

Anthocyanin-Related Gene Expression and Heat Tolerance in Kale

Jingyin Mai, Weixi Li*

Guangdong Country Garden School, Foshan, China

Email: *liweixi@bgyschools.cn

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Abstract

This study investigated the relationship between anthocyanin-related gene expression, pigment accumulation, and heat tolerance in kale (*Brassica oleracea*) with different leaf colors: green, pink, and purple. The expression levels of three key genes—bHLH1, DFR4, and ANS4—were analyzed using qPCR, alongside measurements of anthocyanin content and cell membrane stability under high-temperature stress. Purple kale exhibited the highest expression levels of ANS4 and DFR4, at more than 40-fold and 45-fold, respectively, relative to the green group, and the highest anthocyanin content (18.09 mg/g FW). Heat tolerance tests showed that green kale had the highest critical temperature (Tcrit, 63.26°C), while pink and purple kale showed lower Tcrit values of 59.58°C and 59.29°C, respectively. These findings indicate an association between higher expression of anthocyanin-related genes and greater anthocyanin accumulation, while higher anthocyanin content coincided with lower membrane thermostability under the tested conditions. The observed differences may reflect the combined effects of anthocyanin accumulation and other physiological differences among the kale groups. This study provides molecular and physiological insights into the variation in leaf coloration and heat responses among kale with different leaf colors.

Keywords

Anthocyanin, Gene Expression, Heat Tolerance, Kale (*Brassica oleracea*), Cell Membrane Stability

1. Introduction

Brassica oleracea is a cruciferous leafy vegetable that has gained increasing attention in recent years [1]. In the market, kale is always promoted for its “antioxidant,” “weight loss,” and “laxative” properties [2] and is thus widely cultivated in

various climatic conditions [3]. Different varieties of kale have significant differences in leaf morphology and color, including green, pink, and purple types [4]. These differences not only affect their appearance but also reflect the physiological differences among different varieties to a certain extent [5]. Moreover, with changes in the planting environment, particularly in high-temperature conditions, the growth performance and adaptability of different types of kale may also vary [6]. Therefore, further investigation on these characteristics is necessary.

These differences in leaf coloration are primarily attributed to the accumulation of anthocyanins [7], which are important plant pigments responsible for red, purple, and blue coloration in plant tissues [8]. Anthocyanins are synthesized through a comprehensive and complex biosynthetic pathway regulated by multiple regulatory genes [9], their expression levels are associated with differences in pigment accumulation in leaves [10]. In addition to their role in determining plant coloration, anthocyanins are also involved in plant responses to environmental stress [11]. Previous studies have suggested that environmental factors, particularly temperature, can significantly affect anthocyanin biosynthesis and stability [12]. However, the relationship between anthocyanin accumulation, gene expression, and plant adaptability under high-temperature conditions remains not fully understood.

The *bHLH1* gene, which functions as a transcription factor regulating the expression of anthocyanin-related genes in kale leaves, has been reported to play an important role in the control of anthocyanin biosynthesis. In addition, the *ANS4* and *DFR4* genes, which encode key enzymes involved in the anthocyanin synthesis pathway, are also essential for pigment accumulation in kale [13]. Based on these findings, gene-specific primers targeting *bHLH1*, *ANS4*, and *DFR4* were designed for subsequent qPCR analysis to examine the relative expression levels of anthocyanin-related genes in kale leaves with different coloration. By comparing gene expression patterns among green, pink, and purple varieties, this study aims to further understand how transcriptional regulation contributes to differences in anthocyanin accumulation and provide a molecular basis for explaining the variation in leaf coloration and its potential association with physiological traits such as heat tolerance.

