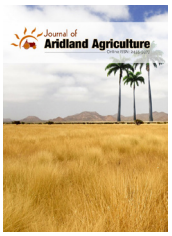
Journal of Aridland Agriculture 2024, 10: 157-163
doi: 10.25081/jaa.2024.v10.9300
https://updatepublishing.com/journal/index.php/jaa

Research Article



ISSN: 2455-9377

Effect of high-temperature stress on phenylpropanoid biosynthesis and antioxidant activities of *Acer palmatum*

Jiwon Yoon[1], Haejin Kwon[2], Jinsu Lim[3], Kihyun Kim[1], Hyewon Seo[2], Md Romij Uddin[4], Kohila Manickam[5], Jae Kwang Kim[6]*, Sang Un Park[1,2,3,7]*

[1]Department of Crop Science, Chungnam National University, 99 Daehak-ro, Daejeon 34134, Republic of Korea, [2]Department of Smart Agriculture Systems, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea, [3]Department of Bio-AI Convergence, Chungnam National University, 99 Daehak-ro, Daejeon 34134, Republic of Korea, [4]Department of Agronomy, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, [5]Department of Computer Science, Shrimati Indira Gandhi College, Trichy-620002, Tamil Nadu, India, [6]Division of Life Sciences and Convergence Research Center for Insect Vectors, Incheon National University, Incheon 22012, Republic of Korea, [7]EuHerb Inc., 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea

ABSTRACT

Acer palmatum is an ornamental tree grown in East Asia and is exposed to the possible hazards of global climate changes and increased temperatures. In this context, a study was conducted in a greenhouse at the experimental farm of Chungnam National University, Daejeon, South Korea from April to August 2023, to explore the consequences of high temperature stress on A. *palmatum* and assess the phenylpropanoid biosynthesis, antioxidant activity, and chlorophyll content in its leaves. One-year-old seedlings were treated at 40 °C for 1-3 days to determine the phenolic and flavonoid content, chlorophyll concentrations, and antioxidant activity at various intervals. The highest phenylpropanoid, total phenolic compound, and total flavonoid contents were observed during the first 2 days of high temperature treatment and reduced afterwards. Regarding the duration of the plant's exposure, 1 day of exposure was associated with the highest antioxidant level assessed with DPPH and ABTS assays, while antioxidant activity was lowest under a long duration. The chlorophyll B content increased 2-fold with 1 day of heating exposure, in contrast to the levels of chlorophyll A and B. However, A. *palmatum* benefitted from a moderate level of heat stress, which stimulated the development of protective biochemical responses without developmental features. At the same time, extended exposure compromised the physiological health of the plant, reducing the phenolic content, antioxidant activity, and chlorophyll content.

KEYWORDS: *Acer palmatum*, Phenolic compounds, Flavonoids, Antioxidant activities

Received: September 04, 2024
Revised: November 12, 2024
Accepted: November 20, 2024
Published: November 27, 2024

***Corresponding authors:**
Jae Kwang Kim
E-mail: kjkpj@inu.ac.kr
Sang Un Park
E-mail: supark@cnu.ac.kr

INTRODUCTION

The palmate maple (*Acer palmatum*), a deciduous tree belonging to the Sapindaceae family, is well-known for its vibrant red leaves in the fall. Trees from the *Acer* genus are widely distributed, particularly in East Asia, North America, and Europe (Sun *et al.*, 2022). A. *palmatum* is an ornamental deciduous tree that is famous for its colourful foliage and extensively cultivated in Japan, Korea, and China (Wan *et al.*, 2023). The leaves of A. *palmatum* contain chlorophyll and accessory pigments, such as carotenoids and anthocyanins. However, these pigments remain hidden by chlorophyll during the spring and summer. As days shorten and temperatures drop in the fall, chlorophyll production ceases, and the existing chlorophyll decomposes. An abscission layer forms between the leaves and branches, allowing the leaves to fall off in the wind. Since starch cannot be transported to the stem due to the abscission layer, it accumulates in the leaves, eventually causing chlorophyll degradation. Pigments, such as carotenoids, then become visible, leading to the characteristic red colour of the leaves (Primka & Smith, 2019).

Global warming has led to rising temperatures each year, making high-temperature stress a significant factor that negatively affects plant development and growth. High temperatures disrupt photosystem II, reduce photosynthetic and respiratory activities, and diminish photosynthetic pigments (Elnaggar *et al.*, 2024). They also lead to metabolic imbalances, oxidative damage, and ultimately plant wilting and death (Haider *et al.*, 2021). Plants respond to heat stress by undergoing various physiological and biochemical changes. High temperatures disturb plant cell homeostasis, ion distribution, and osmotic

balance, leading to reactive oxygen species (ROS). Woody plants possess antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and ascorbate peroxidase (APX), which act as defence mechanisms to remove ROS from plant cells (Rajput *et al.*, 2021). Although antioxidant enzyme activity increases under heat stress, it tends to decrease when the temperature exceeds the plant's tolerance limit, causing structural damage and reducing the antioxidant enzyme activity (Yamane *et al.*, 2022).

