-modulated fluorimeter FluorPen FP 110 (Photon Systems Instruments, Czech Republic) according to the procedure described by Lichtenthaler [23]. For details on the procedure and Chl fluorescence kinetic parameters and their calculation, see Supplementary Material & Methods File.

Estimation of leaf nitrogen (N) deficiency progress

For the nondestructive indication of leaf N deficiency progression, fluorimetry (DUALEX 4 Scientific FLAV fluorimeter; Force-A) was performed to estimate the chlorophyll/flavonoid (Chl/Flv) ratio, called NBI, using a 0.5-cm area, 2 to 3 mm below the leaf tip. The Flv level was estimated from the difference in chlorophyll fluorescence induced by UV (375 nm) and red light (650 nm), as only UV is affected by Flv.

The chlorophyll level was estimated from the transmittance of red light (710 nm). Since leaf structures could interfere with this measurement, the readings were automatically corrected by the transmittance measured at the 850-nm reference wavelength.

Conductometric analysis of the permeability of cauliflower membranes

To measure the relative electrical conductivity (REC), for each treatment, 10 fresh discs (6 mm in diameter) were cut from the second fully expanded leaves and thoroughly rinsed five times with deionized water. The clean leaf discs were placed in closed vials containing 10 mL of deionized water and incubated at 25°C for 24 hours on a rotary shaker (100 rpm) before determining the initial electrical conductivity (C1). Samples were then boiled for 10 min, and the final electrical conductivity (C2) was obtained after attaining equilibrium at 25°C. The relative electrical conductivity (REC) was calculated as follows: REC (%) = C1/C2×100

Whole transcriptome sequencing

RNA extraction, library preparation, and sequencing

The total RNA from cauliflower leaves collected from plants subjected to HS and HR, as well as from Ctr-grown plants, was extracted using the GeneJET Plant RNA Purification Mini Kit (Thermo Scientific), following the manufacturer's instructions, with three biological replicates per variant (for details see Supplementary Material & Methods File). All steps regarding RNA quality checks, library synthesis, amplification, and sequencing, as well as data annotation and differential expression analysis (see below), were performed by Macrogen Inc. (South Korea). The rationale for *de novo* strategy of transcriptome sequencing was described in Discussion.

Library preparation and sequencing

Libraries were prepared using Illumina TruSeq Stranded Total RNA LT Sample Prep Kit (Plant) according to the TruSeq Stranded Total RNA Sample Prep Guide. For paired-end sequencing, both ends of the cDNA were sequenced using the Illumina NovaSeq platform (Macrogen Inc., South Korea). A de novo transcriptome sequencing strategy was employed (for details, see Supplementary Material & Methods File).

Whole proteome shot-gun analysis

For MS/MS analysis, cauliflower leaf or curd proteins were isolated (see Supplementary Material & Methods File) and subsequently standardly digested in solution and 1.5 µg of the protein digest per run was analyzed by nano-LC MS/MS analysis using a Dionex UltiMate 3000 RSLC and Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, USA). The raw files were analyzed using MaxQuant (https://maxquant.org/) [62]. Quantitative analysis between experimental groups was based on label-free quantification (LFQ) intensities.

Whole metabolome analysis

Metabolites were extracted from 100 mg powdered plant material using methanol [63] and analyzed on a TRACE 1310 gas chromatograph (ThermoFisherScientific) connected to a TSQ8000 mass spectrometer (ThermoFisherScientific). Mass spectra were recorded from 35 to 1000 m/z. All spectra were subjected to automatic peak detection, deconvolution, retention index calculation, and library search using MSDial v4.16 [64]. For detailed information on the GC-MS/MS and data analysis, see Supplementary Material & Methods File.

Statistical analysis

DEGs were met following criteria: -2 = > FC > = 2 (the cut-off level of the fold change in accumulation in relation to the control conditions was equal to 2) and the value of the p coefficient in the nbinomWaldTest test < 0.05. Results for abundances of proteins or metabolites, as well as results from measurements of physiological parameters from at least three replicas (n = 3), were submitted to one-way ANOVA to select variants for which CS, HS, CR, and HR treatments had a significant effect ($p < 0.05$) on their values with respect to control values. The most variable variants were also checked using post-hoc Tukey's HSD test (JMP Software v8, SAS Institute, Cary, NC, USA).

Statistical analyses of proteins and metabolites were performed using Perseus v1.6.1.3 [65].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read the content of the manuscript and consent to its publication.

Competing interests:

The authors declare no competing interests.

Funding

Declaration: Financial support was provided by Flora Robotica: A Community of Symbiotic Bio-Hybrid Robot-Plant Architectural Artifacts, funded by the European Union's Horizon 2020 research and innovation program under FET Grant Agreement No. 640959, and by grants for PhD students from the Dean of the Faculty of Biology, Adam Mickiewicz University in Poznań, awarded to Magdalena Taube

Author Contribution

MT (Magdalena Taube) – conceptualization, performing stress experiments, RT-qPCR, collecting plant material, data curation, statistical analysis, writing original draft; AKM – study design, supervision, project administration, critically reviewing and revising the manuscript; DB – transcriptomic data clustering, transcriptomic and proteomic data correlation; ES - electrolyte leakage test, chlorophyll a and leaf N deficiency progress measurement, conductometric analysis of the cauliflower membranes permeability; MT (Michal Taube) – figures preparation and critically reviewing and revising the manuscript; WP – supervision during plants' physiological analysis after stress treatments, critically reviewing and revising the manuscript; WN – critically reviewing and revising the manuscript; MR – supervision, transcriptomic data analysis, critically reviewing and revising the manuscript; PW - securing financial support for the research, critically reviewing and revising the manuscript.

Acknowledgement

The authors would like to express their gratitude to Professor Włodzimierz Krzesiński from the Department of Vegetable Crops, University of Life Sciences in Poznan, for cauliflower curds preparation for experiments, and to Mrs. Liliana Ciszewska and Mr Grzegorz Pietkiewicz for technical support.

