Biochemical Responses of *Brassica rapa* L. to High Temperature Stress

Liam Patrick Mitchell [1*], Mia Charlotte Wilson [2], Jack Harrison Anderson [3]

[1] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia
[2-3] Centre for Cancer Therapeutics Research, The University of Melbourne, Australia

*Corresponding author: **Liam Patrick Mitchell**

Received: 31 May 2021
Revised: 18 Jun 2021
Accepted: 05 Jul 2021
Published: 26 Jul 2021

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2021.1.2.36-40

ABSTRACT

Background: Global warming is increasingly endangering the survival of *Brassica rapa* L., a vegetable with nutritious leaves through disturbances in photosynthesis, cellular membrane integrity, and cellular redox balance.

Aim: It has been aimed in the present study to assess the biochemical response of B. rapa seedlings on exposure to short-term heat stress.

Methodology: The seedlings were subjected to a temperature of 42±2°C in a completely randomized design for 6 hours each day for 5 days along with controls at 25±2°C. Chlorophyll, carotenoids, proline, soluble sugars, proteins, malondialdehyde (MDA), hydrogen peroxide, electrolyte leakage, relative water content, and activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were estimated and results were analyzed using one-way ANOVA ($P<0.05$).

Results: There was a decline in chlorophyll a, chlorophyll b, carotenoids, and the moisture content stemming from heat stress as MDA, H2O2, and ion leakage underwent a surge. Proline and the quantity of sugar present increased as well while SOD, CAT, POD, and APX activity significantly increased relative to the control plants indicating an efficient antioxidant capability and osmotic adjustment mechanism.

Conclusion: In the face of heat stress, B. rapa initiates a concerted biochemical defense strategy via osmotic agent accumulation and inducing antioxidant enzymes, however with just a partial success. These biophysical properties can serve as valuable criteria for developing heat-tolerant varieties amid climatic changes.

Keywords: *Brassica rapa*; Heat stress; Antioxidant enzymes; Oxidative stress; Osmolyte accumulation; Photosynthetic pigments

Introduction

Global climate change is making extreme heat events more and more common, which poses a serious danger to the ability of crops to grow in all parts of the globe. *Brassica rapa* L. (turnip, Chinese cabbage and other variations) is an important Brassicaceae vegetable and is widely grown because of its high nutritional value and economic value, which stem from its vitamins, glucosinolates, and many other phytochemicals rich in antioxidants. It is a cool season crop that grows best in temperatures of around 12–25 °C, so it is sensitive to high temperatures that negatively influence plant germination, growth and yield.

Heat stress affects the way plants work. It breaks down thylakoid membranes and speeds up chlorophyll decomposition, which leads to the reduction of photosynthesis. It also makes membranes more flexible and permeable, leading to increased loss of electrolytes and moisture. One of the most important aspects of heat stress is overproduction of reactive oxygen species (ROS) like superoxide ($O2^{\bullet-}$) and hydrogen peroxide (H2O2), which are formed as a result of the malfunctioning of electron transport in the chloroplasts and mitochondria.

Plants use enzymatic antioxidants such as superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase to protect themselves from oxidative damages. As part of this process, superoxide and hydrogen peroxide are converted into molecular oxygen and water in a stepwise manner. At the same time, plants produce compatible osmolytes including proline and sugars, which facilitate the maintenance of turgidity and protein membrane stabilization in difficult conditions. Thus, the extent of biochemical response will determine the resistance to heat stresses in the particular genotype of the plant.

Although heat-stress physiology is regarded as an area of inquiry, only limited research has been conducted with respect to specific biochemical characteristics of B. rapa in comparison with economic cereal crops. The knowledge about how plants respond biochemically to stress is necessary for the identification of physiological markers of heat resistance. In addition, this knowledge can

be used for breeding processes of plants in a changing climate. Thus, the aim of the present study was to (i) understand how parameters of photosynthetic pigments, osmolytes, and indicators of oxidative damage change under controlled exposure to heat stress and (ii) analyze how antioxidant enzymes respond to the exposure heat stress in the B. rapa seedlings.

