Prolonged heat stress in *Brassica napus* during flowering negatively impacts yield and alters glucosinolate and sugar metabolism

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1. ABSTRACT

Oilseed rape (*Brassica napus*), one of the most important sources of vegetable oil worldwide, is adversely impacted by heat wave-induced temperature stress especially during its yield determining reproductive stages. However, the underlying molecular and biochemical mechanisms are still poorly understood.

In this study, we investigated the physiological, transcriptomic and metabolomic responses to heat stress in *B. napus* plants exposed to a gradual increase of temperature reaching 30 °C in the day and 24 °C at night for a period of 6 days. High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) were used to quantify the content of carbohydrates and glucosinolates respectively. Results showed that heat stress reduced yield and altered oil composition. Heat stress also increased the content of carbohydrate (Glucose, Fructose and Sucrose) and aliphatic glucosinolates (Gluconapin, Progoitrin) in the leaves but decreased the content of the indolic glucosinolate (Glucobrassicin).

RNA-Seq analysis of flower buds showed a total of 987, 1590, 2858, 2922 and 596 genes were differentially expressed at timepoints 0, 1, 2, 6 and 12 respectively. Heat treatment resulted in down regulation of genes involved in respiratory metabolism namely Glycolysis, Pentose Phosphate Pathway, Citrate Cycle and Oxidative Phosphorylation especially after 48 hrs of heat stress. Other down regulated genes mapped to sugar transporters, nitrogen transport and storage, cell wall modification and methylation. In contrast, up regulated genes mapped to small heat shock proteins (sHSP20) and other heat shock factors that play important roles in thermotolerance. Furthermore, two genes were chosen from the pathways involved in the heat stress response to further examine their expression using real time RT-qPCR.

Taken together, the results of this study demonstrated that the changes in carbohydrates and glucosinolates metabolism and transport under heat stress is one of the response mechanisms employed by the plant under heat stress conditions. Moreover, the transcriptomic data will be useful for further understanding the molecular mechanisms of *B. napus* plant tolerance to heat stress and provide a basis for breeding heat-tolerant varieties.

Keywords: *Brassica napus*, oilseed rape, heat stress, RNA-Seq, HPLC, LC-MS, simple sugar, glucosinolates, yield, quality

2. INTRODUCTION

Over the last decade, climate change has led to more extreme climatic events, impacting on crop productivity and threatening global food security (Ismaili et al., 2015; Lamaoui et al., 2018). These extreme events include periods of high temperature stress in the form of heatwaves (Goel et al., 2023), broadly defined as periods of excessively high temperature as compared to the local climate (Dikšaitytė et al., 2020). Previous studies have shown that heat stress can be detrimental during reproduction development due to the damage caused to plant organs and cellular structures (Hedhly 2011; Hinojosa et al., 2019, Goel et al., 2023). During reproduction, high temperature can induce irreversible structural and physiological changes in both male and female floral organs, leading to premature senescence (Lohani et al., 2020; Jagadish et al., 2021). High temperature stress can also decrease chlorophyll synthesis and disrupt photosynthesis and respiration, further reducing the yield potential of crop plants (Hasanuzzaman et al., 2014; Zandalinas et al., 2016). Thus, understanding the impact of heatwaves on crops and particularly during reproductive stages is essential to the development of varieties that can withstand these periods of elevated temperatures.

Traditionally, most heat stress experiments involve a heat shock, where plants are subjected to a high temperature within a very short time (10–15 °C above their optimal temperature, from several minutes to a few hours (Wahid et al, 2007). As a result, plant responses to heat shock have been well studied (Huang et al., 2019; Wang et al., 2020), but relatively little information exists on the responses to heatwaves, especially at the transcriptomic and metabolomic levels (Jin et al., 2011; Glaubitz et al., 2017).

In plants, heat shock reduces photosynthesis and respiratory metabolism, and increases antioxidant activity (Huang et al., 2019; Wang et al., 2020). Heat shock also negatively affects plant growth and productivity (Ismaili et al., 2015). While these experiments revealed the regulatory mechanisms in response to sudden heat stress (Seth et al, 2021; Ikram et al., 2022; Li et al., 2022), they do not fully represent the impact of temperature changes in the field during heatwave conditions. As a result, several studies employed prolonged warming experiments to study heat stress (Jin et al., 2011; Way & Yamori 2014; Glaubitz et al., 2017). In Arabidopsis, studies showed that plants exhibit different response patterns to prolonged warming (7 days) as compared to heat shocks (Jin et al., 2011; Wang et al., 2020). While prolonged warming led to a reduction in stomatal conductance, heat shock increased transpiration. Under both heat shock and prolonged heat stress there was an induction of antioxidant enzymes in Arabidopsis, however,

these were significantly higher under the heat shock treatment compared to the prolonged heat treatment (Wang et al., 2020). Arabidopsis plants grown at 28/23°C (elevated by 5°C from ambient temperature) suffered from significant reduction in life span, total biomass, total weight of seeds, and leaf concentrations of starch, chlorophyll, and proline (Jin et al., 2011). In rice, under prolonged elevated night temperature, transcriptomic analysis showed changes in cell wall, ABA signalling and secondary metabolism related genes, while metabolite profiles revealed a highly activated Citrate Cycle and enzyme activities (Glaubitz et al., 2017).

Oilseed rape (*Brassica napus* L.) is the second-largest source of oilseed after soybean and the third-largest source of vegetable oil worldwide (Lee et al., 2020). Cultivation and breeding practices have resulted in numerous genetically diverse lines with strong agronomic and adaptation traits (Sun et al., 2017). As a result, *B. napus* is widely cultivated around the world for food, biofuel and animal feed (Namazkar et al., 2016). Like other major temperate field crops, *B. napus* can be extremely sensitive to high temperature stress, especially if it occurs during flowering or seed development, threatening yields and quality (Angadi et al., 2000; Koscielny et al., 2018; Kourani et al., 2022). Studies in different Brassica species have found negative relationships between heat stress and seed yield and quality (Yu et al., 2014). For example, under elevated temperature scenarios, four *B. napus* cultivars experienced a significant reduction in seed biomass, resulting in a 58% decrease in the oil yield, and 77% decrease in the essential fatty acid C18:3-ω3 (Namazkar et al., 2016). Heat stress also reduced oil seed content and impaired carbohydrate incorporation into triacylglycerols in *B. napus* (Huang et al., 2019). Additionally, impairment of chlorophyll biosynthesis and disruption of the biochemical reactions of photosystems were exhibited in 10-day-old *B. napus* seedlings subjected to 38 °C (Hasanuzzaman et al., 2014). This was manifested through a significant reduction in chlorophyll and leaf relative water content as well as through an inefficient antioxidant defence system (Hasanuzzaman et al., 2014). Despite current advances in studying the effect of heat on different Brassica species, the impact of heatwaves on *B. napus,* especially during its yield determining reproductive stages, is still poorly understood (Kourani et al., 2022).

To address this, we investigated the physiological, transcriptomic and metabolomic responses to heat stress in *B. napus* under simulated field conditions. To achieve this, a heat treatment experiment simulating heatwave episode during the flowering stage of *B. napus* cv. Westar was conducted in a controlled environment. Global transcriptome profiling using RNA-Seq was employed to identify differentially expressed genes in flower buds of heat stressed plants (30

°C/24 °C day/night for a period of 6 days). This was complemented with HPLC and LC-MS analyses to detect changes in sugar and glucosinolates (GLS) concentrations in leaves in response to the heat treatment. Therefore, this study aims to provide insights into the impact of a future warmer climate on the important oil crop species *B. napus* during its reproductive stages.

3. MATERIALS AND METHODS

3.1 Plant materials and physiological parameters

Seeds of spring oilseed rape (*Brassica napus* L., cv. Westar) were surface-sterilized and sown into seed trays filled with a seed potting mix (Clover Peat, Dungannon, Northern Ireland). Trays were then placed in polyethylene tunnels for four weeks at the Crops and Environment Laboratory, University of Reading, to germinate. Twenty-eight days after sowing (DAS), the plants were transplanted into 3L pots containing a peat based potting mix (Clover Peat).

At green bud stage (38 DAS), the plants were moved to two controlled environment chambers (Fitotron, Weiss Technik (UK) Ltd), for a period of one week to adjust to the new environment before the start of the heat experiment. Each cabinet contained 16 plants and was maintained under control conditions of 20 °C/14 °C day/night and a photoperiod of 16/8-hr light/dark.

3.2 Heat wave experimental design

Since the flowering stage of winter sown *B. napus* occurs during May in the UK, an analysis of daily weather temperatures during this developmentally crucial period was carried out using the UK Meteorological (Met) Office weather data obtained from the Met office (Met office n.d.) and from WeatherOnline (WeatherOnline n.d.) platforms. The data showed an increase in the frequency of high temperature fluctuations during May, ranging between 26 °C and 28 °C over several days (Supplementary Fig. S1). Given the continuous rise in global temperature, it is expected that future heatwaves would increase in severity and duration (Seneviratne et al., 2012). Based on these data, the current study temperature simulates a heat stress event through a temperature increase to 30 °C/24 °C day/night for a period of 6 days and 20 °C/14 °C day/night for control. Forty-five DAS, the temperature in one cabinet was kept under control conditions (20 °C/14 °C) and denoted as control cabinet, while the second cabinet was set to a gradual increase in temperature and denoted as heat treatment (HT) cabinet. To mimic a heatwave, the temperature in the HT cabinet was increased gradually from 20 °C to 30 °C between 9:00 h and 12:00 h in 3 steps: 20 °C at 9:00 h, 24 °C at 10:00 h, 28 °C at 11:00 h, and 30 °C at 12:00 h. The temperature was held at 30 °C until 22:00 h (Supplementary Fig. S2B), before it was dropped gradually and

maintained at 24 °C until 8:00 h of the next day (Supplementary Fig. S2B). This cycle of gradual increase (day) and decrease (night) of temperature was held for the next 5 days (Supplementary Fig. S2C). On the 6th day of treatment, the temperature was gradually decreased to 20 °C/14 °C day/night cycle (Supplementary Fig. S2D) and held for a recovery period of 7 days (Supplementary Fig. S2E). During heat treatment, the plants were frequently irrigated to avoid drought.

2.5 Sample preparation for transcriptomic analysis

Five biological replicates from each treatment condition were collected at days 0, 1, 2, 6, & 12 of the experiment. Each replicate was made up of three individual plants. Three flower buds were collected per plant (nine buds per replicate). All samples were immediately snap-frozen in liquid nitrogen before they were stored at -80°C. Bud samples were ground into fine powder using pestle and mortar, with the frequent addition of liquid nitrogen to stop enzymatic reactions. The ground material was then stored at -80°C until RNA extraction.

2.6 RNA extraction and sequencing

Total RNA was extracted from bud samples using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich) in accordance with the manufacturer's protocol and treated with genomic DNAse using DNASE 70-On Column DNase I Digestion set (Sigma-Aldrich) to eliminate DNA contamination. RNA quantification was estimated on both NanoDrop 2000 (Thermo Scientific) and Qubit 2.0 fluorometer (Invitrogen, USA), and quality was evaluated using 1% (w/v) denaturing formaldehyde agarose gel (MOPS). Samples were shipped over dry-ice to Novogene Europe, where llumina-based RNA sequencing was performed using the Illumina Novaseq™ 6,000 (PE150) platform.

2.7 Differential expression and cluster analysis

Raw sequence reads were assessed for quality using FastQC tools (v0.11.5) (Andrews, 2012). The mean sequence lengths were 150 bp and the mean sequence GC content was 43%. The mean quality scores in each base position were higher than 36 and the mean quality scores per sequence were 36. As a result, sequence trimming was not necessary.

STAR software (v2.7.10a) was used to map the clean reads to the *B. napus* cv. Westar v0 reference genome (Song et al., 2020), and the percentage of aligned reads was calculated by using the flagstat command from Samtools on each alignment file generated by STAR aligner (Supplementary Figure S3). To estimate transcript abundance, RSEM (RNA-Seq by Expectation-Maximization) software (v1.2.18) was used in three steps to prepare the reference, calculate expression and generate the count matrices. Firstly, rsem-prepare-reference script was used with '–gff3' option to extract reference sequences from the genome and '--star' option to build STAR

indices. Next, rsem-calculate-expression script was used with '--paired-end' option to align input reads against the reference transcriptome with STAR and calculated expression values using the alignments. Finally, rsem-generate-data-matrix script was used to generate the count matrices from expression results files.

For differential expression analysis, DESeq2 (v1.41.10) was used in R environment (Love et al., 2014) with default parameters. A pre-filtering step was performed to keep transcripts that have a minimum count of 10 reads in a minimum of four samples. To identify transcripts that were significantly differentially expressed by heat stress, the two treatment conditions (Heat stress versus Control) were contrasted in pairwise and time-series comparisons. Both designs were in general agreement, hereby, we present the output of the pairwise comparison at each timepoint. Thresholds of $|\log2(\text{foldchange})| \geq 0.5$ and adjusted P-value < 0.05 (using the Benjamini and Hochberg method) was considered to identify significantly differentially expressed transcripts between the two treatment conditions.

Significant DEGs in each contrast were further analysed using K-mean clustering based on the kmeans() function in R (distance: Euclidean), with log2FC as the input.

