Data Article

Analytical dataset of short-term heat stress induced reshuffling of metabolism and transcriptomes in maize grown under elevated CO₂

Jemaa Essemine a, Jikai Li b, Genyun Chen a, Mingnan Qu a,∗

a CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 200032, Shanghai, China
b Institute of Grass Research, Heilongjiang Academy of Agricultural Sciences, Harbin, China

ARTICLE INFO

Article history:
Received 27 August 2019
Received in revised form 28 November 2019
Accepted 9 December 2019
Available online 17 December 2019

Keywords:
Metabolism
Transcriptomes
Sudden heat stress
Elevated CO₂
Maize

ABSTRACT

This data article describes the analysis of sudden heat stress (SHS) induced transcriptomes and metabolism in SQ maize cultivar (Zea mays L. cv. Silver Queen). Plants were grown under elevated CO₂ in both field based open top chambers (OTCs) and indoor growth chamber conditions [1]. After 20 days after radicle emergence, intact leaf section of maize was exposed for 2 hours to SHS treatment. Samples were stored in liquid nitrogen immediately and used thereafter for metabolism and transcriptomes determinations. Metabolism consisting of 37 targeted metabolites together with corresponding reference standard were determined by gas chromatography coupled to mass spectrometry (GC-MS). Total RNA was extracted using TRizol® reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). RNA integrity was assessed using RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Transcriptomes were determined by Illumina Hiseq 4000 platform. Further interpretation and discussion on these datasets can be found in the related article entitled “Elevated CO₂ concentrations...”
may alleviate the detrimental effects of sudden heat stress on photosynthetic carbon metabolism in maize" [1].

© 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Specifications Table

| Subject                          | Agricultural and Biological Sciences (General) |
|---------------------------------|-----------------------------------------------|
| Specific subject area           | Heat stress induced modulation in metabolism and transcriptomes in maize |
| Type of data                    | Tables (Microsoft word)                       |
| How data were acquired          | GC-MS: gas chromatography coupled to mass spectrometry (GC-MS; 7890 GC system, 7693 autosampler, 5975C inert XL MSD; Agilent Technologies, Santa Clara, CA, USA) Transcriptomes: Illumina Hiseq 4000 platform |
| Data format                     | Raw, analyzed and formatted                    |
| Parameters for data collection  | Leaves were obtained from maize plants grown under two conditions, field based OTCs and indoor growth chamber, under either elevated (560 μmol mol⁻¹) or ambient CO2 (380 μmol mol⁻¹). Maize plants were grown under two CO2 treatments for 20 days after radicle emergence they were then subjected to a 2 h sudden heat shock stress. |
| Description of data collection  | Following the heat stress, the leaves were immediately immersed into liquid nitrogen for metabolism and transcriptomes. |
| Data source location            | Beltsville Agricultural Research Centre (BARC), United State Department of Agriculture-Agricultural Research Service. |
| Data accessibility              | Data are presented in this article in the form of figures (Figs. 1–5) and tables (Tables 1–6). |
| Related research article        | Li et al., 2019. Roles of heat shock protein and reprogramming of photosynthetic carbon metabolism in thermotolerance under elevated CO2 in maize. Environ. Exp. Bot.168. doi.org/10.1016/j.envexpbot.2019.103869 |

Value of the Data

- The experimental data presented herein as well as in Ref. [1] can be used to better understand the response of global gene expression in maize under multiple stress conditions.
- The generated datasets specifically provide information on the beneficial effect of elevated CO2 on photosynthetic carbon metabolites in response to sudden heat stress treatments.
- The expression of heat shock protein in response to CO2 treatments can be also learned from this study.
- Positive relationship regarding the photosynthetic carbon metabolites between field-based open top chambers (OTCs) and indoor growth chamber was investigated herein.
- The data can be used for reference of metabolite quantification and allow other researchers to extend the statistical analysis.