Data availability statement:

transcriptomic raw data are available in NCBI database under BioProjectID PRJNA1363906 https://dataview.ncbi.nlm.nih.gov/object/PRJNA1363906?reviewer=f8u82lhs3vn2upnh9krn0fred5 (link to NGS data for reviewers); data from metabolomic comparative analysis are available in RepOD repository https://repod.icm.edu.pl/dataset.xhtml?token=2c8aeb8e-cd3e-4dce-afca-b4d79f9e2e0f ; data from proteomic comparative analysis are available in RepOD repository https://repod.icm.edu.pl/dataset.xhtml?token=461d5eed-37ee-43b2-ab52-f52b134a489d

References

1. Wu Z, et al. A Canonical DREB2-Type Transcription Factor in Lily Is Post-translationally Regulated and Mediates Heat Stress Response. Front Plant Sci. Mar. 2018;9:243. 10.3389/fpls.2018.00243.
2. Suzuki N et al. Jan., ABA Is Required for Plant Acclimation to a Combination of Salt and Heat Stress, *PLOS ONE*, vol. 11, no. 1, p. e0147625, 2016, 10.1371/journal.pone.0147625
3. Mizoi J, et al. Heat-induced inhibition of phosphorylation of the stress-protective transcription factor DREB2A promotes thermotolerance of Arabidopsis thaliana. J Biol Chem. Jan. 2019;294(3):902–17. 10.1074/jbc.RA118.002662.
4. Guo W, et al. The Wheat NAC Transcription Factor TaNAC2L Is Regulated at the Transcriptional and Post-Translational Levels and Promotes Heat Stress Tolerance in Transgenic Arabidopsis. PLoS

ONE. Aug. 2015;10(8):e0135667. 10.1371/journal.pone.0135667.

5. Gao F, Ma P, Wu Y, Zhou Y, Zhang G. Quantitative Proteomic Analysis of the Response to Cold Stress in Jojoba, a Tropical Woody Crop. Int J Mol Sci. Jan. 2019;20(2):243. 10.3390/ijms20020243.
6. Sawicki M et al. Mar., On a Cold Night: Transcriptomics of Grapevine Flower Unveils Signal Transduction and Impacted Metabolism, *Int. J. Mol. Sci.*, vol. 20, no. 5, p. 1130, 2019, 10.3390/ijms20051130
7. Beck EH, Heim R, Hansen J. Plant resistance to cold stress: Mechanisms and environmental signals triggering frost hardening and dehardening. J Biosci. Dec. 2004;29(4):449–59. 10.1007/BF02712118.
8. Wang B, Wang G, Shen F, Zhu S, Glycine-Rich A, Protein RNA-B. CsGR-RBP3, Is Involved in Defense Responses Against Cold Stress in Harvested Cucumber (Cucumis sativus L.) Fruit. Front Plant Sci. Apr. 2018;9:540. 10.3389/fpls.2018.00540.
9. Dobrá J, et al. The impact of heat stress targeting on the hormonal and transcriptomic response in Arabidopsis. Plant Sci. Feb. 2015;231:52–61. 10.1016/j.plantsci.2014.11.005.
10. Dong X, Yi H, Lee J, Nou I-S, Han C-T, Hur Y. Global Gene-Expression Analysis to Identify Differentially Expressed Genes Critical for the Heat Stress Response in Brassica rapa. PLoS ONE. Jun. 2015;10(6):e0130451. 10.1371/journal.pone.0130451.
11. Dubouzet JG, et al. *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. Feb. 2003;33(4):751–63. 10.1046/j.1365-313X.2003.01661.x.
12. Deng X, et al. Comparative transcriptome analysis reveals phytohormone signalings, heat shock module and ROS scavenger mediate the cold-tolerance of rubber tree. Sci Rep. Mar. 2018;8(1):4931. 10.1038/s41598-018-23094-y.
13. Kosová K, Vítámvás P, Urban MO, Prášil IT, Renaut J. Plant Abiotic Stress Proteomics: The Major Factors Determining Alterations in Cellular Proteome. Front Plant Sci. Feb. 2018;9:122. 10.3389/fpls.2018.00122.
14. Das A et al. Leaf Proteome Analysis Reveals Prospective Drought and Heat Stress Response Mechanisms in Soybean, *BioMed Res. Int.*, vol. 2016, pp. 1–23, 2016. 10.1155/2016/6021047
15. Ma L et al. Mar., Transcriptome Analysis Reveals Key Cold-Stress-Responsive Genes in Winter Rapeseed (Brassica rapa L.), *Int. J. Mol. Sci.*, vol. 20, no. 5, p. 1071, 2019, 10.3390/ijms20051071
16. Kan Y, Lin H-X. Molecular regulation and genetic control of rice thermal response, *Crop J.*, vol. 9, no. 3, pp. 497–505, Jun. 2021, 10.1016/j.cj.2021.02.008
17. Wang J, Song L, Gong X, Xu J, Li M. Functions of Jasmonic Acid in Plant Regulation and Response to Abiotic Stress. Int J Mol Sci. Feb. 2020;21(4):1446. 10.3390/ijms21041446.
18. Amara I, et al. Insights into Late Embryogenesis Abundant (LEA) Proteins in Plants: From Structure to the Functions. Am J Plant Sci. 2014;05(22):3440–55. 10.4236/ajps.2014.522360.