Materials and Methods

Plant Material and Growth Conditions

Certified seeds of *Brassica rapa* L. cv. 'Purple Top' were surface-sterilized, germinated, and grown in a 3:1 soil–vermiculite mixture inside a controlled-environment growth chamber (16 h light/8 h dark photoperiod, 150 μmol m^{-2} s^{-1} irradiance, 60–65% relative humidity, 25±2°C). Seedlings were arranged in a completely randomized design with three independent biological replicates per treatment.

Heat Stress Treatment

At the four-leaf stage, seedlings were randomly divided into control and heat-stress groups. Control plants remained at 25±2°C, while heat-stress plants were transferred to a growth chamber maintained at 42±2°C for 6 h daily over five consecutive days, after which the third fully expanded leaf was sampled, flash-frozen in liquid nitrogen, and stored at −80°C (Table 1).

Biochemical Analyses

Chlorophyll a, b, and total carotenoids were determined spectrophotometrically in acetone extracts [9, 10]. Free proline was quantified by the ninhydrin method [11]; soluble sugars by the phenol–sulfuric acid assay [12]; total soluble protein by the Bradford dye-binding method [13]. Lipid peroxidation was estimated as MDA content via the thiobarbituric acid reaction [14]. Hydrogen peroxide was measured using the potassium iodide method [15]. Electrolyte leakage was assessed by conductivity before and after boiling, and relative water content (RWC) was calculated from fresh, turgid, and dry weights. SOD activity was assayed by inhibition of nitroblue tetrazolium photoreduction [16, 19]; CAT and POD activities were determined by monitoring H2O2 and guaiacol oxidation, respectively [16, 17]; APX activity was measured by ascorbate oxidation at 290 nm [18].

Statistical Analysis

Data (mean±SD, n=3 biological replicates) were analyzed by one-way ANOVA followed by Tukey's post-hoc test in SPSS v25.0 (IBM Corp.), with $P<0.05$ considered statistically significant.

Table 1: Experimental design and temperature treatments.

Parameter	Control	Heat stress
Growth stage at treatment	Four-leaf stage	Four-leaf stage
Temperature	25±2°C	42±2°C
Duration	Continuous	6 h/day × 5 days
Photoperiod	16 h light / 8 h dark	16 h light / 8 h dark
Relative humidity	60–65%	55–60%
Biological replicates	3	3
Experimental design	Completely randomized design	Completely randomized design

Results

Heat stress significantly altered all measured biochemical parameters relative to control plants (Table 2). Chlorophyll a, chlorophyll b, and carotenoid contents declined by 37.3%, 37.7%, and 29.4%, respectively, consistent with heat-accelerated pigment degradation and chloroplast destabilization. Relative water content decreased by 22.8%, while electrolyte leakage nearly tripled (+171.8%), reflecting compromised membrane integrity under thermal stress.

Oxidative damage markers increased sharply: MDA content rose by 121.4% and H2O2 accumulation by 119.4%, indicating substantial lipid peroxidation and ROS overproduction. In parallel, osmoprotectant accumulation was pronounced, with proline increasing 181.7% and soluble sugars 67.8% above control levels, while total soluble protein declined by 28.1%, likely reflecting heat-induced protein denaturation and altered proteolytic turnover.

All four antioxidant enzymes showed significant activation under heat stress (Table 2; Figure 2). SOD activity increased by 81.6%, CAT by 88.2%, POD by 102.9%, and APX by 106.5% relative to controls, indicating a broad, coordinated enzymatic response aimed at ROS detoxification. Table 3 summarizes the overall direction and functional significance of these biochemical shifts, illustrating a consistent pattern of pigment and membrane deterioration counterbalanced by osmotic and antioxidant adjustment.

Table 2: Biochemical parameters measured under control and heat stress conditions (mean±SD, n=3). *Significant difference from control, P<0.05 (ANOVA, Tukey's test).