2.7 Gene Ontology and KEGG pathway enrichment analysis of DEGs

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted on the DEGs using OmicsBox (BioBam Bioinformatics, 2019). The significantly enriched GO terms and biological pathways in HT samples as compared to control at different timepoints were identified to determine heat stress-related functions and pathways. The analysis was carried out using Fischer exact statistical test with FDR adjustment cut-off <0.05. The background dataset consisted of all *B. napus* cv. Westar identifiers present in the assembly's annotation file (Song et al., 2020).

2.8 Validation of RNA-seq results by real-time quantitative PCR (RT-qPCR)

Sucrose Synthase 5 (SS5) and Heat Shock Protein 20 (HSP20) were chosen from the pathways that were involved in the heat stress response to further examine their expression in real time using RT-qPCR. Five timepoints were chosen for each gene to represent the time course of the experiment. SS5 and HSP20 were not analysed at timepoint 1 as they exhibited similar expression profile at timepoints 0 and 1, therefore one timepoint was selected (Supplementary Table S2).

Total RNA used for transcriptomic sequencing was used for cDNA synthesis. First, cDNA was synthesized from 1 µg total RNA by Invitrogen SuperScript IV VILO Master Mix kit according to the

manufacturer's instructions (ThermoFisher Scientific). The RT-qPCR reaction volume was 20 µL consisting of 3 µL cDNA, 10 µL SYBR Premix Ex Taq II (Takara, China), 1 µL (200 nM final concentration) of 4 µM of each primer (forward and reverse, Supplementary Table S3) and 5 µL RNAse-free water. Three technical and three biological replicates for each sample were measured with the following protocol: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles of 95 °C for 3 s, 60 °C for 30 s, followed by dissociation curve analysis ramping from 60°C to 95 °C with a ramp rate of 0.3 °C s^{-1} on an ABI StepOne Plus RT-qPCR platform (Applied Biosystems, USA). BnaActin was used as housekeeping gene to normalize the data. The relative expression level of all selected genes at each time point was calculated using the $2^{-\Delta\Delta CT}$ method of the StepOne software (v2.2.2).

2.9 Sample preparation for metabolomic analysis

Five biological replicates from each treatment condition were collected at days 0, 1, 2, 5, 6, 8 & 12 of the heat treatment experiment. Each replicate was made up of three youngest fully expanded leaves collected from three plants. All leaf samples were immediately snap frozen in liquid nitrogen before being stored at -80°C until further analysis. Frozen leaf samples were freeze-dried for five days, then ground into a fine powder using a Precellys 24 lysis & homogenization (Stretton Scientific, Alfreton, UK) and stored at -40 °C in sealed sample bags until extraction and analysis.

2.10 Glucosinolates extraction and analysis

GLS extraction was carried out as per the protocol of Bell et al. (2015) as follows: 40 mg of ground leaf powder was heated in a dry block at 75 °C for 2 min. This step was done as a precautionary measure to inactivate as much myrosinase enzyme as possible before extraction (Pasini et al., 2012). Afterwards, 1 mL of preheated (70 % v/v) methanol (70 °C) was added to each sample and placed in a water bath for 20 min at 70 °C. Samples were then centrifuged for 5 min (6000 rpm at 18 °C) to collect the loose material into a pellet. The supernatant was then transferred into fresh labelled Eppendorf tube and stored at -80 °C until further analysis. Before LC-MS analysis, the samples were filtered using 0.25 µm filter discs, diluted in 50% methanol at a 1:4 ratio (dilution factor =5) and spiked with a 100 µL of the internal standard Sinigrin (100 ng mL^{-1}). The GLS content were analysed by SCIEX QTRAP 6500+ LC-MS.

For LC separation, a Waters Acquity BEH C18 column (particle size 1.7µ, 2.1 x 50 mm) with a Security VanGuard system from Waters (UK) was used. The mobile phase consisted of water with 0.1 % formic acid (A) and methanol with 0.1 % formic acid (B). The gradient started at 5 % B and was raised to 90 % B in 3.5 min, held at 90 % B for 0.5 min and re-equilibrated at 5 % B for 1 min.

The total time of the gradient program was 5 min. The flow rate was 0.4 mL min^{-1} with a column temperature of 60 °C. A 1 µL aliquot of sample was injected for analysis.

The LC system was interfaced with a SCIEX QTRAP 6500+ mass spectrometer equipped with an IonDrive Turbo V ion source. Multiquant software was used to control sample acquisition and data analysis. The QTRAP 6500+ mass spectrometer was tuned and calibrated according to the manufacturer's recommendations. For quantification, an external standard calibration curve was prepared using a series of standard samples containing the following GLS: Gluconapin (GNA), Progoitrin (PRO) & Glucobrassicin (GBS), with concentrations: 1000 ng mL^{-1}, 500 ng mL^{-1}, 250 ng mL^{-1}, 100 ng mL^{-1}, 50 ng mL^{-1}, 10 ng mL^{-1}, and 1 ng mL^{-1}. Two-way mixed ANOVA in R was performed at 0.05 significance level to calculate the significance variation in concentration under heat treatment as compared to control.

2.11 Sugar content extraction and analysis

The extraction of soluble sugar (Glucose, Fructose, and Sucrose) was performed as follows: 50 mg of ground leaf powder was extracted with 1 mL of 62.5 % methanol: 37.5% HPLC-grade water (v/v) at 55 °C over a period of 15 min in a shaking water bath and vortexed every 5 min. Afterwards, the samples were allowed to cool for 2 min and then centrifuged at 13000 rpm for 10 min and filtered using 0.25 µm filter discs. The supernatant was then transferred to a clean labelled Eppendorf tube and stored at -80 °C until further analysis. Before HPLC analysis, the samples were diluted in HPLC-grade water in a 1:2 ratio (dilution factor = 3). Chromatographic separation of sugar content was performed using HPLC (Agilent 1260, Infinity Series) equipped with an Evaporative Light Scattering Detector (ELSD) system and an Asahipak NH2P-50 4E column (250 × 4.6 mm; Shodex, Tokyo, Japan) in an isocratic elution mode. The mobile phase consisted of 75 % acetonitrile: 25 % water at a flow rate of 1 mL min^{-1}, a column temperature of 40 °C and an injection volume of 20 µL. The analysis had a total run time of 25 min. For quantification, an external standard calibration curve was prepared using a series of standard samples containing Glucose, Fructose and Sucrose with concentrations: 0.025 mg mL^{-1}, 0.05 mg mL^{-1}, 0.1 mg mL^{-1}, 0.5 mg mL^{-1}, 1 mg mL^{-1}.

2.4 Near-Infra Red Spectroscopy (NIRS) scanning

Following the treatments, the plants were allowed to complete their lifecycle and total aboveground biomass and seed yields were collected from individual plants. Seed samples were scanned with DA 7250 NIR analyzer (PerkinElmer, Beaconsfield, UK). The seed samples were scanned for moisture, oil and protein content as well as for fatty acid composition of the oil.

2.12 Statistical analysis

Two-way mixed ANOVA was performed at 0.05 significance level to calculate the statistically significant differences between the means of sugar and glucosinolate concentrations under heat treatment as compared to control. In addition, NIRS data was analysed for the investigated components using a t-test. All statistical analysis was performed using R.

RESULTS

3.1 Prolonged heat treatment reduced yield and altered *B. napus* oil composition

Heat treatment significantly altered plant growth and appearance, with visible physiological changes on the flowers and buds (Fig. 1A-G). Five days after heat treatment started, flowers displayed smaller and paler petals compared to plants growing under control conditions (Fig.1 A-D). On day 8, three days after the end of heat treatment, many buds suffered from abscission, appeared brown and shrunken in size on plants subjected to the heat treatment (Fig.1 E-G). At maturity, heat-treated plants exhibited less total biomass and seed weight relative to control plants (Supplementary Fig. S4). In terms of oil quality, heat treatment significantly decreased Linoleic Acid, Linolenic Acid and Palmitic Acid concentrations, but increased Oleic Acid and Stearic Acid concentrations in seeds compared to control plants (Supplementary Fig. S5).

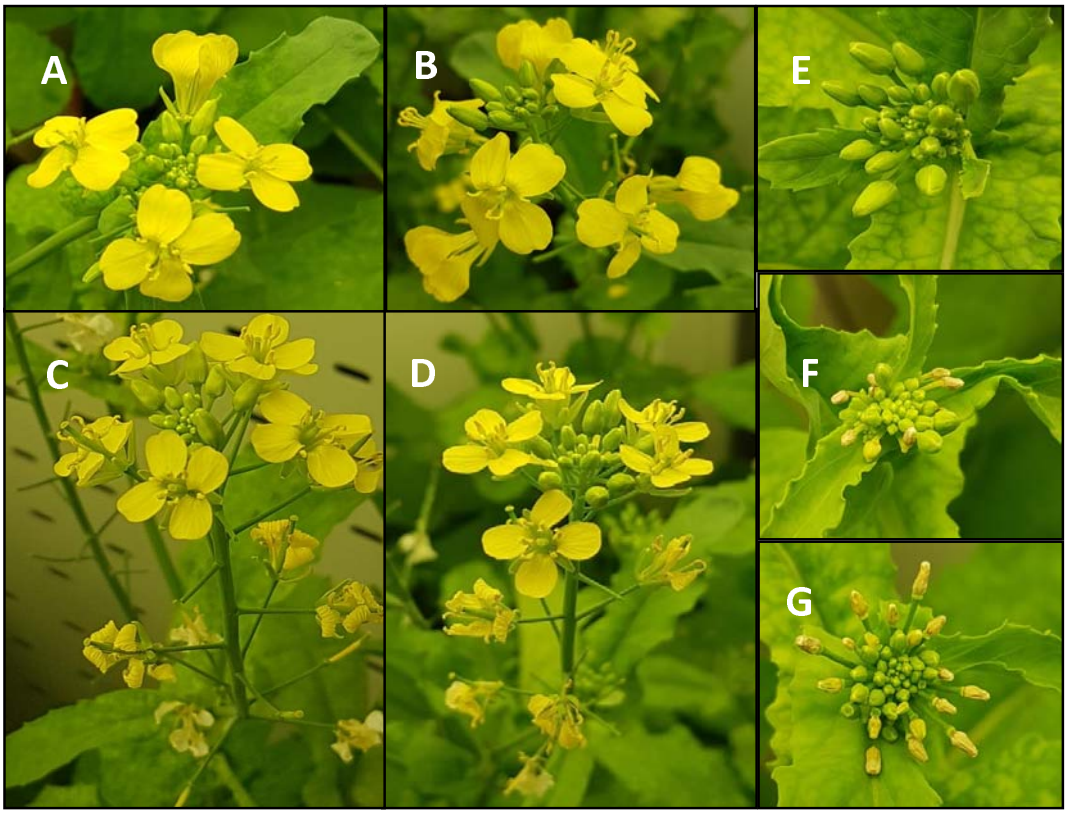

Figure 1. *B. napus* flowers and buds at days 5 & 8 of the heat treatment experiment respectively. (Control: A, B & E; Heat stressed: C, D, F & G).

3.3 Differential gene expression identifies distinct patterns of expression in *B. napus* under heat stress and recovery

Libraries were constructed and sequenced on the Illumina NovaSeq platform. An average of 47 million reads were generated per sample after low quality reads had filtered out (Supplementary Table S1). Following this cleaning step, around 90%- 95.49% total reads per sample were mapped to the *B. napus* cv. Westar v0 reference genome (Supplementary Fig. S3) (Song et al., 2020).

To understand the effect of heat stress on gene expression in *B. napus*, differentially expressed genes (DEGs) were identified in a pairwise comparison between heat treatment and control conditions at five timepoints during and after heat treatment. A total of 987, 1590, 2858, 2922 and 596 genes were differentially expressed at days 0, 1, 2, 6 and 12 respectively (Supplementary Fig. S6). Analysis of the top differentially expressed gene revealed that nine transcripts with logFC ranging between 2-10.6 mapped to small heat shock proteins (sHSP20), in addition to transcripts mapped to other heat shock factors such as DnaJ Homolog Subfamily B member 4, Elongation Factor 1-beta 1-like and Chaperone Protein ClpB1 (Supplementary Table S7). On the other hand, the most down regulated genes featured transcripts mapped to sugar transporters, nitrogen transport and storage, cell wall modification and methylation (Supplementary Table S8).

To identify trends and clusters in the expression of genes across the time course of the experiment, K-means clustering was applied to 6106 DEG with logFC ≥ 0.5. The algorithm randomly assigned each gene into one of the clusters based on the Euclidean distance between the gene and the cluster mean. Six distinctive clusters based on expression changes across the five timepoints were identified (Fig. 2). In cluster 1 (87 genes), the genes had moderately higher expression levels than in cluster 2 (1434 genes), but both show increased expression during the heat treatment (days 0-5), with expression gradually declining after the heat treatment, reaching normal levels at timepoint 12. In clusters 3 & 4, (1807 & 437 genes) genes were down regulated along the course of the experiment, but more pronounced during the heat treatment phase, with cluster 4 exhibiting a greater decease in expression than cluster 3. Interestingly, clusters 5 & 6 showed opposite expression patterns, but both in response to the end of the heat treatment and the start of the recovery phase. In cluster 5 (1089 genes), the expression profile of genes increased at timepoint 6, before returning to normal levels at timepoint 12, while in cluster 6 (1218 genes), a sharp decrease in expression was observed at time point 6, returning to normal levels at timepoint 12 (Fig. 2).