Plant metabolism is greatly influenced by environmental stressors, such as pathogen attack, high light, and heat stress. When stress occurs, growing plants start producing increased amounts of phenylpropanoids because of increased secondary metabolism (Ashraf *et al.*, 2018). Phenylpropanoids are a family of organic compounds synthesised by plants, bacteria, and fungi. They are responsible for the protection of cells against ultraviolet irradiation and resistance to various pathogens (Ortiz & Sansinenea, 2023). Phenylpropanoids give flowers their colour and scent and act as a signal that the organism is under stress. The substances are the result of trans-cinnamic acid synthesis, which is produced as a result of the deamination of L-phenylalanine, and achieved with phenylalanine ammonia-lyase (Kong, 2015).

When plants face stress, secondary metabolism increases the amount of phenylpropanoids (Dixon & Paiva, 1995), that accumulate. Phenylpropanoids comprise a large group of such organic compounds, which are synthesised by plants, bacteria, and fungi (Gumul *et al.*, 2007). These substances help cells resist ultraviolet light; with the help of these compounds, plants inhibit a number of herbivores and pathogens. In addition, phenylpropanoids are a group of compounds that colour flowers as well as give them a scent. Moreover, phenylpropanoids are also an indicator of stress. That is, when the plant faces stress, it starts producing phenylpropanoids, which are synthesised from trans-cinnamic acid, and formed after the deamination reaction of L-phenylalanine with the enzyme phenylalanine ammonia lyase (Herrmann, 1995; Solecka, 1997).

Formation of this acid is a mechanism of connecting the secondary metabolites with the primary metabolites, such as amino acids (Stapleton, 1992). In response to UV, substances such as anthocyanins and other non-flavonoid phenylpropanoids accumulate in the epidermis. Phenylpropanoid biosynthesis is easily modulated, as it depends on plant growth and stress. Generally, phenolic substances accumulate during stress (Liu *et al.*, 2023); the production of secondary metabolites, especially those with antioxidant effects, increases so that the plant can better tolerate these conditions (Cheynier *et al.*, 2013). Recent studies have shown that specific phenylpropanoids and flavonoids in carrot cells are associated with heat damage (Commisso *et al.*, 2016), and treatment of wheat with zinc and plant growth-promoting rhizobacteria (PGPRs) increases phenolic and organic acid content in root exudates (Rehman *et al.*, 2018).

Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. In plants, high temperatures cause oxidative stress by inducing excessive ROS production. Although ROS serve as signal molecules, their excessive accumulation disrupts electron transport in mitochondria and chloroplasts, causing significant damage to cellular components, such as proteins, nucleic acids, and membrane lipids (Aroca *et al.*, 2003; Imlay, 2003). Secondary metabolites synthesised under stress act as antioxidants, protecting cells from the harmful effects of ROS, lipid peroxidation, protein denaturation, and DNA damage (do Nascimento & Fett-Neto, 2010; Król *et al.*, 2015). Phenolic acids are considered to possess antioxidant and radical-scavenging activities and make a significant contribution to reducing ROS-induced damage. The synthesis of intracellular phenolic compounds largely follows induced changes under environmental stress. As a result of the phenolic compounds, ROS are scavenged, the reaction of complex formation with metals is formed, and activation of the oxidative enzymes is increased (Elavarthi & Martin, 2010). This process leads to an increase in the resistance of the plant to stress-inducing factors with an increase in the antioxidant enzyme activity (Hayat *et al.*, 2010). Plants differentially react to stress exposure and have developed various mechanisms to survive and adapt. The given study investigates the biochemical properties in terms of the response of *A. palmatum* to high-temperature exposure, examining the phenylpropanoid biosynthesis, antioxidant systems and the chlorophyll content in the leaves.

MATERIALS AND METHODS

Plant Materials

In April 2023, 1-year-old palmate maple seedlings, approximately 30 cm tall, were purchased from Yeonggwang Botanical Garden, Yeonggwang-gun, Jeollanam-do, Republic of Korea. The seedlings were grown in a greenhouse at the experimental farm of Chungnam National University, Daejeon, South Korea, until August, when they were subjected to high-temperature treatment.

High-Temperature Stress Treatment

To carry out high-temperature treatment, palmate maples were moved to the growth chamber and grown for 4 months. Palmate maples growing in the chamber were treated at a high temperature, 40 °C, for 3 days. Dry, high temperature-treated samples of palmate maples were collected every day for 3 days, stored at -70 °C, then freeze-dried. The samples were then ground into a fine powder for further analysis.

High-Performance Liquid Chromatography (HPLC) Analysis of Phenylpropanoids

A method similar to that of a previous study was applied to analyse the phenylpropanoid content (Sathasivam *et al.*, 2022). For this purpose, 100 mg of powdered plant material was added to 1 mL of 80% MeOH and then sonicated at 25 °C for 1 h. It was then centrifugated at 12,000 rpm for 10 min, and the supernatant was collected. The settings for the HPLC system used and the details of the applied gradient programme were previously described by (Sathasivam *et al.*, 2022).