1. Data

The data collected for SQ maize cultivar exposed to combined effects of elevated CO2 and sudden heat stress is presented in five segments of data: A) The relatedness of biological samples in four combination of CO2 and SHS regarding to transcriptomes and metabolism in field OTCs conditions as shown in Fig. 1; B) Statistical analysis on sequencing quality across all bases from transcriptomes analysis in field OTCs (Figs. 2 and 3; Table 1); C) GO and KEGG analysis on enriched biological pathway involved in SHS and CO2 response (Tables 2–3); D) Abundance of heat shock protein based on transcriptomes in different SHS and CO2 treatments in growth chamber (Table 4); E) Photosynthetic carbon metabolites and the gene expression of their catalysing enzymes induced by SHS and CO2 effects (Fig. 4; Tables 5–6). The data included herein are based on the experimental results provided in a previous publication by present authors [1].
Fig. 1. Relatedness of biological samples of maize leaves exposed to combined SHS and elevated CO2 grown in field. Heatmap of transcriptomes (A) and metabolism (B) in field. Three biological replicates were performed.

Fig. 2. Statistical analysis on quality control of samples for transcriptomes across growth chamber and field. Quality scores (A) and sequence contents (B) across all bases were performed based on transcriptomes analysis. Coverage and distribution of mapped reads across gene body were shown in panels C and D, respectively.
2. Experimental design, materials, and methods

2.1. Materials and growth condition

SQ Corn seeds were supplied by the maize germplasm information resources from the United States of America, USA (GRIN: http://www.ars-grin.gov/). Experiments were conducted in both fields-based open top chambers (OTCs), and indoor conditions. The location of field is at Beltsville Agricultural Research Center (BARC), USDA-ARS (39-00' N, 76-56'W). The designed 4/4 random blocks for the experiment are as displayed in Fig. 5A. After germination, Corn seedlings were sown in 16 OTCs. The dimension for each OTC is: 2 m long, 2 m width and 2 m height (Fig. 5B). The interval between chambers is uniformly spaced by 2 m, to minimize shading effect. Maize seedlings for 7 days after radicle emergence were transplanted and spaced by 15 cm between each other as well. The soil in each OTC keeps moist by watering once a week. Plants in OTC are exposed to ambient air or ambient air plus 180 ppm CO₂, as described elsewhere [2].

For indoor chambers, plants were grown under either ambient CO₂ (380 µmol mol⁻¹) or high CO₂ (560 µmol mol⁻¹) concentrations, as described earlier [3]. Day and night temperatures were 29/17 °C,
Table 2
Gene ontology (GO) analysis on biological pathway enriched from differentially expressed genes induced by SHS with up-regulation of elevated CO2.

| GO ID    | Term                                      | Category              | P value  | Enrichment score |
|----------|-------------------------------------------|-----------------------|----------|------------------|
| GO:0006351 | transcription, DNA-templated             | biological_process    | 1.49E-07 | 1.44015704       |
| GO:0009737 | response to abscistic acid                | biological_process    | 9.24E-07 | 2.43930502       |
| GO:0010,161 | red light signaling pathway              | biological_process    | 2.12E-06 | 19.6231454       |
| GO:0006021 | inositol biosynthetic process            | biological_process    | 2.33E-06 | 10.9017474       |
| GO:0070,413 | trehalose metabolism in response to stress | biological_process    | 4.84E-06 | 5.98038717       |
| GO:0006952 | defense response                          | biological_process    | 5.87E-06 | 1.82692626       |
| GO:0006778 | NADP biosynthetic process                | biological_process    | 1.03E-05 | 15.6985163       |
| GO:0005992 | trehalose biosynthetic process           | biological_process    | 1.95E-05 | 5.10520856       |
| GO:0080,163 | regulation of protein serine/threonine    | biological_process    | 2.65E-05 | 7.69535114       |
| GO:0010,072 | primary shoot apical meristem specification | biological_process    | 2.65E-05 | 7.69535114       |
| GO:0005886 | plasma membrane                          | cellular_component    | 4.63E-05 | 1.34131818       |
| GO:0070,449 | elongin complex                          | cellular_component    | 0.000229,12 | 8.72139796 |
| GO:0005779 | integral component of peroxisomal membrane | cellular_component    | 0.00156,164 | 3.60661297 |
| GO:0005615 | extracellular spacial phosphate           | cellular_component    | 0.00264,417 | 2.25877933 |
| GO:0005887 | integral component of plasma membrane    | cellular_component    | 0.00347,733 | 1.71231635 |
| GO:0005578 | proteinaceous extracellular matrix        | cellular_component    | 0.0044,664 | 3.60885433       |
| GO:0048,046 | apoplast                                  | molecular_function    | 0.00729,448 | 1.60063304 |
| GO:0003700 | transcription factor activity, sequence-specific DNA binding | molecular_function | 1.24E-14 | 1.95021467 |
| GO:0004512 | inositol-3-phosphate synthase activity   | molecular_function    | 8.05E-08 | 16.3526212       |
| GO:0004760 | serine-pyruvate transaminase activity    | molecular_function    | 8.08E-08 | 20.9313551       |
| GO:0050,281 | serine-glyoxlate transaminase activity   | molecular_function    | 8.08E-08 | 20.9313551       |
| GO:004445 | inositol-polyphosphate 5-phosphatase activity | molecular_function | 4.69E-07 | 17.4427959 |
| GO:0052,658 | inositol-1,4,5-trisphosphate 5-phosphatase activity | molecular_function | 4.69E-07 | 17.4427959 |
| GO:0052,659 | inositol-1,3,4,5-tetrakishphosphate      | molecular_function    | 4.69E-07 | 17.4427959       |
| GO:0043,856 | sequence-specific DNA binding            | molecular_function    | 1.39E-06 | 1.85799281       |
| GO:0016,161 | beta-amylase activity                    | molecular_function    | 1.62E-06 | 9.2342136        |
| GO:0003951 | NAD+ kinase activity                     | molecular_function    | 1.03E-05 | 15.6985163       |