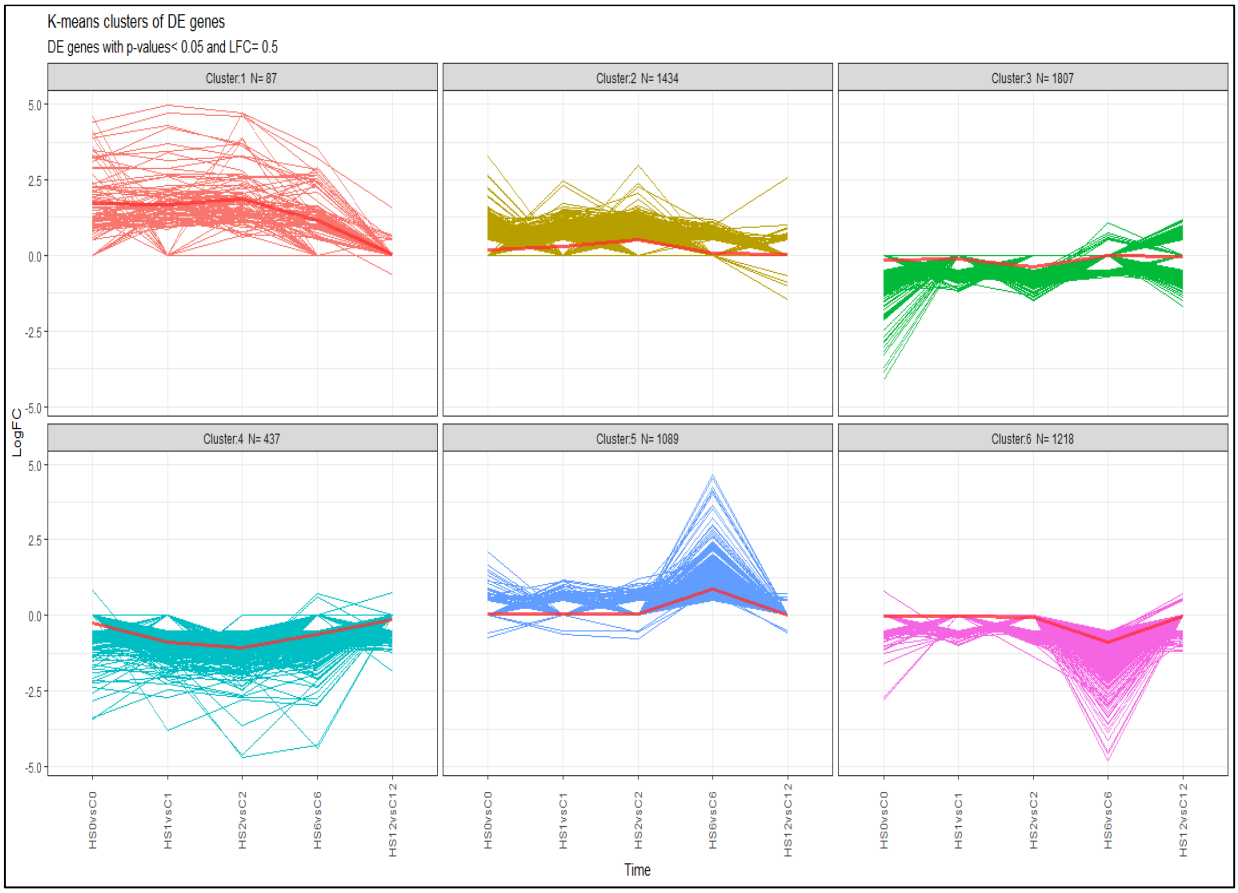

Figure 2. K-means cluster analysis of differential expressed genes in B. napus during (0-3) and after (6-12) heat treatment. K-means clustering was applied to 6106 DEG with logFC ≥ 0.5 using the "kmeans" function in R package "stats" (v. 3.2.2), where k represents a pre-specified number of clusters. Six groups of genes were classified, with N the number of genes in each cluster, and the solid line representing the average expression profile of all genes in each cluster.

Validation of RNA-seq data by quantitative real-time PCR

To verify the reliability of RNA sequencing results, two genes with diverse expression profiles, including up regulated or down regulated at different timepoints of the experiment, were selected for real-time qPCR to measure expression levels. As a result, HSP20 (Heat Shock Protein 20) and SS5 (Sucrose Synthase 5), exhibited similar expression profiles between RT-qPCR and RNA-seq data (Fig. 3 & Supplementary Table S4), with correlation coefficients of r=0.86, and r=0.68 respectively.

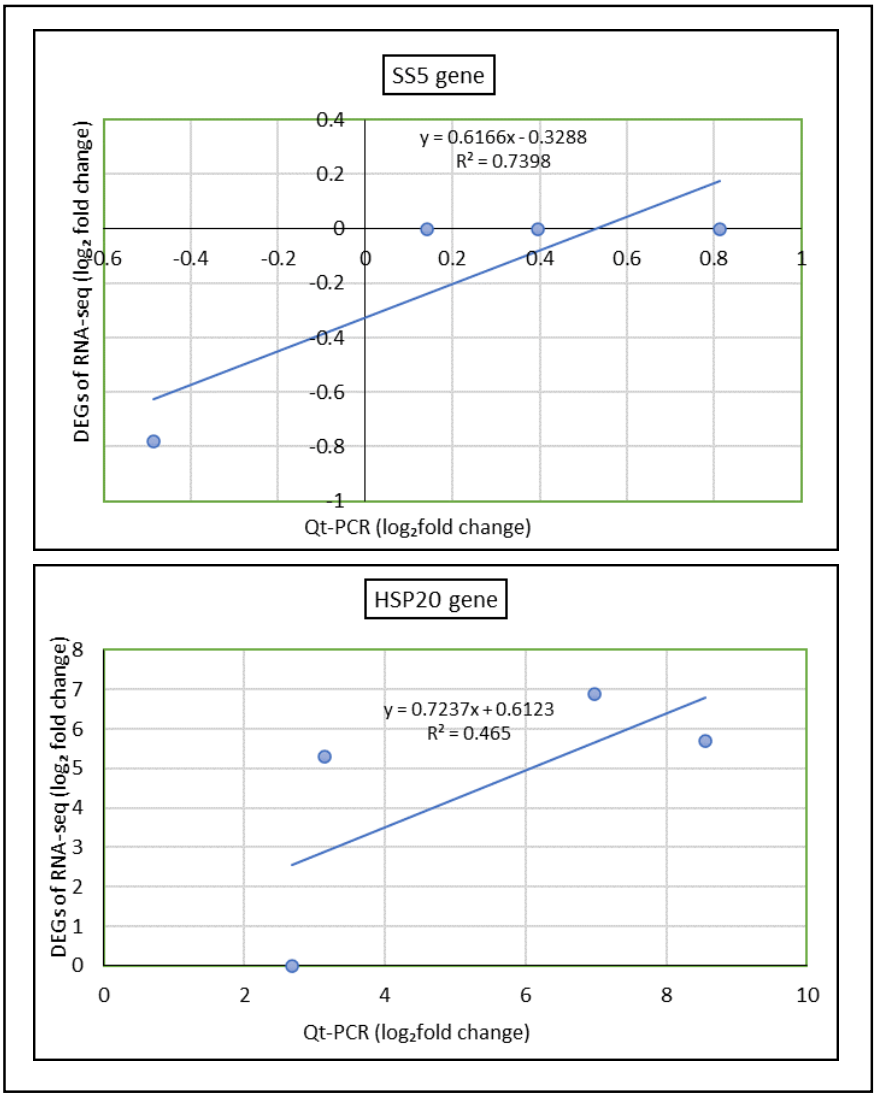

Figure 3. Correlation analysis of the results obtained from RNA-seq and qt-PCR: HSP20 and SS5 exhibited similar expression profiles between RT-qPCR and RNA-seq data with correlation coefficients of r=0.86, and r=0.68 respectively.

3.6 Functional Annotation of DEGs identifies key pathways responding to heat treatment and recovery

Gene ontology (GO) enrichment analysis of the DEGs was performed to identify enriched GO terms. In the category of molecular function, “binding”, “catalytic activity”, “transporter activity”, “transcription regulator activity”, “antioxidant activity” were among the predominant enriched terms at all timepoints with more transcripts mapped to these terms during heat treatment than during recovery (Supplementary Fig. S7). Oxidoreductase, Hydrolase and Transferase were among the top identified enzymes (Supplementary Fig. S8). These results suggest that high temperature

activates several metabolic processes through the expression of numerous enzymes involved in alleviating the impact of heat stress not only during the stress period but also during recovery.

Based on KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis (Kanehisa & Goto, 2000), pathways in the respiratory metabolism namely Glycolysis and Pentose Phosphate Pathway were enriched in all heat treatment comparisons at all timepoints, while Citrate Cycle and Oxidative Phosphorylation were enriched at timepoints 2 and 6 respectively (Supplementary Fig. S9). Other related pathways were also enriched along the course of the treatment and during recovery period, and these include one Carbon Pool by Folate (enriched at timepoints 1, 2 and 6), Carbon Fixation in Photosynthetic Organisms, Cysteine and Methionine metabolism (enriched at timepoints 1 & 2), Glycerolipid and Tryptophan metabolism (enriched at timepoint 2), Fatty Acid Biosynthesis and Pyruvate metabolism (enriched at timepoint 6) and Fructose and Mannose metabolism (enriched at timepoint 12) (Supplementary Fig. S9). In the present study, most of the genes involved in these pathways were responsive to high temperature stress, with their expression declining with the onset of heat treatment, especially after 48 hours (Supplementary Figs. S10-S17).

Heat treatment down regulated the transcript levels of most aliphatic and indolic GLSs synthetic genes

Analysis of the aliphatic GLS synthetic and regulatory genes showed that heat treatment had a significant effect on the expression of 15 out of 28 genes at timepoint 2 (Fig. 3). Eleven genes encoding different GLS synthetic genes including different UDP Glycosyltransferases and Glutathione S-transferases were down regulated while four genes encoding GLS related transcription factors were up regulated. At timepoint 6 which marks 24 hours of recovery, nine genes that were not differentially expressed under heat treatment were found up regulated. These genes include different Cytochrome P450 genes, transcription factors, Branched-Chain Aminotransferase 4.2 and Isopropylmalate Dehydrogenase 2 (Fig. 3).

Analysis of the indolic GLS synthetic and regulatory genes showed that heat treatment altered the expression of 15 and 25 out of 36 genes at timepoints 1 and 2 respectively (Fig. 4). These genes include Sulfotransferases, Cytochrome P450 genes, UDP Glycosyltransferases, Glutathione S-transferases and transcription factors. After one week recovery (at timepoint 12), most of the differentially expressed genes identified during heat treatment were no longer differentially expressed. This is similar to what was seen with the aliphatic GLS encoding genes. Interestingly,

one transcript encoding UDP Glycosyltransferase 74E2-like remained down regulated after one week of recovery.

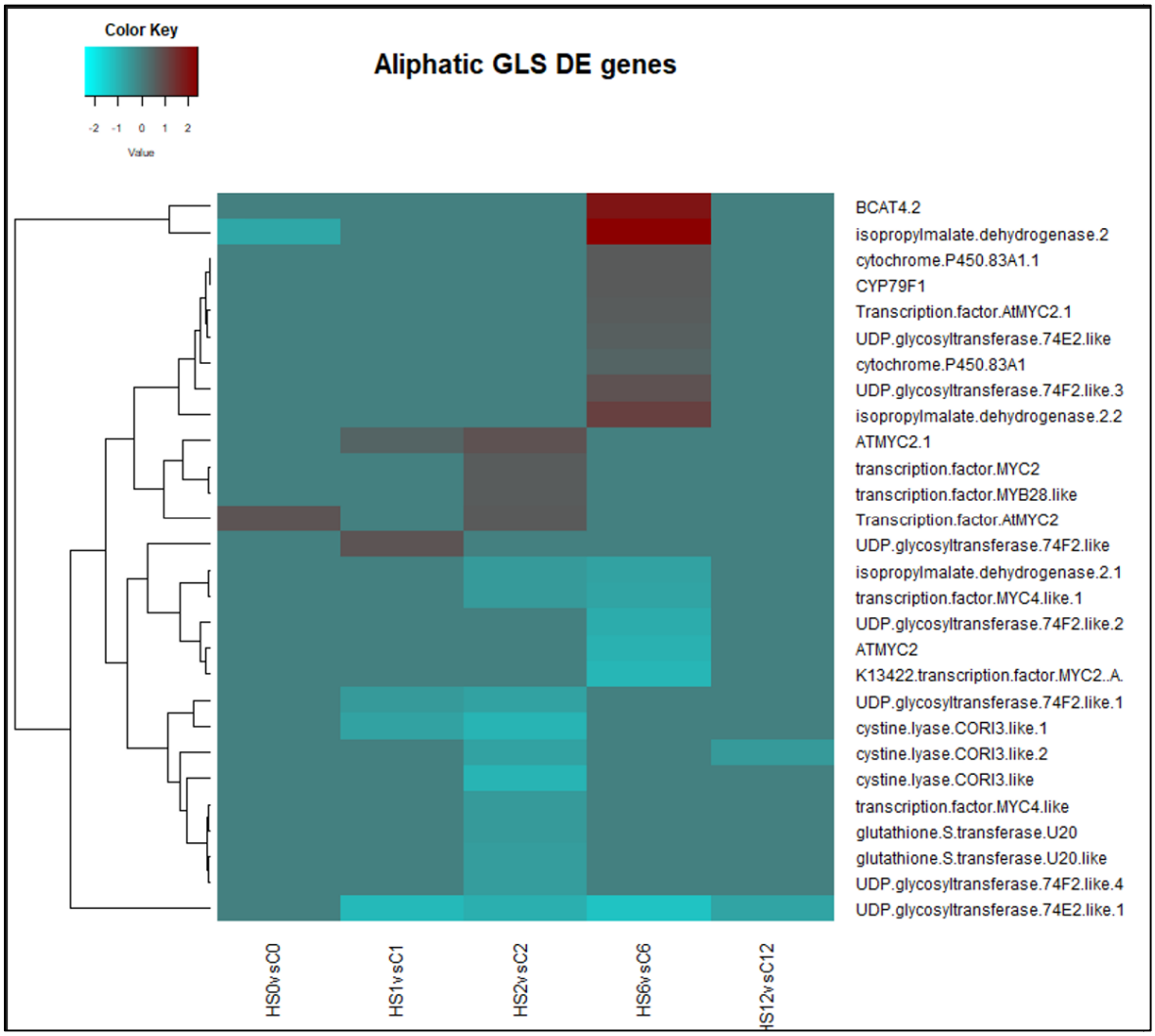

Figure 3. Heatmap showing differential expression of genes involved in the regulation or biosynthesis of aliphatic glucosinolates (GLS) in *Brassica napus*. Eleven genes encoding different GLS synthetic genes including different UDP Glycosyltransferases and Glutathione S-transferases were down regulated while four genes encoding GLS related transcription factors were up regulated.