19. Kan Y, Mu X-R, Gao J, Lin H-X, Lin Y. The molecular basis of heat stress responses in plants. Mol Plant. Oct. 2023;16(10):1612–34. 10.1016/j.molp.2023.09.013.
20. Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci. 2013;4. 10.3389/fpls.2013.00273.
21. Yousef EAA, Müller T, Börner A, Schmid KJ. Comparative analysis of genetic diversity and differentiation of cauliflower (Brassica oleracea var. botrytis) accessions from two ex situ genebanks. PLoS ONE. Feb. 2018;13(2):e0192062. 10.1371/journal.pone.0192062.
22. Lichtenthaler HK, Babani F. Light Adaptation and Senescence of the Photosynthetic Apparatus. Changes in Pigment Composition, Chlorophyll Fluorescence Parameters and Photosynthetic Activity, in *Chlorophyll a Fluorescence*, vol. 19, G. C. Papageorgiou and Govindjee, Eds., in Advances in Photosynthesis and Respiration, vol. 19., Dordrecht: Springer Netherlands, 2004, pp. 713–736. 10.1007/978-1-4020-3218-9_28
23. Lichtenthaler HK, Langsdorf G, Lenk S, Buschmann C. Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system, *Photosynthetica*, vol. 43, no. 3, pp. 355–369, Sep. 2005, 10.1007/s11099-005-0060-8
24. Netto AT, Campostrini E, Oliveira JGD, Bressan-Smith RE. Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves, *Sci. Hortic.*, vol. 104, no. 2, pp. 199–209, Mar. 2005, 10.1016/j.scienta.2004.08.013
25. Zapalska-Sozoniuk M, Chrobak L, Kowalczyk K, Kankofer M. Is it useful to use several 'omics' for obtaining valuable results? *Mol. Biol. Rep.*, vol. 46, no. 3, pp. 3597–3606, Jun. 2019, 10.1007/s11033-019-04793-9
26. Argueso CT, et al. Directions for research and training in plant omics: Big Questions and Big Data. Plant Direct. Apr. 2019;3(4):e00133. 10.1002/pld3.133.
27. Kaplan F, et al. Exploring the Temperature-Stress Metabolome of Arabidopsis. Plant Physiol. Dec. 2004;136(4):4159–68. 10.1104/pp.104.052142.
28. Urano K, Kurihara Y, Seki M, Shinozaki K. 'Omics' analyses of regulatory networks in plant abiotic stress responses, *Curr. Opin. Plant Biol.*, vol. 13, no. 2, pp. 132–138, Apr. 2010, 10.1016/j.pbi.2009.12.006
29. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids. Nov. 2008;35(4):753–9. 10.1007/s00726-008-0061-6.
30. Fahad S, et al. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. Front Plant Sci. Jun. 2017;8:1147. 10.3389/fpls.2017.01147.
31. Sun L, et al. Cold Priming Induced Tolerance to Subsequent Low Temperature Stress is Enhanced by Melatonin Application during Recovery in Wheat. Molecules. May 2018;23(5):1091. 10.3390/molecules23051091.
32. Zhao Y, et al. Integrated transcriptomics and metabolomics analyses provide insights into cold stress response in wheat. Crop J. Dec. 2019;7(6):857–66. 10.1016/j.cj.2019.09.002.

33. Chen R, et al. Genomic analyses reveal the stepwise domestication and genetic mechanism of curd biogenesis in cauliflower. Nat Genet. Jun. 2024;56(6):1235–44. 10.1038/s41588-024-01744-4.
34. Sun D, et al. Draft genome sequence of cauliflower (Brassica oleracea L. var. botrytis) provides new insights into the C genome in Brassica species. Hortic Res. Dec. 2019;6(1):82. 10.1038/s41438-019-0164-0.
35. Wang X, Li M, Liu X, Zhang L, Duan Q, Zhang J. Quantitative Proteomic Analysis of Castor (Ricinus communis L.) Seeds During Early Imbibition Provided Novel Insights into Cold Stress Response. Int J Mol Sci. Jan. 2019;20(2):355. 10.3390/ijms20020355.
36. Wojciechowska R et al. Jun., Alterations in Chlorophyll a Fluorescence and Pigments Concentration in the Leaves of Cauliflower and Broccoli Transplants Subjected to Chilling, *Not. Bot. Horti Agrobot. Cluj-Napoca*, vol. 44, no. 1, pp. 17–24, 2016, 10.15835/nbha44110251
37. Kumar RR, et al. Differential expression of heat shock protein and alteration in osmolyte accumulation under heat stress in wheat. J Plant Biochem Biotechnol. Jan. 2013;22(1):16–26. 10.1007/s13562-012-0106-5.
38. Zhang X, et al. Expression Profile in Rice Panicle: Insights into Heat Response Mechanism at Reproductive Stage. PLoS ONE. Nov. 2012;7(11):e49652. 10.1371/journal.pone.0049652.
39. Fu J, et al. Improved cold tolerance in Elymus nutans by exogenous application of melatonin may involve ABA-dependent and ABA-independent pathways. Sci Rep. Jan. 2017;7(1):39865. 10.1038/srep39865.
40. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. Dec. 2010;48(12):909–30. 10.1016/j.plaphy.2010.08.016.
41. Shi Y-L, et al. Effect of Freezing on Photosystem II and Assessment of Freezing Tolerance of Tea Cultivar. Plants. Oct. 2019;8(10):434. 10.3390/plants8100434.
42. Agati G, Tattini M. Multiple functional roles of flavonoids in photoprotection. New Phytol. Jun. 2010;186(4):786–93. 10.1111/j.1469-8137.2010.03269.x.
43. Mouradov A, Spangenberg G. Flavonoids: a metabolic network mediating plants adaptation to their real estate. Front Plant Sci. Nov. 2014;5. 10.3389/fpls.2014.00620.
44. Dixon RA, Pasinetti GM. Flavonoids and Isoflavonoids: From Plant Biology to Agriculture and Neuroscience, *Plant Physiol.*, vol. 154, no. 2, pp. 453–457, Oct. 2010, 10.1104/pp.110.161430
45. Udvardi MK et al. Jun., Legume Transcription Factors: Global Regulators of Plant Development and Response to the Environment, *Plant Physiol.*, vol. 144, no. 2, pp. 538–549, 2007, 10.1104/pp.107.098061
46. Agarwal P, Agarwal PK, Joshi AJ, Sopory SK, Reddy MK. Overexpression of PgDREB2A transcription factor enhances abiotic stress tolerance and activates downstream stress-responsive genes, *Mol. Biol. Rep.*, vol. 37, no. 2, pp. 1125–1135, Feb. 2010, 10.1007/s11033-009-9885-8
47. Nishizawa A, Yabuta Y, Yoshida E, Maruta T, Yoshimura K, Shigeoka S. Arabidopsis heat shock transcription factor A2 as a key regulator in response to several types of environmental stress, *Plant J.*, vol. 48, no. 4, pp. 535–547, Nov. 2006, 10.1111/j.1365-313X.2006.02889.x