Parameter	Control	Heat stress	% Change
Chlorophyll a (mg g^{-1} FW)	1.42±0.08	0.89±0.06*	−37.3
Chlorophyll b (mg g^{-1} FW)	0.61±0.05	0.38±0.04*	−37.7
Carotenoids (mg g^{-1} FW)	0.34±0.03	0.24±0.02*	−29.4
Proline (µg g^{-1} FW)	18.6±1.9	52.4±4.1*	+181.7
Soluble sugars (mg g^{-1} FW)	12.1±1.1	20.3±1.6*	+67.8
Total protein (mg g^{-1} FW)	8.9±0.7	6.4±0.5*	−28.1
MDA (nmol g^{-1} FW)	9.8±0.9	21.7±2.0*	+121.4
H2O2 (µmol g^{-1} FW)	3.1±0.3	6.8±0.6*	+119.4
Electrolyte leakage (%)	14.2±1.3	38.6±3.2*	+171.8
Relative water content (%)	88.5±2.1	68.3±3.0*	−22.8
SOD (U mg^{-1} protein)	42.3±3.5	76.8±5.4*	+81.6
CAT (U mg^{-1} protein)	18.7±1.6	35.2±2.8*	+88.2
POD (U mg^{-1} protein)	24.1±2.0	48.9±3.9*	+102.9
APX (U mg^{-1} protein)	15.3±1.4	31.6±2.6*	+106.5

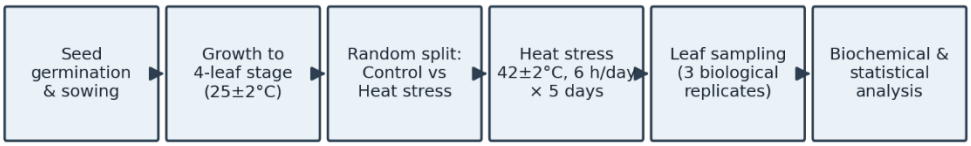

Fig 1: Experimental workflow illustrating heat-stress treatment and biochemical analyses.

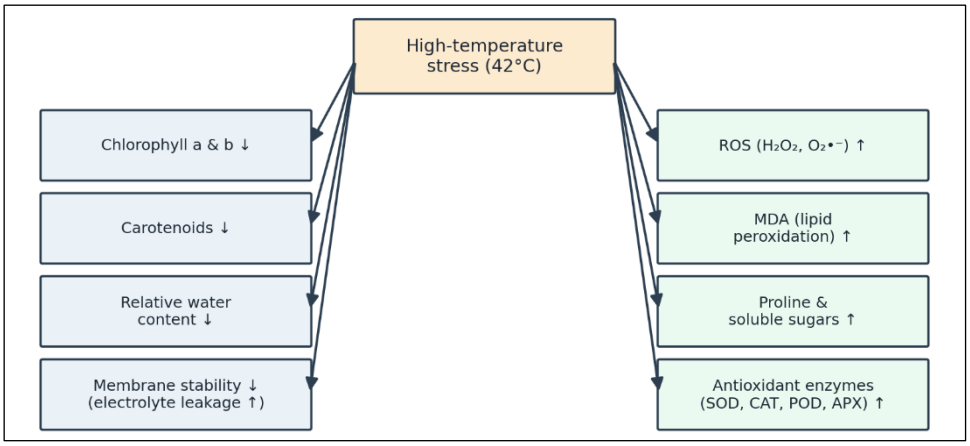

Fig 2: Schematic representation of biochemical responses of *Brassica rapa* under high-temperature stress.

Table 3: Summary of physiological and biochemical responses of *Brassica rapa* to heat stress.