Determination of the Total Phenolic (TPC) and Flavonoid Contents (TFC)

In our experiment, TPC and TFC were determined following the methodology described by Lim *et al.* (2024). The extract of all samples was adjusted to a concentration of 10,000 ppm. TPC was analysed at a wavelength of 760 nm after the reaction of the sample with Folin–Ciocalteu reagent and sodium carbonate. The phenolic content was determined based on the gallic acid calibration curve. The TFC was determined by reaction a diluted extract with sodium nitrite and aluminium chloride, and the absorbance was read at 415 nm. The flavonoid content was calculated based on the quercetin calibration curve. The absorbance was recorded using a spectrophotometer (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany).

Chlorophyll Content

For chlorophyll content analysis, 200 mg of sample powder was mixed with 4 mL of 100% ethanol, vortexed, and incubated at 4 °C for 1 h. The mixture was then centrifuged at 14,000 rpm for 5 min, and 200 µL of the supernatant was mixed with 1 mL of 100% ethanol. After incubating at 4 °C for 5 min, the absorbance was measured at wavelengths of 663.6 and 646.6 nm. The chlorophyll A content was calculated using the formula: (12.25 × absorbance at 663.6 nm) - (2.55 × absorbance at 646.6 nm) and the chlorophyll B content was calculated using the formula: (20.31 × absorbance at 646.6 nm) - (4.91 × absorbance at 663.6 nm). All samples were measured in triplicate.

Determination of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity

A method described by Lim *et al.* (2024) was used to determine the scavenging activity of DPPH. Briefly, 30 µL of each sample concentrated from 31.25 to 1000 µg/mL was mixed with 170 µL DPPH solution (0.2 mM in 99.9% non-denatured MeOH). The solution was mixed and incubated in the dark for 20 min, and the absorbance at 517 nm using a SPECTRO Star Nano plate reader. The percentage of DPPH radicals scavenged by the sample was calculated using the equation described by Lim *et al.* (2024).

Determination of 2,2′-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Free Radical Scavenging

ABTS free radical scavenging activity followed a modified method from a previous paper Lim *et al.* (2024). To analyse ABTS radical scavenging, the ABTS solution was prepared by reacting 7 mM of ABTS powder with 2.5 mM potassium persulfate, incubating for 16 h in the dark, and adjusting to an absorbance of 0.7±0.01 at 734 nm. A 30 µL sample of each extract at a concentration of 0-1000 ppm was mixed with 170 µL of ABTS solution, and the absorbance was measured at 734 nm using a spectrophotometer.

Determination of Reducing Power

Reducing power is the most representative indicator for measuring samples that can be used as antioxidants and refers to the ability to reduce oxidised substances. Reducing power analysis was performed following (Lim *et al.*, 2024). Sample extracts ranging from 31.25 to 10,000 ppm were mixed with sodium phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide ($C_6N_6FeK_3$, w/v) and then incubated. After adding 10% trichloroacetic acid buffer ($C_2HCl_3O_2$, w/v), the samples were centrifugated, and 0.1% ferric chloride ($FeCl_3$, w/v) was added. The absorbance was measured at 700 nm. The higher the absorbance, the stronger the reducing power.

Statistical Analysis

Statistical analysis was performed with analysis of variance (ANOVA) using SPSS Statistics 26.0 (IBM Corporation, New York, NY, USA). Significant differences were determined using Duncan's multiple range test at the $p<0.05$ level. The results are presented as the mean±standard deviation, with all experiments conducted in triplicate.

RESULTS

Analysis of Phenolics in *A. palmatum* Leaves

HPLC analysis was performed to investigate phenylpropanoid accumulation in A. *palmatum* leaves treated at 40 °C. Eight substances were identified and quantified: catechin, caffeic acid, (-)-epicatechin, epicatechin gallate, ferulic acid, sinapic acid, rutin, and trans-cinnamic acid (Table 1). There was a difference in the total amount of phenylpropanoids depending on the number of days of treatment. Compared to samples not treated at high temperatures, the largest amount of phenylpropanoid accumulated in samples treated at high temperatures for 1 and 2 days. Caffeic acid was only detected in samples treated at high temperatures, and phenylpropanoids were detected most frequently at 2.3633±0.0723 mg/g dry weight (DW) in samples treated for 1 day among those treated at high temperatures. Catechin increased almost 2-fold to 0.2325±0.0071 mg/g DW in samples that were not treated at high temperatures and to 0.444±0.0205 mg/g DW in samples that were treated at high temperatures for 2 days. However, sinapic acid showed a higher concentration in samples that were not treated at high temperatures, and rutin increased in samples that were treated at high temperatures. Overall, compared to non-treated samples, plants under high-temperature treatment showed a higher phenylpropanoid content, but the phenylpropanoid content decreased over time. Thus, high-temperature treatment for a certain period affected phenylpropanoid accumulation, and excessive high-temperature treatment reduced phenylpropanoid accumulation.