Kale leaves with different coloration were selected to investigate the relationship between anthocyanin-related gene expression, pigment accumulation, and heat tolerance. The anthocyanin content in different kale varieties was quantified, and their heat tolerance was evaluated by assessing cellular responses under high-temperature conditions. In addition, the expression levels of key genes involved in anthocyanin biosynthesis, including *bHLH1*, *DFR4*, and *ANS4*, were analyzed using the qPCR technique. By investigating molecular data with physiological measurements, we aim to explore how differences in gene expression influence anthocyanin accumulation and further affect heat tolerance among different kale varieties. It is hypothesized that purple kale shows higher expression levels of anthocyanin biosynthesis genes, while higher anthocyanin accumulation may not

necessarily correspond to stronger heat tolerance under high-temperature conditions.

2. Methodology

2.1. Materials Sampling

Kale plants with different leaf colors (purple, green, and pink) were purchased from the same online supplier (Taobao). The three color groups were confirmed to be from the same cultivar and were purchased in the same batch. The plants were approximately 4 - 6 weeks old at the time of sampling and had 3 - 4 fully expanded young leaves. Therefore, the three color groups were treated as different color phenotypes of the same cultivar under the same experimental conditions. The observed differences in leaf coloration were considered to be primarily associated with differences in anthocyanin accumulation. However, other physiological or environmental factors may also have contributed to the observed differences among the color groups.

For each color group, six individual plants were selected. Two plants of the same color were transplanted into one pot, resulting in three pots per color group. Each pot was approximately 15 cm in diameter and contained commercially available nutrient-rich soil. The pots were placed in a controlled indoor environment and maintained for three days at 24°C under a 12-hour light/12-hour dark photoperiod. Light intensity was kept consistent during the daytime, and all plants received equal amounts of water daily to maintain adequate soil moisture. After three days, samples were collected from the third to fourth fully expanded leaves of each plant between 9:00 and 11:00 AM to reduce variation associated with diurnal changes. The distal portion of each leaf was carefully excised using sterilized scissors, immediately transferred into tubes, rapidly frozen in liquid nitrogen, and stored at -80°C until further analysis.

2.2. RNA Extraction

Total RNA was extracted from plant samples using the Plant RNA Kit (Omega, Cat. No. R6827-02) following the manufacturer's instructions. The procedure was as follows:

- 1) All 18 samples (6 of green, 6 of pink, 6 of purple) were removed from liquid nitrogen, and the samples were pulverized with a bead mill to ensure that all the leaf tissues were ground effectively. (*Purpose: to break open cells and release RNA while maintaining low temperature to inhibit RNase activity and prevent RNA degradation.*)
- 2) The ground tissue was transferred into 1.5 mL RNase-free microcentrifuge tubes.
- 3) 500 µL of RCL Buffer was added to each tube, and the samples were vortexed thoroughly, then kept on ice to prevent RNA degradation.
- 4) Samples were incubated at 55°C for 2 minutes, followed by centrifugation at 10,000 × g for 5 minutes.

- 5) The supernatant was transferred to a gDNA Filter Column placed in the corresponding collection tube, and centrifuged at $14,000 \times g$ for 2 minutes.
- 6) The filter column was discarded, and an equal volume of RCB Buffer was added to the collection tube; the solution was mixed thoroughly by pipetting. 700 μL of the mixture was transferred to a HiBind RNA Mini Column placed in a collection tube and centrifuged at $12,000 \times g$ for 1 minute. The flow-through was discarded, and the step was repeated until all the sample solution had passed through the column.
- 7) 400 μL of RWF Wash Buffer was added to the column, followed by centrifugation at $10,000 \times g$ for 30 seconds.
- 8) 500 μL of RNA Wash Buffer was added, followed by centrifugation at $10,000 \times g$ for 30 seconds; this step was repeated once.
- 9) The flow-through was discarded, and the column was centrifuged at maximum speed for 1 minute.
- 10) The column was placed into a new 1.5 mL microcentrifuge tube, and 50 μL of RNase-free water was added to the column membrane. After standing for 5 minutes, the tube was centrifuged at maximum speed for 1 minute. The column was discarded, and the RNA samples were stored at -20°C until further use.