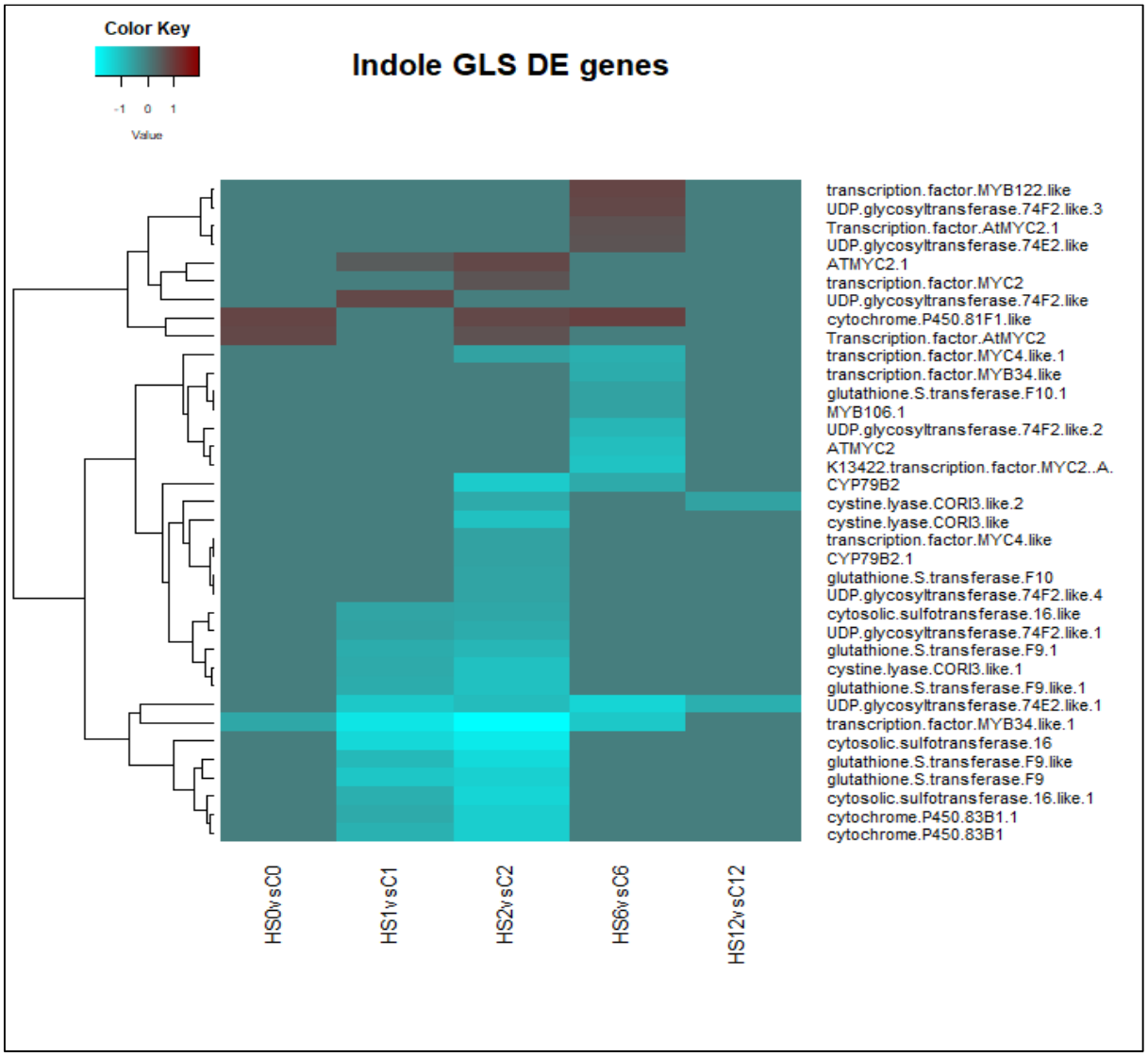

Figure 4. Heatmap showing differential expression of genes involved in the regulation or biosynthesis of indolic glucosinolates (GLS) in *Brassica napus*. Fifteen and twenty-five out of thirty-six genes encoding different Sulfotransferases, Cytochrome P450 genes, UDP Glycosyltransferases, Glutathione S-transferases and transcription factors were down regulated at timepoints 1 and 2 respectively.

3.9.3 Heat treatment down regulated the transcript levels of most Sulphur assimilation and transport genes

Analysis of the genes encoding Sulphur assimilation and transport showed that 18 out of 21 transcripts were down regulated in response to heat at either timepoints 1 or 2 or both (Fig. 5). Two transcripts encoding Sulphate transporter and Adenylyl Sulphate Kinase were up regulated at timepoints 1 & 2, respectively. Interestingly, only four transcripts remained down regulated at timepoint 6, and one transcript encoding Adenylyl Sulphate Kinase was up regulated at timepoint 12.

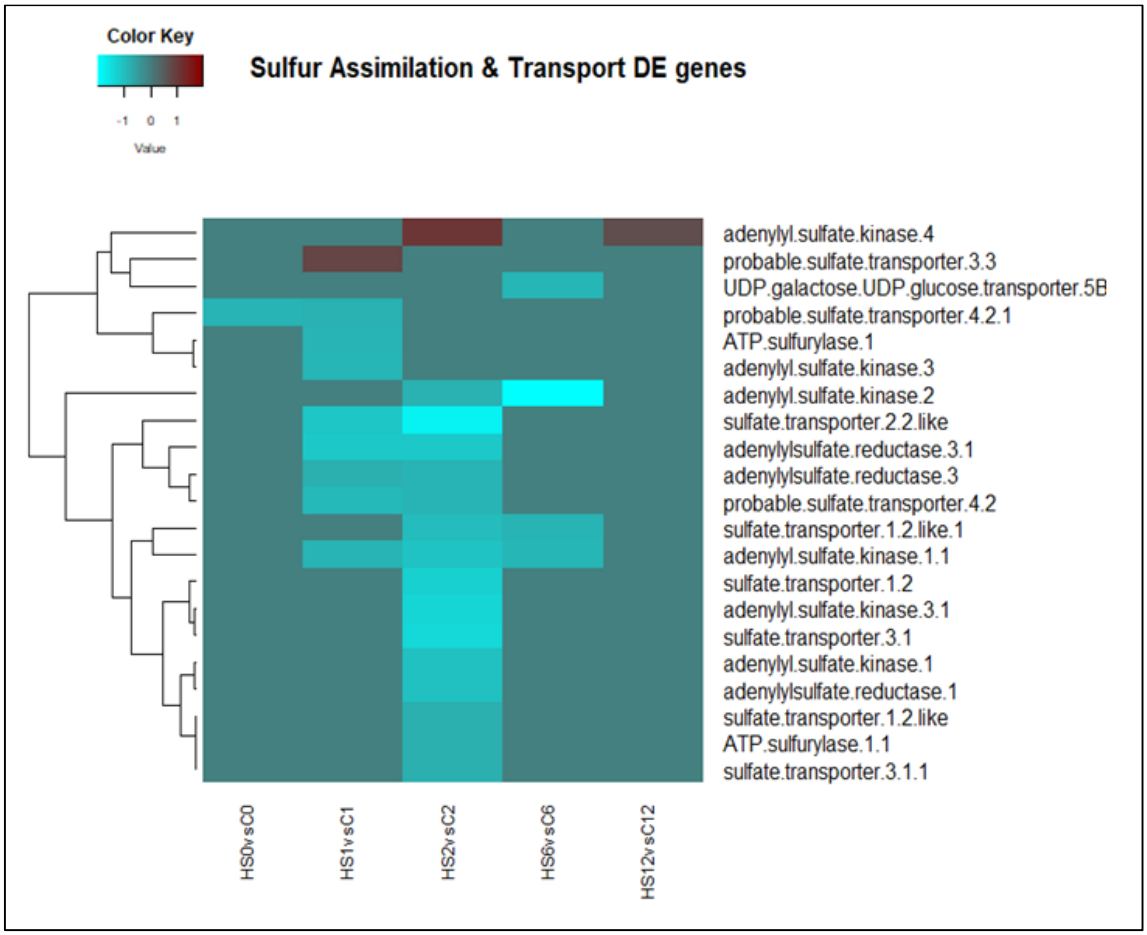

Figure 5. Heatmap showing differential expression of genes involved in the assimilation and transport of Sulphur in *Brassica napus*. Eighteen out of twenty-one transcripts were down regulated in response to heat at either timepoints 1 or 2 or both.

Metabolic analysis revealed altered leaf concentrations of aliphatic and indolic GLS in response to heat treatment

Given the changes observed in the abundance of transcripts associated with GLS metabolism and Sulphur assimilation and transport, the concentrations of two aliphatic GLS Progoitrin (PRO) and Gluconapin (GNA), and one indole GLS Glucobrassicin (GBS) were quantified using LC-MS. Their role in response to different abiotic stress factors has been previously reported (Ljubej et al, 2021; Jasper et al, 2020). Two-way mixed ANOVA pairwise comparisons showed a gradual increase in both PRO and GNA concentrations in response to heat (Fig. 6 A&B). This increase was significant after 48 hours of treatment. After 5 days, the concentration of GLS started to decline but remained higher in heat treated plants than in the leaves of control plants. In contrast, the concentration GBS decreased in response to heat treatment (Fig. 6C). During recovery, the concentration of GBS continued to decrease with the difference being significant after 24 and 72 hours of the end of the

treatment. At 7 days recovery, GBS concentration in heat treated plants remained lower than that of the control.

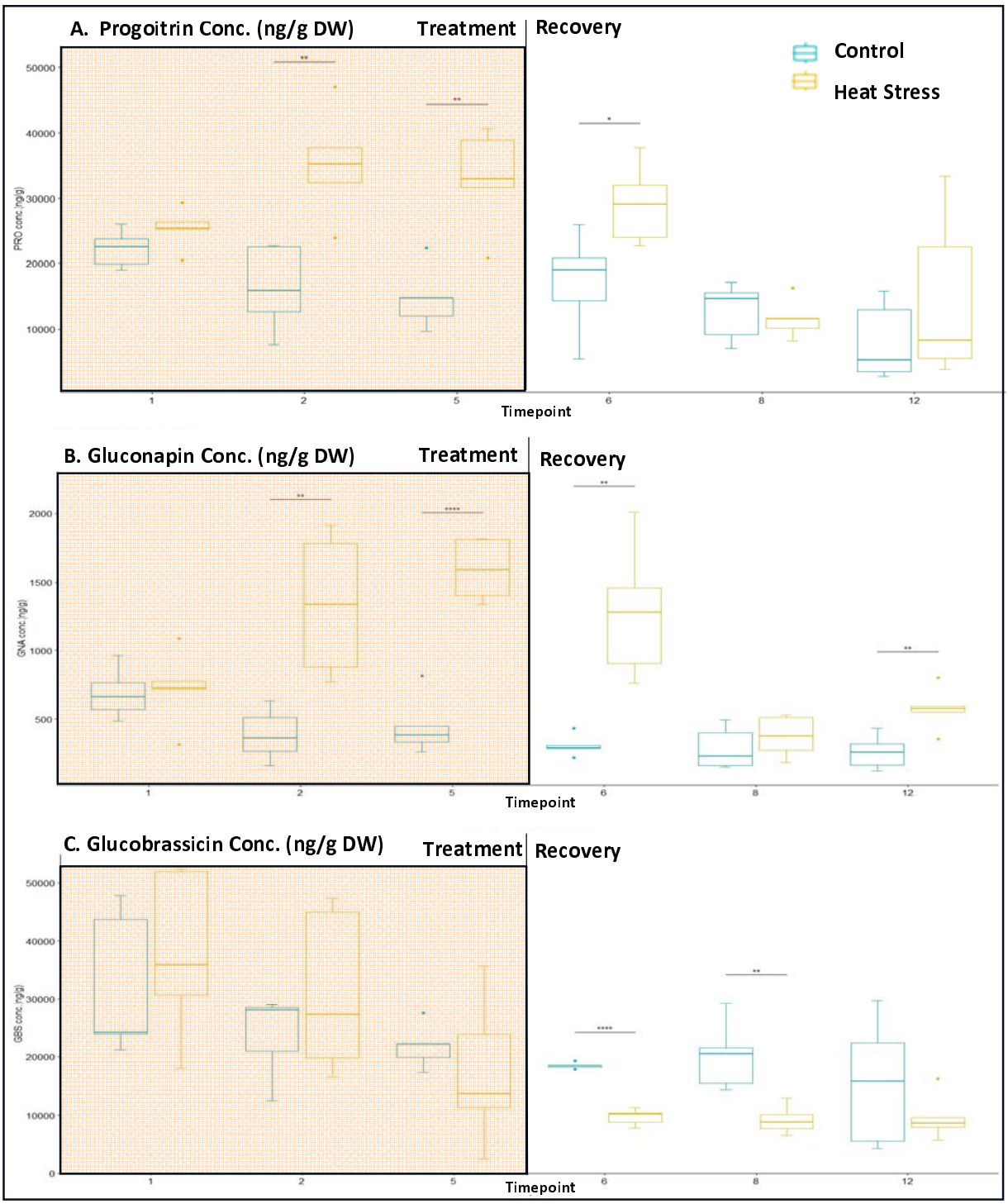

Figure 6. Leaf GLS (PRO, GNA & GBS) concentrations in ng/g of dry weight (DW) in *Brassica napus* plants subjected to heat treatment (30 °C/24 °C day/night) at timepoints 1-5 (yellow boxes) or maintained under control temperatures (20 °C/14 °C day/night) at all timepoints (blue boxes). GLS concentrations were quantified using LC-MS. Data were analysed using two-way mixed ANOVA pairwise comparisons. Each box plot represents data from 5 replicates, showing the median (horizontal bar), 25th and 75th percentiles (the box) and the min and max values (whiskers), with outliers as dots.