Total Phenolic Content and Total Flavonoid Content in *A. palmatum* Leaves

TPC was compared in A. *palmatum* leaves during a period of high-temperature treatment. The TPC of A. *palmatum* leaves

Table 1: Effect of high-temperature stress on the phenylpropanoid content (mg/g dry weight) in *Acer palmatum* leaves

Compound	0 Day	1 Day	2 Days	3 Days
Catechin	0.2325±0.0071^{b}	0.4398±0.0171^{a}	0.444±0.0205^{a}	0.2251±0.0038^{b}
Caffeic acid	ND	0.0259±0.0003^{b}	0.0236±0.0004^{c}	0.0289±0.0001^{a}
(-)-Epicatechin	0.2571±0.0074^{b}	0.3574±0.0263^{a}	0.2363±0.0133^{b}	0.14±0.0041^{c}
Epicatechin gallate	0.0921±0.004^{c}	0.1618±0.0006^{a}	0.0924±0.0023^{c}	0.1048±0.0005^{b}
Ferulic acid	0.0012±0.0001^{c}	0.0018±0.0002^{c}	0.0048±0.0004^{a}	0.0035±0.0002^{b}
Sinapic acid	0.0041±0.0009^{a}	0.0033±0.0007^{ab}	0.0041±0.0014^{a}	0.0008±0.0009^{c}
Rutin	0.3549±0.0021^{c}	1.3969±0.0274^{a}	1.0293±0.008^{b}	0.36±0.0023^{c}
Trans-cinnamic acid	0.0009±0^{c}	0.0024±0^{b}	0.0027±0.0001^{a}	0.0028±0.0001^{a}
Total	0.9427±0.0216^{c}	2.3633±0.0723^{a}	1.8136±0.0459^{b}	0.837±0.0117^{d}

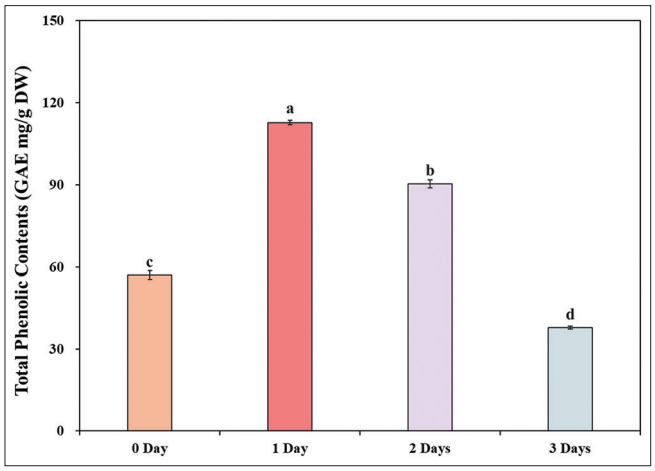


Figure 1: Total phenolic content in *Acer palmatum* leaves. Different alphabetical letters in the values represent statistically significant differences among the means according to Duncan's multiple range test (ANOVA, p<0.05). Values are means±Standard deviation

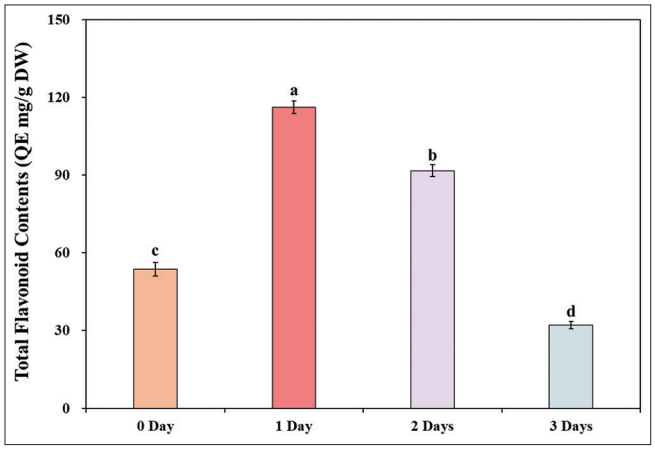


Figure 2: Total flavonoid content in *Acer palmatum* leaves. Different alphabetical letters in the values represent statistically significant differences among the means according to Duncan's multiple range test (ANOVA, p<0.05). Values are means±Standard deviation

treated at 40 °C for 1 day was 112.7±0.83 gallic acid (GAE) mg/g DW, which was two times higher than that in the control group (56.98±1.64 GAE mg/g DW), which was not treated at a high temperature. Similarly, leaves treated at high temperatures for 2 days (90.37±1.45 GAE mg/g DW) showed lower values than those treated at a high temperature for 1 day but about 1.6 times higher values than the control group (Figure 1). However, when

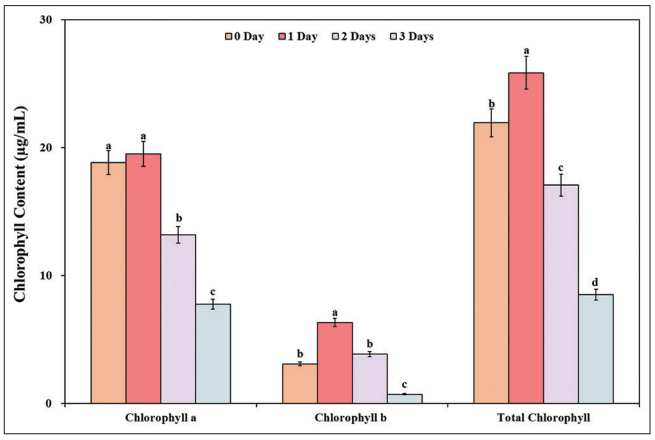


Figure 3: Chlorophyll content in *Acer palmatum* leaves. Different alphabetical letters in the values represent statistically significant differences among the means according to Duncan's multiple range test (ANOVA, p<0.05). Values are means±Standard deviation

treated at a high temperature for 3 days (37.76±0.54 GAE mg/g DW), TPC was lower than that of the control group. The tendency for TPC to decrease as the high-temperature treatment period increased was consistent with the results of phenylpropanoid content using HPLC.