2.3. Synthesis of cDNA

cDNA was synthesized using the **EvO M-MLV RT Mix Kit with gDNA Clean for qPCR** (AG, Cat. No. AG11728) according to the manufacturer's instructions. To eliminate any residual genomic DNA in the RNA samples, preventing false-positive signals during qPCR, the residual genomic DNA was removed by using the reaction mixture prepared as **Table 1**. The reaction was incubated at 42°C for 2 minutes. The reverse transcription reaction was performed in a reagent system as **Table 2** in a PCR thermocycler under the following conditions: 37°C for 15 minutes, followed by 85°C for 5 seconds. After completion, cDNA was stored at -20°C .

Table 1. Reagent system of removing residual genomic DNA.

Component	Volume
5 $\times$ gDNA Clean Reaction Mix	2 μL
Total RNA	1 μL
RNase-free water	10 μL

Table 2. Reaction system of reverse transcription.

Component	Volume
Reaction mixture	10 μL
5 $\times$ Evo M-MLVRT Reaction Mix*	4 μL
RNase free water	6 μL

2.4. Quantitative Real-Time PCR (qPCR)

Quantitative PCR was performed using the **SYBR® Green Premix Pro Taq HS qPCR Kit** (AG, Cat. No. AG11701), with **ACT** used as the internal reference gene. A total of four pairs of primers were designed the NCBI online primer tools (**Table 3**).

The reaction mixture was as prepared as **Table 4**. After preparing the reaction mixture, the PCR plate was sealed with a single-sided adhesive film and centrifuged at $3,700 \times g$ for 5 minutes to remove air bubbles and ensure uniform distribution (**Table 5**).

Ct value measures the number of cycles needed for the detection of gene expression, with lower Ct values representing higher expression levels.

qPCR quality control

RNA quality was assessed by measuring the A260/A280 ratio, which ranged from 1.8 to 2.1 across samples. Each biological sample was analyzed in three technical replicates. Amplification specificity was assessed using melting curve analysis, and each primer pair produced a single sharp melting peak. ACT Ct values ranged from 20.71 to 25.88 across all samples and were used as the reference for normalization. However, reference-gene stability was not formally evaluated using a dedicated stability analysis. No-template controls (NTCs) and no-reverse-transcription controls were not included in the qPCR analysis, which limited independent assessment of potential contamination and genomic DNA amplification.

Table 3. qPCR primers.

Primer pairs	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
1. <i>ACTIN</i>	CTTGACCTTGCTGGTCGTGA	AGTCTCCATCTCCTGCTCGT
2. <i>ANS4</i>	CCTCCAAGGACGTTTGCTCA	AACGTATCACAATGACACAATCCA
3. <i>bHLH1</i>	GCGTATGCGAGGAGGAATCA	GCGACTCTAATGTGGAAAGAGC
4. <i>DFR4</i>	CGTGGTTACTTTGTCCGTGC	TCCGTTTATGGCGTCATCGT

Table 4. qPCR reaction mixture preparation.

Component	Volume
2xSYBR @ Green Pro Taq HS Premix	5 µL
Forward primer	0.4 µL
Reverse primer	0.4 µL
cDNA template	4 µL
RNase-free water	10.2 µL
Total	20 µL

Table 5. qPCR amplification conditions.