48. Yoshida T et al. Apr., Functional analysis of an Arabidopsis heat-shock transcription factor HsfA3 in the transcriptional cascade downstream of the DREB2A stress-regulatory system, *Biochem. Biophys. Res. Commun.*, vol. 368, no. 3, pp. 515–521, 2008, 10.1016/j.bbrc.2008.01.134
49. Schramm F, et al. A cascade of transcription factor DREB2A and heat stress transcription factor HsfA3 regulates the heat stress response of Arabidopsis. Plant J. Jan. 2008;53(2):264–74. 10.1111/j.1365-313X.2007.03334.x.
50. Li S, Zhou X, Chen L, Huang W, Yu D. Functional Characterization of Arabidopsis thaliana WRKY39 in Heat Stress. Mol Cells. May 2010;29(5):475–84. 10.1007/s10059-010-0059-2.
51. Zhao Y, Cheng X, Liu X, Wu H, Bi H, Xu H. The Wheat MYB Transcription Factor TaMYB31 Is Involved in Drought Stress Responses in Arabidopsis, *Front. Plant Sci.*, vol. 9, p. 1426, Sep. 2018, 10.3389/fpls.2018.01426
52. Guo W, et al. The Wheat NAC Transcription Factor TaNAC2L Is Regulated at the Transcriptional and Post-Translational Levels and Promotes Heat Stress Tolerance in Transgenic Arabidopsis. PLoS ONE. Aug. 2015;10(8):e0135667. 10.1371/journal.pone.0135667.
53. Distelbarth H, Nägele T, Heyer AG. Responses of antioxidant enzymes to cold and high light are not correlated to freezing tolerance in natural accessions of *A rabidopsis thaliana*, *Plant Biol.*, vol. 15, no. 6, pp. 982–990, Nov. 2013, 10.1111/j.1438-8677.2012.00718.x
54. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: A review, *Plant Signal. Behav.*, vol. 7, no. 11, pp. 1456–1466, Nov. 2012, 10.4161/psb.21949
55. Díaz ML, Soresi DS, Basualdo J, Cuppari SJ, Carrera A. Transcriptomic response of durum wheat to cold stress at reproductive stage, *Mol. Biol. Rep.*, vol. 46, no. 2, pp. 2427–2445, Apr. 2019, 10.1007/s11033-019-04704-y
56. Templer SE et al. Mar., Metabolite profiling of barley flag leaves under drought and combined heat and drought stress reveals metabolic QTLs for metabolites associated with antioxidant defense, *J. Exp. Bot.*, vol. 68, no. 7, pp. 1697–1713, 2017, 10.1093/jxb/erx038
57. Wang W, Vinocur B, Shoseyov O, Altman A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci. May 2004;9(5):244–52. 10.1016/j.tplants.2004.03.006.
58. Echevarría-Zomeño S, Fernández-Calvino L, Castro-Sanz AB, López JA, Vázquez J, Castellano MM. Dissecting the proteome dynamics of the early heat stress response leading to plant survival or death in Arabidopsis. Plant Cell Environ. Jun. 2016;39(6):1264–78. 10.1111/pce.12664.
59. Zhang Y, et al. Proteomic Analysis of Heat Stress Response in Leaves of Radish (Raphanus sativus L). Plant Mol Biol Rep. Feb. 2013;31(1):195–203. 10.1007/s11105-012-0486-7.
60. Bečková M, Gardian Z, Yu J, Konik P, Nixon PJ, Komenda J. Association of Psb28 and Psb27 Proteins with PSII-PSI Supercomplexes upon Exposure of Synechocystis sp. PCC 6803 to High Light. Mol Plant. Jan. 2017;10(1):62–72. 10.1016/j.molp.2016.08.001.

61. Rurek M, Woyda-Ploszczyca AM, Jarmuszkiewicz W. Biogenesis of mitochondria in cauliflower (Brassica oleracea var. botrytis) curds subjected to temperature stress and recovery involves regulation of the complexome, respiratory chain activity, organellar translation and ultrastructure, *Biochim. Biophys. Acta BBA - Bioenerg.*, vol. 1847, no. 4–5, pp. 399–417, Apr. 2015, 10.1016/j.bbabio.2015.01.005
62. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification, *Nat. Biotechnol.*, vol. 26, no. 12, pp. 1367–1372, Dec. 2008, 10.1038/nbt.1511
63. Szuba A, Marczak Ł, Ratajczak I, Kasprowicz-Maluśki A, Mucha J. Integrated proteomic and metabolomic analyses revealed molecular adjustments in *Populus* × *canescens* colonized with the ectomycorrhizal fungus *Paxillus involutus*, which limited plant host growth, *Environ. Microbiol.*, vol. 22, no. 9, pp. 3754–3771, Sep. 2020, 10.1111/1462-2920.15146
64. Tsugawa H, et al. A lipidome atlas in MS-DIAL 4. Nat Biotechnol. Oct. 2020;38(10):1159–63. 10.1038/s41587-020-0531-2.
65. Tyanova S et al. Sep., The Perseus computational platform for comprehensive analysis of (prote)omics data, *Nat. Methods*, vol. 13, no. 9, pp. 731–740, 2016, 10.1038/nmeth.3901