TFC was similarly compared in A. *palmatum* leaves during a period of high-temperature treatment. TFC tended to be consistent with TPC, with A. *palmatum* leaves treated at 40 °C for 1 day showing the highest flavonoid content at 116.18±2.46 quercetin (QE) mg/g DW (Figure 2). This was about 2.2 times higher compared to the control group (53.68±2.61 QE mg/g DW), and for the sample treated at a high temperature for 2 days, it was 91.71±2.35 QE mg/g DW, which was about 1.7 times higher than the control group. The sample treated at a high temperature for 3 days showed a lower content than the control group at 32.08±1.42 QE mg/g DW.

Chlorophyll Contents in *A. palmatum*

Figure 3 shows the results of the chlorophyll content in A. *palmatum* leaves treated at high temperatures. The chlorophyll A content in the control group was 18.844 µg/mL, and it was 19.532 µg/mL after treatment at a high temperature for 1 day, with no significant difference. The chlorophyll B content was 18.844 µg/mL in the control group. The sample treated at a high temperature for 1 day had a content approximately 2-fold higher at 6.334 µg/mL. The chlorophyll A

and B contents of the sample treated at a high temperature for 2 days were 13.189 and 3.881 μg/mL, respectively, which were lower than those in the control group. Similarly, the chlorophyll A and B contents in the sample treated at a high temperature for 3 days were the lowest at 7.772 and 0.729 μg/mL, respectively. These results show that chlorophyll accumulates the most when treated at a high temperature for one day.

In Vitro Antioxidant Assays

To measure the antioxidant activity of A. *palmatum* leaves treated at a high temperature *in vitro*, DPPH and ABTS assays, which measure the free radical scavenging ability, were used. As shown in Figures 4 and 5, A. *palmatum* leaves treated at 40 ℃ for 1 day showed the highest efficiency in the DPPH scavenging activity assay compared to the control. The sample treated at a high temperature at a concentration of 1000 μg/mL showed a radical scavenging activity of over 62.99±0.01% (Figure 4). The ABTS assay showed similar results to those of the DPPH assay. At 500 μg/mL, the high-temperature-treated sample showed a scavenging ability of approximately 92.36±0.0%, which was higher than that in the control sample (72.56±0.01%).

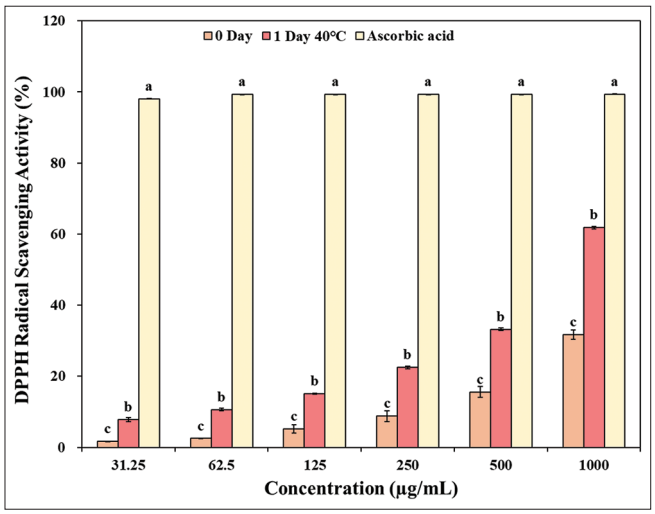


Figure 4: DPPH radical scavenging activity in *Acer palmatum* leaves. Different alphabetical letters in the values represent statistically significant differences among the means according to Duncan's multiple range test (ANOVA, $p<0.05$). Values are means±Standard deviation

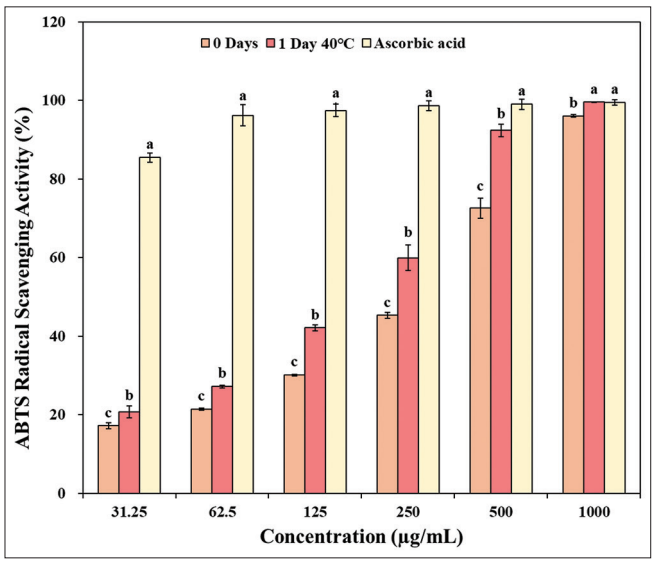


Figure 5: ABTS radical scavenging activity in *Acer palmatum* leaves. Different alphabetical letters in the values represent statistically significant differences among the means according to Duncan's multiple range test (ANOVA, $p<0.05$). Values are means±Standard deviation