Response category	Direction under heat stress	Physiological significance
Photosynthetic pigments	Decreased	Reduced light-harvesting capacity and photosynthetic efficiency
Osmolytes (proline, sugars)	Increased	Osmotic adjustment, membrane and protein stabilization
Oxidative damage (MDA, H2O2)	Increased	Evidence of lipid peroxidation and ROS accumulation
Membrane stability / RWC	Decreased	Impaired cellular water status and membrane integrity
Antioxidant enzymes (SOD, CAT, POD, APX)	Increased	Enhanced ROS-scavenging and stress tolerance capacity

Discussion

The observed decline in chlorophyll and carotenoid content reflects heat-accelerated pigment degradation, likely mediated by chlorophyllase activity and thylakoid membrane disruption, which together diminish light-harvesting efficiency and net photosynthesis. Because carotenoids also serve a photoprotective, ROS-quenching role, their depletion under stress likely compounds oxidative pressure on the photosynthetic apparatus rather than solely reflecting reduced pigment synthesis.

The concurrent rise in MDA and H2O2 confirms that heat stress in B. rapa is accompanied by substantial oxidative damage, arising from ROS overproduction that outpaces basal scavenging capacity. The marked induction of SOD, CAT, POD, and APX activities represents an adaptive enzymatic response: SOD catalyzes the dismutation of superoxide to H2O2, which is subsequently detoxified by CAT (via direct decomposition) and

by POD/APX (via the ascorbate–glutathione cycle). That oxidative markers still rose despite enzyme induction suggests the antioxidant system was upregulated but only partially sufficient to offset ROS accumulation at this stress intensity and duration — a pattern typical of moderate-to-severe, rather than mild, heat stress.

The substantial accumulation of proline and soluble sugars indicates active osmotic adjustment. Beyond osmotic buffering, proline is known to stabilize protein and membrane structures and may act as a ROS scavenger and redox buffer, providing a secondary line of defense complementary to the enzymatic system. The decline in relative water content and the sharp rise in electrolyte leakage together point to heat-induced membrane destabilization, consistent with the lipid peroxidation evidenced by elevated MDA.

From an agricultural standpoint, these biochemical shifts — particularly proline accumulation, antioxidant enzyme induction, and membrane stability indices — represent practical, measurable markers for screening heat tolerance among B. rapa germplasm. Breeding programs could integrate MDA content and antioxidant enzyme activity as selection criteria alongside conventional agronomic traits, or pursue marker-assisted or transgenic approaches targeting HSP and antioxidant-pathway genes to enhance thermotolerance.

This study has limitations: it examined a single cultivar and short-term (5-day) stress exposure under controlled conditions, which may not fully capture field-level heat dynamics, diurnal fluctuation, or genotype-by-environment interactions. Future work should compare multiple cultivars of contrasting thermotolerance, extend exposure duration, and integrate transcriptomic or metabolomic profiling to more precisely resolve the regulatory networks underlying the biochemical responses reported here.

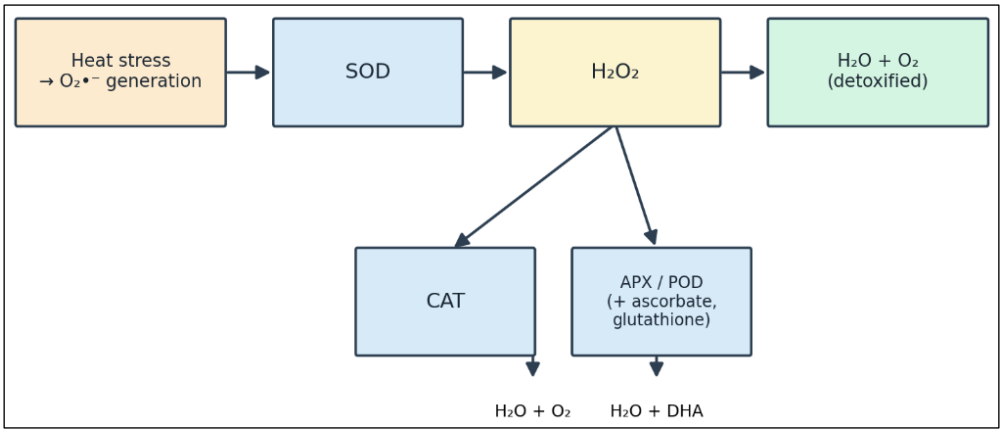

Fig 3: Proposed antioxidant defense mechanism activated during heat stress.