Stage	Step	Temperature	Time
Hold stage	Initial denaturation	95.0°C	2 min
	Denaturation	95.0°C	5 s
PCR stage (40×)	Annealing	60.0°C	10 s
	Extension	72.0°C	15 s

Continued

Melt curve stage	Hold 1	95.0°C	15 s
	Hold 2	60.0°C	1 min
	Dissociation	95.0°C	1 s
Instrument settings	Reaction volume	20 µL	
	Cover temperature	105.0°C	

2.5. Anthocyanin Content Measurement

Approximately 50 mg of plant leaf tissue was weighed and transferred into a 2 mL microcentrifuge tube. A total of 600 µL of extraction solution (methanol:HCl = 99:1, v/v) was added, and the samples were incubated overnight at 40°C in the dark with gentle shaking. After incubation, 400 µL of deionized water and 400 µL of chloroform were added to each sample, followed by gentle inversion to mix thoroughly. The samples were then centrifuged at 12,000 rpm for 2 minutes. The supernatant was collected, and absorbance was measured at 530 nm and 657 nm using a spectrophotometer. Anthocyanin content was calculated using the following formula:

$$\text{Anthocyanin content (mg/g FW)} = (A_{530} - 0.25 * A_{657}) / \text{FW (g)}$$

Results are expressed as cyanidin-3-glucoside equivalents (mg C3GE/g FW) based on the molar extinction coefficient ($\epsilon = 26,900 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$) of cyanidin-3-glucoside. Six biological replicates were analyzed per color group.

2.6. Heat Resistance Measurement

A total of 5 mL of dd water was added into the measurement chamber. The background electrical conductivity was measured and ensured to be below 10 µS/cm before starting the experiment. The measurement chamber was inserted into the aluminum slot with slight pressure to confirm proper positioning and stable contact. A magnetic stirring bar was placed into the chamber to maintain continuous mixing during the measurement. The conductivity probe was also inserted into the solution, ensuring that it was completely immersed and did not attach to the walls of the chamber. Before adding the sample, the instrument parameters were set as: Conductivity range: 0 - 10 µS/cm, Ft parameter: 0 - 100. Leaf samples (approximately 1 cm in diameter) were cut using scissors and fixed in the frame, then inserted into the sample holder. The leaf samples were positioned to maintain a full contact with the measurement solution for effective electrolyte release. After sample insertion, the instrument parameter was adjusted to: Ft = 2000. The experiment was then started from room temperature (approximately 25°C) up to 70°C at a linear heating rate of 3°C /min. During the measurement, the solution was continuously stirred at a low speed, and electrical conductivity was monitored and recorded in time. Excessive stirring was avoided to prevent fluctuations in the readings. Six biological replicates were analyzed per color group, with three leaf discs per replicate. The critical temperature (Tcrit) was determined as the inflec-

tion point of the conductivity-temperature curve, where the rate of electrolyte leakage reaches its maximum. The curve was fitted using a Logistic equation of the form $Y = k/(1 + a \cdot e^{(-b \cdot x)})$, where Y is the relative conductivity, x is the temperature, and k , a , and b are fitted parameters. The changes in electrical conductivity were used to evaluate membrane stability and heat tolerance of the kale plant samples.

3. Results

3.1. qPCR Results Analysis

In this study, the expression levels of the anthocyanin biosynthesis-related genes in three kale color groups (green, pink, and purple) were quantitatively analyzed using qPCR technology, with six biological replicates per color group and three technical replicates per sample. *Actin* (*ACT*) was employed as the internal reference gene, and the green sample was used as the control group to calculate relative expression levels. The expression levels of the *ANS4* gene showed significant variation across the different colored kale samples, as calculated using the $2^{(-\Delta\Delta Ct)}$ method. Green Samples (Control Group): The expression of *ANS4* was lowest comparing to the others in the green samples. The initial relative expression level for this group was defined as the baseline (approximately 1.0). Pink Samples: The expression of *ANS4* was significantly higher in the pink samples comparing with the green samples, with average expression levels approximately 10-fold higher than those of the green samples. Purple Samples: The highest expression levels were observed in the purple samples. Based on the calculated data, the expression of *ANS4* in the purple samples was markedly upregulated ($P < 0.05$), reaching a relative expression level more than 40-fold that of the green sample (**Figure 1**).