As shown in Figure 6, the reducing power increased further in A. *palmatum* leaf samples treated with a high temperature. At 250 μg/mL, the samples treated at high temperatures showed no significant difference (0.208±0.003), but at a concentration of 500 μg/mL, the samples treated at high temperatures showed a higher reducing power. Similarly, at 1000 μg/mL, the control group had 0.363±0.006, while the high-temperature-treated sample had a higher reducing power of 0.533±0.011. The consistent trend observed in the DPPH, ABTS, and reducing power analyses suggests that maple wood treated at high temperatures for 1 day has higher antioxidant activity.

DISCUSSION

This research aimed to determine the physiological and biochemical responses of A. *palmatum* to high temperature stress in phenylpropanoid biosynthesis, antioxidant activity, and chlorophyll content. The results suggest that plants exhibit protective biochemical responses under short-term high-temperature exposure, while long-term exposure diminishes them. Such results indicate a more complex relationship between the length of stress exposure and plant health.

Phenylpropanoid accumulation, particularly 1 and 2 days after heat treatment, indicates that high temperatures likely activate the pathways responsible for secondary metabolite production, acting as a stress response. According to previous studies, phenylpropanoids play an essential role in protecting plants from environmental stress because they are responsible

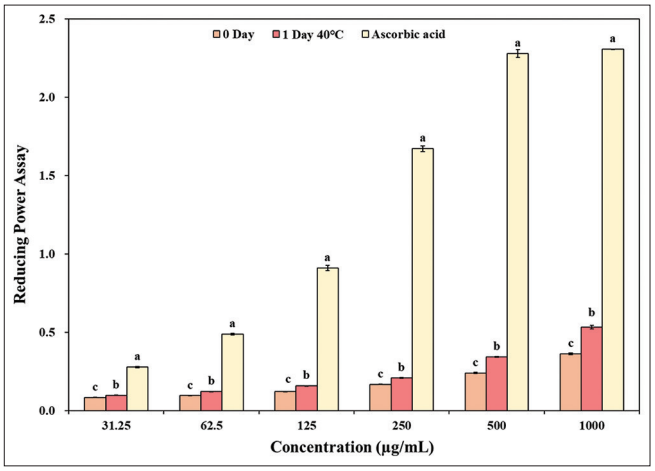


Figure 6: Reducing power in *Acer palmatum* leaves. Different alphabetical letters in the values represent statistically significant differences among the means according to Duncan's multiple range test (ANOVA, $p<0.05$). Values are means±Standard deviation

for scavenging ROS in the cell and increasing the antioxidant capacity of the plant in general (Solecka, 1997; Cheynier *et al.*, 2013). Therefore, long-term heat exposure may overcome the plant's capacity to maintain a high level of biosynthesis for these compounds (Stapleton, 1992; Dixon & Paiva, 1995).

Similarly, the dynamics of the total phenolic content and total flavonoid content followed a similar pattern, peaking after 1 day of heat treatment. The increase in these compounds is most likely related to heat-induced oxidative stress since the plant tries to protect the processes affected by ROS and high concentrations of the toxic compounds (Elavarthi & Martin, 2010). However, the decrease in these compounds after long-term exposure under high temperatures might suggest a depletion of the phenolic pool in A. *palmatum* because of excessive consumption during ROS scavenging or reduced biosynthetic capacity because of a continuous shortage of time (do Nascimento & Fett-Neto, 2010). Such a situation is also characteristic in other studies because the antioxidant capacity of a plant decreases under long-term exposure to stress (Aroca *et al.*, 2003; Sehgal *et al.*, 2017).

The chlorophyll content, another important indicator of plant health, was severely reduced by prolonged heat exposure. The doubling of the chlorophyll B content after 1 day indicates that the plant initially increases its photosynthetic machinery to counteract stress. However, the subsequent significant decreases in chlorophyll A and B after 2 and 3 days of heat treatment showed that prolonged exposure inhibited photosynthesis, and the amount of energy produced by the plant decreased as the overall plant vigour decreased (Primka & Smith, 2019; Yamane *et al.*, 2022). This decrease in the chlorophyll content is likely the result of damage to the photosynthetic proteins and membranes due to the high temperature, as this is well-known deleterious effect of heat stress (Hayat *et al.*, 2010). Antioxidant assays also indicate the effect of high temperatures on the antioxidant capacity of A. *palmatum*. In this study, one day after heat treatment caused the peak antioxidant activity, with increases in both the DPPH and ABTS scavenging activity. This indicates that the ability of the plant to nullify ROS increased with the increase in phenylpropanoid compounds, which are also antioxidants according to Gumul *et al.* (2007). However, the assay also showed that heat treatment for 3 days was detrimental to the plant because the antioxidant scavenging of both compounds and the reducing power were reduced by extended stress exposure (Imlay, 2003; Król *et al.*, 2015).