Table 3
Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on metabolic pathway enriched from differentially expressed genes induced by SHS with up-regulation of elevated CO2.

| KEGG ID | Term                                      | P value  | Enrichment score |
|---------|-------------------------------------------|----------|------------------|
| path:zma00062 | Fatty acid elongation                     | 0.00021,537 | 5.534060847 |
| path:zma00760 | Nicotinate and nicotinamide metabolism    | 0.00068,782 | 6.896291209 |
| path:zma00500 | Starch and sucrose metabolism             | 0.0012,358  | 2.668207908 |
| path:zma02010 | ABC transporters                          | 0.00124,559  | 5.976785714 |
| path:zma00052 | Galactose metabolism                      | 0.00127,888  | 4.038368726 |
| path:zma00650 | Butanate metabolism                       | 0.00206,036  | 5.27364454 |
| path:zma00710 | Carbon fixation in photosynthetic organisms | 0.00258,693  | 3.320436508 |
| path:zma00630 | Glyoxylate and dicarboxylate metabolism   | 0.00551,224  | 3.049386665 |
| path:zma00562 | Inositol phosphate metabolism             | 0.00571,992  | 2.75851648 |
| path:zma00600 | Sphingolipid metabolism                   | 0.10122607  | 3.448145604 |
| path:zma00250 | Alamine, aspartate and glutamate metabolism | 0.01244944  | 2.846088435 |
| path:zma00280 | Valine, leucine and isoleucine degradation | 0.02105207  | 2.80161304 |
| path:zma00051 | Fructose and mannose metabolism           | 0.02133673  | 2.490327381 |
| path:zma00940 | Phenylpropanoid biosynthesis              | 0.02318281  | 1.854864532 |
| path:zma04146 | Peroxisome                                | 0.0239,811  | 2.230143923 |
| path:zma00564 | Glycerophospholipid metabolism            | 0.02896395  | 2.01464687 |
| path:zma00270 | Cysteine and methionine metabolism        | 0.03275973  | 2.075272817 |
| path:zma00416 | MAPK signaling pathway - plant            | 0.03674623  | 1.83497807 |
| path:zma00030 | Pentose phosphate pathway                 | 0.03702845  | 2.359257519 |
| path:zma00260 | Glycine, serine and threonine metabolism  | 0.05842218  | 2.037540584 |
| Maize ID  | Gene annotation                  | Gene abbre. | log2FC (SHS/ck) | Significant | Regulate | Log2FC (eCO2/aCO2) | Significant | Orthologue in Arabidopsis |
|----------|----------------------------------|-------------|-----------------|-------------|----------|--------------------|-------------|--------------------------|
| GRMZM2G458208 | cpn1 - chaperonin 1            | Cpn1        | 1.9             | yes         | up       | −0.284             | no          | no ch.                    | AT3G23990   |
| GRMZM2G416120 | cpn2 - chaperonin2              | Cpn2        | 0.5356          | yes         | up       | 2.4825             | yes         | up                        | AT3G23990   |
| GRMZM2G310431 | hsp1 - heat shock protein1      | Hsp1        | 0.4664          | yes         | up       | 3.9482             | yes         | up                        | AT3G12580   |