Significant differences between heat treated and control plants at each time point are denoted by (*) for p-value ≤ 0.05 or (**) for p-value ≤ 0.01 or (****) for p-value ≤ 0.0001. Heat treatment significantly increased leaf concentration of aliphatic GLS Progoitrin and Gluconapin (Figs. 6A & B) and decreased leaf concentration of the indolic GLS Glucobrassicin (Fig. 6C).

Heat treatment down regulated the transcript levels of most sugar transporter genes

To investigate the effect of heat stress on sugar transport and metabolism, analyses of genes encoding the SWEET and ERD6-like sugar transporters were undertaken. Compared to the control plants, heat stress significantly altered the expression of 18 SWEET genes with logFC ranged between -5 and 1.5 (Fig. 7). These genes belonged to the phylogenetic clades II and III. Members of these clades predominantly transport Hexose and Sucrose respectively. Eight of these transcripts were down regulated after 48 hrs (at timepoint 2) of heat treatment, while two were up regulated. Interestingly, out of the 11 genes that were DE after 24 hrs of recovery (at timepoint 6), five up regulated genes were not DE during heat stress (were not affected by heat treatment) and became up regulated after the removal of stress (Fig. 7).

Analysis of genes encoding the tonoplastic Glucose symporters showed that nine transcripts mapped to different ERD6-like genes (Fig. 8). After 48 hours of heat stress, four were down regulated and two were up regulated.

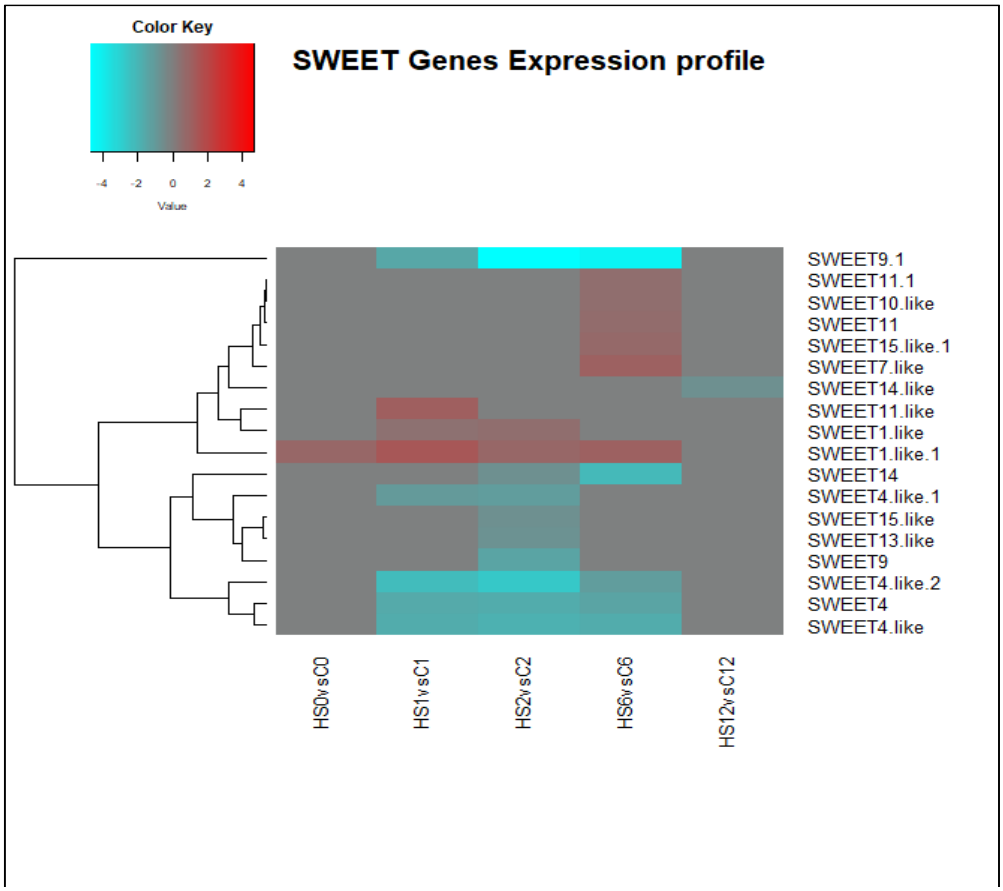

Figure 7. Heatmap showing differential expression of SWEET genes involved in sugar transport in *Brassica napus*. Heat stress significantly altered the expression of 18 SWEET genes with logFC ranged between -5 and 1.5.

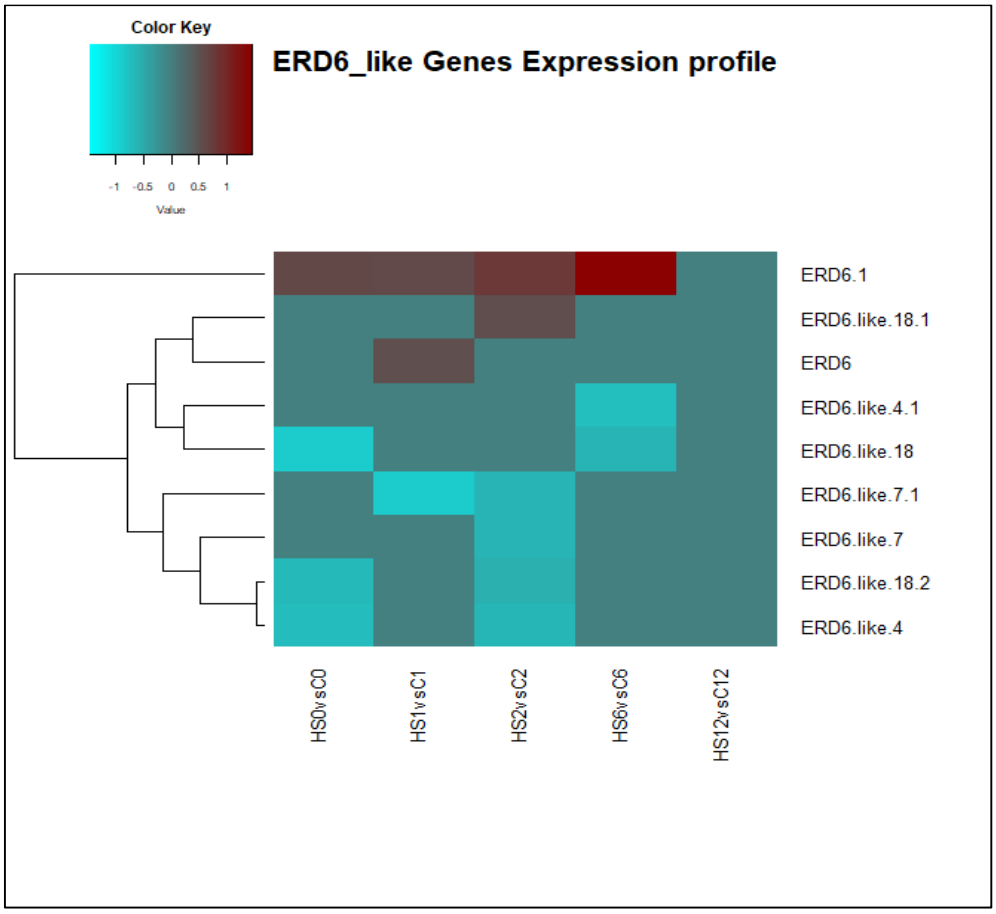

Figure 8. Heatmap showing differential expression of ERD-6 like genes involved in sugar transport in *Brassica napus*. After 48 hours of heat stress, four transcripts were down regulated and two transcripts were up regulated.

In addition, one transcript mapping to a Facilitated Glucose transporter member 8-like showed a LogFC of -9.2 at timepoint 1, and transcripts encoding Sucrose transporters (SUTs) were found up regulated at timepoint 1 & 6 (Supplementary Fig. S18). In addition to their significant role in phloem loading and unloading, these transporters are also involved in sugar influx into the cytosol. Furthermore, the present results showed that the expression of transcripts mapping to the Sucrose synthesizing enzyme, Sucrose Phosphate Synthase (SPS) increased during heat treatment (Supplementary Table S5), while the expression of Sucrose catalysing enzymes, namely Sucrose Synthase and Cell Wall Invertase decreased significantly (Supplementary Table S6).

Metabolic analysis revealed an increase in leaf concentrations of sugar in response to heat treatment.

To investigate the effect of heat stress on the sugar concentrations in the leaves of *B. napus*, the concentrations of Fructose, Glucose and Sucrose were quantified using High Performance Liquid Chromatography. Under control growth conditions, leaf Fructose, Glucose and Sucrose concentrations increased slowly as the plants grew (Fig. 9 A, B &C). In contrast, in the heat-treated

plants, the leaf concentrations of individual sugar increased. Two-way mixed ANOVA pairwise comparisons showed that the change in sugar concentrations was significant at timepoints 2 and 5 for Glucose, and at timepoints 1, 2 and 5 for Fructose and at timepoint 5 for Sucrose.

After the removal of heat stress, the leaf concentration of Sucrose in the treated plants decreased gradually until it reached a lower concentration than that of the control plants 7 days after the heat treatment stopped. In contrast, Fructose and Glucose concentrations showed a slight decrease initially before they increased again significantly 3 days after the end of the heat treatment (at timepoint 8). After 7 days of recovery, the content of Fructose, Glucose and Sucrose dropped substantially, reaching lower concentrations than that of the control plants.