Thus, it can be concluded that A. *palmatum* has a rather intricate response to high-temperature stress. Short-term exposure triggers protective mechanisms that counteract the stress. These include phenylpropanoid biosynthesis, the increased activity of antioxidant systems, and augmented chlorophyll content. However, prolonged exposure leads to a decrease in protective responses, indicating that the plant's longitudinal physiological health deteriorates under stress. Despite the limited scope of the investigation, these findings contribute to the general understanding of how temperature stress impacts ornamental trees. They can also be used in the context of plant breeding or engineering when the goal is to improve the organisms' resilience to climate change.

CONCLUSION

In this study, the physiological and biochemical characteristics of phenylpropanoid biosynthesis, antioxidant activity, and chlorophyll content were observed in A. *palmatum* in response to high-temperature stress. The plant was observed under moderate heat exposure and showed significant phenylalanine ammonia-lyase activity and similar phenylpropanoids accumulation. The contents of phenylpropanoids, such as catechin, caffeic acid, and rutin, in the plant were significantly higher when exposed to heat for 1 or 2 days. Moreover, the measurement of the antioxidant activity using the DPPH and ABTS assays confirmed that 1 day at a high temperature was most favourable for the plant, as its protective activity against oxidative stress was highest. Nonetheless, 3 days of exposure to high temperature reduced phenylpropanoids accumulation, TPC, and TFC, as signs of a reduced response to oxidative stress in A. *palmatum*. In addition, the decrease in the levels of chlorophyll a indicates that prolonged exposure to heat negatively affects the physiological integrity of the plant.

FUNDING

This research was supported by Start up Pioneering in Research and Innovation (SPRINT) through the Commercialization Promotion Agency for R&D Outcomes (COMPA) grant funded by the Korea government (Ministry of Science and ICT) (2710001946) and this research was also carried out with the support of 'R&D Program for Forest Science Technology (Project No. 2021379B10-2123-BD02)' provided by Korea Forest Service (Korea Forestry Promotion Institute).

REFERENCES

Aroca, R., Irigoyen, J. J., & Sánchez-Díaz, M. (2003). Drought enhances maize chilling tolerance. II. Photosynthetic traits and protective mechanisms against oxidative stress. *Physiologia Plantarum, 117*(4), 540-549. https://doi.org/10.1034/j.1399-3054.2003.00065.x

Ashraf, M. A., Iqbal, M., Rasheed, R., Hussain, I., Riaz, M., & Arif, M. S. (2018). Environmental stress and secondary metabolites in plants: an overview. In P. Ahmad, M. A. Ahanger, V. P. Singh, D. K. Tripathi, P. Alam & M. N. Alyemeni (Eds.), *Plant Metabolites and Regulation under Environmental Stress* (pp. 153-167) Cambridge, USA: Academic Press. https://doi.org/10.1016/B978-0-12-812689-9.00008-X

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry, 72*, 1-20. https://doi.org/10.1016/j.plaphy.2013.05.009

Commisso, M., Toffali, K., Strazzer, P., Stocchero, M., Ceoldo, S., Baldan, B., Levi, M., & Guzzo, F. (2016). Impact of phenylpropanoid compounds on heat stress tolerance in carrot cell cultures. *Frontiers in Plant Science, 7*, 1439. https://doi.org/10.3389/fpls.2016.01439

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell, 7*(7), 1085-1097. https://doi.org/10.1105/tpc.7.7.1085

do Nascimento, N. C., & Fett-Neto, A. G. (2010). Plant secondary metabolism and challenges in modifying its operation: an overview. In A. G. Fett-Neto (Eds.), *Plant Secondary Metabolism Engineering: Methods and Applications, Methods in Molecular Biology*; (pp. 1-13) New York, USA: Humana Press. https://doi.org/10.1007/978-1-60761-723-5_1

Elavarthi, S., & Martin, B. (2010). Spectrophotometric assays for antioxidant

enzymes in plants. In R. Sunkar (Eds.), *Plant Stress Tolerance: Methods and Protocols* (pp. 273-280) Stillwater, USA: Springer. https://doi.org/10.1007/978-1-60761-702-0_16

Elnaggar, A., Tsombou, F. M., Hussain, M. I., Almehdi, A. M., Abideen, Z., Yong, J. W. H., & El-Keblawy, A. (2024). *Citrullus colocynthis* regulates photosynthetic and biochemical processes to develop stress resilience and sustain growth under sub-optimal temperatures. *Plant Stress, 12*, 100502. https://doi.org/10.1016/j.stress.2024.100502

Gumul, D., Korus, J., & Achremowicz, B. (2007). The influence of extrusion on the content of polyphenols and antioxidant/antiradical activity of rye grains (*Secale cereale* L.). *Acta Scientiarum Polonorum Technologia Alimentaria, 6*(4), 103-111.