Conclusion

High-temperature condition affects *Brassica rapa* L. and leads to biochemical changes which include reduction of photosynthetic pigments and decrease of membrane stability, but at the same time, it leads to the rise of osmoprotectants content and activity of antioxidant enzymatic system SOD–CAT–POD–APX. These adaptive mechanisms enable the compensation of some oxidative and photosynthetic damages under the adverse high-temperature conditions. Therefore, it is possible to apply the biochemical markers for practical purposes to screen and develop heat-resistant varieties of B. rapa, which is especially important for the improvement of vegetable production in conditions when climate change leads to the rise of adverse high-temperature conditions.

References

1. Zhang L, Dai Y, Yue L, Chen G, Yuan L, Zhang S, *et al*. Heat stress response in Chinese cabbage (*1*L.) revealed by transcriptome and physiological analysis. PeerJ. 2022;10:e13427.
2. Yue L, Li G, Dai Y, Sun X, Li F, Zhang S, *et al*. Gene co-expression network analysis of the heat-responsive core transcriptome identifies hub genes in *Brassica rapa*. Planta. 2021;253:111.
3. Guan Q, Lu X, Zeng H, Zhang Y, Zhu J. Heat stress induction of miR398 triggers a regulatory loop that is critical for thermotolerance in Arabidopsis. The Plant Journal. 2013;74(5):840-851.
4. Song Y, Chen Q, Ci D, Shao X, Zhang D. Effects of high temperature on photosynthesis and related gene expression in poplar. BMC Plant Biology. 2014;14:111.
5. Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science. 2013;4:273.
6. Chen S, Li H. Heat stress regulates the expression of genes at transcriptional and post-transcriptional levels, revealed by RNA-seq in Brachypodium distachyon. Frontiers in Plant Science. 2017;7:2067.
7. Yu J, Li P, Tu S, Feng N, Chang L, Niu Q. Integrated analysis of the transcriptome and metabolome of *Brassica rapa* revealed regulatory mechanism under heat stress. International Journal of Molecular Sciences. 2023;24(18):13993.
8. Jiang J, Bai J, Li S, *et al*. HTT2 promotes plant thermotolerance in *Brassica rapa*. BMC Plant Biology. 2018;18:334.

9. Arnon DI. Copper enzymes in isolated chloroplasts: polyphenoloxidase in Beta vulgaris. Plant Physiology. 1949;24(1):1-15.
10. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology. 1987;148:350-382.
11. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-207.
12. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;28(3):350-356.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72(1-2):248-254.
14. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968;125(1):189-198.
15. Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Science. 2000;151(1):59-66.
16. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany. 1981;32(1):93-101.
17. Aebi H. Catalase in vitro. Methods in Enzymology. 1984;105:121-126.
18. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 1981;22(5):867-880.
19. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971;44(1):276-287.
20. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology. 2004;55:373-399.
21. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002;7(9):405-410.
22. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: an overview. Environmental and Experimental Botany. 2007;61(3):199-223.
23. Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences. 2013;14(5):9643-9684.
24. Intergovernmental Panel on Climate Change. *Climate Change 2021: The Physical Science Basis.* Cambridge: Cambridge University Press; 2021.
25. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 2007;59(2):206-216.
26. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development. 2009;29(1):185-212.
27. Suzuki N, Mittler R. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiologia Plantarum. 2006;126(1):45-51.
28. Larkindale J, Hall JD, Knight MR, Vierling E. Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiology. 2005;138(2):882-897.
29. Camejo D, Rodriguez P, Morales MA, Dell'Amico JM, Torrecillas A, Alarcon JJ. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. Journal of Plant Physiology. 2005;162(3):281-289.
30. Zhu J-K. Abiotic stress signaling and responses in plants. Cell. 2016;167(2):313-324.