| Zm00001d028555 | hsp10 - heat shock protein10    | Hsp10       | 0.3535          | yes         | up       | −1.384             | no          | down                      | AT1G47890   |
| GRMZM2G306679 | hsp11 - heat shock protein11    | Hsp11       | 0.4522          | yes         | up       | −0.9482            | no          | no ch.                    | AT1G53540   |
| GRMZM2G422240 | hsp17.2 - heat shock protein17.2 | Hsp17.2     | 0.2553          | yes         | up       | 3.4858             | yes         | up                        | AT5G12020   |
| GRMZM2G4044249 | hsp18a - 18 kda heat shock protein18a | Hsp18a  | 0.21,093        | yes         | up       | 5.92,874           | yes         | up                        | AT5G59720   |
| GRMZM2G034157 | hsp18c - heat shock protein18c   | Hsp18c      | 0.21,985        | yes         | up       | 0.9482             | no          | no ch.                    | AT5G12020   |
| GRMZM2G083810 | hsp18f - heat shock protein18f   | Hsp18f      | 0.2052          | yes         | up       | 2.4924             | yes         | up                        | AT5G12020   |
| GRMZM2G007729 | hsp22 - heat shock protein22     | Hsp22       | 0.2132          | yes         | up       | 2.94,823           | yes         | up                        | AT5G51440   |
| GRMZM2G149647 | hsp26 - heat shock protein26     | Hsp26       | 0.1942          | yes         | up       | −1.94,824          | no          | no ch.                    | AT4G27670   |
| GRMZM6G199466 | hsp3 - heat shock protein3       | Hsp3        | 0.0942          | yes         | up       | −0.928             | no          | no ch.                    | EFH47634.1  |
| GRMZM2G069651 | hsp4 - heat shock protein4       | Hsp4        | −0.042          | no          | no ch.   | 0.09482            | no          | no ch.                    | AT1G53540   |
| GRMZM2G340251 | hsp70-4 - heat shock protein70-4 | Hsp70       | 0.0486          | no          | no ch.   | 0.0838             | no          | no ch.                    | AT5G56000   |
| GRMZM2G080724 | hsp8 - heat shock protein8       | Hsp8        | 0.095           | yes         | up       | 1.2948             | no          | no ch.                    | AT4G27670   |
| GRMZM2G046382 | hsp9 - heat shock protein9       | Hsp9        | 0.1821          | yes         | up       | 1.94,823           | no          | no ch.                    | AT1G47890   |
| GRMZM5G833699 | hsp90 - heat shock protein, 90 kDa | Hsp90      | 0.1284          | yes         | up       | 0.098,482          | no          | no ch.                    | AT5G52640   |
Fig. 4. Comparison on metabolites involved in serine and threonine metabolic pathways reprogrammed following combined SHS and elevated CO2. Three biological replicates were carried out.

Table 5
Targeted metabolites relevant to metabolic pathways enriched by GO and KEGG analysis with CO2 thermal-mitigation effects in indoor growth chambers.