The expression profile of *bHLH1* revealed striking differences associated with leaf coloration in kale. While the green samples exhibited baseline expression levels, the gene was significantly up-regulated in both pink and purple samples. Specifically, *bHLH1* expression in pink samples was approximately 15-fold higher than that of the green control. The purple samples displayed the highest transcript abundance, with a 21-fold increase relative to the control group. The statistical significance of these elevations was confirmed by independent t-tests ($P < 0.05$) (**Figure 1**).

The relative expression levels of *DFR4* also exhibited significant variation correlating with leaf pigmentation. Using the green samples as a control (baseline expression of 1), a progressive increase in transcript abundance was observed. The pink samples showed a notable 15-fold elevation compared to the control. Notably, the purple samples displayed a dramatic 45-fold increase in expression levels relative to the green samples. *DFR4* expression showed an increasing pattern across the green, pink, and purple groups, with higher expression observed in the more intensely colored groups. ($P < 0.05$ for pink, purple and green) (**Figure 1**).

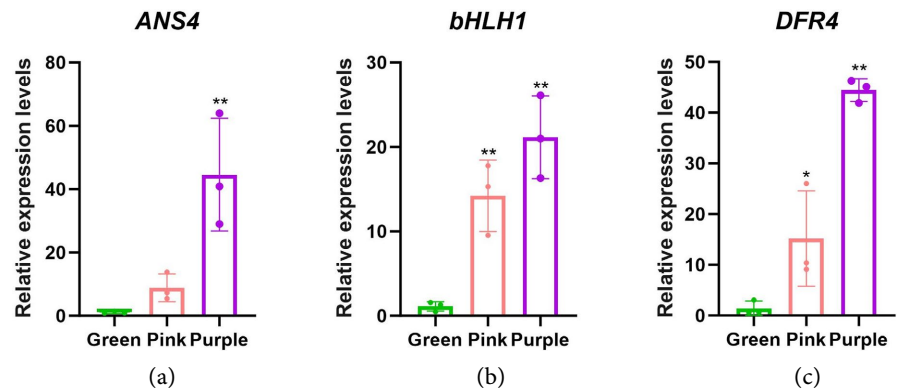


Figure 1. Relative expression levels of *ANS4*, *bHLH1*, and *DFR4* in green, pink, and purple kale. Asterisks indicate significant differences compared to the green group ($P < 0.05$). Data are mean $\pm$ SD.

3.2. The Content of Anthocyanin

The total anthocyanin content was quantified to investigate the physiological basis of the distinct leaf colorations in the three kale variants (**Figure 2**). The results revealed that Anthocyanin content increased with the intensity of leaf pigmentation across the three color groups. In the green kale samples, anthocyanin levels were minimal, serving as the baseline for this study (average: 0.23 0.04 mg/g FW). In contrast, the colored variants showed a substantial increase in pigment deposition. The anthocyanin content in the pink samples was measured at an average of 3.33 0.51 mg/g FW. The most prominent accumulation was observed in the purple samples, which reached an average concentration of 18.09 mg/g FW. Statistical analysis confirmed that these differences were highly significant ($P < 0.05$), demonstrating that the progressive intensification of leaf pigmentation from green to pink and purple was consistent with substantial anthocyanin accumulation (**Figure 2**).

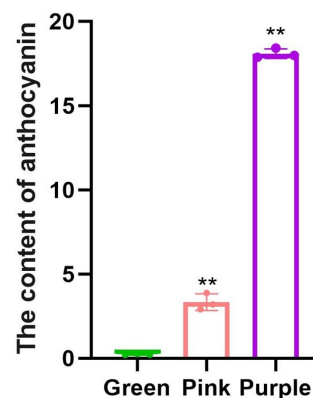


Figure 2. Total anthocyanin content in green, pink, and purple kale leaves. Asterisks indicate significant differences compared to the green group ($P < 0.05$). Data are mean $\pm$ SD.