Haider, S., Iqbal, J., Naseer, S., Yaseen, T., Shaukat, M., Bibi, H., Ahmad, Y., Daud, H., Abbasi, N. L., & Mahmood, T. (2021). Molecular mechanisms of plant tolerance to heat stress: current landscape and future perspectives. *Plant Cell Reports, 40*, 2247-2271. https://doi.org/10.1007/s00299-021-02696-3

Hayat, Q., Hayat, S., Irfan, M., & Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: a review. *Environmental and Experimental Botany, 68*(1), 14-25. https://doi.org/10.1016/j.envexpbot.2009.08.005

Herrmann, K. M. (1995). The shikimate pathway: early steps in the biosynthesis of aromatic compounds. *The Plant Cell, 7*(7), 907-919. https://doi.org/10.1105/tpc.7.7.907

Imlay, J. A. (2003). Pathways of oxidative damage. *Annual Review in Microbiology, 57*, 395-418. https://doi.org/10.1146/annurev.micro.57.030502.090938

Kong, J.-Q. (2015). Phenylalanine ammonia-lyase, a key component used for phenylpropanoids production by metabolic engineering. *RSC Advances, 5*(77), 62587-62603. https://doi.org/10.1039/C5RA08196C

Król, A., Amarowicz, R., & Weidner, S. (2015). The effects of cold stress on the phenolic compounds and antioxidant capacity of grapevine (*Vitis vinifera* L.) leaves. *Journal of Plant Physiology, 189*, 97-104. https://doi.org/10.1016/j.jplph.2015.10.002

Lim, J., Kim, K., Kwon, D. Y., Kim, J. K., Sathasivam, R., & Park, S. U. (2024). Effects of different solvents on the extraction of phenolic and flavonoid compounds, and antioxidant activities, in *Scutellaria baicalensis* hairy roots. *Horticulturae, 10*(2), 160. https://doi.org/10.3390/horticulturae10020160

Liu, S., Zenda, T., Tian, Z., & Huang, Z. (2023). Metabolic pathways engineering for drought or/and heat tolerance in cereals. *Frontiers in Plant Science, 14*, 1111875. https://doi.org/10.3389/fpls.2023.1111875

Ortiz, A., & Sansinenea, E. (2023). Phenylpropanoid derivatives and their role in plants' health and as antimicrobials. *Current Microbiology, 80*, 380. https://doi.org/10.1007/s00284-023-03502-x

Primka, E. J., & Smith, W. K. (2019). Synchrony in fall leaf drop: chlorophyll degradation, color change, and abscission layer formation in three temperate deciduous tree species. *American Journal of Botany, 106*(3), 377-388. https://doi.org/10.1002/ajb2.1247

Rajput, V. D., Harish, Singh, R. K., Verma, K. K., Sharma, L., Quiroz-Figueroa, F. R., Meena, M., Gour, V. S., Minkina, T., Sushkova, S., & Mandzhieva, S. (2021). Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology, 10*(4), 267. https://doi.org/10.3390/biology10040267

Rehman, A., Farooq, M., Naveed, M., Nawaz, A., & Shahzad, B. (2018). Seed priming of Zn with endophytic bacteria improves the productivity and grain biofortification of bread wheat. *European Journal of Agronomy, 94*, 98-107. https://doi.org/10.1016/j.eja.2018.01.017

Sathasivam, R., Choi, M., Radhakrishnan, R., Kwon, H., Yoon, J., Yang, S. H., Kim, J. K., Chung, Y. S., & Park, S. U. (2022). Effects of various *Agrobacterium rhizogenes* strains on hairy root induction and analyses of primary and secondary metabolites in *Ocimum basilicum*. *Frontier in Plant Science, 13*, 983776. https://doi.org/10.3389/fpls.2022.983776

Sehgal, A., Sita, K., Kumar, J., Kumar, S., Singh, S., Siddique, K. H. M., & Nayyar, H. (2017). Effects of drought, heat and their interaction on the growth, yield and photosynthetic function of lentil (*Lens culinaris* Medikus) genotypes varying in heat and drought sensitivity. *Frontiers in Plant Science, 8*, 1776. https://doi.org/10.3389/fpls.2017.01776

Solecka, D. (1997). Role of phenylpropanoid compounds in plant responses to different stress factors. *Acta Physiologiae Plantarum, 19*, 257-268. https://doi.org/10.1007/s11738-997-0001-1

Stapleton, A. E. (1992). Ultraviolet radiation and plants: burning questions. *The Plant Cell, 4*(11), 1353-1358. https://doi.org/10.1105/tpc.4.11.1353

Sun, S., Zhang, Q., Yu, Y., Feng, J., Liu, C., & Yang, J. (2022). Leaf coloration in *Acer palmatum* is associated with a positive regulator *ApMYB1* with potential for breeding color-leafed plants. *Plants, 11*(6), 759. https://doi.org/10.3390/plants11060759

Wan, Y., Zhu, L.-H. & Li, D.-W. (2023). First report of *Diaporthe acuta* causing leaf blight of *Acer palmatum* in China. *Plant Disease, 107*(10), 3316. https://doi.org/10.1094/PDIS-10-22-2425-PDN

Yamane, K., Nishikawa, M., Hirooka, Y., Narita, Y., Kobayashi, T., Kakiuchi, M., Iwai, K., & Iijima, M. (2022). Temperature tolerance threshold and mechanism of oxidative damage in the leaf of *Coffea arabica* 'Typica'under heat stress. *Plant Production Science, 25*(3), 337-349. https://doi.org/10.1080/1343943X.2022.2064309