Figures

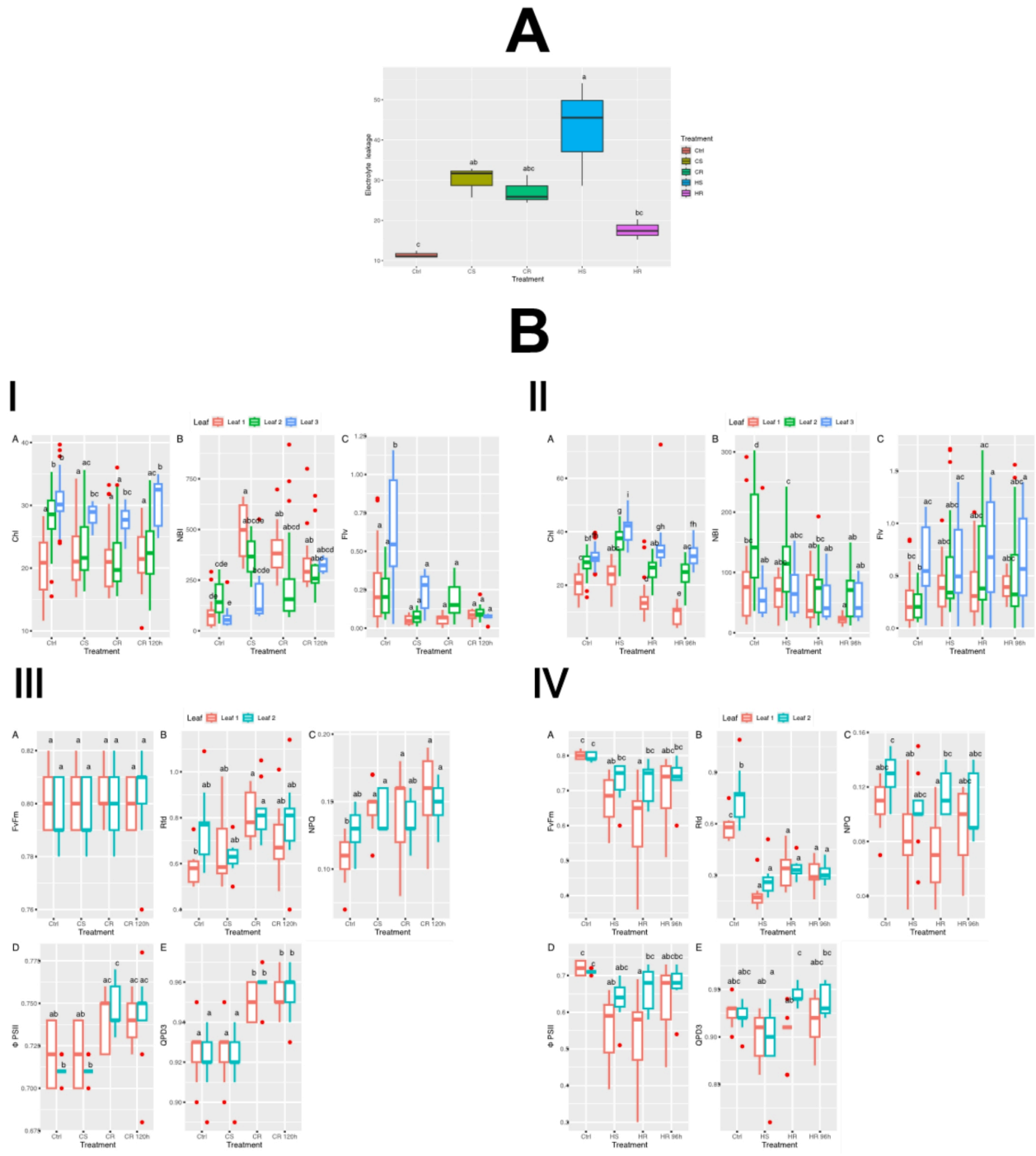


Figure 1

Physiological analysis of cauliflower in response to stress and during the recovery phase after stress.

(A) The level of electrolyte leakage from cauliflower leaves in all experimental variants

(BI-BIV) the relative content of chlorophyll (Chl, panels BIA, BIIA), flavonoids (Flv, panels BIC, BIIC), and the value of the NBI index (panels BIIB, BIIB) in cauliflower leaves. Changes in photosynthetic parameter values (photochemical and non-photochemical) in cauliflower leaves in response to CS and CR are presented on panel BIII and for HS and HR on panel BIV

Statistically significant data with p <0.05 after one-way ANOVA analysis and post hoc Tukey's HSD test. The different letters next to the standard deviation bars indicate statistically significant differences between the variants. Ctr- control, CS- cold stress, CR- cold recovery after 48 h, CR120h- cold recovery after 120 h, HS- heat stress, HR- heat recovery after 48 h, HR 96h- heat recovery after 96 h