3.3. Heat Resistance Test and Cell Membrane Stability Analysis

To evaluate the heat tolerance of different kale variants, the relationship between temperature and electrolyte leakage (measured as conductivity) was analyzed to

identify the critical thermal points (Figure 3). The Conductivity Curvature peak represents the critical temperature (T_{crit}) at which cell membrane integrity begins to fail, leading to rapid electrolyte efflux. As illustrated in Figure 3, significant variations in membrane thermostability were observed among the three samples. The green samples exhibited the highest thermal stability, with a critical point identified at 63.26 degree. The critical temperature for the pink samples was recorded at 59.58 degree, which is significantly lower than that of the green control. Similarly, the purple samples revealed a critical point of 59.29 degree, a value closely aligned with the pink samples but notably lower than the green variant.

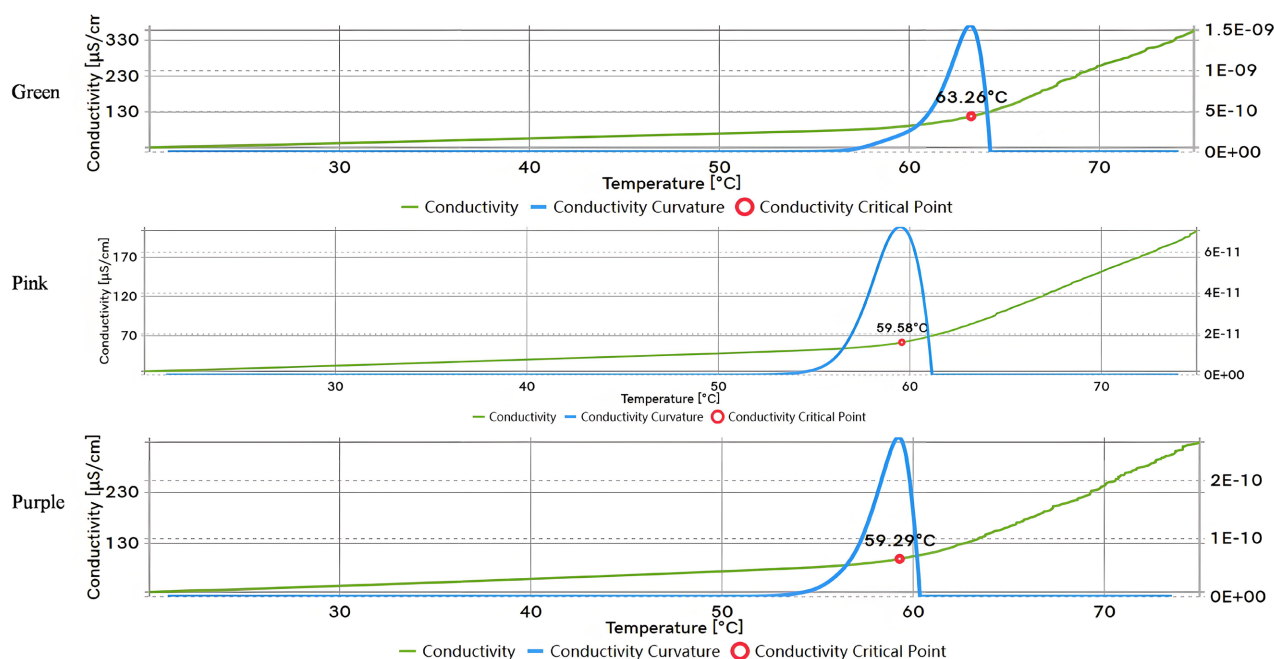


Figure 3. Heat resistance test and cell membrane stability analysis of green, purple, and pink kale leaves.

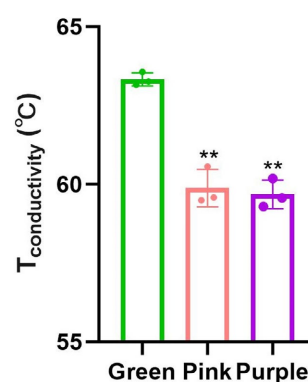


Figure 4. Critical temperatures of green, pink, and purple kale leaves. Asterisks indicate significant differences compared to the green group ($P < 0.05$). Data are mean $\pm$ SD.