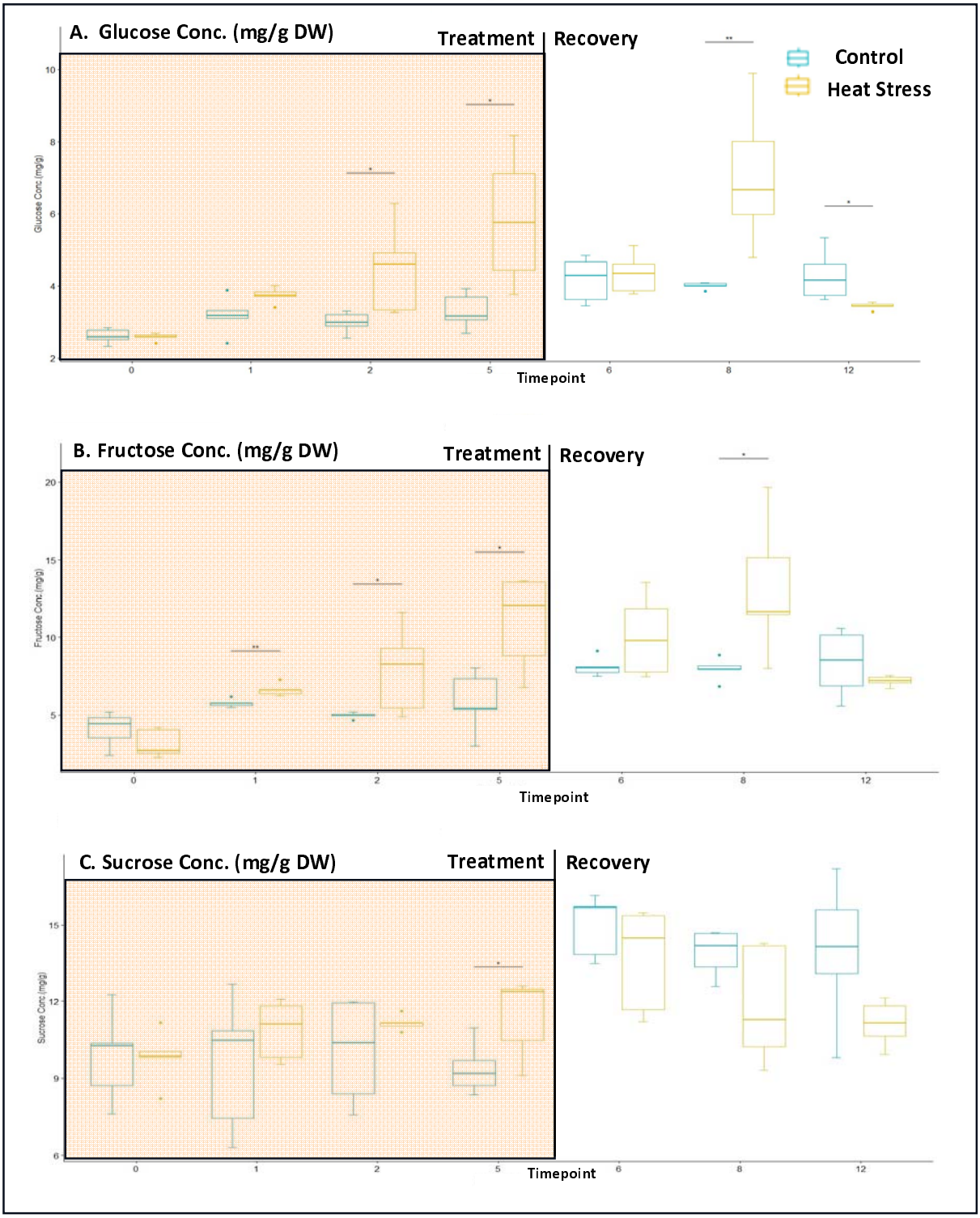

Figure 9. Leaf sugar (Glucose, Fructose and Sucrose) concentrations in mg/g of dry weight (DW) in *Brassica napus* plants subjected to heat treatment (30 °C/24 °C day/night) at timepoints 1-5 (yellow boxes) or maintained under control temperatures (20 °C/14 °C day/night) at all timepoints (blue boxes). Concentrations were quantified using HPLC. Data were analysed using two-way mixed ANOVA pairwise comparisons. Each box plot represents data from 5 replicates, showing the median (horizontal bar), 25th and 75th percentiles (the box) and the min and max values (whiskers), with outliers as dots. Significant differences between heat treated and control plants at each time point are denoted by (*) for p-value ≤ 0.05 or (**) for p-value ≤ 0.01. Heat treatment significantly increased leaf concentration of Glucose (Fig. 9A), Fructose (Fig. 9B), and Sucrose (Fig. 9C).

4. DISCUSSION

To investigate the effect of prolonged high temperatures (heatwaves) on the growth and adaptability of *B. napus,* especially during its yield-determining reproductive stages, heat treatment experiments were designed in a way that mimic field temperature fluctuations. In the present study, *B. napus* plants were exposed to a gradual increase in temperature for a six-day period and samples were collected at different timepoints during treatment and recovery. The results showed a prevailing effect on the plants both physiologically and metabolically. Heat treatment negatively impacted seed weight and total plant biomass (Supplementary Fig. S4), consistent with similar negative impacts documented in *B. napus* and in other plants such as, Arabidopsis and wheat (Aksouh-Harradj et al., 2006; Zhang et al., 2017; Bheemanahalli et al., 2019). This confirms that heat stress during flowering irreversibly damages vital reproductive processes leading to reduced productivity. NIRS showed significant effects of heat treatment on fatty acid concentrations in seeds in response to heat (Supplementary Fig. S5). Several studies also observed heat-induced alteration in lipid composition (Namazkar et al., 2016; Zhou et al., 2018; Zoong Lwe et al., 2021). In *B. napus*, high night temperature has led to overexpression of genes involved in fatty acid catabolism (Zhou et al., 2018). Furthermore, different heat tolerant and susceptible genotypes exhibited different heat-induced changes in their lipid content (Narayanan et al., 2016; Narayanan et al., 2020). Because lipids and proteins are the major constituents of biological membranes, maintaining cellular homeostasis depends mainly on the dynamic nature of lipid composition (Zheng et al., 2011). This suggests an essential role of cellular membrane lipid remodelling under high temperature stress. Additionally, under periods of reduced Carbon availability in plants, the conversion of lipids to organic acids provides an additional source of energy, thus contributing to the plants' acclimation process (Yuenyong et al., 2019).

4.1 Heat stress resulted in down regulation of many genes involved in multiple functional and metabolic processes after 48 hours of treatment.

In this study, comparative transcriptome analysis between heat treatment and control identified 6106 significantly differentially expressed transcripts ($|\log2(\text{foldchange})| \geq 0.5$, FDR ≤ 0.05) (Supplementary Fig. S6-left) with a balanced proportion of up and down regulated genes in contrasts HS0vsC0 & HS1vsC1, and more down regulated genes than up regulated ones in contrasts HS2vsC2 & HS6vsC6 were identified. This suggests the impact of heat treatment on the plant's transcriptome after 48 hours of heat stress, resulted in down regulation of many genes involved in multiple functional and metabolic processes such as Glycolysis, PPP, Citrate Cycle and

Oxidative Phosphorylation. In contrast HS12vsC12, although the proportion of down regulated genes reached nearly double that of the up regulated ones, the total number of DEG decreased dramatically as the plant started to restore its metabolic functions after 6 days of recovery.

4.2 DEGs showed enrichment of pathways related to respiratory metabolism.

The analysis of KEGG enriched pathways revealed the main underlying biochemical pathways altered in *B. napus* in response to heat stress (Supplementary Fig. S9). In accordance with other reports (Estravis-Barcala et al., 2021; Li et al., 2022), DEGs showed an enrichment of pathways related to respiratory metabolism namely Glycolysis, Citrate Cycle, Oxidative Phosphorylation and Pentose Phosphate Pathway (PPP). At timepoint 2, the impact of heat stress on Glycolysis was manifested by the down regulation of the key enzymes Phosphoglucomutase, Aldose 1-Epimerase, Hexokinase, Glucose-6-Phosphate Isomerase, and 6-Phosphofructokinase (Supplementary Fig. S11). Moreover, most of the Citrate Cycle genes were also down regulated at timepoint 2 (Supplementary Fig. S12). In the Electron Transport Chain (ETC), gene expression analysis showed that most of the involved genes were down regulated under heat treatment, except for Succinate Dehydrogenase (SDH) which was up regulated (Supplementary Fig. S13). In Mitochondrial metabolism, SDH also known as complex II, plays a central role in the Citrate Cycle and ETC (Huang & Millar, 2013). Additionally, SDH is thought to impact different components of the plant stress response, including stomatal conductance and ROS scavenging (Huang et al., 2019).

Although respiratory pathways have been found to have potential roles in adaptive response to abiotic stress, mainly through reducing the production of ROS (Van Dongen et al., 2011), under extreme conditions such as severe or prolonged heat stress, respiratory enzymes are deactivated, and proteins are denatured leading to culminating of ROS and a total breakdown of mitochondrial respiration (Scafaro et al., 2021). This negatively impacts Oxygen and Carbon fluxes and eventually leads to more severe yield penalty (Rasmusson et al., 2020). In line with this, results from heat stress experiment on seagrass showed that high midday temperature stress of 40°C was associated with significant decrease in biomass (George et al., 2018). Likewise, Imp and co-workers (2019) found that under high night temperature, wheat tolerant cultivar exhibited smaller reductions in biomass and lower rates of both net photosynthesis and respiration compared to other wheat cultivars (Imp et al., 2019).

In the PPP, the oxidative phase (OPPP) was mainly affected at timepoint 1, and this was indicated by the differential expression of Glucose-6-Phosphate Dehydrogenase (G6PDH) and 6-

Phosphogluconate Dehydrogenase (6PGDH) enzymes (Supplementary Fig. S14). G6PDH and 6PGDH are the key enzymes in the OPPP. They catalyse the first and third steps of the pathway, respectively (Long et al., 2016), and have been reported to be associated with the response to various abiotic stresses in plants (Gong et al., 2012; Tian et al., 2021). Esposito (2016) reported the involvement of the OPPP in the early response to abiotic stress factors, forming a true metabolic sensor to oxidative stress. At timepoint 2, most of the oxidative and non-oxidative phase enzymes were down regulated, including Transketolase (TK), Transaldolase and Phosphofructokinase enzymes (Supplementary Fig. S15). TK has a central role in primary metabolism, where the products of the involved reactions produce the precursors for nucleic acids biosynthesis, aromatic amino acids, and vitamins (Bi et al., 2013). Bi et al (2013) showed that the cucumber TK gene (*CsTK*) was sensitive to temperature and light (Bi et al., 2013; Bi et al., 2019). Likewise, the Transaldolase gene was found to be involved in the regulation of expression of genes involved in the ABA signalling pathway and the enzymes responsive to ROS (Rong et al., 2021). The results of this study add further evidence to the involvement of the PPP enzymes in the heat stress response in *B. napus*.

4.3 Heat stress altered GLS metabolism during treatment.

At the transcriptomic level, heat treatment differentially affected the expression of GLS synthesis and regulatory genes (Figs. 3 & 4). Several genes encoding different UDP Glycosyltransferases and Glutathione S-Transferases appeared down regulated, while others encoding GLS related transcription factors were up regulated, suggesting that these genes might have participated at different stages of the stress response.

The influence of heat stress on individual GLS was further evaluated through metabolomic analysis of two aliphatic GLS (Progoitrin (PRO) and Gluconapin (GNA)), and one indolic GLS (Glucobrassicin (GBS)). The role of these GLS in response to different abiotic stress factors has been previously reported (Jasper et al., 2020; Ljubej et al., 2021). In the current study, the concentration of PRO and GNA significantly increased in response to heat stress before it started to decline after 5 days of treatment (Fig. 6). This decline could have resulted from membrane damage caused by prolonged exposure to heat, leading to GLS degradation. Besides, the removal of heat stress after timepoint 5 could also be another factor for the decline in GLS content. In addition to its primary role in response to plant pathogen interaction (Hopkins et al., 2009; Touw et al., 2020), GLS accumulation in response to heat stress has also been previously reported (Valente Pereira et al., 2002; Martínez-Ballesta et al., 2013; Jasper et al., 2020). When plants are stressed, growth is

reduced, and Carbon utilization is predominantly diverted towards the production of secondary metabolites. As part of the plant defence mechanism, GLS play important role as osmoprotective compounds (Martínez-Ballesta et al., 2013), where their concentration increases in response to stress, providing protection against oxidative damage. At 30/15°C (day/night) temperature regime, *Brassica oleracea* seedlings had significantly higher GLS concentrations than plants cultivated at lower temperatures (22/15°C and 18/12°C) (Valente Pereira et al., 2002). Similarly, an Arabidopsis GLS mutant experienced a remarkable decline in growth and development and was found to be less heat tolerant than wild-type plants upon exposure to high temperature (Ludwig-Müller et al., 2000).

In contrast, indolic glucosinolate GBS showed a downward trend where concentration decreased gradually in response to heat (Fig. 6). Similar results have been found where GBS concentration was reduced in response to heat treatment (Bohinc & Trdan, 2012). Also, during short term high temperature stress, the transcript level of the indolic glucosinolates synthetic genes were predominantly down regulated (Rao et al., 2021). Several studies reported that indole GLS are much more sensitive to heat treatment (Bones & Rossiter, 2006; Bohinc & Trdan, 2012) and can demonstrate more thermal degradation than aliphatic GLS at lower temperatures (Orlemans et al., 2006).

The results of our study indicate that the concentration of GLS in the leaves of *B. napus* is influenced by high temperature stress, which was previously reported in other Brassica species (Rask et al., 2000; Bohinc & Trdan, 2012). It also shows that different groups of GLS (aliphatic versus indolic) differ in their response to heat stress. As reported by Ljubej et al., (2021), the different accumulation trends of these groups could correspond to different protective mechanisms played by individual GLS (Ljubej et al., 2021). Additionally, the variety of enzymes involved in GLS synthesis and regulation could be another factor for the difference in heat response (Schonhof et al., 2007).

4.4 Heat stress altered the expression of Sulphur assimilation and transport genes

In the current study, the transcript levels of Sulphur assimilation and transport genes were significantly down regulated especially at timepoint 2 (Fig. 5). This included several Adenylyl Sulphate Kinases and Reductases, ATP Sulphurylase (ATPS) and Sulphate transporter genes. These genes are important for the activation, catalysis and transport of Sulphur compounds (Capaldi et al., 2015). Moreover, enrichment pathway analysis showed that Cys and Met metabolism pathway

was among the top significantly enriched pathways at timepoints 1 and 2 (Supplementary Fig. 9). In this pathway, ten transcripts mapping to S-Adenosylmethionine Synthetase and 11 transcripts mapping to Homocysteine S-Methyltransferases were down regulated (Supplementary Fig. S10). These enzymes are among the key enzymes that control the Methionine pool (Capaldi et al., 2015). Given that GLS compounds are rich in Sulphur, assimilation of Sulphur is closely associated with GLS biosynthesis. Cysteine (Cys) which is the terminal metabolite in Sulphur assimilation, acts as a Sulphur donor for Methionine (Met), a precursor for aliphatic GLS. This renders Cys an important intersection point between Sulphur assimilation and GLS biosynthesis (Rao et al., 2021). Such proximity between Sulphur and these amino acids makes Cys and Met metabolism pathway directly related to GLS biosynthesis.