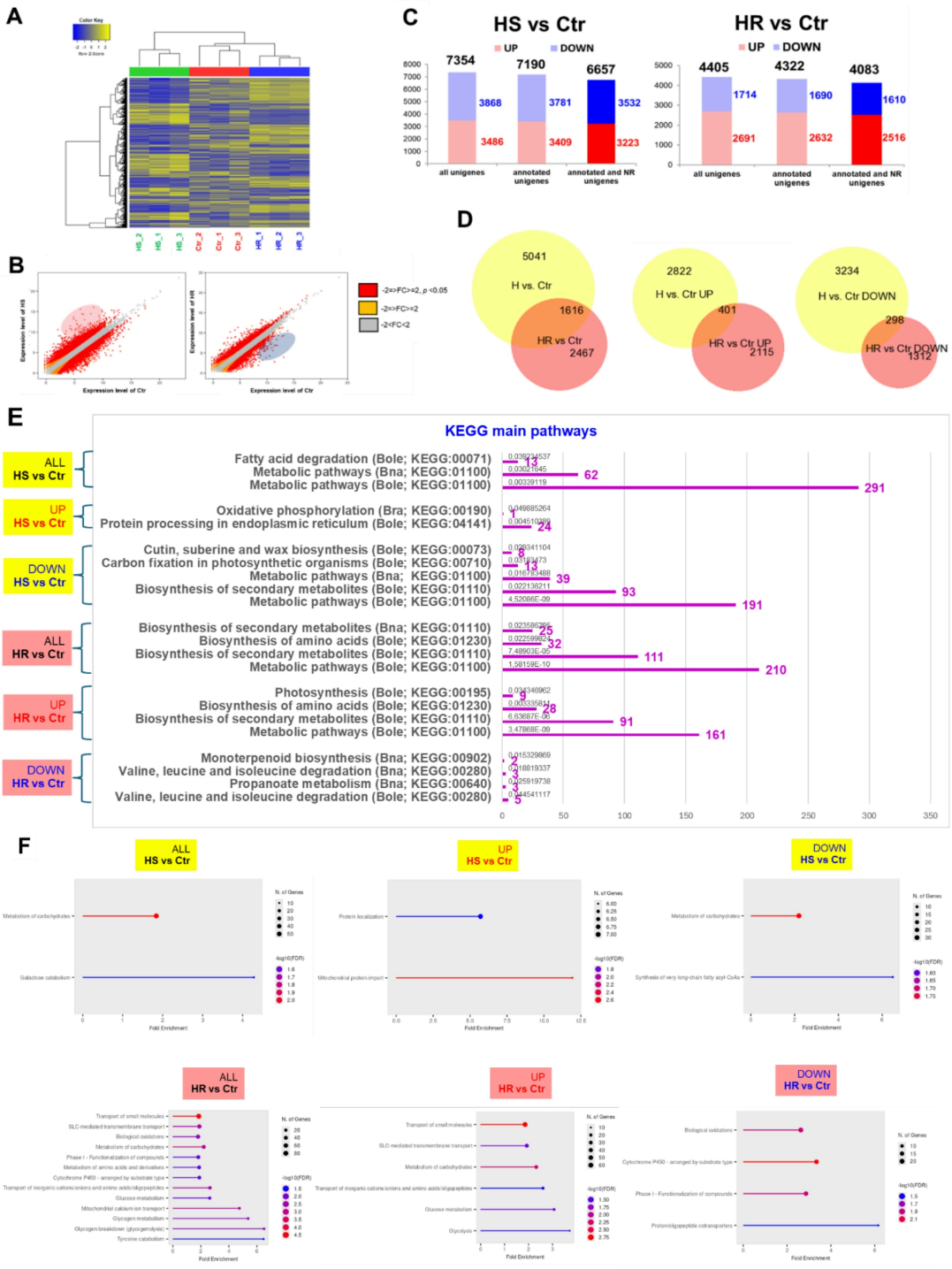


Figure 2

Transcriptional profiles of cauliflower leaves and the affected unigenes under HS and HR response.

(A) Heat map of the one-way clustering using Z-scores for normalized expression values (log_2-based) of 3000 unigenes that were randomly selected from the total pool of 12757 raw contigs, satisfying with FC 2 and raw.*p*. value (details in the text)

(B) Changes in the level of gene expression in cauliflower leaves in response to HS and HR compared to the expression level under control conditions (Ctr). Ovals indicate gene pools with particularly higher (*the pink oval*) or lower (*the blue oval*) expression levels than the average expression level under HS (*left plot*) or after HR (*right plot*). Gray dots aligned with the diagonal line indicate genes whose expression level does not change under investigated conditions. FC- fold change in accumulation: stress conditions (HS or HR)/control. Filtered (red and orange dots) as well as unfiltered (gray dots) data were displayed (conditions shown at the right)

(C) Statistics on all, annotated, and annotated and unique (non-redundant) unigene quantities (upregulated and downregulated pools are indicated by red and blue on histograms)

(D) The number of unique (non-redundant) unigenes regulated during heat stress (HS; yellow circles) and heat recovery (HR; red circles); on the left - the total pool of unique (non-redundant) unigenes, in the center - unigenes upregulated or (right) downregulated during HS and HR

(E) Summary of main GO: terms related to KEGG pathways that appeared enriched among HS- and HR-responsive cauliflower contigs comparing to *Brassica* data. For the analysis *g: Profiler* was used. Only significant GO: terms (adjusted $p < 0.05$) are presented. In this graph, *p*-values for each term were also indicated. ALL- all contigs, *Bole- Brassica oleracea*, *Bna- Brassica napus*, *Bra- Brassica rapa*, DOWN- downregulated contigs, UP- upregulated contigs. Full data is available in Supplementary Table 2g.

(F) Enriched reactome pathways among HS- and HR-responsive cauliflower contigs comparing to *Brassica* data from ShinyGO tool. NR protein sequence accession.version numbers were used as a query (*column D, Supplementary Table 1a*). Only significant pathways (adjusted $p < 0.05$) were presented. Results for all HS-/ HR-regulated contigs (ALL) as well as for the upregulated (UP) and downregulated (DOWN) contigs were shown. For the clarity, as reference only *Brassica oleracea* STRING database resources were used (full results are presented in *Supplementary Table 2g*). Due to the lack of enriched pathways for upregulated HS-responsive contigs with *B. oleracea* as reference, the data were compared to *B. rapa* resources the biosynthesis of glutathione, as well as relevant amino acids (Trp, Val, Leu, and Ileu), and the metabolism of ubiquinone, were also observed. According to KEGG and reactome pathway analysis, under HS unigenes for proteins necessary for fatty acid degradation and carbohydrate metabolism are specifically enriched. In contrast, HR engages particularly broadened list of functional categories, including transport of small molecules as well as aminoacid and secondary metabolism that are particularly enriched. Among upregulated unigenes in HS, the ones coding for proteins ER-processed and responsive for protein import contrast with the downregulated genes for secondary metabolite synthesis and carbohydrate metabolism. On the other hand, after HR, among upregulated unigenes the ones for proteins of secondary metabolism and transmembrane transport are distinctive and downregulated ones code proteins for biological oxidations and aminoacid degradation (Figure 2, Supplementary Table 2).