The thermal stability of the cell membranes across the three kale varieties was further evaluated by determining the critical temperature ($T_{conductivity}$), defined as the threshold where membrane integrity is compromised (Figure 4). Consistent

with the conductivity-temperature profiles, the green kale samples demonstrated the highest thermal threshold, with a $T_{\text{conductivity}}$ of approximately 63.26°C. In contrast, both the pink and purple samples exhibited significantly lower critical temperatures, recorded at 59.58°C and 59.29°C, respectively. Statistical analysis revealed that the reduction in thermal tolerance for both colored variants was highly significant compared to the green control ($P < 0.05$) (**Figure 4**).

4. Discussion

4.1. qPCR Discussion

Through the data analyzed in qPCR process, there is a positive association between leaf coloration intensity and the expression of anthocyanin-related genes [14]. The darker the color of the leaves of kale, the expression of anthocyanin-related genes will increase accordingly [15]. The green samples with less color content show relatively low expression of anthocyanin-related genes, and as the color gets darker, the purple samples indicate the highest expression of anthocyanin-related genes. The results of this study suggest that the expression levels of anthocyanin biosynthesis-related genes (*ANS4*, *bHLH1*, and *DFR4*) increase with the intensity of leaf coloration in kale [16]. This relationship can be explained by the role of these genes in the anthocyanin biosynthesis pathway. *bHLH1* serves as a transcription factor regulating the expression of anthocyanin-related genes. *ANS4* and *DFR4* are employed as key enzymes for anthocyanin synthesis in kale [17]. The increased expression of *bHLH1* likely played a regulatory role in anthocyanin biosynthesis by activating *ANS4* and *DFR4*, which encode key enzymes responsible for anthocyanin synthesis. Therefore, their regulation promotes more intense darker coloration, which is extensively observed in the purple samples.

4.2. The Content of Anthocyanin Discussion

As shown in **Figure 2**, the highest content of anthocyanin occurs in purple samples, medium in pink samples, and lowest in the green samples. This reveals a positive association between leaf coloration intensity and anthocyanin content in the leaves [15]. This discovery can be explained by anthocyanin being a plant pigment that controls the coloration of the leaves, with most anthocyanin concentrated in the leaves, consistent with previous studies [18]. Darker colored leaves are associated with higher levels of anthocyanin, regulated by the upregulation of genes involved in the biosynthesis pathway.

4.3. Heat Resistance Test and Cell Membrane Stability Discussion

The heat resistance measurements showed that the green group had the highest T_{crit} (63.26°C), whereas the pink and purple groups had lower T_{crit} values of 59.58°C and 59.29°C, respectively. These results indicate greater membrane thermostability in the green group under the tested conditions [19] [20]. The lower T_{crit} values observed in the pink and purple groups coincided with their higher anthocyanin contents. Anthocyanins are known to be sensitive to temperature

and may undergo degradation under high-temperature conditions [21] [22]. Therefore, the observed association between higher anthocyanin content and lower membrane thermostability may be related to differences in pigment stability. However, because the specific cultivars and genetic backgrounds of the plants were not available or controlled, the lower Tcrit values cannot be attributed solely to anthocyanin accumulation. Other physiological differences among the kale groups may also have contributed to the observed variation in heat tolerance [23] [24].