4.5 Heat stress altered sugar metabolism during treatment and recovery periods.

In the present study, heat treatment altered carbohydrate concentration as the amounts of Glucose, Fructose and Sucrose increased with heat treatment (Fig. 9). At the transcriptional level, the starch hydrolysing enzyme Alpha-Amylase was induced. Conversely, the Starch synthesizing enzymes such as Phosphoglucomutase (PGM), AGPase and Hexokinase were inhibited (Supplementary Figs. S11 & S17). Likewise, heat induced the up regulation of Sucrose synthesizing enzymes and the down regulation of Sucrose hydrolysing enzymes such as Cell Wall Invertase and Sucrose Synthase (Supplementary Tables S5 & S6). Together with the inhibition of many sugar transporters such as SWEET and ERD6-like (Figs. 7 & 8) suggest that heat induced Starch hydrolysis and promoted Sucrose synthesis and accumulation in the leaves. Several reports showed that different environmental stress affect sugar metabolism and transport (Xalxo et al., 2020; Mathan et al., 2020). For example, drought and salinity stresses increased Sucrose content in leaf and root tissues of rice (Mathan et al., 2020). Similarly, a significant accumulation of total sugar and Proline was observed in different genotypes of heat stressed seedlings of moth bean (Harsh et al., 2016). Marias et al. (2017) also reported an accumulation of Glucose and Fructose under heat stress in the leaves of *Coffea arabica* (Marias et al., 2017). In maize ear leaf, the ^{13}C export rate was decreased by high temperature treatment, resulting in growth enhancement of vegetative plant parts and reduction in grain yield (Suwa et al., 2010). During heat stress, leaves are the first tissues to sense and experience damage by heat. Consequently, leaves use sugar to efficiently scavenge ROS and alleviate oxidative damage. In high respiration environment, it has been hypothesized that sugar acts as energy supply to ensure survival (Burke, 2007), as their role in osmotic readjustments helps to maintain cell-water balance and membrane integrity. Thereby, maintaining

leaf sugar content through reducing source to sink export is considered a tolerance strategy (Kaushal et al., 2013; Zho et al., 2017). Additionally, the decrease in sink demand due to growth limitation, could also contribute to the accumulation of sugar in the source leaves under stressful conditions (Hummel et al., 2010; Lemoine et al., 2013).

5. CONCLUSION

Through a comprehensive transcriptomic and metabolomic analysis, this study brings evidence for the effect of high temperature stress and in particular heatwaves on different functional and metabolic pathways, highlighting its role in the plant stress defence system. More specifically, our results indicated that heat treatment:

1) Inhibited important metabolic pathways such as respiratory metabolism namely Glycolysis, Pentose Phosphate Pathway, Citrate Cycle and Oxidative Phosphorylation especially after 48 hours of heat treatment.
2) Reduced yield and plant biomass; and altered seed composition.
3) Altered sugar and glucosinolates levels in leaves.
4) Induced the expression of 6106 genes which were differentially regulated during heat treatment and recovery. Most of the top up and down regulated genes involved in key biological processes such as heat shock proteins, cellular processes regulation, transcription factors, cell wall remodelling, sugar and secondary metabolites transport and metabolism.

Finally, the gradual increase in temperature adapted in the heat experiment helped to create an environment that mimics field conditions. Thus, this study represents an important step towards developing an understanding of the heat stress response and tolerance mechanisms, a knowledge that could possibly be transferred to other plants.

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Authors Contributions: All authors contributed to the experimental design of the study. MK undertook the research, carried out the lab work, conducted the bioinformatics and data analysis, and drafted the manuscript. FM supervised the bioinformatics analysis. MA supervised the metabolomic lab work and data analysis. JPH supervised the molecular genetics lab work. All authors contributed to and revised the manuscript, given the opportunity to comment, and approve the final version of the manuscript.

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Data Availability Statement: Raw sequencing for the RNA-Seq analysis have been deposited at the NCBI's Sequence Read Archive, under the submission number SUB14296957. All other datasets supporting the results of this article are included within the article and supplementary materials.

Conflict of Interest: The authors declare no conflict of interest.

References:

1. Angadi, S. V., Cutforth, H. W., Miller, P. R., McConkey, B. G., Entz, M. H. & Brandt, S. A., et al. (2000). Response of three Brassica species to high temperature stress during reproductive growth. *Canadian Journal of Plant Science,* 80, 693–701.
2. Aksouh-Harradj ,N., Campbell, L. & Mailer, R. (2006). Canola response to high and moderately high temperature stresses during seed maturation. *Canadian Journal of Plant Science,* 86, 967–980.
3. Bell, L., Oruna-Concha, M. J. & Wagstaff, C. (2015). Identification and quantification of glucosinolate and flavonol compounds in rocket salad (*Eruca sativa*, *Eruca vesicaria* and *Diplotaxis tenuifolia)* by LC–MS: Highlighting the potential for improving nutritional value of rocket crops. *Food Chemistry*, 172, 852–861. https://doi.org/10.1016/j.foodchem.2014.09.116.
4. Bheemanahalli, R., Sunoj, V. S. J., Saripalli, G., Prasad, P. V. V., Balyan, H. S., Gupta, P. K., Grant, N., Gill, K. S. & Jagadish, S. V. K. (2019). Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. *Crop Science*, 59(2), 684–696. https://doi.org/10.2135/cropsci2018.05.0292.
5. Bi, H., Wang, M., Dong, X. & Ai, X. (2013). Cloning and expression analysis of transketolase gene in Cucumis sativus L. *Plant Physiology and Biochemistry*, 70, 512–521. https://doi.org/10.1016/j.plaphy.2013.06.017.
6. Bi, H., Li, F., Wang, H. & Ai, X. (2019). Overexpression of transketolase gene promotes chilling tolerance by increasing the activities of photosynthetic enzymes, alleviating oxidative damage and stabilizing cell structure in *Cucumis sativus* L. *Physiologia Plantarum*, 167, 502–515. https://doi.org/10.1111/ppl.12903.

7. Bohinc, T. & Trdan S. (2012). Environmental factors affecting the glucosinolate content in Brassicaceae. *Journal of Food, Agriculture & Environment,*10 (2), 357-360.
8. Bones, A. M. & Rossiter, J. T. (2006). The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry,* 67, 1053-1067.
9. Burke, J. J. (2007). Evaluation of source leaf responses to water-deficit stresses in cotton using a novel stress bioassay. *Plant Physiology,* 143, 108121. https://doi.org/10.1104/pp.106.087783.
10. Dikšaitytė, A., Viršilė, A., Žaltauskaitė, J., Januškaitienė, I. & Juozapaitienė, G. (2019). Growth and photosynthetic responses in *Brassica napus* differ during stress and recovery periods when exposed to combined heat, drought and elevated CO_2. *Plant Physiology and Biochemistry*, 142, 59–72. Https://doi.org/10.1016/j.plaphy.2019.06.026.
11. Duan, H., Wu, J., Huang, G., Zhou, Sh, Liu, W., Liao, Y., Yang, X., Xiao, Z. & Fan, H. (2017). Individual and interactive effects of drought and heat on leaf physiology of seedlings in an economically important crop. *Annal of Botany Plants,* 9(1), plw090. Https://doi.org/10.1093/aobpla/plw090.
12. Esposito S. (2016). Nitrogen assimilation, abiotic stress and glucose 6-phosphate dehydrogenase: The full circle of reductants. *Plants (Basel, Switzerland),* 5(2), 24. https://doi.org/10.3390/plants5020024.
13. Estravis-Barcala, M., Heer, K., Marchelli, P., Ziegenhagen, B., Arana, MV. & Bellora,N. (2021). Deciphering the transcriptomic regulation of heat stress responses in *Nothofagus pumilio*. *PloS ONE,* 16(3), e0246615. https://doi.org/10.1371/journal.pone.0246615.
14. Andrews S. (2012). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
15. Finkelstein, R.R. & Gibson, S.I. (2002). ABA and sugar interactions regulating development: Cross-talk or voices in a crowd? *Current Opinion in Plant Biology*, 5, 26–32.
16. George, R., Gullström, M., Mangora, M. M., Mtolera, M. S. P. & Björk, M. (2018). High midday temperature stress has stronger effects on biomass than on photosynthesis: A mesocosm experiment on four tropical seagrass species. *Ecology and Evolution,* 8, 4508–4517. doi: 10.1002/ece3.3952.
17. Glaubitz, U,. Li, X., Schaedel, S., Erban, A., Sulpice, R., Kopka, J., Hincha, DK. & Zuther, E. (2017). Integrated analysis of rice transcriptomic and metabolomic responses to elevated night temperatures identifies sensitivity- and tolerance-related profiles. *Plant Cell and Environment,* 40, 121–37. Https://doi. Org/10.1111/pce.12850.

18. Goel, K., Kundu, P., Sharma, P. *et al.* (2023). Thermosensitivity of pollen: a molecular perspective. *Plant Cell Reports,* 42, 843–857.
19. Gong, H., Chen, G., Li, F., Wang, X., Hu, Y. & Bi, Y. (2012). Involvement of G6PDH in heat stress tolerance in the calli from *Przewalskia tangutica* and *Nicotiana tabacum. Biologia plantarum*, 56, 422–430. https://doi.org/10.1007/s10535-012-0072-8.
20. Harsh, A., Sharma, Y.K., Joshi, U., Rampuria, S., Singh, G., Kumar, S. & Sharma, R. (2016). Effect of short-term heat stress on total sugars, proline and some antioxidant enzymes in moth bean (*Vigna aconitifolia*), *Annals of Agricultural Sciences*, 61(1), 57-64.
21. Hasanuzzaman, M., Nahar, K., Alam, M. M. & Fujita, M. (2014). Modulation of antioxidant machinery and the methylglyoxal detoxification system in selenium-supplemented *Brassica napus* seedlings confers tolerance to high temperature stress. *Biological Trace Element Research*, 161(3), 297–307. Https://doi.org/10.1007/s12011-014-0120-7.
22. Hedhly, A. (2011). Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environment and Experimental Botany,* 74, 9–16.
23. Hinojosa, L., Matanguihan, JB. & Murphy, KM. (2019). Effect of high temperature on pollen morphology, plant growth and seed yield in quinoa (*Chenopodium quinoa* Willd.). *Journal of Agronomy and Crop Science,* 205, 33–45.
24. Hopkins, RJ., Van Dam, NM. & Van Loon JJ. (2009). Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology,* 54, 57–83.
25. Huang, W., Ma, H.Y., Huang, Y., Li, Y., Wang, G.L., Jiang, Q., Wang, F. & Xiong, A.S. (2017). Comparative proteomic analysis provides novel insights into chlorophyll biosynthesis in celery under temperature stress. *Physiologia Plantarum,* 161, 468–485.
26. Huang, S. & Millar, A. H. (2013). Succinate dehydrogenase: the complex roles of a simple enzyme. *Current Opinion in Plant Biology*, 16(3), 344–349. https://doi.org/10.1016/j.pbi.2013.02.007.
27. Huang, R., Liu, Z., Xing, M., Yang, Y., Wu, X. & Liu, H., et al. (2019). Heat stress suppresses *Brassica napus* seed oil accumulation by inhibition of photosynthesis and BnWRI1 pathway. *Plant Cell Physiology,* 60, 1457–1470. doi: 10.1093/pcp/pcz052.
28. Hummel, I., Pantin, F., Sulpice, R., Piques, M., Rolland, G. & Dauzat, M., et al. (2010). Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiology,* 154, 357–372 10.1104/pp.110.157008.

29. Ikram, M., Chen, J., Xia, Y., Li, R., Siddique, K. H. M. & Guo, P. (2022). Comprehensive transcriptome analysis reveals heat-responsive genes in flowering Chinese cabbage (*Brassica campestris* L. ssp. chinensis) using RNA sequencing. *Frontiers in Plant Science,* 13. https://doi.org/10.3389/fpls.2022.1077920.
30. Impa, S. M., Sunoj, V. S. J., Krassovskaya, I., Bheemanahalli, R., Obata, T. & Jagadish, S. V. K. (2019). Carbon balance and source-sink metabolic changes in winter wheat exposed to high night-time temperature. *Plant Cell and Environment,* 42, 1233–1246. doi: 10.1111/pce.13488.
31. Ismaili, A., Salavati, A. & Mohammadi, PP. (2015). A comparative proteomic analysis of responses to high-temperature stress in hypocotyl of canola (*Brassica napus* L.). *Protein & Peptide Letters,* 22, 285-299.
32. Jagadish, SK., Way, DA. & Sharkey, TD. (2021). Plant heat stress: concepts directing future research. *Plant Cell and Environment,* 44, 1992–2005.
33. Jasper, J. Wagstaff, C. & Bell, L. (2020). Growth temperature influences postharvest glucosinolate concentrations and hydrolysis product formation in first and second cuts of rocket salad. *Postharvest Biology and Technology,* 163, 111157. doi:10.1016/j.postharvbio.2020.111157.
34. Jin, B., Li, W., Jing, W., Jiang, KZ., Yang, W., Jiang, XX., Ni, CY., Wang, YL. & Teng, NJ. (2011). The effect of experimental warming on leaf functional traits, leaf structure and leaf biochemistry in *Arabidopsis thaliana*. *BMC Plant Biology,* 11, 35. Https://doi.org/10.1186/1471-2229-11-35.
35. Kanehisa, M. & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research,* 28, 27–30. https://doi.org/10.1093/nar/28.1.27 PMID: 10592173.
36. Kaushal, N., Awasthi, R., Gupta, K., Gaur, P., Siddique, K.H.M. & Nayyar, H. (2013). Heat-stress-induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. *Functional Plant Biology,* 40:1334–1349. doi: 10.1071/FP13082.
37. Kourani, M., Mohareb, F., Rezwan, F. I., Anastasiadi, M. & Hammond, J. P. (2022). Genetic and physiological responses to heat stress in *Brassica napus*. *Frontiers in Plant Science,* 13. https://doi.org/10.3389/fpls.2022.832147.
38. Koscielny, C. B., Hazebroek, J. & Duncan, R. W. (2018). Phenotypic and metabolic variation among spring *Brassica napus* genotypes during heat stress. *Crop Pasture Science,* 69, 284–295. doi: 10.1071/CP17259.