| Cluster     | # Metabolites | Amb_noSHS | Elv_noSHS | Amb_SHS | Elv-SHS |
|-------------|--------------|-----------|-----------|---------|---------|
|             | Mean         | S.E.      | Mean      | S.E.    | Mean    | S.E.    | Mean    | S.E.    |
| Carbohydrates| 1 starch     | 9.845     | 0.041     | 11.682   | 0.046   | 1.517   | 0.020   | 3.330   | 0.027   |
|             | sucrose      | 77.723    | 1.330     | 66.872   | 0.062   | 0.072   | 0.044   | 0.044   | 0.003   |
|             | trehalose    | 0.331     | 0.003     | 0.437    | 0.006   | 0.166   | 0.004   | 0.346   | 0.003   |
|             | fructose     | 12.148    | 0.323     | 16.621   | 0.437   | 5.292   | 0.349   | 11.518  | 0.358   |
|             | mannose      | 1.618     | 0.113     | 1.422    | 0.121   | 0.586   | 0.012   | 0.67    | 0.020   |
| Amino acids | 1 valine     | 0.213     | 0.021     | 0.254    | 0.005   | 0.894   | 0.139   | 1.364   | 0.136   |
|             | leucine      | 0.346     | 0.035     | 0.301    | 0.030   | 0.765   | 0.077   | 1.038   | 0.104   |
|             | isoleucine   | 0.138     | 0.014     | 0.158    | 0.006   | 0.183   | 0.018   | 0.249   | 0.025   |
|             | glycine      | 1.427     | 0.019     | 1.267    | 0.015   | 1.938   | 0.033   | 2.108   | 0.031   |
|             | threonine    | 2.747     | 0.315     | 2.821    | 0.322   | 3.206   | 0.321   | 3.399   | 0.340   |
|             | alanine      | 2.014     | 0.295     | 2.083    | 0.302   | 3.010   | 0.301   | 3.564   | 0.319   |
|             | serine       | 0.850     | 0.109     | 0.967    | 0.117   | 1.102   | 0.110   | 1.474   | 0.118   |
| Organic acids| 1 glyoxylate | 0.551     | 0.019     | 0.548    | 0.017   | 1.837   | 0.007   | 2.216   | 0.011   |
|             | aspartate    | 7.891     | 0.037     | 7.379    | 0.033   | 6.289   | 0.078   | 6.121   | 0.071   |
|             | glutamate    | 5.232     | 0.064     | 3.867    | 0.052   | 8.807   | 0.127   | 6.604   | 0.101   |
|             | pyruvate     | 0.648     | 0.017     | 0.773    | 0.021   | 0.106   | 0.018   | 0.155   | 0.018   |
|             | citrate      | 1.063     | 0.016     | 1.027    | 0.019   | 0.105   | 0.016   | 0.255   | 0.017   |

Note: Metabolic responses of maize leaves to CO2 and heat stress treatments were presented as: ambient CO2 with non-heat stress (Amb_noSHS), elevated CO2 with non-heat stress (Elv_noSHS), ambient CO2 with heat stress (Amb_SHS), elevated CO2 with heat stress (Elv-SHS). One-way ANOVA was used to estimate the significant effects of CO2 and heat stress on each metabolite in maize leaves, while different alphabet letters represent significant difference at $P < 0.05$. 


with soil temperature average of 25.7 ± 0.33 °C/14.8 ± 0.41 °C day/night. The light intensity and photoperiod were 1000 μmol m⁻² s⁻¹ and 12/12 h, respectively. Local air humidity was 60% during the day time.

2.2. Experimental design

SQ corn variety grown in fields OTCs and growth chambers for 20 days under ambient and high concentrations of CO₂ as mentioned above. The marked part of the whole intact leaves is placed in a water jacketed leaf chamber (Fig. 5C), with the internal radiator and fan for 2 hours of SHS treatment as described earlier [4]. By circulating heated water from the temperature control tank to the leaf cuvettes