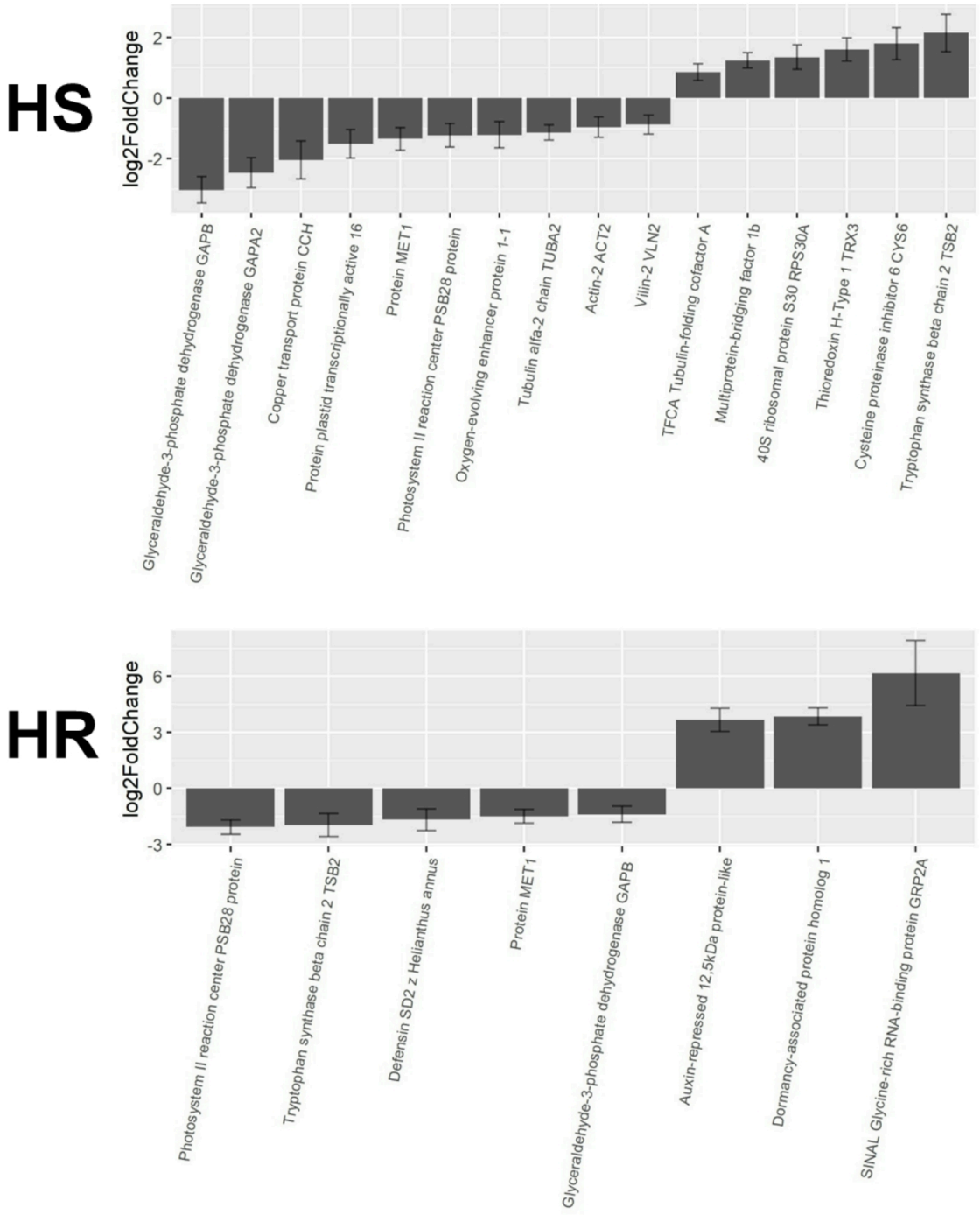


Figure 3

Summary of the accumulation level of transcripts encoding proteins identified in proteomic analysis under conditions of elevated temperature stress (HS) and the recovery after heat stress (HR).