39. Lamaoui, M., Jemo, M., Datla, R. & Bekkaoui, F. (2018). Heat and drought stresses in crops and approaches for their mitigation. *Frontiers in Chemistry*, *6*. Https://doi.org/10.3389/fchem.2018.00026.
40. Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, JL., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J. & Durand, M. (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science,* 24, 4-272. doi: 10.3389/fpls.2013.00272.
41. Li, M., Li, J., Zhang, R., Lin, Y., Xiong, A., Tan, G., Luo, Y., Zhang, Y., Chen, Q. & Wang, Y. et al. (2022). Combined analysis of the metabolome and transcriptome to explore heat stress responses and adaptation mechanisms in celery (*Apium graveolens* L.). *International Journal of Molecular Science,* 23, 3367. https://doi.org/ 10.3390/ijms23063367.
42. Ljubej, V., Radojčić, Redovniković I., Salopek-Sondi, B., Smolko, A., Roje, S. & Šamec, D. (2021). Chilling and freezing temperature stress differently influence glucosinolates content in *Brassica oleracea* var. *acephala*. *Plants* (Basel), 27, 10(7), 1305. doi: 10.3390/plants10071305.
43. Lohani, N., Singh, MB. & Bhalla, PL. (2020). High temperature susceptibility of sexual reproduction in crop plants. *Journal of Experimental Botany,* 71:555–568.
44. Long, X., He, B., Fang, Y. & Tang, C. (2016). Identification and characterization of the glucose-6-phosphate dehydrogenase gene family in the para rubber tree, *Hevea brasiliensis. Frontiers in Plant Science,* 7. https://doi.org/10.3389/fpls.2016.00215.
45. Love, MI., Huber, W. & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 550. doi:10.1186/s13059-014-0550-8
46. Marias, D., Meinzer, F.C., & Still, C. (2017). Impacts of leaf age and heat stress duration on photosynthetic gas exchange and foliar nonstructural carbohydrates in *Coffea arabica*. *Ecology and Evolution,* 7, 1297–1310.
47. Martínez-Ballesta, M. del C., Moreno, D. A. & Carvajal, M. (2013). The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. *International Journal of Molecular Sciences,* 14, (6), 11607–11625. https://doi.org/10.3390/ijms140611607.
48. Mathan, J., Singh, A. & Ranjan, A. (2020). Sucrose transport in response to drought and salt stress involves ABA-mediated induction of OsSWEET13 and OsSWEET15 in rice. *Physiologia Plantarum,* 171(4), 620–637. https://doi.org/10.1111/ppl.13210.

49. Met Office (n.d.). *UK temperature, rainfall and sunshine time series*. https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-temperature-rainfall-and-sunshine-time-series.
50. Müller, JL., Krishna, P. & Foreiter, C. (2000). A glucosinolate mutant of *Arabidopsis* is thermosensitive and defective in cytosolic Hsp90 expression after heat stress. *Plant Physiology*, 123, 949–958.
51. Namazkar, S., Stockmarr, A., Frenck, G., Egsgaard, H., Terkelsen, T., Mikkelsen, T., Ingvordsen, C. H. & Jørgensen, R. B. (2016). Concurrent elevation of CO_2, O_3 and temperature severely affects oil quality and quantity in rapeseed. *Journal of Experimental Botany*, 67(14), 4117–4125. https://doi.org/10.1093/jxb/erw180.
52. Narayanan, S., Tamura, PJ., Roth, MR., Prasad, PVV., & Welti, R. (2016). Wheat leaf lipids during heat stress: I. High day and night temperatures result in major lipid alterations. *Plant Cell and Environment,* 39, 787–803.
53. Narayanan, S., Zoong-Lwe, ZS., Gandhi, N., Welti, R., Fallen, B. & Smith, JR et al. (2020). Comparative lipidomic analysis reveals heat stress responses of two soybean genotypes differing in temperature sensitivity. *Plants,* 9, 457.
54. OmicsBox – Bioinformatics Made Easy, BioBam Bioinformatics, March 3, 2019, https://www.biobam.com/omicsbox.
55. Orlemans, K., Barrett, D.M., Bosch Suades, C., Verkerk, R. & Dekker, M. 2006. Thermal degradation of glucosinolates in red cabbage. *Food Chemistry,* 95, 19-29.
56. Pasini, F., Verardo, V., Caboni, M.F. & D'Antuono, L.F. (2012). Determination of glucosinolates and phenolic compounds in rocket salad by HPLC-DAD-MS: Evaluation of *Eruca sativa Mill.* And *Diplotaxis tenuifolia* L. genetic resources. *Food Chemistry*, 133(3), 1025–1033.
57. Pereira, F. M. V., Rosa, E., Fahey, J. W., Stephenson, K. K., Carvalho, R. & Aires, A. (2002). Influence of temperature and ontogeny on the Levels of glucosinolates in broccoli (*Brassica oleracea* Var. italica) sprouts and their effect on the induction of mammalian phase 2 enzymes. *Journal of Agricultural and Food Chemistry*, 50(21), 6239–6244. https://doi.org/10.1021/jf020309x.
58. Prasad, P. V., Bheemanahalli, R. & Jagadish, S. K. (2017). Field crops and the fear of heat stress — opportunities, challenges and future directions. *Field Crops Research,* 200, 114–121. doi: 10.1016/j.fcr.2016.09.024.

59. Rao, S.-Q., Chen, X.-Q., Wang, K.-H., Zhu, Z.-J., Yang, J. & Zhu, B. (2021). Effect of short-term high temperature on the accumulation of glucosinolates in *Brassica rapa*. *Plant Physiology and Biochemistry*, 161, 222–233. https://doi.org/10.1016/j.plaphy.2021.02.013.
60. Rask, L., Andréasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B. & Meijer, J. (2000). Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Molecular Biology,* 42:93-113.
61. Rasmusson, L. M., Buapet, P., George, R., Gullström, M., Gunnarsson, P. C. B. & Björk, M. (2020). Effects of temperature and hypoxia on respiration, photorespiration, and photosynthesis of seagrass leaves from contrasting temperature regimes. J. Norkko (Ed.), ICES *Journal of Marine Science,* 77(6), 2056–2065. Oxford University Press (OUP). https://doi.org/10.1093/icesjms/fsaa093.
62. Rong, Y., Li, T., Liu, X., Shi, S., Wang, X. & Tu, pengfei. (2021). A transaldolase from *Aquilaria Sinensis* involves in ABA-mediated seed germination and root growth. https://doi.org/10.21203/rs.3.rs-234062/v1.
63. Scafaro, A. P., Fan, Y., Posch, B. C., Garcia, A., Coast, O. & Atkin, O. K. (2021). Responses of leaf respiration to heatwaves. *Plant, Cell & Environment,* 44(7), 2090–2101. https://doi.org/10.1111/pce.14018.
64. Schonhof, I., Kläring, H. P., Krumbein, A., Clauβen, W. & Screiner, M. (2007). Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. *Agriculture, Ecosystems and Environment,* 119, 103-111.
65. Seth, R., Maritim, TK., Parmar, R. & Sharma, RK. (2021). Underpinning the molecular programming attributing heat stress associated thermotolerance in tea (*Camellia sinensis* (L.) O. Kuntze). *Horticulture Research,* 8(1). Https://doi.org/10.1038/s41438-021-00532-z.
66. Song, JM., Guan, Z. & Hu, J. *et al* (2020). Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. *Nature Plants,* 6, 34–45. https://doi.org/10.1038/s41477-019-0577-7.
67. Seneviratne, SI. et al (2012). Changes in climate extremes and their impacts on the natural physical environment. *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation* [Field C. B. et al. (eds)]. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge, UK, and New York, NY, USA, 109–230.

68. Sun, F., Fan, G., Hu, Q., Zhou, Y., Guan, M. & Tong, C., et al. (2017). The high quality genome of *Brassica napus* cultivar 'ZS11' reveals the introgression history in semi-winter morphotype. *The Plant Journal,* 92, 452–468. doi: 10.1111/tpj.13669.
69. Suwa, R., Hakata, H., Hara, H., El-Shemy, HA., Adu-Gyamfi, JJ., Nguyen, NT., Kanai, S., Lightfoot, DA., Mohapatra, PK. & Fujita, K. (2010). High temperature effects on photosynthate partitioning and sugar metabolism during ear expansion in maize (Zea mays L.) genotypes. *Plant Physiology Biochemistry,* 48, 124-30. doi: 10.1016/j.plaphy.2009.12.010.
70. Touw, AJ., Verdecia Mogena, A., Maedicke, A., Sontowski, R., van Dam, N. M. & Tsunoda, T. (2020). Both biosynthesis and transport are involved in glucosinolate accumulation during root-herbivory in *Brassica rapa*. *Frontiers in Plant Science,* 10. https://doi.org/10.3389/fpls.2019.01653.
71. Van Dongen, J. T., Gupta, K. J., Ramírez-Aguilar, S. J., Araújo, W. L., Nunes-Nesi, A. & Fernie, A. R. (2011). Regulation of respiration in plants: A role for alternative metabolic pathways. *Journal of Plant Physiology,* 168(12), 1434–1443. https://doi.org/10.1016/j.jplph.2010.11.004.
72. Wahid, A., Gelani, S., Ashraf, M. & Foolad, MR. (2007). Heat tolerance in plants: An overview. *Environment and Experimental Botany,* 61:199–223. Https://doi.org/10.1016/j.envexpbot.2007.05.011.
73. Wang, L., Ma, K.B., Lu, Z.G., Ren, S.X., Jiang, H.R., Cui, J.W., Chen, G., Teng, N.J., Lam, H.M. & Jin, B. (2020). Differential physiological, transcriptomic and metabolomic responses of Arabidopsis leaves under prolonged warming and heat shock. *BMC Plant Biology,* 20(1). Https://doi.org/10.1186/s12870-020-2292-y.
74. Way, DA. & Yamori, W. (2014). Thermal acclimation of photosynthesis: On the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis Research,* 119, 89–100. Https://doi.org/10.1007/s11120-013-9873-7.
75. WeatherOnline (n.d.). *Max temperature London - Observations 05.2017 interval: 02 Weeks | United Kingdom Weather History*. https://www.weatheronline.co.uk/weather/maps/city.
76. Xalxo, R., Yadu, B., Chandra, J., Chandrakar, V. & Keshavkant, S. (2020). Alteration in Carbohydrate Metabolism Modulates Thermotolerance of Plant under Heat Stress. *Heat Stress Tolerance in Plants,* 77–115. https://doi.org/10.1002/9781119432401.ch5.
77. Yu, E., Fan, C., Yang, Q., Li, X., Wan, B. & Dong, Y. et al. (2014). Identification of heat responsive genes in *Brassica napus* siliques at the seed-filling stage through transcriptional profiling. *PLoS One* 9:e101914. doi: 10.1371/journal. pone.0101914.

78. Yuenyong, W., Sirikantaramas, S. & Qu, LJ. *et al.* (2019). Isocitrate lyase plays important roles in plant salt tolerance. *BMC Plant Biology,* 19, 472. https://doi.org/10.1186/s12870-019-2086-2.
79. Zandalinas, SI., Rivero, RM., Martínez, V., Gómez-Cadenas, A. & Arbona, V. (2016). Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. *BMC Plant Biology,* 16, 1-16.
80. Zhang, S. S., Yang, H., Ding, L., Song, Z. T., Ma, H., Chang, F. & Liu, J. X. (2017). Tissue-specific transcriptomics reveals an important role of the unfolded protein response in maintaining fertility upon heat stress in Arabidopsis. *Plant Cell*, 29(5), 1007-1023. https://doi.org/10.1105/tpc.16.00916.
81. Zheng, G., Tian, B., Zhang, F., Tao, F. & Li, W. (2011). Plant adaptation to frequent alterations between high and low temperatures: Remodelling of membrane lipids and maintenance of unsaturation levels. *Plant Cell and Environment,* 34, 1431–42.
82. Zhou, L., Yan, T., Chen, X., Li, Z., Wu, D., Hua, S. & Jiang, L. (2018). Effect of high night temperature on storage lipids and transcriptome changes in developing seeds of oilseed rape. *Journal of Experimental Botany*, 69(7), 1721-1733. https://doi.org/10.1093/jxb/ery004.
83. Zoong Lwe, Z., Sah, S. & Persaud, L. *et al* (2021). Alterations in the leaf lipidome of *Brassica carinata* under high-temperature stress. *BMC Plant Biology,* 21, 404. https://doi.org/10.1186/s12870-021-03189-x.