| Maize ID       | Gene name                        | Abbreviation | Amb_noSHS | Elv_noSHS | Amb_SHS | Elv_SHS | log2FC (SHS/ck) |
|----------------|----------------------------------|--------------|-----------|-----------|---------|---------|-----------------|
| GRMZM2G069486  | β-amylase                        | AMY8         | 9.088     | 18.398    | 2.682   | 14.324  | 0.537           |
| GRMZM2G068943  | Threahloase 6-phosphate synthase | TPS          | 0.381     | 0.414     | 0.147   | 0.314   | 0.572           |
| GRMZM6G477257  | Phosphoglucone isomerase         | PGI          | 12.682    | 18.701    | 6.325   | 17.263  | 0.711           |
| GRMZM2G129246  | Glycolate oxidase                | G01          | 0.402     | 0.449     | 0.846   | 1.170   | 2.356           |
| GRMZM2G382914  | Phosphoglycerate kinase          | PK2          | 0.613     | 0.759     | 0.182   | 0.290   | 0.340           |
| GRMZM2G438998  | Mannose phosphate isomerase      | MPI          | 1.550     | 2.257     | 0.639   | 1.801   | 0.605           |
| GRMZM2G053939  | Alanine transaminase             | GPT2         | 2.208     | 2.260     | 2.130   | 2.200   | 0.969           |
| GRMZM2G452630  | Serine hydroxymethyltransferase  | SHMT         | 1.363     | 1.197     | 1.853   | 1.910   | 1.477           |
| GRMZM2G473001  | PEP kinase                       | PEPc         | 1.181     | 1.159     | 1.110   | 1.015   | 0.908           |
| GRMZM2G407044  | Acetolactate synthase            | ALS          | 0.349     | 0.312     | 0.621   | 0.730   | 2.059           |
| GRMZM2G094939  | Pyruvate dehydrogenase           | PDH          | 0.289     | 0.408     | 0.275   | 0.203   | 0.724           |
| GRMZM2G064023  | Citrate synthase                 | CS1          | 1.353     | 1.582     | 0.654   | 1.365   | 0.673           |
| GRMZM2G142863  | 2-oxoglutarate dehydrogenase     | OGDH         | 1.015     | 1.048     | 0.278   | 0.097   | 0.184           |
| GRMZM2G178415  | Glutamate dehydrogenase          | GLUD1        | 4.893     | 4.864     | 7.371   | 6.991   | 1.472           |
| GRMZM2G146677  | Aspartate transaminase           | AST          | 7.736     | 7.547     | 6.397   | 6.566   | 0.848           |
| GRMZM2G002205  | Threonine synthase               | TS2          | 0.270     | 0.250     | 0.275   | 0.267   | 1.043           |

Table 6. FPKMs from RNAseq relating to carbon assimilation metabolic pathways in indoor growth chambers.

Fig. 5. Field experimental design and set-up. (A) 4 × 4 randomized block design for field-open top chamber (OTCs) experiments. Ambient and elevated CO₂ chambers were shown in grey and yellow cells, respectively. (B) Image of field OTCs. (C) Image of water-jacketed leaf cuvettes. (D) Image of maize grown under ambient (left) and elevated (right) CO₂ conditions for 20 days.
(Fig. 5C), the air temperature in the cuvette could increase to approximately 45 °C. Air from the OTCs is constantly flushed through each leaf cuvette. Untreated or heat-treated leaves were immediately stored in liquid nitrogen for transcription and metabolic analysis.

2.3. Metabolism measurements

Leaves from six different plants around 20-day old were used (Fig. 5D) for metabolic measurements. ~30 mg leaf tissue with frozen dried is squashed by adding 3.2 mm ceramic beads and 100 μl fine pomegranate powder in 2.0 mL Eppendorf tube, followed by homogenization with a Tissue Lyzer ball mill at 30 cycles s⁻¹ as previously described [4]. The squashed samples were subsequently dissolved using 50 μl mixture consisting of 2.5 mM alpha-aminobutyric acid, 2.0 mg ribitol and 1.4 mL cold 70% methanol and vortexed. Then the mixture was incubated in a water bath at 45 °C for 15 min. After centrifugation for 5 min at 12,000 g, super-fluid was gently transferred to a 15 mL fresh conical plastic centrifuge tube. The particles are washed once with 70% methanol, and the supernatants were combined with previous step. Finally, the mixed supernatants were air-dried overnight and used for determination of starch as previously described [5]. Organic acids, amino acids and soluble carbohydrates were measured by gas chromatography coupled to mass spectrometry (GC-MS) as described elsewhere [6]. Derived samples are performed by GC-MS equipped with mass selective detection (7890 GC system, 7693 automatic sampler, 5975C idle XL MSD). Total ion chromatograms obtained were quantified using Agilent MSD Chemstation software program. Independent standard curves were prepared for each set of extractions with known mixtures of organic acids, amino acids and soluble carbohydrates. Ribitol added during extraction process as internal standard. Compounds in organic acid fraction: 2-oxoglutaric, quinic acid, adipic acid, shikimate, pyruvate, citrate, aconitate, maleic acid, malate, oxalic acid, malonic acid, glyoxyxlate, fumarate and succinate. Compounds in soluble carbohydrate fraction were: ribose, fructose, glucose, myo-inositol, sucrose, maltose, mannose, trehalose, raffinose and starch. The compounds present in amino acids fraction: leucine, Isoleucine, alanine, glycine, serine, valine, threonine, proline, putrescine, aspartate, glutamate and phenylalaine. Five biological replicates, with three technique replicates for each biological one, were conducted for metabolic measurements. Values of standard error (SE) were calculated based on data from three technique and five biological replicates. One-way analysis of variance (ANOVA) via software SPSS 10.0 (SPSS Inc., USA) was applied to identify significant differences between heat stress and CO₂ treatments for specific metabolite in SQ maize cultivar leaves.