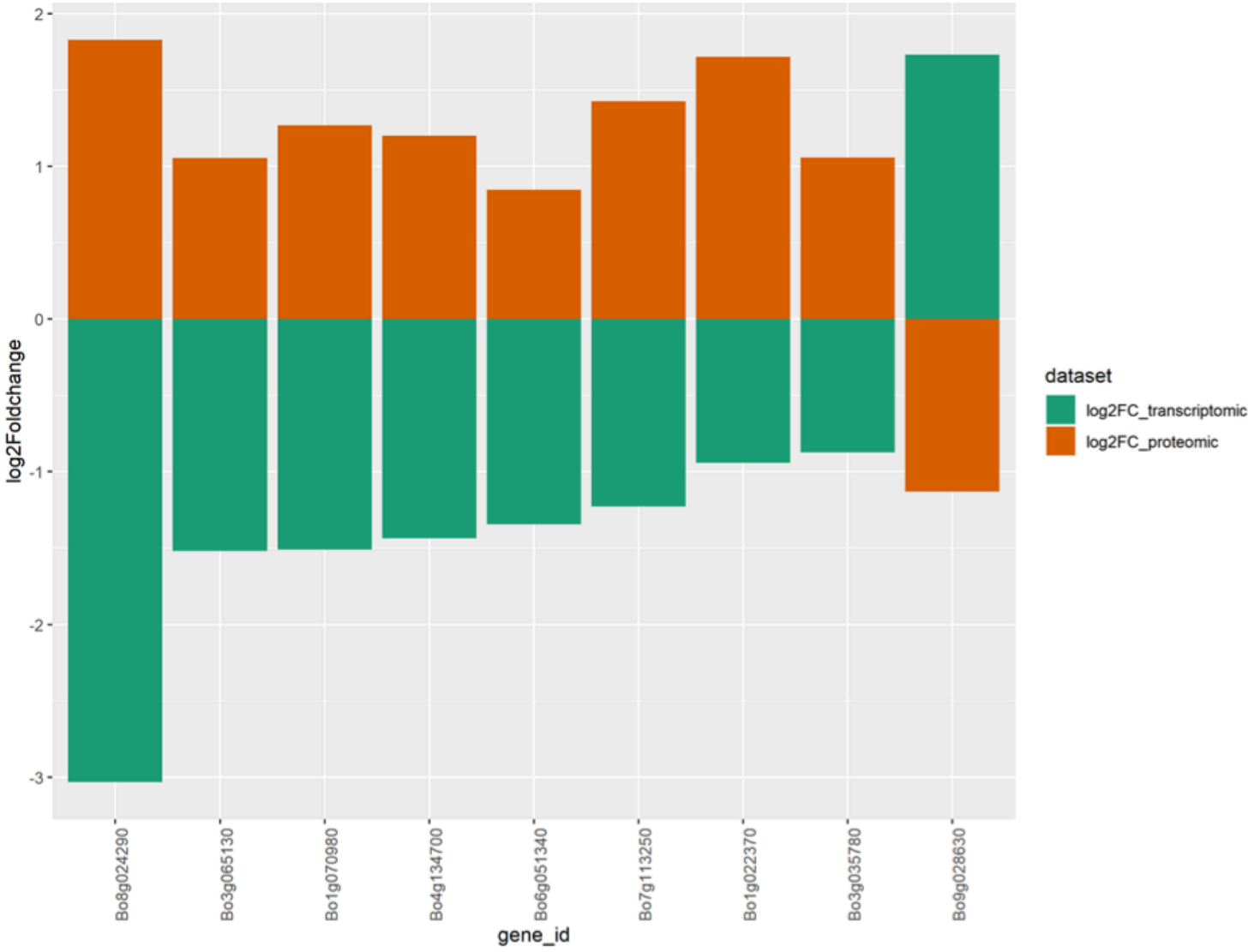


Figure 4

Relationship of the level of proteins and their transcripts in Brassica oleracea var. botrytis under heat stress (HS). Bo8g024290, Glyceraldehyde-3-phosphate dehydrogenase GAPB; Bo3g065130, Phosphoglycerate kinase 1; Bo1g070980, Protein plastid transcriptionally active 16; Bo4g134700, Glutamine synthetase; Bo6g051340, Protein MET1; Bo7g113250, Photosystem II reaction center PSB28 protein; Bo1g022370, Elongation factor Tu; Bo3g035780, Villin-2; Bo9g028630, Hsp70-Hsp90 organizing protein 2

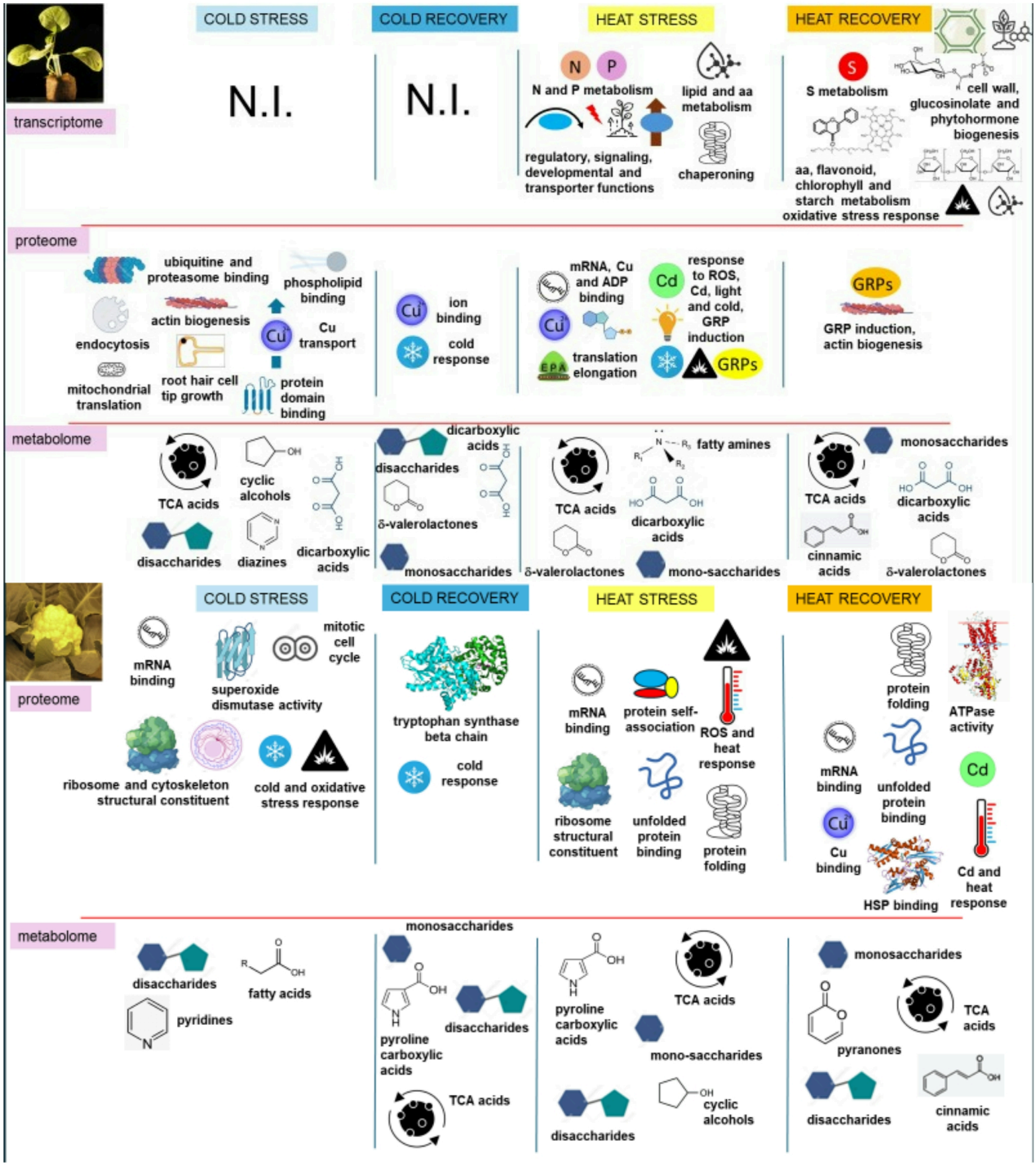


Figure 5

The general summary of cauliflower responses to various temperature stress treatments at the investigated levels. CS- cold stress, CR- cold recovery phase, HS- heat stress, HR- heat recovery phase; N.I.- not investigated (high throughput transcriptomic analyses were not conducted in CS and CR); TCA- tricarboxylic acid cycle. Only the most important responses were considered (with respect to the given

biomolecule families, their molecular functions, or biological processes). Relevant responses were depicted by small icons with short comments.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.pptx
- SupplementaryFigure3.pptx
- SupplementaryFigure2.pptx
- SupplementaryFileS2.xlsx
- SupplementaryFileS4.xlsx
- SupplementaryFileS1.xlsx
- SupplementaryTable2.xlsx
- SupplementaryFileS3.xlsx
- SupplementaryTable3.xlsx
- SupplementaryTable1.xlsx