4.4. Relationships between Gene Expression, Pigment Accumulation, and Heat Tolerance

This study investigated the relationship between anthocyanin-related gene expression, pigment accumulation, and heat tolerance in kale and found significant differences among green, pink, and purple samples. The qPCR results showed that the upregulation of the anthocyanin biosynthesis pathway was associated with more intense leaf coloration, which helps explain the higher anthocyanin content observed in purple samples [25]. In addition, anthocyanin content was closely related to leaf coloration, indicating that darker leaves generally accumulate higher levels of anthocyanin [26]. However, in the present study, higher anthocyanin-related gene expression and anthocyanin accumulation coincided with lower Tcrit values, indicating lower membrane thermostability under the tested conditions [27]. Green leaves showed relatively higher heat tolerance, while pink and purple leaves, which contained higher anthocyanin levels, exhibited lower tolerance to elevated temperatures [28]. This pattern may be related to the thermal instability of anthocyanins, although other physiological differences among the kale groups may also have contributed to the observed variation [29].

4.5. Limitations of This Study

The initial experimental design aimed to investigate the relationship between anthocyanin-related gene expression and antioxidant capacity in kale, with the goal of exploring the nutritional value of different kale varieties [30]. However, due to time constraints and the unavailability of the antioxidant capacity assay kit, the research focus was shifted from anthocyanin gene expression and antioxidant capacity to anthocyanin gene expression and heat tolerance, as the latter could be more readily measured in the laboratory. As a result, the relationship between anthocyanin gene expression and antioxidant capacity in kale remains to be further elucidated. The study also analyzed only a limited number of anthocyanin biosynthesis-related genes, which may not fully represent the complexity of the anthocyanin biosynthetic pathway.

4.6. Future Study Direction and Applications in Cultivation

Future studies could further investigate the relationship between anthocyanin-related gene expression and antioxidant capacity to provide a more comprehensive understanding of the nutritional characteristics of kale. RNA sequencing could

also be used to examine global gene expression changes under heat stress and identify pathways associated with differences in heat responses among kale groups [26]. Protein-level analyses, such as Western blotting, could be conducted to determine whether differences in transcript abundance are reflected at the protein level for key enzymes involved in anthocyanin biosynthesis, such as *DFR4* and *ANS4* [31]. The findings of this study may provide a preliminary reference for investigating kale responses to high-temperature environments such as those found in Guangdong [32]. However, the observed differences in Tcrit were obtained under the specific experimental conditions used in this study and may provide only limited implications for kale cultivation under the high-temperature conditions of Guangdong [27]. Further studies using larger sample sizes, independent experimental units, longer-term heat treatments, and field or greenhouse experiments are needed to evaluate the cultivation performance of different kale color phenotypes under high-temperature conditions.

5. Conclusion

This study aims to study the relationship between anthocyanin-related gene expression levels, pigment accumulation, and heat tolerance in green, pink, and purple kale leaves. By designing primers, conducting qPCR experiments, and measuring anthocyanin content, the results showed a positive relationship between gene expression levels and pigment accumulation. In contrast, an opposite trend was observed in heat tolerance, where samples with higher anthocyanin content tended to exhibit lower heat tolerance based on measurements of cell membrane stability. These findings suggest that while anthocyanin accumulation contributes to leaf coloration, it may not enhance heat resistance under high-temperature conditions. This study provides a clearer understanding of how molecular-level changes are associated with physiological responses in plants. In addition, the results have certain value in the field of agriculture. From these findings, it can be inferred that varieties such as purple kale may not be suitable for cultivation in regions with consistently high temperatures, such as Guangdong, and that crop selection should consider both environmental conditions and physiological traits.

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Author Contributions

Conceptualization, Jingyin Mai and Weixi Li; methodology, Jingyin Mai and Weixi Li; software, Jingyin Mai; validation, Jingyin Mai; formal analysis, Jingyin Mai; investigation, Jingyin Mai; resources, Jingyin Mai; data curation, Jingyin Mai; writing—original draft preparation, Jingyin Mai; writing—review and editing, Jingyin Mai and Weixi Li; visualization, Jingyin Mai; supervision, Weixi Li;

project administration, Jingyin Mai and Weixi Li; funding acquisition, Jingyin Mai. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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