2.4. Transcriptomes measurements

Total RNA was extracted using TRIzol® reagents, following manufacturer’s instructions (Invitrogen, Carlsbad, California). Quality and purity of RNA were determined by 1% of agarose gels and nano-drop (IMPLEN, California, USA), respectively. RNA integrity was evaluated via Agilent Bioanalyzer 2100 system (Agilent Technologies, California, USA). The total amount of RNA per sample was normalized to 1.5 μg, which was used as an input for RNA sequencing. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA). Sequencing libraries was featured by Illumina Hiseq 4000 platform with 150bp pair-read was generated [7]. The original read was aligned with B73 reference genome (RefGen_v3), using TopHat2.0.8 and STAR, with a minimum inner length set to 20bp. The gene and heterogeneous are quantified using the GTF annotation file generated by PacBio sequencing. To reduce transcription noise, gene is included only if FPKM value is < 0.01. The value is selected based on the genetic coverage saturation analysis as reported previously [8].

Acknowledgments

We thank Shanghai Orizmes Biotech Co. Ltd. and Shanghai Applied Protein Technology Co., Ltd. for technical service on metabolic determinations and transcriptomes analysis. This work was supported in part by Chinese Strategic Leading project category B (XDB27020105), National Natural Science Foundation of China (31700201) and Sailing Project, Shanghai Municipal Science and Technology Commission, China (17YF1421800), Chinese Strategic Leading project category A (XDA08020301).
Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J. Li, J. Essemine, J.A. Bunce, C. Shang, H. Zhang, D. Sun, G. Chen, M. Qu, Roles of heat shock protein and reprogramming of photosynthetic carbon metabolism in thermotolerance under elevated CO2 in maize, Environ. Exp. Bot. 168 (2019), https://doi.org/10.1016/j.envexpbot.2019.103869.

[2] M. Qu, G. Chen, J.A. Bunce, X. Zhu, R.C. Sicher, Systematic biology analysis on photosynthetic carbon metabolism of maize leaf following sudden heat shock under elevated CO2, Sci. Rep. 8 (2018) 7849, https://doi.org/10.1038/s41598-018-26283-x.

[3] J.A. Bunce, How do leaf hydraulics limit stomatal conductance at high water vapour pressure deficits? Plant Cell Environ. 29 (2006) 1644–1650.

[4] S. Koussevitzky, N. Suzuki, S. Huntington, L. Armijo, W. Sha, D. Cortes, V. Shulaev, R. Mittler, Ascorbate peroxidase 1 plays a key role in the response of Arabidopsis thaliana to stress combination, J. Biol. Chem. 283 (49) (2008) 34197–34203.

[5] R.C. Sicher, J.A. Bunce, Growth, photosynthesis, nitrogen partitioning and responses to CO2 enrichment in a barley mutant lacking NADH-dependent nitrate reductase activity, Physiol. Plant. 134 (1) (2008) 31–40.

[6] U. Roessner, C. Wagner, J. Kopka, R.N. Trethewey, L. Willmitzer, Simultaneous analysis of metabolites in potato tuber by gas chromatography–mass spectrometry, Plant J. 23 (1) (2000) 131–142.

[7] C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D.R. Kelley, et al., Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks, Nat. Protoc. 7 (3) (2012) 562–578.

[8] H.R. Wang, X. Xu, F.G. Vieira, Y.H. Xiao, Z.K. Li, J. Wang, et al., The power of inbreeding: NGS-based GWAS of rice reveals convergent evolution during rice domestication, Mol. Plant 9 (7) (2016) 975–985, https://doi.org/10.1016/j.molp.2016.04.018.